RESEARCH ARTICLE

A Cost Analysis on Multi - Item Inventory Model for Factory Outlets with Constraint under Ranking Asteroid Fuzzy Set

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ABSTRACT

A multi-item inventory model for factory outlet in crisp and fuzzy sense are formulated in the fuzzy environment with stowage space under one constraint has been considered. In this model, demand is constant and is related to the unit stowage space. The asteroid fuzzy set is defined and is properties are given. The parameters involved in this model represented by asteroid fuzzy set. The average total cost is defuzzify by ranking method. The analytical expressions for maximum inventory level and average total cost are derived for the proposed model by using nonlinear programming technique. A numerical example is presented to illustrate the results.

Keywords: Factory outlets, Asteroid fuzzy set, Multi items, Stowage space constraint, Maximum Stowage space, ranking fuzzy set.

INTRODUCTION

Cost parameters, objective functions, and decision makers' constraints are all imprecise in most real-world situations. The classical (EOQ) inventory problem is defined as the problem of determining the optimal order quantity under relatively stable conditions. This EOQ problem with varying variance had been solved for several years and

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published since 1915 by a number of researchers. F. W. Harries (1913) [1], E.W. Taft (1918) [2], and G. Hadley & T.M. Whit in (1958) [3] discuss two major assumptions in the classical EOQ models: the demand rate is constant and deterministic. Uncertainties are treated as randomness in conversional inventory models, and they're dealt with using probability theory. However, in some cases, uncertainties are caused by fuzziness, and the fuzzy set theory can be used in these situations. The fuzzy inventory model with storage space and budget constraints was discussed by Shuo-yan Chow and Peterson C. Julian (2009)[4]. Reza mogh dani (2019)[5] discussed a multi-item EPQ model that was developed for multi-packaging delivery by taking into account warehouse space constraints and the number of total orders, and the resulting fuzzy model was simplified using α-cuts and variable changes.

A factory outlet is outlined as a factory shop and it is a store where manufacturers sell their products directly to the public at steep discounts. Because not all of a company's products are of high quality, they cannot be sold in retail stores. Manufacturers have initiated to produce products specifically for outlet centres in order to avoid competing with their retail outlets, thanks to the industrial development of outlet centres. Adriana F.Gabor,jan-kees van Ommeren, Ondrei Sleptchenko [9] discussed an inventory model for an Omni channel retailer, that is, a retailer that sells items both via brick-and-mortar stores and online. In the crisp environment, all the parameters related to the model such as consumable cost, employee cost, security cost, wastage cost, admin cost, marketing cost, demand rateareknown and have uncertain value. While some trading scenarios apply to such conditions, in fact most scenarios and parameters and variables are very uncertain in fast changing market conditions. These parameters and variables are referred to as ambiguous parameters in such cases. Clarification acknowledges the reliability of the model by allowing ambiguity throughout the system, which brings it closer to reality.

A variety of methods have been proposed in the literature for sorting obscure numbers. Nirmal Kumar Mandal [10] proposed vague numerical cost parameters. A. Faritha Asma [6] decribed the fuzzy inventory model subject to constraints has been transformed in to the crisp inventory problem using Robust's ranking indices. P.Kasthuri, P. Vasanthi (2011)[7] developed with three constraints and have been solved by Kuhn tucker conditions. Roy and Maiti [8]. explored existing problems in their solution procedure for Kuhn–Tucker's method . Although there are some comparative studies, it is not yet known whether similar ranking methods are still in use today, and they have the potential to introduce an ambiguous set of ranking asteroids. The obscure inventory sample for factory sales outlets was obtained using the ranking asteroid ambiguous set.

Till now, there is no literature by using asteroid fuzzy sets. This paper developed the fuzzy inventory model by using asteroid fuzzy set. In a realistic situation, the total expenditure for an inventory model and the space available to store the inventory may be limited. The inventory costs, consumable cost, employee cost, security cost, wastage cost, admin cost, marketing cost, and temporary stowage space cost, may be flexible with some vagueness for their values. The ambiguity of the above parameters necessitates analyzing the inventory problem in a fuzzy environment. The inventory of multiple items for factory outlets is the subject of this article. The asteroid ambiguous is used to represent the cost parameters. The model is distorted by the ranking system, which determines the average total cost. There was a stowage space constraint in this situation. Finally, a numerical example of the sample and sensitivity analysis is given.

1. PRELIMINARIES

Definition (Consumable cost)
The cost associated with consumer goods (e.g., food and clothing) used for everyday life is used up or exhausted during their consumption, retailers often make more profit by selling consumer goods ranging from non-recycled staples. The demand for this specialty has increased as a consequence of current corporate scandals.

Definition (Security cost)
It means the cost of reimbursing the City for ordinary, necessary and reasonable direct costs of providing security services within the District.

Definition (Employee cost)
The total costs of hiring a person are known as employee expenses. It varies depending on the country, industry, and profession.

Definition (Admin cost)
Admin costs are the costs incurred only by running a business or hotel called overhead costs or fixed costs. Examples of administrative expenses include taxes, rent, insurance, license fees, utilities, accounting and legal boards, administrative staff, and facility maintenance.

Definition (Marketing cost)
Cost associated with marketing is the system of selling an item. For example, determining its price, the areas in which it should be offered and how it should be advertised. The cost of marketing explains the cost of changing the title and the cost of moving the goods to the customer.

Definition (Wastage cost)
Waste is the amount of raw material lost in the production process. This may include losses due to shrinkage, scraping or evaporation. When the actual amount of waste exceeds the standard waste cost, it appears as a negative amount or utility variance in the business expense statement.

Definition (Factory outlet)
Factory Outlet, also known as factory Shop, is a store where manufacturers sell their products directly to the public at steep discounts. The Factory outlet center is a manufacturer-owned store that sells shares of the company directly to the public. A factory stores inventory could be first class inventory or cancelled, irregular, cancelled orders with extremely low prices.

ASSUMPTION AND NOTATIONS
ASSUMPTION

i. Multi item will be considered.
ii. Demand rate is uniform.
iii. Shortages are not allowed
iv. Time horizon is finite.
v. The production rate is always greater than demand rate.
vi. Stowage space constraint allowed.

NOTATION

The following are for the ith item (i= 1,2,3,....N)

N	-	No. of items
Q_i	-	Outlet quantity (Decision variable)
q_i	-	Sales quantity (Decision variable)
R_i	-	Demand is constant
C_{c_i}	-	Consumable cost per unit per unit time

S_{c_i}	-	Security cost per unit per unit time
E_{c_i}	-	Employee cost
A_{c_i}	-	Admin cost
M_{c_i}	-	Marketing cost
W_{c_i}	-	Wastage cost
s_i	-	Fuzzy stowage space required by each unit (in sq.mt)
T_c(or) C (q$_i$,Q$_i$)		- Fuzzy Total cost per unit time
$\tilde{C}_{c_i}$	-	Fuzzy Consumable cost per unit per unit time
$\tilde{S}_{c_i}$	-	Fuzzy Security cost per unit per unit time
$\tilde{E}_{c_i}$	-	Fuzzy Employee cost
$\tilde{A}_{c_i}$	-	Fuzzy Admin cost
$\tilde{M}_{c_i}$	-	Fuzzy Marketing cost
$\tilde{W}_{c_i}$	-	Fuzzy Wastage cost
$\tilde{s}_i$	-	Stowage space required by each unit (in sq.mt)
$\tilde{s}_w$	-	Maximum available stowage space for factory out let (in sq.mt)
$\tilde{T}_c$ (or) $\tilde{C}(q_i, Q_i)$	-	Fuzzy Totalcostperunittime

The factory outlet model is constructed by using the above assumptions and notations.

MATHEMATICAL MODEL IN CRISP ENVIRONMENT

Total cost = $\displaystyle\sum_{i=1}^{n}$ [Consumable cost + Security cost + Employee cost + Admin cost +

Marketing cost + Wastage cost]

$$T_C(or)C(q_i,Q_i) = \sum_{i=1}^{n}\left(\frac{1}{2}C_{C_i}\frac{q_i^2}{Q_i} + \frac{1}{2}S_{C_i}\frac{q_i^2}{Q_i} + \frac{E_{C_i}R_i}{Q_i} + \frac{A_{C_i}R_i}{Q_i} + \frac{M_{C_i}R_i}{Q_i} + \frac{1}{2}W_{C_i}\frac{(Q_i-q_i)^2}{Q_i}\right) \qquad 4.1$$

The problem is stated that minimize the total cost (TC), subject to constraint

$$\sum_{i=1}^{n}s_iQ_i \leq S_m$$

Minimize TC

$$\sum_{i=1}^{n}s_iQ_i \leq S_m \qquad\qquad 4.2$$

Using Lagrange multipliers method, the Lagrange function is

$$L(q_i,Q_i,\lambda) = \sum_{i=1}^{N}\left(\frac{1}{2}C_{C_i}\frac{q_i^2}{Q_i} + \frac{1}{2}S_{C_i}\frac{q_i^2}{Q_i} + \frac{E_{C_i}R_i}{Q_i} + \frac{A_{C_i}R_i}{Q_i} + \frac{M_{C_i}R_i}{Q_i} + \frac{1}{2}W_{C_i}\frac{(Q_i-q_i)^2}{Q_i}\right) - \lambda(\sum_{i-1}^{N}s_iQ_i - S_m) \qquad 4.3$$

By using Kuhn-Tucker necessary condition in (4.3)
Differentiate the equation 4.3 with respect to q$_i$ and equal to zero

ie) $\dfrac{\partial L(q_i,Q_i,\lambda)}{\partial q_i} = 0$

$$q_i = \left(\frac{W_{C_i}}{C_{C_i} + S_{C_i} + W_{C_i}}Q_i\right) \text{, i=1,2,3…N} \qquad 4.4$$

Differentiate the equation 4.3 with respect to Q$_i$ and equal to zero

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ie) $\dfrac{\partial L(q_i, Q_i, \lambda)}{\partial Q_i} = 0$

$W_{C_i} Q_i^2 - 2\lambda s_i Q_i^2 = (C_{C_i} + S_{C_i} + W_{C_i}) q_i^2 + 2R_i(E_{C_i} + A_{C_i} + M_{C_i})$　4.5

Substitute the expression $q_i = \left(\dfrac{W_{C_i}}{C_{C_i} + S_{C_i} + W_{C_i}} Q_i \right)$ in the equation　　　　　　　4.5

ie) $Q_i^* = \sqrt{C_{C_i} + S_{C_i} + W_{C_i}} \sqrt{\dfrac{2R_i(E_{C_i} + A_{C_i} + M_{C_i})}{W_{C_i}(C_{C_i} + S_{C_i}) - 2\lambda s_i(C_{C_i} + S_{C_i} + W_{C_i})}}$　　　4.6

Substitute equation 4.6 in 4.4

$q_i = \left[\dfrac{W_{C_i}}{C_{C_i} + S_{C_i} + W_{C_i}} \left[\sqrt{C_{C_i} + S_{C_i} + W_{C_i}} \sqrt{\dfrac{2R_i(E_{C_i} + A_{C_i} + M_{C_i})}{W_{C_i}(C_{C_i} + S_{C_i}) - 2\lambda s_i(C_{C_i} + S_{C_i} + W_{C_i})}} \right] \right]$

$q_i^* = \left[\sqrt{\dfrac{W_{C_i}}{C_{C_i} + S_{C_i} + W_{C_i}}} \sqrt{\dfrac{2R_i(E_{C_i} + A_{C_i} + M_{C_i})W_{C_i}}{W_{C_i}(C_{C_i} + S_{C_i}) - 2\lambda s_i(C_{C_i} + S_{C_i} + W_{C_i})}} \right]$

Differentiate the equation 4.3 with respect to λ and equal to zero

ie) $\dfrac{\partial L(q_i, Q_i, \lambda)}{\partial \lambda} = 0$

$\dfrac{\partial L(q_i, Q_i, \lambda)}{\partial \lambda} = \sum_{i=1}^{n} (s_i Q_i - S_w) = 0$

$\left(s_i \sqrt{C_{C_i} + S_{C_i} + W_{C_i}} \sqrt{\dfrac{2R_i(E_{C_i} + A_{C_i} + M_{C_i})}{W_{C_i}(C_{C_i} + S_{C_i}) - 2\lambda s_i(C_{C_i} + S_{C_i} + W_{C_i})}} \right) = 0$　　　4.7

Substitute the expression of $Q_i *$ and $q_i *$ in equation 4.1, the minimum average cost is derived

$C(q_i, Q_i) = \sum_{i=1}^{n} \left[\left(\dfrac{W_{C_i}}{C_{C_i} + S_{C_i} + W_{C_i}} \right)^2 \dfrac{Q_i^2}{2Q_i} (C_{C_i} + S_{C_i}) + \dfrac{R_i}{Q_i}(E_{C_i} + A_{C_i} + M_{C_i}) + \dfrac{1}{2} W_{C_i} \left[Q_i - \left(\dfrac{W_{C_i}}{C_{C_i} + S_{C_i} + W_{C_i}} \right) Q_i \right]^2 \right]$

$\Rightarrow C(q_i, Q_i) = \sum_{i=1}^{n} \left[\sqrt{\dfrac{(C_{C_i} + S_{C_i})W_{C_i}}{C_{C_i} + S_{C_i} + W_{C_i}}} + \sqrt{2R_i(E_{C_i} + A_{C_i} + M_{C_i})} \right]$　　　4.8

ASTEROID FUZZY SET

DEFINITION AND ITS PROPERTIES

An Asteroid fuzzy set $\tilde{A}$ described as a fuzzy subset on the real line R whose membership function $\mu_{\tilde{A}}(x)$ is defined as follows.

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$$\mu_{\tilde{A}}(x) = \begin{cases} w\left[1+\left(\dfrac{x-a}{b-a}\right)^{\frac{3}{2}}\right] & a \leq x \leq b \\[2em] w\left[1+\left(\dfrac{x-c}{b-c}\right)^{\frac{3}{2}}\right] & b \leq x \leq c \\[2em] \alpha-base & x=w \\[1em] w\left[1-\left(\dfrac{x-c}{b-c}\right)^{\frac{3}{2}}\right] & c \leq x \leq b \\[2em] w\left[1-\left(\dfrac{x-a}{b-a}\right)^{\frac{3}{2}}\right] & b \leq x \leq a \end{cases}$$

Where $0.1 \leq w \leq 0.5$ and a, b, c, are real numbers.

This type of fuzzy set be denoted as $\tilde{A}$ =[a,b,c; ω]

PROPERTIES OF ASTEROID FUZZY SET

$\mu_{\tilde{A}}(x)$ satisfies the following conditions:

1. $\mu_{\tilde{A}}(x)$ is a continuous function from R to the closed interval [0,1].

2. $\mu_{\tilde{A}} = 0$, $0 < x \leq a$

3. $\mu_{\tilde{A}} = L(x)$ is strictly increasing and decreasing on (a, b)

4. $\mu_{\tilde{A}} = R(x)$ is strictly decreasing and increasing on (b ,c)

5. $\mu_{\tilde{A}} = 1, x = b$.

RANKING ASTEROID FUZZY SET OF COST PARAMETERS WITH BEST APPROXIMATION LEVEL[11]

Let $\tilde{A}$ = (a,b,c) be a Asteroid fuzzy set. The α -level interval of $\tilde{A}$ is defined as $A_\alpha \in \left[A_L(\alpha), A_R(\alpha)\right]$. When $\tilde{A}$ is a fuzzy set , the left and right α cuts are

$$A_L(\alpha) = \begin{cases} a+(b-a)\left(1-\dfrac{\alpha}{w}\right)^{\frac{2}{3}} & if\, 0 \leq \alpha \leq w \\[2em] a+(b-a)\left(\dfrac{\alpha}{w}-1\right)^{\frac{2}{3}} & if\, w \leq \alpha \leq 2w \end{cases}$$

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$$C_L(\alpha) = \frac{\int_0^{2w} A_L(\alpha)f(\alpha)d\alpha}{\int_0^{2w} f(\alpha)d\alpha}$$

$$C_L(\alpha) = \frac{\int_0^{w} C_{L_1}(\alpha)f(\alpha)d\alpha + \int_w^{2w} C_{L_2}(\alpha)f(\alpha)d\alpha}{\int_0^{w} f(\alpha)d\alpha + \int_w^{2w} f(\alpha)d\alpha}$$

$$C_L(\alpha) = \frac{\int_0^{w} a+(b-a)\left(1-\frac{\alpha}{w}\right)^{\frac{2}{3}}.d\alpha + \int_w^{2w} a+(b-a)\left(\frac{\alpha}{w}-1\right)^{\frac{2}{3}}\alpha d\alpha}{\int_0^{w} 1 d\alpha + \int_w^{2w} \alpha d\alpha}$$

$$C_L(\alpha) = \frac{2}{2+3w}\left\{a\left(1+\frac{3}{2}w\right)+\frac{3}{5}(b-a)\left(1+\frac{13}{8}w\right)\right\} \qquad 5.3.1$$

$$A_R(\alpha) = \begin{cases} c+(b-c)\left(1-\frac{\alpha}{w}\right)^{\frac{2}{3}} & if\, 0 \le \alpha \le w \\[2em] c+(b-c)\left(\frac{\alpha}{w}-1\right)^{\frac{2}{3}} & if\, w \le \alpha \le 2w \end{cases}$$

$$C_R(\alpha) = \frac{\int_0^{2w} A_R(\alpha)f(\alpha)d\alpha}{\int_0^{2w} f(\alpha)d\alpha}$$

$$C_R(\alpha) = \frac{\int_0^{w} C_{R_1}(\alpha)f(\alpha)d\alpha + \int_w^{2w} C_{R_2}(\alpha)f(\alpha)d\alpha}{\int_0^{w} f(\alpha)d\alpha + \int_w^{2w} f(\alpha)d\alpha}$$

$$C_R(\alpha) = \frac{\int_0^{w} c+(b-c)\left(1-\frac{\alpha}{w}\right)^{\frac{2}{3}}.d\alpha + \int_w^{2w} c+(b-c)\left(\frac{\alpha}{w}-1\right)^{\frac{2}{3}}\alpha d\alpha}{\int_0^{w} 1 d\alpha + \int_w^{2w} \alpha d\alpha}$$

$$C_R(\alpha) = \frac{2}{2+3w}\left\{c\left(1+\frac{3}{2}w\right)+\frac{3}{5}(b-c)\left(1+\frac{13}{8}w\right)\right\} \qquad 5.3.2$$

Ranking Asteroid fuzzy set of cost parameters with best approximation level is

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$$R_\alpha(\tilde{C}) = \alpha C_L(\alpha) + (1-\alpha)C_R(\alpha)$$

Using the equations 5.3.1& 5.3.2

$$R_\alpha(\tilde{C}) = \frac{2}{2+3w}\left\{\left(1+\frac{3}{2}w\right)[\alpha(a-c)+c] - \frac{3}{5}\left(1+\frac{13}{8}w\right)[\alpha(a-c)-(b-c)]\right\}$$

$$\frac{2}{2+3w}\left\{\left(1+\frac{3}{2}w\right)(\alpha a - c\alpha + c) - \frac{3}{5}\left(1+\frac{13}{8}w\right)[\alpha a - \alpha c - b + c]\right\}$$

INVENTORY MODEL IN FUZZY ENVIRONMENT

The above crisp model (4.1) is fuzzified by asteroid fuzzy set, then

$$\tilde{C}(\tilde{q}_i,\tilde{Q}_i) = \sum_{i=1}^{n}\left(\frac{1}{2}\tilde{C}_{C_i}\frac{\tilde{q}_i^2}{\tilde{Q}_i} + \frac{1}{2}\tilde{S}_{C_i}\frac{\tilde{q}_i^2}{\tilde{Q}_i} + \frac{\tilde{E}_{C_i}\tilde{R}_i}{\tilde{Q}_i} + \frac{\tilde{A}_{C_i}\tilde{R}_i}{\tilde{Q}_i} + \frac{\tilde{M}_{C_i}\tilde{R}_i}{\tilde{Q}_i} + \frac{1}{2}\tilde{W}_{C_i}\frac{(\tilde{Q}_i-\tilde{q}_i)^2}{\tilde{Q}_i}\right)$$

Subject to

$$\sum_{i=1}^{n}\tilde{s}_i\tilde{Q}_i \le \tilde{S}_w$$

Minimize TC

$$\sum_{i=1}^{n}\tilde{s}_i\tilde{Q}_i \le \tilde{S}_w$$

The total cost is defuzzified by ranking method

$$R_{\tilde{C}}(\alpha,\tilde{q}_i,\tilde{Q}_i,\lambda) = \sum_{i=1}^{n}\left(\begin{array}{c}\frac{1}{2}R_{\tilde{C}_{C_i}}(\alpha)\frac{\tilde{q}_i^2}{\tilde{Q}_i} + \frac{1}{2}R_{\tilde{S}_{C_i}}(\alpha)\frac{\tilde{q}_i^2}{\tilde{Q}_i} + \frac{R_{\tilde{E}_{C_i}}(\alpha)\tilde{R}_i}{\tilde{Q}_i} + \frac{R_{\tilde{A}_{C_i}}(\alpha)\tilde{R}_i}{\tilde{Q}_i} + \frac{R_{\tilde{M}_{C_i}}(\alpha)\tilde{R}_i}{\tilde{Q}_i} + \\ \frac{1}{2}R_{\tilde{W}_{C_i}}(\alpha)\frac{(\tilde{Q}_i-\tilde{q}_i)^2}{\tilde{Q}_i}\end{array}\right) - \lambda(\sum_{i-1}^{N}\tilde{s}_i\tilde{Q}_i - \tilde{S}_w)$$

$$R_{\tilde{C}}(\alpha,\tilde{q}_i,\tilde{Q}_i,\lambda) = \sum_{i=1}^{n}\left(\begin{array}{c}\frac{1}{2}\left(\alpha\tilde{C}_{C_{i_L}}+(1-\alpha)\tilde{C}_{C_{i_R}}\right)\frac{\tilde{q}_i^2}{\tilde{Q}_i} + \frac{1}{2}\left(\alpha\tilde{S}_{C_{i_L}}+(1-\alpha)\tilde{S}_{C_{i_R}}\right)\frac{\tilde{q}_i^2}{\tilde{Q}_i} \\ + \frac{\left(\alpha\tilde{E}_{C_{i_L}}+(1-\alpha)\tilde{E}_{C_{i_R}}\right)\tilde{R}_i}{\tilde{Q}_i} + \frac{\left(\alpha\tilde{A}_{C_{i_L}}+(1-\alpha)\tilde{A}_{C_{i_R}}\right)\tilde{R}_i}{\tilde{Q}_i} \\ + \frac{\left(\alpha\tilde{M}_{C_{i_L}}+(1-\alpha)\tilde{M}_{C_{i_R}}\right)\tilde{R}_i}{\tilde{Q}_i} + \frac{1}{2}\left(\alpha\tilde{W}_{C_{i_L}}+(1-\alpha)\tilde{W}_{C_{i_R}}\right)\frac{(\tilde{Q}_i-\tilde{q}_i)^2}{\tilde{Q}_i}\end{array}\right) - \lambda(\sum_{i-1}^{N}\tilde{s}_i\tilde{Q}_i - \tilde{S}_w)$$

6.1

Differentiate the equation 6.1 with respect to α

$$\frac{\partial R_{\tilde{C}}(\alpha,\tilde{q}_i,\tilde{Q}_i,\lambda)}{\partial\alpha} = \frac{1}{2}\left(\tilde{C}_{C_{i_L}}-\tilde{C}_{C_{i_R}}\right)\frac{\tilde{q}_i^2}{\tilde{Q}_i} + \frac{1}{2}\left(\tilde{S}_{C_{i_L}}-\tilde{S}_{C_{i_R}}\right)\frac{\tilde{q}_i^2}{\tilde{Q}_i} + \left(\tilde{E}_{C_{i_L}}-\tilde{E}_{C_{i_R}}\right)\frac{\tilde{R}_i}{\tilde{Q}_i} + \left(\tilde{A}_{C_{i_L}}-\tilde{A}_{C_{i_R}}\right)\frac{\tilde{R}_i}{\tilde{Q}_i}$$

$$+ \left(\tilde{M}_{C_{i_L}}-\tilde{M}_{C_{i_R}}\right)\frac{\tilde{R}_i}{\tilde{Q}_i} + \frac{1}{2}\left(\tilde{W}_{C_{i_L}}-\tilde{W}_{C_{i_R}}\right)\left(\frac{Q_i-\tilde{R}_i}{\tilde{Q}_i}\right)^2 = 0$$

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Differentiate the equation 6.1 with respect to $\tilde{q}_i$

$$\frac{\partial R_{\tilde{C}}(\alpha,\tilde{q}_i,\tilde{Q}_i,\lambda)}{\partial \tilde{q}_i} = \left(\alpha\tilde{C}_{C_{i_L}} + (1-\alpha)\tilde{C}_{C_{i_R}}\right)\frac{\tilde{q}_i}{\tilde{Q}_i} + \left(\alpha\tilde{S}_{C_{i_L}} + (1-\alpha)\tilde{S}_{C_{c_{i_R}}}\right)\frac{\tilde{q}_i}{\tilde{Q}_i}$$

$$- \left(\alpha\tilde{W}_{C_{i_L}} + (1-\alpha)\tilde{W}_{C_{i_R}}\right)\frac{(\tilde{Q}_i - \tilde{q}_i)}{\tilde{Q}_i} = 0$$

Differentiate the equation 6.1 with respect to $\tilde{Q}_i$

$$\frac{\partial R_{\tilde{C}}(\alpha,\tilde{q}_i,\tilde{Q}_i,\lambda)}{\partial \tilde{Q}_i} = -\frac{1}{2}\left[\alpha\tilde{C}_{C_{i_L}} + (1-\alpha)\tilde{C}_{C_{i_R}}\right]\frac{\tilde{q}_i^2}{\tilde{Q}_i^2} - \frac{1}{2}\left[\alpha\tilde{S}_{C_{i_L}} + (1-\alpha)\tilde{S}_{C_{i_R}}\right]\frac{\tilde{q}_i^2}{\tilde{Q}_i^2}$$

$$- \left[\alpha\tilde{E}_{C_{i_L}} + (1-\alpha)\tilde{E}_{C_{i_R}}\right]\frac{\tilde{R}_i}{\tilde{Q}_i^2} - \left[\alpha\tilde{A}_{C_{i_L}} + (1-\alpha)\tilde{A}_{C_{i_R}}\right]\frac{\tilde{R}_i}{\tilde{Q}_i^2}$$

$$- \left[\alpha\tilde{M}_{C_{i_L}} + (1-\alpha)\tilde{M}_{C_{i_R}}\right]\frac{\tilde{R}_i}{\tilde{Q}_i^2} + \frac{1}{2}\left[\alpha\tilde{W}_{C_{i_L}} + (1-\alpha)\tilde{W}_{C_{i_R}}\right]\left[\frac{2\tilde{Q}_i(\tilde{Q}_i - \tilde{q}_i) - (\tilde{Q}_i - \tilde{q}_i)^2}{\tilde{Q}_i^2}\right] - \lambda(\tilde{s}_i - \tilde{S}_w) = 0$$

Differentiate the equation 6.1 with respect to λ

$$\frac{\partial R_{\tilde{C}}(\alpha,\tilde{q}_i,\tilde{Q}_i,\lambda)}{\partial \lambda} = -\frac{1}{2}\left[\alpha\tilde{C}_{C_{i_L}} + (1-\alpha)\tilde{C}_{C_{i_R}}\right]\frac{\tilde{q}_i^2}{\tilde{Q}_i^2} - \frac{1}{2}\left[\alpha\tilde{S}_{C_{i_L}} + (1-\alpha)\tilde{S}_{C_{i_R}}\right]\frac{\tilde{q}_i^2}{\tilde{Q}_i^2}$$

$$- \left[\alpha\tilde{E}_{C_{i_L}} + (1-\alpha)\tilde{E}_{C_{i_R}}\right]\frac{\tilde{R}_i}{\tilde{Q}_i^2} - \left[\alpha\tilde{A}_{C_{i_L}} + (1-\alpha)\tilde{A}_{C_{i_R}}\right]\frac{\tilde{R}_i}{\tilde{Q}_i^2} - \left[\alpha\tilde{M}_{C_{i_L}} + (1-\alpha)\tilde{M}_{C_{i_R}}\right]\frac{\tilde{R}_i}{\tilde{Q}_i^2}$$

$$+ \frac{1}{2}\left[\alpha\tilde{W}_{C_{i_L}} + (1-\alpha)\tilde{W}_{C_{i_R}}\right]\left[\frac{2\tilde{Q}_i(\tilde{Q}_i - \tilde{q}_i) - (\tilde{Q}_i - \tilde{q}_i)^2}{\tilde{Q}_i^2}\right] - (\tilde{s}_iQ_i - \tilde{S}_w) = 0$$

NUMERICAL EXAMPLE

Develop a mathematical program to minimize the average total cost. Consider a factory outlet shop which produces three type of items. The three items are readymade, carpets and suits. The items are produced in lots. The shop has only 250 sq. feet's of storage space. The demand ratio for each item is constant. The appropriate data given,

Items	1	2	3
$\tilde{S}_{C_i}$	(25, 30, 35)	(40, 42, 44)	(51, 54, 57)
$\tilde{C}_{C_i}$	(10, 11, 12)	(13, 14, 15)	(16, 17, 18)
$\tilde{E}_{C_i}$	(100, 130, 160)	(150, 151, 159)	(170, 180, 190)
$\tilde{A}_{C_i}$	(140, 145, 150)	(140,150,160)	(200,220,240)
$\tilde{M}_{C_i}$	(110, 115, 120)	(112, 113, 114)	(130, 136, 142)
$\tilde{W}_{C_i}$	(5, 5.5 ,6)	(7, 7.1, 8)	(9, 9.2, 9.4)
$\tilde{s}_i$	0.3	0.6	0.8 (per sq. feet)
$\tilde{R}_i$	200	250	300

w = 0.5 and, S_w = 190, i = 1,2,3.

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Using MATLAB software, the optimal values $Q^*, q^*, \alpha^*, \lambda^*$ and T_c^* are tabulated.

OBSERVATION

In table 7.1 shows the optimal values for ambiguous models and smooth models. Since our allowable spending range is (Rs. 3000 to Rs. 3700) only the two crisp model costs in Table 7.1 fall within this range, although these values are higher than the ambiguous models. The average total cost obtained in the ambiguous model is lower than that obtained in the crisp model, as shown off in the table above. In comparison to the crisp model, the fuzzy model is more effective. In table 7.2, numerically compares the clear and fuzzy models. A sensitivity analysis was performed on the data to see how much the output of the model was affected by changes in the input parameter, the demand rate. When we reduce the sales quantity, the demand decreases, and the inventory cost decreases. As a consequence of the decreased the magnitude of these variables, it will. The fuzzy cost will be reduced as a consequence, and the fuzzy cost will be increased as the magnitude of these variables increases.

CONCLUSION

In this paper, it developed a fuzzy inventory model for multi-item in our numerical experiments, The inventory level in the fuzzy environment is high compared to the crisp value, for the fuzzy inventory model with stowage space constraint, Moreover, the fuzzy inventory model subject to the constraints has been transformed in to crisp inventory problem using ranking indices. Numerical example shows that by this method we can have the optimal total cost. Ranking asteroid fuzzy set method we have shown that the total cost obtained is optimal. Moreover, one can conclude that the solution of fuzzy problems can be obtained by ranking method effectively. The expected minimum total cost in the crisp environment is high compared to the fuzzy value. Finally, conclude that the fuzzy model can be executable in the real work.

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Table 1. Optimal Solution

Model		Item	C_c	S_c	A_c	E_c	M_c	W_c	S	R	Q*	q*	λ*	T_c*
Crisp	1	1	25	10	140	100	110	5	0.3	200	179.3046	22.4131	0.8172	3374.8
		2	40	13	140	150	112	7	0.6	250	181.1361	21.1325		
		3	51	16	200	170	130	9	0.8	300	197.8573	27.3956		
Crisp	2	1	30	11	145	130	115	5.5	0.3	200	180.3467	21.3313	0.7488	3595.8
		2	42	14	150	151	120	7.1	0.6	250	182.7213	20.5598		
		3	54	17	220	180	136	9.2	0.8	300	199.7673	22.9160		
Crisp	3	1	35	12	150	160	120	6	0.3	200	181.3513	20.5303	0.8032	3793.6
		2	44	15	160	159	128	8	0.6	250	183.5583	19.9640		
		3	57	18	240	190	142	9.4	0.8	300	204.0135	22.7219		

Table 2. Sensitivity Analysis (Ranking method)

Fuzzy	$\tilde{C}_{C_L}$	$\tilde{C}_{C_R}$	$\tilde{S}_{C_L}$	$\tilde{S}_{C_R}$	$\tilde{A}_{C_L}$	$\tilde{A}_{C_R}$	$\tilde{E}_{C_L}$	$\tilde{E}_{C_R}$	$\tilde{M}_{C_L}$	$\tilde{M}_{C_R}$	$\tilde{W}_{C_L}$	$\tilde{W}_{C_R}$	S	R	Q*	q*	α*	λ*	T_c*
Item 1	28.1071	31.8929	10.6214	11.3786	143.1071	146.8929	118.6429	141.3571	113.1071	116.8929	5.3107	5.6893	0.6	200	180.0351	62.3570	0.8701	0.9863	3248.67
Item 2	41.2429	42.7571	13.6214	14.3786	146.2143	153.7857	150.6214	154.0286	116.9714	123.0286	7.0621	7.4407	0.7	250	182.0143	61.5089			
Item 3	52.8643	55.1357	16.6214	17.3786	212.4286	227.5714	176.2143	183.7857	133.7286	138.2714	9.1243	9.2757	0.8	300	199.1973	60.7601			

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Model	Percentage	Item	$\tilde{C}_{C_h}$	$\tilde{C}_{C_{l_s}}$	$\tilde{S}_{C_l}$	$\tilde{S}_{C_p}$	$\tilde{A}_{C_h}$	$\tilde{A}_{C_r}$	$\tilde{E}_{C_l}$	$\tilde{E}_{C_r}$	$\tilde{M}_{C_l}$	$\tilde{M}_{C_p}$	$\tilde{W}_{C_l}$	$\tilde{W}_{C_p}$	S	R	Q*	q*	α*	λ*	Tc*
Fuzzy	+50%	1	42.1607	47.8394	15.9321	17.0679	214.6607	220.3394	177.9644	212.0357	169.6607	175.3394	7.9661	8.5340	0.9	300	270.0527	93.5355			
		2	61.8644	64.1357	20.4321	21.5679	219.3215	230.6786	225.9321	231.0429	175.4571	184.5429	10.9661	11.5340	1.05	375	273.0215	92.26335	0.7564	0.67945	5362.5
		3	79.2965	83.7965	24.9321	26.0679	318.6429	341.3571	264.3215	275.6786	200.5929	207.4071	13.685	13.916	1.2	450	298.796	91.14015			
	-25%	1	35.1339	39.8662	13.2768	14.2233	178.8839	183.6161	148.3036	176.6964	141.3839	146.1161	6.6384	7.1116	0.75	250	225.0439	77.94625			
		2	51.5536	53.4464	17.0268	17.9733	182.7679	192.2321	188.2768	192.5358	146.2143	153.7858	9.1384	9.6117	0.875	312.5	227.5179	76.88613	0.87625	0.632875	4468.75
		3	66.0804	69.8304	20.7768	21.72323	265.5358	284.4643	220.2679	229.7321	167.1608	172.8393	11.4054	11.5946	1	375	248.9966	75.95013			
	-25%	1	21.0803	23.9197	7.9661	8.5334	107.3303	110.1697	88.98218	106.0178	84.8303	87.6697	3.9830	4.2670	0.45	150	135.0263	46.76775			
		2	30.9322	32.0679	10.2161	10.7840	109.6607	115.3393	112.9661	115.5215	87.7286	92.2715	5.48303	5.76640	0.525	187.5	136.5107	46.13168	0.892575	0.739725	2681.25
		3	39.6482	41.8982	12.4661	13.0340	159.3215	170.6786	132.1607	137.8393	100.2965	103.7036	6.8432	6.9568	0.6	225	149.398	45.57008			
	+50%	1	14.0536	15.9465	5.3107	5.6893	71.55355	73.44645	59.32145	70.67855	56.5536	58.4465	2.6554	2.8447	0.3	100	90.01755	31.1785	0.89505	0.49315	1787.5

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Figure 1. Graphical representation of asteroid fuzzy set where w = 0.5

RESEARCH ARTICLE

In-vitro Studies on Phytochemical Screening and Anti-Diabetic Activity of *Gelidiella acerosa* Macro-Algae Aqueous Extract

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ABSTRACT

Macroalgae are an integral part of coastal ecosystems is known to have various health-promoting effects. Since macroalgae is one of the wealthiest and largest amount potential resources with bio-functional compounds in pharmaceutical industry they have been utilized as drug and food in the world. They have bioactive compounds with enormous health prospective which interests the pharmaceutical industries. All over the world, Diabetes mellitus as the greatest non-transmittable disease has motivated search for new ant diabetic approaches due to its serious condition caused in human body. Researchers have taken various steps to discover the potential benefits by using natural resources like macroalgae. So, in the present study, the red macroalgae *Gelidiella acerosa* from Mandapam coast, Tamilnadu was selected as experimental species and tested for the presence of secondary metabolites and later characterized using UV-Vis spectroscopy, FT-IR and XRD to identify the functional groups present in it and for its anti-diabetic potential. The purpose of the research was to determine the phytochemical properties and antidiabetic effect using aqueous fraction of marine red algae *Gelidiella acerosa*. The phytochemical analysis showed the presence of the compounds flavonoids, alkaloids, terpenoids, carbohydrates, proteins, tannins, steroids, coumarins, quinines and glycosides. Glycosides, Terpenoids and alkaloids have the potential anti-diabetic properties. Characterization revealed the presence of alcohol, phenolic group, alkenes, amines, alkynes.

Keywords: *Gelidiella acerosa*, Phytochemical analysis, Characterization, Anti-diabetic activity.

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INTRODUCTION

Diabetes mellitus, one of the multifarious, most problematic diseases causing public health issues is prevalence worldwide. It has doubled over the past 30 years due to no satisfactory effective therapy is yet available to cure this chronic hyperglycemia. Moreover, in modern medical field such patients are advised to go for long-term consumption of medicines which causes severe side (Akram Kharroubi and Hisham Darwish, 2015). Thus, treating Diabetes mellitus still todayis a very big predicament. So, researchers all over the world are in search of novel medicine. In current years, a significant number of novel metabolites with potent pharmacological properties have been discovered from the marine organism. Marine macroalgae, a renewable natural resource, found growing in large quantities along the Indian coast that stretches about 5700 km covering about 7500 km including islands and union territorieshave been discovered important number due to presence of novel secondary metabolites with potent pharmacological properties. Most of the marine macroalgae have contributed to the global search for novel medicinal agentsas they are rich source of carotenoids, proteins, oligosaccharides, fatty acids, antioxidants, vitamins, and minerals, which are useful for medical and pharmaceutical industries.

It has been reported that bioactive compounds extracted from seaweeds have the potential for anti-coagulation, anti-oxidation, anti-tumor, anti-viral, and anti-bacterial. Thus, seaweeds can be considered for developing new drugs and functional foods, with few or no side effects (Ponthier *et al.*, 2020). Hebsibah Elsie *et al.*, 2011made invitro screening on secondary metabolites of the seaweeds *Gelidiella acerosa*. Navya Poulose *et al.*, 2021**,** using the seaweed *Gelidium spinosum* observed stigma sterol characterized under FTIR and GC-MS had reduced hyperglycemic effects and therefore could be used as a supplement in diets for diabetic patients. The ant obesity, anti-diabetic, anti-oxidative, and anti-hyperlipidemic activities of various bioactive compounds in the seaweed was examined and identified by Zhanyi Sun *et al.*, 2018.Marine macroalgae, the major component of ecosystems are considered natural dietary bio-resources for various organisms (Cermeño *et al.*, 2020; Dietz *et al.*, 2021) and also one of the largest producers of chemically active metabolites with valuable chemical and pharmaceutical properties, and therefore can be used as new chemotherapeutic agents or source of inspiration to develop new ones. Especially, red algae for have yielded an immense quantity of attention in medicinal manufacturing as a fresh source of bioactive compound with enormous therapeutic prospective. Generally red seaweeds are high in vitamins, minerals and anti-oxidants which can easily be taken by the body. It's important benefits includes promoting healthy circulation in the body, regulate blood sugar level and lower bad cholesterol level since its high in dietary fibre. Hence the present study suggests that the red seaweed *Gelidiella acerosa* can be further considered as an anti-diabetic agent.

These marine benthic algae, are an affluent source of various bioactive compounds, including phytochemicals and antioxidants thus exhibiting various health promoting properties. Seaweed extracts and its bioactive compounds have anti-diabetic potential as they inhibit carbohydrate hydrolyzing enzymes in vitro and exhibit blood glucose lowering effect in random and post prandial blood glucose tests in vivo. Their beneficial effect has been seen in serum and hepatic lipid profile and antioxidant enzymes indicating the protective role of seaweeds against free radicals mediated oxidative stress induced hyperglycemia and associated hyperlipidemia. However, the detailed and in-depth studies of seaweeds as whole, their bioactive isolates and their extracts need to be explored further for their health benefits and wide application in food, nutraceutical and pharmaceutical industries. Surbhi Agarwal *et al.*, 2022.Alpha glycosidase inhibitor functioned as medically oralanti-hyperglycemic agents, like acarbose and voglibose however often instigate harsh gastrointestinal side impact. Anf in addition consequently, the medication of postprandial hyperglycemia have developed into an attractive method for explore new α-glucosidase inhibitors from natural resources. polysaccharides drawn from seaweed mostly in low molecular weight oligosaccharides are also potential as stimulant of insulin secretion(Zhang *etal.*, 2008).From the previous researches like Domettila *etal.*, 2013; Kumar and Sudha,2013; Sharifuddin *etal.*, 2015, it is well known that seaweed contain antibacterial, antiviral, ant diabetic. Due to their potentially beneficial activities, seaweed has been intensive studied. Hence the present study aimed at exploring the phytochemical constituents of *Gelidiella acerosa*, characterization of the macro algae

under UV-Vis spectroscopy , FT-IR and XRD, and also to evaluate the experimental species anti-diabetic activity in-vitro.

MATERIALS AND METHODS

Collection and characterization of experimental macroalga

The live and healthy macro algal sample was collected by handpicking method at a depth of 1-2m during the month Oct 2021, from spots like Mandapam (Latitude: 9.2770392 and Longitude: 79.1252174) and Pamban bridge (Latitude: 9.2761; Longitude: 79.1867). The macro alga thus collected was identified with the help of algologist from Tamilnadu Agricultural University (TNAU), Coimbatore, Tamilnadu, India. The collected macroalgal smaples were perfectly packed in polyethylene Ziplock bags with neat label of time of collection and landmark. After reaching laboratory, the samples were then thoroughly washed with tap water followed by distilled water. The washed macro algae were then spread on the smudging paper, for 5-6 hours and then cut into small pieces. The pieces of macro algal samples were shade dried for two weeks in aseptic condition under room temperature. The fortnight dried samples were grounded into a fine powder utilizing tissue blender. The powdered samples were then stored in polyethylene zip lock bag and stored in the refrigerator at 4ºC for further experimental analysis.

Aqueous extract of the shade dried macroalga samples was extracted using soxhlet apparatus in which powdered seaweed of 10g was soaked in 100ml of water to obtain crude extract. The mixture was then centrifuged at 5000rpm for 20 minutes at 4ºC and filtered using sterilized 0.2μm membrane syringe. A fraction of seaweed aqueous extract of selected marine macroalgae *Gelidiella acerosa* was subjected to phytochemical analysis using standard procedure as reported by (Janarthanan and Senthil Kumar, 2013).Following the phytochemical analysis the aqueous extract of the seaweed *G.acerosa* was subjected for characterization under UV spectrophotometric analysis (Suresh *et al.*, 2010), FT IR (Suresh *et al.*, 2010) and XRD analysis. Under the UV analysis the extract was centrifuged at 3000rpm for 10 min and filtered through Whitman No.1 filter paper for UV spectrophotometer analysis by using high vacuum pump. The sample was diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900nm using Perkin Elmer spectrophotometer and the characteristics peaks were detected.

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Eberhardt *et al.*, 2007; Hazra *et al.*,2007). The macro algal extracts was subjected to Fourier transform infrared spectrometry (Nicolet iS5 iD7 ATR; Thermo Scientific, Germany) equipped with OMNIC software was used in the analysis.10mg of dry powder sample was mixed with 100mg of potassium bromide (Kbr) and compressed to prepare as a salt disc. The disc was then read spectrophotometrically. The frequencies of different components present in each sample were analyzed. X-ray diffraction (XRD) is a powerful nondestructive technique for characterizing crystalline materials. It provides information on structures, phases, preferred crystal orientations (texture), and other structural parameters, such as average grain size, crystallinity, strain, and crystal defects. The instrument was operated at 20 to 40 kV at a wavelength of 1.5418 A° in θ - 2θ configurations, which gave the diffracted intensities in 2θ angle range of 10 ° to 90°C.

Application of Experimental Macro Algal Sample for Anti - Diabetic Assay

α- AMYLASE INHIBITORY ASSAY

The α- amylase inhibitory activity was assessed by the method described by Dong et al., 2012, with suitable modification. Briefly, (500, 250, 100, 50 and 10 μg/ml) of the test seaweed sample was remixed with 200 μl of α-amylase solution (1.0 U/ml in phosphate buffer pH 6.9), and incubated at 25ºC for 30 min. After pre-incubation, 400 μl of 0.25 % starch solution in the phosphate buffer (pH 6.9) was added to each tube to start the reaction. The reaction was carried out at 37ºC for 5 min and terminated by the addition of 1.0 ml of the DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The test tubes were then kept over a boiling

water bath for 10 minutes and cooled to room temperature. The reaction mixture was then diluted by making up the volume to 10 ml of distilled water and absorbent (A) was measured using spectrophotometer at 540 NM. Control incubations representing 100% enzyme activity were conducted in a similar way by replacing extracts with buffers. For blank incubation (to allow for absorbance produced by the extracts), enzyme solution was replaced by buffers solution and absorbance was recorded. The α- amylase inhibitory activity was expressed as percent inhibition and was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{A control} - (\text{A test} - \text{A background}}{\text{Control}} \times 100$$

Where Acontrol, Atest, Abackground represented the absorbance of 100% enzyme activity, test sample with the enzyme and test sample without the enzyme, respectively.

α - GLUCOSIDASE INHIBITORY ASSAY

The α-glucosidase inhibitory activity will be measured as described by Ahamad et al., 2010. Briefly, a volume of 60 µl of G.acerosa sample (500, 250, 100, 50 and 10 µg/ml) and 50 µl of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/ml) was incubated in 96 well plates at 37□ for 30 minutes. After pre-incubation, 50 µl of 5 Mm p nitrophenyl –α –D - glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) will be added to each well and incubated at 370 □ for another 20 minutes. Then the reaction will be stopped by adding 160 µl of 0.2 M NaCO 3 into each well, and absorbance reading (A) will be recorded at 405 nm by micro – plate reader and compared to a control which had 60 µl of buffer solution in place of the of the extract. For blank enzyme solution was replaced by buffer solution and absorbance was recorded. The α-glucosidase inhibitory activity is expressed as inhibition % and will be calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Aco} - \text{At}}{\text{Aco}} \times 100$$

Where, Aco is absorbance of the control and A1 is absorbance of the sample.

RESULTS AND DISCUSSION

Diabetes mellitus is a disorder of carbohydrate metabolism characterized by impaired ability of the body to produce or respond to insulin and thereby maintain proper levels of sugar (glucose) in the blood. The 3 main type of diabetes are: Type 1 diabetes, Type 2 diabetes and gestational diabetes. People with diabetes have the possibilities of getting affected by other diseases such as cardiovascular disease, nerve related problems, kidney damage etc., The allopathic drugs used for treating diabetes again have adverse effects on health. Natural resources are being used worldwide for pharmacological purposes. Marine algae are a great source of various medicinal properties. Each type of seaweed may contain slightly different nutrients and minerals. Eating marine algae may be a simple way to boost a person's intake of vitamins and minerals without adding many calories. Compounds like carotenoid, polysaccharides, fatty acids, glycoprotein's, halo forms, halogenated alkenes, alkenes, alcohols, aldehydes, hydroquinone's, ketones, phlorotannins, pigments, lectins, alkaloids, terpenoids, sterols and some heterocyclic and phenolic compounds are among the most important seaweed substances that receive attention from pharmaceutical companies for use in drug development Rai Abdelwahab, 2017.

The present investigation of aqueous extract of *Gelidium acerosa* was used for the qualitative analysis and is summarized in the Table.1 respectively. Preliminary phytochemical screening of 14 different chemical compounds (proteins, carbohydrates, lipids, alkaloids, phenols, flavonoids, tannins, terpenoids, steroids, saponins, coumarins, quinones glycoside and anthroquinones) were tested in aqueous extracts of *G.acerosa*. Thus 14 tests were executed for the presence or absence of the above mentioned compounds. Out of the 14 tests performed the different chemical

compounds like proteins, carbohydrates, alkaloids, phenols, flavonoids, tannins, terpenoids, steroids, coumarins, quinones and glycoside showed positive results while metabolites like lipids, saponins, anthroquinones proved to be negative. Several phytoactive compounds such as flavonoids, lignans, prophenylphenols, are also found to combat the complications of diabetes. Various significant bioactive compounds such as polysaccharides (aginate, agar, and carrageenan), lipids and polyphenols, steroids, glycosides, flavanoids, tannins, saponins, alkaloids, triterpenoids, antheraquinones and cardiac glycosides have been reported in red algae. (Ejaz Aziz *et al.*, 2020). Seaweed proteins have antioxidant, anti-inflammatory, antibacterial, antithrombotic and immune stimulating activities. Subsequently, they can be used for treating diabetes, hypertension and hepatitis (Francavilla *et al.*, 2013).Seaweeds are measured as a source of bioactive compounds and a prodigious variety of secondary metabolites categorized by abroad spectrum of biological and pharmacological activities (Sachindra *et al.*,2010). The pharmaceutical analysis by UV-Visible Spectroscopy comprises the procedures necessary to determine the "identity, strength, quality and purity" of compounds as well as for the analytical evaluation of the different types of the solvents and substances (Govinda Verma and Manish Mishra, 2018).The aqueous extract was examined under UV visible spectral analysis and the profile showed the compounds separated at 198.30 nm, 279.85 nm and 339.30 nm with the absorption rates at 0.822, 0.199, 0.126. The peak occurrence at 234-676 nm exposes the presence of phenolic and alkaloids compounds in the seaweed *Sargassum wightii*, Rajeswari and Jeyaprakash, 2019. The results of the UV- vis spectroscopy are presented in (Fig.1.).

The aqueous extract of *Gelidiella acerosa* sample was done and associated functional group was observed using FT-IR spectral analysis. The FTIR spectra of *Gelidiella acerosa* showed a peak at 3443.98, 2074.24, 1634.07 and 665.45 cm^{-1}.It is related to alcohol, phenol group, carboxylic acids, alkenes, alkynes and amines. The outcomes of FTIR spectrum and its peak values with functional groups of bioactive components are denoted in(Fig.2.).The broad bands centered at 3443.98 (Cm^{-1}) assigned to hydrogen-bonded O–H and N–H stretching vibrations, which correspond to polysaccharides and amino acids. This bond is a strong bond which also corresponds to alcohol. The strong C=O stretching from1634.07 (Cm^{-1}) proved the presence of COOH i.e. Carboxylic groups. The peaks at 1634.07 (Cm^{-1}) was due to the C=O bond stretching of the polysaccharides. The weak stretching band C=C=H 665.45 (Cm^{-1}) corresponds to Alkenes are acyclic (branched or unbranched) hydrocarbons having one carbon-to-carbon double bond (C=C) and the general molecular formula CnH2n.The peak near 2074.24 (Cm^{-1}) was due to the H- bond predicted the presence of phenol group.The presence of O–H and N–H stretching vibrations clearly states that the selected seaweed *Gelidiella acerosa* has antioxidant antiviral and antibacterial property which is due to the presence of CH$_2$OH acid group. The N–H stretching vibrations proved the presence of amino acids and glycoproteins which confirm that the present selected *Gelidiella acerosa* possess functional amino acid CNH$_3^+$ which provides anticoagulant, immunomodulatory, and functional food. The essential biocompounds of seaweeds based on the FTIR peak values and biological activities reported in previous studies Tanna *et al.* (2019) and Kumar *et al.* (2019).X-ray diffraction peaks are produced by constructive interference of a monochromatic beam of X-rays scattered at specific angles from each set of lattice planes in a sample. The peak intensities are determined by the distribution of atoms within the lattice. Consequently, the X-ray diffraction pattern is the fingerprint of periodic atomic arrangements in a given material. Andrei Bunaciu *et al.*, 2015.The dosage fraction was increased to 250 μg/ml and upto 500 μg/ml in which a maximum inhibition percentage of 69.25 to 79.27 % inhibition was recorded and it is denoted in (Fig.3.).

α- AMYLASE ACTIVITY

In the present study, the results of in vitro *α* – amylase inhibitory assay of the experimental sample *G.acerosa* was compared on basis of the different concentrations and their percentage of inhibition. Acarbose, the positive control used in this study inhibited the activity of *α* – amylase with an (IC $_{50}$ = 60.98 μg/ml) and at 72 %, and the following are the different concentrations and its inhibition percentage: at 10 μg/ml – 4.89 % , 50 μg/ml – 11.32 %, 100 μg/ml – 14.48 %, 250 μg/ml – 16.63% and 500 μg/ml – 18.99%. Vinoth *et al.*, 2015 (*Champia parvula*) The crude extract of *C. Parvula* was carried out for *α*-amylase inhibitory activity at different concentrations (100 to 900 μg/mL), and it showed the inhibitory activity at the dose of 173 μg/mL for *α*-amylase activity. Acarbose, crude methanol extract, and the four fractions exhibited a dose-dependent enzyme inhibition. Thilina Gunathilaka *et al.*, 2019.

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α – GLUCOSIDASE ACTIVITY

Glycosidases are well known targets in the design and development of antidiabetic, antiviral, antibacterial, and anticancer agents. In type II diabetes, delaying glucose absorption after meals by inhibition of a-glucosidase is known to be beneficial in therapy (Tewari *et al.*, 2003). Seaweeds are known to contain a-glucosidase inhibitors (Kurihara *et al.*, 1995, 1999a, b; Xu *et al.*, 2003; Kurata *et al.*, 1997).The present study shows the α – Glucosidase inhibitor effectiveness of aqueous extract of the seaweed *G.acerosa* which was compared on the IC $_{50}$ values. Acarbose the positive control showed a potent inhibitory activity at (IC $_{50}$ = 100.9 µg/ml) at 88 % and at different concentrations it showed the following inhibition percentages at 10 µg/ml - 50.76 %, 50 µg/ml - 58.92 %, 100 µg/ml - 66.33%, 250 µg/ml - 69.25%, 500 µg/ml - 79.27%.In vitro antidiabetic activity of native and commercial carrageenans from the red seaweed *Kappaphycus alvarezii* against a-glucosidase enzyme showed a dose dependent inhibitory activity. At a concentration of 100mg/ml, both native and commercial carrageenans showed 27.61 ± 0.69 and 24.55 ± 0.68% inhibition of a-glucosidase, whereas at the same concentration, the a- glucosidase inhibitory activity of standard drug acarbose was found to be 50.73 ± 1.39%, Arumugampillai Manimehalai Suganya *et al* ., 2016.

CONCLUSION

The present study revealed the presence of major bioactive compounds such as Alkaloids, Tannins, Steroids, Terpenoids, Flavonoids, Carbohydrates, Proteins, Phenols, Coumarins, Glycosides and Quinines. These bioactive compounds have been shown to possess a wide range of pharmacological properties, such as anti – diabetic, anti – oxidant, anti – bacterial etc., Therefore UV- Vis spectroscopy, FT – IR and XRD analysis was done to identify and characterize the functional groups compounds. The *in vitro* anti-diabetic activity of α-amylase and α-glucosidase was dose dependent and the study showed that the positive control acarbose showed a higher inhibition capacity. Therefore further studies are required to determine more beneficial pharmaceutical uses of this marine red algae *Gelidiella acerosa.*

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Table .1. Preliminary phytochemical screening of aqueous extracts of *Gelidiella acerosa*

S.no	Name of the compounds	G.acerosa
1	Alkaloid	+++
2	Saponins	-
3	Tannins	+++
4	Steroid	++
5	Flavonoids	+++
6	Terpenoids	+++
7	Phenols	++
8	Carbohydrates	+++
9	Proteins	+
10	Lipids	-
11	Coumarins	+
12	Quinines	+
13	Glycoside	+
14	Anthroquinones	-

-- = Negative, + = Positive, ++ = Moderate, +++ = Highly Positive

Fig .1. UV-Visible spectrum of aqueous extract of *Gelidiella acerosa*	Fig. 2. FTIR spectrum of aqueous extract of *Gelidiella acerosa*

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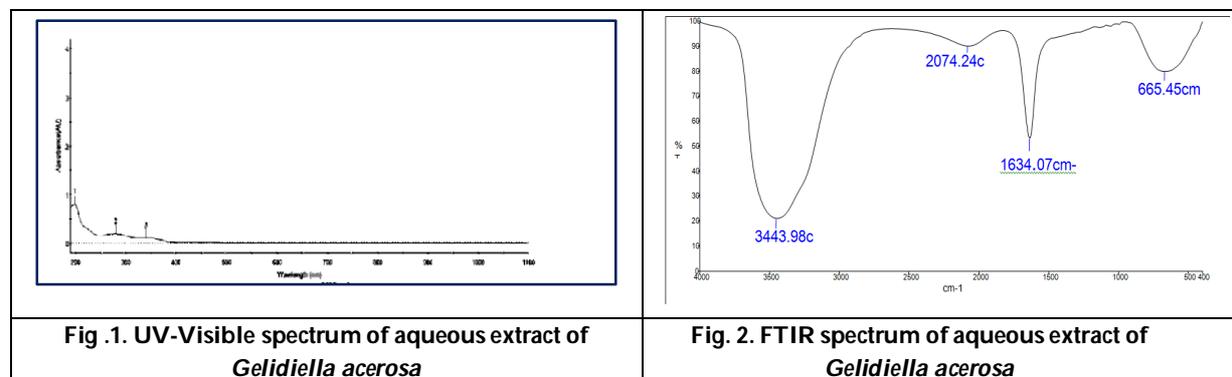
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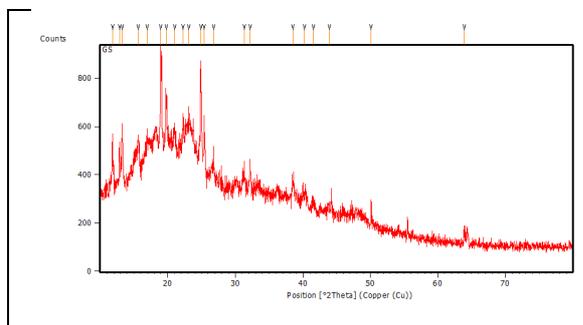
**Fig.3. XRD analysis of aqueous extract of *Gelidiella acerosa***
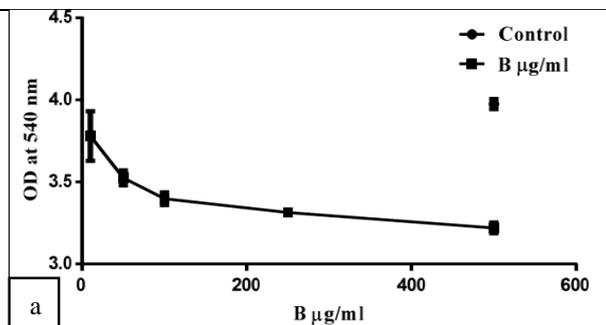
Fig.4. IC ₅₀ values for α-amylase of aqueous extract of *G.acerosa*
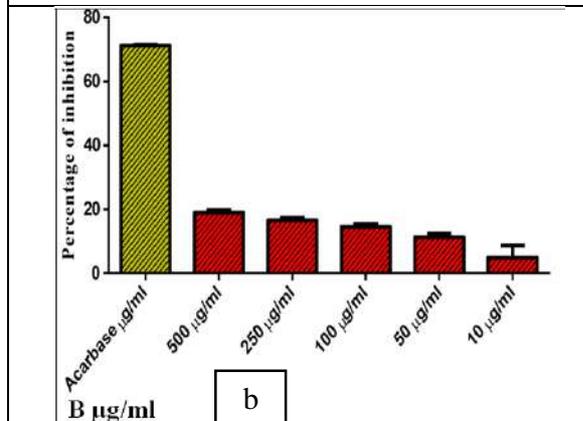

Fig.5. α-Amylase inhibition activity using aqueous extract of *G.acerosa*
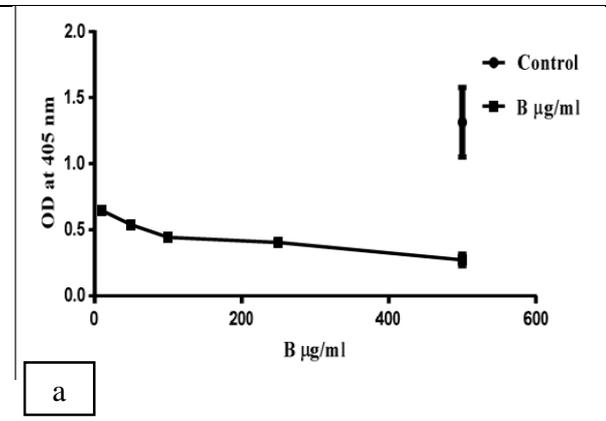

**Fig.6. IC ₅₀ values for α- glucosidase of aqueous extract of *G.acerosa***
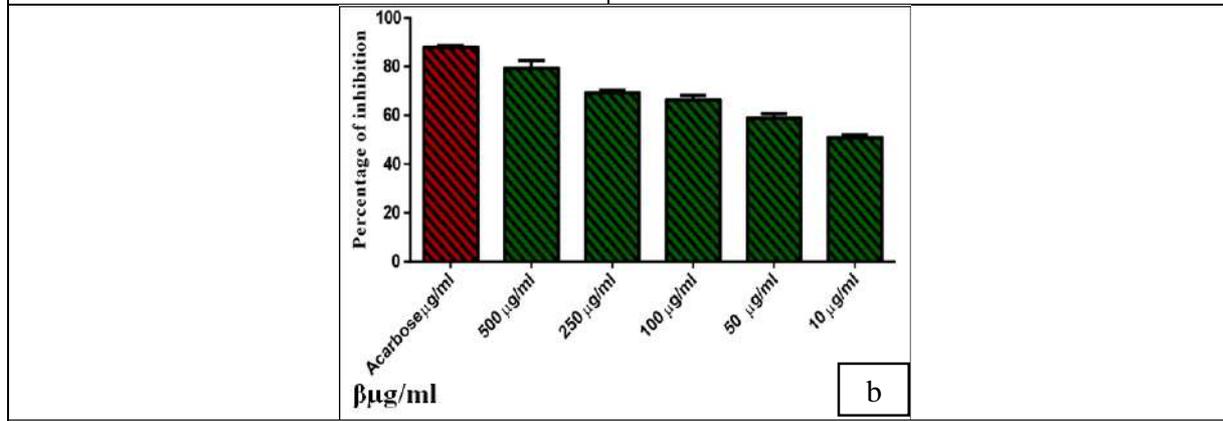
Fig.7. α-glucosidase inhibition activity using aqueous extract of *G.acerosa*

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RESEARCH ARTICLE

Lipid Content Enhancement in Diatoms for Production of Bio-Fuels in Clean and Sustainable Energy Sector

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ABSTRACT

Diatoms are microalgae which are ubiquitously present across all aquatic ecosystems and have the potential and capacity to produce bio-fuel from the lipid content which is present in them. This very property makes the study of diatoms very interesting and it has become the icon of the bio-fuel industry. The presence of diatoms ranges from fresh water to marine ecosystem, as well as the culturing of diatoms on an industrial level is also very easy and it doesn't take much time and resources to cultivate diatoms on an industrial level. This makes diatoms an apt choice in the sector of bio-fuel industry as for production of bio-fuel the amount of capital (diatoms) needed should be in abundance. Also, the lipid content in diatoms can be improved by methods ranging from nitrogen deprivation to genetic engineering using CRISPR Cas9 and also researches show that diatoms seem to produce more lipid content under stressed condition. From the light of fuel industry, diatoms can contribute a lion's share in the sector of clean energy as the lipid content produced from diatoms is very organic and eco-friendly as well as diatoms can lead the way in entering a new area of green economy thus making the fuel industry more sustainable and it will definitely reduce the dependence of fossil fuels and give some respite to the environment. Hence, scopes and opportunities galore in the study and research of diatoms, like generating a novel technique/ method to improve and enhance the production and quality of lipid content in diatoms but there are challenges and hurdles for the mass production of bio-fuel from it. This

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review discusses about the distribution of diatoms across the globe, methods to enhance production of bio fuel from diatoms, scopes and opportunities and its subsequent challenges.

INTRODUCTION

Diatoms are microalgae which are present all over the globe ranging from marine to fresh water ecosystem, with a total species of 200000 spread globally across different aquatic ecosystem [1]. The entire carbon cycling done by diatoms is as equivalent to all the rainforests combined together of this planet and diatoms also produce one- fourth of the total net primary productivity of this planet [2]. Diatoms act as a bridge by linking our past and present with their remarkable fossil records which can persist up to 120 million years because of their siliceous nature in the cell wall [3]. Diatoms have a unique property to accumulate lipid/fat droplets in their body, but it is interesting to note that this lipid storage property in diatoms stretches a very long evolutionary history and is defined on the principle of secondary endo-symbiosis theory of evolution [4].

The adaptation of diatoms ranging from turbulent to smooth aquatic conditions is phenomenal; this is by virtue of its silicified cell wall and this very tolerant nature of diatom makes it a perfect candidate for the manufacturing and commercialization of various bio-products ranging from bio- fuels to bio plastics and many more [5]. The role of diatoms in bio remediation leading to environmental sustainability is praise worthy as it is ubiquitous, it is present across all water bodies and moreover it is photosynthetic, so, it can fix the atmospheric carbon-dioxide and other dissolved carbon-dioxide, which are harmful for other aquatic animals, hence diatom converts the carbon-dioxide into various other bio-molecules like carbohydrates, proteins etc. Apart from this diatom are also involved in the bio remediation of heavy metals like Hg, Cd, etc. with the help of its siliceous shells, it acts in conjugation with the ligand part of harmful and carcinogenic heavy metals [6]. The taxonomic level of study of diatoms is governed by certain protocols, as to determine the specific taxonomic positions in diatoms we need 18S DNA sequences and 28S r RNA partial sequences as gene markers [7]. Diatoms' preservation is also very important, the best option available is cryopreservation as it is beneficial in long term preservation of diatoms and simultaneously it guarantees genetic stability of diatoms [8]. When the salinity of the environment is very high, diatoms are quick to response, as they immediately alter their lipids and fatty acids make up in order to reset their membrane permeability [9].

As the fossil fuels present in earth are depleting day by day, finding a new alternative for it is the need of the hour and diatoms can pave the way in the production of fossil fuels, as they have a significant amount of lipid deposition in their body and under certain controlled conditions, they escalate the production of lipids many folds [10]. Diatoms are qualified enough to be placed under the category of third generation energy feedstock and they can be cultivated/cultured very easily all throughout the season and the specialty of diatom culturing is that it requires very less amount of water and also it doesn't affect any arable land like other higher plant-based bio fuels, which makes it more sustainable [11]. The comparison of economy factor of diatoms and fossil fuels is worth studying, as the investment on diatoms for fuel production can be the next sensation in the energy sector and thus providing a sustainable option [12].

DISTRIBUTION OF DIATOMS ACROSS THE GLOBE IN MARINE AND FRESHWATER ECOSYSTEM

Being a dominant photosynthetic group, diatoms are crucial phytoplankton, and they contribute significantly to our environment. They are an essential part of the food web in case of marine waters and they are food sources for certain specific organisms which cannot survive without them. Furthermore, diatoms are also accountable for driving silicate cycle and they also contribute largely in the nitrate pool (25 – 30 %) especially in the large water bodies namely oceans [13]. Almost one-fourth of the total primary production on earth accounts to the presence of diatoms only and if only marine ecosystem is considered, they are responsible for roughly forty percent of it. Not

only they release significant amount of oxygen but also play a vital role in carbon fixation (approximately 20%) which cannot be ignored as such [13]. Hence, diatoms are very crucial for the maintenance of the ecosystem as a lot of organisms depends on them for the carbon.

They are distributed throughout the world, be they in any water ecosystem, fresh or marine. Their concentration is often more as compared to other phytoplankton as they can reproduce relatively faster [14]. Most of the studies provide insights that diatoms have a marine origin, accounting for the high number of diatoms found in the marine ecosystem (saltwater) [15,16]. Marine water genera of diatoms are almost ubiquitous as they are found in four or more continents but some exceptions are also there, such as *Tursiocola* (found only in South America) and *Actinocyclus* (found in less than 3 continents) [17]. Distribution in the case of freshwaters is also considerable, and some groups like eunotioids are found only in freshwater ecosystems [17,18]. Cymbelloids, as well as *thalassiosiroids*, also show a similar trend. *Aulacoseira, Pleurosira, Urosolenia,* and *Hannaea* are the genera that are widely distributed in freshwaters; despite this, the majority of freshwater genera are endemic to one or two continents only which in turn hinders the process of their study due to regional availability [15,17].

A similar trend is observed in lipid content synthesized by the diatoms, as most of the high lipid-producing diatoms' genera are concentrated in saline waters only [15,19]. This could be due to the high salt concentration present in the oceans and seas as it can cause osmotic differences, which leads to the synthesis of more lipid content [16]. However, research is still ongoing in this aspect, and no conclusive evidence has been found yet. *Cyclotella cryptica* (marine water) and *Navicula saprophila* (freshwater) are the two species of diatoms that are readily available across the planet and thus, are being widely studied for their application in biofuel production, and researchers are trying to increase their lipid content further [14,19].

Overall, the Asian continent contains the vast majority of genera owing to different types of water bodies surrounding the continent along with the variations in climatic conditions throughout the year [20]. Additionally, some regions of this continent lie near the equator and the warm temperature in those areas also contributes directly and indirectly (by changing viscosity of water) to the abundance of diatoms [20]. Monsoon from the South-West direction is another important factor which is responsible for variety of diatoms in the Indian and Chinese seas and oceans. Hence, it is the best place to study them and their various application [21]. Then comes North America and Europe, and almost equal amounts of Bacillariophyceae genera are found in them [17]. Antarctica contains the least number of genera as compared to other continents owing to the hostile living conditions present there and especially it experiences a lack of freshwater species [18].

METHODS TO ENHANCE AND IMPROVE THE LIPID CONTENT IN DIATOMS

Diatoms, one of the precious microalgal species, are a crucial candidate for the production of biofuels [22,23]. There are several approaches in which they can be made more efficient and productive for their use in fuel industry, since the production of biofuels is mostly reliant on the lipids generated by an organism, thus diatoms' lipid content would have to be boosted many times if they were to be used for the same. The production of lipids can be enhanced by natural methods as well but it consumes a lot of time to get them adapted to new environmental conditions [16]. However, advances in biotechnological field have enabled us to use genetic engineering tools to get the desired results in much shorter span of time which is also the dire need of today as the dependence on fossil fuels has to be reduced as soon as possible [24]. Although a significant amount of lipids is produced by diatoms in natural conditions; however, synthesis can be further stimulated by the use of the following two methods which are highly efficient

Nitrogen deprivation method to improve lipid content in diatoms

Diatoms which are classified under the category of unicellular algae have a very unique and significant property of accumulating triglycerides (TAGs) in the form of stored lipids when their growth is halted by depriving them from essential nutrients like nitrogen [25]. The exposure of diatoms under stressed condition for accumulation of more

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lipids (TAGs) should be done prior to harvesting/culturing them in the desired culture conditions [26]. The culturing of diatoms was carried out on f/2 medium along with vitamins and artificial sea salts, along with-it temperature and light intensity (physical parameters were also controlled and fixed), which were 22-degree Celsius and 80 micro mol/square meter/ sec respectively and all this setup was done under the fixed culturing regime of 16-hour day and 8-hour night [27]. When diatoms were exposed to nitrogen deficient conditions prior to pre harvesting/culturing, it was observed that it led to protein degradation, which resulted the growth medium into alkaline condition and ultimately it generated ammonia gas, which paved the way for exponential decrease in the metabolism and growth of diatoms but simultaneously it resulted in the increase of accumulation of lipid content in them. But to the contrast of protein degradation, carbohydrate amount in the diatoms increased under the stressed condition and accumulation of carbohydrate was not very streamlined and it resulted in the hyper accumulation of lipids in the diatoms [28].

CRISPR Cas9 technique to improve lipid content in diatoms

The entire mechanism or modus operandi of CRISPR Cas9 is classified into three main tiers viz. i) adaptation stage, ii) expression stage & iii) interference stage. In the adaptation stage, the unique sequence of the foreign DNA gets assembled with the cr RNA and Cas protein of the CRISPR system. In the expression stage, the nascent crRNA and Cas protein gets transcribed, eventually the nascent crRNA gets matured and possesses the unique sequence called spacer to identify the foreign DNA. In the interference stage, crRNA and Cas proteins twin together to degrade and distort the foreign DNA at specific sites or targeted sites [29]. CRISPR Cas9 technology has increased the precision of gene editing technology manifolds because of its high specificity and precise site directed approach, it has set new high standards in the area of precision gene editing and by virtue of its biomedical sector has highly benefitted from it [30].

Recent studies have shown that using CRISPR technology, gene knock out method is implemented in the regulatory sequence of the lipid producing genes in diatoms. But, gene editing in microalgae is equally cumbersome, but simultaneously there is a respite as generally microalgae have single cells and their genome organization is haploid in nature as far as their vegetative cells or asexual cells are concerned, hence gene knock out method using CRISPR Cas9 technique can be smoothly carried out [31]. Work using CRISPR Cas9 in diatoms has embarked from the year 2013 and it is continuing since then, successful experiments have been conducted on two categories of diatoms, they are *P. Tricornutum* and *T. Pseudonana* [32]. In the first successful experimentation of gene editing in diatoms using CRISPR Cas9 technology, it was observed that four genes were manipulated with great success, via the optimized activity of Cas9 protein which was thoroughly guided by s g RNA [33]. The genetically engineered diatoms to enhance the lipid content come under the category of fourth generation bio-fuel, to produce more lipids from diatoms at industrial scale, controlled and optimized growth of competent strains all over the year is very important and this can be achieved by besieged mutagenesis or through heterologous gene expression which is aided and processed efficiently by CRISPR Cas9 assembly [34]. An observation was done in research, wherein, when diatoms were growing exponentially, the mutation by virtue of which gene knock out was carried with the help of antisense guided by CRISPR Cas9 technology yielded 3.3 folds more lipid content as compared to its wild varieties [35]. Recent researches have suggested that a mutated version of Cas9 protein, which is also termed as dCas9 exhibits scarcity of nuclease action and this dCas9 with the combination of CRISPR assembly can alter the expression of distinct gene which has been targeted. Functional classification of a newly synthesized gene is yet another brownie points of CRISPR technique above gene editing, and via this classification, the newly synthesized genes which encode proteins for lipid generation can elevate the level of bio-fuel industry [36].

ROLE AND APPLICATION OF DIATOMS IN BIOFUEL INDUSTRY

Diatoms have very unique metabolic in addition physiological features and they give a proposal of exciting prospects used for a wide-ranging of the profitable plus industrial bid [37]. Biofuels are sorted into first, second, as well as in third-age biofuels relying upon the kind of unrefined substance that is utilized for their creation. First-age biofuels are created from agriculture yields; one illustration is bioethanol creation from sugarcane. Second-age biofuels created out of woody left-over as well as unpalatable food slices, for example, biofuels from lignocellulose

biomass resources, ought to propose as an additional for first-age biofuels. Third-age biofuels, for example, bio sustainable creation by microalgae, this manner been researched to supplement first and second-age biofuels [38]. Biofuel creation includes transformation of algal biomass into energy sources and the change cycles can be isolated into three classifications; biochemical, substance, and thermo chemical [39].

Microalgae, for example, diatoms remain the favorable feedstock toward, supplant non- inexhaustible causes of power. The principal biomarker used for diatoms is in the proportion of C28 and C29 steranes in addition remarkably diverged to is oprenoid alkene which is situated at great oil fields all over the planet [40]. Focusing on diatom lipids (fat) via controlling as well as improving, development plus culture environments like light, supplements, in addition to stress could give a fascinating choice to assist with fulfilling the current needs of industry manufacturer of biofuel. Knowing the significant capability about diatoms to gather great lipids besides different structures as well as the composition of unsaturated fats, diatoms are an under-exploited region of the biofuel business [40,41].

The measurable information upholds the utilization of microalgae biofuel however there can be different limits in a mechanical stage for enormous scope plus execution of this undertaking. Subsequently, there can be the choices of utilizing the mixed type biodiesel. It can be extra proficient to create a mixed adaptation of petroleum plus diesel in addition to microalgae i.e., diatoms-based fuel aimed at a huge scope of action [42].

DIATOM: GREEN ECONOMY, SCOPES, LIMITATIONS, AND FUTURE PERSPECTIVE
Algae are an assorted collecting of prokaryotic as well as eukaryotic living organisms starting from unicellular categories, for illustration, Chlorella plus diatoms to multicellular organizations, for example, the giant kelp, and a huge plain stained alga that might produce near 50 m in length [43]. Under ideal development conditions, green growth through the course of Trans-esterification, alter unsaturated fats into glycerol-based film lipids, establishing 5-15% of their dry cell weight [44].

The primary restrictions engaged with microalgae biofuel creation are the low deliberation of biomass in the way of life and slight oil content. What's more, slight size of microalgae cells creates the collecting system very expensive. Gatherings as well as drying away the microalgae biomass from great volume of H_2O are an energy polishing off procedure. Contrasted with the traditional horticulture practice, microalgae cultivating is all the more exorbitant as well as convoluted. These challenges could be limited or overwhelmed with the enhancement of the gathering innovation [12]. A portion of the financially savvy innovative systems proposed to create microalgae biofuel creations are:
1) Improvement of bio processing plant or coproduce technique
2) Planning extraordinary photosynthesis productivity photo bioreactors
3) Advancement of practical advances for biomass collecting in addition to drying
4) Improvement of hereditary designing innovation to adjust metabolic passage ways for microalgae biomass as well as lipid creation
5) Comprehension of cooperative associations among microalgae plus microorganisms that additionally influences the biomass in addition to lipid creation in microalgae.

The green economy is a feasible option in contrast to the present financial system, which enrages disparity, invigorates contamination, prompts asset shortage, and represents various ecological wellbeing chances. As per the UNEP a green economy is "low-carbon, asset viable plus socially impartial", with a definitive objective of decreasing ecological effect and biodiversity misfortune as well as further developing human prosperity and civil rights. The various advantages related with algal energy, for example, eco-cordiality and high usefulness, lead to a green economy and economic development by working on human wellbeing and personal satisfaction [45]. Scopes and future perspective of microalga-diatom biofuel: Diatom biomass, the biodiesel doesn't supplement to air CO_2 outflow. It tends to be utilized in existing diesel motors without change. It is non-poisonous and exceptionally

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biodegradable [46]. Maintaining this multitude of benefits at the top of the priority list we can say that diatom-based biofuel is a cleaner option and hold incredible guarantee for the fruitful substitution of petrol fuel. Existing interest for fluid vehicle powers could be met economically with biodiesel from diatoms. Diatom biomass required for creation of huge amounts of biodiesel could be filled in photo bioreactors; however, a thorough appraisal of the financial matters of creation is important to lay out seriousness with oil inferred powers. Accomplishing the ability to cheaply produce biodiesel from diatoms is of vital importance to an ecologically supportable society [47].

CONCLUSION

Diatoms have displayed a lot of potential in the sector of bio-fuel industry. The key highlight of this paper is the abundance and culturing phenomena of diatoms. Also, emphasis to highlight the surplus production of lipids in diatoms and improving its lipid content was discussed with emphasis on altering physical and chemical conditions, inducing method like nitrogen deprivation and also genetic engineering method like CRISPR Cas9 was discussed. The discussion continued further on to scopes and opportunities of diatoms in the clean energy sector. Environment friendly and sustainability of bio fuel production from diatoms was the main goal of this discussion alongside generating a green economy. Although challenges will be there in the process of generation of bio fuel from diatoms, the main challenge is the competence of bio fuel produced by diatoms with that of fossil fuel. The answer to this is best kept with time and the upcoming days will reveal the consequences. But as of now, the study and discussion on this topic is very much required and its high time we create an alternative for fossil fuel for the sake of our planet.

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RESEARCH ARTICLE

Diversity of Pesticide Tolerance Algae from Agriculture Fields in Thiruvannamalai District

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ABSTRACT

The distribution of microalgae in relation to seasonal variation of paddy fields of Arni (site-I), Thimiri (site-II) and Kalambur (site-III) Thiruvannamalai district. This study examined algal diversity in an environment contaminated by pesticide. We isolate 52 algae taxa representing six phyla, among which Chlorophyceae, Chroococcaceae, Oscillatoriaceae, Nostocaceae, Bacillariophyceae and Euglenophyceae are prevailed. We found that Nostocaceae can survive and show high species diversity in the presence of Imidacloprid pesticide contaminated field in both sites. The high tolerance species present in most soil were *Chlorella vulgaris*, *Oscillatoria annae* and *Anabaena azollae* in 3 sites and followed by *Chlamydomonas* sp., *Closterium gracile* were represented. It is unclear whether the algae have a broad tolerance that evolved in pesticide contaminated soil and are adapted to this environment.

Keywords: Nostocaceae, Distribution, Microalgae, Imidacloprid and Agriculture filed.

INTRODUCTION

Algae are a diverse category of eukaryotic creatures that include unicellular genera like *Chlorella* and multicellular forms like the giant kelp, a huge brown alga that may grow up to 50 metres in length. When compared to other photosynthetic organisms of higher plants, this is distinguished by great productivity per unit area. They feature high photosynthetic efficiency, a small number of internally competitive physiological processes, quick reproduction

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cycles, low nutritional requirements, and a wide range of temporal and spectrum irradiance adaptability [1]. Furthermore, because microalgae are primary producers, the foundation of the aquatic food chain, and respond to the environment and chemicals in the aquatic ecosystem, their herbicide sensitivity is crucial by [2 and 3].In general, there are 20 wild and two domesticated species of the annual agricultural plant paddy (*Oryza sativa*), which belongs to the Poaceae family [4 and 5]. The yield of the paddy crop is heavily influenced by the surrounding environment. Rice cultivation on almost 45 million hectares of agricultural land in India is heavily reliant on the use of a substantial number of agrochemicals [6].Rice production takes place in moist, temperate climates, making it susceptible to different fungal and insect pests, resulting in significant losses in rice crop productivity. More than 70 noxious insects have been discovered as having a major effect on crop growth and productivity, either directly or indirectly. To combat insect and fungal pest infestations, as well as nutritional deficiencies, indiscriminate application of agrochemicals such as insecticides, pesticides, and fertilizers has increased dramatically in agro ecosystems. Because of significant differences in soil qualities and prevailing environmental circumstances, the Northern region, for example is more reliant on the usage of such pesticides than the rest of the country [7].Microalgae have been continually used in hazard assessments of pollutants since they are easy to cultivate have high growth rate and are responsive to a wide range of pollutants [8, 9, 10 and 11]. In this study, we conducted a survey of soil algal diversity in different sites of Imidacloprid pesticide polluted agriculture field in Thiruvannamalai district using morphological observations with optical electron microscopes.

MATERIALS AND METHODS

Soil samples were collected from Imidacloprid pesticide polluted paddy fields of Thiruvannamalai district namely, Arni, Thimiri and Kalambur 2020 to 2021.

Isolation and Identification of samples

Nine soil samples (3 sites × 3 replicates per site) were collected at the three sites on winter, summer and Moonsoon 2020 to 2021. In each sampling site, an area of 500–1000 m^2 was investigated to select three representative soil sampling points, showing a range of biocrust colonization states [12]. Samples were collected in clean polyethylene bags and maintained at 4°C until further analysis [13] (Rehakova *et al.*, 2011). After sieving and grinding soil samples, a series of 5 dilutions ranged from 10^{-1} to 10^{-5} were prepared. Under aseptic conditions, 1 ml of the soil suspension of each dilution was inoculated in triplicate on solid (1.5% agar) and then on liquid media. BG-11 medium. The cultures were maintained under controlled conditions (temperature 26 °C ± 1, light intensity 60 μmol/m-2/s, light-dark cycle of 15/9 h with continuous aeration) for 8 to 12 days. After the growth of the cyanobacteria, a series of successive transplants of the isolated strains on a new medium was carried out to obtain a purified algal culture. The algal strains were utilized for in-depth morphological identification of the species under light microscopy. The identification of isolate microalgae was done using the key given by [14 and 15]. The results are given in mean ± standard error (SE). Two-way Analysis of Variance (ANOVA) was performed to assess the significant effects of main factors (type of disturbance and sampling time) and their interaction. Significant differences among these two factors were calculated at 5%. When the interaction between factors was significant according to the ANOVA analysis, post hoc comparisons with Tukey's HSD test were used to find differences between groups. The ANOVA analysis was carried out using SPSS version 22.0 statistical software [16].

RESULTS AND DISCUSSION

We identified 52 algal taxa representing six phyla (Table-1) were reported from the three different study sites of Imidacloprid pesticide polluted rice fields in Thiruvannamalai district of Arni (site-I), Thimiri (site-II) and Kalambur (site-III) and three different season of agro climatic zone. Nostocaceae dominated among the observed phyla in every sampling site, and they also were the group with the highest diversity, numbering 16 taxa. Among the some of other five algal phyla were 10 taxa of Oscillatoriaceae, 9 of Chroococcaceae, 7 of Bacillariophyceae, 5 of

Euglenophyceae and 5 of Chlorophyceae are noted above three sites. Nostocaceae stream showed great number, including 9 taxa of *Anabaena*, 7 and taxa of *Nostoc* are often reported from both Imidacloprid pesticide polluted sites. In general, a total of 52 algal taxa have been observed from each study area were found to be the most tolerance represented by *Chlorella vulgaris* of Chlorophyceae, *Oscillatoria annae* of Oscillatoriaceae and *Anabaena azollae* of Nostocaceae. In soils contaminated by insecticides, there was a decrease in the diversity of soil algal colonies and native Cyanobacteria were replaced with more resistant green algae [17]. After using Thiodan and Karate at high concentrations, the population of protozoa is reduced, has been reported by [18]. Furthermore, after 24 hours of exposure to glyphosate herbicide, *C. sorokiniana* microalgae in South African waters were shown to be the most sensitive species [19].

Microalgae diversity: morphological identification

Using a field based on approach, a total of 52 algae taxa were identified in the agriculture field samples belonging to 2 orders, 5 families, and 18 genera (Fig. 1b, c). The Nostocaceae are the most diverse order with 35% of all taxa, followed by the Chroococcaceae 14% and lowest one is Euglenophyceae (10%). The species richness was significantly different ($p < 0.05$) between soils (Fig. 1a and Table-2). The highest species number was observed in Arni agricultural soils polluted either by Imidacloprid (SI, 48 taxa), Thimiri (SII, 44 taxa) while the lowest one was observed in Kalambur (SIII, 43 taxa). The two way ANOVA showed a significant effect ($p < 0.05$) of the sampling time on the algae diversity of the agricultural soils (Fig. 1a). However, no significant effect of sampling time on diversity was observed in Arni and Kalambur. The three genera *Anabaena* (9 taxa), Nostoc (7 taxa), and *Oscillatoria* (5 taxa) were the most represented (Fig. 3c). In order of occurrence, the high tolerance species present in most soil were *Chlorella vulgaris*, *Oscillatoria annae* and *Anabaena azollae* in 3 sites and followed by *Chlamydomonas* sp., *Closterium gracile* were represented. With 66 species, the algal flora of the contaminated Graniczna Woda stream was weaker. Heavy metal contamination has been shown to impact algal diversity and community structure. Different species may be more or less tolerant to pollution, and some may even thrive in it [20 and [21].

CONCLUSIONS

This survey provides a first inventory (52 taxa) of the soil microalgae communities and shows their high sensitivity to the Imidacloprid pesticide in agriculture soil. The agriculture soils were characterized by species highly associated with pesticide, such as Nostocaceae, Oscillatoriaceae and Chlorophyceae. Microalgae are a naturally occurring biological agent that was described as one of the most suitable techniques for pollution control and efficient at eliminating pesticide contaminants from agricultural fields. In further research, it would be interesting to develop a bioremediation system that resembles something in-between a biofilter and wetland approach.

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Table -1. Tolerant studies of micro algal species from three different sites at pesticide contaminated paddy field in Thiruvannamalai district.

S. No	Name of Species	Site-I			Site-II			Site-III			Total
		Win	Sum	Mn	Win	Sum	Mn	Win	Sum	Mn	
	1. Family: Chlorophyceae										
1.	*Chlorella ellipsoidea* Gerneck	+	-	-	-	+	-	+	+	-	4
2.	*Chlorella vulgaris* Beyerinck	+	-	+	+	+	+	+	-	+	7
3.	*Chlamydomonas* sp.	+	-	+	+	-	+	+	-	+	6
4.	*Chlorococcum humicola*	-	+	-	-	-	+	+	-	-	3
5.	*Closterium gracile* Bréb. ex Ralfs	+	-	+	+	-	+	+	-	+	6

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	2. Family- Chroococcaceae										
6.	*Microcystis aeruginosa* Kütz	-	-	+	-	-	-	-	-	+	2
7.	*Microcystis bengalensis* Banerji	+	-	-	+	-	-	+	-	+	4
8.	*Microcystis pulverea* var.incerta (Lemm.) Crow.	-	-	-	+	+	-	-	-	-	2
9.	*Microcystis flos-aquae* (Wittr.) Kirchner.	+	+	-	+	-	-	+	-	+	5
10.	*Chroococcus cohaerens* (Bréb) Näg.	-	+	-	-	-	-	+	-	-	2
11.	*Chroococcus dispersus* Lemm.	+	-	-	+	-	-	-	-	+	3
12.	*Chroococcus indicus* Zeller.	-	-	+	-	-	-	-	-	-	2
13.	*Chroococcus macrococcus* (Kütz.) Rabenh.	+	-	-	+	-	-	-	+	+	4
14.	*Chroococcus minimus* var. crassa Rao, C.B.	-	-	+	-	-	+	-	+	-	4
	3. Family: Oscillatoriaceae										
15.	*Arthrospira spirulinoides* Ghose.	+	-	-	-	-	-	-	-	-	1
16.	*Spirulina gigantea* Schmidle.	-	-	+	-	-	-	+	-	-	2
17.	*Spirulina major* Kütz. ex Gomont.	-	-	-	-	+	-	-	-	+	2
18.	*Spirulina meneghiniana* Zanard. ex Gomont.	+	+	-	-	-	-	-	+	-	3
19	*Spirulina princeps* West.	-	-	+	-	+	-	+	+	-	4
20.	*Oscillatoria acuta* Bruhl et Biswas.	+	-	-	+	-	+	-	-	-	3
21.	*Oscillatoria anguina* (Bory) Gomont.	-	+	+	+	-	-	-	-	+	4
22.	*Oscillatoria annae* van Goor.	+	+	-	+	+	-	+	+	+	7
23.	*Oscillatoria chalybea* var. insularis Gardner.	-	+	-	-	+	-	-	-	+	3
24.	*Oscillatoria chilkensis* Biswas.	+	-	+	-	-	+	+	-	+	5
	4. Family: Nostocaceae										
25.	*Nostoc calcicola* Brébisson.	+	-	-	-	-	+	-	-	-	2
26.	*Nostoc ellipsosporum (Desm.) Rabenh.*	+	-	+	-	+	-	+	-	+	5
27.	*Nostoc linckia* (Roth) Bornet.	-	+	+	-	-	+	-	-	+	4
28.	*Nostoc muscorum* Ag. ex Gomont.	-	-	+	+	-	-	-	+	-	3
29.	*Nostoc paludosum* Kützing.	+	-	+	-	-	+	-	-	+	4
30.	*Nostoc punctiforme* (Kütz) Hariot.	-	+	-	+	-	-	-	-	-	2

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No.	Species										Total
31.	*Nostoc spongiaeforme* Agardh.	+	-	-	+	-	+	-	+	+	5
32.	*Anabaena ambigua* Rao, C.B.	-	+	+	-	-	+	-	+	-	4
33.	*Anabaena anomala* Fritsch.	+	-	-	+	+	+	-	-	+	5
34.	*Anabaena azollae* Strasburg.	+	-	+	+	+	+	+	-	+	7
35.	*Anabaena doliolum* Bharadwaja.	-	-	+	+	-	+	-	-	-	3
36.	*Anabaena fertilissima* Rao, C.B.	+	-	-	-	-	-	+	+	-	3
37.	*Anabaena gelatinicola* Ghose.	-	-	+	+	-	-	+	-	-	3
38.	*Anabaena iyengarii* var. tenuis Rao, C.B.	-	+	-	+	+	-	+	-	+	5
39.	*Anabaena oryzae* Fritsch.	+	-	-	-	-	+	+	-	+	4
40.	*Anabaena sphaerica* Bornet et Flah.	+	-	-	+	-	+	-	-	+	4
	5. Family:Bacillariophyceae										
41.	*Achnanthes inflata*	+	-	+	-	-	+	-	+	+	5
42.	*Amphora ovalis*	-	-	+	-	-	-	+	+	-	3
43.	*Fragillaria leptostaurum*	-	+	-	+	-	-	+	-	-	3
44.	*Gyrosigma spencerii*	+	-	-	-	+	-	-	-	-	2
45.	*Nitzschia sps.*	-	-	-	+	-	+	+	-	+	4
46.	*Diatoma vulgaris*	+	+	-	-	-	+	-	-	+	4
47.	*Nitzschia sps.*	+	-	+	-	-	+	+	-	-	4
	6. Family:Euglenophyceae										
48.	*Euglena elonata*	-	+	+	-	-	+	-	+	+	5
49.	*Euglena sanguine*	+	-	-	+	-	-	+	-	+	4
50.	*Euglena oblonga*	-	-	-	+	-	-	-	-	-	1
51.	*Euglena viridis*	+	-	-	+	+	-	+	-	-	4
52.	*Euglena minuta*	+	-	+	-	+	+	-	-	-	4

Table 2. Shanon-Weaver diversity indices in microalgae of soil sample from the Imidacloprid pesticide polluted field.

| Sites	Total Species	Relative Abundance	Diversity Index (Shannon Weaver)
Arni (Site-I)	16	1.422077922	5.356977946
Thimiri (site – II)	12	1	5.296043329
Kalambur (Site-III)	10	0.830769231	5.045314546

Nirmal Nevedhana and Ayyappan
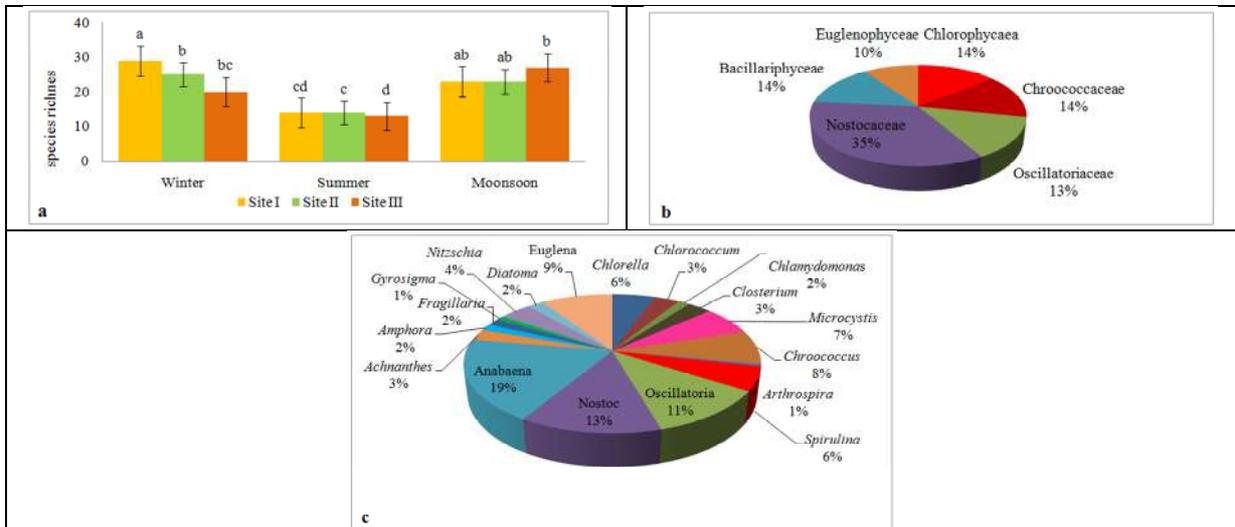
Fig. 1 Species richness (a), spectrum families (b), and spectrum genera (c) of micro algae in the Imidacloprid pesticide polluted soils. Data are means ± standard error (n = 3). Different letters indicate significant differences (p < 0.05 by Tukey's HSD test) between sampled sites.

RESEARCH ARTICLE

Therapeutic Potential of *Musa balbisiana colla*: with Reference to Its Antioxidant Properties

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ABSTRACT

The present study was performed to determine the antioxidant activity of *Musa balbisiana colla* seed, peel, pulp and flower extract .In addition to antioxidants, phytochemical analysis revealed *Musa balbisiana colla* flower extract were rich in phenol and flavonoid content64.5 ± 1.2 mg equi./ Gallic acid/g dw152.42 ± 0.5 mg equi./Quer/g dw . The methanolic and petroleum ether extract of seed ,peel and flower were also evaluated for antioxidant assay using 1,1-diphenyl-2-picrl-hydrazyl(DPPH), ferric reducing antioxidant power(FRAP), 2,2-azino-bis(3-ethlbenzothiazoline-6-sulfonic acid)(ABTS), Nitric oxide(NO) assays, Hydrogen radical activity(HRA) which exhibited the metabolic seed extract posses highest scavenging activity in all the assays. Thus the result indicates that extracts of *Musa balbisiana colla* seed and flower posses potent antioxidant effect.

Keywords: Antioxidant, IC_{50}, Total Phenolic Content,Total Flavonoid Content

INTRODUCTION

Musa balisiana colla belongs to *Musaceae* species classified as monocotyledons having number of seeds.It is commonly known as Bhimkol or Athiyakol in the state of Assam (Borborah *et al.*,2016). Bhimkol classified as triploid (BBB) is cultivated in every household of the people of Assam due to its medicinal and therapeutic uses (Uma *et*

*al.,*2000).Various reports illustrates the importance of different parts of *Musa balbisiana* colla which shown to possesses antioxidant activity, antihyperglycaemic, hepatoprotective effect. (Revadigar *et al.,*2017;Nofianti*et al.,* 2021;Arumugam *et al.,* 2021). Studies conducted however describes the potential of ethanolic extract of different parts of banana. Therefore in this study we have evaluated the antioxidative activity of methanolic extract of *Musa balbisiana* seed and flower.

METHODS

Collection of samples
Bulk number of samples were procured from local sellers of Guwahati. Different parts like fruit, peel, seed, edible flower and inedible part of banana were collected for the further studies.

Drying and extraction of different parts of banana
Drying of the samples was done in hot air oven at the temperature of 40ºC. After drying, the samples were ground to homogeneous powder. Continuous hot extraction was performed using a Soxhlet apparatus and allowed to stand at room temperature for a period of 3 days with frequent agitation until the soluble matter of the samples have dissolved. The solvents like Petroleum ether, Methanol were used in the extraction process.

Evaluation of the *In vitro* Antioxidant Activity
Different *In vitro* Antioxidant Assays includes

1. DPPH radical scavenging Activity
2. ABTS radical scavenging Activity
3. NO radical scavenging Activity
4. Hydrogen radical Activity
5. Ferric reducing antioxidant capacity

F All the *In vitro* Antioxidant Activities were performed by using standard protocol with some minor modifications. NO radical scavenging activity was carried out by using griesh reagent method. UV visible spectrophotometer was used for the analysis purposes for all assays. IC50 (μg/ml**)** value was calculated by plotting the standard graph using graph pad.

RESULTS

Analysis of total phenol and flavonoid
The Total Phenol Content (TPC) and Total Flavonoid Content(TFC) is depicted in Table 1.TPC quantified in seed , peel ,stem, flower,flowerinedibleextract were 33.88 ± 0.5 ,7.3 ± 1.0,13.51± 2.1,64.5.2 ,27.11 ± 1.15 mg equi./ Gallic acid/g dw respectively. TPC of methanolic extract of flower was significantly high compared to seed, peel, stem .The flower depicted high total phenol content of 64.5.2 mg equi./ Gallic acid/g dw .Total flavonoid content quantified in seed extract, peel extract ,stem extract and flower extract, flower inedible were 38.1±1.1, 10.15±0.78, 4.61±1.5,152.42±0.5 and 72.5±1.1mg equi./Quer/g dw respectively. Among all the extracts flower methanolic extract exhibited highest flavonoids content of 152.42±0.5mg equi./Quer/g dw.

Antioxidant capacity of the selected sample extracts
Fig 1,2,3,4,5.*In vitro* antioxidant assay of ethanolic extract of *Musa balbisiana.* Antioxidant effect of methanolic extract of *Musa balbisiana* determined by DPPH, ABTS, HRA, NO, FRAP assays expressed in IC$_{50}$.(1)DPPH activity (DRC) of methanolic extract of banana seeds and flower, (2)ABTS activity (DRC) of methanolic extract of banana seed and flower,(3) HRA activity of the methanolic extract of banana seed and flower,(4) Nitric oxide scavenging activity, and (5)FRAP assay of methanolic extract of banana flower and seed. The methanol and petroleum ether extracts of peel, flower and seeds of *Musa balbisiana* were screened for antioxidant activity at five different concentrations using 1,1-

diphenyl-2-picrylhydrazyl (DPPH),2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate (ABTS), hydrogen peroxide (H_2O_2) and Nitric Oxide (NO) assays to determine free radical scavenging activity and ferric reducing antioxidant power (FRAP) to determine reducing power capacity. Ascorbic acid was used as the standard antioxidant i.e the positive control for the assays. The IC_{50} or EC_{50}values (the concentration of extract or standard that can inhibit 50% of the antioxidant capacity) were determined from regression analysis of a plot of percent inhibition against the extract concentration (μg/mL). The results indicate a dose-dependent increase in antioxidant scavenging activity. A comparison of the IC_{50} values (μg/mL) obtained from the DPPH,ABTS,NO,$H_2O_2$and FRAP assays for the different extracts and ascorbic acid is presented in Table 3.The highest DPPH, scavenging potency was recorded for the seed methanolic extracts with IC_{50} = 1000 μg/mL. In ABTS and H_2O_2 assay the highest scavenging potency was recorded for the seed methanolic extracts with 190.47μg/mL, 2300 μg/mL respectively followed by the flower methanolic extracts with IC_{50} = 371.72 μg/Ml and 3295μg/Ml respectively. Similarly EC_{50} value of 568.18μg/mL was recorded for seed methanolic extracts in nitric oxide (NO) assay. Highest reducing power in FRAP assay was recorded for seed methanolic extracts (60.27 Equivalent ascorbic acid /gm extract) followed by flower methanolic extracts (16.22 Equivalent ascorbic acid /gm extract) and peel methanolic extracts (13.53 Equivalent ascorbic acid /gm extract). The extract with highest percentage inhibition and IC 50 value for all the five assays has also been depicted in the figure 1, 2, 3, 4 and 5.Based on above results, it can be said that seeds of *Musa balbisiana* colla possess significant antioxidant capacity as compared to other part of the plant. The present study can also be compared with previous studies done by (Nghia 2016) which suggested that the seed ethanolic extract of *Musa balbisiana* colla showed a high antioxidant value with IC_{50} = 301.8 μg/m .Earlier various studies (Sheng *et al.,* 2011;Arya Krishnan and Sinija 2016; Thaweesang 2019) had reported the high antioxidant properties of flower part of different banana varieties. However limited reports have been found on the antioxidant activity of the seed part of *Musa balbisiana* colla.

DISCUSSIONS

The antioxidant data demonstrates that methanolic extract of *Musa balbisiana* colla seed and flower is a more potent antioxidant than methanolic extracts of other samples and this observation was further supported by total phenolic and total flavonoid content of methanolic extract of *Musa balbisiana* colla seed and flower.The higher antioxidant potential of *Musa balbisiana* colla seed and flower may be attributed to its flavonoid content. We performed multiple radical scavenging assays (DPPH,FRAP,ABTS,HRA,NO) to investigate the free radical scavenging activity of *Musa balbisiana* seed, flower, peel, stem .The results demonstrated high amount of oxidant radical scavenging power in Mb seed in all the assays .The antioxidant activity thus can prevent oxidative damage in normal cells (Lee *et al.,*2003).Further evaluation was done to investigate the relationship between antioxidant activity and polyphenolic compounds,therefore its phenolic and flavonoid content was established. The study showed MbC flower possess high amount of phenol and flavonoid content .Various literature showed the relationship of phenol and flavonoid and its effect in controlling antioxidants (Firuzi*et al.,* 2005). Phenols acts as antioxidant by transferring H atom to free radicals (Sakihama *et al.,* 2002) .This antioxidant activity of phenolic compound has ability to chelate metal ions involved in the generation of free radicals (Perron and Brumaghim 2009). Polyphenolic compounds are major compound of *Musa balbisiana* and thus has effective role in treating certain diseases such as diabetes mellitus (Kalita *et al.,* 2016).This inhibition mechanism was studied further by analysis of total phenol and flavonoid content and antioxidant activity of the extracted sample. The total phenolic content of stem, peel, flower,seed is presented in Table 1 where the good amount of total phenolic content and total flavonoid content was expressed by flower methanolic extract 64.5 ± 1.2 mg equi./ Gallic acid/g dw and 152.42 ± 0.5 mg equi./Quer/g dw respectively and seed methanolic extract 33.88 ± 0.5 mg equi./ Gallic acid/g dw and 38.1 ± 1.1 mg equi./Quer/g dw respectively. Good phenol content in extract can have significant role in inhibition of α-glucosidase (Mai and Chyen 2007) and α -amylase therefore such inhibitory activity of seed extract can have a potential role in dietary management in controlling postprandial blood glucose level (Wongsa, Chaiwarit, Zamaludien 2012). The results of the study showed that *Musa balbisiana* colla seed and flower is an potential antioxidant the methanolic extract exhibited high amount of phenol and flavonoid content which validates its potential. The *Musa balbisiana* colla seed and flower methanolic extract showed powerful free radical scavenging activity.

CONCLUSION

The study has illustrated the methanolic extract of *Musa balbisiana* colla seed and flower posses antioxidant activity. The antioxidant activity showed strong scavenging power against DPPH, ABTS, FRAP, HRA, NO assays. Therefore high antioxidant potential of *Musa balbisiana* colla seed and flower can be beneficial in preventing oxidative damage in cells and related diseases. Also phytochemical analysis exhibited the high amount of total phenolic content and total flavonoid content. Thus further investigations are required to establish its other therapeutic potential of different parts of the plant.

Abbreviations

DPPH - 1,1-diphenyl-2-picrl-hydrazyl; FRAP-Ferric reducing antioxidant power ;ABTS- 2,2-azino-bis(3-ethlbenzothiazoline-6-sulfonic acid; NO- Nitric oxide; HRA-Hydrogen Radical Activity;TPC-Total phenol content; TFC-Total flavonoid content.

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Table no 1: Quantitative Analysis of total phenol and flavonoid

SI.no	Name of the extract	Total Phenol (mg equi./ Gallic acid/g dw)	Total flavonoids (mg equi./Quer/g dw)
1	Seed methanolic extract	33.88 ± 0.5	38.1 ± 1.1
2	Peel methanolic extract	7.3 ± 1.0	10.15 ± 0.78
3	Stem methanolic extract	13.51± 2.1	4.61 ± 1.5
4	Flower methanolic extract	64.5 ± 1.2	152.42 ± 0.5
5	Flower inedible methanolic extract	27.11 ± 1.15	72.5 ± 1.1

Phytochemical analysis of methanolic extract of *Musa balbisiana*colla.Data represent means of triplicate determination ± S.D .Values of TPC and TFC is expressed in mg equi./ Gallic acid/g dw and mg equi./Quer/g dw.TPC=Total Phenol Content, TFC=Total Flavonoid Content.

Table no 2: Antioxidant assays of the selected hot extracted samples

SI.no	Name of the extract	DPPH IC50 (μg/ml)	ABTS IC50 (μg/ml)	HRA IC50 (μg/ml)	NO assay (EC50)	FRAP(Equivalent ascorbic acid /gm extract)
1	Peel methanolic extract	>1mg/ml	>1mg/ml	>20mg/ml	>1mg/ml	13.53
2	Flower methanolic extract	>1mg/ml	371.72	3295	>1mg/ml	16.22
3	Seed methanolic extract	1000	190.47	2300	568.18	60.27
4	Peel Pet ether extract	>1mg/ml	>1mg/ml	>20mg/ml	>1mg/ml	7.31
5	Flower edible Pet ether extract	>1mg/ml	>1mg/ml	>20mg/ml	>1mg/ml	6.33
6	Flower inedible pet ether extract	>1mg/ml	>1mg/ml	>20mg/ml	>1mg/ml	6.97
7	Seed Pet ether extract	>1mg/ml	>1mg/ml	>20mg/ml	>1mg/ml	7.97
8	Ascorbic acid	129.17	45.34	445.54	307.23	-

Antioxidant activity of *Musa balbisiana*colla as measured by DPPH ,ABTS,HRA,NO,FRAP assays and compared with ascorbic acid .Values are expressed as Mean ±SD of triplicate experiments. DPPH=1,1-diphenyl-2-picrl-hydrazyl, ABTS=2,2-azino-bis(3-ethlbenzothiazoline-6-sulfonic acid, HRA= Hydrogen Radical Activity, NO=Nitric Oxide ,FRAP=ferric reducing antioxidant power.

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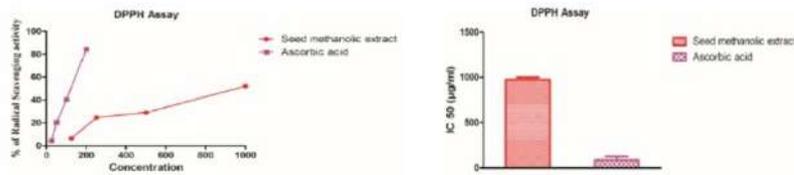

Fig 1: DPPH activity (DRC) of the Methanolic Extract of Banana Seeds
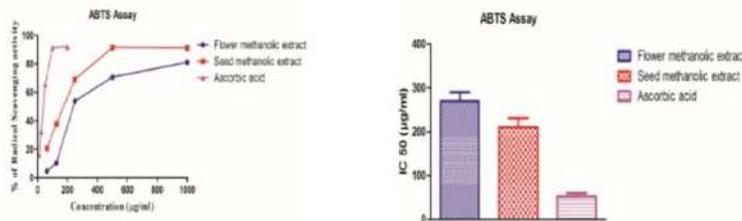
Fig 2: ABTS activity (DRC) of the Methanolic Extract of Banana Seeds
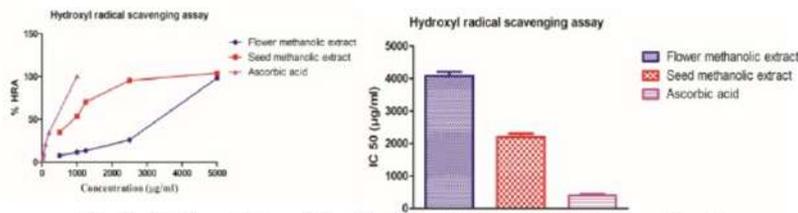

Fig 3: HRA activity of the Methanolic Extract of Banana Seeds
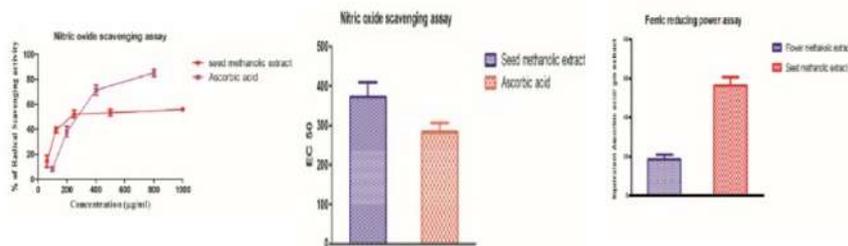
Fig 4: Nitric oxide scavenging activity of the Methanolic Extract of Banana Seeds

Fig 5: FRAP assays of the Methanolic Extract of Banana Flower & Seeds

RESEARCH ARTICLE

A Study on the Positive Impact of the Merging of Public Sector Banks on the Indian Economy

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ABSTRACT

The Indian government announced in August 2019 its decision to consolidate ten public sector banks into four for business growth and organizational efficiency. The decision taken by the government is for the betterment of our society's welfare by increasing lending efficiency to the public. The objective of merging public sector banks is to prevent weaker banks from getting wiped out of the market. This paper aims to discuss how RBI Rules and regulations will govern business entities and individuals' everyday business transactions. Merging of public sector banks will result in the efficient usage of resources very effectively. one of the positive aspects of this consolidation will lead to the banking sector may be able to fund big projects very quickly and easily.

Keywords: Assets, Bank, Merger, Non-Performing Assets, Shares

INTRODUCTION

Public sector banks mean the significant shareholding that is more than 50% of shares lies with the central and state government. The financial disparity in the banking sector is one of the major causes of the emergence of public sector banks in 1969. In 1969, the state bank of India was the only public sector bank nationalized in 1955. Nationalization means consolidating privately owned business entities' assets pooled and organized by the government. Nationalization usually occurs in developing countries and can reflect a nation's desire to control assets. Currently, in India, there are 12 banks in number that are nationalized, and their names are PNB, Bank of Baroda, Bank of India, Central Bank of India, Canara Bank, Union Bank of India, Indian Overseas Bank, Punjab and Sind Bank, Indian Bank, UCO Bank, and Bank of Maharashtra, State Bank of India.

One of the main objectives of nationalization is to diversify the flow of credit towards the primary sectors such as agricultural industries and to provide the best guidance to the banking authorities. Despite the many advantages in the banking sector, the increasing defaults, frauds, and non-performing businesses forced the adoption of the policy of merging. The main aim of merging is to increase the efficiency of the banking sector. A merger is an agreement between two business entities where they can pool their assets and liabilities and bring them into one business entity. Today, India has 12 public sector banks, including Bank of Baroda and State bank of India.

REVIEW OF LITERATURE

The effects of bank mergers and acquisitions in India were studied by Sonia Singh et al. (2018). Three of India's largest banks have been selected for an in-depth analysis of the effects of mergers and acquisitions. In this research, we compare the performance of the Indian banking sector before and after six years of merger and acquisition activity. The report used financial metrics such as net profit margin, operating profit margin, return on capital used, return on equity, earnings per share, capital adequacy ratio, dividend per share, etc., to examine the merged banks' post-merger financial performance. In light of these findings, it is clear that tactics and policies in procedural, physical, and socio-cultural contexts have a significant role in the post-merger and acquisition period. Some critical concerns about the consolidation of government-owned banks were examined by Benazir Banu.T (2021). The study relied on a descriptive approach to research. The primary research topic was the effect of a merger on key performance indicators for financial institutions. Existing problems with bank mergers were examined.

The effects of the consolidation of India's public sector banks were studied by Yasmin K. et al. (2021). The period from the 1990s to 2020 was marked by the dominance of new-generation private banks, the availability of a wide range of products and services delivered via several different channels, coordinated marketing efforts within financial institutions, widespread adoption of cutting-edge technological advancements, etc. The Central Government gave this issue some serious thought in response to rising NPAs (Non-Performing Assets), frequent re-capitalization in State Sector Banks, poor earnings, a lack of competition in the global banking market, and subpar customer service at public banks. The country's problem was solved by consolidating its public sector banks to streamline the financial system.

OBJECTIVES

- To understand the concept of merging public sector banking institutions.
- To identify the positive impact of merging public sector banks on society.
- To analyze the advantages of public sector banks towards the bankers.
- To study the benefits of public sector banks to the customers.

RESEARCH METHODOLOGY

- This study is based on secondary data, and it's a descriptive study.
- This fact is extracted from various journals, articles, websites, and bulletins of RBI.

POST-MERGER PUBLIC SECTOR BANKS

There is an improvement in PSBs, the profitability in the year ended March21, despite the coronavirus pandemic disruptions. The key reason for attaining a profit of 31.817 crores is to lower the cost of frauds, reduce operating expenses, and raise gains in bond portfolios amid declining bond yields. The benefits of a merger could be highlighted in figures related to profitability, the employees of the big Bank merger are tremendous, and it cannot be measured in financial numbers both to bankers and customers.

The positive impact on the bankers is as follows:

- There is an improvement in the reported gross non - performing assets(NPA) (9.4% as on Mar2021 from 10.7% as of March 2020) and net NPA (3.1% VS 3.8%) in FY21, overdue loan book across the bank remains high.
- PSB started improving its lending capabilities and turnaround time by digitally sanctioning the loans by enhancing its customers through online tools.
- Merger leads to the possibility of maximizing the utilization of infrastructure and workforce efficiency.
- A merger of public sector banks creates a better platform to expand its operations widely.
- Where one bank has a broad customer base with other banks.
- Increased geographical locations
- Merger creates excellent control and also reduces the burden on the government.
- A merger facilitates better Risk management with a more extensive capital base.
- Merger helps abolish redundant posts and designation in the banks, increasing savings.
- Larger banks can finance big projects for companies which leads to increased returns.
- It helps to prevent the closure of unprofitable businesses.
- Merger creates to increase the market share.
- Mergers may improve the service quality, which ultimately benefits the customers.
- The merged banking sector could achieve price cuts by operating more efficiently, reducing redundancies in staffing.
- Merged banks will lead to higher customer safety as the government of India possesses verifiable aspects.
- Customers' loans can be sanctioned very fast.
- Lower-income groups also can be benefitted more and more.
- Loans with lower interest rates would be more beneficial to the customers.
- Bank employees could attain job security.
- Better service facility to the rural customers
- Bank employees are also eligible for pension after Retirement

SUGGESTIONS

- Merger facilitates scope for more extensive scale operations for the public, but the employees' position is questionable, and the government should also take necessary steps for employees' job security.
- The size of the board committee can be increased to increase the accountability aspects.
- A separate ethical committee has to be formed to assess the risk in the market.
- Government should take adequate steps to prevent the dominance of the acquired bank by the acquiring bank and monitor the usage of resources.
- Employees can be provided better training programs to adapt themselves for efficient management.
- Government should also allocate an adequate budget for the welfare of the merged bank to serve the nation better.

CONCLUSION

Though the above study concludes that merging was done to save the interest of bankers and customers from the weaker banks, in the later stage, the critical objective is to build a strong image over the public sector bank to facilitate a better economy and to increase the standard of living for the better economy. Merging helped most of the time to protect the weaker bank and met its societal expectations. After the merger, SBI increased its turnover to 52.05 lakh crores, PNB Stood second largest public sector bank with 17.94 crores, and the bank of Baroda achieved its business target of 16.13 crore. To increase financial services to society, we need government support just like a steering wheel in the form of public sector banks.

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RESEARCH ARTICLE

Importance of Mathematics in Civil Engineering

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ABSTRACT

In this world engineering is essential tool to solve any problem. Mathematics is required to find solution in innovative ways. Combination of civil engineering and applied mathematics makes wonderful result. Discrete mathematics is the finest application to solve structure related issues. Road making process, traffic management, Route regarding issues, buildings, elevator and many more issues will be solved using discrete mathematics.

Keywords: Civil engineer, Structure, Mathematics, Modeling, Analysis.

INTRODUCTION

A civil engineer uses mathematics at one point in time for work. Algebra is used on a daily basis, and civil engineers will have to deal with discrete mathematics, statistics, and calculus occasionally. A good portion of a civil engineer's time is not spent doing math, but when the time comes civil engineers have to be very comfortable with all the forms of math, especially those that deal with physics. Civil engineers have to use math equations that are derived from chemistry on a daily basis. Chemistry's equations are used to measure the strength of materials, and engineers must use these equations to select the right material for a project [1].Civil engineers use trigonometry often when surveying a structure. Surveying deals with land elevations as well as the various angles of structures [2].Basic math skills such as accounting and statistics must be utilized during the planning phase of any project. Figuring out the financial side of a project is an important part of a civil engineer's job and he must figure out how much a project is going to cost its investors [3].Good civil engineers have good command in number system. They have to use calculation for each time for how many quantities they have used for their work. Ancient architects had to be mathematicians because architecture was part of mathematics. Using math and design principles, they built pyramids and other structures that stand today. Because angles are an intricate part of nature, sines, cosines and tangents are a few of the trigonometry functions ancient and modern architects use in their work [4]. Surveyors also

use trigonometry to examine land and determine its boundaries and size. Although surveyors perform this task, architects may rely on surveys when designing structures.

Structure

All engineering needs maths. A building, a bridge, a port, a railway line they all need to be built somewhere. That location will require surveying, accurate measurement. The site might need to be changed regarding its topography: earthworks will require to be moved. These will need to be set out, as will the building. The building needs to be designed. The foundations need to be designed. The ground conditions need to be understood before foundations can be designed. Some geological survey is often necessary, especially where other conditions exist - disused mine working and quarries for example. Yet more surveying, but this time also soil and possibly rock samples. These need to be tested for their strength. Even the simplest of buildings needs to have a structure of some kind. It needs to support the loads on it for its purpose: furniture, flooring, any mechanical and electrical services, and something for the people in it too. The roof might need to sustain snow loads or other windblown material. Then there are wind loads, which can be very significant. Establishing the loads on the building requires maths, as does the analysis. Trigonometric functions are used to determine structures.

NEW ARCHITECTS AND TECHNOLOGY

In these days, updates are required for better results. In ancient time, civil engineers have made shelters according to situations. Mathematics has been a fundamental tool for designing process. Right from drawing or making plans, execution till finalization of building projects, architects, builders, and construction experts use it intentionally or unintentionally. If we peek into history, mathematicians were architects and vice-versa. One of the famously known mathematicians, Vitruvius, was also a well-known architect. Pythagoras readings were then used in the building proportions. Leonardo Da Vinci, who worked and used the golden ratio widely, was also an architect. The importance and presence of mathematics in architecture can be seen right from site analysis to the final facade design. Mathematics, though a technical science, is a crucial part of architectural design. One can never realize its enormous extent unless employed and worked upon. It can be beautifully applied both artistically and practically while generating a design proposal. Mathematics has a two-fold function, firstly it serves as the economic factors relevant to the proposed design solution. Through this method, one can decide the budget for the construction and maintenance with the help of floor area, heights, materials, and developments. New architectures design plan is to be made with comfortable to the pocket friendly as well as contains less space.

STATISTICS

Modelling traffic using hourly rates utilizes the Poisson Distribution. Using this as well as other techniques one is able to simulate current and projected traffic. Normal distribution is used to determine traffic queue lengths, which by and large is the basis of determine how long a turn lane bay length needs to be to accommodate traffic. In geotechnical analysis, statistical models are used to in conjunction with Monte Carlo schemes to determine the probability of failure in specific designs, which in terms help define Factor of safety. Conversely the LRFD concepts used in structural design developed by apply statistics to valid previously used under allowable stress design. The newer techniques offer more certainty as well as shown better economy in structural component selection.

CONSTRUCTION MANAGEMENT

Estimating In Construction Management

One common part of construction management that requires math is estimating. There's a science behind allocating enough resources to a construction project to actually do the job, without going overboard.

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Another essential math skill in construction management is budgeting. Without a good budget, the project will not be profitable. Budgeting for a construction project requires math. The process of construction budgeting requires comprehension of the work taking place, understanding what is available and what is not included in work.

Accounting In Construction Management

The most important math-related skill in construction management is Project Accounting. Project accounting consists of many subcategories, including client billing, vendor payments, subcontractor invoices, revenue collected, etc. Construction managers must also know how much has been spent and how much will be spent to complete the project. This includes accounting for payments being made to vendors and subcontractors along with any upcoming costs in these arenas. While this can be an involved and lengthy process, it really isn't math-heavy stuff.

Dimensioning-Scaling in Construction Management

Construction projects exist on the paper first. Throughout the construction process, drawings are constantly referred to. In some instances, construction managers must calculate dimensions based on these drawings using scales. Dimensions on drawings are important for estimating quantities, site surveying, onsite coordination and a ton of other project-specific reasons. When it comes to the math, though, it doesn't get more complicated than basic geometry and multiplication.

NUMERICAL METHODS

Numerical methods are used to find approximate solution to a problem whose analytical solution is not available or the analytical solution can't be generalized. Starting from solution to system of linear equations viz. Jacobi method or the more used Gauss elimination method is used for solving the structures involving the linear-elastic assumption. Software's like Staid Pro uses the LU decomposition for solving system of equations for linear elastic problems because the size of stiffness matrix is not too big. Second is numerical differentiation. Numerical differentiation such as the forward or the backward difference for solving the fluid structure interaction problems. They are also incorporated in forward Euler and backward Euler. Third is the numerical integration. In general, the numerical integration methods such as the Gauss Quadrature is used to compute the global LHS and the global RHS in the finite elements (FEM). The trapezoidal method is widely used in the reservoir modeling and surveying. The extension of numerical integration such as the forward Euler or the backward Euler is widely used for solving the fluid continuum problems using the forward difference method (FDM). Last but not the least is the solution to system of non-linear equations. Methods such as Newton-Rap son or the Modified Newton-Rap son are used to solve the nonlinear structural problems. They are also used to simulate the non-Newtonian fluids.

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RESEARCH ARTICLE

A Study on Consumer Preference towards Electronic Food Ordering

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ABSTRACT

A study of consumer preferences for electronic food orders has, for many reasons, the adaptation of shopping facilities, technological advances, increased revenue and education for shoppers, and rapid profitability at several stages of the world. With the improvement, it will be 10 years later. The use of the internet for shopping and shopping has grown to an extraordinary number over the last century. The significance development of online businesses and the distinctive characteristics due to the growth of Internet many companies are attracted that have entered 12 established businesses via the Internet without knowing the motivation for buyers to buy products and services through online. Is collecting. Many companies agree that successful internet marketing will definitely increase buyers spending and engagement on both online and offline things. There are many advantages by using the Internet f marketing, as it provides marketers with a great opportunity to embark on innovative initiatives that were previously impossible. Marketers have not yet gained a keen understanding of consumer online buying behavior. This information helps sales managers plan marketing mixes and offers to better meet customer requirements. In this way, businesses increase, maintain, and improve customer satisfaction, build strong brand loyalty, and ultimately provide consumers with a solid explanation of why they continue to buy the same brand. Therefore, this research is important because it is a major attempt to identify the factors that influence consumers and their relative strength. Online purchase decision of health food. The controversial issues and objectives of this study are described below.

Keywords: Consumption, e-Food, E-Commerce, Lifestyle.

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INTRODUCTION

The Internet is involved in various organizations such as z-Commerce, e-Business, e. -CRM, e-Supply Chain, e-Marketplace, e-Payment, e-Entertainment, e-Ticket, e-Learning, Togo Government. The Internet has many sales and promotions as data collection, product discussions, revenue tools, sales channels, and customer service for explanatory purposes, from collecting valuable statistics to distributing data to various stakeholders. It has been used astronomically for coordination. A window of almost absolute all occasions. It could be a viable gesture to do business in cyberspace or connect people around the world without geographical restrictions. Consumers can order pure details and victims 24 hours a day, 7 days a week, anywhere. 7 days a week without the hassle of tracking clocks, time zones and caller points. It also opened new opportunities for entrepreneurs by providing consumers with data about themselves. This is mainly due to the great advantages to buyers and sellers' communication on the Internet and the capable to transfer information faster and cheaper than other traditional mass media that use only one-way communication. The current rapid rate at which consumers abandon certain computers and network systems is encouraging and encouraging companies to deploy online business. In fact, some experimenters predict that the need for physical stores will disappear, and that electronic merchandising may replace it within about 40 years (Management1996). While many marketers recognize the importance of using the Internet in their marketing mix, few experimenters have investigated factors that encourage or prevent buyers from purchasing products and services online. Despite the growing popularity of the Internet, most Internet marketing knowledge is based on stories and real justifications from television, radio, popular news outlets, or magazines.

PROBLEM OF THE STUDY

Service is a website with an interactive menu that allows guests to order original coffee and food co-operatives. As important as ordering consumer goods online, guests often have an account and can order frequently. Customers search for their favorite restaurants and choose from available dates to decide on consumer choice. Payments can be made by credit card or cash, and restaurants will try online food companies. The online meal ordering service is a website with an interactive menu that allows guests to order authentic Kaftan dishes. Many of them allow guests to have an account so that they can place frequent orders. Customers search for their favorite restaurants and choose from available dates to decide on delivery or pick-up. Voice of mode of payment. The shops return the opportunity to the online food company. Online business has been around for over 10 years, but it took time to bridge the gap between grocery stores and the Internet. Caff's first online credit for grocery orders was a commercial vote similar to Domino.

COMPOSITION AND RESEARCH IMPORTANCE

Today, if existence in the digital world is essential, small cafes and manufacturer also need to benefit from it. The online grocery ordering site only offers the Saionji Baldur option. This applies not only to hospice, but also to catering services. From the organized chain cuffs, there is the largest cuff feed depending on the original needs, often in the micro needs position. In such scenarios, building a dedicated customer base is not difficult and may not require technical marketing and sales force. By David Buttress. Access to the online grocery ordering site. This website was developed for the convenience of consumers above all else. However, they open many opportunities for SMEs to grow their business. Ordering food online has been a cross-border wonder for some time. There are also many variations in India that have taken root at different times but have not been successful. One of those early game sites was HungryZone.com. It started in Bangalore in 2006 and there were more than 650 cuff sins in big cities. The company was recently acquired by BritishJustEat.com and launched in India as Just Eat. in. This will eventually be released. This place was previously active in Bangalore, Mumbai, and Delhi. David Buttress, MD of JustEat.com, said: Just-Eat Group operates in the United Kingdom, Denmark, Sweden, Belgium, Spain, the Netherlands, Ireland, Norway, Canada, and India. Just-Eat.com (innovated in 2000) has invested $ 5 to $ 10 million in India's reality, Aditi Tarija, over the next three years.

ELECTRONIC FOOD ORDER APP

Zomato Started as Foodie bay, Zomato was founded in July 2008 by two graduates of IIT Delhi, Deepinder Goyal and Pankaj Chadah. The idea came to Deep Inder as he continued to seek paper circulations that offered a variety of dishes ordered by his employees. July 11, 2018. Swiggy Introducing Swiggy, a new destination for online food. Swiggy is a grocery ordering and delivery company based in Bangalore, India. Swiggy was inspired by a study that provided civilian recipients with a complete record of food orders and deliveries from stylish neighborhood cafes. Uber Eats Uber Eat's parent company, Uber, was innovated in 2009 by Garrett Camp and Travis Kalanick.The company launched Uber FRESH in Santa Monica, California, and launched grocery delivery in August 2014. In the UK and Ireland, the number of deliveries is based on the amount of the order.

RESEARCH METHODOLOGY

This survey aims to develop an "online meal ordering system".
Design and build to give easy easy access when entering orders and payments.
To analyze how guests perceive electronic meal orders and understand what influences their purchasing decisions.
Analyze the channels that are increasingly being used for electronic meal orders. Hypothesis
There is no significant association between grocery ordering and ease of satisfaction and accessibility.
There is no significant correlation between age and frequency of electronic meal orders.

SAMPLING DESIGN PATTERN

A preferred cutoff system with non-probability cutoffs is employed. For primary data, a random diner was nominated to use electronic meal orders, especially in the Tiruchirappalli area.

SAMPLE SIZE

A sample of 100 respondents, both genders, drawn from a pot in the capital Tiruchirappalli, returned a properly filled-in questionnaire. From the total guest population of Tiruchirappalli, 100 answers were used as the sample size.

BENEFITS OF ONLINE ORDERING

These benefits for both the buyer and seller when ordering online. First, customers can place orders when they have time and when they want. It is also convenient because you can adjust your order according to your wishes without disturbing the communication between you and the recipient of your order. In addition to the benefit of the customer, the dining room is also suitable for other work with few staff. The restaurant does not require waiters or customers to order by phone. Orders can go directly to the kitchen. The above data depicts that, the samples were collected from 100 respondents, out of 100 respondents, 54 % belongs to male category and 46% comes under the female category. From the above table reveals that, 46% of the respondents were 21-30 years, 24% of the respondents were below 20 years, 24% of the respondents were 31-40 years, and 6% of the respondents were above 40years.

The above table shows that, out 100 respondents, 34% respondents were Under Graduate, 30% of the respondents were Professional, and 24% of the respondents were from Post Graduate, 12% of the respondents were Higher Secondary Level. The above table shows that, out of 100 respondents, 56% of the respondents are married, 44% of the respondents are single. The above table shows that, out of 100 respondents, 36% of the respondents were Self – employed, 26% of the respondents were Student, 20% of the respondents were Professional, 16% of the respondents were Employee, 2% of the respondents were Others.

The above table shows that, out of 100 respondents, 34% respondents were from the family whose monthly income is below Rs.15,000, 30% respondents were from Rs.20,000-25,000, 22% respondents were from the family whose income is Rs.25,000 – 30,000, 14% respondents were from the family whose income is Rs.30,000 &above. The above table shows, out of 100 respondents, all of the respondents have knowledge about Electronic Food Ordering. The above table shows that, out of 100 respondents, 36% of the respondents are know about friends, 28% of the respondents are

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know about advertisement, 24% of the respondents are know about internet, 12% of the respondents are know about Newspaper. The above table shows that, out of 100 respondents, 34% were using recently, 26% were using the One month, 20% were using 3 to 6 months, 16% were using 2 to 3 months, 4% were using more than 6 months. The above table shows that, out of 100 respondents,32 respondents choose that the mobile or telephone is the convenient electronic channel, 30 respondents were used restaurant site, 20respondents used all of the above option, 12 respondents used multiple restaurants, 6 respondents used restaurant app. The above table show that, out of 100 respondents, 34respondents were spent more than 3000, 30 respondents were spent Rs 1000, 22 respondents were spent Rs 2000, 14 respondents were spent Rs 3000. The above table shows that, out of 100 respondents, 30 respondents ordered for lunch, 26 respondents were ordered food for dinner, 16 respondents ordered for snacks, 13 respondents ordered for breakfast and 15 respondents were ordered food for desserts. The above table show that, out of 100 respondents, 40 respondents used Swiggy, 38 respondents used Zomato, 14 respondents used other company,8 respondents used Uber Eats.

The above table show that, out of 100respondents, 34 respondents choose that company service for on time delivery, 30 respondents were choosing that company service for best offers and discounts, 28 respondents were choosing that company service for good packaging, 10 respondents were choosing the company service for easy to order. The above table show that, out of 100 respondents, 66 respondents are choose completely safe, 22 respondents choose unsafe, 6 respondents choose neither unsafe or safe, and the remaining 6 respondents choose somewhat safe. The above table show that, out of 100 respondents, 52respondents are satisfied with services, 26 respondents are highly satisfied with services, 12 respondents are neutral, 10 respondents are dissatisfied with services. The above table reveals that,outof100respondents,34respondentswereonly neutral with the Electronic Food Ordering, 24 respondents were Highly Dissatisfied, 20 respondents were satisfied, and another 20 respondents were dissatisfied and there mainingonly 2 respondents were Highly Satisfied with Electronic Food Ordering.

Testing of Hypothesis

H0: There is no significant relationship between the easy and convenient of electronic food ordering and satisfaction.

$\chi^2 = (O – E)^2 /E$ =11.3696

Degree of freedom   = (r – 1) (c -1)

                               = (5- 1)(4-1)

                               = (4)Calculation value= 11.3696 Table value=21.026

The calculated value is less than the table value. So, the null hypothesis is accepted.

Hence, there is no significant relationship between the easy and convenient of electronic food ordering and satisfaction.

FINDINGS OF THE STUDY

- The majority of 54% of respondents are women.
- 46% of respondents between the ages of 21 and 30 are the majority.
- The majority of 34% of respondents have a college degree.
- Most of the 56% of respondents are married.
- The majority of 36% of respondents are self-employedThe majority of respondents, 34%, earn 20,000 to 25,000 rupees per month.
- All respondents have a general understanding of digital channels for ordering meals. The majority of 36% of
- respondents implement digital food orders through friends. Almost 36% of respondents generally ordered a week's meal and it was easiest to give up. 34% of respondents spent more than Rs 3000.
- Most of the respondents ordered food for lunch.
- Most respondents like Swiggy. Up to 44% of respondents choose coins for shipping.
- The majority of 66% of respondents find it completely secure even when sharing credit / debit card information.
- Most of the 92% of respondents agree that food will come on time. 52% of respondents are happy with the digital

app for ordering meals.

- Most of the 32% of respondents who chose the other option are interested in the situations we face, such as ordering meals. The majority of 38% of respondents may face terrible fines from airlines
- The majority of 34% of respondents to electronic orders report fairness.

SUGGESTIONS

For customers who no longer accept orders, the cafeteria may not always accept orders or conveyor belts. There is no minimum charge for each order and each order will be shipped untied. Respondents' recommendations for transportation fines should be traditional. This helps organizations identify areas that need improvement. We learned from the answers of the respondents, decided, and analyzed them, and made follow-up proposals based on this. The market is new and growing rapidly. It's also a very aggressive setting. Enterprises want to constantly improve and optimize their applications for smoother, seamless, and faster operations. Distractions like pop-up ads are annoying. Instead of using analytics, it may be desirable to make the decision-making process quick and practical. Since it is a distribution channel, it is necessary to work on distribution from the perspective of software programs as well as from the perspective of human transportation. Both together affect the overall experience

CONCLUSION

After investigating the client's perception of digital meal orders, it was concluded that each device has its strengths and weaknesses. The reason behind this online grocery ordering feature is primarily to get customers to buy. Time, especially when they must ask people for some opportunity. The main purpose of digital ordering is convenience. The best definition characteristic of unmarried digital orders is accuracy. To be fair, see how online food orders have become famous to some citizens of Tiruchirappalli city Corporation. Almost 90% of the people surveyed knew about digital food orders. Customers between the ages of 31 and 35 became regular customers because they ordered additional digital meals and usually didn't have to cook dinner on weekends. Customers who check porter fines primarily based solely on staff interaction require women to use their own porter orders. Similarly, customers who are dissatisfied with a generation may hesitate to try a digital self-carrier website for fear of ruining it within a generation. This finding shows that perceived management and convenience are the keys to getting customers to use online orders, which leads to greater satisfaction. My findings need to recognize that food service operators provide customers with a higher level of perceived control and convenience as they relate to better reasons for online orders with an internal future. It suggests that there is. Young customers are much more likely to use online, mobile, or SMS content orders. Younger customers want more comfort and speed than older customers. In conclusion, respect that customers no longer need to attend, and other prepared customers can be inspired to try digital food orders

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Table 1 Gender wise respondent

Gender	Number of Respondent	Percentage
Male	54	54
Female	46	46
Total	100	100

Source: Primary data

Table 2. Age wise respondents

Age	Number of Respondent	Percentage
Below 20 years	24	24
21-30 years	46	46
31-40 years	24	24
Above 40 years	6	6
Total	100	100

Source: Primary data

Table 3. Educational Qualification

Qualification	Number of Respondents	Percentage
Higher Secondary	12	12
Under Graduate	34	34
Post Graduate	24	24
Professional	30	30
Total	100	100

Source: Primary data

Table 4. Marital Status

Marital Status	Number of Respondents	Percentage
Single	44	44
Married	56	56
Total	100	100

Source: Primary data

Table 5.Occupation

Occupation	Number of Respondents	Percentage
Student	26	26
Self – employed	36	36
Professional	20	20
Employee	16	16
Others	2	2
Total	100	100

Source: Primary data

Table 6. Monthly Income

Monthly Income	Number of Respondents	Percentage
Below Rs. 15,000	30	30
Rs. 20,000-25,000	34	34
Rs. 25,000-30,000	22	22
Rs. 30,000&above	14	14
Total	100	100

Source: Primary data

Table 7. Knowledge about Electronic Food Ordering Channels

Knowledge about Electronic Food Ordering	Number of Respondents	Percentage
Yes	100	100
No	0	0
Total	100	100

Source: Primary data

Table 8. Knowledge about Electronic Food Ordering Process

Sources	Number of Respondent	Percentage
News paper	12	12
Internet	24	24
Advertisement	28	28
Friends	36	36
Total	100	100

Source: Primary data

Table 9. Duration of Electronic Food Ordering

Duration	Number of Respondent	Percentage
Recently	34	34
1 Month	26	26
2 to 3Months	16	16
3 to 6 Months	20	20
More than 6 months	4	4
Total	100	100

Source: Primary data

Table 10. Electronic Channels to Food Ordering

Channels	Number of Respondents	Percentage
Mobile or Telephone	32	32
Restaurant Site	30	30
Restaurant App	6	6
Multiple – Restaurants	12	12
All of The Above	20	20
Total	100	100

Source: Primary data

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Table 11. Amount Spent on Electronic Food ordering

Amount	Number of. Respondents	Percentage
Rs.1000	30	30
Rs.2000	22	22
Rs.3000	14	14
More than Rs.3000	34	34
Total	100	100

Sources: Primary data

Table 12. Food Order on Online

Food	Number of Respondent	Percentage
Break fast	13	13
Lunch	30	30
Dinner	26	26
Snacks	16	16
Desserts	15	15
Total	100	100

Sources: Primary data

Table 13. Preference of Electronic Food Ordering

Company Name	Number of Respondent	Percentage
Zomato	38	38
Uber Eats	8	8
Swiggy	40	40
Others	14	14
Total	100	100

Sources: Primary data

Table 13. Quality Services regarding Electronic Food Ordering

Particulars	Number of Respondent	Percentage
Good Packaging	28	28
On Time Delivery	34	34
Best offer and Discount	30	30
Easy to Order	8	8
Total	100	100

Sources: Primary data

Table 14. Risk in Sharing Credit/Debit Card Information through Online

Reason	Number of Respondent	Percentage
Completely unsafe	22	22
Neither unsafe or safe	6	6
Somewhat safe	6	6
Completely safe	66	66
Total	100	100

Sources: Primary data

Table 15 .Satisfaction regarding services

Satisfaction	Number of Respondent	Percentage
Highly Satisfied	26	26
Satisfied	52	52
Neutral	12	12
Dissatisfied	10	10
Highly Dissatisfied	-	-
Total	100	100

Sources: Primary data

Table 16. Rate of electronic food ordering

Particulars	Number of Respondent	Percentage
Highly Satisfied	2	2
Satisfied	20	20
Neutral	34	34
Dissatisfied	20	20
Highly Dissatisfied	24	24
Total	100	100

Sources: Primary data

Table 17. Testing of Hypothesis

Satisfaction level	Easy and convenient				Total
	Yes	No	Some Extent	Quite extent	
Highly Satisfied	5	6	2	1	14
Satisfied	1	2	1	4	8
Neutral	5	11	10	10	36
Dissatisfied	7	10	10	13	40
Highly Dissatisfied	1	1	-	-	2
Total	19	30	23	28	100

O	E	$(O - E)^2$	$(O - E)^2 / E$
5	2.66	5.4756	2.058
6	4.2	3.24	0.771
2	3.22	1.4884	0.462
1	3.92	8.5264	2.175
1	1.52	0.2704	0.178
2	2.4	0.16	0.067
1	1.84	0.7056	0.383
4	2.24	3.0976	1.383
5	6.84	3.3856	0.495
11	10.8	0.04	0.003
10	8.28	2.9584	0.357
10	10.08	0.0064	0.0006
7	7.6	0.36	0.047

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10	12	4	0.333
10	9.2	0.64	0.069
13	11.2	3.24	0.289
1	0.38	0.3844	0.012
1	0.6	0.16	0.267
-	0.46	0.2116	0.46
-	0.56	0.3136	0.56
Total			11.3696

RESEARCH ARTICLE

Yield Performance of Finger Millet *(Eleusine coracana* L.*)* as Influenced by Integrated Nutrient Management in Rainfed Condition of Uttarakhand

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ABSTRACT

A field experiment was carried out at Crop Research Centre, School of Agricultural Sciences, Shri Guru Ram Rai University, Pathribhag, Dehradun, Uttarakhand. The treatments consist of two establishment methods in main plots (consisting of two cultivars: V1: VL Mandua- 347 and V2: VL Mandua- 352) and six nutrient management practices in sub plots (twelve combinations) was laid out in split plot design (SPD) with three replications. The result revealed that the growth attributes *viz.* number of ear head/ m^2, number of ear head/plant, earhead weight (g), test weight (1000 grains), grain yield (kg/ha), straw yield (kg/ha) and biological yield (kg/ha) were significantly influenced by the application 50% RDF (NPK@ 30:15:15) + Vermicompost (1t/ha) and was on par with the application of 75% RDF (NPK@ 30:15:15) + FYM (4t/ha). The highest observed grain yield (2162 kg/ha), straw yield (4421 kg/ha), biological yield (6583 kg/ha) in 2019 and grain yield (2232 kg/ha), straw yield (4592 kg/ha), biological yield (6824 kg/ha) in 2020 was found with 50% RDF (NPK@ 30:15:15) + Vermicompost (1t/ha). The lowest yield was found in the control treatment. There was no significant difference between the cultivars in the establishment method.

Keywords: Finger millet, establishment method, cultivars, PSB, vermicompost.

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INTRODUCTION

Among the millets of the world, finger millet (*Eleusine coracana* sub species *coracana*) ranks fourth in importance after sorghum, pearl millet, and foxtail millet [1]. Millet is a collective term referring to several small-seeded annual types of grass that are cultivated as grain crops, primarily on marginal lands in dry areas in temperate, subtropical, and tropical regions [2]**.** Grains of finger millet is consumed as unleavened bread, porridge, and making beer [3]**.** Finger millet is an African native that originated in the highlands of Ethiopia and Uganda, where subsistence farmers have been growing it for thousands of years. It is estimated that, in India, the crop covered an area of 1.27 million hectares with a total production and productivity of 1.93 million tonnes and 1.60 tonnes per hectare respectively [4]. It is a staple food crop in many hilly regions of Uttarakhand and Himachal Pradesh.

Ragi may be grown as a hot weather crop, from May to September. The crop is well adapted to very poor and marginal lands where other Kharif crops cannot be grown successfully. The crop has extraordinary potential to adapt itself to multiple stresses encountered in rainfed to dry areas. Finger millet is the principal tropical millet with adaptations to survive drought and nutrient deficiencies in the dry and semi-dry regions of India [5]. To improve productivity, integrated nutrient management (INM) is an important practice in modern agriculture where conjunctive use of chemical fertilizer and organic amendments would prove advantageous to the soil health management and improvement of soil fertility as well as the overall crop productivity[6].

Continued use of fertilizers alone can have some adverse effects on the physical, chemical, and biological properties of the soil, which in turn affect yield levels and soil health. In recent years, there has been growing concern that the use of chemical fertilizers, especially inorganic ones, can lead to serious environmental consequences. For this reason, the balanced use of organic and inorganic fertilizers is important not only to improve growth and yield but also to maintain soil health[7]. It is suggested to optimize the use of inorganic fertilizers along with the organic manures such as farmyard manure (FYM) and vermicompost for getting the higher yields of better quality and keeping the cost of production at a sustainable level. Hence the present study was undertaken to optimize the FYM and vermicompost requirement of rainfed transplanted finger millet in conjunction with biofertilizers (PSB).

MATERIALS AND METHOD

The field experiment was conducted on sandy loam texture soil Department of Agronomy, School of Agricultural Sciences, Shri Guru Ram Rai University (30º30´ N latitude and 78º30´E longitude), Dehradun, Uttarakhand during the *Kharif* season in 2019 and 2020. The soil of the experimental site was sandy loam, having neutral in soil reaction (pH 7.1), high in organic carbon (2.22%), very low in available nitrogen (0.007 kg/ha), high in available phosphorus (23.16 kg/ha), low in available potassium (69.20 kg/ha). The maximum and minimum temperature recorded during the growing season of the crop in 2019 from June to December was 37 and 2.77 respectively whereas in 2020 from June to December was 35.60 and 1.5. It was recorded that Dehradun received 1489.6mm and 1032.8mm of rainfall from June to December 2019 and 2020 respectively. The experiment was laid out in split plot design (SPD) with establishment method in main plots and nutrient management practices in sub-plots with three replications.

The finger millet cultivars V1: VL Mandua- 347 and V2: VL Mandua- 352 with a seed rate of 5 kg/ha each are used for nursery and it was transplanted 25 days after sowing (DAS) with the spacing of 25 cm ×10 cm which was an establishment method. Nutrient management practices consisted of treatment of **T1**. Control, **T2.** 100% Recommended dose of fertilizers (RDF: NPK@ 40:20:20), **T3**. 75% RDF (NPK@ 30:15:15) + FYM (4t/ha),**T4**. 75% RDF (NPK@ 30:15:15) + Vermicompost (1t/ha), **T5**. 75% RDF (NPK@ 30:15:15) + 50% Phosphate solubilising bacteria (PSB) (20g/seed), **T6.** 50% RDF (NPK@ 20:10:10) + FYM (2t/ha) + Vermicompost (0.5t/ha) + PSB (20g/seed). Inorganic sources of nutrients used in the experiment were N, P, and K consisting fertilizers such as Urea, Single super phosphate, and Murate of potash.

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Organic sources of nutrients were farm yard manure (FYM) and vermicompost and biofertilizer used for seed inoculation was phosphate solubilizing bacteria (PSB) which was executed in the nursery. Organic nutrients FYM and vermicompost were applied in requisite quantity as per the treatment and incorporated well in advance i.e. two weeks before transplanting of the crop. One-third of urea, full dose of SSP, and Murate of Potash were applied at the time of transplanting as basal dose, and the remaining urea was applied in two split doses *viz.,* 1/3 at tillering stage and 1/3 before ear head initiation as per various treatments. The experimental data recorded for yield and yield attributes were statistically analyzed using the method of analysis of variance (ANOVA) as described by Gomez and Gomez (1984).

RESULT AND DISCUSSION

Yield Parameters

The observations on yield parameters *viz.* number of ear head/m^2, number of ear head/ plant, ear head weight (g), test weight (1000 grains), grain yield (kg/ha), straw yield (kg/ ha) and biological yield (kg/ha) were taken at harvest in 2019 and 2020. Number of ear head/m^2 was highest in T4: 50% RDF (NPK@ 30:15:15) + Vermicompost (1t/ha) and T6: 50% RDF (NPK@ 20:10:10) + FYM (2t/ha) + Vermicompost (0.5t/ha) + PSB in both the years, whereas in establishment method V2 showed slightly higher result than V1. Number of ear head/ plant was significantly higher in T6 and T4 during 2019 and 2020 respectively, at par with 100% RDF (Table 1).

In case of ear head weight (g), test weight (1000 grains) (Table 2) similar trend was observed as noted earlier in ear head/ m^2 and ear head/ plant, whereas V2 showed higher number of ear head weight and test weight compared to V1. Control expressed the least value in all the above mentioned parameters which is significantly at par T5: 75% RDF+ PSB. Grain, straw and biological yield of finger millet differed significantly with varying level of inorganic fertilizers in association with FYM and vermicompost over control. Application of 50% RDF (NPK@ 30:15:15) + Vermicompost (1t/ha) recorded maximum grain yield (2162 kg/ha), straw yield (4421 kg/ha) and biological yield (6583kg/ha) in 2019 and grain yield (2232 kg/ha), straw yield (4592 kg/ha) and biological yield (6824 kg/ha) in 2020 and at par with 75% RDF + FYM (4t/ha).

It was significantly superior to rest of the treatment under investigation. V1 was significantly similar in grain yield as compared to V2 in 2019 whereas V2 was slightly higher in grain yield in 2020 (Table 3). Higher yield with combined application of inorganic fertilizer, FYM, vermicompost and biofertilizer may be regards as formation of humus and organic carbon which increased availability of nutrients and improved the soil properties. Organic manure provide a good environment for microorganisms, ie. *Azospirillum,* which fixes atmospheric nitrogen available to plants. Furthermore, PSBs are one of the most important nutrient-dissolving microorganisms, converting insoluble phosphates to soluble forms by secreting several organic acids. Earlier researchers also noted similar type of observation. [8][9]

CONCLUSION

Based on the above research it clearly stated that different yield parameters were found significantly higher with the use of RDF along with vermicompost and FYM. Use of organic manure not only enhances the yield but also improved the soil condition. PSB + RDF alone showed no influence on yield of finger millet. It is well proved that use INM improves the production of finger millet.

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Table 1: Number of ear head m^{-2} and number of ear head plant^{-1} as influenced by the treatments at different crop growth stages

Treatments	2019		2020	
	Number of ear head m^{-2}	Number of ear head plant^{-1}	Number of ear head m^{-2}	Number of ear head plant^{-1}
Establishment methods				
V$_1$: VL Mandua- 347	87	3.5	87	3.8
V$_2$: VL Mandua- 352	88	3.71	89	4
S.E.m (±)	0.03	0.06	0.10	0.38
C.D. (5%)	0.25	NS	0.68	1.13
Nutrient Management				
Control (N:P:K)	70	2.58	72	3.0
100% RDF	89	4.0	87	4.10
75% RDF+ FYM(4t/ha)	91	3.96	92	4.01
75%RDF+Vermicompost(1t/ha)	96	4.10	97	4.59
75% RDF+50%PSB	84	3.01	82	3.28
50% RDF + FYM (2t/ha) + Vermicompost (0.5t/ha) +PSB	96	4.16	97	4.55
S.E.m (±)	0.43	0.10	0.05	0.05
C.D. (5%)	1.29	0.30	NS	0.15

Table 2: Effect of INM treatments on earhead weight (g) and test weight (1000 grains) of finger millet at harvest

Treatments	2019		2020	
	Earhead weight (g)	Test weight (1000 grains)	Earhead weight (g)	Test weight (1000 grains)
Establishment methods				
V₁: VL Mandua- 347	6.11	3.25	6.37	3.35
V₂: VL Mandua- 352	6.18	3.26	6.42	3.41
S.E.m (±)	0.3	0.3	0.35	0.01
C.D. (5%)	NS	NS	NS	NS
Nutrient Management				
Control (N:P:K)	4.80	2.90	4.92	3.0
100% RDF	6.35	3.4	6.49	3.49
75% RDF+ FYM(4t/ha)	6.6	3.54	6.72	3.6
75%RDF+Vermicompost(1t/ha)	7.74	3.68	7.80	3.74
75% RDF+50%PSB	5.9	2.9	6.1	3.20
50% RDF + FYM (2t/ha) + Vermicompost (0.5t/ha) +PSB	6.20	3.1	6.34	3.24
S.E.m (±)	0.08	0.07	0.05	0.04
C.D. (5%)	0.25	0.23	0.17	0.12

Table 3. Effect of INM treatments on Grain yield (kg ha⁻¹), Straw yield (kg ha⁻¹) and Biological yield (kg ha⁻¹) of finger millet at different growth stages

Treatments	2019			2020		
	Grain yield (kg ha⁻¹)	Straw yield (kg ha⁻¹)	biological yield (kg ha⁻¹)	Grain yield (kg ha⁻¹)	Straw yield (kg ha⁻¹)	biological yield (kg ha⁻¹)
Establishment methods						
V₁: VL Mandua- 347	1751	3868	5608	1798	3971	5432
V₂: VL Mandua- 352	1750	3874	5612	1801	3973	5774
S.E.m (±)	0.77	4.77	5.53	1.80	7.70	7.79
C.D. (5%)	NS	NS	NS	NS	NS	NS
Nutrient Management						
Control (N:P:K)	1062	2721	3783	1074	2801	3875
100% RDF	1684	3744	5428	1710	3789	5499
75% RDF+ FYM(4t/ha)	2124	4392	6516	2210	4400	6610
75%RDF+Vermicompost(1t/ha)	2162	4421	6583	2232	4592	6824
75% RDF+50%PSB	1764	4078	5842	1810	4289	6099
50% RDF + FYM (2t/ha) + Vermicompost (0.5t/ha) +PSB	1710	3801	5511	1764	3964	5728
S.E.m (±)	8.52	5.42	10.18	2.17	6.44	6.96
C.D. (5%)	25.31	16.12	30.26	6.45	19.15	20.70

RESEARCH ARTICLE

Determination of Efficient Crop Identification using WAA Algorithm

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ABSTRACT

In the agriculture environment, sustainability refers to the successful management of agricultural resources to satisfy human needs while at the same time maintaining or enhancing the quality of the environment and conserving natural resources for future generation. Farmers typically select crops for cultivation based on their conventional methods and previous agricultural experience; however this may go wrong because of a natural disaster. Hence, a decision making model must be established to enable farmers to make consistent decisions on farming. In this paper, we have shown the comparative analysis of identify the finest crop using RC tool with weight Aggregation Analysis Algorithm which is used to be cultivated. Each crop in the data set can be analyzed by the developed model. The model is able to accomplish the optimum agricultural development strategy. This strategy generates better than the current production plan.

Keywords: Fuzzy soft set, Weight Aggregation Analysis Algorithm, Relative Closeness.

INTRODUCTION

The one important way of getting revenue in India as Agriculture. India's geographical area covered forty three percent by the agricultural sectors. The current cultivation system will increase the productivity by applied the technological changes in farming activities. Although, optimizing the choice now seems to be a wonderful solution to current crop problems. This is a mathematical process which focuses on optimizing the output of many input variables which influence the outcome relatively.

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For occasion, if we get optimize profit, more water-intensive crops will be required. In drought areas, it is important to minimize the use of water, however it may conflict the benefit target. The objective of this paper is to apply the decision making optimization strategy for the crop selection.

RELATED WORKS

(Ananthi, 2020) Suggested the idea focused on fuzzy sets. When the fluidity can occur, the brightness amount should be assumed in-pixel in the images for the calculated degree. When the ambiguity of the images is treated effectively in the fuszy package, when particularly IFSs is processed. When the activation of the segmentation can be calculated by the satellite by which the unknown capture images can be decreased. Then, the segmentation of the deficit of the crops for the clustering technique will fuse an image, since it depends on the interval between the intuitionist fuzzy set .(Cui, Zhang, Wang, & Wang, 2020) suggested a novel extract approach for the optimized sub-set function.

Cultivation depending on the forms to be tracked for algorithm items dependent on the vector machine help classification. The performance provided by the technologies can be best rated with a total accuracy of about 89.6 percentage points. (Baidar, 2020) using a chart rice crop based neural network algorithm and forecast the yield in the district of Terai. (Garg, 2019) develop relevant operating laws and the required weighted aggregation operators. In it the characteristics of a neutral type in the membership degrees of the group and the sum of probability are specified by a new neutral addition and scalar multiplication operational rules. Any aspects of the legislation introduced are analyzed.(Arii, Van Zyl, & Kim, 2010) In this article, the methods of extension are relying on the de-composition of the system as they establish a decomposition-based methodology for adaptive model, which helps them to approximate for the mean randomness that the scattering canopy is based on each pixel of the image. (Fowler et al., 2015) describe a system that only utilizes its corresponding Input and Result sources to combine these multiple components. (Deepa & Ganesan, 2019) in which priority weights for variables is calculated using a rough set method centered on superiority.(Nielsen, Vigil, & Benjamin, 2011) evaluated production, water output (WEE), output in precipitation usage (PUE) and net crop returns based on proven crop responses to the use of water by integrating the rotation of grass / broadleaf.(Lavanya, Jain, & Jain, 2019**)** emphasizes the degree to which different environmental factors influence rainfall and also uses the decisions taken on crop development, including the identification of diseases and crop selection.(Tamsekar et al., 2019) treats the comparative study of the GIS-based crop prediction interface machine learning algorithms (CSPM). (Song et al., 2019) evaluated the viability of the z-score process, and categorized and decreased input functionality using a technique of backward removal. (Lagos-Ortiz et al., 2019) Presents an professional knowledge support program for rice, coffee and cocoa production, focused on the user's input, and external details, such as position and environment, which will assist the selection processes, tracking, surveillance, detection, pest prevention, selection of fertilizer, among others.

METHODS AND PROPOSED ALGORITHM

In this part presents a comparative analysis of various crop selection methods are discussed. Hence, the data analysis and research process used by the obtained data. The Model questionnaire of the crop cultivation includes the following features which is tabulated in Table1. This questionnaire helpful to take the information table from the different farmers suggestions for crop cultivation process.

PROBLEM DESCRIPTION

Regarding the decision making problem involving eleven features of each crop F_i(i=1,2,….11) and Nine Crops C_j =(j=1,2,…9) all the crop feature values are the maximum number of acceptance of the Farmers in cultivation. The measurement of weight value is calculated the Maximum value of the outer texture. Try to compare the various decision methods Relative closeness(R_i) value of the each crop and make the effective decision depending on the maximum value of R_i for the selection of the crop.

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K-Means Algorithm

The partitions of a database of N objects into a set of k clusters are created using Partitioning algorithms. The creation involves formative the optimal partition with respect to an objective function. K-Means algorithms, where each cluster is represented by the center of gravity of the cluster [22]. The basic algorithm is given below.1.Select k points as initial centroids.2.Repeat3.Form k clusters by assigning all points to the closest centroid.4.Recompute the centroid of each cluster5.until the centroids don't change.

Relative Closeness Method

The Effective of decision making is measured using RC method. For calculating the relative closeness, first determine the weight value, this value is multiplied by the features of the crop and also find the ideal score and Negative of each crop. Relative closeness (R_i) not only indicates how good the solution is but also corresponds to how close the features to each crop. This can be evaluated by $R_i = NS_i'/(IS_i + NS_i')$.

Proposed WAARC Algorithm

The proposed algorithm for crop selection is detailed below.

STEP 1 Pick the collection of crops depending on decision of Farmer by applying K-Means algorithm.

STEP 2 Form the Decision table in three ways

i)   Having Selected (All) maximum value of the Features Information table
ii) By applying the Quick Reduce Algorithm to form the Reduce features Information table
iii) By categorizing the Information table and applying the Fuzzy Membership function OR.

STEP 3 Construct the All Features and Reduce Features Weight Analysis Aggregation Table by applying maximum weight for the decision parameters.

i)   For AFWAA calculate $Si = eijVfi*Wi$. The Weights (Wi) are Max(f1)=0.7,Max(f2) = 0.7 and Max(f3) = 0.8
ii) For RFWAA calculate $Si = eijVfi*Wi$. The Weights (Wi) are Max(f1)=0.7, Max(f2) = 0.8
iii) For MFWAA (OR) calculate $Si = eijVfi*Wi$.The Weights (Wi) are Max(d1)=0.7, Max(d2) = 0.8

STEP 4 Determine the All Features, Reduct Features and Membership Relative closeness value of each crop
STEP 5 Finally crop selection can be optimized by Maximum value of R_i using AFRC,RFRC and FMFRC Tool. The decision is S_k if $S_k = Max\ R_i$. Lastly, comparative $R_i$value for AFWAA, RFWAA and MFWAA RC Tool is represented by Relative closeness Chart which is used to show the best method to take the effective decision making for all kinds of problems.

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Proposed Model
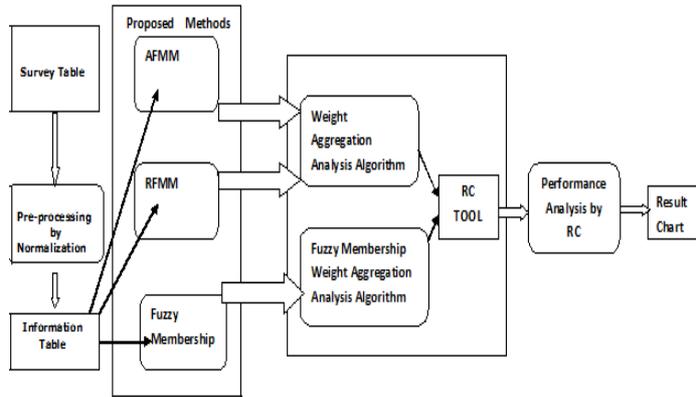
EXPERIMENTAL RESULTS

In this section shows the comparative analyzes of AWAARC, RFWAARC and MFWAARC by using proposed RC Tool for effective decision making to which crop is to be cultivated by the farmer. Here we get the acceptance from 100 Farmers in Nallampatti village. The content or input of the Table 2 can be created by the choice of the farmers' decision from the Table 1 with the maximum acceptance of the farmer.

STEP 1 Pick the collection of crops depending on all the features by applying K-Means algorithm with k=3
After applying the K –Means algorithm and the final Clusters arecluster1 = {C1,C4,C5,C7}; cluster2 = {C2,C8} and cluster3 = {C3,C6,C9}.The above cluster values are tabulated in Table 3. This Table 3 contains three input features with one " decision value" column which can be yielded from K-Means algorithm.

STEP 2 Construct the AFWAA, RFWAA and MF Weight Aggregation Analysis Table by applying the maximum weight of the decision parameters. $Si= eijVfi*Wi$. The Weights (Wi) are Max(f1)=0.7, Max(f2) = 0.7 and Max(f3) = 0.8

STEP 3 Determine the Relative closeness value (Ri)of the parameters for each crop by using the Ideal score(ISi)= Max(C1) and Negative score(NSi') =Min(C1).Ri = NSi'/(ISi+ NSi') and its tabulated in Table 4 for AFWAA, Table 5 for RFWAA by using RC tool and Table 6 for FMFWAA by using RC tool.

From the table 4, Crop Tomato (C5) has First Maximum Value, Maize (C3) has second Maximum Value and Groundnut, Tapioca (C7 & C9) has Third Maximum Value. From the Table 5, Crop Tomato (C5) has First Maximum Value, Maize (C3) has second Maximum Value and Groundnut (C7) has Third Maximum Value. From the Table 6, Crop Tomato (C5)&(C7) has First Maximum Value, Maize (C3) has second Maximum Value.

STEP 4 Finally crop selection can be optimized by Maximum value of Ri using AFWAA, RFWAA and FMWAA
The decision is Sk if Sk=Max Ri . By both the Method, Thus proved, Tomato (C5) is suitable for cultivation depending on the farmers decision and also RFWAA having Max (Ri = 0.5000) comparing to the AFWAA.

STEP 5: The decision is represented by Relative closeness Chart and its represented in

CONCLUSION

Integration of science and technology in agriculture is considered to be judicious action. The proposed work of this paper, K-Means algorithm was applied to group the crops, which is taken from the questionnaire depending on the motivating features of the Farmers. In this paper we used a Relative Closeness tool with WAAA that can be helpful to categorize the variety of crops based on the schedule. To make the effective decision for the particular crop selection done by the proposed methods like AFWAA, RFWAA and MFWAA with Weight Aggregation analysis algorithm, depending on the features which can be optimized by Relative Closeness (R_i) Method. In this crop data set among 9 crops Tomato crop was selected for cultivation and also observed Crop C5(Tomato) & C7(Groundnut) coming under the same group resulted from the K-means algorithm. This study clearly shown that the proposed approach ended in effective manner to select the particular crop. Further research is required for the application of the above mentioned technology to improve agricultural conditions.

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Table 1. Sample survey features of the various crops

Features/ Crops	Minimum Capital (f1)	Short duration (Yield) (f2)	Minimum Irrigation (f3)	Low Manpower (f4)	Easy Cultivation (f5)	Minimum Fertilizer (f6)	Low Risk (f7)	Low Machinery (f8)	Easy Harvesting (f9)	Flexible Marketing (f10)	High Profit (f11)
Sugarcane(C1)	6	5	4	5	4	4	5	2	6	7	8
Paddy (C2)	4	2	1	3	2	3	2	2	3	5	6
Maize (C3)	7	6	4	2	4	2	4	3	3	6	6
Turmeric(C4)	5	3	3	4	3	4	2	4	4	6	7
Tomato (C5)	7	6	3	3	4	5	4	1	5	7	7
Cotton (C6)	7	6	2	4	2	5	3	1	2	4	6
Groundnut (C7)	6	5	2	4	3	4	3	1	2	7	7
Plantain(C8)	2	1	2	3	1	4	3	2	5	7	8
Tapioca (C9)	7	4	2	3	2	3	2	3	3	6	7

Table2. Selected Features Information Table

Features/ Crops	Minimum Capital (f1)	Flexible Marketing (f10)	High Profit (f11)
Sugarcane(C1)	6	7	8
Paddy (C2)	4	5	6
Maize (C3)	7	6	6
Turmeric(C4)	5	6	7
Tomato (C5)	7	7	7
Cotton (C6)	7	4	6
Groundnut(C7)	6	7	7
Plantain(C8)	2	7	8
Tapioca (C9)	7	6	7

Table3. Decision Table

Features/ Crops	Minimum Capital (f1)	Flexible Marketing (f10)	High Profit (f11)	Decision Value
Sugarcane(C1)	6	7	8	C1
Paddy (C2)	4	5	6	C2
Maize (C3)	7	6	6	C3
Turmeric(C4)	5	6	7	C1
Tomato (C5)	7	7	7	C1
Cotton (C6)	7	4	6	C3
Groundnut(C7)	6	7	7	C1

Plantain(C8)	2	7	8	C2
Tapioca (C9)	7	6	7	C3

Table 4. AFWAA- Weight Aggregation Analysis Table & Relative Closeness Method

Crops	S1	S2	S3	Isi	Nsi	Ri
Sugarcane(C1)	42	49	64	64	42	0.3962
Paddy (C2)	28	35	48	48	28	0.3684
Maize (C3)	49	42	48	49	42	0.4615
Turmeric(C4)	35	42	56	56	35	0.3846
Tomato (C5)	49	49	56	56	49	0.4667
Cotton (C6)	49	28	48	49	28	0.3636
Groundnut(C7)	42	49	56	56	42	0.4286
Plantain(C8)	14	49	64	64	14	0.1795
Tapioca (C9)	49	42	56	56	42	0.4286

Table5. RFWAA- Weight Aggregation Analysis Table & Relative Closeness Method

Crops	S1	S2	Isi	Nsi	Ri
Sugarcane(C1)	42	49	49	42	0.4615
Paddy (C2)	28	35	35	28	0.4444
Maize (C3)	49	42	49	42	0.4615
Turmeric(C4)	35	42	35	35	0.4545
Tomato (C5)	49	49	49	49	0.5000
Cotton (C6)	49	28	49	28	0.3636
Groundnut(C7)	42	49	49	42	0.4615
Plantain(C8)	14	49	49	14	0.2222
Tapioca (C9)	49	42	49	42	0.4615

Table 6. FMFWAA- Weight Aggregation Analysis Table & Relative Closeness Method

| Crops	S1	S2	S3	S4	Isi	Nsi	Ri
Sugarcane(C1)	0.64	0.64	0.49	0.49	0.64	0.49	0.4336
Paddy (C2)	0.48	0.48	0.35	0.35	0.48	0.35	0.4217
Maize (C3)	0.56	0.48	0.42	0.42	0.56	0.42	0.4286
Turmeric(C4)	0.56	0.56	0.42	0.42	0.56	0.42	0.4286
Tomato (C5)	0.56	0.56	0.49	0.49	0.56	0.49	0.4667
Cotton (C6)	0.56	0.48	0.28	0.35	0.56	0.28	0.3333
Groundnut(C7)	0.56	0.56	0.49	0.49	0.56	0.49	0.4667
Plantain(C8)	0.64	0.64	0.49	0.49	0.64	0.49	0.4336
Tapioca (C9)	0.56	0.56	0.42	0.42	0.56	0.42	0.4286

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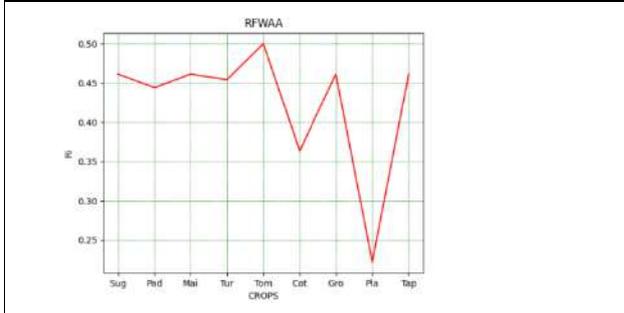
Figure.1 Shows RC for AFWAA
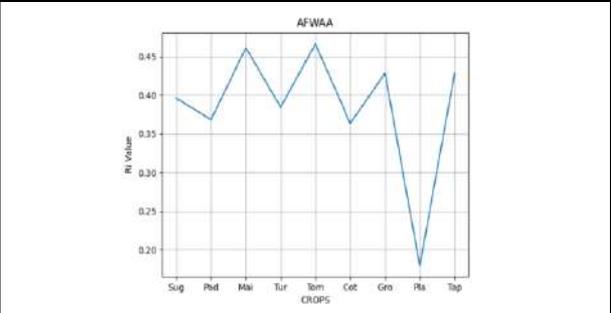

Figure 2.shows RC for RFWAA
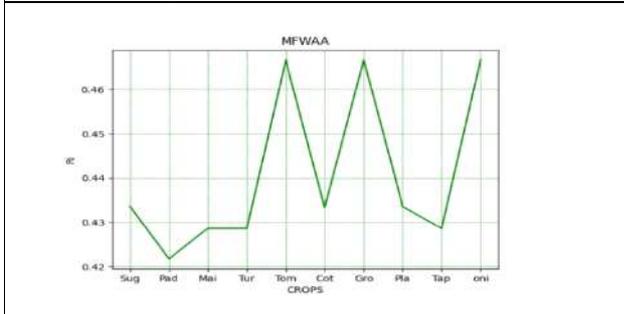
Figure 3, Shows RC for MFWAA
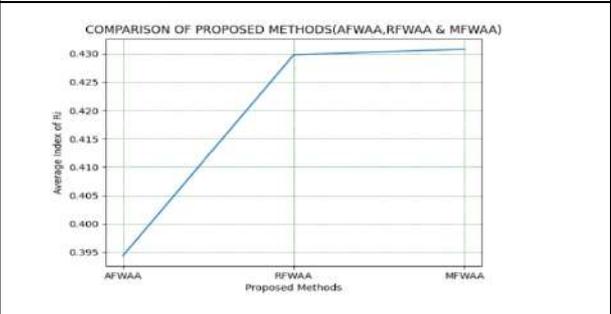
Figure 4.shows comparative analysis of RC for AFWAA, RFWAA & MFWAA

RESEARCH ARTICLE

Spiritual Intelligence: Construction and Development of Tool among the Employees of Private Insurance Sector for the Sustainable Work Culture

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ABSTRACT

Spiritual intelligence (SI) is the ability to behave with wisdom and compassion irrespective of the situation. The study of spiritual intelligence is becoming more popular among academics and researchers today. This essay aims to foster a positive attitude toward the idea of spiritual intelligence and to pinpoint the elements that either support or have an impact on it. A person is more prepared for their psycho-social-physical surroundings with spiritual intelligence. In today's world, spiritual intelligence is highly needed to manage oneself and be resilient. In the service sector work, pressure is high in that the Insurance sector is facing more challenges in retaining employees. Work pressure, work target, and attrition are more among the insurance sector employees. There is no such workplace where pressure is almost nil. The only solution today to lead a balanced life is to increase our inner strength. The investigator has chosen 18 Private Insurance sector employees for the study. The primary intention of the study is to construct a tool to measure employees' spiritual intelligence levels. A pilot study has been conducted considering a sample size hundred.SI tool has been constructed by considering Mindfulness, *Transcendence*, connecting with others, and value systems as significant components. Each component was taken based on answers to the interview schedule on spiritual intelligence and with the support of a literature review. SI tool was constructed with the help of a reliability test and validity test. Forty-six statements have been constructed in this tool. The tool will be helpful for the workforce to check on their level of spiritual intelligence and work upon it to improve.

Keywords: Spirituality, work place spirituality, spiritual intelligence, Reliability, Validity.

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INTRODUCTION

Spiritual Intelligence Quotient was given much weight over the world. The assumption was that the higher the number, the greater the intelligence. This mindset dates back to the early twentieth century when psychologists introduced cognitive tests. The primary goal of these examinations was to assess intellectual or reasoning intelligence (used to solve logical problems). Daniel Goleman published results in neuroscience and psychology in the mid-1990s that emphasized the importance of Emotional Quotient (EQ). It makes us aware of our own and others' sentiments. Goleman claimed that EQ was a prerequisite for using IQ. However, in 2000, authors Dana Zohar and Ian Marshall presented Spiritual Quotient (or SQ), the ultimate intelligence, as a new dimension to human intellect. This is the intellect that is employed to address meaning and value difficulties. "Does my employment provide me with the satisfaction I seek?" "Am I relating to the people in my life in a way that helps them and me to be happy?" The answers to these questions determine whether or not we will be happy. In such a situation, IQ and EQ are insufficient. Our ability to think correctly is harmed if the parts of our brain feel are destroyed.

Spirituality is present in the hearts and minds of both men and women worldwide, regardless of their affiliation with a particular religion. Theologian Paul Tillich asserts that everyone is spiritual if we consider spirituality to be the object of ultimate anxiety. This is because everyone has similar worries. The term "ultimate concern," on the other hand, has several different meanings. Some people reject the idea that they or their problems are spiritual. Similar to emotion, there are different levels of spiritual expression. It may or may not be aware, developed, healthy or unhealthy, complex or simple, beneficial or dangerously distorted. Spirituality is present in the hearts and minds of both men and women worldwide, regardless of their affiliation with a particular religion. Theologian Paul Tillich asserts that everyone is spiritual if we consider spirituality to be the object of ultimate anxiety. This is because everyone has similar worries. The term "ultimate concern," on the other hand, has several different meanings. Some people reject the idea that they or their problems are spiritual. Similar to emotion, there are different levels of spiritual expression. It may or may not be aware, developed, healthy or unhealthy, complex or simple, beneficial or dangerously distorted.

Spirituality is defined as a "sense of ultimate connection or belonging to the transcendental root of existence." Some people define spirituality as having a relationship with God, other people, or the natural world. Others characterise it as zeal and devotion to a certain idea or activity. The good life is characterised in humanistic terms as living up to one's full potential as a human (Anastoos, 1998) honestly. To comprehend how Spirituality could contribute to this, it is important to distinguish between healthy Spirituality and beliefs and practises that may harm one's wellbeing. As a result, it becomes challenging to define and cultivate spiritual intelligence. When we consider the significance of queries like "Who am I? ", "Does it matter? ", we rely on spiritual intelligence. "Why am I here?" and "What am I doing here?" Perhaps through spiritual intelligence, one can uncover sources of love and joy that are concealed behind the pressure and chaos of daily life.

According to to Forman (1997), Spirituality is more about experiencing it rather than philosophical theory. "The adaptive application of spiritual information to enhance everyday problem solving and goal fulfillment," according to SI (spiritual intelligence) (Emmons, 2000). SI refers to a person's capacity to act prudently and compassionately while maintaining inner and outer harmony (Wigglesworth, 2002). Spirituality is simply knowing oneself, and spiritual intelligence is knowing who I am and being conscious of who I am. SI is the ability to put one's actions and life in a larger, more meaningful context and the intelligence with which one confronts and resolves difficulties of purpose and value (Zohar & Marshall, 2000). In order to apply, actualize, and embody spiritual resources, values, and traits in ways that are beneficial to their daily lives and well-being, people use a set of abilities known as SI. (2007, Amram). Observing things from multiple perspectives and comprehending the connections between perception, belief, and behavior. Despite being inextricably linked, most people are expected to take responsibility for their actions, not for their beliefs or perceptions; like any other form of intellect, spiritual intelligence requires training and discipline to refine. When we investigate the meaning of questions like "Who am I?" we rely on spiritual

intelligence. "What matters?" and "Why am I here?" Perhaps spiritual intelligence can also assist an individual in discovering hidden wellsprings of love and joy beneath the stress and turbulence of daily life.

Greedy, immoral behavior, individualism, and disappointments have pervaded corporate life in the first two decades of the twenty-first century, resulting in a growing need for Spirituality. In truth, the insatiable needs of today's world are unstoppable. Workplaces worldwide put a lot of pressure on employees to keep their emotions under check. Control, obtaining higher levels of performance, and honing self-management abilities. Such a thing exists. This scenario usually leads to an imbalanced and undesirable work-life balance. Personal life, In essence, the next frame is clearly outlined: if a worker is dissatisfied with a large number of unemployed people, he/she is free to leave his/her work at any time. Workers may be able to rapidly and cheaply replace him. When seen as a whole, various types of problems. More specifically, "Inner space exploration and resolution is a spiritual process! Inner space refers to one's spiritual center" (Guillory, 2001, p. 220).

Spiritual awareness and the development of spiritual strengths lead to excellence in corporate decision-making, interpersonal interactions, and culture, helping businesses to grow and last. Spirituality comprises enlightening one's thoughts, emotions, and lower impulses. It enlightens the intellect and cultivates sensitivity to one's fellow man and nature's beauty. According to Vaughan (2002), spiritual intelligence encompasses more than one brain capability. It builds a connection between the creator and the individual, as well as between the soul and others. Spiritual intelligence goes beyond traditional psychological development; it opens the heart, enlightens the mind, inspires the soul, and allows a person to distinguish between reality and imagination, allowing them to discover hidden springs of love and joy amid daily stress and problems. We can also see things as they are, free of unconscious distortion, and can be expressed in any culture as love, wisdom, and service. To put it another way, to cope with the tensions and obligations they experience, workers must awaken their inner strengths. According to Mitroff (2003), people also want to evolve. Individuals should be able to display their faith at work because they spend so much of their lives there. However, even though work in and of itself usually provides people with a feeling of meaning and purpose in life, the other side of this argument is that organizations have overwhelmed personnel. "Unfortunately, the word 'work' has become a curse for many people nowadays," writes East (2005, p. 16).

Similarly, Twenge and Campbell (2008) argue that the modern workplace is not psychologically safe – or, to put it another way, it is not spiritually healthy – due to quick work demands, productivity goals, and stressful feelings. In this situation, people feel pressured to accomplish more and more. Work should not be allowed to become a destructive force in an ideal world (Evers & Reid, 2009), but because work is such a vital part of our lives, we must learn to deal with all obstacles.

Sustainable work culture

Work culture generally refers to the views, ideological perspectives, thought processes, and attitudes shared by the individuals who make up a company or organization. Workplace culture can emerge naturally or be shaped and impacted by company practices and rules. Opening up and creating opportunities for team members to form strong working connections and build trust is key to creating a more positive and sustainable work culture. It entails fostering their development and providing constructive and encouraging feedback. In a nutshell, work culture refers to an organization's collective personality. Working without a sense of purpose and meaning leaves people dissatisfied, and it makes it difficult for businesses to establish themselves in the market and contribute to the communities they serve (Covey, 1990; Deming, 1981). Yahyazadeh-Jeloudar and Lotli-Goodarzi (2012) found a strong association between work satisfaction and spiritual intelligence in a study of master's (MA) and bachelor's (BA) program teachers. Spiritually inclined one can create a positive work culture, and individuals can build upon it through daily prayers, meditation, Yoga, Pranayama, Reading spiritual books, and family influence; these can influence spiritual paths. If we try to focus on our inner thoughts and feelings, we will help solve conflicts within ourselves. "Mindfulness means paying attention in a particular way on purpose, in the present moment, and non judgmentally" (Kabat-Zinn,1994). Our mind should be trained to stay in the present, which can be achieved through mindful meditation, breath control techniques, and life experiences. Understanding the proper purposes of life and

expanding our horizons by connecting with others and involving ourselves to serve society also helps us to walk on a spiritual path. To be spiritual, empathetic, and create a positive environment is impacted by the individual value system. Our education system, practices followed at home, spiritual guru's guidance and grandparents' guidance all support an individual to shape the personality.

SI and Psychological health

Since it connects workers, the organization's strategies, and its long-term goals, leadership is crucial in every business. It manages problems and crises within the organization and creates answers to them since it is the melting pot in which concepts, policies, and strategies are fused. To achieve harmony and balance between the needs and desires of his group and the needs and desires of the organization to which they all belong, as well as to have a positive effect on individual morale and productivity, and to strike a balance between personal and organisational goals, is therefore the leader's primary responsibility. The leader must possess distinctive personal traits that enable him to fulfill this vital task (Alkhatib&Arnout, 2019; Arnout, 2008, 2017). The potential of spiritual intelligence to affect people and transform civilizations and communities has led to its recognition as the most significant type of intellect. (2019b, 2016). In addition to the immense stress of contemporary family, work, and school life, a desire to cultivate spiritual intelligence can aid individuals in adopting a positive outlook and achieving inner peace by enhancing motivation for self-control (Arnout, 2007, 2008, 2015, 2016, 2019b).

The health of an organization is intrinsically tied to both spiritual well-being and intelligence. In actuality, goal-setting and Spirituality at work may coexist. Spirituality has been perceived throughout history as a concept dimension (Yang & Moa, 2007). People who feel connected, in solidarity with their coworkers, and believe that their beliefs and objectives align with the firm have a strong sense of purpose in their job. Some experts define "spirituality" as an employee's experiences of working energetically, being content with and having meaning and goals in their work, and developing positive relationships with coworkers (Emmons 2000:3). Spiritual intelligence is frequently linked to psychological well-being, even though some types of Spirituality are dysfunctional or pathological (Deikman, 1990). Denial and projection, as well as fear and conflict, can be poisonous and dangerous spiritual beliefs. When a cult leader, for example, plays on people's fears and guilt, the community can take on the characteristics of a dysfunctional family. A person's recovery from making a spiritual commitment without thinking can take years.

SI and Resilience

According to Masten (2009), successfully leading life irrespective of the advice situations is termed resilient. According to Singh and Yn (2010), psychological resilience refers to effective coping and adaptation in the face of loss, difficulty, or adversity. People with a high level of resilience are strong enough to fight life adversities using positive emotions to survive in the environment (Carle AC,2004). Highly resilient individuals are aware of life purposes and spiritually inclined (Eksi et al. 2019). Spirituality, on the other hand, provides people with the strength to comprehend and overcome the traumas created by unpleasant events, as well as giving these wounds meaning, giving people hope and making them more mentally resilient (Eksi et.al. 2019; Gultekin et al.,, 2019; Jannati et al., 2017). those who are spiritually inclined are more resilient, and stress level is also considerably low. Stress is also likely to influence a person's coping mechanisms in ways that affect their life paths, and their level of resilience can directly influence their health and well-being (Ong AD, 2004).

SI and Employee Retention

Organizations should focus on employee retention and must try to reduce employee turnover (Michaud, 2000; Bussin, 2002). Today's employee's expectation is different from the old generation; employees now need better working conditions. They give more importance to their quality of life and better prosperity in terms of career at a specific time (Heneman& Judge,2003; Daniel, 2010). Organizations need to focus more on employee focused activities to create a positive atmosphere to retain them and keep them happy (Box all & Macky,2009). Old study says that a combination of extrinsic and intrinsic motivation may help the organization retain employees

(Halil&Cem, 2010 ;Hafiza et al., 2011). Employees' psychological satisfaction from being assigned relevant job tasks and completing those duties as necessary is linked to intrinsic rewards. Proper and meaningful work engagement increases employee commitment toward work, bringing job satisfaction. Meaningful work helps to create a positive environment and binds the relationship among the human resources (Miller et al., 2001). Psychological satisfaction can be enhanced among employees if the organization follows workplace spirituality. A definition of workplace spirituality that emphasized the idea of spiritual orientation in the workplace was provided by Pradhan, Jena, and Soto (2017). "Where work goes beyond transactional boundaries to foster a spiritual bond among employees, leading one's alignment of values to business aims" (p. 47). Workplace spirituality is a potential strategy for luring and keeping personnel in the corporate setting. If there is one term that best captures the idea of Spirituality at work, it is connectedness, claim Mitroff and Denton (1999). Work Performance and Spiritual Intelligence

For many years, researchers have been looking at factors that influence work performance and will continue to do so in the future. Workplace performance is crucial to a company's or organization's ultimate goal of product development, service delivery, and competitiveness advantage. It also can work faster while maintaining accuracy towards the goal (Wichen et al., 2000). Various sorts of influence on work have been proven in the previous study. Performance. Hard work, as well as a person's talents and abilities, influence labor performance. Favorable conditions and the task's relative simplicity (Rosenthal, 1995); work quality task contextual performance (Farh et al., 1991), as well as the amount of it (Farh et al., 1991). (Farh et al., 1991). Borman and Motowilda (2007). Employees may achieve their goals without sacrificing their creativity, and they can cope with a demanding work environment with the support of workplace spirituality (Altaf. A & Awan, N.A, 2011). A person who has internalized spiritual ideals will have more self-assurance. Spirituality's primary purpose is to achieve a highly evolved individual state or to realize one's full potential, leading to increased creativity, drive, and institutional commitment. Those who are guided by spiritual beliefs are confident in their abilities, and this mindset may help them multitask more effectively. Declarative knowledge, procedural knowledge, and adaptable performance are all examples of declarative knowledge (Hesketh& Neal, 1999).abilities and motivation (Campbell, McHenry & Wise, 1990).

SI and Employee Loyalty
It has been discovered that workplace spirituality positively correlates with intuition, creativity, honesty, trust, personal fulfillment, organisational commitment, organisational performance, customer orientation, adaptability, service orientation, ethical selling behaviour, job satisfaction, organisation citizenship behaviour, job involvement, and decreased negative job attitudes like intention to leave the organisation. Employee loyalty is impacted by employee empowerment, contentment, organizational culture, interpersonal trust, and work-life policies (Yee et al., 2010). When companies foster a feeling of community by emotionally tying employees to the organization's main objectives, employee motivation and loyalty increase (Brown,1999). As a result, when firms establish a feeling of community by emotionally linking employees to the organization's primary objectives, employee motivation and loyalty increase (Milliman et al. 1999)

SI and Work-Life Balance
Spirituality has always been present in human history, even if it is not always obvious. Some have described it as simply being in the presence of higher and more complete consciousness. In most cultures, work is considered sacred and provides purpose to people's lives. It is hardly surprising that people's personalities, or ideas of who they are, are shaped by their occupations. Most of us spend a significant portion of our day working. Spiritual intelligence is one of the newest types of intelligence that can aid problem-solving. Spiritual intelligence has numerous consequences on a person's life, particularly at work and home. Spiritual intelligence is a solid link to work-life balance, a crucial aspect influencing life quality and work-life balance (Maryam et al., 2012).

Objective
To construct and standardize Spiritual Intelligence Scale using reliability and validity tests.

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RESEARCH METHODOLOGY

Eighteen private insurance companies from throughout India were covered in the analysis. The information was gathered using a basic random sampling technique. The people being polled are from the department of sales. The sample size for the study has been established at 100 persons. A systematic questionnaire was used to collect responses. A five-point scale was employed to divide the questionnaire into two portions. Section A is concerned with data categorization based on respondents' demographic traits, while Section B is concerned with spiritual intelligence. Construction of tool has been done through 1) Reliability test 2) Validity test. Table 1 shows the results of two tests: the Kaiser-Meyer-Olkin sample adequacy score and Bartlett's Test of Sphericity to see if the link between the variables is significant. The test statistic for the Kaiser-Meyer-Olkin measure of sampling adequacy is 0.811, indicating that the factor analysis for the specified variables is suitable. The significant result of Bartlett's Test of Sphericity is 0.000, indicating that the selected variables are statistically significant and that there is a strong association between the factors of spiritual intelligence among Insurance sector personnel.

Content Validity: A long list of components and structures make up the research instrument. These first items and structures are derived from the available review of literature. The exhaustive research of the literature is where the variables needed to gauge spiritual intelligence are gathered. Three research researchers were first given the tool, and their feedback on the questionnaire was gathered. The three professors who were authorities in the fields of management, commerce, and statistics were then asked to review the instrument and their comments were noted. These recommendations were carefully considered, and the variables in the questionnaire were appropriately adjusted, eliminated, and added. As a result, the topic experts' opinions and suggestions were used to confirm the validity of the questionnaire's content, and some adjustments were made to the questionnaire to make it clearer, easier to comprehend, and more useful.

The degree to which the study's constructs genuinely measure the anticipated performance in comparison to the planned measurement standards is known as construct validity (Herl et al., 1996)
1. Clustering the constructs using Exploratory Factor Analysis
2. Convergent Validity ensuring through Confirmatory Factor Analysis
3. Composite Reliability

Table 2 reveals that out of the 46 parameters of spiritual intelligence among the Insurance sector employees. These four factors explain the total variance of factors affecting spiritual intelligence to 68.75 percent. The 11 parameters of spiritual intelligence such as 'I don't try to spend time to know myself, 'I believe in living in the present rather than worrying about past or future', 'My spiritual practices help me to keep work- life balanced', 'At the time of controversies I always try to remain calm, 'I always try to maintain good relationship with my superiors to avoid conflicts with them' , 'I always try to attach meaning to the events which happens in my life, 'When I am sad I avoid people who celebrate their victory', 'I can't able to achieve my work targets during mental stress', 'I don't humiliate anybody in any place', 'I don't wait for the right time to sensitize others mistake', 'I guide my colleagues during their tough times' were clustered together as factor 1 (**Mindfulness**) with **24.66 percent variance** . And then other 11 parameters of spiritual intelligence such as 'I practice meditation regularly, 'I developed my spiritual interest through reading spiritual books, 'In the process of my spiritual practice I experience a sense of stability in my mind', 'I am interested in listening to spiritual discourses', 'I feel meditation is a difficult task and leads nowhere in life', 'I am too tensed in my work to focus on spiritual practices', 'Spending time to practice Yoga is not possible always', 'I feel grace factor plays major role in my life accomplishments', 'I believe that this job is given to me to achieve something better in life, 'I have developed my spiritual beliefs through my own life experiences, 'I am not emotionally stable to withstand pressure' were grouped as factor 2 (Transcendence) with variance of 19.53 percent variance. Another ten parameters of spiritual intelligence such as 'I don't like to involve myself in social services, 'Love to my duty provides me the confidence to accomplish better', 'I believe that main purpose of life is to be content, 'I keep grudges towards people who hurt me, 'I can't be loyal to my organization at times, 'Acquiring

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material wealth gives me a sense of satisfaction, 'I can find solutions to any kind of problem when I sit in solitude', 'I believe that I am accountable to the supreme power for all my actions', 'I don't like to involve myself in unnecessary arguments', 'Spiritual influence helps me to generate new ideas' were constituted as factor 3 (Connecting with others) with 15.07 percent variance. Another 14 parameters of spiritual intelligence such as 'My inclination towards spirituality is attributed to my family members', 'I don't like to visit spiritual places', 'I don't get sound sleep during hectic schedules', 'I consult spiritual Gurus during my tough times', 'My educational institutions played a major role to inculcate spiritual beliefs in me', 'I believe that my food habit has an impact of way of thinking', 'Social media plays a critical role in my spiritual journey', 'Thinking about the outcome of the work, I get tensed even before starting it, 'This organization does not focus on empowering employee by trying to maintain a positive environment', 'I seek to elderly guidance on a regular basis to calm myself', 'I am very impulsive and I use harsh words at times in anger', 'I don't believe that celebrating festivals help in inculcating value in us', 'I believe that it is natural to die as it is to be born', 'I am not sentimental about anything because life is like a river and it flows' were clustered as factor 4 (Value System) with 9.49 percent variance. All of these variables are found to be influencing the level of spiritual intelligence among the workers in the insurance industry, according to the loading patterns of the factors, which point to a strong link between the parameters.

It was determined that an item statement's factor loading must be more significant than .40 to be retained in its respective scale based on the CFA models used in the studies of Paré& Tremblay (2007) and Nasurdin, Ahmad, & Lin (2009). Insignificant and negatively significant/insignificant statements (.40) were eliminated. Because of this, 46 claims on spiritual intelligence have been kept in their respective scales because their factor loadings are under .40, but other assertions that were deemed to be unimportant have been removed. GFI =.925, AGFI =.923, NFI =.985, TLI=.987, CFI =.956, RMSEA =.022, RMR=.026, chi-square = 54.785, CMIN/df = 1.154, and Probability level (p-value) =.142 were all acceptable indices for the CFA model for spiritual intelligence (Anderson and Gerbing, 1988 Hair et al., 1995, Kline, 2005). Spiritual intelligence's Average Variance Explained (AVE) is greater than 0.5, indicating that the measure has convergent validity (Nunally & Bernstein, 1978). The construct assures unidimensionality because the components loaded for spiritual intelligence are over the prescribed level.

COMPOSITE RELIABILITY
The Cronbach coefficient is commonly used to evaluate the survey instrument's reliability. Only instruments with a Cronbach reliability score of 0.70 or higher are considered reliable. However, it has been found that the Cronbach score does not give each item in the construct equal weight, which could cause the dependability results to be off. A separate composite reliability test is necessary as a result. Table 3 shows that the overall reliability of the constructs of spiritual intelligence is more than 0.7, indicating that the construct's dependability is well established.

CHECKING ASSUMPTIONS
The procedure for verifying the multivariate data analysis assumptions is described in the next section. A number of assumptions behind multivariate approaches are based on basic statistical theory. Three of them may have an impact on all multivariate statistical analyses, despite the fact that multivariate statistical procedures involve various assumptions or requirements. (Hair and others, 2010) 23.

1. Normality
2. Homogeneity
3. Multicollinearity
It depicts the shape of the distribution of data and diagram of the histogram that compares the observed data with normal distribution. The data come under normal distribution curve and hence, the data set is confirmed to be possessing normality (Epps & Pulley, 1983) [24].

Framework of Analysis
The framework of analysis contains several statistical procedures for data analysis that are in line with the study's aims and aid in the development of meaningful findings. Various statistical tests were used to validate and

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demonstrate the dependability of the acquired data. The data were placed under a normal distribution curve after validating the normality assumptions, and appropriate statistical methods were employed to analyse the data. The collected data was coded using the SPSS package, and various appropriate statistical tools such as exploratory factor analysis and confirmatory factor analysis, As a result, the researcher used a variety of statistical methods for analysis, including the statistical packages SPSS 22 and AMOS 20, which are more suited to making inferences about the study's subject matter.

RESULTS AND DISCUSSION

The current study was done with the objective to construct and standardise the Spiritual Intelligence tool through reliability and validity constructs. The tool consisted of 50 statements in the beginning and was reduced to 46 statements, the reduction of the statement was done during the pilot study and with the help of EFA. The tool consisted of four factors they are Mind fullness, Transcendence, Connecting with others and Value System. The standardised tool will help to check the spiritual intelligence of employees especially belong to the service sector and can be used in every sector.. This tool will help to check their SI capability in varied aspects and researchers can use this tool to measure the level of Spiritual Intelligence.

CONCLUSION

As a result of globalization, today's workplaces have a diverse workforce. As a result of this diversity, issues like the coalition, cooperation, and teamwork have become increasingly important for management to address. People with a heart, spirit, and soul operate on the job. Moreover, there must be relationships between people. Empathy is a necessary component of spiritual intelligence since it allows one to reflect more deeply on one's fellow beings and their circumstances, which is proof of spiritual intelligence.

Those at work constantly look for ways to improve themselves and feel invested in their jobs. Every employee needs to have a sense of accomplishment and fulfillment at work. Workplace harmony fosters a more vital link and enjoyable working environment, allowing employees to weather the ups and downs of this fast-paced industry. This type of spirit exemplifies the need for spiritual intelligence. To create workplace serenity, spiritual intelligence encourages both intrapersonal and interpersonal communication. Intrapersonal communication aids in maintaining a positive relationship with oneself, whereas interpersonal communication aids in maintaining a positive and easy relationship with co-workers. As a result, spiritual intelligence aids in the reduction of conflicts that obstruct development and enhances the ability to have a purposeful relationship with the organization. Work would become more important to employees due to spiritual intelligence, resulting in the betterment and enhancement of spiritual inner being. As a result, employees' values are identified and supported with a strong sense of purpose. Learning from one's flaws and pains is a critical attitude that allows one to view things from a fresh perspective, resulting in a more holistic approach to life. Life is a riddle, and the goal is to figure out how to solve it. Employees must be able to solve problems. Spiritual intelligence makes it easier to identify solutions to challenges by facilitating essential information. When goals are set correctly (REALISTIC Goals) and personnel focus on achieving them, goal attainment becomes simple. This becomes their work and life's purpose and meaning. Enhancement of Spiritual Intelligence should be a goal of each individual as well as of the organisation. Work place spirituality helps to bring sustainable growth in the organisation.

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Table: 1 Reliability analysis of Spiritual Intelligence

KMO and Bartlett's Test		
Kaiser- Mayer- Olkin Measure of Sampling Adequacy		0.811
Bartlett's Test of Sphericity	Approx. Chi-Square	42378.672
	Df	1035
	Sig.	.000

Source : Computed from primary data

Table: 2 Clustering the factors of spiritual intelligence using EFA

Factor	Parameters	Rotated Factor Loadings
	I don't try to spend time to know myself. (M1)	.787
	I believe in living in the present rather than worrying about past or future (M2)	.834
	My spiritual practices help me to keep work- life balanced (M3)	.699

Factor 1:	At the time of controversies I always try to remain calm (M4)	.642
	I always try to maintain good relationship with my superiors to avoid conflicts with them. (M5)	.574
	I always try to attach meaning to the events which happens in my life (M6)	.817
	When I am sad I avoid people who celebrate their victory (M7)	.652
	I can't able to achieve my work targets during mental stress (M8)	.712
Mindfulness	I don't humiliate anybody in any place. (M9)	.746
	I don't wait for the right time to sensitise others mistake (M10)	.532
24.66 % of Variance	I guide my colleagues during their tough times (M11)	.589
	I practice meditation on a regular basis. (T1)	.833
	I developed my spiritual interest through reading spiritual books (T2)	.714
Factor 2:	In the process of my spiritual practice I experience a sense of stability in my mind (T3)	.586
	I am interested in listening to spiritual discourses. (T4)	.699
	I feel meditation is a difficult task and leads nowhere in life. (T5)	.752
Transcendence	I am too tensed in my work to focus on spiritual practices. (T6)	.809
	Spending time to practice Yoga is not possible always. (T7)	.533
	I feel grace factor plays major role in my life accomplishments. (T8)	.746
	I believe that this job is given to me to achieve something better in life. (T9)	.822
	I have developed my spiritual beliefs through my own life experiences. (T10)	.749
19.53 % of Variance	I am not emotionally stable to withstand pressure (T11)	.830
	I don't like to involve myself in social services. (C1)	.845
	Love to my own duty provides me confidence to accomplish better (C2)	.664
	I believe that main purpose of life is to be content. (C3)	.652
	I keep grudges towards people who hurt me (C4)	.726
	I can't be loyal to my organisation at times. (C5)	.867
Factor 3:	Acquiring material wealth gives me a sense of satisfaction. (C6)	.532
	I can find solutions to any kind of problem when I sit in solitude. (C7)	.541
	I believe that I am accountable to the supreme power for all my actions (C8)	.632
Connecting with others	I don't like to involve myself in unnecessary arguments. (C9)	.887
15.07 % of Variance	Spiritual influence helps me to generate new ideas (C10)	.674
	My inclination towards spirituality is attributed to my family members. (V1)	.703
	I don't like to visit spiritual places. (V2)	.564
	I don't get sound sleep during hectic schedules (V3)	.687
	I consult spiritual Gurus during my tough times (V4)	.692
Factor 4:	My educational institutions played a major role to inculcate spiritual beliefs in me (V5)	.519
	I believe that my food habit has an impact of way of thinking. (V6)	.556
	Social media plays a critical role in my spiritual journey (V7)	.856
	Thinking about the outcome of the work, I get tensed even before starting it. (V8)	627
Value System	This organisation does not focus on empowering employee by trying to maintain a positive environment (V9)	.552
	I seek to elderly guidance on a regular basis to calm myself (V10)	.578
9.49 % of Variance	I am very impulsive and I use harsh words at times in anger. (V11)	.833
	I don't believe that celebrating festivals help in inculcating value in us. (V12)	.689

	I believe that it is natural to die as it is to be born. (V13)	.694
	I am not sentimental about anything because life is like a river and it flows. (V14)	.651
Total Variance Explained : 68.75 % Variance		

Table: 3 Results of Goodness of Fit test for Spiritual Intelligence

Indices	CMIN/df	P	GFI	AGFI	NFI	TLI	CFI	RMSEA	RMR	AVE
Model Value	1.154	.142	.925	.923	.985	.987	.956	.022	.026	.54
Recommended Value	<3.0	>0.05	>0.90	>0.90	>0.90	>0.90	>0.95	<0.05	<0.05	>0.50

Table: 3 Assessment of Composite Reliability

Construct	Indicators	Composite Reliability
Spiritual Intelligence		
Mindfulness	11	0.89
Transcendence	11	0.75
Connecting with others	10	0.84
Value system	14	0.78
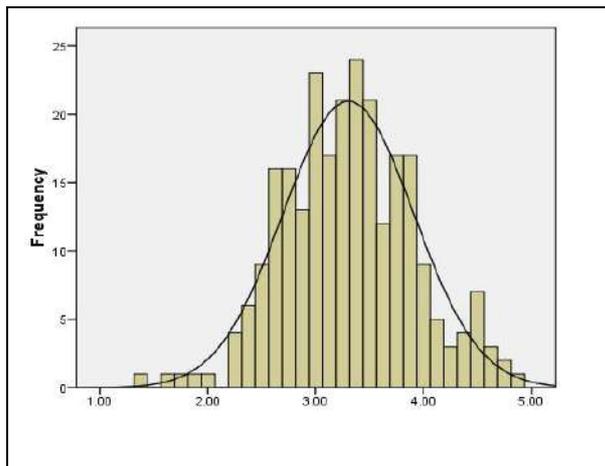
Figure.1. Normality
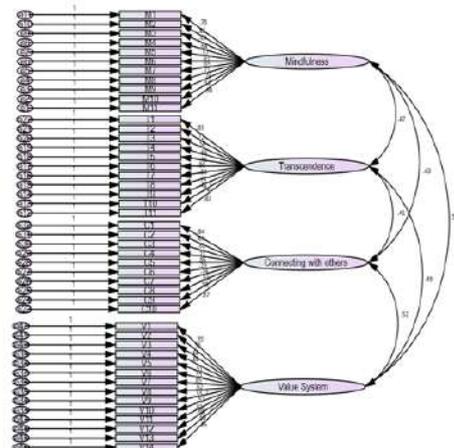
Figure.2. CFA Measurement Model of Spiritual Intelligence

RESEARCH ARTICLE

Comparative Analysis of Biochemical Composition of Commercial Freshwater and Marine Fishes from Tiruchirappalli, Tamil Nadu

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ABSTRACT

Many parameters impact the biochemical compositions of fishes, including biological state in the life cycle, food patterns, and environmental elements, as well as sex, size, maturation stages, and season, all of which have an impact on the compositions and quality of aquacultured or wild fish. The Present study is carried out to find the biochemical composition of five species of Freshwater fishes namely, *Etroplus suratensis, Cirrhinus reba, Oreochromismos sambicus, Cyprinus carpio, Channa striata* and 5 species of Marine fishes namely, *Lethrinus nebulous, Lutjanus compechanus, Caranx melampygus, Sardinella longiceps,* and *Elops saurus* from Tiruchirappalli, Tamil Nadu. This study aims to estimate the amount of Protein, Glucose, Cholesterol and Urea present in tissues of vital organs like gill, muscle, kidney and liver in this ten species of Freshwater and Marine fishes. Protein content was found to be lowest in muscles of *Sardinella longiceps* (0.148 ± 0.02) gm/ dl and highest in muscles of *Channa striata* (78.973 ± 0.01) gm/dl. Glucose content in liver was estimated highest in *Sardinella longiceps* (208.8 ± 0.02) mg/dl and lowest in kidneys of *Caranxme lampygus* (0.473 ± 0.03) mg/dl. Urea content was predicted to be highest in the muscles of *Sardinella longiceps* (249.51 ± 0.01) mg/dl and lowest in gills of *Elopes saurus* (1.28 ± 0.02) mg/dl. Cholesterol is highest in gills of *Etroplus suratensis* (381.062 ± 0.03) mg/dl and lowest in liver of *Caranxme lampygus* (28.77 ± 0.01) mg/dl. The results revealed that highest amount of protein content present in muscles of *Channa striata* which is a freshwater fish, higher levels of Glucose and Urea were present in liver and muscles of *Sardinella longiceps*, which is a marine fish and highest cholesterol content was estimated in gills of *Etroplus suratensis*, which is a freshwater fish.

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INTRODUCTION

Fish is a key source of food for humans, supplying a considerable portion of the animal protein diet as well as great dietary supplies of highly unsaturated fatty acids and polyunsaturated fatty acids, particularly the omega-3 fatty acids, eicosapentaenoic acid, and docosahexaenoicacid [1]. Fish intake is becoming more popular as a result of its high polyunsaturated fatty acid content. Furthermore, fish intake has been linked to health benefits, as the long-chain chain polyunsaturated fatty acid has gained attention due to the control of chronic coronary artery disease[2], enhancement of retina and brain development[3], reduced risk of developing breast cancer, rheumatoid arthritis, multiple sclerosis, asthma, psoriasis, inflammatory bowel disease [4], and control of prostaglandin synthesis [5]. A clear idea on the biochemical makeup of fish is highly useful in a variety of situations. Today, there is a growing awareness of the need of eating nutritious foods, and fish is gaining popularity as a result of its unique nutritional benefits. In this context, nutritionists and dieticians must have a thorough grasp of the biochemical elements of fish. Animal meals contain fish and fisheries products. A thorough survey on the biochemical makeup is also an indispensible factor in this situation for the formulation of fishery products and goods.

Another critical area where correct biochemical composition information is required is in the processing and preservation of fish and fishery products. Fish is a perishable item, and quality decline is caused by changes in numerous elements such as proteins, cholesterol, etc. Information on the biochemical ingredients will surely assist a processing technologist in determining the best processing and storage conditions to ensure optimal quality preservation. Hence, the present investigation is done to study the biochemical components of fishes such as proteins, glucose, urea and cholesterol in vital tissues such as gills, muscles, liver and kidney in ten species of fishes (5 Freshwater species and 5 Marine species) from Tiruchirappalli, Tamil Nadu.

MATERIAL METHODS

FISH COLLECTION AND PREPARATION
In December 2021, a total of 200 individual fish, 20 of each species, were caught from four different fish markets in Tiruchirappalli namely, Kallanai, Cauvery Paalam, Gandhi Market and Vengur Market. These species were chosen because of their diverse habitat, year-round availability, and economic significance (www.fishbase.org), According to local fishermen knowledge, as people living around the shoreline have consumed the fish species on a regular basis throughout the years. Ten different species of fishes belonging to freshwater and marine habitat were sampled (Fig 1 & Fig 2). Table 1 contains information about the fish species. The fishes were wrapped in aluminum foil, stored in an icebox, and brought to the Department of Zoology laboratory, National College, Tiruchirappalli, where they were dissected or frozen (–20 C).

BIOCHEMICAL ANALYSIS
The Biochemical Analysis such as quantitative analysis of proteins, glucose, cholesterol and urea were estimated by standard procedures in four tissues of vital organs namely gill, muscle, liver and kidney of the experimental fishes given in Table 1.Glucose estimation was done by Enzymatic (GOD/POD), Photometric, Trinder Reaction, Fixed Time and End Point, Urea was estimated according to Photometric test enzymatic according to modified Berthelot, End point, Cholesterol was estimated according to "CHOD-PAP": enzymatic photometric test, Trinder, End Point and Protein was estimated according to Photometric test corresponding to Biuret method, end point. All the analysis were carried out in ROBONIK semi auto analyser (VER – RBK 2.2.010(ATP1200219RPK)).

QUALITY ASSURANCE AND QUALITY CONTROL

Throughout the experiment, medical gloves, glassware, and metal instruments which were utilised to treat the samples, and the dissecting tools were cleaned three times with distilled water before use.

STATISTICAL ANALYSIS

For statistical analysis and charting, Microsoft Office Excel 2010 and SPSS 16 were used. To investigate interspecies and geographic differences, a one-way ANOVA test was performed at significance level of 0.05.

RESULTS

The Freshwater and Marine fish species used in the present investigation are given below in Table 1. The Glucose content in the tissue extract of liver in *Sardinella longiceps* was estimated to be highest (208.8 ± 0.04) mg/dl and lowest in the tissue exracts of Kidney in *Caranxme lampygus* which was estimated to be (0.473 ± 0.04) mg/dl. The Glucose content measured in the ten species of Freshwater and Marine Fishes were indicated in table 2 and Graph 1 respectively. The Protein Content in the tissue extracts of muscles in *Sardinellalongiceps* was found to be the least (0.148 ± 0.04) gm/dl and reported to be the highest in the tissue extracts of muscles in *Channastriata* (78.973 ± 0.04) gm/dl. The Protein content analysed in the ten species of Freshwater and Marine Fishes were indicated in table 3 and Graph 2 respectively.

The Urea Content was reported to be high in the tissue extracts of muscles in *Sardinella longiceps* (249.512 ± 0.05) mg/dl and lowest in tissue extract of gills in *Elopssaurus* (1.28 ± 0.05) mg/dl. The Urea content analysed in the ten species of Freshwater and Marine Fishes were indicated in table 4 and Graph 3 respectively. The Cholesterol levels were estimated to be highest in tissue extracts of gills in *Etroplus suratensis* (381.062 ± 0.02) mg/dl and lowest in tissue extracts of liver in *Caranxme lampygus* (28.775 ± 0.03) mg/dl. The Cholesterol content estimated in the ten species of Freshwater and Marine Fishes were indicated in table 5 and Graph 4 respectively.

DISCUSSION

The ten Species of fish harvested in local aquatic bodies by fishermen and widely consumed by local rural people in Tiruchirappallimay provide substantial sources of affordable nourishment, particularly for rural people. The nutritional value of various fishes fluctuates, based on their biological contents such as protein, fat, vitamins, and Mineral content. Fish is said to be a great source of omega-3 fatty acids, contains high-quality protein, especially important amino acids, Methionine and lysine. Aside from the excellent nutritional value, In addition to their monetary worth, fish proteins offer beneficial functional qualities, including water-holding capacity, gelling, emulsification, and textural qualities for goods such as fish mince and surimi. Quality criterions like water retention capacity and gelling characteristics is critical to establish the textural properties of the items.

Proteins are necessary for the body's growth and development, as well as its repair and replacement of worn-out tissues. The protein composition of fish varies according to species, nutrient intake, and muscle type. Protein plays a crucial function in biological processes such as metabolic activation, diffusion, and structural support, as well as growth and differentiation regulation.

Protein depletion suggests fast use of energy stores to fulfil the energy demands of the environment, and protein content degradation may be attributed to increased proteolytic activity [6] or to changes in membrane porosity. After different pollutant treatments, there was a decrease in content in *Heteropneustes* fossils and *Anabas testudineus*[7].Fish proteins have a significant health benefits because they include all amino acids in the proper proportions, particularly lysine, as well as sulphur-containing amino acids like methionine and cysteine, which are lacking in plant proteins. In the present investigation, the fresh water fish *Channa striata* had the highest concentrations of proteins in its muscular tissues based on the type of protein distribution in tissues of vital organs like gill, muscle,

liver and kidney among the 10 fishes scrutinized in the current study. The cholesterol content of fish varies according on species and season. Fish, on the other hand, has less fat than red meat. Lipoproteins are the most common and cost-effective type of energy storage in fish, and they may be stored in a variety of organ [8]. The main component of fat is cholesterol, which typically varies from 0.2 to 255. As the fat level increases, so does the water content, and vice versa. It is worth noting that the decrease of cholesterol from the liver, which leads to a rise of cholesterol in the gonads through circulation, is dependent on the spawning phase [9]. In the present investigation, the freshwater fish, *Etroplussuratensis* had the highest concentrations of Cholesterol in its gill tissues based on the type of cholesterol distribution in tissues of vital organs like gill, muscle, liver and kidney among the 10 fishes evaluated in the study.

Monosaccharides are protoplasmic fundamental chemicals that play a role in energy storage and release. They are chemically described as aldehyde or ketone derivatives of higher polyhydric alcohols or substances that give these derivatives upon hydrolysis. The major sugars in animal cells are glucose, fructose, mannose, sucrose, galactose, maltose, lactose, and glycogen. Shobana Manoharan *et al.,* 2017 investigated *Lepidocephalichthys thermalis*, an IUCN least care category consumable freshwater fish with indigenous taste and aroma identified native to Tamil Nadu [10].According to Sumi *et al.,* 2016, seafood is a good source of metabolically important proteins, Glucose, vitamins, trace minerals, and polyunsaturated fatty acids [11].In the present research study, the Marine fish, *Sardinella longiceps* had the highest concentrations of Glucose in its liver tissues based on the type of Glucose distribution in tissues of vital organs like gill, muscle, liver and kidney among the 10 fishes analysed in the study.

The rise in urea level in fishes provides solid evidence for the activation of the second process of ammonia detoxification, ureogenesis by fishes. The current study made on 10 species of both Freshwater and Marine fishes from Tiruchirrappalli, reported an highest concentration of Urea levels in the muscles tissues of Marine fish, *Sardinella longiceps.* The significance of the present study lies in fact that alterations in the level of biomolecules such as Glucose, Protein, Cholesterol and nitrogenous waste material like Urea could be used in risk assessment of pollutant exposure in both Freshwater and Marine ecosystem of Tiruchirappalli, Tamil Nadu.

CONCLUSION

The Present investigation provides a tool for assessing the quality of both Freshwater and Marine ecosystem in Triuchirappalli, Tamil Nadu, which in turn may result in pronounced changes in the biochemical parameters like Protein, Glucose, Cholesterol and nitrogenous waste products such as Urea, with respect to increase in concentration of environmental pollutants and their exposure time periods. Hence, it may be concluded that this changes may probably affect the enzyme mediated bio-defense mechanism of these fish population in the future.

DECLARATION OF COMPETING INTEREST
The authors state that they have no known competing financial interests or personal ties that may seem to have influenced the work described in this study.

ACKNOWLEDGEMENTS

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Table 1: Depicting Fish Species used in Present Investigation

Species Name	Sample	Habitat
Etroplus suratensis	20	Freshwater
Cyprinus carpio	20	Freshwater
Oreochromis mossambicus	20	Freshwater
Channa striata	20	Freshwater
Cirrhinus reba	20	Freshwater
Elops saurus	20	Marine
Lutianus compechanus	20	Marine
Caranx melampygus	20	Marine
Sardinella longiceps	20	Marine
Lethrinus nebulous	20	Marine

Table 2: Representing Glucose Concentration of Ten species of Marine and Freshwater Fishes

S.No.	Fish Species Name	Glucose Concentration in mg/dl			
		Gill	Liver	Muscle	Kidney
1.	*Sardinella longiceps*	105.739 ± 0.02	208.8 ± 0.04	185.11 ± 0.05	204.327 ± 0.06
2.	*Lutjanus campechanus*	23.2 ± 0.04	33.286 ± 0.05	22.498 ± 0.03	48.082 ± 0.02
3.	*Caranx melampygus*	2.89 ± 0.06	8.294 ± 0.07	6.09 ± 0.05	0.473 ± 0.04
4.	*Lethrinusnebulosus*	59.314 ± 0.02	20.445 ± 0.03	42.612 ± 0.07	35.51 ± 0.06
5.	*Elopssaurus*	23.755 ± 0.04	41.845 ± 0.05	46.759 ± 0.06	14.988 ± 0.02
6.	*Etroplussuratensis*	20.571 ± 0.02	26.906 ± 0.04	7.706 ± 0.03	11.837 ± 0.05
7.	*Cirrhinusreba*	20.506 ± 0.01	6.596 ± 0.03	18.335 ± 0.02	13.388 ± 0.06
8.	*Oreochromismossambicus*	29.731 ± 0.03	21.388 ± 0.02	15.282 ± 0.04	18.498 ± 0.06

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9.	*Cyprinuscarpio*	30.645 ± 0.02	45.853 ± 0.04	50.269 ± 0.03	20.024 ± 0.01
10.	*Channastriata*	40.453 ± 0.05	76.69 ± 0.04	35.665 ± 0.03	50.616 ± 0.01

Table 3: Representing Protein Concentration of Ten Species of Marine and Freshwater Fishes

S.No.	Fish Species Name	Protein Concentration in gm/dl			
		Gill	Liver	Muscle	Kidney
1.	*Sardinella longiceps*	0.424 ± 0.02	0.625 ± 0.03	0.148 ± 0.04	0.199 ± 0.05
2.	*Lutjanus campechanus*	0.836 ± 0.06	1.2 ± 0.07	0.309 ± 0.03	1.556 ± 0.02
3.	*Caranx melampygus*	0.972 ± 0.03	0.677 ± 0.02	1.041 ± 0.01	0.877 ± 0.04
4.	*Lethrinus nebulosus*	8.639 ± 0.06	4.373 ± 0.04	0.922 ± 0.03	1.196 ± 0.02
5.	*Elopss aurus*	0.176 ± 0.05	0.484 ± 0.04	0.279 ± 0.02	0.097 ± 0.03
6.	*Etroplus suratensis*	0.745 ± 0.01	6.584 ± 0.02	0.042 ± 0.04	0.409 ± 0.03
7.	*Cirrhinus reba*	0.36 ± 0.03	0.029 ± 0.04	0.091 ± 0.05	0.714 ± 0.02
8.	*Oreochromis mossambicus*	1.149 ± 0.04	1.903 ± 0.05	0.23 ± 0.03	0.922 ± 0.02
9.	*Cyprinus carpio*	0.439 ± 0.03	2.877 ± 0.02	0.395 ± 0.01	1.924 ± 0.05
10.	*Channas triata*	50.744 ± 0.02	40.452 ± 0.03	78.973 ± 0.04	30.638 ± 0.05

Table 4: Representing Urea Concentration in Ten Species of Marine and Freshwater Fishes

S.No.	Fish Species Name	Urea Concentration in mg/dl			
		Gill	Liver	Muscle	Kidney
1.	*Sardinella longiceps*	179.397 ± 0.04	163.096 ± 0.03	249.512 ± 0.05	201.509 ± 0.06
2.	*Lutjanus campechanus*	6.695 ± 0.07	8.41 ± 0.06	3.213 ± 0.03	10.793 ± 0.04
3.	*Caranx melampygus*	10.024 ± 0.05	71.391 ± 0.06	21.138 ± 0.04	31.172 ± 0.02
4.	*Lethrinus nebulosus*	113.053 ± 0.06	238.678 ± 0.02	185.659 ± 0.01	126.355 ± 0.03
5.	*Elopss aurus*	1.28 ± 0.05	10.43 ± 0.04	3.953 ± 0.03	2.696 ± 0.06
6.	*Etroplus suratensis*	69.431 ± 0.03	71.833 ± 0.02	11.989 ± 0.01	10.658 ± 0.04
7.	*Cirrhinus reba*	19.839 ± 0.02	6.546 ± 0.01	11.198 ± 0.03	6.085 ± 0.06
8.	*Oreochromis mossambicus*	39.865 ± 0.03	40.638 ± 0.04	30.511 ± 0.02	24.412 ± 0.05
9.	*Cyprinus carpio*	7.51 ± 0.05	17.692 ± 0.04	13.757 ± 0.03	21.166 ± 0.02
10.	*Channas triata*	16.071 ± 0.03	14.769 ± 0.02	18.254 ± 0.01	10.22 ± 0.04

Table 5: Representing Cholesterol Concentration of Ten species of Marine and Freshwater Fishes

| S.No.	Fish Species Name	Cholesterol Concentration in mg/dl			
		Gill	Liver	Muscle	Kidney
1.	*Sardinella longiceps*	140.266 ± 0.03	137.879 ± 0.02	130.813 ± 0.01	146.051 ± 0.06
2.	*Lutjanus campechanus*	43.187 ± 0.08	99.538 ± 0.05	71.594 ± 0.03	47.113 ± 0.02
3.	*Caranx melampygus*	40.619 ± 0.04	28.775 ± 0.03	99.769 ± 0.02	70.39 ± 0.05
4.	*Lethrinus nebulosus*	180.84 ± 0.06	145.725 ± 0.07	116.567 ± 0.08	122.402 ± 0.02
5.	*Elopss aurus*	142.263 ± 0.01	169.977 ± 0.04	101.617 ± 0.05	139.03 ± 0.03
6.	*Etroplus suratensis*	381.062 ± 0.02	215.038 ± 0.03	215.243 ± 0.04	236.951 ± 0.05
7.	*Cirrhinus reba*	264.203 ± 0.03	294.688 ± 0.04	186.143 ± 0.02	323.788 ± 0.01
8.	*Oreochromis mossambicus*	196.767 ± 0.04	215.243 ± 0.02	142.956 ± 0.01	138.106 ± 0.03
9.	*Cyprinus carpio*	174.365 ± 0.03	291.917 ± 0.02	139.954 ± 0.01	276.674 ± 0.04
10.	*Channas triata*	174.207 ± 0.02	183.869 ± 0.01	161.397 ± 0.03	130.107 ± 0.04
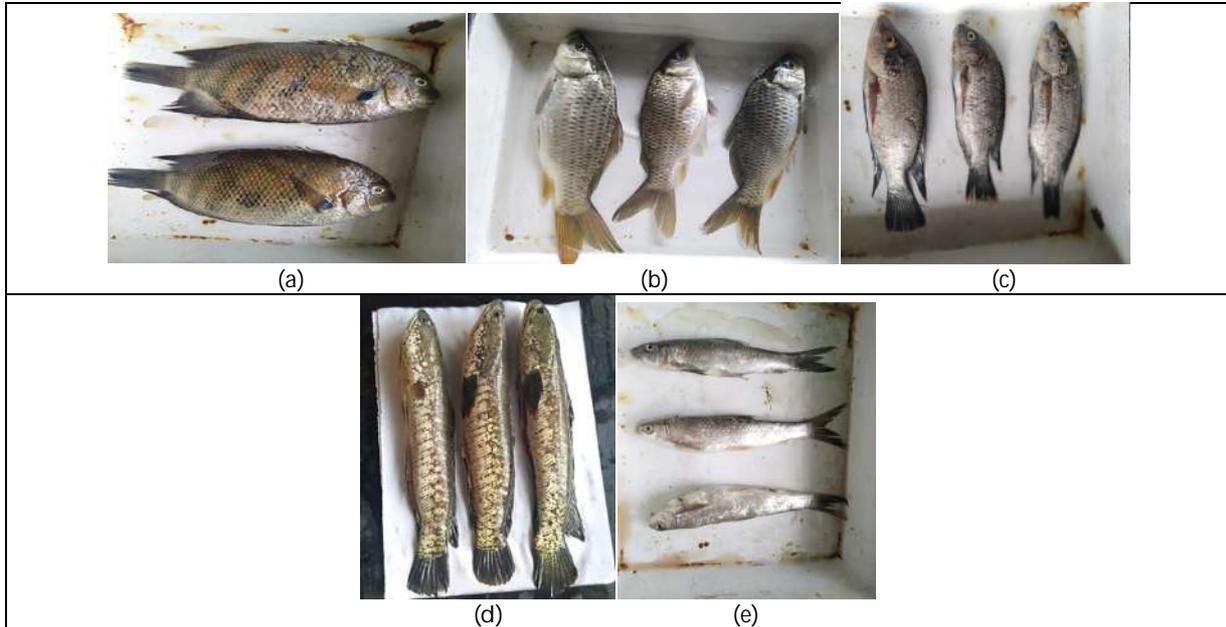
Fig. 1: Freshwater fish species collected from different sampling sites –
a) *Etroplus suratensis* b)*Cyprinus carpio* c) *Oreochromismos sambicus* d)*Channa striata* e) *Cirrhinus reba*
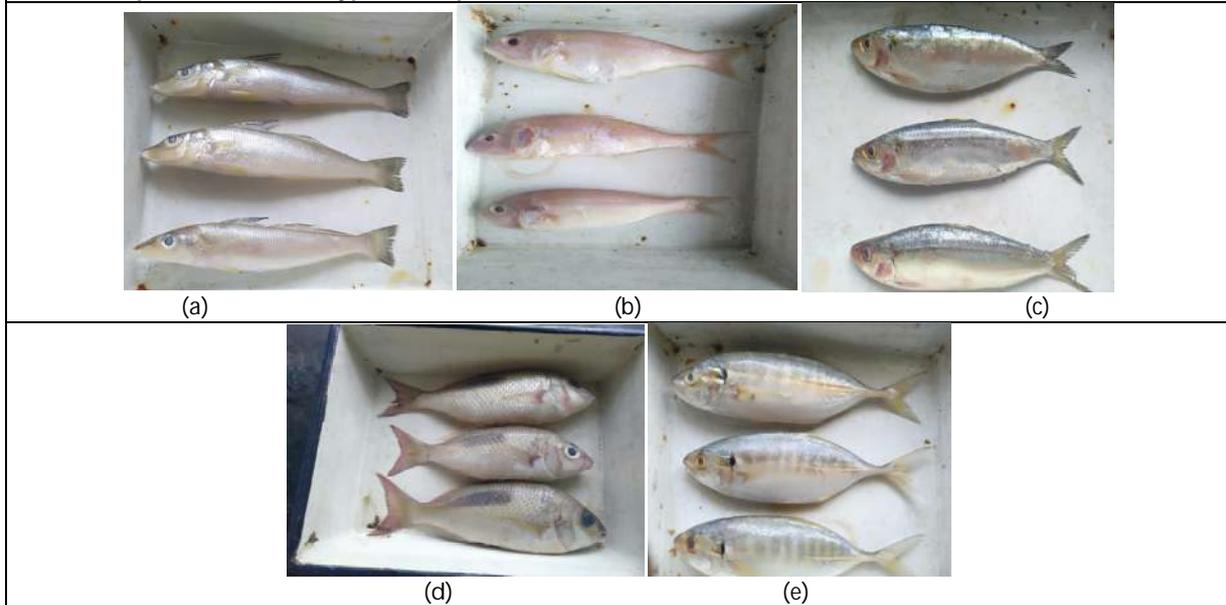

Fig. 2: Marine fish species collected from different sampling sites –
a)*Elops saurus* b)*Lutianus compechanus* c)*Caranxmelam pygus* d) *Sardinella longiceps* e)*Lethrinus nebulous*

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Graph 1: Representing Glucose Concentration in Ten Species of Freshwater and Marine Fishes
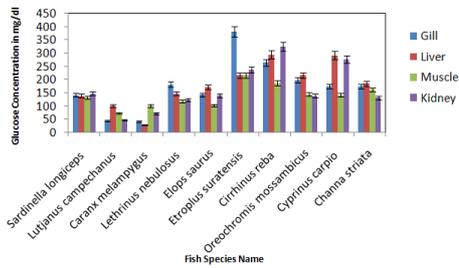
Graph 2: Representing Protein Concentration in Ten Species of Freshwater and Marine Fishes
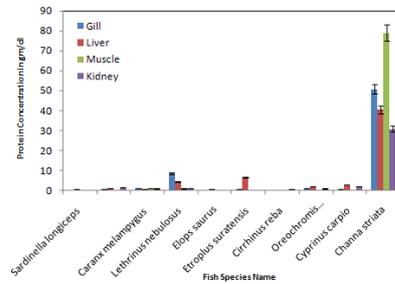
Graph 3: Representing Urea Concentration in Ten Species of Freshwater and Marine Fishes
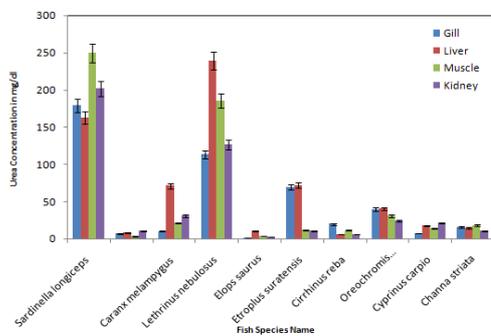
Graph 4: Representing Cholesterol Concentration in Ten Speices of Freshwater and Marine Fishes
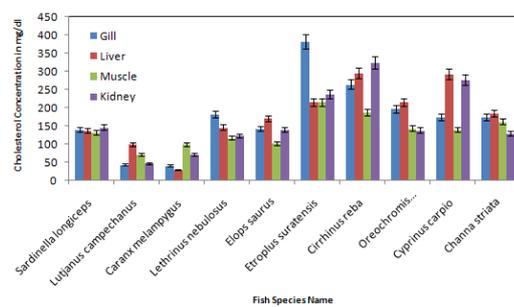
RESEARCH ARTICLE

Effectiveness of Video Assisted Teaching Module on Knowledge Regarding Vaginal cone among Postnatal Mothers at Selected Hospitals UP

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ABSTRACT

Vaginal cone is a medically designed tool its outer shell is made from polypropylene (PP)and internal weights are manufactured from stainless steel, it's far applied in obstetrics and gynecology physiotherapy set up for treating pelvic floor ailment amongst women, it facilitates to repair the pelvic muscle power brings more advantageous the exceptional lifestyles to the patients .Vaginal cones are innovated devices it really is designed to improvement the strength and tone of the pelvic floor muscle. These cones are readily available in a fixed of five with different shape and volume but increased weight (i.e20g,32.5g,45g, 60g,75g). The samples accrued by using Convenient Sampling from Non-Probability Sampling Technique, size was 40 Postnatal Mothers of CHC Chaubeypur, Kalyanpur Kanpur UP. The statistics was accumulated by the use of self-structured questionnaire. While assessing the knowledge mean of pretest was 10.175 and mean of post-test was 16.675 and mean difference was 6.5 and table value become 2.02 and T value was 13.46. The study concludes and significantly proofs that there is exceedingly brilliant improvement in the information of postnatal mothers concerning the vaginal cone.

Keywords: Vaginal cone, Postnatal Mothers, Effectiveness, VATM.

INTRODUCTION

The postpartum (postnatal) duration starts off evolved at once after infant start as mom frame together with hormones tiers and uterus size, returns to a pregnant state. The phrases puerperium , puerperal duration or instant post-partum duration are normally used to consult the primary six weeks following infant births [1]. The World

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Health organization (WHO) describes the post-natal duration because the maximum important and but the maximum left out segment within side the lives of mothers and babies most maternal and new baby deaths rise up in some unspecified time in the future of this length [2]. In medical literature the time period is generally abbreviated to PX, in which X is numerous for example "day P5" ought to be observe as "the fifth day after start". The post-partum duration may be divided into 5 awesome stages, the preliminary or acute segment,eight-19 hours after infant start, sub-acute post-partum period –which closing to six weeks, behind schedule post-partum period that would last as long as eight months, within side the sub-acute post-partum duration 87% to 94% of women file at the least one health problem [3]. The use of vaginal cone to bolster pelvic ground muscle turned into first of all proposed with the aid of using Plevnik in 1985, the affected person have been told to stroll for 15 mins two times an afternoon with a cone within side the vagina without developing a voluntary manufacturing however the sensation of enduring the cone, this outrage but plan of action an involuntary contraction of the pelvic ground musculature as display through electromyography of the pelvic ground in the course of using vaginal cone. four In systematic review, Her bison and dean discovered that remedy with vaginal cones is useful while as compared to no lively remedy; but, there is no consensus on the superiority of the usage of the ones devices even as in comparison to distinctive remedy. Vaginal cones are weighted gadgets designed to growth the power of the pelvic ground muscle he cones are be had in devices of five with identical shape and amount but developing weight (i.e20g,32.5g,45g, 60g ,75g) [5].

Vaginal cone is a medically designed tool its outer shell is made from polypropylene (PP) an inner weights are manufactured from stainless steel, it's miles applied in obstetrics and gynecology physiotherapy set up for treating pelvic floor ailment among women, it facilitates to repair the pelvic ground muscle power and brings greater the pleasant existence to the patients [6]. As a part of a innovative resistive workout program, a single cone is inserted into the vagina and held in area through tightening the leveater ani muscle for so long as 15 mines because the levater ani muscle turn out to be more potent the workout period can be accelerated to 30 mins. Several remedy alternatives are to be had for dealing with SUI strategies aims' at strengthening the pelvic ground muscular tissues are regularly taken into consideration the primary-desire remedy due to their non- invasive person the opportunity of mixing them with different treatments [7]. Thus, pelvic ground musculature, composed of each kind 1 (slow) and kind 2 (fast) muscle fibers, is vital within side the mechanism of urinary continence. Vaginal cone is scientific gadgets mainly designed and formed to exercising pelvic ground muscle mass so as to give a boost to them and repair right bladder features in ladies with urinary pressure incontinence. The lively segment become to start with accomplished with the cone the affected person turned into capable of hold within side the vagina in a status function for one minute thru non-mandatory diminution of the pelvic floor muscle to locate the suitable cone, the affected individual commenced out via introducing the following heaviest devise used at the forestall of the passive segment [8].

The principle at the back of using cones is that the sensation of dropping the cone makes you settlement your pelvic ground in an try to hold the cone in region. Stress UI may be outstanding among 3 stage, segment 1- occurs most effective at some point of a main attempt including sneezing vomiting or giggling inflicting in elevated intra- belly strain, segment 2- signs stand up throughout mild workout which includes walking, lifting or jogging, main to a moderate growth in intra- stomach strain and segment 3- with minimum bodily exertion signs and symptoms may be located and intra - stomach pressure increases [9]. A look at with the aid of using Norwegian scientist in 1995 used E 194 (electromyogram) recording and vaginal cones, it confirmed that once cone turned into used there has been an boom in muscle tone, whether or not the ladies have been capable of keep the cone or now no longer this examine additionally confirmed stepped forward coordination as pelvic ground muscle activity. Later research additionally display that it is higher to apply cone remedy than no remedy in any respect and the use of a cone can be as powerful as pelvic ground muscle exercising achieved but the research confirmed that the usage of cones does not have a higher impact than pelvic ground muscle exercising [10].

Ten girls with SUI have been examined on events separated through one week to decide the interclass correlation coefficient (ICC) and trendy mistakes of dimension for all variables. The 1-h pad take a look at became completed compare urinary leakage in keeping with the protocol proposed with the aid of using Abrams and associates the

ladies have been informed to put on pad which were formerly weighted on a precision balance. Women have been informed exercise day by day for six weeks Kegels workout and two times /week workout with vaginal cone turned into given beneathneath supervision of the investigator a hard and fast of 10 seconds settlement and 10 seconds loosen up with vaginal cone turned into persevered for 15 min within side the subsequent consultation heavier weight vaginal cone become used however best after warranty that required a voluntary contraction became robust sufficient to give the cone from slipping out of vagina and keep for six weeks [11].

Objectives of the Study
- To assess the knowledge regarding vaginal cone among postnatal mother.
- To assess the effectiveness of video assisted teaching module on knowledge regarding vaginal cone among postnatal mother.
- To associate the Knowledge score with their selected demographic variable.

Hypothesis
H01: There is no significant gain knowledge in post test score of the higher postnatal mother after attending video assisted teaching programme on knowledge regarding vaginal cone.
H02: There is no significant association between pretest knowledge score regarding knowledge about vaginal cone.
H1: There is significant gain knowledge in post test score of the postnatal mother after attending video assisted teaching programme on knowledge regarding vaginal cone.
H2: There is a significant association between pretest knowledge score regarding vaginalcone with selected demographic variable.

METHODOLOGY

Research Approach
Quantitative method Evaluatory Research Approach
Research Design
Pre Experimental research type of pretest and posttest in only experimental group
Study Setting
Selected hospital in Kanpur Uttar Pradesh
Population
The population of present study consist post-natal mother.
Sampling and sampling size
Convenient Sampling from Non-Probability Sampling Technique and the sample size of this study will consist of 40 post-natal mothers.

Variables
Research Variable
Knowledge level of the post-natal mother on vaginal cone.

Demographic Variable
Age, Educational status, are a of residence, religion, income of family, obstetrical history, previous knowledge and source of information.

Inclusion Criteria
Postnatal mother who was:
Postnatal mother in selected hospital
Postnatal mother who was present at the time of data collection
Postnatal who are willing to participate

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Exclusion Criteria
The postnatal mothers who were completely ill or complicated with any serious medical condition.

DATA ANALYSIS AND MAJOR FINDINGS
Section A: Assessing the knowledge regarding vaginal cone among postnatal mother
(Table 1, Fig 1) shows that differentiation of pretest and posttest level of knowledge. in pretest among the subject 23 sample (57.5%) had inadequate level of knowledge, 17 sample (42.5%) had moderately adequate level of knowledge and in posttest among the subject, none of them adequate level of knowledge, 35sample (87%) had over adequate level of knowledge and 5 sample (12.5%) had adequate level of knowledge.

Table 2 explains the mean and standard deviation of knowledge level regarding vaginal cone were in pretest 10.175 and 2.56, with regard to post test it was 16.675 and 3.05 respectively.

Section B: The Effectiveness of Video Assisted Teaching Module on knowledge regarding vaginalcone among postnatal mother.
Table 3 shows that the calculated t value about the video assisted teaching module effectiveness regarding vaginal cone was 13.46. which was more than the table value. Hence it is proved that video assisted teaching module is effective in increasing the knowledge level among post natal mothers..

Section C: Association between the knowledge score with their selected demographic variables.
On the basis of findings of analysis there was no statistically significant association of the pre- test knowledge score of subjects with their selected socio demographic variables such as age, education status, area of residence, religion, family monthly income, number of parity, previous knowledge regarding vaginal cone, source of information..

Nursing Implication
Location of the study can be seen in the area of nursing practices, nursing education, nursing administration, and nursing research, community health nursing Adolescent girls.

Nursing Services
- The implication of nursing services that the nurses play an important role in the awareness video assisted teaching module program help the postnatal mother, adolescent girls to update their knowledge.
- The finding of the study can be disseminated to motive motivate nurses to awareness programme.

Nursing Education
- The nurse has an important role in giving education the teacher can utilise. The content of the Video assisted teaching module program teach the students in the Classroom and clinical setting and update the knowledge of the student.
- The findings will help the nursing students to understand the need to be equipped with adequate knowledge.

Nursing Administration
- The nurse administrated can utilise the content of awareness programme for an Inservice education programme to nurse to update their knowledge.
- Continuous quality assessment can be done to assist the quality of education provided to the postnatal mother.

Nursing Research
- Nursing practice need to be based on scientific knowledge because nurses are facing lots of challenges while delivering health service the people.
- That has to encourage further study regarding vaginal cone and

- The study finding help to motivate and initiate for the research related to the effectiveness of the awareness VATM programme regarding vaginal cone.

Community Health Nursing
- Nurses have expanded and ex tended role of promotion prevention, curative and rehabilitation of individual family and community level.
- Nurses can provide centralise approach regarding prevention and management of the vaginal cone by taking action to impart knowledge to the committee people.

CONCLUSION

The present observe was aimed toward determine the effectiveness of video assisted Teaching application post Natal mother the current information was accrued and analyzed statically primarily based totally on Hospital Kanpur Uttar Pradesh. On basis of findings of the observe following conclusion have been drawn: In the pre-test knowledge primarily based totally on Hospital Kanpur Uttar Pradesh regarding vagina cone in order that among 40 postnatal mother 17(42.5) % had moderate knowledge 23(57.5) % has inadequate knowledge and no one had adequate knowledge in pre-test and 5(12.5%) had adequate knowledge 35(87.5) had moderate knowledge in post-test. It was inferred that, most of the postnatal mother had inadequate knowledge in pre- test & most of had moderate knowledge in post-test. There was no significant affiliation among pre-test knowledge rating fit their decided on demographic variable. The dependent effectiveness of video assisted teaching module progressed the knowledge level of postnatal mother regarding vaginal cone.

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Table 1: Distribution of postnatal mothers according to the pretest and posttest level of knowledge on vaginalcone

					N=40
SI. No	Level of knowledge	Pretest		Posttest	
		F	%	F	%
1	Inadequate knowledge (0-10)	23	57.5%	00	00%
2	Moderately adequate knowledge (11-20)	17	42.5%	35	87.5%
3	Adequate knowledge(21-30)	00	00%	5	12.5%

Table2: Table 2: Comparison of pretest and posttest knowledge level regarding vaginal cone among postnatal mothers   n=40

S. N	Knowledge level	Mean	Mean difference	Mean percentage	Standard Deviation
1	Pretest	10.175	6.5	33.91%	2.56
2	Posttest	16.675		55.58%	3.05

Table 3: The Effectiveness of Video Assisted Teaching Module regarding vaginal cone.

| Test	Mean	Standard Deviation	Calculated t value	Table Value	Inference
Pre Test	10.175	2.56	13.46	2.02	Significant
Post Test	16.675	3.05			
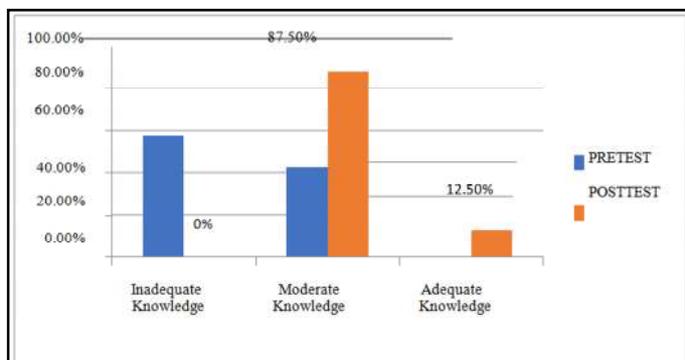
Fig1: column bar diagram showing percentage wise distribution of postnatal mothers according to level of knowledge in pretest and posttest.

REVIEW ARTICLE

Procurement of Blended Learning Mode in Learning and Development in (IT) Sector: Literature Review

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ABSTRACT

The aim of this study is based on the procurement of blended learning mode in learning and development in IT sector. In this review paper numerous articles has been taken to incorporate ideology about blended learning. This paper shows the effectiveness of learning and its importance for the institution which provide student centric mode of learning. By the procurement of blended learning in the study it has been visualize blended learning development in various modes and also positive impact towards learner with comparison of any other mode of learning. This study takes sufficient articles to find information about the problem. The discussion of the study shows about the overall analyzing and interpretation about the problem of the study. The objectives are given in this study that aim to achieve the goals. At last, this study represents recommendation for future association fulfillment.

Keywords: Blended learning, procurement, consequences, online learning, offline learning.

INTRODUCTION

Informational technology (IT) industries are ruling in business sector in today's era. Human resource management came up with the concept of learning and development. Learning programs has various modes like traditional face-to-face learning and online learning, blended learning and mobile learning. Online teaching or learning has three approach: enhanced approach, blended learning approach and online approach. Online course during covid-19 pandemic some learners view that online education was not much effective than face-to-face learning (Almahaseen.Z, Qassem.M 2021). In a study shown students/learners are strongly agreed for the introduction of blended learning mode closely to their peer groups, which enhanced peer learning and reflective thinking. The blended learning improves presentation knowledge of learner. During the e-learning sessions a study mentions

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learner find more query to ask freely. (Tso.A.W.B 2015). Various courses are adopted for blended learning; it consists of moodle, supplementary audio-video lessons. In this way there are limited numbers of face-to-face interaction lectures and in the other side scholars point out that blended learning which increase active learning in strategies. Blended learning can also apply to compel on-campus and off-campus. Learner's priority is blended learning which choose to deliver blended learning. While teaching OUSL give compulsory facility which is related to knowledge and technology to teach mixed/hybrid learning mode(Selvaras.J 2019)

OVERVIEW

Procurement of Blended Learning

To obtaining blended learning in learning and development in IT industry. The involvement of blended learning is the learning platform makes learner's attitude towards blended learning is positive as compared to traditional course of learning. This blended mode of learning has various advantages like the better access can get by learner with learning materials and greater independence in study, used e-learning platform for convenience and time-saving, motivation, control and effectiveness (Fenech.R, Baguant.P, et. al. 2021). The efficacious planning will eventually guide to learning to provide support for learner to the engagement of blended learning (Amponsah.S, Benjamin.K.A et. al. 2021). This mode of learning improves active participation and motivation, (Josephine.M 2016). The method of blended learning has outstanding effect towards the performance of learner when compare to traditional method. Learner has positive perception on self-learning mode and technology. The achievements score of student effectively improve when teaching a course. The development of educational platforms and process it is important to on-going improvement which implement quality education (Josephine.M 2016).

Consequences of Blended Learning

There are various consequences of blended learning, study shown blended learning is never contemplating a stable model. This mode design based on the institution setting based on subject and needs of student. All the factors must be linked based on institution's plan that is balanced entirely (Cobo-Rendon R, Bruna Jofre.C, et. al. 2022). The design, course and teaching instructions, and various other factors are not in online platform (Estacio.R.R, Raga.R.C, 2017). In a study shown during the e-learning sessions a study mentions learner find more query to ask freely. (Tso.A.W.B 2015).

LITERATURE REVIEW

The author has done his research on use technology for teaching and learning in open and distance learning. In their study it shows that more number of students has accessed technology by mobile device and is aware of blended learning. to learning law course but not accept full learning in online. In blended learning social media and mobile application mostly acceptable mode in blended learning. Author also found that for teaching to access the technology internal staff has enough knowledge to enter in digitalize learning environment but external staff face challenges for the same. (Selvaras.J 2019)

The researcher's study based on a reflection on blended learning: a case study at the open university, their study is based on the case study, researcher identify that presentation skills get learnt through video lectures and online lecture as well as traditional face-to-face teaching. The results of their study report was being suggest the students learnt course ENG LA122F by blended learning they develop positive attitude. Blended learning approach improved learner's learning, proposition knowing and experimental knowing, presentational knowing were enhanced, sharing online assignment and allow students to comment freely on their work were concern to other student's. (Tso.A.W.B). Researcher conduct a research on open university Malaysia self-managed and face-to-face learning are prefer, huge efforts and resources given in e-learning make in ensuring that OUM (Open university Malaysia) right combination of blended learning mode. She mentions in her research that, the OMU uses blended learning which they consist of three different methods such as (a)Self-Managed Learning (b)Face-to-Face Learning (c)e-Learning. By using various

data researcher clear blend of learning approach can be provided to the institution more approaches like online and offline discussion are made(Kaur.A, Ahmed.A). This study is based on acceptance of online study of student in Punjab. Both online and off-line mode of learning process have some advantages and disadvantages. Researcher found that the sudden changes from regular mode to online mode not so easy for students during Covid-19 pandemic. They also found that both online and offline study modes was accepted by students based on availability of IT infrastructure as based on the analysis that half respondent perceived online and half for offline mode.(Chadda.I, Kaur.H 2021)

The researcher's study is based on UGC to the pandemic where IQAC(Institutional Quality Assurance Cells in Bangladesh) gave in the direction to shift to full online education during covid-19 pandemic. He also mention in her research that the conduction of the survey from IQAC to those faculty and student regarding access to internet, financial difficulties, and mental health situation, then from here IQACs made academic policies like, attendance, student assessment and online teaching. Then organize capacity building activities, monitoring faculty performance, formulating guidelines on student behavior in online performance, make guidelines on student online behavior uplift stakeholders to believe the online system. She also find out that as per UGC, some university shift to full online learning and some university are not have experience in blended learning.(Genilo.J.W.R)

Their study is based on faculty perception of teaching translation courses online during covid-19 author found that effectiveness of online education but in case of face-to-face learning it found ineffective as per respondent's view. Its identified that student face difficulty in online learning because of lack of interaction and motivation, and deficiency of data connections. During covid-19 online education works as an aid but not replace face-to-face learning. So as per the funding their study recommended blended learning. Author also mention online and face-to-face plus synchronous and asynchronous, would result in a perfect online learning environment. (Almahasees.Z, Qassem.M).
The author reviewed that covid-19 pandemic come-up with opportunity to implement of blended learning mode in higher education. They also mention that blended learning is a suitable or perfect during pandemic. In post pandemic blended learning is a proper and suitable education delivery mode(Cobo-Rendon.R, Jofre.C.B et al. 2022).
Author found in their study that student's attitude are changed towards blended learning from positive prospective. Researchers also found that the student's attitude towards blended learning is learning flexibility, study management, classroom learning, online learning, and online interaction but lack of exposure does not make improvement in blended learning. (Ferech.R, Baguant.P, 2021)

The study on based on student's online learning behavior in blended courses using Moodle. In the dynamic explosion of information in web based educational system is suitable for learners. Blended learning implement in institution where learning can done in online but still rely on face-to-face learning as compared to online learning (Estacio. R.R and Raga.R.C, 2017). Author mentions in their study that student's engagement in blended learning an analytical study. In their study students prefer minimal usage of technology and mostly face to face are accepting. Author found from this study that it's a self-paced learning. At last author said   (Sairaj.K.K, Al Maskari.A 2019). Researcher's study is based on design and develops of blended learning program in student teacher academic achievement and perception of blended learning. Blended learning give the opportunity to develop cognitive concept.(Josephine.M 2016).

OBJECTIVES
This study makes prominent objectives which focuses on aim to achieve for research outcomes:
• To determine the procurement of a blended learning mode in learning and development and their consequences.
• To assess which learning platform is efficacious for learner and then inculcate learner's adaptability based on it.

METHODOLOGY

This study will be view in-detail review of diverse articles and books about procure of blended learning for learner's perspective. In this study more focus is given on reviewing articles, the adjacent to consider several methods which will be intend to carry out the research in logical, systematic plan to solve research problem (Indeed Editorial Team, 2022). The creation of reliable research certain methods draw for research driven like Descriptive Research Design, Data collection, Structured Undisguised Questioners, Systematic Sampling, Statistical tools, ANOVA, Chi Square Regression, Correlation , Sample Size, Pilot Questioner etc.,.

DISSCUSSION

Blended pedagogy is made up of three different methods for delivery of learning is self-managed learning, face-to-face learning and e-learning. The clear pedagogical approach for blended learning is utilizing more systematic manner with online and offline discussion are implemented. (Kaur.A, Ahmed.A 2006). In covid-19 pandemic teacher and student accept different digital platform where students can participate without any practical sessions. (Chadda.I, Kaur.H 2021). For better learning experience from learning effectiveness perspective, before pandemic some private and public universities use blended learning to delivered learning sessions. It was imperative due to numerous reasons such as Political turmoil(nationwide, strikes and protest actions) and environmental issues (flooding and fire) that disrupt academic activity. The University Liberal Arts Bangladesh Moodle (Learning Platform) used for (blended learning, distance education, flipped classroom and other e-learning modes). In other institutes, which not have enough experience in blended learning apparently faces struggle with the changes. (Genilo.J.W.R 2021)

Blended learning has been found to positively influence students' engagement in the course, ownership and flexibility, learner's productivity, participation, feedback and interactions, access to relevant course data, a sense of belongingness, motivation, and relating the learning the real life applications.(Siraj.K.K, Al Maskari.A, 2019). In this table shows the student preferences on the mode of instructions and what students preferred most. (Siraj.K.K Al Maskari.A 2019). It is been find that blended learning has positive as well as negative impact depends on learner preferences.

RECOMMENDATION

As per the study the generation of few recommendations for the further implementation in the learning program:
- If learners are not interested to continue blended learning or pure e-learning details of the course delivery pattern must be discussed with learner. Most of the institutions may lack of technology unfamiliar with blended learning, these things are recommend that proper guidance and facility is mandatory.
- For growing blended learning mode new models are required to make learning more productive like entertainment, pastime, games etc.

CONCLUSION

The study focused on the procurement of blended learning in learning and development in IT industries. This study meant learners are very efficacious while used blended learning sessions in their institutions. There are various articles which are taken for this study and those reviews are positive as well as negative. Mainly two objectives are being mention to achieve the aim. This review paper mainly concentrates on whether learner engaged in pure online learning or offline learning or blended learning. Therefore, through this review paper the concept of blended learning procurement can be represented and recommendations are given for future assessment.

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Table 1. Learner got the opportunity to choose preferred mode

Instructions	Weighted Average	Rank
Complete face-to-face (lecture) instruction	3.26	2
Minimal use of technology, mostly face-to-face	3.65	1
Equal mix of technology and face-to-face instruction	3.13	3
Extensive use of technology, less face-to-face instruction	3.00	4
Completely online with no face-to-face instruction	1.91	5

RESEARCH ARTICLE

A Review on Biofertilizers with Special Reference to Liquid Biofertilizers

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ABSTRACT

Chemical fertilisers are overused, which not only costs money but also pollutes the environment. Today, biofertilizer have developed as a highly strong type of fertilizer due to their environment-friendly, easy-to-handle, non-poisonous, and cost-efficient characteristics. In India, the government is attempting to promote the use of different biofertilizers such as liquid biofertilizers and carrier-based biofertilizers along with contemporary agrochemicals. When compared with liquid formulations, carrier-based biofertilizers are not very desirable due to certain disadvantages. The beneficial effects of liquid biofertilizers without damaging the environment have made them a daily need in this 21stcentury, where urbanization and industrialization are quickly developing to meet society's needs. This paper emphasizes on effect of liquid biofertilizers on crops and the promotion of biofertilizers by the Indian government through various schemes.

Keywords: Agrochemicals, Carrier-based biofertilizers, Crop productivity, Government schemes, Liquid biofertilizer

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INTRODUCTION

Judicious utilization of chemical fertilizers (for instance urea, calcium nitrate, ammonium sulphate, diammonium phosphate, and others) is essential for global food production as well as for farmers' income because they not only act as fast food for plants, helping them to develop more quickly and efficiently [1], but also essential for improving soil fertility and crop output [2-4], Unfortunately, various studies have confirmed that excessive and long-continued use of chemical fertilizers is expensive and pollutes the environment, causing adverse impacts on human health, crop productivity as well as on soil (texture, fecundity and capacity to hold water)[5], and water quality (eutrophication) [6,1,7]. Keeping in view its negative impacts, the introduction and use of eco-friendly and alternative methods like biofertilizers (Organic farming) have become a necessity and boon for farmers. Only about 1.5 percent of the world's agricultural land is organic in 2020. It has experienced a tremendous rise from 2005 to 2018, rising at an annual growth rate of 7.05 percent, with worldwide organic land reaching 71.5 million hectares in 2018. However, during the same period, India experienced a phenomenal growth of 10.62 percent, placing it tenth among other countries in terms of organic agricultural land (2018-19) [8].

Biofertilizers are one of the most effective agricultural technologies and using them in the field is extremely beneficial as they contain beneficial living microorganisms that improve the soil fertility by colonizing the plant's rhizosphore, or interior and boost plant growth by increased nutrient supply (N, P, K, S, etc.) to the host plant through natural processes including nitrogen fixing, phosphorus solubilization; when used to seeds, plants, or soil [9-11].Plant growth-promoting rhizobacteria (PGPR) and microbes such as nitrogen fixers, phosphate solubilizers, and mycorrhizae are few sources of biofertilizers [12-13].Few examples of microorganisms employed in biofertilizers are *Bacillus, Pseudomonas, Lactobacillus,* Photosynthetic bacteria, *Trichoderma* fungus, Yeast, etc. As compared to chemical fertilizers, biofertilizers are an economical, ecologically benign, and sustainable source of plant nutrients, and as a result, they are gaining worldwide acceptance and value in agricultural production [14]. These have shown considerable promise as a renewable and environmentally acceptable plant nutrient source (Fig:1)[15]

TYPES OF MICROBIAL FORMULATIONS

Depending upon the physical properties and carrier material used, a large variety of biofertilizers are available in the global market. They are fluidized bed dried formulations, polymer entrapped formulations, solid carrier based formulations, and liquid formulations [16]. A proper microbial formulation is one of the most important factors for determining the quality of biofertilizers containing alive microbial cells. The main functions of formulating inoculants are (i) to provide protection throughout the time, in order to minimize a rapid loss of survivability of the cells during storage (ii) to decrease in losses caused by predators after soil application (iii) to help the microbes compete with the local adapted soil microbes. To fulfill all these functions and to get the predicted good reaction from the created inoculums, adequate number of cells (10^6–10^7 cells per plant) are needed. Although various types of biofertilizers are available in the market but in India majority of biofertilizers used are carrier-based (solid) and liquid-based biofertilizers [17-18]. Both liquid and carrier based bio-formulations have positive effects on soil health and crop productivity, however this review paper have focused upon the effects of liquid biofertilizer, status of biofertilizers market and government's role in biofertilizer promotion.

Carrier based biofertilizer

These are carrier-based formulations containing beneficial bacteria that are bounded with a delivery vehicle that boost crop plant development and yield [19]. Carrier-based bio-fertilizers use carrier as a medium that 1) helps in transporting large amounts of beneficial microorganisms from lab to plants in the field [20], 2) has a significant impact on sustaining microorganism survivability and increasing the effectiveness of biofertilizers by establishing a short term protective covering and supplying substrates that stimulate bacterial development, taking into account the significant diversity of the soil's microenvironment [21-22] and 3) maintain them active under specific conditions, for extending the shelf life of bacteria that have been injected and 4) due to solid formulation, their transport to the farmers is convenient.

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Carrier materials used can be organic, inorganic, or synthetic with suitable physicochemical properties for continuing the microbial strains in fine physiological conditions under storage and on the ground[12-13].Different materials are used as carrier and each carrier material possesses specific characteristic (Table 1)[23-28]. Carrier based biofertilizers although undoubtedly have numerous benefits when used in agricultural sector but they too have various disadvantages such as short shelf life, poor quality, competition with native soil microbes for energy, vital nutrients, and inhabitable area and unpredictably poor field performance [29-30]. Considering the drawbacks, liquid formulations have been created as a substitute to carrier-based biofertilizers and are claimed to be one of the best solutions to the concerns listed above. In liquid formulations no solid carrier is needed for transporting beneficial microorganisms.

Liquid based biofertilizers

Liquid biofertilizers are liquid formulations that are modified with water, oil, or polymers to improve cell suspension viscosity and stability [31].They contain the dormant form of desired microorganisms and their nutrients, as well as chemicals that induce the creation of resting spores or cysts for increased shelf life and resistance to adverse conditions [32-34]. Traditionally, liquid biofertilizers made from effective microorganisms' fermentation were advised to be used within three months [35-36]. But, now ready-to-use liquid biofertilizer made from effective microorganisms has become accessible on the market [37].

Liquid biofertilizers possess the following characteristics

1. The dormant form of desired microorganisms and their nutrients, induce the creation of resting spores that increase the shelf life and resistance of the microbes. The shelf life is estimated to be 15-24 months which is comparatively more than carrier based biofertilizers [38].
2. There is no impact of high temperature (55°C) on the properties of liquid biofertilizer [39-40].
3. Several types of polymers are utilized for inoculants development in liquid bioinoculants because of their capacity to prevent heat transfer their great rheological properties and high water activities [18].
4. High population i.e. more than 10^9 cells/ml for 12-24 months can be maintained [40,41-44].
5. Liquid biofertilizer are easy to use by the farmers rather than using carrier based biofertilizers [40,45].
6. The required dosage of liquid biofertilizer is 10 times less than carrier based biofertilizer [46-49,34,40].
7. Quality control protocols of liquid biofertilizers are easy and quick [40].
8. Moisture retention capacity and enzymatic activity of liquid biofertilizer are very high [7,50-51]. When compared with carrier based biofertilizers, using liquid biofertilizers is quite beneficial for the increase of crop productivity and for the good health of soil and its microorganisms (Table 2)[33,35,45,52-65].

CURRENT STATUS OF BIOFERTILIZERS MARKET IN INDIA

Negative impacts of chemical fertilizers have forced the farmers to switch to an environment friendly and sustainable method. As discussed above one of such methods is using biofertilizers. As far as India is concern, it is an important country in terms of production and consumption of biofertilizers. In 1956, prominent scientist N.V. Joshi launched the first commercial biofertilizer in India [66]. The Government of India has also largely implemented the popular National Project on Development and use of Biofertilizers (NPDB) for both production and consumption of biofertilizer. The country has a combined capacity for production of different biofertilizer of more than 10,000 t per year [67]. As per the latest data of production in different states during the year 2020-21, Chhattisgarh is topping the list, producing 63 % of total production (24,52,061.46 MT), Karnataka produced 8% (3,15,834.00 MT), Assam produced 5% (1,74,943,82 MT) and all other states cumulatively produced 24.14% (9,36,649.47 MT) of total production of organic fertilizer (Fig 2) [68].

For better production and increased growth, the production rate of different categories of biofertilizers is also increasing. For instance, according to Ministry of Agriculture and Farmers Welfare of the Government of India [69], carrier-based biofertilizer output increased from 88029 MT in 2015-16 to 121066 MT in 2017-18. In the same way, liquid-based biofertilizer production grew from 6241 KL in 2015-16 to 9033 KL in 2017-18.

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Day by day the demand of biofertilizer' s is increasing and in coming years it will reach to 27.3 million tonnes but its supply is not at par to its demand and has to be increased to fulfill the increasing demand (Table 3) [70]. The National Bio-fertilizer Development Centre (NBDC), Ghaziabad, determined the total bio-fertilizer demand of different biofertilizers in India on the basis of farmed areas of the country and treatment of seed sown at a rate of 200g bio-fertilizer per 10 kg seed (Table 4) [67].

ROLE OF GOVERNMENT OF INDIA IN BIOFERTILIZER PROMOTION

The production rate of the current year is although higher than the previous five years, but still it requires some more efforts and increase in production to achieve the target for zero hunger (2nd Sustainable Development Goal). To accomplish this task, the Government of India has taken certain initiatives by promoting more economical, ecofriendly bio-fertilizers through various schemes such as the National Mission on Oilseeds and Oil Palm (NMOOP), Rashtriya Krishi Vikas Yojana (RKVY), Paramparagat Krishi Vikas Yojana (PKVY), and National Mission for Sustainable Agriculture (NMSA), National Food Security Mission (NFSM), and Indian Council of Agricultural Research (ICAR)[71].

The goals of the scheme included organising training courses for extension workers and field demonstrations, as well as offering quality assurance services (Ministry of Agriculture and farmers Welfare, 2019)[72]. According to press release of Ministry of Agriculture & Farmers Welfare, 2020 [73] the government is assisting in the installation of state-of-the-art liquid/carrier based bio-fertilizer units with a competency of annually, 200 tonnes (TPA) under the Capital Investment Subsidy Scheme (CISS) of the Soil Health Management Scheme (SHM) of the National Mission of Sustainable Agriculture (NMSA). State governments and government agencies receive 100 percent assistance up to a maximum of Rs.160.00 lakh per unit for the promotion of biofertilizers. National Bank for Agriculture and Rural Development (NABARD) also offers assistance to individuals and private organisations up to 25% of the cost of a unit, up to a maximum of Rs. 40 lakhs per unit, as a capital investment. According to NABARD data, 61 biofertilizer/fruit and vegetable compost production facilities were created in various states under the Capital Investment Subsidy scheme. In Pune, the Natural Input Complete Utilization (NIKU) Bioresearch lab was founded in 1996.

One national and seven regional centres were formed under the seventh five-year plan. For the establishment of a biofertilizer factory, various grants of 20 lakh/unit of 150 tonnes/year were made available. Since the plan's commencement, more than 83 biofertilizer manufacturing units have been set up [71]. The Soil Health Management Scheme has also funded 10 biofertiliser production units and 16 organic/biofertiliser testing labs since 2015.At the national level, the National Biofertilizer Development Centre in Ghaziabad, Uttar Pradesh, is being promoted for conducting biofertilizer development training programmes.

CONCLUSION

It is concluded from the study that liquid biofertilizers have the potential to replace traditional chemical fertilisers and carrier-based biofertilizers. They have an important influence on the soil health restoration, augment the rate of increase and yield parameters of different crops when applied alone or in combination with fertilizers in appropriate amount, but many other measures in terms of technology, government aid, subsidies, and encouraging awareness among agrarians are still required. Biofertilizers has been generally adopted in numerous parts of India due to which they have grown by double digits in the last five years. Despite this rapid development, India's average consumption is far lower than that of most arising and evolved countries. Although all Indian states have not adopted biofertilizers as a necessary component of crop production, but there is still plenty of potential for future growth and modernization. An increase in the number of government and non-government activities is anticipated in near future and aimed at educating and spreading biofertilizer literacy and relevance among farmers.

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Table 1: Carrier material used for biofertilizers and their characteristics

Carrier material	Inoculant bacterium	Characteristics	References
Alginate-perlite dry granule	*Rhizobium* sp.	• Used for Soil inoculation • In dry granule form, rhizobium strains have a survival rate of more than 180 days • The inoculant can be kept dry without losing much of its potency.	[23]
Vermiculite	*Aspergillus* spp.	• Vermiculite is used as soil amendment and fertilizer carrier • This could be a potentially promising carrier for micromycetes' solid-state fermentation because of its appropriate size. • It increases the yield of proteases by solid-state fermentation conditions (SSF)	[24]
Peat	*Bradyrhizobium/ Rhizobium* strains (for *Phaseolus, Hedysarum, Lupinus* and *Glycine* max.)	• Used for Seed inoculation • Better storage survival at 4°C (refrigerated temp.) and 25°C room temperature • For all strains, the survival rate of sterile peat-based inoculants was higher and maintains a robust Rhizobia population after 6 months of storage	[25]
Coal and Charcoal	*Rhizobium meliloti, Rhizobium trifolii*	• Used for Seed inoculation • Enhancing the seedling biomass and the number of nodules • Improve biological nitrogen fixation by rhizobial inoculants	[26]
Perlite	*Bradyrhizobium* sp., *Rhizobium* sp., *Pseudomonad, Bacillus*	• Used to inoculate Seeds • Bacterial survival is maximised when a sucrose adhesive is paired with a perlite carrier.	[27]

Wastewater sludge	*Sinorhizobium meliloti*	• Used for Seed inoculation • The results demonstrated that sludge can be used as a carrier since it has the same or higher capability than peat to promote *S. meliloti* survival	[28]

Table 2: Effect of liquid biofertilizer and its combinations on Soil health and crop productivity

S. No	Types of Biofertilizer used	Dose	Crop plant	Effect	References
1.	Liquid *Rhizobium* inoculants (LRI) and Carrier based *Rhizobium* inoculants (CRI) and inorganic N	60 g carrier based and 20 ml liquid inoculant/kg of seed and 20 and 40 kg h^{-1} of inorganic N	Urdbean (*Vigna mungo* L.)	Seed yield was high using liquid inoculants as compared to carrier inoculants in urdbean and the authors recommended the use of liquid biofertilizers because of its extra advantage.	[52]
2.	Liquid and carrier based *Rhizobium* inoculants in combination with different level of phosphorus	• Liquid bioinoculant and carrier based cultures: double the recommended dose (20 ml or g kg-1 of seed) • Fertilizer N: P_2O_5:K_2O (20:80:20 kg ha^{-1})	Soybean (*Glycine max*)	The nodules number, fresh and dry weight, seed yield and stover yields of soybean crop was found to be highest in the treatment P$_{80}$+LRh (80 kg P_2O_5+liquid inoculum of *Rhizobium*). Same treatment enhanced the soil properties like available N and P at the time of harvesting.	[53]
3.	*Azotobacter* and PSB carrier-based and liquid bioinoculants in conjunction with NP fertilisers	Recommended dose of N and P fertilizes 100%, 75% and 50% combined level (120 kg N & 60 kg P_2O_5 ha^{-1}) with carrier biofertilizer-10kg ha^{-1} and 0.625 and 1.25 L ha^{-1} of liquid biofertilizer.	Wheat (*Triticum aestivum* L.)	The growing rates of liquid and carrier biofertilizers utilised in soil improved the various measured characteristics moderately. Application of 0.625 L ha-1 liquid biofertilizers in soil containing 75 percent NP was shown to be best for growth, yield, nutrient uptake, and accessible NPK in soil after harvesting.	[54]
4.	Different treatment combinations of chemical fertilizers and different combination of biofertilizers (Liquid and Carrier based)	NPK fertilizers: 400:160:400 g/ tree and carrier based biofertilizer: 100 g/plant and liquid biofertilizer: 5 ml/plant.	Guava (*Psidium guajava* L.) cv. Taiwan White	The liquid biofertilizer treatment T2 [(100% RDF (recommended dose of fertilizers) + NFB (*Azotobacter chroococcum)*, PSB (*Bacillus megaterium*) and KSB (*Bacillus mucilaginosus*) liquid biofertilizers)] was found to have better results in terms of fruit length, number of fruit/plant, fruit diameter,	[33]

				weight of fruit, volume of fruit, fruit yield/plant and yield/acre	
5.	Liquid biofertilizer and Carrier based biofertilizer of *Rhizobium* and PSB	Carrier based biofertilizers: 250 g each/10 kg of seed and liquid based biofertilizers: 10ml for 10 kg seed and PSB 300-500 ml/acre.	Mungbean (*Vigna radiate*)	The seeds treated with liquid biofertilizer at the time of sowing and soil application at 40 DAS (days after sowing) together in Mungbean crop was found to have highest seed yield calculated per hectare and the crop was found to have highest nodules number and positive nitrogen fixation ability of the plant	[55]
6.	Liquid form of *Azotobacter* spp., Carrier based *Azotobacter* and combined form of liquid and carrier based *Azotobacter*		Green Leafy Vegetables	The results showed that using a combination of liquid biofertilizer and a carrier-based biofertilizer like *Azotobacter* might improve morphological parameters including plant height, number of leaves, shoot length, and root length on the 20th day.	[35]
7.	*Mesorhizobium ciceri* liquid and carrier inoculants, as well as PGPR (*Pseudomonas diminuta*)	Carrier-based *Mesorhizobium* sp. and PGPR inoculants 20g/kg seed, liquid inoculants: 4.0ml/kg seed.	Chickpea	Liquid inoculants of *Mesorhizobium* sp. and PGPR were found to be superior than carrier-based inoculants in terms of root nodulation, nodule quantity, and nodule dry weight.	[56]
8.	Azotobacter and PSB bioinoculants in liquid and carrier form	250 g 10 kg-1 seed for carrier-based bioinoculants and 100 ml 10 kg-1 seed for liquid-based bioinoculants	Sunflower (*Helianthus annus*)	Highest results were found in (T8) liquid form of *Azotobacter* and PSB along with RDF bioinoculants over other treatments. Phosphorus content, nitrogen content, potassium content, ferrous content and zinc content and there uptake was enhanced as compared to all other treatments in Sunflower.	[57]
9.	Liquid based biofertilizers and Carrier based biofertilizers (*Rhizobium*, PSB)	Liquid based biofertilizer 50 ml /10 kg and 100 ml/10 kg (PSB and *Rhizobium*) Carrier based biofertilizer: PSB and *Rhizobium*	Green Gram (*Vigna radiata* L.)	The results revealed that liquid based *Rhizobium* + PSB @ 100 ml/10 kg resulted in significantly higher plant height, number of branches/plant, number of leaves/plant, leaf area/plant,	[58]

			and dry matter/plant growth characteristics. Similarly, when compared to carrier-based biofertilizer treatment and others, the same treatment gave considerably increased numbers of pods/ plant, seeds/ pod, weight of pods /plant, seed yield /plant, seed yield /ha, and straw yield /ha.	
10.	Liquid biofertilizer and Carrier based biofertilizers and three levels of recommended dose of fertilizers (RDF) (100%, 80% and 60%)	Carrier based biofertilizers: 5 kg/ha, liquid biofertilizer: 1.25 L/ha and recommended dose of farm yard manure: 25 t/ ha	Tomato (*Solanum lycopersicum* L.)	Maximum plant height, number of primary branches/plant, leaf chlorophyll content, and leaf area (vegetative characters) were recorded after using 80 percent RDF in combination with liquid biofertilizer (nitrogen fixing bacteria, phosphate solubilizing bacteria, and potassium solubilizing bacteria), and this increase in vegetative growth eventually resulted in higher yield/plant and yield/ha in tomato.	[59]
11.	Liquid and Carrier based biofertilizers (*Rhizobium* inoculants)	Seed treatment with liquid *Rhizobium* (25 mL/kg seed) and seed treatment with *Rhizobium* carrier (25 g/kg seed).	Mung bean (*Vigna radiata*)	Inoculation with a liquid Rhizobium solution improved mung bean growth and yield. Further, the population of Rhizobium was dramatically increased when liquid formulations were inoculated.	[60]
12.	Liquid and Carrier based biofertilizers (*Rhizobium* and PSB)	-	-	When comparing carrier and liquid-based biofertilizers (*Rhizobium* and PSB), the microbiological investigation found that carrier-based biofertilizers had a lower population (viable count-sustained for the first three months) and higher contamination. However, liquid biofertilizers maintained a consistent viable count upto six months.	[45]
13.	Liquid based biofertilizers (LBBF) and	Liquid *Rhizobium*-10ml and PSB-10ml and inorganic	Green gram (*Vigna radiata* L.) variety (MGG –295)	Seed treatment based on liquid biofertilisers with a 100% RDF combination significantly	[61]

	carrier based biofertilizers (CBBF) with combination of recommended dose of fertilizers (NPK)	fertilizers dose: 20:50:00 NPK kg/ha		increased plant height, root length and dry matter output at 30, 45, and 60 DAS, as well as during harvest. In case of soil, the soil treated with LBBF and 100% RDF had the largest nodule dry weight and quantity of root nodules.	
14.	Carrier-based and liquid bioinoculants of *Azotobacter* and PSB	10 kg ha^{-1} soil application of carrier-based biofertilizer and 625 and 1250 mL ha^{-1} soil application of liquid-based biofertilizers	Wheat (*Triticum aestivum* L.)	A 75 percent NP liquid biofertilizer treatment resulted in the largest population of *Azotobacter* and PSB, microbial biomass C, and acid and alkaline phosphatase activity in soil at various crop ages.	[62]
15.	Liquid formulation of *Azotobacter* (MPKV and Commercial firm) and Carrier based (*Azotobacter*)	Liquid formulation of *Azotobacter* dose: 1 ml/ kg seeds and Carrier based (*Azotobacter)*: 25 g/ kg of seeds.	sorghum (var. Maldandi-35) and maize (var. African tall)	In sorghum and maize, MPKV liquid *Azotobacter* had the highest germination percentage, number of leaves, plant height and dry matter/green fodder weight respectively.	[63]
16.	Liquid (*Azotobacter* liquid) and carrier based biofertilizers (*Azotobacter* powder)	Liquid agar slant *Azotobacter* culture dose: 20 ml/ kg seeds and carrier based culture (powder): 10% was placed in jaggery solution.	Amaranth cv. GA-2	The treatment N$_{60}$ + *Azotobacter* liquid outperformed the other treatments in terms of water consumption and water efficiency. In the same combination, the maximum grain production of 1780 kg ha^{-1} was also achieved.	[64]
17.	Liquid, gel and carrier formulations of *Azotobacter chroococcum* MAC- 4	Liquid based inoculants: 15 ml/ kg of seed and carrier based inoculants: 20 g/ kg of seeds.	Maize var. Co 1	It was found from the study that inoculating maize with a liquid-based formulation could improve growth and yield characteristics by fixing a higher amount of nitrogen. When compared to the gel and carrier based formulation, maize secretes a higher amount of plant development stimulating chemicals such Indole acetic acid (IAA) and Gibberellins in liquid-based formulation.	[65]

Alka Kataria *et al.,*

Table 3: Biofertilizers' demand and supply (year wise in million tonnes):

Year	1990	2000	2011	2031	2041
Demand	16.0	19.0	20.2	27.3	31.1
Supply	12.8	14.9	15.8	20.9	23.9
Gap	3.2	4.1	4.4	6.4	7.2

Table 4: The estimated demand for bio-fertilizers in India

| Bio-fertilizers	Estimated requirement (tonnes)
Rhizobium	34,999
Azotobacter	145,953
Azospirillum	74,342
Blue-green algae	251,378
P solubilizer	25,534
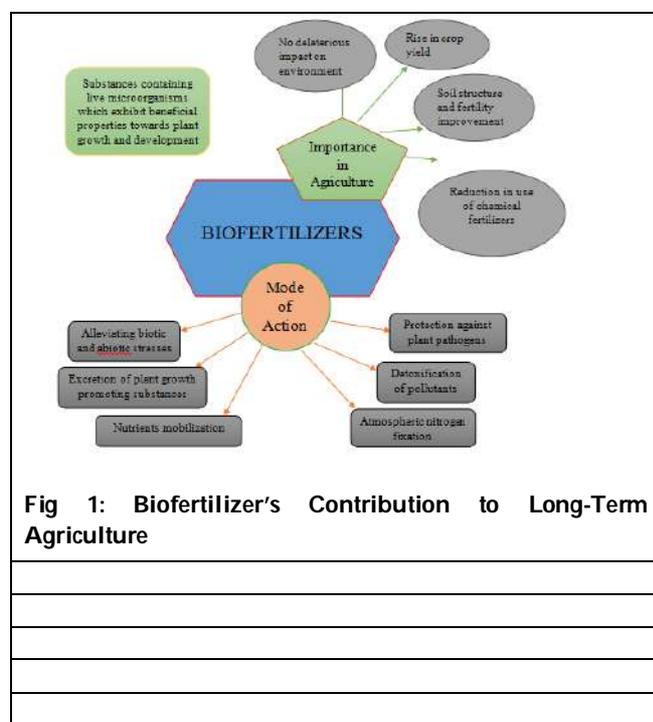
Fig 1: Biofertilizer's Contribution to Long-Term Agriculture
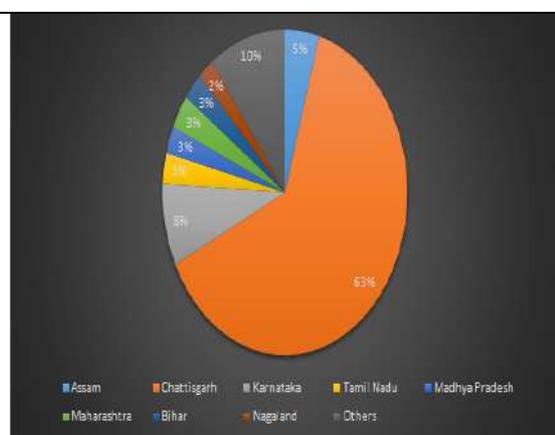
Fig 2: Production of Organic Fertilizer in different states during the year 2020-21 (Total Production 3879488.93 MT)

RESEARCH ARTICLE

Hospital Selection Process based on Graph Theory

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ABSTRACT

Hospitals play a crucial role in diagnosing and treating patients with diseases or illness. Generally, a hospital is selected based on parameters such as Surgeons, Staffs, Infrastructure and location, among others. In this work an attempt has been made to classify the different types of hospital based on the factors and its associated sub factors. Also, a graph theoretic model based on digraphs and Variable Permanent Function (VPF) was adopted to choose the best hospital. A fishbone diagram was utilized in representing the factors and the corresponding cofactors to illustrate the process in a simpler manner. Then, a digraph was sketched between the various factors and the cofactors to depict the interdependencies which ultimately led to the formulation of a weight matrix with numerical values which were provided by an experienced professional. Furthermore, an individual matrix was formed for the three different hospitals namely rural, suburban and metropolitan to aid in the decision-making process.

Keywords: Graph Theory, Digraph, Fish Bone, Variable Permanent Function

INTRODUCTION

Healthcare systems and their numerous facilities are complicated and multi-faceted in today's world. In recent years, people have become more concerned about the quality of healthcare, prompting hospital owners to devise plans and policies to improve medical services by establishing new hospitals. One of the most critical policy considerations for government and health officials is the location of a hospital. The most vital point in health care is to put all patients in the right place, give outstanding service, and be fair. For the efficacy, quality, and equity of health care, selecting the best hospital location is critical. Hospitals are classified based on their geographic location namely rural, suburban and urban (metropolitan). Rural hospitals, which often lack modern equipment or specialized procedures and techniques, help a smaller population. Rural hospitals play a critical role in ensuring the availability of health services in rural areas, and are an important part of the social and economic identity of rural communities. They have had mixed success in responding to environmental threats, and are extremely sensitive to public policies due to their small size. Suburban areas are mostly residential regions with a population density higher than rural areas. Medicare payment policies assist low-volume hospitals stay afloat so that vulnerable populations can get medical care without having to travel to cities. Urban hospitals, which range in size from 100 to 500 beds, provide services in densely populated areas. With several hospitals and medical institutions covering the same geographic areas, many metropolitan hospitals confront fierce competition. Thus, urban hospitals serve greater metropolitan regions and must frequently provide a wide range of treatment options and patient experiences.

Graph theory is the study of graphs, which is a collection of vertices (nodes or points) connected to each other through a set of edges (lines or links) [1,2]. Graphs are classified into directed and undirected graphs. A graph in which the direction of the edge is defined to a particular node is a directed graph or digraph. Undirected graphs have edges that do not have a direction. They merely show whether or not there is a relationship between two vertices. Graphs can be used to model many types of relations and processes in physical, biological, social and information systems, and has a wide range of useful applications. Graph Theory is basically a mathematics concept with widespread applications in every discipline for modeling and analysis. Graph theoretic and matrix approach serves as a strong tool for calculating a single numerical index for interpretation of significant factors relevant to any engineering challenge. This approach was used to select the best power plant among three major power plants during day time and night time and a fishbone diagram was used to represent the factors and cofactors. The cofactors for each major factor were listed using a fishbone diagram and factors were represented in a digraph. The VPF of the individual matrices was calculated with weighted matrices and the power plant with highest VPF was selected by Sriganesh *et al.,* [3]. A similar work was conducted by Sangeetha *et al.,* using digraph to select one among three types of concrete based on their mechanical properties [4]. In this work, the concept of graph theoretical approach has been implemented to select the best hospital based on different factors. Directed graphs are used to show the interaction between the factors and its sub-factors. Matrix representation and Variable Permanent Function (VPF) is also used in this work.

LITERATURE SURVEY

Paramasivam *et al.,* described three multi-attributes decision-making methods, namely, digraph and matrix approach, analytical hierarchical process and analytical network process for equipment selection. The matrix representation permits analysis, storage and retrieval of information [5]. In a similar study, Paramasivam *et al.,* studied a digraph and matrix technique in the analysis, selection, and evaluation of product design, obtaining a product design evaluation function and evaluating the index [6]. Omit Singh *et al.,* provided a digraph for assessing and selecting vendors in which the attributes that contribute to the vendor's quality were produced and its representation in matrix form was utilized to calculate a numerical index of the vendor's quality [7]. Graph theory and matrix method for the evaluation of reliability index for a Combined Cycle Power Plant (CCPP) and a Reliability Attributes Digraph was developed by Nikhil Dev *et al.,* [8]. Gandhi *et al.,* proposed methodology to analyze the failure causes of a machine tool used on the mechanical and hydraulic systems using digraph [9]. Prabhakaran *et al.,*

[10] proposed a digraph and matrix approach for developing a composite product by considering all attributes responsible for design and production. The matrix representation is a powerful tool for storage and retrieval of composites in computer databases. Graph Theory has also been used in healthcare, with research by Guru *et al.,* suggesting the possibility for enhancing the operational, tactical, and strategic decision-making of health care inventory systems by using graph theory methodologies [11]. Graph theory matrix was adopted by Geetha *et al.,* to analyze and evaluate the optimal combination of operating parameters on a diesel engine [12]. The digraph is represented in a matrix form called performance attributes matrix that considers all the attributes and their relative importance. Rao devised a 'material suitability index' that evaluates and ranks the materials for a given engineering component. The graph was developed considering material selection factors and their relative importance for the application [13]. This methodology can be extended to any type of selection problems.

FACTORS IN CONSIDERATION FOR SELECTION PROCESS
There are numerous factors that influence the provision of hospitals and their ability to provide equitable access to care services.

Location
Selecting an optimal location is one of the first and most important steps in developing a healthcare facility. Site selection is a major factor that can have a huge impact on the rest of the project and, ultimately, the facility's success. Health-care organizations should select a piece of land that can fit their requirements, parking infrastructure, and other factors without requiring a large amount of extra space. The hospital should be in a location that is easily accessible to the general public and biomedical waste should be disposed of in a way that does not harm the environment. Adding to this, surplus amounts of water and power supply are the critical factors in determining the location of the healthcare facility.

Cost of land and taxes
The cost of land and tax plays a major role in site selection for healthcare development. The main attributes include quality of location, topography, area in square feet, availability of water, sewer lines etc., More and better facilities are attributed to a higher price of land. Furthermore, topography has a direct impact on construction costs and, as a result, overall development costs. Land costs and taxes would be greater in commercial suburbs than in rural areas.

Population
Population directly affects the healthcare facility. Hospitals located in a densely populated region have the necessary infrastructure and amenities that greatly benefit the public residing in the region. The variability among older age groups is the most crucial aspect in projecting hospitality demands and prospects.

Facilities
The fundamental facilities required for smooth functioning of the hospital includes, continuous supply of electricity and water, sufficient and sanitized wards, proper infrastructure, skilled staffs and standardized critical care equipment. A hospital may also have a pharmacy, a laboratory, adequately working imaging and diagnostic systems, physical therapy departments, and an obstetrical unit in addition to the necessary services linked to patient care, depending on its size and location.

Surgeons/Staffs experience
An experienced surgeon/staff is, in the end, a basic requirement for any hospital. The surgeon is in charge of determining the patient's diagnosis, performing the operation, and providing postoperative care and treatment. Each surgeon should be well-trained, able to communicate effectively, retain professional skills and knowledge, and keep patient information updated. They should also have effective decision-making skills, experience, leadership and management skills, maintain professionalism, creativity and courage. Figure 1 shows the fishbone diagram to represent the factors that influence the selection of hospitals. The four factors identified are Surgeons (F1), Staffs (F2),

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Infrastructure (F3) and Location (F4). The interaction between the factors is shown as a digraph as plotted in figure 2. The value of the assigned factor for qualitative measure of the various parameters are listed in Table 1 and Table 2.

REPRESENTATION AS MATRIX AND VPF CALCULATION

The digraph technique is utilized to visualize the aforementioned factors and sub factors. Calculation of Variable Permanent Matrix (VPM) becomes a tedious process with an increasing number of sub factors. To reduce complexity and increase understanding, the cofactors associated with the factors are represented in the form of a N x N matrix, where N represents the number of factors that occur along a matrix's main diagonal and the individual elements in in the matrix represent the dependency of factor j on i. From the fish bone diagram, it can be seen that the major factors that affect the selection process are the Surgeons in the hospital (K1), working staff (K2), Infrastructure (K3) and location of the hospital (K4). These factors play a vital role in determining the value of the Variable Permanent Matrix (VPM).

The VPM is represented as,

$$\begin{pmatrix} K1 & K12 & K13 & K14 \\ K21 & K2 & K23 & K24 \\ K31 & K32 & K3 & K34 \\ K41 & K42 & K43 & K4 \end{pmatrix}$$

Matrix representation helps in more clear visualization and determination of the factors and the dependence. But it does not provide information about the decision-making process. Hence, the VPF is calculated for this purpose. VPF is obtained in a way similar to that of a matrix determinant but with a positive sign.

VPF is calculated from VPM as,

VPF = $K1K2K3K4 + K12K21K3K4 + K13K31K2K4 + K14K41K3K2 + K23K32K1K4 + K24K42K3K +$ $K34K43K1K2 + K12K23K31K4 + K13K32K21K4 + K12K24K41K3 + K14K21K3K42 + K13K2K34K41 + K14K2K31K43 +$ $K1K23K34K42 + K1K24K43K32 + K12K21K34K43 + K13K31K24K42 + K14K41K23K32 + K12K23K34K41 +$ $K14K43K32K21 + K12K24K43K31\ K13K34K42K21 + K14K42K23K31 + K13K32K24K41$

DIGRAPH REPRESENTATION OF FACTORS

Surgeon based Digraph

The Surgeon or Physician factor has three sub factors that are associated with it namely Experience or Expertise (P1), Complexity (P2), Capability (P3) and Confidence level (P4). For the purpose of determining the interdependency between the cofactors a digraph was drawn. The factors mentioned play an important role in the selection of the hospital. Surgeons are critical components of every hospital. The surgeon's expertise demonstrates his ability to handle increasingly difficult cases. If the surgeon is experienced and has already faced complex problems, he'd be able to treat the patients with more confidence. For example, if a surgeon performs cystectomy on a regular basis, he will have more knowledge and confidence in treating the same condition, resulting in a higher number of in-patient visits at the hospital. Hence, all the cofactors are interlinked in a similar fashion and their interdependencies are shown in Figure 3. Based on the mentioned cofactors (P1, P2, P3 and P4) a weight matrix was formed based on the viewpoints of an experienced professional and by incorporating values from Table 1. The weight matrix for surgeon-based digraph is shown below.

$$P = \begin{pmatrix} P1 & 5 & 5 & 5 \\ 5 & P2 & 4 & 4 \\ 5 & 5 & P3 & 4 \\ 5 & 4 & 4 & P4 \end{pmatrix}$$

Staff based Digraph

Now, the second important factor considered is the Staff. The cofactors that are associated with the main factor are Trainings (Q1), Communication (Q2), Hospital Etiquette (Q3) and Leadership (Q4) and the same can be viewed in the fish bone diagram. In order to simplify the relationship between the cofactors a suitable graph was drawn. The Quality of care provided in a hospital depends on the working staff which includes nurses, clerks, attendees, caretakers etc., If a person working in a hospital can demonstrate leadership characteristics, improved communication, coordination, and basic manners, it is immediately assumed that the person is highly trained. Hence, all the cofactors are interlinked in a similar fashion and their interdependencies are shown in Figure 4. Furthermore, A weight matrix was formed with the cofactors Q1, Q2, Q3 and Q4 from the values in Table 1.

$$Q = \begin{pmatrix} Q1 & 5 & 5 & 3 \\ 2 & Q2 & 2 & 3 \\ 2 & 3 & Q3 & 3 \\ 1 & 2 & 2 & Q4 \end{pmatrix}$$

Infrastructure based Digraph

In this case, the main factor is the infrastructure. The cofactors that are associated with the main factor are Departments (I1), Equipment (I2), Information systems (I3) and Safety (I4) and the same 8 is shown in the fish bone diagram. In order to simplify the relationship between the cofactors a suitable graph was drawn. The infrastructure of a hospital is yet another important factor in the hospital selection process. The factors such as departments, Equipment, Information systems and Safety play a crucial role in the diagnosis of the patient. A hospital has several departments, the most important of which being Cardiology, Pulmonologist, Radiology, Orthopedics, and others. The operation of a department will depend on the number of equipment available in order to promote better and accurate diagnosis. Furthermore, it is reliant on information and security technologies that allow it to store patient data in a virtual environment or cloud and to safeguard the data from misuse through the use of encryption codes. The safety of the patients is also considered to be an important parameter. Hence, all these factors were interlinked as stated and their interdependencies are depicted in the digraph in Figure 5. Also, a weight matrix was formed with the cofactors I1, I2, I3 and I4 along with the values from Table1.

$$I = \begin{pmatrix} I1 & 5 & 5 & 4 \\ 4 & I2 & 3 & 4 \\ 4 & 2 & I3 & 3 \\ 4 & 4 & 3 & I4 \end{pmatrix}$$

Location based Digraph

In this case, the main factor is the Location. The cofactors that are associated with the main factor are Price of land (L1), Accessibility (L2), Distance from raw material suppliers (L3) and parking space (L4). A similar weight matrix was formed with the cofactors L1, L2, L3 and L4 along with values from Table 1. Location of an hospital is a deciding factor for the in-patient counts. Location depends on cofactors such as price of land, distance from raw material suppliers, accessibility and transport. The cost of land impacts whether the hospital can be built close to or distant from hotspots. It also determines the amount of area that can be utilized to construct the hospitals. The hospitals should also be accessible by people and be within reach from material suppliers so as to ease transportation and to increase patient visits. Hence, a digraph depicting the interdependency and linking the cofactors was drawn as shown in Figure 6. Access to healthy food, physical exercise and recreation facilities, water and sewer utilities, health care, transportation, and even digital connectivity varies by region.

$$L = \begin{pmatrix} L1 & 5 & 5 & 5 \\ 5 & L2 & 4 & 4 \\ 4 & 5 & L3 & 3 \\ 5 & 4 & 4 & L4 \end{pmatrix}$$

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HOSPITAL SELECTION

Rural Hospital

Health is greatly influenced by place, because of the environmental and cultural characteristics of neighborhoods and communities. Rural areas are places of insanitation, poverty and diseases. Healthcare is a vital necessity for indigents who reside in rural areas. Rural hospitals are located in areas which have less population. In addition to traditional hospital services such as emergency treatment, inpatient care, and laboratory testing, small rural hospitals offer rehabilitation, long-term care, maternity care, and home health care. These hospitals, on the other hand, are understaffed and lack essential amenities such as beds, competent surgeons, operating rooms, laboratories, and emergency services.

Suburban Hospital

Hospitals in the suburbs are placed in lower-density locations that separate residential and business districts. People who live in the suburbs face significant health-care barriers. For low-income adults, the suburbs enjoy a slight edge over urban and rural locations. Consumers from the suburbs must frequently travel great distances to urban safety-net providers since fewer suburban physicians appear prepared to handle uninsured patients. After accounting for demographic differences by region, rates of access to care were comparable across all regions.

Urban Hospital

Urban hospitals are hospitals located in areas with high population density. An urban area is often the main area of employment. Urban hospitals cater to domestic and international patients and offer their best in-class medical and surgical services through skilled team of doctors and specialists. These hospitals are well-equipped with 24-hour emergency services, enhanced cleanliness, continuous water and electricity supplies, and high-class amenities such as a critical care unit, well-trained personnel and surgeons, and a sterile supply department, among other things. The hospital is accredited based on how it provides quality health care. Urban hospitals also benefit from having a large number of research programmers, which helps them gain popularity and efficiency. They are also the first to receive the most recent technological developments. This is a great assistance in terms of being able to treat large groups of individuals swiftly and effectively.

DIGRAPH AND MATRIX REPRESENTATION OF SELECTION PROCESS

The selection of the hospital is done based on the factors and its sub factors as mentioned above. The interaction between the three different types of hospital namely, Rural hospital (R), suburban hospital (S) and Urban/Metropolitan hospital (M) is plotted as a digraph as shown in figure 7.Prioritizing the operations of various hospitals allows for more efficient use of resources, which has an impact on the hospitals operating costs and also allows for more effective treatment programmers for patients. Taking into account the discussed parameters, the hospital's infrastructure, staff, and surgeons are chosen depending on the hospital's quality of care.

The matrix representation of the three hospitals Rural (Hr), Suburban (Hs) and Metropolitan (Hm) are given below.

Rural	Suburban	Metropolitan
$H_r = \begin{pmatrix} Pr & 1 & 1 & 1 \\ 2 & Qr & 1 & 1 \\ 1 & 2 & Ir & 1 \\ 0 & 1 & 0 & Lr \end{pmatrix}$	$H_s = \begin{pmatrix} Ps & 3 & 4 & 2 \\ 3 & Qs & 3 & 3 \\ 3 & 3 & Is & 2 \\ 2 & 2 & 2 & Ls \end{pmatrix}$	$H_m = \begin{pmatrix} Pm & 5 & 5 & 4 \\ 5 & Qm & 5 & 4 \\ 5 & 5 & Im & 5 \\ 4 & 4 & 4 & Lm \end{pmatrix}$

In order to select the best option from the three hospitals namely rural, suburban and metropolitan identification of the significant factors is a crucial stage. The weight matrices of each factor were calculated using the VPF. The VPF of the distinct matrices are evaluated and the highest one was selected. The weighted values are determined by

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feedback from an experienced professional in the field. In this case of selection, all the four factors were considered as significant and a matrix representing the selection is given below.

Surgeon – Experience, Complexity, Capability and Confidence

P_r, P_s and P_m values for three different hospitals are calculated below.		
Rural	Suburban	Metropolitan
$P_r = \begin{pmatrix} 2 & 5 & 5 & 5 \\ 5 & 1 & 4 & 4 \\ 5 & 5 & 3 & 4 \\ 5 & 4 & 4 & 2 \end{pmatrix} = 5933$	$P_s = \begin{pmatrix} 3 & 5 & 5 & 5 \\ 5 & 3 & 4 & 4 \\ 5 & 5 & 4 & 4 \\ 5 & 4 & 4 & 4 \end{pmatrix} = 8352$	$P_m = \begin{pmatrix} 3 & 5 & 5 & 5 \\ 5 & 3 & 4 & 4 \\ 5 & 5 & 4 & 4 \\ 5 & 4 & 4 & 4 \end{pmatrix} = 15918$

Staff – Trainings, Communication, Hospital Etiquette and Leadership

Q_r, Q_s and Q_m values for three different hospitals are calculated below.		
Rural	Suburban	Metropolitan
$Q_r = \begin{pmatrix} 1 & 5 & 5 & 3 \\ 2 & 2 & 2 & 3 \\ 2 & 3 & 1 & 3 \\ 1 & 2 & 2 & 1 \end{pmatrix} = 616$	$Q_s = \begin{pmatrix} 3 & 5 & 5 & 3 \\ 2 & 3 & 2 & 3 \\ 2 & 3 & 4 & 3 \\ 1 & 2 & 2 & 3 \end{pmatrix} = 1356$	$Q_m = \begin{pmatrix} 7 & 5 & 5 & 3 \\ 2 & 6 & 2 & 3 \\ 2 & 3 & 6 & 3 \\ 1 & 2 & 2 & 6 \end{pmatrix} = 4323$

Infrastructure – Departments, Equipment, Information systems and Safety

Q_r, Q_s and Q_m values for three different hospitals are calculated below.		
Rural	Suburban	Metropolitan
$I_r = \begin{pmatrix} 2 & 5 & 5 & 4 \\ 4 & 2 & 3 & 4 \\ 4 & 2 & 1 & 3 \\ 4 & 4 & 3 & 1 \end{pmatrix} = 2460$	$I_s = \begin{pmatrix} 3 & 5 & 5 & 5 \\ 5 & 4 & 4 & 4 \\ 4 & 5 & 3 & 3 \\ 5 & 4 & 4 & 3 \end{pmatrix} = 4074$	$I_m = \begin{pmatrix} 7 & 5 & 5 & 5 \\ 5 & 7 & 4 & 4 \\ 4 & 5 & 7 & 3 \\ 5 & 4 & 4 & 7 \end{pmatrix} = 11252$

Location – Price of Land, Accessibility, Distance from raw material suppliers and parking space

L_r, L_s and L_m values for three different hospitals are calculated below.		
Rural	Suburban	Metropolitan
$L_r = \begin{pmatrix} 1 & 5 & 5 & 5 \\ 5 & 1 & 4 & 4 \\ 4 & 5 & 2 & 3 \\ 5 & 4 & 4 & 1 \end{pmatrix} = 4434$	$L_s = \begin{pmatrix} 4 & 5 & 5 & 5 \\ 5 & 3 & 4 & 4 \\ 4 & 5 & 3 & 3 \\ 5 & 4 & 4 & 3 \end{pmatrix} = 6866$	$L_m = \begin{pmatrix} 7 & 5 & 5 & 5 \\ 5 & 7 & 4 & 4 \\ 4 & 5 & 7 & 3 \\ 5 & 4 & 4 & 5 \end{pmatrix} = 14353$

RESULTS

The obtained values are now substituted in the selection matrices Hr, Hs and Hm respectively and the highest value was chosen as the best one.

Rural	Suburban	Metropolitan
H_r = $\begin{pmatrix} 5933 & 1 & 1 & 1 \\ 2 & 616 & 1 & 1 \\ 1 & 2 & 2460 & 1 \\ 0 & 1 & 0 & 4434 \end{pmatrix}$	H_s = $\begin{pmatrix} 8352 & 3 & 4 & 2 \\ 3 & 1356 & 3 & 3 \\ 3 & 3 & 4074 & 2 \\ 2 & 2 & 2 & 6866 \end{pmatrix}$	H_m = $\begin{pmatrix} 15918 & 5 & 5 & 4 \\ 5 & 4323 & 5 & 4 \\ 5 & 5 & 11252 & 5 \\ 4 & 4 & 4 & 14353 \end{pmatrix}$
=39864549110595	=316793730564648	=11113395732237159

ACKNOWLEDGEMENTS

CONFLICT OF INTERESTS
The authors declare no conflict of interest.

CONCLUSION

In this work, a graph-theoretical approach was used to select a hospital among rural, suburban and urban/metropolitan. The methodology for determining the best hospital by considering all factors responsible for offering best quality and care is proposed using a digraph, matrix approach and VPF. It is a dynamic tool for modelling the degree to which the sub-factors interact with each other. The present work identifies four factors, surgeon, staff, infrastructure and location which parameterize the hospital selection system. Digraph is the mathematical representation of interdependence between factors. Digraph was converted to a weighted matrix by using qualitative metrics. The variable permanent function is a mathematical model characterizing the selection of the best facility and also helps one to determine the best healthcare facility. The variable permanent function values are calculated for each of the four factors. By observing the VPF values, it can be concluded that urban hospitals have the highest VPF when compared to rural and suburban, and thus, urban hospitals are preferred to acquire the best quality health care.

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Table 1. Interdependence value within factors (Wij) for off-diagonal entries

S. No	Qualitative Interdependency Measure	Assigned value
1	Very, very good	5
2	Good	4
3	Average	3
4	Low	2
5	Very, very low	1

Table 2. Value of interdependence between factors (Pij) (For diagonal entries)

| S. No	Qualitative Interdependency Measure	Assigned value
1	Very, very poor	1
2	Poor	2
3	Below average	3
4	Average	4
5	Above average	5
6	High	6
7	Exceptionally good	7

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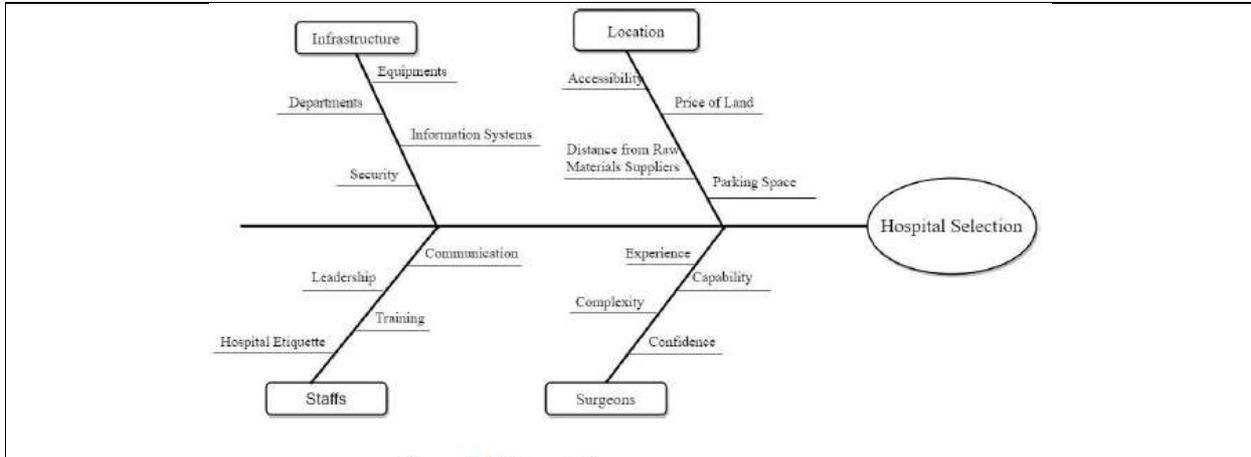
Figure.1 Fishbone Diagram
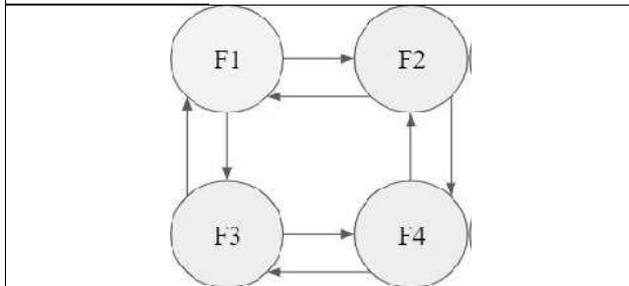
**Figure 2. Digraph for the interaction of factors**
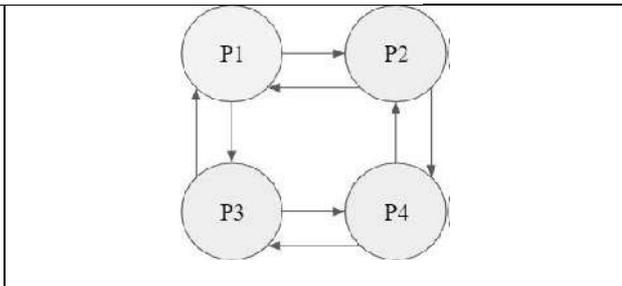

Figure 3. Digraph for Surgeon
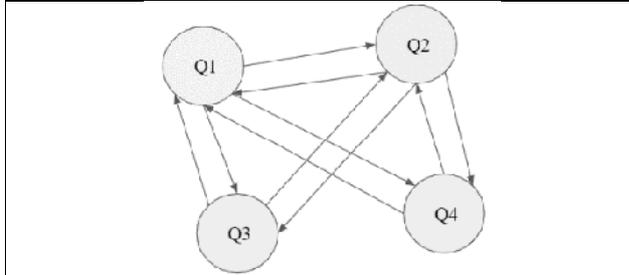
Figure 4. Digraph for staffs
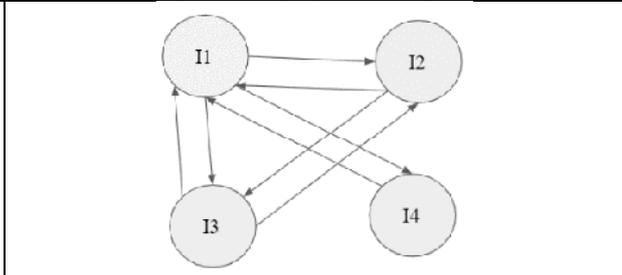
Figure 5. Infrastructure based Digraph
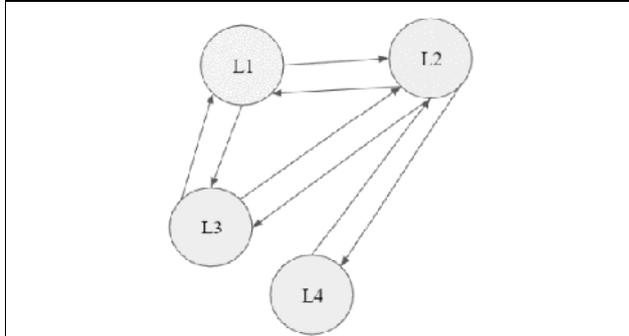
**Figure 6. Location based Digraph**
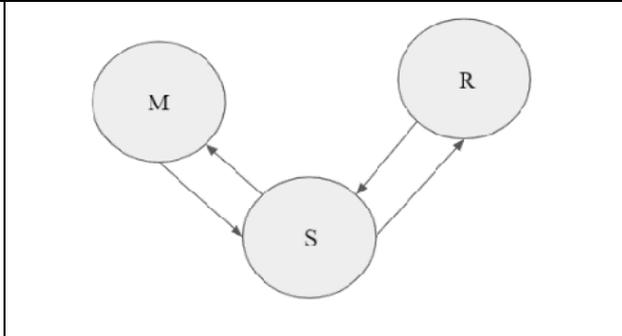
Figure 7. Digraph for hospital selection

REVIEW ARTICLE

Identification of Risk Factors and their use for Predicting Developmental Delay in Neonates – A Narrative Review

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ABSTRACT

Early identification is important for early intervention in children with developmental delay; Many NICU discharged infants identified as high risk for developmental delay facing delayed diagnosis due to a lack of specialized assessments. The objective of the present study is to review the literature relevant to identification of risk factors and their use for prediction of developmental delay in neonates. Various electronic search engines were used for literature by entering Key words synonymous with predictive tool, predictive model, developmental delay, cerebral palsy, NICU, early diagnosis, early prediction, early intervention, causes of developmental delay, risk factors and predictive factors. Articles published in between 2010 to 2021 in English language have been included in the study. From selected 33 relevant research reports, 11 articles met with the inclusion criteria and were critically appraised and reviewed in this study. In current review it is evident that majority of available articles of predictive tool include only few of the risk factors. It is important to identify responsible risk factor for delay in the development and use them in predictive formula, so that child at risk of delay can be provided with early intervention.

Keywords: Developmental delay, Predictive tool, Risk factors

INTRODUCTION

The purpose of this article is to provide an overview of the risk factors influencing developmental delay. Infants discharged from the Neonatal Intensive Care Unit (NICU) are at increased risk for poor neurodevelopment outcomes [1-4]. though, the developmental surveillance of NICU patients is highly variable, ranging from none to greater than 90% in research studies supporting systematic NICU follow-up [5]. Many NICU discharged infants identified as high

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risk for developmental delay facing delayed diagnosis due to a lack of specialized provider or assessments .Cerebral Palsy (CP) is challenging to diagnose in young children due to the complexity of signs, symptoms and developmental progression involved [2]. However, for an individual infant, early identification is important for early intervention in children with developmental delay to optimize the effectiveness of rehabilitative interventions. Infancy and early childhood are periods of maximal neural plasticity during which therapeutic interventions have the greatest potential for long-term effectiveness [6-10]. However there are no supporting literatures available for an individual prediction model based on independent predictors of CP. So, having disposition of an individual prediction on the development of CP would help neonatologists in the early decision-making process and parental counseling, as well as prediction of the CP characteristics would enable therapists to define more precisely short and/or long term intervention strategies. Although numerous tools for predicting the functional outcomes of neonates have been developed in the past three decades, no studies have provided a comprehensive overview of these tools.

NEED OF THE STUDY

- Most of Research has focused mainly on highly specialized tools, from imaging to complex neurological assessments, while few studies examine the value of more basic developmental milestone trajectories.
- Implications and challenges of early diagnosis of CP by developing simple tools for early identification and screening in high-risk infants is a priority.
- The purpose of this study is to provide review of the current Predictive models available for predicting the developmental delay in high risk neonates.

OBJECTIVE

The objective of the present study is to review the literature relevant to identification of risk factors and their use for prediction of developmental delay in neonates

METHODOLOGY

- Various electronic search engines were used for literature by entering Key words
- Articles since 2010 to 2021 years were searched.
- Search for literature on predictive model based on risk factors for Developmental delay

Search engines	Key words
1.Google scholar	Predictive tool,
2.Pubmed	Predictive model, developmental delay, cerebral palsy(CP),
3.EBSCO	NICU,
4.Medline	early diagnosis,
5.sciene direct	early prediction,
	early intervention,
	causes of developmental delay,
	Pre,Peri Neonatal risk factors and
	predictive factors

Inclusion criteria

- Articles published in English language
- Articles between 2010 to 2021
- Developmental delay or CP was the primary outcome
- The primary aim of the study was to identify Predictive model for developmental delay or CP in all births

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Exclusion criteria
- Articles published in languages other than English
- Case reports
- Comments and letters or report information
- Models were also excluded if they limited their prediction to the outcome of survival

RESULT

From selected 33 relevant research reports, 10 articles met with the inclusion criteria and were critically appraised and reviewed in this study

No	Author name and year	Study	Type of study	Population	Outcome measure	Predictors	Conclusion
1	Ushida T, Moriyama Y et.al, 2021[11]	Antenatal prediction models for short-and medium-Term outcomes in preterm infants	Population based study. (cohort study)	Born alive between January 2006 and December 2015 at <32 weeks of gestation and weighed ≤1500 g, JAPAN	short-term outcomes at the end of each infant's stay in the NICU: eg. Neonatal death ,RDS,IVH etc and medium-term Outcomes eg.CP, DQ etc. were assessed when children 3 years of age:	Maternal age, gestational age, parity, delivery mode, diabetes mellitus/gestational diabetes mellitus, hypertensive disorders of pregnancy, clinical chorioamnionitis, premature rupture of membranes, antenatal corticosteroid treatment, plurality of pregnancy (singleton or twin), chorionicity (monochorionic or dichorionic), infant's sex and Birth weight	They have established and validated risk prediction models for short and medium-term outcomes in extremely and very preterm infants. They suggest estimating the likelihood of infants' adverse outcomes with the use of multiple risk factors, not gestational age or birth weight alone.
2	Crilly CJ, Haneuse S, Litt JS et.al, 2021[12]	Predicting the outcomes of preterm neonates beyond the neonatal intensive care unit: What are we missing?.	literature review and narrative analysis	preterm neonates		Preterm	Risk assessment and outcomes prediction are valuable tools in medical decision-making.

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3	van Dokkum NH, Reijneveld SA et.al, 2020[13]	Development of a prediction model to identify children at risk of future developmental delay at age 4 in a population-based setting.	Longitudinal cohort	4 years age children from community-based consisting of both <u>full-term and preterm children</u> of Netherlands	Dutch version of Ages and Stages questionnaire	Perinatal factors, i.e., GA and birth weight, sex , being born small-for-gestational-age (SGA), multiple birth , and Apgar score ; (2) parental factors, i.e., maternal educational level , pre-existing maternal obesity, and maternal smoking during pregnancy (3) growth, i.e., weight for height z score at 1 and 2 years and (4) developmental milestones, i.e., smiling , speaking 2–3 word sentences , head-lifting, standing, and walking	In conclusion, They developed a prediction model based on only seven easily obtainable variables sex , maternal educational level , pre-existing maternal obesity , smiling, speaking 2 to 3-word sentences ,standing and BMI at 1 year to be predictors.
4	Licia Lugli1 , Marisa Pugliese et.al, 2020[14]	Neuroprem : the Neuro-developmentaloutcome of very low birth weight infants in an Italian region	Area-based prospective cohort study	Surviving VLBW infants from an Italian network of 7 neonatal intensive care units (NICUs)	Griffiths Mental Developmental Scales (GMDS-R) or the Bayley Scales of Infant and Toddler Development (BSDI III) and neuro-functional evaluation according to the International Classification of Disability and Health (ICF-CY).	Very low birth weight , Preterm infants	They have concluded that Neuroprem represents an Italian network of NICUs aiming to work together to ensure preterm neuro developmental assessment and supports the implementation of a preterm follow-up programme from a national network perspective
5	Caesar R, Boyd RN et.al, 2016[15]	Early prediction of typical outcome and mild developmental delay	Prospective cohort study	(<32 weeks and/or <1500 g) admitted to the Special Care Nursery	Neurological, neuromotor, neurobehavioral and perceptual assessments including	very preterm/VLBW Social risk and environmental impact, Perinatal factors and different outcome measure values at neonatal period	Infants will be enrolled in the study between 34 and 36 weeks Post term , they have a series of

		for prioritisation of service delivery for very preterm and very low birth weight infants: a study protocol.		(SCN), Nambour General Hospital (NGH)in Queensland	General Movement Assessment at preterm, writhing and fidgety age. At 24 months, corrected age infants will be assessed using standardised tools including the Bayley Scales of Infant and Toddler Development —Third Edition (Bayley III		Assessment at preterm, to predict typical or delayed neurodevelopment outcome at 24 months, corrected age.
6	Granild-Jensen JB, Rackauskaite G et.al, 2015[16]	Predictors for early diagnosis of cerebral palsy from national registry data. Developmental Medicine & Child Neurology.	Exploratory study	Children registered with CP and born between 1 January 1995 and 31 December 2003 in the Denmark using the Danish National Cerebral Palsy Register (NCPR)	Type of CP-unilateral spastic CP, bilateral spastic CP, dyskinetic CP, and ataxic CP. The degree of motor disability and Cognitive disability at age of 6.	Type of CP, degree of motor disability, cerebral Ultrasonography results, epilepsy, gestational age, and degree of cognitive impairment.	They found the corrected diagnostic age of CP to be 11 months. Early diagnosis was associated with the type of CP, presence of epilepsy, a high degree of motor disability, and abnormalities in the cerebral Ultrasonography. A late diagnosis was associated with less severe symptoms, and gestational age did not influence the diagnostic age.
7	Maitre NL, Slaughter JC et.al, 2013[17]	Early prediction of cerebral palsy after neonatal intensive	Retrospective study	NICU Follow-up Clinic at Vanderbilt University (USA) with	Neurologic exams, the Developmental Assessment of Young Children	Birth weights 1500 g and those with a diagnosis of hypoxic ischemic encephalopathy (HIE)	They concluded that Standardized assessments of motor milestones quantitatively predict the risk of

		care using motor development trajectories in infancy		birth weight b1500 g or a diagnosis of hypoxic ischemic encephalopathy	(DAYC), the Bayley Scales of Infant Development (BSID) and the Gross Motor Function Classification Scale.		CP in former NICU patients by 12 months before complex neurodevelopment tests and specialized neurologic exams can be performed.
8	**Grazyna H,Cyrylo wski L et.al, 2010**[18]	The role of magnetic resonance imaging in early prediction of cerebral palsy.	Prospective study	Neonates with symptoms of perinatal asphyxia in Poland	Magnetic Resonance Imaging (MRI)findings	Term neonate -hypoxic - ischemia and Preterm neonates with and perinatal asphyxia, intrauterine hypoxia, fetal distress and postnatal hypoxia	These results support the hypothesis that MRI performed in the neonatal period plays an essential role in predicting cerebral palsy in both term and preterm neonates, regardless of their gestational age.
9	Charitou S, Asonitou K et.al, 2010[19]	Prediction of infant's motor development	Explorat ory study	46 infants were tested with Participants recruited from public and private nursery school, greece	Alberta Infant Motor Scale (AIMS) (Piper & Darrah, 1994	Motor development or early motor performance supine position and Prone position	They concluded , prediction of infant's motor development by using indicators from early motor performance is possible.
10	Himpens E, Oostra A, Franki l et.al, 2010[20]	Predictability of cerebral palsy in a high-risk NICU population.	Cohort study	1099 NICU-admitted high-risk infants, Centre for Developmental Disorders Ghent'.Belgium	Ultra sonography	Perinatal asphyxia, mechanical ventilation >7 days, white matter disease except for transient echo densities <7 days, intraventricular haemorrhage grades III and IV, cerebral infarction and deep grey Matter Perinatal characteristics	The presented model based on Perinatal characteristics and neonatal US-detected brain injuries is a useful tool in identifying specific infants at risk for developing CP.

DISCUSSION

In this article, narrative review of the current research on Predictive models available for predicting the development in high risk neonates, evaluating their relative strengths and weaknesses in Predictor and outcome selection, and considering how Predictive model development and validation can be improved in the future has been done. Development of the central nervous system (CNS) is a dynamic process that occurs as a cascade of events, with each step dependent on the completion of the previous step. The most rapid periods of CNS maturity in humans occur in utero, during infancy, and at puberty [21] in the study it is found that most of studies have used similar physiologic variables and standard regression techniques to develop models that primarily predict the risk of poor neurodevelopment outcomes.

Antenatal risk factors
Maternal age, gestational age, parity, delivery mode, diabetes mellitus/gestational diabetes mellitus, hypertensive disorders of pregnancy, clinical chorioamnionitis, premature rupture of membranes, antenatal corticosteroid treatment, plurality of pregnancy (singleton or twin), chorionicity (monochromic or dichorionic), infant's sex and Birth weight these risk factors [11] parental factors, i.e., maternal educational level , pre-existing maternal obesity, and maternal smoking during pregnancy [13], developmental delay is considerably high in these antenatal risk factors identified in this study.

Perinatal risk factors
Most of the studies have included Preterm and Low Birth weight Neonates in their study [5,6,8,9,11]. While van Dokkum NH et.al, included both full-term and preterm children in their study [13] Gestational Age and Birth weight, sex , being born small-for-gestational-age (SGA), multiple birth , and Apgar score , Very low birth weight , Preterm infants [14] Birth weights 1500 g and hypoxic ischemic encephalopathy (HIE) [17] Term neonate -hypoxic-ischemia and Preterm neonates with and Perinatal asphyxia, intrauterine hypoxia, fetal distress and postnatal hypoxia[18] mechanical ventilation >7 days, white matter disease except for transient echo densities <7 days, intraventricular hemorrhage grades III and IV, cerebral infarction and deep grey Matter Perinatal characteristics [20] are found high risk factors for developmental delay While other factors like Type of CP, degree of motor disability, cerebral Ultrasonography results, epilepsy, gestational age and degree of cognitive impairment.[16] Social risk and environmental impact [15] growth, i.e., weight for height z score at 1 and 2 years and developmental milestones, i.e., smiling , speaking 2–3 word sentences , head-lifting, standing, and walking[7]are other factors identified in different studies

Outcome measure
2 of studies used Imaging techniques [18,20] for prediction while 2 studies concluded that motor Performance includes milestone only can predict cerebral palsy [17,19] while other studies have used different developmental scale as Griffiths Mental Developmental Scales (GMDS-R),Dutch version of Ages and Stages questionnaire, Neurologic exams, the Developmental Assessment of Young Children (DAYC), the Bayley Scales of Infant Development (BSID) Gross Motor Function Classification Scale. Alberta Infant Motor Scale (AIMS) (Piper & Darrah, 1994, Development (BSDI III) and neuro-functional evaluation according to the International Classification of Disability and Health (ICF-CY).In few of the study outcome measure taken at age between 1 to 4 years [11,13,16,17] but from these few outcome measure can predict developmental delay at later age while few outcome measure are complicated and expensive In this review most of studies are longitudinal cohort studies from that 4 studies are prospective cohort study while 2 are retrospective cohort study in our review and only one review literature that is on preterm only while other 2 are exploratory study .This review provides a comprehensive review and critique of risk prediction models developed for neonates, specifically predicting functional outcomes instead of mortality, to reveal areas of improvement for future studies aiming to develop risk prediction tools. Infancy and early childhood are periods of maximal neural plasticity during which therapeutic interventions have the greatest potential for long-term effectiveness [22,26]. As early diagnosis of motor development will be done for high-risk infants, we can start early intervention. (Early intervention means providing effective early support to children who are at risk of poor

outcomes.) As it is seen that less number of studies are focusing on finding various risk factors and using them for prediction of developmental delay it seems necessary that exploration of different risk factors, their role and weightage in prediction of developmental delay in high risk neonates can be more useful.

CONCLUSION

In current review it is evident that majority of available articles of predictive tool include only few of the risk factors and more studies have focused mainly on highly specialized tools, from imaging to complex neurological assessments, while few studies examine the value of more basic developmental milestone trajectories. It is important to identify responsible risk factor for delay in the development and use them in predictive formula, so that child at risk of delay can be provided with early intervention. Predictive Model can be helpful for Pediatric therapists working with Neonatal and CP patients to set appropriate goals regarding motor development and can guide their family to plan for future realistically.

Further Implication
Implications and challenges of early diagnosis of developmental delay by developing simple tools for early identification and screening in high-risk infants is a priority.

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33 articles found through Google scholar, Pubmed,EBSCO, Medline, science direct,CINAHL

2 articles found through manual search

31 articles after duplicates removed

31 articles screened

21 articles excluded

1. Articles published in languages other than English (n=2)
2. Case reports(n=1)
3. Models were also excluded if they limited their prediction to the outcome of survival (n=2)
4. articles found before2010 (n=13)
5. Only abstract(n=3)

10 articles included in the narrative review

Fig.1: PRISMA flow diagram explaining the result of search

REVIEW ARTICLE

Stress and Stress Management: A Review

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ABSTRACT

Stress is a major individual and public health issue that are linked to a variety of physical and mental health issues. Stress-related disorders are thought to account for 75 % to 90 % of primary care physician visits. Some of the health issues linked to stress include cardiovascular disease, obesity, diabetes, depression, anxiety, immune system suppression, headaches, back and neck pain, and sleep issues. Stress management is a commonly used phrase with a simple definition. Many studies testing its effectiveness exist in the literature, however it is unclear how many different types of Stress management exist or how helpful they are for which particular problem.

Keywords: stress, crises, eustress, distress, exercise, yoga, massage.

INTRODUCTION

Stress is defined as a person's physical, mental, and emotional reaction to a certain stimuli, often known as a "stressor." Stress is our bodies' way of responding to any type of demand.[1] An agent or stimulus that creates stress is referred to as a stressor. Noises, disagreeable people, a speeding car, a job, finances, and family difficulties are some of the stressors. Any situation might cause stress. The feeling is first affected by stress, which leads to psychological disorders. Anxiety, distracting anxiety, excessive worry, changes in sleep patterns, impatience, anger, sadness, intolerance, thoughts of harming oneself or others, palpitation, stress headache, and internal pressure are all early

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signs of stress. Headaches, severe fatigue, nausea and vomiting, diarrhoea, tachycardia, chest discomfort, elevated blood pressure, flushing or disorientation, shortness of breath, restlessness, choking sensation, or hyperventilation are some of the other symptoms [2]. Eustress, distress, acute stress, and chronic stress are some of the several types of stress. Our bodies respond to stress by going into fight or flight mode [3].

The fight/flight reaction is divided into two stages:
1. Short term fight/flight responses
2. Long term fight/flight responses
Stress hormones such as adrenaline and cortisol are released during this reaction.[4]When this occurs, our body prepares for action by increasing heart rate, decreasing digestion, shunting blood flow to main muscle groups, and altering numerous other autonomic nerve activities, providing a burst of energy and strength to the body. As a result, stress management is critical. Stress management encompasses a wide range of approaches and psychotherapies aimed at controlling a person's stress levels, particularly chronic stress, with the goal of controlling and reducing the tension that arises in stressful situations while also affecting emotions and bodily changes.[5]
STRESSOR
The term "stressor" refers to situations that are thought to be stressful. There are four basic sources of stress that we can encounter.[6]

THE CAUSE OF STRESS
- The Environment - The environment can present you with a barrage of competing demands for you to adjust. Weather, noise, crowding, pollution, traffic, dangerous and poor housing, and crime are all examples of environmental stressors [7].
- Social Stressors - The pressures of the various social positions we fill, such as parent, spouse, caregiver, and employee, might cause us to feel several stressors. Deadlines, financial troubles, job interviews, presentations, conflicts, demands for your time and attention, loss of a loved one, divorce, and co-parenting are all instances of social stressors [8].
- Physiological - Physiological stresses are situations and conditions that have an impact on our bodies. Adolescent rapid development, menopause, disease, ageing, giving birth, accidents, lack of exercise, poor nutrition, and sleep disorders are all examples of physiological stressors [9].
- Thoughts – Our brain interprets and sees situations as stressful, challenging, painful, or enjoyable. Some life situations are stressful, but it is our perception of them that determines whether or not they are a problem for us.[10]
- The many sorts of stressors are often classified into four groups by psychologists [11].
- Crises/catastrophes, major life events, daily hassles/micro stressors, and ambient stressors are the four types of stressors.

Crises/catastrophes
This form of stressor is unforeseeable and unpredictable, and as a result, it is completely beyond the individual's control. Devastating natural disasters, such as massive floods or earthquakes, as well as wars, are examples of crises and catastrophes. Despite its rarity, this type of stressor can generate a lot of anxiety in a person's life [12].

Major life events
Marriage, college, the death of a loved one, the birth of a child, and other big life events are examples of major life events. These occurrences might be both positive and unpleasant. Substantial life events, according to research, are relatively rare major sources of stress since they occur seldom [13].

Minor annoyances/micro-stressors [14]
This category comprises little annoyances and inconveniences that occur on a daily basis. Making decisions, meeting deadlines at work or school, traffic congestion, interactions with irritable personalities, and so on are all examples. Conflicts with other individuals are frequently a source of this type of stressor. Daily stresses, on the other hand,

differ from person to person, as not everyone regards the same occurrence as stressful. For example, most people find public speaking stressful; but, a seasoned politician is unlikely to do so. This is not to say that daily annoyances are unimportant or harmless-the accumulation of minor annoyances has been related to a number of psychiatric illnesses.

There are three sorts of psychological conflicts that can create stress:
- An approach-approach conflict occurs when a person must choose between two equally appealing options, such as going to a movie or a concert.
- The avoidance-avoidance conflict, in which a person is forced to choose between two equally unpalatable options, such as taking out a second loan with un favourable terms to pay off the mortgage or facing foreclosure on one's home.
- The battle between approaching and avoiding.

When a person is faced with the decision of whether or not to participate in something that has both attractive and unattractive characteristics, such as whether or not to attend an expensive college, this occurs (meaning taking out loans now, but also meaning a quality education and employment after graduation).
Delayed time, surprises (unexpected events such as lost or delayed baggage), and routine breakers are the three main sources of travel-related stress (inability to maintain daily habits).

Ambient stressors
As the name implies, they are low-grade stressors that are a part of the background environment rather than specific stressors. They are defined as stressors that are "chronic, adversely valued, non-urgent, physically noticeable, and intractable to people' efforts to change them." Pollution, noise, crowding, and traffic are all examples of environmental stresses. Unlike the other three types of stressors, ambient stressors can (but do not have to) have a detrimental impact on stress without the person being aware of it. As a result, they have little "perceptual salience," as Stokols defined it.[15]

TYPES OF STRESS
Stress is four types,
1.Eustress
2.Distress
3.Acute stress
4.Chronic stress

Eustress:[16]
Eustress is the "good" or "positive" stress that occurs in pleasurable settings. It may come as a surprise, but certain stressors can have a positive impact. Remember how you felt earlier when you were considering the possibility of being awarded that wonderful new job? That is only one instance of eustress. Other examples are the thrill of purchasing a new home, getting married, or the sensation you receive after a good workout. Eustress can help you gain confidence, learn new abilities, and be more motivated. Understanding, positive coping techniques can help you address "distress" more effectively, even though this type of stress is often overlooked when thinking about stress management.

Distress:[17]

Distress is the negative or "bad" form of stress that arises when you consider stress to be hazardous, unusual, unjust, or painful. Job loss, the death of a loved one, long-term illness, catastrophic injury, divorce, and depression are all examples.

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Eustress vs. Distress: What's the Difference?[18]
Eustress is derived from the Greek root "eu," which means "excellent" in the sense of euphoria. When a person considers a stressor as pleasant, it is referred to as eustress. Distress is derived from the Latin root "dis," which also refers to disharmony or disagreement. Distress has a negative impact on one's quality of life. It occurs when a demand much exceeds a person's abilities.

Acute Stress: [19]
Acute stress is a type of stress that lasts for a short period of time. It could be either eustress or distress. A presentation in front of a group of people is an example of acute stress. Stress causes you to sweat, your heart to accelerate, and your breathing to speed. These feelings only endure a short time, and the body swiftly recovers. The most common type of stress is acute stress. It stems from previous demands and tensions, as well as predicted needs and pressures in the near future. In small doses, acute stress is thrilling and exciting, but too much is draining. Early in the day, for example, a quick run down a steep ski slope is exhilarating. Late in the day, the same ski run is hard and exhausting. Skiing beyond your capabilities might result in falls and shattered bones. Overdoing short-term stress, on the other hand, can cause psychological distress, tension headaches, upset stomach, and other symptoms.

Chronic stress is a type of stress that lasts for a long time. This sort of stress can be caused by major events, but it can also arise when minor stressors pile and you are unable to recover from them. Long-term disease, company downsizing or reorganisation, and long-term marital problems are all examples of chronic stress. Chronic stress is the most hazardous to your health, as it increases your risk of burnout and other bad consequences [20]. Acute stress might be exhilarating and fascinating, but persistent stress is not. This is the kind of nagging tension that wears individuals down day after day, year after year. Chronic stress wreaks havoc on our bodies, minds, and lives. It causes devastation by attrition over time. It's the stress that never-ending "troubles" have brought to the people of Northern Ireland, the tensions in the Middle East have brought to Arabs and Jews, and the never-ending ethnic rivalries have brought to the people of Eastern Europe and the former Soviet Union. Chronic stress occurs when a person cannot see a way out of a difficult situation. It's the strain of never-ending demands and pressures that lasts for what seems like an eternity. When a person loses hope, he or she stops looking for answers.

Some chronic pressures are the result of traumatic early childhood experiences that become internalised and stay painful and present for the rest of one's life. Some events have a significant impact on one's personality. A worldview, or belief system, is formed that leads the individual to experience everlasting stress. Recovery necessitates active self-examination, frequently with professional assistance, when personality or deep-seated convictions and beliefs must be reformulated. The worst part about persistent stress is that people become accustomed to it. They are oblivious to the fact that it is present. Acute stress is seen right away because it is new; chronic stress, on the other hand, is ignored because it is old, familiar, and sometimes almost comforting.[21]

THE EFFECT OF STRESS
Your body produces stress hormones when you are stressed. Infection and disease may be exacerbated by stress and the accumulation of stress hormones over time.[22]

Effect of stress on body
Heart disease
High blood pressure, irregular heartbeats, high cholesterol, heart attacks, heart failure, and coronary artery disease are all possible outcomes. Researchers have long thought that people with a stressed-out attitude had a higher risk of heart disease and high blood pressure. We're not sure why, exactly. Stress induces the release of cholesterol and triglycerides into the bloodstream, which increases heart rate and blood flow. It's also probable that stress is linked to other issues, such as an increased possibility of smoking or obesity, which raises cardiac risks indirectly. Sudden emotional stress, according to doctors, can be a cause for significant cardiac problems, including heart attacks. Chronically ill people with cardiac problems must avoid acute stress and learn to cope with life's inevitable stressors [23].

Asthma

It can aggravate asthma and COPD symptoms (chronic obstructive pulmonary disease).[24]Stress has been demonstrated in numerous research to aggravate asthma. According to some data, a parent's chronic stress may raise the probability of their children having asthma. In one study, researchers looked at how parental stress influenced the asthma rates of young children who were exposed to pollution or whose mothers smoked during pregnancy. Children whose parents were stressed had a significantly increased risk of having asthma [25].

Obesity

For some people, it might lead to overeating and inactivity. If this continues for an extended length of time, these habits may become difficult to break, resulting in weight gain and obesity. Excess belly fat appears to be more dangerous to one's health than fat on the legs or hips, and regrettably, that's where people who are under a lot of stress tend to keep it. "Higher amounts of the hormone cortisol are caused by stress," explains Winner, "and this appears to increase the amount of fat accumulated in the abdomen"[26].

Diabetes

Diabetes can be aggravated by stress in two ways. For starters, it increases the likelihood of harmful habits like unhealthy eating and binge drinking. Second, stress appears to directly boost glucose levels in persons with type 2 diabetes [27].

Headaches

One of the most prevalent causes of headaches—not just tension headaches, but migraines as well—is stress.[28]

Anxiety and depression

It can cause anxiety, personality changes, sadness, irritability, insomnia, and memory loss, among other mental and emotional issues. Chronic stress is linked to higher incidence of depression and anxiety, which comes as no surprise. According to a review of recent studies, those who experience stress at work, such as rigorous labour with few incentives, have an 80 percent higher chance of getting depression within a few years than people who experience less stress [29].

Gastrointestinal problem

It can cause nausea, diarrhoea, and other digestive issues. (peptic ulcers or irritable bowel syndrome) One thing stress does not do: it does not induce ulcers. However, it has the potential to aggravate them. Many other gastrointestinal diseases, such as chronic heartburn (GERD) and IBS, are linked to stress, according to research.[30]

Alzheimer's disease

According to one animal study, stress may exacerbate Alzheimer's disease by hastening the formation of brain lesions. According to some researchers, lowering stress may help to decrease the growth of the disease [31].

Accelerated ageing

Stress has been shown to have an impact on how you age. One study contrasted the DNA of mothers who were under a lot of stress—caring for a chronically unwell child—and mothers who weren't [32].

Reproductive organs

Menstrual irregularities, diminished fertility, and erectile dysfunction are all possible side effects. Stress hormones can make it more difficult to conceive since they impact oestrogen and progesterone levels as well as menstrual cycles. Dealing with infertility may be extremely stressful, ranging from the sadness of negative pregnancy tests to the physical and mental toll of undergoing therapies in the hopes of becoming pregnant. The loss of a baby during pregnancy, known in the medical field as a spontaneous abortion, is a pregnant woman's worst dread [33].

PTSD and pregnancy

PSTD, or post-traumatic stress disorder, is an anxiety disorder that arises after a psychologically distressing experience. Flashbacks, insomnia, and difficulty functioning in daily life are all symptoms of PSTD. They may also have additional issues, such as drug usage, that make it difficult to sustain a safe pregnancy. PTSD can be successfully treated with a variety of therapies ranging from medication to talk therapy for mothers who are experiencing other difficulties in their everyday lives. They may also have additional issues, such as drug usage, that make it difficult to sustain a safe pregnancy.[34,35]

SIGNS AND SYMPTOMS

Cognitive symptoms [36]
- Memory problems
- Inability or difficulty concentrating
- Poor judgment
- Seeing only the negative
- Anxious racing or ruminating thoughts
- Constant worrying

Emotional symptoms [37]
- Moodiness
- Irritability or short tempered
- Agitation, inability to relax
- Feelings overwhelmed
- Sense of loneliness or isolation
- Depression or general unhappiness

Physical symptoms[38]
- Aches and pains, muscle tension
- Diarrhea or constipation
- Nausea, dizziness or butterflies in the stomach
- Chest pain or rapid heart beat
- Loss of sex drive
- Frequent colds
- Shallow breathing and sweating

Behavioral symptoms[39]
- Eating more or less
- Sleeping too much or too little
- Isolating yourself from others
- Procrastinating or neglecting responsibilities
- Using alcohol, cigarettes, or drugs to relax
- Nervous habits (nail biting, pacing).

STRESS MANAGEMENT

Stress is a natural reaction to the demands of our ever-changing world. Although we are constantly confronted with change and demands, how we interpret these internal and external changes has a direct impact on how much stress we experience.[40,41,42]

METHOD FOR IDENTIFYING THE CAUSES OF STRESS

```
┌─────────────────────────────────────────┐
│ Identify the source of stress in your    │
│ life.                                     │
└─────────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────────┐
│ List and priority the source of stress.  │
└─────────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────────┐
│ Identify appropriate stress management    │
│ techniques.                               │
└─────────────────────────────────────────┘
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┌─────────────────────────────────────────┐
│ Create your stress management plan.       │
└─────────────────────────────────────────┘
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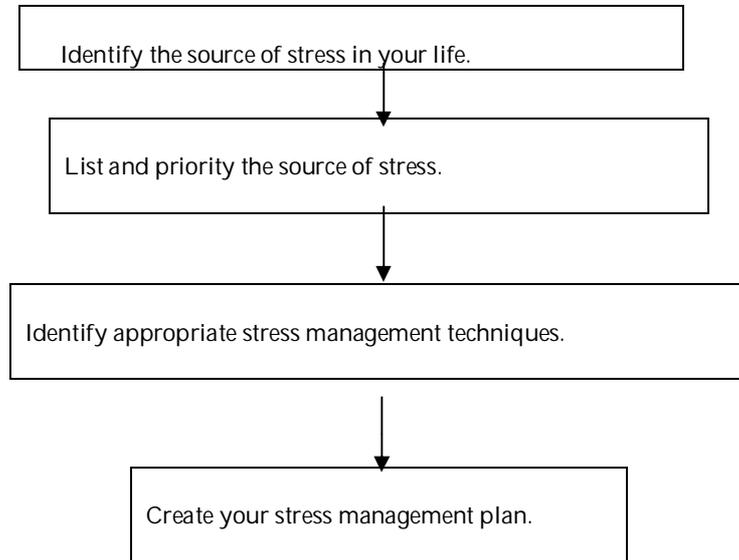
STRESS MANAGEMENT STRATEGIES[43,44]

Learn how to say "no" - Know what your boundaries are and don't cross them. It is not a good idea to take on more than you can handle. It's fine if you don't participate in every activity organised by your club, fraternity, sorority, or friends.

Attitude - It is natural for people to want to freak out. Your mind is a powerful weapon; utilize it to your advantage. Thinking in terms of relationships can help you get a long way.

Laugh - Do something you enjoy, pick up a hobby, spend time with friends, and learn to maintain a healthy balance in your life. Express your dissatisfaction if you are upset. Keep them to yourself since keeping them to yourself will just add to your tension.

Avoid alcohol and smoking - this is only a temporary remedy. You'll feel agitated again once the chemicals have left your body, and you'll probably be worse off than you were before.

Healthy eating- Get the essential nutrition by eating a healthy diet. Every day, eat at least one hot, home-cooked meal.

Exercise - Physical activities can aid in the burning of calories as well as the release of tension. Tension can be relieved by exercise. Exercising for 30 minutes three times a week is recommended.

Take a few deep breaths to relax your mind and body. Visualize yourself succeeding. Set aside some "extended time" to do something that you enjoy. Focus your attention on the current moment by practicing "mindfulness."[45]

Sleep - In for your brain and body to function at their best, you'll need at least 7 hours of sleep. Avoid naps that last more than an hour.

Health relationship - Talk to your pals and hang out with them for a healthy relationship. Find someone with whom you can relate and with whom you can share your issues.

Time management- Get a planner, make a timetable, or even a to-do list to help you manage your time. Make a plan for how you'll spend your quarter. Make a timetable for each week after you've completed that. Then, for each day, make a timetable. Be as precise as possible. Make a schedule that includes class meetings, study time for a given subject, mealtimes, enjoyable activities, and sleep.[46]

Organization- Learn how to organize your notes, keep track of your assignments, and make a calendar of critical due dates and exam dates. Make a list of your daily priorities.

Budget - Make a monthly budget for your costs. Distribute your funds according to the bills that must be paid during the quarter (i.e. rent, tuition, groceries, personal items, house, bills, gasoline, etc.) Determine how much money you will be able to spend on "fun" activities.

Spirituality - Spirituality is defined as the ability to connect with others and the ability to find purpose in one's life.

Find out whether you're a visual, auditory, or kinesthetic learner by determining your learning type.

Slow down- take a deep breath, and be aware of your limitations. Take your time so that you can do the task correctly.

Locate a support system - Whether it's your mother, sister, brother, friend, or counselor, find someone with whom you feel comfortable sharing your emotions. Sometimes all we need is to let our frustrations out.

Make changes to your environment - If you're having trouble studying at your dorm, try transferring to a location with less noise and brighter lighting.

Delegate obligations - When school or job becomes too much for you, splitting up the work or tasks can help relieve tension and strain.

RELAXATION TECHNIQUE
The body's natural relaxation response is an effective stress reliever. Deep breathing, visualization, gradual muscle relaxation, meditation, and yoga are among the relaxation strategies that can help you trigger this relaxation response. When you do these things regular basis, you'll notice a decrease in your daily stress levels and an increase in your sentiments of joy and peace. Furthermore, they have a protective quality in that they teach you how to remain calm and composed in the face of life's unexpected twists and turns.

The relaxation response, in addition to its calming physical benefits, has been shown to raise energy and focus, combat disease, relieve aches and pains, improve problem-solving abilities, and promote motivation and productivity. Best of all, anyone may reap these benefits with a little practice.[47,48,49]

Starting a relaxation practice
You can achieve a relaxation response using a variety of relaxing techniques. Deep breathing, gradual muscle relaxation, meditation, visualization, yoga, and tai chi are some of the stress-relieving techniques that have been extensively researched. It's not difficult to pick up the fundamentals of these relaxation techniques. To properly harness their stress-relieving effect, though, consistent practice is required. The majority of stress experts advise devoting at least 10 to 20 minutes every day to your relaxation routine. If you want to relieve even more stress, set aside 30 minutes to an hour.

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Make the most of your relaxing technique. Make time on your regular agenda for this. Incorporating a relaxation technique into your daily routine is the greatest approach to getting started and keeping it going. Schedule your practice time once or twice a day. You might find that doing your practice first thing in the morning, before other tasks and responsibilities get in the way, makes it simpler to remain with it [50]. When you're tired, don't practice. These strategies can make you feel so relaxed that you fall asleep quickly, especially if it's close to bedtime. If you practice these techniques when you're fully awake and attentive, you'll get the most out of them.

Deep breathing for stress relief
Deep breathing is a simple yet effective relaxation method that focuses on large, cleansing breaths. It's simple to learn, can be done practically anywhere, and is an effective approach to reduce tension quickly. Deep breathing is also an important part of many other relaxation exercises, and it can be paired with other relaxing factors like aromatherapy and music. All you need are a few minutes and a comfortable place to stretch.[51,52].

Progressive muscle relaxation for stress relief
Another effective and widely used stress-relieving strategy is progressive muscle relaxation. It entails a two-step procedure in which you systematically tense and relax various muscle groups throughout your body.
Progressive muscle relaxation gives you a deep understanding of how tension—as well as complete relaxation—feels in different parts of the body with regular practice. This awareness helps you spot and counteract the first signs of the muscular tension that accompanies stress. Your thoughts will relax as your body does. Deep breathing can be combined with increasing muscular relaxation to provide extra stress reduction.[53].

Most progressive muscle relaxation practitioners start at the feet and work their way up to the face. Also:

1. Loosen your clothing, take off your shoes, and get comfortable.
2. Take a few minutes to relax, breathing in and out in slow, deep breaths.
3. When you're relaxed and ready to start, shift your attention to your right foot. Take a moment to focus on the way it feels.
4. Slowly tense the muscles in your right foot, squeezing as tightly as you can. Hold for a count of 10.
5. Relax your right foot. Focus on the tension flowing away and the way your foot feels as it becomes limp and loose.
6. Stay in this relaxed state for a moment, breathing deeply and slowly.
7. When you're ready, shift your attention to your left foot. Follow the same sequence of muscle tension and release.
8. Move slowly up through your body — legs, abdomen, back, neck, face — contracting and relaxing the muscle groups as you go.

Mindfulness meditation for stress relief
Mindfulness meditation is very useful in reducing stress, anxiety, despair, and other negative emotions. Mindfulness is the state of being completely immersed in the present moment, without analyzing or otherwise "over-thinking" it. Rather than worrying about the future or dwelling on the past, mindfulness meditation switches the focus to what's happening right now.[54,55]
For Stress Relief, Try The Following Mindfulness Meditation Techniques.[56,57]

Body scan - By focusing your attention on different parts of your body, you can create awareness. You begin with your feet and work your way up, similar to progressive muscle relaxation. Instead of tensing and releasing your muscles, you simply concentrate on how each area of your body feels without categorizing them as "good" or "bad."

Walking meditation - You don't have to be seated or completely still to meditate. Mindfulness in walking meditation is focusing on the physicality of each step, such as the sensation of your feet contacting the ground, the rhythm of your breath as you move, and the feel of the wind against your face.

Mindful eating – Try eating consciously if you find yourself reaching for food when you're stressed or gulping down your meals in a hurry. Take a seat at the table and give the food your whole attention (no TV, newspapers, or eating on the run). Slowly eat, allowing yourself to completely appreciate and concentrate on each bite. Zen meditation is not the same as zoning out. When your mind wanders or you begin to drift off, it requires work to retain your concentration and bring it back to the current moment. Mindfulness meditation, on the other hand, affects the brain over time, increasing the areas connected with joy and relaxation while weakening the areas associated with negativity and stress.

Guided imagery for stress relief
Visualization, often known as guided imagery, is a type of meditation that can help you relax. Guided imagery is a relaxing technique that includes envisioning a setting in which you feel at ease, free of all tension and anxiety. Choose a relaxing location, whether it's a tropical beach, a favorite childhood haunt, or a peaceful forested glen. This visualization exercise can be done on your own, with the guidance of a therapist, or with the help of an audio recording [58].

- Close your eyes and let your worries drift away. Imagine your restful place. Picture it as vividly as you can—everything you can see, hear, smell, and feel. Guided imagery works best if you incorporate as many sensory details as possible. For example, if you are thinking about a dock on a quiet lake:
- See the sun setting over the water
- Hear the birds singing
- Smell the pine trees
- Feel the cool water on your bare feet
- Taste the fresh, clean air

Yoga for stress relief
Yoga is a fantastic way to relieve tension. It entails a combination of dynamic and motionless poses, as well as deep breathing. Yoga's physical and mental advantages act as a natural stress reliever and help to develop the relaxation response in your daily life.[59]

Tai chi for stress relief
T'aichi – (tie chee), also known as "moving meditation," is a Chinese form of exercise and energy training that consists of a series of coordinated, slow motions combined with mental concentration and coordinated breathing.[60] You've probably seen tai chi if you've ever seen a group of people slowly moving in unison in the park slowly moving in unison. Tai chi is a gradual, flowing set of body motions that can be done at your leisure. Concentration, relaxation, and the conscious circulation of vital energy throughout the body are all emphasized in these movements. Though tai chi has its origins in martial arts, it is now mostly used to relax the mind, condition the body, and reduce stress. Tai chi practitioners, like meditation practitioners, concentrate on their breathing and staying in the present moment. Tai chi is a low-impact exercise that is suitable for people of all ages and fitness levels, including the elderly and those recovering from injuries. You can practice the techniques anywhere, at any time, by yourself or with others once you've learned them.[61]

Massage therapy for stress relief
Getting a massage promotes deep relaxation, and as your body's muscles relax, so does your overworked mind. You don't even have to go to a spa to reap the benefits of massage. You may relax and relieve stress by using a variety of basic self-massage techniques.[62,63]

Self-Massage Techniques
Soothing Scalp Spread your fingers on top of your head and place your thumbs behind your ears. For 15-20 seconds, gently move your scalp back and forth by forming circles with your fingertips. Simple to Look at Place your ring fingers directly under your brows, at the bridge of your nose, while closing your eyes. Slowly increase the pressure

for 5-10 seconds before releasing it softly. Repeat 2-3 times more. Relief from Sinus Pressure Places your index and middle fingers on the bridge of your nose. Slide your fingertips down the bridge of your nose and across the tops of your cheekbones to the outside corners of your eyes. Tension Relief in the Shoulders Reaches across the front of your body to the opposite shoulder with one arm. Rep on the opposite side. Press forcefully on the muscle above your shoulder blade in a circular motion. Rep on the opposite side.[64]

Swedish massage is the most frequent style of massage, a relaxing technique that is specifically meant to relax and rejuvenate. Shiatsu, often known as acupressure, is another popular style of massage. Shiatsu massage therapists manipulate the body's pressure points with their fingers. Although self-massage can help relieve tension, getting a massage from a professional massage therapist can be far more calming and thorough than self-massage. When planning a massage, choose one that promotes general relaxation, such as Swedish or Shiatsu. Sports massages and deep tissue massages are more vigorous. They frequently target specific regions and may leave you hurting for a few days, making them less helpful for stress release and relaxation.[65,66]

CONCLUSION

For many people, exercise can be a useful part of a psychological intervention, and it should be suggested to those who are suffering with acute, acute episodic or chronic stress. The well-documented physical and psychological health advantages of exercise are an advantage of adding exercise into a crisis management plan over other stress management strategies. However, it's important to remember that exercise is only one part of a stress-management strategy, and there may be times when you need help from someone who isn't fitness professional, such as when working with people who are dealing with acute episodic or chronic stress. Although exercise may assist a person who is struggling with these sorts of stress feel calmer, it will not alleviate the problem of large chronic or recurring stresses. It may be important to refer these persons to resources such as a psychologist or other health care experts who may assist them in dealing with their pressures.

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Table 1: signs of unmanaged and managed stress

| Signs of Unmanaged Stress	Signs of Managed Stress
Physical: ➢ Fatigue ➢ insomnia Emotional, mental and behavioral: ➢ negative thinking ➢ mind racing ➢ worry ➢ lack of motivation ➢ anger ➢ over eating ➢ sedentary lifestyle	Physical: ➢ good energy level ➢ feeling rested Emotional, mental and behavioral: ➢ positive self-image ➢ setting realistic limits ➢ feeling calm or reassured ➢ sense of accomplishment ➢ feeling content or happy ➢ eating balanced meals ➢ being physically active
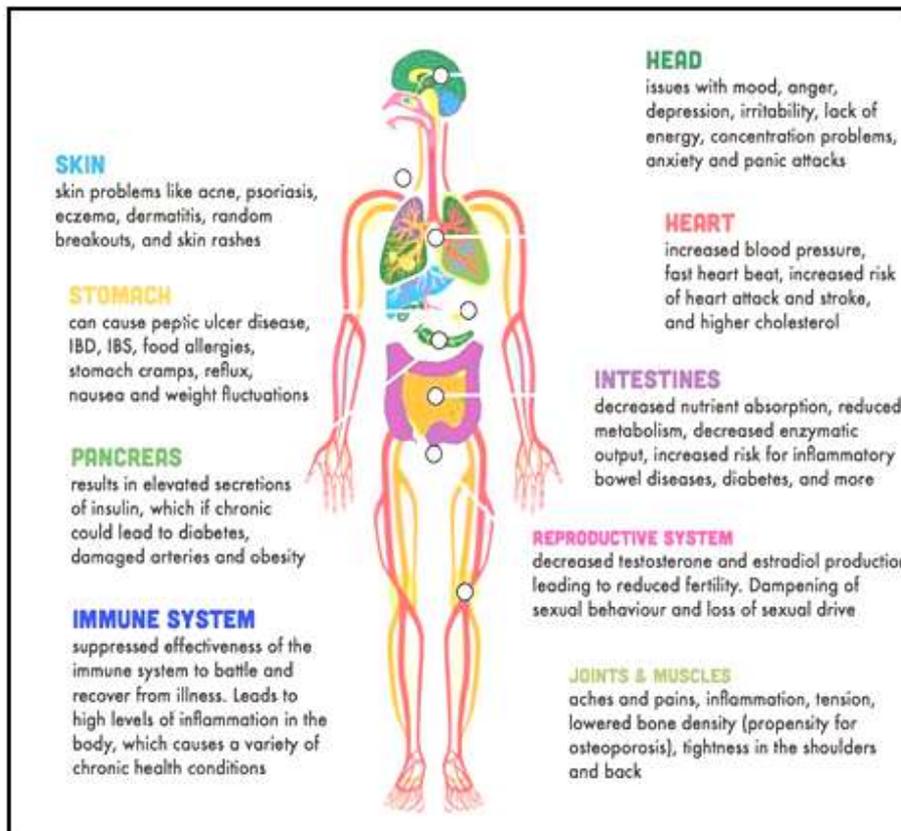
Figure 1: Effect of stress on human organs

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REVIEW ARTICLE

Therapeutic Potential of *Musa balbisiana* Colla as a Super Food –A Review

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ABSTRACT

Research and studies on potential health benefits of fruits and vegetables are in a demand stage to make their sustainable use in medicine. Fruits such as carambola, dates, wood apple have been earlier studied in literature for their therapeutic potential. Health benefits properties of fruits and bioactive compound are explored recently to understand its activity. Fruits contain bioactive compounds such as flavonoids, phenols and phytochemicals. They can help in radical scavenging and prevention of many diseases. Such beneficial properties of various underutilized fruits can be explored for its new possibilities and can bring novel addition in treating various diseases. Such beneficial fruit found widely in Assam has been discussed in this review paper. *Musa balbasiana* colla is widely grown in North East India. It is widely consumed and its various parts such as fruit, peel, flower, stem, roots are used as traditional medicine. This review elaborates the recent data in relation to nutritional and pharmacological benefits also understanding the medicinal value and folklore treatment associated with *Musa balbisiana*. We have discussed its pharmacological benefits namely antidiabetic, antiurolithic, antibacterial properties in this review. Many researchers has studied its application in pharmacological view and discussed the most beneficial characteristics which can be a good approach for determining its ultimate potential. *Musa balbisiana* colla effective role as a source of bioactive compounds has been addressed in earlier reports. Also in vitro and in vivo applications were carried out in recent reports to acknowledge its various health benefits.

Keywords: *Musa balbisiana* colla, bioactive compounds, therapeutic benefits, antioxidant, antidiabetic .

INTRODUCTION

Banana is one of the oldest cultivated plants classified as elongated, edible fruit –botanically a berry. Banana (*musa spp*) is a tropical fruit and fourth most important dietary staple food crop in the world [1]. The edible banana and plantains are derived from progenitor species *Musa acuminate* and *Musa balbisiana* .This genome originated in dried ecology and potential to survive in drought environment [2]. Assam shares a place as one of the major banana producing state in the country. *Musa balbisiana* colla (genome group BB) a monocotyledons herb commonly known as Bhimkol or Athiyakol is widely spread in the state of Assam of the North eastern part of India [3]. It belongs to the family Musaceae and suitably grows in tropical rain forest habitat [4]. The fruits of *Musa balbisiana* colla appear to be same as that of other found bananas with exception of having seeds within it [5]. Bhimkol classified as triploid (BBB) is cultivated in every household of the people of Assam due to its medicinal and therapeutic uses [6]. Described by [7], Bhimkol serves as a healthy and nutritional food for baby with determining it to be rich source of minerals, amino acids, energy, Vit C. Generally in Assam this particular variety is practiced as a local folklore treatment for various diseases such as dysentery, diarrhea [8].

Various studies has shown that parts of *Musa balbisiana* colla seeds ,leaves ,roots, inflorescence and juice, ash of banana leaves has incredible benefits such as having medicinal properties with a large possibilities of treating many illness. Dietary minerals are of equal importance and should be included in our daily intake which can enhance our immune system to fight against several disease referring to this context *Musa balbisiana* colla contains a good amount of iron, calcium, phosphorus, potassium[9]. Also kolakhar obtained from peel of Bhimkol preferably contained a higher amount of carbonate and potassium [10]. As described by [3] bhimkol each and every part is utilized either as food and/or for rituals religious affairs or social ceremony or occasions such as koldil which means inflorescence so people of Assam consider koldil as one of favourite vegetables because of less acidic and sharp taste whereas fruit is also used as a prasad, bhog during religious gathering. The nutritional status analyzed by [11] quantified the nutritional content in blossoms and ripened fruit which were calculated as in blossoms the protein content was 1.63(g/100g) , carbohydrate(g/100g)- 8.79, fat(g/100g) –0.50, β-carotene (g/100g)– 0.19, phosphorous(g/100g) –76, potassium(g/100g) – 176, calcium(g/100g) – 105, magnesium(g/100g) -56, iron(g/100g) – 3.5, zinc(g/100g) -1.4, and quantified protein, carbohydrate and fat (g/100g) in ripened fruits as 1.29,22.14,0.31 respectively.

Scientific classification

Kingdom	: Plantae
Division	: Angiospermae
Class	: Scitaminae
Order	: Zingiberales
Family	: Musaceae
Genus	: Musa
Species	: *M. balbisiana colla*

Potential Source of bioactive compounds

Musa balbisiana colla is a potent source of flavonoids, tannins, saponins, diterpenes, triterpenes, and phenol [12]. **Table 1** represents the functional action associated with bioactive compounds. According to [13] indicated the presence of phenolic antioxidant such as catechol, kaempferol, quercetin, rutin, chlorogenic acid. Apiforol a flavanol which is a natural bioactive was shown to be found in the seeds of *Musa balbisiana* colla [14]. Flavonoid ,phenols, steroid/ triterpenoid secondary metabolites were detected in the extract of *Musa balbisiana* flower, peel, pulp, seed with tannins widely detected only those in flower and peels also phytochemical screening detected the presence of saponin in peel, pulp, and seeds [15]. Another study carried out by [16] revealed the presence of compounds identified as steroids, fatty acids, long chain saturated and unsaturated hydrocarbons. Three triterpenes were extracted from the inflorescence of the plant which was confirmed by [17] as 31-norcyclolaudenone (1), cycloartenol (2) and (24R)-4a, 24-trimethyl-5a-cholesta-8,25(27)-dien3b-ol (3). Also another study revealed the presence of high

protein, carbohydrate, phenolic, and flavonoid content in the metabolic corm extract of the plant [18]. Work carried out by [19] on the liphophilic components from the ripe pulp of banana cultivators of *M.acuminata* and *M. balbisiana* species represented the pulps to be a good source of ω-3 and ω-6 fatty acids, α-tocopherol, long chain aliphatic alcohols which enhance human nutrition. Analysis carried out by [20] the extract of root, shoot, inflorescence were estimated for photochemical analysis thus having polyphenol and flavonoid quantified in three extract and root extract amounting higher for poyphenol than inflorescence and shoot. The presence of alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, carbohydrate , protein ,amino acids, vitamin C ,starch ,pectin and tryptophan was demonstrated by [21] in his qualitative chemical test. [22] also described the inflorescences extract were a source of flavonoids ,diterpenes ,phenol and demonstrated detection of alkaloid in flower extract.

Effect on human health
Antidiabetic
Musa balbisiana colla earlier studied by various researchers depicted the antidiabetic factor in the roots, seeds of this particular species. Detailed phytochemical analysis detected the occurrence of inhibitory factor α- amylase and α-glucosidase enzyme from the seeds of *Musa balbisiana* colla which has an anti hyperglycemic effect and a natural bioactive achieved from the seed extract persist to have a hypoglycemic activity thus its reduction ability in the postprandial hyperglycemia can be a potent antidiabetic agent studied by [23]. The mechanism of action includes inhibition of digestive enzymes such as α-amylase and α-glucosidase which help in inhibiting glucose absorption [24]. As analyzed by [25] the presence of α- glucosidase and α-amylase in the food can effectively control post prandial blood sugar level so consumption of such food can be a sustainable benefits in management of type II diabetes, such mechanism has been depicted in Fig 1. Moreover peel extract of MB intensively detected a high reduction % in blood glucose level in the rats with glucose level more than 200 mg/dl [26].

Blood glucose level can be lowered by inhibiting α-glucosidase enzymes [27]. Experimental study performed by in jamun seeds investigated that presence of flavonoid is responsible for antidiabetic property which they further confirmed with high alpha amylase inhibitory effect. Another investigations carried out by [28] determined the effect of *Musa balbisiana* colla flower extract on correction of diabetic and diabetic induced oxidative stress where the study revealed on orally administration of the extract at the dose of 10mg/100g body weight on STZ induced diabetic male rat significantly elevated the serum insulin level. Evaluation done with respect to *Musa balbsiana* flower and inflorescence stalk extract showed a significantly reduction in blood glucose level in STZ induced type 1 diabetic rat on oral administration of 250mg/kg for 2 weeks and also prevented the weight loss and increase in serum insulin level on those with extract treated groups [22]. Lipid abnormalities is commonly linked in diabetic condition in support to that [20] experimental observation in STZ induced rats treated with MB root extract depicted a appreciable reduction in TG, TC, LDL level thus their study presented MB root exerts a potential lead as antidiabetic and antilipidemic.

Antioxidant
Banana has a rich antioxidant property and thus having beneficial health factor by removing free radicals and subsequently preventing arise of disease such as diabetes, heart disease, and cancer etc, [28]. Oxidative stress usually occur when there is an increase shift in cellular free radicals, these free radicals cause tissue damage leading to numerous chronic disease such as diabetes mellitus, cardiovascular disease, hepatic damage [29]. Earlier reported *Musa balbisiana* colla inflorescences methanol extract indicated antioxidant property followed by DPPH and FRAP assays, where it was observed that the DPPH free radical scavenging activity proportionally elevated with the concentration of extract resulted in highest inhibition of (62.268± 1.43 %) which ascertain to be useful property in treating free radical related pharmacological problems [30]. Antioxidant test conducted by [24] from the peel of klutuk banana showed the ability of scavenging DPPH free radicals with using Vit C as the standard. The inflorescences extract of *Musa balbisiana* colla has chemotherapeutic potential cited by [16] subjected to having antioxidant activity, with cytotoxicity towards HT-29 cell lines and comprising of apoptosis inducing nature confirming the antioxidative and cytotoxic attributes.

Prevention of cardiac hypertrophy

Cardiac hypertrophy is associated with myocardial inflammation and oxidative stress thus to complement its effect on prevention an *In vitro* study in H9c2 cells was further enhanced and resulted in carrying antihypertrophic and anti-inflammatory in the aqueous extract of *Musa balbisiana* colla fruit pulp powder, thus concluding it to be an useful supplement for prevention of cardiac hypertrophy by inhibiting the associated inflammation and oxidative stress [13].

Antiurolithic

Urolithiasis commonly known as kidney stone disease is prevalent in India, therefore prevention and treatment is a necessary step to be taken. The nucleation and aggregation assay carried out by [31] in crude extract of *Musa balbisiana* colla fruit displayed an outstanding effect on Cao$_x$ crystal morphology such as change in crystal structure, size and number thus outcomes were possessing anti urolithic ability. A study established among the Swedish women determined that those who ate banana 4-6 times per week lowered the risk likely to develop kidney disease [32].Therefore banana help in proper functioning of the kidney in body.

Anti ulcer property and anti bacterial

Banana is one of the few fruit patient suffering from ulcer can safely consume. Experimental observation carried out using unripe fruit extract of MB displayed a positive potential against peptic ulcers and constituting ulcer protection ability as such of Omeprazole [21]. Thus further investigation can be a assurance of MB pertaining its antiulcer potential. Phytochemical test revealed the role of bioactive secondary metabolites as antibacterial by determining ethanol extract of klutuk banana fruits thus found to have antibacterial activity against *S. dysenteriae* ATCC 13313 [34]. Fig 2 depicts the various health benefits of *Musa balbisiana.*

Traditional Medicinal uses

Among the tribal people known as Sonowal kacharis of Assam has a strong belief on herbal medicine among which *Musa balbisiana* colla fruit decoction is consumed during dysentery on early morning for 3 days. Moreover during pin worm infection they have exudates of rhizomatous stem of banana [35]. Kolakhar prepared from ashes of Bhimkol stem, peels, rhizomes, has potential medicinal uses as such the liquid extract (filtrate) is used as antiseptic in cut and wounds, good for healing cough and also serves as antacid [36]. Findings acquired by [37] were that kolakhar obtained from MbC pseudo stem can be a tremendous salt substitute as the extract showed the presence of 46 % K and 26% Na thus making it an excellent replacement of table salt and lowering the certainty of Na associated health problems. [22] cited that stem extract of Bhimkol is believed to enhance male fertility among the Morans people of Tinsukia district, Assam. Recent review ascertain that banana ash extract contain high alkalinity so it can have potential role in inactivation effect on respiratory viruses/infection .Thus Bhimkol kolakhar can have future prospects of pharmacotherapy and diet supplement in the prevention of globally affected pandemic Covid -19 [38].

Post harvest Uses

Musa balbisisna colla fruits ash has been a promising products in the field of industry as reported the trunk ,peels , rhizome ,stem of *Musa balbisiana* colla was introduced as a novel catalyst in preparing high quality biodiesel and can be a source for large scale preparation due to vast availability and being a renewable catalyst [39 ; 40; 10; 41]. Study carried out by [42] concluded *Musa balbisiana* colla leaf extract on addition in making films can be a good ingredient for active packaging. *Musa balbisiana* colla an agricultural bio waste has an ability to act as potent antibiofilm and antibacterial therefore possessing applications as water disinfectant in food conservation and drug industry reported by [43].

Kolakhar the alkali extract of *Musa balbisiana* colla plant contain a generous amount of potassium and carbonate thus analyzed by [44] Kolakhar can be an alternative support for commercial production of potassium carbonate. Inferences led by [45] determined the high output of antioxidant activity and Vitamin C content in *Musa balbisiana* colla blossom (kolphul) thus their study revealed it can be key constituents in promoting antioxidant and Vit C enriched food formulations. As described by [46] Bhimkol plant has a potential as a substrate to produce pectinase, a

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widely used enzyme for industrial purpose therefore bhimkol plant which is mostly believed to be less productive after post harvest of fruit can be an efficient low cost and readily accessible raw product for the production of pectinase. Other reports contributed by [47] determined the waste *Musa balbisiana* colla pseudo stem biomass as a potential candidature for biochemical conversion via fermentation for bioethanol production due to appreciable hemicellulose content in it (58. 67%) also determining waste *Musa balbisiana* colla fibre acts as a natural filler so can be used as reinforcing agent in PVC polymer composites rendering it to have wide use in structural components like window, window profiles, partition wall. Studies conducted by [48] on developing extruded product using low amylose rice incorporated with Bhimkol flour identified bhimkol to be a potential food ingredient in third degree processing industry with outcomes having preferable texture, colour, pasting property therefore Bhimkol possess opportunities in food industry to enhance products nutritionally and healthier as addons.

CONCLUSION

Musa balbisiana colla not only being used as a source of food it encompasses numerous benefits within its leaves, seeds, roots, stem, peel, inflorescences therefore creating opportunities to be explored in area of product development, food packaging, bio waste, commercial sector, therapeutic potential etc. Also its plant extract and the presence of bioactive compound can be natural, safe, and effective application and enlightening its interest in pharmacological studies. This plant contains rich phytochemical and secondary metabolites thus will be a potential source for high value phytochemicals and its application in nutraceuticals, pharmacological industry. People belief and therapeutic use of traditional medicinal value of *Musa balbisiana* colla can be further check for their multiple efficiency. *Musa balbisiana* colla stands a place as an emerging food with multidisciplinary perspective. However more research has to be achieved to satisfy its candidature and explore further with respect to its recent identified potential.

ACKNOWLEDGEMENTS

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Abbreviation

Abbreviation	Full name
Musa spp	Musaceae species
LDL	Low density lipoprotein
MB	*Musa balbisiana*
STZ	Streptozotocin
TG	Triglycerides
TC	Total cholesterol
DPPH	1,1 – diphenyl-2-picrylhydrazyl
FRAP	Ferric reducing ability of plasma
Vit	Vitamin
CaO_x	Calcium oxalate
S. dysenteriae	*Shigella dysenteriae*
MbC	*Musa balbisiana* colla
K	Potassium
Na	Sodium

Covid -19	Corona virus disease 2019
PVC	Polyvinyl chloride

Table 1 Functional action associated with bioactive compounds

Bioactive compounds	Health benefits	Source
Chlorogenic acid	Reducing rates in acute and chronic hypertensive, cardio protective, cholesterol lowering	[49]
Quercetin	Anti carcinogenic, anti inflammatory, antiviral	[50]
Rutin	Anticancer, antidiabetic, organ protection	[51]
Kaempeferol	Antidiabetic,hepatoprotection, anti oxidant, anti-inflammatory	[52]
Tannins	Lowering blood glucose level thus help in treatment of diabetes, promotes preventing cardiovascular diseases	[53]
Saponins	Hyperlipidemia, hypertension	[54]
Diterpens	Cytotoxic, antitumor, anti-inflammatory	[55]
Catechin	LDL resistance to oxidation, antioxidant activity, fat oxidation	[56]
Cycloartenol	Antidiabetic	[57]
Alkaloids	Antihypertensive, mild anti diuretic, anti inflammatory	[58]

*the table depicts various bioactive compounds present in *musa balbisiana* colla and contemporary benefits in human health of each bioactive compound. sources [49,50,51,52,53,54,55,56,57,58].

Fig1: schematic antidiabetic mechanism of action. source [59]. the diagram illustrates the mechanism of action of the antidiabetic factor in human health	**Fig. 2: physiological advantages of *Musa balbisiana* on multiple organ systems. the graphical fig depicts the significance of different parts of *Musa balbisiana* colla and related health benefits. sources [30]; [14];[35]; [13]; [34]; [31];[37]; [24] ; [21]**

RESEARCH ARTICLE

Preparation and Characterization of Zinc oxide (ZnO) and Iron Oxide (FeO) Nanoparticles by Precipitation Method

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ABSTRACT

Deficient of micronutrients in Indian soil and reduced yield and poor quality grain production of crops are most common. The use efficiency of approved chemical fertilizers to alleviate the deficiency of micro nutrients is very less ranging from 5-10 per cent. Nano fertilizer has a greater scope to increase the nutrient efficiency and ensure quality grain and yield increase. Zinc oxide (ZnO) nanoparticles (NPs) and Iron oxide nanoparticles (NPs) has been synthesized by precipitation method using NaOH and $ZnSO_4$ and $FeSO_4$ as starting material. Synthesized nanoparticles (NPs) have been characterized by using XRD (X-ray diffraction), SEM (Scanning electron microscopy) and FT-IR (Fourier Transformation Infrared Spectroscopy). XRD studies of synthesized nano nutrients showed that zinc was formed as ZnO and iron formed as FeO, it has hexagonal structure crystallite sizes in nanometer range and diameter (D) was calculated by Debye- Scherre'S formula and the crystalline size found to be ZnO is 36 nm and FeO is 21 nm. The FT-IR band of ZnO nanoparticle at 788.89 cm^{-1} indiacted Zn-O stretching and FeO nanoparticle at 794.67 cm^{-1} indicated Fe-O stretching. The observation of some larger nanoparticles in ZnO and FeO SEM image is attributed to agglomeration. Zn and O, Fe and O are the main constituents of the sample and no trace of impurities could be found within the detection limit of EDX, percentage of Zn and Fe constitutes 64% and 68%.

Keywords: Micro nutrient Deficiency, ZnO and FeO nano particles, Bio fortification.

INTRODUCTION

Micronutrient such as Zn and Fe plays an important role in crop physiology, metabolic and enzyme activities in plants. Micronutrient determines the nutrient content and yield of the crop. The crop grows in micronutrient deficient soil produce lesser yield as well as poor nutritional quality of crops. Soil salinity is a global problem that affects approximately 20 per cent of the irrigated land and reduces crop yield significantly (Quadir *et al.*, 2014). High salinity and pH, low organic matter and poor CEC, leaching etc contribute to the deficiency of micro nutrient in soils. Zn deficiency in Indian soils is likely to increase from 49% to 63% by 2025 (Arunachalam *et al.,* 2013) and 12% deficient in Fe, Singh, (2001). Deficiency of Zn and Fe is a well-documented public health issue especially children and pregnant women, It leads to impairment of immune system, Physical growth retardation and reproductive health etc. (Stein *et al.*, 2007). The production and consumption of Zn and Fe fortified cereals such as rice could be most appropriate weapon to fight hidden hunger of micronutrients (Zhao and McGarth, 2009 ; Rehman *et al.*,2015).

The farmers usually apply micronutrients as $ZnSO_4$ and $FeSO_4$ to alleviate micro nutrient deficiency in soil. The efficiency of the nutrients in soil varies from 5-10%. Nutrient losses from conventional fertilizers are one of the challenges being faced in the agriculture. This necessitates for an alternate strategy to improve the Zn and Fe use efficiency. Under such circumstances nano technology proved to be an alternative technology. Nanotechnology has been described as the next great frontier of agricultural science and occupies a prominent position in transforming agriculture and food production through efficient management of soil nutrients. Nano fertilizer is the promising candidate which slowly released its nutrient content over a period of up to 60 days compared to only 30 days exhibited by conventional counterpart (Ullah et al., 2012). Nano nutrients are proved for increasing the efficiency in soil and also uptake by plant. Thus nano nutrients is an effective tool to produce fortified grains with improved quality which can solve the nutrients deficiency among human especially for children and women. In this paper to enhance the Zn and Fe nano nutrient to the soil and were synthesized by co-precipitation method and the obtained nano ZnO and FeO are characterized using XRD, SEM, EDX and FTIR techniques.

EXPERIMENTAL

MATERIALS

All chemicals viz., Zinc Sulphate ($ZnSO_4$), Iron Sulphate ($FeSO_4$), Sodium Hydroxide (NaOH), Deionized water was used throughout the study without further purification process.

Synthesis of Nano-zinc oxide and Fe oxide
In co-precipitation method, homogeneous solutions of inorganic salts viz., Zinc sulphate and Iron Sulphate) are used as a precursor. By reaction with an appropriate precipitating agent, these salts are precipitated as hydroxide when the concentration of ions is attained followed by nucleation and growth phase. The resulting hydroxides are calcinated to transform into oxides with a crystalline structure. pH, temperature and concentration of salt solution greatly affect the shape and particle size of nanoparticles (Reddy, 2011). This method is simple, low cost, flexible and effective in particle size control (Jadhav et al., 2009).

RESULTS AND DISCUSSION

Characterization of Micronutrients
X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy dispersive absorption X-ray analysis (EDAX), and Fourier Transform Infrared Spectroscopy FT-IR were used to identify the morphology, particle size, crystalline structure, basal spacing, functional groups, (Akbari et al., 2011; Prabhu et al., 2014) structural features and surface

area of the nanoparticles synthesized. The identification of the crystalline phase and size of the nano particles was measured by XRD. Morphological information has been obtained by SEM (Karkare, 2008) EDAX was used to confirm the various elements present in the sample. The morphological studies were performed by SEM and the elemental composition was analyzed by EDX. Fourier Transform Infrared Spectroscopy (FTIR) is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint" (Mudunkotuwa et al., 2014).

X-ray Diffraction Analysis
XRD patterns of synthesized ZnO nano particles revealed that all the diffraction peaks of ZnO nano particles matches with the standard ZnO nanoparticles data as shown in Fig 1 (Jian *et al.* 2006). The diffraction peak of XRD very well matched with the hexagonal structure by comparison with data from JCPDS card number 79-2205. The observed sharp and intense peaks in the XRD indicated that the sample are highly crystalline in nature, This finding corroborates the similar view of Tamus, (2003). The XRD patterns can be indexed for diffraction from the 100, 002, 101, 102, 110, 103, 200, 112 and 201 planes. From the XRD data, it was found that the peaks are broad suggesting the crystallite sizes in nanometer rang. The diameter (D) was calculated by Debye- Scherre formula and the crystalline size found to be 36 nm, (JICDD, 2003). These findings corroborates the earlier results (Zhou et al., 2007 and Khoshhesab et al., 2011).

Fourier Transmission Infrared Spectroscopy (FT-IR)
FT-IR pattern of the synthesized ZnO nanoparticle were studied and it ranges from 400-4000 cm^{-1}. The strongest adsorption of the zinc oxide nano particle was seen at 1720 cm^{-1} as 1720 corresponds to O-H and C-H stretching. The band at 1294 cm^{-1}corresponds to C-C stretching. The FT-IR band of ZnO nanoparticle at 788.89 cm^{-1} indicated Zn-O stretching. The Presence of FT-IR peaks for ZnO nano particles and other metal oxides are well supported by Mudunkotuwa et al. (2014). FTIR spectral studies give information regarding the chemical bonding shown in Fig 3. The band of FTIR spectra peaks observed in the range of 1676.14 between Zn and O. The spectrum showed a broad peak around 457 cm¡1 and shoulder around 545 cm^{-1}, which corresponds to ZnO nanoparticles reported by Kalpana Handore *et al.*(2014).

FT-IR pattern FeO nanoparticle were studied and it ranged from 400-4000 cm^{-1}. The strongest adsorption of the Iron oxide nano particle was seen at 1708 cm^{-1}. The IR spectra of nano iron oxide particle recorded peak at 1708, 1442, 1101, 794 cm^{-1} as shown in Fig 4. The band of FTIR spectra peaks observed in the range of 1442.75 to 1708.93 corresponds to O-H and C-H stretching. The band at 1101.35 cm^{-1} corresponds to C-C stretching. The FT-IR band of FeO nanoparticle at 794.67 cm^{-1}indicated Fe-O stretching. The Presence of FT-IR peaks for FeO nano particles and other metal oxides are well supported Nazari et al. (2004). Chakrabarti et al.(2004) reported that Fe_2O_3 nanoparticle IR peak observed at 1621 cm^{-1} and 3387 cm^{-1} absorption peak corresponded to the O-H Stretching. Presence of hydroxyl group indicated crystallization of water in the powder. Hence, the result of the absorption band for O-H group was successfully achieved.

Scanning Electron Microscope (SEM)
The SEM image of ZnO nanoparticles is shown in **Figure 5.** A high degree of agglomeration is clearly visible in agreement with previous studies (Chou et al. 2007). (Caglara and Yakuphanoglub, 2012). The selected area electron diffraction (SAED) pattern shows distinct bright rings which confirm the preferential orientation of nanocrystals instead of irregular. These findings are similar to Khoshhesab et al. (2011) and Gupta et al. (2006) EDX spectrum of ZnO nanoparticle samples is shown in **Figure 6.** The names and percentages of the elements for the ZnO sample are shown in the labeling. The EDX spectrum indicated that Zn and O are the main constituents of the sample and no trace of impurities could be found within the detection limit of EDX reported by(Rana et al. 2003). The ZnO nanoparticles were also investigated with EDAX, which affirmed an atomic ratio of Zn 1:1. The chemical composition of ZnO nanoparticles was confirmed by using EDAX measurement reported by Kalapana Handore et al. (2014).
The SEM image of FeO nanoparticles is shown in Figure 7. A high degree of agglomeration is clearly visible in agreement with previous studies. The observation of some larger nanoparticles in SEM image is attributed to

agglomeration, These findings are similar to earlier observation by Caglara and Yakuphanoglub (2012). As the molecule contained main elements of iron and oxygen, existence of Fe-O group is necessary. Absorption peak for Fe-O group was observed for the tested sample powder. EDX spectrum of FeO nanoparticle samples is shown in Figure 8. The names and percentages of the elements for the FeO sample are shown in the labeling and it indicates that Fe and O are the main constituents of the sample and no trace of impurities could be found within the detection limit of EDX. EDX spectral analysis reveals the presence of carbon, oxygen, iron in the synthesized nanoparticles (Li and Smith, 2002)

CONCLUSION

Nanotechnology is act as a next revolutionary technology in agriculture in which nano Zinc and Fe nutrients provide sustainable tools to conventional farming practice. Nanotechnology offers a great potential to adapted fertilizer production with the desired chemical composition, improve the nutrient use efficiency that may reduce environmental impact and enhance the plant efficiency. Zinc oxide and Iron Oxide nano nutrients used as an alternate of conventional agricultural. The development of nano fertilizer nutrient offer a great scope for increasing crop productivity and bio-fortification of zinc in rice and produce fortified grains with improved quality which can solve the micro nutrients deficiency especially zinc and iron among human. The synthesized nano materials are characterized with XRD, SEM, EDX and FTIR techniques and the observed results confirmed the formulation of ZnO and Fe nano materials.

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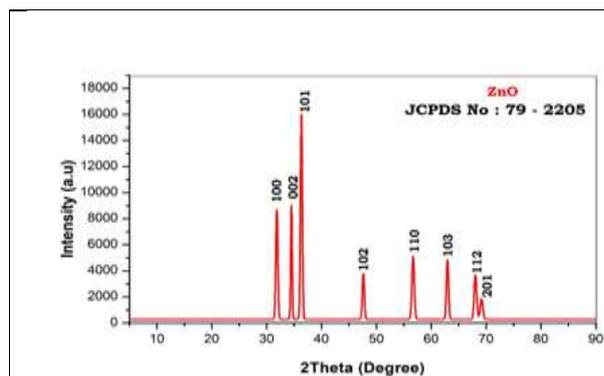
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Fig 1. XRD patterns of synthesized ZnO nano particles.	**Fig 2. XRD patterns of synthesized nano FeO nano particles.**
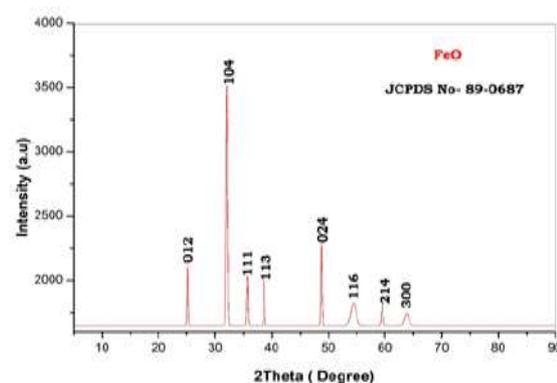
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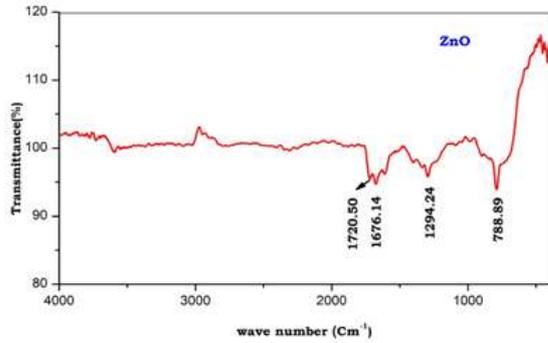
**Fig 3. FTIR spectra of synthesized ZnO nano particles.**
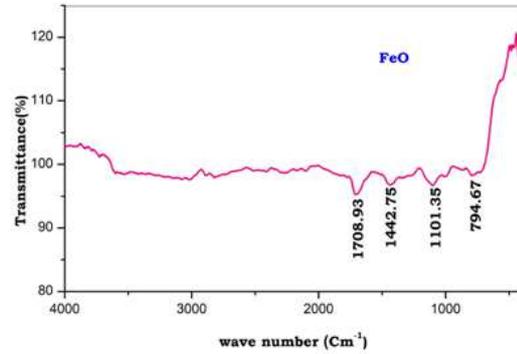
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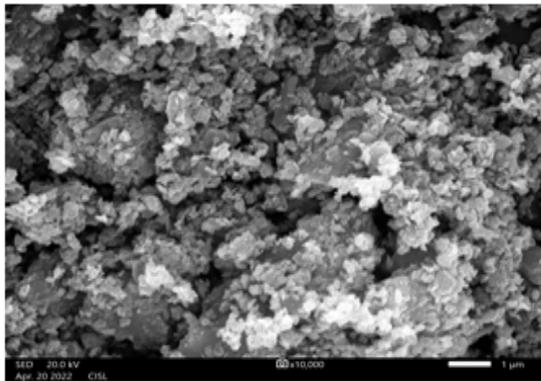

Fig 5. SEM image of ZnO nano particles.
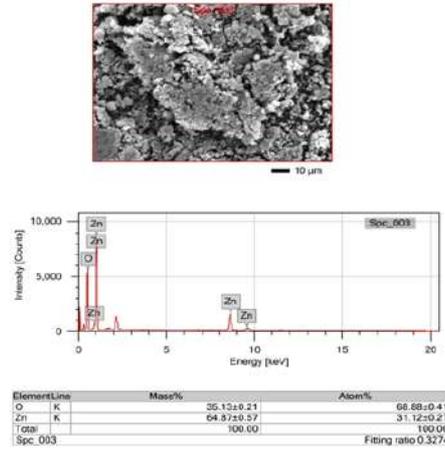
Fig 6. EDX image of ZnO
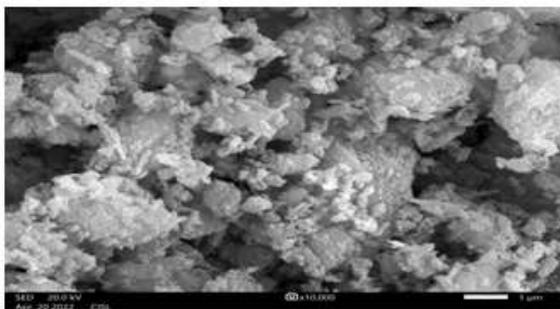
**Fig 7. SEM image of FeO nano paticles**
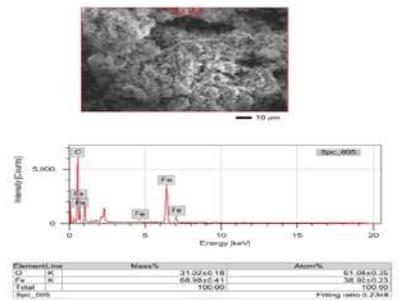
Fig 8. EDX image of FeO

RESEARCH ARTICLE

COVID-19 Vaccination Status and Hospitalization: A Cross-Sectional Study

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ABSTRACT

Utilization of vaccine campaigns to control corona virus 2019 (COVID-19) disease is not only dependent on vaccine's effectiveness and safety. Vaccine acceptance among the general public appears to play a vital role in the successful control of the COVID-19 pandemic. A cross sectional study was done on all admitted patients suffering from moderate to severe COVID-19 disease at a COVID hospital in Rajkot. The outcome measure was to assess the association of being fully vaccinated with the rate of hospitalization. Vaccination reduces the risks of Covid-19 disease, especially severe infection that leads to hospitalization. It is important to be immunized as soon as possible when the vaccine is offered to you, and continue to practice preventive measures to break the chain of spread of infection.

Keywords: Covid-19, Hospitalization, Vaccination.

INTRODUCTION

COVID-19 stands for Corona virus Disease and 19 because it was first seen within the year 2019. COVID-19 disease is caused by infection with the SARS-CoV-2 virus which belongs to the Corona virus family. SARS-CoV-2 stands for Severe Acute Respiratory Syndrome (SARS) and CoV for corona virus. Transmission of Covid-19 Infection occurs primarily from person to person through small droplets from the nose or mouth which is expelled when an individual with COVID-19 coughs, sneezes, or speaks. People can get infected from COVID-19 if they breathe in these droplets from an individual infected with the virus or by touching contaminated objects or surfaces, then touching their eyes, nose or mouth.

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Symptoms of Covid-19 may vary with age but some of the main symptoms of it are: coughing, fever and a loss or change in smell or taste sensation. Other symptoms are shortness of breath, headache, sore throat, aching muscles, fatigue and lethargy. Symptoms may begin gradually and are usually mild. Around 80% i.e, the majority of people have asymptomatic to moderate infection from COVID-19 disease recover without need of hospitalization. Around 15% may get severe disease including pneumonia. Old age people and those with underlying medical conditions such as heart and lung problems, hypertension, diabetes, or cancer are more likely to develop serious illness. And around 5% become critically unwell. This may include septic shock and/or multi-organ and respiratory failure. [1]

COVID-19 vaccine prepares the immune system of a person so that when there is an exposure to the virus, it is able to respond and prevent or reduce the severity of infection. Social distancing and wearing of masks helps to lower the chance of getting the virus or spreading it to others, but these measures are not enough. The combination of getting vaccinated and following COVID-19 guidelines to protect ourselves will offer the best protection from COVID-19. When more number of people will get vaccinated, the faster we can get back to our normal lives.

In clinical trial of vaccination it was noted that significant immunity was achieved in both previously infected and naive subjects and varied from 92% for documented infection, 87% for hospitalization, and 92% for severe disease. [2,5]During the second wave of COVID-19 pandemic in India, beginning in March 2021, large number of patients with more severe disease were hospitalized. At the same time, as part of the vaccination drive that was started from 16 January 2021, most of the healthcare and frontline workers of the Indian Armed Forces had been vaccinated with COVISHIELD. Then the process of vaccinating the common people was being undertaken thereafter with COVIDSHIELD and COVAXIN. So the purpose of this study is to determine the association of vaccination status with rate of hospitalization in COVID-19 patients.

MATERIALS AND METHODS

Study design: Cross-sectional study
The present study was conducted at Civil Hospital, Rajkot. Many patients were getting admitted in the hospital and we have included 572 patients of SARS-CoV-2 infection who had their RTPCR or rapid antigen positive test confirmed and had moderate to severe illness. Patient's records were reviewed to obtain clinical and demographic data. The data of gender, age, co morbid conditions and vaccination status of the patient was extracted. Dates of vaccination, onset of symptom, laboratory confirmation of the diagnosis, hospital admission and clinical outcome were recorded. Patients were discharged at least ten days from onset of symptoms and being a febrile for 3 days irrespective of RT-PCR status and SPO2 >94% on room air (sustained for > 24 hours) or with negative status of RT-PCR with clinical recovery.

STATISTICAL ANALYSIS
All Statistical analysis was done by Microsoft Office Excel 2007 version & SPSS version 20.0 software. Mean & Standard Deviation (SD) were used as a measure of central tendency & measure of dispersion respectively.

RESULTS

Data of 572 patients who were infected with COVID-19 disease was taken for the study. Out of 572 patients 336 were male patients whereas 236 were female patients. The data showed that 47% of patients belonging to age group of 45-64 years, 31% of patients belonging to age group 65 years or older and 22% of patients belonging to age group of 18-44 years got infected by COVID-19 and were hospitilized. From these, only 7% of patients had received the first (single) dose of vaccination. Thus a single dose of vaccination reduces the chances of hospitalization due to corona virus by 93%. In addition to it, taking two doses reduces the chances of hospitalization due to corona virus by 98 to 99 %. After taking both doses, few people got infected by COVID-19 and most of them got recovered by taking home

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isolation. Table 1 demonstrates Mean and SD of age. Table 2 demonstrates Age group wise distribution of the patients. Graph 1 shows Gender Distribution for the study. Graph 2 shows Patients infected with Covid-19 and their vaccination status.

DISCUSSION

The aim of the study was to know about the rate of hospitalization in patients infected with COVID-19 disease and their vaccination status (single or double dose) with COVID-19 vaccine so that we can come to know that what is the effect of vaccination on severity of the disease and can patient get recover with home isolation only or do they require hospitalization. In the present study total 572 patients of SARS-CoV-2 infection, were taken and it has been observed that among them 533 patients did not take any dose of covid-19 vaccine, 36 patients took single dose of vaccine whereas only 3 patients had taken both the dose of covid-19 vaccine. Muthukrishnan J et al. did a cross sectional study on vaccination status and COVID-19 related mortality, in that it was concluded as vaccination with two doses of COVISHIELD was associated with lower odds of mortality rate among hospitalized patients with moderate to severe COVID. [6] These findings are consistent with the result of our present study.

Sowpati DT et al. did a study on clinical outcomes in vaccinated individuals hospitalized with Delta variant of SARS-CoV-2 and it was found out that both COVISHIELD and COVAXIN offer protection and have comparable efficacies against the Delta variant of Covid-19 resulting in reduction of severity of the disease and mortality rate among the hospitalized patients with two doses of vaccination.[7] So rapid and complete vaccination of the population, therefore, remains our only hope in mitigating the deadly covid-19 pandemic. This supports the results of our study that vaccination reduces the risks of infection, especially severe infection that leads to hospitalization.

Abhilash KP et al. did a cohort study to determine the impact of prior vaccination with COVISHIELD and COVAXIN on mortality among symptomatic COVID-19 patients during the second wave of the pandemic in South India during April and May 2021. From the study it was concluded that among symptomatic COVID-19 patients, prior vaccination with COVISHIELD or COVAXIN impacted the severity of illness and reduced mortality during a period of widespread delta variant circulation. [8] These findings are consistent with the result of our present study that full vaccination (both dose) gives greater protection than partial vaccination (single dose) and it also reduces the rate of hospitalization. From the present study we found out that majority of the patients those who are hospitalized have not taken vaccine for COVID-19. Probable reasons for not taking the vaccine can be trust in efficiency of the vaccines, doubt on the health system and the vaccines, fear regarding adverse reactions of the vaccines and preference for natural immunity compared to the vaccines.[9] To overcome this Government of India needs to plan effective public health strategies regarding mass vaccination. Vaccine hesitancy should be tackled by awareness campaigns. [10]Looking at the emerging SARS-CoV-2 variants, the government should focus on maintaining a high pace of vaccination in the whole population of India. Limitation of the study was Male: Female ratio was not equally distributed in the sample and mortality was not taken into consideration. This study is not generalizable to different variants of corona virus.

CONCLUSION

Among COVID-19 patients, prior vaccination with either COVISHIELD or COVAXIN reduces the severity of illness and hospitalization when compared with unvaccinated patients. Full vaccination gives a substantially higher protection over partial vaccination against COVID-19 disease. So we can say that it is important to be immunized as soon as possible, and continue to practice preventive measures to break the chain of spread of infection.

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Table 1: Mean (in years) and SD of age

Number of Patients	Mean (years)	SD
572	55.00	15.42

Interpretation of Table 1: The above table shows the mean age of subjects i.e. 55.00 ± 15.42 (SD) years taken for the study.

Table 2: Age group wise distribution of the patients

Age Group (years)	Number of patients	Percentage of patients (%)
0-17	1	0
18-44	126	22
45-64	271	47
65+	174	31

Interpretation of Table 2: The above table shows age group wise distribution of the patients for the study.

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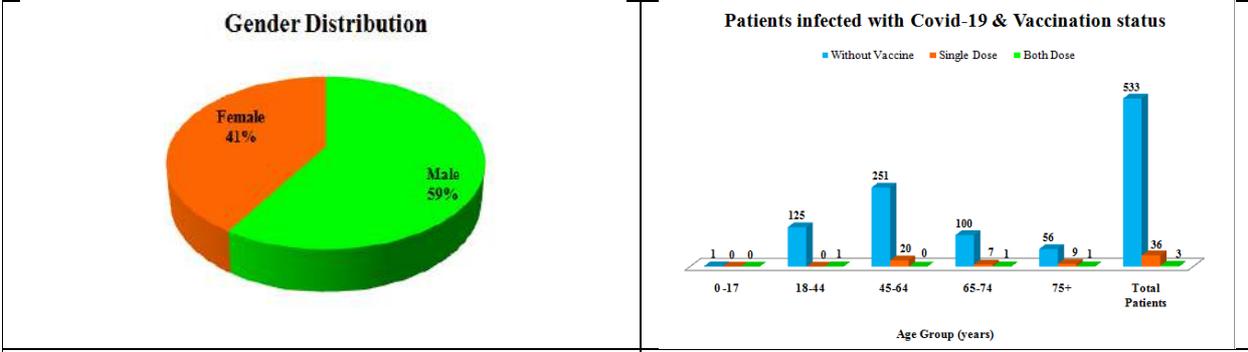
Graph 1: Gender Distribution for the study
Interpretation of Graph 1: The above graph shows gender wise distribution for the study.

Graph 2: Patients infected with Covid-19 and their vaccination status.
Interpretation of Graph 2: The above graph shows vaccination status of patients infected with Covid-19 who were hospitalized.

RESEARCH ARTICLE

Inventory Model for Deteriorating Items with Linear Time Dependent Demand with Shortage Cost Under Trade Credits; A Fuzzy Approach

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ABSTRACT

In this study, we frame an EOQ model for deteriorating items with linear time dependent demand with shortage costin crisp and fuzzy notions. We have to determine the optimal relevant profit and optimum order quantity under two different circumstances i.e for Case I; the credit period is less than the cycle time for settling the account and for Case II; the credit period is greater than or equal to the cycle time for settling the account. To attain this type of techniques, we have been establishing the trapezoidal fuzzy numbers. The working out of optimal profit and economic order quantity (EOQ) are granted out during defuzzification process. Graded mean integration method is used for defuzzification process. To validate the results of this proposed model, we give a numerical example and sensitivity analysis.

Keywords: Linear time –dependent demand, trade credits, Deteriorating Items, Shortage Cost, Trapezoidal Fuzzy Numbers, Graded Mean Integration Method, Defuzzification.

INTRODUCTION

Inventory Management is a procedure through which stocked goods, inventories, and non-capitalized resources are kept in a suitable custom according to their explicit shape and employment. An Inventory can be any item that a business holds to obtain the goal of resale or restoration. Inventory Management is a process of ordering, storing and using inventories. This stock management contains engendering the lead on raw materials, components, and finished products, along-side warehousing and processing of such items in your company. Inventory can be classified into

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four categories: (1) raw materials (2) work-in-process (3) maintenance, repair and operations goods and (4) finished goods.

Inventory management helps establishments identify which and how much stock to order at what time. It tracks inventory from purchase to the sale of goods. The practice identifies and responds to trends to ensure there's always enough stock to fulfill customer orders and proper warning of a shortage. Perhaps the most important advantage of inventory management is saving company money. Inventory is often the largest asset a company has. Inventory is also expensive to purchase, putting a company in the red until it sells those products for a profit. Dependent demand items means items whose demand is set depending on other items. Here we have taken the dependent demand as in quadratic form.

Trade credit is a business-to-business agreement in which a customer can purchase goods without paying cash up front, and paying the supplier at a later scheduled date. In 1995, Aggarwal and Jagg established the ordering policies of deteriorating items under permissible delay in payments. In 2001, Chang, Hung and Dye developed an inventory model for deteriorating items with linear trend in demand under conditions of permissible delay in payments. In 1985, Davis and Gaither determined optimal ordering policies under conditions of extended payment privileges. In 1985, Goyal derived an EOQ under condition of permissible delay in payments. In 1995, Hariga coined an EOQ model for deteriorating items with shortages and time-varying demand. In May 2021, Jayanthi have recognized the production inventory models for deterioration items using penalty, transportation and shortage cost: A Fuzzy Approach. In September 2020, Jayanthi and Yasotha Nanthini recognized Fuzzy production inventory model for deterioration items with shortages and lead time using penalty cost. In January 2020 Jayanthi and Yasotha Nanthini framed the production inventory model with allowed shortages: A fuzzy approach. In December 2019, Jayanthi, Maragatham and Ananthi demonstrated the production inventory model with shortages for deteriorating items with two uncertain rates of deterioration. In 2011, Khanna, Ghosh, and Chandhuri established an EOQ model for deteriorating items with time-dependent demand under permissible delay in payment. In 2000, Lin, Tan and Lee formulated an EOQ model for deteriorating items with time-varying demand and shortages. In 1995, Wee, introduced a deterministic lot size inventory model for deteriorating items with shortages and a declining market.

Here we nurture an inventory model for deteriorating items with linear time dependent demand with shortage cost under trade credits in a crisp and fuzzy sense. The optimal cycle time, optimal relevant profit and optimal order quantity are derived in both crisp and fuzzy environment.

METHODOLOGY

Fuzzy Numbers

Any fuzzy subset of the real line R, whose membership function μ_A satisfied the following conditions, is a generalized fuzzy number $\tilde{A}$.

(i) μ_A is a continuous mapping from R to the closed interval [0, 1].

(ii) $\mu_A = 0, -\infty < x \le a_1,$

(iii) $\mu_A = $ L(x) is strictly increasing on [a_1, a_2]

(iv) $\mu_A = $ w$_A$, $a_2 \le x \le a_3$

(v) $\mu_A = $ R(x) is strictly decreasing on [a_3, a_4]

(vi) $\mu_A = 0, a_4 \le x < \infty$

where $0 < w_A \le 1$ and a_1, a_2, a_3 and a_4 are real numbers. Also this type of generalized fuzzy number be denoted as $\tilde{A} = \left(a_1, a_2, a_3, a_4 : w_A\right)_{LR}$; When w$_A$ = 1, it can be simplified as $\tilde{A} = \left(a_1, a_2, a_3, a_4\right)_{LR}$.

Trapezoidal Fuzzy Number

A trapezoidal fuzzy number $\tilde{A}$= (a, b, c, d) is represented with membership function $\mu_{\tilde{A}}$ as follows:

$$\mu_A(x) = \begin{cases} 0, & x > d \\ \dfrac{x-a}{b-a}, & a \le x \le b \\ 1, & b \le x \le c \\ \dfrac{d-x}{d-c}, & c \le x \le d \end{cases}$$

Graded Mean Integration Method

If $\tilde{A} = \left(a_1, a_2, a_3, a_4\right)$ is a trapezoidal fuzzy number then the graded mean integration representation of $\tilde{A}$ is,

$$p\left(\tilde{A}\right) = \frac{a_1 + 2a_2 + 2a_3 + a_4}{6}$$

Notations and Assumptions

The mathematical model of this paper is developed on the basis of the following notations and assumptions.

Notations

R = R (t) = p + qt; p > 0, 0 < q < 1; the annual demand

A   : the ordering cost per order

C   : the unit purchase cost

s   : the unit selling price

r   : the backlogging cost per unit quantity per unit time

θ : the deterioration rate

Q: the order quantity

M   : the permissible credit period offered by the supplier to the retailer for settling the account

I_c : interest rate at which the interest is charged

I_e: interest rate at which the interest is earned

I(t)   : inventory level at any instant of time

T: replenishment cycle time

F_i (T)   : total profit per unit time; i = 1,2

Q_i^* : optimal order quantity; i = 1,2 for Case I and Case II respectively

T_i^* : optimal replenishment cycle time; i = 1,2

$F_i\left(T_i^*\right)$: optimal profit per unit time; i = 1,2

$\tilde{A}$: the fuzzy ordering cost per order

$\tilde{C}$: the fuzzy unit purchase cost

$\tilde{s}$: the fuzzy unit selling price

$\tilde{r}$: the fuzzy backlogging cost per unit quantity per unit time

$\tilde{Q}$: the fuzzy order quantity

$\tilde{Q}_i^*$: fuzzy optimal order quantity; i = 1,2 for Case I and Case II respectively

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$\tilde{T}_i^*$:fuzzy optimal replenishment cycle time; i = 1,2

$\tilde{F}_i\,(T_i^*)$ 　　: fuzzy optimal profit per unit time; i = 1,2

Assumptions

- The inventory system under consideration deals with the single item.
- The planning horizon is infinite.
- The demand of the product is a linear increasing function of time.
- Shortages are allowed.
- Lead time is zero.
- Inventory is continuously reviewed.
- The retailer can deposit generated sales revenue in an interest bearing account during the permissible credit period. At the end of the period, the retailer settles the account for all the units sold keeping the difference for day to day expenditure and paying the interest charges on the unsold items in the stock.

Model Formulation

Proposed Inventory Model in Crisp Sense:

From the above notations and assumptions, we obtain the optimal cycle time, optimal relevant profit and optimal order quantity for the inventory model for deterioration items under trade credit with shortages in crisp environment.

The inventory level I(t) depletes to meet the demand and deterioration. The differential equation governing the rate of change of inventory at any time t is given by,

$$\frac{dI(t)}{dt}+\theta\,I(t)=-\,R(t),0\le t\le T \qquad \text{------------------------ (1)}$$

With the initial condition I (0) = Q and boundary condition I(T) = 0　---------------------- (2)

The solution of (1) is given by,

$$I(t)=\frac{1}{\theta}\left(p-\frac{q}{\theta}\right)\left(e^{\theta(T-t)}-1\right)+\frac{1}{\theta}q\left(Te^{\theta(T-t)}-t\right) \qquad \text{---------------------------- (3)}$$

And the order quantity Q is given by,

$$Q=\frac{1}{\theta}\left(p-\frac{q}{\theta}\right)\left(e^{\theta T}-1\right)+\frac{1}{\theta}q\left(Te^{\theta T}\right) \qquad \text{---------------- (4)}$$

The total profit per unit time of inventory system consists of the following:

Ordering Cost:

$$OC=\frac{A}{T} \qquad \text{----------------------- (5)}$$

Sales Revenue:

$$SR=\frac{s}{T}\int_0^T R(t)\,dt$$

$$SR=s\left(p+\frac{qT}{2}\right) \qquad \text{--------------------- (6)}$$

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Deterioration Cost

$$DC = \frac{C}{T}\left[q - \int_0^T R(t)\,dt \right]$$

$$DC = \frac{C}{\theta T}\left(p - \frac{q}{\theta} \right)\left(e^{\theta T} - 1 \right) + \frac{C}{\theta} q\, e^{\theta T} - pC - \frac{qTC}{2} \qquad \text{---------------------- (7)}$$

Holding Cost

$$HC = \frac{h}{T}\int_0^T I(t)\,dt$$

$$HC = \frac{h}{\theta T}\left[\left(p - \frac{q}{\theta} \right)\left(\frac{e^{\theta T} - 1}{\theta} - T \right) + qT\left(\frac{e^{\theta T} - 1}{\theta} - \frac{T}{2} \right) \right] \qquad \text{--------------------- (8)}$$

Shortage Cost

$$SC = \frac{r}{T}\int_M^T I(t)\,dt$$

$$SC = \frac{r}{\theta T}\left[\left(p - \frac{q}{\theta} \right)\left(\frac{e^{\theta(T-M)} - 1}{\theta} \right) + q\left(\frac{Te^{\theta(T-M)} - T}{\theta} - \frac{T^2}{2} + \frac{M^2}{2} \right) \right] \qquad \text{------------------ (9)}$$

Now there are two cases arise by considering interest charged and interest earned based on length of T and M.

Case:1 M < T

The interest charged per unit time for above case is,

$$IC = \frac{CI_c}{T}\int_M^T I(t)\,dt$$

$$IC = \frac{CI_c}{\theta T}\left[\left(p - \frac{q}{\theta} \right)\left(\frac{e^{\theta(T-M)} - 1}{\theta} \right) + q\left(\frac{Te^{\theta(T-M)} - T}{\theta} \right) - \left(p - \frac{q}{\theta} \right)(T - M) - q\left(\frac{T^2 - M^2}{2} \right) \right]$$

$$\text{--------------------- (10)}$$

During [0, M] retailer sells the product and deposits the revenue into an interest earning account at the rate I_e per unit per year. Therefore, the interest earned IE_1 per unit time is given by,

$$IE_1 = \frac{s\,I_e}{T}\int_0^M R(t)\,t\,dt$$

$$IE_1 = \frac{s\,I_e}{T}\left[\frac{pM^2}{2} + \frac{qM^3}{3} \right] \qquad \text{----------------------- (11)}$$

Hence, the total profit $F_1(T)$ of an inventory system per unit time is,

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$$F_1(T) = SR + IE_1 - OC - DC - HC - IC - SC$$

--------------- (12)

Substitute the values of SR, IE₁, OC, DC, HC, IC and SC in equation (11) and solving by using truncated Taylor's series for approximating the exponential function, we get,

$$e^{\theta T} = 1 + \theta T$$

$$e^{\theta(T-M)} = 1 + \theta T - \theta M$$

$$F_1(T) = ps - 2pC + \left(\frac{qs}{2} - \frac{3qC}{2} - \frac{hq}{2\theta} - \frac{qCI_c}{2\theta} - \frac{rq}{2\theta}\right)T + \left(\frac{qCI_c}{\theta} + \frac{rq}{\theta}\right)M + \left(psI_e - \frac{qCI_c}{\theta} - \frac{rq}{\theta}\right)\frac{M^2}{2T} +$$

$$\frac{qsI_e M^3}{3T} - \frac{A}{T}$$

------------------ (13)

Case II: T ≤ M

Hence the retailer sells R(t) t – units in all by the end of the cycle time and has C R(t) t to pay the supplier in full by the end of the credit period M. Hence the interest charges,

IC = 0   ---------------------------- (14)

The interest earned per unit time is

$$IE_2 = s I_e \left[(p + qT)M - \left(\frac{p}{2} + \frac{2qT}{3}\right)T \right]$$

------------------ (15)

Hence the total profit F₂(T) of an inventory system per unit time is,

$$F_2(T) = SR + IE_2 - OC - DC - HC - SC$$

--------------- (16)

Substitute the values of SR, IE₁, OC, DC, HC and SC in equation (16) and solving by using truncated Taylor's series for approximating the exponential function, we get,

$$e^{\theta T} = 1 + \theta T$$

$$e^{\theta(T-M)} = 1 + \theta T - \theta M$$

$$F_2(T) = ps - 2pC + \frac{qsT}{2} + \frac{2qC}{\theta} + \frac{qCT}{2} - \frac{q^2T}{2\theta} + psI_e M + qsTMI_e - \frac{psI_e T}{2} - \frac{2qsI_e T^2}{3}$$

$$- \frac{rT}{2\theta} + \frac{rM}{\theta} - \frac{rM^2}{2\theta T} - \frac{A}{T}$$

-------------------- (17)

Determination of optimal solutions

To find the optimal solution for the problem, we maximize F (T) for case I and case II respectively and then compare them to obtain maximum value. Our aim is to find the maximum average profit per unit time for both the cases w.r.to T. The necessary and sufficient condition to maximize $F_1(T)$ and $F_2(T)$ for given values of T are respectively.

$$\frac{dF_1}{dT}=0, \qquad \frac{dF_2}{dT}=0 \qquad and \qquad \frac{d^2F_1}{dT^2}<0, \qquad \frac{d^2F_2}{dT^2}<0$$

Differentiating equation (13) w.r.to T, we get,

$$\frac{dF_1(T)}{dT}=\left(\frac{qs}{2}-\frac{3qC}{2}-\frac{hq}{2\theta}-\frac{qCI_c}{2\theta}-\frac{rq}{2\theta}\right)-\left(psI_e-\frac{qCI_c}{\theta}-\frac{rq}{\theta}\right)\frac{M^2}{2T^2}-\frac{qsI_eM^3}{3T^2}+\frac{A}{T^2}$$

------------- (18)

$$\frac{dF_1(T)}{dT}=0$$

Optimal cycle Time for Case I:

$$T=\sqrt{\frac{\left(psI_e-\frac{qCI_c}{\theta}-\frac{rq}{\theta}\right)\frac{M^2}{2}+\frac{qsI_eM^3}{3}-A}{\left(\frac{qs}{2}-\frac{3qC}{2}-\frac{hq}{2\theta}-\frac{qCI_c}{2\theta}-\frac{rq}{2\theta}\right)}}$$

-------------------------------------- (19)

Again differentiate equation (18) w.r.to T, we get,

$$\frac{d^2F_1(T)}{dT^2}=\left(psI_e-\frac{qCI_c}{\theta}-\frac{rq}{\theta}\right)\frac{M^2}{T^3}-\frac{2qsI_eM^3}{3T^3}-\frac{2A}{T^3}<0$$

--------------------- (20)

Differentiate equation (17) w.r.to T, we get,

$$\frac{dF_2(T)}{dT}=\frac{qs}{2}+\frac{qC}{2}-\frac{q^2}{2\theta}+qsMI_e-\frac{psI_e}{2}-\frac{4qsI_eT}{3}-\frac{r}{2\theta}+\frac{rM^2}{2\theta T^2}+\frac{A}{T^2}$$

------------ (21)

$$\frac{dF_2(T)}{dT}=0$$

Optimal cycle Time for Case II:

$$T=\sqrt{\frac{\left(\frac{rM^2}{2\theta}+A\right)}{\left(\frac{q^2}{2\theta}+\frac{psI_e}{2}+\frac{r}{2\theta}-\frac{qs}{2}-\frac{qC}{2}-qsI_eM\right)+\frac{4qsI_e}{3}}}$$

---------------- (22)

Again differentiate equation (21) w.r.to T, we get,

$$\frac{d^2 F_1(T)}{dT^2} = \frac{-4qsI_e}{3} - \frac{2rM^2}{2\theta T^3} - \frac{2A}{T^3} < 0$$

--------------- (23)

3.2 Proposed Inventory Model in Fuzzy Sense:

From the above notations and assumptions, we obtain the optimal cycle time, optimal relevant profit and optimal order quantity for the inventory model for deterioration items under trade credit with shortages in fuzzy environment.

The inventory level I(t) depletes to meet the demand and deterioration. The differential equation governing the rate of change of inventory at any time t is given by,

$$\frac{dI(t)}{dt} + \theta\, I(t) = -R(t),\, 0 \le t \le T$$

----------------------- (24)

With the initial condition I (0) = Q and boundary condition I(T) = 0 ---------------------- (25)

The solution of (24) is given by,

$$I(t) = \frac{1}{\theta}\left(p - \frac{q}{\theta}\right)\left(e^{\theta(T-t)} - 1\right) + \frac{1}{\theta}q\left(Te^{\theta(T-t)} - t\right)$$

------------------------------ (26)

And the order quantity Q is given by,

$$Q = \frac{1}{\theta}\left(p - \frac{q}{\theta}\right)\left(e^{\theta T} - 1\right) + \frac{1}{\theta}q\left(Te^{\theta T}\right)$$

--- (27)

The total profit per unit time of inventory system consists of the following:

Fuzzy Ordering Cost:

$$O\tilde{C} = \frac{\tilde{A}}{T}$$

--- (28)

Fuzzy Sales Revenue:

$$S\tilde{R} = \frac{\tilde{s}}{T}\int_0^T R(t)\, dt$$

$$S\tilde{R} = \tilde{s}\left(p + \frac{qT}{2}\right)$$

--- (29)

Fuzzy Deterioration Cost:

$$D\tilde{C} = \frac{\tilde{C}}{T}\left[q - \int_0^T R(t)\, dt\right]$$

$$D\tilde{C} = \frac{\tilde{C}}{\theta T}\left(p - \frac{q}{\theta}\right)\left(e^{\theta T} - 1\right) + \frac{\tilde{C}}{\theta}q\, e^{\theta T} - p\tilde{C} - \frac{qT\tilde{C}}{2}$$

----------------- (30)

Holding Cost:

$$HC = \frac{h}{T}\int_0^T I(t)\, dt$$

$$HC = \frac{h}{\theta T}\left[\left(p - \frac{q}{\theta}\right)\left(\frac{e^{\theta T} - 1}{\theta} - T\right) + qT\left(\frac{e^{\theta T} - 1}{\theta} - \frac{T}{2}\right)\right]$$

---------------- (31)

Indian Journal of Natural Sciences

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Vol.13 / Issue 73 / August / 2022

International Bimonthly (Print)

ISSN: 0976 – 0997

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Fuzzy Shortage Cost:

$$S\widetilde{C} = \frac{\widetilde{r}}{T} \int_{M}^{T} I(t)\, dt$$

$$S\widetilde{C} = \frac{\widetilde{r}}{\theta T}\left[\left(p - \frac{q}{\theta}\right)\left(\frac{e^{\theta(T-M)} - 1}{\theta}\right) + q\left(\frac{Te^{\theta(T-M)} - T}{\theta} - \frac{T^2}{2} + \frac{M^2}{2}\right)\right]$$

-------------- (32)

Now there are two cases arise by considering interest charged and interest earned based on length of T and M.

Case:1 M < T

The interest charged per unit time for above case is,

$$I\widetilde{C} = \frac{\widetilde{C}I_c}{T} \int_{M}^{T} I(t)\, dt$$

$$I\widetilde{C} = \frac{\widetilde{C}I_c}{\theta T}\left[\left(p - \frac{q}{\theta}\right)\left(\frac{e^{\theta(T-M)} - 1}{\theta}\right) + q\left(\frac{Te^{\theta(T-M)} - T}{\theta}\right) - \left(p - \frac{q}{\theta}\right)(T - M) - q\left(\frac{T^2 - M^2}{2}\right)\right]$$

------------------ (33)

During [0, M] retailer sells the product and deposits the revenue into an interest earning account at the rate I_e per unit per year. Therefore, the interest earned IE_1 per unit time is given by,

$$I\widetilde{E}_1 = \frac{\widetilde{s}\, I_e}{T} \int_{0}^{M} R(t)\, t\, dt$$

$$I\widetilde{E}_1 = \frac{\widetilde{s}\, I_e}{T}\left[\frac{pM^2}{2} + \frac{qM^3}{3}\right]$$

-- (34)

Hence, the total profit $F_1(T)$ of an inventory system per unit time is,

$$\widetilde{F}_1(T) = S\widetilde{R} + I\widetilde{E}_1 - O\widetilde{C} - D\widetilde{C} - HC - I\widetilde{C} - S\widetilde{C}$$

----------------------------------- (35)

Substitute the values of SR, IE_1, OC, DC, HC, IC and SC in equation (34) and solving by using truncated Taylor's series for approximating the exponential function, we get,

$$e^{\theta T} = 1 + \theta T$$

$$e^{\theta(T-M)} = 1 + \theta T - \theta M$$

$$F_1(T) = p\widetilde{s} - 2p\widetilde{C} + \left(\frac{q\widetilde{s}}{2} - \frac{3q\widetilde{C}}{2} - \frac{hq}{2\theta} - \frac{q\widetilde{C}I_c}{2\theta} - \frac{\widetilde{r}q}{2\theta}\right)T + \left(\frac{q\widetilde{C}I_c}{\theta} + \frac{\widetilde{r}q}{\theta}\right)M + \left(p\widetilde{s}I_e - \frac{q\widetilde{C}I_c}{\theta} - \frac{\widetilde{r}q}{\theta}\right)\frac{M^2}{2T} +$$

$$\frac{q\widetilde{s}I_e M^3}{3T} - \frac{\widetilde{A}}{T}$$

------------------------ (36)

Case II: T ≤ M

Hence the retailer sells R(t) t – units in all by the end of the cycle time and has C R(t) t to pay the supplier in full by the end of the credit period M. Hence the interest charges,

$$IC = 0 \qquad\qquad\qquad \text{------------------------- (37)}$$

The interest earned per unit time is

$$IE_2 = \tilde{s}\, I_e \left[(p+qT)\,M - \left(\frac{p}{2} + \frac{2qT}{3} \right) T \right] \qquad \text{-------------------------------------- (38)}$$

Hence the total profit F$_2$(T) of an inventory system per unit time is,

$$F_2(T) = S\tilde{R} + I\tilde{E}_2 - O\tilde{C} - D\tilde{C} - HC - S\tilde{C} \qquad \text{------------------------------------- (39)}$$

Substitute the values of SR, IE$_1$, OC, DC, HC and SC in equation (39) and solving by using truncated Taylor's series for approximating the exponential function, we get,

$$e^{\theta T} = 1 + \theta T$$

$$e^{\theta(T-M)} = 1 + \theta T - \theta M$$

$$F_2(T) = p\tilde{s} - 2p\tilde{C} + \frac{q\tilde{s}T}{2} + \frac{2q\tilde{C}}{\theta} + \frac{q\tilde{C}T}{2} - \frac{q^2 T}{2\theta} + p\tilde{s}I_e M + q\tilde{s}TMI_e - \frac{p\tilde{s}I_e T}{2} - \frac{2q\tilde{s}I_e T^2}{3}$$

$$\qquad\qquad - \frac{\tilde{r}T}{2\theta} + \frac{\tilde{r}M}{\theta} - \frac{\tilde{r}M^2}{2\theta T} - \frac{\tilde{A}}{T} \qquad\qquad \text{------------------ (40)}$$

Determination of optimal solutions:

To find the optimal solution for the problem, we maximize F (T) for case I and case II respectively and then compare them to obtain maximum value. Our aim is to find the maximum average profit per unit time for both the cases w.r.to T. The necessary and sufficient condition to maximize F$_1$(T) and F$_2$ (T) for given values of T are respectively.

$$\frac{d\tilde{F}_1}{dT} = 0, \qquad \frac{d\tilde{F}_2}{dT} = 0 \qquad and \qquad \frac{d^2\tilde{F}_1}{dT^2} < 0, \qquad \frac{d^2\tilde{F}_2}{dT^2} < 0$$

Differentiating equation (36) w.r.to T, we get,

$$\frac{d\tilde{F}_1(T)}{dT} = \left(\frac{q\tilde{s}}{2} - \frac{3q\tilde{C}}{2} - \frac{hq}{2\theta} - \frac{q\tilde{C}I_c}{2\theta} - \frac{\tilde{r}q}{2\theta} \right) - \left(p\tilde{s}I_e - \frac{q\tilde{C}I_c}{\theta} - \frac{\tilde{r}q}{\theta} \right) \frac{M^2}{2T^2} - \frac{q\tilde{s}I_e M^3}{3T^2} + \frac{\tilde{A}}{T^2}$$

$$\text{-- (41)}$$

$$\frac{d\tilde{F}_1(T)}{dT} = 0$$

Optimal cycle Time for Case I:

$$T = \sqrt{\dfrac{\left(p\tilde{s}I_e - \dfrac{q\tilde{C}I_c}{\theta} - \dfrac{\tilde{r}q}{\theta}\right)\dfrac{M^2}{2} + \dfrac{q\tilde{s}I_e M^3}{3} - \tilde{A}}{\left(\dfrac{q\tilde{s}}{2} - \dfrac{3q\tilde{C}}{2} - \dfrac{hq}{2\theta} - \dfrac{q\tilde{C}I_c}{2\theta} - \dfrac{\tilde{r}q}{2\theta}\right)}}$$

----------------------------------- (42)

Again differentiate equation (41) w.r.to T, we get,

$$\frac{d^2 \tilde{F}_1(T)}{dT^2} = \left(p\tilde{s}I_c - \frac{q\tilde{C}I_c}{\theta} - \frac{\tilde{r}q}{\theta}\right)\frac{M^2}{T^3} - \frac{2q\tilde{C}I_c M^3}{3T^3} - \frac{2\tilde{A}}{T^3} < 0$$

------------------ (43)

Differentiate equation (39) w.r.to T, we get,

$$\frac{d\tilde{F}_2(T)}{dT} = \frac{q\tilde{s}}{2} + \frac{q\tilde{C}}{2} - \frac{q^2}{2\theta} + q\tilde{s}M I_e - \frac{p\tilde{s}I_e}{2} - \frac{4q\tilde{s}I_e T}{3} - \frac{\tilde{r}}{2\theta} + \frac{\tilde{r}M^2}{2\theta T^2} + \frac{\tilde{A}}{T^2}$$

----------- (44)

$$\frac{d\tilde{F}_2(T)}{dT} = 0$$

Optimal cycle Time for Case II:

$$T = \sqrt{\dfrac{\left(\dfrac{\tilde{r}M^2}{2\theta} + \tilde{A}\right)}{\left(\dfrac{q^2}{2\theta} + \dfrac{p\tilde{s}\tilde{I}_e}{2} + \dfrac{\tilde{r}}{2\theta} - \dfrac{q\tilde{s}}{2} - \dfrac{q\tilde{C}}{2} - q\tilde{s}\tilde{I}_e M\right) + \dfrac{4q\tilde{s}\tilde{I}_e}{3}}}$$

--------------------------- (45)

Again differentiate equation (44) w.r.to T, we get,

$$\frac{d^2 \tilde{F}_1(T)}{dT^2} = \frac{-4q\tilde{s}\tilde{I}_e}{3} - \frac{2\tilde{r}M^2}{2\theta T^3} - \frac{2\tilde{A}}{T^3} < 0$$

--- (46)

4. Numerical Example

4.1 Numerical Example in Crisp Sense:

4.1.1 Example:1

Case: I

p = 1250, q = 0.5, M = 36/365, Θ = 0.3, h = 3, s = 50, C = 30, A = 250, I_c = 0.25, I_e = 0.2 and r = 0.5 in approximate units.

Optimal Cycle Time T = T_1^* =3.1386 years, Optimal total relevant profit F_1 (T) = F_1^*(T) = Rs. 12619.462 and Optimal order quantity = Q = Q_1^* = 4173.4760 units.

4.1.2 Example: 2

Case: II

p = 800, q = 0.4, M = 50/365, Θ = 0.3, s = 50, C = 15, A = 60, I_e = 0.16 and r = 0.4 in approximate units. Optimal Cycle Time T = T_2^* = 0.137 years, Optimal total relevant profit F_2 (T) = F_2^*(T) = Rs. 16042.209 and Optimal order quantity = Q = Q_2^* = 112.003 units.

4.2 Numerical Example in Fuzzy Sense:

4.2.1 Example:1

Case: I

$$\tilde{s} = (40, 50, 60, 70), \tilde{C} = (20, 30, 40, 50)$$

p = 1250, q = 0.5, M = 36/365, Θ = 0.3, h = 3, I_c = 0.25, I_e = 0.2

$\tilde{A} = (240, 250, 260, 270)$ and $\tilde{r} = (0.4, 0.5, 0.6, 0.7)$ in approximate units. Optimal cycle Time T = T_1^* = 2.942

years, Optimal total relevant profit F_1 (T) = F_1^*(T) = Rs. 13832.716 and Optimal order quantity = Q = Q_1^* = 5912.311 units.

4.1.2 Example: 2

Case: II

$$\tilde{s} = (40, 50, 60, 70), \tilde{C} = (10, 15, 20, 25) \ \tilde{A} = (50, 60, 70, 80)$$

p = 800, q = 0.4, M = 50/365, Θ = 0.3, I_e= 0.16

and $\tilde{r} = (0.3, 0.4, 0.5, 0.6)$ in approximate units. Optimal Cycle Time T= T_2^* = 0.143

years, Optimal total relevant

profit F_2 (T) = F_2^*(T) = Rs. 16138.625and Optimal order quantity = Q = Q_2^* = 117.337 units.

Sensitivity Analysis
Sensitivity analysis in Crisp Sense

5.1.1 Sensitivity Analysis for Case I:

S.No	C	T_1^*	F_1^*(T) (Rs.)	Q*(T) (units)
1.	20	3.7145	12714.579	4259.7601
2.	30	3.1386	12619.462	4173.4760
3.	40	3.0528	12472.814	4025.8112
4.	50	3.0121	12245.913	3969.7124
5.	60	2.9923	12034.414	3876.3146

5.1.2 Sensitivity Analysis for Case II:

S.No	C	T_1^*	F_1^*(T)	Q*(T) (units)
1.	10	0.139	16347.987	119.245
2.	15	0.137	16042.209	117.337
3.	20	0.135	15829.476	116.389
4.	25	0.132	15639.865	114.614
5.	30	0.130	15426.344	112.903

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Sensitivity analysis in Fuzzy Sense
Sensitivity Analysis for Case I

S.No	C	T_1^*	$F_1^*(T)$ (Rs.)	$Q^*(T)$ (units)
1.	$\tilde{C} = (10, 20, 30, 40)$	2.794	13947.801	6003.481
2.	$\tilde{C} = (20, 30, 40, 50)$	2.942	13832.716	5912.311
3.	$\tilde{C} = (30, 40, 50, 60)$	3.038	13793.293	5890.273
4.	$\tilde{C} = (40, 50, 60, 70)$	3.121	13692.654	5702.154
5.	$\tilde{C} = (50, 60, 70, 80)$	3.257	13554.329	5612.6643

Sensitivity Analysis for Case II:

S.No	C	T_1^*	$F_1^*(T)$	$Q^*(T)$ (units)
1.	$\tilde{C} = (5, 10, 15, 20)$	0.145	16200.497	118.370
2.	$\tilde{C} = (10, 15, 20, 25)$	0.143	16138.625	117.337
3.	$\tilde{C} = (15, 20, 25, 30)$	0.141	16049.576	116.418
4.	$\tilde{C} = (20, 25, 30, 35)$	0.140	15937.003	114.235
5.	$\tilde{C} = (25, 30, 35, 40)$	0.139	15847.902	112.408

CONCLUSION

In this paper, we develop an inventory model for deteriorating items with linear dependent demand rate with shortage cost under trade credits in both crisp and fuzzy sense. Here we establish the numerical solution for finding the solution of optimal cycle time, optimal order quantity and optimal total relevant profit in both crisp and fuzzy forms. From the sensitivity analysis we determine that the results are quite sensitive with respect to the variation of different parameters. Truncated Taylor's series expansion is used for finding the closed form of this optimal solution. For further studies, we apply the fuzzy provisions for all the parameters and compare the result between the crisp and fuzzy sense.

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Figure 1. Trapezoidal Fuzzy Numbers

RESEARCH ARTICLE
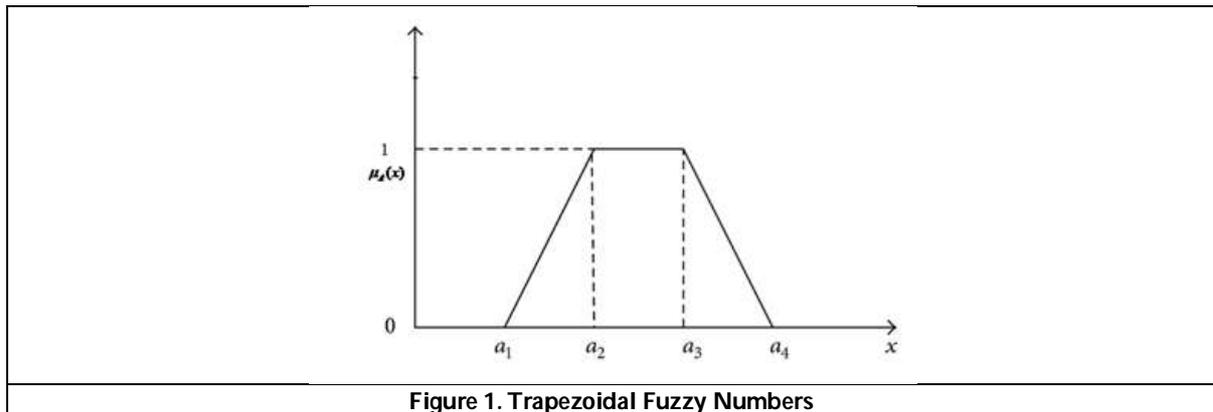
Analysis of Chat bots based Artificial Intelligence (AI) Marketing

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ABSTRACT

Artificial intelligence (AI) is a platform that allows marketers to develop highly tailored client experiences, improve organisation response, and solve customer problems. In this study, the chatbot is examined as an artificial intelligence tool in marketing, as well as its current application and future potential in the sector. A survey of respondents' behaviours, habits, and expectations when utilising various communication channels was undertaken, with a focus on chatbots and their benefits and drawbacks in comparison to other communication channels, with a total of 60 survey respondents. The findings revealed that the greatest benefit of employing chatbots in marketing services was when offering simple, quick information, but they also revealed respondents' worry of chat bots supplying them with incorrect information. Chatbots should be considered by businesses, particularly if they face communication issues with clients, but also if they want to keep up with the changing lifestyles of their customers.

Keywords: Artificial Intelligence, Customer, Chat bots, Pattern recognition,

INTRODUCTION

Artificial intelligence is garnering greater attention from the scientific community and the general public as technology advances, resulting in the development of technical skills. A rising number of initiatives are attempting to integrate artificial intelligence into business processes in order to profit from the implementation described above. According to the author of this paper, marketing's purpose is to resell a product or service. A delighted client who receives added value from the product or service purchased and then returns to make another purchase is referred to as a continuous sale. Artificial intelligence's advent into the marketing sector opens up new possibilities. It will be feasible to offer customised products and services using a machine that can track client behaviour, document observed behaviour, and spot patterns, giving marketers significantly more options. The role and potential of artificial intelligence in marketing will be discussed in the following section, with a focus on chatbots. A survey was conducted to examine respondents' behaviour, habits, and expectations while using various communication channels, as well as the benefits and drawbacks of chat bots compared to other communication channels.

Marketing of Artificial Intelligence

Artificial intelligence has a number of different expert definitions. Artificial intelligence is defined as "the science of making smart machines," according to Demis Has ibis, co-founder and CEO of Google Deep Mind. In this case, smart computers refer to the degree to which they have succeeded in imitating human thought patterns [1-2]. Marketers may use artificial intelligence to offer highly personalised customer experiences at a lower cost than traditional marketing strategies. Any interaction a user has with a product or service is recorded and utilised to improve and customise it in the future. Artificial intelligence has evolved from a science-fiction concept to a technological reality in today's world.According to a PwC poll 72 percent of marketers believe that artificial intelligence can help them grow their business. Companies can get a competitive edge by using customer data to make their offer more relevant: 1. They can create items that are more consumer-oriented. 2. They can deliver services that are more focused on the customer. 3. They can pinpoint the target market with more accuracy, resulting in a higher conversion rate. 4 They are able to totally satisfy their clients' requirements. Data has evolved into a company's most important asset. The utility of data for artificial intelligence development through machine learning grows in tandem with the amount of data available. To construct, test, and prepare artificial intelligence, huge amounts of data are required.

When it comes to boosting industrial sectors, artificial intelligence has a lot of potential. Financial institutions, the automotive industry, law companies, and others have all employed it. For organisations trying to beat their competitors, owning data and analysing it using artificial intelligence has become important[3-4]. Artificial intelligence, as previously said, is not a recent invention. However, the capabilities of artificial intelligence and machine learning remained a mystery for a long time after their discovery because we were unable to collect huge amounts of data from various sources and across categories in order to cross them subsequently.Because enormous volumes of data were required to design and test artificial intelligence-based computers, no significant advances in artificial intelligence development were made at the time. With the passage of time, however, the situation has shifted. We now have the ability to not only gather and store vast volumes of data, but also to cross and analyse it in order to make conclusions and link it. The evolution of artificial intelligence and its tools is a result of changing and developing BDs.

Chatbots

Bots are software that conducts automated activities, and chatbots are bots that may be found on a variety of messaging sites. Because the purpose of chatbots is to have a discussion with humans, the major role of chatbots is to communicate with humans. It is desirable for individuals to have as simple access to information as possible, therefore messaging systems have been chosen as convenient platforms for people to utilise for daily communication. A. Market segmentation breakdown by type of chatbot The market for chatbots is classified into three segments.

• Market segmentation based on the type of chatbot o Rules-based chatbot o Artificial intelligence-based chatbot It's worth noting that rule-based chat bots are a subset of chat bots that use artificial intelligence. Rules-based chatbots are typically intended to serve as an interactive FAQ page. They've been trained to identify particular concepts and patterns, allowing them to respond with a set of predetermined responses. Artificial intelligence-based chatbots use complex algorithms to operate like an artificial brain. They understand not just the user's request, but also the context, intention, and emotion, and they continue to "learn" about the user through each individual discussion.
• Segmentation of the market based on the chatbot's user interface o Web-based chatbots o Application-based chatbots
• Chatbot end-user segmentation o News and media o E-commerce o Banking and insurance o Health care o Telecommunications o Gaming and entertainment B. Chatbots are judged based on the following criteria. Does the chatbot react with the requested and proper information? The emphasis is on quantitative outputs, with no consideration given to the quality of the user experience. Accuracy, precision, and reaction are the three measures used. Is the chatbot capable of natural and lengthier discussions due to language principles? It is necessary to have a court of non-numerical expert witnesses.

Is the chatbot engaging and interactive. Qualitative experience is quantified and based on task completion and customer satisfaction surveys, but determining these criteria can be complex and costly. Artificial Intelligence - Does a chatbot behave like a human, and how intelligent is it? Artificial intelligence's "humanity" is determined by observing logical and abstract thought processes.

Survey of chatbots communication in organizations
The data was gathered using a survey. Google's tool, Google Forms, was used to construct it and then evaluate it. The survey was shared with many Faculty of Organizational Sciences student groups on Facebook. The purpose of the survey is to look into respondents' interactions with organisation chatbots, such as their behaviour, expectations, and habits when using various communication channels, particularly the Internet, and then reflect on the benefits and drawbacks of chatbots in comparison to other communication channels, as well as assess how respondents view chatbots. Students, or the most active millennials, were chosen as the target demographic since they are the most frequent users of social media and the Internet in general (pewresearch.org, 2018). At the start of the poll, a few basic demographic questions were asked. The respondents' ages were initially looked at. Because the survey link was posted on the Faculty of Organizational Sciences' Student Groups, it was projected that 97 percent of the responders would be between the ages of 19 and 25. The gender of the responders was the next factor to consider. Females make up somewhat more than two-thirds of the responders, while males make up slightly less than a third. The respondents' occupation was the subject of the third question.

Students account for over 64 percent of responses, while employees account for 32.8 percent. When asked, "How have you communicated with organisations over the past 12 months. 77 percent of respondents said they communicated with them by phone, 56 percent by email, 55 percent via social media (not including chatbots), 43 percent live with some of their employees at their organisations, and 21 percent communicated with a chat bot. Despite the fact that chat bot contact is not particularly common, it is nevertheless more extensive than communication via the website or mobile app. When asked if they start a conversation with an online chat, 62 percent answered they do. The following questions are presented in a textual format. The following step was to select from a list of pre-defined internet difficulties that they had encountered in the previous month. The lack of functioning of the site search option, difficulty accessing important information on the site, and the organization's non-responsiveness to user requests are the most prevalent complaints respondents have with the internet. Users expect a chatbot to obtain a speedy answer (89 percent), reserve a table in a restaurant or café (65 percent), and provide product or service information when they are asked what they think a chatbot would do (58 percent). The next question was about when they would prefer not to speak with the chatbot if they required specific information.

When requested for information, the vast majority of respondents are concerned that the chatbot would provide incorrect information (almost 50 percent of respondents).With 47 percent of respondents, the second most common

response is that they would prefer to interact with a live person. The third most popular response (33 percent) is that they would be annoyed if the chat bot did not speak in a polite manner. Then they had to link the communication channels at the desired response time.

When it comes to instant communication, the chatbot is the most popular option, followed by the phone and mobile apps. When given a 24-hour time frame, however, around 40% of respondents expect a response by email, website, or social media. In terms of responding after more than 24 hours, 20% of respondents expect to respond to the email within that time frame, while 17% expect to answer via the website. After that, individuals had to choose which of the specified benefits they expected to receive while dealing with businesses. Respondents to chatbots demand a quick response to simple questions, availability 24 hours a day, seven days a week, and uncomplicated communication. When it comes to difficult questions and professional responses, a chat bot is not the ideal mode of contact.

Future of Machine Intelligence Techniques in the Financial Markets: A Systematic Analysis
Artificial intelligence is a wide and promising field that assists people in a variety of ways, including medicine, education, telecommunications, finance, and the economy. The financial market is an important component of any country's finances; understanding how it works can significantly enhance the country's economy and, as a result, people's lives. We propose in this paper to provide the most recent research on deep learning techniques applied to the financial market industry, which can assist investors in making informed decisions. This paper contains all recent studies on deep learning algorithms for financial market forecasting, including stock market, stock index, commodities forecasts, and Forex. The main purpose is to identify the most recent models used to solve the prediction issue using RNN, as well as its features and uniqueness. We'll go over every step of the forecasting process, from pre-processing to input features, deep learning approaches, and the metrics used for evaluation.

The practise of forecasting the financial market dates back to the beginning of the stock market's existence. The stock market fluctuates at random and is difficult to forecast. Financial analysts have never ceased looking for new ways to predict this chaotic and nonlinear domain. Analysts tried three different approaches to solve this challenge. Fundamental analysis was the first. The technical analysis, which is based on historical data and some indicators, was the second. Researchers have recently attempted to obtain more valuable features, such as sentiment index, by extracting the trend from historical data and using it in the input features. The third strategy, fundamental analysis and technical analysis [combines the two preceding techniques. The rate of employment, political statistics, and the firm's report are all used in fundamental analysis. Deep learning is challenging to apply directly due to the unstructured nature of the data. Because technical analysis is entirely based on historical data, it is easier to apply Artificial Intelligence (AI) in this arena. To estimate the movement of the market, various methodologies were used. Statistics, machine learning, pattern recognition, sentiment analysis, and hybrid are the five most well-known types

CONCLUSION

After going over the theoretical and practical aspects of marketing, artificial intelligence, and chatbots, as well as their impact on today's marketing practises, one gets the idea that the influence will continue to grow. Humans are already using chatbots, despite the fact that they are rule-based. Artificial intelligence will become more available and studied as technology advances, thus its practical use will expand, and at some point, a large portion of operational communication will be conducted using artificial intelligence-based robots. The results of the poll revealed that the greatest benefit of utilising chatbots in marketing is the providing of basic, quick information, but they also revealed respondents' worry of receiving incorrect information from chatbots, which has to be addressed in the future. Organizations should consider the benefits of artificial intelligence technology and its application to the realm of chatbots, especially in the face of challenges in communicating with customers, but also if they intend to keep up with the lifestyle of a growing consumer, given the aforementioned increase in the growth of artificial intelligence technology and its application to the realm of chatbots.

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RESEARCH ARTICLE

On Pythagorean Fuzzy Rough Continuous Functions and Separation Axioms

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ABSTRACT

Present paper is an concept of Pythagorean fuzzy topology deals with continuous function, separation axioms and obtain of their theorems and properties respectively.
2010 AMS Classification:54A40.

Keywords: Pythagorean fuzzy rough continuous functions, separation axioms, Hausdorff space, regular and normal spaces.

INTRODUCTION

Fuzzy set was introduced by Zadeh. Intuitionistic fuzzy set (IFS) was introduced by Atanassov it deals with condition $o \leq (\mu_{IF}) + (\lambda_{IF}) \leq 1$. Pythagorean fuzzy set (PFS) was introduced by yager [5] it deals with the condition $o \leq (\mu_{PyF})^2 + (\lambda_{PyF})^2 \leq 1$. Fuzzy rough topological spaces was introduced by Anita Shanthi et al. [1,2]. Pythagorean fuzzy rough connected spaces was introduced by Revathi et al.[3].In this article, the concept of Pythagorean fuzzy rough (P yFR) topology deals with continuous function, separation function and obtain their theorems respectively.

PYTHAGOREAN FUZZY ROUGH CONTINUOUS FUNCTIONS:
Definition of PyFR topological space is given in [3].

Definition:
Let (U,PyFR(X), τ) and (U*,PyFR(Y), τ`) be PyFR topological spaces, where

$\tau = \{U, \phi, PyFR(X)\}$, $\tau^{\cdot} = (U^{*}, \phi, PyFR)(Y))$ and

h $= ((f^{\mu}, f^{\lambda}), (g^{\mu}, g^{\lambda}))$: PyFR(X) $\longrightarrow$ PyFR(Y)

where membership (μ) and non- membership (λ)

$$f^{\mu} : \underline{PyFR}(X) \to \underline{PyFR}(Y)$$

$$f^{\lambda} : \underline{PyFR}(X) \to \underline{PyFR}(Y)$$

$$g^{\mu} : \underline{PyFR}(X) \to \underline{PyFR}(Y)$$

$$g^{\lambda} : \underline{PyFR}(X) \to \underline{PyFR}(Y)$$

Are PyFR mappings. Then h is said to be PyFR continuous if the inverse image under h of any PyFR(Y) $\in \tau^{\cdot}$ is a PyFR set PyFR(X) $\in \tau$, i.e. h^{-1} (PyFR(Y)) $\in \tau$,whenever PyFR(Y) $\in \tau^{\cdot}$.

Definition :
Let (U,PyFR, τ) be a PyFR topological space and PyFR(Y$_s$) $\subseteq$ PyFR(X).Then the PyFR topology $\tau_{PyFR} (Y_s) = \{PyFR(Y_s) \bigcap PyFR(O) / PyFR(O) \in \tau\}$ is called PyFR subspace topology and $(PyFR (Y_s), \tau_{PyFR}(Y_s)$ is called PyFR subspace of (U,PyFR(X), τ).

Theorem :Let $(U, PyFR(A), \tau)$ and (U*,PyFR, $\tau^{\cdot}$) be two PyFR topological spaces. Let

$$h((f^{\mu}, f^{\lambda}), (g^{\mu}, g^{\lambda})) : PyFR(X) \to PyFR(Y),$$

$$f^{\mu} : PyFR(X) \to PyFR(Y), f^{\lambda} : PyFR(X) \to PyFR(Y),$$

where $g^{\mu} : PyFR(X) \to PyFR(Y), g^{\lambda} : PyFR(X) \to PyFR(Y)$ are PyFR mappings.

Then the following statements are equivalent:

1.The PyFR function $h((f^{\mu}, f^{\lambda}), (g^{\mu}, g^{\lambda})): PyFR(X) \to PyFR(Y)$ is PyFR continuous.

2.The inverse image of every PyFR closed set is PyFR closed.

3.For each PyFR point neighborhood of h(xPyFR(X)) under h is a PyFR neighborhood of xPyFR(X).

4.For each PyFR point xPyFR(X) in PyFR(X) and each PyFR neighborhood PyFR(B) of h(xPyFR(A)), there is a PyFR neighborhood PyFR(Z) of xPyFR (X) such that h(PyFR(Z)) $\subseteq$ PyFR(Y).

5.h$\overline{PyFR(X)} \subseteq \overline{h(PyFR(A))}$

Proof :
$1) \Rightarrow 2)$ Let h(f,g):PyFR(X) $\to$ PyFR(Y) be PyFR continuous and PyFR(Y) $\in \tau^{\cdot}$. Then $h^{-1}(PyFR(Y)) \in \tau$. PyFR(Y) $\in \tau^{\cdot}$

$\Rightarrow [PyFR(B)]^{c}$ is PyFRclosed in (U,PyFR(Y), $\tau^{\cdot}$).

Again $h^{-1}[PyFR(Y)]^{c} = [h^{-1}PyFR(Y)]^{c}$ and h being PyFR continuous,

$h^{-1}(PyFR(Y)$ is PyFR open in (U,PyFR, τ) .Hence $[h^{-1}PyFR(B)]^{c}$ is PyFR closed in (U,PyFR, τ)

i.e) $h^{-1}(PyFR(B)^{c})$ is PyFR closed in (U,PyFR(Y), τ)

$2) \Rightarrow 3)$

Let PyFR(Z) be a PyFR neighborhood of h(xPyFR(X)).Then there is a PyFR open set PyFR(Y)$\in \tau^{'}$ such that h(xPyFR(X))$\subseteq$PyFR$\subseteq$PyFR(Z).

$$Now, \; xPyFR(X) \in h^{-1}(h\,(xPyFR(X))) \subseteq h^{-1}(PyFR(Y)) \subseteq h^{-1}(PyFR(Z)).$$

$$i.e, xPyFR(X) \in h^{-1}(PyFR(Y)) \subseteq h^{-1}(PyFR(Z)),$$

where $h^{-1}(PyFR(Y))$ is PyFR open in (U,PyFR(Y),τ).

$3) \Rightarrow 4)$

Let $XPyFR(X) \in PyFR(X)$ and PyFR(Y) be PyFR neighborhood of h(xPyFR(X)).Then h^{-1}(PyFR(Y)) is a PyFR neighborhood of xPyFR(X).Thus there exists a PyFR open set PyFR(Z) in PyFR(X) such that,

$$xPyFR(X) \in PyFR(Z) \subseteq h^{-1}(PyFR(Y))\,PyFR(Z) \subseteq h^{-1}(PyFR(Y))$$

$$\Rightarrow h(PyFR) \subseteq h(h^{-1}(PyFR(Y))) \subseteq PyFR(Y)$$

$i.e, h(PyFR(Z)) \subseteq PyFR(Y).$

$4) \Rightarrow 5)$

Since $\overline{h(PyFR\ (X))}$ is PyFR closed in PyFR(Y),

$h^{-1}(\overline{h(PyFR(X))})$ is PyFR closed in PyFR(X).

Thus $h^{-1}(\overline{(h(PyFR(X)))} = \overline{h^{-1}((\overline{h(PyFR(X))})}.$

Now $PyFR(X) \subseteq h^{-1}(h(PyFR(X))) \subseteq h^{-1}((\overline{h(PyFR(X))}),ash\,(PyFR(X) \subseteq \overline{h(PyFR(X))}.$

$\Rightarrow PyFR(X) \subseteq h^{-1}(\overline{(h(PyFR(X)))} = \overline{h^{-1}((\overline{h(PyFR(X))})}$

$\Rightarrow PyFR(X) \subseteq \overline{h^{-1}((\overline{h(PyFR(X))})}$

$\Rightarrow \overline{PyFR(X)} \subseteq \overline{h^{-1}((h(PyFR(X)))}$

$\Rightarrow h\overline{PyFR(X)} \subseteq h(h^{-1}(\overline{h(PyFR(X))})) \subseteq h\overline{PyFR(A)}.$

$i,e, h(\overline{PyFR(X)}) \subseteq \overline{h(PyFR(X))}.$

$5) \Rightarrow 1).$ Let [PyFR(Y)]c be PyFR closed in PyFR(Y) and let PyFR(X)$\in h^{-1}(PyFR(Y)).$

By assumption $h[\overline{h^{-1}(PyFR(Y))}] \subseteq \overline{h[h^{-1}(PyFR(Y)]}$

$i.e, h[\overline{h^{-1}(PyFR(Y))}] \subset \overline{PyFR(Y)} = PyFR(Y).$

$\Rightarrow [\overline{h^{-1}(PyFR(Y))}] \subset [\overline{h^{-1}(PyFR(Y))}] = [h^{-1}(PYFR(Y))]$

$\Rightarrow [\overline{h^{-1}(PyFR(Y))}] = [h^{-1}(PyFR(Y))].$

Therefore, $h^{-1}[PyFR(Y)]$ is PyFR closed in PyFR(X) whenever[PyFR]c is PyFR closed v in PyFR(Y).Let PyFR(Z) be a PyFR open set of PyFR(Y).

$\Rightarrow [PyFR]^{c}$ is PyFR closed in PyFR(Y)

$\Rightarrow [PyFR]^{c}$ is PyFR closed in PyFR(X)

$\Rightarrow h^{-1}(PyFR)$is PyFR open in PyFR(X),whenever PyFR(Z)is PyFR open in PyFR(Y).Therefore h is PyFR continuous.

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Theorem: Let $(U, PyFR(A), \tau)$ and (U*, PyFR τ') be two PyFRTS. If h:PyFR(X)$\rightarrow$PyFR(Y) maps all of PyFR(X) into a single PyFR point (constant)xPyFR(Y)of PyFR(Y).Then h is PyFR continuous .

Proof: Let h: PyFR(X) $\rightarrow$ PyFR(Y)be aPyFR mapping such that f(xPyFR(x))=xPyFR(Y) for every xPyFR(X)$\in$PyFR(X). Consider PyFR(Y) a PyFR open set in PyFR(X).

$$f^{-1}(PyFR(Y)) = \begin{cases} \phi, if\ xPyFR(Y) \notin PyFR(Y) \\ U, if\ xPyFR(Y) \in PyFR(Y) \end{cases}$$

Then U and ϕ are PyFR open in PyFR(X).Therefore $f^{-1}(PyFR(Y))$ is PyFR open in PyFR(X),whenever PyFR(Z) is PyFR open in PyFR open in PyFR(Y).Therefore any constant function is PyFR continuos.

Theorem: Let (U,PyFR,τ)be a PyFRTS. If PyFR(Y$_s$) is a PyFR subspace of PyFR(X),the inclusion function i:PyFR(Y$_s$) $\rightarrow$PyFR(X) is PyFR continuous.

Proof: Let PyFR(Y$_s$)be a PyFR subspace of PyFR(X) and i:PyFR(YS) $\rightarrow$ PyFR(X). Since PyFR(Y$_s$) is a PyFR subspace of PyFR(X),for any PyFR open set PyFR(V) of PyFR(A) ,i^{-1} (PyFR(V))=PyFR(V)$\bigcap$PyFR(Ys) is PyFR open in PyFR subspace to plogy of PyFR(Y$_s$).i^{-1} (PyFR(V)) is PyFR open in PyFR(Ys), whenever PyFR(V) is PyFR open in PyFR(X).Therefore, every inclusion map is PyFR continuous.

Theorem: Let (U,PyFR(X),τ),(U*,PyFR(Y),τ^*) and(U**,PyFR(Y),$\tau^{..}$)be PyFR topological spaces. If h:PyFR(X)$\rightarrow$ PyFR(Y) and j:PyFR(Y) $\rightarrow$PyFR(Z) are PyFR Continuous, then the map $j \circ h : PyFR(X) \rightarrow PyFR(Z)$ is PyFR Continuous.

Proof: Let PyFR(D) be a PyFR open in PyFR(C). As j is PyFR continuous, j^{-1} (PyFR(D)) is PyFR open in PyFR in PyFR(Y).As h is PyFR continuous, $h^{-1}(j^{-1}$(PyFR(D)))=$(j \circ h)^{-1}$ (PyFR(D)) is PyFR open in PyFR(X),whenever PyFR(D) is PyFR open in PyFR(Z).Therefore, composition of two PyFR continuous function is PyFR continuous.

Theorem: Let (U,PyFR(X),τ),(U*,PyFR(Y),τ^*) be a PyFR topological spaces. If h: PyFR(X) PyFR(Y) is PyFR continuous and if PyFR(Y$_s$) is a subspace of PyFR(X),then the restricted function h /PyFR(Y$_s$):PyFR(Y$_s$) $\rightarrow$PyFR(Y) is PyFR continuous.

Proof: Let i:PyFR(Ys) $\rightarrow$ PyFR(X) be the inclusion map of PyFR(Ys)into PyFR(X).i being the inclusion map is PyFR continuous. Given h: PyFR(X) $\rightarrow$PyFR(Y) is PyFR continuous. Therefore it follows that $h \circ i$:PyFR(Ys):PyFR(Ys)$\rightarrow$ PyFR(Y) is PyFR continuous. Hence h/PyFR(Ys):PyFR(Ys) $\rightarrow$PyFR(Y) is PyFR(Y) is PyFR continuous.

Theorem: Let (U,PyFR(X),τ) and (U*,PyFR(Y),τ^*) be a PyFR topological spaces. If h: PyFR(X) $\rightarrow$PyFR(Y) is PyFR continuous and PyFR(Y$_s$) is a PyFR subspace of PyFR(Y) containing the image set h(PyFR(X)),then the function j:PyFR(X) $\rightarrow$PyFR(Y$_s$) obtained by restricting the range of h, is PyFR continuous. If PyFR(Z) has PyFR(Y) as a subspace ,then the function k:PyFR(X) $\rightarrow$PyFR(Y)obtained by expanding the range of h is PyFR continuous.

Proof: Let h:PyFR(X) $\rightarrow$PyFR(Y) be PyFR continuous.
If h(PyFR(X))$\subset PyFR(Y_s) \subset PyFR(Y)$,we show that the function

J:PyFR(X)$\rightarrow PyFR(Y_s)$ obtained from h is PyFR continuous. Let PyFR(F)be PyFR open in $\tau_{PyFR(Y_s)}$,PyFR(F)=PyFR(Ys)$\bigcap$PyFR(E) , for some PyFR open set PyFR(E)of (U*,PyFR(Y),τ^*).

Since h^{-1}(PyFR(E))is PyFR open in $(U^*, PyFR(B), \tau')$ .since h^{-1}(PyFR(E)) is PyFR open in $(U, PyFR(A), \tau)$ h^{-1}(PyFR(E)) = h^{-1}(PyFR(F)),because PyFR(Ys).Therefore j:PyFR(X) $\longrightarrow$ PyFR(Ys) is PyFR continuous. h :PyFR(X) $\longrightarrow$ PyFR(Y)is PyFR continuous and the inclusion map i: PyFR(Y) $\longrightarrow$ PyFR(Z) being the composition of two PyFR continuous functions PyFR continuous.

Theorem :(Pasting lemma)

Let (U,PyFR(X), τ) and (U*,PyFR(Y), τ^*) be a PyFR topological spaces. If f:PyFR(X) $\longrightarrow$ PyFR(Z) and g:PyFR(B) $\longrightarrow$ PyFR(Z) are PyFR continuous mappings, then f and g combine to give a PyFR continuous function h:(PyFR(X),PyFR(Y)) $\longrightarrow$ PyFR(Z),defined by setting

$$h(PyFR(X),PyFR(Y))(y) = \begin{cases} f(PyFR(X))(y) if PyFR(Z) = f(PyFR(X)) \\ g(PyFR(Y))(y) if PyFR(Z) = g(PyFR(Y)) \end{cases}$$

Proof: Consider PyFR(Z) aPyFR open set in τ'.

Then $h^{-1}(PyFR(Z)) = f^{-1}(PyFR(Z)) \bigcup g^{-1}(PyFR(Z))$

$f : PyFR(X) \to PyFR(Z)$ is PyFR continuous and PyFR(Z) is PyFR open in τ'.But PyFR(X) is PyFR open in τ.Therefore f^{-1}(PyFR(Z)) is PyFR open in τ.g:PyFR(Y) $\longrightarrow$ PyFR(Z) is PyFR continuous and PyFR(Z) is PyFR open in τ'.But PyFR(Y)is PyFR open in τ.Therefore g^{-1}(PyFR(Z)) is PyFR open in τ.

Therefore $h^{-1}(PyFR(Z)) = f^{-1}(PyFR(Z)) \bigcup g^{-1}(PyFR(Z))$ is PyFR open in τ.whenever PyFR(Z) is PyFR open in τ'.Therefore h:(PyFR(X),PyFR(Y)) $\longrightarrow$ PyFR(Z) is PyFR continuous.

PYTHAGOREAN FUZZY ROUGH T₀ AND T₁ SPACE

Definition: A PyFRTS (U, PyFR(X), PyFR(Y), τ) is called a PyFR T_0 space if for every pair of distinct PyFR points xPyFR(P), yPyFR(Q),there exists PyFR open set PyFR(X) $\in \tau \ni$ xPyFR(P) $\in$ PyFR(X), yPyFR(Q) $\notin$ PyFR(X) or there exists a PyFR open set PyFR(Y) $\in \tau \ni$ yPyFR(Q) $\in$ PyFR(Y),xPyFR(P) $\notin$ PyFR(B).

Theorem: A PyFR subspace of a PyFR T_0 space is a PyFR T_0 space.

Proof: Let (PyFR(Ys), τ(PyFR(Ys))) be a PyFR subspace of a PyFR T_0 space (U, PyFR(X), τ) and xPyFR(P), yPyFR(Q) be two distinct PyFR points of PyFR(Ys). Then these PyFR points are also in PyFR(X).

⇒∃ a PyFR open set PyFR(Z) ∈ τ containing one PyFR point but not the other.

⇒ PyFR(Ys) ∩ PYFR(Z) is a PyFR open set in τPyFR(Ys) containing one PyFR point but not the other. Hence (PyFR(Ys), τ(Py FR(Ys))) is a PyFR T_0 space.

Definition: A PyFRTS (U, PFR(X), PFR(Y), τ) is said to be a PyFR T1 space if for every pair of PyFR points xPyFR(P), yPyFR(Q) such that xPyFR(P) $\neq$ yPyFR(Q), there exist PyFR open sets PyFR(X) and PyFR(Y) such that xPyFR(P) $\in$ PyFR(X), xPyFR(P) $\notin$ yPyFR(Q) $\in$ PyFR(Y), yPyFR(Q) $\notin$ PFR(X).

PYTHAGOREAN FUZZY ROUGH HAUSDORFF SPACE

Definition: A PyFRTS (U, PyFR(A), PyFR(B), τ) is said to be a PyFR Hausdorff if for every pair of distinct PyFR points xPyFR(P), yPyFR(Q) there exists PyFR(A), PyFR(B) ∈ τ such that, xPyFR(P) ∈ PyFR(A), yPyFR(Q) ∈ PyFR(B) and PyFR(A)$\overline{}^{q}$PyFR(B).

Theorem: A PyFR subspace of a PyFR Hausdorff space is a PyFR Hausdorff space.

Proof: Let (PyFR(Bs), τPy FR(Bs)) be a PyFR subspace of a PyFR Hausdorff space (U, PyFR(X), τ) and xPyFR(P), yPyFR(Q) be two PyFR points of PyFR(Bs). (U, PyFR(X), τ) is a PyFR Hausdorff space. Hence there exists PyFR(C),

PyFR(D) $\in$ τ ,such that xPyFR(P)$\in$PyFR(C),yPyFR(Q) and PyFR(C)$\overline{q}$PyFR(D), It follows that there exist PyFR(Bs) ∩ PyFR(C), PyFR(Bs) ∩ PyFR(D) $\in$ $\tau_{P\,yFR(Bs)}$ such that xPyFR(P) $\in$ PyFR(Bs) ∩ PyFR(C), yPyFR(Q) $\in$ PyFR(Bs) ∩ PyFR(D) and (PyFR(Bs) ∩ PyFR(C)) ∩ (PyFR(Bs) ∩ PyFR(D)= PyFR(Bs)$\overline{q}$PyFR(D). Hence (PFR(Bs), $\tau_{P\,FR(Bs))}$ is a Pythagorean fuzzy rough Hausdorff space.

PYTHAGOREAN FUZZY ROUGH REGULAR AND NORMAL SPACES

Definition: A PyFRTS (U, PyFR(A), PyFR(B), τ) is said to be PyFR regular, if for each pair consisting of a PyFR point xPyFR(P) and a PyFR closed set

[PyFR(E)]c$\ni$ xPyFR(P)$\notin$ [PyFR(E)]^c, there exists PyFR open sets PyFR(A), PyFR(B) $\in$ τ such that xPyFR(P) $\in$ PyFR(A), [PyFR(E)]c $\subseteq$ PyFR(B) and PyFR(A)$\overline{q}$PyFR(B).

Theorem: A PyFR subspace of a PyFR regular space is a PyFR regular.

Proof: Let (PFR(Bs), $\tau_{PyFR(B_s)}$) be a PyFR subspace of a PyFR regular space(U,PFR(X),τ). Let xPFR(P), [PFR(E)]^c be PyFR point and PyFR closed set respectively in $\tau_{PyFR(B_s)}$ such that

$$xPyFR(P) \in PyFR(C), [PyFR(E)]^c \subseteq PyFR(D)$$ and PyFR(C)$\overline{q}$PyFR(D).It follows that there exists

$$PyFR(B_s) \bigcap PyFR(C) \,(PyFR(B^s)), [PyFR(E)]^c \subseteq PyFR(B_s) \bigcap PyFR(D)$$ and

$$(PyFR(B_s) \bigcap PyFR(C)) \bigcap (PyFR(B_s) \bigcap PyFR(D)) = PyFR(B_s)\overline{q}(PyFR(C)\overline{q}PyFR(D)).$$

Hence (PyFR(Bs), $\tau_{PyFR(B_s)}$) is a PyFR regular space.

Theorem: A PyFRTS (U,PyFR(A),PyFR(B), τ) is a PyFR regular if and only if for every PyFR open set PyFR(E) and xPyFR(P) $\in$PyFR(E) there exists PyFR(A) $\in$PyFR(A)$\subseteq \overline{PyFR(A)} \subseteq PyFR(E).$

Proof: Let (U,PyFR(A),PyFR(B), τ) be a PyFR regular space. Hence there exists PyFR(A),PyFR(B)$\in \tau.$
Such that xPyFR(P)$\in$PyFR(A),[PyFR(E)]^c$\subseteq PyFR(B)$ and $PyFR(A)\overline{q}PyFR(B).$Now we have xPyFR(P)
$\in PyFR(A) \subseteq \overline{PyFR(A)} \in PyFR(A) \subseteq \overline{PyFR(A)} \subseteq [PyFR(B)]^c \subseteq PyFR(E).$Hence there exits
PyFR(A)$\in \tau$, such that xPyFR(P) $\in PyFR(A) \subseteq \overline{PyFR(A)} \subseteq PyFR(E).$
Conversely, let [PyFR(E)]^c be a PyFR closed set, xPyFR(P) be a PyFR point such that xPyFR(P)$\notin$ [PyFR(E)]^c. PyFR(E) is a PyFR open set, xPyFR(P)$\in$PyFR(E).Then there exists PyFR(A)$\in \tau,$
$\ni xPyFR(P) \in PyFR(A) \subseteq \overline{PyFR(A)} \subseteq PyFR(E).$
Since $\overline{PyFR(A)} \subseteq PyFR(E)$ and $[PyFR]^c \subseteq [\overline{PyFR(A)}]^c$ and $PyFR(A)\overline{q}[\overline{PyFR(A)}]^c.$
Thus (U,PyFR(A),PyFR(B), τ) is a PyFR regular space.

Definition: A PyFRTS (U, PyFR(A), PyFR(B), τ) is said to be a PyFR normal if for every pair of disjoint PyFR closed sets [PyFR(E)]^c, [PyFR(F)]^c, there exist PyFR open sets PyFR(A),PyFR(B)$\in \tau$),such that[PyFR(E)]^c $\subseteq$PyFR(A), [PyFR(F)]^c $\subseteq$PyFR(B)and PyFR(A)$\overline{q}$PyFR(B)

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Theorem: A PyFRTS(U,PyFR(A),PyFR(B), τ) is PyFR normal $\Leftrightarrow$ for any PyFR closed set [PyFR(E)]c and PyFR open set PyFR(D) containing[PyFR(E)]^{c}, there exists a PyFR open set PyFR(A) $\in \tau$ such that[PyFR(E)]$^{c} \subset PyFR(A)$ and

$$\overline{PyFR(A)} \subset PyFR(D).$$

Proof: Let [PyFR(E)]^{c} and[PyFR(D)]^{c} be disjoint PyFR closed sets.

Let (U,PyFR(A),PyFR(B), τ) is PyFR normal space

$\Leftrightarrow \exists$ disjoint PyFR open sets PyFR(A),PyFR(B)

Such that [PyFR(E)]$^{c} \subset$ PyFR(A) and [PyFR(D)]$^{c} \subset$ PyFR(B)

Now   PyFR(A) $\subset$ [PyFR(B)]^{c}

$\Rightarrow \overline{PyFR(A)} \subset [\overline{PyFR(B)}]^{c} = [PyFR(B)]^{c}.$

Also, [PyFR(D)]$^{c} \subset PyFR(B)$

$\Rightarrow [PyFR(B)]^{c} \subset PyFR(D) \Rightarrow \overline{PyFR(A)} \subset PyFR(D).$

CONCLUSION

In this paper ,we have introduced compactness on Pythagorean fuzzy rough topology with continuous function ,separation axioms and some theorems ,properties with suitable examples. In further study, we will analysis the concepts of compactness for Pythagorean fuzzy rough topology.

ACKNOWLEDGEMENT

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RESEARCH ARTICLE

Use of Vegetables Processing Waste as Fish Feed on the Haematological and Biochemical Parameters of *Oreochromis mossambicus*

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ABSTRACT

An investigation was carried out to study the use of vegetables processing waste as fish feed on the haematological and biochemical parameters of Oreochromis mossambicus. Four Experimental feeds were prepared by replacing the constituents of control feed at 6.25, 12.5, 25, 50 gm/100gmrespectively. Oreochromismossambicus in the control and treatments were the Respective feeds for a period 45 days. On 45th day after haematological Parameters such as RBC,WBC and Hb. biochemical parameters such as serum protein, serum glucose and serum cholesterol Observed. The data obtained were analysed using one way analysis of variance (ANOVA).The Fishes fed with vegetables waste 50gm/100gm feed showed significantly higher (p<0.05%) in RBC, WBC, Hb and serum protein. maximum decrease in serum glucose and serum cholesterol when compared to control.

Keywords: Oreochromis mossambicus, vegetables waste, erythrocyte count, leucocyte count, haemoglobin, protein, glucose, cholesterol.

INTRODUCTION

Aquaculture has been one of the fastest growing agricultural industries worldwide (Hishamunda and Ridler, 2002). In recent years, Aquaculture has been growing at an annual rate of 3.2%. This is mostly Attributed to developments in fish feed manufacture, which is the biggest cost factor, and the use of more productive aquaculture systems (FAO, 2016). If plant sources can be used as a supplement to animal protein sources, it will not only Reduce the production cost and also increases the growth and production (Schieber *et al.,* 2001)in recent research, it has been shown that many plant protein sources has potential protein source in fish feed and was utilized successfully in many

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experimental aquaculture diets (Booth, 2001). Waste streams can include the peel, stems, cores, and skin of fruits, and seeds, husks, bran and straw from cereals. Food waste has traditionally been viewed as an undesirable material to be disposed of, at considerable expense, via landfill or incineration, or used as animal feed. However, such wastes are now increasingly considered to be a promising source of valuable nutraceuticals (Galanakis, 2012).Vegetables are rich in fibres, vitamins and minerals, so their waste is rich in bioactive compounds. These wastes are not being used appropriately, so with this point of view in the present study vegetables processing waste are used as partial replacement in the preparation of fish feed and the study was conducted in *Oreochromis mossambicus*.

Objectives of this study,
- To study the haematological parameters of Oreochromis mossambicus supplemented with fruits and vegetables processing waste.
- To analyse the biochemical parameters of Oreochromis mossambicus supplemented with fruits and vegetables processing waste.
- To recommend the efficient feed farm level.

MATERIALS AND METHODS

O. mossambicus fingerlings were collected from fish farm. They were acclimatized to fresh water condition for 2-3 weeks in the laboratory and transferred to experimental tanks. Aerators were used to raise the dissolved oxygen level. The tanks were maintained neat and clean. Care was taken to avoid contamination. Fishes were fed initially for a week with control feed. The excess of food and faecal matter were cleaned daily in order to prevent fungal and other microbial growth.

Preparation of fish feed
Fish feed was prepared by adding of wheat flour, soybean meal, coconut oil cake in the ratio of 1:2:1 and corn flour was added as a binder. These ingredients were mixed using water and made into soft dough. The dough was dried room temperature for one day. Four experimental feeds were prepared by adding the vegetables processing waste. The pellets were prepared by using domestic appliances with pore size 0.5mm diameter and stored for further use.

Blood sample collection
The blood samples were collected from fish caudal vein by a sterile syringe containing EDTA as an anticoagulant.

Statistical analysis
The data obtained in haematological and biochemical parameters on RBC, WBC, Hb, serum protein, serum glucose and serum cholesterol content were subjected to one way ANOVA.

RESULTS AND DISCUSSION

Haematological parameters
Haematological parameters of fish are used as indicators of their physiology and stress condition (Logambal *et al.,* 2000).Haematological techniques, including RBC, WBC, Hb concentration, have provide an understanding and knowledge for the biologist in the field of aquaculture in assessing the health status of fishes and to observe response of fishes towards stress (Soivio and Oikari, 1976).

Erythrocyte count of *O. mossambicus* fed with different concentration of vegetables processing waste
Erythrocytes or red blood cells count is abundant in the blood. The abundance of erythrocytes in the blood shows the great oxidative potential of fish (Revin *et al.,* 2019). (Table 2) illustrate the total erythrocyte count of *Oreochromis mossambicus* fed vegetables waste at four different concentrations. Maximum erythrocyte count (5.24 ± 0.0781 million cell/cubic mm) was obtained in T_4fishesfed with vegetables waste (50gm/kg of feed) when control showed minimum

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value of ($3.56 \pm 0.1217 \times 10^6$ million cell/cubic mm). The values of erythrocyte count were significantly higher ($p<0.05\%$) in experimental fish when compared to control. These results were supported by Pratheepa and Sukumaran (2014) who reported that *E.hirta* induced erythropoesis in *Cyprinus carpio*. The results were also in accordance with (Sahu *et al.,* 2007),who stated that erythrocyte count was significantly higher in *L.rohita* fingerlings fed with *M.indica* kernel.

Leucocyte count of *O. mossambicus* fed with different concentration of vegetables processing waste
Leucocytes are useful for the assessment of the immune system and variations in the proportion of these cells are considered normal (Dias, 2006). The total Leucocyte count of *Oreochromis mossambicus* fed with vegetables waste at four different concentrations are presented in (**table 2**),Maximum leucocyte count ($9650 \pm 55.677 \times 10^3$ Thousand cells / cubic mm) was obtained in T_4 fishes fed with vegetables waste(50gm/kg of feed) when control showed minimum value of ($6840 \pm 78.102 \times 10^3$ Thousand cells /cubic mm). The values of leucocyte count were statistically significant ($p<0.05\%$) in fish fed with experimental feed when compared to control.

This increase in leucocyte count might be due to the presence of alkaloid and phenol. (Rajaram and Janardhanan, 1991). A significant increase ($P<0.05\%$) in WBC of cat fish brood stock treated with ethanolic extract of *G. cambogia* was observed by (Dada and Ikuerowo, 2009). Similar increase in leucocyte count was observed in common carp fed with feed incorporated with plants like and infected with *Aeromonas hydrophila* (Absali and Mohammad,2010).

Haemoglobin content of *O. mossambicus* fed with different concentration of vegetables processing waste
(**Table 2**),illustrate the haemoglobin content of *Oreochromis mossambicus* fed with fruits and vegetables waste at four different concentrations. Maximum haemoglobin content (11 ± 0.5568 g/dl) was obtained in T_4 fishes fed with vegetables waste (50gm/kg of feed) When control showed minimum value of (9 ± 0.5292 g/dl).The values of haemoglobin count were significantly higher ($p<0.05\%$) in experimental fish when compared to control. Significant increase ($P<0.05\%$) In the level of haemoglobin might be due to the presence and action of chemical constituents in the fruits peel which influenced the binding of iron in the blood. Similar increase in haemoglobin content was observed in common carp fed with feed incorporated with plants materials and infected with *Aeromonas hydrophila* (Absali and Mohammad, 2010). (Shalaby *et al.,*2006) reported that an increased haemoglobin content (Hb) in Nile tilapia after administration of garlic. Feed containing lupin, mango and stinging nettle showed increased haemoglobin content in rainbow trout, *Oncorhynchus mykiss* (Awad, 2010).

Biochemical parameters
Serum biochemical data are very important in observing the health condition of fishes, especially in management programs (Dias and Moraes,2007), The vegetable waste at 4 different concentrations (vegetables waste 6.25, 12.5,25 and 50gm/kg of feed) were given as feed supplement to for a period of 45 days and the serum biochemical parameters such as protein, glucose and cholesterol content were analysed initially before the experiment and after 45 days of treatment. The data obtained for serum biochemical parameters are presented in (table 2)

Serum protein content of *O. mossambicus* fed with different concentration of vegetables processing waste
Proteins are the important constituents of serum that form defensive molecules and help the body against infection. Serum protein is a labile biochemical molecule that reflects the condition of organism and changes under the influence of internal and external factor (Metwally *et al.,* 2009). The total serum protein concentration of *Oreochromis mossambicus* fed with vegetables waste at four different concentrations. The maximum increase in serum protein concentration was found in T_4 fishes (1.82 ± 0.0529 mg/dl) fed with fruits when compared to control (0.48 ± 0.0346 mg/dl).The values of serum protein concentration were statistically significant ($p<0.05\%$) in fish fed with experimental feed when compared to control.

(Misra *et al.,* 2006) stated that increase in total protein might be due to elevated white blood cell counts (WBC) as a major source of serum protein. High protein and globulin in blood plasma indicates high level of safety in fish that is a result of stimulation of leucocytes and secretion of immunoglobulins (Nayak *et al.,* 2014).

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Serum glucose content of *O. mossambicus* fed with different concentration of vegetables processing waste

Glucose is one of the most important energy substrates used by fish to cope up with physiological stress; therefore, plasma glucose levels have been used as an indicator of the stress response. Cortisol and glucose have been reported to increase in teleosts exposed to stress (Mommsen *et al.,* 1999). (Table2),illustrate the serum glucose levels of *Oreochromis mossambicus* fed with fruits and vegetables waste at four different concentrations. Minimum serum glucose level (6 ± 1.7321 mg/dl) was noticed in T_4fish fed with vegetable waste at (50gm %) and control showed maximum values of (36 ± 1.7321 mg/dl). The values of serum glucose level were significantly lower ($p > 0.05\%$) in experimental fish when compared to control.

In our studies, there is a decrease in the glucose level in the T_4 fishes which were fed with 50% concentration of vegetables waste. The decrease in the glucose level in T_4 might be due to the action of vegetable waste under high levels which acted as non-stressors. So, the stress of T_4 fish were avoided by vegetable waste feed. The results of the present study correlate with (Toutou *et al.*, 2019). Whoreported that fishes fed with 2% lemon peel had the lowest glucose values may be because of stress was avoided under high levels of vitamin C (lemon peel).

Serum cholesterol content of *O. mossambicus* fed with different concentration of vegetables processing waste

The concentration of cholesterol in the blood can be influenced by nutrition, activity level, and hepatic activity and can be changed by the sexual cycle of fish. Triglycerides are the major lipids of adipose tissue and are the most important form of fat storage in the body.

The concentration of triglycerides in the blood is influenced by the fat content in the diet and is therefore an important blood component to be evaluated in the use of new diets (Van der Boon *et al.,* 1991). Serum cholesterol level of *Oreochromis mossambicus* fed with vegetable waste at eight different concentrations. The maximum decrease in serum cholesterol level was found in T_4 fish value (82 ± 3.6056 mg/dl) fed with vegetables waste (50gm/kg of feed) when compared to control (168 ± 11.269 mg/dl).The values of serum cholesterol level were statistically significant ($p > 0.05\%$) in fish fed with experimental feed when compared to control. In this present study, the haematological and biochemical parameters of *O.mossambicus* fed with experimental feed were significantly (T_1– T_4) different from the control fishes. The results of present work were supported by the findings of Nahak and Sahu (2014) who stated that *Clarias batrachus* treated with 2.5% and 5% *Ocimum basilicum* incorporated diet for 15 and 30 days of treatment had lower cholesterol content than control. Stated that feeding aquatic organisms with feed containing phytochemical compounds might effect the fat metabolism.

CONCLUSION

Hence it is concluded that *O.mossambicus* fed with vegetables waste at four different concentrations (6.25, 12.5,25,50 gm/kg of feed) for a period of 45 days influenced the haematological and biochemical parameters in all treatments. Feed prepared from vegetables waste at four different concentrations (6.25, 12.5,25,50 gm/kg of feed) is optimum for enhancing the RBC, WBC, Hb, serum protein, reduced in serum glucose and serum cholesterol. Therefore this study is very useful in the field of aquaculture for the production of less expensive and eco-friendly aqua feeds.

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Table-1. Ingredients of experimental feed

Constituents	Wheat flour (gm)	Soya bean meal(gm)	Coconut oil cake (gm)	Vegetables (gm)
Control	25	50	25	-
T_1	23.43	46.87	23.43	6.25
T_2	21.87	43.75	21.87	12.5
T_3	18.75	37.75	18.75	25
T_4	12.5	25	12.5	50

Table: 2Haematological and biochemical parameters of *O. mossambicus* fed with different concentration vegetables waste

TREATMENT	RBC	WBC	HAEMOGLOBIN	SERUM PROTEIN	SERUM GLUCOSE	SERUM CHOLESTOL
Control	3.56 ± 0.121	6840 ± 78.102	9 ± 0.5292	0.48 ± 0.0346	36 ± 1.7321	168 ± 11.2694
T1	4.12 ± 0.529	7880 ± 130.88	10.6 ± 0.4583	1.19 ± 0.0436	12.6 ± 2.886	102 ± 2.6458
T2	4.56 ± 0.121	8350 ± 45.825	11.3 ± 0.3606	1.58 ± 0.0794	12 ± 1.7321	89 ± 14
T3	5.02 ± 0.043	8900 ± 10.0	10.9 ± 0.4583	1.75 ± 0.0361	11 ± 2	86.33± 3.7859
T4	5.24 ± 0.078	9650 ± 55.677	11 ± 0.5568	1.82 ± 0.0529	6 ± 1.7321	82 ± 3.6056

RESEARCH ARTICLE

Synthesis, Biological Evaluation and Molecular Docking of Indole Based 1,3,4-Oxadiazol Derivative

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ABSTRACT

The synthesis of organic compounds depends on the various atoms in their compounds and they are linked in the rings to show various properties encompassing at minimum single atom in organic synthesis of an element apart from carbon molecule. The present study designed to synthesize the heterocyclic compound as one of the derivative of in dole based 1,3,4- oxadiazol using complex pre-materials, characterized by spectroscopic studies like infrared (IR) and nuclear magnetic resonance (NMR) and evaluated their biological activities to determine antioxidant potential and molecular docking studies. A new class of potentially biologically active heterocyclic molecule was synthesized and and exhibited antioxidant activity of compound (E)-N-[5-(1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]-1-(4-methoxyphenyl) methanimine maximum (65.63%) at 250µg/ml concentration showing satisfactory DPPH free radical scavenging potential with the IC50 concentration compared to the standard drug, ascorbic acid with 6ug/ml used for the study and synthesized compound interacts with DNA helix at regions of DT-9, DA-17, DG-16, with binding energies of -8.66 kcal/mol.

Keywords: Heterocyclic, Indole, 1,3,4-Oxadiazole, Antioxidant property, Molecular docking.

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INTRODUCTION

Heterocyclic compounds have significant role to play immense role in medicinal chemistry and are commonly found in huge percentage in bio molecules such as enzymes, vitamins, natural products and biologically active compounds. Five-membered heterocyclic compounds are considered an imperative group of biologically active moieties. 1,3,4-oxadiazoles have been used as precursors in the synthesis of an assortment of pharmaceutical drugs for therapeutic purposes and in synthetic organic chemistry [1]. 1,3,4- oxadiazole is a heterocyclic scaffold used as a key component in a range of therapies including anti-inflammatory, anticonvulsant, anti diabetic, antibacterial, antifungal and anti-HIV drugs, amid others. Because of their various therapeutic qualities [2-5], unsymmetrically disubstituted derivatives of 1,3,4-oxadiazole have anticipated much notice among 1,3,4-oxadiazole derivatives [6, 7].

Likewise, the 1,3,4-oxadiazoles moiety has a long history of pharmacology, as evidenced by its wide range of biological applications, including anticonvulsant, anticancer, anti-inflammatory, antifungal and antibacterial effects [4, 5]. In addition, the synthesis of heterocyclic compounds by means of varied spacer-linkers has involved consideration as an outcome of increasing activity comparing with monoanalogs. More distinctively, the biological activity of heterocyclic compounds and their derivatives have been well premeditated and reported in the literature [8].

In this context current work designed to synthesis of heterocyclic compound name as (E)-N-[5-(1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]-1-(4-methoxyphenyl) methanimine and as preliminary investigation of biological action studied their antioxidant potential and also explored compound molecular docking to know their interaction with DNA.

MATERIALS AND METHODS

Materials
Indole based 1,3,4-oxadiazol derivative were synthesized in Department of Pharmaceutical Chemistry, Karnataka State Akkamahadevi Women's University, Vijayapur, Karnataka, India as per the designed scheme. Mortar, pestle, cell culture plastic flasks ,test tubes, etc use as material for the studies to synthesize.

Experimental Design
Synthesis of Heterocyclic Compound
Compound was synthesized using a literature procedure [8] semicarbazide hydrochloride (50 mg, 1 mmol) were mixed with sodium acetate (50 mg). Following this, an indole-3-carbaldehyde (1 mmol) was added. And then mixing is continued until to get smooth paste. The crude semi Carbazone get separates out when cold water is added to the mixture and then it is recrystallized from solvent ethanol.

***In Vitro* Antioxidant Study**
DPPH Free Radical Scavenging Assay
DPPH assay was carried out as described by Blois [9] method. The reaction mixture was well mixed and incubated at room temperature for 30 min and the absorbance was recorded at 517 nm. The control was prepared by adding 2 ml of DPPH solution and 1 ml of methanol [10]. The IC_{50} value was determined by using linear regression equation i.e.
$Y = Mx + C$
where, Y = 50, M and C values were derived from the linear graph trend line.
% Scavenged [DPPH] = [(AC−AS)/AC] ×100
Where AC is the absorbance of the control and
AS is the absorbance in the presence of the sample of extracts or standard.

Molecular Docking Studies

Docking studies was initialized using Auto Dock Vina 1.1.2. by doing synchronize files of protein and its respective compounds in PDBQT format [11]. To compound, torsions were place and the grid box was fixed at the dimension of 25×21×25 and grid center at 15.1267, 21.4908, 8.8193 fitting around the active pocket of 1BDNA and grid parameter file was written. The Auto Dock Vina 1.1.2., files were prepared based on a precise compound by adding up its individual parameters. Finally, docking calculations were done to attain the best conformational binding energy. Further docked molecules interaction patterns were visualized in Py MOL [12]. As illustrated in Table.1

This is useful in developing potential drug candidates and understanding binding nature. Auto Dock Vina 1.1.2. is the most version which has been widely used for virtual screening, due to its enhanced docking speed [13]. Its default search function is based on Lamarckian Genetic Algorithm (LGA), a hybrid genetic algorithm with local optimization that uses a parameterized free energy scoring function to estimate the binding energy [14]. The main objective of the study is to define in silico activity of novel in dole based 1,3,4-oxadiazol derivative.

RESULTS AND DISCUSSION

DPPH Free Radical Scavenging Activity

The novel in dole based 1,3,4-oxadiazol derivative exhibited a significant concentration dependent inhibition of DPPH scavenging activity. A concentration-dependent assay was carried out with the synthesized compound and the results were presented in Graph 1. Among five graded concentrations were used in the study along with blank, cell control and standard control. Synthesized Compound showed scavenging activity as 24.67%, 38.37%, 46.35%, 58.34% and 65.63% inhibition at 15.62, 31.25, 62.50, 125.00 and 250.00 μg/ml concentrations respectively. On the other hand, standard ascorbic acid showed 52.80% inhibition. The inhibitory concentration (IC$_{50}$) value of the novel indole based 1,3,4-oxadiazol derivative showed 50.23μG/mL against the DPPH (Graph 1).

The outcome of the synthesized molecules reveals the competence as an antioxidant molecule exhibited. Results of the DPPH radical assay of the compound showed is capability to a snatch the chain mechanism of the free radicle potentially with inhibition. It exhibited 65.63% inhibition, proved that to it is capable to form a stable free radical. The synthesized molecules bearing the pi donating and lone pair of electrons exhibited better anti-oxidant property. Similar compound described by Bala et al, [15] and reported for their antibacterial activity synthesized derivatives was correlated with their physicochemical and structural properties by QSAR analysis using computer assisted multiple regression analysis and four sound predictive models were generated with good R2, R adj 2, and Fischer statistic.

Characterization of Synthesized Heterocyclic Compound
(E)-N-[5-(1H-Indol-3-yl)-1,3,4-Oxadiazol-2-yl]-1-(4-Methoxyphenyl) Methanimine

M.P. 129 °C; yield 79%; FT-IR (KBr, ν cm^{-1}): 3348 in dole (NH), 3168 amine (N–H), 3046 aromatic (C–H), 2954-2867 aliphatic (C–H), 1675(C=O). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ (ppm): 10.8 (bs, 1H, -NH, in dole), 9.2 (s, 1H, -CH=N), 8.6 (d, 1H, indoleAr-H), 8.14 (d, 1H, Ar-H), 7.84 (dd, 2H, Ar-H), 7.54 (d, 1H, Ar-H), 7.38 (dd, 2H, Ar-H), 7.24 (t, 1H, Ar-H), 7.02 (t, 1H, Ar-H), 3.84 (s, 3H, -O-CH$_3$) Anal. Calculated for (C$_{18}$H$_{14}$N$_4$O$_2$) C, 67.91; H, 4.43; N, 17.60. Found (%):C, 67.98; H, 4.54; N, 17.67. ESI-MS (m/z): 319.33 (M+H) $^+$

Molecular Docking Studies

For the prediction of the desired configuration of the compounds with the DNA helix of newly constructed N- [(4-methoxyphenyl), methyl]-5-(1H-indol-3-yl)-1,3,4-oxadiazol-2-amine molecule Docking was performed with the DNA sequence d(CGCGAATTCGCG)$_2$dodecamer (PDB ID:1BNA). Synthesized compound interacts with DNA helix at regions of DT-9, DA-17, DG-16, with binding energies of -8.66 kcal/mol. Similarly, the derivatives with potent antibacterial activity were subjected to molecular docking studies to investigate the interactions between the active

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derivatives and amino acid residues existing in the active site of peptide deformylase to assess their antibacterial potential as peptide deformylase inhibitor [16].

CONCLUSION

In this article, we discuss the synthesis of a compound indole based 1,3,4-oxadiazol, its biological action and molecular docking to know their interactions. At the molecular level, the docking experiments demonstrate 1BNA's inhibitory property binding energies of -8.99. Because of the compound is having lower energy, it is stable one. Due to its strong antioxidant potential we explore various other biological properties. In future we are planning to study on anticancer and cardiovascular diseases in in vivo condition.

ACKNOWLEDGMENT

Conflict of Interest
The authors declare no conflict of interest.

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Table 1: The binding scores (kcal/mol) of compounds-DNA docking complex along with RMSD values and compounds interactions with specific nucleotides for prioritization of the compound.

Rank	Binding energy	Inhibition constant	cIRMS	RefRMS	Ligand efficiency	Hbonds
1	-8.99	256.47	0.0	23.91	-0.41	1, DA6
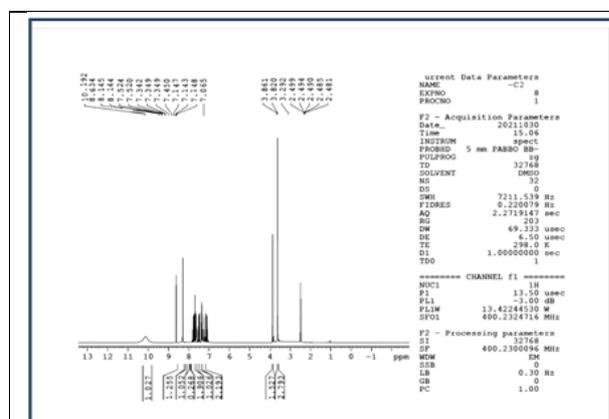
**Figure 1: NMR Spectra:(E)-N-[5-(1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]-1-(4-methoxyphenyl) methanimine**
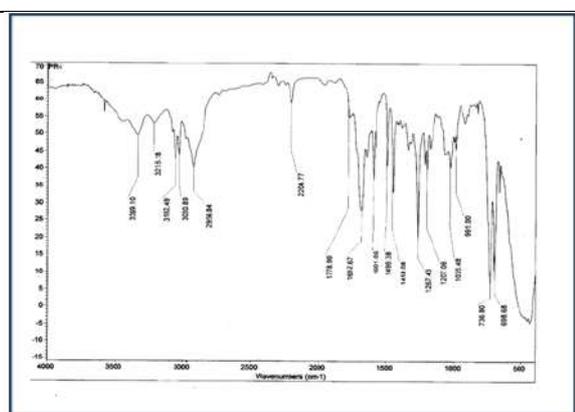

Figure 2: IR Spectra:(E)-N-[5-(1H-indol-3-yl)-1,3,4-oxadiazol-2-yl]-1-(4-methoxyphenyl) methanimine
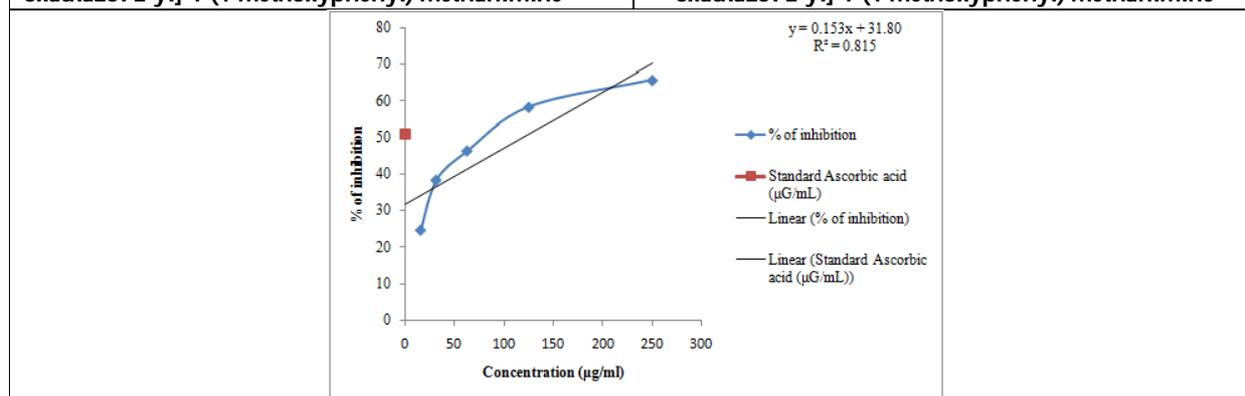
Graph 1: Scattered graph showing the % of inhibition of indole based 1,3,4-oxadiazol derivative against the DPPH and the inhibitory concentration (IC$_{50}$ Value) observed is 50.23μg/mL.

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RESEARCH ARTICLE

COVID-19 Pandemic: Analysis of Impediments of Online Classes by Single-Valued Neutrosophic Environment

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ABSTRACT

Impediments of online classes during the COVID 19 pandemic situation is analysed among the students of age group 17-18 by conducting a survey of the neutrosophic environment in terms of a single-valued neutrosophic environment using multi-criteria decision-making, the many choices and attributes are ranked by using the TOPSIS method. The short comings, suggestions, recommendations and improvements were discussed and analysed using neutrosophic sets with a single value.

Keywords: Neutrosophic sets, Single-valued neutrosophic sets, pandemic, MCDM, TOPSIS.

INTRODUCTION

Smarandache [8] proposed the neutrosophic set (NS), which is a generalisation of fuzzy sets by Zadeh [14] and the fuzzy intuitionistic sets by Atanassov [1]. Neutosophy is a philosophical branch that examines the origin, nature, and scope of the environment using a range of logical and linguistic attributes. Further, in order to solve certain real-time imprecise, vague, in deterministic decision making situations, truth membership degree ($\tilde{T}$), indeterminacy

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membership degree ($\tilde{I}$), and falsehood membership degree ($\tilde{F}$) characterise the proposed single-valued neutrosophic set (SVNS) by Smarandache and Wang et al. [12]. Mutiple attribute decision making (MADM) situations have wider applications in managerial sciences, optimization techniques, medical sciences, industrial sector, urban planning, etc. It is not always possible to get the crisp numbers to evaluate a survey, based on the complexity of situations and attributes Hwang and Yoon [4] devised a method for determining order preference based on similarity to the ideal solution (TOPSIS). Chen [3] improved the TOPSIS approach for solving MADM in a fuzzy environment. This paper includes preliminaries of NS and SVNS, research methodology and survey for the data were discussed, followed by linguistic variables and TOPSIS method. Impediments of online classes during the pandemic situation (COVID-19) is analysed by taking the survey and the linguistic data has been converted in terms of single valued neutrosophic values. Results were discussed and lead to the conclusion.

PRELIMINARIES

This section provides a brief review about neutrosophic and single-valued neutrosophic sets were discussed.

NEUTROSOPHIC SET [NS]

Definition 1.[2] "Let U be the Universe of Discourse, and x denote a generic element in X. In X, the Neutrosophic Set (NS) A is an object with the following frame :

$\hat{A} = \left\{ \left(x, \hat{T}_{\hat{A}}(x), \hat{I}_{\hat{A}}(x), \hat{F}_{\hat{A}}(x) \right) ; x \in X \right\}$, whereas $\hat{T}_{\hat{A}}(x) : X \to]^{-}0, 1^{+}[$,

$\hat{I}_{\hat{A}}(x) : X \to]^{-}0, 1^{+}[$, $\hat{F}_{\hat{A}}(x) : X \to]^{-}0, 1^{+}[$ and $^{-}0 \leq \hat{T}_{\hat{A}}(x) + \hat{I}_{\hat{A}}(x) + \hat{F}_{\hat{A}}(x) \leq 3^{+}$, where $\hat{T}_{\hat{A}}(x)$ is the truth membership function, $\hat{I}_{\hat{A}}(x)$ is the indeterminacy membership function, $\hat{F}_{\hat{A}}(x)$ is the falsity membership function".

Definition 2.[2] "$\hat{A}^{C}$ is the complement of a neutrosophic set $\hat{A}$ and is defined as
$\hat{T}_{\hat{A}}^{C}(x) = 1^{+} - \hat{T}_{\hat{A}}(x)$, $\hat{I}_{\hat{A}}^{C}(x) = 1^{+} - \hat{I}_{\hat{A}}(x)$, $\hat{F}_{\hat{A}}^{C}(x) = 1^{+} - \hat{F}_{\hat{A}}(x)$ for all x ∈ X" .

Definition3.[2] "A neutrosophic set $\hat{B}$ contains a neutrosophic set $\hat{A}$, $\hat{A} \subseteq \hat{B}$ iff
$\inf\hat{T}_{\hat{A}}(x) \leq \inf\hat{T}_{\hat{B}}(x)$, $\sup\hat{T}_{\hat{A}}(x) \leq \sup\hat{T}_{\hat{B}}(x)$, $\inf\hat{I}_{\hat{A}}(x) \geq \inf\hat{I}_{\hat{B}}(x)$,
$\sup\hat{I}_{\hat{A}}(x) \geq \sup\hat{I}_{\hat{B}}(x)$, $\inf\hat{F}_{\hat{A}}(x) \geq \inf\hat{F}_{\hat{B}}(x)$, $\sup\hat{F}_{\hat{A}}(x) \geq \sup\hat{F}_{\hat{B}}(x)$ for all x ∈ X".

SINGLE-VALUED NEUTROSOPHIC SETS [SVNS]

A neutrosophic set is a specific case of SVNS. It is used in a variety of fields, notably engineering, healthcare, industrial engineering, natural science, and indeed the military. The basic definitions, operations, and features of SVNSs are described in this section.

Definition 4. [10] "Let X be a set which is non-empty set. In X, the SVNS $\tilde{A}$ is defined as follows:
$\tilde{A} = \{\langle x, \tilde{T}_{\tilde{A}}(x), \tilde{I}_{\tilde{A}}(x), \tilde{F}_{\tilde{A}}(x)\rangle | x \in X\}$, where $\tilde{T}_{\tilde{A}}(x) : X \to [0,1]$; $\tilde{I}_{\tilde{A}}(x) : X \to [0,1]$; $\tilde{F}_{\tilde{A}}(x) : X \to [0,1]$ and $0 \leq \tilde{T}_{\tilde{A}}(x) + \tilde{I}_{\tilde{A}}(x) + \tilde{F}_{\tilde{A}}(x) \leq 3$, where $\tilde{T}_{\tilde{A}}(x)$ is the truth membership function, $\tilde{I}_{\tilde{A}}(x)$ is the indeterminacy membership function, $\tilde{F}_{\tilde{A}}(x)$ is the falsity membership function".

Definition5. [5] "A SVNS $\tilde{A}$ complement is represented by $\tilde{A}^{C}$ and is defined as
$\tilde{T}_{\tilde{A}}^{C}(x) = \tilde{F}_{\tilde{A}}(x)$, $\tilde{I}_{\tilde{A}}^{C}(x) = 1 - \tilde{I}_{\tilde{A}}(x)$ and $\tilde{F}_{\tilde{A}}^{C}(x) = \tilde{T}_{\tilde{A}}(x)$ for all x ∈ X.
$\tilde{A}^{C} = \{x, \tilde{F}_{\tilde{A}}(x), 1 - \tilde{I}_{\tilde{A}}(x), \tilde{T}_{\tilde{A}}(x) : x \in X\}$".

Definition 6. [5] "If $\tilde{T}_{\tilde{A}}(x) \leq \tilde{T}_{\tilde{B}}(x)$, $\tilde{I}_{\tilde{A}}(x) \geq \tilde{I}_{\tilde{B}}(x)$ and $\tilde{F}_{\tilde{A}}(x) \geq \tilde{F}_{\tilde{B}}(x)$ for all x ∈ X then a single-valued neutrosophic set $\tilde{A}$ is contained in the other SVNS $\tilde{B}$, $\tilde{A} \subseteq \tilde{B}$".

Definition 7. [13] "If $\tilde{A} \subseteq \tilde{B}$ and $\tilde{B} \subseteq \tilde{A}$, Two SVNSs $\tilde{A}$ and $\tilde{B}$ are equivalent written as
$\tilde{A} = \tilde{B}$".

Definition 8. [6] "Let $\tilde{x}_j = (\tilde{t}_j, \tilde{\imath}_j, \tilde{f}_j)$ be a set of SVNS and $\tilde{W} = (\tilde{w}_1, \tilde{w}_2, \dots \tilde{w}_n)^T$ denote the related weighting vector. The Single Valued Neutrosophic Weighted Average (SVNWA) operator of $\tilde{a}_j$ is defined as follows:

$\text{SVNWA}(\tilde{a}_1, \tilde{a}_2, \dots \tilde{a}_n) = \sum_{j=1}^{n} \tilde{w}_j \tilde{a}_j = \left(1 - \prod_{j=1}^{n}(1 - \tilde{t}_j)^{\tilde{w}_j}, \prod_{j=1}^{n}(\tilde{\imath}_j)^{\tilde{w}_j}, \prod_{j=1}^{n}(\tilde{f}_j)^{\tilde{w}_j}\right)$, where $\tilde{w}_j$ is the element j of the weighting vector, $\tilde{w}_j \in [0,1]$ and $\sum_{j=1}^{n} \tilde{w}_j = 1$".

Definition 9. [5] "Let $\tilde{x}_j = (\tilde{t}_j, \tilde{\imath}_j, \tilde{f}_j)$ be a set of SVNS and $\tilde{W} = (\tilde{w}_1, \tilde{w}_2, \dots \tilde{w}_n)^T$ denote the related weighting vector. The Single Valued Neutrosophic Geometric (SVNWG) operator of a_j is as follows:

$\text{SVNWG}(\tilde{a}_1, \tilde{a}_2, \dots \tilde{a}_n) = \prod_{j=1}^{n}(\tilde{a}_j)^{\tilde{w}_j} = \left(\prod_{j=1}^{n}(\tilde{t}_j)^{\tilde{w}_j}, 1 - \prod_{j=1}^{n}(1 - \tilde{\imath}_j)^{\tilde{w}_j}, 1 - \prod_{j=1}^{n}(1 - \tilde{f}_j)^{\tilde{w}_j}\right)$, where $\tilde{w}_j$ is the element j of the weighting vector, $\tilde{w}_j \in [0,1]$ and $\sum_{j=1}^{n} \tilde{w}_j = 1$".

Definition 10. [9] "A Single-Valued Neutrosophic Number (SVNN) $\tilde{A} = (\tilde{t}, \tilde{\imath}, \tilde{f})$, is a particular case of an SVNS on the set of real numbers $\mathcal{R}$, with $\tilde{t}, \tilde{\imath}, \tilde{f} \in [0,1]$ and
$\tilde{t} + \tilde{\imath} + \tilde{f} \leq 3$".

Definition 11. [9, 2] " Let $\tilde{x}_1 = (\tilde{t}_1, \tilde{\imath}_1, \tilde{f}_1)$ and $\tilde{x}_2 = (\tilde{t}_2, \tilde{\imath}_2, \tilde{f}_2)$ be two SVNNs, then:
(i) $\tilde{x}_1 \oplus \tilde{x}_2 = (\tilde{t}_1 + \tilde{t}_2 - \tilde{t}_1\tilde{t}_2, \tilde{\imath}_1\tilde{\imath}_2, \tilde{f}_1\tilde{f}_2)$
(ii) $\tilde{x}_1 \otimes \tilde{x}_2 = (\tilde{t}_1\tilde{t}_2, \tilde{\imath}_1 + \tilde{\imath}_2 - i_1 i_2, \tilde{f}_1 + \tilde{f}_2 - \tilde{f}_1\tilde{f}_2)$
(iii) $\alpha\tilde{x}_1 = \left(1 - (1 - \tilde{t}_1)^\alpha, \tilde{\imath}_1{}^\alpha, \tilde{f}_1{}^\alpha\right), \alpha > 0$
(iv) $\tilde{x}_1{}^\alpha = (\tilde{t}_1{}^\alpha, \tilde{\imath}_1{}^\alpha, 1 - (1 - \tilde{f}_1)^\alpha, \alpha > 0$
(v) $\tilde{x}_1 \cup \tilde{x}_2 = (\max(\tilde{t}_1 . \tilde{t}_2), \min(\tilde{\imath}_1, \tilde{\imath}_2), \min(\tilde{f}_1, \tilde{f}_2)$, for all $x \in X$.
(vi) $\tilde{x}_1 \cap \tilde{x}_2 = (\min(\tilde{t}_1 . \tilde{t}_2), \max(\tilde{\imath}_1, \tilde{\imath}_2), \max(\tilde{f}_1, \tilde{f}_2)$, for all $x \in X$.
(vii) $\tilde{x}_1 = \tilde{x}_2$ iff $\tilde{x}_1 \subseteq \tilde{x}_2$ and $\tilde{x}_1 \supseteq \tilde{x}_2$ for all $x \in X$".

Definition 12. [6] "Consider that $\tilde{x}_1 = (\tilde{t}_1, \tilde{\imath}_1, \tilde{f}_1)$ is an SVNN. The SNNN score function $\tilde{S}$ is based on the degree of truth membership, degree of indeterminacy membership and degree of falsity membership function is defined by
$\tilde{S}(\tilde{x}) = \frac{1 + \tilde{t}_1 - 2\tilde{\imath}_1 - \tilde{f}_1}{2}$ where $\tilde{S}(\tilde{x}) \in [-1,1]$".

Distance between the two SNNSs:
Definition 13. [2] "Let the two SVNSs are
$\tilde{A} = \left\{\left(\tilde{T}_{\tilde{A}}(x_1), \tilde{F}_{\tilde{A}}(x_1), \tilde{I}_{\tilde{A}}(x_1)\right), \dots \left(\tilde{T}_{\tilde{A}}(x_n), \tilde{F}_{\tilde{A}}(x_n), \tilde{I}_{\tilde{A}}(x_n)\right) : x_1, \dots x_n \in X\right\}$ and
$\tilde{B} = \left\{\left(\tilde{T}_{\tilde{B}}(x_1), \tilde{F}_{\tilde{B}}(x_1), \tilde{I}_{\tilde{B}}(x_1)\right), \dots \left(\tilde{T}_{\tilde{B}}(x_n), \tilde{F}_B(x_n), \tilde{I}_{\tilde{B}}(x_n)\right) : x_1, \dots x_n \in X\right\}$.
The following is the description of the Euclidean distance between $\tilde{A}$ and $\tilde{B}$:

$$D(\tilde{A}, \tilde{B}) = \sqrt{\sum_{i=1}^{n} \left\{\left(\tilde{T}_{\tilde{A}}(x_i) - \tilde{T}_{\tilde{B}}(x_i)\right)^2 + \left(\tilde{I}_{\tilde{A}}(x_i) - \tilde{I}_{\tilde{B}}(x_i)\right)^2 + \left(\tilde{F}_{\tilde{A}}(x_i) - \tilde{F}_{\tilde{B}}(x_i)\right)^2\right\}}$$

And here is how the normalised Euclidean distance between the two SVNSs is evaluated:

$$D^N(\tilde{A}, \tilde{B}) = \sqrt{\frac{1}{3n}\sum_{i=1}^{n} \left\{\left(\tilde{T}_{\tilde{A}}(x_i) - \tilde{T}_{\tilde{B}}(x_i)\right)^2 + \left(\tilde{I}_{\tilde{A}}(x_i) - \tilde{I}_{\tilde{B}}(x_i)\right)^2 + \left(\tilde{F}_{\tilde{A}}(x_i) - \tilde{F}_{\tilde{B}}(x_i)\right)^2\right\}}$$ "

MATERIALS AND METHODS

In this section, the steps involved in the evaluation of the survey data are proposed.
Step 1: Analysing the survey data for the problem.
Step 2: Preparing the questionnaire for the survey.
Step 3: Collecting the data and grouping it.
Step 4: Converting the data in terms of SVNNs.
Step 5: Implementing the MCDM technique.
Step 6: Ranking the attributes.

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Data were collected by questionnaire, by using the Google Forms which was sent to the respondents through the WhatsApp groups. The respondents are the students of age group 17-18 years of both genders. The survey is conducted on the Panimalar Engineering College students who were admitted in the academic year 2021-22 to different engineering departments like Computer Science and Engineering(CSE), Electronics and Communication Engineering (ECE), Information Technology(IT), Electrical and Electronics Engineering (EEE), Civil Engineering (CIVIL), Computer Science and Business Systems(CSBS), Computers and Communication Engineering(CCE).

The students belong to different board of education like Central Board of Secondary Education, State Board mode of education, Indian Certificate of Secondary Education of different social and economic status and the parents of different livelihood like employed, self-employed and others.
The survey questions are listed below:

Survey Questions
A1. Are you satisfied with your marks which you got in 12 th grade?
A2. With your 12 th marks, are you satisfied of what you got into your preference of your college education?
A3. Were you satisfied with a gadget you used to handle your online classes during COVID-19 pandemic?
A4. Does your network connection was satisfactory during online classes.
A5. The money you spent monthly for your network charges was satisfactory?
A6. Are you satisfied with the amount of money you spent for your gadgets.
A7. Did the electricity supply was satisfactory during online classes.
A8. The network connection was satisfactory during online classes.
A9. Whether you were satisfied with the online classes without any disturbances on both the ends. (Noise of TV, music, vehicle, siblings, family members...)
A10. Did you satisfy with your mathematics classes online?
A11. Whether the Physics classes were satisfactory.
A12. Whether the Chemistry classes were satisfactory.
A13. Did the Laboratory Sessions were satisfactory.
A14. Did the Language classes were satisfactory.
A15. Whether you satisfied with your assignment works which you uploaded online.
A16. Are you satisfied with the study material you got it from your teachers.
A17. Did the teachers approach was satisfactory.
A18. Whether your psychological stress were satisfactory during online classes.
A19. Whether your physical stress (headache, eye pain, ear ache, backache) were satisfactory during online classes.
A20. Are you satisfied with the fee concession given by the schools?

LINGUISTIC VARIABLES AND TOPSIS METHOD
Linguistic variables are the expressions of our thoughts and decisions in terms of natural and artificial language. For instance, or attribute ratings are represented in terms of linguistic variables such as " Highly Satisfied", "Moderately Satisfied", "Satisfied", "Less Satisfied" and "Poorly Satisfied". These linguistic variables are represented in terms of SVNNs.

Algorithm
Step 1: Define a problem in terms of SVNNs
Step 2: Formulate the problem by MCDM with different attributes and alternatives using TOPSIS SVNNs.
Step 3: Linguistic variables are formulated for the alternatives and attributes.
Step 4: SVNNs are allotted to the linguistic variables.
Step 5: The score function is used to generate SVNNs into fuzzy numbers.

$$\tilde{S}(\tilde{x}) = \frac{1 + \tilde{t} - 2\tilde{\imath} - \tilde{f}}{2}$$

Step 6: TOPSIS SNNNs method is applied.

Step 7: By ranking method, results have been discussed to arrive at the conclusion.

TOPSIS METHOD IN NEUTROSOPHIC ENVIRONMENT
Ching-Lai Hwang and Yoon created TOPSIS (Technique for Order Preference by Similarity to Ideal Solution) in 1981 [4], which was further improved by Yoon in 1987 and Hwang, Lai, and Liu in 1993. It's an aggregation method that evaluates a set of alternatives by determining weights for each criterion, normalising the score, and then using geometric distance between each option and the ideal structure. TOPSIS chooses the option that is the most similar to the ideal best solution and the most dissimilar to the ideal worst solution. This method is well suited to all practical approaches to MCDM problem solving.

The TOPSIS approach is described in the following manner. Let $\tilde{A} = \{\tilde{A}_1, \tilde{A}_2, \tilde{A}_3, \dots \tilde{A}_m\}$ be the possible alternatives set, $\tilde{C} = \{\tilde{C}_1, \tilde{C}_2, \tilde{C}_3, \dots \tilde{C}_n\}$ and $\widetilde{D} = \{\tilde{d}_{ij}\}, i = 1,2, \dots m, j = 1,2, \dots n$, respectively, be the result with the criterion weight. $\widetilde{W} = \{\widetilde{w}_j, j = 1,2, \dots n\}$.

Algorithm for TOPSIS SVNNs Method :
1. Create the SVNNs choice matrix based on the characteristics' and alternatives' responses.

$$\widetilde{D} = \begin{bmatrix} (\tilde{T}_{11}, \tilde{I}_{11}, \tilde{F}_{11}) & (\tilde{T}_{12}, \tilde{I}_{12}, \tilde{F}_{12}) & \dots & (\tilde{T}_{1n}, \tilde{I}_{1n}, \tilde{F}_{1n}) \\ (\tilde{T}_{21}, \tilde{I}_{21}, \tilde{F}_{21}) & (\tilde{T}_{22}, \tilde{I}_{22}, \tilde{F}_{22}) & \cdots & (\tilde{T}_{2n}, \tilde{I}_{2n}, \tilde{F}_{2n}) \\ \cdots & \cdots & & \cdots \cdots \\ (\tilde{T}_{m1}, \tilde{I}_{m1}, \tilde{F}_{m1}) & (\tilde{T}_{m2}, \tilde{I}_{m2}, \tilde{F}_{m2}) & \dots & (\tilde{T}_{mn}, \tilde{I}_{mn}, \tilde{F}_{mn}) \end{bmatrix}$$

2. To determine the number of decision makers α:
Create a group of decision makers to scale the evaluations according to their own preferences. It is calculated by

$\alpha\tilde{x}_1 = \left(1 - (1 - \tilde{t}_1)^\alpha, \tilde{\iota}_1{}^\alpha, \tilde{f}_1{}^\alpha\right)$, if $\alpha > 0$ and
$\alpha\tilde{x}_1 = (0, 1, 1)$, if $\alpha = 0$.

3. To find the score value for SVNNs : $\tilde{S}(\tilde{x}) = \frac{1 + \tilde{t} - 2\tilde{\iota} - \tilde{f}}{2}$

4. Normalize the matrix: $\tilde{X}_{ij} = \frac{X_{ij}}{\sqrt{\sum_{j=1}^n X_{ij}{}^2}}$

5. Calculate the weighted normalized matrix $\tilde{V}_{ij} = \tilde{X}_{ij} * w_j$
6. Calculate the ideal best($\tilde{V}_j{}^+$) and ideal worst($\tilde{V}_j{}^-$) values for each of the attributes
7. For Beneficial Criteria: Ideal best will be the maximum of the values.
   Ideal worst will be the minimum of the values.
8. For Non-Beneficial Criteria: Ideal best will be the minimum of the values.
   Ideal worst will be the maximum of the values.

9. Calculate the Euclidean distance from the ideal best $\tilde{S}_i^+ = \sqrt{\sum_{j=1}^m (\tilde{V}_{ij} - \tilde{V}_j^+)^2}$

10. Calculate the Euclidean distance from the ideal worst $\tilde{S}_i^- = \sqrt{\sum_{j=1}^m (\tilde{V}_{ij} - \tilde{V}_j^-)^2}$

11. Calculate the performance score. $\tilde{P}_i = \frac{\tilde{S}_i^-}{\tilde{S}_i^+ + \tilde{S}_i^-}$

12. Based on the performance score, rank the alternatives.

Illustrative example
Our whole world underwent the pandemic situation in the year 2019 due to the deadly virus COVID-19. Everyone was locked up at their homes. Among the various sectors, imparting education to the children was very much challenging for the academicians, teachers and especially the learners who are the students. We conducted a survey by creating thequestionnaire and sending it to the students to analysis their state of nature which includes financially, emotionally, physically and psychologically. By using TOPSIS SVNNs technique we ranked the different attributes of the survey.

RESULTS AND DISCUSSIONS

This survey gives us a deep understanding of what the students felt about the online classes which they under went for their higher secondary school education and also helped to analyze their psychological and emotional stress and also the challenges and difficulties they faced during the online education. Based on their ranking preference, we arrived at the following conclusions:

- Most of the students were not highly satisfied with their 12 th grade marks, only the CCE girls were found satisfactory.
- Only students of EEE, Civil, CCE girls, were found that they got into their preference of their higher education underwent by the online classes.
- Students of all streams are pretty much satisfied with the gadget they used to handle the online classes during the pandemic situation.
- ECE, EEE, CSBS boys were not bit satisfied of the network connection they had for their online classes.
- Boys students of Civil department, followed by boys of CCE and also girls of CCE, ECE and CSE were found that they had tough time to spend money for the network charges monthly.
- Most of the students, especially girls of ECE, CCE, boys of CCE, EEE, Civil, ECE were not satisfied with the amount they spent for their gadget.
- The students of IT- Boys followed by EEE-Boys, CSBS-Girls, ECE-Boys, CSE- Boys and Girls were not much satisfied with the electricity supply they had in their locality during online classes.
- Almost most of the students were convinced with their network connection, only few of them especially girls of ECE and CSBS department found bit lagging.
- Students were able to manage to attend their online classes without any disturbances like noise of Television, music, vehicles, siblings, family members etc. except few of the girls from ECE and CSBS and also few of the boys of IT and Civil found bit disturbed.
- Only girls of CCE and Civil and also boys of CCE were found difficult to manage with mathematics classes whereas other students were found comfortable.
- Online physics classes were found bit challenging for girls of CCE followed by girls of Civil then boys of CSBS and girls and boys of IT and EEE department students.
- It seems clearly that most of the students found difficult in chemistry classes especially girls of CCE followed by CSBS-Girls, CSE-Girls & Boys, CCE-Boys, Civil-Girls, It-Girls, ECE-Boys, Civil-Boys and IT-Boys.
- CSE- Boys, ECE-Girls, IT-Boys, CSBS-Boys and Girls are well satisfied with the laboratory sessions, whereas IT-Girls, Civil-Girls,-Boys and CSE-Girls are not pretty satisfied with the laboratory sessions.
- Students of ECE-Boys and CCE-Boys are satisfied with the language classes, whereas other students have not given the positive feedback.
- Girls of ECE, EEE and CCE are satisfied with the assignment work they have uploaded online, whereas other students are not most satisfied.
- Study materials given by the teachers were satisfied by only Civil and CCE-Boys and Girls, whereas others found it was not satisfactory.
- Girl students of Civil and CSBS are well satisfied with their teachers approach rather than that they found tough time with their teachers.
- While analyzing the psychological stress among the students, EEE, Civil, CSBS, and CCE-Girls and also boys of CCE had psychological stress, whereas others are pretty well able to balance their stress.
- Students of CSE-Boys and Girls, ECE-Boys, IT-Boys and EEE-Boys were able to manage the physical stress, other than these all others had a more physical stress like headache, eye pain, ear ache, backache during their online classes.
- CSE-Boys, EEE and Civil-Girls and CCE- Boys and Girls were not satisfied with the fee concession given by the students, whereas others are pretty much satisfied with the fee concession given by the school.

CONCLUSION

While analyzing the impediments of online classes, students have faced some shortcomings, it has been discussed below:

- Most of them felt that offline classes were much motivating and interesting than the online classes.
- Lack of concentration and laziness as the students were exposed to the electronic gadget for long period of time.
- It was stressful as the internet connection gets cut-off, some of them faced power shut-downs especially during Saturdays.
- They felt that there should be adequate amount of break timings in between the classes.
- There was very less interaction between the students and teachers. They missed the practical knowledge which will be taught in schools during physical mode.
- During online exams, they were very panicked as there will be network issues, power shutdowns.
- Students felt that teaching should have been more interesting, as sometimes they felt very monotonous way of teaching.
- Some of the students had back-ache, irritation in eyes, mental stress as the environment was noisy and also, they felt disturbed by their family members.

While analysing all the issues TALK AND CHALK is the best and right mode of education at all levels.

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Table 1: Linguistic variables in terms of SVNNs

Linguistic Variables	SVNNs
Highly Satisfied	(0.90, 0.10, 0.10)
Moderately Satisfied	(0.80, 0.20, 0.15)
Satisfied	(0.50, 0.40, 0.45)
Less Satisfied	(0.35, 0.60, 0.70)
Poorly Satisfied	(0.10, 0.80, 0.90)

Table 2: Survey data for Computer Science and Engineering Department (Boys & Girls)

	COMPUTER SCIENCE AND ENGINEERING (CSE)									
	BOYS (93)					GIRLS (43)				
	HS	MS	S	LS	PS	HS	MS	S	LS	PS
A1	12	14	51	15	1	5	11	18	6	3
A2	19	17	47	6	4	8	7	20	8	0
A3	19	12	41	16	5	7	7	19	5	5
A4	22	9	32	22	8	7	5	15	9	7
A5	11	9	30	29	14	4	2	12	19	6
A6	15	5	41	19	13	5	3	15	14	6
A7	16	8	51	13	5	7	2	17	11	6
A8	16	8	42	16	11	4	3	19	11	6
A9	23	12	34	18	6	6	7	15	18	7
A10	19	6	41	20	7	4	5	20	8	6
A11	19	7	44	16	7	8	4	19	6	6
A12	23	10	42	14	4	7	7	21	5	3
A13	15	8	38	13	19	2	5	19	9	8
A14	22	12	47	9	3	8	6	21	5	3
A15	18	20	45	5	5	7	3	21	10	2
A16	24	15	44	8	2	13	4	20	5	1
A17	23	8	51	7	4	9	6	20	7	1
A18	12	8	35	20	18	6	2	18	8	9
A19	9	9	32	18	25	4	2	14	11	12
A20	8	3	38	19	25	5	3	16	11	8

Table 3: Survey data for Electronics and Communication Engineering Department (Boys & Girls)

	ELECTRONICS AND COMMUNICATION ENGINEERING(ECE)									
	BOYS (99)					GIRLS (88)				
	HS	MS	S	LS	PS	HS	MS	S	LS	PS
A1	8	18	62	7	4	6	11	55	16	0
A2	14	15	60	7	3	13	10	55	7	3
A3	9	14	55	12	9	11	9	43	19	6
A4	8	13	50	18	10	7	10	36	24	11
A5	6	7	46	20	20	2	6	36	29	15
A6	6	9	53	22	9	1	5	45	29	8

A7	10	11	53	20	5	4	4	53	16	11
A8	9	8	49	24	9	5	3	45	24	11
A9	16	13	47	12	11	10	10	45	12	11
A10	10	12	46	21	10	10	7	38	20	13
A11	10	12	51	15	11	9	8	40	22	9
A12	10	13	54	12	10	10	10	43	16	9
A13	7	7	46	17	22	5	5	47	15	16
A14	17	9	54	8	11	13	7	51	10	7
A15	14	11	58	9	7	7	8	49	15	9
A16	12	21	58	6	2	10	10	53	12	3
A17	18	16	55	6	4	17	10	52	8	1
A18	10	5	46	22	16	4	4	40	28	12
A19	13	5	35	18	28	2	2	31	32	21
A20	8	6	40	22	23	5	6	48	17	12

Table 4: Survey data for Information Technology Department (Boys & Girls)

INFORMATION TECHNOLOGY										
	BOYS (80)					**GIRLS (75)**				
	HS	**MS**	**S**	**LS**	**PS**	**HS**	**MS**	**S**	**LS**	**PS**
A1	7	8	52	11	2	9	11	45	10	0
A2	10	7	51	9	3	8	11	46	9	1
A3	13	6	38	16	7	10	10	40	11	4
A4	10	10	30	18	12	8	8	36	17	6
A5	6	5	35	19	15	5	6	37	18	9
A6	3	2	40	24	11	2	6	42	16	9
A7	8	9	45	16	2	6	6	50	8	5
A8	7	8	36	23	6	7	4	41	16	7
A9	10	10	35	21	4	9	13	36	13	4
A10	5	11	33	21	10	6	3	43	18	5
A11	8	14	33	21	4	5	5	51	11	3
A12	9	12	34	21	4	6	4	54	8	3
A13	11	6	32	15	16	8	1	33	17	16
A14	20	10	37	9	4	9	10	49	5	2
A15	12	11	39	15	3	5	13	47	7	3
A16	17	14	35	11	3	11	7	51	3	3
A17	14	15	43	5	3	13	9	48	3	2
A18	6	7	36	20	11	4	5	41	15	10
A19	8	7	29	22	14	1	3	34	20	17
A20	5	4	33	23	15	3	3	46	10	13

Table 5: Survey data for Electrical and Electronics Engineering Department (Boys & Girls)

ELECTRICAL AND ELECTRONICS ENGINEERING(EEE)										
	BOYS (86)					**GIRLS (44)**				
	HS	**MS**	**S**	**LS**	**PS**	**HS**	**MS**	**S**	**LS**	**PS**
A1	8	13	59	6	0	2	7	28	6	1
A2	11	15	49	5	6	3	5	23	8	5
A3	11	9	43	15	8	7	8	23	3	3
A4	9	10	42	18	7	6	6	6	20	6

A5	6	5	34	28	13	4	2	18	8	12
A6	4	5	51	19	7	4	3	20	10	7
A7	8	7	55	12	4	6	7	24	5	2
A8	4	6	46	18	12	6	4	21	8	5
A9	10	11	41	15	9	4	7	21	6	6
A10	6	6	38	22	14	2	7	18	9	8
A11	9	9	44	16	8	5	9	17	11	2
A12	17	8	38	14	9	6	9	17	8	4
A13	11	4	39	17	15	2	5	20	12	5
A14	18	7	47	7	7	8	10	21	5	0
A15	13	6	48	11	8	5	6	21	7	5
A16	18	7	52	5	4	10	11	16	6	1
A17	15	8	55	5	3	9	7	21	7	0
A18	10	4	50	12	10	2	1	24	9	8
A19	9	4	34	23	16	2	1	16	13	12
A20	11	5	37	18	15	4	1	22	10	7

Table 6: Survey data for Civil Engineering Department (Boys & Girls)

	CIVIL ENGINEERING(CIVIL)									
	BOYS (14)					GIRLS				
	HS	MS	S	LS	PS	HS	MS	S	LS	PS
A1	1	1	12	0	0	0	2	4	1	0
A2	1	0	12	0	1	0	3	4	0	0
A3	1	1	6	3	3	0	2	5	0	0
A4	1	1	9	0	3	1	2	3	1	0
A5	1	0	9	1	3	1	1	4	0	1
A6	2	0	6	5	1	1	1	4	1	0
A7	1	1	9	2	1	1	0	4	1	1
A8	1	1	9	1	2	1	0	4	2	0
A9	2	1	7	2	2	1	0	4	2	0
A10	1	1	6	1	5	0	1	3	2	1
A11	1	1	7	2	3	0	1	3	2	1
A12	1	1	9	1	2	0	2	3	1	1
A13	1	1	9	0	3	0	1	3	1	2
A14	1	2	8	1	2	0	2	3	1	1
A15	2	0	8	2	2	0	2	4	1	0
A16	2	2	9	1	0	1	3	3	0	0
A17	3	0	10	0	1	2	1	4	0	0
A18	1	2	8	2	1	0	1	3	2	1
A19	3	0	7	2	2	0	0	4	3	0
A20	2	1	8	2	1	0	2	3	2	0

Table 7: Survey data for Computer Science and Business Systems Department (Boys & Girls)

	COMPUTER SCIENCE AND BUSINESS SYSTEMS(CSBS)									
	BOYS (70)					GIRLS (46)				
	HS	MS	S	LS	PS	HS	MS	S	LS	PS
A1	9	7	45	9	0	1	5	31	7	2
A2	11	11	37	7	4	4	6	28	5	3

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A3	12	8	28	15	7	7	4	20	10	5
A4	9	10	31	14	6	3	2	22	15	4
A5	4	4	31	17	14	2	2	16	16	10
A6	6	5	34	16	9	2	5	19	14	6
A7	8	6	38	14	4	3	1	31	8	3
A8	7	7	29	19	8	3	2	21	17	3
A9	13	13	25	13	6	4	5	23	11	3
A10	5	14	32	13	6	2	4	18	10	12
A11	8	11	34	15	2	4	3	18	17	4
A12	6	16	38	8	2	3	4	28	10	1
A13	9	8	26	11	16	4	4	17	13	8
A14	14	12	38	2	4	4	6	29	5	2
A15	13	10	35	8	4	0	11	24	8	3
A16	15	18	30	5	2	7	6	26	5	2
A17	16	11	31	10	2	4	5	32	4	1
A18	7	3	32	16	12	1	2	20	14	9
A19	8	1	24	19	18	1	1	14	14	16
A20	5	3	36	12	14	2	4	14	11	15

Table 8: Survey data for Computer and Communication Engineering Department (Boys & Girls)

COMPUTER AND COMMUNICATION ENGINEERING(CCE)										
	BOYS (25)					GIRLS (22)				
	HS	MS	S	LS	PS	HS	MS	S	LS	PS
A1	5	0	18	2	0	1	3	11	5	2
A2	2	6	13	3	1	1	4	12	2	3
A3	1	6	7	8	3	3	0	10	8	1
A4	3	3	11	2	6	4	2	6	5	5
A5	1	3	8	5	8	0	1	11	6	4
A6	0	2	11	7	5	0	1	9	10	2
A7	2	3	13	5	2	3	3	9	5	2
A8	2	4	10	8	1	1	2	7	8	4
A9	3	2	11	5	4	0	3	8	8	3
A10	3	1	12	5	4	0	0	11	7	4
A11	3	3	11	7	1	0	1	13	6	2
A12	4	2	10	7	2	0	1	13	6	2
A13	4	1	7	8	5	0	2	5	10	5
A14	5	5	8	6	1	0	2	16	3	1
A15	6	2	12	1	4	1	1	14	5	1
A16	5	4	12	3	1	2	3	11	6	0
A17	4	5	13	3	0	2	4	11	5	0
A18	2	4	9	7	3	1	0	8	10	3
A19	2	1	10	8	4	0	0	5	10	7
A20	2	1	14	4	4	0	0	11	8	3

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Table 9: Score value of SVNNs

CSE-BOYS					
	HS	MS	S	LS	PS
A1	1	1	1	0.9964	-0.7
A2	1	1	1	0.8568	-0.0657
A3	1	1	1	0.9976	0.08183
A4	1	1	1	0.9997	0.4017
A5	1	1	1	0.99998	0.7272
A6	1	0.9995	1	0.9992	0.6908
A7	1	1	1	0.992	0.08183
A8	1	1	1	0.9976	0.6003
A9	1	1	1	0.9989	0.2065
A10	1	0.9999	1	0.9995	0.312
A11	1	0.99999	1	0.9976	0.312
A12	1	1	1	0.9946	-0.0657
A13	1	1	1	0.992	0.8505
A14	1	1	1	0.9594	-0.241
A15	1	1	1	0.7802	0.08183
A16	1	1	1	0.9385	-0.45
A17	1	1	1	0.9063	-0.0657
A18	1	1	1	0.9995	0.8319
A19	1	1	1	0.9989	0.4962
A20	1	0.7343	1	0.9992	0.4962

Table 10: Normalized matrix for SVNNs

CSE-BOYS					
	HS	MS	S	LS	PS
A1	0.223607	0.226235	0.223607	0.229248	-0.33159
A2	0.223607	0.226235	0.223607	0.19713	-0.03112
A3	0.223607	0.226235	0.223607	0.229525	0.038763
A4	0.223607	0.226235	0.223607	0.230008	0.190284
A5	0.223607	0.226235	0.223607	0.230072	0.344473
A6	0.223607	0.226122	0.223607	0.229893	0.32723
A7	0.223607	0.226235	0.223607	0.228236	0.038763
A8	0.223607	0.226235	0.223607	0.229525	0.284361
A9	0.223607	0.226235	0.223607	0.229824	0.097819
A10	0.223607	0.226213	0.223607	0.229962	0.147794
A11	0.223607	0.226233	0.223607	0.229525	0.147794
A12	0.223607	0.226235	0.223607	0.228834	-0.03112
A13	0.223607	0.226235	0.223607	0.228236	0.40288
A14	0.223607	0.226235	0.223607	0.220736	-0.11416
A15	0.223607	0.226235	0.223607	0.179506	0.038763
A16	0.223607	0.226235	0.223607	0.215927	-0.21316
A17	0.223607	0.226235	0.223607	0.208519	-0.03112
A18	0.223607	0.226235	0.223607	0.229962	0.394069
A19	0.223607	0.226235	0.223607	0.229824	0.235049
A20	0.223607	0.166125	0.223607	0.229893	0.235049

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Table 11: Ideal best, ideal worst and performance ratings of SVNNs

CSE-BOYS									
	HS	**MS**	**S**	**LS**	**PS**	$\tilde{S}_i^+$	$\tilde{S}_i^-$	$\tilde{P}_i$	**Rank**
A1	0.16771	0.03394	0.01342	0.00688	-0.0033	0.00735	0.00914	0.55443	19
A2	0.16771	0.03394	0.01342	0.00591	-0.0003	0.00445	0.00952	0.68138	16
A3	0.16771	0.03394	0.01342	0.00689	0.00039	0.00364	0.00986	0.73035	11
A4	0.16771	0.03394	0.01342	0.0069	0.00190	0.00213	0.01053	0.83199	7
A5	0.16771	0.03394	0.01342	0.00690	0.00345	0.00058	0.01137	0.95115	3
A6	0.16771	0.03392	0.01342	0.00690	0.00327	0.00076	0.01126	0.93700	4
A7	0.16771	0.03394	0.01342	0.00685	0.00039	0.00364	0.00986	0.73022	12
A8	0.16771	0.03394	0.01342	0.00689	0.00284	0.00119	0.01102	0.90290	5
A9	0.16771	0.03394	0.01342	0.00690	0.00098	0.00305	0.0101	0.76803	10
A10	0.16771	0.03393	0.01342	0.00690	0.00148	0.00255	0.01032	0.80182	8
A11	0.16771	0.03394	0.01342	0.00689	0.00148	0.00255	0.01032	0.80183	8
A12	0.16771	0.03394	0.01342	0.00687	-0.0003	0.00434	0.00962	0.68907	14
A13	0.16771	0.03394	0.01342	0.00685	0.00403	0.00001	0.01172	0.99532	1
A14	0.16771	0.03394	0.01342	0.00662	-0.0011	0.00518	0.00936	0.64376	17
A15	0.16771	0.03394	0.01342	0.00539	0.00039	0.00395	0.00975	0.71191	13
A16	0.16771	0.03394	0.01342	0.00648	-0.0021	0.00618	0.00916	0.59731	18
A17	0.16771	0.03394	0.01342	0.00626	-0.0003	0.00439	0.00954	0.68504	15
A18	0.16771	0.03394	0.01342	0.00690	0.00394	0.00001	0.01167	0.99250	2
A19	0.16771	0.03394	0.01342	0.00690	0.00235	0.00168	0.01076	0.86502	6
A20	0.16771	0.02492	0.01342	0.00690	0.00235	0.00917	0.00587	0.39003	20
$\tilde{V}_j^+$	0.16771	0.03394	0.01342	0.00690	0.004029				
$\tilde{V}_j^-$	0.16771	0.02492	0.01342	0.00539	-0.00332				

Table 12: Overall ranking of all the departments.

	CSE-B	CSE-G	ECE-B	ECE-G	IT-B	IT-G	EEE-B	EEE-G	CIVIL-B	CIVIL-G	CSBS-B	CSBS-G	CCE-B	CCE-G
A1	19	14	17	19	20	18	20	14	14	11	19	18	19	6
A2	16	20	19	16	17	16	16	4	20	9	13	10	10	7
A3	11	13	14	13	8	9	11	12	6	15	7	2	18	9
A4	7	3	10	5	5	5	13	2	12	2	10	8	9	1
A5	3	10	4	14	2	3	5	6	19	5	2	6	17	14
A6	4	6	13	20	9	10	15	1	15	3	5	7	20	15
A7	12	11	16	7	19	6	17	11	8	8	11	16	6	2
A8	5	7	12	8	10	4	6	3	10	6	6	11	7	5
A9	10	2	7	6	11	8	8	20	2	6	8	9	1	11
A10	8	5	9	2	7	7	4	8	9	16	8	4	14	19
A11	8	9	6	9	11	11	10	10	7	16	14	5	5	16
A12	14	15	11	9	11	12	9	7	11	13	16	14	3	16
A13	1	12	3	1	1	19	3	9	12	19	1	1	13	12
A14	17	16	9	12	14	14	14	16	16	13	18	12	4	13
A15	13	17	15	9	15	13	12	5	17	11	12	20	12	8
A16	18	19	20	15	16	15	18	13	1	4	17	12	8	3
A17	15	18	18	18	18	17	19	15	18	1	15	15	11	4
A18	2	8	5	4	6	2	7	19	4	16	4	17	12	10
A19	6	4	1	17	4	20	1	18	15	20	20	19	15	18
A20	20	1	2	3	3	1	2	17	3	10	3	3	16	20

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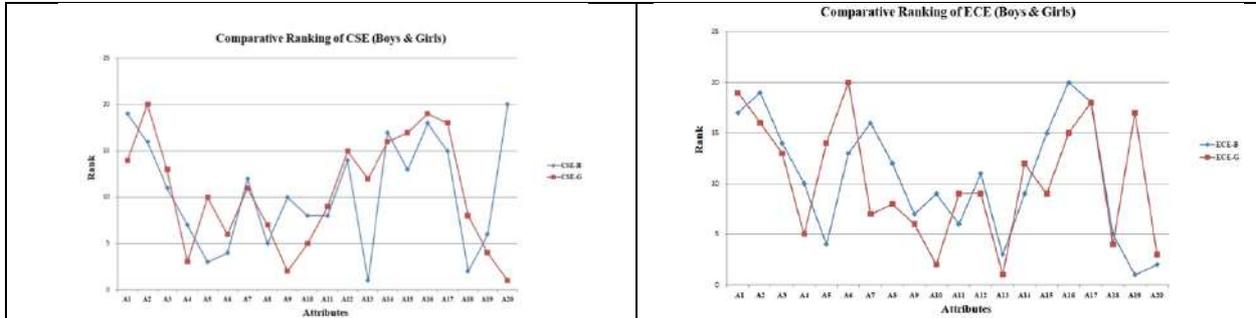
**Figure 1. Comparative Ranking of CSE (Boys & Girls)**


Figure 2. Comparative Ranking of ECE (Boys & Girls)
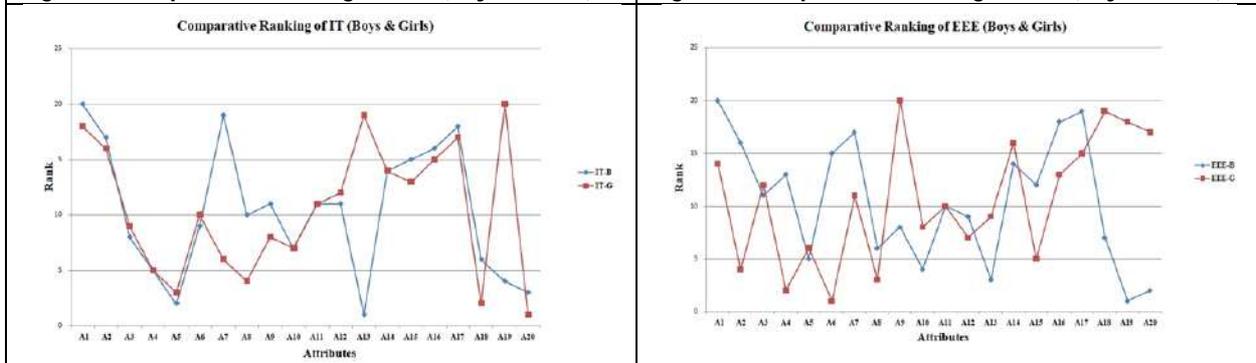

Figure 3. Comparative Ranking of IT (Boys & Girls)


Figure 4. Comparative Ranking of EEE (Boys & Girls)
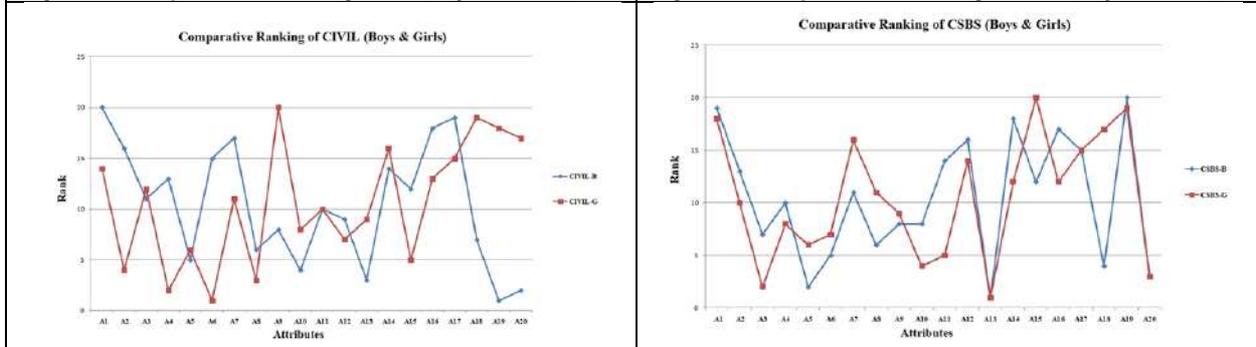

Figure 5. Comparative Ranking of CIVIL (Boys & Girls)


Figure 6. Comparative Ranking of CSBS (Boys & Girls)
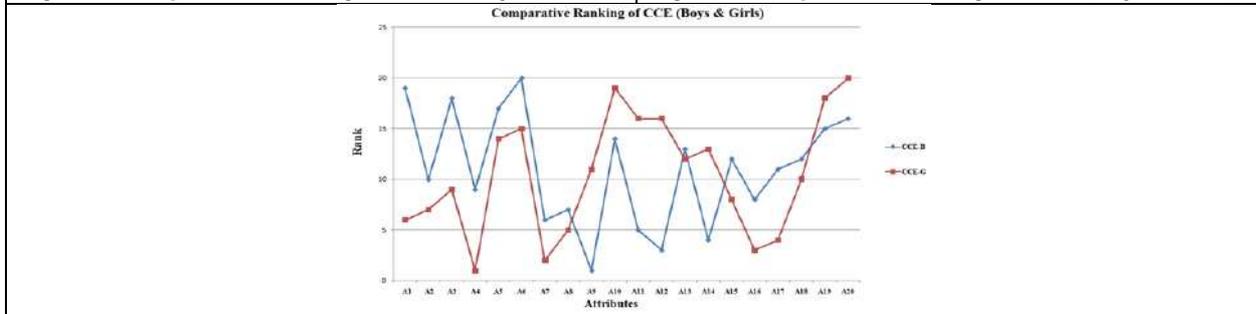
Figure 7. Comparative Ranking of CCE (Boys & Girls)

RESEARCH ARTICLE

Mapping Scientific Literature Trend on Sustainable Development Goals at Global Perspectives: A Scientometric Insight

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ABSTRACT

The purpose of this manuscript is to find related SDG literature variation in features such as readability scores, publication rates, the effect of title format, and multi authorship over 22 years. Sustainable development goals used as a keyword to search terms of titles, abstracts, and keywords, by searching the sources listed in the SCI, SSCI, and A&HCI databases. The 17780 scientific papers were scanned between 2000 and 2021 and later scientometric analysis was performed using the Bib excel and R studio software. The study critically examines RGR, authorship pattern, degree of collaboration, collaborative index, time series, and predominant areas of research in SDG. Major findings reveal that the maximum AGR was recorded in the year 2015 (59.23). The Doubling Time increases from 0.89 in 2001 to 4.40 in 2004. the result also found based on citations received by authors, Reed MS received the highest citations of 2005 and Holling CS citations of 1836. Most of the articles have been published by the USA, the UK, and China on SDG research. The effects of field empirical findings have been thoroughly discussed, and further analysis is proposed to better visualize this assessment.

Keywords: Scientometric, Web of Science, Annual Growth Rate, Relative Growth Rate, Doubling Time.

INTRODUCTION

Scientometric is the study of quality and impact measurement of research, understanding citation processes, scientific mapping domains, and the use of indicators in management, sociology of science, met science, information systems, information science, and research policy [1-2].The frequency of published articles and citations is a well-known application for assessing the output of research and quality [3].

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In recent decades, the field of scientrometric has grown in prominence. All of the research is documented and preserved in massive databases. Scientometric analysis is used to generate quantitative results from such documented knowledge. These studies determine the growth of knowledge in various fields [4]. Scientmetric is a field of research that focuses on the study of research activities such as authors, keywords, citations, and institutions in a published database[5]. It is a useful tool for determining a country's or territory's research pattern [6]. Moreover, scientmetricanalysis can analyze a huge number of documents simultaneously, both methodically and quantitatively [7]. They can highlight research hotspots and identify literature trends by exploring the time, source, and regional distribution of the research. As a result, scientmetricanalyses are frequently researchers to assist novice scholars in a discipline in quickly grasping the scope of a subject [8].

The first step in performing a scientometric study is to choose the optimal data source that matches our research area's scientific coverage [9]. It's worth noting that while there are several bibliographic databases such as PubMed, INSPEC, Chemical Abstract, LISA, Sociological Abstract, and EM base, etc., not all of them include information that makes it easy for scientometric analysis. Web of Science, Scopus, Cochrane database, Google Scholar, Microsoft Academic, and Dimensions are the main bibliographic databases used in the scientometric analysis [10-11-12]. We have to use the Web of Science database for the manuscript in scientometric analysis [13]. This study mainly analyses the research publication trend of SDG research indexed in Web of Science during the period 2000 to 2021.

This study carried out based on the following objectives
▪ To analyze the Annual Growth Rate from 2000-2021 in SDG research.
▪ To determine the degree of collaboration and collaborative index of SDG research.
▪ To examine the distribution of the journals according to Bradford's law.
▪ To find out the major contributions by authors, sources, and countries in SDG research through the R studio tool.

LITERATURE REVIEW
Our literature review covers the latest challenges in SDG literature. Few of the literature the work are reviewed, in a study conducted by Turcea & Ion [14], they studied the value of the research and emphasized the importance of the UN's goals (17), such as no health and wellness, quality education, no poverty, no hunger, gender equality and so on. The purpose of this paper is to demonstrate the importance of sustainable development by examining it in literature and the daily media. Armitage et al .[15] analyzed mapping scholarly papers related to sustainable development goals. The goal of this study considered to find scholarly documents based on SDG targets to discover academic publications linked to SDG 1, SDG 2, SDG 3, SDG 7, SDG 13, and SDG 14. Bautista-Puig et al.[16] identified indexed in Web of Science by using search terms for titles, abstracts, and keywords. The results reveal the growing participation of these organizations in this study (from 660 organizations in 2000-2005 to 1744 organizations involved in 2012-2017).

Olawumi& Chan [17] observed the global trend and structure of sustainability research from 1991 to 2016 based on scientometric analysis, utilizing approaches such as co-authorship, co-wording, co-citation, clustering, and geographical analysis. The study's research power networks and topographic map were created using a total of 2094 bibliographic records from the Web of Science database. Benavides et al.[18] found 102 scientific papers published between 2015 and 2020 from the WoS and Scopus databases. Furthermore, they investigated the development of networks for the selected papers using bibliographic coupling analysis. Petrushenko et al.[19] used the VOS viewer tool to identify important patterns such as dynamics by year, nation, authors, subject area, and so on as well as clusters that graphically depict the data. They also found 8 clusters from the Scopus database and 11 clusters in the WoS database with the search term "sustainable development". Meschede [20] identified the corresponding sources, study topics, affiliated nations, thematic foci, and funding acknowledgment availability. The result found based on publications, almost a third of all articles are international publications, meaning they were co-authored by authors from multiple countries. Zhu & Hua[21] used Cite Space to find and visualize cited references and keyword networks, as well as the distribution of categories and countries and highly cited references in SD literature.

The United States and the United Kingdom hold leading positions, whereas China has the biggest number of publications. Keyword analysis reveals the most lucrative terms using BC 9 CB as a metric, as well as citation maps and observable hot study regions. Sweileh[22] concluded the study will benefit researchers, institutes, governments, funding agencies, and policymakers related SDGs literature. In the realm of SDGs, countries in Africa, the Middle East, and Southeast Asia need to enhance their funding and research partnerships.

METHODOLOGY

Data source and search strategies

We searched all types of documents (articles, review articles, reviews, editorial materials, early access, proceedings papers, meeting abstracts, book chapters, etc.) published by all languages during the study period 2000–2021 in the field of sustainable development goals from Web of Science database. The scientometric data was chosen and downloaded from the Clarivate Analytics' Web of Science database (https://www.webofscience.com/). The database covers more than 8500 notable journals of science and technology across 156 research fields, with new records being updated daily [23]. It is the most often utilized data source for assessing scientific achievements. We selected the Web of Science Core Collection, which included Science Citation Index (SCI), Social Science Citation Index (SSCI), and Arts & Humanities Citation Index (A&HCI) articles to focus on SDG literature[24].

The process for obtaining data on the publications of sustainable development goals. Searches were done in the fields, Topic "sustainable development goals" and Publication Date "2000-01-01 to 2021-12-31" was retrieved using WoS analytical tools. The data was scanned on January 11, 2022, the metadata of the 17780 articles was found, between 2000 and 2021 (publication date). Most of them are articles published in 29976 different sources.

In this manuscript, we used the research tools such as Bibexcel, Bibliometrix R package, and have been created for scientometric analysis. We also used VOS viewer software is adopted. Bibexcel is a free program that helps a user in the analysis of bibliographic data. The idea is to create data files that can be imported into Excel or any other tool that can handle tabbed data records for further analysis. Bibliometrix R package provides a convenient scientometric analysis package for Web of Science, Scopus, Dimensions, and PubMed databases[25], constructing data matrices for co-citation, coupling, scientific collaboration analysis, and co-word analysis. VOS viewer program is a useful tool for visualizing co-occurrence networks, which helps in mapping a scientific field's knowledge structure.

RESULTS

Table 1 depicts the Annual Growth Rate (AGR) of the research result from the marked period of study in SDG research from the Web of Science during 2000-2021. The AGR of the total records is calculated year-wise. Fluctuation is seen throughout the field study of SDG literature. The maximum AGR was recorded in the year 2015 (59.23) followed by 2017 (47.44), 2005 (45.45), 2016 (45.33), and 2020 (43.48). There has been volatility year after year as shown in Table 1. The explanation for the fluctuations is that the number of publications does not grow at the same rate every year. This AGR is calculated[26] using Formula 1.

Annual Growth Rate (AGR)

$$AGR = \frac{end\ valu - first\ value}{first\ vale} \times 100 \tag{1}$$

We started our scientometric analysis by looking at a year-by-year overview of research articles published on SDG which we found on the Web of Science. Following that, we calculated two helpful and informative parameters, the Relative Growth Rate and Doubling Time, for the years2000 to 2021 for 22 years. RGR is the increase in the number of publications per unit time. Doubling time is the given period that is required to double the size or value of the quantity. It can be calculated using the formula. We used, Dt (A) = average DT of articles[27-28]. In RGR given in

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Table 2, RGR has decreased from the year 2001 (0.700) to 2012 (0.149). In 2013, it increased to 0.173 and in 2014 it decreased to 0.143. we observed, from 2015 to 2021 has continuously increased. The highest 0.700 RGR was found in the year 2001, This RGR is calculated using Formula 2. This is also constrained by the fact that the increase of the literature is not in an exponential but rather in an arithmetic ratio, and that the SDG research explosion did not occur during the study period. Based on Doubling Time, 4.855 DT was observed in the year 2014 during the study period (see Table 2). The doubling time (DT) has increased when calculated year-wise. The Doubling Time increases from 0.89 in 2001 to 4.40 in 2004. In 2005, it slightly decreased to 2.633 and again it increased from 2006 to 2012 (2.798 to 4.660). DT has continuously decreased from the year 2013 to 2021, this DT is calculated using Formula 3. Though the DT is getting longer, it is still not growing at exponential growth, as shown (Table 1) in the AGR.

Relative Growth Rate (RGR)

$$RGR = (1 - 2^R) = (Log_e W_2 - Log_e W_1)/(T_2 - T_1) \tag{2}$$

Doubling Time (DB)

$$DT = 0.693/R \tag{3}$$

The co-authorship index (CAI) is calculated proportionally for publications by single, two, multiple, and mega-written articles for different branches. CAI = 100 indicates that the number of publications corresponds to the global average, CAI > 100 indicates that the number of publications is higher than the world average, and CAI <100 reflects less than the world average in a co-authorship pattern[29-30]. This CAI is calculated using Formula 4.

Co-authorship Index (CAI)

$$CAI = \left(\frac{N_{ji}}{N_{oj}}\right) \div \left(\frac{N_{io}}{N_{oo}}\right) \times 100 \tag{4}$$

Table 3indicates the co-authorship effort which is found from CAI on SDG literature from 2000 to 2021, 17780 papers were contributed by 60899 authors. In the solo and second authors, CAI is more than 100 from 2000 to 2017. CAI is greater than 100 in the 3 authors found from nine years. In the four authors, four CAI is greater than 100 out of sixteen years. In the more than five authors, CAI 100 is more than from 2017 to 2021 were found during the study period. The data shows that CAI has been increasing and decreasing during the study period. This means that multi-authored papers, rather than single contributions are the primary source of collaboration in SDG literature. Figure 2 shows the findings of the analysis, and it indicates that the articles published by 5<authors are more than the papers of a single, two, three, and four authors. Authorship and collaboration patterns of authors by year wise related to SDG research are presented in Table 4. We computed standard parameters degree of collaboration (DC), collaborative index (CI), and collaborative coefficient (CC) in addition to year-by-year authorship patterns (solo, two, three, four, and more than five authors). The degree of collaboration is the ratio of collaborative research articles to the overall number of research publications in the discipline over a specific period[31]. The highest DC 0.92 value was recorded in 2021 while the lowest DC 0.53 value was recorded in 2000. This DC is calculated using Formula 5.

Degree of Collaboration (DC)

$$DC = \frac{N_m}{N_m + N_s} \tag{5}$$

CI calculates the average number of authors per article. It has the drawback of being difficult to understand as a degree because it has no upper limit and grants a non-zero weight to single-authored publications that do not include collaboration[32]. CI 3.58 highest was in the year 2021 and minimum CI 2.01 in 2000. This CI is calculated using Formula 5.

Collaboration Index (CI)

$$CI = \frac{\sum_{j=1}^{k} jf_j}{N} \tag{6}$$

In the case of collaborative coefficient, each manuscript is assigned a credit that is divided among all authors, i.e., each author receives a credit of 1/j for the article with j authors. It goes away for a collection of solo-authored articles, and it distinguishes between solo-authored, two-authored, third-authored, and so on. CC, on the other hand, does not yield 1 for maximum collaboration unless the number of authors is infinite[33-34]. This CC is calculated using Formula 7.

Collaborative coefficient(CC)

$$CI = \frac{\sum_{j=1}^{k}\frac{1}{j}f_j}{N} \tag{7}$$

The top 10 cited publications in SDG research

Table 5 highlights the global citation structure of SDG research publications between 2000 to 2021, a list of the top 10 most cited papers from the Web of Science. All the papers in this list got more than 600 citations. There are 9 titles that received more than 1000 citations. The year 2008 stands at the top position for highest citation. It is found that the Lancet journal has been publishing quality and impactful research papers. Reed MS received the highest citations of 2005 for the article titled "Stakeholder Participation for Environmental Management: A Literature Review". The second position is acquired by the title "Understanding the complexity of economic, ecological, and social systems" authored by Holling CS since 2001. Between the last two years in 2017 found authors for a citation received, they are Hughes K (2017), and Pecl GT (2017), respectively.

Sankey diagram

The plotting of a Sankey diagram (three-field) in biblioshiny is a useful feature for seeing numerous attributes at the same time. Figure 3 shows a Sankey diagram with the names of the top 20 authors (left side), sources (middle side), and countries (right side) in the field study of SDG literature. R Studio was used to carry out the analysis for the diagram. Shiny is a sophisticated tool developed by the R Studio Team to facilitate web applications for interactive analysis.

Country collaboration

The authors Bhutta ZA (n-48 papers), Liu Y (n-42 papers), and Kumar A (n-39 papers) showed a maximum collaborative trend for both sources and countries. Sustainability (n-2099 papers), Journal of Cleaner Production (n-637 papers), and Science of The Total Environment (n-200 papers) sources/journals were the major articles published. Most of the articles that discussed consent have been published by the USA (n-3959 papers), the UK (n-2782 papers), and China (n-2185 papers) on SDG research.

CONCLUSION

To summarize, the goal of this study is to look at the number of contributions made by academics in the field of SDG research that was published in the Web of Science database between 2000 and 2021.According to the analysis, there were 17780 publications on the subject of SDG literature. This study analyzed quantitatively examines RGR, authorship pattern, degree of collaboration, collaborative index, time series, and predominant areas of research in SDG using well-established scientometric indicators. The number of publications on the topic under investigation has gradually increased, and recent years have produced a large number of articles compared to previous years. The result founda maximum number of 4617papers were published in the year 2021. The highest number of AGR 47.74% in 2017 has been noticed. Out of 17780 articles, 5665 articles were contributed by more than five authors. Highest DC, CC, and CI have been calculated in 2021, respectively. It also examined author organizations that demonstrate that countries such as the United States, the United Kingdom, and China, are actively engaged in field research. The most popular source titles for publishing the field's authors' writings were identified, and it was discovered that "Stakeholder Participation for Environmental Management: A Literature Review" topped the list with the most citations (2005) written by Reed MS published in 2008, source in Biological Conservation. The output of research has

become a key issue for scientists, researchers, and library professionals as they try to keep up with the latest developments in their field, and information profess endeavor to organize this knowledge.

DECLARATION

Declaration of interest My co-authors and I don't have any interest that might be interpreted as influencing the research "conflicts of interest: none".
Contributors It is acknowledgment that all the authors contributed equally.
Ethical approval Not Applicable
Funding None

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Table 1: Annual growth rate (AGR) in SDG literature from 2000-2021 for 22 years

Year	Publications	%	Cumulative	AGR
2000	73	0.41	-	-
2001	74	0.42	147	1.37
2002	64	0.36	211	-13.51
2003	84	0.47	295	31.25
2004	77	0.43	372	-8.33
2005	112	0.63	484	45.45
2006	136	0.76	620	21.43
2007	168	0.94	788	23.53

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2008	193	1.09	981	14.88
2009	212	1.19	1193	9.84
2010	239	1.34	1432	12.74
2011	283	1.59	1715	18.41
2012	275	1.55	1990	-2.83
2013	376	2.11	2366	36.73
2014	363	2.04	2729	-3.46
2015	578	3.25	3307	59.23
2016	840	4.72	4147	45.33
2017	1241	6.98	5388	47.74
2018	1761	9.90	7149	41.9
2019	2470	13.89	9619	40.26
2020	3544	19.93	13163	43.48
2021	4617	25.97	17780	30.28
Total	17780	100	-	-

*AGR- Annual Growth Rate

Table 2: Growth of research productivity on sustainable development goals from 2000-2021

Year	Pub.	Cum.	W1	W2	RGR	Mean RGR	DT	Mean DT
2000	73	73		4.290				
2001	74	147	4.290	4.990	0.700		0.990	
2002	64	211	4.990	5.352	0.361		1.917	
2003	84	295	5.352	5.687	0.335		2.068	
2004	77	372	5.687	5.919	0.232		2.988	
2005	112	484	5.919	6.182	0.263	0.271	2.633	2.435
2006	136	620	6.182	6.430	0.248		2.798	
2007	168	788	6.430	6.669	0.240		2.890	
2008	193	981	6.669	6.889	0.219		3.163	
2009	212	1193	6.889	7.084	0.196		3.542	
2010	239	1432	7.084	7.267	0.183		3.795	
2011	283	1715	7.267	7.447	0.180		3.843	
2012	275	1990	7.447	7.596	0.149		4.660	
2013	376	2366	7.596	7.769	0.173		4.004	
2014	363	2729	7.769	7.912	0.143		4.855	
2015	578	3307	7.912	8.104	0.192		3.607	
2016	840	4147	8.104	8.330	0.226	0.229	3.062	3.271
2017	1241	5388	8.330	8.592	0.262		2.647	
2018	1761	7149	8.592	8.875	0.283		2.451	
2019	2470	9619	8.875	9.171	0.297		2.335	
2020	3544	13163	9.171	9.485	0.314		2.209	
2021	4617	17780	9.485	9.786	0.301		2.305	

Table 3: Growth of co - authorship index on SDG from 2000-2021

Year	1 Authored		2 Authored		3 Authored		4 Authored		> 5 Authored		Total
	Papers	CAI	Papers	CAI	Papers	CAI	Papers	CAI	Papers	CAI	
2000	34	348.53	20	140.50	9	62.27	4	35.40	6	25.80	73
2001	32	323.60	21	145.53	8	54.61	8	69.85	5	21.21	74
2002	24	280.62	23	184.30	7	55.25	4	40.38	6	29.42	64

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2003	31	276.16	27	164.84	11	66.15	5	38.46	10	37.36	84
2004	23	223.52	25	166.50	9	59.04	6	50.34	14	57.06	77
2005	37	247.21	23	105.31	26	117.26	15	86.53	11	30.83	112
2006	39	214.59	42	158.38	28	103.99	9	42.76	18	41.54	136
2007	51	227.17	58	177.05	25	75.17	14	53.84	20	37.36	168
2008	48	186.11	52	138.17	37	96.84	26	87.04	30	48.79	193
2009	48	169.43	51	123.37	44	104.83	31	94.47	38	56.26	212
2010	52	162.81	60	128.75	51	107.79	34	91.91	42	55.15	239
2011	50	132.21	77	139.53	61	108.88	46	105.02	49	54.34	283
2012	55	149.66	78	145.46	53	97.35	29	68.13	60	68.48	275
2013	77	153.25	98	133.66	78	104.78	43	73.89	80	66.78	376
2014	62	127.81	77	108.78	71	98.80	55	97.89	98	84.73	363
2015	107	138.53	113	100.26	122	106.62	93	103.95	143	77.65	578
2016	147	130.96	184	112.34	150	90.20	112	86.14	247	92.29	840
2017	182	109.75	250	103.31	215	87.51	178	92.67	416	105.21	1241
2018	226	96.04	303	88.24	338	96.95	267	97.96	627	111.75	1761
2019	301	91.19	468	97.17	479	97.96	382	99.92	840	106.74	2470
2020	361	76.23	646	93.48	758	108.04	570	103.91	1209	107.07	3544
2021	389	63.05	771	85.64	940	102.84	821	114.89	1696	115.29	4617
Total	2376		3467		3520		2752		5665		17780

Table 4: Authorship and collaboration patterns on SDG from 2000-2021

Year	Authorship Pattern					Total Authors	Collaborative Indices		
	1	2	3	4	> 5		D.C	C.I	C.C
2000	34	20	9	4	6	73	0.53	2.01	0.33
2001	32	21	8	8	5	74	0.57	2.09	0.35
2002	24	23	7	4	6	64	0.63	2.14	0.37
2003	31	27	11	5	10	84	0.63	2.24	0.39
2004	23	25	9	6	14	77	0.70	2.52	0.44
2005	37	23	26	15	11	112	0.67	2.46	0.44
2006	39	42	28	9	18	136	0.71	2.45	0.45
2007	51	58	25	14	20	168	0.70	2.37	0.43
2008	48	52	37	26	30	193	0.75	2.68	0.49
2009	48	51	44	31	38	212	0.77	2.81	0.51
2010	52	60	51	34	42	239	0.78	2.81	0.52
2011	50	77	61	46	49	283	0.82	2.88	0.54
2012	55	78	53	29	60	275	0.80	2.86	0.52
2013	77	98	78	43	80	376	0.80	2.87	0.52
2014	62	77	71	55	98	363	0.83	3.14	0.57
2015	107	113	122	93	143	578	0.81	3.09	0.56
2016	147	184	150	112	247	840	0.83	3.15	0.56
2017	182	250	215	178	416	1241	0.85	3.32	0.59
2018	226	303	338	267	627	1761	0.87	3.43	0.61
2019	301	468	479	382	840	2470	0.88	3.40	0.61

2020	361	646	758	570	1209	3544	0.90	3.46	0.63
2021	389	771	940	821	1696	4617	0.92	3.58	0.65
Total	2376	3467	3520	2752	5665	17780	NIL	NIL	NIL

Table 5: The top 10 cited publications in SDG research

Rank	Author/Year	DOI	Sources	Title	GC	NGC
1	Reed MS, 2008	10.1016/j.biocon.2008.07.014	Biological Conservation	Stakeholder Participation for Environmental Management: A Literature Review	2005	32.58
2	Holling CS, 2001	10.1007/s10021-001-0101-5	Ecosystems	Understanding the complexity of economic, ecological, and social systems	1836	28.24
3	Liu L, 2016	10.1016/S0140-6736(16)31593-8	Lancet	Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals	1255	35.91
4	Griggs D, 2013	10.1038/495305a	Nature	Sustainable development goals for people and the planet	1235	32.41
5	Kassebaum NJ, 2016	10.1016/S0140-6736(16)31460-X	The Lancet	Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015	1149	32.88
6	Hughes K, 2017	10.1016/S2468-2667(17)30118-4	Lancet Public Health	The effect of multiple adverse childhood experiences on health: a systematic review and meta-analysis	1105	37
7	Pecl GT, 2017	10.1126/science.aai9214	Sciences	Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being	1053	35.26
8	Simon P, 2013,	10.1021/ar200306b	Accounts of Chemical Research	Capacitive Energy Storage in Nanostructured Carbon Electrolyte Systems	1041	27.32
9	Yoshimura A, 2016	10.1021/acs.chemrev.5b00547	Chemical Reviews	Advances in Synthetic Applications of Hypervalent Iodine Compounds	1013	28.98
10	Rebitzer G, 2004	10.1016/j.envint.2003.11.005	Environment International	Life cycle assessment: Part 1: Framework, goal and scope definition, inventory analysis, and applications	974	16.39

*DOI- Digital Objective Identifier GC- Global Citation NGC- Normalized Global Citation

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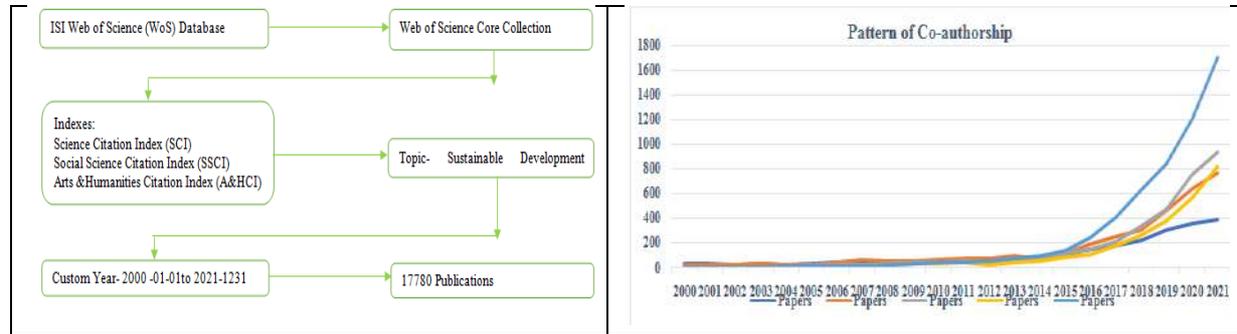
Figure 1: Methodological workflow on sustainable development goals
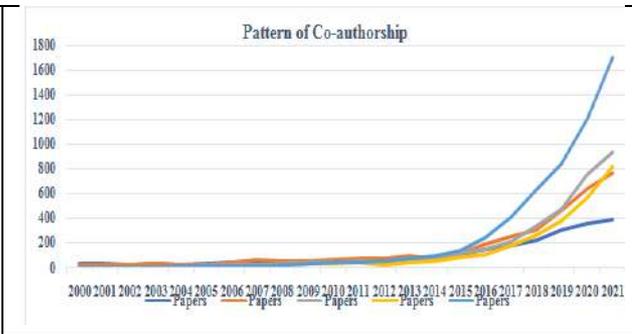
Figure 2: Co-authorship pattern on SDG literature
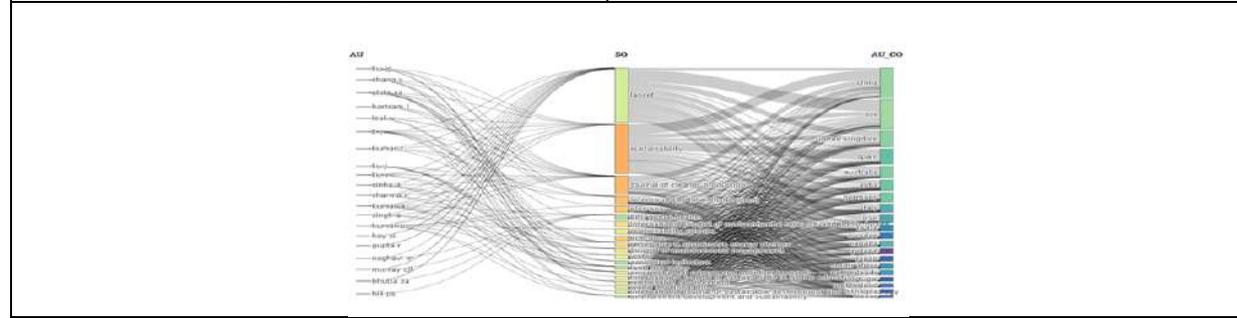
Figure 3: Sankey diagram (three-field) in SDG research

RESEARCH ARTICLE

Time Synchronization with Data Accuracy in Under Water Sensor Network

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ABSTRACT

Time synchronization is crucial for wireless sensor networks to function properly (WSNs). However, because of the severe environment and the movement of nodes, temporal synchronization is difficult for underwater WSNs (UWSNs). Large-scale networks are incompatible with current UWSN time-synchronization approaches focused on point-to-point synchronization. Some also require special equipment and deployment settings. Here, we suggest using TSDA, which stands for "time synchronization with data accuracy." TSDA uses the broadcast's acoustic communication properties to implement a sync communication technique. All timing messages, including those on the downlinks of two-way timing message exchanges between active nodes, are taken into consideration by ROS due to the clock skews of Nodes. Skew and offset are calculated using the MMLE and linear function approaches. Multi-source beacons' time reference information is utilized to the fullest extent possible using a time estimate and SVM approach. According to simulation tests, for throughput and energy consumption, TSDA has a high degree of accuracy.

Keywords: TSDA, UWSN, MMLE, ROS, SVM, Synchronization

INTRODUCTION

Human life depends on the availability of water. Marine resources such as oil, natural gas, and mining have piqued humanity's interest in the ocean's depths [1]. Scientific and technological advancements have only lately allowed humans to investigate the ocean floor fully. However, only around 10% of the 71% aquatic globe has been studied, while the remaining 61% is still undiscovered. Sensor and communication technologies for autonomous exploration of the Earth's surface have become more popular in recent years [2] [4] Sensor technology's major goal is to perform

collaborative monitoring responsibilities in a certain region and convey this information to the user using advanced wireless or wired communication technologies [5][6].

This problem has been researched extensively for many years and draws in many researchers. [7] A synchronization protocol's energy consumption and accuracy are two of the most important elements to consider while designing one. Synchronization energy accounted for up to 17% of the total energy utilized by a node in a terrestrial wireless sensor network. Underwater, the major means of communication is sound; there is a considerable increase in consumption. Nonetheless, batteries have a finite amount of energy and are difficult to replace or charge while traveling, necessitating the development of an energy-efficient synchronization mechanism [9].

There are three main synchronization protocols, all of which use statistical approaches to increase accuracy. An example of Radio Resource Sharing (RRS) is when nodes communicate information regarding the arrival of broadcast messages from a single source. The most used RRS-based protocol is RBS, which means that all nodes get the broadcast virtually simultaneously [11]. Delays are inherent throughout the message exchange process; hence ROS synchronization accuracy suffers from signal processing's uncertainty about message delays. When two nodes communicate with one other, they use Sender-Receiver Synchronization (SRS) [12]. Examples of SRS systems (LTS) are TPSN (Timing-synchronization Protocol for Sensor Networks), tiny-sync and mini-sync, and lightweight time synchronization [13] [14]. In recent years, SRS-based synchronization algorithms have included more advanced statistical signal processing approaches to reduce the uncertainty associated with the delays. An exponential delay model was initially used to develop a minimal variance unbiased estimate (MVUE) of clock offset variance, which was then used to unify all of the MLEs for clock offset variance based on Gaussian, exponential, or log-normal random delays [15]. The re-synchronization period must be reduced since the processes given above do not incorporate clock skew. Several scholars have accepted the idea of combining synchronization with localization because of the significant link between delays and distances between nodes [17].

The TSDA model is suggested in this study for time synchronization in UWSNs with exponential delays. TSDA features a synchronization communication strategy that uses the broadcast's acoustic communication characteristics. During two-way timing messages, ROS takes into account clock skews on nodes as well as all timing messages, including downlinks. The MMLE and linear function techniques are used to calculate skew and offset.
The remainder of this research is structured in the following manner. Section

BACKGROUND STUDY
Y. Dong et al. [2] introduced a unique time synchronization approach incorporating mobility prediction. Anchors may forecast their velocity using historical data in this way. Unsynchronized nodes determine their velocity using spatial correlation. M. Faheem et al. [3] Bio-inspired multi-objective evolutionary routing protocols for event-driven applications using UWSNs have been proposed. The proposed method uses a clustering methodology that builds exceptionally stable, dynamic clusters of varied sizes to distribute the network's data traffic burden. The proposed routing strategy maintains a high degree of stability in connection quality between cluster heads, allowing robust data transfer in UWSNs. By constraining data flow in the network's uphill and downhill directions, the proposed approach prevents data route loops and needless multi-hop packet transmission. It dramatically decreases energy usage 2 summarizes prior work, routing protocol design issues, and reasons. Section 3 details the TSDA system. Section 4 compares the TSDA scheme's simulation metrics, parameters, and outcomes to previous schemes. Finally, Section 5 summarizes the article by outlining future research directions malformed data packets, and network lifespan.

The authors described F. Hong et al. [5] UWSNs may be synchronized using MulSync, a technique that requires just a few beacons. For local skew or offset estimation, MulSync makes use of three linear regressions rather than the acoustic signal component of earlier approaches. The MulSync team also didn't assume any particular hardware or deployment requirements. Simulated data show that MulSync has greatly improved synchronization accuracy, message overhead, and time consumption.

N. Ilyas et al. [6] the authors provide SMDBR (Sink-Mobility in Depth-Based Routing Protocol), a routing protocol for UWSNs based on node depth information. It uses the MS to maximize network longevity and data collection efficiency when submerged. The authors know the considerable distance between the underwater sensor nodes and the surface sink. As a result, many transmissions and receptions use a lot of energy and result in a protracted end-to-end delay.

Liu et al. [10] mentioned in A time-synchronization solution for mobile UWSNs, Mobi-Sync, is discussed in this article. An underwater object's spatial correlation features were used to improve synchronization accuracy and efficiency in Mobi-initial Sync's attempt at synchronization. With this new approach we can get higher accuracy while spending less money each message. The authors of Wang, S., and Gao, M. [16] present a dynamic clock synchronization system used in conjunction with the current ocean model. Unlike current techniques for underwater sensor network clock synchronization, this approach corrects the clock skew using a high-precision clock, eliminating the difficult linear estimation and correction. Along with its pace of movement, the undersea node was influenced by the flow velocity. The node's traveling speed in water was determined using the current ocean model. Simultaneously, the Doppler Effect determined the relative velocity between nodes and the propagation delay B. Zhang et al. [18] It was designed to find only one normal node in mind. However, energy limits have restricted the underwater acoustic communication range for large-scale UWSNs. The difficulty of locating underwater nodes in large-scale UWSNs is made substantially more difficult as a result. D. Zhang et al. [19] A long-term study on energy allocation for monitoring targets in UWSNs with energy harvesting constraints was conducted. The authors started with a non-myopic energy allocation method to enhance tracking precision and efficiency. Increasing the precision of tracking over time was a focus of the authors' research. Measurement of energy allocation and tracking accuracy was studied using Fisher Information Matrix (FIM) and quantized data. The energy allocation optimization challenge was built by employing FIM as a performance indicator to optimize tracking performance under energy constraints

PROBLEM FORMATION

The conversion of Time Of Arrival (TOA) data into range estimations is a common practice in underwater acoustic localization. Temperature and pressure may affect sound, speed when the aqueous medium isn't uniform; however this is not always the case. The development of a two-way message exchange system with a combined clock offset, clock skew, and fixed delay estimator. For the joint maximum likelihood estimation issue, an initial LP formulation (linear programming) is utilized. Despite the fact that the solution to the LP issue is absolutely the greatest option worldwide, fixing it directly is inefficient. The time information transferred between the nodes has been used to estimate clock skew, clock offset, and fixed delay using both one-way and two-way message exchange protocols. Only one form of communication can accurately forecast the clock offset and fixed latency. Additionally, a two-way message exchange system is required to reduce the uncertainty created by the rank deficit while computing the estimated value.

TIME SYNCHRONIZATION

Due to the sequencing of message processing operations at the transmitter and receiver, as well as message queuing in the Medium Access Control (MAC) layer, there are both fixed propagation delays and random delays, should be considered. Nodes must be located simultaneously to ensure that all messages have the same propagation time. As the active nodes exchange N messages, q is the passive node that just listens. Syncing the clocks of both nodes is the main purpose of this project The ROS scheme's purpose is accurate message conveyance. The CVX toolboxes provide a computationally straightforward approach to generate the same effect as the combined MLE of clock skew and offset for quiet and active nodes.

Network Model

As part of ROS' standard model, all timing messages, including those on the downlink of two-way timing message exchanges between active nodes, are included. The Support Vector Machine (SVM)predicts the active node's clock offset and skew.SVM is both accurate and durable in alg1. We also develop and provide the same findings as CVX toolboxes, but with a lot less effort in the message exchange rounds, an MLE of clock skew and offset for active and passive nodes. In addition, we demonstrate that the active node's MLE clock offset estimate is equivalent to the SVM clock offset computation previously stated. For both active and silent nodes, including the method into our current model improves the MVUE clock offset calculation accuracy. Figure 1 depicts the proposed design.

Support Vector Machine Algorithm

Plot the decision boundary for a non-linear SVM problem

```
def plot_decision_boundary(model, ax=None):
 if ax is None:
 ax = plt.gca()
 xlim = ax.get_xlim()
 ylim = ax.get_ylim()
 # create grid to evaluate model
 x = np.linspace(xlim[0], xlim[1], 30)
 y = np.linspace(ylim[0], ylim[1], 30)
 Y, X = np.meshgrid(y, x)
            # shape data
 xy = np.vstack([X.ravel(), Y.ravel()]).T
# get the decision boundary based on the Model

P=Model.decision_function(xy).reshape(X.shape)
  # plot decision boundary
 ax.contour(X, Y, P,levels=[0], alpha=0.5, linestyles=['-'])
```

ROS Model

Between the active nodes and node q, which is only listening to what's going on, there are N rounds of message exchange. P and Q are synchronised with node m using the standard clock as their target. Entire messages are replaced during message exchange cycles in the ROS system. Node m broadcasts a message with its own time-stamp, $T(s)1;i$, and the time-stamp generated in d) at the (i-1)th round, $T(s)4;i-1$, both of which refer to the ordinary clock; Upon receiving the message from node m, node p records its current clock reading, $T(r)2;i$; c) Node R replies to node m in a broadcast manner. Node O records its local clock readings, $T(o)5;i$ and $T(o)6;i$, when it receives messages from node R and node O, respectively, across the two-way link.

Node 31 sends messages to its neighbor, and CH1, CH2, CH3 are represented in figure 2.

Linear Function Algorithm:
        Preliminaries
        build overlapping mass windows across joint runs
        1.Cluster examination
        for each mass-window do
                utilize p peaks with maximum intensities
                calculate space matrix of pairs of peaks(j,h)
                    $$d_{j,h} = \left\{ \frac{diff(mass)}{\infty} \right\}$$
                    if diff(rt) < k1 ^ diff(log10(intensity))<k2
                    if diff(rt)≥ k1 ∨ diff(log10(intensity))≥k2

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hierarchical common linkage cluster analysis
       cut cluster-tree at swarm accuracy   m

$\triangle$ if n_{dup} < threshold$_1$ ^ n_{miss} < threshold$_2$ then
           cluster is 'well-behaved'
knead out duplicated 'well-behaved' clusters
for each 'well-behaved' cluster do
        $\acute{r}t$= median(rt)
        for each peak i do
             $dev_i = rt_i - \acute{r}t$
        End For
    End for
End for

Multi-Hop Maximum Likelihood Estimation (MMLE)
In a more realistic multi-hop network, not every normal node will be able to reach three anchor nodes. In this instance, a multi-hop technique is required to provide network-wide localization and synchronization. In this section, we'll go through how to expand the network to encompass several hops and how to deal with synchronization and location concerns at the network level. Anchor nodes may not cover all ordinary nodes that need to be synchronized and localized in order to function properly. In order to accomplish multi-hop or even network-wide synchronization and localization, additional reference points may be necessary. After three anchor nodes have located and synced ordinary nodes, they might be prospective candidates. More than three reference nodes may span an ever-increasing region as more synchronized and localized nodes are added to the reference set. The reference nodes must be carefully picked in light of the widespread network localization and synchronization error. A reference node must meet accuracy requirements before it can help other nodes be synced and located promptly. Mobile Sink Node 23 delivers various events to node 42, and the sensed data is shown in Figure 3.

Time Estimation Algorithm
Parameters: envelope averaging time Te(0.05 s),
Percentile calculation time Tp(the 20s),
Percentile offset K,
event detection threshold $\Upsilon1$ (9dB),
event validation time window Tv(1/8 s)
event validation energy threshold $\Upsilon2$(6 dB).
Outputs: event start and end times Ts,Te
for all times t in the recording
        Compute the signal envelope a(t) (average $x(t)^2$ over Te)
        Estimate a noise floor n(t) (find 10th percentile of a(t)
            over a time interval Tp; scale by K)
        Initial detection of events
            -event is detected if a(t) > n(t) * $\Upsilon1$
end for
for all events in list of initial detections
        Refine estimate event start
            -adjust event start time early until a(t) stops
                Decreasing or reaches noise floor n(t)
        Validate the event (rapid increase in energy)
            -event is valid if median energy before, after
                event start(calculated over Tv) increases by $\Upsilon2$
        Merge any events that overlap after refinement
        Update list of event start, end times Ts, Te

end for When it comes to network-wide synchronisation and localisation, we offer a hierarchical approach that divides the task between a primary and a secondary process. At least three anchor nodes must be present in order for MMLE to locate and synchronise the ordinary nodes. And this is true for each and every ordinary node.

Synchronization The accuracy approach is used. Despite this, the TSDA approach has better synchronization accuracy than the other methods. This situation is consistent with the analysis after hypothesis, which states that clock measurements from the quiet node do not aid in precisely estimating the active node's clock parameters since they contribute more unknown variables than valuable information. Additionally, the TSDA approach is substantially more accurate than the MMLE method.

RESULTS AND DISCUSSION

We simulate our proposed protocol in NS2 Simulator. We compare our TSDA Model with the joint maximum likelihood estimator (JMLE) and generalized maximum likelihood-like estimator (GMLLE) method. The Network size is 550 x 480 m. MMLE of the TSDA technique is explored if a random delay distribution function exists. In place of the exponential distribution, a gamma distribution is used to create random delays. In comparison to the exponential distribution, it has one extra degree of freedom and is one of the most often used delay models. Figure 4 illustrates the time synchronization with PDR. The accuracy of TSDA is increasing the message spread through the medium and remains. It shows the representation of the JMLE and GMLLE methods of inactive nodes' performance. In the X-Axis, the time in seconds is represented by the Y-axis, and the percentage of PDR is shown on the X-axis. Figure 4 illustrates the time synchronization with PDR. The accuracy of TSDA is increasing the message spread through the medium and remains. It shows the representation of the JMLE and GMLLE methods of inactive nodes' performance. In the X-Axis, the time in seconds is represented by the Y-axis, and the percentage of PDR is shown on the X-axis.

Figure 6 illustrates the time synchronization with energy consumption. The TSDA method energy consumption is very less. The JMLE and GMLLE methods are high energy consumption of active nodes. The X-axis is the time in seconds, and the Y-axis is the level of energy in that period. Figure 7 illustrates the time synchronization with throughput. The accuracy of TSDA is increasing the message communication. It shows the throughput comparison; the TSDA has a better throughput than JMLE and GMLLE methods. In X-axis represents the time, and Y-axis represents the throughput levels. Figure 8 illustrates the time synchronization with bandwidth frequency. The JMLE and GMLLE methods were used as high bandwidth frequency levels. The TSDA method has less usage in bandwidth. In the X-axis, the average energy and the Y-axis, the bandwidth frequency are shown.

CONCLUSION

We introduce TSDA for time synchronization in UWSN delays in this article. It uses ROS to extend the network's life and collect data efficiently under unfavorable underwater conditions. The TSDA approach maximizes time efficiency by combining SVM, Time estimation, and Linear Functions algorithms. As a result, data transmission is quicker than with standard JMLE and GMLLE techniques. Obtaining information about the ocean floor regarding hazard alerts and response time would benefit an underwater sensor network. The experimental findings suggest that TSDA outperforms current protocols when used with an underwater sensor network. The work will be expanded in two ways in the future: 1) compare our architecture to various underwater mobility patterns, including one that incorporates vertical movement; and 2) analyze the effect of errors on supernode localization and velocity estimates, as well as on MAC layer activities such as packet loss and re-transmission.

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Network Model

Parameters	Value
Simulation Time	500(s)
Number of Nodes	0 to 42
Mobility	10-50m/s
Routing Protocol	DSR
Pause Time	10 (m/s)
Simulation Area	550 x 480 m
Transmission Range	250m
No. of Active Node	6
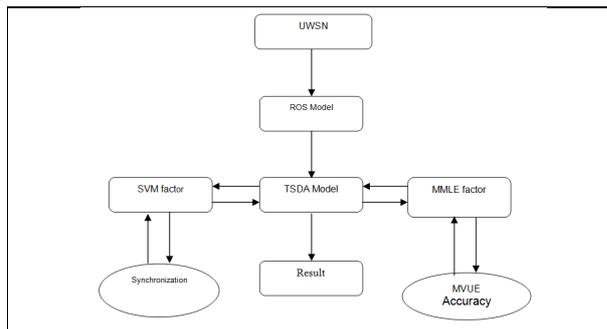
Fgure 1: Architecture Diagram for TSDA Model
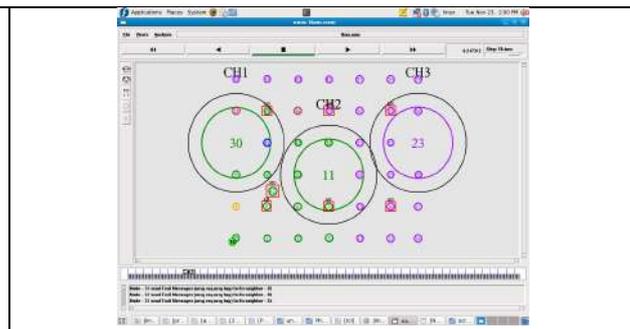
Figure 2: Sending messages to a neighbor node in TSDA model
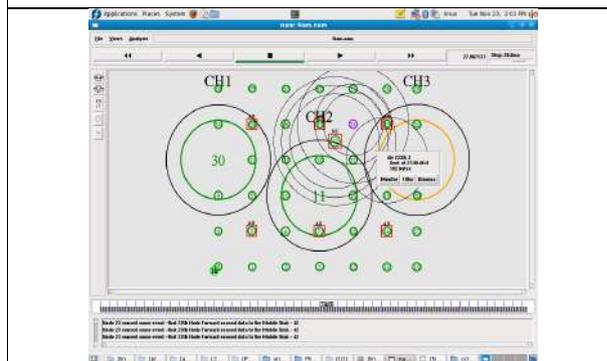
Figure 3: Data forwarding to the Mobile Sink
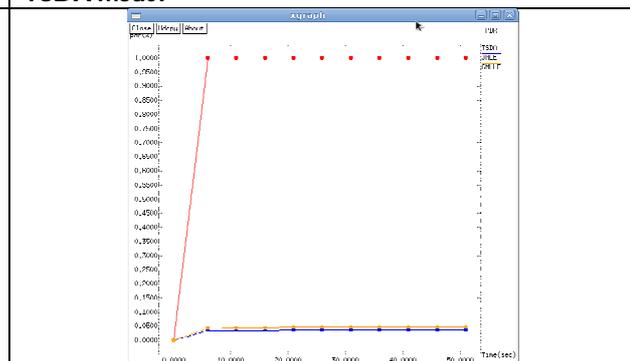
Figure 4: Packet Delivery Ratio (PDR) Comparison Chart

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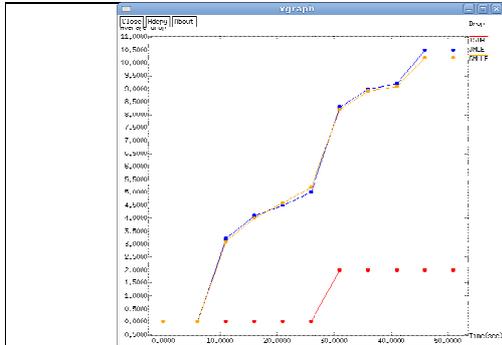
Figure 5: Packet Drop Comparison Chart
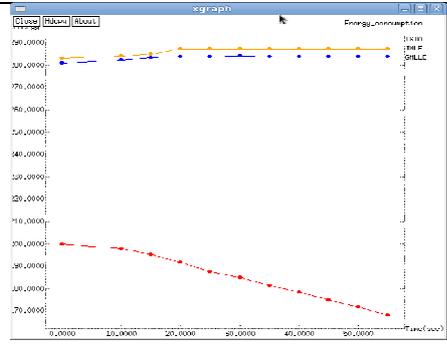
Figure 6: Energy consumption Comparison Chart
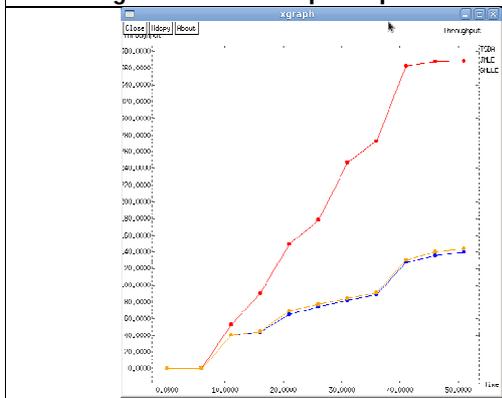
Figure 7: Throughput Comparison Chart
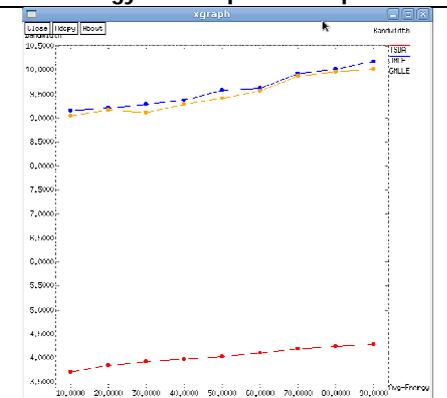
Figure 8: Band width

RESEARCH ARTICLE

Effect of Combination of Natural and Synthetic Polymers on the Formulation of Nifedipine Gastro-Retentive Floating Tablets

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ABSTRACT

The aim of the present study was to formulate and evaluatenifedipine into an effervescent gastro retentive floating tablet, which can extend its release up to 12 h in gastric pH, and simultaneously to study the effect of the combination of synthetic hydroxypropylmethylcellulose (HPMC K15M / K100M) with natural (sodium alginate, guar gum, and xanthan gum) polymers in extending the release of nifedipine. The drug-excipient compatibility studies of the drug and the polymers were carried out by FT-IR studies. The effervescent nifedipine floating tablets were preparedby the non-aqueous wet granulation method. All Formulations were evaluated for pre-compression, post-compression, and *invitro* buoyancy studies. Accelerated stability studies were conducted for the optimized formulation (F7). The drug-excipients compatibility studies reveal that the drug and the polymers used in the study are compatible. Pre-and post-compression parameters were within the acceptable limits for all formulations. *In vitro* dissolution studies, showed the formulation F7 (10% w/w sodium alginate and 15% w/w HPMC K100M in the ratio 1: 1.5 respectively) is exhibiting better extended-release of nifedipine up to 12 h, with a floating lag time (FLT) of01 min: 05 sec ± 0.12, total floating time and matrix integrity maintained up to 12 h. Drug release kinetics of formulation (F7) reveals it follows zero-order kinetics (r^2= 0.993), drug release is predominantly by diffusion and the release mechanism is by non-Fickian diffusion.

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Comparative DSC & FT-IR studies of pure NF and optimized formulation (F7) accelerated stability 3 M samples further confirmed the integrity of the drug and it passes the test for stability as per ICH guidelines. It was finally concluded that nifedipine floating tablets were formulated and evaluated.

Keywords: Nifedipine, gastro retentive floating tablets, hydroxypropylmethylcellulose, sodium alginate, xanthan gum, guar gum, *in vitro* buoyancy studies.

INTRODUCTION

A gastro-retentive drug delivery system (GRDDS) can be defined as a system that remains in the stomach for a sufficient time interval against all the physiological barriers, releasing the active moiety in a controlled manner[1]. A GRDDS can be a useful tool in the delivery of drugs that are primarily absorbed in the duodenum and upper jejunum or those that have an absorption window in the gastrointestinal tract[2]. Various GRDDS include floating systems, bioadhesive, swelling, expanding, and high-density systems. Floating systems are more popular in comparison with the other described GRDDS because they do not have any adverse effect on the motility of the GIT[3,4]. Based on the mechanism of floating, two systems of FDDS are effervescent and non-effervescent systems. Effervescent systems contain carbonates (eg. sodium bicarbonate) and/or organic acids (eg. citric acid / tartaric acid) in their formulation to produce carbon dioxide (CO_2) gas, when comes in contact with gastric fluids.

The CO_2 gas entrapped in the matrix system reduces the density of the system and makes it buoyant. The non-effervescent systems are based on the mechanism of swelling of polymer or bio-adhesion to the mucosal layer in GIT [5]. Carbopols and cellulose derivatives are synthetic polymers established for the controlled release of various GRDDS. The combination of natural and synthetic polymers in the formulation of GRDDS has the advantages of inexpensiveness and enhanced extended release of the drug[6]. Nifedipine (NF) is a calcium channel blocking agent used in the treatment of various cardiovascular diseases, long-term treatment of hypertension [7], and angina pectoris[8]. Earlier, short-acting nifedipine was used sublingually in emergency management of severe hypertension. Later it was established that there is an increased risk of myocardial infarction or mortality in patients receiving short-acting nifedipine for hypertensive emergencies [9,10].In the present investigation, the gastro retentive effervescent floating tablets of NF are prepared by the non-aqueous wet granulation method. The objective of the present study is to study the effect of the combination of synthetic (HPMC K15M / HPMC K100M) with natural (sodium alginate/xanthan gum/ guar gum) and to optimize their ratio in extending the release of NF up to 12 h from its floating tablets.

MATERIALS

Nifedipine is a gift sample received from M/s Dr. Reddy's Laboratories, Hyderabad, India. Hydroxypropyl methyl celluloses (HPMC K15M & K100M) and lactose were received as gift samples from Colorcon Asia Pvt. Ltd., Mumbai, India. Sodium alginate (SA), guar gum (GG), and xanthan gum (XG) are purchased from Arihant Trading Co. Ltd., Bangalore. Magnesium stearate, sodium bicarbonate, talc, polyvinyl pyrrolidone (PVP K30), and isopropyl alcohol (IPA) were purchased from S.D. Fine-Chemicals Ltd., Chennai, India. The function of excipients: HPMC K15M / K100M are synthetic controlled-release (CR) polymers, GG, XG, and SA are natural CR polymers, lactose is a diluent, sodium bicarbonate is an effervescent agent, magnesium stearate is a lubricant and talc is a glidant.5% w/v PVP K30 in IPA is used as a binder solution. All the excipients used in the study are of pharmaceutical grade.

METHODS

Drug-excipient compatibility (FT-IR) studies

FT-IR spectra of pure drug and drug: polymer (1:1) physical mixtures were recorded, in the region of 400-4000 cm^{-1} at a spectral resolution of 1 cm^{-1}, obtained by the KB r pellet method, by *FTIR* spectrometer (Bruker MATRIX-MF FT-IR, Germany)[11].

Standard calibration curve

100 mg of NF pure drug was dissolved in 0.1 N HCl in a 100 mL volumetric flask and the volume was adjusted with 0.1 N HCl and then placed in a sonicator for 10 min to obtain stock solution-I (SS-I) of conc. 1000 µg/mL. 1 mL of SS-I was taken into a 100 mL volumetric flask and the volume was adjusted with 0.1 N HCl to obtain an SS-II (10 µg/mL). Aliquots of 2, 4, 6, 8, and 10 mL of stock solution-II were transferred to a series of 10 mL volumetric flasks and diluted with 0.1 N HCl up to the mark to obtain the conc. of 2, 4, 6, 8, and 10 µg/mL NF working dilutions respectively. The median conc. 6 µg/mL NF solution was scanned by using a double beam UV-Vis spectrophotometer (T60, PG Instruments, UK) with a limit of 1nm in the spectral range of 200 to 400 nm, to determine the maximum absorbance (λ_{max}). The working dilutions were analyzed at the λ_{max}. The standard calibration curve was plotted by taking conc. on X-axis and absorbance on Y-axis[11].

Preparation of NFFTS

All the batches were prepared by the non-aqueous wet granulation method, by keeping the amount of NF constant at 5 mg per tablet. NF and all other excipients excluding magnesium stearate and talc were co-sifted through sieve No. # 40 (ASTM), and blended in a poly bag for 5 min. the blend was granulated with 5% w/v PVP K30 in IPA binder solution and the obtained wet mass was sifted through sieve No. # 16 (ASTM); obtained wet granules were dried at 100 °C in a hot air oven for 30 min. the dried granules were sifted through sieve No. # 30 (ASTM) and lubricated with sieve no. # 60 (ASTM) passed magnesium stearate and talc by mixing in the same poly bag, for an additional 2 min. Tablets were compressed on a tablet punching machine (CMD 3-16MT, Cadmach, India.) fitted with 5 mm standard flat circular punches with an avg. wt. of 200 mg and avg. hardness of 6.0 kg/cm². The composition of NFFTs is tabulated in (Table 1).[12]

Pre-compression studies

Directly compressible NFFTs blends were evaluated for angle of repose (AR), bulk density (BD), tapped density (TD), Carr's index (CI), and Hausner's ratio (HR) as per the standard procedures[13,16].

$\theta = tan^{-1}$ **(h/r)**   **Eq. No. 1**

Where, θ = angle of repose, h = height of pile, and r = radius of the base of the pile

BD = M/V$_b$ **Eq. No. 2**

Where, BD = bulk density, M = mass of the powder, and V$_b$ = bulk volume of the powder

TD = M/V$_t$ **Eq. No. 3**

Where, TD = tapped density, M = mass of the powder, and V$_t$ = tapped volume of the powder

CI = $\frac{(TD-BD)}{TD}$ x 100   **Eq. No. 4**

Where, CI = Carr's index, TD = tapped density and BD = bulk density

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HR = TD/BD   **Eq. No. 5**

Where, HR = Hausner's ratio, TD = tapped density and BD = bulk density

Post-compression studies
Weight variation test
As per IP, 20 tablets (n = 20) were selected randomly from each batch, and weighed on an electronic balance (AX-200, Shimadzu Corporation, Japan). The mean and % deviation of wt. were calculated. The batch passes the wt. variation test if NMT wt. of two tablets deviates from the mean tablet wt. of the batch and no tablet should deviate more than twice the % mentioned (acceptable % deviation is 7.5% when the Avg. wt. of the tablet is more than 80 mg but less than 250 mg) [17].

% Deviation $= \frac{(Wt. - Avg.\ wt.)}{Wt.}$ **x 100**   **Eq. No. 6**

Thickness
Was calculated with the digital Vernier calipers (RK Industries, India) for three randomly picked tablets from each batch (n =3)[18].

Hardness
Was calculated with the hardness tester (Monsanto hardness tester, Singla, India) for three randomly picked tablets from each batch (n =3)[18].

Friability
Was calculated with the friability apparatus (EF2-Fribilator, Electrolab, India) operated for 4 min at a speed of 25 rpm, for ten randomly picked tablets (n = 1) in each batch[17].

% Friability $= \frac{(W1 - W2)}{W1}$ **x 100**   **Eq. No. 7**

Where, W_1 = initial wt. and W_2 = final wt. after friability test

Assay
Six tablets from each batch (n = 6), were randomly selected and crushed in a mortar with a pestle; the quantity of blend equivalent to 100 mg of NF was suspended in 100 mL of 0.1N HCl in a volumetric flask and sonicated for 2 min. The dispersion was filtered through a 0.45µm membrane filter, suitably diluted, and analyzed by a UV-Vis spectrophotometer (T60, PG Instruments, UK)[18].

***In vitro* buoyancy studies**
Were characterized by floating lag time (FLT), total floating time (TFT), and matrix integrity (MI) up to 12 h, as per the method described by (Rosa et al., 1994). Three tablets from each batch (n = 3), were randomly selected. A tablet was dropped into 100 mL of 0.1 N HCl in a beaker. The time required for the tablet to rise to the surface and the duration of time it constantly floated on the medium was noted as FLT and TFT, respectively. During this study, whether the swollen matrix was intact or disintegrated was observed, to confirm the MI[19].

***In vitro* dissolution studies**
Three tablets from each batch, were randomly selected (n=3). Dissolution was performed with the USP-II (paddle) dissolution apparatus (Disso 2000, Labindia, India), each flask was filled with 900 mL of 0.1N HCl; the speed of the paddle was maintained at 50 rpm, and the temperature was kept constant at 37 ± 0.5°C. At time points: 0, 1, 2, 3, 4, 6, 8, 10 and 12 h. Aliquots of 5 mL of dissolution media were withdrawn, filtered through a 0.45µm membrane filter, suitably diluted, and analyzed at the predetermined λ_{max} at 269 nm for NF, using a double beam UV-Vis

spectrophotometer (T60, PG Instruments, UK). Each sample withdrawn was replaced with an equal amount of fresh 0.1 N HCl, to keep the volume of the dissolution medium constant[18].

In vitro drug release kinetics

The *in vitro* drug release data of all batches were fitted into zero-order, first-order, Higuchi, and Korsemeyer-Peppas models to ascertain the drug release kinetics. The drug release from the hydrophilic matrix whether depends on the drug's concentration or not was explained by zero and first-order models. The Higuchi model describes whether the drug release is predominantly by diffusion or not. The Korsemeyer-Peppas model further explains the mechanism of diffusion. The respective models were defined by the equations below.

Zero order: $Q_t = Q_0 + K_0 t$ ***Eq. No. 8***

First order: $Log\ Q = log\ Q_0 - K_1 t / 2.303$ ***Eq. No. 9***

Higuchi model: $Qt = K_H t^{\frac{1}{2}}$ ***Eq. No. 10***

Korsemeyer-Peppas model

$M_t / M_\infty = K t^n$ ***Eq. No. 11***

Where Q_t is the amount of drug dissolved at the time, t; Q_0 is the initial amount of drug in the solution at time t = 0, Q is the amount of drug remaining at the time, t; M_t/M_∞ is the fraction of drug released at the time, t and n is diffusion exponent. K_0, K_1, K_H, and K refer to the rate constants of respective kinetic models [20,23]

Accelerated Stability Studies

Stability studies of the optimized NFFTs (F6), packed in 10 CC HDPE containers for up to 3 months were carried out according to International Conference on Harmonization (ICH) guidelines; in a humidity chamber (PCI 87, Thermo Lab, India) maintained at 45°C ± 2°C and 75% ± 5% RH. At the end of every month for up to 3 months, the samples were withdrawn and evaluated for post-compression and in vitro buoyancy studies. The chemical stability of the drug in the 3M-accelerated stability sample of optimized NFFTs (F7); which will influence its *in vitro* and *in vivo* dissolution characteristics, was investigated using FT-IR studies as per the procedure mentioned earlier and by Differential Scanning Calorimetry (DSC) studies, thermal properties of the pure drug and the 3M-accelerated stability sample of optimized NFFTs (F7) were analyzed by (Shimadzu DSC60, Shimadzu Ltd. Japan). The samples were heated in hermetically sealed aluminum pans. Heat runs for the sample were set from 0 °C to 300 °C at a heating rate of 10 °C / min, using nitrogen as blanket gas[24].

RESULTS AND DISCUSSION

Drug-excipient compatibility (FT-IR) studies

The characteristic peaks of nifedipine are identified at 3096 cm^{-1} (aromatic group) - νAr-H (-CH) bands, 1680 cm^{-1} (aryl carboxylic group) - ν (C=O) stretching vibration, 1622 cm^{-1} (pyridine group) - ring breathing band and 1226 cm^{-1} (carbonyl group) – ν C-O/ δ O-H. The IR bands of pure NF and NF: polymer (1:1 ratio physical mixtures) show no significant shifts or reduction in the intensity of the characteristic peaks. Hence there was no incompatibility problem between the drug and polymers used in the study[25]. Comparative FT-IR spectra of NF, NF + HPMC K100M, NF + xanthan gum, NF + guar gum ,and NF + sodium alginate are shown in(Fig.1).

Standard calibration curve

The Λ_{max} is observed at 269 nm. The standard curve is defined by a straight line equation, y = 0.0343x - 0.002, following linearity with a regression coefficient (r^2 = 0.999). This method obeyed Beer's law in the conc. range of 0-10

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µg/mL. This method was suitable for the estimation of NF[9]. The standard calibration curve of NF in 0.1 N HCl was shown in (Fig.2).

Pre-compression studies

The angle of repose of all the directly compressible blends of NFFTs are ranging between 24°.10′±0.41 to 25.35° ± 0.52′, CI and HR were found to be in the range of 38.96to 43.04 % and 1.64 to 1.76 respectively, indicating excellent flow properties and compressibility of the blends[13-16]. The consolidated results of pre-compression studies were tabulated in (Table 2).

Post-compression studies

As the % wt variation of all batches is within ± 7.5% w/w, they passed the % wt. variation test as per USP 30-NF 25. The thickness of tablets was found to be between 3.20±0.04 to 3.72±0.06 mm. The hardness of tablets was found to be between 5.4±0.32 to 6.0±0.32 kg/cm^2, indicating satisfactory mechanical strength. The % friability was NMT 1.0% w/w for all the formulations, which is an indication of good mechanical resistance to the physical erosion of the tablet. As the % assay of all batches is within 96.11±0.56 to 98.24±0.53 %, they passed the content uniformity test as per USP 30-NF 25[17-18].

In vitro buoyancy studies

The FLT was found to be NMT 2 min for all the formulations. Among all the formulations, F7 with 10 % SA and 15% HPMC K100M and the ratio of SA: HPMC; 1: 1.5 respectively, had the lowest FLT of 1 min: 05 sec ± 0.04 and retained its MI and TFT up to 12 h[19]. The consolidated results of post-compression and *in vitro* buoyancy studies are tabulated in (Table 3). *In vitro* floating images of optimized formulation NFFTs (F7) were shown in (Fig.3).

In vitro dissolution studies

As the conc. of synthetic polymer HPMC K100M increases, there is increased viscosity of the gel matrix and a decrease in the effective diffusion coefficient of the drug[26]. Other factors that may contribute to differences in drug release profiles include; differences in water penetration rate, water absorption capacity, polymer swelling, and drug: polymer ratio[27,28]. Among all factors, drug: polymer ratio is an important factor affecting the rate of drug release from the matrix, which has to be optimized[29]. The pH-independent, zero-order release profile of bio pharmaceutics classification system (BCS) class-II drug (low solubility and high permeability) like NF can be attained from the hydrophilic matrix systems, by combining the synthetic polymers (HPMC K100M) with natural polymers like (guar gum/xanthangum/sodium alginate). In the combined matrix when exposed to gastric fluids, HPMC hydrates first to form a gel layer at the surface of the tablet, while the natural polymers, due to lesser hydration rate than HPMC remains less hydrated. The resulting matrix system acts as a barrier for the diffusion of highly soluble drugs like NF and extends its release[29,30]. Among all the formulations, F7 with 10% SA and 15% HPMC K100M and the ratio of SA: HPMC; 1: 1.5 respectively is able to extend NF release up to 12 h, with a better zero-order release profile (r^2 =0.993). *In vitro* dissolution profiles of NFFTs were shown in (Fig.4).

In vitro drug release kinetics

Among all the formulations, F7 fitted best to the zero-order kinetics (as zero-order, r^2 = 0.993), indicating the drug release from the matrix does not depend on the drug conc. is considered as an optimized one. Drug release process is predominantly by diffusion (as Higuchi, r^2 = 0.941; i.e. r^2> 0.9); and the mechanism of diffusion is by Non-Fickian diffusion (as Korsemeyer-Peppas, n=0.746 and the n value is 0.45 < n < 0.89)[20,23]. Drug release mechanisms based on n-values, for cylindrical shape, as per the Korsmeyer-Peppas model, were tabulated in (Table 4). The consolidated drug release kinetics data of NFFTs were tabulated in (Table 5).

Accelerated stability studies

As there were no significant differences in post compression studies (wt. variation, thickness, hardness, friability, and *in vitro* dissolution studies) and floating characteristics (FLT, TFT, and MI) of initial and accelerated stability samples of optimized NFFTs (F7) in the HDPE pack, it passes the test for stability. Comparative FT-IR spectra in (Fig.

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6), reveals there is no significant change in the functional groups of the NF due to interaction with polymers and other excipients used in the formulation[25]. DSC is useful in the investigation of solid-state interactions. DSC thermogram of NF (pure drug) showed a sharp endothermic peak at 166.74°C corresponding to its melting point, and the DSC thermogram of optimized NFFTs (F7) accelerated stability 3M sample (Fig.7) revealed a negligible change in the melting point of NF in the presence of other excipients and the absence of solid-state interactions were confirmed by DSC studies[25]. The consolidated results of accelerated stability studies were tabulated in (Table 6). Comparative *in vitro* dissolution profiles of initial and accelerated stability samples were shown in (Fig.5). Comparative FT-IR spectra of NF (pure drug) and optimized NFFTs (F7) accelerated stability 3M sample were shown in (Fig.6). Comparative DSC thermo grams of NF (pure drug) and optimized NFFTs (F7) accelerated stability 3M sample were shown in (Fig.7).

CONCLUSION

In the view of above findings, the effect of a combination of synthetic and natural polymers, in extending the release of NF from its effervescent GRFTs was better understood. It was further concluded that the optimization of the ratio of SA and HPMC K100M, had a significant effect on extending the release profiles of NF in the gastric region from the hydrophilic matrix tablets. Among the various combinations of synthetic (HPMC K15M / HPMC K100M) and natural polymers (guar gum/xanthangum/sodium alginate); formulation F7 with 10% SA and 15% HPMC K100M and the ratio of SA: HPMC; 1: 1.5 respectively forms a better matrix for the extending the release of NF in gastric pH up to 12 h, with a zero-order release profile (r^2 = 0.993), FLT of 1 min: 05 sec ± 0.12, TFT and a better MI up to 12 h. Hence it is an optimized formulation. A combination hydrophilic matrix design of this kind can serve as a suitable strategy for extending the release of other BCS class II drugs like NF (low solubility and high permeability) and their salt forms, which are having shorter elimination half-life ($t_{1/2}$ < 5 h).

ACKNOWLEDGEMENTS

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Table 1: Formulation Table of Nefidipine Floating Tablets

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Intra-granular									
Nefidipine	5	5	5	5	5	5	5	5	5
HPMC K15M	40	60	80	-	-	-	-	-	-
HPMC K100M	-	-	-	20	30	40	30	30	30
Sodium alginate	-	-	-	-	-	-	20	-	-
Xanthan gum	-	-	-	-	-	-	-	20	-
Guar gum	-	-	-	-	-	-	-	-	20
Sodium bicarbonate	20	20	20	20	20	20	20	20	20
Lactose	131	111	91	151	141	131	141	141	141
5% w/v PVP K30 in IPA	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Extra-granular									
Mg. stearate	2	2	2	2	2	2	2	2	2
Talc	2	2	2	2	2	2	2	2	2
Total	**200**	**200**	**200**	**200**	**200**	**200**	**200**	**200**	**200**

***The quantities are expressed as mg/tablet

Table 2: Results of Pre-Compression Studies of Nefidipine Floating Tablets *

F. Code	Angle of repose (θ)	Bulk density (gm/cm³)	Tapped density (gm/cm³)	Carrs Index (%)	Hausner's Ratio ( )
F1	24.10±0.41	0.47±0.01	0.79±0.02	40.51	1.68
F2	24.12±0.22	0.46±0.02	0.78±0.02	41.03	1.70
F3	25.32±0.32	0.48±0.02	0.79±0.02	39.24	1.65
F4	25.16±0.45	0.47±0.09	0.77±0.01	38.96	1.64
F5	24.48±0.41	0.45±0.04	0.79±0.01	43.04	1.76
F6	25.35±0.52	0.47±0.05	0.78±0.03	39.74	1.66
F7	24.11±0.31	0.45±0.01	0.79±0.02	43.04	1.76
F8	25.21±0.24	0.48±0.03	0.79±0.04	39.24	1.65
F9	25.31±0.24	0.47±0.01	0.79±0.03	40.51	
					1.68

*The values are expressed as mean ± S.D, where n=3. Hausner's ratio and Carr's index values are calculated from the mean values of bulk and tapped densities

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Table 3: Results of Post-Compression and *In vitro* Buoyancy Studies of Nefidipine Floating Tablets *

F. Code	Post-compression studies					*In vitro* buoyancy studies		
	Wt. variation (mg)	Thickness (mm)	Hardness (kg/cm²)	Friability (%)	Assay (%)	FLT (min: sec)	TFT (h)	MI (up to12h)
F1	±1.51	3.71±0.08	5.6±0.18	0.12	97.27±0.26	01:12± 0.04	Up to 12	+
F2	±2.12	3.51±0.04	5.5±0.06	0.72	96.11±0.56	01:05± 0.06	Up to 12	+
F3	±1.42	3.62±0.05	5.6±0.48	0.51	97.46±0.83	01:08± 0.13	Up to 12	+
F4	±1.21	3.72±0.06	5.7±0.38	0.22	96.57±0.63	01:20± 0.09	Up to 12	+
F5	±2.48	3.67±0.04	5.4±0.32	0.24	97.89±0.94	01:07± 0.11	Up to 12	+
F6	±1.49	3.46±0.06	5.6±0.44	0.37	96.49±0.61	01:02± 0.08	Up to 12	+
F7	±2.26	3.69±0.05	6.0±0.32	0.54	98.23±0.79	01:05 ±0.12	Up to 12	+
F8	±2.75	3.44±0.05	5.6±0.34	0.22	96.33±0.82	01:09± 0.07	Up to 12	+
F9	±1.72	3.20±0.04	5.7±0.53	0.64	98.24±0.53	01:10± 0.15	Up to 12	+

*All the values are expressed as mean ± SD, where n=3 except for Avg. wt. (n=20) and test for friability test 10 tablets per batch were taken (n=1)

Table 4: Drug Release Mechanism for Cylindrical Shape In Korsmeyer-Peppas Model

Diffusion Exponent (n)	Overall solute diffusion mechanism
0.45	Fickian diffusion
0.45 < n < 0.89	Non-Fickian diffusion
0.89	Case II transport
n > 0.89	Super Case II transport

Table 5: Results of Drug Release Kinetics of Nefidipine Floating Tablets

F. Code	Zero order r²	First order r²	Higuchi r²	Krosmeyer-Peppas r²	n
F1	0.765	0.898	0.936	0.929	1.607
F2	0.815	0.975	0.955	0.937	1.501
F3	0.865	0.977	0.973	0.969	1.429
F4	0.979	0.905	0.967	0.996	1.14
F5	0.988	0.838	0.876	0.997	0.607
F6	0.942	0.783	0.795	0.972	0.549
F7	0.993	0.723	0.941	0.997	0.746
F8	0.983	0.875	0.982	0.945	1.019
F9	0.973	0.870	0.992	0.956	1.045

Table 6: Results of Accelerated Stability Studies of Optimized Nefidipine Floating Tablets (F7)*

Time Interval	Post compression studies					*In vitro* buoyancy studies		
	Wt. variation (mg)	Thickness (mg)	Hardness (kg/cm²)	Friability (%)	Assay (%)	FLT (min: sec)	TFT (h)	MI (up to12h)
Initial	±2.26	3.69 ± 0.05	6.0 ± 0.32	0.54	98.23 ± 0.79	1:05 ± 0.12	Up to 12	+
1 Month	± 2.28	3.93 ± 0.08	5.6 ± 0.12	0.55	98.12 ± 0.08	1:09 ± 0.09	Up to 12	+
2 Month	± 2.32	3.91 ± 0.10	5.7 ± 0.13	0.57	97.98 ± 0.21	1:10 ± 0.11	Up to 12	+
3 Month	± 2.37	3.89 ± 0.21	5.8 ± 0.21	0.61	97.64 ± 0.11	1:14 ± 0.15	Up to 12	+
6 Month	± 2.42	3.88 ± 0.32	5.6 ± 0.21	0.62	97.52 ± 0.21	1:17 ± 0.12	Up to 12	+

*All the values are expressed as mean ± SD, where n=3 except for Avg. wt. (n=20) and test for friability test 10 tablets per batch were taken (n=1)

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Fig.1. FT-IR spectra of A) Nefidipine, B) Nefidipine + HPMC K100M, C) Nefidipine + xanthan gum, D) Nefidipine + guar gum and E) Nefidipine + sodium alginate

Fig.2. Standard calibration curve of Nefidipine in 0.1 N HCl

Fig.3. *In vitro* floating images of optimized Nefidipine Floating Tablets (F7)

Fig.4. *In vitro* dissolution plots of Nefidipine Floating Tablets with; A. HPMC K15M; B. HPMC K100M; C. HPMC K100M + SA and HPMC K100M + XG and HPMC K100M + GG

Fig.5. *In vitro* dissolution profiles of initial, 1, 2 and 3M accelerated stability samples of optimized Nefidipine Floating Tablets (F7)

Fig.6. Comparative FT-IR spectra of a A) Nefidipine (Pure drug) and B) Optimized Nefidipine Floating Tablets (F7) accelerated stability 3M sample

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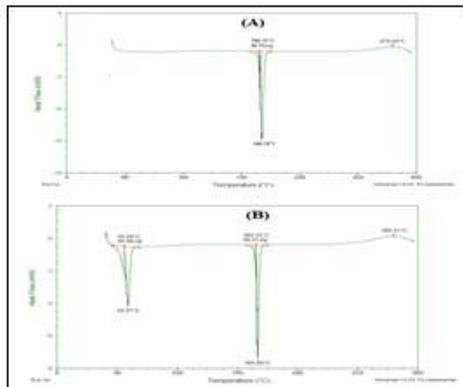
Fig.7. Comparative DSC thermograms of a A) Nefidipine (Pure drug) and B) Optimized Nefidipine Floating Tablets (F7) accelerated stability 3M sample.

RESEARCH ARTICLE

A Study on Modified Weighted Kaplan – Meier using Medical Data

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ABSTRACT

In this paper we have studied the modified weighted Kaplan Meier estimator for estimating the survival probabilities for the censored observations. The Kaplan Meier, Weighted Kaplan Meier, Modified Kaplan Meier have been discussed and analysed with a censored data. It is observed from the results that the latter method is better than modified weighted Kaplan Meier method. From the study, we revealed that Kaplan-Meier provides a better survival probability but for the last censored observation it gives zero survival probability. Modified Weighted Kaplan Meier provides non-zero survival probability for last censored observation.

Keywords: Survival Analysis, Kaplan – Meier, Weighted Kaplan – Meier, Modified Kaplan Meier.

INTRODUCTION

Survival analysis is a tool which is used to analyse time – to – event data (time means years, months, weeks or days). The Survival analysis plays a vital role in statistics which deals with the death in biological creature and failure in machine – like systems, equipments, items etc. Meanwhile, it is very much useful in follow – up an individual until any event (i.e., birth, death, recurrence of disease, rehabilitation etc.,) occurs. Censoring is the form of mislaid information about the individuals who either die of any other causes than particular disease for which they was getting treatment or are lost to follow – up. Censoring broadly classified into five types namely Right Censoring, Left Censoring, Interval Censoring, Random censoring. Right censoring occurs when a subject leaves the study ends before the event has occurred. Left censoring occurs if an individual entered into the study when the milestone of interest occurred prior to study entry but the age at that individual is unknown. Interval censoring mean that a

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random variable of interest is known only to lie within an interval instead of being observed exactly. Human Immuno Virus (HIV) is a virus transmitted from one person to another thus involving serious risk factors. It damages the cells in immune system and greatly weaken's one's ability to fight infections and diseases. An individual affected with HIV is capable of leading a healthy life provided very effective drug treatments. In India 23.19 lakh people are affected with HIV. As there is no cure for HIV it is still one of the dangerous diseases of India. AIDS refers to the number of life threatening infections that happen when the immune system is damaged by HIV and it is non – contagious. With early diagnosis and required treatments most of the people with HIV won't develop any AIDS related illness. Since there is not a standard and prolonged symptom of HIV. Other ways include sharing syringes between patients and transmission during pregnancy, delivery and breast feeding. HIV is found in body fluids (semen, vaginal and anal fluid, blood and breast milk). It is an individual's responsibility to be cautious towards this fragile yet serious virus.

Jiaqiet al. (2021) demonstrated Kaplan Meier estimator as M-estimator and discussed confidence interval as well as confidence bands. Liu et al. (2021) introduced two stage approach to determine the individual patient data IPD from Kaplan – Meier curves and discussed about the flexibility accuracy of IPD from Kaplan – Meier. Guyot et al. (2021) proposed the model to increase the quality of secondary data analysis which acquire from the Kaplan - Meier survival curves. Mishiro et al. (2008) studied and revealed that Kaplan Meier survival analysis is useful for recurrence of Cholesteatoma. Friedo et al. (2011) studied patients with ERA – EDTA primary reneal disease with multiple myloma and non – multiple myloma and revealed there is need to adjust the potential confounders in patients. Shafiqet al. (2007) proposed a new modification of weighted Kaplan Meier estimator by providing a new weight function for estimating the probability of censored observation.

Kaplan – Meier Estimator

Kaplan – Meier estimator was introduced by Edward L. Kaplan and Paul Meier in 1958 for estimating the survival probability of the censored observations.Kaplan–Meier curve is that the method can take into account some types of censored data, particularly right-censoring, which occurs if a patient withdraws from a study, is lost to follow-up, or is alive without event occurrence at last follow-up.

$$KM = S(t) = \prod_{x \leq t} \left[1 - d(x)/n(x) \right]$$

where,
d(x) – the number of deaths at time x
n(x) – the number of individuals at risk just prior to time

Weighted Kaplan – Meier

Weighted Kaplan – Meier was introduced by Bahrawar Jan (2004) by modifying the Kaplan – Meier to a greater extent. It is used in the case of heavy censoring. Thus, Weighted Kaplan – Meier estimates can be expressed as

$$S*(t) = \prod_{x \leq t} W_j \left[1 - d(x)/n(x) \right]$$

where $W_j = \left(\dfrac{n_j - c_j}{n_j} \right)$ is known as non- censoring rate.

Weighted Kaplan – Meier gives zero weight to the last censored observation considered as a greatest drawback whereas a new weight function is developed to remove the deficit.

Modified Weighted Kaplan Meier

The Modified Weighted Kaplan – Meier estimator was developed by Mohammad Shafiq et al. (2007) by adding a new weight function to the Weighted Kaplan – Meier to remove the inadequacy.

The Modified Weighted Kaplan – Meier is defined by

$$S**(t) = \prod_{x \le t} W_j \left[1 - d(x)/n(x)\right]$$

Where the weight function W_j is given by

$$W_j = 1 - Sin\left(\frac{C_j * P_j}{n_j}\right)$$ is known as non – censoring rate.

C_j = Censored
P_j = Probability of Survival
n_j = number of individual survive at time t

Data Analysis

The data set given below represents the survival times (in months) of 100 HIV patients reported by David W. Hosmer& Stanley Lemeshow.

5,6*,8,3,22,1*,7,9,3,12,2*,12,1,15,34,1,4,19*,3,2,2*,6,60*,11,2*,5,4*,1*,13,3*,2*,1*,30,7*,4*,8*,5*,10,2*,9*,36,3*,9*,3*,35,8*,1*,5*,
11,56*,2*,3*,15,1*,10,1*,7*,3*,3*,2*,32,3*,10*,
11,3*,7*,5*,31,5*,58,1*,2*,1,3*,43,1*,6*,53,14,4*,54,1*,1*,8*,5*,1*,1*,2*,7*,1*,10,24*,7*,12*,7*,60*,4*,57,1*, 12*.
(* - Represents Censored observation)

Figure 1 shows that Kaplan Meier estimator gives the highest probability when compared with Weighted Kaplan Meier and Modified Kaplan Meier. Thus, the above figure reveals that Kaplan Meier gives the better fit.

CONCLUSION

In this study, survival probabilities has been determined by the approach of Kaplan Meier, weighted Kaplan-Meier and Modified Weighted Kaplan Meier. These models has been analysed by using real life data and observed that the Kaplan – Meier provide high survival probability for the observations. From the study, it is clearly seen that for the last censored observation, the weighted Kaplan Meier method provides a probability of survival zero. From the table 1 we conclude that it is better to use the Modified Weighted Kaplan – Meier when the last observation is censored because it provide non – zero survival probability.

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Table: 1 Estimation of Survival probability using different methods.

S.no	Time	No. of. Death	No. of censored	No. of. Risk	Prob. of. Survival	Weighted KM weights	Modified weight	Kaplan Meier S(t)	Weighted Kaplan Meier S(t)	Modified Weighted Kaplan Meier S(t)
1	1	3	14	100	0.970	0.860	0.8646	0.970	0.834	0.8386
2	2	1	9	83	0.987	0.892	0.8931	0.957	0.734	0.7392
3	3	2	10	73	0.973	0.863	0.8671	0.932	0.616	0.6230
4	4	1	4	61	0.9836	0.934	0.9335	0.91580	0.567	0.5716
5	5	2	5	56	0.9642	0.911	0.9140	0.883	0.4977	0.5026
6	6	1	2	49	0.979	0.959	0.9600	0.864	0.467	0.4733
7	7	1	6	46	0.978	0.869	0.8727	0.845	0.397	0.4039
8	8	1	3	39	0.974	0.923	0.9251	0.823	0.357	0.3639
9	9	1	2	35	0.971	0.943	0.9445	0.799	0.327	0.3337
10	10	3	1	32	0.906	0.968	0.9716	0.724	0.287	0.2937
11	11	3	0	20	0.8928	1	1	0.647	0.256	0.2619
12	12	2	2	25	0.920	0.92	0.9264	0.595	0.217	0.2232
13	13	1	0	21	0.952	1	1	0.566	0.206	0.2124
14	14	1	0	20	0.95	1	1	0.538	0.196	0.2017
15	15	2	0	19	0.895	1	1	0.482	0.175	0.1805
16	19	0	1	17	1	0.941	0.9412	0.482	0.165	0.1698
17	22	1	0	16	0.938	1	1	0.452	0.155	0.1592
18	24	0	1	15	1	0.933	0.9333	0.452	0.144	0.1485
19	30	1	0	14	0.928	1	1	0.419	0.134	0.1378
20	31	1	0	13	0.923	1	1	0.387	0.124	0.1279
21	32	1	0	12	0.917	1	1	0.355	0.113	0.1165
22	34	1	0	11	0.909	1	1	0.322	0.103	0.1058
23	35	1	0	10	0.90	1	1	0.290	0.093	0.0952
24	36	1	0	9	0.889	1	1	0.258	0.083	0.0846
25	43	1	0	8	0.875	1	1	0.225	0.072	0.0740
26	53	1	0	7	0.857	1	1	0.1934	0.062	0.0634
27	54	1	0	6	0.833	1	1	0.1612	0.052	0.0528
28	56	0	1	5	1	0.8	0.8013	0.1612	0.0412	0.0423
29	57	1	0	4	0.750	1	1	0.121	0.0309	0.0317
30	58	1	0	3	0.667	1	1	0.081	0.02063	0.0211
31	60	0	2	2	1	0	0.1585	0.081	0	0.0033
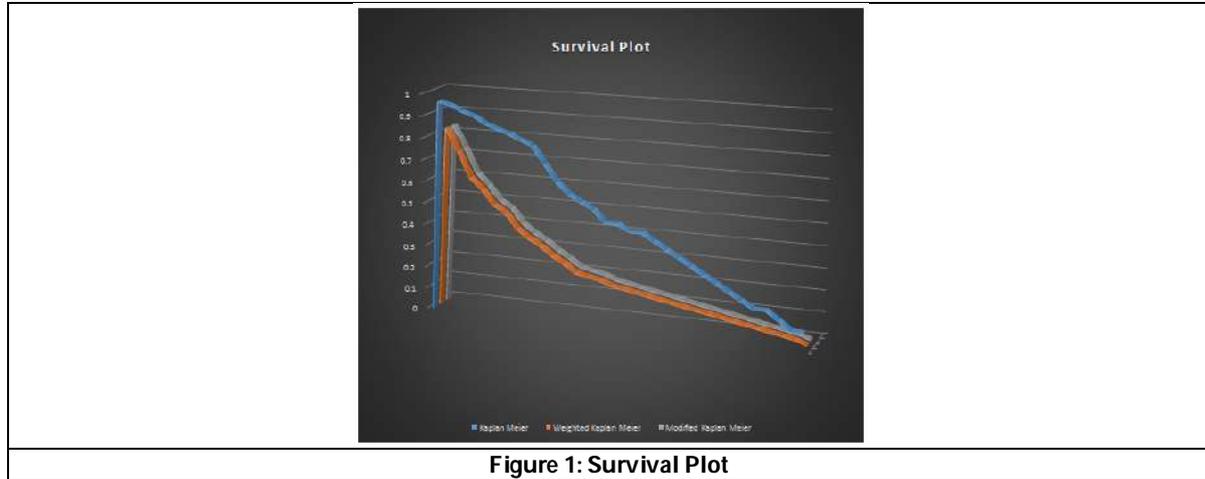
Figure 1: Survival Plot

RESEARCH ARTICLE

In silico Approaches to Characterize the Biophysical and Chemical Properties and Molecular Docking Studies of Phenol Degradation Enzymes (Phenol Hydroxylase and Catechol 1,2 Dixoygenase) from *Corynebacterium glutamicum* ATCC 13032

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ABSTRACT

Corynebacterium glutamicum is a non-pathogenic, gram-positive, non-spore forming facultative anaerobic bacterium and for decades, it is widely used for the production of amino acids. In addition, the potential of *C. glutamicum* in the biodegradation of toxic environmental compounds is well appreciated. In the current study, we explored the biophysical and chemical properties (theoretical pI, extinction coefficients, estimated half-life, instability index, aliphatic index and grand average of hydropathicity) of phenol degradation enzymes from *C. glutamicum* using *in silico* approaches. GRAVY scores of both the enzymes were -0.456 and -667, respectively suggesting their hydropthay nature. The aliphatic index and pI values of these enzymes suggested that they were thermo stabile and acidic nature. Further, molecular docking studies indicated that the amino acid residues of phenol hydroxylase showed interactions with phenol were van der waals bonds (Pro 155), Pi-cation (Met 14), Pi-Pi bonds (Phe 150) and Pi-alkyl (Met 16) bonds. The other residues that were associated with ligand to protein interactions were Ser 52, Arg 53, Asn 153, and Ser 154, while, the amino acid residues of catechol 1,2 dioxygenase showed interactions with catechol were Leu 80, Asp 83, Val 84, Ile 107, Pro 110, Tyr 111, Tyr 201, Arg 222, His 227, Gln 241 and Ala 255 and Glu 108 and Gly 109 showed hydrogen bonding. This information will be useful for scientists to design experiments in the removal of other phenolic compounds.

Keywords: *Corynebacterium glutamicum*; catechol, catechol 1,2 dioxygenase, phenol, phenol hydroxylase

INTRODUCTION

Corynebacterium glutamicum is a non-pathogenic, gram-positive, non-spore forming facultative anaerobic bacterium and for decades, it is widely used for the production of amino acids (Tsuge and Yamaguchi, 2021). As per the estimates, the annual production of L-glutamate and L-lysine from *C. glutamicum* is 2,160,000 and 1,480,000 tons, respectively. Thus, *C. glutamicum* is well acknowledged microorganism in the area of white biotechnology (Wolf et al., 2021). *C. glutamicum* is used as a cell factory for the production of amino acids and value-added products (Wolf et al., 2021), a preferred choice to develop therapeutic approaches against corynebacteriales pathogens (Si et al., 2014) and also used in the bioremediation of toxic aromatic compounds phenol, resorcinol, benzyl alcohol, benzoate, 4-cresol and heavy metals including arsenic metalloid (Shen et al., 2012). Based on these facts, *C. glutamicum* is a versatile model system to design experiments related to over production of desired products or design eco-friendly strategies that are helpful in the degradation of aromatic compounds that cause environmental pollution.   Phenol contamination is believed to be due to improper treatment of industrial effluents and agricultural pesticides (Mohammadi et al., 2015). Phenol and its related compounds have ability to interfere with ecosystem equilibrium and eventually targets biogeochemical pathways of organic matter and nutrient cycling (Cordova-Rosa et al., 2009). Thus, removal of phenol is of prime importance. Earlier many approaches have been employed to environmental clean-up of phenol such as adsorption, chemical oxidation, solvent extraction and incineration. However, production of hazardous secondary products and economic constraints limited the use of these techniques (Loh and Wang, 1998). In addition to its primary function, *C. glutamicum* has ability to degrade various aromatic compounds including phenols (Lee et al., 2010a; 2010b), forming an ecofriendly approach. Two enzymes have been identified, phenol hydroxylase (Phe) and catechol dioxygenases (C12O) of *C. glutamicum* related to phenol degradation (Shen et al., 2012). However, the biophysical and chemical characteristics of phenol degradation enzymes are yet to be resolved. Therefore, an *in silico* approach was used to depict the biophysical and chemical characteristics of phenol. Further, docking studies were performed to investigate the interactions between the ligands (phenol and catechol) and the proteins (Phe and C12O) selected.

MATERIALS AND METHODS

Biophysical and chemical properties
The amino acid sequences of phenol hydroxylase (Phe) and catechol 1,2 dioxygenase (C12O) from *C. glutamicum* were retrieved from NCBI (National Center for Biotechnology Information) database (URL: http://www.ncbi.nlm.nih.gov) and Bio Cyc database (URL: http://BioCyc.com/) in FASTA format for computational analysis. The amino acid sequences of Phe and C12O were subjected to primary (expasyprotparum: https://web.expasy.org/protparam/) and secondary structural prediction analysis tools PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) and CFSSP (http://cho-fas.sourceforge.net/index.php) servers to gain insights into the biophysical and chemical properties and information related to protein folding, respectively *in silico*.

Homology modeling
After structural analysis of these enzymes, the amino acids were subjected to SWISS Homology-Modelling server (https://swissmodel.expasy.org/) to predict 3D protein models. The structural evaluation and validation of predicted models was analyzed by Ramachandran Plot analysis, PROCHECK, ERRAT, VERIFY3D and ProSAanalysis (SAVES Programme: http://nihserver.mbi.ucla.edu/SAVES), and QMEAN. Finally, functional and protein-protein interactions of selected enzymes were determined by String database (STRING v10.0 (http://string-db.org/) and motif finder server (http://www.genome.jp/tools/motif/).

Molecular docking
Molecular docking of Phe and C12O against the ligands, phenol and catechol, respectively were performed using Galaxy WEB server and the docked models were visualized by Discovery studio and Chimera tools. Briefly, the protein sequences of Phe and C12O were subjected to BLASTP analysis and asked to search against PDB database to

determine the suitable models and accordingly, two structures corresponding to Phe and C12O were retrieved from PDB database and used as templates for homology modeling (SWISS database). The ligands such as phenol [Phenol; 108-95-2; Carbolic Acid; Hydroxybenzene; Phenic Acid; Oxybenzene; Phenylic Acid; Benzenol; Compound CID: 996; MF: C_6H_6O; MW: 94.11g/mol; IUPAC Name: phenol; Isomeric SMILES: C1=CC=C(C=C1)O] and catechol [Pyrocatechol; Catechol; 120-80-9; 1,2-Benzenediol; 1,2-Dihydroxybenzene; Benzene-1,2-Diol; Pyrocatechin; 2-Hydroxyphenol; Compound CID: 289; MF: $C_6H_6O_2$; MW: 110.11g/mol; IUPAC Name: benzene-1,2-diol; Isomeric SMILES: C1=CC=C(C(=C1)O)O] was obtained from pubchem database (https://pubchem.ncbi.nlm.nih.gov/). The selected ligands were fetched into the Chimera server (https://www.cgl.ucsf.edu/chimera/) and saved in mol2 formats. For molecular docking analysis, the protein in pdb format and ligands in Mol2 formats were subjected to a web-based molecular docking server, Galaxy WEB server (https://galaxy.seoklab.org/). The Galaxy WEB server provides a platform for protein structure prediction, refinement and related methods (Shin et al., 2013). The docked files were fetched into the discovery studio for visualization (https://discover.3ds.com/discovery-studio-visualizer-download).

RESULTS AND DISCUSSION

Biophysical and chemical parameters of the phenol degradation related enzymes like Phe and C12O were determined by using Expasy protparum tool. The parameters like number of amino acids, molecular weight, theoretical pI, extinction coefficients, estimated half-life, instability index, aliphatic index and grand average of hydropathicity of phe and C12O were shown in Table 1. The number of amino acids in Phe and C12O were found to be 627 and 285 with MW 70.11 kDa and 32.42 kDa and pI value of 5.10 and 4.69, respectively. Extinction coefficient of Phe and C12O was 69915 $M^{-1}cm^{-1}$ and 73340 $M^{-1}cm^{-1}$, respectively when assuming all pairs of Cys residues form cystines and 69790 $M^{-1}cm^{-1}$ and 73340 $M^{-1}cm^{-1}$, respectively when all Cys residues were reduced. The estimated half-life of Phe and C12O were calculated to be 30 hours in mammalian reticulocytes, in vitro; >20 hours in yeast, in vivo and >10 hours in *E. coli*, in vivo, showing it can be stable in prokaryotic cells. The instability index of the phe and C12O enzyme was 33.65 and 32.49, respectively thus classified as stable *in vitro* (values below 40 indicate stability of enzyme *in vitro*). The aliphatic index of phe and C12O were found to be 73.27 and 71.16, respectively, while the grand average of hydropathicity (GRAVY) of the phe and C12O were -0.456 and -667, respectively. Studies of Xiao et al. (2015) indicated that the protein encoded for Phe from *C. glutamicum* RES167 comprised of 627 amino acids with a molecular weight of 70 kDa and pI of 4.92. On a comparative note, biophysical and chemical properties of Phe from *Pseudomonas* species were documented. It has been shown that Phe from these species comprises of 427-576 amino acids with a molecular weight around 61.3 kDa. Further, it has been indicated that the isolectric point of Phe from *Pseudomonas* species ranges from 4.93 to 6.03 suggesting that Phe from these species were mostly acidic in nature, with an exception of *Pseudomonas* species KZNSA wherein the pI of 9.03 was reported suggesting the alkaline nature of this enzyme. Studies of Xiao et al. (2015) showed that the phylogenetic analysis revealed that the Phe from *C. glutamicum* RES167 exhibited close relationship to yeast over bacteria and shared 31%, 19%, 15% and 15% identity to fungus, *Trichosporon cutaneum* (Enroth et al., 1998), and bacteria *Pseudomonas pickettii* PKO1 (Kukor and Olsen, 1990), *Bacillus thermoglucosidasius* A7 (Kirchner et al., 2003) and *Bacillus stearothermophilus* BR219 (Kim and Oriel, 1995), respectively. With respect to catechol 1,2 dioxygenase (catA), the biophysical and chemical parameter studies reported that the number of amino acids for CatA from *C. glutamicum* ATCC 13032 was 285 amino acids with a molecular weight of 32 kDa and pI of 4.69 suggesting the acidic nature of catA. Studies from other *Pseudomonas* spp. have shown that the number of amino acids, molecular weight and pI of catA range from 314-327, 34-36 kDa and 4-11, respectively. Though most of the catA from *Pseudomonas* sps. was found to be acidic, catA from *Pseudomonas chlororaphis* PCL1606 was found to be alkaline in nature with a pI of 11.37 (Al-Hakim et al., 2015). Interestingly, studies of Setlhare et al. (2018) reported that the cat A in *P. chloroaphis* UFB2 strain was found to be acidic in nature with pI of 5.3.

The secondary structure of Phe and C12O were predicted using PSIPRED and CFSSP (Fig 1). Both the proteins did not contain disordered binding sites indicating that the three dimensional structure could be well defined and

reflects their functionality. The predicted secondary structure of Phe and C12O from *C. glutamicum* ATCC 13032 comprised of helix, strands and turns in the order of 69.4%, 58.5% and 12.6% and 66.7%, 30.2% and 13.7%, respectively. STRING database (Fig 2) was used to predict the protein-protein interaction network of phenol hydroxylase and catechol 1,2 dioxygenase. With respect to the Phe of *C. glutamicum* ATCC 13032, ten nodes were predicted by the server and the nearest node was C12O with a score of 0.923, whereas the distant node with a score of 0.422 was found to be C12O3. With respect to the C12O of *C. glutamicum* ATCC 13032, ten nodes were predicted by the server and the nearest node was catB, chloromuconate cycloisomerase with a score of 0.994, whereas the distant node with a score of 0.730 was found to be pcaG. Different motifs of Phe and C12O were determined using motif finder. With respect to the phenol hydroxylase of *C. glutamicum* ATCC 13032, 13 motifs were predicted by the server and out of which two domains were significantly recognized by the motif server *viz.,* FAD binding domain (PF01494) and phenol hydroxylase, C-terminal dimerization domain (PF07976) with E values of 2.2e-69 and 3.4e-19, respectively and the position of amino acids were 33 to 403 and 435 to 580, respectively. With respect to the catechol 1,2 dixoygenase of *C. glutamicum* ATCC 13032, 5 motifs were predicted by the server and out of which three domains were significantly recognized by the motif server *viz.,* dixoygenase domain (PF00775), catechol dioxygenase N terminus (PF04444) and carboxypeptidase regulatory domain (PF13620) with E values of 5.3e-56, 4.8e-13 and 1.8e-06, respectively and the position of amino acids were 107 to 282, 35 to 98 and 140 to 203, respectively.

SWISS Modelling database was used to build models for Phe and C12O of *C. glutamicum* ATCC 13032 (Fig 3). A total of 50 templates were predicted by the server for each protein and out of which one protein with good resolution, percent identity, GMQE and QMEANDisCo. With respect to the phenol hydroxylase of *C. glutamicum* ATCC 13032, the template 3-hydroxybenzoate hydroxylase from *Comamonas testosteroni* with pdb id 2dki.1.A was selected based on the X-ray resolution: 2.50Å, GMQE value: 0.78, QMEANDisCo global value: 0.77 ± 0.05, sequence identity of 42.67%. With respect to the catechol 1,2 dixoygenase of *C. glutamicum* ATCC 13032, the template catechol 1,2 dixoygenase from *Rhodococcus opacus* with pdb id 3i51.1.A was selected based on the X-ray resolution: 1.8Å, GMQE value: 0.85, QMEANDisCo global value: 0.85 ± 0.05, sequence identity of 67.66%. The quality of predicted models were analyzed by SAVES server. The server provides various quality check databases like ERRAT, PROCHECK and VERIFY3D. With respect to the modeled catechol 1,2 dioxygenase of *C. glutamicum* ATCC 13032, VERIFY 3D showed that the model shows 80.93% residues have an average 3D-1D score >=0.2 and ERRAT value of 88.47 indicates that the overall quality factor of the model was good. In general, ERRAT value represents the overall quality factor for non-bonded atomic interactions, with higher scores indicating higher quality and the accepted range of ERRAT value is >50. Modeled C12O showed 95.69% residues fall under allowed regions or Ramachandran favored region (Fig 4), while 1.18% residues fall under Ramachandran outliers and 2.74% fall under Rotamer outliers. Based on the analysis of Ramachandran plot, the modeled protein was of high quality and stable. The overall quality of model can be predicted through Z-scores in ProSA. The z-score of modeled C12O protein of *C. glutamicum* ATCC 13032 was found to be -5.41 suggesting that the modeled protein was non-erroneous. With respect to the modeled phenol hydroxylase of *C. glutamicum* ATCC 13032, VERIFY 3D showed that the model shows 86.18% residues have an average 3D-1D score >=0.2 and ERRAT value of 97.188 indicates that the overall quality factor of the model was good. Modeled phenol hydroxylase showed 94.14% residues fall under allowed regions or Ramachandran favored region, while 0.65% residues fall under Ramachandran outliers and 1.16% fall under Rotamer outliers. Based on the analysis of Ramachandran plot, the modeled protein was of high quality and stable. The z-score of modeled phenol hydroxylase protein of *C. glutamicum* ATCC 13032 was found to be -9.95 and the local model quality which represent residue energies indicate that the most of the amino acids with negative energies, suggesting that the modeled protein was non-erroneous.

After structure validation of both the modeled enzymes of *C. glutamicum* they were used for molecular docking analysis (Fig 5) using Galaxyweb server with an in built docking tools. When the modeled C12O in the PDB format and ligand in Mol2 format was subjected to the Galaxy server, 10 docked models were obtained and out of which the docked model with highest binding energy (-8.74) was visualized using Discovery studio. The molecular docking revealed that the C12O of *C. glutamicum* ATCC 13032 forms two conventional hydrogen bonds with amino acids were Glu and Gly and their positions were 108 and 109, respectively. The other residues that were associated with

ligand to protein interactions were Leu 80, Asp 83, Val 84, Ile 107, Pro 110, Tyr 111, Tyr 201, Arg 222, His 227, Gln 241 and Ala 255. On the other hand, when the modeled Phe in the PDB format and ligand in Mol2 format was subjected to the Galaxy server, 10 docked models were obtained and out of which the docked model with highest binding energy (-9.18) was visualized using Discovery studio. The molecular docking revealed that the modeled Phe of *C. glutamicum* ATCC 13032 show van der waals bonds (Pro 155), Pi-cation (Met 14), Pi-Pi bonds (Phe 150) and Pi-alkyl (Met 16) bonds. The other residues that were associated with ligand to protein interactions were Ser 52, Arg 53, Asn 153, and Ser 154. The biophysical and chemical properties of Phe and C12O provide value insights into the stability, and pH of enzyme activities. While, molecular docking studies provide insights into the mechanistic properties of the Phe and C12O.

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Table 1: **Biophysical and chemical properties of phenol hydroxylase and catechol 1,2 dioxygenase from** *C. glutamicum* **ATCC 13032 analyzed by Exapsy protparum**)

Properties	Phenol hydroxylase	Catechol 1,2 dioxygenase
Formula	$C_{3092}H_{4772}N_{864}O_{960}S_{22}$	$C_{1459}H_{2161}N_{383}O_{450}S_5$
Total number of atoms	9710	4458
Number of amino acids	627	285
Molecular weight	70114.44	32426.81
Theoretical pI	5.10	4.69
Extinction coefficients		
Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water		
Abs 0.1% (=1 g/l)　0.997, assuming all pairs of Cys residues form cystines	69915	73340
Abs 0.1% (=1 g/l)　0.995, assuming all Cys residues are reduced	69790	73340
Estimated half-life:	30 hours (mammalian reticulocytes, *in vitro*). >20 hours (yeast, *in vivo*). >10 hours (*Escherichia coli, in vivo*).	30 hours (mammalian reticulocytes, *in vitro*). >20 hours (yeast, *in vivo*). >10 hours (*Escherichia coli, in vivo*).
Instability index >30 value indicate that the protein as stable.	33.65	32.49
Aliphatic index >70 value indicate the protein as thermostable	73.27	71.16
Grand average of hydropathicity (GRAVY) Negative value indicate an interaction between water and protein	-0.456	-0.667

Table 2: Z-scores, Errat, verify 3D values of phenol hydroxylase and catechol 1,2 dioxygenase from *C. glutamcium* **ATCC 13032**

Parameter	Phenol hydroxylae	Catechol 1,2 dioxygenase
Z-scores	>-9	> -5
ERRAT	97.18%	88.477%
Verify 3D	86.18% (3D-1D >=0.2)	80.93% (3D-1D >=0.2)

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Phenol hydroxylase [% of helices: 69.4; % of strands: 58.5; % turns: 12.6]
Catechol 1,2 dioxygenase [% of helices: 69.7; % of strands: 30.2; % turns: 13.7]
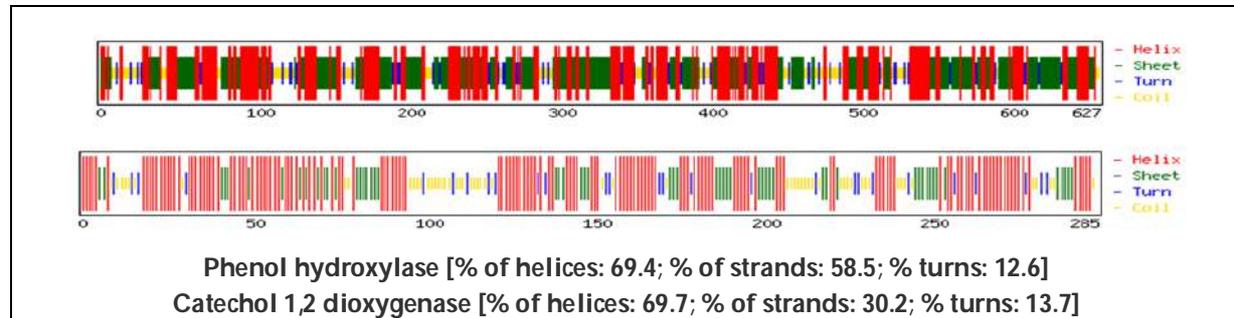
Figure 1. Secondary structure prediction of phenol hydroxylase (upper panel) and catechol 1,2 dioxygenase (lower panel) from *C. glutamicum* ATCC 13032 using CFSSP server
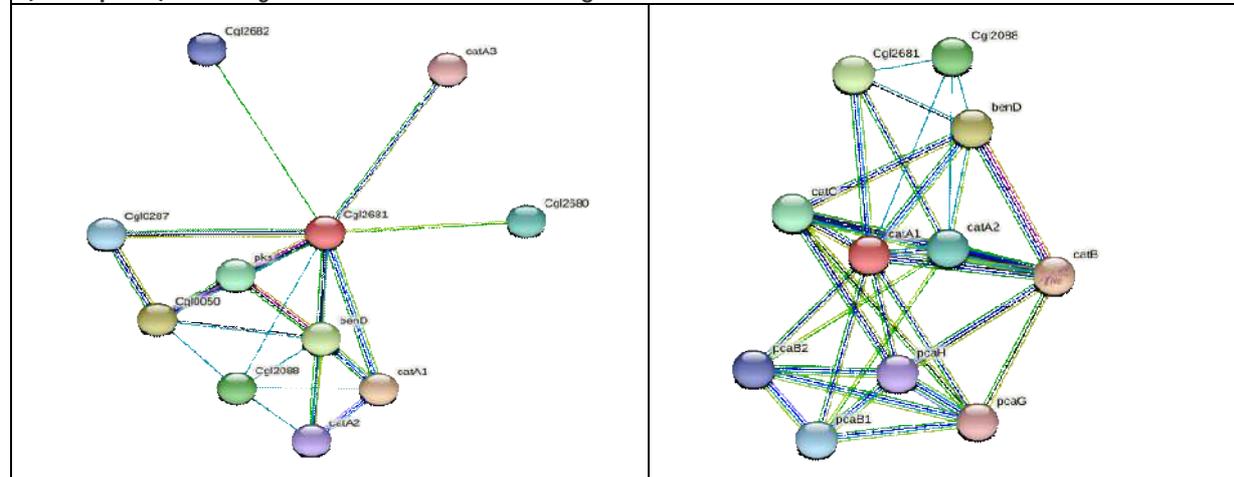
Figure 2. Functional analysis of phenol hydroxylase (left) and catechol 1,2 dioxygenase (right) of *C. glutamicum* ATCC 13032using STRING database reveals protein-protein interaction map
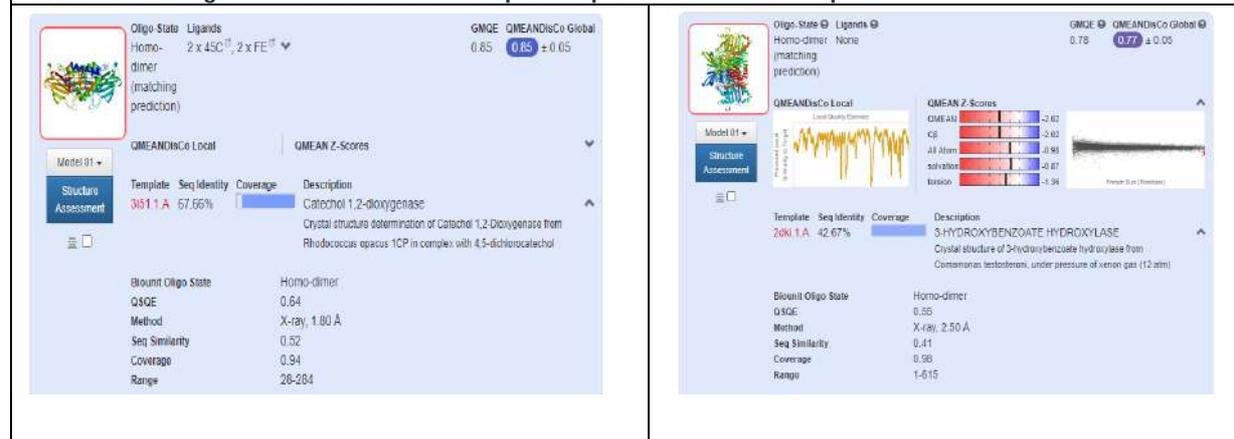
Figure 3. SWISS modelling of phenol hydroxylase (left) and catechol 1,2 disoxygenase (right)from *C. glutamicum* ATCC 13032

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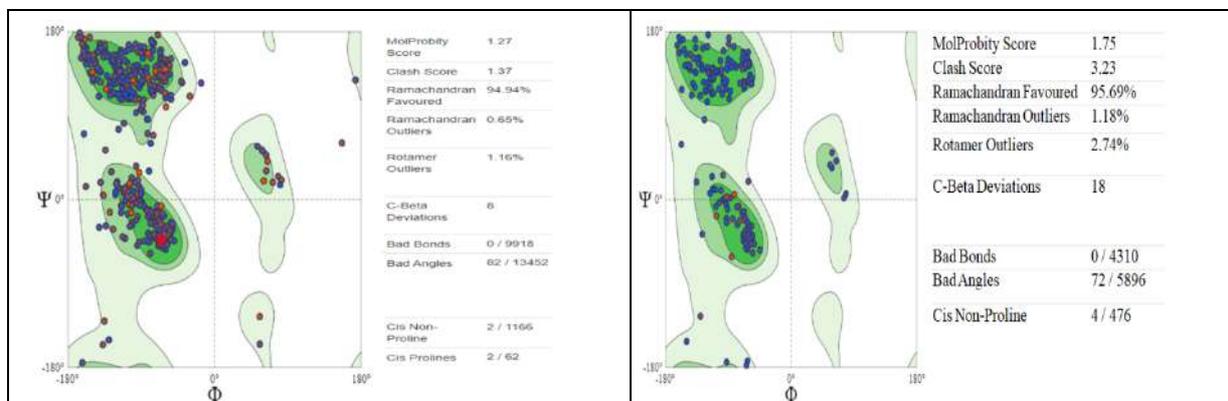
Figure 4. Ramachandran plot of phenol hydroxylase (left) and catechol 1,2 disoxygenase (right) from *C. glutamicum* ATCC 13032
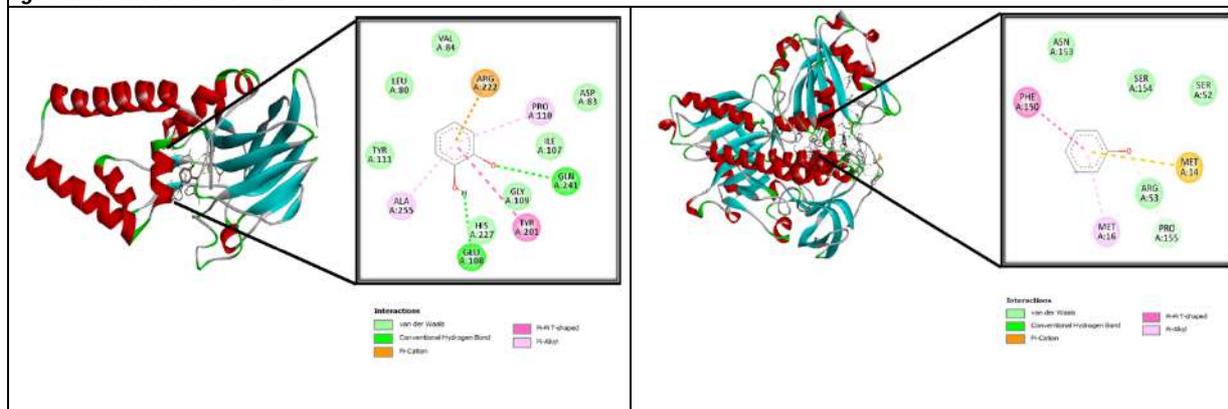
Figure 5: Molecular docking of catechol 1,2 dioxygenase (upper panel) and phenol hydroxylase (lower panel) from *C. glutamicum* ATCC 13032 against catechol and phenol, respectively

RESEARCH ARTICLE

An Efficacy of Shaman Chikitsa on Amlapitta: A Systemic Appraisal

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ABSTRACT

Increase prevalent rate of Amlapitta is a constant challenge to field of research. Here is an attempt has made to analyse the research work for management of Amlapitta. The aim the study was to review the efficacy of Shaman Chikitsa on Amlapitta. The review of clinical study using different polyherbal or mineroherbal formulation in Amlapitta was carried out. Extensive search were done using Internet search engine. Eleven articles were reviwed in which Madhur, Tikta Rasa Pradhan &SheetaVirya quality formulations are very useful in treating Amlapitta.

Keywords: Amlapitta, polyherbal or mineroherbal, Madhur, Tikta

INTRODUCTION

In Ayurveda, It is said that Agnimadya is the fundamental cause of all diseases. In this Modern era, fast rhythm of the present day made many people adopt fast food as their meal. "Hurry, worry and Curry are the biggest enemies to the body." Due to the present lifestyle and unawareness of one s Prakriti, digestive disorders are very common in all age groups and highly ignored. Amlapitta is a commonest functional disorder of Annavahastrotas. In Brihatrayi, Charak has not explained Amlapitta as an individual entity, but discussed at many places like kulattha causes Amlapiita, Excessive use of Lavana rasa cause Amlapitta, rajmasha has quality of suvsiding symptoms of Amlapitta, enlisted as indication of Mahatiktak Ghrita, enlisted as indication kansaharitaki etc. Sushruta has mentioned about "Amlika" while discussing about diseases arise due to excessive use of lavana rasa. Acharya Kashyap has explained about the Amlapitta as individual entity. Madhava Nidana is the first available text which gives importance to Amlapitta and describes its aetiopathogenesis and symptomatology in detail along with two clinical sub types viz. 1)

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Urdhvaga and 2) Adhoga Amlapitta. Chakradatta : Chakrapani in Ch. Chi. 15 has given the symptomatology of Amlapitta without mentioning its references. He discussed its treatment in Chakradatta. Shrikanthadatta in his commentary on the relevant chapter has defined that Amlapitta is a condition where excessive secretion of Amla Guna Pitta takes place causing vidahyadi conditions. Madhava Nidana has given a clinical definition of Amlapitta that presence of Avipaka, Klama, Utklesha, Amlodgara, Gaurava, Hrit-KanthaDaha and Aruchi should be termed as Amlapitta. (M. N. 51/2) this is a disease mainly due to vitiation of Pitta (Pachaka-Pitta) but Kapha (Kledaka) and Vata vitiation may be there as mentioned by Shrikanthadatta. In Samhita, some other words have also been mentioned in the reference of Amlapitta. These are Amlaka, Dhumaka and Vidaha.

Signs and symptoms of Amlapitta can be correlated with different modern terms like acute or chronic gastritis, GERD, Non ulcer dyspepsia. All the above terminologies being treated with H2 blockers, Proton pump inhibitors. They shows remarkable relief in symptoms only but long term use of the same has side effects. Madhavakara stated that, Amlapitta can be easily managed in early stage. In chronic conditions of Amlapitta, treatment should be given for very long time. In addition, for this long term treatment, there is need for herbal, eco friendly, toxicity free medicine to use.[1]

MATERIALS AND METHODS

Related Research Article published on different journals are reviewed.
1) Study Title : THE EFFECT OF VASA (ADHATODA VASICA) ON AMLAPITTA[2]
   No. Of Patients: 20 patients
   Drug Given: Vasa Syrup
   Dose: 60 ml syrup in four divided doses for 6 weeks
   Result: Out of 20 patients of non-ulcer dyspepsia (Amlapitta) having hyperchlorhydria and hyperacidity and treated with Vasa syrup 35% patients were cured, 50% were improved and remaining 15% patients were found unchanged.
   Discussion: Vasa has beneficial effect on Sign& symptoms of Amlapitta & decrease the free HCL. Vasa has Sheetavirya that may have helped in reliving the symptoms.

2) Study Title: A comparative study of efficacy of Tugaksheeree [*Curcuma angustifolia* Roxb. and *Maranta arundinacea* Linn.] in management of Amlapitta (Oct 2010)[3]
   No. Of Patients: 67 patients of Amlapitta
   Drug Given: Tugakshiri
   Grouping
    Group I: *Curcuma angustifolia* Roxb. (Fam. Zingiberaceae)
   Group II: *Maranta arundinacea* Linn. (Fam. Marantaceae)
   Dose: 4gm thrice daily for 30 days
   Result: Between the two, *C. angustifolia* Roxb. was found more effective on the symptoms Gaurava (61.75%↓) and Udaradhmana (92.65%↓) against *M. arundinacea* Linn. (51.50%↓) and (74.25%↓) respectively. The difference between the effect of the therapy on cardinal and associated symptoms of Amlapitta is statistically non significant, and hence both the drugs *C. angustifolia* Roxb. and *M. arundinacea* Linn. are equally potent in treating the disease

   Discussion: Both the drugs *C. angustifolia* Roxb. and *M. arundinacea* Linn. are highly effective in treating Amlapitta. They are highly effective in relieving the cardinal symptoms viz., Avipaka, Tiktaamlodgara, Daha, Shoola, and Chardi and also effective against the associated symptoms viz., Aruchi, Gaurava, Udaradhmana, Antrakujana, Vitbheda, Shiroruja, Angasada, and Trit. The drugs have the capacity to correct the Agni and act as Brihmana and Dhatupushtikara as observed by increase in body weight. Both the drugs *C. angustifolia* Roxb. and *M. arundinacea* Linn. are free from side effects and can be used as substitutes for each other.

3)   Study Title: A comparative clinical study of Shatapatrayadichurna tablet and Patoladi yoga in the management of Amlapitta (july 2011)[4]

No. Of Patients: Group A- 20 patients
Group B – 15 patients
Drug Given: Group A – Shatpatryadi tablet
Group B- Patoladi Yoga tablet
Dose: 2 tablets of 500 mg t.i.d. For 30 days with milk in both group
Result: Shatapatrayadi tablet shows better result in symptoms such as Kukshi Daha, Hrida Daha, and Kantha Daha. It also shows better results on Amlo and Tikta Udgara. Quality of sleep is more improved in group A. Feeling of well-being improved more in group A and Deha Bala also improved more in group A. While Patoladi Yoga shows better result in Gaurava, Avipaka, Shirashula and Klama.

Discussion: Shatapatrayadi tablet showed better result. Ingredients of Shatapatrayadi tablet inlcude Tikta, Kashaya, and Madhura rasa. Madhura, Tikta, and Kashaya Rasa are Pitta Shamaka. Tikta rasa and Kashya Rasa also subside Kapha Dosha. Madhura Rasa having soothing effect promotes strength and pacify Pitta and Vata Dosha and useful in Daha. All four drugs has Tikta rasa and Kashaya Rasa which are Pittashamaka and Kaphanashaka. In pathogenesis of Amlapitta, Mandagni leads to Ama formation and Sunthi is the best medicine for Amapachana and alleviates the Srotorodha by UshanTikshana Guna. All the drugs have DeepanaPachana property, which improves the status of Agni.

4)   Study Title: A comparative clinical trial of Chinchakshara and Kadalikshara on Amlapitta (Oct 2011)[5]
No. of Patients: 30 patients
Drug Given: Grouping: Group C- 10 patients – Chinchakshar
Group K- 10 patients – Kadalikshar
Group P – 10 patients – Friend wheat powder (Placebo group)

Dose: 500 mg thrice in a day after food with warm water for 30 days
Results: Statistical analysis inferred that avipaka (indigestion), hrit-kanthadaha (heart burn), tikta-amlaudgara (acid eructation), utklesa (nausea), adhmana (flatulence), klama (tiredness) and aruchi (anorexia) were statistically reduced (P<0.01). but reduction in udarasula was less statistically significant as compared to the aforesaid symptoms. No improvement was detected in the placebo group patients (P>0.05). It is also found that the signs of gastritis was reduced satisfactorily in group C (P<0.05) and group K (P<0.05) but unsatisfactory in Group P (P>0.05).

Discussion: Chinchakshara was more effective than Kadalikshara due to its more alkaline nature. The kshara preparations have been used since the time of Charaka[16] and Sushruta[3] in diseases related to gastrointestinal system such as amlapitta, gluma, sula, etc. It is an applied fact that kshara gets neutralized in combination with amla. Five rasas are present in kshara except amla. Lavana rasa, being anurasa, is converted to madhura when mixed with amla. So, the teekshnaguna of lavana rasa present in kshara is neutralized due to its combination with amla as the water extinguishes the fire.

5)   Study Title: Fundamental approach in the management of Drava Bahula Amlapitta with Bhringaraja (*Eclipta alba*) (Oct 2011)[6]
No. Of Patients: 22 Patients
Grouping – Group A – Treatment group
Group B – Placebo group
Drug Given: Group A – Bhringraj tablet Group B- Fried rice tablet
Dose: 4gm per day for before food with water for 28 days

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Result: Moderate to marked (summation of moderate+marked) improvement was observed in more than 80% and 14.29% of patients in groups A and B, respectively, when data were presented in percentage improvement method.

Discussion: The above explanation proves that Bhringaraja has efficacy in treating the Drava Bahula Amlapitta in comparison to placebo. Bhringaraja can give moderate to marked improvement in Drava Bahula Amlapitta patients. Statistically significant results observed in Group A (Bhringaraja) specify that the applied concept of Drava Guna plays an important role in the pathogenesis of disease. Hence, Bhringaraja having Drava opposite properties, i.e. Ruksha–Ushna, can be a choice of remedy for the physician while treating diseases of Pitta Drava Guna Vriddhi like Amlapitta.

6)   Study title: Clinical evaluation of the efficacy of Dashanga Kwatha Ghana Vati in the management of Urdhwaga Amlapitta (Non-ulcer dyspepsia)[7]

No. Of Patients: Group A – 82 patients
                        Group B – 56 patients
Drug given: Group A – Dashangkwath Ghana vati
                  Group B – Placebo tablet prepared with Gram flour

Dose: 250 mg twice a day after food with water for 8 weeks
Result: Dashanga Kwatha Ghana Vati has shown significant relief in Urdhwaga Amlapitta in comparison to the placebo group. No clinically significant changes were observed in hematological or biochemical parameters after the study. No adverse drug reactions were noted with administration of the trial drug for 8 weeks of duration.
Discussion: The chief Dosha involved in the Urdhwaga Amlapita is Pitta and Kapha. Majority of the drugs of this formulation havTikta and Kashaya Rasa, Katu and Madhura Vipaka, Sheeta Virya, Pitta Kapha Shamaka property. In Urdhwaga Amlapitta, Rasa Dhatu is affected. The property of Deepana and Pachana Karma and Tikta Rasa of the drugs will directly act on the vitiated Rasa Dhatu. Due to Tikta Rasa property, Sama Pitta will become Nirama and Agni will be increased. Charaka says that Manadagni and Ajirna creates Annavisha, when it gets mixed with Pitta Dosha, creates Pittaja Vyadhis [12] like Amlapitta. So, the Tikta Rasa property of the drugs purifies the Pitta by Niramikarana. Acharyas have also given the treatment of Pittaja disorders and says that first of all Tikta Rasa Dravyas have to be used for Niramikarana of Pitta and then after Madhura Rasa and Kashaya Rasa should be used to pacify Paittika Vyadhis.

7)   Study title: Evaluation of the efficacy of avipattikarchurna in the management of amlapitta[8]
No. of patients: 10 patients
Drug Given: Avipattikar Churna
Dose: 3 gm twice a day with water for 21 days
Result: Patients response of the treatment was evaluated on all the subjective criteria and significant improvement were found among 70% patients and 30% of the patients showed moderate response.
Discussion: In Avipattikarchurna the drugs are madhurtiktakasaya, katu rasa yukta and madhuravipaka and sitavirya. In Amlapitta, tiktaamlataudgara, hritkanthadaha due to vidagda pitta which is relived by pittasamak rasa, virya, vipaka present in ingredients of Avipattikarchurna. Shunthi, pippali, maricha act as dipana and pachana which relive a ma like symptom aruchi, gourava, avipakautklesha.

8)   Study title: A comparative clinical study of Jethimala (*Taverniera nummularia* Baker.) and Yashtimadhu (*Glycyrrhiza glabra* Linn.) in the management of Amlapitta. (randomized clinical trial)[9]

No. of Patients: 40 patients
Drug Given: group A- Yashtimadhu moola Churna
                  Group B- Jethimala moola churna

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Dose: 2gm thrice a day with water for two weeks.

Result: The effectiveness of the treatment is considered positive on the basis of scoring pattern before treatment and after the completion of treatment. Yashtimadhu (G. glabra) group provided a significant improvement in Avipaka 63.11%, Klama 61.11%, Utklesha 65.35%, Tikta-amlodgara 67.64%, Gaurava 61.11%, Hrit-kanthadaha 69.00%, and Aruchi 40.42% .

Jethimala (*T. nummularia*) provided significant relief in Avipaka (47.11%), Klama (53.19%), Utklesha (61.70%), Tikta-Amlodgara (60.00%), Gaurava (43.18%), Hrit-Kanthadaha (66.06%), and Aruchi (78.94%)

Discussion: Yasthimadhu (G. glabra Linn.) and Jethimala (*T. nummularia* Baker.) are effective in relieving the cardinal symptoms. Significant improvement observed in almost all the symptoms in both the groups but group A (*G. glabra*) showed better effect than group B and but in Aruchi *T. nummularia* showed better effect than G. glabra.

9) Study title: Study on avipattikarchurna and kapardikabhasma in the management of hyperacidity (amlapitta)(An open, non comparative randomized clinical study)[10]
   No. Of Patients: 33 patients
   Drug given: Avipattikar Churna & Kapardikabhasma
   Dose: Avipattikar Churna 1gm along with Kapardika Bhasma 500 mg twice in a day orally with normal water after food for 30 days.

   Result: Both the drugs have efficacy in reducing the symptoms of hyperacidity (Amlapitta) in patients. It shows a relief of 82.85% in Burning sensation in upper abdomen (Udaradaha), 76.73% in Burning sensation in chest (Urodaha), 89.47% in vomiting (Chardi), 77.77% in acid eructation (amlotklesha), 80% in water brash, 87.50% in nausea after the treatment. 82.35% also relief was achieved in vertigo (Bhrama). 100% relief was observed in Flatulence (Adhamana). 69.56% relief was seen in constipation (Vibandha) and 87.50% relief in tenderness in upper abdomen respectively . Overall the result on 33 patients was 82.196% (Table-3) and according to response 81.81%, 15.15%, 3.03% good, fair and poor respectively.

   Discussion: it is concluded that drug Avipattikarchurna and Kapardikabhasma holds potent neutralizing effect of pitta dosha in stomach and symptoms of Amlapitta. It is safe and tolerated drug for the treatment of Amlapitta (hyperacidity).

10) Study title: Efficacy of GuduchiSattwa with Karvellakswarasa (Rasayan therapy) in the management of Amlapitta (Open non comparative single blind study)[11]
    No. Of Patients: 30 patients
    Drug given: Guduchi Sattva &Karvellak Swarasa
    Dose: Guduchi Sattva 1 gm & Karvellak swarasa 40 ml was administered after meal twice a day for 30 days.
    Result: the above drug is more useful urahkanthadaha then Klama. It has shown marked increase in appetite with weight gain.
    Discussion: Guduchi Sattva with Karvellakswarasa has reduced the clinical symptoms of amlapitta.

11) Study title: Clinical efficacy of Baladi Manduramin the management of Amlapitta (open clinical trial)[12]
    No. Of Patients: 33 patients
    Drug given: Baladi Manduram
    Dose: 500 mg twice a day after meals with hot water as Anupana for a duration of 30 days.
    Result: The therapy had shown marked improvement in 67% of patients, mild improvement in 27% of patients, and complete remission was noticed in 6% of patients.
    Discussion: Baladi Manduram has provided significant results on the parameters of Amlapitta.

CONCLUSION

In this reasearch article , eleven research studies have been reviewed. All the works had been carried out on shaman chikitsa of Amlapitta. All the chosen classical medicine has good overall effect on amlapitta.

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RESEARCH ARTICLE

Synthesis, Characterization, Molecular Docking and Biological Screening of 1-(3-amino- 7-methoxy-1-benzofuran-2-yl) Ethanone and their Derivatives

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ABSTRACT

The benzofuran nucleus possesses remarkable pharmaceutical properties. It has been observed that when benzofuran moiety is attached or coupled with five or six-membered nitrogen and oxygen heterocycles, the resulting compounds exhibited enhanced biological activities. In view of this and in continuation of our search for biologically potent benzofuran compounds we now report the synthesis of some benzofuran derivatives substituted with diazole, oxazole and pyrimidines heterocycles. The initial step involves the synthesis of 1-(3-amino-7-methoxy-1-benzofuran-2-yl) ethanone (1). Compound 1 was converted to compounds 2a-f by treating with various aromatic aldehydes. The compounds 2a-f were converted into corresponding hydrazones 3a-f by treating with hydrazine hydrate. These hydrazones when treated with hydroxylamine hydrochloride we obtain compounds 4a-f which were finally converted compounds5a-fby treating with malanonitrile and ammonium acetate. All the synthesized compounds were concurrent with the assigned structures which were supported by their spectral data such as FTIR, ^{1}HNMR and Mass spectra. The synthesized derivatives were subjected to molecular docking studies and also they were screened for antibacterial and antifungal activity and some of the new compounds exhibited moderate to appreciable activity.

Keywords: Benzofuran, diazole. Oxazole, Pyrimidine, Molecular docking, Antibacterial, and Antifungal activity.

INTRODUCTION

Heterocyclic compounds are of very much interest in our daily life because of their wide range of their biological properties. Many heterocyclic compounds are found as key components in biological processes[1-3].Oxazole units can be found in a large number of natural products and bioactive molecules. As a result, a lot of attention has been paid to the synthesis of highly functionalized oxazoles and several efficient methods were also reported[4,5].The molecules of many biological materials consist in parts pyridine and pyrrole rings and such compounds yield small amounts of pyridine and pyrrole upon strong heating[6,7]. Pyrrole as a model of the Π–excessive ring and pyridine as a model of the Π -deficient one. Several pyrimidines such as uracil, thymine and cytosine have been isolated from the nucleic acid hydrolysis [8]. Pyrimidines comprise a relatively large, growing, and most interesting group of compounds with antibacterial properties which have made a major impact in the field of antibacterial chemotherapy [9].

Pyrimidines and condensed pyrimidines are important classes of heterocyclic compounds that exhibit a broad spectrum of biological activities such as antibacterial, antifungal and anti-inflammatory activity [10,11]. The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behaviour of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes [12]. Molecular docking and scoring via computational approach provide the most promising route for drug designing and discovery. Computational drug designing and discovery is more economic, time saving and effective over conventional technologies[12].

Various pharmacologically important benzofuran derivatives have been reported from this laboratory which is involved in the synthesis and characterisation of benzofuran derivatives [13-17]. The present work involves the synthesis of key intermediate 1-(3-amino-7-methoxy-1-benzofuran-2-yl) ethanone (1). It was synthesized from o-vanillin as the starting material. Compound I was achieved by first converting o-vanillin into 2- [(E)-(hydroxyimino)methyl]-6-methoxyphenol(oxime derivative) using hydroxylamine hydrochloride which was then treated with Sodium ethoxide and acetic anhydride to obtain2-hydroxy-3-methoxy benzonitrile as per the procedure established in the literature [18]. This nitrile when refluxed with chloroacetone in presence of potassium carbonate and acetone yielded compound 1[19,20]. The compound 1 was converted to compounds 2(E) -1-(3-amino-7-methoxy-1-benzofuran -2-yl)-3-arylprop-2-en-1- ones (2a-f) by treating with various aromatic aldehydes in presence of ethanolic sodium hydroxide.

The compounds 2a-f were converted into 4H-Pyrazol-3-yl-7-methoxybenzofuran-3-amine (3a-f) by the reaction with hydrazine hydrate in ethanol. Further, the compounds 2a-f were converted into 4,5-dihydro-1,2-oxazol-3-yl-7-methoxy benzofuran-3-amine (4a-f) by reaction with hydroxylamine hydrochloride in the presence of ethanol. The compounds 2a-f were also used to get 2-amino-6-(3-amino-7-methoxy-1-benzofuran-2-yl-4-pyridine-3-carbonitrile (5a-f) by treating with malanonitrile and ammonium acetate in the presence of ethanol. All the synthesized compounds were confirmed by spectral and analytical data. Hence in the present work the synthesized derivatives 5a, 5b and 5c were subjected to molecular docking in order to study the mode of interaction between the compounds and protein molecules.

MATERIAL AND METHODS

All the chemicals and solvents used were of Sigma Aldrich (analytical grade). The ethanol was distilled and then used as a solvent for synthesis. Melting points determined are uncorrected and TLC (Thin layer chromatography) was used to test the compound for the completion of reaction. FTIR spectra were recorded on Shimadzu model IR-435 spectrophotometer using KBr pellets method.^{1}HNMR spectra was obtained by IISC, Bangalore and University of Mysore. TOF ES+ Mass spectra (m/z) were recorded on micro mass Auto spec LCTKC455.

Experimental procedures

Preparation of 2- [(E)- (hydroxyimino) methyl]-6-methoxyphenol

o - Vanillin and hydroxylamine hydrochloride were dissolved in the solution of ethanol and this solution was continuously stirred for about 30 min. A solution of sodium hydroxide (2 .4 g in 5ml water) was added in small proportions, during addition care was taken so that the solution doesn't turn vigorous. Later the solution was cooled to room temperature under running water and then the solution was completely transferred to ice-cold water. The solution was then acidified with dilute HCl and precipitate was observed. The obtained product was oxime, it was filtered, washed, dried, and recrystallized in ethanol. The melting point was found to be 112^0C. (yield 65%)

Preparation of 2-hydroxy-3-methoxy benzonitrile

A mixture of oxime and acetic anhydride was refluxed for about one hour. The remaining acetic anhydride was distilled off. The solution was cooled to room temperature and then kept for stirring. During stirring, sodium ethoxide was added in small portions. After complete addition, the solution was transferred to ice-cold water and acidified with dilute HCl for precipitation to occur. The obtained product was nitrile. It was filtered, washed several times with water dried and recrystallization was done using ethanol. The melting point was found to be 202^0 C. (yield 60%)

Preparation of 1-(3-amino-7-methoxy-1-benzofuran-2-yl) ethanone (1)

A mixture of nitrile and anhydrous potassium carbonate in dry acetone and chloroacetone was taken in a round bottom flask and this mixture was refluxed for about 8 hours. Later, potassium carbonate was filtered off and from the filtrate, acetone was distilled off under reduced pressure. The remaining product was allowed to dry. The obtained dried product was furan, it was recrystallized using ethanol. The melting point of the product was found to be 175^0 C. (yield 62%)

Preparation of 2(E) -1-(3-amino-7-methoxy-1-benzofuran -2-yl)-3-arylprop-2-en-1- ones (2a-f)

A mixture of compound (2) (0.01 mole) and appropriate aldehyde (0.01 mole) was dissolved in 50 ml of ethanol and cooled to 5 to 100 C. To this cooled aqueous NaOH solution (5 ml, 20 percent) was slowly added. The mixture was stirred for two hours and kept overnight. The product was collected and Melting points, FT-IR data of compounds 2a-f, 3a-f,4a-f and 5a-f. are recorded in table -1

Preparation of 4H-Pyrazol-3-yl-7-methoxybenzofuran-3-amine (3a-f)

A mixture of compound (2)(0.01 mole) in ethanol and hydrazine hydrate was taken in a round bottom flask to this solution add a drop of concentrated H_2SO_4 was refluxed for 3 hours. The reaction mixture was cooled the solid separated was collected and recrystallized from ethanol. Melting points, FT-IR data of compounds 2a-f, 3a-f,4a-f and 5a-f. are recorded in table -1

Preparation of 4,5-dihydro-1,2-oxazol-3-yl-7-methoxy benzofuran-3-amine (4a-f)

A mixture of compound (2)(0.01 mole) in ethanol and hydroxylamine hydrochloride (0.01mole) and a drop of hydrochloric acid was refluxed for 2 hours. The reaction mixture was cooled the solid separated was collected and recrystallized from ethanol. Melting points, FT-IR data of compounds 2a-f, 3a-f, 4a-f and 5a-f. are recorded in table -1

Preparation of 2-amino-6-(3-amino-7-methoxy-1-benzofuran-2-yl-4-pyridine-3-carbonitrile (5a-f)

A mixture of compound (2) (0.01mole) in ethanol, malanonitrile (0.01 mole), and ammonium acetate (0.01 mole) were added and the reaction mixture was refluxed for 3 hours was cooled, the solid separated was collected and recrystallized from ethanol. Melting points, FT-IR data of compounds 2a-f, 3a-f,4a-f and 5a-f. are recorded in table -1

RESULT AND DISCUSSION

All the newly synthesized compounds are analyzed by the spectral data such as FTIR, 1HNMR, MASS, Biological activity and Molecular docking.

Compound3a

FTIR: in cm-1 -2834 (OCH_3), 2915 (N-H stretching), 1605 (C=N), 831 (C-Cl).
^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 8.01 (s,NH_2,2H),4.79 (s,1H),7.13-7.47(m,Ar-H,7H),1.94-2.17 (t,1H).
Mass the calculated mass is 339 m/z and observed mass is 340.23 m/z $(M+1)^+$.

Compound 3b

FTIR: in cm-1 - 2854 (OCH_3), 2909 (N-H stretching) , 1639 (C=N), 1469 (NO_2).
^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 3.82 (s, OCH3,3H),4.01 (s, NH2,2H), 6.88-7.12 (m , Ar-H,6H), 2.08-2.12 (d, CH=CH, 3H) ,3.93 (s, CH,IH).
 Mass the calculated mass is 350 m/z and observed mass is 351.22 m/z $(M+1)^+$.

Compound4a

FTIR: in cm-1- 2847 (OCH_3), 3343 (N-H stretching), 1707(N-O stretching), 1605 (C=N), 817 (C-Cl).
^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 3.76 (s, OCH_3,3H), 8.05 (s,NH_2,2H), 7.40-7.59 (m, Ar-H, 8H), 3.95-4.06 (d, CH=CH 1H). Mass the calculated mass is 342.77 m/z and observed mass is 342.17 m/z.

Compound 4d

FTIR: in cm-1-2847(OCH_3), 2909 (N-H stretching), 1735 (N-O), 1612 (C=N), 844 (C-Cl).
1H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.31 (s,NH_2,2H),7.10-8.03 (m,Ar-H,8H), 3.71-3.73 (d, CH=CH 1H), 2.09-2.17 (t, CH=CH-CH,2H),1.42 (s,1H), 1.11 (s,1H).
Mass the calculated mass is 342.77 m/z and observed mass is 342.17 m/z.

Compound 5a

FTIR: in cm-1- 2861 (OCH_3),2922 (N-H stretching), 1272 (C-CN), 1639 (C=N), 763 (C-Cl).
^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.59 (s,2H,NH_2), 7.45-7.57 (m,Ar-H,8H), 3.92 (s, OCH_3,3H), 4.055-4.065 (d, -CH=CH,1H). Mass the calculated mass is 390.82 m/z and observed mass is 391.24 m/z $(M+1)^+$.

Compound 5c

FTIR: in cm-1-2834 (OCH_3), 2909 (N-H stretching), 1252 (C-CN), 1605 (C=N).
^{1}H-NMR ($CDCl_3$, 400 MHz): δ ppm 7.63 (s,NH_2,2H),7.59-8.30 (m,Ar-H,7H), 3.82 (s,1H),2.169-1.656 (d, CH=CH,4H), 1.25-1.366 (d, OCH_3, 3H), 4.014-4.135 (t,CH=CH-CH,1H).
Mass the calculated mass is 357.36 m/z and observed mass is 357.17 m/z.

Biological activity

Anti-bacterial activity

The newly synthesised compounds were subjected antibacterial activity against bacteria *Escherichia coli, Klebsiella pneumonia and Citrobacter spp.* Dispersion method was used to perform the anti-bacterial activity. The bacteria were grown in Muller-Hinton agar (Hi-media) plate (37 ^{0}C, 24h). The stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with that of compound and antibiotic at different concentrations. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone (mm) were noted [21]. (Table-2,3,4 and 5)

Anti-fungal activity

The newly synthesized compounds are subjected to anti-fungal activity against *Candida albicans and Aspergillus flavus* by dispersion method. The stock cultures of fungi were revived by inoculating in broth media and grown at 27ºC for 48 hrs. The plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 48 h old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with that of compound and antibiotic at different concentrations. All the plates were incubated at 27ºC for 96 h and the diameter of inhibition zone (mm) were noted [22]. (Table.7)

Molecular Docking

The molecular docking was carried out for compounds 5a, 5b and 5c using Auto-DockVina software. Auto-Dock Vina is designed for protein-ligand docking, utilizing multiple CPU's at a time, making it exceptionally faster and accurate. Vina is based on a gradient optimization algorithm along with local optimization and automatically clusters the results [23]. For Auto-Dock Vina, the input required was generated using Auto-Dock tools that were the pdbqt files of the ligands, protein target, and the configuration file containing dimensions of the grid box that is x, y, and z coordinates. The target protein in PDB format was loaded in MGL Tools, the water molecules were deleted, non-polar hydrogen and Kolloman charges were added, it was then saved in pdbqt format [24]. The ligand also was saved in pdbqt format. Auto-Grid was used for the preparation of the grid map using a grid box. The grid size was set to $40 \times 40 \times 40$ xyz points with grid spacing of 0.375 Å and grid centre was designated at dimensions (x, y, and z) [25,26].The protein molecules chosen were 3EWH, 2VF5 and 3EQM (PDB ID's). The protein molecules were retrieved from RCSB Protein data bank. The synthesized compounds 5a, 5b and 5c were subjected to docking studies with the above three protein molecules. The results of Aauto-Dock Vina were analysed and visualized using BIOVIA discovery studio visualizer. The docking results are represented in table 7-8.

CONCLUSION

The newly synthesized benzofuran derivatives like pyrazole, oxazole and pyrimidine they shows high to moderate antibacterial activity and antifungal activity. these newly synthesised compounds are in concurrent with the assigned structures which are confirmed by IR, NMR and Mass spectral data. Among all the subjected compounds i.e.5a, 5b and 5c with the proteins molecules 3EWH, 2VF5, 3EQM (PDB ID), the compound 5a showed highest binding affinity towards all three proteins with binding affinity values of -9.5, -8.6 and -9.9 respectively. The compounds 5b and 5c also exhibit excellent binding affinities towards the docked proteins. A further investigation in this area of study may provide good results for use of these compounds as therapeutic agents.

ACKNOWLEDGEMENTS

Conflict of Interest

We declare that we have no conflict of Interest.

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Table-1: Melting point, FT-IR data of compounds 2a-f, 3a-f, 4a-f and 5a-f.

compound	Substituent R	Molecular formula	MP°C	FT-IR data in cm-1					
				NH	OCH₃	C=O	C=N	N-O	C-Cl
2a	$C_6H_4Cl(p)$	$C_{18}H_{14}ClNO_3$	152	3445	2827	1639	--	--	838
2b	$C_6H_4NO_2(p)$	$C_{18}H_{14}N_2O_5$	165	3431	2834	1612	--	--	--
2c	$C_5H_4N(p)$	$C_{17}H_{14}N_2O_3$	174	3418	2854	--	1639	1462	--
2d	$C_6H_4Cl(o)$	$C_{18}H_{14}ClNO_3$	154	3418	2834	1605	--	--	743
2e	$C_6H_4Cl(m)$	$C_{18}H_{14}ClNO_3$	156	3431	2868	1605	--	--	776
2f	$C_7H_7O(p)$	$C_{19}H_{17}NO_4$	180	3445	2841	1612	--	--	--
3a	$C_6H_4Cl(p)$	$C_{18}H_{14}ClN_3O_2$	182	2915	2834	---	1605	---	831
3b	$C_6H_4NO_2(p)$	$C_{18}H_{14}N_4O_4$	188	2909	2854	----	1639	1469	--
3c	$C_5H_4N(p)$	$C_{17}H_{14}N_4O_2$	176	2915	2834	---	1620	---	--
3d	$C_6H_4Cl(o)$	$C_{18}H_{14}ClN_3O_2$	184	2922	2847	---	1602	---	756
3e	$C_6H_4Cl(m)$	$C_{18}H_{14}ClN_3O_2$	186	2922	2941	---	1713	---	784
3f	$C_7H_7O(p)$	$C_{19}H_{17}N_3O_3$	190	2909	2834	---	1595	----	---
4a	$C_6H_4Cl(p)$	$C_{18}H_{15}ClN_2O_3$	165	3343	2847	---	1605	--	817
4b	$C_6H_4NO_2(p)$	$C_{18}H_{15}N_3O_5$	170	2922	2847	---	1639	1469	--
4c	$C_5H_4N(p)$	$C_{17}H_{15}N_3O_3$	177	2930	2861	---	1602	--	--
4d	$C_6H_4Cl(o)$	$C_{18}H_{15}ClN_2O_3$	166	2909	2847	---	1612	--	844
4e	$C_6H_4Cl(m)$	$C_{18}H_{15}ClN_2O_3$	168	2929	2847	---	1598	--	770
4f	$C_7H_7O(p)$	$C_{19}H_{18}N_2O_4$	181	2930	2834	---	1612	--	--
5a	$C_6H_4Cl(p)$	$C_{21}H_{15}ClN_4O_2$	146	2922	2861	---	1639	---	763
5b	$C_6H_4NO_2(p)$	$C_{21}H_{15}N_5O_4$	155	2930	2843	---	1612	1472	--
5c	$C_5H_4N(p)$	$C_{20}H_{15}N_5O_2$	173	2909	2834	---	1605	---	--
5d	$C_6H_4Cl(o)$	$C_{21}H_{15}ClN_4O_2$	148	2922	2847	---	1605	---	756
5e	$C_6H_4Cl(m)$	$C_{21}H_{15}ClN_4O_2$	150	2922	2854	---	1603	---	770
5f	$C_7H_7O(p)$	$C_{22}H_{18}N_4O_3$	178	2929	2847	---	1612	---	--

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Table 2: Represents the anti-bacterial activity against the *Escherichia coli.*

Sample compound	62.5 μg	250 μg	500 μg	1000 μg	MIC μg
2b	15	17	20	23	62.5
3f	15	18	20	25	62.5
4a	15	18	20	25	62.5
4b	13	15	18	23	62.5
4d	14	18	20	23	62.5
4e	18	20	24	28	62.5
4f	13	15	20	25	62.5
5b	16	18	20	26	62.5
5d	15	20	23	26	62.5
5f	13	18	20	23	62.5
	25 μg	50μg	100μg	250μg	MIC μg
Ciprofloxacin (standard drug)	25	28	32	34	25

Table 3: Represents the anti bacterial activity against *Klebsiella pneumonia*

Bacteria: *Klebsiella pneumonia*

Sample compound	62.5 μg	250 μg	500 μg	1000 μg	MIC μg
2b	18	20	23	25	62.5
3f	15	17	20	23	62.5
4a	15	18	20	25	62.5
4b	16	18	22	25	62.5
4d	17	20	22	26	62.5
4e	17	19	23	26	62.5
4f	15	18	25	28	62.5
5b	18	20	23	28	62.5
5d	15	17	19	22	62.5
5f	18	19	21	23	62.5
	25 μg	50μg	100μg	250μg	MIC μg
Ciprofloxacin (standard drug)	20	22	28	31	25

Table 4: Represents the antibacterial activity against *Citrobacter spp.*

Bacteria: *Citrobacter spp.*

Sample compound	62.5 μg	250 μg	500 μg	1000 μg	MIC μg
2b	16	19	22	25	62.5
3f	16	20	25	28	62.5
4a	14	17	20	22	62.5
4b	12	15	17	19	62.5
4d	15	19	21	25	62.5
4e	20	24	28	31	62.5
4f	18	20	21	25	62.5
5b	13	15	18	22	62.5
5d	20	22	25	28	62.5
5f	12	17	20	22	62.5
	25 μg	50μg	100μg	250μg	MIC μg

Ciprofloxacin (standard drug)	20	22	28	31	25

Table 5: Represents the antifungal activity against *Candida albicans*

Fungi: *Candida albicans*

Sample compound	62.5 µg	250 µg	500 µg	1000 µg	MIC µg
2b	10	10	10	10	62.5
3f	15	15	15	16	62.5
4a	16	17	18	18	62.5
4b	12	12	15	15	62.5
4d	13	15	16	20	62.5
4e	18	20	21	22	62.5
4f	15	15	15	18	62.5
5b	12	15	16	15	62.5
5d	11	12	12	13	62.5
5f	15	18	18	20	62.5
	25 µg	50µg	100µg	250µg	MIC µg
Amphotericin (standard drug)	15	18	20	22	25

Table 6: Represents the antifungal activity *Aspergillus niger*

Fungi: *Aspergillus niger*

Sample compound	62.5 µg	250 µg	500 µg	1000 µg	MIC µg
2b	15	16	16	16	62.5
3f	12	13	14	16	62.5
4a	15	15	18	20	62.5
4b	12	15	16	18	62.5
4d	16	18	19	21	62.5
4e	18	18	19	20	62.5
4f	15	16	16	18	62.5
5b	10	15	18	20	62.5
5d	15	17	18	19	62.5
5f	11	13	15	17	62.5
	25 µg	50µg	100µg	250µg	MIC µg
Amphotericin (Standard drug)	19	22	25	28	19

MOLECULAR DOCKING

Table 7: Represents the docking results of compound 3(a-c)

Compounds	Protein molecules	Binding affinity kcal/mol
3a	3N8Y	-10.0
	1M17	-8.6
	1PXX	-9.3
3b	3N8Y	-10.4
	1M17	-9.0
	1PXX	-8.8
3c	3N8Y	-8.9
	1M17	-8.5
	1PXX	-8.1

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Table 8: Represents the docking results of compound 5(a-c)

Compounds	Protein molecules (PDB ID)	Binding affinity kcal/mol
5a	3EWH	-9.5
	2VF5	-8.6
	3EQM	-9.9
5b	3EWH	-9.0
	2VF5	-8.4
	3EQM	-9.5
5c	3EWH	-9.1
	2VF5	-8.2
	3EQM	-8.4
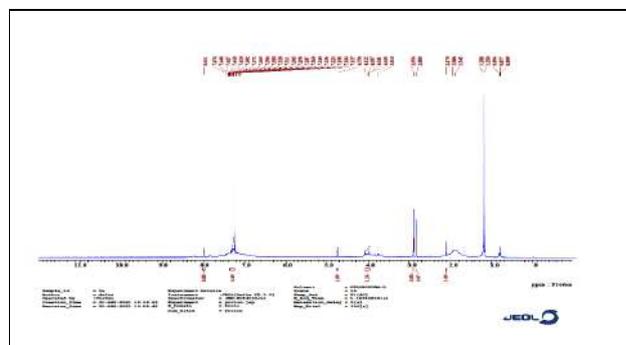
Fig 1:¹HNMR for the compound3a
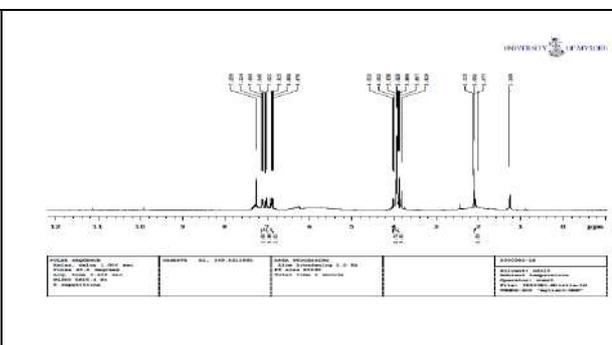
Fig 2: ¹HNMRof the compound 3b
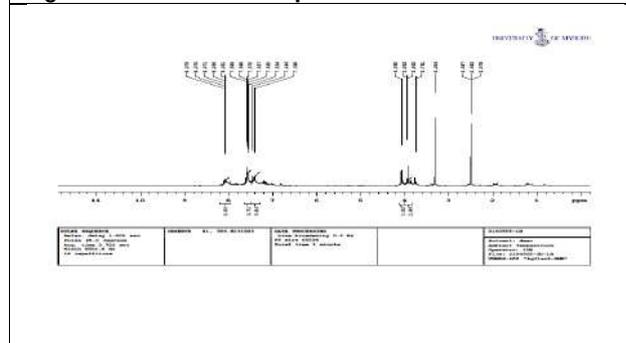
Fig 3:¹HNMR of the compound4a
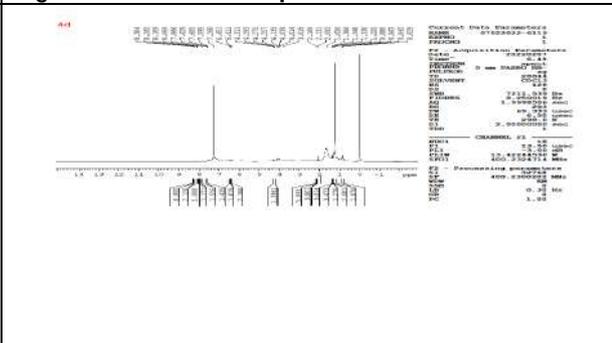
Fig 4:¹HNMR of the compound 4d
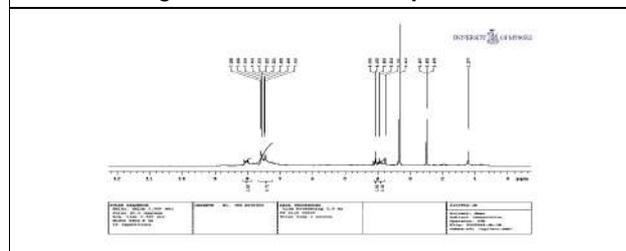
Fig 5:¹HNMR of the compound 5a
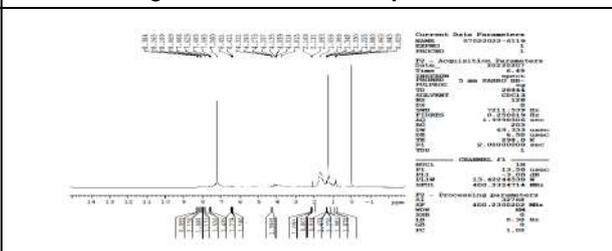
Fig 6:¹HNMR of the compound 5c

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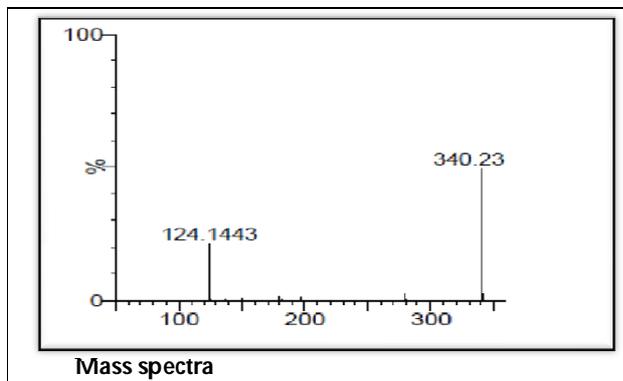
Mass spectra
Fig 7: The mass spectra of Compound 3a
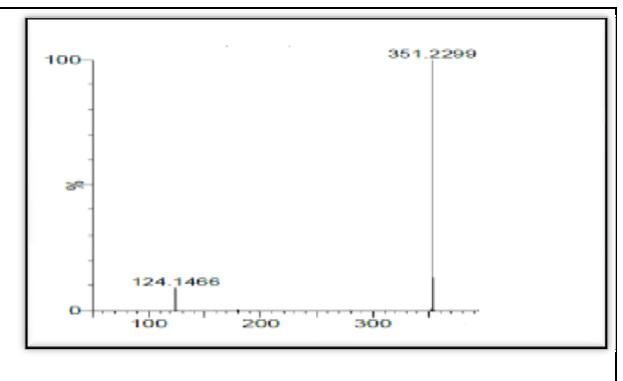
Fig 8: The mass spectra of Compound 3
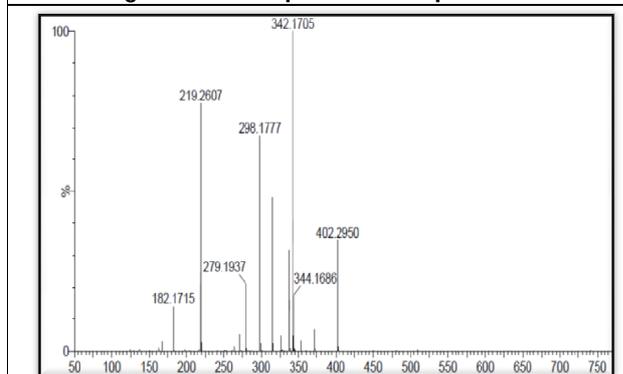
Fig 9: The mass spectra of Compound 4a
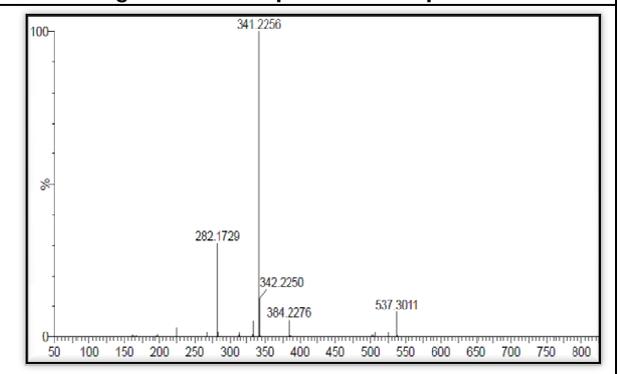
Fig 10: The mass spectra of Compound 4d
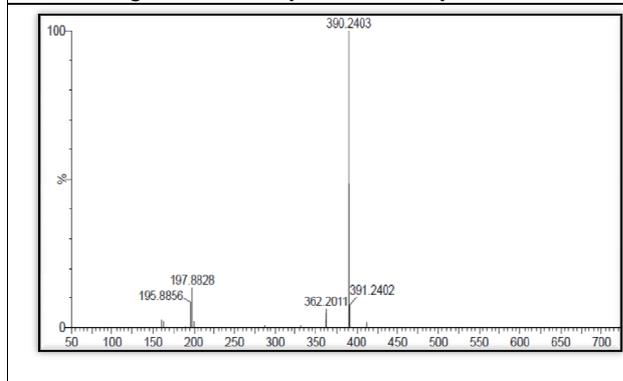
Fig 11: The mass spectra of Compound 5a
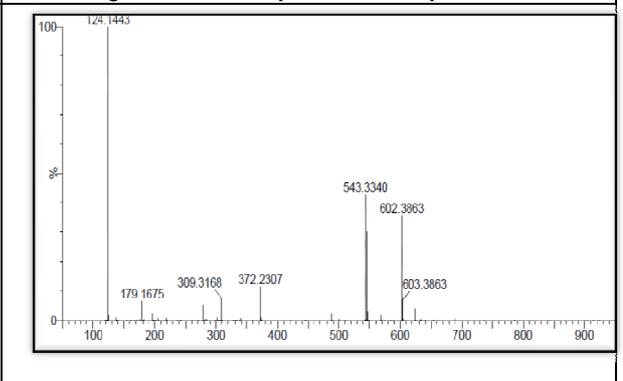
Fig 12: The mass spectra of Compound 5c
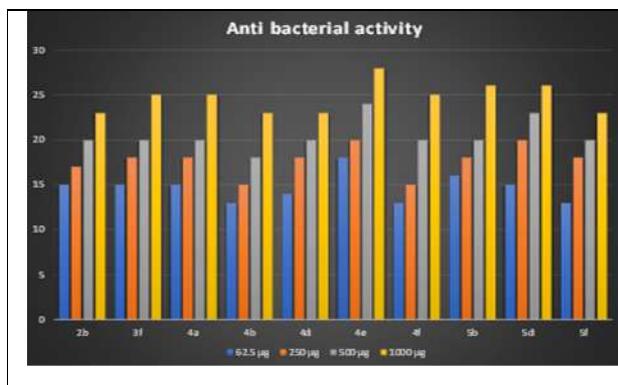
Fig 13: The graph represents the antibacterial activity of synthesized compounds against the *Escherichia coli* in which the compound 4e shows very high activity compare to the other compounds.
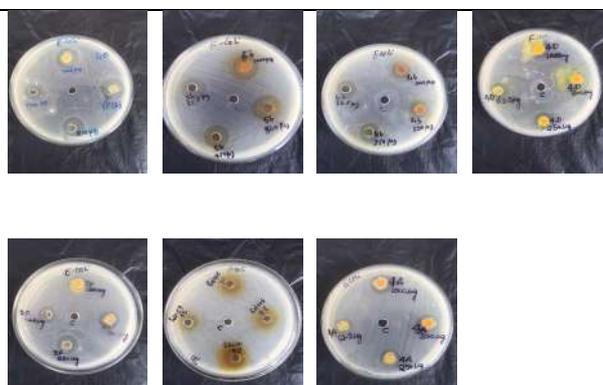
Fig 14: Images showing antibacterial activity against *Escherichia coli*.
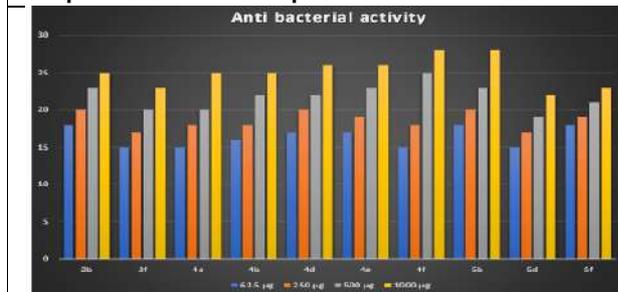
Fig 15: The graph represents the antibacterial activity of the compounds against *Klebsiella pneumonia*in which compound 4f and compound 5b show very high activity
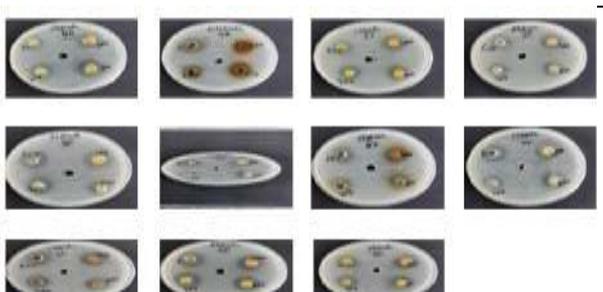
Fig 16: Images shows the antibacterial activity against *Klebsiella pneumonia*
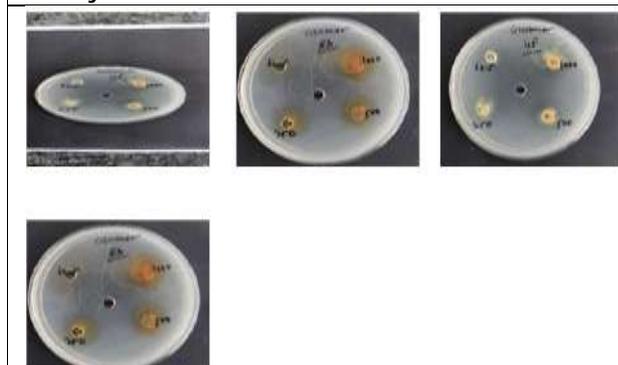
Fig 17: Images shows the antibacterial activity against *Citrobacter spp*
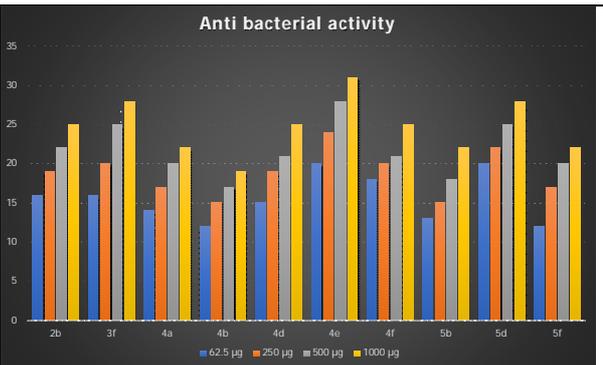
Fig 18: The graph represents the antibacterial activity against *Citrobacter spp*in which compound 4e shows very high activity and compounds 3f and compound 3d are show moderate activity.

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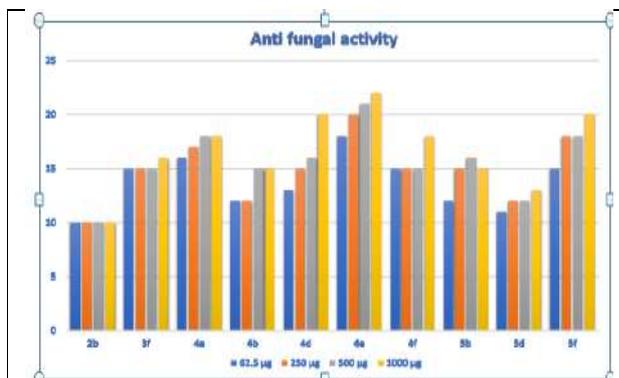
Fig 19:The graph represents the ant fungal activity of the newly synthesized compounds against *Candida albicans* **in which compound 4e shows good fungal activity and the compounds 4d and 5f are shown moderate fungal activity.**
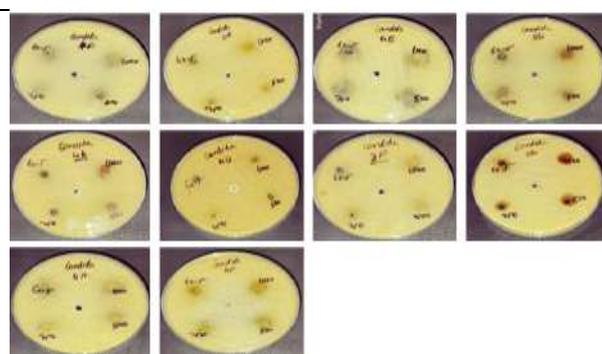
Fig 20: Images shows antifungal activity against *Candida albicans*
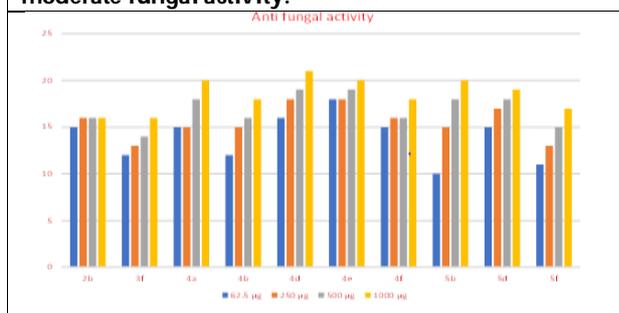
Fig 21:The graph represents the antifungal activity of the compounds against *Aspergillus niger* **in which compound 4d shows good fungal activity and the compounds 4a,4e,5b and 5d show moderate fungal activity.**
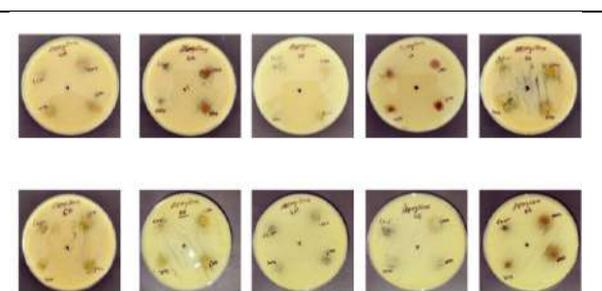
Fig 21: Images shows antifungal activity against *Aspergillus niger*
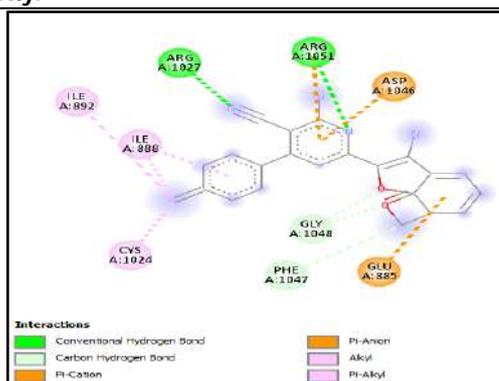
Fig 22: 2d image of compound 5a docked with protein 3EWH showing various amino acid residue interactions with compound 5a
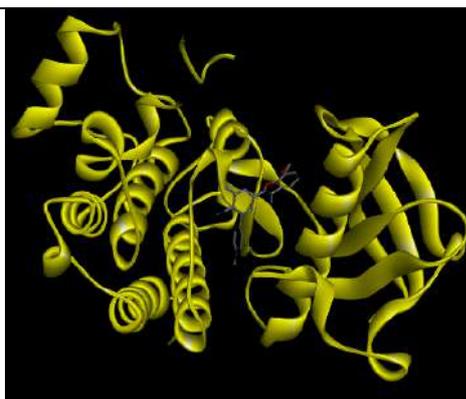
Fig 23: showing docking image of compound 5a with protein 3EWH

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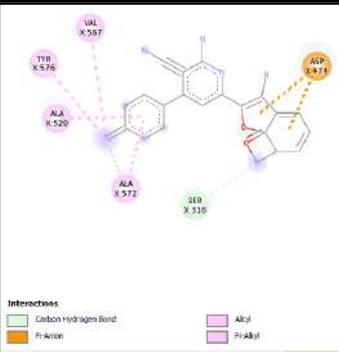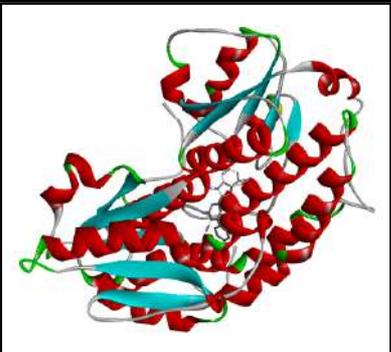
Fig 24: 2d image of compound 5a docked with protein 2VF5 showing various amino acid residue interactions with compound 5a

Fig 25: showing docking image of compound 5a with protein 2VF5
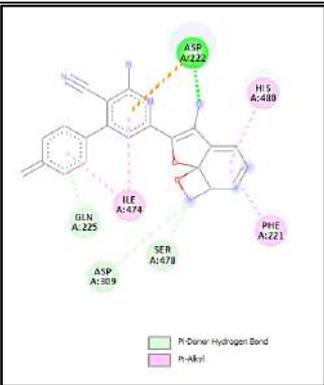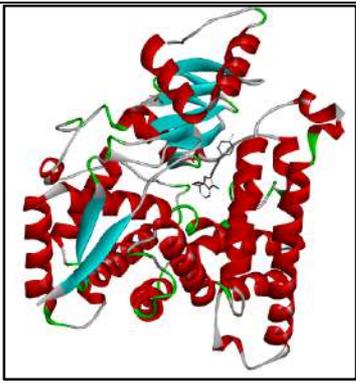
Fig 26: 2d image of compound 5a docked with protein 3EQM showing various amino acid residue interactions with compound 5a

Fig 27: showing docking image of compound 5a with protein 3EQM
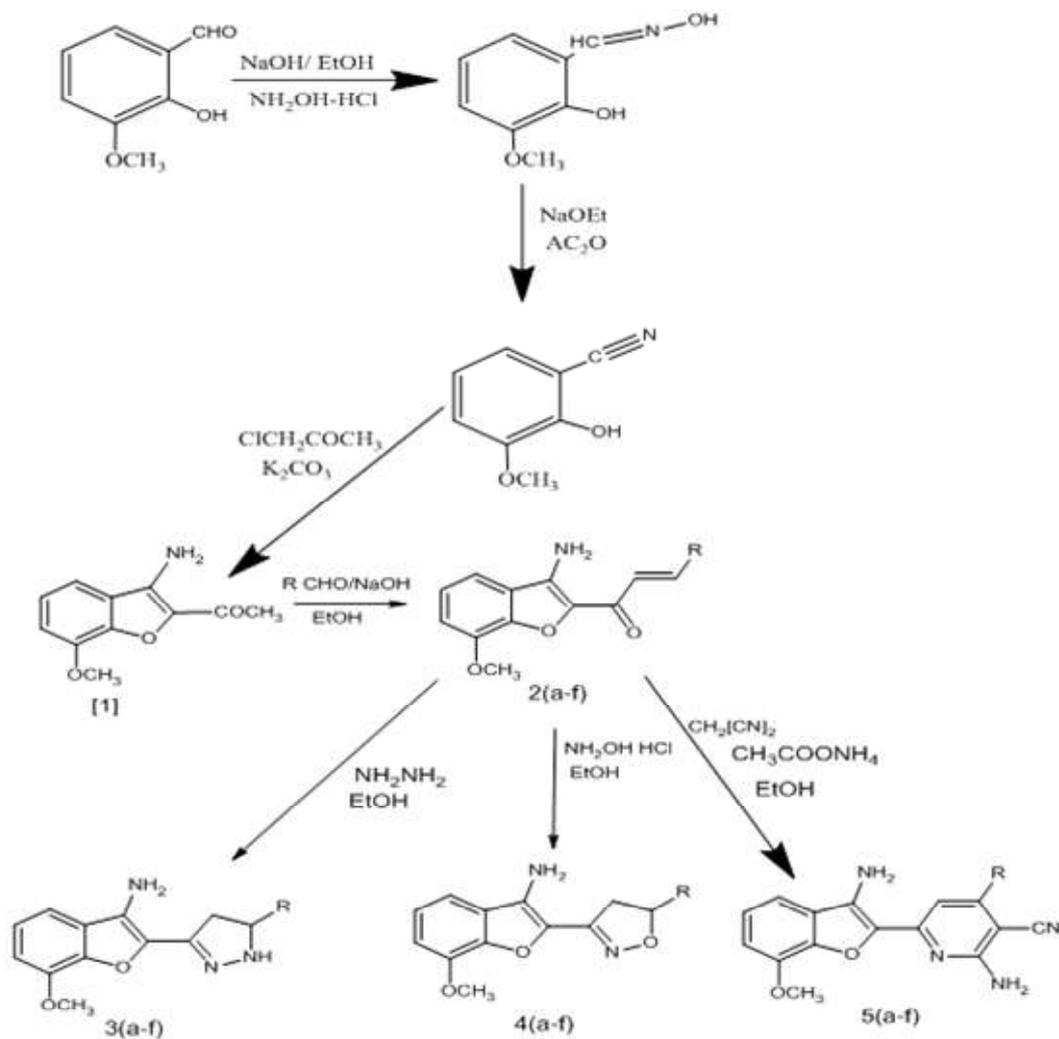
R:　　a= 4-Chlorobenzaldehyde ,　b=4-Nitrobenzaldehyde,　c=4-Pyridinecarboxaldehyde
　　d=2-Chlorobenzaldehyde,　　e=3-Chlorobenzaldehyde,　　f= 4-Anisaldehyde

SCHEME 01

RESEARCH ARTICLE

Identification of Suitable Sites Selection for Artificial Recharge of Groundwater using Geospatial Techniques in Varattar Sub-Bain, Dharmapuri District.

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ABSTRACT

The groundwater scarcity is due to increasing population, urbanization, and expansion of agricultural activities, so need to obtain more attention to groundwater resources. Artificial recharge of groundwater plays a vital role in the sustainable management of groundwater resources. In the present study, suitable sites for the artificial recharge of groundwater were delineated using geospatial techniques in the Varattar sub-basin. Weighted overlay analysis was carried out in the following thematic layers viz. Land use/land cover, soil, slope, drainage density, lithology, and lineament density. Each thematic layer and individual classes were assigned proper weightage and a score based on their relative contribution to groundwater recharge. Finally, all the thematic layers were integrated by the weighted index overlay (WIO) method. The groundwater recharge map thus obtained was divided into five zones as very high (115.99 km^2), high (175.19 km^2), moderate (173.16 km^2), poor (58.25 km^2), and very poor (4.66 km^2). The highest groundwater recharge sites are located towards western and middle regions because of high infiltration rates caused due to the distribution of 0-7° gentle slope areas, red loamy soil, flood plains, shallow buried pediment, and cropland. The least effective recharge potential is in the eastern parts of the study area due to the low infiltration rate. This is in order to overcome the water shortage and to improve the storage capacity of the groundwater aquifer as well as increase the level of the groundwater table.

Keywords: Artificial recharge sites, groundwater, weighted index overlay, Varattar sub-basin.

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INTRODUCTION

Groundwater is one of the most valuable natural sources for irrigation, drinking, and other purposes of water requirements. In this regard, groundwater plays a vital role in overcoming this shortage. Due to over-exploitation of groundwater without proper recharge mechanism and scanty rainfall, the water table of the open or dug wells in the region are being gone into deeper parts of the surface and many a time even some of them were dried up. In order to increase the level of the water table of the regions, it was necessary to adopt newer methods of groundwater recharge. Artificial recharge and rainwater harvesting are suitable solutions to address groundwater scarcity apart from demand-side management. Groundwater recharge is the replenishment of an aquifer with water from the earth's surface. Groundwater recharge occurs naturally and artificially. Natural recharge occurs through the process of infiltration of water percolates from the surface into the aquifer layer. Artificial recharge is the use of water to artificially replenish the water supply in an aquifer. In the recharge studies selection of sites for artificial recharge is a very important task (Das, 2003). Several studies have been conducted for the determination of areas most suitable for artificial recharge (Krishnamurthy and Srinivas 1995; Krishnamurthy et al. 1996 and Saraf and Choudhury 1998; Balachandar et al. 2010; Sarup et al. 2011; Amanpreet Singh et al. 2013; Rajasekhar et al. 2018; Mohanavelu Senthilkumar et al. 2019). The majority of the studies involving the identification of suitable sites for artificial recharge have utilized weighted index overlay methods (Saraf and Chowdary 1998; Agarwal et al. 2013). The weighted overlay index method has been implemented in several key groundwater provinces in India (Srivastava and Bhatacharya 2006; Ravishankar and Mohan 2005). A set of weights for the different themes and their individual features was decided based on expert knowledge considering their relative importance from the artificial recharge viewpoint (Alivia Chowdhury et al. 2010).

Geospatial technology is an important strategy for the water resources management system for the identification and exploration of groundwater recharge zones. In recent years, the role of Remote sensing and GIS techniques has received much attention with regard to artificial recharge (Shobharam Ahirwar et al. 2020; Suresh et al. 2015). They considered a varying number of thematic maps, such as geology, geomorphology, drainage density, slope, aquifer, permeability, fluctuations of water level or depth to groundwater level and lineament density, etc. Various layers are prepared and weighted overlay analysis is performed to determine the sites suitable for artificial recharge. Therefore, the present study was carried out in the Varattar sub-basin, wherein seven thematic layers viz. geomorphology, geology, land use/land cover, slope, drainage density, soil, and lineament density were integrated on weighted index overlay by GIS techniques.   The groundwater in the major part of the area is being continuously exploited for the purposes of domestic and irrigations.   Thus, proper planning for groundwater resources management of the area is required.

Study area
The study area lies between 78°24' to 78°38'E longitude and 11°51' to 12°11'N latitude covering a total area of 528.88km2 in Dharmapuri district (Fig. 1). The area falls in the Survey of India top sheets 57 L/8, L/12, 58 I/5, and I/9. The Varattar sub-basin is one of the minor sub-basins in the River Ponnaiyar basin and it originates from the Vellaikkalmalai in the southern part of the study area with an elevation of 1173 m. Varattar joins with Vaniyar in the right flank near Harur town. Geomorphologically the study area is covered by hills and pediplains. The study area is underlain by Archaean formations of crystalline rock types such as Charnockite, Gneiss, etc. The area receives an average annual rainfall of 835mm.

METHODOLOGY

The methodology followed in this study to delineate suitable sites for groundwater recharging has been illustrated in Fig. 2. In the present study, geospatial techniques were used for the identification of suitable sites for artificial recharge zones by considering various thematic layers such as geomorphology, lithology, drainage density, slope, land use/land cover, lineament density, and soil have been prepared in ArcGIS obtained from various data sources.

Each thematic map and class were given a suitable weight and ranking respectively as per their relative significance in groundwater occurrence, movement, and infiltration. Then, all the thematic maps were converted into raster format so that they can be easily integrated and processed in a GIS environment. Finally, all the thematic maps were integrated and reclassified into very high, high, moderate, poor, and very poor groundwater recharge sites by weighted overlay analysis in ArcGIS 10.2.

RESULTS AND DISCUSSION

Preparation of thematic layers and assigning ranks

From the IRS LISS-III satellite imagery, geomorphology, lineament density, and land use/land cover map were prepared. The lithology map of the study area is prepared by using the Geological Survey of India District Resource map. A soil map of the study area is prepared from the soil survey department. The drainage density map is prepared from the survey of India topographical maps and the slope map was derived from the Shuttle Radar Topographic Mission (SRTM) DEM data with a resolution of 90m. Integrating these thematic layers in terms of the weighted overlay index (WOI) method, the potential groundwater recharge sites were demarcated in the area. A numerical rating system ranging from 1 to 10 was given to each class of individual themes, based on the degree of influence of individual categories on the groundwater regime of the area. Scores of 1-10 were given to feature categories of each parameter. Further, weight age has been assigned to each parameter as per their relative significance in the identification of the potential recharge zones of groundwater, in which a maximum weight age of 25 % was given to geomorphology, 20% to lithology and land use/land cover, 15 % slope, 10 % of drainage density and 5% to lineament density and soil. A brief description of each data layer with its significance in the delineation of potential groundwater recharge zones is given below.

Geomorphology

In the study area, geomorphic features have been interpreted from the IRS LISS III satellite image (Fig. 3). Geomorphologically, the study area consists of hills and plain landforms. Hill landforms like a dissected structural hills and residual hills act as runoff zone.   In the plain landforms, flood plain, pediplain, and shallow buried pediment that influences groundwater recharge to occupy a large portion. The score and weights were allotted to the parameter and individual landforms, according to their respective features influencing the groundwater recharge, holding, and its occurrence Table 1. The maximum weights of 10 were given to the water body and the minimum weights of 1 has been given to the residual hill and dissected structural hill.

Lithology

The lithology is an important factor that controls infiltration, runoff, and storage of the precipitated water.   The study area lithology map was prepared from the district resource map of GSI, 2002. The different lithological units are charnockite, hornblende biotitic gneiss, epidotic-hornblende gneiss, ultramafic rock, and pyroxene granulite (Fig. 3). The major part of the study area is covered by charnockite and epidote-hornblende gneiss. In these litho units, groundwater recharge capability varies. Thus, scores and weightages have been given to each rock unit, according to the properties of rocks (Table 2). The highest weights of 5 are given to hornblende biotitic gneiss and low weights of 1 and 2 were given to ultramafic rock and charnockite respectively, because of their impervious to nature.

Land use/land cover

Land use/land cover is an important indicator of the extent of groundwater requirements and utilization as well as in the selection of sites for artificial recharge of groundwater. IRS LISS III satellite image were used for land use/ land cover mapping (Fig. 3). The land use of the study area is classified into nine classes: Built-up land, agriculture land, fallow land, waste land, crop land, scrub land, barren rocky/rocky knob, a forest plantation, and water bodies. The weights were devised to the individual classes, according to their individual features influencing the groundwater recharge, holding, and its occurrence (Table 3). Built-up lands are typically accompanied by a reduction in the infiltration rate due to a lack of permeable surfaces. In contrast, agricultural land and fallow lands allow more

infiltration of water since the vegetative cover can retain water and facilitate infiltration. In the study, maximum weights of 10 were given to water bodies because of their highest infiltration rate next to agricultural land, cropland, and fallow land respectively.

Slope

The slope gradient directly controls the surface water infiltration. Considering the slope factors the gentle slope, decreased will be the runoff and thus, higher is the groundwater recharge and steeper slope having increased runoff having lesser in groundwater recharge. The slope map was prepared from the Shuttle Radar Topography Mission (SRTM) satellite image. Slope in the area varies from 0 to 35° with more gradient in the southeast than the northwest. On the basis of the slope, the study area was divided into five classes (Fig. 3). The area where the slope is minimum and red loamy soil tended to retain water longer had a greater chance of infiltration and recharge. The score and weights were assigned to the respective slope class, according to its respective features influencing the groundwater recharge, holding, and its occurrence (Table 4).

Drainage density

The drainage density is an expression of the closeness of spacing of channels, thus providing a quantitative measure of the length of stream within a square grid of the area in terms of km/km^2 (Strahler 1957). Drainage density is an inverse function of permeability. The drainage density indicates the relative runoff in an area. In places where the density is high runoff would be more and of less drainage density runoff would be less. In the study area, the density ranges from 0 to 577 km/km^2, and it's classified into five classes which are shown in Fig. 3.

Lineament density

A lineament is a linear characteristic that expresses basic structural features such as fractures, faults, cleavages, and discontinuity surfaces. Lineament density indicates the relative infiltration ability of an area. Places, where the density is high infiltration, would be more and of less lineament density infiltration would be less. Lineament density represents the total length of lineaments per unit area. A lineament map was converted to lineament density using line density in the spatial analysis tool. Then, classified into five classes based on the lineament density (km/km^2) and shown in Fig. 3.   The highest weights of 5 have been given to high lineament density (281-351) and low weights of 1 are given to (0-70) are shown in Table 6.

Soil

Soil has a significant control on the infiltration and percolation rates into an aquifer (Anbazhagan et al. 2005). The study area soil map was obtained from the soil survey department and classified into three classes (Fig. 3). The study area mostly covered by red loamy soil and the score and weights given to each soil class are shown in Table 7. Based on the groundwater recharge capability the higher weightage 9 is given to red loamy soil.

Site selection for artificial recharge

The identification of the artificial recharge sites is based on the integration of various thematic maps considering their behaviour with respect to groundwater control. The weighted index overlay method was adopted for combining the various thematic maps in the study area. This method is quite powerful as human judgement can be incorporated into the analysis. In this model, different scores are assigned to the map classes that occur in each input map, as well as to the maps themselves that receive weights. The average score is then defined by:

$S = \Sigma S_{ij}W_i/\Sigma W_i$

Where, S is the weight score of an area object (polygon, pixel), Wi is the weight for the ith input map and Sij is the rating score of the jth class of the ith map. The value of j depends upon the class occurring in the current location. After assigning the score and weightages for each thematic map with classes integrating all these layers were carried out applying weighted overlay analysis in a GIS environment. The artificial recharge site map has been categorized into five zones that are anchored in the prospects for artificial recharge of groundwater (Fig. 4). These areas very

high (116.29 km^2), high(175.49 km^2), moderate(173.46 km^2), poor(58.55 km^2), and very poor (4.97 km^2). The graphical representation of the artificial recharge sites is shown in Fig. 5.

CONCLUSION

The study reveals that the use of the geospatial technique is very helpful to delineate the artificial recharge sites. A new integrated approach to the identification of the artificial recharge sites is efficiently enabled for quick decision-making and sustainable water resources management. All the thematic layers assigned a proper weightage, scores, and integrated into the GIS environment to finally prepare the recharge sites map. The groundwater recharge map thus obtained was divided into five zones as very high (115.99 km^2), high (175.19 km^2), moderate (173.16 km^2), poor (58.25km^2), and very poor (4.66km^2). The results obtained can be used for the sustainable management of groundwater resources in the area in terms of artificial recharge. The suitable sites for artificial recharge to groundwater show an area having 0-7° gentle slope areas, red loamy soil, cropland and water holding capacity in the floodplain, and shallow buried pediments are suitable sites for constructing an artificial recharge structure. Various groundwater recharge structures like boulders dams, check dams, percolation tanks, recharge pits, etc., were suggested in appropriate locations of Varattar sub-basin.

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Table 1. Geomorphology classes with score and weightage

S.No.	Classes	Weightage (%)	Score	Area (km²)
1.	Water body	25	10	7.23
2.	Dissected structural hill	25	1	120.12
3.	Residual hill	25	1	6.34
4.	Shallow buried pediment	25	7	158
5.	Linear ridge/dyke	25	3	3.87
6.	Pediplain	25	8	89.12
7.	Flood plain	25	9	128.37
8.	Valley fill/filled valley	25	5	15.71

Table 2. Lithology classes with score and weightage

S.No.	Classes	Weightage (%)	Score	Area (km²)
1.	Charnockite	20	2	233.24
2.	hornblende biotite gneiss	20	5	7.69
3.	Epidote- hornblende gneiss	20	4	238.62
4.	Ultramafic rock	20	1	0.74
5.	Pyroxene granulite	20	3	48.47

Table 3. LU/LC classes with score and weightage

S.No.	Classes	Weightage (%)	Score	Area (km²)
1.	Water body	20	10	7.33
2.	Forest plantation	20	7	115.12
3.	Barren rocky/rocky knob	20	1	92.44
4.	Scrub land	20	4	145.71
5.	Crop land	20	9	121.05
6.	Waste land	20	2	7.89
7.	Fallow land	20	8	18.83
8.	Agriculture land	20	9	18.28
9.	Built-up land	20	6	2.11

Table 4. Slope classes with score and weightage

S.No.	Classes	Weightage (%)	Score	Area (km²)
1.	0 - 7°	15	8	430.15
2.	7 - 14°	15	6	42.97
3.	14 - 21°	15	4	31.66
4.	21 - 28°	15	3	19.87
5.	28 - 35°	15	1	4.11

Table 5. Drainage density classes with score and weightage

S.No.	Classes	Weightage (%)	Score	Area (km²)
1.	0 – 115	10	1	115.12
2.	115 – 231	10	3	204.26
3.	231 – 346	10	5	141.69
4.	346 – 462	10	6	60.8
5.	462 – 577	10	7	6.89

Table 6 Lineament density classes with score and weightage

S.No.	Classes	Weightage (%)	Score	Area (km²)
1.	0 – 70	5	1	156.94
2.	70 – 140	5	2	233.82
3.	140 – 211	5	3	110.59
4.	211 – 281	5	4	25.05
5.	281 – 351	5	5	2.36

Table 7 Soil classes with score and weightage

S.No.	Classes	Weightage (%)	Score	Area (km²)
1.	Red loamy soil	5	9	326.58
2.	Red gravelly soil	5	1	86.31
3.	Red sandy soil	5	5	115.87
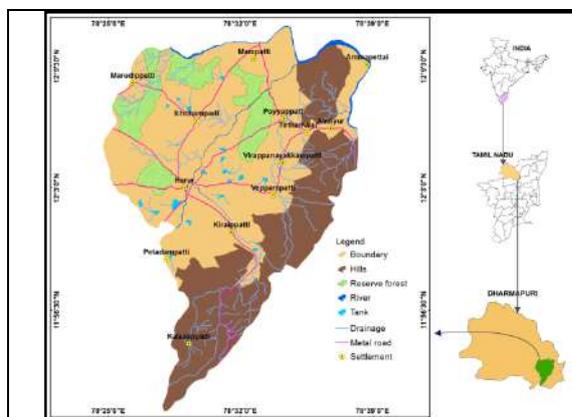
| Fig. 1 Location map of the study area | Fig. 2 Methodology flow chart |
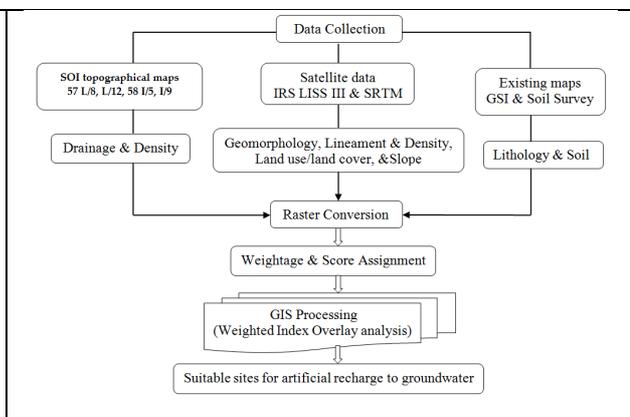
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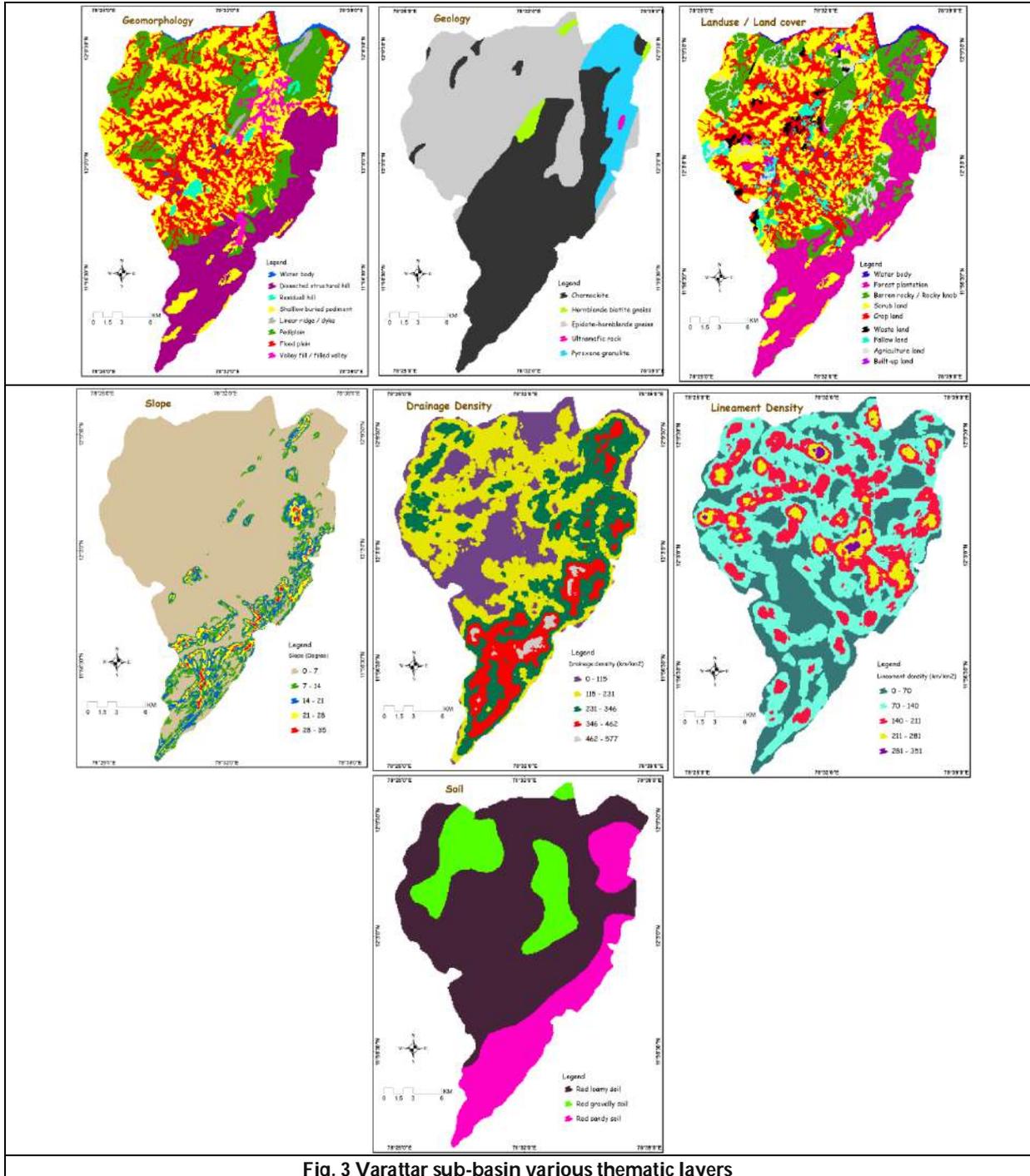
Fig. 3 Varattar sub-basin various thematic layers

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	very poor	poor	moderate	high	very high
■ %	0.94	11.07	32.81	33.19	21.99
■ Area	4.97	58.55	173.46	175.49	116.29
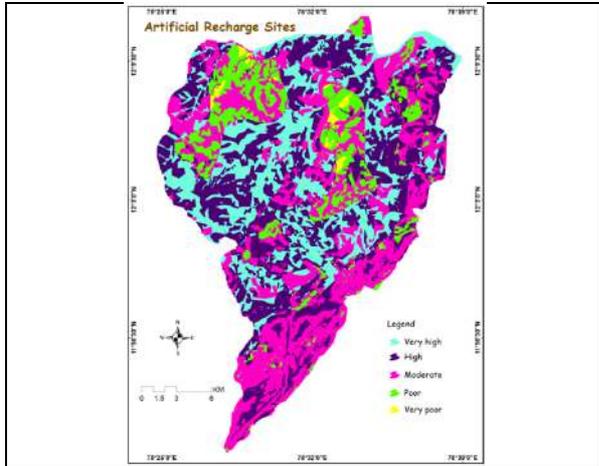
Fig. 4 Artificial Recharge Sites for Varattar sub-basin	Fig. 5 Graphical representation artificial recharge sites
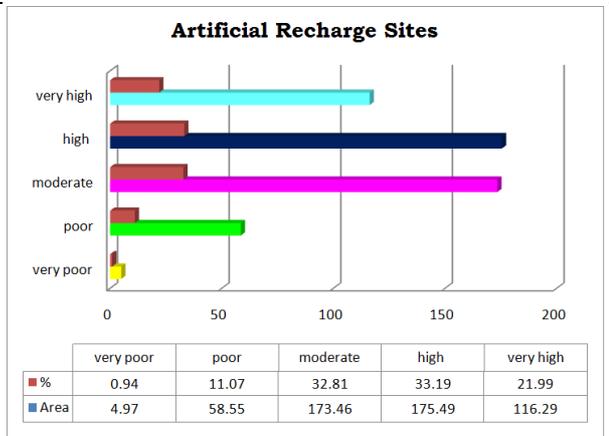
RESEARCH ARTICLE

Caesarean Section One Hundred One years (1920-2021): It's Journey from Ancient to New Technology

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ABSTRACT

In the United States, one out of every three women has a caesarean delivery, whereas in other areas of the world, up to four out of every five women have one. Caesarean section has a long history dating back over four centuries. Up the operation was carried out till the end of the nineteenth century. Because of its high mortality rate, it should be avoided. In 1926, the Munro Kerr was the first to use a modest transverse uterine incision. For the following 50 years, it was the standard method. Since the 1970s, novel surgical procedures have steadily gained popularity. Because of this, it has become the most often utilised approach today benefits during and after surgery. Concurrently, despite past initiatives to favour vaginal birth, Cesarean delivery rates have gradually climbed. Over the last ten years, from 5% to 30–32 per cent, with over the last ten years, expenses have risen, as have short- and long-term maternal, neonatal, and developmental difficulties. Efforts to minimise Because of the apparent safety of the procedure, the rate of caesarean deliveries has been generally unsuccessful. With the legal climate, and short-term postpartum benefits in the absence of indicators, a maternal request is made. In the United States, as the rate of caesarean births has risen in the United States, maternal health has suffered. Over the previous few decades, both mortality and morbidity have progressively increased. Disproportionately affecting black women over three decades in relation to other races prenatal data in abundance Cesarean-related abnormalities: diagnosis and treatment Placentation has helped altered women's results. However, there isless evidence available regarding the improvement of Caesarean-related gynaecological conditions' outcomes, in this review, the authors discuss the obstacles and opportunities for study, education, and change in the health impacts of caesarean birth for all women.

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INTRODUCTION

Caesarean section (CS), which was once shunned because of its high mortality rate, is now used by one out of every three women in the United States [1] and up to four out of every five women in other parts of the world [2]. Its long history reflects changing surgical opinion throughout time on topics such as bleeding, infection, pain, sutures, and, more recently, hospitalization time and cost reductions.

HISTORY

Caesarean section has been thoroughly researched. Interestingly, historians have refuted the claim that Julius Caesar was born by this procedure, concluding that the operation's name does not come from his birth. Instead, they claim that King Numa Pompilius (715–673 BC) established the surgery in Roman legislation to be performed on women dying in the latter weeks of pregnancy. During Julius Caesar's rule, the Lex Regia was renamed Lex Caesarea, and the technique became known as the caesarean operation. Through an incision in the abdominal and uterine walls, a kid, either alive or dead, was removed from a dead or dying mother. The surgery was traditionally carried out by the patient, her husband, sow gelders, barbers, midwives, surgeons, or tribal natives. From razors to axes, a range of devices were used. Gored lacerations of a woman by horned animals, for example, resulted in primitive abdominal births occurring spontaneously during tough labour or inadvertently. Surprisingly, some women are said to have survived [3].Caesarean section (CS), which was once shunned because of its high mortality rate, is now used by one out of every three women in the United States [1] and up to four out of every five women in other parts of the world [2]. Its long history reflects changing surgical opinion throughout time on topics such as bleeding, infection, pain, sutures, and, more recently, hospitalisation time and cost reductions..

The uterine walls were not sutured following the uterine incision and the removal of the baby during this time, instead relying on contractions and retraction to prevent haemorrhage. The majority of women died from haemorrhage or infection; maternal death rates after the procedure ranged from 52.5 to 100%, and the treatment was avoided at all costs. The tremendous progress in science and medicine during the nineteenth century made caesarean section a viable option for obstructed labour, though it was still dangerous. In 1876, Italian doctor Eduardo Porro published his method of amputating the body of the pregnant uterus and sewing the cervical stump as a way of delivery, recognising infection rather than haemorrhage as the primary cause of death [4]. In 1882, gynaecologist Max Sanger described the use of a second layer of sutures to close the vertical uterine incision and introduced approximation of the peritoneal borders to protect the uterus following delivery [5]. In contrast to the hysterectomy used in the Porro approach, the procedure was dubbed "conservative caesarean section.

" Although the conservative operation reduced maternal mortality significantly, the outcomes were restricted by the risk of infection. Sanger's relevance was recognised, and his work became the norm for classical operations today. For better results, an extra peritoneal caesarean approach was described in the early twentieth century, in the absence of antibiotics, as well as many changes to existing techniques for women with protracted labours or probable infections. The use of antiseptic method in abdominal surgery, as well as the selection of patients and specific operations, lowered maternal mortality to 1–2%, especially when done at a predetermined time before labour (or shortly after its commencement) and on uninfected women. As maternal mortality decreased and the procedure became better known in the 1920s, uterine rupture during labour, bleeding, and infection became the primary concerns. Because future conception was conceivable following a conservative CS, successful repeated CSs on the same person was documented. Following an unsuccessful attempt at vaginal birth, the occurrence of uterine rupture via the site of a prior classical incision led to the maxim "Once a caesarean, always a caesarean" [6].

With the absence of scar tissue by gross and histology at the site of a previous CS, even in the presence of a previous CS, such a belief was challenged and deemed to be incorrect, even if the anterior uterine wall has a slight vertical

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furrow on the exterior and interior surfaces Intra-abdominal adhesions, as well as omental and intestinal adhesions, were frequently produced between the uterus and the abdominal wall. The placenta was found to be abnormally adherent. in rare cases, by placental attachment that is solid and broad, as evidenced by the absence of decidua and profound chorionic effusion Invasion of villi into the myometrium [7, 8].

Caesarean surgical Technique Evolution1920–1970
The Sanger procedure used interrupted deep sutures that went through the myometrium's thickness, avoiding the decidua. Muscles that are visible on the surface a continuous layer connects the peritoneum and the layer suture. The peritoneum, muscles, fascia, and parietal peritoneum are next. In distinct layers, the skin is closed [5]. The success of the for the next 40 years, the classic Sanger operation continued. The obstetrician John Martin Munro Kerr's procedure of double closure of the lower abdomen was introduced in 1926 by obstetrician John Martin Munro Kerr.[9, 10]In honour of the German gynaecologist who invented the abdominal incision in 1900, the Kerr incision included the creation of a bladder flap in the lower uterine segment, double layer closure of the uterine incision, emphasis not to include the decidua in the uterine closure, re-approximation of the bladder flap, and approximation of the parietal peritoneum. This lower segment approach was seen as the most important of the several caesarean operations at the time, and is considered the most significant advancement in obstetrical treatment.

The operation's popularity remained unchallenged, and it became the procedure of choice after that, as it minimised the risks of haemorrhage and infection [11] during this time, maternal mortality in most major hospitals in the United States continuously declined to near 1%, and the foetal salvage rate climbed. During this time, surgical skill became more refined, and blood banks, aseptic procedures, and antibiotics were introduced. From 1937 until 1944, the classical and low flap surgeries were performed in equal numbers. The lower segment method, on the other hand, was taught to all residents in training and covered in all major teaching texts beginning in the 1950s. When faced with obstetrical complications, it was widely acknowledged by all obstetricians as the best way of delivery for mother and baby. Cesarean section complications from 1920 to 2020, with immediate treatments. . With the development of antibacterial medicines, extra peritoneal surgery became less common. The principal disadvantage was the limited operative field and the unavoidable extension into the peritoneal cavity, whose integrity was being preserved. Only some indications, such as placenta previa and severe abruptio placentae, were treated with the classical section. From 1932 to 1963, the overall incidence of CS in the United States increased from 2.5 to 5.1 percent, then to 8% in 1970, as perinatal death fell from 9.8 to 2.9 percent and maternal mortality fell from 1.8 to 0.04 percent. [12]

1970–2021
In the 1970s, there was an increase in the CS rate, which was explained by better mother and foetal outcomes and increased safety From 5.5 percent of deliveries in 1970 to 16.5 percent in 1980 [13], the rate continued to rise until stabilising around 31–32 percent in the United States in 2019 . The widely used Pfannenstiel–Kerr approach has been challenged by new surgical procedures or adjustments to current ones. Joel-Cohen progressed blunt dissection of the abdominal incision and parietal peritoneum [14] The Misgav–Ladach modified Misgav–Ladach approaches were then merged with blunt uterine incision as the cornerstone of a new generation of caesarean techniques.[15,16] The multiple processes used since 1926 have now been adjusted to incorporate one or two layer uterine closure with no peritoneal layer approximation.[17] When compared to the Pfannenstiel–Kerr method, the new procedures provided advantages such as shorter operating times, less blood loss, quicker hospitalisation and recovery times, less postoperative pain, and lower costs [18, 19]. These discoveries prompted prominent medical centres in the United States and overseas to teach the newer techniques exclusively, resulting in a new generation of obstetricians who specialise in them.

The previous method has been replaced by a procedure with far fewer steps and a shorter operational time. These steps do not include endometrium or decidua care during uterine closure, whether single or double layer. The American College of Obstetricians and Gynaecologists, as well as other governing bodies that support physician

autonomy in selecting preferred treatments, have recognised the safety of these novel approaches for post-partum outcomes [20]. Peritoneal closure's efficacy has been questioned and mostly abandoned [21]. Currently there is a lack of data on the impact of these contemporaneous techniques on subsequent pregnancies and long term maternal outcomes [22].worldwide c section rates have risen from around 7% in 1900 to 21% today, and are projected to continue increasing over this current decade. If this trend continues by 2030 the highest rates are likely to be in Eastern Asia (63%), Latin America (54%), western Asia (50%), Northern Africa (48%), Southern Europe (47%), Australia and New Zealand (45%), the research suggests [23].

Abnormal placentation
The rise in aberrant placentation that is proportional to the number of CSs appears to be a recent phenomenon. The rising caesarean birth rate, as well as the perceived safety of doing more caesareans, coincides with an increase in aberrant placentation. Cesarean delivery (CD) rates have risen drastically from 5.8% in 1970 to 31.9 percent in 2016, [24], resulting in an increase in placenta previa (PP), placenta accreta (PA), and caesarean scar pregnancy (CSP). Years after the previous CD, the emergence of these long-term diseases resulted in a substantial increase in maternal death and morbidity. The maternal mortality rate rose from 7.2 per 100,000 live births in 1987 to 17.4 per 100,000 in 2018 [26]. PA and PP account for a significant portion of maternal morbidity and mortality in modern obstetrics, accounting for 1.7 percent of all maternal fatalities in the United States [27, 28]. If the CD rate continues to climb, there will be an extra 6,236 instances of PP, 4,504 cases of PA, and 130 maternal deaths every year by 2020 [29]. In earlier observational studies, the prevalence of PA was estimated to be 1 in 4,000 deliveries in the 1970s [30], 1 in 2,500 deliveries in the 1980s [31], and 1 in 533 deliveries in 2002 [32]. According to a recent study, the total rate of PA in the United States is closer to 1 in 272 live births among women who had a birth-related hospital discharge diagnosis. [33]. A number of studies have documented a strong association between PP and previous CD with the incidence of PA. The risk is significantly increased when both factors are present. Clark concluded that a woman with one prior cesarean and a PP has a 24% risk of PA. The risk increases to 67% with four or more CSs and a PP [34]. Silver and many others have also reported an increase in PA with the number of previous CDs. In women with PP and prior CDs, the risk of PA is 11% after one procedure, 40% after two procedures and 61% after three procedures [35]. Systematic reviews and meta-analysis of PP and PA have emphasized the prevalence and incidence of PP complicated by PA, and found evidence of regional variation [36, 37].

The placenta accreta spectrum (PAS) describes the range of aberrant placental trophoblast adhesion to the uterine wall, as well as invasion into and through it. Morbidly adherent placenta was the previous name for it. Since its implementation in 1978, the frequency of CSP has risen in lockstep with the increase in the CD rate. Its true incidence is unknown, Antoine and Young: Cesarean section 100 years 1920–2020 7 possibly because the condition is underreported or under diagnosed [38]. CSP is projected to affect 1 in every 1,688 normal pregnancies [39]. The placenta accrete spectrum (PAS) describes the range of aberrant placental trophoblast adhesion to the uterine wall, as well as invasion into and through it. Morbidly adherent placenta was the previous name for it. Since its implementation in 1978, the frequency of CSP has risen in lockstep with the increase in the CD rate. Its true prevalence is unknown, most likely because to underreporting or under diagnosis [38]. CSP is projected to affect 1 in every 1,688 normal pregnancies [39].

CSP and PAS are thought to be symptoms of the same aberrant implantation spectrum, with identical histological findings of a pregnancy implanted in a previous CS scar [40]. Severe haemorrhage, uterine rupture, peripartum hysterectomy, organ injury, intensive care admission, protracted hospitalisation, and even mortality have all been linked to CSP and PAS. Both hysterectomies associated with CD and peripartum hysterectomy are most commonly indicated by PAS. Between 1969 and 2009, the rate of peripartum hysterectomy in the setting of previous CD grew from 27 to 57 percent, with PA as the primary indication rising from 5.4 to 46.5 percent [41]. . PA was noted to be the indication for peripartum hysterectomy in 33–50% of cases in reviewed studies [42]. PA, first described in 1937, occurs after manual removal of the placenta, endometritis or uterine curettage [43]. Today, it is primarily the result of uterine scar as a result of damage to the endometrium-myometrium interface of the uterine wall secondary to cesarean delivery [44]. The CS approach has changed through time and has become more stable in the last 50 years.

Despite advances in surgical procedures, anaesthetic techniques, blood banking, and antibiotic therapy, caesareans remain a high-risk procedure. Both women and children may experience short- and long-term health consequences as a result of the surgery. Every CD-owning woman doubles her chances of experiencing these unwanted outcomes. Joel-Cohen pioneered an abdominal entrance technique that emphasises blunt dissection [14]. Several variants of his technique grew more widespread than the Pfannenstiel– Kerr era [15–17]. As a result, providers are educated to improve the perceived short-term benefits while ignoring the potential long-term dangers.

Uterine rupture
Following a prior CS, uterine rupture is an uncommon but possibly dangerous complication of a subsequent pregnancy. It can occur before or during delivery and is frequently linked to the need for emergency CS, severe bleeding, placental or foetal evacuation into the abdomen, hysterectomy, or uterine repair. Because the new-born is frequently distressed, admittance to a neonatal critical care facility is required. The risk of uterine rupture varies depending on the clinical situation and is estimated to be between 0.5 and 4%.[45] After attempting vaginal birth after two previous CSs, uterine rupture is more common, increasing the likelihood of caesarean hysterectomy. Although uterine scar dehiscence is more common, it rarely causes life-threatening maternal and foetal problems [46]. The outcome of a trial of labour after a CS has been linked to uterine suture method, with no differences reported between single and double layer closure procedures [47]. With rising CDs, the risk of uterine rupture is expected to rise. Understanding the differences in uterine closure techniques used by different obstetricians could help with the inquiry.

Long-term gynaecological complication
In modern obstetrical practice, the relative safety of CD is based on short-term rather than long-term mother outcomes. Women are at a higher risk for a number of chronic gynaecological disorders after a CD, though. Surgical adhesions, pain, infertility or sub fertility, irregular bleeding, difficult intercourse, painful menses, and endometriosis are just a few of them [48–51]. These chronic disorders frequently necessitate close monitoring or surgical intervention, such as laparotomy or endoscopic exploration using hysteroscopy, laparoscopy, or robotic assistance [52, 53].These operations come with an increased risk of consequences due to unexpected difficulties.

Chronic pain
There have been a few studies published on the risk of chronic pain in women after CD. Sun et al. [54] discussed the difficulties in anticipating discomfort following CD. Other researchers point to the impact of surgical technique on the risk of pain. Nerve entrapment, pelvic adhesions, uterine scar abnormalities, and endometriosis may occur as a result of emergency CD, abdominal incision selection, closure versus non closure of the visceral or peritoneal incision, and uterine closure technique[55– 58]. The likelihood of pain increases as the number of CDs increases. Women with repeated repeat CDs have a significantly higher risk of organ injury after surgery. Cesarean scar endometriosis is a rare complication. , it often presents as cyclic abdominal pain and incisional mass favouring a Pfannenstiel incision more than a midline vertical incision [50].

Pelvic adhesions
The cause of pelvic and abdominal adhesions following CD is poorly understood. Surgical approach appears to influence the risk of post cesarean adhesion formation, just as it does with pain. Adhesions are frequently linked to persistent discomfort and infertility in non-pregnant women [59]. Pregnant women are frequently confronted with difficult repeat CDs, organ injury, high blood loss, and prolonged operating time during delivery [35].

Fertility
There are very few studies in which the authors assessed the effect of CD on infertility or sub fertility. Pelvic adhesions might interfere with tubal function and patency. Cesarean scar defects have been related to infertility. Surgical intervention with laparoscopy, hysteroscopy or both combined has been successful in restoring fertility [60]

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Irregular bleeding
The prevalence of caesarean uterine scar defect, or "niche," is rising at the same time as the long-term gynaecological consequences, which include dysmenorrhea, irregular uterine bleeding, infertility [61, 62], and improper placentation in subsequent pregnancies [63].

Tanos analysed five studies that looked at 5,123 patients with specialised symptoms. 65 per cent of patients had a uterine haemorrhage, 46.2 percent had chronic pelvic discomfort, 52per cent had dysmenorrhea, 71.5 percent had infertility, and 24.2 percent had dyspareunia, according to him [46]. Long-term research on the impact of uterine closure procedures on these unexpected gynaecological and reproductive problems is desperately needed.

Neonatal morbidity
There is growing evidence that neonates born via CS have a different immune system, have a smaller intestinal micro biome, have late childhood obesity, and asthma [64–67]. To reinforce the evidence, more inquiry is required [68]. Prematurity and its consequences are the main causes of adverse perinatal outcomes in women who have had previous caesareans [69, 70]. Infants born by CD are more likely to develop respiratory problems and require NICU care than those born vaginally [71].

Healthcare-associated costs
Cesarean delivery expenses are frequently mentioned as significant advantages of various surgical methods employed over the last five decades. These prices usually include short-term advantages but exclude long-term complications, which are sometimes neglected as CD side effects.

However, the treatment of women with PA and the subsequent maternal and neonatal morbidity can be costly to the health care system. From 2001 to 2011, Mogos et al. showed a higher mean cost of inpatient care, adjusted for inflation, resulting in almost $115 million in increased inpatient costs compared to non-PA impacted deliveries [33]. In addition, the expenditures of gynaecological exams and antenatal consultations in regard to niche and aberrant placentation must be considered.

Reducing cesarean section rates
The goal of "Healthy People 2020," the United States' federal prevention program me for establishing a healthier population, is to reduce caesarean births to 24.7 percent. Or Low-risk females have a lower risk [72]. The relative safety, though, and the short-term benefits of CD often urge obstetricians to proceed without hesitation. The long-term hazards that are often unknown to the public pregnant women are rarely considered or discussed. Prior to surgery in the absence of a medical need, some people will. In part, pregnant women opt for a caesarean birth. Because of a lack of awareness of prospective dangers and fear of pelvic floor dysfunction and discomfort Women must be strong. Schooled on the potential short- and long-term hazards of a Both mother and child were born via caesarean section. Avoiding a CD and attempting a vaginal delivery after a previous CS are the most recommended methods for reducing PAS risk and should be promoted. Edward Cragin reported on the hazards of vaginal birth in women who had previously been delivered by CS in a 1914 speech and again in 1916, saying "once a caesarean, always a caesarean." [6]. Cragin was forecasting that a group of women who failed to deliver vaginally after several days in active labour would almost certainly need repeat CS.

Rickets and pelvic deformity were common at the time, oxytocin for labour augmentation was non-existent, there were no blood banks or antibiotics, and surgery was risky. The primary CS was performed in order to preserve the life of a disabled mother. In those days, fetal distress was not an indication for CS was used only to confirm a living fetus, and fetal monitoring did not exist. This is, in large part, why obstetricians and hospitals have refused to let women try natural labour following a CS for the past half-century. Changes in labour management in the United States kept the CS rate low throughout the 1960s. The use of forceps, oxytocin, vaginal delivery of breeches, and foetal monitoring by auscultation alone [73, 74] all contributed to a 4–6% reduction in the rate. Studies showing the relative safety of a trial of labour after a low transverse incision CD (TOLAC) resulted from a reconsideration of

Cragin's paradigm. [75–77]. Despite studies from greater numbers of patients confirming the relative safety of a labour trial following CS, the US CS rate continued to climb, rising from 5% in 1970 [78] to 20% in 1996, 31% in 2006, and remaining around 32% in recent years [1, 24]. The rise in labour following CD trials was reflected in an increase in the vaginal birth after caesarean (VBAC) rate (VBAC per 100 women having a prior CD) from 18.9 to 28.3 percent between 1989 and 1996, [35], resulting in a decrease in the total CS rate from 25 to 20.7 percent in 1996 [79]. Several published reports describing uterine ruptures and accompanying neonatal and maternal problems [80] questioned this significant fall in the CS rate in the mid-1990s.

VBAC was not recommended by practising obstetricians because of these dangers and the risk of professional liability. VBAC had reversed its trend by 2006, and the rate had dropped to 8.5 percent, resulting in a substantial increase in the total CD rate to 31.1 percent [79, 81, 82]. In some hospitals, policies have restricted or banned TOLAC altogether [83]. There are a number of reasons for this the current caesarean rate in the United States is due to clinical and social factors. For starters, CS is one of the safest and most reliable options. In the United States, the most common procedures are there were some in 2018 =1,208,176 caesareans were done, accounting for 21.7 percent of all deliveries.

The caesarean rate is influenced by a desire for a caesarean birth for convenience, patient views of social standing, and concern of pelvic anatomic disruption. Fertility treatment-induced pregnancies put a lot of pressure on the mother to have a caesarean birth. Electronic foetal monitoring became the standard of care for all patients as CS became a very safe and common procedure, and the focus switched to the foetus. Due to a misunderstanding of the significance of intrapartum hypoxia as a cause of cerebral palsy, this resulted in massive cash awards.

Any negative outcome for the new-born became indefensible in a lawsuit, which would similarly result in enormous monetary judgments for alleged misconduct. Vaginal delivery of breeches was abandoned, and the use of forceps and suction reduced. In the attempt to minimise the CS rate, appropriate labour management and consistent interpretation of foetal monitoring have long been promoted, albeit with little success. The CD rate in the United States has been in the 30–32 percent range over the past ten years. Other treatments, such as requiring a second opinion and following stringent standards, have been demonstrated to reduce the caesarean birth rate safely [86, 87]. Despite mounting pressure to reduce the rate of CSs, experts believe it will take at least 15 years for a significant reduction. However, as midwives, doulas, and other continuous labour assistance become more widely recognised, we hope to see a drop in caesarean births [88–90].

Some of the following steps might limit caesarean births and obtain the best outcomes
- Uniform criteria for diagnosing fetal jeopardy in labor using fetal monitoring.
- Diagnosing dystocia by standardized criteria.
- Using oxytocin for induction of labor and stimulation of arrested labor in a defined protocol.
- Extensive training, including simulation in operative vaginal delivery of vertex presentations [91, 92].
- Extensive training, including simulation in vaginal delivery of breech presentations [93].
- Developing ultrasound pelvimetry to supplement clinical evaluation of the pelvis prior to operative vaginal delivery, a new concept based on now-obsolete X-ray pelvimetry
- Counselling patients requesting primary or repeat CS regarding risks and suggesting a limit to three, recognizing progressively increasing risks [94].
- Employing proper surgical techniques to minimize complications at subsequent pregnancies and Malpractice law reform.

Maternal mortality/morbidity and disparate racial outcomes
CDs had a higher maternal mortality and morbidity rate than vaginal births [95, 96]. Clark discovered that CD was associated with an 8–10 times higher risk of maternal death than vaginal birth [97]. In comparison to other Westernized countries, the United States has extremely high rates [98]. Maternal mortality in the United States climbed from 7.2 per 100,000 live births in 1987 [25] to 17.4 per 100,000 live births in 2018, with 658 women dying

[26]. These higher death rates are largely due to racial and ethnic differences. After childbirth, black and African American women are three to four times more likely to die than women of other races and ethnicities.

In the United States, severe maternal morbidity has increased in recent years, with over 50,000 women suffering in 2014 [100]. Racial and ethnic disparities are also prominent in severe maternal morbidity. Severe maternal morbidity increased by 170 percent in each racial/ethnic group studied between 1997 and 2014, with black women (1.63 percent) having the highest frequency and white women (0.84 percent) having the lowest [101]. Mothers' comorbidities, such as hypertension, diabetes, obesity, and caesarean birth, are frequently cited as key contributors to increased morbidity [102, 103].Cesarean birth was linked to racial and ethnic differences in maternal mortality and morbidity. These inequalities, which date back to 1935, when Title V of the Social Security Act was enacted, were emphasised in a trend analysis from 1935 to 2007 [104]. Recognizing the multifaceted nature of the disparity, improving health care outcomes for women of colour begins with equitable, high-quality health care services that consider co-morbid health conditions and socioeconomic status, as well as the importance of respect, dignity, and communication in the delivery of care. To reduce maternal fatalities and improve outcomes for women of colour, it is critical to recognise the impact of individual and structural racism on maternal health.

Future directions: cesarean surgical techniques
Currently, researchers are concentrating on the rising prevalence of PAS, as well as its prenatal diagnosis, epidemiology, and various techniques to improving maternal outcomes [63, 105]In addition to CD, research focusing on discovering and preventing additional risk factors may help to reduce the substantial morbidity and economic burden associated with PA [33]. There are no research on the long-term effects of caesarean surgery methods on mothers. Several investigators have made recommendations to see if changing surgical procedures can reduce the likelihood of improper implantation in subsequent pregnancies [107]. Regardless of the number of subsequent pregnancies when the procedure was performed, no evidence of aberrant placental implantation was found in a study of a specific "endometrium-free double closure technique," or EFCT [108]. EFCT focuses on prevention and provides a surgical method that has the potential to minimise PAS and CSP, which are conditions that develop years after a caesarean birth. Unlike previously published data, preliminary review reveals that when EFCT is paired with peritoneal closure, a reduction in the incidence of debilitating disorders such as infertility, chronic pelvic discomfort, irregular bleeding, dyspareunia, and dysmenorrhea is conceivable.

The method of uterine closure appears to have an impact on niche development, which takes on its final shape with healing [108]. According to this study, needle placement through both the endometrium and myometrium causes endometrium to be present at the superficial level of the uterine closure, resulting in the formation of a uterine scar defect or "niche." [109]. the patho physiology of CS-related aberrant placentation has long been linked to uterine scar defect and damaged endometrium [63, 106]. This new study provides the path for more research into the impact of caesarean procedure, namely the endometrium or decidua, on remote obstetrical and gynaecological issues in women who have had previous CDs. As a result, Sholapurkar recommends doing targeted research into the finer points of surgical technique in order to uncover and design preventive treatments [61].

Individual caesarean procedures are not monitored by any local or national agencies, despite the fact that the dangers and complications associated with them are increasing. For the performance of a CD, there are no protocols or conventional approaches. Currently, the operation is entirely based on individual autonomy and preference. "A physician should conduct her or his technique as it makes no difference in short-term maternal outcomes," according to the American College of Obstetricians and Gynaecologists (ACOG) and other governing bodies. [110–112]. The choice of individual physician technique is influenced by a variety of factors. The type of practice (private solo or group), faculty group practice, hospital employees, insurance plan (in and out of network), and patient preference are all factors contributing to cost and operating time. Surgical standardisation, while not yet investigated, is most certainly a contributing cause to unanticipated chronic adverse effects following a CD, as it allows some obstetricians to accomplish the surgery in the quickest possible timeframe while others take longer. A call to action is used to standardise the procedures required to achieve the best results. To accomplish change for the benefit of women's

health and safety, a challenge to medical autonomy will be essential. It may be necessary to evaluate individual provider and institutional performance across the country, as well as to monitor complications by an independent committee made up of midwives, nurses, and doulas, as well as operating room technicians, doctors, and government agents, in order to implement safety parameters and risk-reduction methods.

The impact of technique on a repeat CS is currently being investigated using video footage of CSs [108]. It's utilised to document and compare surgical techniques performed during a previous caesarean section with intra-abdominal findings during a future CD. It is found to be useful for transparency, education, documentation, and the planning of a subsequent operation, including repeat CS [109], in the same patient. The creation of a common platform for CS videos should be viewed as a collaborative learning experience for all obstetrical surgeons. Video documentation could be incorporated to the CS operative report as an additional source of documentation. The narrative report, as it is currently utilised for documenting in the form of a handwritten, typed, or multiple choice template, frequently overlooks critical finer nuances that could aid in understanding or predicting future pregnancy outcomes.

CONCLUSIONS

The function of caesarean births in obstetrics has been evolving during the past century. CSs are still the most common procedure performed on women of reproductive age worldwide, accounting for one in every three American women, and are the primary cause of maternal mortality and morbidity in the United States. In rare situations, the operation can save the life of both the foetus and the mother. However, before a CS is recommended or performed, the justification, including potential risks and benefits, should be presented to the patient and discussed. Both maternal and foetal risks should be considered when discussing potential damage. Uterine rupture, significant blood loss requiring hysterectomy or transfusion, harm to nearby organs (bladder or intestine), and thromboembolic illness are the most serious maternal concerns. Infant harm is unlikely, but not impossible. With increased awareness of the ultimate goal of returning uterine function to normal physiology during the operation, maternal morbidities can be averted in large part. Obstetricians hold a big part of the solution to reducing cesarean-related complications in their hands. Increasing CS performance should never be considered the easy solution to most obstetrical problems. The link between a previous CS and later problems that are far apart necessitates more research and the use of certain surgical features to improve long-term outcomes.

The majority of research on caesarean morbidity has focused on immediate rather than long-term maternal risks and outcomes. Women who have a caesarean birth are more likely to develop a number of chronic and life-threatening diseases. As the number of CDs grows, these problems worsen. These include discomfort, adhesions, abnormal bleeding, and infertility in the non-pregnant condition. CSP and PAS, the most lethal maternal complications in obstetrics, are among the pregnancy-related dangers. Changes in the baby micro biome caused by CS compared to vaginal birth have recently received increased attention, with long-term implications for obesity and immunological diseases such asthma, allergy, and atopic dermatitis.

Cesarean techniques have improved over the last century, allowing for a reduction in maternal morbidity and mortality. Despite this improvement, black women continue to have far higher rates of maternal death and morbidity than white women. The health-care system has been challenged by these gaps, which indicate inequality in access to high-quality obstetrical care.

Surgical procedures have been linked to an increased risk of all long-term problems. Recent research suggests that preserving the endometrium after uterine closure may reduce aberrant placentation in later pregnancies. A new study linked the cause of caesarean scar deformity to the uterine closure procedure used after a caesarean birth. The authors emphasised the importance of the endometrium's position during the uterine incision's closure.

More research is needed to determine the best surgical approaches for reducing caesarean scar abnormalities, which are thought to be the most common cause of incorrect placental implantation. Surgical procedures are carefully woven to reduce the risk of chronic maternal morbidity. Video documentation of individual physicians' approaches may provide a clear picture of the wide range of surgical techniques in use. This should make subjective evaluation of complications in research trials obsolete. The autonomy of physician for surgical technique selection must also be reassessed in favor of a more structured, standardized optimal cesarean technique to curb these long-term maternal complications. Identification of all patients undergoing a cesarean birth and subsequent follow-up would create a registry and database for tracking and a statistic driven understanding of complications related to CDs. These steps could potentially lead to the discovery of the association between surgical technique and related complications.

Patients must be informed about the dangers of a caesarean delivery as part of their pregnancy education, and doctors must consider long-term risks when deciding whether or not to perform a CD. The success of this goal depends on women's education on the short and long-term risks of a CD to both mother and child. The best caesarean procedure will also allow us to level the playing field so that no one racial or ethnic group suffers from unequal reductions in cesarean-related problems. Our aim has not changed one hundred years later as we seek a twenty-first-century answer to the alarming rates of obstetrical bleeding, peripartum hysterectomy, mother death, and racial disparities in health care.

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Fig.1. C section in 1950 and silk worm gut sutures and Michel's clips used for skin closure

No deep suture
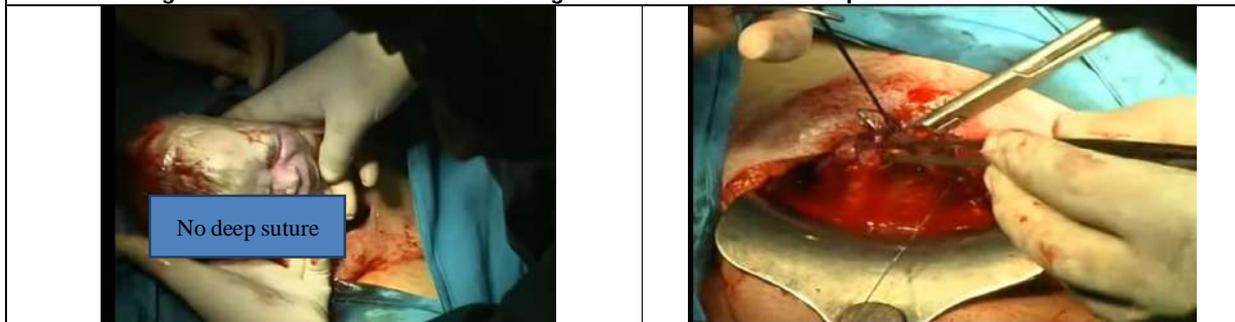
Fig. 2. C section and skin closure in 2021
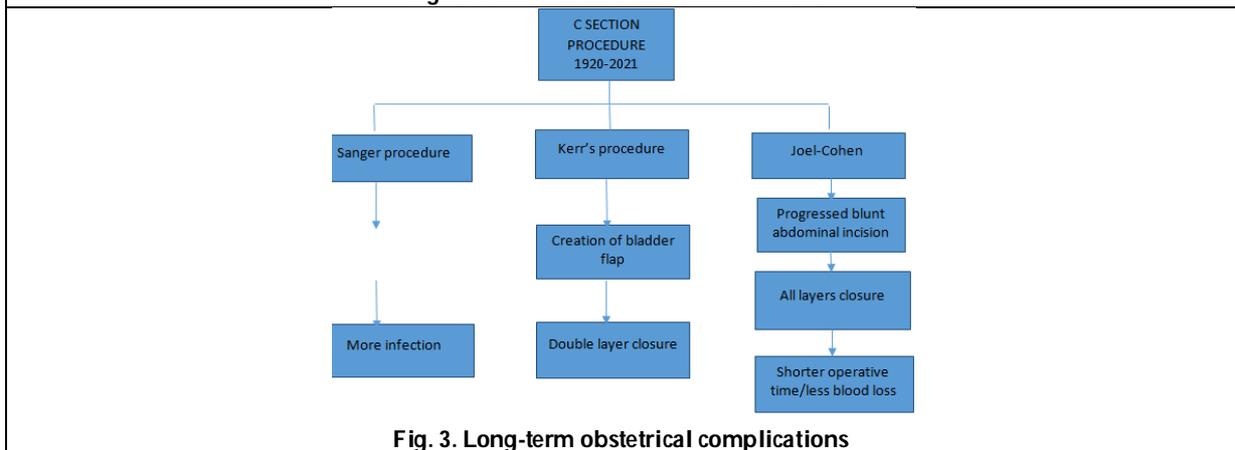
Fig. 3. Long-term obstetrical complications

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RESEARCH ARTICLE

Assessment of Air Pollution Sources in Hyderabad City using Multilinear Regression

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ABSTRACT

The paper intends to quantify the role played by each air pollutant component in the Air Quality Index (AQI) of Hyderabad. For this purpose, the daily data of air pollutants Particulate Matter (PM 2.5 and PM 10), Nitrogen Oxides (NOx), Ammonia (NH_3), carbon monoxide (CO), Sulphur dioxide (SO_2) and Ozone(O_3) is downloaded from Kaggle database along with AQI for 30 months. The data from different localities of Hyderabad, Industrial areas (Bollaram and Pashmyalaram), Residential area (Hyderabad Central University (HCU)), Residential cum industrial areas (Zoopark and ICRISAT) is considered to study the variation in the contribution of specific air pollutants. Statistical software IBM SPSS version 23 is employed to identify the contribution levels of various air pollutant components to air pollution. Multilinear regression of the data infers the major pollutant component as Particulate matter (PM) contributing to AQI in all the areas, however; the amount of contribution depends on the nature of the location. The other significant pollutants contributing to AQI are found to be CO, O_3 and Nitrates. The quantitative results confirmed around 60% of AQI is from PM in Bollaram and ICRISAT, around 50% at Pashmylaram and Zoo Park however, it was observed that at residential areas (HCU) the contribution of PM was less than 50% and Ozone was found to be the second-highest contributor (30%). This study helps to identify local sources affecting the air pollution to implement necessary steps by the authorities concerned to reduce it and improve the human health standards of residents of Hyderabad.
MSC2020: 62

Keywords: Air Quality Index, air pollutants, multilinear regression.

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INTRODUCTION

One of our era's biggest curses is air pollution which has a direct impact on climate change as well as on public health that leads to an increase in mortality. It is a major threat to today's growing world and the rate of air pollution in the atmosphere is getting to a higher rate. Undoubtedly, atmospheric pollution affects human health negatively causing premature deaths [5]. Hyderabad, 400 years old metropolitan city in the state of Telangana, is an industrial hub in southeast India – hosting many heavy industries, pharma industries, technology SEZ industries and research centers. It is one of the fastest-growing cities in India with a population density of ~18000 per sq km. Rapid industrialization and urbanization have encouraged migration to the twin cities which led to an increase in vehicular traffic, infrastructure and industrial output.

There are many major pollutants responsible for the decline of air quality across the globe. Most of the pollution comes from tiny solid and liquid suspended particles, nitrogen dioxide, sulfur dioxide, carbon monoxide, and lead emissions from gasoline exhausts.

Particulate Matter Pollution
Among many air pollutants, anthropogenic suspended particles in the air of very small sizes, called Particulate matter, can penetrate the respiratory system through inhalation, causing cardiovascular and lung diseases. Particulate pollution is one of the fatal types of air pollution in India and on a global level. Particulate matter can fall into two categories based on their size: fine particles (PM 2.5 – size range up to 2.5 microns) and coarse particles (PM10 – size range from 2.5 to 10 microns). These particles can be released into the air due to human activities such as vehicle emission, smoke, or dust particles, burning of garbage, etc., Moreover, they can also generate due to a reaction of air with gases released from industries, automobiles, forest fires and power plants. The adverse effect of these particles varies according to their size. Larger particles could enter and block the nasal passage whereas fine particles can enter directly into the lungs and affect health severely. The tolerable limits of PM 2.5 and PM 10 are 60 micrograms/cm^3 and 100 micrograms/cm^3(24hrs average) respectively.

Ground-level Ozone Pollution
Even though stratospheric ozone in the atmosphere protects human life, ground-level ozone (GOL) has an adverse effect on health. It is a highly reactive secondary pollutant formed when primary pollutants, like hydrocarbons and nitrogen oxides, react with sunlight. It irritates people's lungs and is a major contributor to photochemical smog. This pollutant greatly affects people with asthma or other lung alignments, senior citizens, and infants. The acceptable limit of ozone is 100 micrograms/ cm^3 (8 hrs average).

Air Pollution by Nitrogen Oxides
Furthermore, nitrogen oxides, sulfur dioxide, Carbon monoxide and hydrocarbons are also considered air pollutants that can harm human health. Inhaling higher levels of carbon monoxide is very dangerous and may cause severe health issues. Breathing air with a high level of carbon monoxide lowers the amount of oxygen that can be transported in the bloodstream to critical organs like the brain and heart. Burning fossil fuels (like gasoline) and vehicle exhaust are responsible for a high level of CO and nitrogen oxides both indoor and outdoor. Diesel-operated machinery and vehicles are liable for the rise of nitrogen dioxide into the atmosphere. Power plants release the majority of sulfur dioxide and nitrogen oxides when they burn fossil fuels, such as coal to generate electricity. These pollutants can also cause acid rains when combined with water vapour. The standard limits of nitrogen oxide, sulfur dioxide, and CO are 50 micrograms/ cm^3(24 hrs average) 40 micrograms/ cm^3(24 hrs average)and 02 micrograms/ cm^3(8 hrs average) respectively.

In brief, exposure to these human-caused and natural pollutants leads to many chronic diseases such as asthma, bronchiolitis, lung cancer, cardiovascular events, pulmonary disease, and many other incurable illnesses [7]. Last but not the least, the changes in the climate that result from environmental pollution affect the geographical distribution

of many infectious diseases. AQI is the numerical measurement of air pollution that easily helps everyone to understand the quality of air that they breathe. It is a tool to caution people about the quality of air to take necessary precautions to protect their health and also help authorities to take necessary steps to keep it in check.

The long exposure of an individual to a higher concentration of certain air pollutants is highly dangerous than the short exposure. Hence daily averages of AQI are considered for the study than one-hour or eight-hour average data. Moreover, the accuracy of a 24-hour interval average is higher when compared to other averages which helps in the better assessment of AQI. Daily data from Kaggle is used for this purpose.

Brief Literature survey on Air Pollution in Hyderabad

The overall contribution of vehicular pollution in Hyderabad is fifty percent to Particulate Matter (PM) in the form of direct emissions and thirty percent in the form of indirect emissions along with O_3 and CO [13]. In Hyderabad, two-wheelers are the major contributors to air pollution in the city. Most of the auto-rickshaws run on adulterated fuel, which is also responsible for air pollution. Apart from this, lack of awareness among the drivers, controlling mechanism, public awareness on air pollution, idling of vehicles at traffic intersections, etc. are also the reasons for rising pollution load[1].

The rapid increase of urbanization and industrialization led to deteriorating air quality in Hyderabad resulting in thousands of premature deaths. Hyderabad is now listed in the top ten cities with the worst air quality in India. Levels of PM, NOx and CO are of prime concern. Whereas a growing number of vehicles and increasing vehicle exhaust emissions are contributing to the ground level ozone exceeding the prescribed norms [4].The study conducted by [11] on the Diwali festival air pollution load in Hyderabad location has shown that the PM10 and PM2.5 concentrations levels are high in post-Diwali days; the pollutants are distributed and transformed in other regions. Investigations of [12] showed a significant variation of AQI concerning the station as well as seasons. Industrial activity, transportation and other allied activities are the major air pollutant contributors that determine the ambient air quality in that particular area. Analysis of the odd-even vehicular transportation schemes implemented in Delhi during the year 2016was done by [8].

It was concluded that the effectiveness of the scheme entirely depends on meteorological conditions like low temperatures and low wind speeds. They recommended the implementation of the scheme periodically throughout the year to control air pollution and also public health. Review of the air pollution level in the megacities of India was reported that the concentration of air pollutants is well above the permissible limits [6]. They emphasized the necessity of continuous monitoring of air pollution as it is hazardous to public health. This helps in designing effective strategies pertaining to various cities targeting vulnerable regions.[10] suggested that control of indoor air pollution in the localities of low-income groups will drastically reduce premature deaths due to pollution in India. A recommendation was made for the supply of clean cooking fuels as the most effective way to reduce indoor pollution.

METHODOLOGY

Multilinear regression analysis is the fundamental tool that helps to relate the quantity and type of the pollutant released into the atmosphere with an activity associated with the release of that pollutant. It also facilitates estimating the emissions from various sources of air pollution and to establish the contribution of each pollutant to the air quality index. It is used for assessing the strength of the relationship between each independent variable and a single dependent variable. The multilinear regression model for a dependent variable y with observed values $y_1, y_2, \ldots \ldots y_n$ (where n is the sample size) and q independent variables $x_1, x_2, \ldots \ldots x_q$ with observed values $x_{1i}, x_{2i}, \ldots \ldots x_{qi}$ for $i = 1, 2, \ldots \ldots n$ is

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots \ldots + \beta_q x_{qi} + \varepsilon_i \qquad (1)$$

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The term ε_i is the residual or error for individual i and represents the deviation of the observed value of the dependent variable for this individual from that expected by the model. These error terms are assumed to have a normal distribution with variance σ^2. The regression coefficients $\beta_0, \beta_1, \ldots\ldots \beta_q$ are generally estimated by least squares [2]. A measure of the fit of the model is provided by the multiple correlation coefficient R, defined as the correlation between the observed values of the dependent variable and the values predicted by the model

$$\hat{y}_i = \hat{\beta}_0 + \hat{\beta}_1 x_{i1} + \hat{\beta}_2 x_{i2} + \cdots\ldots\ldots + \hat{\beta}_q x_{iq} \qquad (2)$$

The value of R^2 gives the proportion of the variability of the dependent variable accounted for by the independent variables. Individual regression coefficients can be assessed using the $\hat{\beta}_j \big/ SE(\hat{\beta}_j)$ [9].

Data details

Daily data of seven major air pollutant components (PM 10, PM 2.5, CO, O_3, NO_x, NH_3, SO_2) was downloaded from the Kaggle website for the period of 30 months from January 2018 to June 2020.Significant outliers were removed from the data before the analysis. The pre-processed data of the afore-said five localities were correlated using Karl Pearson's Coefficient of correlation and the results are reported in Table-1a, b, c, d and e respectively. From the correlation matrix, it is observed that most of the variables are moderate to highly correlated, they have been highlighted in bold.

RESULTS AND DISCUSSIONS

As the correlation coefficient of air pollutants does not exceed 0.9 except for PM 2.5 and PM 10the multilinear regression modal was fitted to the data to identify the contribution of individual air pollutants, with AQI as the dependent variable and other pollutants as independent variables. Durbin-Watson test confirms there is no linear autocorrelation in the data hence fit for Multiple Regression analysis. From the values of R-square [Table 2], it can be predicted that PM 2.5, PM 10, NO_x, NH_3, CO, SO_2, and O_3 contribute to 88.6% of the variability of AQI at Bollaram, 90.3% at HCU, 92.3% at ICRISAT, 85.3% at Pashmylaram and 84.2% at Zoo Park. The *F*-ratio in the **ANOVA** tests established the overall regression model is a good fit for the data with p<0.0001.

The standardized beta values[Table 3] tell us the number of standard deviations that the AQI will change as a result of one standard deviation change in the pollutant. PM 10 is the major significant contributor in all the localities followed by PM 2.5. However, at HCU O_3 is the second major contributor. The destruction of atmospheric O_3 is done by the action of solar radiation (SR) on NO_x to break it down to Nitrogen monoxide (NO) and atomic oxygen (O), This atomic oxygen synthesizes with molecular oxygen(O_2) to form tropospheric ozone (O_3), which in turn reacts with NO to form new NO_2 and O_2, maintaining the concentrations of reagents and products in equilibrium (Yurdakul et al., 2013). Error bars corresponding to O_3in Figure-1 are in agreement with the results of the aforesaid paper.

The standardized regression coefficients eliminate this problem by expressing the coefficients in terms of a single, common set of statistically reasonable units so that comparison may at least be attempted. The absolute values of the standardized regression coefficients are compared in Figure-2 in line graphs, giving a rough indication of the relative importance of the variables. The Un-standardized tells us about the relationship between AQI and each component. They tell us to what degree each component affects the outcome of the effects of all other components are held constant.

AQI (at Bollaram) = 0.623(PM 2.5) + 0.554 (PM 10) -0.348 (NO_x) + 0.097 (NH_3) + **4.728 (CO)** -0.116 (SO_2) + 0.039 (O_3)

AQI (at HCU) = 0.440 (PM 2.5) + 0.429 (PM 10) -0.028 (NO_x) + 0.316 (NH_3) + **8.563 (CO)** -0.383 (SO_2) + 0.527 (O_3)

AQI (at ICRISAT) = 0.518(PM 2.5) + 0.544 (PM 10) +0.107 (NOx) + 0.527 (NH$_3$) – **5.936 (CO)** -0.024 (SO$_2$) + 0.474 (O$_3$)

AQI (at Pashmylaram) = 0.483(PM 2.5) + 0.446 (PM 10) + 0.103 (NOx) + 0.128 (NH$_3$) + **12.983** (CO) + 0.396 (SO$_2$) + 0.114 (O$_3$)

AQI (at Zoo Park) = 0.598 (PM 2.5) + 0.394 (PM 10) +0.017 (NOx) + 0.170 (NH$_3$) + **14.816** (CO) -2.195 (SO$_2$) + 0.444 (O$_3$)

The Un-standardized Coefficients emphasize the highest contribution of CO in all the areas when all the other pollutant effects are suppressed. Pashmylaram is a hub of chemical and pharmaceutical industries air pollutants generated by pharmaceutical industries predominantly consisting of SO$_2$ [3], positive relation of AQI with SO$_2$ confirms it.

CONCLUSIONS

Multilinear Regression is successful in demarcating the contributions of various air pollutants to AQI. However, the contribution levels were found to be differing locality-wise. The overall contribution of pollutants to the total variability was found to be highest in ICRISAT and lowest at Zoo Park. PM 10 was the major contributor in all the localities followed by PM 2.5 except at HCU where O$_3$ was the second major contributor. Un-standardized coefficients, a measure of the relation between AQI with each pollutant diminishing other pollutant effect states that the impact of CO is large on AQI when compared to other pollutants.

ACKNOWLEDGEMENTS

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Table 1a. Correlation matrix of air pollutants at Bollaram

	PM 2.5	PM 10	NOx	NH$_3$	CO	SO$_2$	O$_3$
PM 2.5	1.000	**.843**	.339	-.114	**.616**	-.110	.410
PM 10		1.000	.473	.033	**.590**	.043	.432
NOx			1.000	.228	.415	.362	.361
NH$_3$				1.000	-.024	.414	-.063
CO					1.000	-.048	.399
SO$_2$						1.000	.162
O$_3$							1.000

Table 1b. Correlation matrix of air pollutants at HCU

	PM 2.5	PM 10	NOx	NH$_3$	CO	SO$_2$	O$_3$
PM 2.5	1	**.902**[**]	**.688**[**]	**.550**[**]	.498[**]	**.513**[**]	.494[**]
PM 10		1	**.709**[**]	**.521**[**]	**.503**[**]	**.600**[**]	**.581**[**]
NOx			1	**.579**[**]	**.522**[**]	**.553**[**]	.344[**]
NH$_3$				1	.193[**]	.258[**]	.281[**]
CO					1	.301[**]	**.506**[**]
SO$_2$						1	.402[**]
O$_3$							1

Table 1c. Correlation matrix of air pollutants at ICRISAT

	PM 2.5	PM 10	NOx	NH$_3$	CO	SO$_2$	O$_3$
PM 2.5	1	**.907**[**]	**.748**[**]	.316[**]	**.780**[**]	.313[**]	.158[**]
PM 10		1	**.769**[**]	.320[**]	**.774**[**]	.410[**]	.252[**]
NOx			1	.251[**]	**.816**[**]	.290[**]	.065
NH$_3$				1	.317[**]	.159[**]	.160[**]
CO					1	.213[**]	.195[**]
SO$_2$						1	.260[**]
O$_3$							1

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Table 1d. Correlation matrix of air pollutants at Pashmylaram

	PM 2.5	PM 10	NOx	NH$_3$	CO	SO$_2$	O$_3$
PM 2.5	1	.923**	.379**	.110**	.361**	.513**	.405**
PM 10		1	.405**	.149**	.333**	.541**	.343**
NOx			1	.141**	.162**	.330**	.114**
NH$_3$				1	.247**	.091**	.002
CO					1	.163**	.374**
SO$_2$						1	.235**
O$_3$							1

Table 1e. Correlation matrix of air pollutants at Zoo Park

	PM 2.5	PM 10	NOx	NH$_3$	CO	SO$_2$	O$_3$
PM 2.5	1	.930**	.715**	.394**	.695**	.309**	.212**
PM 10		1	.748**	.432**	.723**	.472**	.280**
NOx			1	.486**	.700**	.439**	.172**
NH$_3$				1	.538**	.516**	.298**
CO					1	.574**	.195**
SO$_2$						1	.244**
O$_3$							1

Table 2. Model Summary

	Bollaram	HCU	ICRISAT	Pashmylaram	Zoo Park
R	.942[a]	.950[a]	.961[a]	.936[a]	.918[a]
R Square	.886	.903	.923	.853	.842
Adjusted R Square	.886	.902	.923	.876	.840
a. Independent Variables: PM 2.5, PM 10, NOx, NH$_3$, CO, SO$_2$, O$_3$					
b. Dependent Variable: AQI					

Table 3.Standardized Coefficients

	Bollaram		HCU		ICRISAT		Pashmylaram		Zoo Park	
	β	Sig.	β	Sig.	β	Sig.	β	Sig.	β	Sig.
PM 2.5	.362	.000	.232	.000	.278	.000	.285	.000	.346	.000
PM 10	.614	.000	.465	.000	.617	.000	.538	.000	.468	.000
NOx	-.059	.000	-.008	.680	.018	.328	.049	.001	.007	.746
NH$_3$	.028	.028	.063	.000	.049	.000	.037	.006	.031	.083
CO	.029	.053	.072	.000	-.026	.170	.101	.000	.141	.000
SO$_2$	-.021	.123	-.027	.065	-.004	.728	.038	.017	-.110	.000
O$_3$	.019	.159	.305	.000	.178	.000	.042	.005	.123	.000

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Figure 1. Error Bars of PM 2.5 & PM 10, NOx, NH₃, SO₂, CO, O₃, at Bollaram, HCU, ICRISAT, Pashmylaram and Zoo Park

Figure 2. Line Plots of PM 2.5 & PM 10, NOx, NH₃, SO₂, CO, O₃, at Bollaram, HCU, ICRISAT, Pashmylaram and Zoo Park

RESEARCH ARTICLE
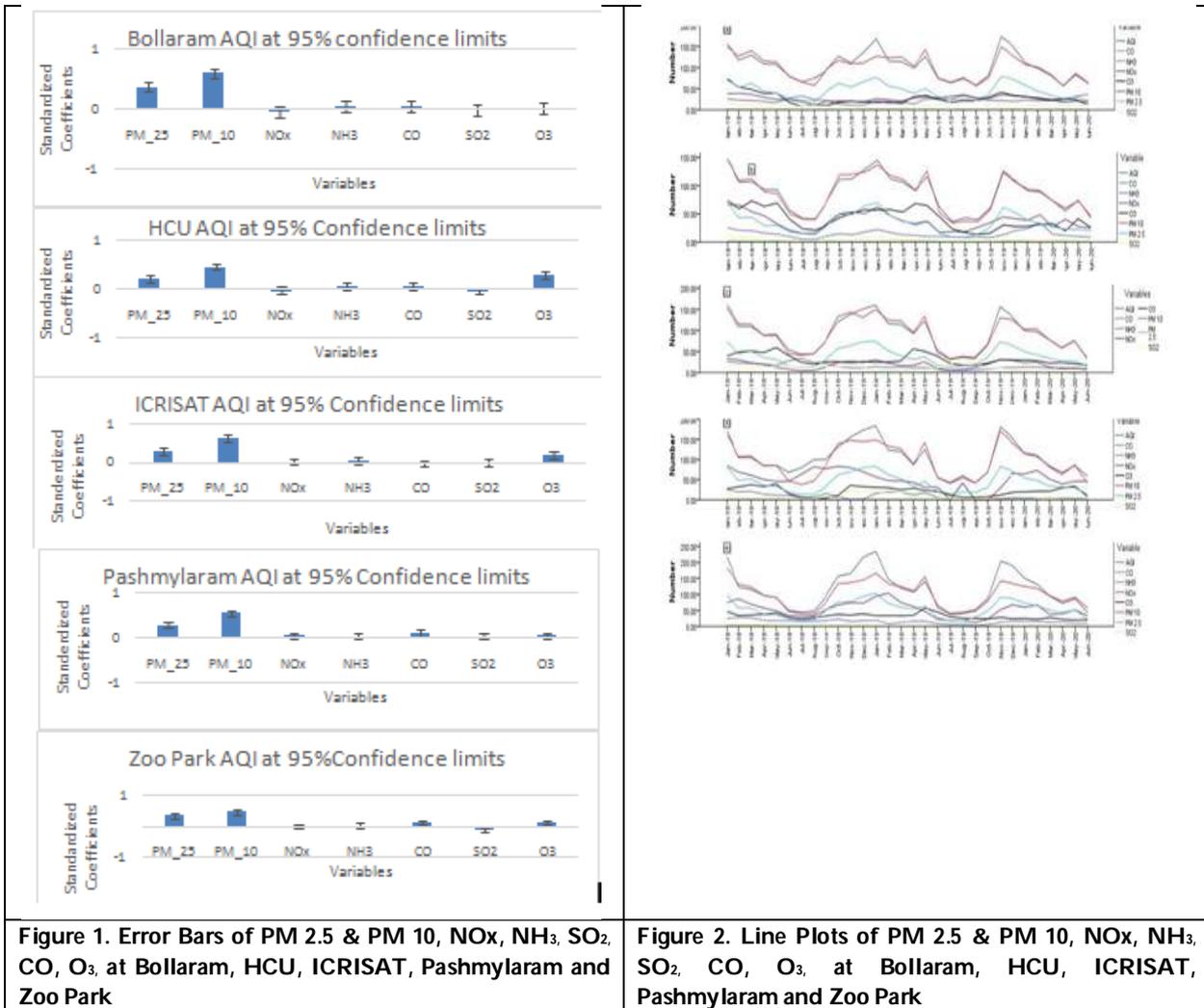
A Investigation on the Waste Water Treatment for the Green Belt Application on Vegetation Lands

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ABSTRACT

In the ancient times reuse of waste water for domestic and agricultural purposes has been occurring. Municipal sewage problems are more complex as the volume of the wastewater is considerable and it requires significant amount for treatment i.e., Sewage Treatment Plant. The objective of this work to determine whether domestic wastewater treated by various wetlands can be successfully recycled to irrigate commercial crops in order to reduce or minimize the sludge level. The physicochemical parameters of the complicated wastewater were determined. Microbial species were to be isolated from a variety of potential sources, and their degrading capacity was to be assessed both individually and in groups. As a result, laboratory tests were conducted in a biological treatment system with aerobic microbial consortia and individual microbial species in separate reactors. The study was carried out to assess individual microbial genera' and consortia's abilities, with the ultimate goal of creating safer

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environmental standard. The research study's main goal, however, is to confirm the importance of microbial flora in municipal sewage treatment.

Keywords: Microbial consortia, Wastewater, Microbial consortium, STP, Plant Crops

INTRODUCTION

Wastewater reuse in agriculture is growing rapidly around the world as a cost-effective and environmentally sustainable way to deal with rising freshwater contamination and demand, especially in agriculture. Agricultural irrigation ranks first in terms of water use, accounting for about 70% of worldwide freshwater supply and projected to extract 15% more by 2030 exacerbating global water scarcity issues, particularly in arid and semi-arid countries. In another perspective, wastewater contains essential minerals and organic compounds that are good to plants, making it a viable input resource for improved agricultural productivity at lower prices (Egbuikwem *et al.,*2020).People have been migrating from rural and semi-urban areas to cities and towns in large numbers throughout the years. The percentage of the population living in cities has risen from 27.8% in 2001 to 34.7 % in 2020. Many Indian towns lack infrastructural services such as water supply, sewerage, storm water drainage, and solid waste management as a result of unregulated urbanization. The primary goal of wastewater treatment is to reduce the impact of pollution on water supplies while also safeguarding public health by protecting water sources from transmission disease. This is carried out through a variety of treatment systems, which could be onsite treatment systems or offsite treatment systems. This section is therefore aimed at describing the offsite (activated sludge, trickling filters, stabilization ponds, constructed wetlands, membrane bioreactors) wastewater treatment systems (USEPA, 2005).

The activated sludge method involves a high concentration of microorganisms, primarily bacteria, protozoa, and fungi, that are present as a loose clumped mass of tiny particles retained in suspension by stirring, with the goal of eliminating organic materials from wastewater (Templeton *et al.,* 2011). A typical activated process consists of an aeration tank, a means of transferring oxygen to the microorganisms presents in the aeration tank, a means of stirring the mixture of fluid dispersed in the aeration tank, a means of separating the microorganisms from the treated water and a system of recycling some of the microorganisms back to the reactor (Qasim *et al.,* 1998).

During the early stage of treatment, the large floating materials in wastewater are first screened out, before the sewage is allowed to pass through the settling chamber, which helps in the removal of sand and other materials, while the floating debris is shredded and ground. In the aeration tank of the system, air is passed through the effluent for primary treatment. It is indicated that an activated sludge is an efficient system that has the ability to remove 75-90% of the biological oxygen demand (BOD) from sewage (Schmidt *et al.,* 2002 and Tortora *et al.,* 2010). Despite the presence of other microorganisms, the bacteria are typically considered to be the significant organisms consuming the organic matter in wastewater. Algae, because of their need for light rarely exist in mixed liquor (Gray, 2002). In a trickling filter system, the microorganisms are attached to the media in the bed and form a biofilm over it. As the wastewater passes through the media, the microorganisms consume and remove contaminants from the wastewater (Tchobanoglous *et al.,* 2003). Recirculation is the process by which filter effluent is returned and reapplied on the filter. This recycling of the wastewater increases the application of the waste with microorganisms thereby making the effluent to undergo proper treatment (Van Haandel *et al.,* 2007).

Membrane bioreactors are used to treat biologically active wastewater feeds from municipal or industrial sources. In the submerged, the membranes are immersed in and integral to the biological reactor while in the external side stream, the membranes are a separate unit process that requires intermediate pumping steps (Singhnia *et al.,* 2012 and Lofrano *et al.,* 2013). The membrane bioreactor system are made up of polymers or inorganic substances. They are made of several small pores, which can only be seen with the help of a microscope. Because of their small pore size, only very tiny particles and water is allowed to pass through the membrane (Tom *et al.,* 2020). The hollow fiber

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and flat sheet membranes are usually dipped in water while the tubular membrane is usually placed outside the bioreactor (Cornel *et al.,* 2008). On the other hand, sludging is caused by the accumulation of thick soft wet mud of industrial wastes. This is avoided by ensuring a sufficient flow of water in the medium (Radjenovic *et al.,* 2008). A wastewater stabilization pond is one of the most important natural methods for wastewater treatment. It is usually a shallow man-made pond that consists of single or several series of anaerobic, facultative or maturation ponds (Mbwele*et al.,* 2006). In this system, the treatment of the wastewater starts from the anaerobic pond, which is designed for the removal of suspended solids and some other organic matter present. In the second stage, also known as the facultative pond, the remaining organic matter is removed through the activity of algae and the heterotrophic bacteria e.g., *Arthrobacter* sp*., Rhodococcus* sp., *Pseudomonas* sp. (Diep *et al.,* 2013). During the last stage, treatment is achieved through the maturation pond, whose main function is the removal of pathogens and nutrients. The system is said to be a cost-effective wastewater treatment system for the removal of pathogenic microorganisms.

The treatment of the wastewater is gained through the use of natural disinfection mechanisms. Stabilization ponds are suitable for tropical and subtropical countries because of the intensity of sunlight and the temperature which aids the effectiveness of the removal processes (Pena *et al.,* 2002). In the subsurface flow, the system is designed to create subsurface flow through a permeable medium, keeping the water that is treated below the surface. This helps in avoiding the development of odors and other problems (Reed *et al.,* 1995). The media, which is typically soil, sand, gravel or crushed rock usually, affects the hydraulics of the system. The two types of wetlands treatment systems are constructed in basins or channels with a natural or constructed subsurface barrier to limit leaching (Brix *et al.,* 1994).

MATERIALS AND METHODS

Sample Collection
Sample were collected from different sites of three different countries, namely the samples were collected from the sewages of colleges, apartments and sewage treatment plants. The Sample-1 was collected from SS Engineering College, Chennai, India, the latitude and longitude as follows (N 13° 5' 29.3964", E 80° 6' 18.6048"). Sample-2 was collected from Golf court and Residential Apartment located in Kaula Lumpur, Malaysia, the latitude and longitude as follows (3.0428° N, 101.8014° E) and The Sample-3 was collected from Jakarta, Indonesia, the latitude and longitude as follows (6.2088° S, 106.8456° E). Each sample were collected and specified based on their KLD levels such as the Sample-1 was 75KLD, Sample-2 was 250KLD and Sample-3 was 550 KLD. All the samples were taken for the further studies such as Physical and Chemical parameter analysis and other Characterization processes.

Physical and Chemical Parameter of Sewage
Sewage slime delivered is either applied to land or utilized as compost for yields or discarded in landfills, causing a few ecological issues. Late investigations uncovered that treating the soil is a demonstrated innovation in lessening natural substance, weighty metals, and unsafe microbes, improving the dietary benefit of sewage ooze, which is helpful for crops (Jain *et al* 2019). In any case, concentrates on variety in actual properties are uncommon. Fertilizing the soil physical science or actual properties during fertilizing the soil assumes a fundamental part. The experiments for the physical and chemical parameter analysis of the samples were under analyzed in NABL accredited laboratory. Various parameters such as pH, Temperature, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Oil & Grease, Ammonical Nitrogen, Total Kjeladahl Nitrogen, Free Ammonia NH_3,Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)were studied.

Development of Microbial Consortium
The most common objective of developing microbial consortium is to capitalize both the capabilities of individual microbes and their interactions to create useful systems in tune with enhanced productivity and soil health improvements through efficient metabolic functionality (Brenner *et al.,* 2008). Two major underlying principles are applied in the whole process of development of microbial consortium. The first one is resource ratio theory, which uses both qualitatively and quantitatively to assess the outcomes between component microorganisms competing for

shared limiting resources. This permits coexistence of multiple microbes or the competitive exclusion of all but a single microbe (Brauer*et al.,* 2012).

Characterization of Sewage Water Treatment:
Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)
ICP-MS is a kind of mass spectrometry that utilizes inductively coupled plasma to ionize the example. It atomizes the example and makes nuclear and little polyatomic particles, which are then recognized. It is known and utilized for its capacity to distinguish metals and a few non-metals in fluid examples at extremely low focuses. It can identify various isotopes of a similar component, which makes it a flexible instrument in isotopic naming. The ICP-MS procedure has a multi-component character and a high example throughput, as ICP-OES, yet it permits one to perform more delicate estimations. Impediments and shortcomings of the ICP-MS location are the event of ghostly and non-phantom obstructions and the significant expenses.

Liquid Chromatography - Mass Spectrometry(LC-MS)
The LC-MS innovation includes utilization of a HPLC, wherein the individual parts in a blend are first isolated followed by ionization and partition of the particles based on their mass/charge proportion. The isolated particles are then coordinated to a photograph or electron multiplier tube indicator, which recognizes and measures every particle. The particle source is a significant segment in any MS investigation, as this fundamentally helps in proficient age of particles for examination. To ionize unblemished atoms, the particle source could be APCI (Atmospheric Pressure Chemical Ionization), ESI (Electrospray Ionization), and so on to give some examples famous ones. The decision of particle source additionally relies upon the substance idea of the analyte of interest for example polar or non-polar. The significant benefits of this innovation incorporate affectability, explicitness and exactness as investigation is done at atomic level. Likewise, underlying subtleties of the analyte can be unraveled.

Gas Chromatography - Mass Spectrometry (GC-MS)
Gas Chromatography/Mass Spectrometry (GC-MS) is essentially used to guarantee item quality and shopper security by dissecting for explicit mixtures, making it a well-known answer for some producers. Joining parts of the two gas-fluid chromatography and mass spectrometry, GC-MS examination is a materials-investigation strategy used to play out a particular test to emphatically recognize a particular substance in an example. Therefore, it is likewise viewed as the "highest quality level" for scientific substance distinguishing proof. Gas Chromatography Mass Spectrometry (GC-MS) is principally used to guarantee item quality and customer wellbeing by breaking down for explicit mixtures, making it a mainstream answer for some makers. Joining parts of the two gas-fluid chromatography and mass spectrometry, GC-MS investigation is a materials-examination method used to play out a particular test to decidedly distinguish a particular substance in an example. Thus, it is likewise viewed as the "best quality level" for criminological substance recognizable proof. Infuse test into Gas Chromatograph (GC), the example is infused into a port which is warmed to up to 300° C where the material is then volatilized) Separation of vaporous segments as they course through the section – The segment is twisted inside a unique broiler which controls temperatures from -20° to 320°. The data is shipped off a PC and a mass range, a circulation of particles of various sizes, is created the mass range is utilized to distinguish the segments by contrasting each with reference libraries of more than 275,000 special spectra. To evaluate compounds inside the broke down example, examiners build up a standard bend of known centralizations of every material.

CONCLUSION

Instrumentation analysis gives a detailed comparison i.e.) there is no high value of Heavy Metals, Pesticides or Volatile Organic Compounds present in the Sewage water. It helps to add a desired microbes i.e.) Bacteria, Cyan bacteria or Mixed Culture. It shows this water won't destroy the microorganisms or any plant kingdoms. The effective microbial consortia can be applied in STP's for onsite treatment of sewage wastewater and to prevent pollution in the environment. In terms of sustainability, bioremediation has priority, because it leads to a real reduction of pollutants in wastewater. Crucial modification in the STP process gives a highly effective result to

reduce the sludge biologically as by the natural process without adding any consumables or chemicals on it. Concept of reducing the activated sludge from STP's successfully done by our new process and also with the help of the microorganisms as taken as a trial by a gated community of STP's.

ACKNOWLEDGEMENT

DECLARATION OF COMPETING INTEREST
There is no conflict of interest for this work.

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Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)
Tested with Important numbers of 17 common wastewater Heavy Metals

Table 1: Instrumentation of Wastewater ICP-MS - INLET

Sl. No.	PARAMETERS	UNITS	SAMPLE- I	SAMPLE - II	SAMPLE - III
1	Leas as Pb	mg/l	BLQ (LOQ 0.1)	BLQ (LOQ 0.1)	BLQ (LOQ 0.1)
2	Chromium (Hexavalent) as Cr6+	mg/l	BDL (DL 0.1)	BDL (DL 0.1)	BDL (DL 0.1)
3	Total Chromium as Cr	mg/l	BDL (DL 0.1)	BDL (DL 0.01)	BDL (DL 0.1)
4	Zinc as Zn	mg/l	BDL (DL 0.1)	BLQ (LOQ 0.05)	0.027
5	Nickel as Ni	mg/l	BLQ (LOQ 0.1)	BLQ (LOQ 0.1)	BLQ (LOQ 0.1)
6	Boron as B	mg/l	BLQ (LOQ 0.1)	BDL (DL 0.1)	BLQ (LOQ 0.1)
7	Chloride as Cl-	mg/l	BDL (DL 0.1)	395.9	148.46
8	Fluoride as F-	mg/l	BDL (DL 0.2)	BDL (DL 0.2)	BDL (DL 0.2)
9	Sulphate as SO4	mg/l	BDL (DL 0.2)	200.3	12.1
10	Sulphide as S2-	mg/l	BDL (DL 0.04)	16	BDL (DL 0.04)
11	Particle Size of Suspended Solids	-	Passed through 850 micron	Passed through 850 micron	Passed through 850 micron
12	Arsenic as As	mg/l	BLQ (LOQ 0.05)	BLQ (LOQ 0.05)	BLQ (LOQ 0.05)
13	Mercury as Hg	mg/l	BLQ (LOQ 0.01)	BLQ (LOQ 0.01)	BLQ (LOQ 0.01)
14	Cadmium as Cd	mg/l	BLQ (LOQ 0.01)	BLQ (LOQ 0.01)	BLQ (LOQ 0.01)
15	Selenium as Se	mg/l	BLQ (LOQ 0.005)	BLQ (LOQ 0.005)	BLQ (LOQ 0.005)
16	Residual Sodium Carbonate as Na2CO3	meq/l	BDL (DL 1)	BDL (DL 1)	BDL (DL 1)
17	Cyanide as CN	mg/l	BDL (DL 0.01)	BDL (DL 0.01)	BDL (DL 0.01)

Table – 2: Instrumentation of Wastewater ICP-MS – OUTLET

SI. No.	PARAMETERS	UNITS	SAMPLE - I	SAMPLE - II	SAMPLE – III
1	Leas as Pb	mg/l	BLQ (LOQ 0.1)	BLQ (LOQ 0.1)	BLQ (LOQ 0.1)
2	Chromium (Hexavalent) as Cr6+	mg/l	BDL (DL 0.1)	BDL (DL 0.1)	BDL (DL 0.1)
3	Total Chromium as Cr	mg/l	BDL (DL 0.1)	BDL (DL 0.01)	BDL (DL 0.1)
4	Zinc as Zn	mg/l	BDL (DL 0.1)	BLQ (LOQ 0.05)	BLQ (LOQ 0.1)
5	Nickel as Ni	mg/l	BLQ (LOQ 0.1)	BLQ (LOQ 0.1)	BLQ (LOQ 0.1)
6	Boron as B	mg/l	BLQ (LOQ 0.1)	BDL (DL 0.1)	BLQ (LOQ 0.1)
7	Chloride as Cl-	mg/l	BDL (DL 0.1)	148.46	39.59
8	Fluoride as F-	mg/l	BDL (DL 0.2)	BDL (DL 0.2)	BDL (DL 0.2)
9	Sulphate as SO4	mg/l	BDL (DL 0.2)	163.8	12.1
10	Sulphide as S2-	mg/l	BDL (DL 0.04)	BDL (DL 0.04)	BDL (DL 0.04)
11	Particle Size of Suspended Solids	-	Passed through 850 micron	Passed through 850 micron	Passed through 850 micron
12	Arsenic as As	mg/l	BLQ (LOQ 0.05)	BLQ (LOQ 0.05)	BLQ (LOQ 0.05)
13	Mercury as Hg	mg/l	BLQ (LOQ 0.01)	BLQ (LOQ 0.01)	BLQ (LOQ 0.001)
14	Cadmium as Cd	mg/l	BLQ (LOQ 0.01)	BLQ (LOQ 0.01)	BLQ (LOQ 0.01)
15	Selenium as Se	mg/l	BLQ (LOQ 0.005)	BLQ (LOQ 0.005)	BLQ (LOQ 0.005)
16	Residual Sodium Carbonate as Na2CO3	meq/l	BDL (DL 1)	BDL (DL 1)	BDL (DL 1)
17	Cyanide as CN	mg/l	BDL (DL 0.01)	BDL (DL 0.01)	BDL (DL 0.01)

Table – 3: Instrumentation of Wastewater GC-MS – INTLET

SI. No.	PARAMETERS	UNITS	SAMPLE - I	SAMPLE - II	SAMPLE - III
1	2,4-D	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
2	Isoproturon	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
3	Malathion	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
4	Methyl Parathion	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
5	Monochrotophos	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
6	Phorate	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
7	o,p-DDT	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
8	o,p-DDD	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
9	p,p-DDD	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
10	o,p-DDE	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
11	p,p-DDE	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
12	Endosulphan alpha	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
13	Endosulphan Beta	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
14	Endosulphan Sulphate	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)

15	Alpha HCH	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
16	Beta HCH	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
17	Gamma HCH	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
18	Delta HCH	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
19	Butachlor	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
20	Chlorpyrifos	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
21	Alachlor	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
22	Atrazine	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
23	Aldein	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
24	Dieldrin	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
25	Ethion	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
26	Cypermethrin	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
27	Alphamethrin	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
28	Permethrin	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)

Table – 4: Instrumentation of Wastewater GC-MS – OUTLET

SI. No.	PARAMETERS	UNITS	SAMPLE - I	SAMPLE - II	SAMPLE - III
1	2,4-D	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
2	Isoproturon	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
3	Malathion	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
4	Methyl Parathion	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
5	Monochrotophos	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
6	Phorate	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
7	o,p-DDT	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
8	o,p-DDD	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
9	p,p-DDD	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
10	o,p-DDE	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
11	p,p-DDE	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
12	Endosulphan alpha	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
13	Endosulphan Beta	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
14	Endosulphan Sulphate	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
15	Alpha HCH	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
16	Beta HCH	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
17	Gamma HCH	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
18	Delta HCH	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
19	Butachlor	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
20	Chlorpyrifos	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
21	Alachlor	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
22	Atrazine	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
23	Aldein	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)

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24	Dieldrin	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
25	Ethion	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
26	Cypermethrin	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
27	Alphamethrin	mg/l	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)	BLQ (LOQ 0.00001)
28	Permethrin	mg/l	BLQ (LOQ0.00001)	BLQ (LOQ0.00001)	BLQ (LOQ 0.00001)

Table – 5: Instrumentation of Wastewater – VOC – INLET

SI. No.	PARAMETERS	UNITS	SAMPLE - I	SAMPLE - II	SAMPLE - III
1	Phenolic Compounds as C6H50H	mg/l	BLQ (LOQ 0.001)	BLQ (LOQ 0.001)	BLQ (LOQ 0.001)
2	Percent Sodium as Na	%	BDL (DL 0.1)	BDL (DL 0.1)	52.2
3	Residual Free Chlorine	mg/l	BDL (DL 0.1)	BDL (DL 0.1)	BDL (DL 0.1)
4	Alpha emitters #	microcurie/ml	BDL (DL 0.0000001)	BDL (DL 0.0000001)	BDL (DL 0.0000001)
5	Beta emitters #	microcurie/ml	BDL (DL 0.0000001)	BDL (DL 0.0000001)	BDL (DL 0.0000001)

Table – 6: Instrumentation of Wastewater – VOC – OUTLET

SI. No.	PARAMETERS	UNITS	SAMPLE - I	SAMPLE - II	SAMPLE – III
1	Phenolic Compounds as C6H50H	mg/l	BLQ (LOQ 0.001)	BLQ (LOQ 0.001)	BLQ (LOQ 0.001)
2	Percent Sodium as Na	%	BDL (DL 0.1)	BDL (DL 0.1)	21.5
3	Residual Free Chlorine	mg/l	BDL (DL 0.1)	BDL (DL 0.1)	BDL (DL 0.1)
4	Alpha emitters #	microcurie/ml	BDL (DL 0.0000001)	BDL (DL 0.0000001)	BDL (DL 0.0000001)
5	Beta emitters #	microcurie/ml	BDL (DL 0.0000001)	BDL (DL 0.0000001)	BDL (DL 0.0000001)

Fig. 1. Wastewater Study – Sewage Inlet & Outlet with Microbes growth in MBBR Media

RESEARCH ARTICLE

Green Products Purchases – A Transition towards Sustainable Development

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ABSTRACT

Our Earth is a pretty unique place in the Universe that has great biodiversity of Plants and Animals. It is a wonderful treasure gifted to the human beings with an extraordinary richness of life. Plants and animals live a life of harmony with nature. But, man is going on using the resources and end up with a lot of wastes. With the increase in the technologies, there has been an increase in the industrial activities which has affected the environment adversely and resulted in climate change, global warming, pollution, depletion of the ozone layer, etc. These issues have raised concerns to protect our environment which has led to the concept of going green that raise in demand for Green Products. Green Products are biodegradable, more durable, non-toxic, resource-intensive and safer for the environment, wildlife and people. Green Consumerism refers to recycling, purchasing and using eco-friendly products that minimise damage to the environment. This involves decisions such as using Electrical Appliances which consume less power and zero emissions, use of Solar and Wind Power to generate electricity and buying locally grown vegetables and fruits. Reducing, reusing and recycling waste helps save landfill space by keeping useful materials out. The researcher has observed that the people in Sivakasi has started moving from Conventional Products to the Green Products due to climate change. Hence, this research is carried out to know about the consumers' attitude towards the consumption of Green Products in Sivakasi for sustainable development.

Keywords: Environment, Climate Change, Green Products and Sustainable Development.

INTRODUCTION

Our Earth is a pretty unique place in the Universe that has great biodiversity of Plants and Animals. It is a wonderful treasure gifted to the human beings with an extraordinary richness of life. Plants and animals live a life of harmony with nature. But, man is going on using the resources and end up with a lot of wastes. With the increase in the technologies, there has been an increase in the industrial activities which has affected the environment adversely and resulted in climate change, global warming, pollution, depletion of the ozone layer, etc .These issues have raised concerns to protect our environment which has led to the concept of going green that raise in demand for Green Products. Green Products are biodegradable, more durable, non-toxic, resource-intensive and safer for the environment, wildlife and people.

MATERAILS AND METHODS

Statement of the Problem
The researcher has observed that the people in Sivakasi has started moving from Conventional Products to the Green Products due to climate change. Hence, this research is carried out to know about the consumers' attitude towards the consumption of Green Products in Sivakasi.

Review of Literature
Amit Kumar Bhardwaj et al. (2020) in their article ‚Research Trends in Green Product for Environment: A Bibliometric Perspective` stated that ‚consumer preferences, environmental activism and stringent regulations have forced sustainability-oriented firms to shift their focus to producing green products [1]″.

Silvia Cachero-Martinez (2020) in his article "Consumer Behaviour towards Organic Products: The Moderating Role of Environmental Concern" stated that "The pandemic caused by COVID-19 has changed the mind set of many consumers. The health crisis could trigger the consumption of organic foods, which are foods produced through environmentally friendly agricultural methods and that have not been artificially altered [2]″.

There is no exclusive study on the Consumers' Attitude towards consumption of Green Products regularly in their day-to-day life in Sivakasi for sustainable development. Hence, the present study has made an attempt to fill up the research gap.

Objectives of the Study
The objectives of the study are
1. To study the types of Green Products used by the Consumers in their day-to-day life for the benefit of the environment
2. To analyse the consumer attitude towards the level of satisfaction of Green Products.
3. To offer valuable suggestions on the basis of findings to strengthen the use of Green Products thereby safeguard the environment.

RESULTS AND DISCUSSION

Research Methodology
The present study is a descriptive study based on survey method. This study envisages Primary data and Secondary data. The primary data collected from 250 respondents in Sivakasi using Interview Schedule during January 2022 and analysed using SPSS. Simple Random Sampling Technique is used and to ensure randomness of selection of sampling, the Lottery method is adopted in this research.

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Generation of single-use plastic waste by each person in selected countries

Single-use plastics are most commonly used for packaging such as bottles, wrappers, straws and bags. The single-use plastics contributes to greenhouse gas emissions at every stage of its lifecycle from its production to its refining and the way it is managed as a waste product. Figure 1 shows that the Single-use plastic waste generated per person in selected countries in 2019 were as follows: Australia 59 kilograms per person (Highest Plastic Waste) and India 4 kilograms per person (Lowest Plastic Waste)[2].

Green Products in our Daily Life

The environmental benefits of green products have zero impact. The list of Eco Friendly and Sustainable Products are as follows:

Category of Products	Eco-Friendly and Sustainable Products
❖ Travel Products	❖ Electric Vehicles
❖ Clothing and Accessories	❖ Cloth or Cotton Shopping Bags, Cloth Napkins
❖ Kitchen Products	❖ Precision Induction Stove
❖ Water Bottles	❖ Stainless Steel Water Bottles
❖ Cleaning Products	❖ House Cleaners without using chemicals
❖ Makeup and Beauty	❖ Non-toxic Makeup Brands consists of vitamins and essential oils.
❖ Electronic Items	❖ Rechargeable Batteries, Solar Powered Electronic Devices, LED Bulbs
❖ Personal Care Products	❖ Biodegradable dental floss
❖ Technology Based Products	❖ Use E-Newspaper, E-Books, E-Journals and E-Mails to support Green Environment
❖ Paper	❖ Paper can also be made from agricultural wastes such as Sugarcane Husk, Paddy and Wheat Straw.
❖ Gardening Materials	❖ Biodegradable Flower Pots in the Garden

Demographic Profile of the Respondents

Table 1 shows that majority of the respondents were female belonging to the age group of 20 - 40 years. They got married and Under Graduates. The respondents were employed and earning up to Rs. 10,000 per month in this study.

Types of Green Products used regularly

Table 2 reveals that majority of the respondents often used energy efficient products like LED Bulbs / Tube Lights in their day-to-day life.

Association between Demographic Profile and Types of Green Products

H_{01}: There is no association between the Demographic Profile of the Respondents and Types of Green Products used by the Respondents.

Chi-Square Test reveals that there is an association between the Gender, Age, Marital Status, Education and Occupation and Types of Green Products used by the Respondents. Chi-Square Test reveals that there is no association between the Monthly Income and Types of Green Products used by the Respondents.

Consumers' Satisfaction towards Green Products

During the course of study, the respondents are asked to mark the level of satisfaction towards Green Products agreement with the **TWELVE** statements. For that, the five-point scale ranged from "Highly Satisfied" to "Highly Dissatisfied" is used.

Health Consciousness
1. I select green products a good choice
2. I always aim for safe and healthy activities

Ecological Consciousness
3. I am well aware of climate change and global warming
4. It is the responsibility of every citizen to protect the environment

Social Consciousness
5. I control my consumption behaviour against the environment
6. I regularly participate in community development activities
7. I know about the Green Crackers which safeguard the environment
8. I know about the launch campaign by the Tamil Nadu Government to revive 'yellow cloth bags'

Save Environment for future generations
9. Environmental degradation can be reduced by using green products
10. The use of green product increases life span of human being
11. Our green product consumptions will help future generations
12. Overall satisfaction towards Green Products

To assess the importance of the four dimensions, Multiple Regression is used to predict the Beneficiaries Perception Ratings towards Green Products and their Overall Satisfaction.

The Coefficient Table 6 shows that the two significant coefficients are Health Consciousness and Save Environment for Future Generations. It is concluded that the Save Environment for Future Generations dimension has the highest Standardized Coefficient 0.487 with the lowest significance value 0.000. This significance value is less than 0.05 which means that "Save Environment for Future Generations" dimension is the main predictor for Overall Satisfaction towards Green Products.

The Predicted Regression Equation is $Y = -.013 + .351 x_1 + 0.125 x_2 - 0.024 x_3 + .597 x_4$

Beneficiaries Perception Ratings towards Green Products
Sometimes the satisfaction level of beneficiaries towards the Green Products in various dimensions may not equal each other and leave a gap. Hence, the paired samples t test is used to analyse with the paired dimensions.

H_{02}: There is no significant difference between that the satisfactory level of all the pairs of Beneficiaries Perception dimensions.

The Table 7 measures the gap between the satisfaction levels of beneficiaries towards the various perception dimensions. The calculated t value for the six pairs Health Consciousness - Ecological Consciousness (-1.431), Health Consciousness - Social Consciousness (-7.692), Health Consciousness - Save Environment for Future Generations (.556), Ecological Consciousness - Social Consciousness (-7.203) and Ecological Consciousness - Save Environment for Future Generations (1.885) are less than the tabulated value 1.96. Hence, the null hypothesis is accepted. It is concluded that there is no significant difference between these pairs and satisfactory levels of beneficiaries' perception dimensions. It represents that these pairs of perception dimensions are equally satisfied among the beneficiaries.

The calculated t value for a pair Social Consciousness - Save Environment for Future Generations (8.282) is greater than the tabulated value 1.96. Hence, the null hypothesis is rejected. It is concluded that there is significant difference between the satisfactory levels of a pair of beneficiaries' perception dimensions. So, these pairs of beneficiaries' perception dimensions are not equally satisfied among the beneficiaries.

Beneficiaries Perception towards Green Products

The correlation between the beneficiaries' perception dimensions and the score on overall satisfaction towards Green Products has been examined in the Table 8. The Table 8 shows the area above the diagonal 1's is rejection area. The area below the diagonal 1's is taken into account. The significance value of six elements is less than 0.05. Hence, they are significantly correlated. The positive correlation exists between Ecological Consciousness and Health Consciousness dimension, since r = 0.676 with significance value 0.000. When the consumers consume green products for their Health Consciousness, then the Ecological balance will be automatically enhanced.

Suggestions to develop Eco-Friendly Products for a Sustainable Development

- ❖ Sip your tea and bite the crunchy and yummy Tea Cup of with various flavors like Elachi (Cardamon), Chocolate and Vennila and Strawberry can be used instead of Paper Tea Cups.
- ❖ The Water Sprinkler Device performs 360 degree rotating action, efficient and quick watering providing better coverage of yards and gardens and save our time and water.
- ❖ The Soil Moisture Metre Instruments are used to measure the percentage of water and it can be fit into the soil to determine water requirement for the crops.
- ❖ The solar-powered Buses, Bus Stands, Bus Stops, Railway Stations, Trains, Tolgates, and Street Lamps can be used to save energy.
- ❖ Use Plantable Stationery Seed Pens and Pencils.
- ❖ Many hospitals are overcrowded with concrete buildings, it is difficult for patients to receive artificial oxygen due to a lack of trees and plants. To avoid this, high oxygen emitting trees such as Peepal Tree, Banyan Tree, Neem Tree, Curry Tree, Wood Apple Kaith Tree and Khejri Tree can be planted around the hospitals.
- ❖ Involve students in green programs, such as student-maintained gardens, cafeteria composting and initiatives to reduce energy use and waste. Environmental projects are fun, and will raise awareness in parents, teachers and children alike.
- ❖ Cotton buds with Bamboo Stick does not pollute the environment while also keeping yourself or your items clean and hygienic. They are disposable, biodegradable, organic, and contain all-natural bamboo.
- ❖ The recyclable bamboo toothbrushes are stronger and cheaper than plastic toothbrushes.
- ❖ More plastic is used up in serving take away food. You can still protect the environment by using reusable utensils to carry your takeout.
- ❖ The toys came in plastic and colorful for sale and attracted more and more children. Now-a-days, the number of toys made of straw has increased. These toys are made of straw and bamboo sticks and decorated with velvet fabrics which are colorful and can be used by children to sit and play and decorate the house.
- ❖ In the Polythene Waste Recycling Centre, the collected polythene wastes can be used to produce Value Added Products. The collected polythene wastes can be given for constructing Road Works by Tar.
- ❖ Man can create a natural garden using household vegetable waste as natural compost and wasted water. Biodegradable waste can be dried and burned and used as natural compost. The water used daily at home can be set to go to the garden without letting it drain. In addition, the surrounding area raises the groundwater level.
- ❖ When the Peanuts Shells are mixed with the soil, the plants will grow well. The Peanut Shells give better ventilation for the roots of the plants and it prevents soil thickening.
- ❖ Awareness of saving energy has been promoted among citizens to increase the use of solar, wind, biomass, waste and hydropower energies. It is evident that clean energy is less harmful and often cheaper.
- ❖ The E-Waste Awareness Programme under Digital India has to be arranged for the Industry Associations to create awareness about disposing their e-waste. The programme stresses the need for adopting environment friendly e-waste recycling practices.
- ❖ When you are ready to buy a new computer, buy one with an Energy Star Label to save 35%-65% in energy use, and consider a laptop, which uses much less energy than a desktop computer. Donate your old computer rather than recycling it.
- ❖ The more you do online, the less you need paper. Think about if you can send emails instead of letters or if you need to print files or if you can save them on your computer.

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- ❖ All sorts of unwanted substances are turning into garbage and polluting the earth. The Garbage collected daily is segregated by household waste and industrial waste and despatch it to the respective Processing Centre for recycling. The vegetable wastes and fruits waste are grind and then it has to be set up for bio-degradable.

- ❖ The sanitary pads commonly used by women from all classes contain Super Absorbent Polymers (SAP), which don't decompose. They gradually break down into what are known as micro-plastics, which contaminate soil, water and air. They also enter the food chain injecting toxins into the food humans and animals consume.

- ❖ The Eco-friendly Sanitary Pads are more comfortable and safer for the user as they don't contain bleach which has carcinogenic dioxins, they are all-natural pads not containing any chemicals, thus reducing CO_2 emission of production.

- ❖ Medical waste is broadly classified as any item that comes into contact with body fluids. Specifically, it is any solid waste that is generated in the diagnosis, treatment or immunization of humans. This type of waste was once collected in special bags and plastic boxes in clinical settings and then disposed of like normal trash. However, this process was quickly found to spread diseases and viruses and potentially cause outbreaks.

- ❖ The accumulated wastes could mix with open-water resources, so leading to high levels of water pollution. The effects of mixing agricultural runoff containing wastes, pesticides and fertilizers in the rural water sources would also need consideration.

- ❖ Brewers grains are the solid residue left after the processing of germinated and dried cereal grains. Brewers grains are one of the oldest byproduct feeds for livestock. Brewer's grains are used in all segments of cattle production as a highly digestible protein and energy.

- ❖ Waste collection is the responsibility of the Municipal Corporations in India and different bins are provided for collecting wastes. The Garbage is gold, if it is collected on time. The old dairy cows stop giving milk. These old dairy cows can be given and maintained with the vegetable and fruit wastes collected from the houses and markets. These wastes can be used as raw materials and act as a resource. After eating the vegetable wastes by the cows, ¼ portion of its food comes as cow dung. The garbage collected from the houses and markets are converted into cow dung. The cow dung can be availed after 8 hours and the methane gas is available. The cow dung is put into the Bio-Digester (Bio-Gas Plant). The cow dung comes without methane gas (99%). Afterwards, the cow dung is put into the worm pit. The waste which come from worms are known as vermicomposting. Vermicomposting is the black gold. It is available in 72 hours. This is the fast process to get vermicomposting. No machine can do this.

- ❖ During Autumn Season, the leaves slowly drop from the trees. The compost can be made from the dried leaves and use it for plants. Instead of burning them, the dried leaves can be placed under the trees and the water will stagnate for a long time, creating moisture and vitality for the plant. Compost can help in enriching the soil, holding moisture, suppressing plant diseases and filtering harmful pests.

- ❖ The Green Skill Development Programme like Workshops, Hands-on-Training, Conferences, Seminars and Webinars can be organised for skill development relating to the environment which will enable India's youth to get gainful employment and/or self- employment. These programmers will help to promote environmental skills among the students in Schools and Colleges.

- ❖ The Environmental Science and Sustainability Courses including Pollution Monitoring (Air / Water / Soil / Noise), Effluent Treatment Plant Operation, Waste Management, Forest Management, Water Budgeting and Auditing, Conservation of River Dolphins, Wildlife Management, Marine Taxonomy and Coastal Biodiversity, Mangroves Conservation and Creation of Miyawaki Forest can be introduced for the students.

- ❖ The Green plantable seed ball pens are biodegradable and non-toxic. It is a complete usable ecofriendly stationery product. Plantable recycled paper seed pens have 90% less plastic than regular pens followed by Plant it - Nurture it - Watch it grow!

- ❖ Seed / Biodegradable / Non Toxic Pencils is a complete usable Eco-friendly stationery product not only inculcate gardening skills also inspires to write and inculcate green habits among children. These pencils contain edible colors and chemical free in the body of the pencil, so it is safe if your child to use. The capsule used in the pencil is also edible and biodegradable. Followed Plant it - Nurture it - Watch it grow.

CONCLUSION

The Government can declare "Clean Green Day" on every month and consider this day as "Smoke Free" and encourage the public to walk or use Bicycle on that day to move one place to another to save our Mother Earth and Planets from the Pollution. Environmentalism has fast emerged as a worldwide phenomenon. Marketing organisations too have risen to the occasion and have started responding to environmental challenges by practicing green marketing strategies. The awareness of the Green marketing is increased among the Indian consumers. Many of the manufacturers are following Green marketing practices in India.

When the individual is more aware about the environmental concerns, it could the create favorable behaviour towards the same. The knowledge about the environment is correlated with the attitude and behavior towards the environment. Proper knowledge about environmental problems can make individuals more responsible towards the environment. Consumers who are more aware and conscious about the environment make more green purchases. Otherwise, the awareness has to be created among the public through various channels like Government, Voluntary Organisations, Media and Educational Institutions to save the environment.

ACKNOWLEDGEMENT

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Table 1.Demographic Profile of the Respondents

Demographic Profile	Categories	Number of Respondents	Percentage
Gender	Male	120	48
	Female	130	52
	Total	**250**	**100**
Age Group	Below 20 years	21	8
	20 - 40 years	119	48
	41 - 60 years	98	39
	Above 60 years	12	5
	Total	**250**	**100**
Marital Status	Married	175	70
	Unmarried	75	30
	Total	**250**	**100**
Education	School Level / Diploma	18	7
	Under Graduate	141	57
	Post Graduate	76	30
	Doctorate	15	6

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	Total	250	100
	Total	250	100
Occupation	Student	43	17
	Employer	55	22
	Employee	60	24
	Home Maker	45	18
	Profession	35	14
	Entrepreneur	12	5
	Total	250	100
Monthly Income	Below Rs. 10,000	83	33
	Rs. 10,000 -Rs. 20,000	64	26
	Rs. 20,001 -Rs. 30,000	42	17
	Above Rs. 30,000	61	24
	Total	250	100

Source: Primary data

Table 2. Types of Green Products used by the Respondents Regularly

Types of Green Products used regularly	Number of Respondents	Percentage
Organic Vegetables	15	6
Natural Fertilizers	34	14
Vehicles with Zero Emissions	24	9
LED Bulbs / Tube Lights	**75**	**30**
Solar Panel / Solar Charger	13	5
Stainless-Steel Water Bottles	37	15
Jute Bags / Woolen Bags	40	16
Coir Products	12	5
Total	**250**	**100**

Source: Primary data

Table 3.Demographic Profile and Types of Green Products - Chi-Square Analysis

Demographic Profile	Chi-Square Value	d.f.	Significance Value	Results of Null Hypothesis
Gender	64.903	7	0.000 <0.05	Rejected
Age Group	44.977	21	0.002 <0.05	Rejected
Marital Status	21.150	7	0.004 < 0.05	Rejected
Education	32.870	21	0.048 < 0.05	Rejected
Occupation	192.116	35	0.000 < 0.05	Rejected
Monthly Income	26.090	21	0.203 > 0.05	Accepted

Source: Calculated Value

Table 4. Beneficiaries Perception Dimensions towards Green Products and Overall satisfaction

ANOVA

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Regression	64.890	4	16.223	73.830	.000
Residual	53.834	245	.220		
Total	118.724	249			

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Table 5. Model Summary of Regression

Dependent Variable	R	R Square	Adjusted R Square
Overall Satisfaction of Beneficiaries	.739	.547	.539

Table 6. Regression Coefficients

Dependent Variable	Independent Variables	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
Overall Satisfaction of the Respondents towards Green Products	(Constant)	-.013	.091		-.142	.887
	Health Consciousness	**.351**	**.085**	**.258**	**4.112**	**.000**
	Ecological Consciousness	.125	.087	.097	1.433	.153
	Social Awareness	-.024	.062	-.024	-.393	.694
	Save Environment for Future Generations	**.597**	**.077**	**.487**	**7.745**	**.000**
a. Dependent Variable: Overall Satisfaction						

Source: Calculated data

Table 7. Beneficiaries Perception Ratings towards Green Products Paired Samples t Test

Pairs	Paired Statements	Standard deviation	t value	Sig. t value
Pair 1	Health Consciousness - Ecological Consciousness	.41983	-1.431	.154
Pair 2	Health Consciousness - Social Consciousness	.56320	-7.692	.000
Pair 3	Health Consciousness - Save Environment for Future Generations	.45972	.556	.579
Pair 4	Ecological Consciousness - Social Consciousness	.51805	-7.203	.000
Pair 5	Ecological Consciousness - Save Environment for Future Generations	.45433	1.885	.061
Pair 6	Social Consciousness - Save Environment for Future Generations	.55396	8.282	.000

Source: Calculated data

Table 8.Bivariate Correlation

Beneficiaries Perception Dimensions	Particulars	Health Consciousness	Ecological Consciousness	Social Awareness	Save Environment for Future Generations
Health Consciousness	Pearson Correlation	1	.676**	.569**	.636**
	Sig. (2-tailed)	---	.000	.000	.000
	N	250	250	250	250
Ecological Consciousness	Pearson Correlation	.676**	1	.647**	.658**
	Sig. (2-tailed)	.000	---	.000	.000
	N	250	250	250	250
Social Awareness	Pearson Correlation	.569**	.647**	1	.605**
	Sig. (2-tailed)	.000	.000	---	.000
	N	250	250	250	250
Save Environment for Future Generations	Pearson Correlation	.636**	.658**	.605**	1
	Sig. (2-tailed)	.000	.000	.000	---
	N	250	250	250	250
**. Correlation is significant at the 0.01 level (2-tailed).					

Source: Primary data

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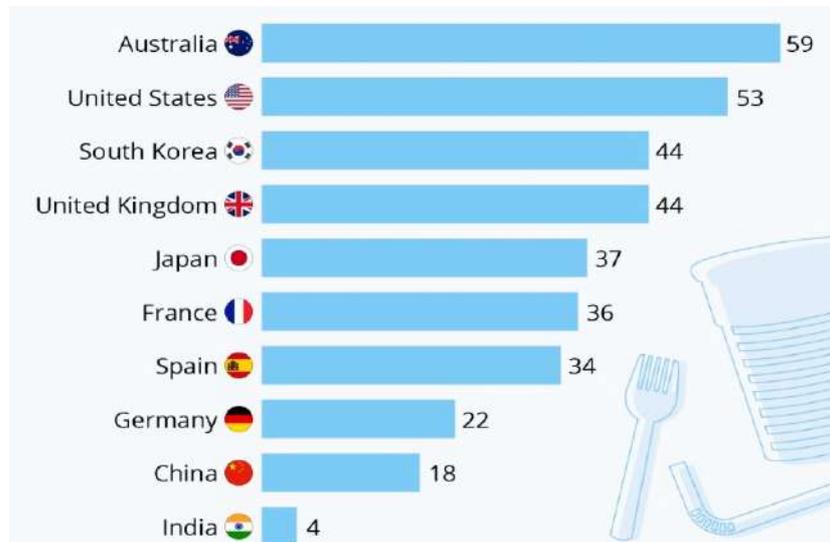
**Figure 1 - Generation of Single-use Plastic Waste by each person in selected Countries
Source: The Plastic Waste Makers Index by The Mindaroo Foundation**

RESEARCH ARTICLE

Knowledge, Attitude and Behavior in Cardiopulmonary Arrest and Resuscitation among Critical Care Nurses in the Rural Tertiary Care Unit

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ABSTRACT

Cardiopulmonary resuscitation is an emergency lifesaving procedure performed when the heart stops beating. Nurses are often the first responders to activate chain of survival when a cardiorespiratory arrest happens in critical care unit. That is why they must keep their knowledge and skills up-to-date and their attitudes to resuscitation are very important. The main objective was to assess the knowledge, attitude and behavior in managing cardiopulmonary arrest and resuscitation among critical care nurses in rural tertiary care unit. This Cross-sectional study was conducted in intensive care unit of our rural tertiary care unit. The nursing staffs working in the intensive care unit of all shifts were selected for the study and one who was ready to give consent included in this study. Basic demographic details of nursing staff were collected. The knowledge based questionnaire contains 7 questions. The nurses' theoretical and practical understanding was assessed by presenting 8 statements that were accompanied by a five-point Likert-type scale ranging from one for strongly disagree to five for strongly agree. Out of a total of 70 nurses,52 nurses responded to all questionnaires by filling out a google form, with a valid response rate of 70% and majority of them were male nurses. Most of the nurses i.e 36(69.23%) had diploma degree. In the knowledge questionnaire, 7 questions were asked. The minimum score was 0. Maximum score was 7. The mean score was 3.55 $\pm$1.85. In behavior perspective, among 52 nursing staff, 18 (35%) had never attended CPR training and workshop. A total of 33 (63.36%) nurses update their knowledge about cardiopulmonary resuscitation by opting regular training.

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The nursing knowledge about cardiopulmonary resuscitation was inadequate. An adequate training program was scheduled using skill and simulation lab. One should do regular auditing about resuscitation practices among critical care nurses to ensure optimal care of patients.

Keywords: Critical care, cardiopulmonary resuscitation, knowledge, nurse.

INTRODUCTION

Cardiopulmonary resuscitation is an emergency lifesaving procedure performed when the heart stops beating. Sudden cardiac arrest is the leading cause of death in many countries. Cardiopulmonary arrest is the most common event which happens in intensive care unit (ICU). Nurses are often the first responders to activate the chain of survival when a cardio respiratory arrest happens in the critical care unit. That is why they must keep their knowledge and skills up-to-date and their attitudes to resuscitation are very important [1]. Cardiopulmonary resuscitation is one of the basic skills that should be performed properly by health care workers [2]. Early initiation of cardiopulmonary resuscitation (CPR) maneuvers and activation of the chain of survival are key factors in the prognosis of patients who have suffered a cardio respiratory arrest (CRA) Therefore, it is important to understand and master CPR techniques, as these are the main determinants of success rates in CRA [3]. Some studies have reported a lack of knowledge among healthcare professionals. Basic aspects of CPR, such as the correct compression and ventilation sequences are not correctly understood by all healthcare professionals and it has been suggested that CPR training needs to be improved in medical professionals.[4] So we conducted this study in our rural set up to assess knowledge, attitude and behavior towards cardiopulmonary arrest and resuscitation in the critical care unit.

MATERIALS AND METHODS

This Cross-sectional study was conducted in our rural tertiary care unit after obtaining institutional ethical clearance (PIMS/DR/RMC/IEC-UG-PG/2021/105). The nursing staffs working in the intensive care unit of all shifts were selected for the study only who accepted to give consent as a part of the study. Out of 70 nurses, 52 nurses showed their willingness to participate in the study. Basic demographic detail like gender, age schedule of a shift of work was collected. The knowledge questionnaire contains 7 questions. The nurses' theoretical and practical understanding was assessed by presenting 8 statements that were accompanied by a five-point Likert-type scale ranging from one for strongly disagree to five for strongly agree. Data were entered in Microsoft excel sheet and data analysis were done by epic -info software.

RESULTS

Out of 70 nurses, 52 nurses responded to all questionnaires by filling out a google form, with a valid response rate of 70%. As depicted in Table no. 1 based on demographic details obtained there were 31(60%) male respondents. 27 (51%) respondents were between 20-29 yrs of age group. 36 (70%) respondents had primary education of diploma in nursing. Almost all nursing staff 49 (94.23%) had all shifts at the workplace. As depicted in Table no. 2 among nursing staff 27 (51%) were working in SICU, 20 (38%) were working in MICU and 5 (10%) were working in CCU. 28 (53%) of the nursing staff had work experience of more than 5 yrs. In behavior perspective, among 52 nursing staff, 18 (35%) had never attended CPR training and workshop. Most nursing staff 26 (50%) had attended cardiopulmonary resuscitation done within a week in ICU at the time of filling the form of the study. A total of 33 (63.36%) nurses update their knowledge about cardiopulmonary resuscitation by opting regular training. In knowledge questionnaire 7 questions were asked in which minimum and maximum score was 0, 7 respectively and mean score was 3.55 $\pm$ 1.85.

As depicted in table no. 3, 2 (3.85%) out of 52 nursing staff did not give correct answer to all 8 questions.1 nursing staff out of 52 correctly answer all questions. Table no. 4 depicts number and proportion of correct responses for knowledge-based questions by nursing staff. Table no. 5 depicts response of nursing staff to attitude related questionnaire.

DISCUSSION

In this cross sectional study out of 70 nurses, 52 were participated who are working in our rural tertiary care unit. 17(32.69%) out of 52 nursing staff had not attended cardiopulmonary resuscitation training for more than 2 years. Study conducted in North Cyprus in 2013 showed that theoretical and skills training should ideally repeated every six months [5]. Our studies showed 18 (35%) nurses had never attended cardiopulmonary resuscitation training which is significant in number. The authorities must make it a compulsory component while posting the nurses to critical care unit. Authors suggest that, the nursing colleges must make compulsory training of all nurses with an exit exam for resuscitation training. In contrast to study conducted in Spain showed 50% of nursing staff never attended cardiopulmonary resuscitation training.70% had primary degree of diploma among nursing staff [1]. There are no legal requirements in our country for nursing staff to perform cardiopulmonary resuscitation in our country. In contrast to study conducted in USA showed that there are mandatory cardiopulmonary resuscitation training in secondary schools only and health care worker need CPR card to perform clinical work [6]. In the Knowledge questionnaire, there was a wide range of correct answers in Knowledge questionnaire 3% to 69% suggest that some of the questions were more difficult than others from individual responses. In contrast to similar study conducted in Spain showed that correct response rate was 10% to 100%. In knowledge questionnaire mean score was 3.55 out of 7.A similar study conducted in Spain showed that mean score was 6.11 out of 11 [1]. In the knowledge questionnaire, only 1.92% had corrected all the answers.

From the attitude questionnaire, we can analyze that only11(21.15%) out of 52 are strongly agreed that they are trained enough to perform cardiopulmonary resuscitation and 7(13.46%) believed that they are not trained enough to perform cardiopulmonary resuscitation. 15 (28.85%) out of 52 were confident enough to perform cardiopulmonary resuscitation and 6(11.54%) are not confident to perform cardiopulmonary resuscitation. 22(42.35%) out of nursing staff believed that hospitals should provide proper cardiopulmonary resuscitation training for nursing staff. So their knowledge can be improved by proper 6 months of cardiopulmonary resuscitation training. This is also ensured study conducted by Um ran D a l and Dilek Sarpkaya in nursing students even after their graduation. [5]. 15(28.85%) strongly believed that CPR can be performed by either Physician or nursing staff in ICU set up.9(17.31%) out of 52 are strongly believed that only health care worker should start cardiopulmonary resuscitation.16 (30.77%) believed that other than healthcare workers any person can start cardiopulmonary resuscitation. Study conducted in South Korea in 2019 showed that because CPR is a psychomotor skill, hands-on clinical training is more effective than lecture - based or video - based education in improving CPR performance [7]. So in our study we can improve knowledge of nursing staff by hands on regular clinical training [8,9] . Study conducted in Botswana in 2018 revealed that there is statistically significant improvement in both knowledge and skills of CPR for all nurses post training [9].

LIMITATION
Although in this study we assessed about cardiopulmonary arrest and resuscitation related knowledge, attitude and behavior of our set up, it cannot be generalized to other places. However, we would like to give a message that, there should be a regular auditing of such important skills, which will help to improve the patient care.

CONCLUSION

The overall knowledge of CPR among critical care nurses is not satisfactory. Although it is inadequate, overall mortality rate among the critical care patient was not more which may be due to involvement of duty doctors who are well trained in CPR and certified by American Heart Association (AHA). All the healthcare workers should

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undergo resuscitation training program. After observing the study results we immediately initiated the training of critical care nurses in skill and simulation lab with exit exam and certification.

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Table 1: Demographic details of nursing staff

	Demographic detail	Numbers	
1)	What is your gender?	Male	31(59.62%)
		Female	21(40.38%)
2)	How old are you?	<20 yrs	0(0%)
		20-29 yrs	27(51.92%)
		30-39 yrs	22(42.31%)
		40-49 yrs	3(5.77%)
		50-59 yrs	0(0%)
3)	Highest Degree of nursing you attained	Masters	0(0)
		Bachelors	16(30.77%)
		Diploma	36(69.23%)
4)	Schedule of shift at work	Morning	2(3.85%)
		Afternoon	1(1.92%)
		Evening	0(0%)
		Night	0(0%)
		All	49(94.23%)

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Table 2: Behavior related questions

	Behavior related questions	Answers	
1)	Current place of work	SICU	27(51.92%)
		MICU	20(38.46%)
		CCU	5(9.62%)
2)	How many years of work experience do you have as nursing staff?	<6 months	0(0)
		6 months-1 yr	6(11.54%)
		1 yr-3yr	11(21.15%)
		3 yr-5yr	7(13.46%)
		>5 yr	28(53.85%)
3)	Last cardiopulmonary resuscitation training attended	<6 months	4(7.69%)
		6 months-1 yr	3(5.77%)
		1-2 yr	10(19.23%)
		>2 yr	17(32.69%)
		never	18(34.62%)
4)	Last cardiopulmonary resuscitation done	Within a week	26(50%)
		<6 months	10(19.23%)
		6 months -1 yr	4(7.69%)
		1-2 yr	2(3.85%)
		>2 yrs	6(11.54%)
		Never	4(7.69%)
5)	How do you update your knowledge about cardiopulmonary resuscitation?	Regular training	33(63.46%)
		Reading guidelines	11(21.15%)
		Information from colleagues	4(7.69%)
		Any other	4(7.69%)

Table 3: Percentage of correct responses answered by nursing staff in knowledge questionnaire

Number of questions correctly answered (out of 7 questions)	Number of nurses (out of 52)	Percent
0	2	3.85%
1	8	15.38%
3	9	17.31%
4	11	21.15%
5	7	13.46%
6	9	17.31%
7	1	1.92%
TOTAL	52	100.00%

Table 4: Number and proportion of correct responses for knowledge-based questions by nursing staff

Knowledge related questions	Correct answers.
Which guideline is followed for adult Cardiopulmonary resuscitation?	33 (63.46%)
How frequently American heart association guidelines are updated for cardiopulmonary resuscitation?	8 (23.08%)
Effective chest compression means?	36 (69.23%)
According to the American Heart Association 2020 recommendations, the correct compression/ventilation relationship in Cardiopulmonary resuscitation is	27 (51.92%)

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How the endotracheal tube placement is confirmed and monitored?	25 (48.08%)
Is Do not resuscitate legally allowed in India?	29 (55.27%)
When should you stop cardiopulmonary resuscitation?	34 (65.38%)

Table 5: Response to attitude related questionnaire

Questions	Strongly disagree	Slightly disagree	Neither agree nor disagree	Slightly agree	Strongly agree
Do you think that you are trained enough to perform CPR?	7 (13.46%)	1 (1.92%)	14 (26.92%)	19 (36.54%)	11 (21.15%)
Do you think yourself to be confident enough for performing CPR?	6 (11.54%)	1 (1.92%)	14 (26.92%)	16 (30.77%)	15 (28.85%)
Do you think that your hospital should provide proper training for performing CPR?	4 (7.69%)	1 (1.92%)	10 (19.23%)	15 (28.85%)	22 (42.31%)
Do you think that CPR can be performed by either physicians or nurses?	4 (7.69%)	0%	13 (25%)	20 (38.46%)	15 (28.85%)
Do you think that only health workers should start CPR?	16 (30.77%)	7 (13.46%)	12 (23.08%)	8 (15.38%)	9 (17.31%)

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REVIEW ARTICLE

Vitamin C 'The Scavenger' and Periodontal Disease A Review

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ABSTRACT

Worldwide the leading cause of tooth loss is periodontal disease. Treatments available currently are limited as it depends on patients learning, good oral hygiene maintenance and repeated visits to oral health care worker. The role of vitamin C is very important in collagen synthesis and immune function. Thus makes it important in wound healing and ultimately periodontal wound healing also. Therefore, Vitamin C deficiency worsens the periodontal disease and outcome of the treatment. Vitamin C is considered as a modulator for periodontal disease. Many studies provide rationale for considering deficiency Vitamin C as a risk factor for periodontal disease.

Keywords: Vitamin C, Periodontal Disease, Wound Healing, Scurvy

INTRODUCTION

In spite of intense research in the field of periodontology, the number of periodontal disease is steadily increasing worldwide. The use of mechanical and chemical treatments yields only momentary improvement of the periodontal

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conditions in some cases. In addition to local factors the environmental factors like nutrition should also be taken into consideration for long term periodontal therapy. Vitamin C (ascorbic acid) is an essential nutrient involved in a variety of bodily processes such as immune function, metabolism and most importantly production of sound collagen. In animals like primates, bats and especially in humans, they completely rely on nutritional sources of vitamin. C because they lack the enzyme required for its synthesis, L-gulono-gamma-lactones oxidize (GLO)Citrus fruit, kiwi, guava, papaya, melon, strawberries, tomatoes, broccoli and Capsicum are some of the rich sources of vitamin. C [1]. Historically, these fresh fruits and vegetables were available scarcely which led to its deficiency. Severe vitamin C deficiency results in scurvy [2]. Symptoms of scurvy include Pain in muscles, fatigue, fever, loss of appetite, nausea, malaise, bleeding gums, tooth loss, skin rashes or red spots, eventual wound re-opening, infections, fractures, hemorrhages, delirium and death.[3] Some other common symptoms are bruising, coiled hair, failure to thrive, irritability, muscle weakness, weight loss. Clinically, vitamin C status is usually assessed by measuring serum or plasma vitamin C levels. Blood levels correlate best with recent vitamin C intake, usually over days, except in the setting of recent supplementation. Commonly, serum vitamin C analysis is used in conjunction with questions about dietary intake of fruits and vegetables. Periodontal disease is a term used for a range of conditions affecting the periodontium. It is the result of chronic bacterial plaque build-up and inflammation resulting in damage to the underlying gingival and alveolar bone.[4-6] Periodontitis is "a chronic and multi-factorial inflammatory disease associated with plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus".[7] This leads to destruction of the alveolar bone, recession of the gums, and, if untreated, eventual tooth loss which is seen in Stage 4 disease.[8] The 2017 American Academy of Periodontology classification system formally incorporates clinical attachment loss around teeth, radio graphical bone loss, tooth loss, probing depth, bleeding on probing, disease progression and presence of systemic diseases. Initially assessment is done and the patient is assigned a stage and grade based on the above consensus guidelines.[9] In short, Stage 1 is the border between simple gingivitis and periodontitis and is associated with early stages of attachment loss. Stage 2 is moderate periodontitis with damage to tooth supporting structures. Stage 3 is present when there is significant damage to the attachment apparatus of teeth and tooth loss can occur without treatment. Stage 4 is considerable damage to tooth support, often accompanied by tooth loss and loss of masticator function. Grade relates to "evidence or rate of progression of disease, anticipated treatment response, and effects on systemic health" and is rated A, B or C. C is considered as the most severe grade.

Role of vitamin C for periodontal health
Vitamin C supports periodontal health through maintenance of gingival, periodontal ligament, cementum and alveolar bone, which all support teeth. This is due to the essential role of vitamin C for the synthesis of collagen which is an integral part for periodontal health. In addition to this role, vitamin C may lower the risk of periodontal disease, and even facilitate healing of the periodontium due to its strong antioxidant capacity that allows it to inactivate ROS that damage structure and function of tissues.

Vitamin C and General Health
Most of us get the recommended levels of vitamin C by consuming fruits and vegetables rich in it. Vitamin. C and vitamin B complex are water soluble vitamins unlike fat-soluble vitamins such as vitamins A, D, E and K. These fat soluble vitamins are stored in fat tissue. But vitamin C is water-soluble and not stored in the body tissues. Excess amount vitamin C is excreted in the urine. Therefore, a continuous supply of vitamin C is required to ensure sufficient levels of it for various processes. If the diet is deficient in vitamin C then the deficiency of vitamin C causes the disease called Scurvy. Consuming fruits and vegetables provides us with a variety of vitamins, minerals and fiber that may help reduce the risk of heart disease and some types of cancer [10].

Recommended Dietary Allowances (RDA) of Vitamin C
India is considered as one of the countries with the lowest RDA for vitamins. Its RDA for vitamin C is 40mg which is much lower than its other APAC neighbors China, Japan, and Singapore, where the RDA is 100mg. Other countries with relatively low RDA are Australia and New Zealand at 45mg for vitamin C. For adults, the RDA for vitamin C is 65 to 90 milligrams (mg) a day, and the maximum vitamin C a person can consume 2,000 mg a day. Consumption of

too much dietary vitamin C is unlikely to be harmful. Remember, for most people, a healthy diet provides an adequate amount of vitamin C. Tables 1 shows the sources of vitamin C like vegetables and fruits and their content of vitamin C. Vitamin C is strong antioxidant agent. Due to its antioxidant property, there is considerable interest in whether higher than recommended levels of vitamin C are associated with better periodontal health or may support healing of periodontal tissues after specific procedures such as deep scaling and root planning. This is called as "sanative therapy".

Requirement of vitamin C in smokers

It is found that the serum levels of vitamin C is substantially low in smokers than non smokers.[11] Oxidative stress is generated through smoking which causes tissue destruction. Poor intake of vitamin C further deteriorates the tissue destruction in periodontal disease. This suggests that the intake of vitamin C should be higher in smokers than non smokers. The Food and Nutrition Board suggests that smokers should modify their diet to include an additional amount 35 mg/day of vitamin C.[12]This extra quantity of vitamin C is easily available with a small serving of vitamin C rich foods

Relationship between Vitamin C intake and Periodontal Disease

Vitamin C intake below recommended levels is not directly related to initiation of periodontal disease, however, some human studies have shown that patients with severe periodontitis have lower vitamin C intake (<47mg/day).[13,14] Low plasma vitamin C levels are related to the development or severity of periodontal disease but the exact mechanism is still under investigation. It is the fact that during times of infection and after surgery, there can be a decrease in blood plasma vitamin C levels and periodontal surgery is no exception. Therefore supplementation of vitamin C is required for tissue regeneration.[15]After periodontal surgery the role of vitamin C becomes important as it helps in formation of new connective tissue in a healing wound and also in collagen synthesis. New tissue is rebuilt around a collagen framework and the enzymes required to form collagen cannot function without vitamin C. Periodontal disease shares a number of similarities with scurvy which is caused by deficiency of vitamin C such as excessive gum bleeding and tooth loss. A low level of serum vitamin C is correlated with more advanced periodontal disease and increased CRP. CRP is a measure of systemic inflammation and this finding is very important. CRP is elevated in some patients with periodontal disease. With non surgical periodontal therapy the level of CRP is lowered. So ultimately levels of vitamin C, CRP and periodontal disease go hand in hand.[16]Intakes of 5 or more servings of fruits and vegetables/day by non-smokers was shown to be associated with a significantly lower percentage of sites with probing depths >3 mm eight weeks following non-surgical scaling and root planning compared to patients with lower dietary intakes of fruits and vegetables (1.5 to 5 servings/day). Dietary vitamin C intakes greater than 150 mg/day were associated with a better response to sanative therapy, suggesting that greater intake of vitamin C i.e. beyond the RDA of 75 or 90 mg/day may be beneficial in periodontal treatment, but cause and effect relationship should be determined first. Coincidently, the association between dietary intake of vitamin C and reduction in probing depth was lessened when total vitamin C intake (dietary and supplement source of vitamin C) was assessed. This finding suggests that the synergistic effect of whole foods rather than supplements may offer a more robust approach to periodontal healing outcomes. However, well-designed, long-term studies involving a whole food approach are needed to determine whether a 'cause and effect' relationship exists between higher vitamin C intakes and improved periodontal health.[17]

Covid 19 and vitamin C supplementation

Theoretical background suggests that vitamin C has very important role in immune regulation during infections. But the therapeutic effect of vitamin C in treatment in respiratory infections and critically ill patients are still controversial. The usefulness of vitamin C treatment in patients with COVID19 is still under investigation worldwide. There are multiple records where COVID 19 patients were treated with vitamin C in different set ups and many of them have experienced clinical improvement after the administration of vitamin C. However, most published data in this regard are observational, case reports and small case series, ruling out the ability to associate any beneficial effect specifically to vitamin C treatment. The results of ongoing RCTs are still pending, and additional high-quality research is essential in view to support the potential benefit of vitamin C in the treatment of patients

with COVID 19 infection. In addition, there is no evidence to support routine use of vitamin C supplementation in healthy individuals for the prevention of COVID-19.[18] In a similar way there is no enough evidence to support the vitamin C supplementation in otherwise healthy individuals to prevent periodontal disease. But definitely it can improve the treatment outcome of the disease not solely.

The Essence

1. Neither the food rich in vitamin C nor the vitamin C supplementation cure the periodontal disease. But vitamin C will definitely modulate the host inflammatory response and support the periodontium
2. The smokers have more oxidative stress so the additional daily dose of vitamin C is required
3. Those who take 7-10 servings of vitamin C containing foods a day, they meet the RDA of vitamin C
4. Sufficient levels of vitamin C does not guarantee of absence of periodontal disease
5. Additional supplement of vitamin C is not the only cure of periodontal disease
6. As far as possible meet the needs of vitamin C through diet. Consume balanced diet as it meet the requirements of all the essential nutrients.
7. Consumption of excess of vitamin C does not mean better periodontal health. "Excess of anything is bad".

CONCLUSION

Vitamin C is strong antioxidant agent. It helps in immune modulation and collagen synthesis as well. It highlights the effects of vitamin C on the periodontal disease onset, progression and treatment. Further studies are required especially randomized clinical trial addressing the use of vitamin C for the treatment of periodontal disease. Vitamin C is easy to get from day to day food we are consuming. In the era of pandemic of COVID 19 the studies should be aimed at use of vitamin C in COVID 19 patients and its effects on the foregoing periodontal disease.

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Table 1: Sources of vitamin C and their nutrient content

Nutrient	Food Groups	Food	Nutrient content for 100g edible portion
Vitamin C	Green leafy vegetables	Agathi, cabbage, coriander leaves, drumstic leaves	120-220 mg
	Other vegetables	Giant chillies (capsicum)	137 mg
		Green chillies	117 mg
	Fruits	Amla	600 mg
		Guava	212 mg

RESEARCH ARTICLE

PCO—Proactive Computation Offloading Method for Maximizing Service Distribution on the Internet of Vehicles

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ABSTRACT

Internet of Vehicles (IoV) provides uninterrupted communication and information sharing for roadside users through smart platform access and user request processing. Multiple vehicles connected to a communication infrastructure require prompt request processing and computation response. By considering this feature, in this article, a Proactive Computation Offloading with Service Realization (PCO-SR) is introduced. This method identifies the connected and un-connected request computations based on the vehicle's communication with the infrastructure. The offloading required computations are distributed eventually by swapping multiple time-constraint processing units. This decision is preferred based on classifier learning; the iterations are modeled for connected and un-connected requests independently. The request processing/ offloading relies on service density and vehicle's request time based on which the classification occurs. This process is congruent in different request-response intervals, maximizing service distribution, reducing computation complexity, and response time.

Keywords: Classifier Learning, Computation Offloading, IoV, Service Distribution.

INTRODUCTION

Internet of Vehicles (IoV) is widely used in Intelligent Transportation System (ITS) to enhance the efficiency and performance of the services among the users. IoV helps to develop communication between the user and the roadside unit system with the help of a wireless network [1]. It helps to identify the vehicle's details such as name, speed, records, etc. IoV mainly focuses on entertainment, safety, and efficiency during the service. Service management in IoV plays a vital role. Quality of service will be increased with the help of the routing algorithm [2].

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Routing is very important for every user to reach their destination. To manage the services in IoV, incentive-based vehicle witness is used. Fog-based service distribution is used to manage the data process in IoV. Software-defined internet of vehicles is used for routing services [3]. Routing is one of the main services in IoV, without proper routing the users may feel difficult to travel. The service-aware routing algorithm is used to identify the shortest path while traveling. With the help of this routing algorithm, services are more improved and efficient when compared with the other algorithm. The routing algorithm will find a proper way by using the roadside unit system via wireless networks [4, 5].

Internet of Vehicles (IoV) is used to improve the features such as safety, reliability, efficiency, and provide comfort for the users in ITS. IoV is used to interact between the vehicles and the users with the help of the roadside unit system [6]. The main challenge in IoV is the security and computing issues. Edge intelligence is used to process the data which leads to IoV. To overcome this, edge computing is used, which increases the computing process and improves the overall performance of the IoV [7]. To avoid privacy and security issues, a computation offloading method which is based on edge computing is used. This is also used in privacy-preserving services. Everything in edge computing is analyzed in a sequenced manner [8]. The computation offloading method is more reliable and efficient. Another challenge in IoV is the delay in services. To avoid delay in IoV, Software-defined networking (SDN) is used. SDN is a machine learning-based process that helps to find a stable routing service in IoV [5, 9]. Latency percentages are decreased and the reliability is increased by using SDN. To address the real-time computation, Vehicle Edge computing is used. To reduce the offload in the system, Road Side Units are well equipped with edge nodes for better performance. Distributed computation offloading is also used to reduce the latency rate in IoV, and it is cost-effective when compared with the other model [4, 10].

In this world of automation, smart automobiles are widely used in many applications and fields. Internet of Vehicles (IoV) is used in the transportation system to create a communication between the vehicles and the public network to know about the environment and the surroundings, which leads to safe and comfortable traveling for the users [11]. In IoV, the vehicles are attached with sensors and wireless networks which helps to identify the information about the nearby vehicle. IoV is mainly used to avoid accidents and to find a comfortable route while traveling. One of the main problems in IoV based computation offloading [12]. The user's personal information is stored in the database and will be attacked by the attacker. Artificial Intelligent (AI) is most widely used to overcome offloading in IoV. AI is based on machine learning techniques to find efficient and smart solutions for problems [13]. AI is used to improve the performance, efficiency, and experience of the users in IoV. The deep reinforcement learning (DRL) method which is based on edge computing for offloading is used to maintain the performance of IoV [14]. Computation offloading will be performed, once the roadside unit receives all the service requests of the vehicles. DRL will increase the quality of the experience and performance of the whole service [15].

Related Works

Vehicle-to-everything (V2X) is widely used as a communication technology to improve the computing process among vehicles. An offloading method named; V2X-COM based on edge computing is proposed by X u et al. [16]. V2X-COM helps to reduce the offloading problems and latency rate during services. V2X-COM validity is high when compared with other methods. Internet of Connected Vehicles (IoCV) has been widely used in many applications in the Internet of Things (IoT). To overcome the offloading issue, 5G is used in IoCV to reduce the latency rate and to increase the quality of performance for the users. With the help of 5G in edge computing, Xu et al. [17] have introduced an adaptive computation offloading method (ACOM) which increases the efficiency of the process. ACOM has experimented with existing methods and results show the effectiveness of the proposed method. Deb et al. [18] have introduced a decentralized computation offloading scheme (DEFT) in IoV, to improve the efficiency and experience of the system. This is done by user entities with is cooperate with the roadside unit in a decentralized manner. An oligopoly game is played to fix the price of service which is based on computation capability. Among user and fog nodes, offloading is mapped with the help of the Stackel berg game. By experimenting with the DEFT

method with existing methods, the latency and power consumption rate are decreased and the quality is increased. Internet of Vehicles (IoV) is widely in applications to improve the efficiency, safety, and comfort of the users. The latency rate can be reduced by using the edge computing method, which reduces the latency rate. Hou et al. [19] have proposed a software-defined internet of vehicles based on edge computing (EC-SDIoV). This mobile edge node and fixed edge nodes are used to improve the low latency rate during the services. The proposed method is improved in reliability and reduces the latency rate.

In Intelligent Transportation System (ITS), Vehicular Fog Computing (VFC) is an improved vision in fog computing. To leverage the latency and energy awareness, Hussain et al. and Beg et al. [20] have introduced a new algorithm named Computation Offloading with Differential Evolution in Vehicular Fog Computing (CODE-V). To perform client tasks, in real-world topological variation is found and sent into fog nodes. When compared with the other offloading methods, CODE-V reduces the latency and energy consumption rate during the services and improves the system efficiency. Vehicle-to-everything (V2X) is widely used in the application for its improved efficiency and performance. Multi-access edge computing (MEC) is one of the best methods to achieve a low latency rate in applications. To overcome the latency rate and mobility rate, Li et al. [21] have proposed a method named, mobility aware dynamic offloading (MADO) algorithm by combining MEC and IoV technologies. It helps to reduce the overall offloading process. MADO algorithm is more effective and reduces the latency rate during the service process.

Applications such as driving, exporting, and video streaming rely on 5G technology for data exchange and seamless services. For providing seamless application support, Tian et al. [22] have introduced a new algorithm named Greedy matching with dynamic computing (GMDC). It is proposed to adapt or suitable the dynamics in a traffic environment. When compared with TOPM algorithm, GMDC is more suitable for server, reduces the latency rate and increase the quality of services to the users. To improve the high quality of service (Qos), the edge service (ES) location and quantity have to be analyzed. To find the problems in ES, Xu et al. [23] have proposed a dynamic edge service placement (DEP) approach to leverage the positions of nodes in ES. The experimented results of DEP result in a lower latency rate and reduce the workload of the service.

Calculating the data storage and computing process in vehicles are the main problems on the internet of vehicle (IoV). To overcome the issues, Wang et al. [24] have proposed an edge computing-based strategy named a highly reliable computing offloading strategy. It is a combination of mobile edge computing (MEC) and software-defined network (SDN) technologies to provide improved control over network and resource management for IoV. The experimented result achieves more coverage, increased efficiency, low latency rate, and reduce system workload when compared with existing methods. Internet of Vehicles (IoV) has transformed a simple system into a smart system network in the internet of things (IoT). To communicate with each other vehicles, intelligent connected vehicles (ICV) are widely used. The main problem is the delay in services. To overcome the problem, Zhang et al. [25] have proposed a vehicular edge computing network (VECH). It is achieved by using mobile edge computing offloading (MECO) technology. MECO is a promising technology to overcome the delay in services. In this world of automation, vehicles are connected by a system named, Connected and Autonomous Vehicle (CAV) to improve the flow of traffic and the safety of the users. Lin et al. [26] have introduced simulated annealing based on a deep Q-network (SA-DQN). SA-DQN reduces the energy consumption rate of the offloading process and increases the quality of service.

By using the 5G system, IoV allows the user to view or share information about the traffics and routing system while traveling. Wan et al. [27] have introduced a computation offloading method (COV) in IoV using 5G architecture. COV will ease to assign the destination of edge nodes while processing. COV is more efficient and effective when compared with other methods of offloading strategies. IoV uses edge computing devices (ECD) to compute the offloading tasks. The main challenge in ECD is to maintain the time and energy consumption rate while having offloading failures. To overcome the problem, Xu et al. [28] have invented a multi-objective computation offloading method (MOC) for IoV which is based on a cloud-edge computing process. The proposed MOC is more effective and

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efficient when compared with the other methods. To increase the reliability and capability, Li et al. [29] have introduced a partial flooding algorithm named computation offloading strategy for clouds. A partial flooding algorithm is used to reduce the task competing time rate of service. The proposed offloading strategy is used to improve the performance of the offloading system and utilize the resources for computation when compared with other methods.

Proposed PCO-SR

This method is designed for improving the service distribution of IoVs. Based on the preemptive classification induced offloading, the request computations are distributed preventing stagnancy. First the time constraint issues are addressed using available vehicles and connectivity between vehicles is ensured for offloading. A schematic representation of the proposed method is given in Fig. 1.

The proposed method is operated between the IoV's three layers. The cloud is responsible for service allocation, offloading requests, and processing. The infrastructure layer interconnects the vehicles and the cloud. In the infrastructure and cloud layer, the constraints are identified and mitigated. These constraints are based on request deadline (time) and vehicle connectivity. The problem definition is defined as in Eq. (1)

$$
\left.
\begin{array}{c}
\forall\ V\ where\ \rho = 1, \\
\alpha_S = \rho.\alpha_R \\
such\ that\ d(\alpha_S) = \frac{R}{N}.dt \\
and\ hence, \sum_{i=1}^{R} d(\alpha_S).N\ dt = \arg\max\left\{0, \frac{S}{R}\right\} \\
provided \\
\sum_{i=1}^{R} t_i = \sum_{j=1}^{S} (t_i)_j
\end{array}
\right\}
\qquad (1)
$$

In Eq. (1), for V vehicles the connectively (ρ) is maximum and hence response (α_S) for α_R requests is high. The assigned N services operate simultaneously in a α_R processing interval (t) that balances the response time (t_s). Hence the problem is the mitigation of $\sum_{i=1}^{R} d(\alpha_S) = 0$. If this case is true, then service distribution is a failure. The variable $N.dt$ refers to the processing instances occupied by the applications in the cloud. Therefore allocation is expected to be concurrent irrespective of vehicle density and α_R. For ease of process definition, the constraint analysis and offloading functions are discussed separately through the following sub sections.

Constraint Analysis

In the constraint analysis, the connectivity and time constraints are handled for preventing failure in Eq. (1). In analyzing the connectivity constraint, a $v's$ request has to be delivered to N provide t is inclusive in this. As the vehicle's posse's mobility, distance and connectivity are subject to change. However the provided condition in equation (1) is to be balanced from V and $N's$ and without maximizing computations. In a first cone first serve analysis, the α_R and α_S are congruent wherein $\rho = 1$. Therefore, for N vehicles in t the t_s is one-to-one mapped as defined in equation (2)

$$
\left.
\begin{array}{c}
\frac{[t]}{N} \forall\ 1\ to\alpha_R = V.[t_s]_{1\ to\alpha_S}.\rho \\
such\ that \\
\begin{bmatrix} \frac{1}{1} \\ \frac{1}{1} \\ \frac{1}{2} \\ \vdots \\ \frac{1}{N} \end{bmatrix}
\begin{bmatrix} t_1 \\ t_2 \\ \vdots \\ t_R \end{bmatrix}
= \rho
\begin{bmatrix} 1 \\ 2 \\ \vdots \\ V \end{bmatrix}
\begin{bmatrix} t_{S_{11}} & t_{S_{12}} & \cdots & t_{S_{1N}} \\ t_{S_{21}} & t_{S_{22}} & \cdots & t_{S_{2N}} \\ \vdots & \vdots & \cdots & \vdots \\ t_{S_{R1}} & t_{S_{R2}} & \cdots & t_{S_{RN}} \end{bmatrix}
\end{array}
\right\}
\qquad (2)
$$

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In Eq. (2), the individual $t_{s_{RN}}$ denotes the α_R assignment to the N. Here the process is congruent wherein the allocation satisfies the time constraint for $\rho = 1$. Therefore the $\rho = 1$ is satisfied between infrastructure and V and N and infrastructure. This ensures rendezvous connectivity between the available components. If a overloading occurs, then vehicle connectivity is less such that $\rho \to 0$. If this case occurs, the offloading fails and hence $R \neq N$ as in equation (2) faces a failure and hence these α_R are to be offloaded with additional computations. In this case, the connectivity between the vehicles is examined for seamless (non-stagnant) request distribution. There in an odd case of connectivity between them for the aforementioned pairs. In this case, if infrastructure and N shares a connectivity whereas V fails in handoff, then $\rho = 0$. This case does not require α_S and hence it is leftout. Contrarily, if the ρ between V and infrastructure is true whereas the infrastructure could not communicate to N. In this scenario, the constraint is the computation allocation; this requires a formal classification. However the classification is for differentiating connectivity and connection-less computations. Second constraint is as defined in Eq. (1), where $\sum_{i=1}^{R} t_i = \sum_{j=1}^{S}(t_s)_j$ is retained between α_R and α_S. In Fig. 2, the constraint analysis for time is presented.

The queued requests are allocated for the available N and hence the α_R satisfies Eq. (1) for $t_s \in (1, S)$. Contrarily, if $t_s \forall R \neq N$, offloading is required for preventing failures. Therefore, the $t_s \notin (1, S)$ instances are filtered from the sequence of $\frac{[t]}{N} \forall 1 \, to \, R$ and this requires a classification. In the classification process, the admitable α_R is extracted from time and connectivity constrained towards new neighbor identification. Therefore, allocation and offloading are the constraint less processes that is to be distinguished for individual analysis. The service distribution increases with the classification instances preventing $R \neq N$ failures. Therefore, the classification is analyzed using machine learning for mitigating different constraints. The prime goal of this process is to retain $t = t_s \forall \alpha_R, \alpha_S$ such that less time is incurred for complexity.

Classification Process

The incoming α_R post the $R \neq N \forall R \in \alpha_R$ is analyzed for its service distribution or offloading. In this process, the classification is performed based on the constraints discussed above. The prime sequence is $(1, \alpha_R)$ wherein the constraint causing $R \in \alpha_R$ is identified at intermediate sequence. First, the sequence is modeled in a linear manner as defined in equation (3)

$$\left. \begin{array}{l} C(\alpha_R) = V[t_s].\rho \, dt - \left(1 - \frac{\alpha_S}{\alpha_R}\right) dt \, \forall (1, R \in \alpha_R) \\ \left(\frac{\alpha_S}{\alpha_R}\right) d \, t_s - \left(\frac{R}{N}\right) d \, t_s \, \forall \, (0, \alpha_S) \end{array} \right\} \quad (3)$$

In Eq. (3), the linear differentiation for different t and t_s of the intervals is presented. The linearity includes failures and distribution for ease of classification. The classification based on t and t_s is presented as in Fig. 3.

The classification is performed for allocated and offloaded R as defined by Eq. (4)

$$\left. \begin{array}{l} C_1 = \frac{t_1}{N} + \frac{\alpha_1}{1} \\ C_2 = \frac{t_2}{N} + \frac{\alpha_2}{2} - \left(1 - \frac{\alpha_1}{2}\right) \\ C_3 = \frac{t_3}{N} + \frac{\alpha_3}{3} - \left(1 - \frac{\alpha_2}{3}\right) \\ \vdots \\ C_R = \frac{t_R}{N} + \frac{\alpha_R}{R} - \left(1 - \frac{\alpha_{R-1}}{R}\right) \end{array} \right| \left. \begin{array}{l} O_2 = \left(1 - \frac{\alpha_S}{\alpha_R}\right)_1 * \frac{2}{N} \\ O_3 = \left(1 - \frac{\alpha_S}{\alpha_R}\right)_2 * \frac{3}{N} \\ O_4 = \left(1 - \frac{\alpha_S}{\alpha_R}\right)_3 * \frac{4}{N} \\ \vdots \\ O_R = \left(1 - \frac{\alpha_S}{\alpha_R}\right)_R * \frac{R}{N} \end{array} \right\} (4)$$

The variables C_R and O_R denote the classification instances and offloading instances respectively. It is to be noted that the proposed method considers the O_R from $2^{nd} \alpha_S$ to R as the linearity based on $C(\alpha_R) \forall (1, R)$ is alone admitted for analysis. This is cross verified by equating Eq. (3) and (4) as follows. The equation is performed for $C_R \forall R \in \alpha_R$ (or) $R = \alpha_R$ and hence,

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$$
\left.
\begin{aligned}
& V\,t_s - \left(1 - \frac{\alpha_S}{\alpha_R}\right) = \frac{t_R}{N} + \frac{\alpha_R}{R} - \left(1 - \frac{\alpha_{R-1}}{R}\right) \; [as\ the\ R\ is\ 1^{st}] \\
& \qquad V\,t_s = \frac{t_R}{N} + \alpha_R \Rightarrow t_s = \frac{1}{V}\left(\frac{t_R}{N} + \alpha_R\right) \\
& \qquad\qquad Contrarily, \\
& V\,t_S - \left(1 - \frac{\alpha_S}{\alpha_R}\right) = \frac{t_R}{N} + \frac{\alpha_R}{R}\left(1 - \frac{\alpha_{R-1}}{R}\right) \; [as\ R > 1] \\
& V\,t_S - 1 = \frac{t_R}{N} + \frac{\alpha_R}{R}\,[as\ \alpha_S \to 0]\,(prior\ to\ offloading) \\
& \qquad\qquad t_s = \frac{1}{V}\left(\frac{t_R}{N} + \frac{\alpha_R}{R}\right) + 1
\end{aligned}
\right\} \quad (5)
$$

The t_s as observed in Eq. (5) for different R is likely to be congruent based on connectivity. Therefore the offloading is required if $\sum_{i=1}^{R} d(\alpha_S) \neq 0$ or< 1. Thus the offloading classification is differentiated in α_1 to α_R sequence, wherein, the intermediate t_s as in equation (5) requires offloading. In this process, the vehicle density with t is responsible for t_s occurrence and hence the classification terminates if $R = N$ is observed. The linearity is however distributed due to ρ and hence t_s exceeds the actual t. Therefore, the $R \neq N$ is again achieved, fluctuating the service distribution. This requires further classification of $t_s = \frac{1}{V}\left(\frac{t_R}{N} + \frac{\alpha_R}{R}\right) + 1$ instances. The limited identification of $t_s \forall (1, R)$ reduces the complexity during V augmentation. In Figure 4, the classification based on t_s is presented.

The classification based on t_s mitigates the time constraints in providing services. This classification identifies R in α_R instances where offloading recommendation is recurrent from C_2 to C_R. This classifies new instances where in the completed and non-completed α_S are identified. The classification for t_s is identified as follows:

$$
\left.
\begin{aligned}
& C_2 = \left(\frac{\alpha_2}{N} \times t\right) & & O_2 = \left(\frac{\alpha_1}{t} - \frac{N}{t_{s_2}}\right) \\
& C_3 = \left(\frac{\alpha_3}{N} * t\right) - (t_{s_2} - t) & & O_3 = \left(\frac{\alpha_2}{t} - \frac{N}{t_{s_3}}\right) \\
& \qquad\quad \vdots & & \qquad\quad \vdots \\
& \underbrace{C_{t_s} = \left(\frac{\alpha_{t_s}}{N} * t\right)\left(t_{s_{t-1}} - t\right)}_{Offloading\ Required} & & \underbrace{O_R = \left(\frac{\alpha_{R-1}}{t} - \frac{N}{t_{s_R}}\right)}_{Completed}
\end{aligned}
\right\} \quad (6)
$$

In Eq. (6) the offloading required $R \in \alpha_R$ in t_s and the α_S succeeded α_R from O_R [Eq. (4)] are identified. The α_R requiring offloading is assigned to the empty O_R observed. This means $C_{t_{s-1}} : \to O_R$ (i.e.) the previously assigned α_R is offloaded to the current $t \in O_R$ (i.e.) available N. Hence the offloading is performed for the completion of α_R that requires high t_s. Now the classification is divided for offloading requiring and completed scenario by equation the last t_s in Eq (6) and (7)

$$
\left.
\begin{aligned}
& \frac{t_R}{N} + \frac{\alpha_R}{R} - \left(1 - \frac{\alpha_{R-1}}{R}\right) = \left(\frac{\alpha_{t_s}}{N} * t\right)\left(t_{S_{t-1_s}} - t\right) \\
& \frac{\alpha_R}{R} - 1 + \frac{\alpha_{R-1}}{R} = 0\,[as\ t_R\ is\ allocated\ for\ O_R\ and\ pra.\ t = 0] \\
& \qquad\qquad \frac{\alpha_R + \alpha_{R-1}}{R} = 1 \Rightarrow \alpha_R + \alpha_{R-1} = R
\end{aligned}
\right\} \quad (7)
$$

The total R observed in a joint manner. These identified R are to be allocated in the complete α_S. This means$\frac{\alpha_S}{\alpha_R} = 1$ for the identified R in t_s; maximizing service distribution. Now the offloading is to be formulated as discussed in the below section.

Offloading Process

The identified constraints based on ρ and t_s are classified based on learning in different t. The finalized R is extracted from t_s that is differentiated from $\sum_{i=1}^{\alpha_R} t_i$ sequence. The first classification is the $(1, R \in \alpha_R)$ and $(0, \alpha_S)$ defined using Eq. (3). Therefore, the offloading is planned for $R \in \alpha_R$ observed a mid t_s for satisfying Eq. (1). In the following process, mapping is performed for the alternating t, R intervals. This requires free $t \in O_R$ identification and C_{t_s} that requires an offloading modeled as per the balancing of equations (4) and (6) as given in Eq. (7). A formal

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representation of the offloading process is given in Fig. 5. The first constraint is the ρ for which $C_{t_s} = O_{R+1}$ is identified and a new t_s from the existing t is allocated. Contrarily, if a new t_s is required then $C_{t_{s-1}} = O_R$ is validated; the sequence from the previous span is utilized for offloading. This means the identified t has completed its allocated α_R and hence is free to handle new α_R. The allocation based on Eq. (3) as satisfying Eq. (2) is derived as

$$\left.\begin{array}{c} \frac{\alpha_{R-1}}{t} - \frac{N}{t_{S_R}} = \frac{\alpha_s}{\alpha_R} = 0 \\ \Rightarrow \frac{\alpha_{R-1}}{t} = \frac{N}{t_{S_R}} \; and \; hence \; t = \frac{t_{S_R}*\alpha_{R-1}}{N} \\ similarly, \left(1 - \frac{\alpha_s}{\alpha_R}\right)*\frac{R}{N} = \frac{\alpha_s}{\alpha_R} = 0 \\ \Rightarrow \frac{\alpha_s}{\alpha_R} = 1 \; (allocated) \\ Therefore, \frac{t_{S_R}*\alpha_{R-1}}{N} = \frac{\alpha_S}{\alpha_R} = 1 \; (in \; t) \end{array}\right\} \quad (8)$$

Here, the linearity is preserved based on the available N and allocated t such that Eq. (1) constraints are satisfied. Therefore, the allocation as in equation (8) ensures completion of α_R and hence $\frac{\alpha_S}{\alpha_R} = 1$ (maximized). Contrarily, the offloading requiring instances as defined in Eq. (6) and (7) isverified for N availability such that Eq. (3) $\forall \, (0, \alpha_S)$ is preserved. Therefore,

$$\left.\begin{array}{c} \left(\frac{\alpha_{t_s}}{N}*t\right) - (t_s - t) = \frac{\alpha_R + \alpha_{R-1}}{R} \\ (or) \left(\frac{\alpha_{t_s}}{N}\right) - (t_s - t) = R \\ and \; N = \frac{\alpha_{t_s}*t}{(t_s - t) + R} \end{array}\right\} \quad (9)$$

As defined in Eq. (9), the N is allocated as linear sequence for admitting offloading α_R. Hence the availability and linearity are retained throughout the offloading t. Therefore, $\frac{\alpha_S}{\alpha_R} = 1$ is the observable response for different O_R instances. This augments the service distribution between the $V's$ reducing request failures. In Fig. 6, the analyses for response rate and offloading ratio for different R is presented   Fig. 6 presents two different analyses for response rate and O_R for different R instances. As R increases, response rate decreases due to classification requiring t. The pending α_R (linearity) is offloading in $t_{S_{t_{s-1}}}$ or t_{R+1} instances, maximizing the response rate. Therefore, for different V densities, the O_R increases as classified through Eq. (4) and (6). The offloaded requests are allocated to the N as in Eq. (9) for which the response rate is high. The classifications increasing the offloading R instances through $R \neq N$ identification and $\alpha_R \notin C(\alpha_R)$ validation. This is differentiated $\forall \, R > 1$ in t_S or t instances for different V. In Fig. 7, the request failure in different offloading instances is analyzed.

Fig. 7 presents the failure rate for different O_R instances under varying classifications. The varying classifications increase the O_R and C_R by mitigating the constraints in Eq. (1). Therefore, the N availability and O_R induced α_R are precisely matched, reducing the failures. In different classification instances, the proposed method ensures N availability in t for admitting $O_R \in (1, R)$, preventing failures. Fig. 8 presents the analyses for offloading and request processing ratio for different classifications.

Fig. 8 presents the offloading and request processing ration for different classifications. As the classification increases, $t_{S_{t_{s-1}}}$ or t_{R-1} is determined and hence allocation is precise without failures. However, the mismatch in t and t_s (failing Eq. (1)) reduces the request processing. Therefore, O_R from t_s is provided from different classifications recommending offloading.

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RESULTS AND DISCUSSION

This section presents the proposed method's analysis using simulated experiments. In the simulation conducted using Van Sim, a real-time scenario covering 4000m highway with 3 intersections is used. In this scenario, the infrastructures are situated at 7 places, for handling 100-1000 requests. The scenario is deployed with 160 vehicles with direct and indirect communication links up to 10Mbps limit. With these features, the metrics service distribution, response time, computation complexity, and request failures are analyzed. A comparative analysis with the existing MOC [28], V2X-COM [16], and MADO [21] methods is performed.

Service Distribution

In Fig. 9, a comparative analysis for service distribution by varying V and α_R is presented. The proposed method identifies the ρ and t_s constraints through repeated classifications. The classifications are initially performed based on V density that is augmented with $R \in (1, \alpha_R)$ instances later. This requires C_R and O_R differentiation as in Eq. (4) and (6) based on ρ and t_s [Condition in Eq. (1) failing].Therefore, by retaining $\frac{[t]}{N}$, $\forall\ 1\ to\ R\ \in (\alpha_R)$, the α_S/α_R is maximized initially. However the allocation and offloading derived from different $R \neq N$ and $\sum t \neq t_s$ instances requires further classification for satisfying equation (1). Therefore, $\alpha_R + \alpha_{R-1}$ and $(t_s - t)$ instances are identified through linear classification learning. This results in new O_R to t_{s-1} and O_R to t_{R+1} mapping, in maximizing $\frac{\alpha_S}{\alpha_R}$, therefore the service distribution on in the first and second cases are retained in the proposed method

Response Time

The proposed method achieves less response time for different V and α_R as presented in Figure 10. The first requirement is the classification of $R \in (1, \alpha_R)$ and $R \in (0, t_s)$ for differentiating N allocation and O_R. First, the $t = t_s \forall (\alpha_R, \alpha_S)$ is retained to verify the linearity. Based on the change in the linearity, C_R and O_R is classified. This does not required additional waiting time for O_R. This process is concurrent, reducing the response time. Contrarily, if $V t_{s-1} = \frac{t_R}{N} + \frac{\alpha_R}{R}$ is observed, then prior offloading in t_s is required. Therefore, further classification as in equation (6) is performed for $\sum t \neq t_S$ instances. Hence a few more R is augmented from (o, t_s) scenario, without requiring additional wait time. The proposed method reduces $\alpha_S \rightarrow 0$ cases by enquing different instances of R in O_R with the available N. Hence the changes in α_R linearity are abruptly adapted, reducing the additional wait time. This is unanimously adapted for different V and α_R wherein in concurrency is retained

Computation Complexity

The proposed method reduces the computation complexity in time allocation by identifying $\sum t \neq t_s$ and $R \neq N$ as in equation (2). In the first constraint, $V * t_s$ and t_s for 1st and last R (identified) is independently assigned. Therefore in a same t, $R \in (1, \alpha_R)$ and $R \in (O, t_s)$ are assigned to N and offloaded concurrently. Here, the time required for classification is alone considered wherein the wait time is null. Therefore, the $R > 1$ conditions are independently classified for reducing break in $\alpha_1\ to\ \alpha_R$ (allocated) or $\alpha_2\ to\ \alpha_R$ (offloaded). Such identification pursues different sequence of processing, reducing the computation time. In the consecutive process, $\left(t_{s_{t_s}-1} - t\right)$ and $\frac{N}{t_{s_R}}$ are sequentially identified for α_R (offloaded) processing. Therefore O_R is mapped to the previous $t = 0$, reducing the slot or t_s (new) requirement. Hence in this case, the time required is comparatively less (Refer to Figure 11).

Request Failure

For different V and α_R, the proposed method achieves less request failure (Refer to Fig. 12). In the proposed method, the t and ρ based constraints are identified and mitigated through different classifications. Both classifications retain the linearity as in equation (3) $\forall (0, \alpha_s)$ and $(1, R) \in \alpha_R$. Further $R = \alpha_R$ and $R \in (1, \alpha_R)$ is independently analyzed for identifying α_S failing instances. Based on the identified instances (of R), the offloading required and completed sequences are distinguished. The O_R in t_{R-1} and C_{t_s} in $\alpha_R + \alpha_{R-1}$ occurences are identified for maximizing α_S. This means, the allocations are performed for O_R in Eq. (4) and (5) followed by the availability check. Through this process, the offloaded α_R in R is allocated to the N, preventing failures. Therefore, the proposed method reduces

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the α_R processing due to ρ and t_s constraints, achieving less request failures. Tables 1 and 2 present the comparative analysis summary for different vehicles and requests.

Inference: The proposed method achieves 8.35% high service distribution, 17.43% less response time, 9.9% less computation complexity, and 9.01% less request failures.

Inference: The proposed method achieves 8.7% high service distribution, 16.22% less response time, 7.97% less computation complexity, and 8.58% less request failures.

CONCLUSION

In this article, a proactive computation offloading with service realization method is introduced for reducing IoV service request failures. The proposed method mitigates the time and connectivity constraints through conditional classification. In the conditional classification process, the offloading requiring instances from the linear request sequence is segregated first. Pursued by this process, the time span based request classification is performed for reducing computational complexity. In the offloading or allocation deciding function, classifier learning is used for reducing request processing failures. Finally, the service provider's availability is verified and the offloaded requests are assigned satisfying the time span constraints, in maximizing service distribution.

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Table 1. Comparative Analysis Summary for Vehicles

Metrics	MOC	V2X-COM	MADO	PCO-SR
Service Distribution	0.867	0.897	0.922	0.951
Response Time (s)	2.38	1.76	1.5	0.897
Computation Complexity (s)	1.02	0.888	0.653	0.342
Request Failure (%)	9.12	7.11	4.89	2.538

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Table 2 Comparative Analysis Summary for Requests

Metrics	MOC	V2X-COM	MADO	PCO-SR
Service Distribution	0.868	0.889	0.925	0.952
Response Time (s)	2.34	1.87	1.55	0.986
Computation Complexity (s)	1.06	0.834	0.685	0.449
Request Failure (%)	9.33	7.1	5.33	2.964
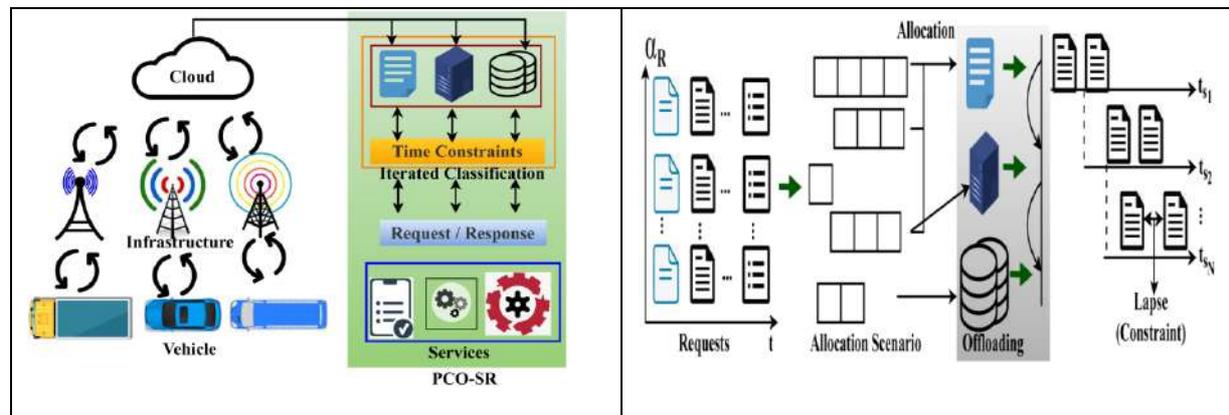
Fig. 1 PCO-SR in IoV Scenario

Fig. 2 Constraint Analyses for t.
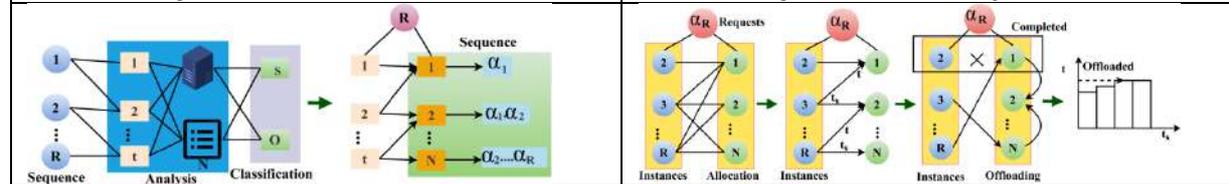
Fig. 3 Classification based on " t

Fig. 4 Classification based on t_s
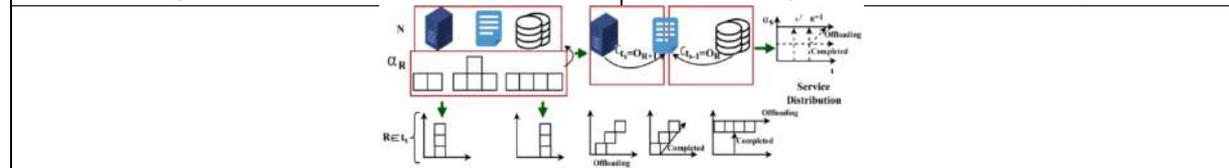
Fig. 5 Offloading Process Representation
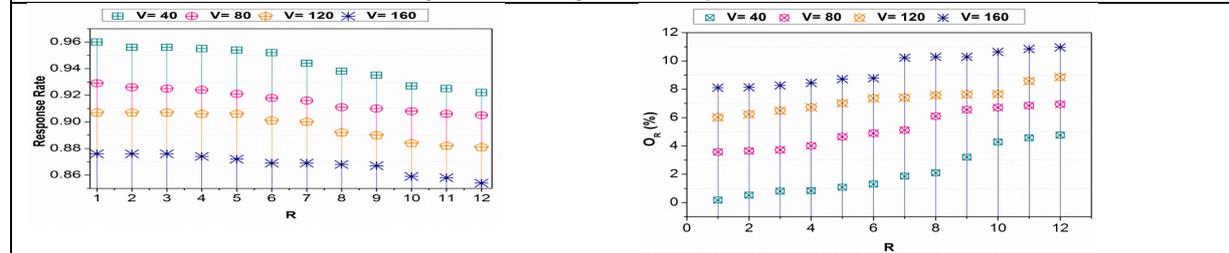
Fig. 6 Response Rate and Offloading Ratio for Different R
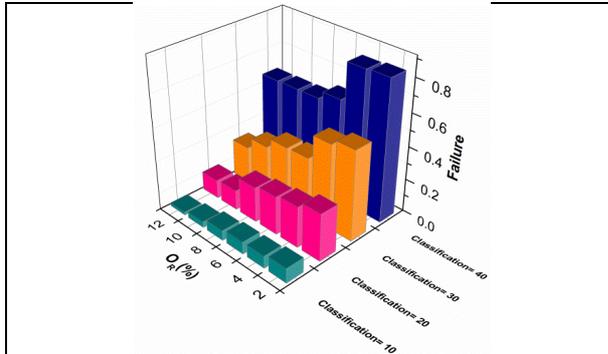
Fig. 7. Request Failures in Different Offloading Instances
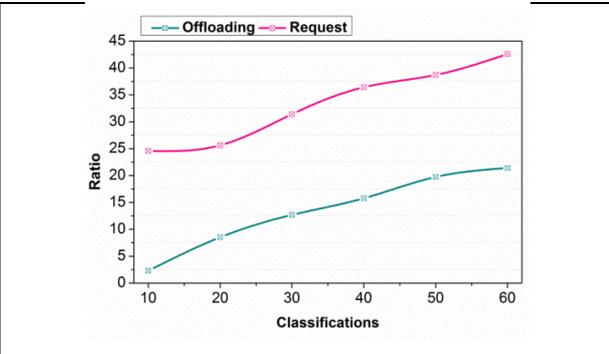
Fig. 8. Offloading and Request Processing Ratio for different Classifications
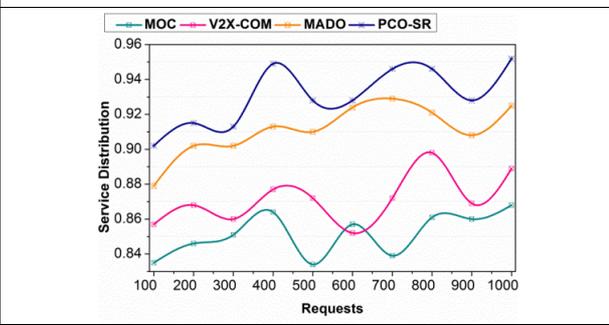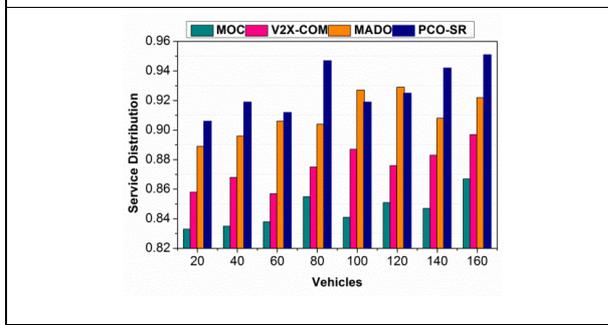
Fig. 9 Service Distribution Analyses
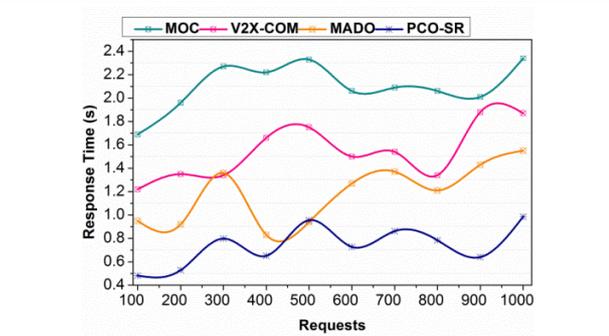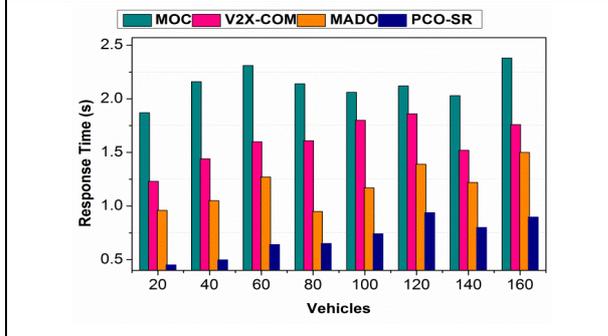
Fig. 10 Response Time Analyses
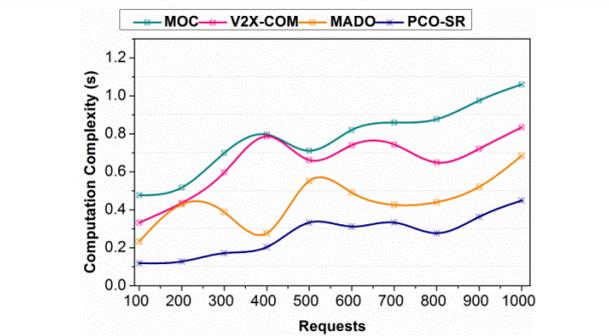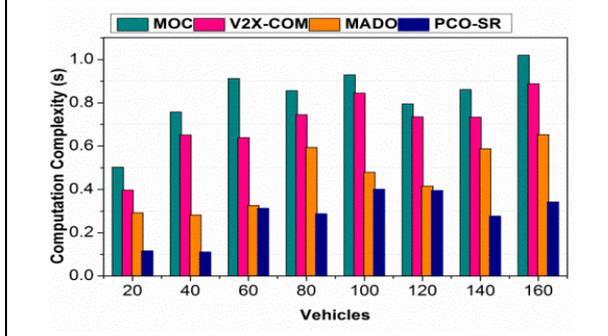
Fig. 11 Computation Complexity Analyses

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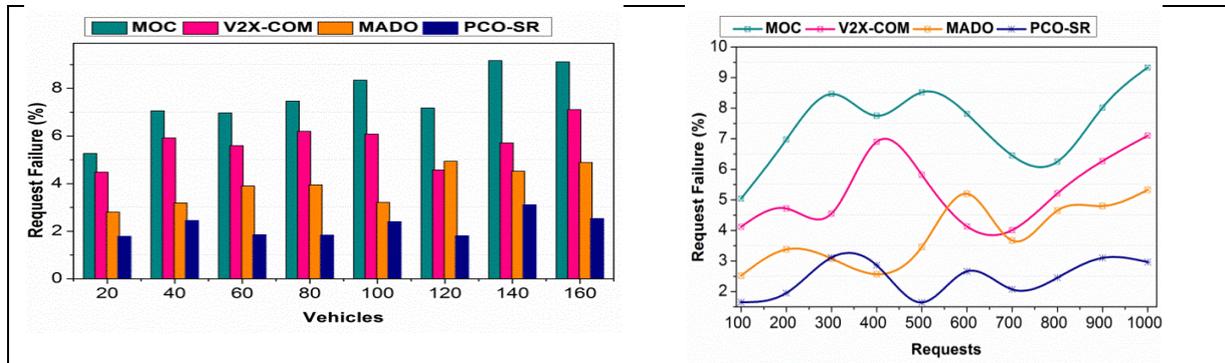
Fig. 12 Request Failure Analyses

RESEARCH ARTICLE

Influence of Composts on Growth, Yield and Quality of Brinjal

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ABSTRACT

Brinjal also known as egg plant is an important and indigenous vegetable crop of India. Pot experiments were carried out at Department of Soil Science and Agricultural Chemistry, Annamalai University to evaluate the influence of composts on growth, yield and quality of brinjal in two different soils (Neutral soil and Coastal saline soil). There were 7 treatments consisting of organics (FYM, Seasoned Pressmud, Poultry manure, Calotropis and Vermicompost) and inorganic (RDF) sources were conducted in FCRD with 4 replications. Among the treatments, highest plant height (113.9 cm), number of leaves plant[-1] (87), number of branches plant[-1] (19.06), leaf area (58.39 cm[2]), LAI (1.9), fruit girth (9.09 cm), fruit length (11.71 cm), no. of fruits (23.62), average fruit weight (41.22 g plant[-1]), fruit yield (972.62 g plant[-1]), Chlorophyll SPAD value (54.37), TSS (4.5°B), Titratable acidity (0.286%) and Ascorbic acid content (12.54 mg 100 g^{-1}) was recorded in treatment receiving 75% RDF + Seasoned Pressmud @ 12.5 t ha^{-1} (S_1T_3) in neutral soil. Application of organic sources of nutrients also produced better quality of fruits compared with RDF.

Keywords: Brinjal, Organics, Seasoned pressmud, Coastal saline, Neutral.

INTRODUCTION

India is regarded as a horticultural paradise with a vast array of vegetable being cultivated in our country, brinjal is considered as one of the leading and the second major vegetable crops next to tomato. Brinjal is highly productive purple fruits have medicinal properties. Egg plant is a long duration crop with high yield which removes large quantities of nutrients from the soil. Now-a-days demand for brinjal as a vegetable is increasing rapidly among the vegetable consumers in view of its better fruit colour, size and taste. In India brinjal is cultivated to an area of 0.72

million ha, production of 1.23 million metric tonnes, productivity of 17.5 Metric Tonnes ha[1](Horticultural Statistics, 2018). Average productivity of brinjal crop is quite low and there exists a good scope to improve its average productivity in India to fulfill both domestic and national needs. The productivity of brinjal can be increased by using, integrated nutrient management. Since the nutrient turnover in soil plant system is considering high in intensive vegetable cultivation, neither the chemical fertilizer nor the organic manures alone can help achieve sustainable production. Moreover, the application of high input technologies such as chemical fertilizers improve the production, but there is growing concern over the adverse effects of the use of chemicals on human health, soil productivity and environment quality (Umalaxmi Thingujam, 2016).

Organic nutrition, although used for thousands of years in agricultural soils has only recently been on the spot due to its positive effects over physical, chemical and biological soil properties. Among the organic manures used in vegetable production, livestock manures standout due to their positive effects on soil conditioning and nutrient availability, especially N. The amount of livestock manure recommended for growing brinjal varies usually 5 to 40 t ha[1] (Adil Rehman, 2015). In case of application of organic manure there is a problem of availability of organic manure at many places. The cane sugar industry has several co-products with immense potential value. The application of sugarcane pressmud is also at low cost with a slower release of nutrients and trace elements and high water holding capacity included in one of our treatments (Padalkar and Raut, 2016).

Rapid population increase, urbanization and industrial growth have led to severe waste management problems in the cities of developing countries like India. Municipal solid waste in Indian mega cities in mainly disposed in land fill by means of open dumping however a small fraction is used for composting. Composting of municipal solid waste is considered as important recycling tool since municipal solid waste would otherwise be land filled and lead to environmental and health issues. The application of municipal solid waste compost improves soil, physical and chemical and biological properties because of higher soil organic matter with the application of municipal solid waste compost in saline conditions crop yield was significantly higher over control (Meena *et al.,* 2019). Poultry industry is one of the largest and fastest growing agro based industries in the world. In India, this industry has attained spectacular growth, especially in the last decade of the twentieth century. Although economically successful, the poultry industry is currently facing a number of highly complex and challenging environmental problems. Poultry manure is a valuable fertilizers and can serve as a suitable alternate to chemical fertilizer (Kannan *et al.,* 2015).

Application of calotropis compost promote plant growth and it may be part of integrated nutrient management. *Calotropis gigantea* is a medicinal plant grown on variety of soil types but it contains poisonous milky juice so it is not used for grazing or other means. Proper plant growth and hence better yield requires supply of all the required nutrients. Compost is one of form of organic fertilizers produced by microbial degradation of a number of degradable organic wastes through activities of aerobic microbes in moist environment (Shah *et al.,* 2015). Sustainable agriculture can be ensured in future with the help of organic farming system, which involves various produces of biological origin such as compost and vermicompost. It is an established fact that earthworms act upon various kinds of wastes including sewage sludge, animal wastes and crop residues etc. Vermicomposting is increasingly becoming popular as an organic farming and solid waste management techniques. It is a dual process by which earthworms of various species derive nourishment from the microbes which are flourishing in the organic waste as well as degrade the complex organic waste into smaller particles in their gizzard, a part of their alimentary canal. The end product is vermicompost is highly humified through the break down of the parent waste materials by earthworm and its is applied as vermicompost as one of the treatments (Kaur *et al.,* 2015).

To have a holistic approach on the response of organic manures on chemical properties and brinjal performance, data on yield and quality of brinjal fruit is imperative. Therefore the study is to compare the different organic manures with RDF doses on growth, yield and quality.

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MATERIALS AND METHODS

Pot experiments were conducted to study the response of composts in yield maximization of brinjal at pot culture in Department of Soil Science and Agricultural Chemistry, Annamalai University during January to April, 2021.

Pot experiment
Pot experiment were carried out to study effect of different composts in two different soils.

Treatment details
S_1 – Neutral soil
S_2 – Coastal saline soil
T_1 – Control (RDF 100:50:30 N, P_2O_5 and K_2O kg ha^{-1})
T_2 – 75% RDF + FYM @ 12.5 t ha^{-1}
T_3 – 75% RDF + Seasoned Pressmud @ 12.5 t ha^{-1}
T_4 – 75% RDF + Municipal Solid Waste Compost @ 12.5 t ha^{-1}
T_5 – 75% RDF + Poultry Manure Compost @ 6.25 t ha^{-1}
T_6 – 75% RDF + Calotropis compost @ 6.25 t ha^{-1}
T_7 – 75% RDF + Vermicompost @ 3 t ha^{-1}

Each treatment is replicated four times in a Factorial Completely Randomized Design (FCRD) Soil samples were collected at the depths of 0-20 cm from 2 sites *i.e.* Vallampadugai, (Neutral soil), Therku Pichavaram (Coastal Saline Soil). Soil samples were air-dried and passed through a 2 mm sieve after removing coarse fragments and roots and stored at room temperature. 20 kg of air-dried processed soil was filled in 35×30 cm cement pot. The composts were applied basally. The chemical compositions of manures are presented in Table 1.

Biometric observations
In each treatment, observations were taken at time of harvest.

Plant height (cm)
Measure the height from the base to the tip of the last leaf using measuring tape and recorded in cm.
Number of primary branches
Count the number of branches emerged on the main stem at final harvest.

Leaf area (cm²)
Leaves in five number from the crop plant are taken and measure the length and breadth of their maximum points. Take leaf samples as described in the above procedure and draw the outlines of all leaves on the graph sheet with pencil. Count the number of squares (up to 1 mm²) that are covered by leaf margin and express the average leaf area in cm².

Number of leaves plant^{-1}
This was determined by counting the total number of leaves produced by each plant.

LAI
Watson (1947) proposed the term, Leaf Area Index

$$LAI = \frac{\text{Leaf area (cm}^2) \times \text{No. of leaves}}{\text{Ground area occupied by the plant}}$$

Yield parameters
Fruit length (cm)
Fully ripened stage of brinjal fruit was measured individually in centimeter from base of calyx to tip of fruit using meter scale and thread, thereafter average was calculated accordingly.

Fruit girth (cm)
Fruit girth was measured by using meter scale and thread, thereafter average was expressed in cm.

Number of fruits plant^{-1}
Average of fruits of all the pickings were counted from the total number of fruits harvested.

Average fruit weight (g plant^{-1})
Fruit weight of mature fruit was weighed individually in each treatment and average weight was worked out.

$$\text{Average fruit weight (g plant}^{-1}\text{)} = \frac{\text{Total weight of fruits plant}^{-1}\text{ (g)}}{\text{Total no. of fruits plant}^{-1}}$$

Fruit yield (g plant^{-1})
The weight of fruits harvested from in each pot at different pickings were added and total yield (g plant^{-1}) was recorded.

Analysis of soil sample
Soil samples were collected just before the start of the pot experiment. The collected soil samples were air dried in shade ground with wooden mallet, passed through 2 mm sieve and stored in polythene bags. The quality parameters were analyzed as per standard procedures (Table 3)

RESULTS AND DISCUSSION

Physico-chemical properties of soil
The soils of Vallampadugai (Sandy loam) and Therkupichavaram (Sandy) were analsyed and the initial soil values were presented in Table 2.

Biometric observations
Analysis of variance (p=0.05) showed significant influence of two different type of soil and compost application. Among two different soil types the biometric observations increased linearly (Table 4). The highest plant height (101.08 cm), number of leaves (74.28), number of branches plant^{-1} (12.60), leaf area (55.32 cm^2), LAI (1.13) registered in sandy loam soil (S$_1$). Among composts application the maximum plant height (107.78 cm), number of leaves (77), number of branches (12.37), leaf area (55.39 cm^2), leaf area index (1.18) recorded in treatment T$_3$ (75% RDF + Seasoned Pressmud @ 12.5 t ha^{-1}). In the interaction effect between soil texture and composts observations were recorded maximum plant height (113.9 cm), no. of leaves (87), no. of branches plant^{-1} (19.06), leaf area (58.39 cm^2) and LAI (1.9) in the treatment receiving 75% RDF + Seasoned Pressmud @ 12.5 t ha^{-1} (S$_1$T$_3$) in neutral soil.

During investigation, plant height and leaf area index were noted to be positively correlated with different treatments by composts in both soils. Vegetative growth is concerned with the expansion of new shoots, leaves and leaf area. The enhancement of vegetative growth may be attributed to the role of nutrients and sugar translocation in the plant cells. It is involved in the cell enlargement and in triggering before tissue or meristematic growth of brinjal crop (Vinod Kumar and Chopra, 2016).

Yield attributes and fruit yield of brinjal

The data pertaining to (Table 5) showed significant difference between two soil types and compost application. The highest fruit girth (7.82 cm), fruit length (10.55 cm), number of fruits (19.86), average fruit weight (37.14 g plant^{-1}) and fruit yield (787.71 g plant^{-1}) was recorded in sandy loam (S_1). Among the composts application yield attributes like fruit girth (8.1 cm), fruit length (11.13 cm), number of fruits (22.12), average fruit weight (40.84 g plant^{-1}), fruit yield (904.16 g plant^{-1}) was registered in treatment T_3 (75% RDF + Seasoned Pressmud @ 12.5 t ha^{-1}). With the interaction of two types of soil and compost application the maximum was recorded in treatment S_1T_3 (75% RDF + Seasoned Pressmud @ 12.5 t ha^{-1} in neutral soil) registered highest fruit girth (9.09 cm), fruit length (11.71 cm), number of fruits (23.62), average fruit weight (41.22 g plant^{-1}) and fruit yield (973.62 g plant^{-1}).

The number of fruits and single fruit weight are the most important traits in determining the yield and these traits were greatly influenced by the application of certain organics and pressmud. It was observed that taller plants with more number of branches, increased photosynthetic area and favourable physiological activities under higher nutrient levels could have resulted in more production and translocation of photosynthates in plants, which accelerated the formation of more number of large sized fruits resulting in higher yields. These results are in conformity with those of Ghulam *et al.* (2012).

Quality parameters

An appraisal of data tabulated in Table 6 represented that sandy loam soil (S_1) recorded highest quality attributes of chlorophyll SPAD (46.29), Total Soluble Solids (4.3), Titratable Acidity (0.2662%) and Ascorbic acid content (12.54 mg 100 g^{-1}) in Sandy loam soil (S_1). Among the composts application of 75% RDF + Seasoned Pressmud application of 12.5 t ha^{-1} (T_3) registered maximum (52.71), Total Soluble Solids (4.26°B), titratable acidity (0.276) and Ascorbic content (12.42 mg 100 g^{-1}). There is no significant different with interaction effect in quality parameters. The chlorophyll content was found to be higher due to application of Seasoned pressmud @ 12.5 t ha^{-1} in both soils and is likely due to NPK contents in the sugarcane pressmud, which was associated with chlorophyll synthesis (Zhou *et al.,* 2012). The quality parameters like total soluble solids is also increased with the compost application which is only due to higher content of metabolites in fruit accumulation of reserve substances in fruit (Singh *et al.,* 2013). The increase in acidity was reported by Mahmud *et al.* (2018).

CONCLUSION

It is concluded that the treatment consisting of 75% RDF + Seasoned Pressmud @ 12.5 t ha^{-1} recorded the highest plant height, no. of leaves plant^{-1}, no. of branches plant^{-1}, LAI, yield attributes, fruit yield and quality attributes in sandy loam soil. The application of seasoned pressmud supplementation with in organics was found to be superior in all parameters of brinjal.

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Table 1. Composition of organic manures

Nutrient (%)	FYM	Seasoned Pressmud	Municipal Solid WasteCompost	Poultry Manure	Calotropis Compost	Vermicompost	References
N	0.5	1.525	0.07	3.03	2.06	1.68	Jackson (1973)
P	0.08	1.09	0.02	1.19	0.24	1.06	Page *et al.* (1982)
K	0.91	0.99	0.32	1.16	0.15	1.30	Jackson (1973)

Table 2. Physico-chemical properties of initial soil

S. No.	Parameters	Neutral soil	Coastal saline soil	Methods Employed
A	Physical properties			
1.	Mechanical Analysis			
	Sand (%)	65	76	
	Silt (%)	28	19	
	Clay (%)	6	4	
	Textural class	Sandy loam	Sandy	Bouyoucos (1962)
	Taxonomical class	*Typic Ustifluvents*	*Typic Ustipsamments*	
2	Bulk density (Mg m^{-3})	1.50	1.55	Soil Survey Staff (1966)
3	Particle density (Mg m^{-3})	2.62	2.61	Soil Survey Staff (1966)
B	Chemical Properties			
4	pH	7.2	8.4	Jackson (1973)
5	EC (dSm^{-1})	0.75	4.2	Bower and Wilcox (1965)
6	Organic carbon (g kg^{-1})	4.7 (Medium)	0.99 (Low)	Walkley and Black (1934)
7	CEC [(cmol (p+)) kg^{-1}]	16.8	2.9	Chapman (1965)
8	KMnO$_4$–N (kg ha^{-1})	159.6 (Low)	103.6 (Low)	Subbiah and Asija (1965)
9	Olsen–P (kg ha^{-1})	50 (High)	23 (High)	Olsen *et al.* (1954)
10	NH$_4$OAC-K (kg ha^{-1})	135.5 (Medium)	197 (Medium)	Hanway and Heidel (1952)
11	DTPA-Fe (mg kg^{-1})	25.13 (Sufficient)	19.13 (Sufficient)	Lindsay and Norvell (1978)
12	DTPA-Mn (mg kg^{-1})	12.56 (Sufficient)	9.56 (Sufficient)	Lindsay and Norvell (1978)
13	DTPA-Zn (mg kg^{-1})	0.28 (Deficient)	0.22 (Deficient)	Lindsay and Norvell (1978)
14	DTPA-Cu (mg kg^{-1})	1.53 (Sufficient)	0.55 (Sufficient)	Lindsay and Norvell (1978)
15	Hot water soluble B (mg kg^{-1})	0.47 (Deficient)	0.32 (Deficient)	Hatcher and Wilcox (1950)

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Table 3. Methods of quality parameters analysis

S. No.	Parameters	Methodology	References
1	Chlorophyll	SPAD-502 meter	Konica Minolta (2012)
2	Total Soluble Solids (°B)	Refractometer method	AOAC Official Method (2007)
3	Titratable acidity	Potentiometric method	Manual of Methods of Fruits, Vegetable Products (2015)
4	Ascorbic acid	Volumetric method	Sadasivam and Manickam (2008)

Table 4. Effect of composts on Plant height, no. of leaves plant^{-1}, no. of branches plant^{-1}, leaf area (cm^2) and LAI at harvest

Treatment	Plant height (cm)			No. of leaves plant^{-1}			No. of branches plant^{-1}			Leaf area (cm^2)			LAI		
	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean
T$_1$	72.1	67.1	69.4	67.0	61.0	64.0	11.2	8.5	9.9	52.01	47.19	49.60	0.961	0.791	0.87
T$_2$	105.3	97.1	100.9	71.0	65.0	68.0	12.5	9.7	10.4	55.29	49.29	52.30	1.082	0.882	0.98
T$_3$	113.9	102.3	107.7	87.0	67.0	77.0	13.4	10.3	12.3	58.39	55.92	55.39	1.901	0.951	1.18
T$_4$	102.3	95.1	98.4	69.0	64.0	66.5	11.7	9.0	11.0	53.73	49.28	51.29	1.022	0.861	0.941
T$_5$	108.1	99.3	103.4	83.0	66.0	74.5	14.0	10.5	11.9	56.79	51.30	53.79	1.30	0.922	1.11
T$_6$	101.7	98.9	101.9	73.0	65.0	69.0	12.6	9.8	11.2	55.31	48.48	52.00	1.13	0.911	1.021
T$_7$	103.9	95.8	99.5	70.0	65.0	67.5	12.4	9.5	11.1	55.00	48.48	51.63	1.06	0.872	0.96
Mean	101.0	93.65	97.3	74.2	64.7	69.5	12.6	9.6	11.1	55.32	49.29	52.28	1.311	0.884	1.011
	S	T	S×T	S	T	S×T	S	T	S×T	S	T	S×T	S	T	S×T
S.Ed.	0.3	0.5	0.8	0.2	0.4	0.5	0.03	0.07	0.1	0.16	0.30	0.42	0.006	0.012	0.017
CD (P=0.05)	0.8	1.1	1.6	0.4	0.8	1.1	0.08	0.15	0.2	0.32	0.61	0.87	0.013	0.024	0.034

Table 5. Effect of composts on fruit girth, fruit length, No. of fruits plant^{-1}, Average fruit weight plant^{-1} and Fruit yield g plant^{-1} in brinjal

Treatment	Fruit girth (cm)			Fruit length (cm)			No. of fruits plant^{-1}			Average fruit weight (g plant^{-1})			Fruit yield (g plant^{-1})		
	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean
T$_1$	7.16	6.95	7.06	9.75	9.56	9.55	17.30	15.12	16.11	37.96	35.96	36.96	657.11	543.73	600.92
T$_2$	7.64	7.36	7.21	10.46	10.05	10.25	19.50	16.90	18.20	38.76	36.56	37.66	750.25	613.13	684.69
T$_3$	9.09	7.75	8.10	11.71	10.55	11.13	23.62	28.63	22.12	41.22	40.56	40.84	973.62	834.69	904.16
T$_4$	7.44	7.05	7.39	10.05	9.76	9.90	18.31	16.11	17.21	38.36	36.16	37.26	704.66	582.92	642.79
T$_5$	8.63	7.66	8.37	11.23	10.36	10.79	21.01	18.03	19.52	41.09	37.36	39.23	866.59	673.60	770.09
T$_6$	7.61	7.46	7.94	10.52	10.12	10.32	20.41	18.01	19.21	40.35	37.16	38.76	538.83	729.10	783.97
T$_7$	7.53	7.25	7.46	10.17	9.96	10.06	18.91	15.91	17.41	38.56	36.36	37.96	741.59	578.86	660.23
Mean	7.82	7.36	7.594	10.55	10.05	10.30	19.86	19.81	18.56	39.41	37.41	38.31	787.71	691.33	714.38
	S	T	S×T	S	T	S×T	S	T	S×T	S	T	S×T	S	T	S×T
S.Ed.	0.023	0.048	0.0623	0.03	0.05	0.08	0.05	0.10	0.15	0.11	0.21	0.30	4.38	8.19	11.58
CD (P=0.05)	0.044	0.090	0.127	0.06	0.12	0.14	0.11	0.22	0.31	0.23	0.44	0.62	8.97	16.78	23.74

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Table 6. Effect of composts on chlorophyll SPAD of brinjal of leaf, Total Soluble Solids, Titratable acidity and Ascorbic acid on brinjal fruit

Treatment	Chlorophyll SPAD value			Total Soluble Solids (°B)			Titratable acidity (%)			Ascorbic acid (mg 100 g⁻¹)		
	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean	Normal soil (S$_1$)	Coastal saline soil (S$_2$)	Mean
T$_1$	42.59	40.79	41.69	4.1	3.7	4.21	0.246	0.226	0.236	12.08	11.00	11.54
T$_2$	43.88	42.19	42.97	4.3	3.9	4.06	0.273	0.246	0.260	12.56	11.47	12.01
T$_3$	54.37	51.07	52.71	4.5	4.1	4.26	0.286	0.266	0.276	12.93	11.92	12.42
T$_4$	42.89	41.79	42.34	4.2	3.8	4.21	0.256	0.236	0.246	12.39	11.20	11.79
T$_5$	52.00	50.19	51.69	4.4	4.0	4.16	0.276	0.256	0.270	12.70	11.51	12.10
T$_6$	45.17	42.99	44.07	4.3	3.9	4.11	0.260	0.246	0.256	12.62	11.42	12.01
T$_7$	43.16	41.79	42.48	4.2	3.8	4.0	0.256	0.236	0.246	12.50	11.33	11.91
Mean	46.29	44.38	45.33	4.3	3.9	4.17	0.266	0.246	0.256	12.54	11.91	11.97
	S	T	S×T	S	T	S×T	S	T	S×T	S	T	S×T
S.Ed.	0.12	0.23	0.33	0.01	0.03	0.09	0.002	0.003	0.005	0.03	0.05	0.07
CD (P=0.05)	0.26	0.49	NS	0.03	0.06	NS	0.009	0.008	NS	0.06	0.11	NS

RESEARCH ARTICLE

Green Synthesis and Characterization of Zinc Oxide Nanoparticles using *Eclipta prostrata* Flower Extract as Reducing Agent

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ABSTRACT

Eclipta prostrata (Asteraceae)   a traditional herbal medicine plant commonly known as false daisy, karisalankanni and bhringaraj is widely used in different regions of India for the treatment of skin problems, hepatic problems such as jaundice, gastrointestinal problems, respiratory problems such as asthma etc. Zinc oxide is an n-type semiconducting metal oxide, with a large band gap (3.37 eV) and high excite on binding energy (60 meV)   exhibits a large number of semiconducting properties like high catalytic activity, optic, UV filtering properties, anti-inflammatory, wound healing etc. Among the inorganic NPs, ZnO nano particles are of particular interest because they can be prepared easily and inexpensively, safe material for human beings and animals. The objective of the present study is to design alternative, safer, energy efficient and less toxic routes towards synthesis of zinc oxide nano particles from locally available plant resources. *Eclipta prostrata* extract mediated synthesized Zno Nps were characterized using several techniques such as SEM-EDX, FT-IR and UV–Vis. UV-Vis absorption peak at 349.5 nm confirmed the presence of ZnO in the nano scale. FTIR spectrum indicated the involvement of different functional activities in the reduction of ZnO NPs. FT-IR studies showed an absorption peak at 601.79, 555.50 and 509.21 cm-1 (Zn-O linkage) which indicated the formation of zinc oxide nano particles. SEM analysis of ZnO NP established the the morphology of the samples. EDX analysis result disclosed the presence of metallic zinc oxide in biosynthesized ZnO NPs.

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INTRODUCTION

Eclipta prostrata commonly known as false daisy, karisalankanni and bhringaraj is a species of plan in the sunflower family. It is a common plant and abundantly grows throughout India up to 6000 feet height of hills [1-4]. *Eclipta prostrate* (Figure 1) is a traditional herbal medicine has been long used in Asia and south America for the therapy of haemorrhagic diseases(e.g, hemoptysis, Hematesis, hematuria, epistaxis and Uterine bleeding), skin diseases, respiratory disorders, coronary heart disease, hair loss, viltligo, snake bite and those caused by the deficiency of liver and kidney [5-7]. Externally, the plant is used as an oil to treat hair loss and it is applied to athletes' foot, eczema, dermatitis, wound etc [8]. It is applied to cuts, bruises and sores in order to stop bleeding and relieve pain [9]. The extract of the plant has the ability to act as an antidote for snake venom [10-11]. It also regards as the best remedy for hair problem in Ayurvedic medicines and act as haematinic, diuretic and anthelmic [12]. This plant is widely used in different regions of India for the treatment of skin problems, hepatic problems such as jaundice, gastrointestinal problems, respiratory problems such as asthma and other symptoms such as fever, hair loss and whitening of hair, cuts, and wounds, spleen enlargement, etc [13-14].

Zinc oxide is an inorganic compound with formula ZnO (Figure 2) Zinc oxide has use in semiconductors, concrete use, ceramic and glass composition. It is an n-type semiconducting metal oxide, had large band gap (3.37 eV) and high excite on binding energy (60 meV) so it exhibit a large number of semiconducting properties like high catalytic activity, optic, UV filtering properties, anti-inflammatory, wound healing [15]. Zinc oxide Nano particles (NP) have drawn interest in recent years due to its wide range of applicability in the field of electronics, optics, and biomedical systems. Many of the metal oxides like Fe_3O_4, TiO_2, CuO are used for nano particle synthesis of all these metal oxides, ZnO NPs is of maximum interest because they are easy to produce, low production cost, safe and can be prepared easily .US FDA has enlisted ZnO as GRAS (Generally Recognized As Safe) metal oxide.

Although ZnO is used for targeted drug delivery, it still has the limitation of cytotoxicity that is yet to be resolved. ZnO NPs have a very strong antibacterial effect at a very low concentration of gram negative and gram-positive bacteria as confirmed by the studies, they have shown strong antibacterial effect than the ZnO NPs synthesized chemically[16-17]. ZnO NPs have a large surface area-to-volume ratio that results in a significant increase in the effectiveness in blocking UV radiation as compared to the bulk material [18-19]. Metal oxide nano particles have environmental applications as it can act as catalyst that is helpful in reduction or elimination of the toxic hazardous chemicals from environment [20-21].

The objective of the present study,
i) To design alternative, safer, energy efficient and less toxic routes towards synthesis of zinc oxide nano particles from locally available plant resources.
ii) To develop a novel approach for the bio nano-synthesis of zinc oxide nano particles using *Eclipta Prostrata* extract, zinc nitrate as the zinc precursor, without any surfactant and reducing agent.
iii) To characterize the synthesized zinc oxide nano particles using UV-Visible , FT-IR and SEM – EDX analyses.

MATERIALS AND METHODS

Collection of *Eclipta prostrata*
The yellow flower of *Eclipta prostrata* were collected from Ramapattinam Pudur, Palani, Dindigul District, South India in January 2021.The taxonomic identification was authenticated by Department of Botany, Arulmigu Palaniandavar College of Arts and Culture, Palani and they dried in dark at room temperature for one week.

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Preparation of plant extract

Eclipta prostrata flowers were washed several times with double distilled water to remove any debris or particulates then shade dried at room temperature. The aqueous extract was prepared by the cold maceration method [22]. The plant powder (50 g) was soaked in 1 L of distilled water, kept in a shaker at 20 °C for 24 h for continuous agitation at 100 rpm for thorough mixing. Then the extract was filtered and stored for further investigations.

Plant mediated synthesis of ZnO nano particles

Green synthesis of zinc oxide nano particles were done with *Eclipta prostrata* yellow flower extract.

Preparation of *Eclipta prostrata* Flower extract

10% of extract was prepared (by dissolving 10 gm in 100ml) in the distilled water. This extract was incubated at 40^0C for 24hrs in the rpm of 60 – 70 in an orbital shaker. After incubation, the extract was filtered through Whatmann No.1 filter paper and used for further study.

ZnO Synthesis from plant extract

For the synthesis of ZnO nano particles, 50ml of 0.5M zinc nitrate solution was prepared using distilled water.1ml of aqueous yellow flower extract was introduced into the above solution after 10 minutes stirring. In order to maintain the pH 12 KOH solution was added drop by drop, which resulted in a pale yellow aqueous solution .This was then placed in a magnetic stirrer for 2 hrs. The pale yellow precipitate was then taken out and washed repeatedly with distilled water and then with ethanol to remove impurities. Then a pale white powder of ZnO nano particles was obtained after drying in oven [23].

Characterisation of nano particles of plant mediated synthesis [24]

Characterization of nano particles of plant-mediated synthesis was done by different methods. UV-Visible spectral analysis was used to analyse the absorbance. Fourier Transform Infra Red spectroscopy (FT-IR) gives specific signals for NPs and shape of the nano particle. Scanning Electron microscopy-EDAX analysis was used to topography and roughness of nano particles characterized.

UV –Visible

UV-visible spectroscopy is usually conducted to confirm the synthesis of ZnO NPs peaks that studied in the range of 200-500 nm .

FTIR

Specific signals obtained by IR spectroscopy according to the vibrations of the molecule. FTIR spectra and functional group involved in ZnO NPs synthesis illustrated peak in the range of $400\text{-}4000cm^{-1}$.The sample pellet was placed into the sample holder and FT-IR spectra were recorded.

SEM-EDX

SEM analysis is used to visualize shape and size of nano particles. Scanning electron microscope was adjusted in different magnification and used to determine the shape of ZnO NPs.SEM images in different magnification ranges can be evaluated [25].

RESULT AND DISCUSSION

ZnO NPs have attracted great attention because of their superior optical properties and its applications in different fields like medicine, agricultural etc., and visual color change is the preliminary test for nano particle synthesis. The results of the present study of Zinc oxide nano particles synthesized from *Eclipta prostrata* yellow flower extract are presented below.

Plant mediated synthesised ZnO NPs (ZnO NP-EP)
The use of green synthesis method by the researchers is rapidly increasing due to usage of less toxic chemicals, eco-friendly nature and one-step synthesis of nano particles. The biological system involved in the green synthesis of nano particles is plants and their derivatives, microorganisms as if bacteria, fungi, algae, yeast [24]**.** Here the synthesis was done with the flower extract of *Eclipta prostrata,* a locally available flowering plant in the species of Asteraceae. The results of the visual observations of ZnO NPs synthesized using flower extract of *Eclipta prostrata* extract shows a colour change from half-white to pale yellow ,after drying for long time the colour again changed to pale white as shown in Figure 3. The yield of product was 200mg.

Optical characterization
UV –visible analysis
UV–VIS absorption spectrum of ZnO NP synthesized by chemical reduction method using 0.1% starch as stabilizing agent in the range of 200-500 nm. Peak obtained at 349.5 nm clearly demonstrates the presence of ZnO NPs in the reaction mixture [25] was shown in Figure 4. This characteristic band may be attributed to the intrinsic band – gap absorption of ZnO nano particles owing to the e^- transition from the valence band to the conduction band (O2p$\rightarrow$Zn 3d).

Recent studies highlight that ZnO NPs had intensive absorption in the ultraviolet band of about 300–700 nm. In addition, it has also been reported that the presence of secondary metabolites in plants reduces zinc ions in the solution to zinc oxide and also act as stabilizing agent. For ZnO nanoparticles, the absorbance peak was reported between 310 nm and 360 nm of wavelength [26].

Surface characterization
FT-IR analysis
FT-IR Spectral studies provide information regarding chemical bonding between Zn and O. Figure 5 represents FT-IR spectra of the synthesized ZnO nano particles in the range 400–4000 cm^{-1}. The broad band observed at 3276.70 cm^{-1} corresponds to O–H stretching of phenolic compounds [27-28]. The C=O stretching group presence was attributed to 1643.35 cm^{-1} and the band at 1095.57 cm^{-1} corresponds to C–N bonds in PO$_3$ stretching and the multiple sharp bands at 447.49 cm^{-1} , 419.62cm^{-1} ,401.19 cm^{-1} may be attributed to the presence of Zn–O stretching bands [29-30] . Peak obtained at 601.79, 555.50 and 509.21 demonstrated the probable presence of C- Alkyl chloride and Hexagonal phase ZnO [31].

FT-IR spectral peak of ZnO –EP indicate that plant metabolites such as alkaloids , carboxylic acids and flavanoids [25] are bound to the surface of ZnO NP and these compounds are responsible for the reduction of Zn ions to ZnO NP. Further, the stability of the ZnO NP is expected to be presence of free amino acids and carboxylic groups that have interacted with the Zn surface.

SEM- EDX analysis
SEM result demonstrated that (Figure 6) the aqueous flower extract of *E. prostrata* was an effective reducing agent for the formation of ZnO NPs alongside a hexagonal phase with a Wurtzite structure. More recently, reported a facile and eco-friendly synthesis of ZnO NPs employing Bauhinia *tomentosa* leaf extract with a hexagonal morphology observed and a size range of 22–94 nm [31]. The energy dispersive X-ray spectroscopy (EDX) spectroscopy of synthesized ZnO NPs depicted the chemical components present in the sample. EDX analysis showed 82% of zinc and 18% of oxides, which confirmed the elemental composition of ZnO NPs. EDX analysis result showed (Figure 7) the presence of metallic zinc oxide in biosynthesized ZnO NPs. The composition obtained from EDAX analysis was Zinc (74.6%), Oxygen (23.46%), and Carbon (2.29%). The presence of carbon in trace amount indicates the involvement of plant phyto chemical groups in reduction and capping of the synthesized ZnO NPs [32].

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All these studies successfully revealed that the use of plants allow for the noncomplex, environmentally friendly, and cost-effective production of valuable ZnO NPs with antimicrobial properties. These green ZnO NPs displayed a comparative advantage over traditionally synthesized ZnO NPs offering a cheap, alternate method of synthesis.

CONCLUSION

The study conclusively reported eco friendly approached for synthesize of ZnO nano particles. A novel approach for one pot biosynthesis of ZnO nano particles using *Eclipta prostrata* extract has been demonstrated. *Eclipta prostrata* extract mediated synthesized Zno Nps were characterized using several techniques such as SEM-EDX, FT-IR and UV–Vis. The UV-Vis absorption peak at 349.5 nm confirmed the presence of ZnO in the nanoscale. FTIR spectrum indicated the involvement of different functional activities in the reduction of ZnO NPs. The FT-IR studies showed an absorption peak at 601.79, 555.50 and 509.21 cm^{-1} (Zn-O linkage) which indicated the formation of zinc oxide nanoparticles. SEM analysis confirmed the ZnO's size and structure. EDX analysis result showed that the presence of metallic zinc oxide in biosynthesized ZnO NPs .

Biosynthesized ZnO nano particle prepared from *Eclipta prostrata flower* extract are expected to have notable applications in pharmaceutical bio medical fields such as drug delivery and in paints and cosmetics industries etc. A variety of plant species and plant parts have been successfully extracted and utilized for the synthesis of NPs since they contain a wide range of metabolites, including carbohydrates, alkaloids, terpenoids, phenolic compounds, and enzymes. Considering the promise of nanotechnology applications in everyday life, more research is warranted to unravel the long-term chemical and physical properties of biosynthesized nano particles.

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Table.1 Vernacular names of *Eclipta prostrata*

Languages	Common Names
English	Bhringaraj
Hindi	Madhukar
Assamase	Kesaraja
Malayalam	Kaikeshi
Marathi	Bhringaraj
Sanskrit	Kesharanjana
Tamil	Karisilaanganni
Gujarati	Bhangara
Oriya	Kesarada
Kannada	Garagadasappu

Figure 1. Flower of *Eclipta prostrata* (Karisilaanganni)

Figure 2. Image of Zincite
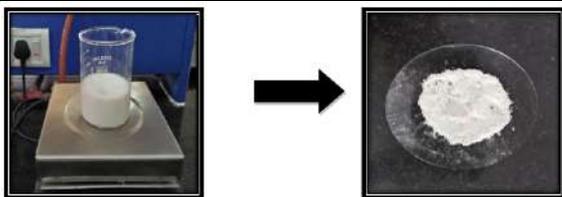
Figure 3. Plant mediated synthesised ZnO NPs (ZnO NP-EP)
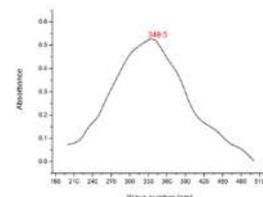
Figure 4. UV-Visible spectrum of *Eclipta prostrata* extract mediated synthesized Zno Nps
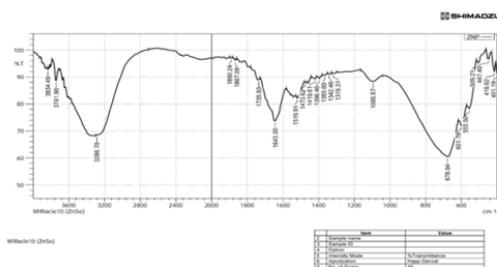
Figure 5.FT-IR image of *Eclipta prostrata* extract mediated synthesized ZnO NPs
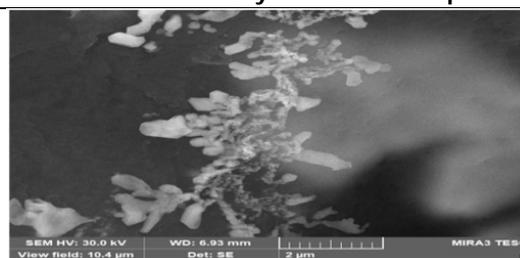
Figure 6. SEM image of *Eclipta prostrata* extract mediated synthesized Zno nanoparticle
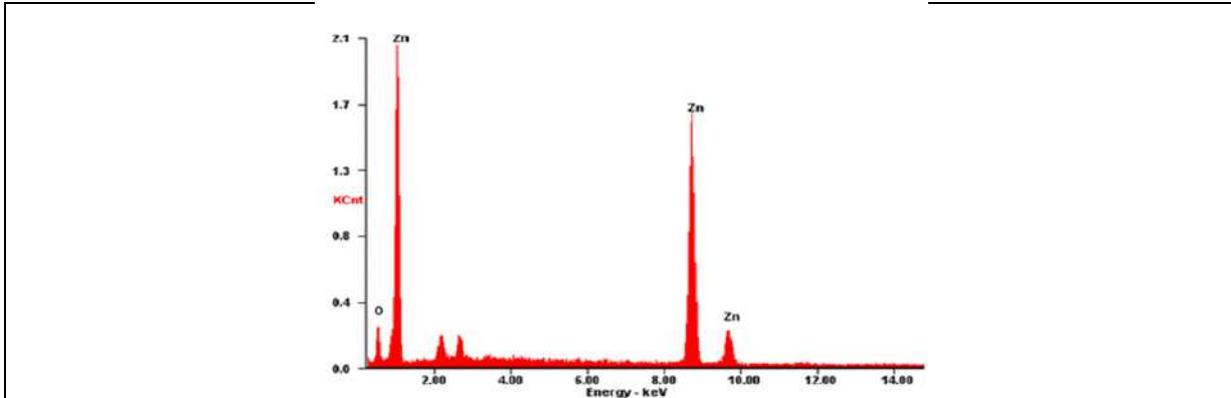
Figure 7. EDX analysis showing the chemical composition of *Eclipta prostrata* extract mediated synthesized Zno Nps

RESEARCH ARTICLE

Proficient and Innovative Internet Based Education Framework through Massive Open Online Courses (MOOCs)

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ABSTRACT

Massive open online courses (MOOCs) are a free online distance learning program that is intended for enormous quantities of topographically scattered understudies. Massive because enlistments are limitless and can run into many thousands. Open since anybody can select that is, there is no affirmation cycle. Online considering the way that they are passed on through the web. Course on the grounds that they will likely show a particular subject. MOOCs commonly contain pre-recorded video examples, readings, appraisals, and conversation gatherings. At long last, some quit being offered altogether. Some MOOCs are independent that progress through them as fast or gradually as required while others run on a timetable. All the course material may not be accessible from the very beginning. All things considered, it is delivered in pieces after quite a large number of weeks, driving understudies to find a steady speed. Evaluations might have cutoff times, keeping understudies away from holding up behind. In any case, in any event, when they include a timetable, MOOCs stay adaptable, may concentrate on when it suitsgreatest, day or night. Ordinarily, each MOOC will incorporate a course supplier and a course stage. A course provider is every now and again a school, which supplies the course materials and educators. In this paper the familiar MOOCs like EdX, Canvas, Coursera, Udacity, Udemy with their benefits, foundation to course modules, client access and other learning assets are discussed.

Keywords: MOOCs, online courses, innovation, modules, modern education.

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INTRODUCTION

The abbreviation of MOOC (fig. 1) represents Massive Open Online Course. And keeping in mind that those words together may seem like an undeniable kind of online schooling, every one of these words is quite loaded with regards to imagining exactly what MOOCs may involve educationally, innovatively, etc. Open specifically is deciphered in numerous ways open as in open enlistment, transparently authorized substance, or open-endedness. MOOCs are a sort of online course that are in their beginning phases of development and are probably going to keep creating later on. They will presumably shape an extremely durable piece of the training scene however they appear far-fetched to altogether disturb numerous parts of regular advanced education.

FEATURES
- ✓ Course members are probably going to appropriated everywhere.
- ✓ Course content is not situated in any one spot, yet tracked down all around the web.
- ✓ The web-based homeroom is one of numerous centers where collaboration happens, which can likewise incorporate individual sites or portfolios, sites, informal communication destinations, and that's just the beginning.
- ✓ Members and teachers total, remix and reuse the substance during the course.
- ✓ The courses don't have explicit necessities; however, members are needed to keep awake to date with unpleasant timetables.
- ✓ Most MOOCs are free; there might be a charge in the event that the member is running after a type of certification.

VARIOUS MOOC TYPES

xMOOC

The most widely recognized kind of MOOC, coordinated around a focal teacher and main subjects of predefined learning materials.

cMOOC

"Availability" MOOCs look like alumni workshop courses; course materials give a beginning stage to understudy conversations with the center of the gaining coming from understudy to-understudy communications.

DOCC

Distributed Online Collaborative Courses will be courses in which a similar course material is dispersed to understudies at numerous foundations, yet the specific organizations of the material can shift. Understudies can likewise draw in with one another across establishments by means of the web-based part.

BOOC

Big Open Online Courses are like MOOCs however restricted to fewer understudies.

SMOC

Synchronous Massive Online Courses contrast from x MOOCs in that the talks are communicated in real time expecting understudies to sign in at explicit occasions to hear the talks.

SPOC

Small Private Online Courses are like BOOCs, in that the class sizes are restricted, yet the understudy's instructor cooperation's are all the more firmly displayed after customary study hall collaborations. SPOCs are likewise referred to in the "flipped study hall" model.

Corporate MOOCs

MOOC courses intended for representative preparing or proceeding with instruction commonly sponsored or interestingly certify by managers.

edX

edX (fig.2) is an American massive open online course (MOOC) supplier made by Harvard and MIT. It has online college level courses in a wide scope of disciplines to an overall understudy body, including a few courses at no charge. It additionally directs investigation into learning in view of how individuals use its foundation. edX runs on the free Open edX open-source programming stage. edX courses comprise of week by week learning groupings. Each learning grouping is made out of brief recordings mixed with intelligent learning works out, where understudies can quickly rehearse the ideas from the recordings. The courses frequently incorporate instructional exercise recordings that are like little nearby conversation gatherings, a web-based reading material, and an internet-based conversation discussion where understudies can post and audit questions and remarks to one another and educating aides. edX courses are by and large comprised of week-by-week modules with pre-recorded recordings that watch on a timetable or at our own speed. There are supplemental readings and understudy conversation discussions, just as schoolwork tasks and appraisals like short tests or tests.

BENEFITS

Expanded attractiveness

Add new abilities and authentications to resume to build appeal to future bosses.

On time

Dates are accessible all through the year to ensure have different choices and adaptability.

No movement important

Never again need to leave loved ones or get some much-needed rest work to go to on location, more costly courses and gatherings.

More reasonable

Most courses are for a portion of the expense of their on-location counterparts.

Added esteem

A large number of our courses accompany Continuing Education Units (CEUs), affirmations, unmistakable items and all the more all notwithstanding an institutional marked declaration

CANVA

Canva (fig.3) is a course the executive's framework that upholds internet learning and instructing. It permits teachers to post grades, data, and tasks on the web. Material gives a secret phrase ensured internet-based study hall in which submit work and speak with educator and colleagues. This cooperative apparatus permits understudies to share their work and solicitation input from their cohorts. Understudies can utilize Canva to develop and show information. They can likewise catch photographs or recordings of their group work, schoolwork, tasks, school exercises, post those in their own record, or a class account, and have conversations about the posts. Educators can utilize Canva to configuration outwardly engaging info graphics, banners, and pictures to supplement text-weighty growth opportunities. Canva for Education is a strong plan instrument, permitting the understudies to make outwardly dazzling plans for any subject or age level. Understudies can make their own plans without any preparation, or browse our library of more than 50,000 superior grade, instructive layouts. From introductions, banners, and info graphics, to worksheets, project-based learning, and illustration designs, the conceivable outcomes are huge. Probably the best thing about Canva is that it permits us to take visual figuring out how to another level: Students and educators can turn into plan scholars, which means we can become gifted at envisioning and making

answers for issues rather than recognizing existing ones. Learning and educating occur as we imagine, make, offer, and overhaul. All we want are the right apparatuses for the gig.

FEATURES
- ✓ Practice configuration thinking
- ✓ Configuration own example plan
- ✓ Find other teachers' example plans
- ✓ Assist understudies with making learning portfolios
- ✓ Make introductions and recordings
- ✓ Assemble a brand for homeroom
- ✓ Make bunch projects really engaging
- ✓

COURSERA

Coursera (fig. 4) is a web-based learning stage established by two Stanford University software engineering teachers. It offers great many web-based courses in organization with north of 200 of the world's driving colleges and organizations, including Yale, Princeton, U Penn, Google, IBM, Amazon, Face book, and the sky is the limit from there. Coursera courses last roughly four to twelve weeks, with one to two hours of video addresses seven days. These courses give tests, week by week works out, peer-evaluated and audited tasks, a discretionary Honors task, and in some cases a last venture or test to finish the course. Courses are additionally given on-request, in which case clients can require some investment in finishing the course with all of the material accessible on the double. The site offers individual courses just as lone wolf's and graduate degree programs that decrease obstructions to advanced education. There are likewise proficient testament programs intended to help laborers in getting new jobs or advancements. The short courses have a week after week timetable of a few recordings and readings, trailed by at least one tests for the week. The recordings have supportive slides and notes that further clarify the substance of the video examples. Alongside the video illustration, there is additionally a full video record accessible in numerous language choices such German, Chinese and some more. However, everything is obviously clarified, there is a ton of data that should be absorbed each week. This is particularly so on the grounds that large numbers of the Coursera courses begin from semester-long college examples at a portion of the world's driving colleges. Along these lines, learning on Coursera and effectively finishing the courses will surely will take a lot of discipline and consistency over the whole length of the course.

FEATURES
- ✓ Joined forces With Educational Institutions
- ✓ Huge Catalog of Courses
- ✓ Gain From Anywhere
- ✓ Reasonable Prices
- ✓

BENEFITS
- ✓ The courses are all around organized and the substance is of exceptionally excellent from presumed establishments and organizations.
- ✓ There is support for some European and Asian dialects in the video records and captions. This will permit more individuals to profit from Coursera.
- ✓ Coursera course suggestions and course content have a reasonable profession center and empower students to give the abilities they create in Coursera something to do in their vocation.
- ✓ There are various delicate updates while advancing through the course to progress forward to the following illustration or test. This is propelling and assists students with pushing ahead with the impending examples and recordings.

✓ Monetary guide is promptly accessible for lower-pay students from a portion of the emerging countries. However, this requires around fourteen days for endorsement, there is no limitation concerning the number of understudies can apply for monetary guide per course.

UDACITY

Udacity (fig. 5) is an internet-based schooling supplier that offers online courses, famously known as MOOCs or Massive Open Online Courses. When contrasted with other MOOC suppliers, Udacity has a more grounded center around work preparing. Udacity offers a miniature certification called Nanodegree. Now and again the courses that are important for Nanodegrees are delivered free of charge. Udacity is a web-based learning stage that trains understudies for sought after, innovation-based vocations. It offers both short internet-based courses and Nanodegree programs in computerized reasoning, independent frameworks, business, distributed computing, online protection, information science, and programming. Nanodegrees are centered around popular abilities by and large in the area of innovation. They comprise of video courses just as undertakings.

A portion of these courses and Nanodegrees are created in organization with colleges and industry specialists like Georgia Tech and Google. Udacity is the fourth most famous MOOC supplier on the planet in light of the quantity of understudies and has a functioning inventory of around 150 internet-based courses.

FEATURES
✓ Udacity Nanodegree
✓ Udacity Nanodegree Plus
✓ Udacity Pricing
✓ Paid Courses
✓ Free Courses

BENEFITS
Content Quality
Nothing is a higher priority than the nature of content in making an internet learning stage and this part is the place where Udacity sparkles. Udacity's substance is custom fitted to address the issues of their understudies. They're breathtaking and very much organized in a supportive organization for dynamic students.

Coach Quality
Udacity ensures that a positive growth opportunity from industry specialists. The guides are drawing in and proficient and will help continue going on the way of learning. Furthermore, example materials are likewise evolved by specialists from huge names like Facebook, Google, Amazon, and GitHub.

Project-based Approach
At Udacity, learn through genuine activities. Udacity gives total help with the activities by explicitly telling to do in a bit-by-bit design. At last, these undertakings can be an exceptionally applicable portfolio that can pitch to expected businesses.

Associations
Innovative courses are created in association with notable tech organizations like Google, Facebook, Mercedes Benz, and World Quant. This multitude of courses are trailed by one-on-one scholarly tutoring and genuine ventures.

Profession Section
This recently added segment offers an incredible profession arrangement. In each progression of the gig hunting process there are numerous steady assets, for example, continue creation apparatuses, introductory letter editors, practice meetings and explicit inquiries questions. Now and again, they can set up 1 on 1 meetings for to cooperate with tutor.

DRAWBACKS
Cost
Udacity's costs are not actually modest for an internet learning stage. Thus, Udacity courses are generally for business people or workers who look to grow their abilities and information, rather than fledglings or specialists.

Profoundly Focused
The pertinence of the substance is a blade that cuts both ways. The instruments and subjects that the stage offers are unmistakable

Testament
The testaments that Udacity offers look pretty just, they are very simple to counterfeit. This is the sort of thing that the organization should address, particularly given the expenses required for understudies.

UDEMY
Udemy (fig.6) is a web-based learning stage that proposals north of 130,000 individual web-based courses and can be an incredible choice and keen on an oddball course in a particular subject or range of abilities. The stage's contributions cover classes like turn of events; business; finance and bookkeeping; IT and programming; office efficiency; self-improvement; plan; advertising; way of life; photography and video; wellbeing and wellness; music, and educating and scholastics.

FEATURES
✓ Experts hoping to acquire abilities
✓ Individuals searching for a lifelong change
✓ Understudies hoping to supplement their studies
✓ Those searching with the expectation of complimentary courses
✓ People hoping to distribute their own courses
✓ Global understudies searching for courses not simply in the English language

BENEFITS
Up skilled Workforce
Recognizes staff with the right capacities and explores the consistently changing obligations in the cutting-edge working environment. Gives a simple, reasonable ability redesign for associations with skill holes. Courses are ability based and educated by specialists.

Culture of Learning
Instructs and prepares workers with an assortment of independent classes. This permits an association to advance schooling reasonably. Permits organizations to give additional time and concentration to advancement amazing open doors. Makes a culture of persistent improvement.

Expanded Efficiency
Permits laborers to further develop abilities and become more productive at their particular employment. Expanded computerization levels make it an enormous time and cash saver, particularly given the quick rate at which occupations change in the advanced period. We should HR staff center less around manual data information and more on cutting edge staff improvement.

More joyful Workforce
We should representatives procure new abilities and fill in their work, one of the top solicitations for workers in any labor force. Through a course library, clients can grow their psyches, meet vocation objectives and rest easy thinking about their work environment.

DISCUSSION

The five greatest MOOC stages on the web like Coursera, EdX, Udemy, Canva and Udacity give right around 600 web-based training courses to over 7.5 million understudies from in excess of 225 nations and regions. Numerous noticeable colleges are proceeding to board the MOOC fad, accumulating materials from nearby projects to extend their range, advance their projects and, by the way, to progress cultural information. Regardless of whether it's an early on course or a more particular point held for those definitely proficient with regards to the branch of knowledge, taking a MOOC in front of selecting a full college course is a decent method for getting to find out about the different specializations in the field and to find new expected employments and interests. It might likewise assist with persuading affirmations staff of the commitment and enthusiasm for the subject. As per Coursera information, 65% of MOOC understudies hold something like a four-year certification, 63% of clients are in regular work and the normal time of clients is somewhere in the range of 24 and 38. Many are looking for proficient turn of events, while some are hoping to review their current information.

While this image of the normal MOOC-taker might leave a severe desire for the mouths of the individuals who supported free internet-based courses as a method of stretching out admittance to advanced education, it likewise shows exactly how truly MOOCs are being taken by the people who have as of now acquired authority capabilities, however decide to keep on considering voluntarily. A few free web-based courses are starting, for students needing to dunk their toes in obscure waters, and some are more mind boggling, offering materials that are examined as a component of genuine college courses, at both undergrad and expert's level. With the ascent of free web-based courses, the significance of giving a local area to internet learning has happened to most extreme significance across all MOOC stages. Gatherings, peer audit and constant conversation during video addresses have all had a vital influence in making on the web training courses more intuitive and easier to use.

CONCLUSION

Online Education has acquired a positive effect the existences of understudies and working experts. It has offered a chance to take up extra courses alongside their studies or job according to their comfort. Online training has additionally helped the personnel in the establishments to request that understudies concentrate on some piece of schedule online which do not need quite a bit of homeroom guidelines. So, the internet-based review assists the personnel with saving time in which they can connect with the students more. The nature of schooling has improved by online courses and even it has become simple for understudies to allude the substance according to their relaxation. In the period of digitalization, the extent of online training increment considerably more and will be valuable for understudies, experts and furthermore organizations.

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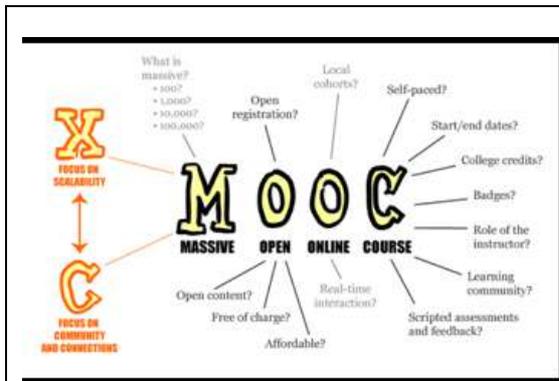
Fig. 1. Structure of MOOC

Fig. 2. edX Education
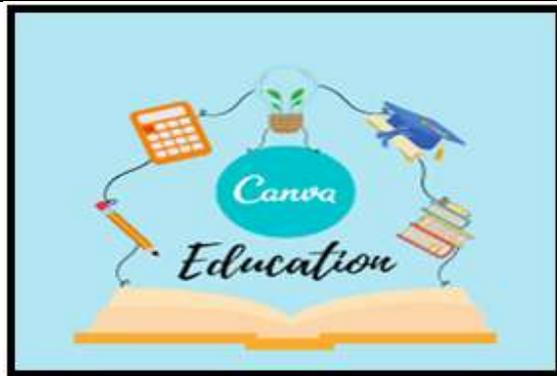
Fig. 3. Canva Education

Fig. 4. Coursera Education

Fig. 5. Udacity Education

Fig. 6. Udemy Education

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RESEARCH ARTICLE

A Study to Assess the Effectiveness of Selected Yoga Asanas to Improve Cognitive Function among School Children in Selected Schools at Puducherry

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ABSTRACT

An experimental study was conducted to assess the effectiveness of selected yoga asanas to improve the cognitive function among school children in selected school, at Puducherry. A total of 120 study participants who fulfils the inclusion criteria were selected by non-probability purposive sampling technique in national school at Thavalakuppam, Puducherry. The data were collected through demographic & Montreal cognitive assessment scale and analysed through descriptive & inferential statistics. The result reveals that majority of the study participants had in mild level of cognitive function before received the yoga intervention .The study findings concluded that majority of the study samples had improved their cognitive level after continuous intervention of yoga practices. The health care sector has to create awareness on continuous implementation of yoga practices in order to improve their cognitive function among school children.

Keywords: Yoga asanas , Cognitive function, School children .

INTRODUCTION

School age is the beginning of a human being's life span. School is an important part of a person's life. The importance of family and school in the development of a person's personality cannot be overstated. In human existence, school is regarded as the major external environment. Exposure in the school setting has a direct impact on a child's development. School provides a learning environment in which students may acclimatise to a group of people, engage with them, and reach out to them in a variety of scenarios. School has a significant impact on a child's personality development [1],[2]. Higher mental activity and processes are referred to as cognitive function. Perception, attention, memory, focus, decision-making, and language understanding are among the mental functions covered [3]. The importance of cognitive function in psychosocial functioning cannot be overstated. From childhood until young adulthood, cognitive functions evolve. These executive mental functions are necessary for the development of social abilities including living, learning, and working. It is the foundation for all academic accomplishment in youngsters [4].

Yoga with mindfulness, specifically pranayama, activates the parasympathetic nervous system, according to science. This sort of meditation improves body perception, induces functional connection within the basal ganglia, and increases grey matter volume and amygdale activation with regional expansion [5]. Yoga has been shown to help children develop their personalities at a young age. Some of the benefits of yoga for school-aged children include managing anxiety, improving emotional control, boosting self-esteem, and increasing body awareness and mindfulness. Strengthens and stretches the body, improving focus and memory. Yoga encourages personal growth on all levels: physical, intellectual, cognitive, emotional, social, and spiritual in terms of progress as a whole [6].

NEED FOR THE STUDY

The importance of cognitive function in psychosocial interactions cannot be overstated. Yoga program me may help to boost cognitive abilities. In children, yoga provides systematic practice on activities related to cognitive functioning such as memory, attention, language, and executive function. From childhood until young adulthood, cognitive functions evolve. These executive functions are necessary for the development of social abilities like living, learning, and working. Yoga has aided in the development of these higher functions. It serves as the foundation for all academic endeavours. [7]. According to one study, children's attention improved after they were given yoga [8]. Yoga provides favorable changes in concentration, energy, and wellbeing, according to an experimental study published in 2011. [9]. According to a study conducted on the impact of yoga and meditation on cognitive function, students who practiced yoga and meditation had higher levels of cognitive skills related to sustained and divided attention and concentration, short term memory, visual information processing and working memory, and complex cognitive speed and flexibility than those who did not. [10]. According to the preceding literature and supportive studies, yoga should be taught to school-aged children as a preventative measure. In order to improve cognitive performance, The researcher wanted to see if yoga had a wide range of benefits for school-aged youngsters.

STATEMENT OF THE PROBLEM

A study to assess the effectiveness of selected yoga asanas on cognitive function among school children in selected schools at Puducherry

OBJECTIVES

- To assess the level of cognitive function among school children.
- To evaluate the effectiveness of selected yoga asanas among school children.
- To find out the association between the pretest level cognitive function among school children with selected demographic variables.

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ASSUMPTION

- The researcher assumes that the selected yoga asanas (Surya Namaskar, Tadaasana, Simhasana, Vajrasana,, Utana Mandukasana, Makarasana, Savasana And Pranayama) may improve the cognitive function among school children.

- The student can able to perform yoga asanas in very easy and effective manner.

DELIMITATION

- The school children for the age group between 9-12 yrs

- The study is limited to 60 school children

- The duration of the study is delimited to 6 weeks

METHODLOLOGY

Research Approach - Quantitative research approach
Research Design - Pre experimental research design one group pretest/ posttest design.
Setting National School, Thavalakuppam, Puducherry
Population Both male and female school children who were willing to participate inthe study.
Sample Both male and female school children who are residing in and around Thavalakuppam, Puducherry, fulfills the inclusion criteria.
Sample size 120 male and female schxool children.
Sampling Technique Non probability purposive sampling technique

Criteria for sample selectionInclusion
Criteria
Children those who
- willing to participate in this study

- those can know write and understand Tamil and English

- present on the day of data collection
Exclusion Criteria
Children those who
- sick on the day of data collection

- any physical and mental illness

- who are less than age of 09 yrs and more than 12 yrs

DESCRIPTION OF THE TOOL
The tool used for data collection was an interview technique. It consists of two parts:
Part I - Demographic Data (age ,gender, parents educational status, parents occupationalstatus, income, religion, family system, location of family, health status)

Part II – Montreal cognitive assessment scale
The standardized scale used to assess the cognitive function which contains 9 items. That are visuaspatial/executive ,Naming, Memory, Attention, language, Abstraction, Delayedrecall, and orientation. Total scoring of MOCA is 30.

Interpretation of score
SCORING
25 – 30: No cognitive impairment
21 -26: Mild cognitive impairment

11 – 20: Moderate cognitive impairment
<10   : Severe cognitive impairment

DATA COLLECTION PROCEDURE

- Prior to data collection a **formal written permission** was obtained from the concerned school authorities of the selected school at Puducherry.
- A written **informed consent** was obtained from the parents or guardian and teachers of the sample with assurance of confidentiality.
- **All participants were informed** about the study
- **Sixty study participants** who met the inclusion criteria were selected by using purposive sampling technique.
- The researcher **assessed the demographic variables** and pre-test level of cognitive assessment by using the Montreal Cognitive Assessment Scale was used..
- The researcher **performed the intervention** to the school children for one month every day on one hour from (07 -08 am) in the morning. The intervention was selected yoga asanas (like Surya Namaskar, Tadaasana, Simhasana, Vajrasana,, Utana Mandukasana, Makarasana, Savasana And Pranayama etc) given to selected school children.
- The researcher was assessed **the post-test** after **one month** by used with Montreal Cognitive Assessment Scale .

DATA ANALYSIS & INTERPRETATIONS

ORGANISATION AND PRESENTATION OF DATA: The analysis of data wasorganized and presented under the following sections.

SECTION A: Distribution of the study participants by their demographic variables
SECTION B: Frequency and percentage distribution of pretest and posttest level ofcognitive functions among school children.
SECTION C: Association between the pretest level cognitive function among school children with selected demographic variables

The table 1 shows that most of the school children in both the groups, 44(73.3%) inthe experimental group and 33(55%) in the control group were aged between 11 – 12 years, 36(60%) and 33(55%) were male, 28(46.7%) and 24(40%) of parents were educated up to 9 – 12th std, 25(41.7%) and 27(45%) of parents were salaried worker, 37(61.7%) and 31(51.7%) had monthly income of <Rs.5000, 40(66.7%) and 48(80%) were Hindus, 54(90%) and 47(78.3%) belonged to nuclear family, 34(56.7%) and 22(36.7%) were residing in urban area,42(70%) and 37(61.7%) were married and 47(78.3%) and 38(63.3%) were healthy. The above table shows that the demographic variable type of family (t=5.850, p=0.0001) had shown statistically significant association with post test score of cognitive function among school children at p<0.001 level and the other demographic did not shown statistically significant association with post test score of cognitive function among school children.

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DISCUSSION

OBJECIVE	RESEARCH FINDINGS
Assess the level of cognitive function among school children.	Pretest of experimental group, 47(78.33%) had no cognitive impairment 13(21.67%) had mild impairment whereas in the post test, after the administration of Yoga Asana on cognitive functions 58(96.67%) had no cognitive impairment and 2(3.33%) had mild impairment. In the pretest of control group, 46(76.67%) had no cognitive impairment and 14(23.33%) had no mild impairment whereas in the post test, 48(80%) had no cognitive impairment and 12(20%) had mild impairment.
Evaluate the effectiveness of selected yoga asanas among school children.	In the experimental group, the pretest mean score was 26.60 ± 1.26 and the post test mean score of 29.08 ± 1.27. The mean difference was 2.47. The calculated paired 't' test was t = 13.237 was found to be statistically significant at p<0.001 level. This clearly infers that administration of Yoga Asana on cognitive functions among children in the experimental group was found to be effective in improving the level of cognitive function among them. Whereas in the control group, the pretest mean score was 26.73 ± 1.35 and the post test means score was 26.83 ± 1.28. The mean difference was 0.10. The calculated paired 't' test was t = 1.941 was not found to be statistically significant. This shows that there was no significant difference in the level of
	cognitive functions among children in the control group. The pretest calculated student independent 't' test score of t =0.558 between the experimental and control group was not found to be statistically significant which infers that there was no significant difference between the two group at the pretest level. But in the post test the calculated student independent 't' test score of t=0.558 between the experimental and control group was found to be statistically significant at p<0.001 level which infers that administration of Yoga Asana on cognitive functions among children in the experimental group was found to be effective in improving the level of cognitive function among them in the experimental group than the children in the control group.
Find out the association between the pretest level cognitive function among school children with selected demographic variables	Demographic variable type of family (t=5.850, p=0.0001) had shown statistically significant association with post test score of cognitive function among school children at p<0.001 level and the other demographic did not shown statistically significant association with post test score of cognitive function among school children.

IMPLICATIONS FOR NURSING

NURSING EDUCATION	Nurse educators engage, support, and coordinate nursing students to increase their desire to participate in various intervention programmes and public awareness initiatives related to their increased role as nurses.
NURSING SERVICE	It aids the health care professionals in the development of therapies for increasing cognitive function in school children in both clinical and community settings.
NURSING ADMINISTRATION	In-service education can be arranged for both nursing students and nurses to promote awareness among parents of children regarding cognitive development.

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NURSING RESEARCH	Nurse researchers may be able to create innovative strategies to help youngsters enhance their cognitive performance.

CONCLUSION

The effectiveness of several therapies in increasing cognitive function among school pupils has been quantitatively proven. This is a simple, low-cost, non-invasive, non-pharmacologist, complementary, and alternative therapy for improving cognitive function.

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Table 1: Frequency and percentage distribution of demographic variables of school children in the experimental and control group.

N= 120(60+60)

Demographic Variables	Experimental Group		Control Group		Chi-Square for Homogeneity
	F	%	F	%	
Age					χ^2=4.909 d.f=2 p = 0.086 N.S
10 – 11	9	15.0	12	20.0	
11 – 12	44	73.3	33	55.0	
>12	7	11.7	15	25.0	
Gender					χ^2=0.307 d.f=1 p = 0.580 N.S
Male	36	60.0	33	55.0	
Female	24	40.0	27	45.0	

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Parents education					χ²=1.735
Illiterate	13	21.7	14	23.3	
1 – 8th std	8	13.3	13	21.7	
9 – 12th std	28	46.7	24	40.0	d.f=3
Graduate / Post Graduate	11	18.3	9	15.0	p = 0.629 N.S
Parents occupation					
Not working	6	10.0	0	0	χ²=10.160
Self employed	14	23.3	18	30.0	
Agriculture	7	11.7	9	15.0	d.f=5
Salaried worker	25	41.7	27	45.0	
Business	8	13.3	4	6.7	p = 0.071
Government employee	-	-	-	-	N.S
Homemaker	0	0	2	3.3	

Demographic Variables	**Experimental Group**		**Control Group**		**Chi-Square for Homogeneity**
Monthly income					χ²=4.613
<Rs.5000	37	61.7	31	51.7	
Rs.5001 – 10000/-	23	38.3	25	41.7	
Rs.10001 – 20000/-	0	0	4	6.7	d.f=2
>Rs.20000/-	-	-	-	-	p = 0.100 N.S
Religion					χ²=2.861
Hindu	40	66.7	48	80.0	
Christian	12	20.0	8	13.3	
Muslim	8	13.3	4	6.7	d.f=2
Others	-	-	-	-	p = 0.239 N.S
Type of family					χ²=3.064
Nuclear family	54	90.0	47	78.3	d.f=1
Joint family	6	10.0	13	21.7	p = 0.080 N.S
Residence					χ²=5.457
Rural area	15	25.0	18	30.0	
Semi-urban area	11	18.3	20	33.3	d.f=2 p = 0.065
Urban area	34	56.7	22	36.7	N.S
Marital status					χ²=5.968
Married	42	70.0	37	61.7	
Unmarried	7	11.7	4	6.7	d.f=3
Widow/Widower	4	6.7	2	3.3	p = 0.113

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Demographic Variables	Experimental Group		Control Group		Chi-Square for Homogeneity
4	7	11.7	17	28.3	N.S
Health status					χ^2=3.267
Healthy	47	78.3	38	63.3	d.f=1
Unhealthy	13	21.7	22	36.7	p = 0.071N.S

N.S – Not Significant

Table 2: Association of cognitive function among school children with their selecteddemographic variables in the experimental group.

N= 60

Demographic Variables	F	Mean	S.D	One Way ANOVA / Unpaired "t test Value
Age				F=0.61 8 p=0.5 43N.S
10 – 11	9	29.22	0.83	
11 – 12	44	29.11	1.40	
>12	7	28.57	0.79	
Gender				t=1.822 p=0.0 74N.S
Male	36	28.83	1.32	
Female	24	29.42	1.14	
Parents education				F=0.83 5 p=0.4 80N.S
Illiterate	13	29.07	1.26	
1 – 8th std	8	29.38	0.74	
9 – 12th std	28	29.18	1.33	
Graduate / Post Graduate	11	28.55	1.44	
Type of family				**t=5.85 0 p=0.0 001 S*****
Nuclear family	54	28.96	1.30	
Joint family	6	30.00	0.00	
Urban area	34	29.12	1.01	
Health status				t=0.702 p=0.4 91N.S
Healthy	47	29.13	1.28	
Unhealthy	13	28.85	1.28	

***p<0.001, S – Significant, N.S – Not Significant
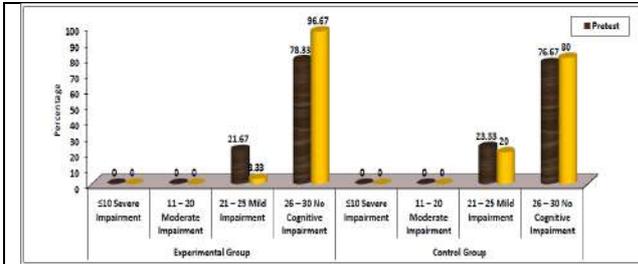
Figure 1. Percentage distribution of pretest and post test level of cognitive functions among school children in the experimental and control group
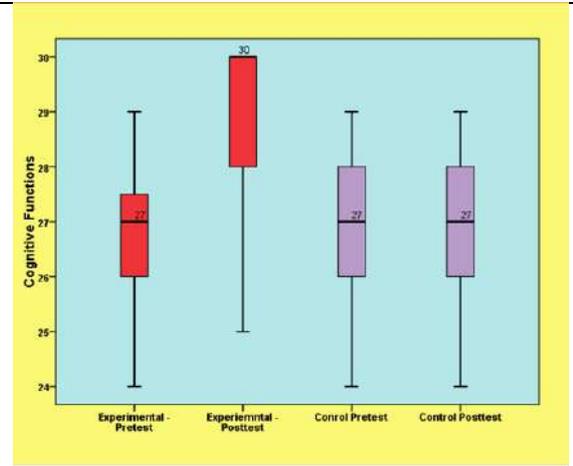
Figure 2. Boxplot showing the effectiveness of Yoga Asana on cognitive functions among school children in experimental group and comparison of pretest and post test level of cognitive functions among school children in control group

RESEARCH ARTICLE

Periodontal Microbiota in Irradiated Patients - An Elusive Truth?

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ABSTRACT

Irradiation causes tissue oxidation with inflammation, altering the regional microenvironment and promoting dysbiosis. Radiation damages the epithelium, leading to cellular DNA as well as RNA injury, which induces cell destruction by apoptosis, ulcerations, and also results in microbial translocation and colonization, further expanding the inflammatory reaction. The precise effects of radiation on periodontal micro flora is uncertain and needs to be explored.

Keywords: Irradiation, Radiotherapy, Periodontal, Micro flora, Cancer.

INTRODUCTION

Head and neck cancers (HNC) harm over millions per year throughout the world.[1] Radiation therapy (RT) is a very important therapeutic tool for HNC. RT monotherapy or in combination with chemotherapy (CRT)can be used as a main treatment, as an adjunct after surgery, and also as a palliative therapy for HNCs that are un resectable.[2] The tumoricidal impact of RT incidentally can damage normal cells within the vicinity of the designated location.[3] Oral

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mucositis (OM), decreased salivation, dysphagia, and odynophagia are among the adverse effects that have a major influence on the health outcomes HNC.[4]

Microbes in oral cavity

Gram-positive bacteria make up the majority of the microorganisms in a healthy mouth. The frequently present microbes are *Actinomyces species* (sp.), *Corynebacterium sp.* ,*Bifidobacterium sp.,* *Lactobacillus sp.*, *Propionibacterium sp.*, *Peptostreptococcus sp.*, *Streptococcus sp.* and *Stomatococcus sp.*, respectively. Gram-negative pathogens such as *Moraxella sp.*, *Neisseria sp*, *Veillonella sp*, *Campylobacter sp*, *Capnocytophaga sp*, *Eikenella sp*, *Fusobacterium sp*, *Hemophilus sp*, *Leptotrichia sp*, *Prevotella sp*, and *Treponema sp*, protozoa such as *Entamoeba gingivalis* as well as *Trichomonas tenax*, yeast such as *Candida sp.,* and viruses also cohabit oral cavity.[5]

Effects of radiotherapy

A bidirectional impact has been documented, in which RT affects the micro biome, which then compromises with the efficacy of RT due to its role in modulating immune response.[6] The alteration of the micro biome caused by radiation is considered to be caused by two main pathways including inflammation.[7] Irradiation causes tissue oxidation with inflammation, altering the regional microenvironment and promoting dysbiosis. Micro biome dysbiosis is hypothesised to disturb the immune response by causing pro-inflammatory and anti-inflammatory compounds to be up regulated and anti-inflammatory substances to be down regulated. Radiation damages the epithelium, leading to cellular DNA as well as RNA injury, which induces cell destruction by apoptosis, ulcerations, and also results in microbial translocation and colonisation, further expanding the inflammatory reaction.

Complication in wound healing in irradiated patients

Wound healing of soft tissue and bone wounds are delayed after radiation therapy that can lead to major clinical problems even after radiation therapy. Present day of tissue damage mainly relies on the theory of hypo cellular, hypo vascular and hypoxic tissues after radiation [8,9]. Studies from seventies and eighties emphasize on correlation between xerostomia, low pH and acidophilic organisms, which leads to radiation caries[10–13].Due to the advances in diagnostic technique, use of molecular biological techniques, the pathogenesis of periodontal disease has been related to major pathogens. With regards to periodontal pathogens, no data for radiotherapy patients is found. Based on the existing data on periodontitis it seems to be direct damage to the periodontium rather than a long-term microbial change of the entire mouth.

Influence of radiation on oral micro biota

There is strong indication that RT alters the balance of the oral micro biota, mostly by increasing the amount of gram-negative microorganisms such as Klebsiella species, Pseudomonas sp., and Candida alb i cans, as well as certain gram-positive organisms, most notably Lactobacillus sp. [14–16] While previous study has found a link between bacteria found in RT-treated oral cavities of HNC patients and RT-induced toxicity, the behaviours and capabilities of the head and neck micro biomes (HNM) as a significant predictor mechanisms are still to be thoroughly characterised.[17]

Molecular investigations have shown a number of RT and CRT-induced alterations in HNM throughout the last decade. In addition to Candida, a greater number and diversity of fungi such as *Peniophora sp., Stereum sp., Cladosporium sp.* have been discovered. The overall proportion of bacteria decreases post treatment and gradually returns, although the comparative abundance of particular species such as Streptococcus mutans and genera such as Bifidobacterium and Lactobacillus increases, indicating the presence of gut-associated anaerobic microorganisms. Other oral research have looked at the variety of RT-induced alterations in the micro biota of supra gingival plaques in HNC patients, as well as probable links to the prevalence of dental caries. [18,19]

One of the most debilitating adverse consequences of RT, CT, or CRT for HNC is OM, which has been researched significantly.[20] Studies have documented alterations in oral microbial proportional diversification and abundance following RT/CRT treatment, advancing our understanding of OM pathogenesis, occurrence, and severity. The oral

micro biota of forty one patients undergoing RT independently or in varying combinations with CT treatment for nasopharyngeal cancer was examined by Zhu et al [21]. They discovered that higher OM severity was linked to a reduction in microbiological diversity and a rise in the number of *Actinobacillus sp.*, *Mannheimia sp.*, *Streptobacillus sp.*, and unclassified Pasteurellaceae. Surprisingly, increasing Candida abundance was not linked to OM incidence as well as severity, but particular gram-negative bacteria such as *Fusobacterium sp.* and *Haemophilus sp.* were linked to OM vulnerability, while others such as *Porphyromonas sp.* and *Tannerella Sp.* were linked to OM severity.[22]

In another research, the development of severe OM was linked to a rise in the quantity of comparable bacteria like *Prevotella sp.*, *Fusobacterium sp.*, *Treponema sp.*, and *Porphyromonas sp.* identified in necrotizing ulcerative gingivostomatitis. By discovering uncultivable microorganisms such as *Filifactor sp.*, *Selenomonas sp.*, *Parvimonas sp.*, *Peptostreptococcus sp.* were discovered in periodontal conditions, the authors reaffirmed the relevance of culture-independent approaches.[23] Researchers observed that OM appeared 21 days after CRT and that distinct genera appeared at different phases of OM progression: *Prevotella sp.*, *Fusobacterium sp.*, and *Streptococcus sp.* just before OM appeared, and *Megasphaera sp.* and *Cardio bacterium sp.* just before extreme OM appeared [24]. One of the most difficult aspects of investigating the oral micro biome is that microbial diversity is simultaneously individual but also site-specific. Distinct bacteria prefer to live in specific microbial locations or sub-niches within the oral cavity.[25] Sub gingival plaque has a distinct micro biota than dorsal tongue, and the tongue has a distinct microbial uniqueness than buccal mucosa. Within the oral cavity the micro biota in sub gingival plaque differs depending on the tooth surfaces [26].

Effect of Periodontal therapy for patients before and after radiotherapy:
Patients who are irradiated in the head and neck region have increased risk for periodontal disease, since its associated with hypo salivation and oral micro biome alterations; moreover periodontitis has been considerate as a trigger point for osteoradionecrosis(ORN)[27,28]. At the molecular level oral keratinocytes reveal loss of proliferative capacity and increased production of proinflammatory cytokines in a radiation dose dependent manner [29].The local effect on periodontal tissue when high radiation dose fraction is used involves alteration in the cellularity, vascularity, and reduced healing/ remodeling potential of the periodontium [30].

Impact of radiation on periodontal micro flora
Periodontal microbial topography is very distinct owing to its complex ecology and microbial in habitation .However, the exact status of period ontalmicrobiota in patients undergoing radiotherapy is not clearly reported in literature and needs to be explored further. The changes in salivary secretion and flow, the resultant lesser volume of salivary immunoglobulins and other defense agents can significantly modify the microbial behaviour. There is a high probability of mutational alteration in the deep pockets. Also the status of qualitative, quantitative and micro genetic variations are highly likely under these scenarios and needs to be investigated more comprehensively.

CONCLUSION

Radiotherapy in or of acial regions can have severe adverse effects. The alterations in salivary flow and concomitant micro bicidal effect of radiotherapy can cause rigorous fluctuation in periodontal microbial population and this carries an increased risk of periodontal disease. More comprehensive research should be initiated to understand the exact effects on periodontal micro flora and their impact on periodontal health in patients receiving radiotherapy.

Conflict of interests: The authors declare that they have no conflict of interest
Ethical statement: Not applicable.
Data availability: Not applicable

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RESEARCH ARTICLE

Solving Diophantine Equations using Golden Ratio

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ABSTRACT

Diophantine Equations has been a great source of interest both for professional and amateur mathematicians. In this paper, I will introduce a special type of pair of Diophantine Equations and will solve them using the continued fraction expansion of golden ratio. In particular, the successive convergent of the continued fraction discussed in this paper provide the complete solutions to the given equations. Incidentally the solutions depend on one of the most important real numbers called Golden Ratio. The method adopted to solve the given equations is novel and provide insights to solve many more similar equations.

Keywords: Diophantine Equations, Golden Ratio, Continued Fraction, Convergent.

INTRODUCTION

Diophantine Equations were named after 3rd century CE mathematician Diaphanous, who suggested solving equations whose solutions are integers. Ever since his famous book Arithmetical got released huge amount of research were carried out in number theory and several mathematicians to this day were immersed in solving different types of Diophantine Equations. In this paper, I will introduce pair of equations and provide their solutions using the successive convergent of continued fraction expansion corresponding to that of golden ratio.

Describing the Diophantine Equations

The main purpose of this paper is to solve the equations $x^2 - xy - y^2 = \pm 1$ (1) where *x, y* are positive integers. In (1), we notice that we have pair of equations as when right hand side of (1) is either – 1 or 1. For solving two equations described in (1), we consider the following method.

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Continued Fraction Expansion

In this section, I will derive the continued fraction expansion of the number $\dfrac{\sqrt{5}-1}{2}$ which is reciprocal of the most

famous real number called golden ratio given by $\dfrac{1+\sqrt{5}}{2}$. Notice that golden ratio is special case of class of metallic

ratios (see **[1–4]**).

First we begin with noticing that $\dfrac{\sqrt{5}-1}{2} \times \dfrac{\sqrt{5}+1}{2} = 1$ (2).

From (2), we have the following computations:

$$\frac{\sqrt{5}-1}{2} = \frac{1}{\frac{\sqrt{5}+1}{2}} = \frac{1}{1+\left(\frac{\sqrt{5}-1}{2}\right)} = \frac{1}{1+\cfrac{1}{1+\left(\frac{\sqrt{5}-1}{2}\right)}}$$

$$= \frac{1}{1+\cfrac{1}{1+\cfrac{1}{1+\left(\frac{\sqrt{5}-1}{2}\right)}}} = \cdots = \frac{1}{1+\cfrac{1}{1+\cfrac{1}{1+\cfrac{1}{1+\cdots}}}}$$

$$\frac{\sqrt{5}+1}{2} = 1 + \frac{\sqrt{5}-1}{2} = 1 + \cfrac{1}{1+\cfrac{1}{1+\cfrac{1}{1+\cfrac{1}{1+\cdots}}}} = [1;\overline{1}] \quad (3)$$

Equation (3) provides the desired continued fraction expansion of the golden ratio $\dfrac{1+\sqrt{5}}{2}$.

Extracting the Solutions

Now using the continued fraction expansion (3), we can derive the required solutions of the given Diophantine Equations. For this, first we will compute the successive convergent of (3).
In doing so, we obtain the following sequence of rational numbers

$$\left.\begin{array}{l} c_0 = \dfrac{1}{1}, c_1 = [1;1] = 1+\dfrac{1}{1} = \dfrac{2}{1}, c_2 = [1;1,1] = 1+\dfrac{1}{2} = \dfrac{3}{2}, c_3 = [1;1,1,1] = 1+\dfrac{2}{3} = \dfrac{5}{3}, \\[4mm] c_4 = [1;1,1,1,1] = 1+\dfrac{3}{5} = \dfrac{8}{5}, c_5 = [1;1,1,1,1,1] = 1+\dfrac{5}{8} = \dfrac{13}{8}, \cdots \end{array}\right\} \quad (4)$$

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Now considering the numerators and denominators of rational numbers in the successive convergent of (4) as *x* and *y* respectively, we notice that $c_0, c_2, c_4, \ldots$ form solutions to $x^2 - xy - y^2 = -1$ and $c_1, c_3, c_5, \ldots$ provide solutions to $x^2 - xy - y^2 = 1$.

In particular, $(x, y) = (1,1); (3,2); (8,5); (21,13); \ldots$ (5) provides solutions to $x^2 - xy - y^2 = -1$ and $(x, y) = (2,1); (5,3); (13,8); (34,21); \ldots$ (6) provides solutions to $x^2 - xy - y^2 = 1$. Since, there are infinite convergent that can be generated from (3), we have infinitely many solutions to pair of equations described by (1). Moreover, for $n \geq 1$ we notice that the subsequent solutions for both (5) and (6) satisfy the recurrence relations $x_{n+2} = 3x_{n+1} - x_n, y_{n+2} = 3y_{n+1} - y_n$ (7).

CONCLUSION

Considering pair of Diophantine equations as described in (1), in this paper, I had determined solutions in a novel way. In particular, noticing the fact the solutions which are directly connected to the golden ratio $\dfrac{1+\sqrt{5}}{2}$, I had derived its continued fraction expansion as in (3). Using the successive convergent of the continued fraction expansion of golden ratio, I had determined all possible solutions in positive integers for (1). Though there are several different approaches that can be adopted to solve the given pair of Diophantine Equations, in this paper, I had used a simplified idea through continued fractions. The method used in this paper can very well be extended to solve similar equations.

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RESEARCH ARTICLE

Tannase Mediated Products from Food Stuffs: Trends, Scope and Therapeutic Relevance

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ABSTRACT

Tannin acyl hydrolase enzyme hydrolyses hydrolysable tannins to produce gallic acid and other enzyme mediated products . The hydrolysable tannins are present in various food stuffs and can act as a suitable substrate to produce Tannase-mediated products including gallic acid and its derivatives. In the current article, we present a new approach/concept to produce these derivatives from food tannins by utilizing the Tannase enzyme. Further, the applications of the by products have been discussed. Gallic acid and its derivatives, therefore could be further developed as promising lead molecules for novel drug development catering to the needs of industrial and pharmaceutical sectors.

Keywords: Tannase, tannins, hydrolysable tannins, gallic acid, derivatives, applications.

INTRODUCTION

Tannins are secondary metabolites (polyphenolic compounds) which possess molecular weights from 500 to 30000 Da, are omnipresent in almost every plant tissue[1]. According to the structure of the monomer moiety, these are of the following types: proanthocyanidins, hydrolysable tannins, phlorotannins [2] and complex tannins. Hydrolysable tannins are polyesters of a sugar moiety (glucose fructose, xylose, saccharose) and organic acids. Hydrolysable tannins are of two types: gallotannins and ellagitannins. Hydrolysable tannins occur in berries (cloudberry, raspberry and strawberry), fruits, nuts, fruit juices, fruit beverages, spices, cereals and pulses. Hydrolysable tannins are hydrolysed to gallic acid, and various other derivatives by different bacterial enzymes [3]. Tannase, an enzyme that catalyses the hydrolysis of ester bonds between phenolic acid and an alcohol, has been reported in fungi,

bacteria and yeast [4]. Tannase mediated products show marvellous antioxidant, anticarcinogenic, antimutagenic, antimicrobial, anti-apoptotic properties. These properties are die to the cleavage of ester bond between gallic acid and various polyols present in tannins. Choubey et al [5] had illustrated gallic acid's various roles, antioxidant potential, mechanism of action and the wide range of medicinal and pharmacological applications. Gallic acid seems to have broader range of therapeutic applications inclusive of anti-fungal, antioxidant and anti-viral activities. The selective cytotoxicity of this key molecule against cancer cells only is extremely important for its wide spectrum role as an anticancer which requires to be explored further to derive its full magnitude. Gallic acid is also attributed to possess anti-diabetic potential and can be successfully employed to treat albuminuria as well. Almost all plants have been known to possess this key molecule having more abundance in grapes, tea, hops and oak bark where it occurs either as a free moiety or embedded in a tannin molecule. Keeping in view of its widespread occurrence, Gallic acid and its derivatives, therefore could be further developed as promising lead molecules for novel drug development catering to the needs of industrial and pharmaceutical sectors.

Tannins – the omnipresent polyphenols
Tannins are a kind of phenolic metabolites which have wide array of molecular weights, present in every plant part, from leaves to fruits. They react characteristically with phenols and precipitate proteins [1]. Tannins possess numerous phenolic groups in their typical structure; therefore they can make complexes with both bimolecular and other elements such as metals.[6] They are a special class of phenolic secondary metabolites with a relatively high molecular weight and the potential to form strong complex compounds with various carbohydrates and proteins. [7]. Depending on the structure of the monomer present, tannins belong to four major categorie condensed tannins, hydrolysable tannins, phlorotannins (marine brown algae)and complex tannins. Proanthocyanidins also called condensed tannins have complexes in which the basic structure moiety is in ester linkage with gallic acid and/or at times with sugar moiety. Hydrolysable tannins have less complex structures than the former [8]. Condensed tannins are a product of the shikimate pathway which further forms anthocyanins. Hydrolysable tannins are biosynthesized via intermediates namely pentagalloyl glucoses which are further converted to Gallo tannins or Ellagitannins, respectively [9].

Types of tannins and their presence in food stuffs
Hydrolysable tannins possess a common galloyl group(s) motif [6]. These are of two types: Ellagitannins and Gallotannins. Ellagitannins are a kind of hydrolysable tannins, that are formed by esterification of hexahydroxydiphenoic acid and monosaccharide moiety (generally glucose). Gallotannins are another type of hydrolysable tannins in which there is an ester linkage between gallic acid and glucose (or some other polyol) [8].
When treated with dilute acids, hydrolysable tannins are cleaved to the respective sugar i.e. glusose and acid moiety upon treatment with diluted acids. Low molecular weight hydrolysable tannins are separated instantly by making extracts with water or many organic solvents. Another variety of tannins which binds with biomolecules to form complex structures remain suspended in extracts [4].

Gallotannins are present in dicot trees as well as herbs. Plants belonging to the families Hamamelidae, Dilleniidae, and Rosidae have been reported to contain elllagitannins [1]. Thushydrolysable tannins are found in fruits, nuts and berries for eg. *Fragaria ananassa*, *Rubus occidentalis*, *Rubus idaeus*, *Rubus chamaemorus*,*Carya illinoinensis*,*Juglans regia*, *Bertholletia excelsa*, *Prunus domestica*, *Prunus granatum* , *Citrus paradisi*, *Citrus reticulata*, *Citrus tangelo*, *Prunus persica*, *Actinidia deliciosa* and beverages like oak-aged wines, tea, cognac, hops in beer. Hydrolysable tannins have also been reported to be found in spices and condiments like nigella seeds, clove, cumin etc[10,11]. Sanguiin H-6 and lambertianin C constitute the major hydrolysable tannin fraction in raspberry [12]. (Daniel et al. 1989)had reported that walnuts contain more ellagitannins than pecan nuts [13]. Ethyl acetate extracts of bahera fruit (*Terminalia bellirica)* contain ellagic acid which is very effective against hepatotoxicity caused by diclofenac [14]. Pedunculagin is the main tannin present in walnuts [15]. The simplest hydrolysable tannins, commonly named gallotannins, consist of gallic acid molecules esterified to a core polyol [16]. The cyclohexane extracts of the two species of medicinal plant *Cytinus hypocistis* and *C. ruber* contained gallotannins, 1-*O*-galloyl-β-D-glucose along with pentagalloyl-*O*-β-D-

glucose.[17] Microorganisms convert Gallotannins to gallic acid and other derivatives whereas ellagitannis are hydrolysed to ellagic acid residues and other esters due to the presence of certain tannin degrading enzymes [16].

Tannin acyl hydrolase- the enzyme that hydrolyses hydrolysable tannins

Tannase, mediates the biocatalysts of the hydrolysis of ester bonds between phenolic acid and an alcohol, has been reported in fungi, bacteria and yeast [18]. The enzyme has the ability to hydrolyze tannins to produce various tannase mediated products, along witb gallic acid and monosaccharide's present in the tannin being degraded as it possesses both esterase and depsidase activities[11], (see figure 1.) It breaks down the ester linkage between the monosaccharide and gallic acid moiety, and the depside bond present between two galloyl residues[7].Tannase works via three routes: first it forms gallic acid and esterifies it to galloyl esters or it esterifies substrate to gallolyl ester. In another way, it may transesterify galloyl esters which leads to the formation of other esters[19].

Tannase mediated products and their medicinal applications

Tannase mediated derivatives have great potential to stop myeloma cells from migrating and invading other tissues. They can inhibit the cancer cells following different pathways and can regulate the expression of various genes involved in cell cycle, metastasis, angiogenesis and apoptosis. These products can trigger apoptosis in various cancer cell lines by down regulating anti-apoptotic proteins and thus up regulating pro-apoptotic proteins. Gallic acid derivates such as alkyl esters are more effective against cancer cells as compared to gallic acid. The increased effectiveness of alkyl esters is related to increased hydrophobicity induced by their side chains[19-21]. Indanone derivatives have shown cytotoxicity against a number of human cancer cell lines, for example KB403, WRL68, Caco2, HepG2 and MCF7 [22]. Hexyl esters derivatives of gallic acid, like cis-2'-hexenyl-3,4,5-trimethoxygallate has dipicted remarkable cytotoxicity against MCF7 cell line [23]. Gallic acid derivatives also contain the anti-inflammatory activity, as reported by [24] Lin and Lin .[25]Rangel et al. suggested that gallic acid ester derivatives can inhibit the ABC transporter Pdr5p in *Saccharomyces cerevisiae*. The Pdr5p transporter confers the multidrug resistance in *S. cerevisiae*. They suggested that ester derivatives with longer side chains (8-16 carbon) and derivatives with side chains of 8-12 carbons that retained hydroxyl groups on the benzene ring are potent inhibitors of Pdr5p ATPase [25]. Gallic acid acts as an antioxidant at higher concentrations due to its scavenging ability [26]. Gallic acid is more hydrophilic than its esters and thus it shows weaker antioxidant activity. Other than hydrophobicity, hydroxyl groups especially at the para position to the carboxylic group maintain the radical scavenging activity in methylated gallic acid derivatives. Steric effects were also found to play a role in the antioxidant activity of esters of gallic acid [27].Gallic acid derivatives isolated from *Loranthus micranthus* parasitic on *Hevea brasiliensis* are great antioxidants possesses ant oxidative potentials and can be imployed to sift free radicals from the human body [28]. The derivatives not only exhibit excellent antioxidant, anticarcinogenic activities but also antimicrobial, anti-hyperglycaemic and anti-inflammatory properties [5]. It has been illustrated through QSAR studies that ester and amide derivatives are more effective than anilides, against several bacteria and fungi[29].The gallic acid compounds like 5-substituted-3-(phenylamino)-1H-pyrazol-1yl and (3, 4, 5-trihydroxyphenyl)-methanone had shown excellentanti-inflammatory effect against paw edema induced by carrageenan[30]. Ethyl acetate extracts of bahera fruit (*Terminalia bellirica)* contain ellagic acid which is very effective against hepatotoxicity caused by diclofenac [14]. Table 1. Shows the Tannase sources, plant parts and threrapeutic potential of various Tannase mediated products.

Methods employed

Various tannin containing food stuffs, like berries, spices, pulses, cereals, legumes and fruits etc., have been screened with respect to their tannin acid content and the food stuffs high in tannic acid content could be of interest for further investigations. The pretreatment method involves the lyophilization of food stuffs using a lyophilizer at -40^{0}C. After freeze-drying, the samples need to be converted to a fine powder, sieved and then could be stored in a dissicator and could be used for further analysis. Food stuff aqueous extracts can be prepared using acetone ddH$_2$O method by Noratto *et al.* (2010)[42] while the total phenolic content could be assessed by the Folin–Ciocalteu method (Singleton and Rossi, 1965)[43]. Tannin amount could be evaluated by protein precipitation method (Hangerman and Butler, 1978) [44]. Hydrolyzable tannins have been determined by KIO$_3$ method of Bossu et al. (2006)[45]. Results are

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expressed as mg tannic acid equivalent (TAE) per g of dry weight (DW). For extraction of tannins, the method by Hagerman (1988)[46] has been widely followed. For the tannase-mediated products synthesis from food extracts, food tannins could be hydrolysed by tannase for the formation of various derivatives with the optimization of various physico-chemical parameters including temperature, pH, enzyme concentration, extract volume (substrate concentration), incubation period etc. The tannase enzyme activity assay can be calculated using a spectrophotometer as suggested by Mondal et al 2001 [47]. Gallic acid derivatives obtained after hydrolysis of food tannins could be further characterized using standard chromatographic techniques including thin layer chromatography, high performance liquid chromatography[48] (Dueñas et al. 2002) etc. While for structural analysis various techniques including nuclear magnetic resonance spectroscopy, Fourier-transform infrared spectroscopy and Mass spectral analysis have been widely employed.For the antioxidant activity of gallic acid derivatives, FRAP Assay has been employed[49].The Free radical scavenging activity could be measured by 2, 2-0-diphenylpicrylhydrazyl (DPPH) spectrophotometric assay using the stable radical DPPH reagent[50]. The antifungal activity has been evaluated by the protocol described by Kotan et al.,[51]. Turbidity measurements have been employed to determine the MIC of the gallic acid derivatives for each of the tested organisms[52].Similarly, the sensitivity of selected bacteria has been assessed by agar diffusion assay[53] (Cushnie and Andrew, 2005). While the MIC of the gallic acid has been determined by the method of Andrews (2001)[54]. Anti-cancer activity has been analysed by determining the inhibitory effects of synthesized compounds against colorectal cell line by using MTT (3-(4, 5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay[55]. While the anti-inflammatory activity has been ascertained by Carrageenan induced paw edema test in rats [28]. Moreover the anti-hyperglycaemic activity has been determined by Streptozotocin induced diabetic rat method [56].

CONCLUSIONS

Tannase enzyme is widely known to hydrolyse hydrolysable tannins to produce gallic acid and other derivatives of gallic acid. There are a variety of food stuffs in which hydrolysable tannins are present which further can act as a suitable substrate to produce Tannase-mediated products including gallic acid and its derivatives. Tannase enzyme could be effectively employed to produce the above derivatives from food tannins underlying the importance of such derivatives. Futuristic studies could further develop gallic acid and its derivatives as promising lead molecules for novel drug development catering to the needs of industrial and pharmaceutical sectors.

ACKNOWLEDGEMENTS

Conflict of Interest

It is hereby certified that there is no conflict of interest concerning the publication of this manuscript in your esteemed Journal.

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Table1. Varoius authors have reported many Tannase mediated products produced ny Tannase enzyme (produced from microorganisms or commercially available enzyme) from a vast range of food stuffs, alongwith their therapeutic applications.

Source of tannins(Hydrolysable tannins)	Source of Tannase	Tannase mediated derivatives	Therapeutic activity	Reference
Pu-erh tea (*Camelliasinensis*)leaves	*Aspergillus niger*	Ethyl- gallate,catechin-catechin-catechin, epiafzelechin and epicatechin-epiafzelechin	Anti-oxidant	[31]
Soy-milk	*Paecilomyces variotii*	Aglycones	Anti-oxidant, anti-inflammatory, anti-carcinogenic	[32]
Green tea	Commercial Tannase(bison corp Korea)	16 Flavanol glycosides	Anti-oxidant, anti-inflammatory, anti-carcinogenic	[33]
Mangifera indica fruit pulp	*Lactobacillus plantarum,Streptococcus gallolyticus*(intesti nal microbiota)	Pyrogallol-O-sulfate , deoxypyrogallol-O-sulfate,4-O-methylgalic acid, 4-O-methylgalic acid-3-O-sulfate, pyrogallol-O-sulfate, methylpyrogallol-O-sulfate, catechol-O-sulfate	Anti-microbial, anti-inflammatory, probiotic effects	[34]
Fruits and nuts	Gut bacterial tannase	Urolithin A	Anti-ageing	[35]

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Green tea extract	Commercial Tannase (Kikkoman, Japan)	Non galllated EC, EGC, gallic acid	Anti-carcinogenic	[36]
Extracts from *Myrtus communis* and *Myrtus nivellei*	Gut tannase from *L. plantarum*	Ellagic acid, gallic acid	Anti-oxidant	[37]
Sumac (*Rhus coriaria*)	Commercial tannase	6 digalloyl glucose, 4 trigalloyl glucose, and 1 tetragalloyl glucose,5 monogalloylg lycose, 7 digalloyl glucose, 2 trigalloyl glucose, and 1 tetragalloyl glucose	Anti-oxidant, anti-carcinogenic, anti-inflammatory	[38]
Mangifera indica beverage	Rats gut microbiota *Lactobacillus plantarum* and *Lactococcus lactis*	Monogalloyl glucoside , gallic acid , p-hydroxybenzoic acid glycoside , coumaric glycoside , and dihydrophaseic acid glucoside	Anti-colitis(DSS-induced), anti-inflammatory	[39]
Soy milk	*Paecilomyces variotii* (CBMAI 1157)	Aglycones (daidzein, genistein, and glycitein)	Anti-oxidant, production of bioactive compounds	[40]
Commercial tannic acid	*Lactobacillus plantarum*	Gallic acid and Pyrogallol	Anti-obesogenic and anti-inflammatory	[41]
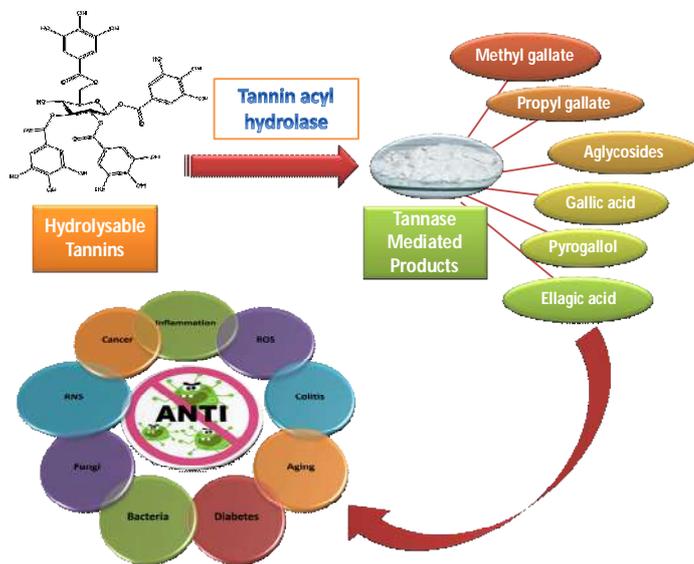
Figure1. Hydrolysable tannins are hydrolysed by Tannin acyl hydrolase enzyme to produce tannase mediated products . These derivatives of gallic acid can further be explored for various therapeutic applications such anti-microbial, anti-oxidant, anti-inflammatory, anti-colitis, anti-hyperglycemic, anti-carcinogenic, anti-aging etc.

RESEARCH ARTICLE

Seismic Analysis of a Reinforced Concrete Structure with and without Shear Wall at Zone-II with Etabs

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ABSTRACT

Shear wall systems are one of the most commonly used lateral load resisting strategies in high-rise buildings, and they are also one of the most expensive. The in-plane stiffness and strength of shear walls are high, and this enables them to withstand considerable horizontal stresses while still supporting gravity loads, making them valuable in a wide range of structural engineering applications. Typically, shear walls are seen in high-rise buildings to prevent the structure from collapsing as a result of lateral loads.. Shear walls are primarily flexural elements that are designed to withstand lateral stresses induced by earthquake and other phenomena. The seismic study in this research is based on an 11-story RCC structure in Zone II. There is a wealth of information available to help you design and analyze a shear wall. However, there is little discussion in the literature on where the shear wall should be placed in a multi-story building. The major goal of this work is to find a solution for shear wall placement in multi-story buildings. The construction is an RCC structure in HYDERABAD that is susceptible to seismic loads in zone II. IS 1893 (PART–I):2002 is used to compute an earthquake load utilizing the seismic coefficient technique. ETABS was used to conduct these analyses.

Keywords: Multi-storey, RC structure, seismic analysis, RC with shear wall and without shear wall, ETABS.

INTRODUCTION

As the construction's mass grows, we must choose for heavier sections to counteract the seismic forces, which will increase the structure's mass, resulting in increased seismic forces. Ductility may be simply produced in a enclosed building with suitable reinforcing details, then when the building rises over a certain height, massive sections are necessary to counter forces, making it practically impracticable. Control of lateral displacement caused by lateral forces is currently one of the most important requirements in building RCC structures in seismic zones. The influence of Shear Wall location on lateral displacement and Storey. Drift in RCC Structures was investigated in this thesis.

Why High Rise Buildings?

The increasing urban people expansion with the resulting demand on restricted space has had a significant impact on city residential construction. The high cost of land, the desire to avoid ongoing urban development, and the necessity to protect key agricultural output have all contributed to the increasing trend in residential construction. Local topographical limits kind towering structures the only viable answer for housing demands in some places, such as Hong Kong and Rio de Janeiro.

Structural System in High Rise Building

Columns and walls are the two principal vertical load acting components of tall structures, with the latter serving as shear walls or shear wall cores in assemblies. The supply of everything to divide and enclose space, as well as cores to hold and carry utilities like elevators, would automatically follow the building purpose. Gravity loads and, in some types of constructions, horizontal loads will be transmitted via columns in then unsupported locations.

SHEAR WALLS

In adding to slabs, beams, and columns, shear walls are vertically concerned with elements that can withstand lateral stresses. RCC shear walls have a high in-plane stiffness while resisting huge horizontal weights and supporting gravity masses in the way of the walls' alignment, making them useful in a wide range of structural engineering applications and lowering the danger of Building damage.

SHEAR WALL – FRAME BUILDINGS
Shear Walls

A shear wall is a structural system made up of braced panels that are used to mitigate the effects of lateral loads on a structure. Shear walls are typically constructed to withstand wind and earthquake stresses. Exterior wall lines in wood or steel surround structure necessity be braced according to numerous construction requirements. Shear walls, on the other hand, when positioned in a favorable position, constitute an effective lateral force resisting structure.

DIFFERENT SHAPES OF SHEAR WALLS

The form and placement of the shear wall have an effect on the structural behaviour of the structure when subjected to lateral stresses. A horizontal diaphragm serves to convey lateral loads parallel to the force of the action. Lateral loads are delivered to the shear walls, where they are absorbed by the shear walls, which serve as a horizontal diaphragm. When viewed in relation to the structural elements, the eccentrically positioned core is required to fulfil a variety of activities, including torque, bending, and direct shear. These shear walls are able to withstand horizontal pressures while remaining upright due to their high stiffness as deep beams that react to shear and flexure against overturning. While rectangular cross sections are the most common in structural applications, L- and U-shaped cross sections are also common. Shear walls constructed of thin-walled deep RC shafts around the elevator core of the building should be used to resist seismic effects. It is included in this part to provide information on the shear wall forms that were employed in this project, as seen in Fig. 1.

- U Section
- W Section
- H Section
- T Section

BASIC ASPECTS OF SEISMIC DESIGN

Because earthquakes generate inertia forces proportional to the mass of the structure under construction, the mass of the structure under construction has an influence on seismic design in addition to the stiffness of the structure under construction. As a consequence, it is possible that the structure will need to undergo damage in order to release the energy that has been absorbed by the earthquake. To counteract this effect, the traditional earthquake-resistant design philosophy stipulates those typical constructions must be capable of withstanding any of the following:

a)   Mild to moderate shaking that occurs on a regular basis with minimal harm to structural or non-structural components;

b)   Moderate shaking with only minimal structural damage and some non-structural damage; and

c)   Severe (and sporadic) shaking results in structural damage but no collapse (in order to spare lives and property inside and next to the structure).

Under design wind forces, on the other hand, structural damage is unacceptable. As a result, earthquake resistant design, rather than earthquake-proof design, is used to describe earthquake-resistant construction, as shown fig.2,

SEISMIC ZONES OF INDIA

The seismic zone map has been altered, with just four zones instead of five, based on the intensities suffered by severe prior earthquakes. Zone I was once split into two zones, one of which is now known as Zone II. As a result, only Zones II, III, IV, and V are included in the new zoning system. About Seismic Zoning Map of India. The first seismic zoning map of India was produced in 1935 by the Geological Survey of India (G. S. I.). This map was originally based on the amount of damage experienced by various parts of India as a result of earthquakes, with multiple adjustments made afterwards. This map depicts India's four unique seismic zones, which are color-coded in varying degrees of red. The following are the country's several seismic zones, as seen on the map, shown in fig3,

Zone - II: This is said to be the least active seismic zone.

Zone - III: It is included in the moderate seismic zone.

Zone - IV: This is considered to be the high seismic zone.

Zone - V: It is the highest seismic zone.

Function of Shear wall

To be able to withstand horizontal earthquake forces, shear walls must possess the necessary lateral strength. Shear walls would convey horizontal forces to other components in the load channel below them as soon as their strength was determined to be sufficient. Shear walls provide additional lateral stiffness to a structure, preventing the roof or floor from swaying excessively to one side. Shear walls with appropriate stiffness will prevent the sliding away of floor and roof bordering members once their supports have been removed. Furthermore, structures that are sufficiently robust may absorb less non-functional damage as a result of their construction. Shear walls also offer lateral stiffness, which prevents the ceiling or floor from moving excessively in either direction sideways.

Requirements of Structural Element In High Rise Buildings

As high-rise buildings are built, the wind and seismic forces to which they are subjected become more important considerations in the design and construction of the structures. It is now possible to improve tall building structural systems in order to manage their dynamic responses as a result of the dramatic increase in the maximum height of concrete buildings over the last few decades, which includes the incorporation of more appropriate structural elements such as shear walls and tube structures, as well as improved concrete material qualities used in their

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construction. The margins of shear walls are especially sensitive to severe compressive and tensile stresses as a consequence of the massive overturning effects induced by horizontal earthquake pressures, which may cause the wall to collapse. In order for shear walls to be capable of withstanding load reversals while retaining their strength, a certain kind of reinforcement must be employed in the concrete at the end parts of the walls. This is referred to as the "ductile reinforcement strategy" in the technical literature. In construction, boundary components are the terminal elements of a wall that are more constrained in their movement than the remainder of the wall structure. Rather than being employed throughout the frame as a restricting element, transverse reinforcement, similar to that found in the columns of a reinforced concrete frame, is used just in the border components. In certain instances, the thickness of the shear wall in these border components is also increased in order to compensate for the increased loading.

RC Shear Wall

As an alternative to slabs, beams, and columns, they are vertical plate-like reinforced concrete (RC) walls that are often employed in reinforced concrete buildings to give extra structural support in addition to slabs, beams, and columns. Shear walls are made of reinforced concrete (RC), and they have a similar appearance to plate-like walls. These walls are often constructed by beginning at the ground level and ascending to the maximum height of the building in a series of steps. When used in tall constructions, they may be as thin as 150 mm or as thick as 400 mm in thickness, depending on the application and the needs of the structure. A well-known consulting engineer from the United States, Mark Fintel, believes we "can not afford to construct concrete structures meant for severe earthquakes without shear walls." "Concrete buildings intended for severe earthquakes without shear walls" is not an option, he argues. Then he said something like, "We can't afford to create concrete structures that are designed to withstand big earthquakes without including shear walls in the design."In particular, the overturning effects of shear walls are remarkable because they carry significant horizontal earthquake forces to the ground underneath them. Shear walls must be organised in a symmetrical manner in order to prevent the harmful effects of twisting on the structural stability of a building's foundation. Asymmetry in plan along one or both directions is possible, as is symmetrical positioning in plan along one or both directions. It is more advantageous for shear walls to be situated on the external perimeter of a structure than on the inside perimeter of a structure. As a consequence of this configuration, the twisting resistance of the structure is enhanced.RCC shear walls have a high in-plane stiffness while resisting huge horizontal weights and supporting gravity masses in the direction of the walls' orientation, making them useful in a variety of structural engineering applications and lowering the risk of structural damage. Shear walls also provide lateral rigidity to avoid excessive side-sway of the roof or floor above. Shear walls also provide lateral rigidity, preventing excessive side-sway of the roof or floor above. Shear walls come in a variety of shapes and sizes, including rectangular and irregular cores like channel, T, L, barbell, and box. The location of a shear wall in a structure has an impact on the structure's behavior.

Function of Shear Wall

Earthquakes are vibrations that occur under the earth's surface, resulting in the death of people and the destruction of structures. The major goal of the structural systems in the building is to properly transmit gravity loads such as dead load, living load, and snow load. Buildings are susceptible to lateral loads induced by wind, blasting, or earthquakes, which can create significant stresses, sway, or vibration in addition to gravity loads. The combination of sufficient vertical strength and enough stiffness can efficiently withstand lateral forces. hear walls in a structure withstand a mixture of shear, moment, and axial loads resulting from lateral and gravity loads.

Objectives

The objectives are as follows,

1. With the help of the ETABS software, a multi-story structure with a shear wall was analysed.
2. To investigate the behaviour of the structure when the shear wall is placed at various locations.
3. Natural Period, storey shear, storey displacement, and storey drift are all factors that will be investigated and compared in this research.
4. In order to establish the most advantageous site for the construction,
5. Shear walls are used to prevent structures from collapsing due to earthquakes.

LITERATURE REVIEW

[Greifenhagen and Lestuzzi, 2005]
According to the results of the investigation, a squat reinforced shear wall, which was not meant to withstand seismic stimulation, has high strength and deformation capacity under load. The outcomes of a series of static cycle tests performed on a poorly reinforced concrete shear wall of an existing building with the purpose of measuring the deformation capacity of the wall are presented in this paper. Before making any suggestions for earthquake-resistant design, this study will conduct a more realistic seismic assessment of existing shear walls in order to offer better earthquake-resistant design recommendations. A multitude of parameters, including cross section type, reinforcement details and quantity, reinforcing steel characteristics, and boundary conditions, may cause failure modes to manifest themselves, but the most common are as follows:

[Elwood, et-al., 2006]
Using a beam–pillar frame construction, the capabilities of an earthquake-resistant structure are examined in this paper. According to the conclusions of this investigation, a beam pillar construction should be strengthened against earthquake loads by applying compression and tension braces diagonally to it. To test the performance of tension braces with a thickness of 200 mm, connectors and tap bolts are attached to the braces and then released (tap bolts on two perpendicular sides of the connections on the pillar-beam element). As an alternative, we investigate compression braces with the same thickness as the walls, and we find that they are particularly advantageous in such situations since they are simple to install and effective at boosting seismicity. In order to prevent any possible difficulties that may occur during the demolition and subsequent rebuilding of the structure for reinforcement, the major goal of this inquiry was to identify them. According to the findings of this research, tensile braces are efficient whether they are employed globally or just as a last option.

METHODOLOGY

Vibrations caused by earthquakes produce inertia forces in the structure. As a result, a structure must be capable of securely transmitting the horizontal and vertical inertia forces created by the superstructure to the earth via the foundation. Seismic codes will help a designer construct a structure that will be safe for its intended use. There are a variety of approaches for analyzing structures for earthquakes; two of them are discussed below. The approach used to assess the seismic behavior of regular and irregular structures with and without shear walls. There are two types of G+11 Storey building geometry: regular buildings with and without shear walls, and irregular buildings with and without shear walls. ETABS 2016 is used to model and evaluate the buildings. The equivalent static approach and the response spectrum method are used to analyze structures. According to the IS: 1893-2002(Part 1) code of practice, models are used for zone factor V and soil type II (Medium). Maximum displacement, stiffness, and storey drift are among the characteristics evaluated in the study.

a   A thorough analysis of the literature to gain a better understanding of seismic evaluation of building structures and to determine the most effective and efficient placement of shear walls inside the structure.
b   Creating a model of a forty-story structure. Shear walls with apertures are given at the central core and at the centre of each side of the exterior perimeter.
c   Checking the building's design for dead load, live load, and seismic load in accordance with Indian standards.
d   Using linear static dynamic analysis, i.e., Response Spectrum Analysis, to analyze the building.
e   Interpreting the findings and drawing inferences.

The following approaches may be used to model a shear wall: -CAD (computer-aided design)
1.   Using the equivalence frame approach,
2.   The braced frame approach is also known as

3. The finite element approach is a kind of numerical analysis. The shear wall is modelled in ETAB, utilising the Finite Element Method and Surface Meshing to get the desired results.

Load Conditions
1. The dead load criterion for all of the models is one kilonewton per metre.
2. In all cases, the live load criterion is 3KN/M for all of the various types.
3. In accordance with IS 1893 (PART 1):2002, the lateral load is the seismic load that is applied to the mass centre of the building.

MODELLING AND ANALYSIS PROGRAM

MODELING
Four distinct types of infill materials are considered in this study: traditional brick, cement concrete block, hollow block, and light weight brick. The computer software ETABS-2009 was used to develop and evaluate the building models using various types of infill materials, and the results were compared.

Material Specifications
Table: 2 Specifications of Materials

Analysis
The building is being analysed with the help of the programme ETABS. ETABS was responsible for the creation of the models. The programme makes use of a variety of RC shear wall cross sections, including the box type, the L type, and the cross type, and these cross sections are placed in various positions, including the perimeter, the corner, and the centre.

METHODS OF ANALYSIS
When a structure is subjected to earthquake ground motion, earthquake response analysis is an art form that employs dynamics and a mathematical model of the structure to simulate the structure's behavior.To do the proper analysis, precise modelling of the behaviour of the materials, components, and connections to the structure will be necessary. Taking into consideration the study's purpose, the number of degrees of freedom should be properly calculated in the model. Given that each node has its own displacement, a three-dimensional model may be used to simulate any kind of behaviour. Because of the difficulties associated with modelling, verification, and numerical calculation, the three-dimensional model has not yet been included in the most sophisticated design methodologies. This research was carried out with the help of the ETABS 2006 software package.

Frame analysis
The appropriate sort of member requirements are used to define three-dimensional (3D) beam or column components in this study. Each node has six degrees of freedom (Ux, Uy, Uz, Rx, Ry, Rz).

Seismic method of analysis
Following the selection of a structural model, it is possible to conduct an analysis to determine the presence of seismically induced forces in a structure. It is the present emphasis of the study to conduct linear static analysis or a similar kind of static analysis for regular structures with limited height.

ETABS INTRODUCTION
Extended 3D (Three-Dimensional) Analysis of Building Systems (ETABS) is a term that refers to the increased 3D (Three-Dimensional) Analysis of Buildings (ETA This is done using finite element software and the stiffness matrix. The analysis and design are carried out in accordance with Indian standards to ensure that all of the checks are met. Finally, a data base for diverse structural reactions is created. TABS Software: Structural analysis and building design

are made easier with the revolutionary new ETABS software package, which is the most complete software solution available today. This version of ETABS lacks comparable 3D modelling and visualisation tools, as well as quick linear and nonlinear analytical power, complex and comprehensive design capabilities for a wide range of materials, and incisive graphic presentations, reports, and schematic drawings. Direct conversion of CAD drawings into ETABS models is possible. The examination of the G+6 Building exposed to loads is the primary focus of this paper. The methods for structural analysis that we will offer in this study were developed using software (ETABS).

ETABS is a structural and seismic engineering programmed developed by Computer and Structural Inc (CSI). The world's tallest structure, the Burj Khalifa, was also planned and assessed using this software.

The following are some of the reasons we picked ETABS:

◎ Easy to use interface,

◎ Confirmation with the Indian Standard Codes

◎ Versatile nature of solving any type of problem,

◎ Accuracy of the solution

MODELLING IN ETABS

53D View of the Building in ETABS

RESULTS

Storey displacement, storey drift, Natural period, and storey shear are the results of the study, which are produced for all models in both lateral directions (X and Y). For multistory building with bare frame model at various positions of shear wall, results are obtained and graphs are generated.

STOREY DISPLACEMENT

Table: 3 Storey Displacement (mm) , Graph1:Storey Displacement (mm)

Story Drift
Table: 4 Storey Drift (m), Graph2:Storey Drift (m)

STOREY SHEAR
Table: 5 Storey Drift (m), Graph3:Storey Drift (m)

CONCLUSIONS

The current investigation aims to determine the impact of a shear wall on a structure in an earthquake-prone zone. For all models, the load combination 1.5DL+1.5EQX is shown to be the most essential. ETABS was used to conduct the investigation. According on the findings of the study, the following conclusions may be drawn.

1. All models show that when the storey height rises, the displacements increase.
2. Storey displacement is lower for structures with shear walls at corners than for structures with shear walls in the middle and structures without shear walls. As a result, it is possible to construct a shear wall at the corners.
3. In comparison to structures with shear walls in the middle, structures with shear walls at the corners yield greater values. As a result, shear walls at corners are a viable option.
4. It is clear from the preceding observations that constructions with shear walls at corners perform better than other structure.

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Table : 1 Details of building

Number of storeys	11
Story Height	3m
Size of Beam	30X60
Size of column	50X50
Slab thickness	15cm
Support condition	Fixed
Thickness External wall	20cm
Grade of steel	Fe-415
Grade of concrete for Beam	M 25
Grade of concrete for column	M 30
Response Reduction Factor	5
Importance Factor	1
Soil condition	Medium
Type of soil	II
Zone	II
Zone Factor	0.36

Table 2. Specifications of Materials

Material Specifications	
Grade of concrete M 30	f_{ck}=30N/mm^2
Grade of Steel	f_y=415N/mm^2
Density of concrete	Γ_c=25N/mm^2
Density of brick walls concrete	Γ_{brick}=20N/mm^2

Table: 3 Storey Displacement (mm)

Storey No	With Shear Wall	Without Shear Wall
Storey 11	71.9	36.9
Storey 10	68.6	32.8
Storey 9	64.3	28.6
Storey 8	58.7	24.3
Storey 7	51.8	20
Storey 6	43.9	15.8
Storey 5	35.3	11.9
Storey 4	26.3	8.3
Storey 3	17.4	5.1
Storey 2	9.2	2.6
Storey 1	2.8	0.9
Base	0	0

Table : 4 Storey Drift (m)

Storey No	With Shear Wall	Without Shear Wall
Storey 11	0.001073	0.001381
Storey 10	0.001446	0.001424
Storey 9	0.001882	0.001439
Storey 8	0.002291	0.001429
Storey 7	0.002627	0.001391
Storey 6	0.002867	0.001317
Storey 5	0.002992	0.001202
Storey 4	0.002968	0.001044
Storey 3	0.00273	0.000845
Storey 2	0.002145	0.000611
Storey 1	0.000939	0.000284
Base	0	0

Table: 5 Storey Drift (m)

Storey No	With Shear Wall	Without Shear Wall
Storey 11	178.8791	430.9792
Storey 10	455.3334	1104.997
Storey 9	679.2614	1650.951
Storey 8	856.1922	2082.323
Storey 7	991.6548	2412.591
Storey 6	1091.178	2655.238
Storey 5	1160.292	2823.742
Storey 4	1204.525	2931.585
Storey 3	1229.406	2992.247
Storey 2	1240.464	3019.207
Storey 1	1241.01	3020.701
Base	0	0

Fig. 1. Various Types of Shear Walls
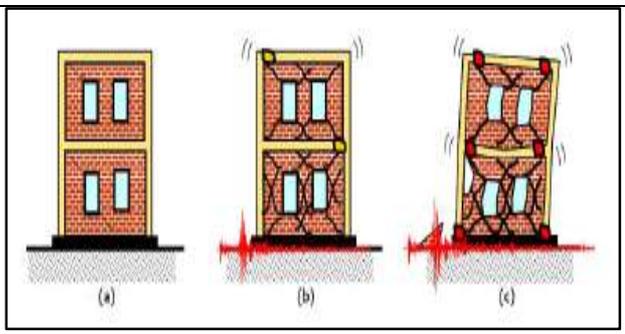
Fig. 2 Earthquake-Resistant philosophy for building
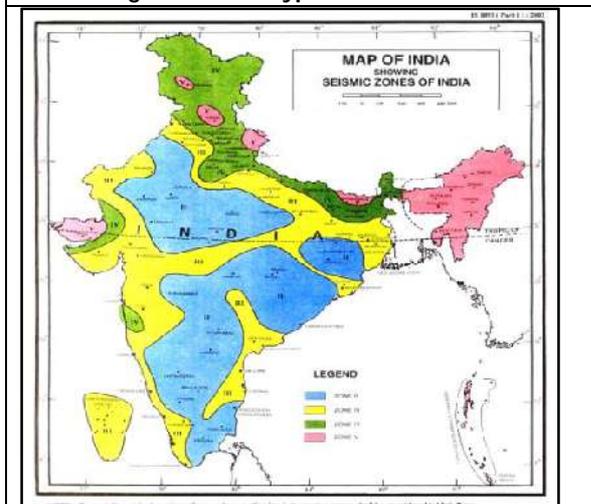
Fig. 3. Modified seismic zones of INDIA (IS 1893-PART 1 2002)
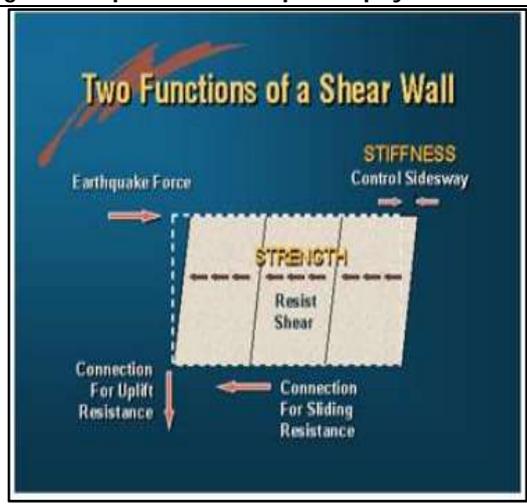
Fig-4: Function of Shear Wall
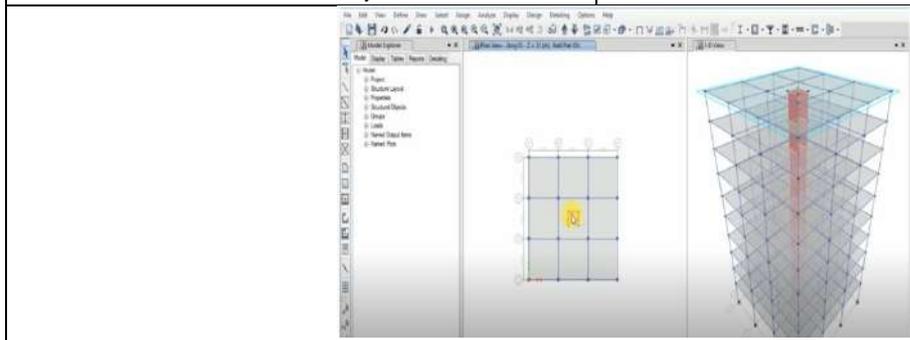
Fig. 5. 3D View of the Building in ETABS

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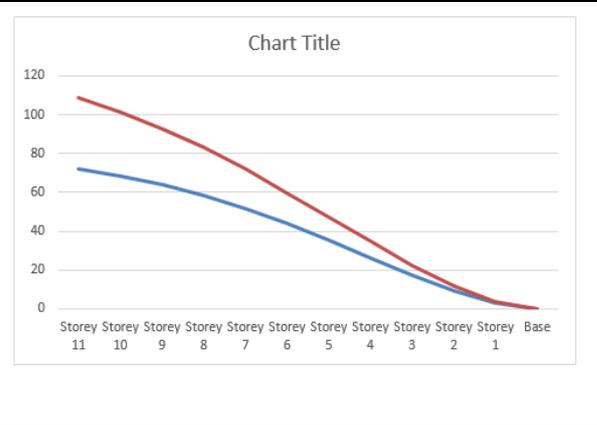
Graph 1. Storey Displacement (mm)
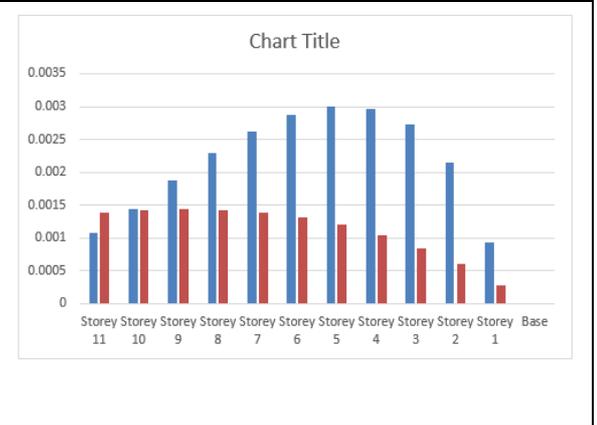
Graph 2 .Storey Drift (m)
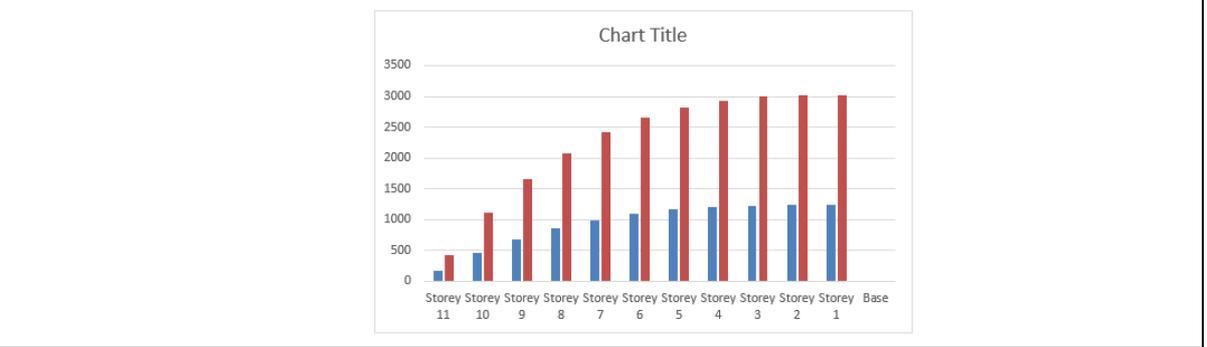
Graph 3 Storey Drift (m)

RESEARCH ARTICLE

Preliminary Study on Correlating Hematological and Hormonal Profile with Microbial Population in the Uropygial Gland of Male and Female Ducks (*Anas platyrhynchos domesticus*)

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ABSTRACT

Healthy Pubertal male and female ducks were chosen for the isolation of bacterial strains and the healthy status was confirmed by checking Haematological, serum Biochemical and Hormone Profiles. Haematological analysis results showed that the RBC, WBC, Haemoglobin and Platelet level was normal and they were not infected. Serum biochemical analysis results provides further evidence that the ducks liver and kidney functioning is normal, which indicates that the physiology of experimental ducks are good. Hormonal analysis (FSH, LH, Testosterone, Estradiol and Progesterone) clearly states that the reproductive physiology of the experimental animals was normal. From the healthy male and female duck 14 different bacterial isolates were obtained from preen gland and cloacal regions. The bacterial isolates were characterized by bacterial biochemical analysis which includes Indole, Methyl red, Vogesproskauer's, Urease, Citrate, TSI, Catalase and Gram staining. From the analysis four different bacterial strains (*Morganella morgani*, *Proteus mirabilis*, *Salmonella typhi* and *E.coli*) were identified. In this study we may conclude that, the healthy male and female pubertal ducks have symbiotic microbes in their Uropygial gland and Cloacal region. These bacterial loads were indirectly controlled by the reproductive hormones.

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Keywords: Uropygial gland, Hormones, Microbes, Pheromone, Reproduction.

INTRODUCTION

These indigenous ducks have innate potential to produce eggs and meat at considerable quantity with lesser input and they are a good dietary source of proteins. However, information on these duck populations, their physical and production characteristics are very scanty and there have been very few systematic studies of ducks in India. The identification of male and female are done using their colouration of feathers. Males are largely dark brown patches on feathers. These feathers attract the females during their breeding season. Males have unique camouflage on greenish colour patches presence in head. Females are comparatively less body size with dull brown colour patches scattered throughout the body. During their reproductive cycle, ducks alternate between periods of sexual quiescence during the summer molt and periods of intense sexual activity in the spring (March-May), when copulations and egg laying occur. Earlier in the breeding cycle (September-October to February), male ducks gather in groups of 4–10 individuals and exhibit a series of stereotyped behaviors called "social displays" directed either to other males or to females. These display express competition between males for access to females and are additionally used to attract the other females. Birds emit many chemical compounds, still ornithologists rarely consider them. Scents consist of volatile chemicals, and birds embrace a remarkable diversity of aromas, such as the musky plumage of storm-petrels (*Hydrobatidae*; Jacob *et al.*, 1982); the tangerine- like perfume of Crested Auklets (*Aethia cristatella*; Humphrey *et al.*,1958); the acrid, sour odor of Hooded Pitohuis (*Pitohui dichrous*) and Variable Pitohuis (*P. kirkocephalus*; Dumbacher *et al.* 1992); the sweet and dusty fragrance of the Kakapo (*Strigops habroptilus*; Butler 1989, J. C. Hagelin pers. obs.*); and the foul stench of the Hoatzin (*Opisthocomus hoatzin*; Weldon *et al.*, 1997).

Researchers found that the chemical composition of uropygial secretions of European hoopoes changed drastically between the breeding and the non-breeding seasons. The composition of uropygial gland secretions of European hoopoes is a result of the combined action of the birds and symbiotic bacteria living within their uropygial glands that provide nestlings and breeding females with a variety of antimicrobial chemicals for their fight against feather-degrading bacteria and pathogens. The uropygial gland secretion of females contained diester waxes throughout the reproductive cycle. The seasonal variation in the composition of the uropygial gland secretion in female birds was shown more recently to be under the control of estrogens (Bohnet *et al*,.1991) Seasonal variation (December to June) of the percentage of male and female domestic ducks synthesizing two types of lipids in their uropygial gland: branched ester waxes and diester axes. Prominent seasonal changes are seen in females. Uropygial secretions are also hypothesized to account for the musky plumage scent of some procellariiforms (De León *et al.*, 2003, Bonadona et al. 2007).

The uropygial gland, however, is not the only source of avian body odor. Several bird groups lack the gland altogether (*Moyer et al.*, 2003). Interestingly, researchers found that the mixture of volatile compounds in individuals with bacteria in their uropygial gland, but not in that of individuals with experimentally induced bacterial clearance, demonstrated antagonistic capacity against all bacterial strains tested, which include the feather-degrading bacilli *B. licheniformis* (Burtt *et al.*,1999) and Bacillus cereus (Burtt 2009,), and several potentially pathogenic bacteria. These compounds were not of a peptide nature, and, therefore, the previous studies suggest that not only bacteriocins (Martın-Platero *et al.*, 2006), but also a variety of chemical defensive products of the uropygial secretion are of bacterial origin. Bacteria are thought to metabolize organic inputs to produce volatile compounds that contribute to host odour. The main model for how bacteria contribute to mammalian chemical communication is known as the fermentation hypothesis for chemical recognition (Albone *et al.*, 1974; Gorman 1976). Hence, the current study was framed to isolate the microbial strains present in the uropygial gland and cloacal region of the male and female pubertal ducks and their role in olfactory communication. Blood parameters were analyzed to confirm the healthy status of the animal to prove that the experimental animals were not infected by any disease and the isolated microbes were in symbiotic relationship with the host. This preliminary study on microbes in duck will helps to carry out further research work to reveal the importance of microbes in avian communication**.**

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MATERIALS AND METHODS

Experimental animals

Healthy Pubertal male and female ducks weighing 2-4 kg were chosen from the Sustainable Integrated Farming System (SIFS), Bharathidasan University, Tiruchirappalli – 620 024. They were maintained in the farm as free roaming from the day they hatched out. These ducks were caged only at night (6 p.m – 6 a.m). They were allowed to roam freely in the farm at day time (6 a.m – 6 p.m). The animals were fed with their natural feeds like grains, rice, etc., no other prepared duck feeds were given. They were fed with water and feed *ad libitum*.

Isolation of micro-organisms from the Uropygial gland and cloacal region of ducks

Healthy Prepubertal male & female chickens were chosen for the isolation of bacterial strains. Sterile cotton swabs were used and smear was obtained from preen gland and cloacal regions of pubertal male and female ducks. Then, the smear was inoculated into a culture tube containing 10 ml of sterile nutrient broth and incubated. After 24 hours, 1ml from each culture (10-4 – 10-7) was transferred aseptically into sterile nutrient agar plates. The plates were further subjected for incubation at 37°C ± 2°C for 24hrs for the isolation of bacterial colonies. The morphologically distinct colonies were picked, streaked and stored for further use.

Biochemical Characterization of Microbes

All colonies were tested for biochemical analysis viz., Indole, Methyl red, Voges proskauer's, Citrate, Urease, TSI (Triple Sugar Iron), Catalase & Gram staining for the preliminary confirmation of species type.

Haematological and serum Biochemical Analysis

Blood was collected from the pubertal male and female ducks by using wing bleeding method. 0.5 ml of blood was mixed with EDTA and it was used for haematological parameters. 2 ml of blood was allowed for serum separation. The serum was separated by centrifugation of 3000 rpm for 20 minutes. The separated serum was used for Biochemical assays**.**

RESULTS AND DISCUSSION

Biochemical characterisation of bacterial strains

The isolated colonies were subjected to biochemical characterisation (Indole, Methyl red, Voges proskauer's, Urease, Citrate, TSI, Catalase, Gram Staining) for preliminary species conformation. The Pubertal male duck contains *Proteus mirabilus,* on the surface of preen gland and cloacal region. The Pubertal female duck contains Morganella morgani and *Proteus mirabilis* on the surface of preen gland and *Salmonella thypi* and *Escherichia coli, Proteus mirabilus* in cloacal region.

Haematological and serum Biochemical Analysis:

Haematological analysis were performed for both male and female Pubertal and adult ducks. The following parameters RBC, WBC, Haemoglobin, HCT, MCV, MCH, MCHC, Platelet, Lymphocytes, MXD, NEVT and RDW results showed near normal range which indicates the test animals are healthy.Serum biochemical analysis were performed for both male and female Pubertal and adult ducks. The liver function tests Billirubin, SGOT and SGPT ALP were analysed in the serum of both male and female and adults ducks. From the analysis it was clearly confirmed that the liver enzymes were in normal range. Hence the liver was functioning good. The kidney function tests Urea, Uric acid and Createnine were performed in the serum of both male and female ducks. The results obtained from these analysis were in the normal range. These analysis results clearly indicate that the kidney functon was normal in the test animals. Lipid profile (Triglyceride, Total Protein, Glucose, Cholesterol, Albumin) was

checked in the serum of both male and female ducks. Results obtained from these results were in near normal range, which clearly confirm that the animal was healthy and physically fit.Serum hormone analysis were performed for both male and female Pubertal ducks to detect the level of reproductive hormones present in it. The level of FSH, LH, Estrodiol, Testosterone, Progesterone were in normal range. These results confirmed that the animals were in Pubertal stage.In this study, both male and female and pubertal ducks are chosen for the isolation of microbes present in preen gland and cloacal regions. Microbes present in the preen gland and the cloacal region are probably the symbiotic relationship and for the odor produced from it. Before isolating the microbes, the healthy status of pubertal ducks should be confirmed because; if the ducks have any infection or disease it may lead to misinterpretation of data. The healthy status of the duck was confirmed by the haematological, biochemical and hormone profiles. Haematological profile provides valuable information on the status of animals. Among all indices, haematological and biochemical parameters are promising indicators of physiological status and environment effect (Ajakaiye *et al.*, 2010). Hence, these indices are important for diagnosis and treatment of diseases and for measuring the environmental stress in ducks. Nonspecific "avian" values are not adequate because hematological and biochemical status is a reflection of many factors such as sex, age, breed, diet, management and stress level. Till today there is no much literature available for our indigenous species *Anas platyrhynchos domesticus.* With consistent to the earlier (Abdi-Hachesoo *et al,*.2011) report, WBC, Platelet, and Haemoglobin count are similar.

The other haematological parameters such as RBC counts were contradictory when compared to the report of Albokhadaim *et al.,* (2012). Sex-dependent variability was observed in blood hematological parameters such as PCV, total erythrocyte count (TLC), and different leukocytes cell counts in Indigenous duck (Abdi-Hachesoo *et al.*, 2013). In support to this in local Saudi duck, these parameters were found higher in male compared to female (Albokhadaim *et al.,* 2012). Most of the hematological parameters were higher in males than females in ducks (Addass *et al.,* 2012). Similarly in the current study, higher level of TEC, PCV, and Hb concentration was observed in males due to effect of androgen. Lipids are the fat that is present in the body. The major lipids in the bloodstream are cholesterol and triglycerides. Cholesterol is an essential part of every cell in the body. It is necessary for new cells to form and for older cells to repair themselves after injury. Cholesterol is also used by the adrenal glands to synthesize hormones such as cortisol, by the testicles to synthesize testosterone, and by the ovaries to form estrogen and progesterone. Triglycerides supply energy for the body. Triglycerides either meet immediate energy needs in muscles or stored as fat for future energy requirements. Elevated serum levels of cholesterol are a major risk factor for coronary artery disease. Elevated triglyceride levels are a milder risk factor for coronary artery disease. Very high triglyceride levels are also a risk factor for acute pancreatitis. This is a condition where the pancreas becomes severely inflamed. In this study the cholesterol level was higher in female than male, similar to the report of (Kumar *et al.*, 2014) and does not confirm the results of (Albokhadaim *et al.*, 2012).

The results of the current study reveals that the triglycerides of duck were similar to the report given by Kumar *et al.*, (2014) and it is contrary to the report of Albokhadaim *et al.*, (2012). Serum enzyme level is a good indicator of physiological status of ducks. Different biochemical parameters - such as total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), Ca and P - are also reflected by the sex and age of the ducks. Higher level of total plasma protein was observed in females in comparison to males. Although a measurement of enzyme activities in serum is very important diagnostic tool of bird diseases, the wide range of activity make it difficult to interpret (Harr, 2002; Perelman, 1999). SGOT is a very sensitive, nonspecific biomarker of liver disease in birds. Conversely, SGPT is of poor diagnostic value in birds due to its existence in many tissues (Harr, 2002; Perelman, 1999). In this study, the mean serum SGOT, SGPT concentrations of our indigenous duck were similar to the results of aseel chicken (Kumar *et al.*, 2014). Total protein, albumin and globulin concentration were higher in female in both season. The total protein level in this study was similar to the finding of former study (Aarif *et al.*, 2014). Similar to our result, increased total protein in female was observed in ducks (Kumar *et al.*, 2015). Nigerian local duck (Isidahomen *et al.*, 2011), and Indigenous ducks (Abdi-Hachesoo *et al.*, 2013). This finding was contrary to the finding in Turkey under arid tropical environment (Gattani *et al.*, 2011). Increased albumin concentration in females was similar to finding in Aseel chicken (Kumar *et al.*, 2015) and Nigerian local duck (Isidahomen *et al.*, 2011). This may be due to many physiological influences in female ducks. High globulin

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concentration in female during both seasons is similar to finding in Turkey (Gattani *et al.*, 2011).Uric acid in birds is the major nitrogen metabolic end product. Many factors such as age, diet and egg laying period affect the concentration of uric acid (Simaraks *et al.*, 2004; Ritchie *et al.,* 1994). It has been reported that serum uric acid of female duck was higher than that of males and serum uric acid of egg laying birds was lower than that in non-reproductive females. However, in the present study serum uric acid levels of indigenous duck were same like that of the local Saudi duck (Albokhadaim *et al.*, 2012). Creatinine, the biomarker of protein metabolism, derived from phosphocreatine in muscle is normally low in birds and its high level is associated with high level of activity (Harr, 2002) and ostriches (Quintavalla *et al.*, 2001) and did not differ among all ducks. Creatinine along with urea, are excellent indicators of protein metabolism and kidney function. In the present study, the value of urea and creatinine was higher in female than male. This was similar to that of Aseel chicken reported by Kumar *et al.,* 2014. The blood chemistry of egg laying birds is different from that seen in nonlaying hens. Domestication has had a definite influence on the hormone cycles of some avian species.

This may lead to certain reproductive problems. Reproductive hormones often have multiple roles and operate via negative feedback systems. The ovary produces estrogens, progestogens, and androgenic compounds. The testes produce testosterones and progesterone. The relationship of the ovarian follicular hormones and the plasma hormones varies depending on the stage of the reproductive cycle and the seasonal photo stimulation. Female birds may conceive in the absence of a mate as a result of the fertile period phenomena. Hormones largely control sexual behavior involving courtship and mating. The stocking density, sex ratio, and environmental conditions of the flock affect aggression, dominance and mating behavior (Mcgovern, 2002).

Some birds, such as ducks, are continuous breeders, reproducing throughout the year. Biologic clocks, known as circadian cycles, control the release of hormones that regulate reproduction, metabolism and behavior. Photoperiod plays an important role in many avian species. Light stimulates a part of the brain, the hypothalamus to produce "releasing factors". These releasing factors stimulate the anterior pituitary to secrete hormones known as gonadotropins. Follicle stimulating hormone (FSH) and Luteinizing Hormone (LH) are two gonadotropins produced by the anterior pituitary which affect the ovaries and testes. FSH, and to a less or degree LH, are responsible for normal ovarian follicular growth. As the follicles increase in size, they produce increasing amounts of estrogen and progesterone. Progesterone acts on LH which then triggers ovulation. Once ovulation occurs, progesterone secretion decreases rapidly. Estrogen is responsible for numerous female secondary sexual characteristics. In the male, FSH initiates seasonal growth and development of the seminiferous tubules in the testes and spermatogenesis. LH promotes the production of testosterone, the male hormone responsible for the production of secondary sexual characteristics and behavior. Supporting to the above said reports in this present study the level of estrogen and progesterone was higher in male than the female; testosterone level was higher in female and lower in male. The same result was obtained in broiler ducks (Hoist-Schumacher *et al.*, 2010).

A number of hormones control sexual maturity, semen production and the behaviors connected with reproduction, aggression and stress in male broiler breeders. As in the female, photostimulation affects the hypothalamus causing the release of luteinizing hormone releasing hormone (LHRH) that affects the pituitary. At the onset of a photo stimulatory photoperiod, there are rapid increases in blood levels of LH and FSH released from the pituitary (Mcgovern, 2002). The obtained results are also evident that the level of LH and FSH was high in male than the female. Follicle stimulating hormone stimulated growth, differentiation and spermatogenic activity of the seminiferous tubules. Luteinizing hormone affects steroidogenic activity of the Leydig cells (Brown *et al.*, 1975). The concentration of LH increased with body weight (Etches, 1996). As sexual maturity is attained, the production of testosterone is stimulated by the rising blood concentrations of the gonadotropin, LH (Sharp and Gow, 1983). Increased LH also stimulates the development and maintenance of accessory sexual organs (Lofts and Massa, 1980). It was trusted that the Uropygial gland of bird involved in sexual communication, through pheromone production (Hirao *et al.*, 2009), and through coloration of the feathers and skin (review in Delhey *et al.*, 2007), as well as in protection against bacteria on the shell surface of eggs (Soler *et al.*, 2008). A secretion from the uropygial gland of mother hens has been isolated on poultry (Gallus gallus) in natural conditions (mother with her ducks). This

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secretion shows a chemical pattern comparable to the appeasing pheromones discovered in mammals (pigs, horses, dogs), which have an appeasing effect on their young (Moltz and Leet, 1981; Mc Glone and Anderson, 2002; Pageat and Gaultier, 2003). Microbial ecology has great potential to reveal how animals recognize individuals, relatives and group members. Recognizing these categories of conspecics is important for mate choice, cooperative relationships, parental care, territory maintenance, dominance rank relationships and group cohesion. Moreover, animals' abilities to recognize others sometimes exceed our expectations; for instance, several studies that animals can recognize unfamiliar relatives, as sometimes occurs among paternal kin (Alberts, 1999; Tang-Martinez, 2001; Widdig *et al.*, 2001; Mateo, 2002; Widdig, 2007). Odour is thought to be the most common mechanism animals use to recognize conspecifics (reviewed in: Burger, 2005; Brennan and Kendrick, 2006; Brennan, 2008; Hurst, 2009).

Odour cues might be derived from an animal's diet or environment, or be synthesized by the animal itself. However, among mammals, many odours are produced by symbiotic bacteria.Uropygial gland secretions usually include chemicals that are active against the growth of some Gram-positive bacteria (Jacob *et al.*, 1997; Shawkey *et al.*, 2003). Interestingly, in our study the bacterial isolates derived from the ducks were gram negative strains. Bacteria living in the uropygial gland might also produce antimicrobial substances that inhibit the growth of feather degrading bacteria. The explanation of basic plumage induction and estrogen secretion during peak testicular activity may lie in the closely related biosynthesis of male and female sex hormones Assenmacher., 1958. Previous studies states that, at high concentrations, part of the male sex hormone is transformed into female sex hormone. Researchers suggest that the spotted and interrupted feather patterns found on the vent and the patterned feathers of the dorsum of females reflect lower estrogen levels in relation to testosterone levels than do the plain and weakly patterned feathers. Furthermore it seems that estrogen induces plain feathers, and that patterns or spots reflect decreasing levels of estrogen. Thus, feather patterns of females suggest that ovarian secretions from December to April are low in estrogen and high in testosterone (Greij, 1973). A relationship between sex hormones and plumage characters is well established (Assenmacher., 1958). In waterfowl the similarity between female plumages and the female like eclipse (basic) plumage of males of sexual dimorphic northern hemisphere anatids. Goodale (1910) found that ovary removal in the Rouen Duck (*Anas platythynchos*) resulted in assumption of the male breeding plumage and also supported the theory that the female plumage of the domestic duck (*A. platythynchos*) was produced under ovarian influence. The above reviews clearly state that the plumage condition of the duck has close relationship with reproductive hormones. The relationship among volatile compound concentrations, sex, and reproductive condition suggests that changes in preen oil concentration may be linked to hormone levels. The uropygial gland contains androgen receptors (Daniel et al., 1977; Shanbhag and Sharp, 1996).

Relatively few progesterone, compared to androgen, receptors have been found in the uropygial gland. Corticosterone, a steroid hormone related to stress, also has been shown to have an effect on cellular activity in the uropygial gland (Maiti and Ghosh, 1969). Whittaker *et al.*, (2011) revealed the role of testosterone on preen oil composition. This clearly state that the uropygial secretion was generally controlled by reproductive hormones.
Birds also host a diversity of avian microbes on feathers and skin for maintaining their plumage (Lucas and Heeb 2005, Shawkey *et al.*, 2005). Microbes influence the expression of secondary sexual traits and the health of infected individuals (Gunderson *et al.*, 2009). Sexual selection theory predicts that microbial infection can indirectly influence mating success by altering the expression of secondary sexual characteristics (Andersson 1994). Through symbioses where bacteria modify or helps to produce secondary sexual traits (Shawkey *et al.*, 2007; Sharon *et al.*, 2010). Birds may manipulate the bacterial composition of their plumage by the selective use of preen oil on feathers (Moyer *et al.*, 2003). In our results we got four different bacterial isolates from both male and female ducks. The pubertal male duck contains *Proteus mirabilus* in both preen gland as well as in cloacal region. The pubertal female duck contains *Morganella morgani* and *Proteus mirabilis* on the surface of preen gland and *Salmonella thypi* and *Escherichia coli, Proteus mirabilus* in cloacal region. According to the recent studies, it is clear that the testosterone and progesterone level influence the uropygial secretion and the uropygial secretion has major role in altering the microbial population. So here we suggest that the testosterone and progesterone level may be the reason for the bacterial load of male and female duck. Further the strains which are isolated from the uropygial gland and the cloacal region of the pubertal ducks were already identified as opportunistic pathogens. These were present in many other vertebrates. In some

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cases, they give high level of death in their host communities. But in our case these strains may not cause any infection in the host (duck). The hematological and the biochemical report gave strong evidence that the experimental animals were healthy and not infected by any disease. The hormonal assay also support that the animal physiology was normal and also control the bacterial community as well as the bacterial load of the host. So our report clearly reported that the isolates were in symbiotic relationship with the host animal and the reproductive hormones play a vital role to control the bacterial population in the uropygial gland of the duck. We need more information or further studies on this to confirm the exact role of reproductive hormones in controlling the symbionts present in the duck

CONCLUSION

Domestic ducks depend predominantly on visual information to function, while olfaction appears to play a major role in their life. Chemical cues from the Uropygial gland may compensate for information that vision is not able to detect (Hirao., 2011). Here we suggest that symbiotic bacterial load may have much impact on their host's reproduction than Uropygial gland. Bacterial loads are responsible for the secondary sexual characteristics as well as for the pheromone communication of birds. Birds may manipulate the bacterial composition of their plumage by the selective use of preen oil on feathers. The uropygial secretions were controlled by the reproductive hormones of the birds. So in this study we may conclude that, the healthy male and female pubertal ducks have symbiotic microbes in their Uropygial gland and Cloacal region. This bacterial load was indirectly controlled by the reproductive hormones. Though more research work is warranted, to provide strong evidence that the microbes are effectively involving in the olfactory communication as well as the role of reproductive hormones in symbiotic microbial load of ducks. It will open a new door in poultry research to save our indigenous breeds.

ACKNOWLEDGEMENTS

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Fig. 1: Indole Test result of isolated microbes from the preen gland and cloacal regions of Pubertal male and female duck (*Anas platyrhynchos domesticus*).

Fig 2: Methyl Red Test result of isolated microbes from the preen gland and cloacal regions of Pubertal male and female duck (*Anas platyrhynchos domesticus*).

Fig 3: Voges Proskauer Test result of isolated microbes from the preen gland and cloacal regions of Pubertal male and female duck (Anas platyrhynchos domesticus).

Fig 4: Citrate Test result of isolated microbes from the preen gland and cloacal regions of Pubertal male and female duck (*Anas platyrhynchos domesticus*).

Fig 5: **Urease Test result of isolated microbes from the preen gland and cloacal regions of Pubertal male and female duck (*Anas platyrhynchos domesticus*).**

Fig 6: TSI Citrate Test result of isolated microbes from the preen gland and cloacal regions of Pubertal male and female duck (*Anas platyrhynchos domesticus*).
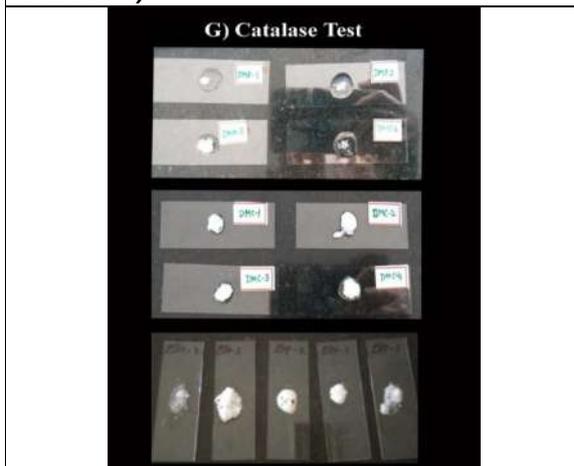
Fig 7: Catalase Test result of isolated microbes from the preen gland and cloacal regions of Pubertal male and female duck. (*Anas platyrhynchos domesticus*)

Fig 8: Gram staining result of isolated microbes from the preen gland and cloacal regions of Pubertal male and female duck (*Anas platyrhynchos domesticus*).
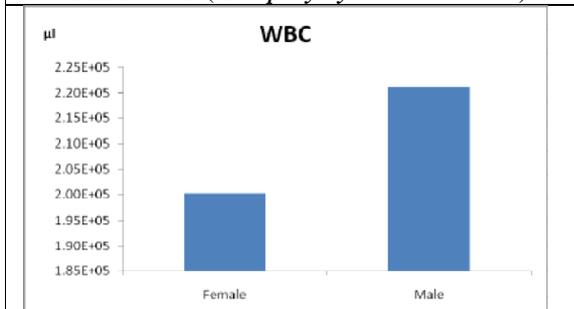
Fig 9: WBC count of pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
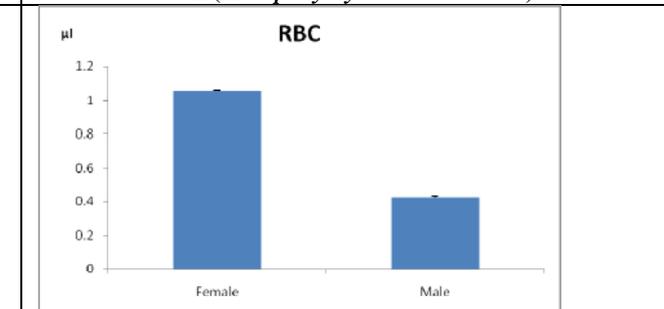
**Fig 10: RBC count of pubertal male and female duck. Values were expressed as mean ± SD (N = 3)**

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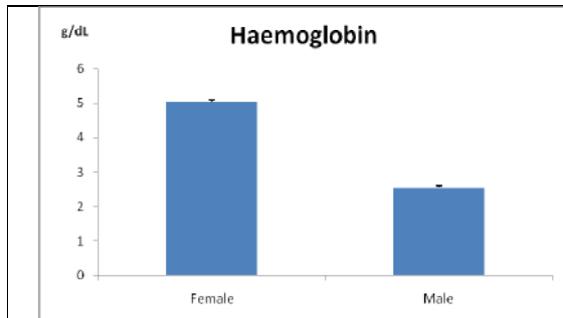
Fig 11: Haemoglobin level of pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
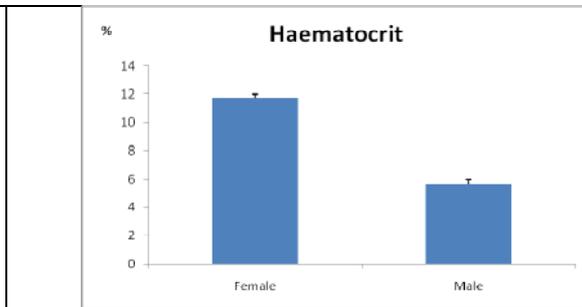
Fig 12: Hematocrit level of pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
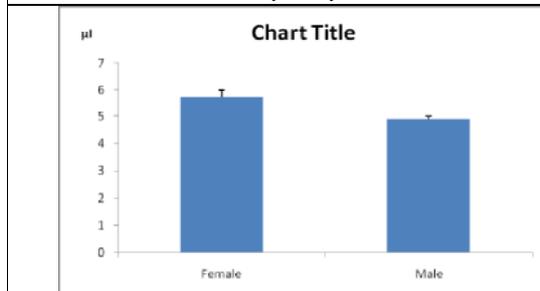
Fig 13: Platelet count of pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
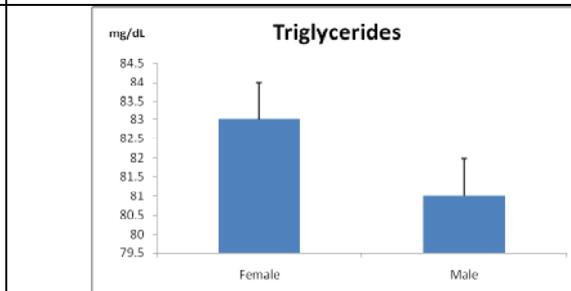
Fig 14: Level of Triglyceride in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
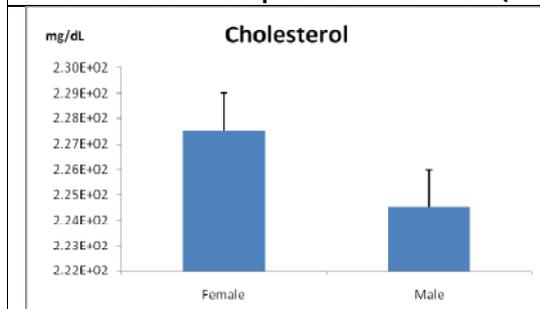
Fig 15: Level of Cholesterol in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
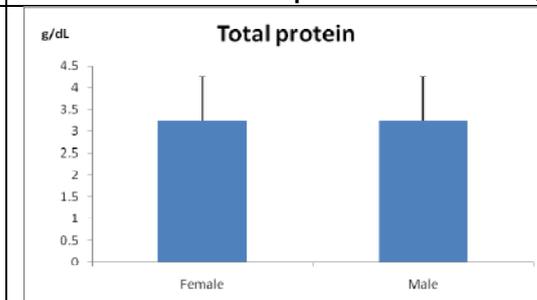
Fig 16: Level of Total protein in pubertal male and female duck. Values were expressed as mean ± SD (N = 3).
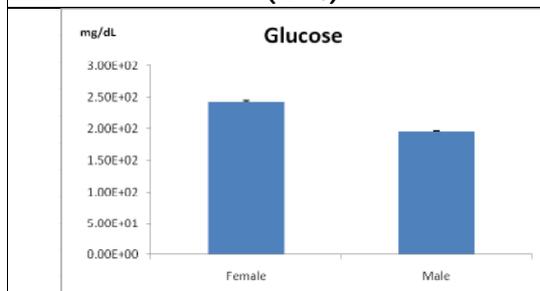
Fig 17: Level of serum glucose in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
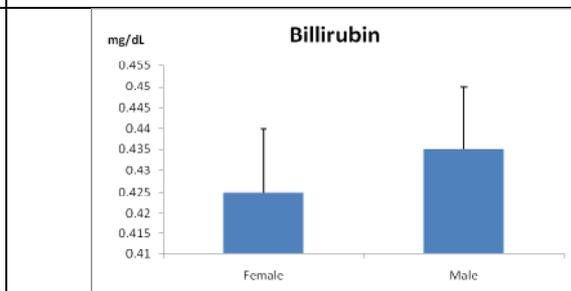
Fig 18: Level of Bilirubin in Pubertal male and female duck. Values were expressed as mean ± SD (N = 3)

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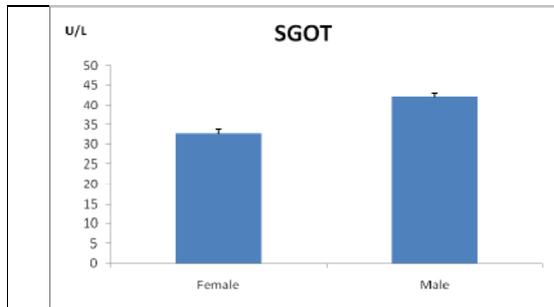
Fig 19: Level of SGOT in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
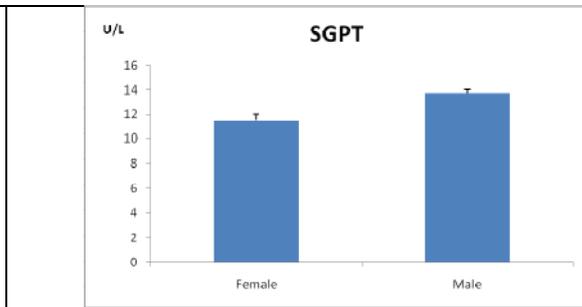
Fig 20: Level of SGPT in pubertal male and female duck. Values were expressed as mean ± SD (N = 3).
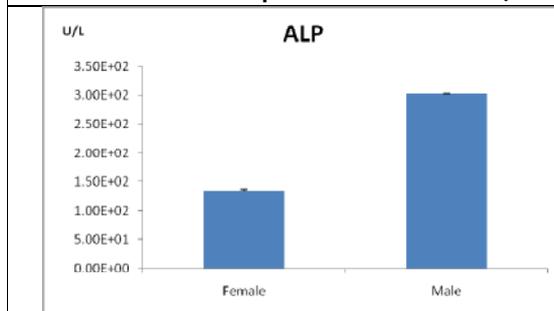

Fig 21: Level of ALP in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
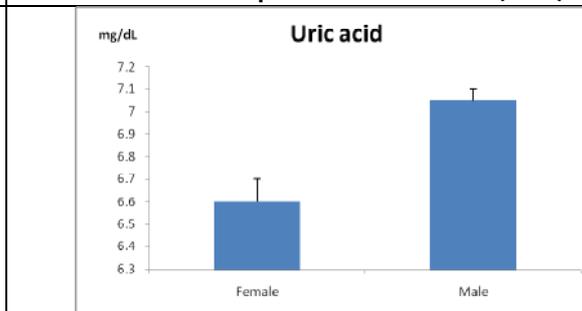
Fig 22: Uric acid level of pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
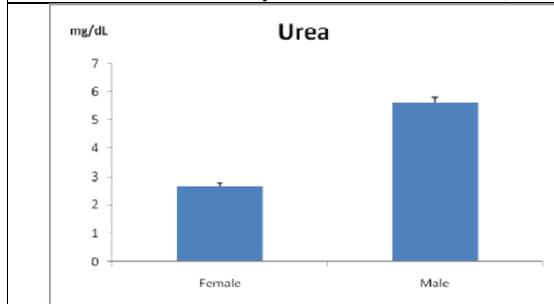
Fig 23: Level of Urea in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
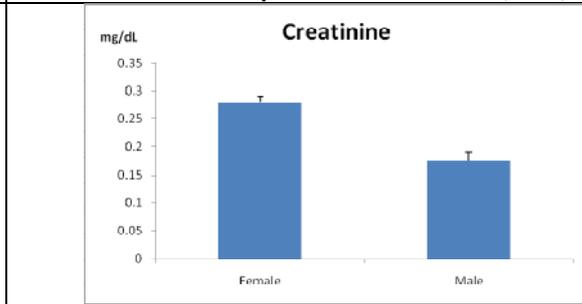
Fig 24: Level of Creatinine in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
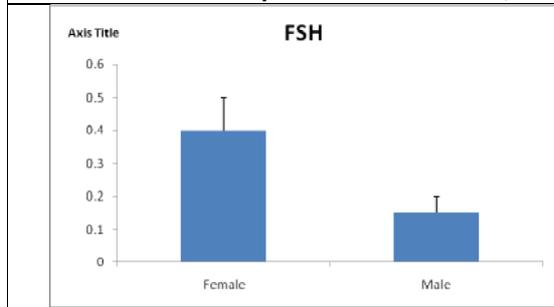
Fig 25: Level of FSH in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)
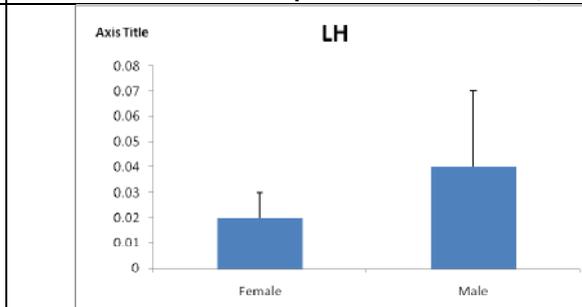
Fig 26: Level of LH in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)

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Fig 27: Level of Estradiol in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)

Fig 28: Level of Testosterone in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)

Fig 29: Level of Progesterone in pubertal male and female duck. Values were expressed as mean ± SD (N = 3)

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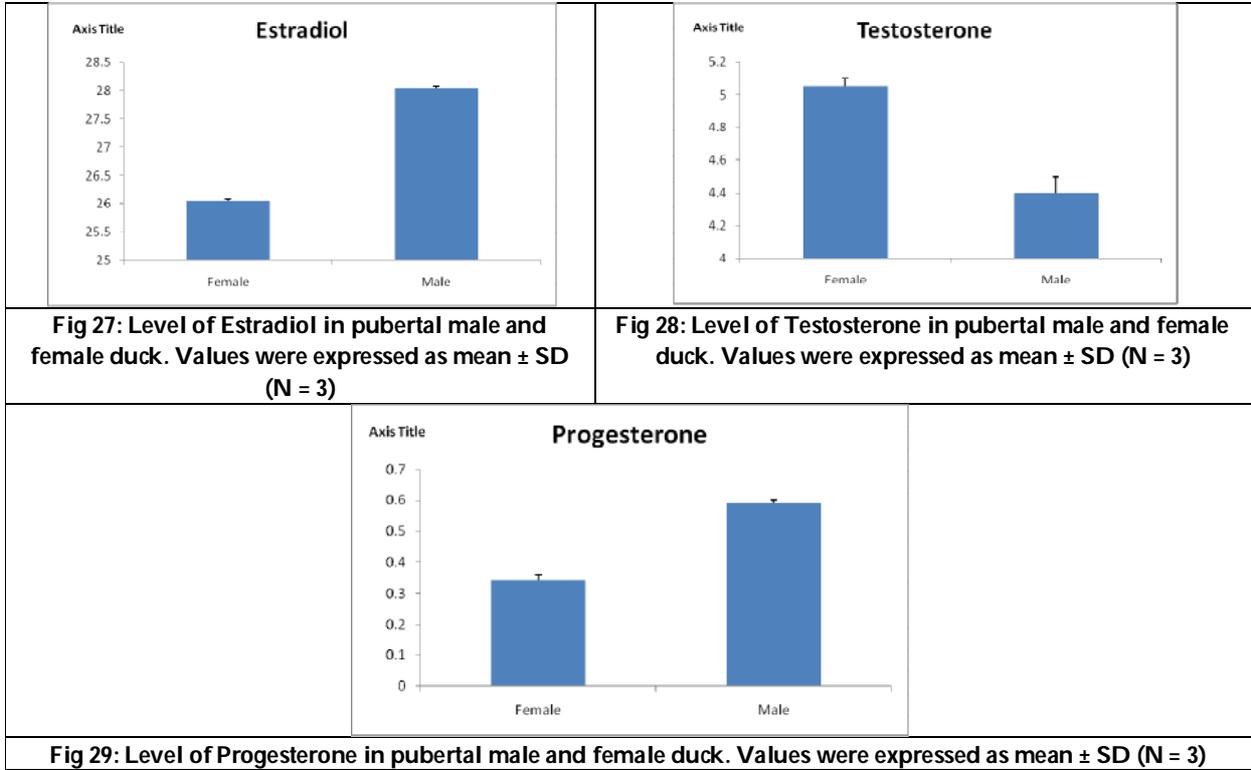
Influence of Trait Emotional Intelligence on Adolescent Stress

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ABSTRACT

The trait emotional intelligence (TEI or Trait EI) framework provides comprehensive coverage of personality facets relating to affect. It is related to behavioral dispositions and self-perceived abilities. It has been observed that people who lack emotional knowledge or regulation skills tend to perceive high stress. The present study was conducted to study the influence of Trait emotional intelligence (TEI) on various facets of stress. A cross-sectional study comprising 374 adolescents (135 males and 239 females) was conducted with a mean age of 15.87 (SD = 1.23). Correlation and regression analyses were performed on the collected data. Findings suggest that well-being and self control of the trait EI dimensions contribute significantly and independently to the prediction of the domains of the stress scores compared to the other two domains of emotionality and sociability. This study could help develop stress management programs to address the problem of stress among adolescents.

Keywords: Emotional Intelligence, TEI, Stress, Adolescence, Wellbeing, Health

INTRODUCTION

Over the past two years, the world has faced unprecedented turmoil, mainly due to the prevailing pandemic. The mental health burden on the young in particular has been enormous. Thus, the need to talk about adolescents' mental health during this pandemic has increased manifolds. With schools remaining closed, classroom teaching replaced by online classes, homebound, family and economic stressors, and not interacting with peers have severely impacted adolescents' developmental and psychological aspects. As per the psychosocial development theory of Erik Erikson [1], adolescence is the most crucial stage of identity formation; it is the phase when cognitive abilities reach their penultimate completion. It is a transitional phase of rapid physiological and psychological development. During this phase, an individual is subjected to many stress-inducing events such as body consciousness, substance abuse, academic pressure, peer pressure, career selection, etc.

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Adolescence is characterized by a phase where the focus of an individual expands from family to peers. It is during this period that interpersonal relationships find new importance. As per the interpersonal stress theorists, individuals do not merely react to stress. On the contrary, individuals may possess typical characteristics or behavior patterns that may increase the potential of suffering stressors, especially in interpersonal relationships [2]. However, it is worth noting that while an individual may act as a contributing factor to a stressor, it does not suggest that the individual is deliberately causing stressful situations in their life. Instead, the above statement means that certain personality characteristics make one more/less predisposed to experiencing stress.

The capacity to repair and recover from a negative emotional state has long-term effects on the quality of social interactions with close interpersonal relationships. It has been demonstrated that adaptive emotion regulation strategies improve interpersonal functioning [3].Many studies back the statement that depressed youth experience more violence/stress with the family, such as fights, conflicts, and arguments, as compared to youth with no symptoms of depression [4,5,6].Research in recent times has built on this and found that adolescents who display anxious traits tend to experience heightened interpersonal stressors [7]. Various studies have started to gauge adolescents' vulnerabilities for anxiety and depression, which may further culminate in a higher risk of internalizing disorders and symptoms. Cognitive vulnerabilities like rumination, negative cognitive styles, excessive reassurance-seeking, co-rumination, and neuroticism have been identified as the key markers of characteristics and behaviors [8,9,10,11,12].

Traditionally, the negative feelings of self-appraisal act as a gateway to substance abuse and delinquency in adolescents. In addition, due to the rapid state of psychological and physiological development, adolescence is marked by intense feelings of confusion and emotional overwhelm. The added academic, social, and other forms of stress may harm adolescents if they cannot handle the situation constructively. A Group of notions about emotional self, located at the lower levels of personality, is known as TEI. The emotional aspects of personality are integrated. They can be assessed through self-report measures [13]. Adolescents who can control their emotions effectively make better social partners. They engage with peers and other close relationships more positively and constructively. A deep analysis of TEI in children has revealed that a higher TEI level is a crucial predictor of children's health-related outcomes. It was observed that children with higher EI had better social interactions and improved well-being throughout development [14]. It was also noted that children with higher EI had fewer somatic complaints [15].

Stress has been defined as a normal reaction to day–to–day pressures but can become unhealthy when it hampers everyday functioning. Both interpersonal and intrapersonal domains serve as sources of psychosocial stress during adolescence. Adolescents' stressful events are associated with maladjustment and psychopathology in the age group [16,17]. The various factors that constitute as stressors for adolescents are primarily in the category of home, school, interpersonal relationships, and financial aspects. For adolescents, tension at home is a common source of stress. For example, adolescents may be troubled by family discord, divorce, a new stepparent, or a new home. Other sources of stress could range from pressure to wear certain types of clothes or jewelry or sport the current hairstyles or experiment with drugs, alcohol, or sex. The pressure of social media to conform to a particular body shape and or body type can also add to the stress levels.

Although having friends or a strong social network can help buffer stress, it can also act as a major source of adding to existing levels of stress. Therefore, it is critical to recognize signs of stress. Observe any changes to behavior patterns such as increased levels of irritability, anger, mood swings, adolescents 'acting out' or appearing 'checked out' or disengaged, changes in sleep patterns or eating preferences or styles - binge or emotional eating on one end of the spectrum and loss of appetite on the other, not completing schoolwork or procrastinating or forgetfulness or suddenly underperforming. Adolescents also get sick more often – stomach or headaches or tiredness, which is a physical manifestation of stress. It has also been observed that the suicide rate and self-harming behavior are at an all-time high among adolescents, and that is one of the major causes of stress and should not be overlooked.

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REVIEW OF LITERATURE

A significant number of research studies have found the influence of TEI on stressful events, both in real life [18,19] and in experimental settings [20]. One of the most fruitful and gratifying research fields in Emotional Intelligence focuses on harnessing evidence for a relationship between psychological well-being and Emotional Intelligence. Higher levels of positive emotional states and a subsequent reduction in negative emotional states can be achieved by optimizing specific emotional strategies [21]. Therefore, to positively impact people's health and welfare, the emotional plan will play a vital and vital role. Numerous findings have revealed the role of TEI during childhood in educational setup. For example, research shows that individual differences between pupils in TEI significantly affect their positive adaptability in the classroom, especially with socio-emotional capabilities and subsequent adaptive behavior with their peers [22]. Research has also established that students who scored high on TEI were less likely to be reprimanded or suspended from school and had a good attendance record[23]. In addition, studies found a negative association among TEI and school burnout levels and academic stress [24,25]. Further, research has successfully established a positive correlation with Trait EI and prosocial behavior as per multiple peer ratings [26].

Well-being is defined as the positive experience of health, happiness, and prosperity. There are five facets of well-being- emotional, physical, social, societal, and workplace. Of these, emotional well-being is of utmost relevance to adolescents. Emotional well-being is the skill that one needs to deal with stressful situations. Skills like positive thinking, resilience, emotion regulation, and mindfulness constitute emotional well-being. Perceived emotional intelligence is a significant predictor of perceived stress and life satisfaction. The findings support the earlier body of research that emphasized understanding one's emotions in developing psychological well-being [27]. A positive relationship between components of TEI and subjective well-being in adolescents has been concluded in research[28,29].Exposure to violence has also been linked to several mental health and behavioral issues. These concepts, as research has shown, are related to TEI. However, neither high EI nor high cognitive skills could protect adolescents' from the said negative impact [30].

The current investigation aimed to study the role of emotional intelligence traits, namely well-being, self-control, emotionality, and sociability, on adolescent stress. The study is essential for adolescent mental well-being and building intervention programs to develop stress management programs. It will also help develop intervention programs implemented within the regular school environment.

METHOD

Participants
The data was gathered from schools situated in and around Delhi and National Capital Region(NCR). The research method used in the study is cross-sectional. The sample comprised of 374 participants (135 males and 239 females) with a mean age of 15.87 (SD = 1.23). Adolescents between the ages of thirteen to seventeen (males and females) were included in the study. The sample was collected from both private and public schools.

Measures
TEIQue-ASF The scale comprises 30 short statements designed to measure *global* trait EI[31] among adolescents. The participant has to show how much he/she agrees or disagrees with each sentence. The scoring is done on a seven-point Likert scale. For example, wherein one strongly disagrees, and seven strongly agree. The internal consistency of the global score usually exceeds .80.

ASQ: The questionnaires a 58-item inventory [32]:.It consists of ten sub domains ranging from the stress of home life to emerging adult responsibility. A Likert scale was used wherein one is given to responses indicated not at all stressful to five for very stressful.

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Data Analysis Procedure

Analyses were performed on the obtained data using SPSS 21. First, we calculated the mean and SD for all the sub domains of the variables. Secondly, the correlation between the five trait EI variables (well-being, self-control, emotionality, sociability, and Global TEI) and the stress domains (home life, school performance, school attendance, romantic relationships, peer pressure, teacher interaction, future uncertainty, school/leisure conflict, financial pressure and emerging adult responsibility). Furthermore, the third analysis involved regressing each of the ten sub-domains of stress onto the five traits EI variables.

RESULTS

Table 1 presents the descriptive statistics, and Table 2 presents the bivariate correlation between the trait of emotional intelligence and different dimensions of stress. Trait emotional intelligence and its four factors, namely well-being, self-control, emotionality and sociability were found to have a significant negative association with all the dimensions of stress. However, regression analysis results (Table 3) revealed the significant role played by various factors of trait emotional intelligence in influencing stress responses among adolescents. Well-being and self-control were the two factors of emotional intelligence that significantly reduced home life stress on adolescents. Well-being and sociability also significantly influenced the stress of performing in school. Further, they also influenced the stress arising due to peer pressure, teacher interaction, future uncertainty, romantic relationships, and emerging adult responsibility. Well-being and emotionality predicted reduced stress due to school leisure conflicts. Self-control was instrumental in managing stress arising due to the pressure of attending regular classes.

Similarly, the well-being factor of trait emotional intelligence predicted reduced stress arising due to financial pressure or constraints. Overall, well-being and self-control were the factors that were instrumental in reducing stress among adolescents. However, emotionality and sociability factors did not determine stress arising from home life, peer pressure, teacher interaction, future uncertainty, romantic relationship, and emerging adult responsibility among adolescents.

DISCUSSION

The present investigation aimed to explore the role of trait emotional intelligence factors, namely, well-being, self-control, emotionality, and sociability, on stress among adolescents. The results revealed that well-being and self-control are the most significant components of TEI that determine the nature of stress perceived by adolescents. Adolescence is characterized by intense feelings of emotional upheaval, physiological changes, and understanding and dealing with the external world. Well-being and self-control were instrumental in reducing stress arising due to peer pressure, teacher interaction, school performance, future uncertainty, and emerging adult responsibility. In addition, well-being and emotionality were responsible for managing stress due to school-leisure conflicts. However, self-control was the only factor that was instrumental in reducing stress due to school attendance pressure. In addition, the well-being factor within the individuals enabled them to manage stress due to financial pressure. When the current outcomes are contrasted with past investigations, it can be interpreted that the findings are similar. For example, EI was negatively related to stress in gifted Chinese adolescents [33,34]. In addition, people who are unsure about their emotional knowledge and emotional regulation abilities experience high perceived stress and low life satisfaction [27]. Similarly, a negative relationship between TEI with school burnout was observed among Italian high school adolescents [25].

At this point, most findings have concentrated on the negative implementation of stress, However, TEI's knowledge and application in an individual's life can be beneficial, primarily to determine and rectify a prosperous future for an adolescent. It has been noted that developing and enhancing TEI does not only help oneself to be successful and productive, but its also helps people around us. The TEI process helps individuals moderate conflict, fostering growth, harmonious relationships, and stability [35].Many students already possess problem-solving and empathic

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skills, and how self-aware one is regarding his / her emotions. However, it becomes difficult to understand and utilize those skills in times of adversity. Numerous studies indicate a well-established relationship between TEI and coping. TEI helps in modifying the coping effectiveness and influences mental health. Data has indicated that children with high Emotional Intelligence are more focused on adaptive coping strategies or positive restructuring techniques as compared to non-adaptive coping mechanisms. TEI emphasizes on reducing distress and cultivating well-being. After three decades, revisions in the New Education Policy (NEP) by the Government of India have been formulated. NEP will not only help to achieve the objective from a Multidisciplinary but also from a cross-disciplinary perspective. It will consider the child's overall development regarding the cognitive domain, emotional stimulation, and teaching the concept of values and traditions. The policy will focus on experiential learning and critical thinking. Students will not just be tested on concepts and knowledge but also on higher-order foundational skills, and what better way to learn those skills and apply in one's routine.

The aim of educators, counselors, and mental health practitioners should be to recognize and make this transitory phase as smooth as possible. Policies and interventions should be designed so that adolescents feel comfortable opening up and communicating their struggles without feeling judged. Students rely on their family and social support for relaxation to channel their emotions more appropriately, especially during stressful situations. Children and adolescents must express their emotions, empathy, and relationships [36]. Working on TEI will aid in developing and implementing interventions that will assist children in improving their socio-emotional competence and help them function effectively in socially and emotionally burdened situations. This would further help make the transition from adolescence to young adulthood smother and equip them to deal with the adversities later in their lives, in interpersonal and intrapersonal domains. The current study has limitations. The global pandemic has influenced certain aspects of the study, which were inconceivable at the onset of the research. The first limitation is collecting data online. Further, the sample population consists of an unequal ratio of females and males as we had limited access to equal participation, which could have seen skewed numbers in a natural setting.

CONCLUSION

Children with emotional intelligence tend to be happier, dedicated to their desired goals, and healthier. Trait Emotional Intelligence is a holistic concept. The majority of the studies focus on Global TEI than individual sub factors. This current study indicates the role of well-being and self-control in managing stress among adolescents. The finding can help develop specific training programs for nurturing well-being and self-control among adolescents to manage their stress. These interventions can improve their socio-emotional ability and help them function effectively in socially and emotionally burdened situations. The competence in handling stress will enable adolescents to deal with future challenges, both in interpersonal and intrapersonal domains.

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Table 1: Descriptive Statistics for Trait emotional intelligence and stress variables (N=374)

	Mean	SD
AGE	15.87	1.232
Well-being (WB)	28.77	8.136
Self-control (SC)	22.97	6.091
Emotionality (EM)	32.78	7.353
Sociability (SO)	27.17	6.040
TOT GTEI	128.42	23.908
Stress of Home Life (SHL)	31.87	9.562
School Performance (SP)	17.43	5.701
School Attendence (SA)	9.90	3.600
Romantic Relationships (RR)	11.06	4.166
Peer Pressure (PP)	16.95	6.013
Teacher Interaction (TI)	14.65	5.183
Future Uncertainty (FU)	7.57	3.153
School / Leisure Conflict (SLC)	10.53	4.404
Financial Pressure (FP)	8.01	3.302
Emerging Adult Responsibility (EAR)	5.72	2.597
Total Adolescent Stress (Tot ASQ)	133.69	39.416

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Table 2: Correlation between TEI dimensions and stress paramenters

S.no	Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	AGE																
2	WB	-.053															
3	SC	-.079	.458**														
4	EM	.009	.339**	.351**													
5	SO	-.041	.374**	.290**	.448**												
6	TEI	-.056	.778**	.701**	.715**	.684**											
7	SHL	.050	-.449**	-.357**	-.225**	-.271**	-.456**										
8	SP	.074	-.328**	-.276**	-.214**	-.270**	-.370**	.739**									
9	SA	.065	-.258**	-.288**	-.243**	-.213**	-.340**	.586**	.613**								
10	RR	.042	-.404**	-.361**	-.226**	-.264**	-.429**	.666**	.683**	.658**							
11	PP	.074	-.527**	-.339**	-.246**	-.259**	-.492**	.719**	.630**	.574**	.748**						
12	TI	.039	-.389**	-.293**	-.244**	-.215**	-.395**	.692**	.710**	.637**	.749**	.708**					
13	FU	.044	-.368**	-.287**	-.230**	-.196**	-.387**	.642**	.559**	.533**	.634**	.630**	.687**				
14	SLC	.031	-.409**	-.233**	-.268**	-.260**	-.411**	.653**	.584**	.507**	.622**	.633**	.737**	.701**			
15	FP	-.011	-.320**	-.228**	-.211**	-.212**	-.338**	.558**	.547**	.386**	.619**	.582**	.685**	.628**	.701**		
16	EAR	.068	-.275**	-.276**	-.149**	-.172**	-.310**	.549**	.534**	.411**	.608**	.559**	.680**	.539**	.592**	.657**	
17	TS	.060	-.474**	-.367**	-.277**	-.294**	-.493**	.881**	.833**	.722**	.851**	.849**	.887**	.785**	.814**	.750**	.717**

* p < .05, ** p < .01;

Table 3: Regression analysis predicting stress among adolescents

Dependent Variable	R	R2	Adj R2	F (df)	TEI	B	β	t
Home Life (SHL)	.488	.238	.23	28.872 (4, 369)	WB	-.390	-.332	-6.180**
					SC	-.274	-.174	-3.313**
					EM	-.014	-.011	-.200
					SO	-.145	-.091	-1.733
School Performance (SP)	.385	.148	.139	16.081(4, 369)	WB	-.143	-.204	-3.599**
					SC	-.121	-.129	-2.325*
					EM	-.028	-.036	-.650
					SO	-.132	-.140	-2.509*
School Attendance (SA)	.349	.122	.112	12.794 (4, 369)	WB	-.050	-.113	-1.957
					SC	-.105	-.177	-3.127**
					EM	-.054	-.111	-1.949
					SO	-.042	-.070	-1.238
Romantic Relationships (RR)	.460	.211	.203	24.722 (4, 369)	WB	-.137	-.268	-4.916**
					SC	-.139	-.203	-3.789**
					EM	-.012	-.021	-.388
					SO	-.065	-.095	-1.765
Peer pressure (PP)	.542	.294	.286	38.330 (4, 369)	WB	-.332	-.449	-8.697**
					SC	-.106	-.107	-2.116*
					EM	-.030	-.037	-.730
					SO	-.043	-.043	-.842

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Teacher interaction (TI)	.421	.177	.168	19.859 (4, 369)	WB	-.187	-.294	-5.266**
					SC	-.100	-.118	-2.156*
					EM	-.063	-.089	-1.613
					SO	-.026	-.031	-.562
Future Uncertainty (FU)	.401	.161	.151	17.640 (4, 369)	WB	-.107	-.275	-4.889**
					SC	-.065	-.126	-2.278*
					EM	-.036	-.084	-1.510
					SO	-.010	-.018	-.333
School/Leisure Conflict (SLC)	.437	.191	.182	21.801 (4, 369)	WB	-.180	-.332	-6.007**
					SC	-.013	-.018	-.341
					EM	-.067	-.112	-2.059*
					SO	-.059	-.080	-1.479
Financial Pressure (FP)	.350	.122	.133	12.840 (4, 369)	WB	-.095	-.235	-4.073**
					SC	-.040	-.074	-1.309
					EM	-.033	-.074	-1.298
					SO	-.038	-.070	-1.231
Emerging Adult Responsibility (EAR)	.327	.107	.097	11.048 (4, 369)	WB	-.055	-.171	-2.946**
					SC	-.077	-.181	-3.177**
					EM	-.001	-.004	-.070
					SO	-.023	-.053	-.932
Total Stress (TS)	.517	.267	.259	33.582 (4, 369)	WB	-1.676	-.346	-6.570**
					SC	-1.041	-.161	-3.113**
					EM	-.339	-.063	-1.217
					SO	-.583	-.089	-1.724

Note. WB = Wellbeing, SC = Self-Control, EM = Emotionality, SO = Sociability. * p < .05, ** p < .01

RESEARCH ARTICLE

Weed Rhizomicrobiome: A Potential Reservoir of Plant - Growth Promoting Rhizobacteria (PGPR)

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ABSTRACT

Soil is known to be a living entity which is not only considered to be a valuable natural resource in agricultural and food security but it is also valuable towards preservation of all life process. Weeds are considered as unwanted plants due to their uselessness in agricultural or any plant growth. However, the rhizobacteria present near the roots of the weeds can be considered useful as they help in fast and unwanted growth of weeds in any environmental condition. Helpful outcomes for plants are essentially a delayed consequence of organic entity's nitrogen-fixation, siderophore production, inorganic phosphate solubilization and stress improvement through an enormous bunch of normal and genuine frameworks. These microorganisms are found inside each the microorganism and parasitic domain, and possess all parts of the plant. The roots and their close to space have an upscale Phyto-microbiome, that in consolidates with the profoundly strong nature of roots makes the rhizosphere a normally and unnaturally powerful region. Subsequently, it impacts factors significant for plant perseverance and achievement, equivalent to enhance openness and presence of microorganisms. Weed rhizosphere can be used for the production, cultivation and growth of various plants as these rhizospheric microorganisms can affect the progression, yield, nature and prosperity of different plants, while simultaneously lowering the requirement of various environmentally un favourable sources like chemical pesticides.

Keywords: Weeds, Plant-Growth Promoting Rhizobacteria (PGPR), Rhizomicrobiome, Rhizosphere, Rhizobacteria.

INTRODUCTION

Not all living organisms blend inside the environment changed by humans. Many species survived alongside the humans, whereas some affected, died and generally fixed by alternative hostile colonizers. However, some unwanted plants managed to grow in groups that are referred to as weeds and sometimes grownup as a crop that doesn't need artificial propagation (1). These are able to establish themselves into new populations inside a man-made surrounding despite continuous efforts to manage and eradicate them. Within the evolution of the weeds, several factors that are measure accountable to face up to the unfavorable conditions, among them, the association of the useful micro flora can be one among the most reasons (2). For sustaining life on the land, every living being depends upon the association with its Neighbors e.g., plant-plant, plant microbes and microbes-microbes, interaction (3). Weeds are considered as undesirable plants, which sometimes flourish in the unfavorable conditions as continuous efforts are created by unpredicted means to manage their growth (4).

Plant growth promoting rhizobacteria are those bacteria which colonize the plant roots and helps in promoting the plant growth (5). Their effects can happen by means of local enmity to soil-borne microbes or by acceptance of foundational resistance against microorganisms all through the whole plant. A few substances created by opposing rhizobacteria are considered to be connected with microbe control and circuitous advancement of development in most plants, for example, antibiotics and siderophores (6). The induced systemic resistance in plants looks like microorganism systemic acquired resistance, where the actuating bacteria and the difficult microbe remain isolated. These two kinds of the induced resistance provide uninfected parts of plant more impervious to microorganisms in different plant species (7-8). They provoke general resistance as the bio-protectant and management in the various plant diseases. PGPR can also promote the plant growth as biofertilizer, however as a bio-stimulant, they manufacture phytohormones i.e., gibberellin, IAA, cytokines and ethylene (9).

Usually, PGPRs promote the plant growth directly either by the kind of nutrient offer (phosphorus, nitrogen, essential minerals and potassium) or by dynamic the amount of phytohormone and indirectly by decreasing the morbific impact in the growth and development of the plant within the types of bio-control mediators, root colonizing bacterium and environmental defender. Plant growth regulator includes hormones as well as additionally artificial compounds (10). The molecular level working site of various plant growth hormones is still unresolved. Every hormone produces an effective physiological response. Out of those responses, many responds to completely different hormones which sometimes acts frequently similar. The response of any plant or any of its structure to plant growth regulators may vary with the variability of the plant.

There are some direct methods as well as indirect methods. The direct methods are the best-known mechanisms for the plant growth promotion of bacteria, which includes providing plants with nutrients/resources that they lack such as phosphorus, fixed nitrogen, iron, potassium and many more. Several agricultural soils require a decent quantity of one or additional of those compounds so that the growth of the plant improves. A number of the direct strategies are nitrogen fixation, phosphate solubilization, sequestering Iron and modulating plant hormone levels (11). The Indirect methods include the ability of the bio control bacterium to indirectly promote the plant growth which has been considered as the supply of rich interest, each in terms of understanding and developing of a number of the underlying mechanisms employed by the bacterium and utilizing these bacteria commercially rather than chemical pesticides (12). It includes use of Antibiotics and Lytic enzymes, ethylene, siderophores, and evoked systemic resistance.

PHYSIOLOGYOFPLANT-GROWTH PROMOTING RHIZOBACTERIA

The co-evolution of plant and microbes results into the turning of many microorganisms into facultative intracellular endophytes (13).Plant-growth promoting rhizobacteria are the non parasitic bacteria which exerts some helpful effects on plants by the means of some direct and indirect methods. Many rhizobacteria are used for the enhancement of nutrients and water uptake, biotic and abiotic stress tolerance. Although various soil

microorganisms are reported for the development and promoting plant growth, their mode of action for exhibiting beneficial activities are typically not understood well. The mechanism responsible for the physiological changes of the plant-microbe interaction are getting down, mainly thanks to the rising "omics" approaches. Phytohormones are the major aspects in control of development and plant growth. In response to various environmental factors which may limit the plant growth or may one day become deadly when uncontrolled ,they conjointly operate as molecular signals (14).Several rhizosphere microorganisms are noted to eject hormones so that they can manipulate hormone balance for root uptake with in the plants tospiceups tress response and growth.

Several PGPRs will turn out to be auxins to exert significantly robust effects on root growth and design. The most studied auxin created by PGPRs is Indole-3-acetic acid (IAA) which is involved in various plant-microbe interactions. The endogenous IAA levels decide the function of exogenous IAA in plants. At optimum Indole acetic acid concentrations in most plants, the application of microorganism Indole acetic acid might have some negative, positive or neutral effects on the plant growth (15). PGPRs that turn out to be auxins are shown to elicit transcriptional changes in cell wall related genes, secretion, defense-related, increase root biomass, induce longer roots (16), reduce stomata size and density as well as activate phytohormoneres ponsegenes that may enhance plant growth. Several PGPR producecy to kin in and gibber ell in show ever the role of hormones that are synthesized by bacteria in plants, and the synthesis mechanism of microorganism, are not fully understood yet. Some of the PGPR strains will promote comparatively large amounts of gibberellins which results in in creased plants hoot growth. The interactions between auxins and those hormones will alter the root design (4). Production of cytokinin by PGPR may cause increased root growth production by the plant (10), doubtless increasing the presence of the PGPRs related to the plant. Ethylene may be a vaporish secretion ,active at extraord in arily low con cent rations and maybea'stress secretion', as its con cent ration spiking through out varied biotic and abioticstresses .During stress, the accumulation of ethylene might increase the plant tolerance or may show some stress response symptoms along with senescence (17). The working of PGPR under stressed and unstressed conditions has been studied and infrequently it provides larger growth stimulation under the stressed conditions, for example under the drought stress conditions (18).

For some PGPRs, ethylene plays a very important and crucial role for improving the plant stress tolerance (19). PGPR sometimes secrete aminocyclo pro panecarboxylase (ACC) deaminase that lowers the production of ethylene in plants (20-21). Several studies have shown increased stress tolerance in the plants through vaccination with PGPR that produces the ACC deaminase. This occurs as PGPR are ready to keep the level of ethylene from reaching levels decent to cut back plant growth. Plant growth promoting rhizobacteria related with different agricultural crops have displayed to advance plant development by lowering malicious micro flora which is pathogenic. *Pseudomonas* and *Bacillus* have risen as the greatest and possibly most encouraging gatherings among PGPR associated with bio-control of illnesses (22). A few examinations on *Pseudomonas* and *Bacillus* revealed their viability as a possible adversary against microorganisms involved to various plant illnesses. Microorganism bio-control embroils different elements of rhizobacteria. Basically, the rivalry for supplements, specialty avoidance, prompted foundational opposition, and creation of antifungal metabolites including anti-microbials, lytic chemicals and bacteriocins are considered the main methods of bio-control movement in PGPRs (8, 23).

RHIZOMICROBIOME

Rhizomicrobiome is a significant part of the plant biological system which impacts the plant wellbeing in normal and dire conditions. In fact, it is made out of different microbial networks whose presence is mainly impacted by the root exudates. The rhizosphere along with its microbial local area is called rhizo micro biome. The term 'rhizosphere' was coined by a plant physiologist "Lorenz Hiltner". The rhizosphere has been depicted as an area around the plant root occupied by some special organisms under the impact of various root exudates (24). "Rhizospheric impact" goes about as the main strength for creating arich and different rhizospheric microbial populace. It is the peculiarity by which dynamic microbial populaces are drawn in toward the natural matter delivered by developing plant roots(25). Archaea, bacteria, protozoa, fungi, oomycetes, nematodes, micro arthropods etc. are the significant parts of the rhizo micro biome. Be that as it may, the bacterial area is the predominant one followed by different protozoa and actinomycetes.

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The rhizosphere can be separated into mainly three districts, viz., ectorhizosphere, rhizoplane and end orhizosphere. The Ectorhizosphere is considered as the peripheral zone and reaches out from the rhizoplane into the mass soil. The rhizoplane is considered as the average zone which incorporates the adhesive and root epidermis. The endorhizosphere incorporates the area of endodermis and cortex involved by microorganisms. The rhizo microbiome plays a significant role in maintaining plant health and is extremely in fluenced by the root exudates of the host plant. Plants choose as et of micro beset completely different s tages of the ir development, and confirm the micro biome community composition in its immediate neighborhood presum ptively for specific functions(26).The Rhizomicrobiome consists of heterogeneous microorganism community with specific functions and is extremely influenced by the plant kind, soil type and environmental conditions. The Rhizomicrobiome communities aside from promoting plant growth conjointly elicit general tolerance to salinity and drought in stressed plants. Microorganism communities manufacturing aminocyclopropanecarboxylate (ACC) deaminase modulate stress ethylene in the Plants and all eviate the consequences of abiotic stress and will increase plant ability to stressed atmosphere. Inter and in traspeciesmi croorganism signals and cross talks modulate plant genes within the rhizo biome and are essential for the functioning and property of plant growth underneath adverse environmental conditions (27). The term rhizosphere to describe the plant-root connection, a word began from the Greek word i.e. rhiza, which means root, the quick zone of soil that encompasses and is affected by plant roots (24). Rhizosphere is a supplement rich zone (28).

It goes about as an intersection for supplement trade between plants, soil and microbes (29-30). It is the problem area for the microbial local area contrasted with mass soil (31-33) and harbors explicitly unequivocal gatherings e.g., ammonifiers, nitrifiers and denitrifiers (34). The micro flora of rhizosphere is dynamic and different, in this manner containing bacteria, nematodes, fungi, algae, protozoa and micro arthropods (33). Yet, the populace shows strength of the species having a place with actinobacteria and proteobacteria (35). Rhizospheric bacteria that have plant development advancement potential are alluded as plant-growth promoting rhizo bacteria's (PGPRs) (36). Variety of rhizospheric micro flora changes with various plant species. *Flavobacteriaceae*, *Comamonadaceae* and *Rhizobiaceae* bacterial families were seen to overwhelm the root micro biota of Barley (13), while that of Thyme was found to be overwhelmed by *Nocardioidaceae*, *pseudomonas*, *Bradyrhizobiaceae* and *Geodermatophilaceae* (37). The rhizospheric microbial networks shift radially including en do-rhizosphere, rhizoplane, and ec to-rhizosphere yet additionally in explicit root areas along the root pivot. Sub-atomic fingerprints have uncovered the presence of unmistakable microbial networks in various root areas like that of arising roots along with their tips and extending site of rise and roots of sidelong roots and more established roots. Rhizospheric microbial networks are additionally observed to be impacted by wholesome stress, age, status or soundness of the host plant (38).

WEED RHIZOMICROBIOME AS A SOURCE OFPGPRs
Soil microorganisms play a significant part in soil processes that decide the usefulness of plant. Rhizosphere microbial networks are affected by the plant roots exudates as mechanical help as well as the contest for various nutrients. Similarly, plants are impacted by rhizosphere microbial networks through their involvement in water dependence, growth promoting metabolites and in quick soil nutrients cycle (39). One of many different processes by which the rhizobacteria advance plant development is by solubilizing the insoluble minerals.

Weeds are undesirable plants normally grow in the horrible climate as nonstop attempts are made to stop and control their development. There are many explanations behind the endurance of weeds in unfriendly conditions, among which relationship of the foundations of helpful micro flora is one reason. Some undesired plants managed to grow alongside home-grown plants which are referred to as weeds and frequently developed as a plant which is not required (40). These can build up new populaces into a man-made environment in spite of ceaseless attempts in controlling them. In the advancement of various weeds, many variables are mindful to withstand the unfavorable conditions, out of them the relationship of the helpful micro flora could be the one of many principle reasons. To support life on the land each organic entity relies on relationship to its neighbors for example microorganisms, plant microorganisms and plant-plant communication (1).

For the improvement in the plant development, advancement, safeguard and method of activity of PGPR include complex systems through the creation of siderophore and anti-toxins. They incite fundamental Obstruction as bioprotectant and help in control the plant illnesses. Inany case ,by further developing supplement obtaining PGPR advance plant development as a biofertilizer however as a bio- stimulant, they mainly produce phytohormones for example gibberellin, cytokines,indole-3-acidic acid and ethylene (41). Through invigorating harmonious N_2 obsession, by a-symbiotic N_2 obsession nodulation, and knob inhabitance PGPR straightforwardly advance development of plants. The biochemical, physiological and morphological characteristics of weed saretraditional means for the PGPR distinguish in g proof and to perceive unidentified bacteria up to species and class level, still the 16s r RNA quality arrangement examination has become a huge technique (42). In an exceptional climate, to development-based methodologies most micro-organisms are hesitant (6).

The PGPRs from the rhizosphere of weeds can be utilized to advance the development of yield plants and their efficiency in unfavorable ecological conditions. It will at last lessen the interest of manures and agrochemicals in farming area. Weeds rhizosphere is mainly studied for various plant growth promoting rhizobacteria that are effectively used to design the rhizosphere of numerous farming harvests. Nonetheless, weeds rhizosphere is still being underexplored. In context of food security and expanding barometrical worth, the usage of plant development advancing rhizobacteria for decreasing synthetics commitment in agro biological system that is possibly a significant issue (26).

PGPR disengaged from the weed rhizosphere are being utilized in various harvests that will help them in better reserve their plant development and improvement credits for example N_2 obsession, organic phosphate solubilization, IAA creation and just as bio-control actives by delivering various compounds metabolites and anti-infection agents. Despite the fact that relationship of rhizobacteria with the non-leguminous plants, for example, grasses are known for quite a long time, they are ineffectively - studied. Weeds provide various opportunities to investigate the soil micro flora for the yield enhancement of crops and its production(43). Weed rhizosphere is considered as a rich source of Plant growth promoting rhizobacteria which helps the crops during the unfavorable environment. However, weeds are still under explored for improvement of the potential PGPR that are valuable bio control and growth stimulator as compared to pesticides and chemical fertilizers. Weedy grass species typically flourish in unfriendly conditions and go about as expected living spaces for the different gatherings of world class microorganisms with various helpful characters stays neglected (44).

CONCLUSION

The use of plant-growth promoting rhizobacteria as part of the agricultural practice can be considered as a technology which becomes a major requirement for the plant growth. These rhizobacteria are already getting used with success in many nations within the world and this is still anticipated to grow more. In the developed world, Wherever agricultural chemicals stay comparatively cheap, the utilization of plant-growth promoting rhizo bacteria occupies a small but growing niche with in the development of the organic agriculture. The rhizo microbiome plays a major role in the development of these PGPB as it is the environment in which these bacteria grow. A good and nutrient rich Rhizomicrobiome will helps in better growth of these PGPB. The network of microbial interaction occurring in Rhizomicrobiome region is highly complex and requires high throughput techniques to analyse the large number of organisms involved in shaping the rhizosphere and influencing plant growth. Discoveries in plant micro biome will advance the development of the microbial inoculants as bio control agents, biofertilizers or stress protectants and to develop relevant bio products for stressed soils. The organization of microbial association happening in Rhizomicrobiome area is exceptionally complicated and requires high throughput procedures to dissect the enormous number of organic entities included in forming the rhizosphere and affecting plant development. Rhizomicrobiome addresses an intricate and dynamic framework including nonstop communications between plant-organism just as microorganism organism. The arrangement and practical job of rhizo microbes is administered by different variables. Current apparatuses and methods are consistently working on our insight

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regarding this complicated specialty. Incorporated omic devices alongside their metaomic accomplices and demonstrating methods can end up being useful in settling the perplexing enigma of rhizosphere framework all in all. Effective understanding and hence attainable double-dealing of rhizobacteria as biofertilizers at the ground level over substance composts will certainly end up being a stage forward toward maintainable improvement overall and maintainable horticulture specifically.

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RESEARCH ARTICLE

On Near Plithogenic Neutrosophic Hypersoft Perfectly Continuous Mappings

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ABSTRACT

The aim of the paper is to bring out the perfect continuity of near plithogenic neutrosophic Hypersoft functions. In addition, some basic and theoretical properties of the near plithogenic neutrosophic Hypersoft perfectly continuous functions are studied.

Keywords: near plithogenic neutrosophic Hypersoft set, near plithogenic neutrosophic Hypersoft topology, near plithogenic neutrosophic Hypersoft continuous functions, near plithogenic neutrosophic Hypersoft strongly continuous functions, near plithogenic neutrosophic Hypersoft perfectly continuous functions

INTRODUCTION

The rough sets were presented by Paw la k [13] in 1982. The theory of near sets was presented by James. Peters [6]. The soft-set concept was developed by as a completely new math tool for solving difficulty in dealing with uncertainty. Molodtsov [9] defined a soft set that is "sub-set as a parameterized family of the set of the universe. In the past few years, the fundamentals of soft set theory have been studied by different researchers. Florentin Smarandache [5] generalized the soft set to Hypersoft set by transforming the function F into a multi-argument function to deal with uncertainty, where one can have multiple parameters and so it can be used in several applications. Florentin Smaradache [4,5] introduces the plithogenic set as a generalization of crisp, fuzzy, intuitionistic fuzzy and neutrosophic sets. A plithogenic set is characterized by one or more parameters" and each parameter may have several values. In 1960, Levine[8] introduced the concept of strong continuity in topological

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spaces. Takashi Noiri[12] defined perfectly continuous functions as one of the strong forms of continuity in topological spaces. Several researchers[2,7,10,11] have extended their work on perfect continuity.

Preliminaries

Definition 2.1[3] Let U be the universal set of objects, A⊆U, **Ω** be a plithogenic Hypersoft set whose degree of appurtenance of each parameter is a "neutrosophic set over U and NAS= (U, Þ, ~$_{Dq}$, Γ$_q$, ζ$_{Γq}$) be the nearness approximation space. The lower and upper near approximations of **Ω** with respect to NAS is given by,

$\underline{\Gamma q(D)}(\Omega)$ = $\cup_{a:[a]Dq \subseteq \Omega}[a]Dq$ and

$\overline{\Gamma q(D)}(\Omega)$= $\cup_{a:[a]Dq \cap \Omega \neq \emptyset}[a]Dq$

respectively. The boundary of Ω with respect to NAS is given by, B$_{\Gamma q(D)}$(Ω) =$\overline{\Gamma q(D)}(\Omega)$ - $\underline{\Gamma q(D)}(\Omega)$. If $\underline{\Gamma q(D)}(\Omega) \neq \emptyset$ and B$_{\Gamma q(D)}(\Omega) \geq 0$, then Ω is a near plithogenic neutrosophic hypersoft set.

Definition 2.2 [3] Let (U, τ$_Þ$) be a near plithogenic neutrosophic Hypersoft topological space over U and Þ$_D$ be a near plithogenic neutrosophic Hypersoft set in U, then,

The set U{C$_D \subseteq$ Þ$_D$: C$_D$ is a near plithogenic neutrosophic Hypersoft open set of U} is called the near plithogenic neutrosophic Hypersoft interior of Þ$_D$" in U. It is denoted by in t$_Þ$ (Þ$_D$).
The set ∩{Q$_D \supseteq$ Þ$_D$: Q$_D$ is a near plithogenic neutrosophic Hypersoft closed set of U} is called the near plithogenic neutrosophic Hypersoft closure of Þ$_D$ in U. It is denoted by cl$_Þ$(Þ$_D$).

Near Plithogenic Neutrosophic Hypersoft Perfectly Continuous Functions
Definition 3.1
A near plithogenic neutrosophic Hypersoft function ϱ : (U, τ$_Þ$) ⟶ (V, τ$_Þ{}^1$) is said to be Near Plithogenic Neutrosophic Hypersoft Perfectly Continuous if the "inverse image of every near plithogenic neutrosophic hypersoft open set of (V, τ$_Þ{}^1$) is near plithogenic neutrosophic Hypersoft c lo pen in (U, τ$_Þ$)

Example 3.2
Let U={a$_1$,a$_2$,a$_3$}; V ={b$_1$,b$_2$,b$_3$}; N= (α_1, α_2, α_3, α_4, α_5)
ϱ (a$_1$)=b$_2$; ϱ (a$_2$)=b$_3$; ϱ (a$_3$)=b$_1$
Þ$_D$ = {(α_1, α_2, α_4)U ,(α_1, α_2, α_5)U, (α_1, α_3, α_4)U ,(α_1, α_3, α_5)U }; Q$_D$ = {(α_1, α_2, α_4)V ,(α_1, α_2, α_5)V, (α_1, α_3, α_4)V ,(α_1, α_3, α_5)V}
τ$_Þ$ = {∅$_Þ$, Þ$_D$,{(α_1, α_3, α_5){ a$_1$, a$_3$}}, (α_1, α_2, α_4){a$_3$},(α_1, α_3, α_4){ a$_1$, a$_2$}}, {(α_1, α_2, α_4){ a$_1$, a$_2$}}, (α_1, α_2, α_5) U ,(α_1, α_3, α_4){ a$_3$},(α_1, α_3, α_5){ a$_2$}} be the near plithogenic neutrosophic Hypersoft topology of Þ$_D$.

τ$_Þ{}^1$= {∅$_Þ$, Q$_D$,{(α_1, α_2, α_4){ b$_2$, b$_3$}}, (α_1, α_2, α_5) V ,(α_1, α_3, α_4){ b$_1$}, (α_1, α_3, α_5){ b$_3$}}, {(α_1, α_2, α_4){ b$_1$}}, (α_1, α_3, α_4) {b$_2$,b$_3$} ,(α_1, α_3, α_5"){ b$_1$, b$_2$}} be the near plithogenic neutrosophic Hypersoft topology of Q$_D$.
ϱ : (U, τ$_Þ$) ⟶ (V, τ$_Þ{}^1$) is near plithogenic neutrosophic Hypersoft perfectly continuous.

Theorem 3.3
If ϱ : (U, τ$_Þ$) ⟶ (V, τ$_Þ{}^1$) be a near plithogenic neutrosophic Hypersoft strongly continuous function, then it is near plithogenic neutrosophic Hypersoft perfectly continuous.

Proof Let ϱ be a near plithogenic neutrosophic Hypersoft strongly continuous mapping and consider a near plithogenic neutrosophic Hypersoft open set Þ$_D$ in (V, τ$_Þ{}^1$). Then ϱ^{-1}(Þ$_D$) is near plithogenic neutrosophic hypersoft clopen in (U, τ$_Þ$).

Since ϱ is near plithogenic neutrosophic Hypersoft strongly continuous every open set in (V, τ$_Þ{}^1$) is near plithogenic neutrosophic Hypersoft clopen in (U, τ$_Þ$).
⇒ ϱ is near plithogenic neutrosophic Hypersoft perfectly continuous.

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Remark 3.4
The converse of the theorem need not be true.

Example 3.5
From Example 3.2, $\varrho : (U, \tau_P) \longrightarrow (V, \tau_P{}^1)$ is near plithogenic neutrosophic Hypersoft perfectly continuous but not strongly continuous.

Theorem 3.6
A near plithogenic neutrosophic Hypersoft function $\varrho: (U, \tau_P) \longrightarrow (V, \tau_P{}^1)$ is near plithogenic neutrosophic Hypersoft perfectly continuous if $\varrho^{-1}(Q_D)$ is near plithogenic neutrosophic Hypersoft cope in (U, τ_P) for every near plithogenic neutrosophic Hypersoft closed set Q_D in $(V, \tau_P{}^1)$.

Proof Let Q_D be a near plithogenic neutrosophic Hypersoft closed set in $(V, \tau_P{}^1)$
Then $Q^C{}_D$ is near plithogenic neutrosophic Hypersoft open in $(V, \tau_P{}^1)$.
$\Rightarrow \varrho^{-1}(Q^C{}_D)$ is both near plithogenic neutrosophic Hypersoft open and closed in (U, τ_P)
Since ϱ is near plithogenic neutrosophic Hypersoft perfectly continuous function, $\varrho^{-1}(Q^C{}_D) = U/ \varrho^{-1}(Q_D)$
$\Rightarrow \varrho^{-1}(Q_D)$ is near plithogenic neutrosophic Hypersoft clopen in (U, τ_P)
Conversely, assume that $\varrho^{-1}(Q_D)$ is near plithogenic neutrosophic Hypersoft clopen in (U, τ_P).
Let Q_D be a near plithogenic neutrosophic Hypersoft open set in $(V, \tau_P{}^1)$. Then $Q^C{}_D$ is near plithogenic neutrosophic Hypersoft closed in $(V, \tau_P{}^1)$
$\Rightarrow \varrho^{-1}(Q^C{}_D)$ is near plithogenic neutrosophic Hypersoft clopen in (U, τ_P).
We know that, $\varrho^{-1}(Q^C{}_D) = U/ \varrho^{-1}(Q_D)$ is near plithogenic neutrosophic Hypersoft clopen in (U, τ_P).
$\Rightarrow \varrho^{-1}(Q_D)$ is near plithogenic neutrosophic Hypersoft clopen in (U, τ_P).
Hence ϱ is near plithogenic neutrosophic Hypersoft perfectly continuous.

Theorem 3.7
Let (U, τ_P) and $(V, \tau_P{}^1)$ be two near plithogenic neutrosophic Hypersoft topological spaces and (U, τ_P) be near plithogenic neutrosophic Hypersoft discrete topology. If $\varrho : (U, \tau_P) \longrightarrow (V, \tau_P{}^1)$ be a near plithogenic neutrosophic Hypersoft mapping, then
 i. ϱ is near plithogenic neutrosophic Hypersoft strongly continuous
 ii. ϱ is near plithogenic neutrosophic Hypersoft perfectly continuous

Proof
 i.   Let R_D be a near plithogenic neutrosophic Hypersoft open set in $(V, \tau_P{}^1)$
      By hypothesis, $\varrho^{-1}(R_D)$ is near plithogenic neutrosophic Hypersoft open in (U, τ_P)
      Since (U, τ_P) is a near plithogenic neutrosophic Hypersoft discrete topological space, $\varrho^{-1}(R_D)$ is near plithogenic neutrosophic Hypersoft closed in (U, τ_P) Therefore, ϱ is near plithogenic neutrosophic Hypersoft strongly continuous.
 ii. By Theorem .4, ϱ is near plithogenic neutrosophic Hypersoft perfectly continuous.

Theorem 3.8
If $\varrho : (U, \tau_P) \longrightarrow (V, \tau_P{}^1)$ and $\delta: (V, \tau_P{}^1) \longrightarrow (W, \tau_P{}^2)$ are near plithogenic neutrosophic Hypersoft perfectly continuous mappings, then their composition is also near plithogenic neutrosophic Hypersoft perfectly continuous.
Proof Let S_D be a near plithogenic neutrosophic Hypersoft open set in $(W, \tau_P{}^2)$. Since δ is near plithogenic neutrosophic Hypersoft perfectly continuous function, $\delta^{-1}(S_D)$ is near plithogenic neutrosophic Hypersoft clopen in $(V, \tau_P{}^1)$.
$\Rightarrow \varrho^{-1}(\delta^{-1}(S_D))$ is near plithogenic neutrosophic Hypersoft clopen in (U, τ_P) (Since ϱ is near plithogenic neutrosophic Hypersoft perfectly continuous)
Since, $\varrho^{-1}(\delta^{-1}(S_D)) = (\delta \circ \varrho)^{-1} (S_D)$
$(\delta \circ \varrho)^{-1} (S_D)$ is near plithogenic neutrosophic Hypersoft clopen in (U, τ_P).

Therefore δ ∘ ϱ is near plithogenic neutrosophic Hypersoft perfectly continuous.

Theorem 3.9

Suppose ϱ :(U, τₚ) ⟶ (V, τₚ¹) and δ: (V, τₚ¹) ⟶ (W, τₚ²) be near plithogenic neutrosophic Hypersoft continuous and perfectly continuous maps respectively, then δ ∘ ϱ : (U, τₚ) ⟶ (W, τₚ²) is near plithogenic neutrosophic Hypersoft continuous.

Proof Let F_D be a near plithogenic neutrosophic Hypersoft open set in (W, τₚ²).

Since δ is near plithogenic neutrosophic Hypersoft perfectly continuous function, $δ^{-1}(F_D)$ is near plithogenic neutrosophic Hypersoft clopen in (V, τₚ¹)

⇒ $ϱ^{-1}(δ^{-1}(F_D))$ is near plithogenic neutrosophic Hypersoft open in (U, τₚ) [Since, ϱ is near plithogenic neutrosophic Hypersoft continuous]

Also, $ϱ^{-1}(δ^{-1}(F_D)) = (δ ∘ ϱ)^{-1} (F_D)$ which is near plithogenic neutrosophic Hypersoft open in (U, τₚ).

⇒ δ ∘ ϱ is near plithogenic neutrosophic Hypersoft continuous.

Theorem 3.10

Suppose ϱ :(U, τₚ) ⟶ (V, τₚ¹) and δ: (V, τₚ¹) ⟶ (W, τₚ²) be near plithogenic neutrosophic Hypersoft perfectly continuous and continuous maps respectively, then δ ∘ ϱ : (U, τₚ) ⟶ (W, τₚ²) is near plithogenic neutrosophic Hypersoft perfectly continuous.

Proof Let G_D be a near plithogenic neutrosophic Hypersoft open set in (W, τₚ²).

Then, $δ^{-1}(G_D)$ is near plithogenic neutrosophic Hypersoft open in (V, τₚ¹) [Since δ is near plithogenic neutrosophic Hypersoft continuous function]

Also, $ϱ^{-1}(δ^{-1}(G_D))$ is near plithogenic neutrosophic Hypersoft clopen in (U, τₚ) [Since, ϱ is near plithogenic neutrosophic Hypersoft perfectly continuous function]

We know that, $ϱ^{-1}(δ^{-1}(G_D)) = (δ ∘ ϱ)^{-1} (G_D)$

$(δ ∘ ϱ)^{-1} (G_D)$ is near plithogenic neutrosophic Hypersoft clopen in (U, τₚ).

⇒ δ ∘ ϱ is near plithogenic neutrosophic Hypersoft perfectly continuous

CONCLUSION

Thus, in this paper the concept of perfect continuity was introduced to near plithogenic neutrosophic hypersoft functions and some of the basic properties and the theoretical properties of the near plithogenic neutrosophic Hypersoft perfectly continuous functions are studied. Further many strong forms of continuity are to be studied in future.

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RESEARCH ARTICLE

Vision-and-Language Transformer using Patch Projection

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ABSTRACT

The performance of Vision-and-Language Pre-training (VLP) on several coupled vision-and-language downstream tasks has been demonstrated. VLP techniques currently rely largely on picture feature extraction processes, the majority of which require region supervision (e.g., object detection) and the convolutional architecture (e.g., ResNet). Although it has been dismissed in the literature, we believe it is problematic in terms of (1) efficiency/speed, as simply extracting input features requires significantly more computation than the multimodal interaction steps; and (2) expressive power, as it is upper bounded by the visual embedder's expressive power and its predefined visual vocabulary.In this study, we introduce the Vision-and-Language Transformer using Patch Projection (VLTPP), a monolithic VLP model in which the processing of visual inputs is dramatically condensed to merely the processing of visual inputs.

Keywords: performance, Vision-and-Language Pre-training (VLP), visual embedder's, Projection (VLTPP),

INTRODUCTION

The Vision-and-Language Pre-training (VLP) belongs to a joint domain of vision and language. There are few popular VLP models like Vi LBERT and Pixel-BERT. In Vi LBERT images will be undergoing CNN Backbone and Region Operation which makes the runtime around 900ms.In Pixel-BERT also image needs to undergo CNNwhich a costly operation makes the runtime around 60ms. So, We thought about removing Convolution or Region Supervision to make modals faster. We are using Patch Projection where we do not perform any costly operations like Convolution Neural Networks (CNN)[4], Regions with Convolutional Neural Networks (RCNN)[13]. While some studies include other aims and data structures, practically every VLP model has these two goals.

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Figure 1 . A visual comparison of traditional VLP structures and the modal we propose. Convolutional neural networks were completely removed from the VLP pipeline without affecting performance on downstream tasks. For multimodal interactions, VLTPP is the first VLP model in which the modal-specific components require less processing than the transformer component. These models are fine-tuned on vision-and-language downstream tasks where the inputs involve two modalities, and are pre-trained with image text matching and masked language modelling objectives1 on images and their matched descriptions.

A visual comparison of traditional VLP structures and the VLTPP we propose. Convolutional neural networks were completely removed from the VLP pipeline without affecting performance on downstream tasks. For multimodal interactions, VLTPP is the first VLP model in which the modal-specific components require less processing than the transformer component. Most VLP research has so far concentrated on boosting the power of visual embedders in order to improve performance. Because area features are often cached in advance during training time to minimize the effort of feature extraction, the drawbacks of having a high visual embedder are often overlooked in academic research. The limitations, however, are still visible in real-world applications, as queries in the wild must go through a laborious extraction process.

To that aim, we'll focus on making visual inputs lightweight and fast to incorporate. Recent work (Dosovitskiy et al., 2020; Touvron et al., 2020) shown that embedding pixels before feeding them into transformers[5] can be accomplished via a simple linear projection of a patch. Transformers (Vaswani et al., 2017) are only recently being employed for images, despite being the established mainstream for text (Devlin et al., 2019). We believe that the transformer module, which is utilised in VLP models for modality interaction, may also process visual information in place of a convolutional visual embedder, in the same way that it processes textual data. The Vision-and-Language Transformer (VLTPP) is a system that handles two modalities in a single unified manner, according to this study. Its shallow, convolution-free embedding of pixel-level inputs sets it apart from earlier VLP models. By design, removing deep embedders dedicated entirely to visual input reduces model size and run time dramatically. Figure 1 indicates that our parameter-efficient model is tens of times quicker than VLP models with region features and at least four times faster than those using grid features, while also performing similarly or better on downstream vision and language tasks.

Our aim is to transformer module extracts and processes visual characteristics instead of a separate deep visual embedder, making it the simplest design for a vision-and-language model. This architecture produces substantial runtime and parameter efficiency by default. We have also designed a web app to demo VLTPP. Where we can pass the image path and a question related to it. In response we will be sending the answer for the question. For User Interface VUE version 3[10], For Backend Python Flask app[1].

System requirements for the training and testing of the Data Sets needs more GPU and RAM to make the process faster, So it's better to do it in google colab by adding a few v RAM and v GPU. We can download the modal to the local system and use it in our flask app.

BACKGROUND KNOWLEDGE

Vision-and-Language Modals
The working of vision-and-language modals based on two points: (1) whether or whether the two modes are equally expressive in terms of specialised parameters and/or computation and (2) In a deep network, whether the two modalities interact. A combination of these two points leads to four archetypes based on runtime of Modality Interaction(MI), Textual Embed(TE) and Visual Embed(VE). Four different archetypes like VE>TE>MI(type 1), VE=TE>MI(type 2),
VE>MI>TE(type 3) and MI>VE=TE (type 4).

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Type 1- In this approach image embed is costly than the remaining two Textual embed and Modality interaction. Modals like VSE++ and SCAN belong to this archetype , here they use separate embedders for image and text, then they represent the similarity of the embedded features from the twoembedders with simple dot products or shallow attention layers. Type 2- In this approach both image and textual are equally costly and same like Type 1 Dot product or shallow of two vectors. Despite CLIP's remarkable zero-shot performance on image-to-text retrieval, we could not observe the same level ofper for- mance on other vision-and-language downstream tasks. This finding supports our hypothesis that simple output fusion,even from high-performing unimodal embedders, may not be enough to master complicated vision and language tasks, emphasising the necessity for a more stringent inter-modal interaction scheme.

Type 3- Here it is the most recent VLP model use a deep transformer to model the interaction of image and text features. This involves convolutional networks to extract and embed image features, Which makes the runtime of image embed takes longer than remaining. Also Modulation-based vision-and-language models also comes under type 3, with their visual CNN[4] stems corresponding to visual embedder, RNN[14] producing the modulation parameters to textual embedder and modulated CNNs to modality interaction. Type 4- Our Modal comes under this type where runtime for text and image embeddings are equal where As with word tokens, the embedding layers of raw images are shallow and computationally light. As a result, the majority of the computation is focused on modelling modality interactions.

Modality Interaction Schema
At this stage we will have a transformer which takes visual and textual embedding sequences as input, model inter-modal and optionally intra-modal interactions throughout layers, then output a contextualized feature sequence. Classifies interaction schema into two categories (1) single-stream approaches(e,g., Visual-BERT,UNITER concentrates on image and text inputs); and (2) dual-stream approaches (e.g., Vi LBERT, LXMERT) where the two modalities are not concatenation of image and text inputs. We follow the single-stream for our modality interaction because the dual approach introduces additional parameters.

Visual Embedding Schema
While all perform ant VLP models use the same textual embedder– a tokenizer from pre-trained BERT, as well as word and position embeddings similar to BERT– the visual embedders are different. Visual embedding is still the bottleneck in most (if not all) extant VLP models. Instead of employing region or grid features, which need expensive extraction modules, we focus on cutting cuts on this phase by adding patch projection.

Region Feature[7]
This VLP model dominantly utilize region features. which are obtained from an off-the-shelf object detection like Faster R-CNN. The following is a general pipeline of generating region features. First, a region proposal network (RPN) suggests regions of interest (RoI) based on grid features aggregated from the CNN backbone. The number of RoIs is then reduced to a few thousand by non-maximum suppression (NMS). RoIsare routed through RoI heads and become region features after being pooled by operations such as RoI Align (He et al., 2017). NMS is then applied to each class, reducing the number of features to under a hundred. Object detectors, no matter how light they are, are less likely to be faster than the backbone or a single-layer convolution. Freezing the visual backbone and caching region features in advance only helps during training and not during inference, and it may hinder performance.

Grid Feature [6]
In addition to detector heads, the output feature grid of convolutional neural networks like Res Nets can beused as visual features for pre-training vision and language. VQA-specific models were the first to suggest direct use of grid features (Jiang et al., 2020; Nguyen et al., 2020), primarily to avoid requiring extremely slow region selection operations. The grid aspects of X-LXMERT (Cho et al., 2020) were revisited by fixing region proposals to grids rather than those from region proposal networks. However, the caching of features prevented the backbone from being fine-tuned any more.

Pixel-BERT[15] is the only VLP model that uses a ResNet version backbone pre-trained with Image Net classification instead of a VG-pre-trained object detector. The backbone of Pixel-BERT[15] is modified during vision and language pre-training, unlike frozen detectors in region-feature-based VLP models. Pixel-downstream BERT's performance withResNet-50 is inferior to region-feature-based VLP models, but it is comparable to other competitors when using a significantly heavier ResNeXt-152.

Patch Projection[8]

To save time, we use the simplest visual embedding approach possible: linear projection on image patches. ViT (Dosovitskiy et al., 2020) developed patch projection embedding for image classification applications. Patch projection reduces the visual embedding phase to that of textual embedding, which likewise involves basic projection (lookup) procedures.

We utilize a 32X32 patch projection, which requires only 2.4 million parameters. Complicated Res Ne(X)t backbones3 and detection components, on the other hand, are extremely complex.

Vision-and-Language Transformer
Model Overview

VLTPP has a straightforward architecture as a VLP model, with a minimal visual embedding pipeline and a single-stream approach. We deviate from the literature in that we use pre-trained ViT instead of BERT to in itialise the inter-action transformer weights. Such initialization makes use of the interaction layers' ability to process visual features in the absence of a separate deep visual embedder.

$$\bar{t} = [t_{\text{class}}; t_1 T; \cdots ; t_L T] + T^{\text{pos}} \qquad (1)$$

$$\bar{v} = [v_{\text{class}}; v_1 V; \cdots ; v_N V] + V^{\text{pos}} \qquad (2)$$

$$z^0 = [\bar{t} + t^{\text{type}}; \bar{v} + v^{\text{type}}] \qquad (3)$$

$$\hat{z}^d = \text{MSA}(\text{LN}(z^{d-1})) + z^{d-1}, \qquad d = 1 \ldots D \quad (4)$$

$$z^d = \text{MLP}(\text{LN}(\hat{z}^d)) + \hat{z}^d, \qquad d = 1 \ldots D \quad (5)$$

$$p = \tanh(z_0^D W_{\text{pool}}) \qquad (6)$$

Vit consists of stacked blocks that includes a multi-headed self-attention (MSA) layer and MLP layer. The position of layer normalization (LN) in Vit is the only difference from BERT:LN comes after MSA and MLP in BERT and before in Vit. the text $t \in RL \times |V|$ is embedded to $\bar{t} \in RL \times H$ with a word embedding matrix $T \in R|V| \times H$ and a position embedding matrix $T^{pos} \in R(L+1) \times H$.

The input image $I \in RC \times H \times W$ is patched and flattened to $v \in RN \times (P2 \cdot C)$, where (P,P) is the patch resolution and N = HW/P2. v is embedded into v RNH after lin- ear projection $v \in RN \times (P2 \cdot C)$ and position embedding V pos $\in R(N+1) \times H$ The text and image embeddings are added together with their responding modal-type embedding vectors t^type, v^type R^H, and then concatenated into a combined sequence z^0. The contextualised vector z is updated iteratively through D-depth transformer layers until it reaches the final contextualised sequence z^D. p is a pooled representation of the entire multimodal input, obtained by performing linear projection Wpool R^HxH and hyperbolic tangent on the first index of sequence z^D.

The text and image embeddings are concatenated into a composite sequence z0 after being summed with their cor- responding modal-type embedding vectors type ,type ®R .Up until the final contextualised sequence zD, the contextualised vector z is iteratively updated using D-depth transformer layers. The first index of sequence zD is used to generate p, which is a pooled representation of the entire multimodal input generated by applying linear

projection Wpool RHH and hyperbolic tangent. We employ weights from ViT-B/32 that have been pre-trained on Image Net for all tests, hence the term VLTPP-B/32.5. The number of attention heads is 12, the hidden size H is 768, the layer depth D is 12, the patch size P is 32, the MLP size is 3,072, and the hidden size H is 768.

Pre-training Objectives
VLTPP is trained using two popular VLP model training objectives: image text matching (ITM) and masked language modelling (MLM).

Image Text Matching. With a chance of 0.5, we replace the aligned image with a different image at random. We compute negative log-likelihood loss as our ITM loss using a single linear layer ITM head that projects the pooled output feature p to logits over binary class. Plus, inspired by the word region alignment objective in Chen et al. (2019), we design word patch alignment (WPA) that computes the alignment score between two subsets of zD : zD it (textual subset) and zD |v (visual subset), and add the approxwas serstein weight multiplied by 0.1 to the ITM loss.

Complete Word Masking
This is a masking technique that masks all consecutive sub word takens that compose a complete word. It is also applied in BERT.

We believe that whole word masking is especially important for VLP to fully utilise information from the other modality. The pre-trained bert-base-uncased tokenizer, for example, tokenizes the word "giraffe" into three word piece tokens["gi", "##raf", "##fe"]. If not all tokens are masked, such as ["gi", "[MASK]", "##fe"], the model may instead depend on the nearby two language tokens["gi", "##fe"] to anticipate the masked "##raf."

Image Augmentation
This improves the generalization power of vision models that builds on ViT experimented with various augmentation techniques and found them beneficial for ViT training. Caching visual features restraining region-features-based VLP models from using image augmentation. Notwithstanding its applicability, neither did Pixel-BERT study its effects. During fine-tuning, we use Rand Augment (Cubuk et al., 2020) to do this. All of the original policies are used, with the exception of two: colour inversion, which is used since texts frequently contain colour information, and cutoff, which is used to remove small but crucial elements that are spread across the image. The hyper parameters are N = 2 and M = 9.

IMPLEMENTATION AND RESULTS

We can run the code as per this paper idea and the modal is ranonly on MSCOCO Data Set[11] and the results are accessible at [16].Now will download the modal to the local system , with which we can start work on User Interface and Flask app. Now, We wrote an api to receive the input and send the answeras output . In the coming sections we will compare our results with other models. screenshots are provided at the results section.

Overview
We use Microsoft COCO[11] (MSCOCO) dataset which has 113000 Images, 567000 Captions and Caption Length of 11.81 ± 2.81. MSCOCO and Flickr30K (F30K) (Plummer et al., 2015) are used, which have been re-split by Karpathy & Fei-Fei (2015). We fine-tune the head and data or- dering three times with various initialization seeds for the classification tasks, then present the mean results. Table 5 shows the standard deviation as well as ablation studies. We just fine-tune the retrieval tasks once. Figure 4 shows the Run Time vs test scores on two VQA Data Sets (VQAv2[16], NLVR2[17]). As discussed Linear model takes very less time approx 15 ms. VLTPP-B/32 was pre-trained on 64 NVIDIA V100 GPUs with a batch size of 4,096 for 100K or 200K steps. We train for 10 epochs for all downstream tasks, with a batch size of 256 for VQAv2/retrieval tasks and 128 for NLVR2[17].

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Retrieval Tasks

On the Karpathy & Fei-Fei (2015) split of MSCOCO and F30K, we fine-tune VLTPP-B/32. We evaluate both zero-shot and fine-tuned performance in image-to-text and text-to-image retrieval8. We use the pre-trained ITM head to in itialise the similarity score head, notably the component that computes true-pair logits. We use 15 random texts as negative samples and use cross-entropy loss to tweak the model so that the scores on positive pairs are maximised.

Table 3 shows the zero shot retrieval results, whereas Table 4 shows the fine-tuned results. Despite Image BERT's pre-training on a bigger (14M) dataset, VLTPP-B/32[9] performs better in general at zero-shot retrieval. VLTPP-B/32 recalls are significantly higher than the second quickest modelwhen retrieval is fine-tuned (Pixel-BERT-R50).

User Interface

We like to provide a User Interface to the users to give inputs and witness the magic of our model. So we built a User interface in Vue version 3 and backend python Flask. Here users can give an image address in http protocol and a question related to the image after that we will send a responseas the answer for the given question.

RESULT

Model Results are available at the [16] . Figure 5 shows the User Interface screenshot.

CONCLUSION

The Vision-and-Language Transformer, a simple VLP architecture, is presented in this work (VLTPP). VLTPP distinguishes itself from competitors who rely primarily on convolutional visual embedding networks (e.g., Faster R-CNN and Res Nets). We request that future work on VLP concentrate on the modality interactions within the transformer module rather than an arms race that just increasesthe power of unimodal embedders. VLTPP-B/32 is more of a proof of concept, demonstrating thatefficient VLP[3] models without convolution and region supervision can still be competent. Finally, we'll mention afew factors that could contribute to the ViLT family.

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18. Patch Projection working image from following address <https://www.analyticsvidhya.com/blog/2021/03/an-image-is-worth-16x16-words-transformers-for-image-recognition-at-sca le-vision-transformers/ />
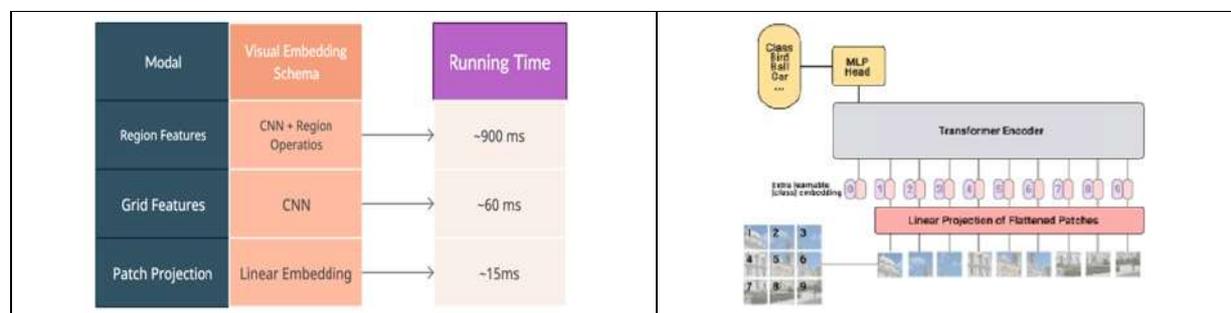
Figure 1 . A visual comparison of traditional VLP structures and the modal we propose.

Figure 2. Patch Projection working image source [18]

Figure 3. Model Overview. Illustration inspired by Dosovitskiy et al. (2020).
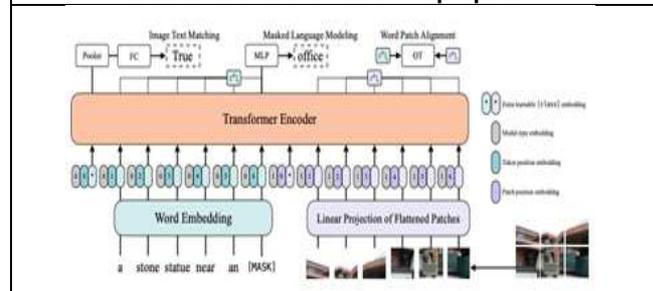
Visual Embed	Model	Time (ms)	VQAv2 test-dev	NLVR2 dev	NLVR2 test-P
Region	w/o VLP SOTA	~900	70.63	54.80	53.50
	ViLBERT	~920	70.55	-	-
	VisualBERT	~925	70.80	67.40	67.00
	LXMERT	~900	72.42	74.90	74.50
	UNITER-Base	~900	72.70	75.85	75.80
	OSCAR-Base†	~900	73.16	78.07	78.36
	VinVL-Base†‡	~650	75.95	82.05	83.08
Grid	Pixel-BERT-X152	~160	74.45	76.50	77.20
	Pixel-BERT-R50	~60	71.35	71.70	72.40
Linear	ViLT-B/32	~15	70.33	74.41	74.57
	ViLT-B/32⊛	~15	70.85	74.91	75.57
	ViLT-B/32⊛⊙	~15	71.26	75.70	76.13

Figure 4. Table of Time comparison

Figure 5. screenshot of User Interface. We passed an image anda question. We got answer as sunset [10]

RESEARCH ARTICLE

Fabrication and Analysis of Hybrid Reinforced Composite Material

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ABSTRACT

Composite materials are formed by reinforcing two or more materials of varying properties. This paper deals with the analysis of hybrid composite materials. Hybridization is a process of incorporating with that of natural fibers in order to yield better strength, stiffness, high strength to weight ratio and other mechanical properties. This paper concerns with the development of new hybrid composite materials using Coconut fiber and Date palm fiber based on the physical, chemical and mechanical properties of the selected materials the required resin, catalysts and accelerators are chosen and fabrication accordingly. Paper will present the mechanical properties of the above mentioned eco-friendly composites and the best possible application of this composite materials

Keywords: composite materials; hybridization; fibers and resins.

INTRODUCTION

Composite materials, often shortened to composites or called composition materials, are engineered or naturally occurring materials made from two or more constituent materials with significantly different physical or chemical properties which remain separate and distinct at the macroscopic or microscopic scale within the finished structure.
A common example of a composite would be disc brake pads, which consist of hard ceramic particles embedded in soft metal matrix. Another example is found in shower stalls and bathtubs which are made of fibreglass. Imitation granite and marble sinks and countertops are also widely used. The most advanced examples perform routinely on spacecraft in demanding environments.

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Wattle and daub is one of the oldest manmade composite materials, at over 6000 years old. Concrete is also a composite material, and is used more than any other man-made material in the world. As of 2006, about 7.5 billion cubic metres of concrete are made each year—more than one cubic metre for every person on Earth

MATERIALS, METHODS AND FABRICATION OF COMPOSITES

Matrixes
Common matrixes include mud (wattle and daub), cement (concrete), polymers (fiber reinforced plastics), metals and ceramics. Unusual matrixes such as ice are also sometime proposed as in pykecrete.

Resins
Typically, most common polymer-based composite materials, including fiberglass, carbon fiber, and Kevlar, include at least two parts, the substrate and the resin. Polyester resin tends to have yellowish tint, and is suitable for most backyard projects. Its weaknesses are that it is UV sensitive and can tend to degrade over time, and thus generally is also coated to help preserve it.

Properties of General Polyester Resin (Table1)
Fabrication (Figure: 4.)
Experimental Method (Table2)

METHODOLOGY

Sample 1
Resin-80g
Accelerator-cobalt napthelene 1ml
Catalyst-methyl ethyl ketone peroxide 2ml
Coconut fiber 10g
Date palm fiber 0g

Sample 2
Resin-80g
Accelerator-cobalt napthelene 1ml
Catalyst-methyl ethyl ketone peroxide 2ml
Coconut fiber 5g
Date palm fiber 5g

Sample3
Resin-80g
Accelerator-cobalt napthelene 1ml
Catalyst-methyl ethyl ket one peroxide 2ml
Coconut fiber 0g
Date palm fiber 10
Initially 5 gm of pure fibers (Date palm, Coconut) are made as composite materials individually. Then the various mixtures are made like Date palm10gm &Coconut 0gm, Date palm5gm & Coconut 5gm, Date palm 0gm & Coconut 10gm, After finishing the manufacturing of piece the mechanical properties like tensile and flexural were analyzed.

RESULT AND DISCUSSIONS

ADVANTAGES
- Low Cost.
- Eco-Friendly.
- Best Alternative.
- Easily Available.
- Good Mechanical Properties.

APPLICATIONS
- Parts like chain cover, mudguard are replaced by this composite.
- Best alternative for asbestos sheet.
- Other Low level applications.

CONCLUSION

Hence this analysis of the various samples their mechanical properties like tensile test and flexural test were tested and the results are obtained. According to the results compare to normal fiber composites hybrid composite materials gives more tensile at yield and break. Its suits for many applications.

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Table: 1. Properties Of General Polyester Resin

PROPERTIES OF GENERAL POLYESTER RESIN		
S.NO	PROPERTIES	RANGE
1	SPECIFIC GRAVITY	1.29-1.40
2	TENSILE STRENGTH Mpa	48.3-72.4
3	TENSILE MODULUS GPa	2.8-4.1
4	YIELD STRENGTH MPa	5.9-3
5	ELONGATION%	30-300

Table: 2. Materials

s.no	MATERIALS
1	Homogenous phase-I (Coconut fiber)
2	Homogenous phase-I (Date palm fiber)
3	GP Resin
4	Accelerator (cobalt napthelene)
5	Catalyst (methyl ethyl ketone peroxide)

Table: 3. Tensile Reading

S. NO	Sample	Length mm	Width mm	Thickness mm	Area mm²	Maximum Force KN	Yield Point N/mm²	Breaking Load kN	Ultimate Tensile Stress Mpa
1	C10 B0	50	28.20	7.70	208.26	3.16	5.2	0.853	14.53
2	C5 B5	50	28.20	7.70	208.26	3.37	6.1	3.37	16.16
3	C0 B10	50	28.20	7.70	208.26	3.09	6	0.93	15.03

Table: 4. Flexural reading

S. No	Sample	Length mm	Width mm	Thickness mm	Area Mm²	Force kN	stress N/mm²
1	C10 D0	100	27.70	7.80	206.06	0.43	1.9
2	C5 D5	100	27.70	7.80	206.06	0.30	1.35
3	C0 D10	100	27.70	7.80	206.06	0.41	1.62

Table: 5. Tensile Test

S. NO	Sample	Length mm	Width mm	Thickness mm	Area 2mm	Maximum Force kN	Ultimate Tensile Stress Mpa	Breaking Load KN
1	C10 B0	50	28.20	7.90	210	2.96	14.02	0.506
2	C5 B5	50	28.20	7.90	210	3.09	16.01	0.474
3	C0 B10	50	28.20	7.90	210	2.80	14.82	0.252

Figure: 1. Materials

Figure: 2. Date palm fiber

Figure: 3. Coconut fiber

Figure: 4. Pattern

Figure: 5. C10D0 Sample

Figure: 6. C5D5 Sample

Figure: 7. C0D10 Sample

Figure: 8. Bar chart .1

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Y axis is flexural

X axis is sample

Figure: 9. Bar chart 2

X axis is sample

Y axis is vickel hardness

Figure: 10. Bar chart 3

Figure: 11. Bar chart 4

RESEARCH ARTICLE

Comparitive Weld Behaviour Analysis of Aluminium AA2024 T3 and Magnesium ZE41 a with Different FSW Tools

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ABSTRACT

Friction stir welding (FSW), a solid-state joining technique, is being extensively used in similar as well as similar joining of Al, Mg, Cu, Ti, and their alloys. In the present study, friction stir welding of dissimilar aluminum joins has to be carried out at various combinations of tool and constant rotation speeds and feed and axial force. In this experimental proper selection of input friction welding parameters is necessary in order to control weld distortion and subsequently increase the productivity of the process. In order to obtain a good quality weld and control weld distortion, it is therefore, necessary to control the input welding parameters. In this research work, experiments has to be carried out on ZE41and AA2024 of 6mm thick using friction welding process with Triangular pin and square pin as a tool. And triangular tool profile pin was executed maximum tensile strength and square tool profile were minimum hardness and higher penetration during the FSW process. Bead appearance of also triangular pin shows that comparatively very better than square pin. The angle distortion only shows almost that equal value during the analysis. So that finally concluded Triangular profile were executed maximum good weld characteristics during the FSW process with AA2024 & ZE41-6mm thickness.

Keywords: Friction stir welding (FSW), joining of Al, Mg, Cu, Ti, and their alloys, FSW process with AA2024 & ZE41-6mm thickness.

INTRODUCTION

The general requirements of a component in the mechanical, automobile and aerospace have given many challenging condition. New materials can be modified and then can be fabricated as to a useful component.One such fabrication technique is solid phase joining process which is the traditional method. Many processes are generally termed as solid phase joining.Each and every process may vary based on time, pressure and temperature usedand the application of heat. In a particular point, cold pressure welding can be done without any heating and several other metals or alloys can be integrated together. Processes such as magnetically impelled arc butt-welding, friction welding, explosive welding, etc., belong to another extreme, employing plastic deformation at higher temperature. The best strategy to overcome the problem is by solid state joining, where melting is not done and hence the defects are eliminated. The integration of immiscible or partially miscible alloy systems which is cumbersome in traditional fusion welding is also possible by this solid state joining. Many solid state joining techniques have been widely used on large scale for industrial processes.

LITERATURE SURVEY

ZHIDA LIANG [1] et.al were evaluated with 5A33 aluminum alloy with AZ31B magnesium alloy bar by continuous drive friction welding. TAIKI MORISHIGE [2] et.al were investigated Dissimilar joint of Al and Mg alloy by laser welding was very brittle because of building up Mg17Al12 inter metallic compounds in fusion zone. SURESH D. MESHRAM [3] et.al were selected the tool material durability, tool material cost, and selection of tool geometry and process parameters. Therefore, the main focus of this paper is towards the selection of tool geometry, tool material, and process parameters that will result in least tool wear/failure in addition to obtaining defect-free welds of mar aging steel using FSW.HUSEYIN TARIK SERINDAG [4] et.al were performed AZ31 Mg-alloy plates were friction stir welded at rotation speed of 1200 rev/min and translational speeds of 80,100,120,140 mm/min.

Temperature evolution in the weld zone during welding was measured by using embedded K-type thermocouples. V. FIROUZDOR [5] et.al were investigated in butt and lap FSW, and conventional lap FSW was modified to improve the joint strength. 6061 Al and AZ31 Mg, the two most widely used Al and Mg alloys, were selected. RAJKUMAR.V [6] et.al was deals with the characterization of friction stir welded dissimilar Aluminum alloys AA 5052 and AA5052. The coupons of above metals were friction - stir welded using cylindrical pin tool using at constant speed of 710 rpm and at two different feed rates of 28 and 20 mm/min. SADEESH PA [7] et.al were carried out by friction stir welding (FSW) technique. Optimum process parameters were obtained for joints using statistical approach.

R. K. KESHARWANIA, [8] et.al were executed optimization of parameters affecting weld quality in tailored friction stir butt welding of 2.0 mm thin dissimilar sheets of AA5052-H32 and AA5754-H22 using Taguchi grey based approach. M. ILANGOVAN [9] et.al were analyzed Joints between two different grades of aluminum alloys are need of the hour in many light weight military structures. K. KIMAPONG [10] et.al were investigated the effects of pin rotation speed, position of the pin axis, and pin diameter on the tensile strength and microstructure of the joint.

TOOL TYPES AND ITS PROCESS

The FSW tool has a probe shaped pin and one shoulder area. The pin plunges into the joining place of the materials it creates the friction similarly deformational heating, afterwards it softens the work piece by connecting with shoulderalong with the material increase the material heat afterwards it expands the zone, after that soft s the material by constrains the aligned materials. The work piece must have the properties such as easy availability, mach inability, thermal fatigue resistance and wear resistance. Some o the materials like aluminum, magnesium and aluminum matrix and its composites are always joined with steel

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tools. AISIH13 was made by chromium molybdenum, whichwas a hot worked air hardening steel and it can be generally used anywhere.

The different types of tools are i) Fixed ii) Adjustable and iii) Bobb in type tool. PCBN tool produces in higher strength and hardness the materials higher temperatures with high temperature stability. Hence this is generally used as a tool material for FSW process for hard alloys like steels and Titanium alloys .Because of its low coefficient of friction a smooth weld surface can be achieved. Similarly a very high temperature and pressure were need in the method of manufacturing for PCBN tool, hence the generation amount for the tool is always very high.

MATERIALS AND METHODS-AA2024 and ZE41 A

2024 aluminum is heat-treatable aluminum alloy with copper as the primary alloying element. It is malleable when in the fully soft, annealed temper and can be heat-treated to high strength levels after forming. Due to its high strength to weight ratio, it is widely used in aerospace applications. Magnesium alloys are light weight and have high mach inability. They are often anodized to improve their corrosion resistance. They are designated using the ASTM and SAE system in which the first part indicates the two main alloying elements in the alloy and the second part represents their percentages

EXPERIMENTAL RESULT AND DISCUSSION

Angle Distortion plate or Bead Straightness were analyzed through auto cad and found above mentioned deviations almost both profiles were weld executed same amount of distortion. Through Image J software found maximum depth of penetration obtained at square tool profile pin compared than triangular pin. In our experimental we found Smooth bead appearance & mild porosity obtained at cylindrical tool with parameter speed of -1000 RPM, tool traverse-14mm/min and axial force-9 KN. Coarse bead appearance & Mild transverse crack shows at square tool profile

CONCLUSION AND FUTURE SCOPE

The FSW constant process parameter was evaluated and compared different tool profile. Generally, tool pin profile has also influenced the weld quality. From this research work, triangular tool profile pin was executed maximum tensile strength and square tool profile were minimum hardness and higher penetration during the FSW process. Bead appearance of also triangular pin shows that comparatively very better than square pin. The angle distortion only shows almost that equal value during the analysis. So that finally concluded Triangular profile were executed maximum good weld characteristics during the FSW process with AA2024 & ZE41-6mm thickness.

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Table: 1. Levels and ranges of FSW process parameters BEFORE WELD BOTH MATERIALS HRB VALUE

2024 – 50 HRB, ZE41 - 70 HRB

SPEED RPM	TOOL-TR Mm/min	AXFC KN	TOOL PROFILE
900	13	8	Square
1000	14	9	
1100	15	10	
900	13	8	Triangular
1000	14	9	
1100	15	10	

Table: 2. hardness value –HRB value

SPEED RPM	TOOL-TR Mm/min	AXFC KN	Avg Hardness	TOOL PROFILE
900	13	8	41	Square
1000	14	9	34	
1100	15	10	37	
900	13	8	54	Triangular
1000	14	9	46	
1100	15	10	50	

Table: 3. Tensile strength value

SL.NO	SPEED RPM	TOOL-TR mm/min	AXFC KN	TENSILE LOAD KN	TENSILE STRENGTH N/mm²	TOOL PROFILE
T_1	900	13	8	1.02	19.57	Square
T_2	1000	14	9	1.42	25.1	
T_3	1100	15	10	2.16	45.2	
T_4	900	13	8	2.04	44.56	Triangular
T_5	1000	14	9	1.09	21.81	
T_6	1100	15	10	2.77	57.71	

Table 4. Angle DISTORTION

TOOL PROFILE	SQUARE			TRIANGULAR		
NO OF PLATES	1st	2nd	3th	4th	5th	6th
ANGLE DISTORTION	1 º	0 º	0 º	1 º	1 º	0 º

DEPTH OF PENETRATION
Table: 5. Depth of Penetration [Square & Triangular

SL.NO	AREA	MEAN	MIN	MAX	ANGLE	DEPTH	WIDTH
1	0.081	62.662	37.667	92	90	4mm plate	14.745
	0.061	60.338	46.667	105.333	90	3.03	
2	0.113	63.889	40.333	87.333	90	4	18.264
	0.083	76.906	53.333	101.333	90	2.87	
3	0.105	71.065	58	100.333	90	4	20.961
	0.092	85.993	58.667	200.667	90	3.529	
4	0.113	60.379	41	131	90	4	15.923
	0.115	71.544	53.333	96.333	90	3.721	
5	0.113	85.358	55.444	252.667	90	4	17.277
	0.121	80.045	56	123	90	3.629	
6	0.105	74.646	55	148.667	90	4	17.642
	0.109	66.123	50	104	90	3.417	

Table: 6. Weld appearances

TOOL PROFILE	SPEED RPM	TOOL-TR mm/min	AXFC KN	RESULT
Square	900	13	8	Rough texture at weld region,
	1000	14	9	Rough texture at weld region, mild cracks found on top portion of advancing side
	1100	15	10	Very fine texture but Flash effects formed at the Retreating side.
Triangular	900	13	8	Mild coarse texture but it has no any surface defects.
	1000	14	9	Very fine texture but excessive flash formed at the Advancing side
	1100	15	10	Very fine texture but it has no any surface defects. But mild flash effect found

Fig. 1. Square tool pin profile & Triangular tool pin profile	Fig. 2. hardness strength graph
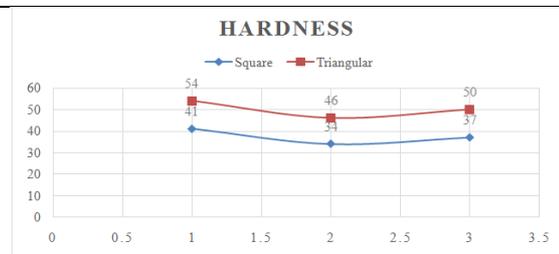
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Fig. 3. Tensile strength graph
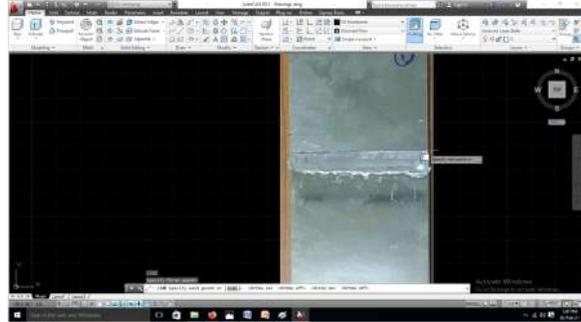
**Fig. 4. Angle Distortion plate-1**
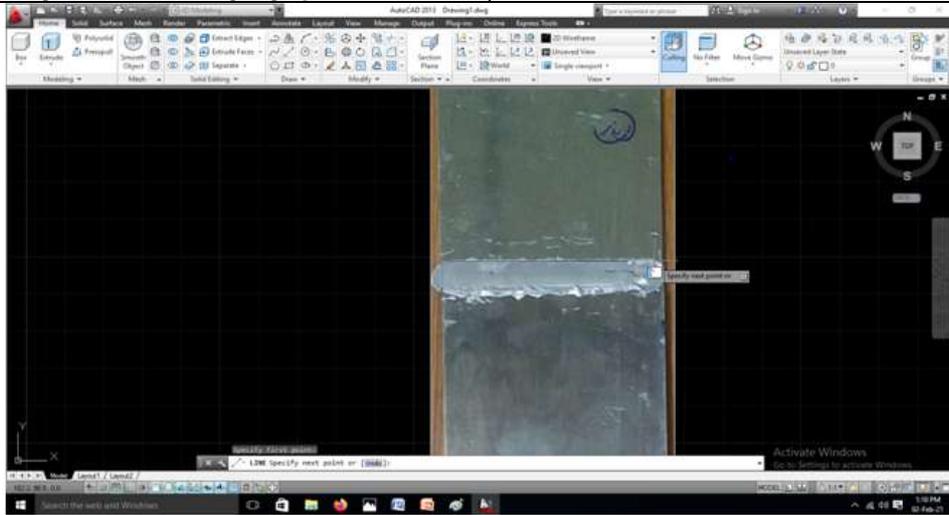
Fig. 5. Angle Distortion plate-1

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RESEARCH ARTICLE

Knowledge of Primary School Teachers Regarding Refractive Errors among School Children

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ABSTRACT

Refractive error (RE) is one of the most common ocular conditions and a major public eye health challenge. According to WHO refractive error is responsible for 42% of visual impairment and 3% of total blindness in the world. School age children constitute a particularly vulnerable group where uncorrected refractive errors may have a dramatic impact on learning capability and educational potential. Lack of knowledge and uncorrected RE can have long-term consequences such as loss of educational and employment opportunities, also interfere with the ability to perform to one's full learning potential which place a greater socio economic burden on the society. Magnitude of RE among school children ranges from 1.75% to 20.7%. The descriptive cross-sectional approach was adopted. Convenient sampling technique was used to obtain the sample of 150 primary school teachers working both government and private schools. The data was collected using semi -structured questionnaire to assess the awareness of school teachers regarding refractive errors in school children. Majority of the primary school teachers (38%)were in the age group of 21-30 years, majority (54%) were Females,(30%) of them were post graduates,(36%) of them had more than 15 years of teaching experience,(54%)f them were using spectacles. Majority (60%) of primary school teachers had moderately adequate knowledge regarding refractive errors in children and (57%) of teachers had previous experience of identifying visual problems among students. Refractive error is a significant problem among school children and has a considerable impact on public health. Early detection by school screening is very much important to prevent blindness and other complications. Therefore the knowledge regarding the refractive errors among school teachers is very much important to detect any manifestations of refractive errors in the early stage

Key Words; Knowledge, Refractive Errors, School children, Snellen's chart ,Visual acuity.

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INTRODUCTION

The ability to see the world clearly can easily be taken for granted. Our eye is a sensitive and highly specialized sense organ subjected to various disorders, many of which lead to impaired vision .Children are more at the risk of developing refractive error (RE) because they are actively growing and subjected to the strain of near work due to demanding academic schedule and E-learining due to Cocid -19 pandemic. It is now a usual routine for our children to spend most of the time (8–12 h per day) attending e- classes in front of a computer or mobile screens. These devices cause harm by emitting short high energy waves that can penetrate eyes and can eventually contribute to photochemical damage to the retinal cells, making an individual vulnerable to a variety of eye problems ranging from dry eye to age- related macular degeneration [11]. Refractive error is an error in the focusing of light by the eye and a frequent reason for reduced visual acuity. The common type of refractive error are Myopia, Hyperopia, Astigmatism and presbiopia

RE can be detected through routine eye examination at hospitals or clinics using snellen's chart or refractometer. Vision screening are not commonly carried out at schools which is a valuable method of identifying potentially treatable eye problems especially myopia , Hyperopia and presbiopia .School screening is performed in various ways either by health professionals or by school teachers .Refractive error can be corrected by spectacles ,Laser therapy and yoga therapy as alternative modalities. Vision problems have been shown to adversely affect a child's achievement in school. Sometimes children are diagnosed as having special educational needs, when actually all they need is a pair of glasses. Vision problems can adversely affect student's ability to function and enjoy learning (3)

There is large evidence that many school children who need glasses do not get them because their refractive errors are not detected, which might not only hamper children's physical, cognitive, and psychosocial development, but also future employability and earning opportunities. Moreover, if refractive error is detected late the child can suffer from amblyopia (lazy eye), a condition where vision remains low even after wearing the glasses. This condition can be treated only till 7 to 8 yrs of age, hence the importance of early detection [2]. School vision screening program can identify and treat or refer children with RE. School based vision screening carried out by teachers and other ancillary staff may be an effective way of detecting children in the early stage .Depending on the prevalence of RE and competing condition, rate of attendance, the need for schools screening is unavoidable and take in to consideration by government. Barriers of service include the cost and quality of available refractive care and misbelieves that spectacles will harm children's eyes.

Need for the study

Globally, uncorrected refractive errors are the main cause of vision impairment in children aged 5–15 years. The prevalence of myopia (short-sightedness) is increasing dramatically among children, particularly in urban areas of South-East Asia [4]. It is estimated that prevalence of Childhood blindness in India is 0.8/1000 children in <16 years age group, implying a total of 300,000 blind children in our country [5]. About 60-80% of visual impairment may be due to refractive erroralone. Children form one of the major age groups requiring attention to refractive errors because of the high prevalence of myopia, hypermetropia and astigmatism. Myopia is a common cause of visual impairment which is usually acquired and nearly progressive. New cases appear throughout the childhood, particularly between the ages of 6-15 years [6]. A study was conducted at Maharashtra (2009) to compare the magnitude and risk factors of

uncorrected refractive error in 6-15 year old school children ,study results revealed that prevalence of uncorrected refractive error especially myopia was significantly higher in school children of urban area compared to children of rural schools. The study concluded that the magnitude and causes of refractive error seem to differ in urban and rural areas of India [7]. With the entrance of the child to the school, the school environment becomes the second family and the school teachers take the role of second parents. Children spend a significant portion of their time in school. Therefore teachers get good opportunity to identify the impairments in the early stage itself. So the researcher

felt the need to assess the knowledge of primary school teachers regarding refractive errors and formulated the following statement.

Statement of the Problem

A study to assess the knowledge of   primary school teachers regarding refractive errors among school children in selected schools at Mahe District

Objectives of the Study

- To assess the level of knowledge regarding refractive errors among primary school teachers.
- To find the association between the level of knowledge of primary school teachers with the selected baseline variables

Hypothesis

➢ H1:There will be significant level of knowledge on refractive error among primary school teachers
➢ H2: There will be significant association between the level of knowledge of school teachers with the selected baseline variables.

MATERIALS AND METHODS

Research Design; Institution based cross –sectional study between November to December 2021
Population; In this study both private and government school teachers were included
Sample; The sample comprised of primary school teachers working at various schools at Mahe
Sampling technique; Simple random sampling   technique was used
Sample size; 150 subjects were selected.

Tool
Semi –structured knowledge questionnaire. It contains 20 questions each correct response was given a score of 1 and a wrong response a score of 0

RESULTS

A total 150 primary school teachers who are working in Mahe District, in 16 schools participated in this study. Table 1 shows that majority of the teachers were in the age group of 21-30 years 57(38%), majority were Females (54%),post graduates 44(30%), 54(36%)of them had more than 15 years of experience Majority of them using spectacles 87(54%) and57% of teacher's had previous experience of identifying visual problems among students Figure 1 shows that majority of the primary school teachers had moderately adequate knowledge (60%) and 26% of them had adequate knowledge, 14% of school teachers had inadequate knowledge on refractive error among students. Table 2 highlights that there was significant association between educational qualification, and year of teaching experience with the level of knowledge (p=<0.05) level.

DISCUSSION

In this study, about 60% of primary school teachers had moderately adequate knowledge regarding refractive error in school children. In similar study done in Rohtak city to assess the knowledge of primary teachers. 55% of them had adequate knowledge. This result is higher as compared to other studies in Singapore (73.4%), Nigeria(66.9%) [8], In this study the knowledge regarding refractive error among subjects with 21-30 years of age is more when

compared to other groups and years of experience. This finding is agreed with a study done at Madhya pradesh in India. by Sudhan A,et al and Joseph N, Rai S, et al. (2016) at south india [9] .

Chacko C, Shetty AP. Conducted study on Role of teaching programme in early detection of visual impairment in children(2009). Result shows that significant association with the source of knowledge about refractive error [10]. A Prospective Study was conducted by Jyothi et al (2015) at Andhra Pradesh to Assess the Quality of Preliminary Eye Screening Done on School Children by Teachers. The result of the study gives emphasis to involve school teachers in detecting vision defects and eye alignments among school children [12].

CONCLUSION

The following conclusions were made based on the findings of the study

➢ Majority of the primary school teachers had moderately adequate knowledge regarding refractiveerrors among students .

➢ The educational qualification and year of teaching experience shows significant   association with their knowledge level.

➢ Eye health education and training to primary school teachers directed towards bringing a significant change in the knowledge regarding refractive error must be stepped-up within school health program

Nursing Implications

The scope of the study brought out the implications for nursing in the areas of practice, education, administration and research.

Nursing Practice

Knowledge on early identification and correction of refractive errors   helps to due the burden of blindness among children and help to perform to one's full learning potential. Educating the teachers and parents regarding refractive errors and the related symptoms by health professionals helps for early identification of visual impairments among children.

Nursing Education

Children are the future of any nation. It is the responsibility of the health care providers especially nurses to prevent the children from avoidable blindness due to uncorrected refractive errors which is an important aspect of various programs at national and international level. Nursing curriculum should be such that it prepares the nursing students to assist the client and the community in all aspects of health care. Nursing curriculum has to be designed accordingly

Nursing Administration

Nurse administrators can take initiatives in organizing school vision screening programs and various school health programs in educating teachers regarding refractive errors. They can also conduct training programs for teachers to test the vision using Snellen's chart.

Nursing Research

Nurses need to be engaged in multidisciplinary research so that it would help them to improve their knowledge and skills in handling various problems related to health and illness. The present study can serve as a baseline data for further nursing and other community based research.

RECOMMENDATIONS

✓ Follow up studies can be done for longer duration in regular intervals

✓ A study can be done t assess the visual impact of E-learning among school children

✓ Same study can be replicated in different settings

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✓ A similar study can be conducted for high school and higher secondary studen

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Table 1.Frequency and Percentage distribution of demographic Variables

(n=150)

S.NO	DEMOGRAPHIC DATA		FREQUENCY	PERCENTAGE
1	Age In Years	20-30 years	49	33
		31-40 years	57	38
		41-5 years	39	26
		51-60 years	5	3
2	Gender	Male	69	46
		Female	81	54
3	Educational Qualification	TTC	31	21
		BSC .BED	37	24
		BA.BED	38	25
		MA.BED	44	30
4	Year of Experience	1-5 yrs	19	13
		6-10 yrs	32	21
		11-15 yrs	45	30
		>15 yrs	54	36
5	Usage of Spectacles	Using	87	58
		Not Using	63	42

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6	Source of information	Social Media and Books	40	27
		Health professional	56	37
		Parents and colleagues	12	8
		School health program	40	27
7	Previous experience of identifying visual problems	Yes	86	57
		No	64	43

Table 2: Association between knowledge scores and selected baseline variables (N=150)

S.No	Demographic variables	Level of knowledge			Chi-square value	p-value
		Inadequate knowledge	Moderately adequate knowledge	Adequate Knowledge	Chi-square value	p-value
1	**Age In years**					
	21-30 years	8	34	7	8.075	0.201 **(N.S)**
	31-40 rears	6	31	20		
	41-50 years	5	22	12		
	51-60 years	2	3	0		
2	**Gender**					
	Male	9	35	25	2.931	.229 **(N.S)**
	Female	12	55	14		
3	**Educational Qualification**					
	TTC	9	18	4	18.67	0.017* **(S)**
	BSC .BED	6	21	10		
	BA.BED	5	21	12		
	MA.BED	1	30	13		
4	**Year of Experience**					
	1-5 years	2	14	3	5.055	0.025* **(S)**
	6-10 years	4	19	9		
	11-15 years	6	27	12		
	>15 years	9	30	15		
5	**Usage of Spectacles**					
	Using	7	53	27	1.529	0.216 **(N.S)**
	Not using	14	37	12		
6	**Source of information**					
	Social media and books	9	14	17	6.89	0.12 **(N.S)**
	Health professionals	6	38	12		
	Parents	2	8	2		
	School health program		30	6		

7	**Previous experience of identifying visual problems**	9 12	51 39	26 13		
	Yes	9	51	26	1.959	0.365 **(N.S)**
	No	12	39	13		
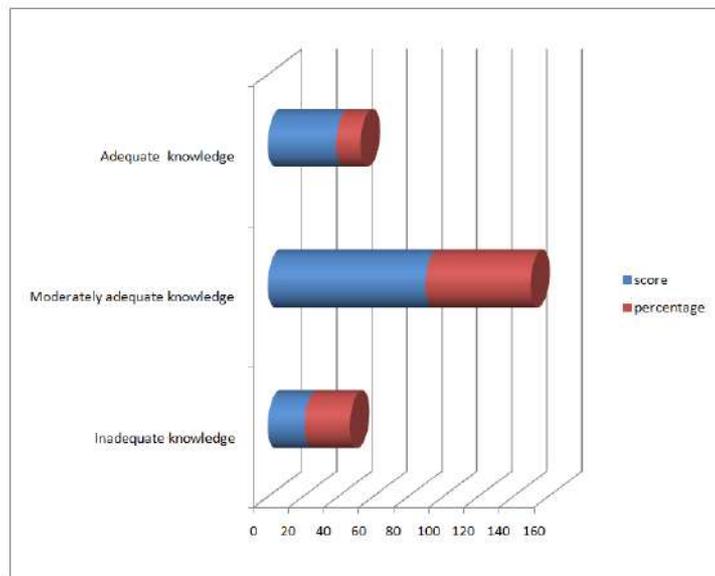
Figure 1 shows that majority of the primary school teachers had moderately adequate knowledge (60%) and 26% of them had adequate knowledge, 14% of school teachers had inadequate knowledge on refractive error among students

RESEARCH ARTICLE

Antioxidant, Antidiabetic, Antimicrobial and Anticancer Activity of SNP from Endophytic Fungi *Rhizopus delemer*

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ABSTRACT

Nanotechnology is a stand-up field in the area of multifaceted research, especially in nanomedicine field. Nanoparticles are ultra-fine molecules that greatly act as activators in many chemical reactions. In this present inspection, silver nanoparticles were synthesised extracellularly from endophyte *Rhizopus delemer* fungi. Ag particle Synthesis was confirmed by colour variation after noticeable time using UV-Visible spectrum peaks at 447 nm. Whereas metabolite coating of Ag nanoparticle was characterized by FTIR Which explores Ag molecules was covered such as O-H, N=C=S, C=C, C=O, CO-O-CO, C-Brcapping agents and their morphology tested by SEM analysis it gives size range in 10-50 nm. The X-RD study shows 2Ø range values are 38.0824, 45.7077, 67.0407, 78.1871 and 78.4575 the peaks of synthesized Ag nanoparticles are cubic structure and crystalline pattern. Further synthesised Ag molecules tested their Antioxidant activity of DPPH and reducing power gives 67.8%, 66.8% while std ascorbic 78.7%, 83.4%. Antidiabetic activity by comparing alpha-amylase 80.2% and alpha-glucosidase 74.2% inhibition assay with std acarbose 88.7%,88.1%. Disease-causing microbes are high-risk vehicle in human beings nowadays. So belonging to us, synthesized Ag were also tested against three gram positive bacterial strains *Staphylococcus aureus* (MTCC 25923), *Streptococcus aureus* (MTCC 457) and *Bacillus subtilis* (MTCC 2451) and two gram negative bacterial strains *Escherichia coli* (MTCC 25922), *Enterococcus aeruginosa* (MTCC 27853)and three fungal strains*Candida albicans* (MTCC 282), *Aspergillus flavus* (MTCC 237) and *Aspergillus niger* (MTCC 1346). which gives 3±1.8257, 5±1.8257, 5±3.3665, 7±4.0824, 8±5.2281 and fungal

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8±4.0824, 6±1.8257, 7±2.3804 inhibition percentile. In the end Anticancer activity of synthesized Ag Nanoparticle displays IC_{50} values at 60.53 % inhibition against MCF-7 Human breast cancer cell line.

Key words: Ag Nanoparticles of *Rhizopus delemer* fungi, Antioxidant activity, Antidiabetic activity, Anti-microbial activity, MCF-7 human breast carcinoma cell line.

INTRODUCTION

Nanotechnology is an interdisciplinary science, which deals with how to synthesis and maintain the particle size nanoscale and how to apply the nanoparticle in various application-oriented field [1]. Nano means dwarf, whereas to obtain the particles in nano ranges various physicochemical methods were available in market to obtain the nanoparticle in preferred use. [2-8] Why this advantage in the (bio) synthesis of nanoparticles, Because of their physical property. It mainly describes their increased surface area with small volumes [9] and increased tensile strength to synthesised particles [10] size-dependent, optical, magnetic, electronic, and catalytic property[10]. Compares to physicochemical processing, extracellular biosynthesis of nanoparticles is very effective, decreases the duration cost to human labour [11] and eco-friendly [12]. And also, this quality encourages the nanoparticle to sustain and chelate the coated metabolites to reach the disease-causing site in a very easy and effective manner. All most of the synthesising nanoparticles are iron oxides, gold, silver, Silicon, quantum dots [13,14]. Mainly in the form of silver and gold nanoparticles used in very competent part in medicine example asthma, anaemia, chronic fever, cough, sleeplessness, muscle weakness,and weak digestion site [15,16]. Various application-oriented researches are ongoing in drug delivery techniques [17], cancer treatment [17,18,19],neurological disorder[20], anti-diabetic [21], and antimicrobial infections [20,22,23,24], cardio vascular disease using Silver nanoparticle. In the synthesis of silver nanoparticles, the filamentous fungi possess some beneficial role, over other plants and bacterial synthesis of nanoparticles, why because of a large group of fungi are easy to maintain, handle to grow in simple nutrients supply, intense high wall binding capacity as well as intracellular metal uptake capability [25]. Amongst fungi, not so much work was done. In fungal endophyte which produces silver nanoparticles very few reports such as, gold nanoparticle extracellularly synthesised from *Colletotrichum sps* which is endophytic fungi isolated from *Pelargonium graveolens* geranium leaves [26]. Another report, silver nanoparticle synthesised from endophytic fungi *Aspergillus clavtus* (Azs-275) from sterilized stem tissues of *Azadirecta indica* plant which act on antibacterial studies effectively [27].Same antibacterial studies especially in gram-negative pathogenic bacteria, silver nanoparticle synthesised from endophytic fungi *Penicillium*sps, isolated in healthy leaves of *Curcuma longua* turmeric plant [28]. Our effort of work also to synthesis the Silver nanoparticle using endophytic fungi *Rhizopus delemer* which is already Isolated, Identified from medicinal plant Abutilon indicum leaf portion and this present examination to test the synthesised Ag nanoparticle physical characterization like UV, X-RD, SEM, FTIR, Particle size distribution and Zeta potentials and finally analyse their application orient activity on anti-diabetic, antioxidant, antimicrobial and cytotoxicity assay on MCF 7 breast cancer cell line.

MATERIALS AND METHODS

Synthesis of silver nanoparticles

1mM silver nitrate was prepared in a 100ml standard flask. 2.5ml of the fungal extract sample is added to 50ml of 1mM silver nitrate with continuous and constant stirring which react at an ambient condition and Ag get reduced in to Ag+ ion [29].

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Characterization of silver nanoparticles

The optical properties of silver nanoparticles were characterized using UV-Vis spectrophotometer. Silver nitrate was added to the fungal extract, UV was taken after 24hours of addition[30]. The absorbance was recorded between 350-500nm[30]. The functional group of the synthesized silver nanoparticles was studied using FT-IR spectrometer. Using KBr pellet method dried powder sample was characterized in the range between 4000-400cm^{-1}[30]. Crystalline nature and grain size of synthesized silver nanoparticles was characterized using X-ray diffraction spectroscopy[30.]The size and morphology of synthesized silver nanoparticles were evaluated using SEM analysis. SEM image confirmed the development of silver nanoparticles[30].The zeta potential was measured by using Zeta Sizer (Malvern Instruments) having zeta cells, polycarbonate cell with gold-plated electrodes and using water as medium for sample preparation. Zeta potential determines the surface potential of silver nanoparticles and it is essential for the characterization of stability of nanoparticles. The stability of nanoparticles are measured when the values of zeta potential ranged from higher than +30 mV to lower than -30 Mv [30].

Antioxidant Activity

DPPH Assay Method

The antioxidant activity of synthesized silver nanoparticles using fungal extracts was examined by stable DPPH free radical activity. Ethanolic solution of DPPH (0.05 mM) (300μl) was added to 1 ml of synthesized silver nanoparticles using fungal extracts with the different concentrations (20, 40, 60, 80, 100μl). The freshly prepared DPPH solution was kept in the dark at 4°C. Then 96% (2.7 ml) of ethanol was added in the mixture and shake vigorously. The mixture was kept to stand for 5 minutes at 540nm, absorbance was measured spectrophotometrically. Absorbance was set to zero by using ethanol. A blank sample contains the same amount of ethanol and DPPH was prepared. They all performed in triplicate. The radical activity of the tested samples, expressed as percentage of inhibition were calculated.

Percent (%) inhibition of DPPH activity = [(A-B)/A] ☐ 100.
Where A and B – absorbance values of blank and sample, respectively.

Reducing power method

The synthesized silver nanoparticles using fungal extracts were taken in different concentrations in phosphate buffer (pH 6.6/ 0.2mol/L), and incubated with potassium ferricyanide (1g/100ml of water) at the 50° C for 20 minutes, then the reaction was terminated by adding TCA solution (10g / 100ml water) and centrifuged at 300rpm for 10 minutes and the ferric chloride was mixed (0.1g / 100ml of water), at 700nm the absorbance was measured. The increased in the absorbance of reaction mixture indicates increases reducing power.

Percent (%) inhibition of reducing power = [(A-B)/A] ☐ 100.
Where A and B – absorbance values of blank and sample, respectively

Antidiabetic activity

Alpha amylase inhibition assay

The alpha-amylase inhibitory assay brings out with the procedure of McCue and Shetty et al., 2004. Into a test tube (20- 100μg/ml) different concentration of synthesized silver nanoparticles was taken and made upto 1ml with ethanol and mixes it. Then a total of 250μL of this solution was transferred into a new tube with different concentrations of synthesized silver nanoparticles using fungal extracts (20- 100μg/ml) and 250μL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5mg/mL) was filled. This solution or result was pre-incubated at 25°C for 10 minutes. 250μL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at regular time intervals and then further incubated at for 25°C for 10minutes. Finally, 500μL of dinitrosalicylic acid (DNS) reagent were added. The test tubes were incubated in boiling water for 5minutes and cooled in room temperature. The reaction mixture was diluted by adding 5mL distilled water and the absorbance was measured at 540nm using

spectrophotometer. The control of process was also prepared as same procedure as 250 µl of distilled water. The α-amylase inhibitory activity was calculated in terms of percentage inhibition as follows;

%Inhibition = [(Abs control – Abs synthesized nanoparticles) / Abs control] x 100.

A and B represents the absorbance value for the test and blank sample. The percent inhibition versus concentration curve and 50% inhibition was determined in a sample graph is plotted.

Alpha glucosidase inhibition assay

The action of silver nanoparticles synthesized using fungal extracts on α-glucosidase was determined by α-glucosidase from *Saccharomyces cerevisiae* (Kim et al., 2005). Into a test tube (20- 100µg/ml) different concentrations of synthesized silver nanoparticles were taken and made upto 1ml with ethanol and mix it. Then, a total of 250µL of this solution was transferred into a new tube with different concentrations (20- 100µg/ml). The P-nitro phenyl glucopyranoside (pNPG) was prepared in 20mM phosphate buffer as a substrate solution and pH 6.9. 100µL of α-glucosidase (1.0 U/mL) was pre-incubated with 50µL of the different concentrations (20-100µg/ml) of the fungal extract of synthesized silver nanoparticles for 10min. After that 50µL of 3.0mM (p-NPG) substrate was dissolved in 20mM phosphate buffer (pH 6.9) were added to start the reaction. At 37oC for 20min and made up with 2mL of 0.1M Na_2CO_3 this reaction mixture was incubated. The α-glucosidase activity was determined by measuring the yellow-colored p-nitro phenol released from pNPG at 405nm. with 2mL of Na_2CO_3 the blank sample was prepared as same as procedure. The α-glucosidase inhibitory activity was calculated by percentage of inhibition as follows.

%Inhibition = [(Abs control – Abs silver nanoparticles) / Abs control] x 100.

A and B represents the absorbance value for the test and blank sample respectively. The percent inhibition versus concentration curve and 50% inhibition was determined in a sample where the graph is plotted.

Antimicrobial Activity
Collection of test pathogens

The antibacterial and antifungal activity of fungal extracts-AgNps were exhibited against three gram positive bacterial strains *Staphylococcus aureus* (MTCC 25923), *Streptococcus aureus* (MTCC 457) and *Bacillus subtilis* (MTCC 2451) and two gram negative bacterial strains *Escherichia coli* (MTCC 25922), *Enterococcus aeruginosa* (MTCC 27853) and for fungal culture used in the study are *Candida albicans* (MTCC 282), *Candida vulgaris* (MTCC 3956) *Aspergillus flavus* (MTCC 237) and *Aspergillus flavus* (MTCC 1346) were prepared as test organisms. All the bacterial strains were purchased from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India and the fungal strains from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

Determination of antibacterial activity by disc diffusion method

The disc diffusion method is used to evaluate the antibacterial activity of the fungal extracts-AgNps. Ten ml of Mueller-Hilton agar medium was poured into sterile petri dishes (diameter 60 mm) and inoculated with test organism. Sterile filter paper disc loaded with various concentrations of sample fungal extracts-AgNps of 60, 80 and 100 µg/ml were placed on the top of Mueller-Hilton agar plates. Filter paper disc loaded with 5 µg of amoxicillin was used as positive control. The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was recorded in millimetre and the experiment was repeated twice.

Determination of antifungal activity by disc diffusion method

Disc diffusion method in order to test the antifungal activity of fungal extracts-AgNps against test pathogens was carried out. In petri dishes (60 mm) filled with Sabouraud's dextrose agar (SDA) and seeded with a 0.3 ml of test organism, a sterile filter paper disc (diameter 6 mm, Whatman paper no.3) was placed. The sterile disc was impregnated with 10 µl of fungal extracts-Ag Nps at varying concentration of 60, 80 and 100 µg/ml respectively. The

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zones of growth inhibition around the disc were measured after 24 h of incubation at 37°C. while, Fluconazole was used as a positive control.

Cell cytotoxicity assay
Cell culture
MCF-7 (Human breast carcinoma cells) cell line was purchased from NCCS, Pune and were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37°C.

MTT Assay
The synthesized silver nanoparticles using fungal extracts was tested for *in vitro* cytotoxicity, using MCF-7cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured MCF-7cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the synthesized silver nanoparticles using fungal extracts in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC_{50} value was calculated using Graph Pad Prism 6.0 software (USA).

RESULT AND DISCUSSION

UV-Vis spectroscopy
The presence of silver nanoparticle was analysed by using UV-Vis spectral technique. In this UV visible absorption spectrum at the range of 447 nm for the fungal extract-AgNPs[30].The gradual change in the colour of samples from colourless to dark brown colour was observed and the bioreduction of Ag+ in the fungal extract. It was the confirmation process for synthesis of Ag Nanoparticle. This is due to the physical property surface plasmon resonance activity. Colour nature varies depends on size and shape of synthesised nanoparticles.

FTIR spectrum band values
Rhizopus delemer synthesised silver nanoparticles undergone to FTIR analysis. It shows that the results of functional groups which are enclosed the Ag nanoparticle, those are some bioactive molecules. Table1 shows the molecules which are stabilize (capping agents) the synthesised silver nanoparticle those are primary amine, isothiocyanate, Alkene, Secondary alcohol, Anhydride, Halo compound. The FTIR fungal filtrates showed the peaks at 3331.07 ,597.93 confirms the existence of primary alcohol O-H Groups, presence of peaks 1637.56, Indicates the presence of isothiocyanate N=C=S groups. The peaks at 659.66,557.43,437.04,414.70 indicates the presence of C=C, C=O, CO-O-CO, C-Br Alkene, Secondary alcohol, Anhydride, Halo compounds respectively [30].

XRD Analysis
The X-ray diffractogram pattern of synthesized silver nanoparticleswas crystalline nature. It was confirmed using X-RD. From this obtained XRD pattern for silver nanoparticles synthesized using fungal extract showed the characteristic peaks 38.0824, 45.7077, 67.0407, 78.1871 and 78.4575 in the 2Θ range. The peak corresponds to 38.0824, 45.7077, 67.0407 following diffraction facets are (111), (200), (311) respectively. This pattern (111),(200),(220) and (311) reflection of the face centered cubicstructure for silver according to (JCPDS, File No. 04-0783)[30].

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Sem analysis

Rhizopus delemer Synthesised Ag Nanoparticle size ranges were detected by Scanning electron microscope. In this SEM results shown the particle size range at 10-50nm. Sem confirms that Endophytic fungi *Rhizopus delemer* synthesised silver nanoparticle was effective particles in nano scale level [30].

Particles size distribution and Zeta potential activity

The particle size distribution, Polydispersity index and average size of synthesised *Rhizopus delemer* extract silver nanoparticles were analysed by a particle size analyser. In PSD And ZPA shows the average particle size diameter of synthesised *Rhizopus delemer* extract silver nanoparticle is 376.4 nm with a polydispersity index of 0.072. From the results of PDI and PSD values, it is found that produced nanoparticles were monodispersed in nature.The Zeta potentiality of Ag nanoparticles was helped to determine the stability of synthesised particles in that particular sample. In this values shows our synthesised *Rhizopus delemer* extract silver nanoparticles have zeta potential value -15.6 mV found to be peak area of 100% intensity. This value indicates that synthesised *Rhizopus delemer* extract silver nanoparticles have stabilized compounds.

Antioxidant activity
DPPH method

The antioxidant activity of synthesised *Rhizopus delemer* fungal extract Ag nanoparticle was measured using the DPPH radical scavenging reduction method. DPPH-is a standard reducing compound to calculate the antioxidant value of synthesized silver nanoparticles[32]. In this our *Rhizopus delemer* fungal extract silver nanoparticle, table 2 clearly differentiate the 100g concentration level have 67.74% inhibition which is a higher level of concentration. When it is compared with figure 1 a (20, 40, 60, 80) μg lower concentration inhibition percentage was stable decreases. Standard of Ascorbic acid in 100μg concentration 78.7 % inhibition. But in the value level of std and sample comparison synthesised *Rhizopus delemer* extract silver nanoparticle have good antioxidant capability.

Reducing power method

Reducing power activity of synthesised *Rhizopus delemer* extract silver nanoparticles were measured with Standard Ascorbic acid. It mainly depends on the Standard as well as our sample concentration. In figure 1 b higher 100 μg concentration of *Rhizopus delemer* extract silver nanoparticle, have an increased 66.8 % of inhibition, towards reducing power. The same Standard solution as in 100 μg table 3 compare to sample increased 83.4 % of inhibition value. Whereas not in bad, comparison over with standard there is our synthesised *Rhizopus delemer* extract silver nanoparticle result shows upright antioxidant and reducing power to radical compound.

Anti-Diabetic activity
Alpha-amylase Inhibitory assay

In this synthesised *Rhizopus delemer* extract silver nanoparticle antidiabetic activity were investigated by the inhibition of alpha-amylase enzyme activity. Whereas depends on concentration, figure 2a synthesised *Rhizopus delemer* extract silver nanoparticle and standard Acarbose higher inhibition values 80.2 %and 88.7 % 100 μg concentration level. There are gradual decreases where it is in lowered concentration (20, 40, 60,80) μg level. Table 4 results, when compares with standard and synthesised *Rhizopus delemer* extract silver nanoparticles have upright antidiabetic activity in the alpha amylase inhibition method. In carbohydrates metabolism depletion of insulin to catabolize the carbohydrates molecules role, alpha-amylase plays to maintain the insulin level in blood. It overcomes the problem of hypoglycaemia in postprandial time [32].

Alpha-glucosidase Inhibitory assay

Synthesised *Rhizopus delemer* extract silver nanoparticle antidiabetic activity was measured with inhibition of alpha-glucosidase activity with standard (Ascorbic acid). Figure 2 b and table 5 shows result of the extract at the higher to lower order concentration there is a decreased level of inhibition. In 100 μg 74.2 % activity ,which is compared with standard 88.1 %. It was upright antidiabetic activity. Ag nanoparticle decreases the absorption of glucose level in the

blood, deformation of kidney crystals in bladder, higher glucokinase activity and boost up the immune system of body [33].

Antimicrobial assay
Antibacterial Activity

Antibacterial activity of synthesised *Rhizopus delemer* extract silver nanoparticles was tested against Gram-positive and Gram-negative pathogenic bacterial culture by disc diffusion method. There are various microgram concentrations (20, 40, 60, 80, 100) µg tested the pathogens. In this table 6 shows the zone of inhibition in percentile gives 3±1.8257, 5±1.8257, 5±3.3665, 7±4.0824, 8±5.2281, when it is in inhibition at increased results of figure 3 ashows that the organism *Staphylococcus aureus* (MTCC 25923), *Streptococcus aureus* (MTCC 457) and *Bacillus subtilis* (MTCC 2451) and two gram negative bacterial strains *Escherichia coli* (MTCC 25922), *Enterococcus aeruginosa* (MTCC 27853). In this a minimum inhibition and its concentration depends on microorganisms it varies the zone of inhibition. Several facts influence the inhibition of growth by the silver nanoparticle. Electron valency of microbial membrane changed by the Ag nanoparticle. It dissolves the cell wall and causes cell lysis [28]. Whereas same author extracellular synthesis of *Penicillium* extract silver nanoparticle tested against to pathogenic bacteria *Klebsiella, Pseudomonas, E. Coli, Salmonella, Enterobacter* showed inhibition at a minimum concentration of Ag nanoparticle at various levels of a zone of inhibition (mm) [28]. Ag nanoparticle mainly attached to the negatively charged bacterial cell wall and rupture it finally denatures the protein formation and lastly cell death happens [22,23]. Other processes were destabilizing the plasma as well as cell membrane affect the proton motive force and ATP formation of cell. It leads to more accumulation of Ag nanoparticles in the cells of sulphur and phosphorus biomolecules of DNA. It denatures the content of genetic materials final results show the death of the cell [22,24].

Antifungal activity

In this table7 and figure 3 b shows Antifungal activity of synthesised *Rhizopus delemer* extract silver nanoparticles, the results was tested against *Candida albicans* (MTCC 282), *Aspergillus flavus* (MTCC 237) and *Aspergillus niger* (MTCC 1346) and it gives 8±4.0824, 6±1.8257, 7±2.3804 inhibition percentile at maximum of 100 µg.And also where tested at various concentration level. Ag nanoparticles have the immense power to state and agglutinate the fungal hyphae and deactivate the mechanism of growth. Several mechanisms are involved in the action against the pathogenic fungi. DNA repaired by Ag nanoparticle and deactivate the needed enzymes for the ATP synthesis and ribosomal subunit formation to protein synthesis for the growth of fungal culture [34]. Ag nanoparticle mainly affects the membrane-bound enzyme action in the respiratory chain of pathogenic fungal culture [34].

Cytotoxicity of the sample against MCF-7 human breast cancer cell line

In figure 4 a graph shows the synthesised *Rhizopus delemer* extract silver nanoparticle was tested their cell viability assay against MCF -7 Human Breast cancer cell line. Depends on the biocompatibility of nanoparticle which acts on control normal cell as well as test samples [35]. The figure 4 b explains there are various concentrations (20, 40, 60, 80, 100) µg of the nano sample tested. OD value 570 nm taken at 10 levels of regular time interval. cell cytotoxicity test gave a result in MTT cell lysis. The IC50 value for the MTT assay was 60.53 %. It describes the minimum concentration of nanoparticles also has cytotoxicity activity against cell line. Increasing the concentration value of nanoparticles there is increased cell death, inhibition of cell growth, cell cycle arrests and leads to apoptotic of the cell.

CONCLUSION

This study reveals that the effort of work was Ag nanoparticles were biosynthesised by the endophytic fungi *Rhizopus delemer* extracellularly. Whereas Endophytic fungi were isolated, amplified and identified already as *Rhizopus delemer* LC514308 by NCBI and GenBank research. Synthesised Ag nanoparticle was characterized in physical property state through UV, X-RD, SEM and Zeta potentiality analysis it given the standard result values as nano range. whether the synthesised particle was capping with active forms such as O-H, N=C=S, C=C, C=O, CO-O-

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CO, C-Br like functional groups, and also it was identified in FTIR study. Synthesised Ag nanoparticles have increased reducing power in Anti-Oxidant analysis. This study further undergoes to application oriented like Antidiabetic, Anticancer, Antimicrobial activity specifically investigated in In-Vitro and also the results were appropriately excellent values in upcoming research. So, from these details end up us to *Rhizopus delemer* fungi synthesised silver nanoparticle were excellent stimulant, activator, catalyst in Nanomedicine discipline.

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Table: 1 FTIR band values of synthesized silver nanoparticles by using SNP from *Rhizopus delemer* fungal extract

Functional group	Band	Frequency. Cm^{-1}
Primary amine	Strong band	3331.07 cm^{-1} corresponds to broad O-H stretching alcohol
Isothiocyanate	Strong band	1637.56 cm^{-1} corresponds to N=C=S stretching vibrations
Alkene	Medium band	659.66 cm^{-1} corresponds to C=C stretching conjugated alkene
Amine	Medium band	597.93 cm^{-1} corresponds to O-H bending alcohol
Alcohol	Strong band	557.43 cm^{-1} corresponds to C=O secondary alcohol
Anhydride	Strong band	437.04 cm^{-1} corresponds to CO-O-CO anhydride
Halo compound	Strong band	414.70 cm^{-1} corresponds to C-Br stretching vibrations

Table: 2 Antioxidant activity of DPPH method for SNP from *Rhizopus delemer* fungal extract

S.NO	CONCENTRATION (µg/ml)	ANTIOXIDANT ACTIVITY DPPH%	
		SYNTHESIZED SILVER NANOPARTICLES USING FUNGAL EXTRACTS	ASCORBIC ACID
1.	20	49.38±0.8211	58.59±0.8288
2.	40	53.47±0.7965	61.73±0.8288
3.	60	59.16±0.8006	64.12±0.8207
4.	80	63.29±0.8083	67.48±0.8126
5.	100	67.74±1.8294	78.63±0.8010

Table: 3 Antioxidant activity of Reducing power method of SNP from *Rhizopus delemer* fungal extract

S.NO	CONCENTRATION (µg/ml)	ANTIOXIDANT ACTIVITY OF REDUCING POWER	
		SYNTHESIZED SILVER NANOPARTICLES	ASCORBIC ACID
1.	20	45.76±2.6001	57.27±0.8288
2.	40	52.68±2.9349	66.43±2.9620
3.	60	55.34±2.6104	75.89±2.9620
4.	80	62.68±2.9552	78.97±2.9620
5.	100	66.72±1.8549	83.34±2.9620

Table: 4 Antidiabetic activity of alpha amylase inhibition assay of SNP from *Rhizopus delemer* fungal extract

S.NO	CONCENTRATIONS	ALPHA AMALYSE	
		SYNTHESIZED SILVER NANOPARTICLES	ACARBOSE
1	20(µg/ml)	61.76±0.8045	76.56±0.8125
2	40(µg/ml)	68.23±0.4356	79.34±0.8167
3	60(µg/ml)	74.98±0.8205	81.48±0.8167
4	80(µg/ml)	78.36±0.8126	85.77±0.8126
5	100(µg/ml)	80.14±0.8207	88.62±0.8416

Table: 5 Antidiabetic activity of alpha glucosidase inhibition assay of SNP from *Rhizopus delemer* fungal extract

S.NO	CONCENTRATIONS	ALPHA GLUCOSIDASE (%)	
		SYNTHESIZED SILVER NANOPARTICLES	ACARBOSE
1	20 (µg/ml)	58.43±0.8043	75.23±0.8416
2	40 (µg/ml)	63.13±0.8207	76.60±0.8288
3	60 (µg/ml)	66.82±0.8253	82.44±0.8288
4	80 (µg/ml)	71.37±0.8005	85.28±0.8288
5	100 (µg/ml)	74.14±0.8330	88.03±0.8288

Table: 6 Antibacterial activity of SNP from *Rhizopus delemer* fungal extract showing inhibition value on *S.aureus, B.subtilis, E.coli, E.aeroginosa.*

Samples	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)				
		Fungal extracts-AgNps				
		Staphylococcus aureus	*Streptococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Enterococcus aeruginosa*
Fungal extracts-AgNps	60	1±0.0000	1±0.0000	1±0.0000	2±0.8165	4±1.8257
	80	2±0.8165	4±1.2909	3±1.8257	4±2.9439	6±2.9439
	100	3±1.8257	5±1.8257	5±3.3665	7±4.0824	8±5.2281
Standard (Std) (Amoxicillin)	10 µl/disc	8±1.8257	9±4.0824	7±1.8257	10±5.2281	10±1.8257

Table: 7 Antifungal activity of *A.niger, A.flavus ,C.vulgaris, C.albicans.*

Samples	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)			
		Fungal extracts-AgNps			
		Aspergillus niger	*Aspergillus flavus*	*Candida vulgaris*	*Candida albicans*
Fungal extracts-AgNps	60	0±0.0000	3±1.8257	0±0.0000	3±2.9439
	80	6±1.8257	4±2.9439	0±0.0000	5±4.0824
	100	8±4.0824	6±1.8257	0±0.0000	7±2.3804
Standard (Std) (Fluconazole)	10 µl/disc	11±1.8257	8±4.3969	5±1.8257	6±1.8257
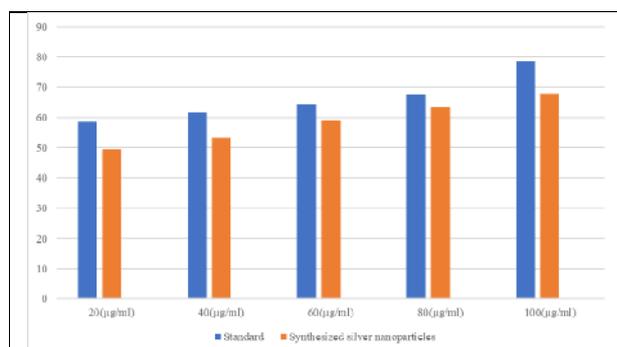
| **Figure: 1 (a) Graph for DPPH Assay of SNP from *Rhizopus delemer* fungal extract** | **Figure :1(b) Graph for Reducing power assay of SNP from *Rhizopus delemer* fungal extract** |
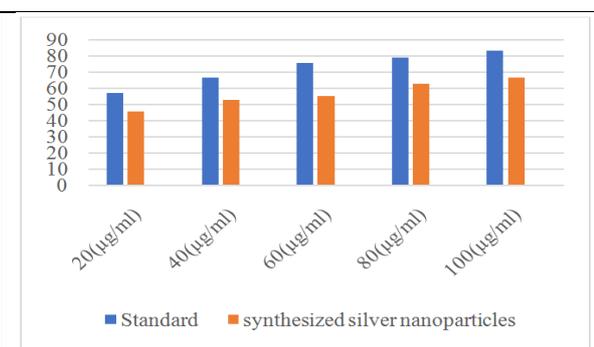
Deepalakshmi and Sebastin Raj
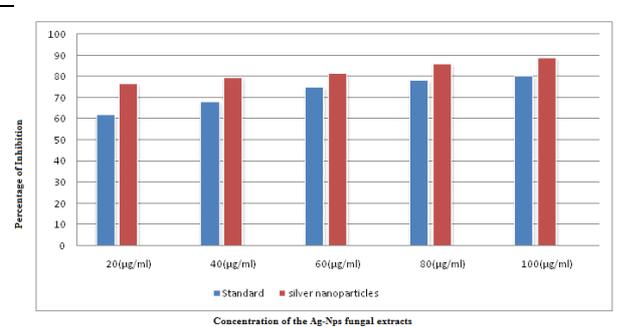
Figure:2 (a) Graph for Alpha amylase inhibition assay for SNP from *Rhizopus delemer* fungal extract
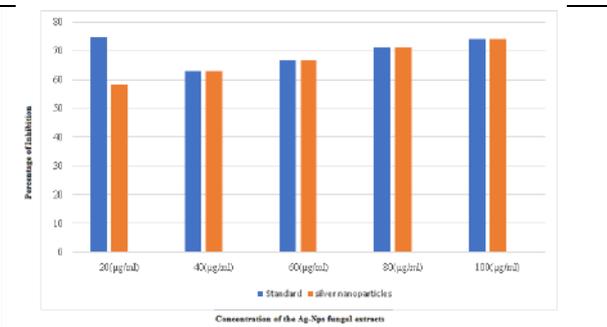
Figure: 2(b) Graph for Alpha glucosidase inhibition assay for SNP from *Rhizopus delemer* fungal extract
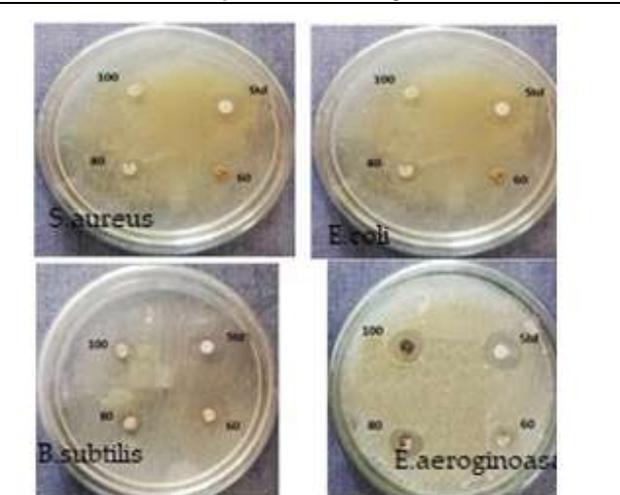

**Figure: 3(a)Antibacterial activity of SNP from *Rhizopus delemer* fungal extract**
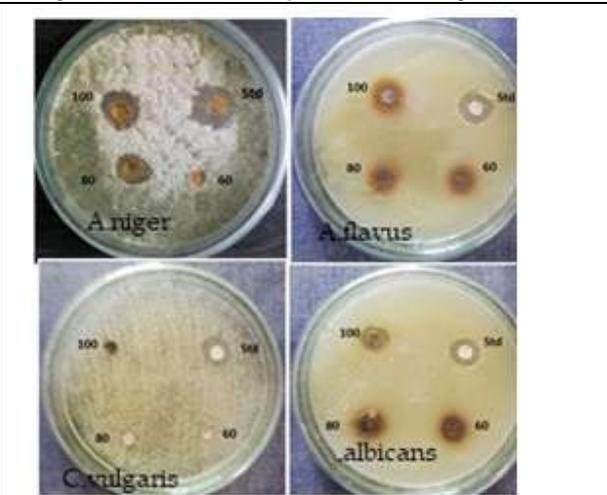
**Figure: 3(b)Antifungal activity of SNP from *Rhizopus delemer* fungal extract**
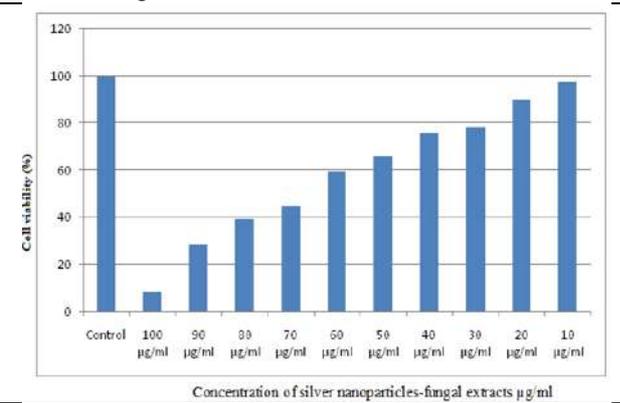

Figure: 4(a) Cytotoxicity assay
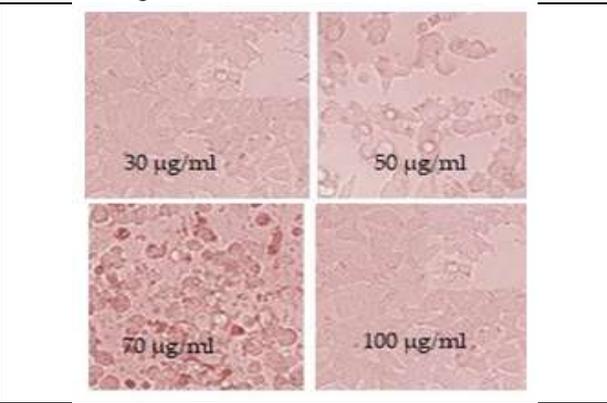
Figure :4(b) Cancer cell line apoptosis at minimum Concentration

RESEARCH ARTICLE

A New Epitome for Cosmetics Regultions in India: 2020

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ABSTRACT

Demand for cosmetic products is increasing owing to various factors. Cosmetic products are used to enhance the beauty of an individual or to alter the appearance or texture of the skin. Cosmetics products in India are governed by the Drug & Cosmetics (D&C) act 1940 and Drug & Cosmetics (D&C) Rules 1945, as well as labeling declarations from the Bureau of Indian Standards (BIS). On 15th December 2020, the Ministry of Health and Family Welfare (MHFW) published the Cosmetics Rules, 2020 under the D&C Act, 1940. This study gives an overview of the difference between old and new cosmetics regulations after the enactment of new cosmetics rules in 2020.

Keywords: Drug and Cosmetics act 1940, Drug and Cosmetics Rules 1945, New Cosmetics Rules 2020, Ministry of Health and Family Welfare, Central Licensing Authority, State Licensing Authority.

INTRODUCTION

Under section 3(aaa) of the Drugs and Cosmetics (D & C) Act, 1940, a cosmetic is defined as "any article intended to be rubbed, poured, sprinkled, or sprayed on, or introduced into, or otherwise applied to, the human body or any

part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and includes any article intended for use as a component of cosmetic "[1]. The New Rules 2020, published by the Indian government in December 2020, removes the rules for cosmetics from those for medications, which were previously governed as per the D&C act 1940. These laws have been amended and defined and apply to the import, production, labeling, sale, and distribution of cosmetics in India [2]. For the first time, the notion of "new cosmetic" was introduced. According to the new legislation, a "new cosmetic" has a "novel substance that has not been utilized somewhere else on the planet or isn't perceived for use in beauty care products in any public or global literature". When producing or importing a new cosmetic, prior authorization from the Central Licensing Authority (CLA) is essential, as is the submission of data concerning the product's safety and efficacy [3].

A candidate for enrolment of a cosmetics products planned for import into India must be submitted through the federal government's website in a prescribed form by the manufacturer, its approved specialist or importer in India, or on the other hand a helper in India supported by the producer (Form COS-1). The CLSA can either provide an import enrollment certificate in the prescribed format (i.e., Form COS-2) or decline any application for a variety of reasons if all requirements have been met that should be reported recorded as a hard copy in no less than a half year of the date of use and stay valid in interminability, dependent upon installment of the enlistment authentication's maintenance expense before the termination Starting from the date of issue, a five-year time limit will be used. Unless a powerful Indian legislative body suspends or repeals it, it will remain in effect [4].

The import registration certificate has a five-year validity period. Form COS-4A has a 3-year validity period. In addition, the Fourth Schedule of the Cosmetics Rules lists a total of 80 product categories that are eligible for import. In the context of cosmetics imports, an "actual manufacturer" is defined as a person who manufactures cosmetics in a facility outside of India that has been approved by the National Regulatory Authority or another government agency. The Central Licensing Authority (CLA) has the authority to cancel or suspend a registration certificate for a while if the manufacturer, authorized agent, or importer fails to comply with any of the Registration Certificate restrictions as the manufacturer, authorized agent, or importer deems appropriate. In addition, if the cosmetics are made at more than one location, the applicant must pay an additional USD 500 for each location where the items are made[5].

The registration certificate's validity [6]
The certificates for cosmetic import registration are substantial for 3 years from the date of issuance as per D&C rules 1940 and it is substantial for 5 years as per cosmetics rules 2020.

RESEARCH METHODOLOGY

The New Cosmetics Rules 2020 consist of Chapters I-IX, Rules 1-71, Schedules 1-13, and Forms COS 1-COS 24.

RESULTS AND DISCUSSIONS

The changes in the New Cosmetics rule 2020 from the existing D&C Act 1940 & Rules 1945 are as follows:[8,9]
1. Cosmetic import and registration are governed by D & C rules 1945 in part XIII and cosmetics rules 2020 in chapter III.
2. The information and undertaking that the producer or his approved importer/wholesaler/specialist should contain the application structure for a registration certificate in schedule D(III) Under the D&C rules 1945, and they are itemized in the Second Schedule of Part-I of the cosmetics rules 2020.
3. According to D&C rules 1945, For the import of cosmetics into India, Rule 129-C is an enrollment declaration in Form 43, and the enlistment declaration is valid for three years from the date of issue. The import enlistment endorsement for beauty care products brought into India is in Form COS-2, and it is significant for a time of five

years from the date of the issue according to cosmetics rules 2020.

4. Part XIV of the D&C rules from 1945 sets the criteria for the manufacturing of cosmetics for sale or distribution. Under cosmetics rules 2020, chapter IV is standard for manufacturing cosmetics for sale or distribution.

5. Cosmetic labeling, packaging, and standards are dealt with in Part XV of the old D&C rules 1945, whereas cosmetic labeling, packaging, and standards for sale or distribution are dealt with in Chapter VI of the Cosmetics Rules 2020.

6. Good laboratory practices and prerequisites for premises and equipment are illustrated in rules 150-C and schedule L-1 under the D&C rules 1945, and it is stated in the eleventh schedule as per cosmetics rules 2020.

Changes in forms

1. Under the D&C rules 1945, form 1 is utilized to provide prescription and cosmetic samples through the courts. As per cosmetics rules 2020, Form COS-20 is a reminder arranged explicitly for moving cosmetics samples to the chief, central cosmetics laboratory, through a general set of laws. The subject is same in the both versions, the only change is that instead of a central drug laboratory, the central cosmetics laboratory is recommended.

2. Under the cosmetics rules 2020, the Central Drugs Laboratory provides a report on Form 2 for both drugs and cosmetics samples provided through the court, and the Central Cosmetics Laboratory provides a report on Form COS-21 for cosmetic samples sent through the court. Form COS-21 purports to be a Report for Cosmetic Samples from the Central Cosmetic Laboratory under the cosmetics rules 2020, whereas Form 2 pretends to be a Certificate from the Central Pharmaceuticals Laboratory for testing or analysis of drugs and cosmetics.

3. On Form 34, the Government Analyst, Central Drugs Laboratory, provides a report for the cosmetics product testing given by the Drug Inspector as per D&C rules 1945. A report from the public authority investigator, Central Cosmetic Laboratory, for remedial examples offered by the pharmaceuticals monitor on Form COS-19, is included in the cosmetics rules 2020.The subject is something similar in the two variants. The report's modification is toward the end, and Form 34 should be endorsed by the Director of the Central Drugs Laboratory/Government Analyst.

4. Form 14A is a request for a drug test or analysis from a purchaser under the D&C standards of 1945. It makes no mention of receiving cosmetic samples. Form COS-13 is an elite application from a buyer for cosmetics products testing or examination under the cosmetics rules 2020. The two forms have similar data.

5. A Certificate of Test or Analysis by a Government Analyst for drug testing was filed on Form 14-A, as required by D&C rules 1945. There is no mention of cosmetic samples. A government analyst's evaluation or analysis of cosmetic samples submitted on Form COS-13 under the cosmetics rules, 2020. The information on both forms is identical. The key difference is that the report on Form COS-14 replaces the certificate on Form 14-B under the cosmetics rules 2020.

6. Under the D&C standards of 1945, Form 15 is a request not to discard supplies of medications or cosmetics items under his control. The cosmetics rules 2020 show Form COS-18 as a request with a similar stated cause but only for cosmetics items. Form15 and Form COS-18 have similar items.

7. Form 17 is a notification sent to the individual from whom a sample (including pharmaceuticals and cosmetics) is taken under the D&C rules of 1945. The individual from whom a cosmetics sample is obtained is sent a notification on Form COS-10 under the cosmetics rules of 2020. Form 17 and Form COS-10 have the same contents. There haven't been any changes.

8. Form 17A is a receipt given to tests of drugs or cosmetics products acquired when a sensible cost is presented under D&C rules, 1945. Form COS-16 is given under the cosmetics rules 2020 for the predetermined reason, however explicitly for cosmetics samples. Structure 17A and COS-16 have similar items.There haven't been any changes.

9. Form 18 is a Memorandum from an Inspector to a Government Analyst to evaluate pharmaceutical and cosmetics samples in line with the 1945 D&C standards. The Inspector's special note for the cosmetic samples drawn in compliance with the cosmetics rules 2020 is Form COS-17. The items in Form 18 and Form COS-17 are identical. There have been no modifications.

10. Licenses to create cosmetics for sale or distribution are now issued on Form 32 under the D&C rules of 1945. However, the cosmetics rules of 2020 propose that Form COS-8 will be issued.

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11. The D&C rules, 1945, have a Form-35 Inspection Book. (This applies to both Drugs and cosmetics.) For this purpose, the 2020 cosmetics rules recommend using Form COS-11. The information on Form 35 and Form COS-11 is identical.

12. Under the D&C guidelines of 1945, Form 37 is utilized to get lab endorsement for testing drugs, cosmetics products, and unrefined substances utilized in their assembling. Under the cosmetics rules 2020, Form COS-23 gives endorsement to directing tests solely on cosmetics products and unrefined components utilized in their manufacture. The data is the same on both forms.

13. Under the D&C rules of 1945, test reports for medicines and cosmetics were issued on Form 39.Under the cosmetics rules of 2020, all cosmetics test results ought to be represented on Form COS-24. The information on Form 39 and COS-24 is indistinguishable.

SCHEDULE MODIFICATIONS

1. The fee information is stated in the corresponding parts for each listed region in the D&C rules 1945. Cosmetics import fees and registration, manufacture, testing laboratory approval, and other fees in one place (i.e., in the Third Schedule) are defined under the cosmetics rules 2020. The proposed fee scheme under the cosmetics rules 2020 differs significantly from the existing charge requirements in all industries.

2. The existing D&C Rules 1945 do not include a list of cosmetics categories that can be imported. The Fourth Schedule of the New Cosmetics Rules 2020 specifies it.

3. According to Schedule B, III of the D&C rules 1945, cosmetics cost between 400 and 1500 rupees. The Director of the Laboratory of the Government Analyst, whichever is appropriate, shall decide the exact amount of the charge. The cosmetics rules 2020 include a set of tests and costs for each.

4. Cosmetics rules 2020 in the Seventh Schedule provide Good Manufacturing Practices and Requirements for cosmetics manufacturing, although D&C rules 1945 in Schedule M-II contain Good Manufacturing Practices and Requirements for cosmetics manufacturing. (Please note that the title has been modified.)

5. Schedule M-II of the D& Crules 1945 says that appropriate waste disposal arrangements must be implemented. The cosmetics rules 2020 say that trash must be disposed of by the Environment Pollution Control Board's approved methods.

6. Schedule MIII of the D&C rules 1945 does not contain a list of cosmetic categories to grant a license to manufacture and sell cosmetics in the country. The cosmetics rules 2020's Seventh Schedule state it under the requirement of plant and equipment.

7. The cosmetics rules 2020's Eighth Schedule, as well as the former Schedule U (I) of the D&C rules 1945, explain the specifications of each batch of cosmetics made and the raw ingredients utilized.

8. The standard for thirty-six cosmetics in finished form is mentioned in schedule S as per D&C rules 1945. As per the cosmetics rules 2020, Ninth Schedule keeps the cosmetics standards for 36 cosmetics in finished form.

9. The ISO standard for cream bleach was ISO 15608 in Schedule S in the previous D&C rules of 1945, and the cosmetics rules 2020 do not incorporate the ISO standard number for a cream fade.

10. The Good Laboratory Practices, Premises, and Equipment Requirements are recorded in Schedule L-I of the D&C Rules 1945. It is referenced in the eleventh Schedule of the 2020 Cosmetics Rules.

Application

• **Existing D&C rules 1945**

✓The application for a permit to make cosmetics for sale or distribution was dealt with in Part XIV of Rule 138, yet the application for an advance permit to loan license cosmetics was tended to in Rule 138-A. The cosmetics rules 2020, Chapter IV, Rule 23, explains how to apply for both a license and a loan license.

✓According to Rule No. 138 of the D&C rules 1945, On Form 31 and Form 31-A, you can apply to the State Licensing Authority for a lending license for up to ten products in each of the categories listed in Schedule M-II.

• **Cosmetics rules 2020:** The following are the modifications:

✓Form COS-5 is used to apply for a cosmetic production license, and Form COS-6 is used to apply for a loan license from the State Licensing Authority. It should also be mentioned that the offline application will be used if the internet portal is unavailable.

✓The Third Schedule specifies the number of items from each category that must be utilized in each application.

✓The Third Schedule states that each cosmetics category may utilize up to ten ingredients in a single application.

Forms of Application

- **Existing D&C rules 1945:** Form 36 is used to submit an application with supplementary information (Both for Drugs & Cosmetics)
- **Cosmetic rules 2020:** Use Form COS-22 to submit an application with supplementary information (Only for Cosmetics).

Fees
- **Existing D&C rules 1945:**
✓Fee for obtaining a license: Rs.2500
✓Fees for inspection: Rs.1000
- **Cosmetics rules 2020:**
✓ Grant of a license in Forms Cos-8 and COS-9 (loan license) for up to ten goods in each cosmetics category: Rs.10000 and Rs.10000, respectively.
✓ A license in Forms Cos-8 and Cos-9 is provided at a fee of Rs. 500 for each additional item in the cosmetics category. (In each successive application, the maximum number of elements is not stated.)
✓ COS-8 (loan licence) and COS-9 (loan application): Rs.10,000 for ten goods from each additional cosmetics category.
✓In the New Cosmetics Rules 2020, the phrase "inspection charge" is not defined.
✓ Sections 138 (4) and 138-E(6) of the old D&C Rules provide that if the original is disfigured, destroyed, or lost, a duplicate copy of a license must be paid Rs. 250, while the Third Schedule suggests a price of Rs. 500 for the New Cosmetics Rules, 2020.

The license's validity is as follows
The license (grant or renewal) had a 5-year validity period previously. Fees and late fees are not paid within six months of their due date. The license has reached the end of its validity period.
- **Existing D&C rules 1945:**As though it was made to last forever.
- **Cosmetics rules 2020**: If license retention costs (given in the Third Schedule) are paid before the 5-year term from the date of issuance, it is asserted that licenses allowed on Form COS-8 or Form COS-9 are legitimate in interminability. In addition to license retention costs, if the license is not paid by the due date, a late fee of 2% of the license will be applied for each month up to 6 months. If the license is not paid within 6 months, it will be considered canceled.
✓ Each excess item in the cosmetics category sold or distributed under a license on Form COS-8 or Form COS-9 for the development of cosmetics is subject to a retention charge of 500 rupees.

License grant
- **Existing D & C rules 1945:**After inspection
- **Cosmetic rules 2020:**Before the Inspection The State Licensing Authority will give a license or loan license in no less than 45 days of the application date if the self-assertion in Form COS-7 and the papers provided in Part II of the Second Schedule agree with the Act and Rules. If the given documents are insufficient, the applicant will be notified within 45 days.

License upload

- **Existing D&C rules 1945**: No
- **Cosmetic rules 2020:**After getting a license or a loan license, the candidate can create cosmetics for sale or distribution by submitting a copy of the license to CDSCO's website.

Inspection

- **Existing D&C rules 1945**: Before the grant of a license
- **Cosmetic rules 2020:**The state licensing authority must analyze and verify the information provided by the firm on Form COS-7 within 30 days of the license or loan being granted (Self-certification). A license or loan license is deemed valid for all purposes if it is not examined within 30 days.

Pre-approval inspection

- **Existing D&C rules 1945**: The State and Central Drugs Inspectors inspected the premises for permission.
- **Cosmetic rules 2020:** The Central Drugs Inspector is the only person who inspects the premises for authorization.

Approval of the Testing Laboratory

- **Existing D&C rules 1945**: In Form 37, approval was provided for a laboratory to evaluate drugs, cosmetics, and their basic ingredients.
- **Cosmetic rules 2020:** Only Form COS-23 received approval for a laboratory to analyze cosmetics and their basic materials. (Only Cosmetics are tested at this laboratory.)

CONCLUSION

The New Cosmetics Rules 2020, enacted under the D&C Act of 1940, have established numerous components for regulating cosmetics in India. During the full assessment of previous and new cosmetics regulations, well-informed and well-defined guidelines are developed. The new rule has nine chapters, thirteen schedules, twenty-four forms, and seventy-one rules. It concerns Import, sale, manufacturing, registration, labelling, packaging, testing, and distribution of cosmetics. The rule's main feature is that it includes a relatively simple procedure for filing for a registration certificate, which eliminates date duplication and allows for faster acceptance.

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Table no 01 Difference between Old and New Cosmetic Rules [7].

OLD COSMETICS RULES 1940	NEW COSMETICS RULES 2020
Form 42 is a new registration application	COS-1 application for a new registration
Form 43 is a certificate of registration	COS-2 is for a Registration Certificate
Renewal fees are determined by the applicable category as well as the variation fee	Fees for renewal are payable, as is a retention fee of 2% for the first 180 days
Schedule D (III) contains information on the company and the cosmetics to be imported	In Second Schedule Part-I, fill in the manufacturer's information and the details of the cosmetics to be imported
The fee for obtaining or maintaining a registration certificate for each cosmetic category is $2,000	The fee for obtaining or maintaining a registration certificate for each cosmetic category is $1,000
Fee for manufacturing site: N/A	Fee for manufacturing site: $500
Schedule S is standard for 28 cosmetics products	The standard for 36 cosmetics products in Ninth Schedule

Figure 01: Overview of cosmetics Rules 2020

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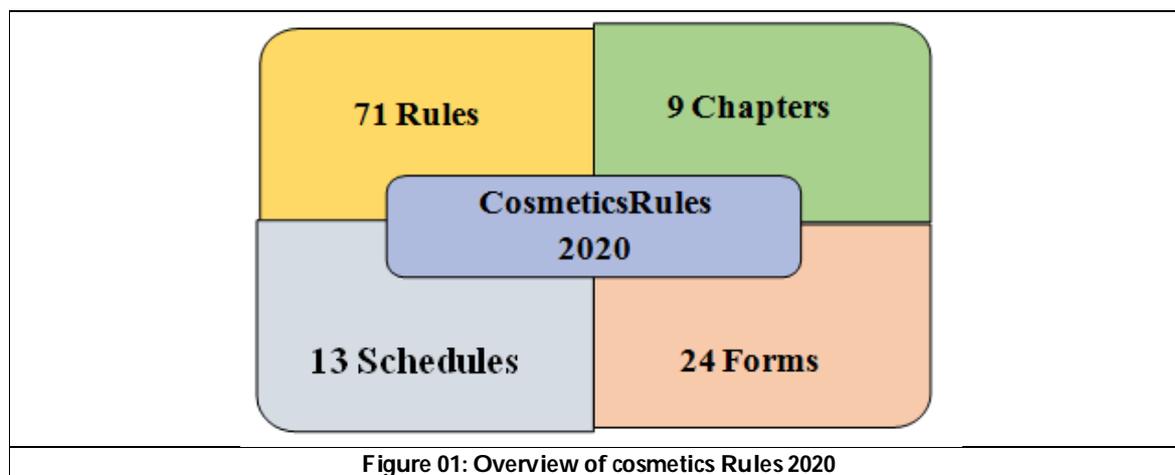
Analysis into Indirect Sinus Lift Procedure Done with Amount of Height Increased or Achieved

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ABSTRACT

Dental implants provide a predictive treatment for prosthetic rehabilitation of edentulous patients. Dental implants are used to replace both the form and the function of missing teeth. Sufficient volume and density of the alveolar bone for implant integration and load bearing are factors that contribute towards the successful placement of an implant. The presence of inadequate bone quality and quantity often complicates implant placement in posterior maxilla. This study was conducted to evaluate the increase in bone height following maxillary sinus lift using indirect technique's .This study is a retrospective observational study conducted in a university hospital Chennai. Data collection was done with the help of the electronic dental record of the university- Dental information archiving software (DIAS). It records all patients' data from initial visit to last visit chronologically. This was followed by Excel tabulation. Data was analysed using SPSS Software. The association of study variables was calculated using Chi Square test. Within the limitations of the study 38.9% of the cases achieved a height of 2 mm followed by 3 mm (22.2%) of indirect sinus lifting procedures in molar regions. It was found that a height up to 4mm could be achieved during the indirect sinus lift procedures. Within the limitations of the study it can be concluded that a height upto 4 mm could be achieved by indirect sinus augmentation procedures in the maxillary molar region. The most common sinus floor elevation height is 2 - 3mm in the 16 and 26 regions. This technique of sinus floor elevation has expanded prosthetic options by enabling the placement of additional implant support in maxillary segments with atrophic ridges and pneumatized sinuses.

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INTRODUCTION

Dental implants provide a predictive treatment for prosthetic rehabilitation of edentulous patients. Dental implants are used to replace both the form and the function of missing teeth. Sufficient volume and density of the alveolar bone for implant integration and load bearing are factors that contribute towards the successful placement of an implant. The presence of inadequate bone quality and quantity often complicates implant placement in posterior maxilla. Reduced bone height below the maxillary sinus is a common problem encountered while placing implants in the posterior maxillary region (1,2). The maxillary sinus also known as antrum of Highmore is a pyramid shaped air filled space lying within the bilateral maxilla , lateral to the nasal cavity, superior to the maxillary teeth, inferior to the orbital floors, and anterior to the infra temporal fossa. It is present at birth and develops until around the age of 14 years (3,4).

The quality and quantity of bone is compromised in edentulous posterior maxilla frequently in the vertical direction due to post extraction ridge atrophy , pneumatization of the maxillary sinus or close proximation of sinus floor to the crestal bone (5,6). The maxillary sinus pneumatization is caused by progressive hallowing out of alveolar process of apical aspect mediated by osteoclasts and by increase in positive intra-antral pressure (7).Maxillary sinus augmentation has emerged as the most common surgical intervention in such cases. It causes detachment of schneiderian membrane from the maxillary sinus floor and creates a space filled with bone graft in order to promote vertical bone augmentation into the maxillary sinus cavity, enabling restoration with dental implants in future(8). There are many techniques that are available for sinus lifting. Basically, they can be divided as two broad categories. The direct method: with lateral antrostomy as a one or two-step proced and The indirect method: with the osteotome technique with a crestal approach. The indirect sinus lift is also called as subantral sinus augmentation, subcrestal augmentation(9). Augmentation of the maxillary sinus was first described by Tatum and published as a clinical study by Boyne and James (10).In the technique described by Tatum access to sinus was through the crest. This technique was later replaced with lateral sinus osteotomy which was considered to be more versatile and practical. There onwards different techniques such as sinus balloon technique was introduced for sinus elevation(11). Currently, in order to reconstruct the atrophic maxillae, different bone grafting methods are used such as autogenous, homogenous and heterogenous grafts(12).

There have been reports of successful bone formation in sinus floor elevation by simply elevating the maxillary sinus membrane without the use of adjunctive grafting materials, however these reports do not address the height of new bone formation possible using this procedure(13,14). The present retrospective study was undertaken to evaluate the gain in height of new bone formation in the maxillary sinus following indirect sinus lift technique.Our team has extensive knowledge and research experience that has translated into high quality publications(15–23),(24–29),(30–34) .

MATERIALS AND METHODS

This study was a retrospective observational study conducted in a university hospital located in South India. The study was conducted with the approval of the Institutional Ethics Committee.All case sheets between June 2020-March 2021 were reviewed. Data collection was done with the help of reviewing patient records. Incomplete records were excluded from the study. Randomisation was done in order to minimise the sampling bias. Patients who have indirect sinus lift were noted. Cases were cross verified by another examiner. This was followed by Excel tabulation. Data analysis was done using SPSS Software.The association between study variables was calculated using chi square test, where $P<0.05$ was considered statistically significant.

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RESULTS AND DISCUSSION

During the past decade, implants have become one of the most exciting and rapidly developing topics in dental practice as they provide a proper treatment alternative to conservative prosthodontics. Anatomical Limitations such as deficiency of maxillary alveolar bone and increased pneumatization of the maxillary sinuses are considered as challenges faced during placement of an implant in the posterior maxillary region . Due to the reduced bone volume in this region, sinus floor elevation is a prerequisite to implant placement(35,36). In the present study indirect sinus lift cases were taken into account. In the present study it is seen that indirect sinus lift augmentation was done in cases in which the residual alveolar bone height was 6 mm and above . 38.9% cases had a residual alveolar bone height of 8mm ,22.2% cases had a residual alveolar bone height of 9 mm, 6mm residual alveolar bone height was seen in 16.7 cases. Similarly 7mm residual alveolar bone height was seen in 16.7 cases. This result is in consensus with the previous study of Watzek G et al 1998 (37). He stated that the most important criteria for selecting an indirect sinus lift is the residual alveolar bone being 6 mm or more. In the present study it is seen that 2mm height was achieved in 38.9% cases 3 mm height was achieved in 22.2% of the cases.1 mm height was achieved in 22.2% of the cases. 4 mm height was achieved in the remaining cases. Previous studies state that the indirect sinus lift is minimally invasive but permits only a limited amount of augmentation up to 4 mm. Milan jurisdiction et al 2008 (38). The most common tooth associated with indirect sinus lift was found to be 26 (44.44%) followed by 16 (27.8%). The distobuccal root of maxillary molar is nearest to the sinus. In old age pneumatization becomes more pronounced, the floor of the sinus moves at a more downward position particularly when the maxillary teeth are lost(39). The limitations of the present study are, it is single centered study, it does not represent ethnic groups and patient satisfaction is not recorded. In future, this research can be done on a larger population. Patient satisfaction could also be recorded.

CONCLUSION

Within the limitations of the study it can be concluded that a height upto 4 mm could be achieved by indirect sinus augmentation procedures in the maxillary molar region. The most common sinus floor elevation height is 2 - 3mm in the 16 and 26 regions. This technique of sinus floor elevation has expanded prosthetic options by enabling the placement of additional implant support in maxillary segments with atrophic ridges and pneumatized sinuses.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

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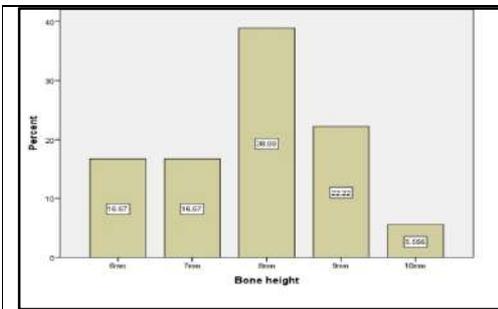
Figure 1. Bar graph shows the distribution of bone height among the patients who underwent indirect sinus lift. The X axis represents the bone height before indirect sinus lift 6mm, 7mm, 8mm , 9 mm and 10 mm and the Y axis denotes the percentage of patients.
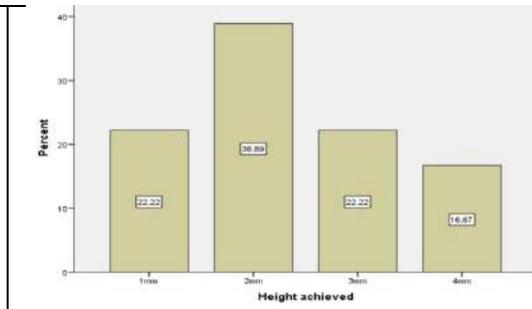

Figure 2. Bar graph showing the distribution of height achieved by indirect sinus lift. The X axis depicts the height achieved and the Y axis denotes the percentage of patients.
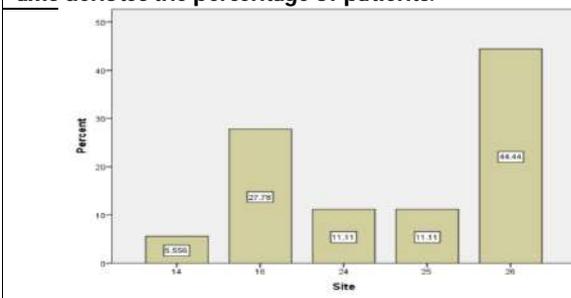

Figure 3. Bar graph shows the distribution of site among the patients who underwent indirect sinus lift. X axis represents the site and the Y axis denotes the percentage of patients.
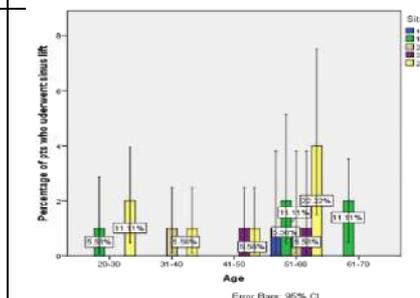

Figure 4. Bar graph showing the association between age and the site of indirect sinus lift. X axis denotes age and the Y axis denotes the percentage of patients who underwent indirect sinus lift. Blue represents 14,green represents 16,beige represents 24, purple represents 25 and yellow represents 26 respectively. Chi square test which found to be statistically not significant. P value =.580 (statistically not significant).
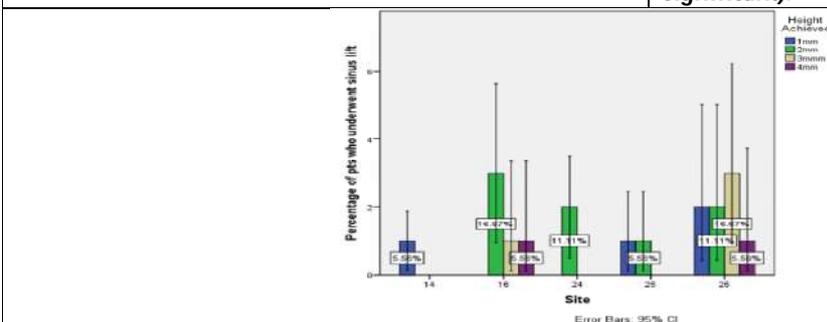

Figure 5. Bar graph showing association between site and the height achieved .X axis denotes site and the Y axis denotes the height achieved. Blue represents 1mm, green represents 2mm, beige represents 3mm and purple represents 4mm. Maximum height was achieved in the molar region.Chi square test which found to be statistically not significant. P value =.055.

RESEARCH ARTICLE

Performance of Hybrid Columns Subjected to Axial, Eccentric and Cyclic Loading

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ABSTRACT

This study deals with the analytical study that investigate the performance of concrete encased hybrid column without reinforcement subjected to axial, eccentric and cyclic loading. The column made up of steel, concrete, and fiber reinforced polymer (FRP). Eight columns of different cross-sectional shape were studied under axial and eccentric loading. Finite element model (FEM) were developed for performing the analysis. Load deflection characteristics, deformation, and ductility of the column were investigated. The result indicate that outer square and inner circular shaped columns have more load carrying capacity. Cyclic analysis was performed for best model. Then the best model is considered for further parametric study. Use of High Strength Concrete (HSC), Radius of inner tube, and hybrid FRP tube are the parameters studied. Cyclic analysis is performed and energy dissipation capacity is studied.

Keywords: Column; Cyclic analysis, Ductility, Energy Dissipation Capacity, Fiber reinforced polymer (FRP), Finite element model (FEM), High Strength Concrete (HSC), Hybrid column.

INTRODUCTION

As a result of the increased need for improved execution at lower prices, there has been an increase in research directed towards enhancing the mechanical behaviour and seismic execution of columns. Diverse column types have been subjected to a number of investigations to better understand their seismic behaviour. Composite elements, also known as hybrid elements, are structural components that are constructed from two or more distinct types of materials. It is the fact that the features of each fabric may be merged to generate a single unit that performs better overall than its partitioned constituent parts that is the most advantageous aspect of hybrid components. According

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to industry experts, a steel-concrete composite frame might be the most widely used hybrid component frame in development right now. Concrete, as a material, performs well in compression, but it performs poorly in tension. Steel is very strong under tension, even when just a tiny quantity of it is employed. Steel-concrete hybrid components combine the compressive strength of concrete with the tensile resistance of steel, resulting in a structurally strong and lightweight unit that is often used in multi-story buildings and bridges.

Due to the great strength of composite columns while having a small cross-sectional area, it will be possible to maximise the amount of usable floor space available for usage. Several different types of composite columns are available, with the most often used being a hollow section steel tube that has been packed with concrete, or an associate degree open steel section that has been encased in concrete. Adding concrete to the steel section increases the compression resistance of the steel, which helps to keep the steel from buckling under compression. Because of its fire resistance, the column may be left uncovered or just lightly shielded if necessary.

The most often seen hollow sections are rectangular and circular in shape. However, rectangular sections are advantageous in that they have flat sides that are suitable for finish plate beam-to-column connections. Fin plates, on the other hand, will be used for rectangular and circular forms. In a vast amount of research on the axial compressive behaviour of FRP-wrapped columns, it has been shown that the external confinement of existing concrete columns by fibre reinforced polymer (FRP) composites is an excellent way of enhancing column ductility. The issues associated with traditional building materials are alleviated by using fibreglass reinforced plastic (FRP). The use of fibre reinforced plastic (FRP) increases the flexural and shear strength. The use of fibre reinforced polymer (FRP) composites to externally restrict existing concrete columns has been shown to be a successful approach for increasing column ductility and extending column life.

OBJECTIVES ANDSCOPE

The Objective of this study is to investigate the behavior of hybrid columns subjected to axial, eccentric and cyclic loading. Hybrid Column is made of an inner tube and outer tube. Concrete is sandwiched between the tubes. Study deals with the comparative study between conventional column and hybrid columns of different shapes, different strength of concrete, radius of inner tube and hybrid FRP. Study includes the load carrying capacity, cyclic behavior, ductility and energy dissipation capacity of columns. Also study the behavior of columns under eccentric loading. This study is to investigate the best shape and material for inner and outer tube. Software used in the study is ANSYS 16.1

METHODOLOGY

A literature survey should be carried out by referring to and reading papers and journals published in the field of study in order to get detailed topic knowledge. Model selection and associated data gathering that will help in the completion of the task, such as validation model detail collecting, earthquake data collection, and so on, must be completed before the work can be completed. A software study is a vital phase in this project; one needs to complete a software study in order to get familiar with the software tools. This aids in the elimination of any and all potential mistakes that may arise throughout the modeling and analysis process. The validation model is being modelled. Validation is accomplished by the use of an RCC column with FRP wrap. The validation process is a critical step in ensuring the accuracy of the final product. It also helps in the evaluation of the product quality received from the software application. Validation should be carried out using the same software as that which is being used to prepare the project study.

The validation process is a critical step in ensuring the accuracy of the final product. It also helps in the evaluation of the product quality received from the software application. Validation should be carried out using the same software as that which is being used to prepare the project study.

Ram Prasanna Kumar Reddy and Mohammed Bilal Badr

SOFTWARE STUDY

ANSYS, Inc. is a publicly traded American corporation with its headquarters in Canonsburg, Pennsylvania. It is a software company that produces and offers engineering simulation software. Designing items and semiconductors using ANSYS software is also possible. The program me may also be used to build simulations that test a product's durability, temperature distribution, fluid motions, and electromagnetic characteristics.

STEPS INVOLVED IN STRUCTURALANALYSIS

The following steps are involved in this structural analysis.

➢ Creation of geometry
➢ Real constants and cross-section properties
➢ Selection of element type
➢ Meshing
➢ Selection of material and properties
➢ Define loads and displacements
➢ Reviewing results
➢ Verify of results

VALIDATIONS

DETAILS OF THE STRUCTUREANALYSED

The column selected for validating the software is 150x150x1000 mm size. The column has eight numbers of 10 mm diameter bars. FRP wrap is provided outside the column. 10 mm clear cover is provided. Lateral ties provided only in two positions. Pushover analysis was performed. Aramid FRP is used for providing outside wrap to the column. Modelling and Analysis were done using ANSYS16.1.

Finite element modeling was done for the RC column with FRP wrap outside. Pushover analysis was performed on this column. And the results were obtained as follows (Table 1). The deformation of the RC column with FRP wrap outside is shown in Figure 3. Buckling is characterized by a sudden sideways deflection of a structural member.
The column shows signs of worldwide buckling. According to the pushover study of the RCC column with FRP wrap performed in ANSYS 16.1, the column has a load carrying capacity of 61.382 k N, which is higher than the load carrying capability found in the validated journal, which was 58 k N. It seems that the result acquired from ANSYS is comparable to the result obtained from the validating journal. The experiment's outcome is only 5.83 percent accurate.

MODELLING AND ANALYSIS

Over the last two decades, the use of fibre-reinforced polymer (FRP) reinforcement to strengthen structures has grown more prevalent. Hybrid FRP-concrete-steel columns are a new kind of hybrid column that is being developed. According to the findings of this investigation, the hybrid column outperformed the traditional RCC column with a FRP wrap. The form of the inner and outer tubes, the presence of high-strength concrete inside and outside the inner tube, the radius of the inner tube, and the presence of a hybrid FRP tube were all considered in the analysis of the hybrid column.

The column was exposed to axial, eccentric, and cyclic loads throughout the experiment. The material characteristics of concrete, steel, and fiber-reinforced plastic (FRP) are listed in Table 2. The element type 20 noded solid 186 is used in the FE modeling process. It is a non-linear analysis, as the name implies. The concrete has multilinear characteristics. Bilinearity is a feature of both fibreglass reinforced plastic and steel.

Axial Loading

Different shaped columns are modeled and analysed under axial loading condition. Different material used for inside and outside tubes. Outside FRP and inside steel tubes and vice versa. The dimension of inner and outer tube is

taken in accordance with the dimension of conventional column and steel table. he compression or tension force exerted on a member is referred to as the axial force. When an axial force is applied via the centroid of a part, this is referred to as concentric loading. Eccentric loading is a term used to describe when a force does not act via the centroid. Due to the fact that the load is located a distance away from the centroid, eccentric loading results in the generation of a moment in a beam.

Eccentric Loading

An eccentric load is applied to the model that performs the best out of all the models. Upon completion of the study, it can be determined that the outer square FRP and inner circular steel column models are the best when applying axial load (later discussed in chapter 6). Eccentricity is measured at 10%, 25%, 50%, and 90% deviations from the centre of the column. Biaxial loading is applied to a column when the eccentricity is measured in both directions (with respect to both axes) of the column (x and y axes). Figure 5.depicts this, with the eccentrically laden column at 90% eccentricity. The inner tube is reinforced with high-strength concrete both inside and outside. The outside tube is made of fibreglass, while the inner tube is made of steel. Steel fibres are added to M40 concrete to provide it with high strength, which amounts to 1.5 percent. M65 concrete is considered to have normal strength. HSC has a yield strength of 43543.08 N/mm2 and is a high-strength material.

Cyclic Loading

Cyclic loading is described as the continuous and repetitive application of a load (fluctuating stresses, strains, pressures, tensions, and so on) on a material or on a structural component that causes deterioration of the material and eventually leads to fatigue of the material or structural component. Cyclic loading causes materials to degrade as a result of fatigue, and this occurs at lower loads and for a shorter period of time than would be anticipated otherwise. Cyclic loading is applied to the columns which have best shape. As per FEMA350 cyclic protocol cyclic loading is given at 0.375%, 0.75%, 1%, 2%, 3%, 4%, 5%, 6%, 8% and 10% drift ratio.

RESULTS AND DISCUSSIONS

PERFORMANCE OF DIFFERENT SHAPEDCOLUMNS

Results from the analysis discussed in this chapter, Maximum load and deflections of each column is discussed here. Also, ductility, deformation of columns, energy dissipation capacity are discussed. Ductility of column calculated from load deformation From this it can conclude that Load carrying capacity is more for outer square FRP and inner circular steel column. It has minimum deflection. Eccentric loading is applied on this column and the results are given in Table4.

Ductility of conventional column = 1.52
Ductility of hybrid column=2.64
Outer square FRP inner circular steel column is more ductile than conventional column. From the hysteresis curve it is clear that outer square FRP inner circular steel column has more ductile behavior. For further parametric study the outer square FRP inner circular steel hybrid column is considered.

PERFORMANCE OF HYBRID COLUMN FILLED WITHHSC

Load and deformation of hybrid columns filled with HSC inside the tube and outside the tube are given in Table 5. HSC in outer tube take more load. The deformation of two models is almost same. For better results, the HSC should give outside the inner tube. Ductility of Column =2.71 At 10% drift the specimen reaches a load value of 200 kN and displacement of 40mm. When unloading the column, it reaches to 15 mm. Its strain recovery is 62.5%. Energy dissipation capacity is good for the column. The column is ductile in nature. It is very clear from the hysteresis curve.

PERFORMANCE OF COLUMN WITH M A X I M U M INNER TUBE RADIUS

Radius of inner tube increases, the confinement increases. As a result, load carrying capacity increased with minimum deformation.

Load   = 6795.6 kN
Deformation   = 22.025mm

From Figure 9 it is clear that the column is subjected to global buckling. It is due to the presence of FRP and steel. Concrete takes the load and fails. Then the load transferred to the steel tube. Steel tube has yielding property. The buckling is occur due to the yielding of steel. FRP enhance the tensile property. There is no crushing due to the presence of FRP. Maximum deformation occurs at outer tube.

Load and deflection of Hybrid column with maximum inner tube radius when subjected to eccentric load is given in Table 6. Ductility is also improved for this column. It is due to the presence of steel tube and FRP tube. FRP enhance the tensile property. From hysteresis curve it can be concluded that the column ductile in nature.

Ductility of the column   =2.72

At 10% drift the specimen reaches a load value of 900 kN and displacement of 100mm. When unloading the column, it reaches to 55 mm. Its strain recovery is 45%. The column has better energy dissipation capacity.

PERFORMANCE OF COLUMNS MADE OF HYBRID FRP TUBE

Carbon and Aramid FRP used for making hybrid FRP tube. Load and deflection of the model subjected to axial loading is given below.

Load   = 7489.3 kN
Deformation= 20.019mm

Maximum deformation occurs on outer FRP tube. The column subjected to global buckling. Load deformation curve for hybrid FRP tube column is given in Figure 6.13. AFRP and CFRP are high strength fibers. They have high modulus of elasticity. Therefore, the column take more load with minimum deflection. Among all models, hybrid column with outer hybrid FRP tube has better performance.

Ductility of the column   =2.58

Ductility of the column is more than conventional one. The use of FRP enhance the ductility.

At 10% drift the specimen reaches a load value of 400 kN and displacement of 80mm. When unloading the column it reaches to 20 mm. Its strain recovery is 75%. The column with hybrid FRP tube have better energy dissipation capacity. It has more strain recovery capacity than all models. It shows good hysteresis behavior and it possess better ductility.

CONCLUSION

Performance of Hybrid Column Subjected to Axial, Eccentric and Cyclic Loading has been conducted in this study. Analytical study is performed in ANSYS 16.1 software. Nonlinear analysis is performed to understand the behavior of the hybrid column under various parameters. Four different parameters are considered in this study. Shape of inner tube and outer tube, High Strength Concrete (HSC) in inner tube and outer tube, Radius of inner tube and Hybrid FRP tube. Load deformation capacity, ductility, failure type, energy dissipation capacity were analysed in this study. The results were compared with conventional column which has a FRP wrap outside the column.

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➤ From the parametric study it is clear that, best shape of hybrid column which has an outer tube and inner tube with sand witched concrete is outer square and inner circular. It has more load carrying capacity than conventional column. It shows good hysteris is behaviour and ductility.

➤ When HSC is filled in inner steel tube and HSC filled in Outer FRP tube, the two models shows increase in load carrying capacity. Hybrid column with HSC in outer tube shows more load carrying capacity than HSC in inner tube. The HSC mixes typically have an increased modulus of elasticity, which increase the stability and reduces deflections. HSC increases the load carrying capacity by 2.64% than above model. It has good ductility and hysteris is behaviour.

➤ When radius of inner tube is in cresed to maximum, the confinement of the concrete increased which results into better load carrying capacity. It shows 2.86% more load carrying capacity than hybrid column with outer square FRP inner circular steel tube.

➤ Use of Hybrid FRP outer tube enhance the load carrying capacity and shows good hysteris is behaviourr and ductility. AFRP and CFRP are high strength fibers. AFRP has high modulus of elasticity and high abrasion resistance. CFRP also enhance the shear strength. This model has very good load carrying capacity and better strain recovery than all models.

➤ Confinement provided by the FRP tube also improve the axial load-carrying capacity

➤ Ductility of all proposed models will have almost same value. Ductility of proposed columns is greater than conventional column. The ductile behaviour is due to the presence of FRP tube.

➤ When column subjected to axial load, it shows good load carrying capacity. And also for eccentric load it exhibit good characteristics. Among them Hybrid Column with Hybrid FRP tube has better performance.

➤ Applying Cyclic load to these columns, all specimens show good hysteresis behaviour and delivered a similar trend of energy dissipation capacity. In case of strain recovery, the specimen with hybrid FRP outer tube has more strain recovery.

➤ From this study, a best model can be predict. Outer square hybrid FRP tube and inner circular steel with maximum radius hybrid column encased with HSC.

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Table 1. Results of Finite Element Analysis

LOAD CARRYING CAPACITY		
Actual Value (from journal) (kN)	**Obtained Value (from ANSYS) (kN)**	**Error(%)**
58	61.382	5.83

Table 2. Properties fibers

Fiber	Nominal thickness(mm)	Tensile strength (MPa)	Ultimate Tensile Strain	Elastic modulus (GPa)
Carbon	0.117	3626	1.44	251
Aramid	0.200	2663	2.12	125.7

Table 3. Load and Deformation

MATERIAL	SHAPE	LOAD (kN)	DEFORMATION (mm)
OUTER FRP INNER STEEL	Both square	5154.5	21.16
	Out square in circle	6606.3	21.02
	Out circle in square	5329.2	21.59
	Both circle	5240	17.95
OUTER STEEL INNER FRP	Both square	2873.2	54.44
	Out square in circle	4103.4	22.16
	Out circle in square	3501.6	19.41
	Both circle	3490	19.40

Table 4. Load and deformation when subjected to eccentric loading

Eccentricity (%)	10	25	50	90
Load (kN)	2837	2525	2172	1123
Deformation(mm)	72.58	77.01	91.96	135.73

Table 5. Load and Deformation

	LOAD (kN)	Deformation (mm)
HSC in inner tube	6663.4	21.023
HSC in outer tube	6780.7	21.024

Table 6. Load and Deformation of Hybrid Column with Maximum Inner Tube Radius Subjected to Eccentric Loading

Eccentricity (%)	10	25	50	90
Load (kN)	3154.5	2868.5	2469.9	2056.6
Deformation(mm)	69.56	75.96	104.95	120.24

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Table 7. Load and Deformation of Hybrid Column with Hybrid FRP Outer Tube Subjected to Eccentric Loading

| Eccentricity (%)	10	25	50	90
Load (kN)	4584.5	4183.1	3687.9	2879.4
Deformation(mm)	65.28	72.78	102.91	118.7
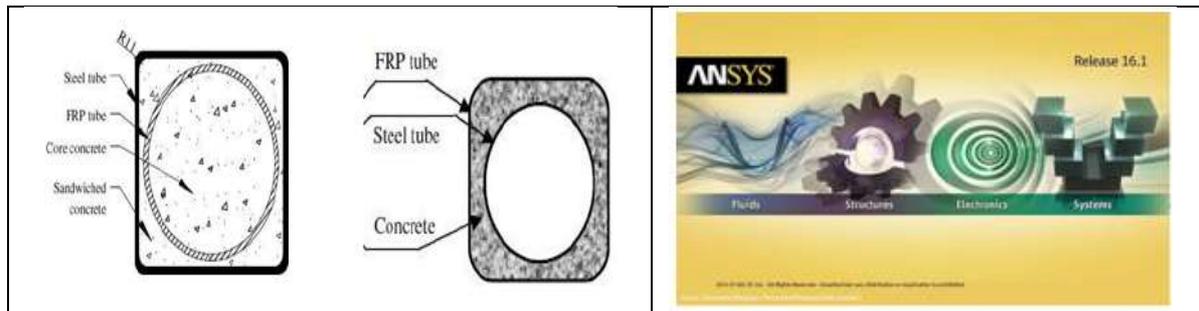
Figure 1. Cross-section layout of SCFC

**Figure 2. ANSYS Software window**
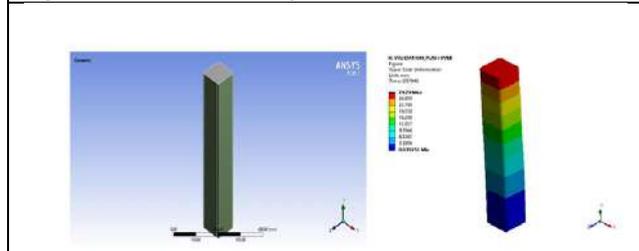
**Figure 3. Isometric view of the model with Total Deformation**
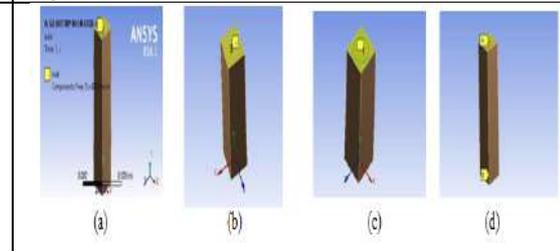
Figure 4. axial loadings (a) Inner and outer tube (b) HSC in column (c) Radius of Inner Tubes (d) Hybrid FRP tubes
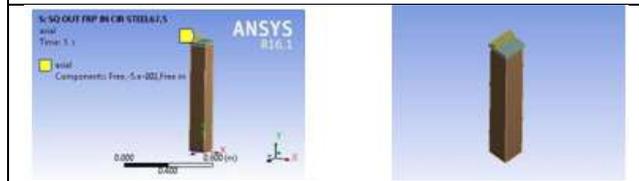

Figure 5.Eccentric loading on column at 90% and 50% eccentricity
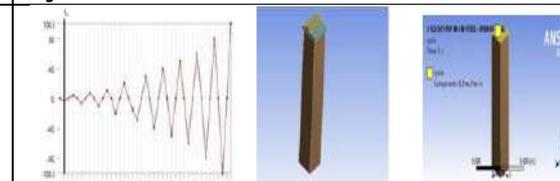
Figure 6. (a) Inner and outer tube (b) Radius of Inner Tubes (c) Hybrid FRP tubes
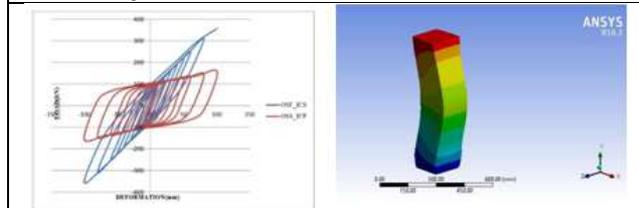

**Figure 7. (a) Hysteresis loop for outer square inner circular columns (b)Deformation of column**
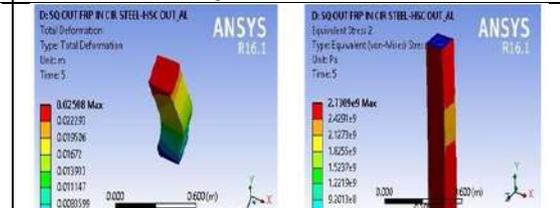
Figure 8. (a) Deformation of Hybrid Column with HSC in Outside Tube (b) Stress on Hybrid Column

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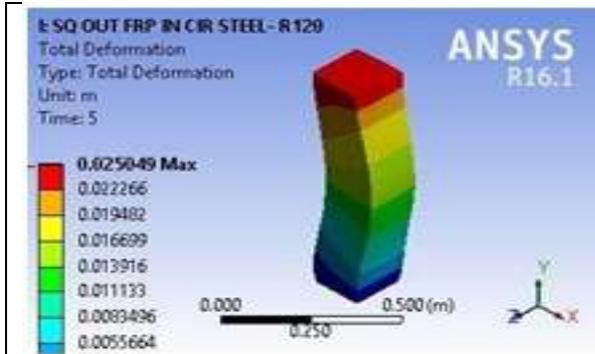
|	
Figure 9. Deformation of column	**Figure 10. Deformation of column**
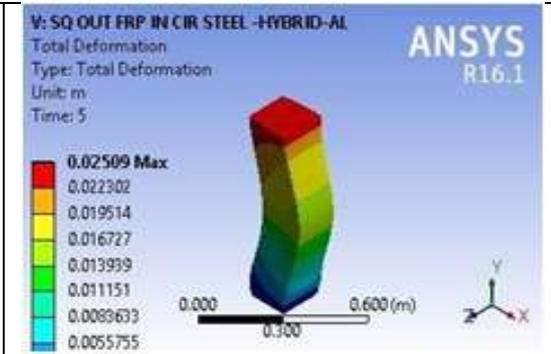
RESEARCH ARTICLE

A Solution for Single – Objective Vehicle Routing Problem with Time Windows using Genetic Algorithms

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ABSTRACT

The Vehicle Routing Problem with Time Windows (VRPTW) is the creation of the least expensive routes between one depot and a group of geographically dispersed depots. Products must be delivered to each consumer within a specific time frame, according to established requests, and at the lowest possible cost and distance. This paper's goal is to cut down on overall journey time. In this instance, a collection of well-known benchmark issues is used to evaluate the effectiveness of the solution. The suggested result offers the best possible solutions when compared to the best solutions discovered, and it also performs better than another heuristic that is utilized in comparison.

Keywords: Vehicle Routing Problem with Time Windows (VRPTW), heuristics, genetic algorithms.

INTRODUCTION

Simon first presented the Vehicle Routing Problem with Time Windows (VRPTW) in 1987[9]. It entails the distribution of a group of constrained-capacity vehicles from a centralized depot to a group of geographically scattered clients with known requirements and predetermined time windows. In VRPTW, the goal is to service a large number of clients within set time intervals at the lowest possible cost while still adhering to each vehicle's capacity and overall trip time restrictions. Given that VRPTW falls under the category of NP-hard combinatorial problems [6], which calls for significant to huge Problems. The primary goals must establish the bare minimum of

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vehicles, expenditures, or trip times on the travelled routes. In VRP, the routes satisfy the following three set of constraints.

(i) Each customer visits exactly once
(ii) All routes begin and end at the depot.
(iii) The total amount of demands along a route shouldn't be greater than the vehicle's capability.
Therefore, a new
VRPTW issue statement will be provided by including a time windows restriction in the VRP problem statement. In essence, the time frame sets a restriction on how often the customers may be visited. This means that each stop the vehicles make will have a start time and an end time. The total of the waiting, travel, and service periods is referred to as each vehicle's route time. When a vehicle arrives at a client,

 (i) Before the earliest start of service time, waiting time will occur.
(ii) After the latest start of service time, tardiness time will occur.
(iii) After the maximum route time, over time will occur.

But it is noted that total customer demand in each route cannot exceed the capacity of the vehicle. The simple example for the solution routing problem is shown in the following figure. Three routes have been produced to a specific vehicle routing problem in the illustration. Except for the start and end stops (depot), it is clear that each route has three stops in total. The route in the top right corner also has the most distance travelled, and its circumference is probably the longest in comparison to the other routes. An order for goods to be transported from a depot to clients at various places is shown in the issue statement in Fig. 1. The issue that needs to be resolved is how to organise them into 3 vehicles. The operation costs can be optimised by ensuring that the total distance and time travelled are kept to a minimum.   The remainder of this essay is structured as follows. The details of how the literature examined the pertinent algorithms and a solution to the VRPTW issue statement are reviewed in Section 2. The approach to solving the desired problem is presented in Section 3 of the study. The genetic algorithm for single-objective VRPTW is thoroughly detailed in section 4. The suggested work's outcome and analysis are reported in section 5. Conclusion is provided in Section 6, and prospects for future employment are recommended in Section 7.

Literature Review

A well-known and challenging combinatorial problem with particular economic implications is the VRPTW [1]. As it involves providing services to consumers with time windows while employing several vehicles, VRPTW is also more complicated than the travelling salesman problem (TSP) [1]. Although the computational time needed to solve the VRPTW optimally is prohibitive for large problems, the optimal solutions to the problem can be obtained utilizing precise approaches [2]. Heuristic techniques are so frequently utilized to solve optimal solutions quickly. Solomon [9] suggested an insertion for a heuristic algorithm after first referring the time frame constraint to vehicle routing and got satisfactory results. Using the insertion heuristic method and the Tabu search algorithm, Garcia et al. [4] created an initial solution for VRPTW. The ant colony algorithm was suggested by Gamberdella et.al [3] to determine the answer to VRPTW. The trip distance and the number of cars are optimized using a hybrid heuristic approach created by Ombuki et al. [8] that is based on the genetic algorithm (GA) and Tabu search (TS).He and Li [5] used the tabu search method to find a solution to the routing optimization problem with the aim of minimizing fuel consumption utilizing a time window and a heterogeneous fleet.

METHODOLOGY: MATHEMATICAL NOTATION

A group of comparable vehicles, a central depot node, a group of customer nodes, and a network that connects the depot and consumers make up the restrictions of the Vehicle Routing Problem with Time Windows (TRPTW). K automobiles and N+ 1 customers are present. Depot node is represented by Customer O. Each route in the network represents the link between two nodes and also displays the direction in which it is travelling. Each route begins at

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the depot, according to the model. In the network, there are an equal number of routes and cars, allowing each route to have its own vehicle. Each route of the network has a cost c_{ij} and a travel time t_{ij} (ij in sub script) in particular. In this section, the notation of the parameters using in the model and the decision variables are explained. There are three types of principal decision variables in VRPTW. The principle variable $x_{ijk}=1$, if vehicle K travels from customer i to j where $i,j \in \{0,1,2,\ldots\ldots N\}$; $k \in \{1,2,\ldots\ldots k\}$; $i \neq j$ } and O otherwise , Then the decision variable named as T_i denoted the time when a vehicle arrives the depot (at the customer) and w_i represents the waiting time at node i. The basic goal is to construct a network that complies with all restrictions. However, the network should also endeavour to keep travel expenses to a minimum. The goal is to reduce the overall amount of time that all the vehicles travel while doing their tours.

Principal Decision Variables

T_i: Arrival time at customer i.
W_i: Waiting time after departure at customer i.
x_{ijk}:the indicator if the customers i & j follow
each other in a route served by vehicle k.
$x_{ijk} \in \{0,1\}$,0 if there is no arc from node i
to node j and 1 otherwise
where $i \neq j$; $i,j \in \{0,1,2\ldots N\}$

Problem Parameters

K : number of vehicles
N : number of customers
C_{ij}: Cost incurred on arc from customer i to j.
t_{ij}: travel time between customer i and j.
m_i: demand of customer i, i=2…N.
q_k: Capacity of each vehicle K
e_i: earliest arrival time at customer i.
l_i: latest arrival time at customer i.
f_i: service time at customer i.
r_k: maximum travel time of each vehicle.

Objective Function

The objective function of the VRPTW is brought up as a single – objective function can be written in terms of the decision variables x_{ijk} as:

$$\text{minimize} \sum_{i=0}^{N} \sum_{\substack{j=0 \\ j \neq i}}^{N} \sum_{k=1}^{K} c_{ij} x_{ijk}$$

subject to

$$\sum_{k=1}^{k} \sum_{j=1}^{N} x_{ijk} \leq k, \text{ for i=0} \ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

$$\sum_{j=1}^{N} x_{ijk}=1 \text{ for i=0 and } k \in \{1,\ldots k\} \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

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$$\sum_{j=1}^{N} x_{jik}=1 \text{ for } i=0 \text{ and } k \in \{1, \ldots, k\} \ldots\ldots\ldots\ldots\ldots(3)$$

$$\sum_{k=1}^{K} \sum_{j=0\,j \neq i}^{N} x_{ijk}=1, \text{ for } i \in \{1, \ldots.N\}\ldots\ldots\ldots\ldots.......(4)$$

$$\sum_{k=1}^{K} \sum_{j=0\,,\,i \neq j}^{N} x_{ijk}=1, \text{ for } j \in \{1,\ldots N\} \ldots\ldots\ldots\ldots.....(5)$$

$$\sum_{i=1}^{N} m_i \sum_{j=0\,j \neq i}^{N} x_{ijk} \leq q_k, \text{ for } k \in \{1,\ldots k\}\ldots\ldots\ldots\ldots..(6)$$

$$\sum_{i=0}^{N} \sum_{j=0\,,\,j \neq i}^{N} x_{ijk}(t_{ij}+f_i+w_i) \leq r_k, \text{ for } k \in \{1, \ldots, k\}\ldots\ldots..(7)$$

$$T_0=W_0=f_0=0\ldots\ldots\ldots\ldots\ldots...............................(8)$$

$$\sum_{k=1}^{K} \sum_{i=0\,,\,i \neq j}^{N} x_{ijk}(T_i+t_{ij}+f_iw_i) \leq T_j, \text{ for } k \in \{1, \ldots, k\}\ldots(9)$$

$$e_i \leq (T_i+w_i) \leq l_i \text{ for } i \in \{1, \ldots, N\} \ldots\ldots\ldots\ldots\ldots\ldots...(10)$$

The above 10 constraint sets proves accordingly. Equation (1) assures that the number of tours is k selected at most k outgoing routes from the depot i= 0. The constraint set 2 ensures that exactly one departure route from the depot is selected for each vehicle. Element (3), which shows that each truck has exactly one route entering in the client node with respect to the depot (i=o), supports the same claim. The two constraint sets (2) and (3) together demonstrate that a full tour for each vehicle is unmistakably guaranteed. Equation (4) ensures that there is only one path from each node i to each vehicle accessing it. The fifth constraint set guarantees that each node has a single path for each vehicle that enters it. Taking this into consideration, constraint sets (4) and (5) state that each vehicle only visits each node once. Equation (6) demonstrates that for each vehicle, the total load (demand) assigned to it is either less than or equal to its capacity. Equation (7) ensures that each vehicle's total travel time along its route is either less than or equal to the maximum route time allotted to it. Each vehicle's arrival time, waiting time, and service time at the depot are all set to zero by the constraint set (8). According to equation (9), it is guaranteed that each vehicle will arrive at the node j earlier than the time allotted for getting there. Finally, the constraint set (10) ensures that the sum of arrival as well as the waiting time of each vehicle at each node j is more than or equal to the earliest arrival time (e_i) at that node, i=1,2,………N. Constraints sets from (8) to (10) define the time windows and the explained formulations clearly specify the desired feasible solutions for the VRPTW.

Genetic Algorithms

The problem with the VRP time window can be efficiently solved using a genetic algorithm. The study of evolutionary computing and artificial intelligence gave rise to the Genetic Algorithms (GA), a family of adaptive heuristics that is based on the Darwinian idea of evolution (survival of the fittest)[11].The initial population of chromosomes is what makes up GA. Each chromosome represents a state of the problem's resolution. Using objective function, potential parents can be chosen based on their fitness value. Vladimir and Travel [11] both provided the Roulette Wheel Selection (RWS) and tournament selection as two selection processes in their works. The potential parents would undergo a crossover operation in the following stage, which would add two new

chromosomes to the population. The logic of the crossover operators is programmed into the algorithm, and the selection method is also changed. The GA –based algorithm for the VRPTW can be summarized as follows. Construct the travel time using Euclidean distances set the parameters for GA which are the maximum number of iterations, the initial population size, the crossover rate (Pc) and the mutation rate (Pm) Generate an initial population. Evaluate the fitness function on the initial population members.

Repeat
Select parent Chromosome using roulette wheel solution. Generate random numbers for the crossover and mutation operators.

If random number for crossover > Pc
then
execute crossover
else
Execute mutation;

Evaluate fitness functions of offspring;
More offspring are produced in the initial solution. Compute the likelihood of selecting each chromosome. Until the allotted number of cycles has been used.

Result Analysis and Discussion

Typically, the new Solomon method is tested using well-known benchmarking issues with well-known properties [9]. Although several writers have given examples, Solomon's VRPTW examples are the most frequently used in experiments for comparison. Location demand, time windows, and service period are some examples. There are three categories identified by the instances: clustered (C-type), random (R-type), and RC-type. There are 100 customer instances represented by Euclidean distance in Solomon's 56 VRPTW [9]. Later, there are 25 and 50 customers. Solomon's VRPTW benchmarking problems with R1&R2, C1&C2, and RC1&RC2 are used to test and experiment with the suggested algorithm, and a total of 56 examples are included[10]. Each of the 56 instances has a single depot and a customer node count of 25, 50, or 100. The solutions have thus been contrasted with those obtained by Manisri et al. (2009)[7] and the single –objective function for total travel time is illustrated in the table 1. The result of the proposed algorithm provides the effectiveness and better solutions than previously published.

CONCLUSION

Vehicle Scheduling issue through efficient customer service delivery, Time Window plays a vital role for productivity enhancement enterprises. This study uses genetic algorithms in an effort to reduce total journey time as a single goal function (GA). In conclusion, the suggested GA offers solutions that are both superior to those that have already been published and competitive in terms of results. Genetic algorithms are effective at minimizing the overall journey time. As the year progresses, the solutions to the issue statement that now exist will undoubtedly get better.

Future Work
As bi-objective functions, there is still considerable work to be done. Future research may therefore concentrate on creating effective hybrid approaches by fusing two or more objective functions to make the problem more difficult to answer with extra real-world constraints from the real-world company that necessitates solving such problems.

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Table 1: The total travel time for 25,50 and100 customer nodes using GA

Problem	Result of GA	Number of Customers			
		25	50	100	All
R1	TT	433.46	702.84	1326.92	821.07
R2	TT	470.54	702.69	1227.61	800.25
C1	TT	252.33	481.91	1563.79	766.01
C2	TT	279.88	520.31	1452.96	751.05
RC1	TT	354.28	711.19	1595.80	887.09
RC2	TT	432.14	739.89	463.30	878.44
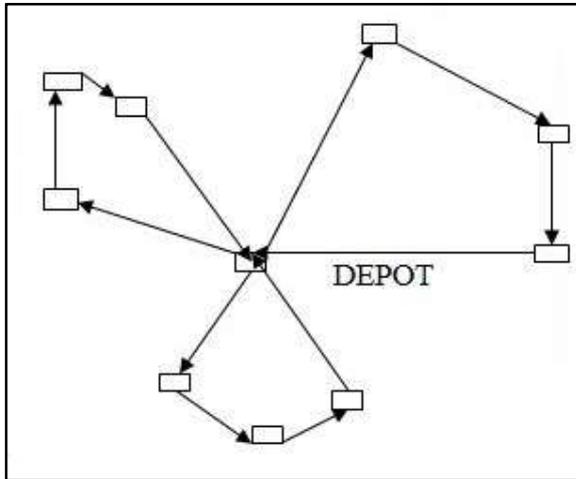
Fig.1 An example for the solution of Vehicle Routing Problem

RESEARCH ARTICLE

Analysis into Skeletal Relationship and Muscle Timing Activity

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ABSTRACT

The aim of this study was to analyze the skeletal relationship and muscle timing activity.A retrospective evaluation was conducted to find out subjects who presented between June 2019- March 2020 at the University Dental Hospital for EMG analysis. For the included subjects, data regarding demographic factors, skeletal relationship, muscle timing activity were extracted. Pearson Chi square test was done to investigate the association of the muscle timing activity to skeletal relationship, age and gender .The results of the study revealed no statistical significance between the muscle timing activity and skeletal relationship. However there is an increased number of the muscle timing activity pattern M(L)-T(R)-M(R)-T(L) in class I skeletal relationship heterogenecity in muscle timing was observed. In the present study, the interdependence between facial morphology and the activity of masticatory muscles was revealed in patients but not statistically significant. More homogenicity in muscle timing was not present between different skeletal relationship.

Keywords: Retrospective, muscle timing activity, skeletal relation, EMG, Invention

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INTRODUCTION

The musculoskeletal system has been determined to play an extensive role in the locomotor function of the body. The force required to provoke the dynamic motion and posture is generated by the excitation of the central nervous system on the skeletal joints. This force provides the joint with the integral summation needed for individual muscle activation [1]. Over the years, the question whether the activity of facial and masticatory muscles has any effect on facial morphology has arisen for understanding of the normal growth and also of morphological abnormalities. If muscle function has any substantial effect, abnormal muscle function may explain certain extreme abnormalities of facial morphology and certain forms of occlusion [2]. One way to study the relation between muscle function and facial morphology is to relate morphology to the electromyographic activity in the facial and masticatory musculature as a measure of the force exerted by the muscle which may perhaps affect the development of the facial skeleton [3]. To date, the electrical response from muscle contraction has been recorded using electromyography (EMG). The Electromyography (EMG) signal is a biomedical signal that measures electrical currents generated in muscles during its contraction representing neuromuscular activities [4]. The electromyography (EMG) has revealed details of the timing and magnitude of muscle activation for many muscles powering a variety of types of locomotion. An integrated analysis of EMG, kinematic, and anatomical information can provide an accurate picture of the function of individual muscles during movement [5]. Most of our understanding of the role of individual muscles in movement is founded on such analysis. Electromyography is unarguably the most commonly used tool for investigating muscle function during locomotion. [6,7]. Since masticatory muscle function has a considerable influence on craniofacial morphology, the evaluation of masticatory muscle activities may be useful in improving orthodontic treatment and in determining a correct retention period after treatment.[8] [8] It may also be useful in order to establish treatment goals and procedures to be adopted in orofacial myofunctional therapy, which has proved to be effective in resolving the muscular and functional disorders frequently associated with an anterior open bite [9–12]. Though there are few previous studies which state that Asymmetric malocclusion can significantly modify jaw muscle activity and reduce muscular efficiency, which will return to normal after orthodontic correction, the available evidence regarding this is inadequate and unclear. The previous literatures also showed that there is considerable variation in the skeletal relationship and muscle timing activity in different populations [13–16] [14-18] With this background and in order to fill lacunae created by previous studies, this study aims to analyse the skeletal relationship and muscle timing activity and also aids in finding out if there is any specific common pattern in the muscle activation order in relation to skeletal relationship. Our team has extensive knowledge and research experience that has translate into high quality publications [17–25],[26–31],[32–36].

MATERIALS AND METHODS

A retrospective cross sectional study was conducted among the patients of University Dental Hospital. All the patients who visited the hospital for EMG Analysis between June 2019 to March 2021 were included in the study. After obtaining the ethical clearance form the institutional ethical committee board of University Dental Hospital, the list of all the subjects EMG analysis results were retrieved by reviewing the 86000 patient's records who have visited the hospital during the study period. The subjects for the study were included based on the inclusions and exclusion criteria. The inclusion criteria are 1) subjects with dental malocclusion referred for EMG analysis between June 2019 - May 2021 2) subjects whose records have complete details of all clinical examinations, orthodontic examinations. The exclusion criteria are 1) subjects with incomplete case sheets. All the case sheets were reviewed and for each patient with EMG analysis results we recorded the demographic variables (age , sex), skeletal relationship, muscle timing activity. Followed by the clinical exploration by the photographs and EMG analysis results available in the image gallery. Cross verification was done by another examiner reviewing the analysis to reduce the data errors available in the case sheet. The main advantage of such data collection is that data is readily available. Data collected were entered into a spreadsheet and analysed subsequently using the Statistical software SPSS (Version 20). Frequency

and percentage were calculated from the study variable. Descriptive statistics was used to summarise the variables in the data set. Chi square test was employed to test the association of muscle timing activity in relation to skeletal relationship, age and sex with the level of significance set at p<0.05.

RESULTS AND DISCUSSION

A total of 15 patients aged between 10-25 years of age were done EMG analysis at the University Dental hospital between study periods as mentioned above. Among these 9 (60%) were females and 6 (40%) were males (Figure1)

With regards to age, 5 (33.3%) patients belonged to the age group of 10-15 years; 7 (46.7%) with the age group of 16-20 years; 3 (20%) with the age group of 21-25 years. (Figure 2) It was noted from the results, that of 15, 9 (60%) had class I skeletal relationship; 5 (33%) had class II Div 1 skeletal relationship; 1 (6.7%) had Class III skeletal relationship (Figure 3).

Figures and Legends

The present study was conducted to analyse the relationship between skeletal relationship and muscle timing activity. The muscles of the maxilla and mandible would seem to be of paramount importance in the aetiology and active treatment of malocclusions and jaw deformities, and also for the stability of such therapies. [37] EMG is a useful tool for diagnosis and for the determination of treatment outcomes [38] and therefore was the method chosen in the present study to determine the activity of the temporal and masseter muscle of subjects. EMG activity has been investigated in subjects with different patterns of skeletal relationship and different types of malocclusion. Direct comparison of the present study with previous studies was limited because none of the previous researchers analysed the muscle timing activity and the skeletal relationship. Very few studies conducted previously showed a significant relationship between masticatory muscle activity and cephalometric variables. [39,40] However, in this study, no significant relationship (p>0.001) has been found between the skeletal relationship and muscle timing activity order and there is no specific pattern of muscle activation noted in patients with the same skeletal relationship. The reason for this variability being Masticatory muscle activity pattern was found to be different or highly associated with respect to the age, malocclusion type, and type and stage of orthodontic treatment. From the results it is also evident that females were the most common gender who reported to the hospital for malocclusion than the males. The reason for this is, females being more concerned about their oral health and their appearance; hence they appeared to be better motivated to demand for oral health care and were found to attend dental services more often for various treatments than males who visit their dental professionals only when it is absolutely necessary. Similar results were seen in the study conducted by a previous researcher who reported more females with malocclusion than males. [41] This study also reveals that 16-20 years is the most common age group to visit the hospital for the treatment of malocclusion; the reason for this is the fact that all permanent teeth in the oral cavity would have been erupted and is the right period for the orthodontic treatment to be initiated. [42]The strength of this study was that the records of patients seen during the period of study were used; therefore there was no issue regarding declined participation from patients and in addition there was no issue regarding improper patient selection. The weakness of the study being a retrospective study, there was no possibility for direct interactions and examinations of the patients and the study relied only on the case sheets and also the sample size was limited and confined to only the South Indian population. However this study will shed light for future studies that study an even larger population. Continued advancements in the field of research can lead to the implementation of correct orthodontic treatment strategies.

CONCLUSION

The results of the study revealed that skeletal morphology (Class 1 & II) and the activity of masticatory muscles in patients have some interdependence, the study showed maseter muscle to be active during functioning but the homogeneity in the muscle timing sequence could not be established. Further studies can be performed in the

direction of muscle activity in younger age groups undergoing functional appliance or other intervention and its relevance.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

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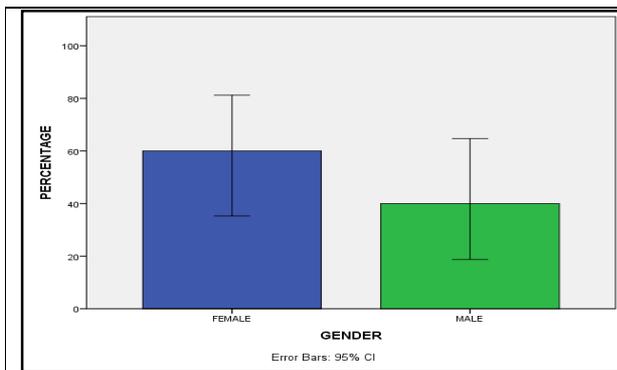
Figure 1 Image representing the frequency distribution of males and females who underwent EMG analysis. X-axis shows the gender while Y-axis shows the no. of children who underwent EMG analysis. Higher number of females had underwent EMG analysis than males.
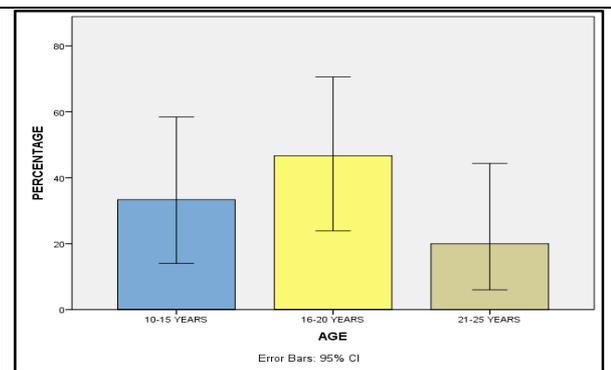
Figure 2 Image representing the frequency distribution of the different age groups that underwent EMG analysis. X-axis shows the age groups while Y-axis shows the no. of patients who underwent EMG analysis. Higher number of patients in the age group of 16-20 years had underwent EMG analysis.
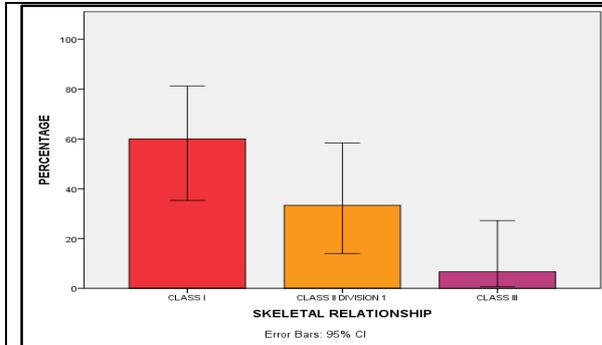
Figure 3 Image representing the frequency distribution of the different skeletal relationship that underwent EMG analysis. X-axis shows the different skeletal relationship groups while Y-axis shows the no. of patients who underwent EMG analysis. Higher number of patients with class I skeletal relationship had underwent EMG analysis.
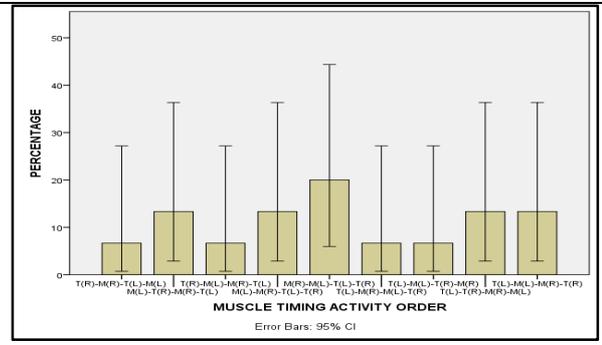
Figure 4 Image representing the frequency distribution of the different muscle timing activity order seen in EMG analysis. X-axis shows the different muscle timing activity order while Y-axis shows the no. of patients. Higher number of patients displayed the muscle activity pattern of M(R)-M(L)-T(L)-T(R).
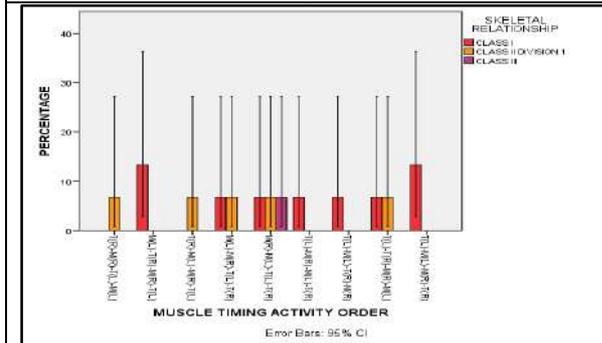
Figure 5: This graph represents the association of muscle timing activity order to the skeletal relationship present. X- axis represents the muscle timing activity order and Y- axis represents the skeletal relationship. Blue colour in the graph represents the Class I skeletal relation ; Green colour represents the Class II skeletal relationship; Grey colour represents the class III skeletal relationship. Correlation was found out using the Pearson Chi-Square test in SPSS software. It is evident from the graph that there is an increased number of the muscle timing activity pattern T(L)-M(L)-M(R-T(R), M(L)-T(R)—M(R)-T(L) in class I skeletal relationship. (but p=0.729; not statistically significant).
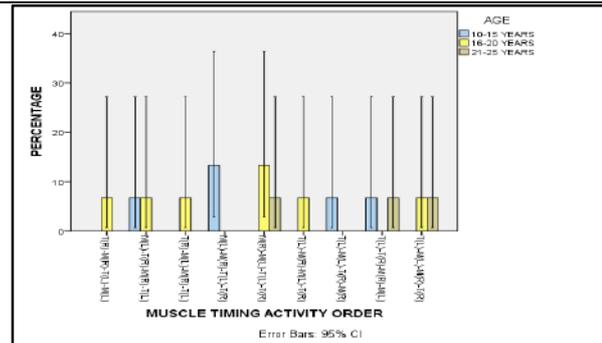
Figure 6: This graph represents the association of muscle timing activity order to the age group. X- axis represents the muscle timing activity order and Y- axis represents the different age groups. Blue colour in the graph represents the age group of 10-15 years; Green colour represents the age group of 16-20 years; Grey colour represents the age group of 21-25 years. Correlation was found out using the Pearson Chi-Square test in SPSS software. It is evident from the graph that there is an increased number of the muscle timing activity pattern M(L)-M(R)-T(L)-T(R) in the age group of 10-15 years, M(R)-M(L)-T(L)-T(R) in 16-20 years (but p=0.729; not statistically significant).
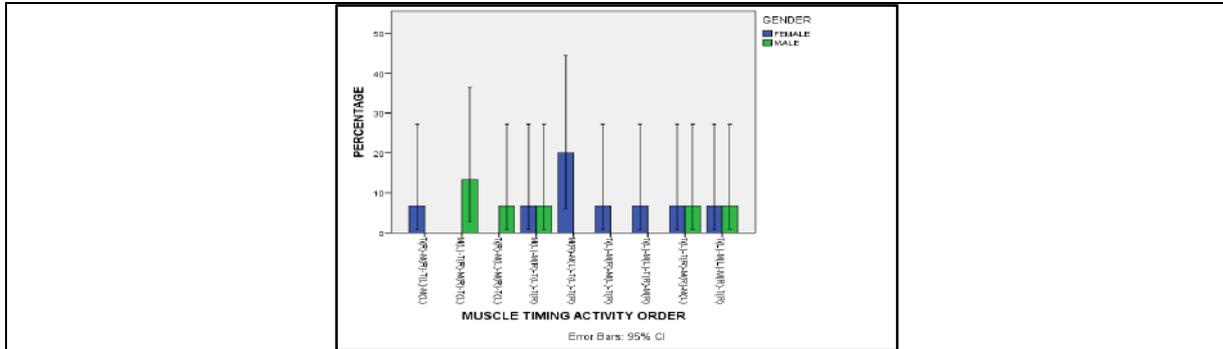
Figure 7: This graph represents the association of muscle timing activity order to the gender. X- axis represents the muscle timing activity order and Y- axis represents the gender. Blue colour in the graph represents the females ; Green colour represents the males. Correlation was found out using the Pearson Chi-Square test in SPSS software. It is evident from the graph that there is an increased number of the muscle timing activity pattern M(R)-M(L)-T(L)-T(R), in females. (but p=0.364; not statistically significant).

RESEARCH ARTICLE

Effectiveness of Story Map Technique on the Level of Comprehension Skills among School Children in SMC Palayam at Coimbatore

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ABSTRACT

The research study was "Effectiveness of story map technique on the level of comprehensive skills among school children in SMC palayam at Coimbatore. In order to evaluate the level of comprehensive skill among children by using story map technique children in SMC palayam, Coimbatore. Quasi experimental research design was adopted. Probability simple random sampling technique was used to selected 25 samples for the study purpose. A score key used to measure the level of knowledge of the children. The data thus collected and were analyzed by both descriptive and inferential statistics and interpretation were made which are based on the objectives of the study. According to the posttest assessment control group 7 (28 %) have inadequate knowledge, 18(72 %) have moderate knowledge. In experimental group 1 (4%) have moderate knowledge, 24 (96 %) of them have adequate knowledge. The calculated paired values of comprehensive skill among children were 1 which is significant at $p < 0.05$, which shows story map technique was effective to improving comprehensive skill. This initiates the acceptance of H1.

Keywords; story map technique, comprehensive skills

INTRODUCTION

Once upon a time a phrase that attracts children and adults to motivate the children to listen to the class. Telling a story is not useful for the children to understand the meaning of the story. If a person's goes to a new place he needs a map to guide him to reach the correct destination. Likewise if a teacher wants to tell a story to the children she needs a guide which makes the children to understand the setting, major characters, problem in the story, events, and the moral of the story. The story map technique is an effective technique in distinguishing significant and insignificant information in the story, directing students (making them focus on important components), providing active participation, transferring information into long term memory, activating foreknowledge, and predicting. By mapping the story, the students will record their thoughts and represent it to the organizer. It will help the students to comprehend the story because the students will focus on what they read. (Akyol, 1999)

Need for the study
The U.S department of education, reveals that one students in four children lacks basic level of reading skills. According to annual survey of education report in 2011 presents a data on learning levels of over 26,000 students cross 29 districts in Tamil Nadu, only about 32% of the students in class 1 could read a simple story in Tamil. To overcome the barrier, story maps are very useful for children to development their comprehension skills. Reading comprehension is the ability to construct and extract meaning from a written text. It is considered to be the most critical skill that is needed to succeed in school. (R. K. Vanathi)

Statement of problem
"A study to assess the effectiveness of story map technique on the level of comprehension skills among school children in selected commounity SMC palayam at coimbatore."

Objectives
► To find out the pretest and posttest level of comprehension skills among school children both in experimental and control group.
► Implement and evaluate the effectiveness of story map technique among the school children both control and experimental group.
► To find out the association between Level of comprehension skills among school children with their selected demographic variables.

Hypothesis
► H1 : Story map technique is effective in improving the comprehension skills among school children.
► H2 : There will be a significant association between the comprehensions skills score of school children with their selected demographic variables.

REVIEW OF LITERATURE

Berkeley (2017) mentioned in her study that almost 11% of learners in the USA public schools attend special education classes because of having specific learning disabilities such as having reading difficulty. Alagozlu, (2011). conduct a study on effectiveness of story map technique among elementary school students in Turkey. The result of this study showed that there was significant difference toward students' reading Comprehensive achievement of narrative text using story mapping technique. In the table of significant, it can be seen that on df=84 and in the degree of significant 0.05%, the value of degree of significance is 1.989. By comparing the value t0 was higher than t, that is 6.987>1.989. Thus, from the data analysis, it can be concluded that story mapping technique was effective and had effect toward students' retelling Comprehensive of narrative text at the fifth-grade students.

METHODOLOGY

Research approach
Quantitative approach was used for this study.

Research design
Quasi experimental research design.

Variables
Independent Variables
Story map technique among school age children.

Dependent Variables
Comprehensive skills of the school age children.

Influencing Variables
Age in Years, Gender, Religion, Parent's Education, Occupation, type of family, Income, Education of Children.

Setting of the study
The present study was undertaken in SMC palayam in Coimbatore.

Population
Children who are 5 to 10yrs.

Sample size
Sample size set of population selected by randomization.
The sample size used for the study was 50 school children's, 25 of each in
experimental and control group.

Sample techniques
Probability simple random sampling technique used.

Description of the tool
Section A
Description of Socio-Demographic Data
This section consists of 08 items Age in year, Religion, gender,
Parent's education, Types of family, Occupation of the parents, Education and
Income.

Section B
Modified Rubric Comprehension Skills Scale to Assess The Comprehension Skills.

Section B consists of modified rubric comprehension skills scale to assess
effectiveness of story map technique.

► Story map technique consists of six elements [Setting, Characters, Conflict (problem), the Plot (events), solution
and Summarizing] through these elements the questionnaires can prepared.
► All elements consists of ten question, each questions has a one marks. So total mark is 10.

45957

Scoring key (Table 1)
Funding By the investigator itself
Etical approval- Ethical committee of ppg nursing college

Major findings of the study
The mean pretest level of control group was 4.48, SD 0.88 and the mean posttest level of the control group were 4.56, SD 0.87. The paired 't' test value of the control group was 3.38* and the table value is <0.0025 and it is significant. The mean pretest level of experimental group was 4.16, SD 0.76 and the mean posttest level of the experimental group was 4.47, SD 0.89. The paired 't' test value of the experimental group was 5.33* and the table value is < 0.0001 and it is significant.

Comparison of the mean pretest score on the level of comprehensive skills in control group and experimental group. The pretest control group mean was 4.48, SD 0.88 and the pretest experimental group mean was 4.16, SD 0.76. Unpaired 't' test value is 1.33 and the table value is 0.1865 and it is found to be Significant.

CONCLUSION

The story map technique was found effective method to improve the level of Comprehensive skills among school children. The findings of this study provided evidence that the use of story map and story map questions was effective in improving the narrative story among school children

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Is Dearth of ERG Needs Articulate for Shortage of Rural CHC Specialists in Tamil Nādu – A Qualitative Study

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ABSTRACT

Deployment of health workforce in rural areas is critical to reach universal health coverage. India's rural public health system has lacked specialists for years. Health policymakers face a difficulty when doctors abandon rural placements after finishing PG. Tamil Nadu has 1 doctor per every 253 people and the highest WHO doctor-patient ratio (4:1000). Since 2015, Tamil Nadu's rural Community Health Centers(CHC) have been shortage more than 1,000 specialists. The study explored rural specialists' perceptions on their needs. In-depth interviews were performed with 12 rural community health center professionals from 4 districts in southern Tamil Nadu. Themes for assessment and analysis included are existential, relatedness, and growth needs (ERG needs) of the specialists as well as their reasons for intent to stay and turnover intention. Insufficient basic amenities, safety hazards, social isolation, and children's education are found to be the existence needs reasoned for leaving the rural placements. The relationship needs are highly cherished by the specialists, either with the working team or with the community. But exertion found in making friends in rural areas within the core group of physicians and family relationship is affected. Communal pride, promotion and altruism were ideal factors identified as growth needs to retain in rural areas.

Keywords: Specialists, Existential needs, Relatedness needs, Growth needs, Turnover intention, Staying intention.

INTRODUCTION

More than 40% of WHO member states have less than 10 doctors per 10000 people, and 26% have fewer than three. India has less than 10 physicians per 10000 people. (Strasser, 2017).Shortages of doctors and specialists, limited infrastructure, and rural-urban health access are the pitfalls. The health workforce aims to deliver enough skilled personnel at the correct moment. But in India, emergency specialists are few and it crippled the public healthcare system. "The number of broad specialists in internal medicine, general surgery is inadequate but can be a manageable proportion. However, the availability of specialists is much less."(Bajpai, 2014) "Lack of specialists is a genuine problem in many states due to an international transition of doctors to corporate hospitals," said T. Sundararaman, former executive director of the National Health Systems Resource Centre.(Kalyan Ray, 2022). The rural public health system lacks professionals who give the finest care in the country's metropolitan districts. For specialist treatment, rural population must travel far from their remote places.(Barik & Thorat, 2015)Although The specialist doctors at CHCs have increased from 3550 in 2005 to 4405 in 2021(Statistics, 2021), rural India suffers from a 68% shortage of specialist doctors like surgeons, pediatricians, obstetricians and gynecologists and physicians, according to the Centre's new rural health report, which depicts the skewed nature of healthcare services available at thousands of community healthcare centers (CHC) in the countryside.(Kalyan Ray, 2022) Tamil Nādu is in first place among 6 states, those are having a greater number of doctors than WHOs 1: 1000 guideline, The density of doctors per 1000 people in Tamil Nādu is as high as 4, (Nagarajan, 2018). Yet the specialists' shortfall figures are going beyond 1000 since the year 2015, Table 1 indicates a massive shortfall of specialists in CHCs. From 2016 the shortfall was on a decreasing trend, but very meagre doctors filled the vacancies. Currently, there is an exceptional high shortfall figure of 1289. The specialists position filled are only 251, despite the required number 1540.(Statistics, 2021) Table 1: Specialists' shortage in Tamil Nādu CHCs.

Even though very high shortfall percentage of specialists found in states of Gujrat, MP, Haryana, Chhattisgarh, but the number of people per doctor also high in these states. In Tamil Nādu, every 253 people, one doctor is available, however shortage of specialists' percentage is high. It indicates that high aversion of Tamil Nādu doctors towards serving in rural areas. (Nagarajan, 2018). NEET exacerbates future rural specialist shortages. The NEET has transformed the profile of MBBS students in Tamil Nadu. Before NEET, more entrants were rural, attended Tamil State Board schools, but Post-NEET, their share fell. More pupils from metropolitan English-medium schools following the CBSE curriculum were admitted. This shift in student demographics might lead to a scarcity of rural doctors and specialists.(Radhakrishnan, 2021) The reason for shortage of specialists is visualized contrarily by researchers and experts as follows:"For the government, the primary or secondary care at rural CHCs is not the priority. Also, there is hardly any push from agencies like WHO or UNICEF. But if there is a dedicated manager at the health ministry and the government is serious, then there are models to improve the CHCs," said Dileep Mavlankar, director, Indian Institute of Public Health, Gandhinagar. According to the observations of professionals, the government did not invest sufficient resources into CHCs and did not explore for novel techniques to entice and keep specialists working in rural areas. "Students who pay huge capitation fees in private medical colleges, would not join government service after passing out. They would not be able to pay their loans if they work for the government. They would look for ways to recover the money only not to work in rural areas" said Sundaraman. (Kalyan Ray, 2022) It is therefore essential to have a comprehensive knowledge of the needs of rural specialists to establish strategies to increase rural health system efficacy and aid in attracting them. Up until this point, there hasn't been any scholarly study in this subject from southern India, specifically from Tamil Nadu. This paper aims to fill this need by examining the needs of CHC specialists while serving in rural areas.

METHODOLOGY

The study was done in Tamil Nadu, India. The study focused on specialists' perceptions of existence, relatedness, and growth (ERG) needs. It also researched the reasons for staying intention and turnover intention in relation to these needs. Between August and November 2020, two researchers interviewed rural CHC specialists. Participants

must have a recognized medical certification and have worked in a "remote" rural location for more than a year. Specialists from four districts who met the requirements were identified using government records. This list of eligible practitioners was used to choose first-round interviewees. Maximum variability principles were applied in this selection to ensure representation of both women and men doctors, those trained in allopathic (western or modern) as well as Indian systems of medicine– collectively categorized as AYUSH, in both categories of employment. (i.e., regular, and contractual)For this study, In-depth interviews conducted with 12 specialists, as the study focused on the heavy shortage of specialists in rural CHCs. The interview was organized with semi-structured questions with probes about their demographic profiles, lifestyle in rural areas, needs requirement, work pressure, family life, and children's education under study. The interview guide was designed to retrieve the information on the needs of doctors based on Alderfer's ERG theoretical framework. The objective of the study is to find out the Existential, relatedness, and Growth needs sufficiency and insufficiencies. And to investigate how these requirements determine the turnover intention and intent to stay of the specialists. The interview conducted with available specialists and interested ones, as the specialists count already low and many of them deputed for COVID camps. The one-on-one interaction noted down with writing short transcripts, as the doctors are hesitating for digital recording. The entire transcripts are coded, classified, and organized manually for themes and sub-themes.

Ethical precautions

Before interviews, participants were told the study's goals and gave verbal agreement. In each case, a signed and witnessed statement of the individual taking consent was presented to the study participant, with a copy kept by the researcher. The statement indicated that s/he had described the context, purpose, methods, and risks involved and taken free and informed consent. Transcripts were locked. Ethics review was undertaken, and clearance obtained from the Information review board of IIHMR University, Jaipur.

RESULTS

Study setting and profile of participants

The study area, the state of Tamil Nādu is in the southern region of India. Four districts were selected for this qualitative study, and all 56 CHCs from the 4 districts have been visited. The specialists who are interested to participate in interview were selected. All interviews were conducted privately in the participants' respective places of work – usually clinics or CHCs. It was often a challenge to ensure the privacy of participants, since superiors and colleagues frequently desired to be in the room at the time of the interview. The researchers were often exhorted by participants to take up their manifold personal and professional concerns in policy circles. It was a challenge for the researchers to manage these expectations and to convince participants.Table2 presents profiles of the 12 participants in the study, based on different characteristics.

Key themes

Key themes to emerge from the thematic analysis included the following needs highly perceived by rural CHC specialists (Fig 1). Key themes: Existential needs (Table 3) shortage of basic amenities, social isolation and low standard of living, grievances on payment and allowances, safety and job security and children education. Relatedness needs – Interpersonal relations in working environments, friends and family relations, relations with patient and community. Growth needs – career progression, promotion opportunities, further education and learning, communal pride, and altruism.

Shortage of basic amenities

Most of the specialists stated that, they are not able to get the potable drinking water, and the water is highly infectious and muddy. The civil department is not fulfilling the necessities of water for usage and drinking purposes, despite the community developed as the sub- urban area. They also stated that the constructed toilet facilities in CHCs were unused infrastructure due to water scarcity. Many of them complained about the poor transport

facilities, and condition of roads which is connecting the remote places. And poor internet facilities, frequent signal problems are inevitable while working in rural areas.

Social isolation and low standard of living

Social isolation is identified as major difficulty for rural doctors. It wasan added burden without any recreation facilities and poor social networking in these areas. many of specialists who are working in rural CHCs, taking accommodation within the CHC premises or nearby location and they are visiting their family once in a week or month. In covid period they encountered with lot of problems to meet their family members.

Grievances on payment and allowances

Payment seems to be a major discouraging factor among rural CHC specialists. Complaints on bribing to get urban placements, no allowances for mandatory rural postings. Even at the time of COVID also the doctors are not provided with any of the special allowances when other professionals like teachers are getting payments for special duties. They are regretting a lot, that urban doctors are provided with City Compensatory Allowance (CCA), but for rural postings they are not getting any allowances.

Safety and job security

Safety of specialists are highly pretentious at the time of COVID, they argued more about the infection, people target frequently on doctors, it adds the burden of vandalism on them. Regretting more on the part, people do not understand health professionals' sacrifice and their illiteracy created a lot of unprecedented incidents. Few doctors talked the danger of dog bites and unavailability of vaccines in CHCs. Many of them told that rowdyism is a threatening cause, especially in villages of southern districts. They said people are casually do these crimes for simple reasons and coming to nearby CHCs coerce the doctors to do the things what they want. Job security issues not uttered by many of the specialists as they got permanent postings, but few contract specialists worried about their future decisions of government and continuation. Some of them are in dilemma to quit the rural places, to get better payments.

Children education

Children education in rural area is seen as a major disappointing factor for every doctor working in rural CHCs. The wards of the specialists interviewed none of them enrolled in rural government schools. Most of them argued the facilities for school education in remote areas are insufficient, and frequently they are searching for best education opportunities in city limits, it propels them to move from rural to urban areas, bribing for the reason and leave the place as soon as possible. Key themes – Relatedness needs (Table 5)

Interpersonal relations in working environment

The AYUSH doctors feel the deviation from allopathic specialists. Some young specialists talk negatively about the associations with colleagues. Even though the meeting time of specialists with the senior people is less, they positively admire the seniors in terms of their leadership, guidance, and support. However, the newly sanctioned young specialists negatively articulated about their seniors.

Relations with family and friends

Making friends in the rural community was highly undesirable among specialists. Many of them said they are not willing to make friends, and their friends are physicians from urban areas. But specialists from rural native and working for long years in the community responded positively to have desire of making friends. Many hindrances for family togetherness spelt out since most specialists are coming for the workplace from their urban residence, and many of them resided in the rural dwellings. Some have their clinic, and rural natives find togetherness with their family and relatives. When the spouse is not working, there are no worries residing in rural areas.

Patient and community relations

The friendly relations found with patients, as the doctors are from DGO and DCH specialization having importance of pregnancy and childbirth, one of the ideal processes in everyone's life, it enhances the reputation of relationship. Some are disappointed with the patient load and their attitudinal change in this COVID situation. The overall perception of a specialist towards the community people is good, but they find snags in educating the rural illiterate Population. Key themes – Growth needs (Table 6).

Career progression

Career progression in the form of opportunities for more practice and exposure of knowledge in rural diseases are possible. In addition to that stipulated period of working in rural places open the prospects of urban placements further. Doing private practice in the same rural community enhances the earning augmentation and enlarges the community relations.

Promotion opportunities

Many specialists disappointed with promotion criteria, they expect the procedural justice in the process. Many recommendations and reprimands given by them overlook the policies. Very few of them having encouraging insights about promotion in relation to training and exams to get the promotion with integrity.

Further education and learning

As most specialists are senior persons, already finished their P.G. and super specialties, they suggested the opportunities of further education through rural postings for the young doctors. Merely one of the specialists expressed that further education is not in the Government's hands or other people working with them, said that it is the sole unique passion of every doctor after their UG program.

Communal pride

The specialists are fulfilled with their esteem needs. Being a doctor in the community, the people consider them key persons and celebrate their auspicious occasions. Few of them talked about pride in being a physician, living in the same community as a doctor, and they said frustration is the result if communal support is absent.

Altruism

One of the hopes of the noble medical profession is self-sacrificing care for needy people. The phrasings of some specialists evidenced that their generous attitude. It reveals their achievement of self-actualization.

Reasons for turnover intention

Most of the specialists are having intention to leave, the major reasons are scarce basic amenities in terms of shortage of water, poor transport facilities and inadequate social networking. Family togetherness is yet a question mark, when the specialists are working in rural placements, no one willing to keep their family and children in the remote areas, due to no recreation, poor housing amenities, very poor education facilities in these areas. In most of the cases the spouse is not willing to reside in the rural areas. The specialists who are waiting for permanent position in near future, enduring to work in remote areas to spend the mandatory time and waiting to leave. Some specialists have no consistency in continuing the with rural placements, as already they have completed their PG or super specialties, so willing to hop from rural to urban, it's a major urge among them. Very few young specialists seeking options for continuing medical education and abroad chances willing to leave the places. Payment issues were the serious ongoing debate for specialists, and this is one of the vital reasons for their frustration while working in remote places.

Reasons for staying intention

Few specialists answered that they wish to continue as they are already spent their maximum service period in rural areas and they are nearing the service to end in near future, and they admire the friendly rural people and said this is the reason to continue further. Some of them expecting for permanent position from contractual, so they are patiently

waiting and willing to continue with the initial rural postings. The study revealed that the specialists of rural natives wish to retain and serve for more years in the same community. Very few AYUSH specialists said that the chances for Indian medicine opened only in rural areas, so no way they can go to serve for metropolitan cities, where people seeking for immediate cure.

DISCUSSION AND CONCLUSION

Compared to relatedness and growth needs, insufficiencies of basic needs drive specialists' leaving intentions. Specialists value interpersonal needs with the working team or community but family relations and togetherness in remote locations are found to be difficult. Career advancement and learning possibilities are positives, but the cumbersome scholarship process frustrates doctors. Specialists mention that expecting performance-based promotion with procedural justice. Senior specialists' comments on communal pride and altruism indicate they've met their growth needs. As per the study results, researchers would like to recommend some valid suggestions in this regard. Upgrading the necessities and facilities in rural regions is important through public private partnership models are being thought of, yet a coordinated joint effort should be essentially taken by public welfare and rural administrative services. It is recommended that priority positions (specialists) should be inspected as per requirements of regions and filling the jobs will be an intelligent move. An annual audit of requirement can be there to doctors who are the willing, not mandatory. For attracting doctors from urban areas and retaining the local talent attractive packages and advancement opportunities must be designed. It is seen that younger workforce has more hedonic needs which do not get fulfilled in rural regions. Therefore, salary and other fringe benefits must be designed to as to not only attract but also retain the workforce.

Most of the specialists do not wish to get their wards admitted to schools in rural regions. Sooner or later this acts as a strong motivator for these doctors to switch. For changing the attitude of doctors, necessary action to improve the quality of education in rural areas is important. This should be further strengthened by giving more focus on digital education, advanced curriculum suiting to the global needs. To enhance the relatedness needs of specialists, Increased community participation in health programs is recommended. Apart from monetary benefits government can encourage private practice of CHC doctors by providing yearly recognition for their health coverage targets. This will informally fulfill the medical needs of people living in rural areas. Also, it results in more community interaction and bridging the gap between private and public care. Specialists are more articulated towards hindrances of family togetherness, this can be improved by good housing facilities, better education system in rural areas. The specialist provided with residential facilities to stay within the community centers which are in extremely rural locations, But the problem of social isolation leads to quick turn over of professionals, so thinking of better quarters for doctors, not within the CHC, but with the communal area is suggested. Most married doctors and their spouses in metropolitan regions are hesitant to move to rural locations owing to work or lack of recreation. They do not wish to stay in rural areas if their spouse works elsewhere, especially in cities. So, it is highly recommended, the public administration should consider creating rural posts with adequate counselling and without delays for married doctors.

Growth needs are highly influenced by specialists–So it is endorsed that medical education programs By NHM should be high skill-oriented and less theoretical to enhance quality of training. Promotion criteria should be enhanced by linking promotion to performance, Tamil Nādu Government should take iron-handed actions to have procedural justice in deciding the parameters for promotion as it deeply affects the motivation of doctors. Accurate and short procedures for getting scholarships for further education by performance level but not by time-bound compulsory rural placements is required. An integrated approach to formulate the policies by health policymakers and educational policymakers to address this problem is suggested. In-depth training modules for future studies and redirection of medical education to support rural health issues will be beneficial. This study supports rural upbringing and reveals that doctors born in rural areas willing to serve for the community and retain the job for longer tenure. Hence, the Government policies should be strengthened for this rural pipeline strategy, and it is imperative to encourage the rural students, not merely admiring but to prepare their minds to serve their community

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from the beginning of their course. Finally, the goal of greater specialists' retention may well be served if authorities were to accord formal recognition and upgrading basic services under difficult conditions to doctors with appropriate credentials and histories of rural service.

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Table 1: Specialists' shortage in Tamil Nādu CHCs

Year	Sanctioned (1540)	Shortfall
2015	0(In position)	1540
2016	76(In position)	1464
2017	78(In position)	1462
2018	210(In position)	1330
2019	179(In position)	1361
2020	228(In position)	1312
2021	251(In position)	1289

Table 2: Characteristics of participants.

Districts	Gender	Background	Status	Designation
Theni	Male	Urban	Permanent	Sr. Medical officer- DCH
	Male	Urban	Permanent	Medical officer – MD
	Female	Rural	Permanent	Block Medical Officer- OB&G
	Male	Urban	Contract	AYUSH M.O. - Naturopathy
	Female	Rural	Permanent	AYUSH M.O – Siddha
Tiruchirappalli	Female	Urban	Contract	Dental surgeon – MDS
	Male	Urban	Permanent	Chief Medical Officer - MD
	Male	Urban	Permanent	Sr. Medical Officer – DCH
Madurai	Male	Urban	Permanent	Block Medical Officer – MD
	Female	Rural	Permanent	Medical officer – OB&G
Siva ganga	Female	Rural	Permanent	Sr. Asst. Surgeon – OB&G
	Male	Urban	Permanent	Medical officer - DA

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Table3: Key themes – Existential needs

Shortage of basic amenities	Water is a major problem in this area, there is no proper drinking water, people travel more distance for water. Being transformed as sub-urban area, people crowd, and their housing amenities increased, yet not able to provide the water facilities, here the water is not potable, but muddy and infectious. The water filters are getting damaged in short frequencies. For three years I'm working in this CHC, the water shortage for toilet necessities is indescribable now as per the government schemes, recently toilets constructed for patients and staff, but insufficient water led to the unused infrastructure. (Sr. Medical Officer – DCH 1) Very few Government buses are plying and cannot rely on this conveyance, all of us coming by our own vehicles, road facilities are very critical, the vehicles getting damaged frequently and transport to interior is difficult. Also, we are facing, frequent signal problems and slow networks in this extreme remote location, it irritates a lot. (Dental surgeon - MDS).
Social isolation and low standard of living	Here we can't live luxury lifestyle, no recreation opportunities, little social networking facilities and we are waiting for a month or weeks to meet our family and other city-based amenities. Loneliness is a major hindrance while working here. My husband is working in Chennai, my daughter also studying there, with her grandparents and father, all my family settled over there. I am waiting to meet them every fortnight. ……. Now I cannot look for transfer or urban placement, since my service is going to end in 2 years, gained well experience working in rural areas. (Block medical officer – OB&G).
Grievances on payment and allowances	In olden days doctors are considered as prideful job as they are doing noble profession, but now the poorest category in terms of earning money are government doctors, there is no rural allowances or any fringe benefits. promotional policies are in the hands of middlemen, as if I want to get urban placements, I should have money in Lakhs, but if I wish to go to rural place, I'm always invited without any hesitation, Government can consider of adding attractive benefits and special salary together. (Medical Officer- MD) Initial rural postings, which are mandatory, but not connected with any of the financial allowances, nonetheless government is providing City Compensatory Allowance for Doctors working in urban cities. ….. The situation is more regrettable when we must attend the Camps compulsorily in schools and temples at this pandemic and came to know that the other professional like teachers and civil workers who are in duty, getting special allowances, but Doctors are getting normal salary even in this tough situation. (Sr. Asst. Surgeon – OB&G).
Safety and job security	Due to pandemic (COVID) we are facing lot of safety issues, when realizing at the boom of emergency, we are not protected. At first when the universe didn't explore what the disease is all about, we face daily cases with such symptoms that is highly contagious and when globe is aware about it, people started to refuse to come near and treat us differently even when we were trying to protect them. Vandalism was more on doctors, the people were against doctors, the village people are not understanding doctors' sacrifice As the COVID emergency spreads a lot, and the people are not well educated, can't handle with their illiteracy. Here the people are lethargic to follow the rules,even though the government has been imposing laws and curfews to lock people inside their homes, despite the ban vegetable markets,

	and meat shops receive the same crowd every day. (Medical officer- DA) In this village dog bite is a very big problem, sometimes we are out of Rabies vaccine and the Municipality is not taking any action to reduce the stray dogs count, mostly children are coming for this cause. (Sr. Medical officer – DCH1) Rural people are highly aggressive, sometimes we attend the problems of rowdies and making the environment threatening, handling these kinds of people is very difficult. Educating them is impossible…. when who will come, what they will ask, what they will do, we don't know. Even for a small problem they will come as society gang and will ask lot of questions. It is nearly one year I am working here, its new and delicate experience, handling with rural illiterate people is very difficult…... there are lot of challenges we are facing everyday basis. (Medical Officer- OB&G). As per the staffing norms, AYUSH doctors are initially in contract position, except Siddha and Homeopathy, if it is considered as a permanent position, we feel secured in our future career. (AYUSH MO- Naturopathy) Payment for contract positions is low……... When compared to permanent and contract staff there is large gap in monetary benefits. Now the government only made the decision to recruit 2 dental surgeons in every rural CHCs, but our retention will be decided by salary and other benefits rather community. (Dental surgeon- MDS).
Children education	I have a son, he is 4 years old, already our relatives are in the same rural community since this is my native place, so no problem of happiness. My son just started the school, currently the education facilities are enough, if he grows, I wish to switch urban schools for better education. (Sr. Asst. Surgeon – OB&G) I have two kids, one son and one daughter, they are studying in Madurai, my In-laws and parents are also there. 'Education is not a priority in these remote areas… but there are good private schools near to city, hope the children can get better education there… so they are living in Madurai. (Chief Medical Officer- MD). The facilities for children education, one cannot expect from this rural area. I have one daughter, she is in primary school, she is studying one of the private schools in Thirupuvanam, if she completes her primary level, I will be looking for better educational facilities in Madurai. None of us willing to give education in this remote area, will be searching urban placements for this special reason. (Asst. surgeon- OB&G).

Table 4: Key themes – Relatedness needs

Interpersonal relations in working environment	We have been quoted as for delayed treatment, not for emergency causes, which makes us feel inferior to allopathic doctors. The benefits of Indian therapy must be understood. If my service continues, I will inspire others and raise awareness about conventional health care. The allopathic colleagues here keep their distance from AYUSH doctors; nevertheless, when administrative collaboration is necessary, a group will be created and the task will be completed; otherwise, we perform our work and depart on time. (AYUSH MO-Siddha). My colleagues, two senior medical officers, and paramedics are friendly, cooperative, and working as a team, despite being from different specialties. My seniors in DMO and DPH provide excellent direction and leadership. All my seniors in separate places, here we may work together and obtain encouraging direction from superiors. We received minimal notice and reprimands during crises and missed objectives. (Block Medical Officer- OB & G). As far as a government job is concerned, we can't anticipate support from the top officials. The duty commitment isn't usually correlated, but coordination is

	needed in crises. I'm used to working without instruction from seniors. (Medical officer-OB & G)
Relations with family and friends	Workplace friendships aren't required. My friends are from urban cities and abroad, not from this community. Why make friends here? (Asst Surgeon-OBG) 2-3 of my friends, they are also medical officers in nearby CHCs, one is working in odaipatti…… another friend, he is a Block medical officer, thevaram CHC. . We three studied in Madurai medical college. my friends are from same circle and some officials from DPH office, nodal officers. (Medical officer- DA) I'm married, spouse is not working, she is in Kerala, having one child, she is in the school age. every week I'm going there and spending time with my family……... waiting for every Saturday to meet our family and other city-based amenities. (AYUSH MO- Naturopathy). My spouse is in occupation, she is also Doctor, gynecologist, she is working in private nursing home in Theni, our family also settled there, I have a son, he is studying in Chennai. Daily I am coming by my vehicle, I settled my family in Theni. (Medical Officer – MD) I am doing private practice, which is giving earning augmentations in this region, my family is near to me, reachable distance, my husband is working as a businessman in KOMBAI, having one son and daughter. (Sr. Assistant surgeon-OB&G) This is my native place, my wife is also a doctor, she is working as an assistant surgeon in Melmangalam, (PHC) one son and one daughter and living together in Dombucherry. (Sr. Medical Officer – MD- Permanent).
Patient and community relations	The respect from patients is good, being as a pediatrician community respect is very high. As they are friendly, I'm staying here, and they trust me and on my care. (Sr. Medical Officer- DCH1). Some patients having long term relationship with us, as it's a slow therapy (siddha) but permanent cure is possible, and many of the village people rely on Indian medicine, and we rely on the people to get the herbs from mountains of these places. (AYUSH MO- siddha). Health units and professionals at certain government hospitals are 'newer untouchables.' People fear catching COVID-19 from medical staff or being stigmatized for possessing it. Even when people are kind and respectful, teaching them won't help. In this epidemic, our duties are extremely scheduled and we're in danger, but we're still trying to help. But we become frustrated if they refuse. (Medical Officer – OB&G).

Table 5: Key themes – Growth needs

Career progression	Now it's vital to tell young physicians, who aren't staying in rural regions. If I can say anything, I'll be optimistic about the chances for practicing in rural illnesses, which will increase knowledge... It's easier to secure urban placements if…… practices in this community. Starting a private practice in rural health institutions will increase exposure. (Chief Medical Officer- MD)
Promotion opportunities	The rules on remuneration and promotion can be overlooked again. Currently, the policy on promotion is not better than a decade ago. By vertical cadres and experience, we got promoted; currently the training and examinations for Assistant surgeon to MO, SMO, and CMO. If I want metropolitan placements, I need Lakhs, but if I want to go rural, I'm always invited. (Sr. Medical officer – DCH2)
Further education and learning	The opportunities of scholarships for continuing medical education, somewhat motivating us, but enduring situations continued to be a problem. Since long procedure for getting the facility. And in remote areas learning opportunities/or

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	more practice cannot be expected. (Sr. Asst. Surgeon- OB&G) I don't perceive a relationship between my education and my friends' encouragement. I've gotten this far because I was committed to study for myself and gain this position, therefore if I must continue with my professional initiatives, I am alone accountable and no one else. (Medical Officer – DA)
Communal pride	if we are friendly and doing timely help in giving care, they are very respectful, it's a fact, rural doctors are getting communal pride among people……. Usually, I attend the communal and school health programs, at times I respected more, have social pride as a pediatrician. In many schools, I have been invited as a chief guest for their health programs. (Sr. Medical officer – DCH2)
Altruism	Accepting rural service with a broad mind is necessary, I feel sometimes, rural population is abandoned when compared to urban health facilities. Here the parents of small children are not well educated and to get the illness free future generation, doctors are mandatory to work here, not only DCH, but also some specialists are required, in times we are crippled with insufficient doctors to handle the emergencies. It is my native place, and I wish to work for this people, like that, if every doctor thinks service for their natives, the rural shortage will not be major difficulty for health system. (Chief Medical Officer- MD)

Fig1: key themes

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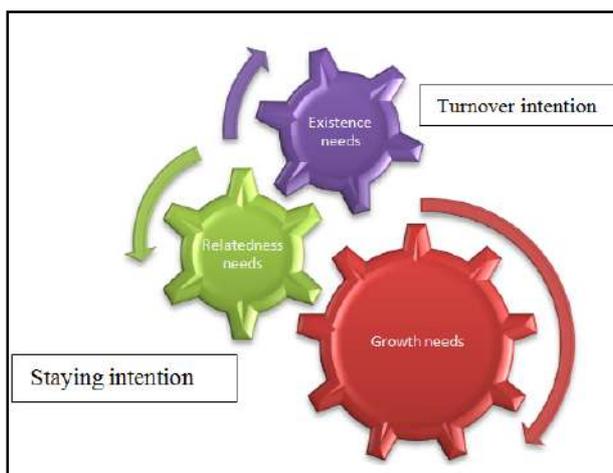
Analysis into Socket Preservation and Requirement of Flap Advancement

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ABSTRACT

Continuous bone resorption is observed at the extraction site. Loss of volume morphology is addressed by bone and soft tissues techniques. Socket preservation and maintenance of optimal alveolar ridge dimension are key factors in implant therapy. This study aims to analyse the implant site and the requirement for socket preservation and flap advancement. The study was designed as a retrospective cross clinical study. In this study, 30 patients suggested for flap advancement during the time period of June 2020-March 2021 at Saveetha Dental College and Hospitals were included in the study. Data collection was done using Dental information archiving software (DIAS). Data were collected; analysed using SPSS Software version 22 and the results were recorded. Out of the 30 patients, 15 were male and 15 were female. Out of which 21 patients underwent flap advancement. Most common implant site for flap advancement was 21 (20%). Flap advancement was more common among female patients (43.3%) compared to male with statistical significance ($p<0.5$) .

Keywords: Dental implant, Socket preservation, Flap advancement, innovation

INTRODUCTION

When a tooth is subjected for extraction, the extraction site undergoes hard and soft tissue remodeling that leads to loss of natural anatomical morphology. The osteoblastic and osteoclastic activity around the extraction socket results in unfavourable bone resorption and its diverse volumetric patterns.(1) There is continuous bone resorption at the extraction site. Loss of bone occurs more during the first month of extraction. Loss of volume morphology is noted such as loss of vertical ridge height and displacement of the buccal wall in the palatal direction in maxilla.(2) In mandible there occurs lingual resorption to such an extent that there is centralised, narrow ridge.(3) Unfavourable bone resorption leads to aesthetic and functional concern during restoration on the impacted site .Loss of volume morphology is addressed by bone and soft tissues techniques.(4) Preservation and maintenance of optimal alveolar ridge dimension are key factors in implant therapy.(5) Dental implants are used for the replacement of teeth in the extracted site. Dental implant is a surgical component that interfaces with the bone of the jaw or skull which supports a dental prosthesis such as a crown, bridge, denture, facial prosthesis or to act as an orthodontic anchor.(6) Implant length and diameter are the factors in success of implant, however loss of neck alveolar bone decreases the success rate and adequate soft and hard tissues are needed for implant healing.(7) Alveolar socket preservation has been described as "a technique in which completely contained extraction sockets are filled with a bone substitute material and/or sealed with membranes.(5)Socket preservation is done after the primary wound closure to reduce bacterial infection, minimise tissue shrinkage and loss of graft materials, and subsequent graft failure. Reduction in the loss of alveolar bone volume is advocated by socket preservation. This helps to achieve high primary stability and generation of new - regeneration tissues for osseointegration during implant placement.(8) Bone sockets are also preserved using both autogenous bone grafts and bone substitutes for the purpose of avoiding future bone grafting.(9)(10) Primary closure results in decreased discomfort and faster healing. Healing by primary intention is carried out by coronal positioning of mucogingival tissues. Tension-free primary closure along the incision line is attained by flap advancement.(11) There are various flap advancement techniques out of which coronal advancement of vestibular flap is the most commonly used flap advancement. Open wounds and soft tissue defects are treated by rotational flaps. Failure of primary closure may result in a soft tissue dehiscence along the incision line that can cause a poor outcome and/or postoperative complications.(4)(12) Our team has extensive knowledge and research experience that has translated into high quality publications. (13–32)

MATERIALS AND METHOD

The study was based on retrospective cross clinical study which analyses patients with dental implants. A data of 86000 patient records during the time period of June 2020 to March 2021 were analysed and reviewed. Out of which 30 patients suggested for flap advancement were identified. Patients records with insufficient medical documentation, improper clinical photographs, duplication of data, incomplete diagnosis were excluded from the study. The demographic and clinical data like age, gender, implant site, requirement for flap advancement were recorded. The data collected were tabulated in excel. Statistical analysis was carried out using Statistical Package for the Social Sciences ,version 22(SPSS). Frequency tables and percentage were calculated using the study variable. Descriptive statistics was used to summarise the variable in the data set. A Chi square test was applied to determine the association and level of significance between groups. p value< 0.05 was considered to be statistically significant .

RESULTS AND DISCUSSION

A total of 30 patients were suggested for socket preservation and flap advancement after implant placement. Among the 30 patients evaluated fifteen were male (50%) and fifteen were female (50%). (Fig 1)In the present study, among the given population there was equal gender predilection. Patients among the age group of 31-40 were predominant. Socket preservation and flap advancement was most common in implant site 21.

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The current study states that there was equal gender predilection and flp advancement ws more common in the age group of 31-40 years. According to study by *Mohammed et al* there was male predilection and the mean age was found to be 50 years.(33)According to the study by *Mardinger et al,* there was equal gender predilection with a mean age of 50 years.(34)The current study states that socket preservation and flap advancement was more common in implant site 21. Salvi *et al. and Grassi et al.* stated that there was a difference in the primary stability between implants placed in the maxilla and mandible. Even though the can cellous bone had similar density in both maxilla and mandible the cortical bone of the mandible was compared to that of maxilla.(35,36)Healing of the extraction site by augmentation of the socket with bone results in primary closure of the socket by preventing the migration of bone particles. There are several methods, procedures and different exogenous materials, soft tissue grafts available for primary closure. (34)

CONCLUSION

The present retrospective cross clinical study indicates that socket preservation and flap advancement was favored more in implant site 21 with more female gender requiring flap advancement procedure and was seen across all the age groups.

AUT   HOR CONTRIBUTION

Priyadharshini S: Literature search, data collection, analysis, manuscript drafting.
Dr.Suresh Venugopal: Data verification, manuscript drafting.
Dr.Vaishnavi Rajaraman : manuscript drafting

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

All the authors declare that there was no conflict of interest in present study.

SOURCE OF FUNDING

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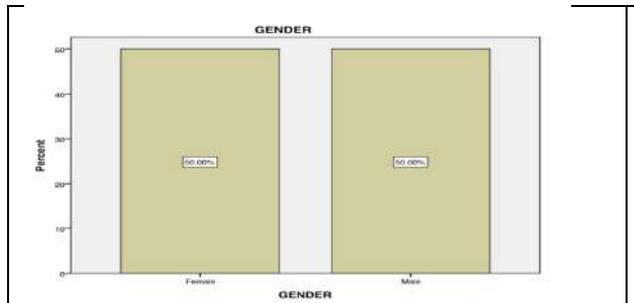
Figure 1: The bar graph represents the frequency distribution of flap advancement based on gender. X-axis shows the gender. The Y-axis shows the percentage of population. Equal gender distribution.
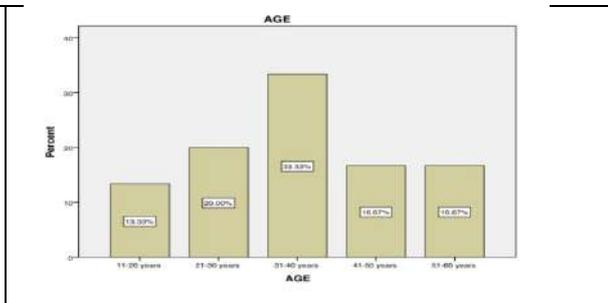
Figure 2: The bar graph represents the frequency distribution of flap advancement based on age. X-axis shows the distribution of age. The Y-axis shows the percentage of population. Patients aged 31-40 years were predominant (33.3%).
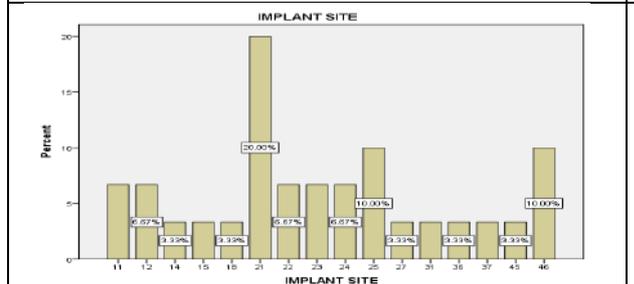
Figure 3: The bar graph represents the frequency distribution of flap advancement based on implant site. X-axis shows the implant site. The Y-axis shows the percentage of population. Most common implant site for flap advancement was 21 (20%).
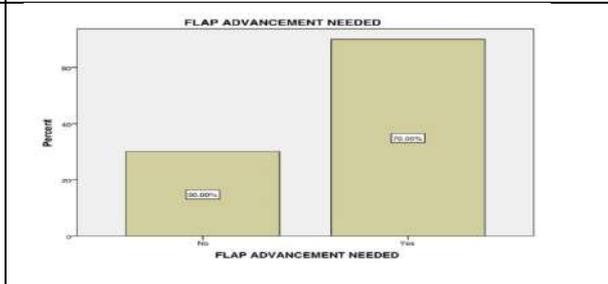
Figure 4: The bar graph represents the frequency distribution of flap advancement based on requirement of flap advancement . X-axis shows the requirement of flap advancement . The Y-axis shows the percentage of population. Majority of 21 patients underwent flap advancement (70%).
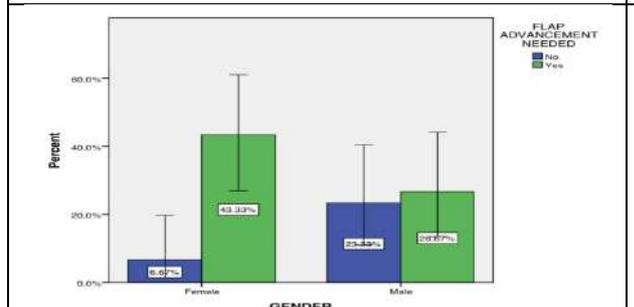
Figure 5: Bar graph showing the association between gender and requirement for flap advancement. X-axis represents the gender of the population based on requirement for flap advancement as Required (Green) and Not required (blue). Y-axis the percentage of patients. Requirement of flap advancement in females was observed to be higher. This was
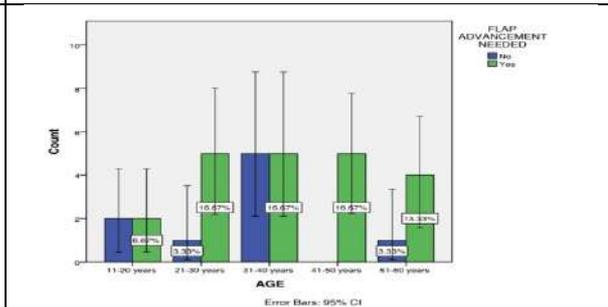
Figure 6: Bar graph showing the association between age group and requirement for flap advancement. X-axis represents the age group of the population based on requirement for flap advancement as Required (Green) and Not required (blue). Y-axis the percentage of patients. Requirement of flap advancement in the age group of 31-40 years was

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statistically significant. Pearson chi square, P= 0.046 < 0.05.

observed to be higher. This was not statistically significant. Pearson chi square, P= 0.235 > 0.05.
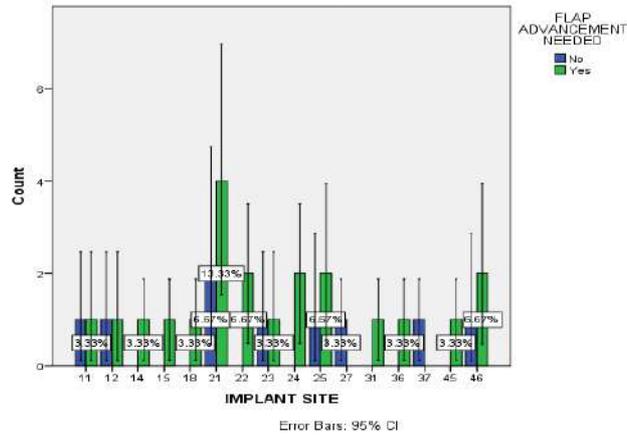
Figure 7: Bar graph showing the association between implant site and requirement for flap advancement. X-axis represents the implant site based on requirement for flap advancement as Required (Green) and Not required (blue). Y-axis the percentage of patients. Requirement of flap advancement in 21 was observed to be higher. This was not statistically significant. Pearson chi square, P= 0.810 > 0.05.

RESEARCH ARTICLE

Core Inverse for a Class of 2×2 Anti-Triangular Block Matrices Over Skew Fields

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ABSTRACT

In this paper, we gave study the necessary and sufficient conditions for the existence and explicit representations of the core inverse of the block matrices $\begin{pmatrix} E & F \\ G & 0 \end{pmatrix}$ in the following three cases, respectively.

(i)$rk(S) = rk(F^\pi E)$,
(ii)$rk(S) = rk(EF^\pi)$,
(iii)$rk(S) = rk(F^\pi E) = rk(EF^\pi)$,
where $E, F, G \in M_{m \times n}(\mathbb{C})$, $F^\#$ exists, $R(F) = R(G)$, $N(F) = N(G)$ and $S = F^\pi EF^\pi$.
AMS Subject Classification: 15A09, 15A24.

Keywords: Skew fields, Block matrix, Core inverse, Range space, Null space, Generalized inverse.

INTRODUCTION

Suppose $\mathcal{K}$ is a skew field. Let $M_{m \times n}(\mathbb{C})$ denote the set of all $m \times n$ matrices over $\mathcal{K}$. The core inverse for a complex matrix was introduced by Baksalary and Trenkler [1] in 2010. Let A be a $n \times n$ complex matrix and $P_{R(A)}$ be the orthogonal projector onto $R(A)$. The symbol ©- denote the core inverse of a matrix. A $n \times n$ complex matrix $A^\#$ satisfying $AA^\# = P_{R(A)}$ and $R(A^\#) \subseteq R(A)$ is the core inverse of A. A complex matrix has core inverse if and only if it is core invertible, and the core inverse is unique when it exists .

In 2015, Jacek Mieliniczuk [5] were investigated C-inverse of a core matrix. Weighted core-EP inverse of an operator between Hilbert spaces was established by Dijana Mosic [3] in 2017. In 2018, Sanzhang Xu et al. [7] developed the concept of new characterization of the CMP inverse of matrices. Three limit representation of the core - EP inverse was studied by Mengmang Zhou et al. [6] in 2018. In 2019, Huihui Zho and Qing - Wen Wang [4] investigated Weighted pseudo core inverses in rings. core invertibility of triangular matrices over a ring was developed by Sanzhang Xu [8] in 2019. In 2019, Yuanyuan Ke et al. [10] extended the core inverse of a product and 2×2 matrices.

Preliminaries

Definition 2.11[1] *A matrix A is Hermitian if $A^* = A$, and A is called an idempotent if $A^2 = A$.* A Hermitian idempotent is said to be a projection.

Definition 2.22[1] *The core inverse of $A \in M_{n \times n}(\mathbb{C})$ is the matrix $X \in M_{n \times n}(\mathbb{C})$ which satisfies*
(1) $AXA = A$ (2) $XAX = X$ (3) $(AX)^* = AX$ (6) $XA^2 = A$ (7) $AX^2 = X$
The matrix X is unique if it exist and is denoted by $A^{\#}$.

Definition 2.33[1] *A matrix A is said to be core EP if $AA^{\#} = A^{\#}A$.*

Definition 2.44[8] *A matrix A is said to be core invertible if $AA^{\#} = A^{\#}A = I$.*

3. Main Results

Lemma 3.15*Let $M = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix}$, $E, F, G \in M_{n \times n}(\mathbb{C})$, $F^{\#}$ exists, $R(F) = R(G)$ and $N(F) = N(G)$, then $G^{\#}$ exists and the following conditions hold:*
(i) $FF^{\#} = GG^{\#}$, $F^{\pi} = G^{\pi}$.
(ii) If $FF^{\#}G = GFF^{\#} = G$, $FF^{\#}G^{\#} = G^{\#}FF^{\#} = G^{\#}$, $FGG^{\#} = GG^{\#}F = F$, $F^{\#}GG^{\#} = GG^{\#}F^{\#} = F^{\#}$.

Proof. From $R(F) = R(G)$, $N(F) = N(G)$ and $F^{\#}$ exists, there exists Invertible matrices $P \in M_{n \times n}(\mathbb{C})$, $F_1, G_1 \in M_{r \times n}(\mathbb{C})$
such that $F = P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$, $F^{\#} = P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$ and $G = P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$, $G^{\#} = P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$ (1)

(i) $FF^{\#} = GG^{\#}$
$FF^{\#} = P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$
$= P \begin{pmatrix} F_1 F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$
$CC^{\#} = P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$
$= P \begin{pmatrix} G_1 G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$
Since $N(E) = N(G)$ and $R(E) = R(G)$ we get $FF^{\#} = GG^{\#}$.
The proof ofconditions (ii) follows. $FF^{\#}C = GFF^{\#} = G$, $FF^{\#}G^{\#} = G^{\#}FF^{\#} = G^{\#}$, $FGG^{\#} = GG^{\#}F = F$, $F^{\#}GG^{\#} = GG^{\#}F^{\#} = F^{\#}$
Now, $FF^{\#}C = P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$
$= P \begin{pmatrix} F_1 F_1^{\#} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} = P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$
$GFF^{\#} = P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$
$= P \begin{pmatrix} G_1 F_1 F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$

$$= P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

Hence $FF^{\#}G = GFF^{\#}$.

Also $FF^{\#}G^{\#} = P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$

$$= P \begin{pmatrix} F_1 F_1^{\#} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$G^{\#}FF^{\#} = P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} G_1^{\#} F_1 F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

Therefore $FF^{\#}G^{\#} = G^{\#}FF^{\#} = P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$

$FGG^{\#} = GG^{\#}F = F$

$$FGG^{\#} = P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} F_1 G_1 G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$GG^{\#}F = P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} G_1 G_1^{\#} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

Therefore $FGG^{\#} = GG^{\#}F = P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$

$F^{\#}GG^{\#} = GG^{\#}F^{\#} = F^{\#}$

$$F^{\#}GG^{\#} = P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} F_1^{\#} G_1 G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$GG^{\#}F^{\#} = P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} G_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} G_1 G_1^{\#} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

$$= P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

Therefore $F^{\#}GG^{\#} = GG^{\#}F^{\#} = P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$

Hence the Lemma

Lemma 3.26 *Let* $M = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix}$ *,* $E, F, G \in M_{n \times n}(\mathbb{C})$, $F^{\#}$ *exists*, $R(F) = R(G)$, $N(F) = N(G)$ *and* $S = F^{\pi}EF^{\pi}$, *then* $(F^{\pi}E)^{\#}$ *exists if and only if* $S^{\#}$ *exists and* $rk(S) = rk(F^{\pi}E)$.

Proof. The only if part, according to equation (1)

$$F^{\#} = P \begin{pmatrix} F_1^{\#} & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$$

Let $E = P \begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix} P^{-1}$, where $E_1 \in M_{r \times r}(\mathbb{C})$, then $S = F^{\pi}EF^{\pi}$

$F^\pi = I - FF^\# = P\begin{pmatrix} I & 0 \\ 0 & I \end{pmatrix}P^{-1} - P\begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix}P^{-1} - P\begin{pmatrix} F_1^\# & 0 \\ 0 & 0 \end{pmatrix}P^{-1} = P\begin{pmatrix} I & 0 \\ 0 & I \end{pmatrix}P^{-1} - P\begin{pmatrix} F_1 F_1^\# & 0 \\ 0 & 0 \end{pmatrix}P^{-1} =$

$P\begin{pmatrix} I - F_1 F_1^\# & 0 \\ 0 & I \end{pmatrix}P^{-1}$

$F^\pi E = P\begin{pmatrix} I - F_1 F_1^\# & 0 \\ 0 & I \end{pmatrix}P^{-1}P\begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix}P^{-1} = P\begin{pmatrix} (I - F_1 F_1^\#)E_1 & (I - F_1 F_1^\#)E_2 \\ E_3 & E_4 \end{pmatrix}P^{-1}$

$\qquad = P\begin{pmatrix} (I - F_1 F_1^\#)E_1 & (I - F_1 F_1^\#)E_2 \\ E_3 & E_4 \end{pmatrix}P^{-1} = P\begin{pmatrix} 0 & 0 \\ E_3 & E_4 \end{pmatrix}P^{-1}$

$F^\pi E F^\pi = P\begin{pmatrix} 0 & 0 \\ E_3 & E_4 \end{pmatrix}P^{-1}P\begin{pmatrix} (I - F_1 F_1^\#) & 0 \\ 0 & I \end{pmatrix}P^{-1} = P\begin{pmatrix} 0 & 0 \\ E_3(I - F_1 F_1^\#) & E_4 \end{pmatrix}P^{-1} = P\begin{pmatrix} 0 & 0 \\ 0 & E_4 \end{pmatrix}P^{-1}$

Since $(F^\pi E)^\#$ exist we get $rk(E_3 E_4) = rk(E_4(E_3 E_4))$.

So $rk(E_4) \geq rk(E_3 E_4)$ (2)

Further $rk(E_4) = rk(E_3 E_4)$ (3)

This implies that there exists X satisfying $E_3 = E_4 X$

in view of equation (2), we can conclude that $rk(E_4) = rk(E_4^2)$, namely $S^\#$ exists combining with equation (3), that means $rk(S) = rk(F^\pi E)$ the if part follows from $rk(S) = rk(F^\pi E)$ and that (3) holds. And note that $S^\#$ exists, then $rk(E_4) = rk(E_4^2)$. This together with equation (3) shows that (2) holds that is $(F^\pi E)^\#$ exists.

Lemma 73.3 *Let* $M = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix}$. $E, F, G \in M_{n \times n}(\mathbb{C})$, $F^\#$ *exists*, $R(F) = R(G)$, $N(F) = N(G)$ *and* $S = F^\pi E F^\pi$, *then* $S^\#$ *exists and the following conditions hold:*

$$(i) F^\pi E S^\# E = F^\pi E$$

$(ii) F^\pi E S^\# = S^\# E F^\pi$

$(iii) F S^\# = S^\# F = F^\# S^\# = S^\# F^\# = 0$

$\qquad (iv) (S^\#)^2 E F^\pi = F^\pi E (S^\#)^2 = S^\#$.

Proof. Suppose $rank(F) = r$. Applying Lemma 3.1, there exist invertible matrices $P \in M_{n \times n}(\mathbb{C})$ and $F_1 \in M_{r \times r}(\mathbb{C})$ such that $F = P\begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix}P^{-1}$ and $F^\# = P\begin{pmatrix} F_1^{-1} & 0 \\ 0 & 0 \end{pmatrix}P^{-1}$

$\qquad$ Let $E = P\begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix}P^{-1}$, where $E_1 \in M_{r \times r}(\mathbb{C})$, $E_2 \in M_{r \times (n-r)}(\mathbb{C}), E_3 \in M_{(n-r) \times r}(\mathbb{C})$ and $E_4 \in M_{(n-r) \times (n-r)}(\mathbb{C})$.

We get $S^\#$ exist and $S^\# = P\begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix}P^{-1}$.

(i) $F^\pi E S^\# E = P\begin{pmatrix} 0 & 0 \\ E_3 & E_4 \end{pmatrix}P^{-1}P\begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix}P^{-1}P\begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix}P^{-1}$

$= P\begin{pmatrix} 0 & 0 \\ E_3 & E_4 \end{pmatrix}\begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix}\begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix}P^{-1}$

$= P\begin{pmatrix} 0 & 0 \\ 0 & E_4 E_4^\# \end{pmatrix}\begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix}P^{-1}$

$= P\begin{pmatrix} 0 & 0 \\ E_4 E_4^\# E_3 & E_4 E_4^\# E_4 \end{pmatrix}P^{-1}$

$= P\begin{pmatrix} 0 & 0 \\ E_3 & E_4 \end{pmatrix}P^{-1}$.

(ii) $F^\pi E S^\# = P\begin{pmatrix} 0 & 0 \\ E_3 & E_4 \end{pmatrix}P^{-1}P\begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix}P^{-1}$

$= P\begin{pmatrix} 0 & 0 \\ E_3 & E_4 \end{pmatrix}\begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix}P^{-1} = P\begin{pmatrix} 0 & 0 \\ 0 & E_4 E_4^\# \end{pmatrix}P^{-1}$

$S^\# E F^\pi = P\begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix}P^{-1}P\begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix}P^{-1}P\begin{pmatrix} 0 & 0 \\ 0 & I_{n-r} \end{pmatrix}P^{-1}$

$= P\begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix}\begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix}\begin{pmatrix} 0 & 0 \\ 0 & I_{n-r} \end{pmatrix}P^{-1}$

$= P\begin{pmatrix} 0 & 0 \\ E_4^\# E_3 & E_4^\# E_4 \end{pmatrix}\begin{pmatrix} 0 & 0 \\ 0 & I_{n-r} \end{pmatrix}P^{-1}$

$\qquad S^\# E F^\pi = P\begin{pmatrix} 0 & 0 \\ 0 & E_4^\# E_4 \end{pmatrix}P^{-1}$.

Therefore $F^\pi E S^\# = S^\# E F^\pi = P\begin{pmatrix} 0 & 0 \\ 0 & E_4^\# E_4 \end{pmatrix}P^{-1}$.

$FS^\# = P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix} P^{-1} = P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} \begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix} P^{-1} FS^\# = 0.$

Similarly,

$S^\# F = 0.$

$F^\# S^\# = P \begin{pmatrix} F_1^\# & 0 \\ 0 & 0 \end{pmatrix} P^{-1} P \begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix} P^{-1} = P \begin{pmatrix} 0 & 0 \\ 0 & 0 \end{pmatrix} P^{-1} = 0.$

Therefore $F^\# S^\# = 0$. Similarly, we can obtain $S^\# F^\# = 0$.

Therefore $FS^\# = S^\# F = F^\# S^\# = S^\# F^\#$.

(iv)$(S^\#)^2 EF^\pi = F^\pi E(S^\#)^2 = S^\#$

$(S^\#)^2 = P \begin{pmatrix} 0 & 0 \\ 0 & (E_4^\#)^2 \end{pmatrix} P^{-1}$

$(S^\#)^2 EF^\pi = P \begin{pmatrix} 0 & 0 \\ 0 & (E_4^\#)^2 \end{pmatrix} P^{-1} P \begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix} P^{-1} P \begin{pmatrix} 0 & 0 \\ 0 & I_{n-r} \end{pmatrix} P^{-1}$

$= P \begin{pmatrix} 0 & 0 \\ 0 & (E_4^\#)^2 \end{pmatrix} \begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix} P^{-1} P \begin{pmatrix} 0 & 0 \\ 0 & I_{n-r} \end{pmatrix} P^{-1}$

$= P \begin{pmatrix} 0 & 0 \\ (E_4^\#)^2 E_3 & (E_4^\#)^2 E_4 \end{pmatrix} \begin{pmatrix} 0 & 0 \\ 0 & I_{n-r} \end{pmatrix} P^{-1} = P \begin{pmatrix} 0 & 0 \\ 0 & (E_4^\#)^2 E_4 \end{pmatrix} = P \begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix}$

$F^\pi E(S^\#)^2 = P \begin{pmatrix} 0 & 0 \\ E_3 E_4 & \end{pmatrix} P^{-1} P \begin{pmatrix} 0 & 0 \\ 0 & (E_4^\#)^2 \end{pmatrix} P^{-1} = P \begin{pmatrix} 0 & 0 \\ E_3 E_4 & \end{pmatrix} \begin{pmatrix} 0 & 0 \\ 0 & (E_4^\#)^2 \end{pmatrix} P^{-1} = P \begin{pmatrix} 0 & 0 \\ 0 & E_4 (E_4^\#)^2 \end{pmatrix} P^{-1} =$

$P \begin{pmatrix} 0 & 0 \\ 0 & E_4^\# \end{pmatrix} P^{-1}.$

Therefore, $(S^\#)^2 EF^\pi = F^\pi E(S^\#)^2 = S^\#$

Hence the Lemma.

Theorem 3.48$Let\ M = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix}$, $E, F, G \in M_{n \times n}(\mathbb{C})$, $F^\#$ exists, $R(F) = R(G)$, $N(F) = N(G)$, $S = F^\pi EF^\pi$ and $rk(S) = rk(F^\pi E)$. Then

(i) $M^\#$ exists if and only if $S^\#$ exists.

(ii) If $M^\#$ exists, then $M^\# = \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix}$, where

$M_{11} = S^\# + (S^\# E - I)G^\# F^\# ESS^\# - (S^\# E - I)G^\# F^\# EG^\pi$

$M_{12} = G^\# - S^\# EG^\#$

$M_{21} = F^\# - F^\# ES^\# + F^\# E(S^\# E - I)G^\# F^\# EG^\pi - F^\# E(S^\# E - I)G^\# F^\# ESS^\#$

$\qquad M_{22} = F^\# ES^\# EG^\# - F^\# EG^\#.$

Proof. (i) Let $rk(F) = r$, by Lemma 3.1, there exists Invertible matrices $P \in K^{n \times n}$, $F_1, G_1 \in M_{r \times r}(\mathbb{C})$ such that

$F = P \begin{pmatrix} F_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}, F^\# = P \begin{pmatrix} F_1^\# & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$

$G = P \begin{pmatrix} G_1 & 0 \\ 0 & 0 \end{pmatrix} P^{-1}, G^\# = P \begin{pmatrix} G_1^\# & 0 \\ 0 & 0 \end{pmatrix} P^{-1}$

Let $E = P \begin{pmatrix} E_1 & E_2 \\ E_3 & E_4 \end{pmatrix} P^{-1}, E_1 \in M_{r \times r}(\mathbb{C})$

It is easy to observe $rk(S) = rk(F^\pi E)$ if and only if $rk(E_3 E_4) = rk(E_4)$. So $E_3 = E_4 X$

The if part, Noting that $S^\#$ exists, we have by using Lemma 4.2.3 $rk(E_4) = rk(E_4^2)$

$rk(M) = rk \begin{pmatrix} E_1 & E_2 & F_1 & 0 \\ E_3 & E_4 & 0 & 0 \\ G_1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}$

$= rk \begin{pmatrix} 0 & 0 & F_1 & 0 \\ 0 & E_4 & 0 & 0 \\ G_1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}$

$= 2r + rk(E_4)$ and

$rk(M^2) = rk \begin{pmatrix} E^2 + FG & EF \\ GE & GF \end{pmatrix}$

$= rk \begin{pmatrix} E^2 + FG - EFF^\# E & 0 \\ 0 & GF \end{pmatrix}$

$$= rk \begin{pmatrix} F_1G_1 + E_2E_3 & E_2E_4 & 0 & 0 \\ E_4E_3 & E_4^2 & 0 & 0 \\ 0 & 0 & G_1F_1 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}$$

$$= rk \begin{pmatrix} F_1G_1 & 0 & 0 & 0 \\ 0 & E_4^2 & 0 & 0 \\ 0 & 0 & G_1F_1 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}$$

$$= 2r + rk(E_4^2).$$

We obtain $rk(M) = rk(M^2)$ that is $M^\#$ exists. As $M^\#$ exists, we know $rk(M) = rk(M^2)$,
so $rk(E_4) = rk(E_4^2)$, and hence $S^\#$ exists.

(ii) Let $M = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix}$ and $X = \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix}$.

$$MX = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix} \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix} = \begin{pmatrix} EM_{11} + FM_{21} & EM_{12} + FM_{22} \\ GM_{11} & GM_{12} \end{pmatrix}$$

$$EM_{11} + FM_{21} = E(S^\# + (S^\#E - I)G^\#F^\#ESS^\# - (S^\#E - I)G^\#F^\#EG^\pi) + F(F^\# - F^\#ES^\# + F^\#E(S^\#E - I)G^\#F^\#EG^\pi$$
$$\qquad - F^\#E(S^\#E - I)G^\#F^\#ESS^\#)$$
$$= E(S^\# + S^\#EG^\#F^\#ESS^\# - G^\#F^\#ESS^\# - S^\#EG^\#F^\#EG^\pi + G^\#F^\#EG^\pi) + F(F^\# - F^\#ES^\#$$
$$\qquad + F^\#ES^\#EG^\#F^\#EG^\pi - F^\#EG^\#F^\#EC^\pi - F^\#ES^\#EG^\#F^\#ESS^\# + F^\#EG^\#F^\#ESS^\#)$$
$$= ES^\# + ES^\#EG^\#F^\#ESS^\# - EG^\#F^\#ESS^\# - ES^\#EG^\#F^\#EG^\pi + EG^\#F^\#EG^\pi + FF^\# - FF^\#ES^\#$$
$$\qquad + FF^\#ES^\#EG^\#F^\#EG^\pi - FF^\#EG^\#F^\#EG^\pi - FF^\#ES^\#EG^\#F^\#ESS^\# + FF^\#EG^\#F^\#ESS^\#)$$
$$= ES^\# + FF^\# - FF^\#ES^\# = (I - FF^\#)ES^\# + FF^\# = F^\pi ES^\# + FF^\#$$

$$EM_{12} + FM_{22} = E(G^\# - S^\#EG^\#) + F(F^\#ES^\#EG^\# - F^\#EG^\#) = EG^\# - ES^\#EG^\# + FF^\#ES^\#EG^\# - FF^\#EG^\#$$
$$= EG^\# - EG^\# + FF^\#EG^\# - FF^\#EG^\# = 0$$

$$GM_{11} = G(S^\# + (S^\#E - I)G^\#F^\#ESS^\# - (S^\#E - I)G^\#F^\#EG^\pi)$$
$$= G(S^\# + S^\#EG^\#F^\#ESS^\# - G^\#F^\#ESS^\# - S^\#EG^\#F^\#EC^\pi + G^\#F^\#EG^\pi)$$
$$= GS^\# + GS^\#EG^\#F^\#ESS^\# - GG^\#F^\#ESS^\# - GS^\#EG^\#F^\#EG^\pi + GG^\#F^\#EG^\pi$$
$$= GS^\# + GG^\#F^\#ESS^\# - GG^\#F^\#ESS^\# - GS^\#EG^\#F^\#EG^\pi + GG^\#F^\#EG^\pi$$
$$= GS^\# - GS^\#EG^\#F^\#EG^\pi + GG^\#F^\#EG^\pi(Since GG^\# = FF^\#) = FS^\# - F^\#ES^\#EG^\pi + F^\#EG^\pi$$
$$= F^\#EG^\pi - F^\#ES^\#EG^\pi = F^\#(I - ES^\#)EG^\pi$$

$$GM_{12} = G(G^\# - S^\#EG^\#) = GG^\# - GS^\#EG^\#(Since GG^\# = FF^\#) = FF^\# - S^\#EFF^\# = FF^\#$$

Consequently,
$$MX = \begin{pmatrix} F^\pi ES^\# + FF^\# & 0 \\ F^\#(I - ES^\#)EG^\pi & FF^\# \end{pmatrix}$$

Similarly,
$$X = \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix} \text{ and } M = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix}$$

$$XM = \begin{pmatrix} M_{11}E + M_{12}G & M_{11}F \\ M_{21}E + M_{22}G & M_{21}F \end{pmatrix}$$

$$XM = \begin{pmatrix} F^\pi ES^\# + FF^\# & 0 \\ F^\#(I - ES^\#)EG^\pi & FF^\# \end{pmatrix}$$

$$(1) MXM = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix} \begin{pmatrix} F^\pi ES^\# + FF^\# & 0 \\ F^\#(I - ES^\#)EG^\pi & FF^\# \end{pmatrix}$$

$$= \begin{pmatrix} E(F^\pi ES^\# + FF^\#) + F(F^\#(I - ES^\#)EG^\pi) & 0 + F(FF^\#) \\ G(F^\pi ES^\# + FF^\#) + 0 & 0 \end{pmatrix}$$

$$= \begin{pmatrix} EF^\pi ES^\# + EFF^\# + F\left(F^\#(I - ES^\#)E(I - GG^\#)\right) & FFF^\# \\ GF^\pi ES^\# + GFF^\# & 0 \end{pmatrix}$$

$$= \begin{pmatrix} E(I - FF^\#)ES^\# + EFF^\# + F\left(F^\#(I - ES^\#)E(I - GG^\#)\right) & FFF^\# \\ G(I - FF^\#)ES^\# + GFF^\# & 0 \end{pmatrix}$$

$$= \begin{pmatrix} EFF^\# & FFF^\# \\ GFF^\# & 0 \end{pmatrix} = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix} = M$$

$$(2) XMX = \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix} \begin{pmatrix} F^\pi ES^\# + FF^\# & 0 \\ F^\#(I - ES^\#)EG^\pi & FF^\# \end{pmatrix}$$

$$= \begin{pmatrix} M_{11}(F^\pi ES^\# + FF^\#) + M_{12}F^\#(I - ES^\#)EG^\pi & M_{12}(FF^\#) \\ M_{21}(F^\pi ES^\# + FF^\#) + M_{22}F^\#(I - ES^\#)EG^\pi & M_{22}(FF^\#) \end{pmatrix}$$

$$= \begin{pmatrix} M_{11}(I - FF^{\#})ES^{\#} + M_{11}FF^{\#} + M_{12}F^{\#}(I - ES^{\#})E(I - GG^{\#}) & M_{12}FF^{\#} \\ M_{21}(I - FF^{\#})ES^{\#} + M_{21}FF^{\#} + M_{22}F^{\#}(I - ES^{\#})E(I - GG^{\#}) & M_{22}FF^{\#} \end{pmatrix}$$

$$= \begin{pmatrix} M_{11}FF^{\#} & M_{12}FF^{\#} \\ M_{21}FF^{\#} & M_{22}FF^{\#} \end{pmatrix} = \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix} = X$$

$(3)(MX)^{*} = MX$

$M = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix}, X = \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix}$

$E = \begin{pmatrix} \sum K & \sum L \\ 0 & 0 \end{pmatrix}$, where $KK^{*} + LL^{*} = I_r$

$(EM_{11})(EM_{11})^{*} + (FM_{11})(FM_{11})^{*} = I_r.$

$F^{\#}(I - ES^{\#})EG^{\pi} = 0$

$MX = \begin{pmatrix} F^{\pi}ES^{\#} + FF^{\#} & 0 \\ F^{\#}(I - ES^{\#})EG^{\pi} & FF^{\#} \end{pmatrix}$

$(MX)^{*} = \begin{pmatrix} F^{\pi}ES^{\#} + FF^{\#} & 0 \\ F^{\#}(I - ES^{\#})EG^{\pi} & FF^{\#} \end{pmatrix}^{*}$

$= \begin{pmatrix} (F^{\pi}ES^{\#} + FF^{\#})^{*} & F^{\#}(I - ES^{\#})EG^{\pi} \\ 0 & (FF^{\#})^{*} \end{pmatrix}$

$= \begin{pmatrix} F^{\pi}ES^{\#} + FF^{\#} & F^{\#}(I - ES^{\#})EG^{\pi} \\ 0 & FF^{\#} \end{pmatrix} (Since F^{\#}(I - ES^{\#})EG^{\pi} = 0)$

$= \begin{pmatrix} F^{\pi}ES^{\#} + FF^{\#} & 0 \\ F^{\#}(I - ES^{\#})EG^{\pi} & FF^{\#} \end{pmatrix} = MX$

$(6)XM^2 = \begin{pmatrix} F^{\pi}ES^{\#} + FF^{\#} & 0 \\ F^{\#}(I - ES^{\#})EG^{\pi} & FF^{\#} \end{pmatrix}\begin{pmatrix} E & F \\ G & 0 \end{pmatrix}$

$= \begin{pmatrix} (F^{\pi}ES^{\#} + FF^{\#})E + 0 & (F^{\pi}ES^{\#} + FF^{\#})F + 0 \\ F^{\#}(I - ES^{\#})EG^{\pi}E + (FF^{\#})G & (F^{\#}(I - ES^{\#})EG^{\pi})F + 0 \end{pmatrix}$

$= \begin{pmatrix} (I - FF^{\#})ES^{\#}E + FF^{\#}E & (I - FF^{\#})ES^{\#}F + FF^{\#}F \\ F^{\#}(I - ES^{\#})E(I - GG^{\#})E + (FF^{\#})G & F^{\#}(I - ES^{\#})E(I - GG^{\#})F \end{pmatrix}$

$= \begin{pmatrix} FF^{\#}E & FF^{\#}F \\ FF^{\#}G & F^{\#}(I - ES^{\#})(0) \end{pmatrix} = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix} = M$

$(7)MX^2 = \begin{pmatrix} F^{\pi}ES^{\#} + FF^{\#} & 0 \\ F^{\#}(I - ES^{\#})EG^{\pi} & FF^{\#} \end{pmatrix}\begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix}$

$= \begin{pmatrix} (F^{\pi}ES^{\#} + FF^{\#})M_{11} + 0 & (F^{\pi}ES^{\#} + FF^{\#})M_{12} + 0 \\ (F^{\#}(I - ES^{\#})EG^{\pi})M_{11} + FF^{\#}M_{21} & (F^{\#}(I - ES^{\#})EG^{\pi})M_{12} + FF^{\#}M_{22} \end{pmatrix}$

$= \begin{pmatrix} F^{\pi}ES^{\#}M_{11} + FF^{\#}M_{11} & F^{\pi}ES^{\#}M_{12} + FF^{\#}M_{12} \\ F^{\#}(I - ES^{\#})E(I - GG^{\#})M_{11} + FF^{\#}M_{21} & F^{\#}(I - ES^{\#})E(I - GG^{\#})M_{12} + FF^{\#}M_{22} \end{pmatrix}$

$= \begin{pmatrix} (I - FF^{\#})ES^{\#}M_{11} + FF^{\#}M_{11} & (I - FF^{\#})ES^{\#}M_{12} + FF^{\#}M_{12} \\ F^{\#}(I - ES^{\#})(EM_{11} - EGG^{\#}M_{11}) + FF^{\#}M_{21} & F^{\#}(I - ES^{\#})(EM_{12} - EGG^{\#}M_{12}) + FF^{\#}M_{22} \end{pmatrix}$

$= \begin{pmatrix} ES^{\#}M_{11} - FF^{\#}ES^{\#}M_{11} + FF^{\#}M_{11} & ES^{\#}M_{12} - FF^{\#}ES^{\#}M_{12} + FF^{\#}M_{12} \\ F^{\#}(I - ES^{\#})(0) + FF^{\#}M_{21} & F^{\#}(I - ES^{\#})(0) + FF^{\#}M_{22} \end{pmatrix}$

$= \begin{pmatrix} 0 + FF^{\#}M_{11} & 0 + FF^{\#}M_{12} \\ 0 + FF^{\#}M_{21} & 0 + FF^{\#}M_{22} \end{pmatrix} = \begin{pmatrix} FF^{\#}M_{11} & FF^{\#}M_{12} \\ FF^{\#}M_{21} & FF^{\#}M_{22} \end{pmatrix} = \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix} = X.$

Therefore by computation we can get $X = M^{\#}$.

In a similar manner, we obtain the following theorem:

Theorem 3.59Let $M = \begin{pmatrix} E & F \\ G & 0 \end{pmatrix}, E, F, G \in M_{n \times n}(\mathbb{C}), F^{\#}$ $exists,$ $R(F) = R(G), N(F) = N(G), S = F^{\pi}EF^{\pi}$ and $rk(S) = rk(EF^{\pi})$ $then$

$(i) M^{\#}$ if and only if $S^{\#}$ exists

(ii) If $M^{\#}$ exists then $M^{\#} = \begin{pmatrix} M_1 & M_2 \\ M_3 & M_4 \end{pmatrix},$

where $M_1 = S^{\#} + SS^{\#}EG^{\#}F^{\#}(ES^{\#} - I) - F^{\pi}EG^{\#}F^{\#}(ES^{\#} - I)$

$M_2 = G^{\#} - S^{\#}EG^{\#} + F^{\pi}EG^{\#} + F^{\pi}EG^{\#}F^{\#}(ES^{\#} - I)EG^{\#} + SS^{\#}EG^{\#}F^{\#}(AS^{\#} - I)$

$M_3 = F^{\#} - F^{\#}ES^{\#}$

$M_4 = FES^{\#}EG^{\#} - F^{\#}EG^{\#}.$

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A Study on the Performance of Physical Models of Permeable Screens in Meandering Reach of Alluvial Channels

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ABSTRACT

Riverbank protection using permeable spurs is necessity in many reaches of rivers where scouring lead to change of course. This work makes an effort to test the efficiency of various models of permeable structures (Porcupines) in the meandering portion of an experimental channel dug out at the field. Efficiency of such models relates to their relative abilities vis. a. viz., velocity reduction within the screen, sediment trapping in near bank, flow diversion towards the mid channel etc. Various models tested in this study are conceived by varying the dimensional parameters associated with them. The experimental results show that a permeable structure with a particular dimensional pattern/ arrangement cannot be expected to perform well for all the purposes sought out of it, such as; velocity reduction, flow diversion and sedimentation etc. Rather, a particular customized pattern may be chosen to suit the specific requirement of any effected reach of a river.

Keywords: Permeable Spur, bank protection, erosion control, flow diversion.

INTRODUCTION

A permeable structure or "Porcupine Screen" is a composite structure that is laid in systematic dimensional arrangement from an affected riverbank towards the river, both longitudinally as well as transversely to the bank. It consists of units called "members" which are clubbed together in three to four converging layers, together called "Spurs"; which in turn are laid in parallels to form the whole structure called "screen".

Porcupine Member: This refers to one unit within the structure, made up of six poles, bound prismatically with one another. RCC porcupines are the most widely used. The members are normally cast at the site or near it with the length of each pole being either 3.0m or 2.0m and with square c/s of 0.1m by 0.1m. The poles are bound together using nut and bolts, nails, wires, ropes etc. These members have extended portions of the poles, beyond their joints, that project outward in radial directions.

Spur: In such an arrangement, the porcupine members are installed in rows, each supporting the adjacent one installed before it. These rows start from deep inside the river bank into the mid channel; across the flow. About three to four rows of porcupine members struck to one another; comprise one spur. The number of rows depends on a lot of factor such as depth and velocity of flow, intensity and time period of occurrence of flood in the channel under consideration. These spurs normally project into mid channel up to about one seventh to one fifth of the channel width, that too again depending on flow depth and velocity.

Screen: provision of a single permeable spur for the purpose of erosion control, flow diversion etc. is generally not found effective in actual practice. The spacing of spurs in a screen depends on channel geometry, with straight reach requiring spacing between three to five times the lengths. On the other hand, for curved portion, the required spacing may vary between twice to thrice the lengths of the spurs, depending on channel curvature. Normally 4 to 6 rows of porcupines are used. This whole arrangement of spurs, laid in conjugate layers and suitably spaced from one another; is called a permeable screen.

The basic objective of this study was to first select a successfully performing porcupine screen along the river Brahmaputra and then test the efficiencies of different scaled down model configurations of the same prototype in an experimental channel. For this purpose, various model screens are conceived by varying the ratio of lateral spreads of individual spurs within them. These ratios are selected in such a way that they fall on either sides of the ratio of lateral spreads of the actual prototype selected. The criteria for study of performance of these model porcupine screens are selected as velocity reduction in near bank, flow diversion towards mid channel and sedimentation within the screen.

MATERIALS AND METHODS

Porcupine member
Prototype (RCC Porcupine)
An RCC porcupine member consists of six RCC poles, bound together by means of bolting at each of their ends, so that the ends protrude out radially. Depending upon the field requirements the length of each member may vary from 2m to 3m and cross section is 15cm×15cm or 10cm x 10cm.

Model
The porcupine models used in this study are prepared in reducing scale to match the dimensions of the prototype porcupine screen as laid in actual rivers. The models are prepared by MS nails of size 5cm in length and 0.5 cm in thickness which bound together and a layer of sand mixed with glue is pasted on the surface of the nail to replicate the surface of the concrete prototype. Extended lengths of 2cm for each member of the model are kept for embedding them into the simulated river bed in the field channel (Figure 1).

Bed material
The bed material was collected from the river Brahmaputra, in Jorhat, Assam, India (Location: 26.862115 N, 94.242466 E). After collecting the river bed material sample, they were air-dried for evaluating the particle size distribution. The bed of the field channel was filled with this collected sample up to a depth of 15 cm from the cut surface. This depth has been selected on the basis of trial runs in the channel without porcupine models with different discharges (that are to be used for various subsequent observations) and observing the scouring level for

such runs. A channel bed with a minimum thickness of 15 cm has been found to withstand significant scouring and subsequent exposure of the cut surface of the channel under any trial run.

The Field Channel
Prototype
The experimental channel is modelled from a stretch of river Bhogdoi, a tributary of River Brahmaputra, in Jorhat, Assam, India. The stretch consists of a straight portion followed by a sharp bend (Location: 26.736528 N, 94.271135 E) and is under severe erosion (Figure 2).

Model
All the experiments for this study were carried out in a field channel that was developed inside the campus of Jorhat Engineering College, Assam, India. The channel is about 27.0 m long with trapezoidal c/s, 1.0 m top width, 0.8 m bottom width and about 0.4m deep. Out of this 0.4m total depth of the channel, 0.2 m were kept available for flow, after preparing the channel bed by filling up the bottom 0.15m with collected bed materials and considering a freeboard of 0.05m. Two 15 HP pumps were installed to collect water from the nearby lake and feed the same into the experimental channel. The water from the pumps was first collected into a chamber, then goes through some energy dissipaters (steps) for reducing the turbulence of the flow before entering the main channel. A foot valve is installed at the bottom of the channel near its u/s face to regulate the quantity of water to be fed to the channel in order to maintain different depths of flow inside it. The d/s of the channel is again fed back to the lake. A steel trolley was installed to support the point gauge and the ADV; above the channel on the side walls (with rails on their tops), constructed on both the sides of the channel (Figure 3).

The modeling of the bend portion in the experimental channel is accomplished through trigonometric calculations on field survey data collected using Theodolite and Ranging on the actual river bend.

During modeling of the bend through scaling down its dimensions in the actual river stretch surveyed, the following ratios are kept constant for both the model as well as the prototype.

i)   (River width / Arc length of the bend) = (69.04 / 329.68) = 0.21 = X (say)
ii) (River Width / Outer radius of the bend) = (69.04 / 195.238) = 0.35 = Y (say)

Thus, for a channel bed width of 0.8 m, the corresponding arc length and outer radius of the channel bend was found to be 3.81 m and 2.28 m respectively. Similarly, the chord length of the channel bend can also be calculated for a chord with radius R and included angle A as under;

Chord length of the channel bend = 2Rsin (A/2) ----------------- (1.0)

For R = 2.28 m and A = 96.4⁰, we get the chord length of the channel = 3.40 m
These values are measured in the ground and the bend is established within the experimental channel

The Porcupine Screen:
Prototype
The Brahmaputra river bank line in and around Jorhat, Assam has been selected to pick on the prototype for the study. Screening of settelite imegary of the target area have revealed that in the year 2008, the river Brahmaputra extensively eroded the southern bank at Aphalamukh and reached almost up to Kamalabari Xatra, that lies along the ridge of Majuli island, However, after some porcupine screens were laid along that affected bank between 2008 and 2013, this rampant erosion was prevented gradually and reclaiming of land was visible from 2015 onwards. This prototype screen is thus taken as the one to be scaled down in to models for performance testing, due to its successful performance in both sediment trapping and flow diversion. The dimensional configurations of these model screens tested in the study are outlined in Table 1.

Model
For preparing the physical trial models of the screen (Table 2.1 and 2.2), the prototype has first been scaled down to fit in the channel and then the dimensional parameters associated with its lateral extend are varied to arrive at different model configurations. These model porcupine screens are installed in the field channel one by one and their performances in terms of parameters like sediment trapping in near bank, flow diversion towards the mid channel etc. are tested experimentally by introducing controlled flow in the channel. Following terminologies are also incorporated taking cue from a previous study [8] to describe various dimensional configurations associated with the screen models.

Field
 The porcupine "field" for different porcupine models is defined as the area that covers the length and breadth of the proposed screen model plus an additional area towards its u/s and d/s of length equal to the spacing between spurs and width equal to that of the nearest screen. The logic behind including the additional portion of the bed at the u/s and d/s face of the screen within the "field" is based on the presumption that some of the sediment load would be deposited on this area as the flow gets obstructed by the screen model.

Submergence Ratio
The term Submergence Ratio is defined by the ratio between the depths of flow within the field of the screen model to the height of the porcupines in the screen.

Observation Grid
An imaginary grid is proposed for the observation and recording of various data during experimentations. The grid lines of this mesh are proposed to be 20 cm apart along the flow and 10 cm apart across it, with intersections as "nodal points". This configuration is adapted to meet the dimensional constraints of the various trial screen models proposed to be tested in the study.

RESULTS AND DISCUSSIONS

Before every experimental run, prior to installing the porcupine models into the channel, the channel bed is leveled and free run was conducted for about 20 minutes and the water is allowed to discharge completely out of the channel, after the closure of the feed. After this, the ripple heights of the channel bed to be covered by the respective "field" of proposed porcupine model are measured by means of a point gauge. After this free run; the porcupine models of required combinations were installed and flow was again introduced by maintaining the depths required to achieve the desired "Submergence Ratios". All these S. R.s are achieved by varying the depths of flow in the channel using different combinations of pump discharge and foot valve positions. During each of such trial runs with different models of screens, the flow velocity data were taken using the ADV at upstream and downstream of every porcupine spur installed within the screen. All the measurements for ripple heights and flow velocity etc. are taken at the nodal points within the "Observation Grid" for the given "field". All the results obtained are tabulated in tables 3.1, 3.2 and 3.3 respectively.

All the experimental findings can thus be summarized in a tabular form as tabulated in Table 4.
Some definitive decisions can be arrived at from this study as presented below:-

1. The staggering pattern of porcupine screen, which is commonly practiced in the field; is not serving the very purpose of lying off those screens vis.-a.-viz, velocity reduction, flow diversion etc. Rather, this pattern hampers those purposes negatively only.
2. The effect of the porcupine screen was found to be more prominent with lower SR for all the criteria tested on the models.

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Table 1: Dimensional Configurations Of Prototype

Spur no. (Serially from East to West)	Length of individual spurs in meter (Source: Brahmaputra Board, Assam)	Relative ratios of individual lengths	Overall Staggering Ratio of Prototype Screen (L1: L2: L3: L4: L5)
1	1870 (L_1)	L1/L5 = 0.607	
2	2070 (L_2)	L2/L5=0.673	
3	2270 (L_3)	L3/L5=0.739	0.61: 0.67: 0.74: 0.84: 1.0
4	2570 (L_4)	L4/L5=0.837	
5	3070 (L_5)	L5/L5=1	

Table 2.1 Combination of Spurs For Screen Models In Terms of Lengths

Trial screen model no.s	Porcupine screen length in cm					submergence ratio (SR)
	1st Spur L_1	2nd Spur L_2	3rd Spur L_3	4th Spur L_4	5th Spur L_5	
1	10	20	30	40	50	
2	20	27.5	35	42.5	50	
3	30	32.5	37.5	42.5	50	3.0, 3.4 and 4.0
4	30	35	40	45	50	
5	40	42.5	45	47.5	50	
6	50	50	50	50	50	

Table 2.2. Combination of Spurs for Screen Models in Terms of Staggering

Trial screen model nos	Relative ratios of spur lengths in the screen models					submergence Ratio (SR)
	1st Spur L_1	2nd Spur L_2	3rd Spur L_3	4th Spur L_4	5th Spur L_5	
1	0.20	0.40	0.60	0.81	1.0	
2	0.40	0.55	0.70	0.85	1.0	
3	0.60	0.65	0.75	0.85	1.0	3.0, 3.4 and 4.0
4	0.60	0.70	0.80	0.91	1.0	
5	0.80	0.85	0.90	0.95	1.0	
6	1.0	1.0	1.0	1.0	1.0	

Table 3.1: Observation on Reduction of Flow Velocity In Near Bank

Trial	Percent Reduction in velocity for		
	SR = 3	SR = 3.4	SR = 4
Model 1	28.54	19.36	10.37
Model 2	29.29	21.27	14.63
Model 3	32.92	25.65	16.50
Model 4	39.76	30.89	18.76
Model 5	42.44	34.79	20.54
Model 6	46.22	36.11	20.88

Table 3.2: Observation on Increase in Velocity Outside Porcupine Field

Trial	Percent Increase in velocity for		
	SR = 3	SR = 3.4	SR = 4.0
Model 1	9.09	2.88	3.75
Model 2	22.72	6.89	4.17
Model 3	33.11	15.15	8.61
Model 4	9.03	18.66	17.72
Model 5	33.87	17.81	13.07
Model 6	52.70	25.80	17.42

Table 3.3: Observations on the Sediment Trapping Capacities within Field

Sl. No.	Model no	Submergence Ratio	Cum. Ht. of Ripples (mm)
1	MODEL 1	3	-1.0*
2		3.4	-1.0
3		4	-1.0
4	MODEL 2	3	2.22
5		3.4	1.81
6		4	0.0
7	MODEL 3	3	4.34
8		3.4	2.38
9		4	1.27
10	MODEL 4	3	5.36
11		3.4	2.38
12		4	1.27
13	MODEL 5	3	0
14		3.4	-3.97
15		4	-4.13
16	MODEL 6	3	3.97
17		3.4	3.11
18		4	1.19

(*: Negative sign represents loss of bed material from initial value)

Table 4: Effects of Dimensional Properties of Modelscreens on their Performance Criteria

Property of the Model Screen tested	Criteria studied		
	Velocity reduction in near bank	Flow diversion towards far bank	Sediment trapping within the field
Submergence Ratio	Inversely proportional	Inversely proportional	Inversely proportional
Degree of stagger	Inversely proportional	Inversely proportional	No effect

Figure 1. Model Porcupine

Figure 1A. Model Screen
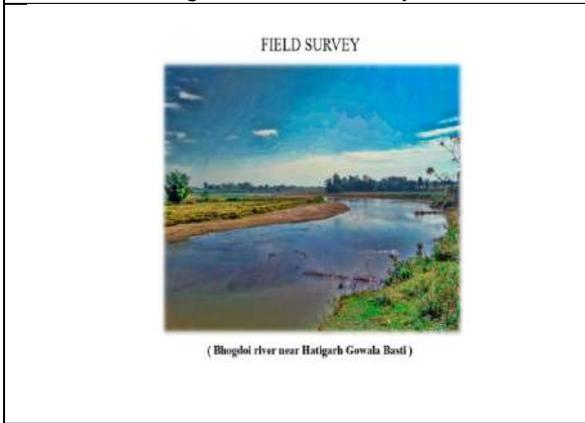
Figure 2. The stretch of the river (Prototype)

Figure 3. Model Field Channel

RESEARCH ARTICLE

Impact and Treatment Methods of Herbal Medicine for Alleviating Premenstrual Syndrome

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ABSTRACT

Premenstrual syndrome is a physical and psychological problem experienced by menstruating women. Due to several reasons, its incidence is increasing recently. Most women in their reproductive years experiences some premenstrual syndrome. The main objective of the present article is to review the potential treatment for PMS. Various treatment have been advocated for PMS. Herbal medicines can be used to cure this problem, and were used since ancient times. The present review aims to provide knowledge of premenstrual syndrome, its causes, and the use of herbals and their compounds to alleviate premenstrual syndrome. The aim of this review was to assess, effectiveness and safety of herbal medicines for treatment of premenstrual syndrome. Article Review was done using the electronic information bases like Pub Med, Scopus, Cochrane and Google Scholar. All articles were investigated to check for a considerable length of time on similar preliminaries.Literature research were performed in electronic databases, covering the period 2009-2019. Out of 30 articles identified and 12 articles were eligible and 6 of them were included.Many women with PMS use alternative therapies, despite the lack of established efficacy (Domoney et al., 2003). The review we studied included 26 trials that assessed the efficacy of herbal remedies for PMS. Trials assessing evening primrose oil produced conflicting findings, whilst insufficient data were found to advocate the use of *vitex agnus castus*, *gingko biloba* or St John's Wort. The current work presumes that botanicals are stronger and safer than synthetic medicines with practically no adverse effects to treat Premenstrual Syndrome. However, more studies are required to account for the heterogenicity of the syndrome .This review demonstrated efficacy and safety of herbal

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medicine in alleviating PMS. Therefore herbal medicine can be regarded as an alternative treatment for women suffering from PMS.

Keywords: : Premenstrual syndrome, Medicinal plants, herbal remedies, *Vitex agnus castus, Hypericum perforatum ,Gingko biloba*, Evening primrose oil, chamomile.

INTRODUCTION

Premenstrual Syndrome (PMS) is a chronic condition of menstruating women that is characterized through distressing physical, behavioral and psychological signs and symptoms that often recur during the lutealsection of the menstrual cycle (from ovulation to the onset of a duration) and that disappear or extensively lessen by way of the end of the length (menstruation)[1,2] . The menstrual cycle generally occurs month-to-month all through a lady's reproductive years as the lining of the womb (uterus) steadily grows in thickness and is shed on the period (menstruation).The common duration of the menstrual cycle (from the primary day of bleeding to the first of some other) is 28 days but that is best an average and might range substantially among ladies. Also, a man or woman might also discover that the duration of her menstrual cycle varies from month to month. The unique reasons of PMS have nonetheless to be identified however there's compelling proof that signs and symptoms are directly associated with the fluctuation of hormone tiers within the monthly cycle. As PMS is absent before puberty (onset of menstruation), in being pregnant and after the menopause there is clear indication that cyclical ovarian process is an important component in explaining PMS .Common symptoms are: tiredness, fatigue or lethargy, feeling out of control, aggression, anger, sleep disorder, food cravings, depression, anxiety, mood swings, irritability, breast tenderness, bloating, weight gain, clumsiness, headaches.

The path physiology of PMS have no longer been hooked up and hypotheses protected hormone imbalances, sodium retention, nutritional deficiencies, strange neurotransmitter responses to everyday ovarian function and atypical hypothalamus pituitary-adrenal axis functio[5]. Many synthetic drugs are available commercially to deal with PMS, however these are involved in inflicting destructive effects and for that reason the necessity of use of medication from nature has become essential. Several plants are known for use to deal with PMS since historical time and has won importance in recent years. Herbal remedy has turn out to be a popular sector of healthcare. Even though numerous variations exist between natural and conventional pharmacological remedies, natural medicinal drug can be examined for efficacy the usage of conventional trial method. Several particular natural extracts have been validated to be efficacious for precise conditions. Even though the general public is regularly misled to trust that each one herbal remedies are inherently secure, herbal medicines do bring dangers. Ultimately, we want to know which herbal remedies do extra harm than true for which situation. Because of the current reputation of herbal medicinal drug, studies on this place must be intensified. The most commonly prescribed medications are SSRIs, based on the hypothesized role of serotonin, which can be taken solely in the Luteal phase . However, women with PMS are often reluctant to take SSRIs, partly due to the side effects produced . Therefore, many women turn to herbal remedies to treat their symptoms [3,6,7]. Herbal remedies may be very effective as a natural alternative to ease menstrual cramps, and other PMS symptoms. Various herbal remedies have been proposed to treat PMS. Ladies use spices and other conventional and reciprocal modalities to treat different diseases for the duration of their life circle. Various herbs in the treatment of premenstrual syndrome (PMS), nausea and vomiting in the first trimester of pregnancy and menopausal hot flushes. Fundamental information support the adequacy of Chaste tree organic product (*Vitex agnus*) in the treatment of PMS, Ginger (*Zingiber officinale*) in the treatment of hyperemesis gravid arum and (*Cimicifuga racemosa*) in the treatment of menopausal hot flushes [8].

Natural agents efficiency in treating Pre Menstrual Syndrome and their action

Chamomile

Chamomile utilized as a viable natural medicine. Eligible investigations were recognized from English and Persian information bases, distributed somewhere in the range of 1990 and 2019.Chamomile has been utilized to treat PMS alleviation as a result of remedial properties like calming impacts (Chamazulene and α-Bisabolol); hostile to uncontrollable impacts (Apigenin, Quercetin, and Luteolin, Metoxicomarin, Matrisin, and Phytoestrogens); against tension impacts (Glycine, Flavonoid).women with PMS are frequently hesitant to take SSRIs, incompletely because of the secondary effects delivered .Chamomile tea has against convulsive properties, which can diminish the agonizing spasms related with the feminine time frames compound named Apigenin found in chamomile tea decreases the effect of excitatory synapse and chemicals on the brain and body, serving to soother the over-terminating thoughtful sensory system. Chamomile tea additionally balances the activities of dopamine and serotonin, assisting with counterbalancing or possibly diminish the effect of burdensome manifestations. Chamomile concentrate can be similarly pretty much as powerful as Mefenamic Acid(MA) when it comes to relieving pain and regulating emotion[11 ,32].

Primrose oil

The helpful adequacy of evening primrose oil (Efamol, Vita-Glow) in the alleviation of 10 side effects related with premenstrual disorder (PMS) just as feminine indications .evening primrose" is containing an important fixed oil. Evening primrose oil has two sorts of omega-6-unsaturated fat including linoleic corrosive (60%-80%) and γ-linoleic corrosive (8%-14%). Fundamental unsaturated fats are considered as fundamental mixtures for body wellbeing, particularly among women. The study was ready by examination in logical articles of electronic assets .Linoleic corrosive advances prostaglandin amalgamation and lightens PMS. The consequences of clinical examinations on the viability of evening primrose oil on ladies 'wellbeing showed its helpful potential for PMS, hot flashes, gestational diabetes, and cervical ripening[16,32].

Chasteberry

Vitex agnus castus or chasteberry is a shrub that originated in Western Asia and Mediterranean. It is widely used in Europe for premenstrual syndrome. It contain a number of essential oils flavonoids and iridoids. It has been proved to increase luteinizing hormone and decrease follicle stimulating hormone levels to increase progesterone and decrease estrogen levels[32]. Adverse effects include acne, early menstrual cycle and gastric upset. Even though it is helpful in decreasing symptoms like headache, sleeplessness etc. It improves both PMS and PMDD. It is used as complementary agent along with serotonin reuptake inhibitors. IIT alleviate stress-induced secretion of prolactin. Patients administered chasteberry extract showed improvement in symptoms when compared to that of placebo. But bloating is not treated by *Vitex agnus* [12,13,15,32].

St. John's Wort

Active constituent of *Hipericum perforatum* is hyperforin. It helps in inhibiting reuptake of serotonin. Only minor side effects are produced by this agent. St John's wort alleviates both physical and behavioral symptoms of PMS. There is no significant effect on pain related symptoms. More than PMS it is also used for PMDD. It is helpful in patients who experience side effects due to administration of selective serotonin reuptake inhibitors[18,19,26,32].

Gingko biloba

Ginkgo leaf extract comes from the oldest living tree species in the international. Useful for improving reminiscence, ginkgo has also been evaluated as an extract which can enhance PMS. Ginkgo leaf extracts comprise many lively materials inclusive of flavonoids and terpenoids. Gingko leaf flavonoids have antioxidant and free radical scavenging properties. Ginkgo also inhibits platelet-activating factor and has anti inflammatory consequences. Some constituents can also loosen up vascular smooth muscle. Ginkgo increases blood circulation by keeping the balance of prostacyclins and thromboxane A2. It also is an inhibitor of monoamine oxidase, which catabolizes many neurotransmitters. In addition, it increases the discharge of catecholamines and different neurotransmitters and decreases their reuptake, thereby improving mood and depressive signs and symptoms.

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The flavonoids in ginkgo inhibit the making of inflammatory prostaglandins and therefore produce an anti-inflammatory impact. This could give an explanation for the relief of congestive signs and symptoms inclusive of edema. abdominal bloating, and mastalgia[20].

Ginger

Ginger is usually used as herbal remedy and historically it used to deal with dysmenorrhea . *Zingiber officinale* is the scientific name of plant Ginger . Ginger rhizome is utilized in traditional remedy . Studies confirmed useful consequences of ginger on vomiting, nausea, motion sickness, arthritis, migraine, headache. Ginger can be the right remedy in decreasing signs of premenstrual syndrome. One of the proposed mechanisms that result in premenstrual syndrome is change in prostaglandin system. Ginger via the inhibition of the metabolism of cyclooxygenase and lipoxygenase prevents the production of prostaglandins. Primary dysmenorrhea is due to immoderate manufacturing of prostaglandins from the endometrial tissue. Because some ginger compounds are prostaglandin inhibitors and are powerful on dysmenorrhea, so possibly through this mechanism they could be powerful on different problems of menstruation cycle. Some of signs of PMS like pain are not unusual with dysmenorrhea (consisting of backache and stomach ache). These signs of PMS have been reduced. Studies confirmed that ginger has anti-inflammatory properties and is powerful in treatment of ache in patients with osteoarthritis, muscle ache . Joint and muscle ache also are signs of PMS; ginger turned into effective in relieving severity of those symptoms of PMS. It is a complex combination of pharmacological compounds containing numerous hundred acknowledged parts, which includes gingerols, beta-carotene, capsaicin, caffeic acid, curcumin [31].

METHODOLOGY

Article Review was led using the electronic information bases like Pub Med, Scopus, Cochrane and Google Scholar. Copy articles, or articles which were irrelevant to the investigation are not included .Reference segment of important preliminaries, precise audits and meta-examinations were checked to perceive further preliminaries missed by electronic inquiry. All articles were investigated to check for a considerable length of time on similar preliminaries. We performed a search period from 2009-2019.The search term include : premenstrual syndrome treatment, alternative treatment, herbal treatment, phytomedicine primrose oil, St. Johns wort, *vitex agnus castus*. Ginkgo biloba. In light of the query items, creators browsed the edited compositions and pertinent records. Full articles were recovered for all possibly huge preliminaries

RESULT

The reviews studied were all written in English and published between 2009 to 2019. Many of the studies shows the result containing. One recent German study of over 1600 women experiencing PMS, showed 93% had a reduction or cessation of their PMS symptoms, after taking chaste tree. Ozgoli G et al., conducted PMS studies on students lived in dormitories of a medical university (Tehran). The result concluded that there was reduction in physical and psychological symptoms after the use of *Ginkgo biloba*. Canning et al.,investigated the effectiveness of *Hypericum perforatum* on symptoms of PMS in UK. They concluded that treatment with *Hypericum perforatum* was more effective than placebo for the most common physical and behavioral symptoms associated with PMS[32]. Systematic literature searches were performed in electronic databases, covering the period January 1980 to September2010. Randomized controlled clinical trials (RCTs) were included. Papers quality was evaluated with the Jadad' scale. A Further evaluation of PMS/PMDD diagnostic criteria was also done .Of 102 articles identified, 17 RCTs were eligible and 10 of them were included. The heterogeneity of population included ,Study design and outcome presentation refrained from a meta-analysis. *Vitex agnus castus* was the more investigated remedy(four trials, about 500 women), and it was reported to consistently ameliorate PMS better than placebo. Single trials also Support the use of either *Gingko biloba* or *Crocus sativus*. On the contrary, neither Evening primrose oil nor St. John Wort Show an effect different than placebo. None of the herbs was associated with major health risks, although the reduced number Of tested patients

does not allow definitive conclusions on safety .Some herb remedies seem useful for the treatment of PMS. However, more RCTs are required to account for the Heterogeneity of the syndrome.(22)

DISCUSSION

Elective medication has been generally utilized in the therapy of premenstrual syndrome. Our audit expected to survey the home grown clinical medicines for PMS.In the audit on the viability and security utilization of explicit natural drugs, phytomedicine like *Vitex agnus castus* (VAC) have been demonstrated of a few adequacy for PMS .when utilized in different spice recipe *Hypericum perforatum* impacts the serotonergic framework and stifles professional fiery cytokine levels .It shows to be a viable treatment for misery which is one of the side effects of disposition related PMS manifestations. Cell reinforcement property of *Ginkgo biloba* prompts its display of remedial movement in congestive manifestations of premenstrual disorder . *Ginkgo biloba* L. is wealthy in flavonoid glycoside and terpenelactone . Bioflavonoid, a functioning element of *Ginkgo*, is known as a pressure modulator which clarifies the use of *Ginkgo* as an anxiolytic medication for PMS . Henceforth, treatment focusing on these components might apply their advantages in PMS by rectifying hidden dysfunctions. This audit shows a wide range of conventional treatment techniques which isn't restricted to one strategy along these lines giving a superior thought of what's in store in treating PMS, with customary medication. Likewise, by inspecting the best treatment techniques for explicit manifestations by auditing the pace of improve sorted by side effects, this survey might be utilized as a rule in treatment strategy determination for the different happening indications customized to every PMS patient (32).

CONCLUSION

The current work presumes that the botanical are more strong with practically no incidental effects to treat Premenstrual Syndrome.This review highlights the effect of herbs in the management of PMS although further studies are needed to investigate this effect. What's more there is shortage of information accessible on the adequacy and security of a large number of these mixtures. Along these lines, it gives an open door to the isolation and portrayal of new dynamic standards and their method of activity associated with treating Premenstrual Syndrome.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare no conflicts of interest

ABBREVIATION

PMS ; Premenstrual Syndrome
VAC: *Vitex agnus castus*
PMDD: premenstrual Dysphoric Disorder
SSRIs:Selective Serotonin Reuptake Inhibitor
MA :Mefenamic Acid

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RESEARCH ARTICLE

Analysis into Socket Preservation and Suturing Techniques

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ABSTRACT

The successful dental implant supported oral rehabilitation required long term biological integration of fixtures with the surrounding tissue as well as establishment of an ideal implant for esthetic results the aim of socket preservation is to preserve the future implant and suturing for better healing.140 university records have been analyzed in this study, data was collected through university data setting and transformed to SPSS and the data was analyzed and the results were prepared. Age, gender, suturing technique and site of implant placement are the parameters analyzed and compared to analyze the results. The most prevalent suturing technique was interrupted suture about 50% followed by horizontal mattress, interrupted suture more commonly prevalent in quadrant 2 undergoing socket preservation. Socket preservation by proper suturing is very useful for future implant prosthesis placement. Interrupted suturing technique is the most commonly used technique in quadrant 2 and more commonly chosen by the operators for its benefits.

Keywords: Extraction, Implant, socket, suture, healing, innovation

INTRODUCTION

A dental implant is one of the treatments to replace missing teeth. Their use in the treatment of complete and partial edentulism has become an integral treatment modality in dentistry. Dental implants have a number of advantages over conventional fixed partial dentures are they have a high success rate and a decreased risk of caries and endodontic problems of adjacent teeth and decreased sensitivity of adjacent teeth. A dental implant is a structure made of alloplastic materials implanted into the oral tissues beneath the mucosa and periosteum and within or through the bone to provide retention and support for a fixed or removable dental prosthesis (1). Alveolar socket preservation has been used to describe "a technique in which completely contained extraction sockets are filled with a bone substitute material and/or sealed with membranes, whereas in alveolar ridge preservation, damaged extraction sockets are also included (2). Conversely, some review articles consider the terms interchangeable and mainly use alveolar ridge preservation, which has been described as "any procedure developed to eliminate or limit the negative effect of post extraction resorption, maintain the soft and hard tissue contour of the ridge, promote bone formation within the socket, and facilitate implant placement in a prosthetic ally driven position (3). The term alveolar ridge preservation will be used throughout this article, and the reviewed literature will involve intact or mild-to-moderately damaged extraction sockets, rather than severely compromised sockets.The primary objective of dental suturing is to position and secure surgical flaps to promote optimal healing. When used properly, surgical sutures should hold flap edges in apposition until the wound has healed enough to withstand normal functional stresses. When the proper suture technique is used with the appropriate thread type and diameter, tension is placed on the wound margins so primary intention healing occurs (4).Accurate apposition of surgical flaps is significant to patient comfort, hemostasis, reduction of the wound size to be repaired, and prevention of unnecessary bone destruction. If surgical wound edges are not properly approximated and are therefore inadequate, hemostasis is present and blood and serum may accumulate under the flap, delaying the healing process by separating the flap from the underlying bone (5). Types of suturing technique included in this study were horizontal mattress, vertical mattress, continuous, interrupted. The aim of the study is to analyze the socket preservation after surgery phase of implant and its suturing techniques.

MATERIALS AND METHODS

This study is a retrospective cross sectional study conducted among patients in a private Dental Institution, Chennai It included demographic data of all patients along with the clinical history of the individual.

Data collection
We reviewed the case record of 140 patients. A customized examination form was used to collect the data and a special table for data collection was prepared. The data collected was classified according to age, gender, site of the suture placement, type of the suturing technique. Dependent variables were sites of suture placement and types of suturing techniques and independent variables were age and gender. Data collected was recorded in an excel sheet which was later transformed to SPSS version 20.0 for statistical analysis. Descriptive statistics and Chi square test was used to determine the correlation between the variables where P value < 0.05 is considered statistically significant. The pros were easy availability of data and the Cons were small sample size and restricted geography.

RESULTS AND DISCUSSION

In this study, about 66% of them were male patients and 34% of them were female patients. 65% of the patients were between 18-30 years of age, 37% of them were 50-70 years of age group. In this study the most commonly implanted site was quadrant 2(54%), followed by quadrant 1(23%). Most commonly used suturing techniques were interrupted suturing techniques(71%), followed by horizontal mattress(24%), vertical and continuous suturing techniques were about 4%. Socket preservation shows better healing in interrupted Suturing techniques(71%), and is most commonly

placed in quadrant 2(39%), followed by quadrant 1(16%). Interrupted suturing techniques were more common, unplaced among male patients(47%) than females.In the study of Patel et al (6) examined success and survival rates with maxillary and mandibular teeth within the region anterior to the premolar teeth. This study consisted of 27 patients randomly assigned into two groups. Fourteen extraction sockets were grafted with Straumann Bone Ceramic (SBC) and 13 were grafted with Bio-Oss; both groups were covered with a resorbable collagen membrane (Bio-gide). Both groups had implant placement at eight months post extraction. At 12 months they had 100% survival and similar success rates according to the criteria of Albrektsson –84.6% in the SBC group and 83.3% in the Bio oss group. In the study of Nortan et al, they observed high degrees of survival and success over observation periods of two to seven years (7). Interestingly in two of these studies, the grafting procedure failed to maintain ridge dimensions to the extent that no simultaneous augmentation was required at implant placement. In fact, 74% of implants placed in 42 sites anterior to the premolar areas required further grafting procedures at surgical placement (8). Therefore, socket grafting does not necessarily eliminate the need for further augmentation at implant placement. On the contrary, in more than half of the cases in the studies reported above, a second augmentation was necessary at implant placement, which can substantially increase the cost and complexity of treatment delivery.Three randomized studies were identified which compared socket grafting and delayed implant placement with immediate implant placement (9) Both treatment modalities appeared to have similar implant treatment outcomes after osseointegration, although Felice et al (10) reported increased difficulty in achieving high insertion torque in sockets grafted with Bio Oss four months post extraction. In this study 75% of implants placed in the grafted sockets did not manage to attain an initial torque of 35 Ncm compared to 35% in the immediate group. This suggests that the quality of the regenerated bone in the socket at four months was still soft. This concurs with manufacturer guidelines and suggests that implants placed into grafted sockets may have a decreased insertion torque compared to native bone if placed too early. This may be regarded as a suboptimal outcome, particularly if the prosthetic plan involves immediate or early loading/temporization as any form of occlusal load beyond the capacity of the degree of primary stability may lead to fibrous scar tissue at the implant interface instead of bone resulting in early failure (11). In socket preservation procedure, the main goal is to preserve the bone volume by filling the socket with the material substitutes. After the extraction process of wound healing has occurred that may result in loss of ridge dimension, compromising ideal imp,any replacement. The greater the width and ,entha that can be used, resulting in better cervical esthetics prognosis and case of oral hygiene procedures. Even though a continuous suturing technique is less time consuming, the failure of any part of suture may result in loosening along its whole length. Horizontal mattress and interrupted suturing technique are recommended for a guide bone regeneration procedure. Our team has extensive knowledge and research experience that has translate into high quality publications(12–20),(21–26),(27–31)

CONCLUSION

The present study indicates the 2nd quadrant to be the most common site undergoing socket preservation (55%). Interrupted suture was the most popular technique while treating for socket preservation in quadrant 2 (38%).

AUTHORS CONTRIBUTION

Vinaya Swetha.T :Literature search, data collection, analysis, manuscript drafting.

Dr. Suresh Venugopal: Data verification, manuscript drafting.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

All the authors declare that there was no conflict of interest in present study.

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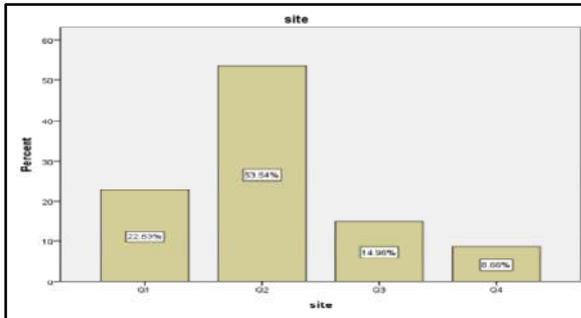
Figure 1: The bar chart depicts the site of suture placement after implant surgery. The X axis denotes the site of suture placement and Y axis denotes the percentage. Suture placed in quadrant 2 were about 53.4%.
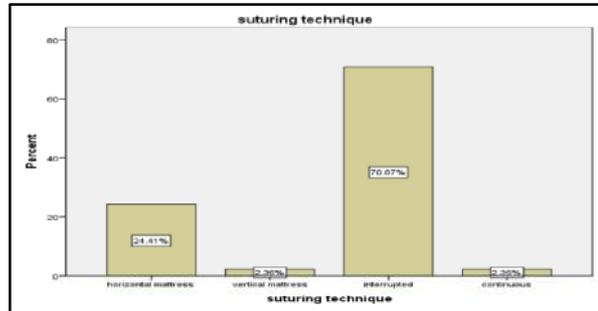
Figure 2: The bar chart depicts the percentage of suturing techniques used after implant surgery. The X axis denotes the suturing technique and Y axis denotes the percentage. Interrupted suturing technique is the most commonly used of about 70.8%.
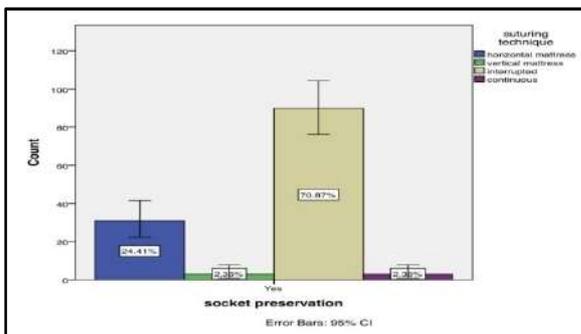
Figure 3: The bar graph depicts the correlation between socket preservation and suturing technique. X axis denotes the socket preservation and Y axis represents the suturing technique.
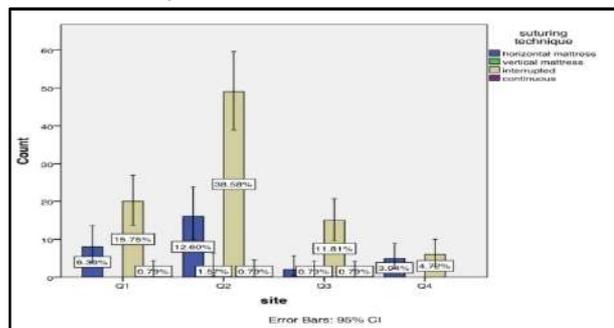
Figure 4: The bar graph depicts the correlation between site of the suture placement and it's technique. X axis denotes the sutured site and Y axis represents the suturing technique.

RESEARCH ARTICLE

Effect of Microplastics on Soil Microorganisms

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ABSTRACT

The widespread presence of micro plastics (MPs) in the environment has increased interesting the interaction between MPs and microorganisms. The diversity and characteristics of MPs in the environment make their interactions with microorganisms more complex. Micro plastics have contaminated all ecosystems as a result of widespread use and poor handling of plastic materials, but the subsequent fate and remediation strategy of these pollutants still poorly understood. Microorganisms, on the other hand, are sensitive to micro plastics due to their adaptability to changes in substrates. Microorganisms play an important role in plastic biodegradation through metabolic and mineralization processes. MP toxicity to microorganisms has been discovered, with the main effects of MPs on microorganisms being the effect on microbial growth, photosynthesis of microorganisms and some single cells. We present the most recent research on MP interactions with microorganisms and summaries their potential environmental impacts. The interaction mechanism and influencing factors between microorganisms and MPs are discussed in this review.

Keywords: Micro plastic (MP), United Nations Environment Programs (UNEP)

INTRODUCTION

Plastic is a type of polymer material with high chemical stability and plasticity that is widely used in all aspect of our lives, including packaging, clothing, construction and construction, automotive manufacturing, telecommunications and agricultural production (Alassali et al., 2020).With the increase in plastic waste over the last decades, plastic pollution is rapidly becoming a serious global eco-environmental problem. Plastic debris can be reduced by external forces to form micro plastic (MP, with a particle diameter of 5 mm) pollutants, which have been named by the United Nations Environment Programs as one of the top environmental concerns (UNEP, 2014). Microplastics are typically

defined as particles smaller than 5 mm in size that have far more harmful physical and chemical properties to the ecosystem than large plastic waste (Thompson et al., 2004).Micro plastics are classified into four types based on their size: macroplastic (>2.5 cm), mesoplastic (5 mm ~ 2.5 cm), micro plastic(large:1 ~ 5 mm, small:1 mm ~ 1 mm) and nanoplastic (<1 mm) (Van Cauwenberghe et al., 2015). MPs are small in size, have a large specific surface area, have strong hydrophobic, and have relatively stable chemical properties, allowing them to survive in the environment for hundreds to thousands of years (Lambert and Wagner, 2016). Micro plastics are classified as granules, fragments, films, pellets, fibers, and foams based on their shape. Because of their small size and bioretention capacity, MPs spread more quickly than traditional plastics (Rochman, 2018; Rillig and Lehmann, 2020). They enter the food chain and are subsequently consumed by a diverse range of living organisms at various trophic levels (Kane et al., 2020; Farrell and Nelson, 2013). Some plastic degradation products, particularly amines and phthalate esters, have been identified as carcinogenic, mutagenic, or toxic to reproduction (Espinosa et al., 2020; Xu et al., 2019), posing serious ecological risks in terms of biodiversity loss and ecosystem function changes. (Eerkes-Medrano et al., 2015; Amaral-Zettler et al., 2020).In fact, the harm caused by MPs to microorganisms in the soil and water environment has been widely reported in recent years. The study of the effect of MPs on microorganisms has primarily focused onemphasised the effects on microbial communities and degradation MPs produced by microorganisms (Bandopadhyay et al., 2018; Sunet al., 2018a; Yang et al., 2018).Microorganisms are an essential component of the natural environment. The interaction between MPs and microorganisms increases with the release of plastics, and its impact on the environment cannot be ignored. However, the complexity of the interaction between plastics and microorganisms is determined by the diversity of MPs and the number of diverse microorganisms. Despite ongoing research, the potential toxic effects of MPs on microorganisms in the environment, as well as whether microorganisms can be used as a solution to MPs pollution, remain poorly understood. The toxicity of MPs to microorganisms, the degradation and uptake of MPs by microorganisms, and the interaction between MPs and microorganisms are all discussed in this paper.

Effects of microplastics in soil

The ubiquity of MPs has become a growing source of concern. As a result, numerous efforts have been made to evaluate their potential effects on the environment and living organisms. The impact of MPs on living organisms is generally caused by the following factors: i) uptake or ingestion of MPs by living organisms and their accumulation in the food chain; ii) adsorption of other pollutants onto the surface of MPs; and iii) toxicity induced by plastic additives. Aside from these, MPs alter soil properties in the terrestrial environment, which may affect the natural habitat of soil organisms as well as plant performance(Boots et al.,2019; Pignattelli et al., 2020).MPs in soil pose a high risk of being transported to and accumulating in terrestrial plants, which are a common source of food for humans. As a result, studying MPs in agricultural soil is critical for the benefit of both the ecological environment and human health.

The effects of MPs on soil microbial habitats

According to the bibliometric analysis, research on MPs in soil environments is still in its early stages, and more extensive studies are needed to investigate the subsequence of MPs in soils. Because of a diverse range of sources and the degradation resistance of most plastics, soils may retain a variety of MPs(Guo et al., 2020; Zhu et al., 2019).A survey was conducted on existing soil-MPs literatures to determine the main MP sources, concentrations, and characteristics associated with different land uses. The results reveal that (1) different locations and land uses have significant differences in MP level and characteristics; and (2) agricultural soils may suffer the most serious MP pollutions due to widespread use of sewage, sludge, and plastic mulches. Furthermore, global soil environments are vulnerable to MP pollution caused by external force factors (e.g., water, wind, or animal transport)(Li et al., 2019; Koutnik et al., 2021). However, whether in soils or transport processes, MPs can age, resulting in embrittlement, cracking, weakening, and chemical group changes over time(Ng et al., 2018; Maity and Pramanick,2020).These processes are carried out through physical, chemical, and biological reactions, which have an impact on soil properties and further microbial habitats.

MP-induced physical changes of habitats

Micro plastics can disrupt the original physical parameters of the soil and have an impact on the water cycle(Rillig and Lehmann, 2020).MPs also affect soil porosity because some MP residues or fragments can disrupt soil pore continuity(Jiang et al., 2017), whereas other particles or powders can easily fill the space of soil pores(Kim and An, 2019).These soil pore changes affect water cycling elements like evaporative or gravitative movement, as well as soil pore water content. Some types of MPs also have an effect on soil aggregation, a critical process that governs soil structure and shapes the habitat of soil microorganisms. Changes in MP aggregates can thus affect soil pore space, water cycling, gas fluxes, and the associated microorganisms(Rillig and Lehmann, 2020). Aggregates, soil organic matter, and even MPs will be protected from rapid decomposition due to their physical protection(Rillig and Lehmann, 2020; Six et al.,1998). Furthermore, changes in soil physical parameters (e.g., water and air content) will cause indirect chemical and biological changes in microbial habitats.

MP-induced chemical changes of habitats

Considering that plastics are chemical compounds that coexist with additives such as antioxidants, flame retardants, plasticizers, and pigments(Bardaji et al., 2020), MPs may leach monomers, oligomers, and additives into soil chemical environments. However, due to the inertness and degradation resistance of most plastics, MPs do not readily or directly release elements that disrupt soil nutrient conditions(Qi et al., 2019). Changes in soil nutrients, the release of toxins, and changes in soil pH all endanger the health of the soil's microbial communities.

Response of soil microorganisms to microplastic

Despite the fact that MPs provide colonization sites, harmful effects on microbes, and other synergistic effects, microorganisms also affect MPs. The effect is mostly evident in the degradation of MPs by microorganisms, accumulation of MPs by microorganisms, and ingestion and sedimentation of MPs by microorganisms. Microorganisms colonized on MPs are more likely to use MPs as a source of nutrition, especially those that can use carbonyl as a carbon source, allowing them to breakdown MPs(Paco et al., 2017;Syranidou et al., 2017b).The production of high cell density biofilms on the polymer surface appears to be linked to the breakdown of MPs by microorganisms. Roughness and hydrophilicity may be crucial characteristics that drive the interaction between MPs and bacteria. Highly adherent cells have an advantage over less adherent cells when MPs are utilised as a food source(Mercieret al., 2017). The effect of nutrient addition on degradation, when compared to merely natural biofilms, glucose accelerates MP degradation by attached biofilms. *Deinococcus-thermus* is the most common bacteria discovered in natural biofilms, according to research. The addition of glucose raises the relative abundance of other bacteria, such as Cyanobacteria, which speeds up the breakdown of MPs to some extent(Shabbir et al., 2020).

Microbial degradation of micro plastics

Microbial degradation is a cost-effective and environmentally acceptable way of removing pollutants from the environment(Singh and Singh, 2016).MPs can be used as a carbon source by the organisms, which then aids in their degradation. MP degradation in the environment is an integrated process influenced by both environmental and biological factors(Paço et al., 2017).Microbial degradation can greatly affect the transformation of MPs. Several bacteria, fungi, and some microbial consortia capable of degrading MPs have been identified from both aquatic and terrestrial ecosystems. Bacteria such as *Bacillus cereus*,*Bacillus gottheilii, Rhodococcus sp.,* are some examples of bacteria which can degrade different types of micro plastics. Degradation by these bacteria also demonstrated their ability to change the appearance of MPs, as well as their functional groups and other properties(Auta et al., 2017a).The low efficiency of naturally occurring plastic-degrading microorganisms emphasizes the need for more effective degrading agents. Genetically modified enzymes and microorganisms are also being developed to improve the efficiency and rate of plastic degradation. PET polymers have been successfully degraded using genetic engineering(Ribitsch et al., 2013; Tournier et al., 2020). Thus, only a few microorganisms' enzymes and degradation pathways for plastic degradation have been identified and characterized. It is critical to understand the microbial degradation pathways for plastic in order to develop highly efficient plastic-degrading microorganisms. As a result, met genomic analysis of microorganisms involved in plastic degradation is an important field of study for understanding and evaluating the potential for improvements in plastic microbial degradation(Purohit et al., 2020).

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CONCLUSION AND FUTURE PERSPECTIVES

The review gives an account of the occurrence, sources, and effects of MPs in the terrestrial environment and the microorganisms present on it anda chance of MP microbial degradation for overcoming this prevalent problem. Recently, there has been a significant increase in the number of studies evaluating the potential for MP contamination in soil, and our understanding of MPs in the terrestrial environment has progressing. There is clear evidence of MPs' harmful effects on various aspects of the terrestrial ecosystem, including soil properties, microbial diversity, and all types of life forms, but many inconsistencies in MP effects have been observed across studies. The variation in the effects induced by MPs can be attributed to MP types (in terms of shape, size, and composition), soil physicochemical properties, and the organisms under observation. As a result, there is still a significant amount of research needed to assess and standardize the MP effects in relation to different parameters. The biodegradation of MPs by microorganisms can be expanded as a research area in the near future, as several microorganisms capable of absorbing and degrading MPs are being discovered. There is also a need to broaden the scope of research on various aspects of MPs, such as their fate, transport, and degradation, from the laboratory to the field scale. Given the current state of MP contamination in the environment, it is also important to implement appropriate regulatory measures to control this widespread contaminant.

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RESEARCH ARTICLE

In-vitro Analysis of *Clerodendrum inerme* as Potential Agent for Psoriasis Management

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ABSTRACT

Herb *C. inerme (L.)* have commonly used medicine since ancient times for treatment of various diseases. Psoriasis is chronic inflammatory skin disease affecting nearly about 2- 5 % of the world population all over world, but due to its persistent occurrence now it became a challenging task for medicinal field to develop safe treatment for management of psoriasis. The present research work deals with extraction, preliminary phytochemical investigation, *In-vitro* antioxidant, anti-inflammatory and antiproliferative activity of ethanolic extract of the leaves of *C. inerme* (L.). Collection and authentification plant *C. inerme (L.)*, extraction of leaves of with soxhlet extraction method using ethanol as solvent. Preliminary phytochemical investigation, *In-vitro* antioxidant, anti-inflammatory, antiproliferative potential was estimated by DPPH, Protein denaturation and MTT assay (A-431 cell line) respectively with standard procedures. The results of preliminary phytochemical investigation of *C. inerme* (*L.*) ethanolic extract shows presence of alkaloids, carbohydrates, proteins flavonoids, tannins and phenolic compounds as phytoconstituents. The TPC and TFC was found 59.23 ± 1.05 mg/g GAE and 36.87 ± 0.45 mg/g QE respectively. Ethanolic extract shows strong antioxidant activity 60.93 ± 1.11 % (Std. Ascorbic acid), anti-inflammatory 50.84 ±1.33 % inhibition at 5 mg/ml (Std. Aspirin), Antiproliferative 26.78 ±1.20% (Std. 5-FU).This research work reports potential of *C. inerme* (*L.*) extract as antioxidant, anti-inflammatory, and antiproliferative agent.

Keywords: Psoriasis, DPPH, Anti-inflammatory, Antiproliferative

Ravindra Ganpati Gaikwad and Anilkumar Jalindar Shinde

INTRODUCTION

In the recent decade, Psoriasis is a chronic inflammatory, oxidative and hyperproliferative skin cells disorder, characterized with the formation of silvery red scaly dry patches [1]. Due to the increased prevalence of psoriasis World Health Organization (WHO) recommended to healthcare professionals to increase awareness among peoples about this disease [2-3]. Although psoriasis is an immune-mediated disease number of genetic and environmental factors are also associated with the formation of Psoriasis. This disease can catch both the genders but some reports suggest that it may be predominant in males than the females.

Current available therapy for psoriasis management includes use of systemic, topical and phototherapy, which are having various serious, and life threatening side effects. Topical corticoids have side effects such as cutaneous atrophy, development of strai decolorizing and thinning of skin, Tarazone having cutaneous irritation [1]. Systemic therapy includes use of methotrexate, cyclosporine, acetrin and antibody therapy having side effect such as bone marrow toxicity, nephrotoxicity and cardio toxicity etc, so the development of newer and selective antipsoriatic agents is a need of time [2]. Herbal formulations used since ancient times for the management of various diseases. Herbs contain secondary metabolites; a rich source of various active medicaments which are in clinical use. Number of phytochemicals demonstrated their prominent significance against psoriasis.

Clerodendrum inerme belongs to the family Verbenaceae found in southern region of India, this plant is used as a medicine to treat cough, scrofulous infection, buboes problem, venereal infection, skin diseases, psoriasis (leaf paste), scabies, ringworm infection vermifuge, beriberi and for ornamental purposes [5]. With respect to isolated phytochemicals of the genus *Clerodendrum*, aerial parts, roots and leaves were the most common targets of investigation for bioactive principles and most of these compounds were reported from *C. serratum*, *C. inerme*, *C. bungei*, *C. infortunatum*, and *C. trichotomum*. Diterpenoids, flavonoids, phenylethanoid glycosides, and steroids are abundant and major bioactive principles of this genus [4,6].

MATERIAL AND METHODS

Chemicals
Aluminium chloride, ethyl acetate, chloroform and ethanol were purchased from Loba chemical, Mumbai, Maharashtra, Butylatedhydroxytoluene (BHT), Trolox, gallic acid and quercetin were purchased from Sigma-Aldrich Mumbai, Maharashtra, India. 2, 2-Diphenyl1-picrylhydrazyl (DPPH), molybdate reagent, dimethyl sulfoxide (DMSO), Methanol, Molychem Mumbai, Maharashtra, India. All the chemicals and reagents used were of analytical grade. A431 cell line purchased from NCCS, Pune.

Plant collection, authentication, and extraction
Leaves of plant *Clerodendrum inerme* (*C. inerme)* were collected in December 2020 from Bagani, Maharashtra. The herbarium sheet was prepared and submitted to Botanical Survey India, Western Regional Centre, Pune. Leaves of plant *C. inerme* were shade dried for 8 days and pulverized with a laboratory grinder. The leaves were extracted using ethanol as a solvent with the soxhlet extraction method. Ethanolic extract was dried under reduced pressure using Rotavap (Heidolph, Germany). The resultant extract was stored at -20°C till further procedure. Preliminary phytochemical screening was performed to detect secondary metabolites in the *C. inerme* ethanolic extract as per the standard procedure.

Preliminary phytochemical investigation
The preliminary phytochemical evaluation of the extract was performed using standard tests procedures.

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Total Phenolic Content (TPC) Determination

The Folin-Ciocalteu reagent (FC) method was slightly modified to determine the total phenolic content of *C. inerme* ethanolic extract [7]. In brief, 200 µl of ethanolic extract was mixed and incubated for 10 minutes with an equal volume of FC reagent, mixture was then neutralised with 1.25 mL of aq. sodium carbonate (Na_2CO_3) and incubated at 37 °C for 45 minutes with occasional shaking to develop colour. Double distilled water (DW) was used as control. The TPC of the extract determined by using UV-Vis spectrophotometer (UV-2700i/ 2600i, Shimdzu, Japan) detecting the blue colour at 765. For calibration of method gallic acid was used as reference standard. The TPC of the extract calculated from the linear equation of calibration curve of GA, in terms of mg/g of gallic acid equivalent (GAE) of dry extract.

Total Flavonoid Content (TFC) Determination

The TFC of *C. inerme* ethanolic extract was estimated as per previously reported method [8]. Briefly, ethanolic extract 0.5 mL was mixed with same volume of 2% aluminium chloride ($AlCl_3$) then the above mixture incubated for 1 hour at room temp. Sample analysis was carried out at 420 nm by measuring absorbance using UV-Vis spectrophotometer (UV-2700i/ 2600i, Shimdzu, Japan), Quercetin was used as reference standard, and TFC was calculated as mg/g quercetin equivalent (QE). The results were carried out in triplicate.

In-Vitro Antioxidant Activity

DPPH method was used to measure the antioxidant potential for the *C. inerme* ethanolic extract for free radical scavenging activity [9, 10]. A microtiter plate was filled with 100µL of varying concentration of ethanolic extract (200, 400, 600, 800, 1000 ug/ml). Methanolic DPPH (100µL of 0.1 %) was added to the samples and samples were incubated for 30 minutes in the dark. The samples were then examined for colour change, discoloration ranging from purple to yellow and pale pink were regarded strong and weak positives, respectively, and the plate was read at 490nm on an Elisa plate reader. Radical scavenging activity calculated with the following equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100

In-vitro anti-inflammatory activity

In-vitro anti-inflammatory potential of *C. inerme* ethanolic extract determined with the protein denaturation method [11, 12]. The reaction mixture (2 mL) consisted of 0.1 mL 5 % aqueous Bovine serum fraction, 1.8 mL of (PBS, pH 6.4) and 0.1 mL of aspirin (5mg/ml). Double-distilled water served as control in similar volume. In the step mixture was heated for 30 min at 57°C followed by incubation at (37°C ±2) in incubator for 15 min. After cooling, absorbance was measured at 660 nm by using vehicle as blank. Aspirin was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula [13].

% inhibition = absorbance of control - absorbance of test / absorbance of control x100

Anti-proliferative

Anti-proliferative potential of *C. inerme* ethanolic extract was determined with MTT assay method on A-431 with standard protocol [14, 15, 16]. *C. inerme* ethanolic extract was examined for cytotoxicity study with MTT assay method. Triplicate samples were analyzed by measuring the absorbance of each sample with the microplate reader at 550 nm.

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RESULT AND DISCUSSION

Plant collection authentication and extraction

The plant was identified by Dr. Priyanka Ingle's BSI, WRI, Pune, (BSI/WRC/100-1/Tech./2020/96), total yield of crude ethanolic extract reported in (Table 1).

Preliminary phytochemical investigation

Preliminary phytochemical analysis of *C. inerme* ethanolic extract shows presence secondary metabolites (Table 2).

Total Phenolic Content (TPC) Determination

The TPC of *C. inerme* ethanolic extract was determined with FC reagent method and expressed as mg/g of (GAE). The TPC obtained in ethanolic extract was 59.23 ± 1.05 mg/g GAE. The result calculated using linear regression equation of gallic acid (Figure 1)

Total Flavonoid Content (TFC) Determination

Total flavonoid content (TFC) of *C. inerme* ethanolic extract was found to be 36.87 ± 0.45 mg/g QE quercetin (Figure 2).

In-vitro Antioxidant activity

The pathophysiology of psoriasis is complex and dynamic where oxidative stress is the major cause for the same [1]. Antioxidants which present in plants act as antioxidant agent by inhibiting or preventing oxidative stress. Herbs contain antioxidant compounds such as poly phenols, flavonoids and phenolic compounds. In the current research, we have determined the free radical scavenging activity of *C. inerme* ethanolic extract. Phenolic and flavonoid components found to be present in *C. inerme* ethanolic extract. The *C. inerme* ethanolic extract showed significant antioxidant potential antioxidant (free radical scavenging) activity of *C. inerme* ethanolic extract at the concentration of 200-1000 ug/ml ethanolic extract of plant *C. inerme* shows promising antioxidant potential (Figure 3). The extract found to show dose dependant antioxidant activity. The pathophysiology of psoriasis is complex and dynamic where oxidative stress is the major cause for the same.

In-vitro anti-inflammatory activity

In the process of protein denaturation proteins lose their structure with the application of external stress or strong of weak chemicals, a concentrated inorganic salt, an organic solvent or heat. Majority of the proteins lose their function (Biological) when they denatured [12, 13]. Inflammation occurs due to the denaturation of the proteins and it is well known cause for th same. In this research the anti-inflammatory action of *C. inerme* ethanolic extract to inhibit protein denaturation was determined. *C. inerme* ethanolic extract was effective for inhibiting heat induced albumin denaturation. Maximum inhibition of *C. inerme* ethanolic extract was observed at 500 µg/ml 50.84 %. A standard anti-inflammatory drug Aspirin showed the maximum inhibition 77.11 % at the concentration 500 µg/ml compared with control (Figure 4).

Anti-proliferative activity (MTT Assay)

Studies of *in-vitro* antiproliferative efficacy of *C. inerme* ethanolic extract was studied using A-431 cell lines have been carried out as this cells show significant hyperproliferative and impaired differentiation as like psoriatic epidermal keratinocytes cells. [14, 15]. The *C. inerme* ethanolic extract of plant shows 56.51 % ±1.31 at 250 of cell viability as compared with standard 5-FU which shows 35.59 % ±1.31 at 250 ug/ml (Figure 5). As compared with standard 5-FU *C. inerme* ethanolic extract shows antiproliferative effect. IC 50 value for *C. inerme* ethanolic extract was found to be 277 ug/ml.

CONCLUSION

This study reports potential of *C. inerme* ethanolic extract as antioxidant, anti-inflammatory, and antiproliferative activity. *C. inerme* ethanolic extract leaves contain phytoconstituents like alkaloids, flavonoids, tannins, steroids, and phenols which are responsible for its strong antioxidant and anti-inflammatory activities. The ethanolic extract of plant *C. inerme* (*L.*) shows strong antioxidant, anti-inflammatory and antiproliferative activity which will be promising for the management of psoriasis. Oxidative stress, inflammation, and proliferation of skin cells are underlying cause of psoriasis. Hence, this will be a promising candidate for future research in case of psoriasis treatment.

ACKNOWLEDGEMENTS

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Table 1: Percentage yield *C. Inerme* ethanolic extract

Sr. No	Sample	Solvent (ml)	Weight sample taken(g)	Weight of extracts obtained(g)	% yield(w/w)
1.	*C. inerme* Leaf powder	Ethanol	50	9.26	18.52

Table 2: Phytochemical screening of plant extract

Sr. No.	Test	Ethanolic extract
1	Alkaloids (Wagners test)	+
2	Carbohydrates	+
3	Glycosides (Legals test)	+
4	Steroids (Salkowski test)	+
5	Flavonoids	+
6	Glycoside	-
7	Proteins	+
8	Tannins and phenolic compounds	+

(+ Present, - Absent)

Figure 1: Calibration curve using gallic acid in *C. inerme* ethanolic extract for TPC determination

Figure 2: Calibration curve using quercetin in *C. inerme* ethanolic extract for determination of TFC
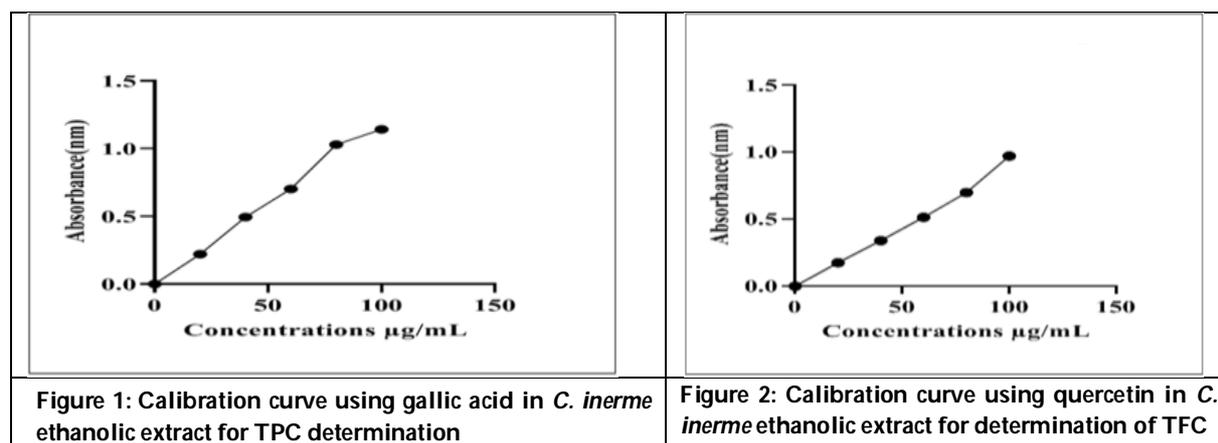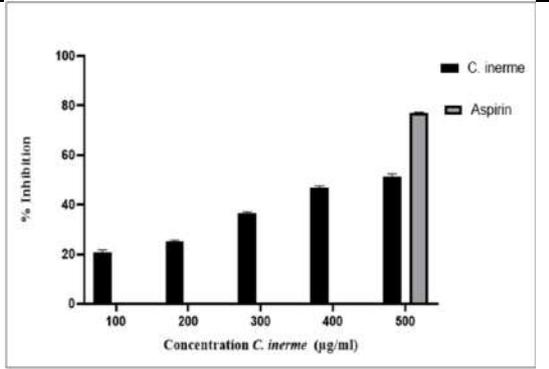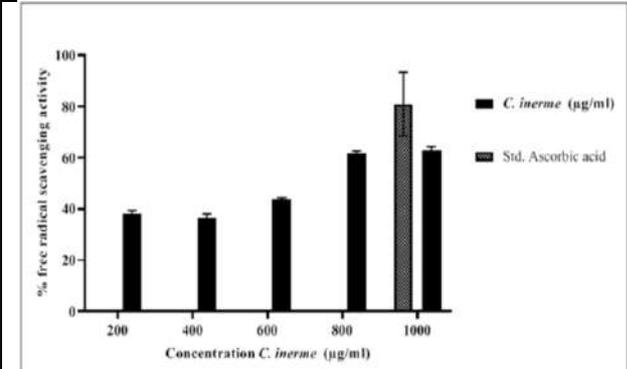
Figure 3: Free radical antioxidant activity of *C. inerme* ethanolic extract (DPPH method)

Figure 4: Anti-inflammatory activity of *C. inerme* ethanolic extract (Protein denaturation method)
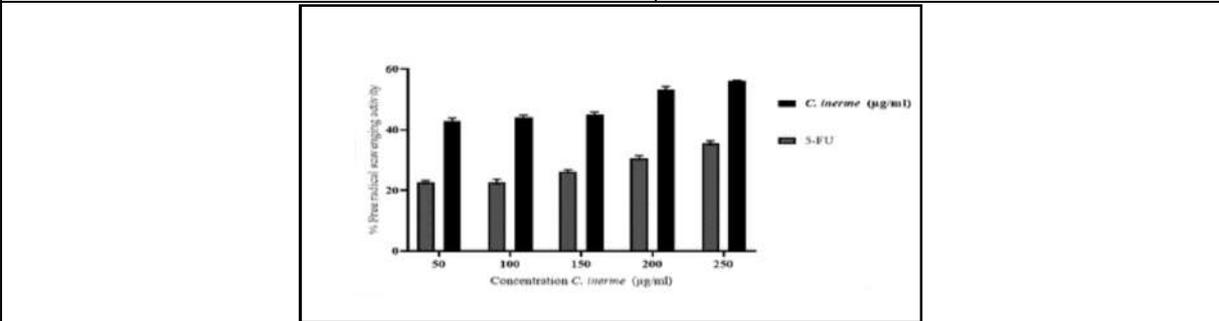
Figure 5: Antiproliferative activity of *C. inerme* ethanolic extract (A431 cells)

RESEARCH ARTICLE

Experimental Study on Usage of Wood Ash in Concrete

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ABSTRACT

This paper is aimed to present the properties of concrete by using wood ash as a mineral admixture and partial substitute for OPC 53 grade Portland cement. The properties examined are compressive strength, dynamic modulus of elasticity, splitting tensile strength, ultrasonic pulse velocity, and flexural strength. The partial substitution levels of wood ash are 0%, 10%, 15%, and 20%. Wood ash concrete results are compared with the results of M30 grade concrete. A good relationship between average compressive strength, ultrasonic pulse velocity,dynamic modulus of elasticity, and flexural strength is observed. Concrete with wood ash up to 15% showed better strength results than conventional concrete.

Keywords: Pozzolanic, Sustainable concrete, Eco-friendly concrete, Wood ash, Dynamic elastic modulus, Filler material.

INTRODUCTION

In the present years, the increase in the energy demand and its insecurity augmented the utilization of renewable energy and its related resources. The most common energy resources are agricultural and forestry wastes or biomass resources are promising renewable energy resources with a continuous regeneration, economical and easy in handling nature. These resources are considered neutral energy resources by considering their consumption rate to growth rate. Further, the effective utilization of the by-products such as hard chips, sawmill scraps, wood bark, wood chips, and sawdust in the production of energy entrusts an effective method of handling these by-products. The combustion of these by-products reduces the volume and mass thus providing economically safe and environmentally friendly solid waste management. In most industries, the chief source of energy for maintaining small-scale boiler units is wood or wood waste [1-3].Wood ash (WA) is a waste fine powder obtained after the burning of waste wood particles such as bark, sawdust, and chips. In general, wastes from wood related sources are

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used as combustion fuel in producing heat energy for industrial and household purposes. The physicochemical properties of WA primarily depend on the species of the wood, incineration temperature, supplementary fuels used, and the efficiency of the boiler. On average, the incineration of wood produces nearly 6% to 10% of ashes [3-5]. The viability of use of WA in concrete is governed by its chemical compounds. The major compounds found in WA are magnesium (1% - 2%), carbon (4% - 34%), manganese (0.3% - 1.3%), potassium (3% - 4%), calcium (7% - 34%), sodium (0.2% - 0.5%), and phosphorus (0.3% - 1.4%). Some traces of molybdenum, copper, zinc, and boron are also found at per million levels. Typically, WA also consists of SiO_2, $CaCO_3$, and $K_2Ca(CO_3)_2$ which confirms the alkaline nature and will have a positive effect on the hydration process of cement [5]. In this study, an effort is made to experimentally study the viability of the use of WA as mineral admixture and partial substitute to cement for producing sustainable and eco-friendly concrete. The properties examined are compressive strength, dynamic modulus of elasticity, splitting tensile strength, ultrasonic pulse velocity, and flexural strength. Also, to identify the optimum partial substitution level of WA in concrete. The experimentally obtained results are compared with the results of M30 grade concrete. For this aim, the locally available WA is employed in the present research.

MATERIALS AND METHODS

Table 1 shows the mix proportions of concrete. Locally available OPC 53 grade Portland cement in agreement with IS 12269:2013 [6]with a specific gravity of 3.15 is used. The water used in mixing the concrete constituents is potable and is collected from the laboratory water grid which is free from organic materials and suspended solids. Crushed coarse aggregate with 20 mm of maximum size is used in accordance with IS 383: 2016 [7] with specific gravity and fineness modulus of 2.86 and 6.82. Clean river sand is obtained from the local resources and collected from the Krishna river. WA is collected from a hotel central cooking system in Guntur city. M30 grade concrete is designed as per IS 10262: 2019 [8]. Figure 1 shows the locally available WA that is used for the experimental study. The tests conducted on WA concrete are shown in Table 2. The constituents of concrete are mixed homogeneously by an electrically operated pan mixer and then the freshly mixed concrete is shifted into beams (50 x 10 x 10 cm), cubes (10 cm), and cylinders (20 cm in height and 10 cm diameter). The fresh concrete samples are left undisturbed for 24 hours and are then transferred into the water for curing. A computerized universal testing machine is used for obtaining the strength tests results. The UPV tests equipment has two transducers with 54 kHz capacity is used for obtaining the UPV values and E_d. A set of fresh concrete samples are shown in figure 2.

RESULTS AND CONCLUSIONS

Compressive Strength
Table 3 presents the three concrete cubes that are cast and tested for each average compressive strength value and the average compressive strength results (W0, W10, W15, and W20). The compressive strength of W0, W10, W15, and W20 at the age of 7 days is 28.62, 33.88, 34.19, and 23.91 MPa, respectively. The average compressive strength of W10 and W15 are increased by 18.38% and 19.46% when compared with the W0 mix. At 28-days, the compressive strength results of W0, W10, W15, and W20 are 42.17, 46.14, 48.07, and 37.85 MPa, respectively. From Table 3, it is noticed that the average compressive strength of W10 and W15 are increased by 9.41% and 13.99% when compared with the W0 mix. The highest compressive strength is observed for the W15 mix. This is justified as WA particles act as filler material in the cement paste and enhance the density of concrete. The highest rate of gain of compressive strength is found at 7 days of curing duration for the W15 mix. This could be due to the reactive nature of amorphous silica and alumina that are present in WA.For mix W20, the strength is decreased by the addition of WA. This may be due to the dilution effect of WA in concrete and the lessening in the cement content.

Splitting Tensile Strength
Three concrete cylindrical specimens are cast and tested at 28-days for each splitting tensile strength result and are presented in Table 3. For the W0 concrete, the splitting tensile strength is 3.42 MPa. For W10, W15, and W20 the splitting tensile strength obtained is 3.65, 3.41, and 3.29 MPa, respectively. The highest splitting tensile strength is

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observed with W10 concrete mix and a negligible loss is observed with W15 concrete mix. The gain of strength shows the positive reactive nature and filling capability of WA in concrete. Further, the improvement in the interaction transition zone of concrete also improves with the addition of filler material or WA. The reduction in strength of W15 and W20 mixes may be attributed to the poor bondingby WA in the cement matrix and also by the high surface area of WA. For the W20 mix, the decrease in the strength may be due to the increase in WA content that affects the chemical composition of the cement matrix.

Flexural Strength

Table 3 shows the average flexural strength results obtained at 28-days. For W0 concrete the average flexural strength is 4.66 MPa. With the addition of WA, for W10, W15, and W20 the flexural strengths obtained are 5.39, 5.55, and 4.65 MPa, respectively. It is apparent that the average flexural strength results of W10 and W15 are increased by 15.67% and 19.10% when compared with the W0 concrete. From Table 3, the results show that the addition of WA in concrete improved flexural strength. The improvement in theflexural strength results of WA blended concrete may possibly be by the filling potential and pozzolanic reactivity of WA in concrete and also to the densification of the cement matrix. For the W20 concrete mix, the flexural strength is 99.79%of W0.Figure 4 shows the correlation between the compressive and flexural strength results.

Dynamic Elastic ModulusandUltrasonic Pulse Velocity

Table 4 presents the UPV and E_d test results. These properties mainly depend upon the modulus of elasticity, density, soundness, curing, and aging of concrete. The setup of UPV testing is shown in figure 3. According to IS 13311 (Part 1): 1992 [9], UPV test results of above 4.5 km/s designate the excellent quality of concrete. All concrete mixes (W0, W10, W15, and W20) exhibited excellent quality concrete. This signifies that the WA concrete does not have any voids or cracks that may lead to damage tothe internal microstructure of the concrete. The correlation among the average compressive strength results and UPV values is shown in figure 5. The E_d results are calculated using UPV test results and as per IS 13311 (Part 1): 1992 [9]. The E_d value of W0 is 52.49 GPa. The E_d values of W10, W15, and W20 are increased by 16.04%, 14.62%, and 4.04%, respectively. The highest E_d value is observed for W10 concrete of 60.91 GPa. At all the partial substitution levels of WA in concrete, E_d results of WA concrete are higher than the E_d result of W0 concrete. The development of E_d results of WA concrete signifies that the WA has the good filling ability and improves the density of concrete. The correlation between E_d and UPV test results is shown in figure 6.

CONCLUSIONS

Considering the findings obtained from the present experimental study, the conclusions are drawn are:
1. WA concrete is prepared from the locally available WA resources. This directly helps in decreasing the quantity of use of cement and indirectly helps in reducing the consumption of raw materials that are used for producing cement.
2. It is evident that the addition of WA in concrete improved dynamic elastic modulus, flexural strength, and ultrasonic pulse velocity.
3. The optimum substitution level of WA in concrete is found to be 15% with no adverse effects on the strength properties. This helps in producing sustainable and eco-friendly concrete.
4. At 20% WA, the strength results of WA concrete are reduced due to the raise in water to binder ratio and also by the reduction of cement content.
5. All concrete mixes (W0, W10, W15, and W20) exhibited excellent quality concrete with UPV values above 4.5 km/s.

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Table 1: Concrete mix proportions

S.No.	Mix ID	Wood ash(%)	Wood ash	Cement	Natural Sand	Crushed Coarse aggregate	Water (lts)
1.	W0	0%	0.0	420.0	634.2	1184.4	168
2.	W10	10%	42.0	378.0	634.2	1184.4	168
3.	W15	15%	63.0	357.0	634.2	1184.4	168
4.	W20	20%	84.0	336.0	634.2	1184.4	168
*The constituents are in (kg/m^3)							

Table 2: Tests conducted on WA concrete

S.No.	Standard/Reference	Name of the test
1.	IS 516: 1959 [10]	Compressive strength
2.		Flexural strength
3.	IS 5816: 1999 [11]	Splitting tensile strength
4.	IS 13311 (Part I): 1992 [9]	Ultrasonic pulse velocity
5.	IS 13311 (Part I): 1992 [9]	Dynamic elastic modulus

Table 3: Average strength results of WA blended concrete

S.No.	Mix ID	Average compressive strength (MPa)		Flexural Strength (MPa)	Splitting Tensile Strength (MPa)
		@ 7 days	@ 28 days	@ 28 days	@ 28 days
1.	W0	28.62	42.17	4.66	3.42
2.	W10	33.88	46.14	5.39	3.65
3.	W15	34.19	48.07	5.55	3.41
4.	W20	23.91	37.85	4.65	3.29

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Table 4: E$_d$ and UPV values of WA concrete

S.No	Mix ID	UPV (km/s) @ 28 days (quality)	E$_d$ (GPa)
1.	W0	4.97 (excellent)	52.49
2.	W10	5.20 (excellent)	60.91
3.	W15	5.12 (excellent)	60.16
4.	W20	5.00 (excellent)	54.61

Figure 1: WA used for experimental study

Figure 2: A set of fresh concrete specimens

Figure 3: Setup of UPV testing
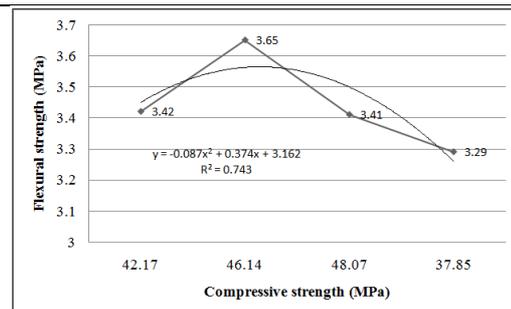
Figure 4: Correlation among compressive and flexural strength results
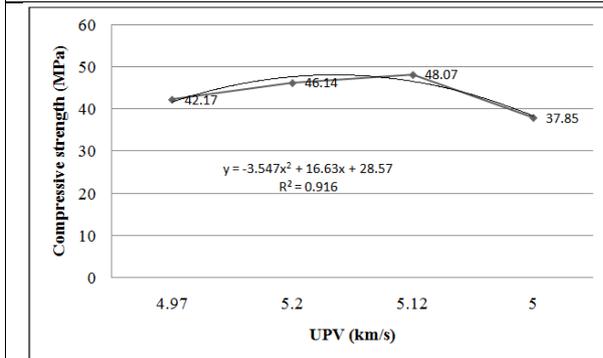
Figure 5: Correlation among UPV values and average compressive strength
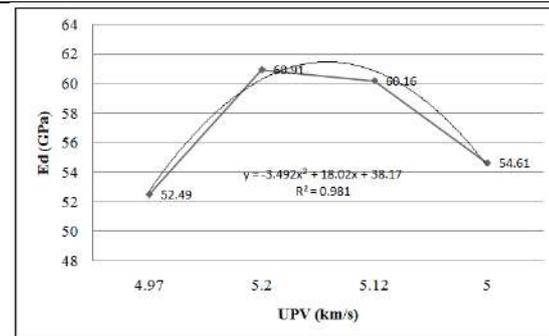
Figure 6: Correlation among UPV and E$_d$

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RESEARCH ARTICLE

A Study on the Influence of Covid19 Pandemic on Mutual Fund Investment with Reference to Bangalore

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ABSTRACT

The main aim of this research paper is to understand how COVID 19 has affected investor's preferences on investing in mutual fund. This Research paper also aims to analyze their willingnessto invest in mutual fund during pandemic. Certain measures were taken to overcome pandemic issues such as wearing masks, using sanitizers, maintaining social distance & shutting down of institutions. The mutual fund sector is booming in overall economy but still during pandemic investors has to face certain challenges. Mutual funds pools money from different investors and same money will be invested in securities such as bonds, stocks & short term debt; hence this combination of holdings is called as portfolio where investors can buy shares in mutual fund. In Mutual fund sector we have more diversified way of investing money where as in this sector investment will be diversified in to different investments in a professional method. The research is carried out by both primary and secondary data. Secondary data was collected through AMFI website. Government has to make certain changes based on the preferences and needs of the investors. Hence it helps investors to be aware about the challenges faced during pandemic.

Keywords: Pandemic, Mutual Fund, Portfolio & Investment avenue,

INTRODUCTION

The episode of the profoundly irresistible COVID-19 through and through upset human life. Measures to fight the pandemic included social eliminating, self-disengagement, shutting down of establishments and establishments,

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restricting strategies for transport, and cross country lockdowns. The COVID-19 pandemic has been seen as a justification for productive bet, subsequently, its impact on money related trades ought to be examined. There are Five extraordinary phases of overall monetary emergency: 1) the beginning of the subprime contract calamity; 2) the expansion of recognize risk for mounting hardships of financial establishments; liquidity crunch; 4) the product esteem air pocket; and 5) a conclusive freeze of credit markets.Enormous assessment thought has also been paid with the effects of the COVID-19 pandemic on gold expenses, crude petrol costs, digital forms of money and market documents. Regardless, the effect of the COVID-19 on individual monetary sponsor approach to acting is a locale that remains fairly under-investigated. An Investment is an asset or thing obtained completely plan on makingpay on appreciation. According to a monetary point of view, a hypothesis is the securing of genuinethat are not consumed today yet are used in the likelihood that the asset will turn out revenue later on. The entrance of Mutual assets is to examine the inclination of Mutual Funds as on Investmentchoice and to break down the sorts of plans picked in shared reserves and the target of Investors. It is Globally demonstrated venture Avenue Muttual supports currently address maybe most suitable speculation opportunity for most financial backers. As monetary business sectors become more modern and complex, financial backers need a monetary delegate who gives the expected information and expert mastery on fruitful money management. As the financial backer generallyattempt to boost the profits the gamble. Common Fund fulfills these prerequisites by giving alluring returns reasonable dangers.

Objective

- To analyze impact of COVID 19 on investors preferences on investing in mutual fund
- To determine willingness of investors to invest in mutual fund during pandemic.
- The study establishes the relationship between the profit and loss earned through MutualFunds.
- To Analyze the saving pattern of investors through Mutual funds during pandemic.

REVIEW OF LITERATURE

Rajat Mehrotra, *et al.* (February2019) Conducted A Case Study on Penetration and Investment patterns ofmutual funds in selected Regions in India. The objective of the study is to analyze the penetration of Mutual Funds, to analyze the preference of Mutual Funds as on Investment option and to analyze the types of schemes opted in Mutual funds, risk taken and the objective of Investors. Suman Chakraborty (April 2013) An Analysis of Investment Patterns of Mutual Funds Investors. It is anEmpirical Study in Orissa. The Objective of the Study has been conducted, to explore the preferred investment avenue of retail investors, to determine the investor's preference for different mutual funds schemes and to evaluate fund qualities that affects the selection of mutual funds. DR S.M. Tariq Zafar, etal. (August 2013) It is an International Journal on A Strategic Study on Investors Preferences of Mutual Funds in India. The main objective of the study is that to emerge the economy of the nation and to analyze the investors' perceptions and their buying behavior and to create awareness of the various Mutual Funds products, the influencing factors in preferring particular brands among the various schemes of different Mutual fund companies which is compared and finding out the market leaderin mutual fund companies on certain level of parameters. Ms. Shalini Goyal, etal. (May 2013) An International Journal was conducted on A Study on Mutual Funds in India. It focusses on mutual fund organizations which are needed to develop their skills and technology. The success of mutual funds depends on the implementation of suggestions with regards tomutual fund investor and the investors needs to adopt for crucial skills for successful investing and investment discipline both needs to be adopted hand in hand at the same time. Reshma, etal. (January 2020) It is an International Journal on A Study on The Awareness of Mutual Funds Investment in India. The imperial of the study is focused to study the extent the awareness on mutual funds which is in related to the age, education, income and to find out the investment preference of mutual funds with regard to other opportunities in investment and with the help of 100 sample collections using the questionnaire further it analyse whether investment in this medium has any impact in investment with respect to demographic profile.

SAMPLE SIZE: 75 Respondents

RESEARCH METHODOLOGY

Methodology used for this study:

PRIMARY DATA
Data was collected by distributing questionnaire to the investors and also by the source of AMFI website.

SECONDARY DATA:
Data was also collected through Internet, Newspaper, Magazines , Records etc.

SIGNIFANCE OF STUDY

The meaning of this study is to comprehend the way that financial backers are impacted before Covid-19 pandemic and furthermore during the flare-up of Covid-19 pandemic concerning their gamble and gets back from shared store.

THEORETICAL FRAMEWORK
Corona virus effect on individuals, organizations, and affiliations is rapidly creating. While COVID-19's impact on the speculation the board business isn't almost pretty much as high fundamental as defending people's prosperity and thriving, adventure specialists really ought to acquire from these challenges. Eventually, when people direct their fixation toward their endeavors, they will inclined to believe that their theory specialists were vigorously endeavoring to safeguard their portfolios during these troublesome times. A Mutual resource is a venture vehicle for monetary patrons for placing assets into upgraded plan of securities with the mark of appreciation in their value. As indicated by Mutual Fund Book, conveyed by adventure Company Institute of the U.S. "A typical Fund is a money related help affiliation that gets cash from financial backers, contributes it, and obtains returns on it, tries to make it create and agrees to pay the financial backer money premium for the continuous worth of his endeavor." The hypothesis managers of the resource manage these saving so the bet is restricted and steady return is ensured. Securities and Exchange Board of India (Mutual Funds) Regulations, 1996 portray 'Shared store' as "a resource spread out as trust to raise support through the proposal of units to general society under something like one designs for adventures. So a typical resource is phenomenal sort of foundation, a trust or an endeavor association which goes probably as a hypothesis go between and channelizes the saving of gigantic number of people to the corporate insurances so monetary supporters gets reliable returns, capitals appreciation and a generally safe.

Idea of Mutual Funds
A Mutual Fund is a trust enlisted with the Securities and Exchange Board of India (SEBI) which pools up the cash from individual/corporate financial backers and contributes similar for the financial backers/units holders, in value shares, government protections, securities, call currency market and so on. The pay acquired through these speculations and the capital appreciations acknowledged are shared by its unit holders with respect to the quantity of units claimed by them. This pooled pay is expertly overseen for sake the unit-holders, and every financial backer holds an extent of the portfolio.

Different types of Mutual Fund Schemes
Different venture roads are accessible to financial backers. Common supports additionally offer wise venture open doors to the financial backers. Like all speculations, they additionally convey specific dangers. The financial backers ought to look at the dangers and anticipated yields after change of expense on different instruments while taking speculation choices. The financial backers might look for counsel from specialists and advisors including specialists and merchants of shared reserves plans while settling on venture choices.

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Based on Ownership
1. Public Sector
2. Private Sector
B. Based on Scheme of Operation:-
1. Open-ended Scheme
2. Close-ended
C. Based on Portfolio
1. Equity Scheme
2. Debt Scheme
3. Balanced Fund
4. Money Market
5. Gilt Fund
6. Index Funds
D. Based on Location
1 . Domestic Funds
2. Off-shore Fund

DATA ANALYSIS AND INTERPRETATION

INTERPRETATION

From the above table it is shown that 57% of investors are in to private jobs where as 16% are doing government jobs , it also indicate that investors who are self employed and retirement was only 10%.It tells that many of them who is in to mutual fund investors are from private job.

INTERPRETATION

The table shows that 40% of respondents' income is between 1-1.5 lakhs, where 37% of respondents' income is below 1 lakhs and between 1.5 to 4lakhs the income level of respondents is 11% and only 12% of respondents income is more than 4lakh.

INTERPRETATION

39% of investors save their annual income about 5% to 10%. 10-20% of investors save 24% of their income whereas 30% & above investors save 21% of income earned but 20% to 30% of investors savesonly 16% of their annual income.

INTERPRETATION

43% of respondents are aware about mutual funds through the means of advertisement given by AMFI. 24% of investors invest in mututal fund with the help of the financial advisors who will helpthem in making profit where as 20% of investors are aware about mutual funds through their peers and only 13% of investors takes opinon of investing in mutual fund from their family.

INTERPRETATION

From the above table we can understand that there was a huge change in their investment during COVID 19 where 77% of investors says there was much fluctuation in their investment plan of mutual fund because of uncertainty caused by nature. But very few investors specifies COVID 19 asnot affected any of their investment plans.

INTERPRETATION

Investors invest in mutual fund because they think that mutual fund is a safe investment .33% of investors assumes mutual fund is less bearing risk because of its diversification, where as 17% of investors believe they get higher returns and only 11% of investors invest because of its low costcompared to other investment.

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INTERPRETATION
The above table indicates that 46% of investors believe that there was a severe impact of COVID19 on mutual fund investments because there was more volatility in the market and all the money aspects were curbed by the government. Rest 16% of investors thinks that the impact of COVID 19 was quite moderate and neutral because they were at least getting few returns when they lost their job during pandemic. Only 5% says there was no impact of COVID 19 on mutual funds.

INTERPRETATION
39% of investors feel more weight age in getting good returns from mutual fund where as 21% of investors say the stability of risk in mutual fund is less and 12% of investor prefer mutual fund because of the tax benefits from mutual fund. 18% of inventors gives more weight age because of liquidity.

INTERPRETATION
From the above table it indicates that 55% of investors says they had a enormous loss during COVID 19 because of the money market but 45% of investors says that they were getting returnsfrom mutual funds.

INTERPRETATION
53% of investors prefer close ended mutual funds because they can redeem the units based on predetermined dates. 47% of investors goes with open ended mutual fund because of its flexibilityto exit and enter. Assets managed by the Indian mutual fund industry has increased from Rs. 33 trillion in May 2021 toRs. 37.37 trillion in May 2022. Which says that there is a increase of 13.26% in That represents 13.26 %increase in assets over May 2021

RESEARCH FINDINGS
The finding of this research is that more than 57% of investors were in to the private job so the income which they were earning was very low so that they could save only 20% of their income . It is also revealed that many investors are not aware about their investment, they are aware about mutual fund through means of advertisement. It is also found that COVID 19 has affected their investment plans and their earnings. It is found that investors invest in mutual fund mainly for thehighest return. Many investors revealed that they had a huge loss due to COVID19, 53% of investors preferred to invest in close ended mutual funds. As per AMFI reports it is found that there is an increase in AUM of about 13.26%.

CONCLUSION

Speculations are future security of individuals. Different sorts of adventure are available in the economy. Both essential and Secondary information was used for compelling culmination of the endeavor. From our audit it assumes that most of the monetary benefactors get hardship from shared save hypothesis during COVID 19. The COVID-19 episode has out and out affected the economy. Given the immense people and hazardous states of the economy, especially the financial region and lockdown and social isolating have displayed to upset. In light of measures taken by the public power to control the spread of COVID-19, for instance, lockdown and the monetary trade crash, individual monetary benefactor's excitement to place assets into normal resources and the monetary trade has been impacted unfavorably. In present times, monetary sponsor seem to have become more bet reluctant, and favor commonly secure endeavor decisions offering moderatereturn.

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Table 1. Data Regarding The Occupation Of Investors

Type of Job	NO OF RESPONDENTS	PERCENTAGE %
Private Job	43	57
Govt Job	12	16
Self Employed	10	13
Retired	10	13
Total	75	100

Table.2 :Distribution Of Respondants Based on Their Income

Annual Income	NO OF RESPONDENTS	PERCENTAGE %
Below 1 Lakhs	28	37
1-1.5 Lakhs	30	40
1.5 to 4Lakhs	8	11
4 Lakhs and Above	9	12
Total	**75**	**100**

Table 3: Data Regarding The Saving Percentage Of Investors

Factors	NO OF RESPONDENTS	PERCENTAGE %
5 to 10%	29	39
10 to 20%	18	24
20% to 30%	12	16
30 and Above	16	21
Total	**75**	**100**

Table.4: Data Regarding How Investors Are Aware About Mutual FundScheme

Influence	NO OF RESPONDENTS	PERCENTAGE %
Advertisement	32	43
Peer Group	15	20
Family	10	13
Financial Advisor	18	24
Total	**75**	**100**

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Table 5: Distribution of Respondents Based on The Changes In TheirInvestment During Covid19

Opinion	NO OF RESPONDENTS	PERCENTAGE %
Yes	58	77
No	17	27
Total	**75**	**100**

Table 6: Data Regarding Reason Behind Investing In Mutual Fund

REASONS	NO OF RESPONDENTS	PERCENTAGE %
low Risk	25	33
Safe investment	29	39
High returns	13	17
Low Costs	8	11
Total	**75**	**100**

Table 7: Data Regarding Impact Of Covid19 On Investing In Mutual Fund

INVESTOR PERCEPTION	NO OF RESPONDENTS	PERCENTAGE %
Severe impact	46	61
Moderate Impact	12	16
Neutral	12	16
No impact	5	7
Total	**75**	**100**

Table8: Data Regarding Highest Weightage For Their Investment

INVESTORSADOPTION	NO OF RESPONDENTS	PERCENTAGE %
Risk	16	21
Return	29	39
Tax Benefit	12	16
Liquidity	18	24
Total	**75**	**100**

Table 9: Data Regarding Profit Or Loss In Mutual Fund Investment DuringCovid 19

BASIS	NO OF RESPONDENTS	PERCENTAGE %
Yes(Profit)	34	45
No(Loss)	41	55
Total	**75**	**100**

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Table 10: Data Regarding The Scheme Opted In Mutual Fund

CHOICE	NO OF RESPONDENTS	PERCENTAGE %
Close Ended	40	53
Open Ended	35	47
Total	**75**	**100**
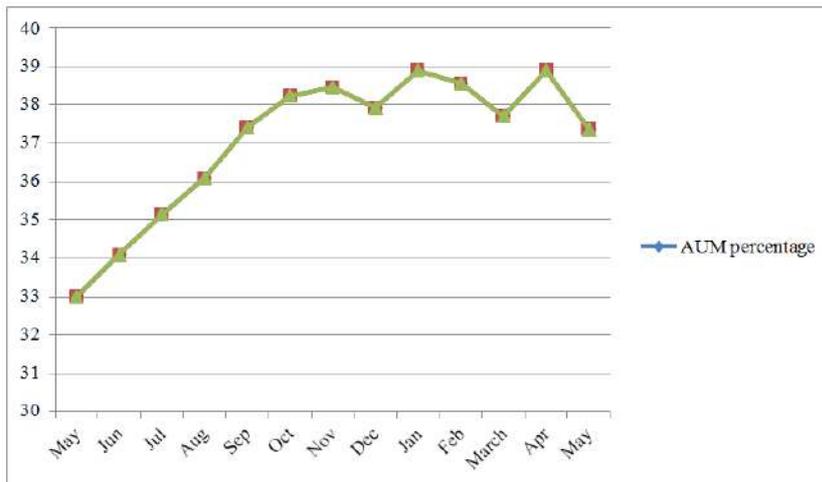
Fig 1: Total Assets under Management of Mutual fund (Source AMFI) 2021-22

RESEARCH ARTICLE

Analysis into Age and Type of Partial Edentulism in Lower Arch

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ABSTRACT

Introduction: Partial edentulousness is a dental arch in which one or more but not all natural teeth are missing. Generally, it occurs by caries, periodontal problems, traumatic injuries, impactions, supernumerary teeth, hypoplasia, over eruption neoplastic, cystic lesions, and extraction due to orthodontic reasons have also been reported. Also age correlates positively with partial edentulism. Hence the aim of the study is to analyse the age and type of partial edentulism in the lower arch. Materials and method: The data of patients reporting to Saveetha Dental College and Hospitals was reviewed and patients with partial edentulism in lower arch were selected for the study. It included parameters like age, gender, and type of partial edentulism. Chi square tests were carried out using age as independent variable and type of partial edentulism as dependent variable. The statistical analysis was done by Pearson chi square test. P value < 0.05 was considered statistically significant. Results: the most common age group with partial edentulism in lower arch was 41-60 years (58.1%), and Kennedy's class I was the type of partial edentulism present in majority of the patients (34.7%).Conclusion: With an increase in age, there is an increase in the Class I & Class II dental arch tendency. Age had a significant relationship with the pattern of partial edentulism.

Keywords: partial edentulism, caries, periodontal diseases, Kennedy's classification, innovative analysis, innovative database

INTRODUCTION

Partial edentulousness is a dental arch in which one or more but not all natural teeth are missing. Generally, it occurs by caries, periodontal problems, traumatic injuries, impactions, supernumerary teeth, hypoplasia, over eruption neoplastic, cystic lesions, and extraction due to orthodontic reasons have also been reported.(1–3) Some studies have reported caries as the main causative agent for tooth loss. According to Zaigham et al., and Abdel Rahman et al., dental caries and periodontal disease were the major causes of tooth loss in early childhood and adolescence.(4,5) Also, studies have documented that age correlates positively with partial edentulism. (6,7)Partial edentulism leads to several drawbacks to the subjects including clinical challenges and lifestyle compromises. Clinically, partial edentulism results in drifting and tilting of adjacent teeth, supra eruption of opposing teeth, altered speech, changes in facial appearance and tempero-mandibular disorders.(1,4) Also, the loss and continuing degradation of the alveolar bone, the adjacent teeth and also the supporting structures will influence the difficulty to achieve an adequate restoration in a partially edentulous patient.(8,9) On the lifestyle compromises, partial edentulism restricts dietary options, which leads to weight loss. Further, it leads to lack of confidence and confined social activities, which may adversely affect the quality of life and lead to psychological dissatisfaction.(1,4).

Partially edentulous arches have been Classified by various methods. The possible combinations of partial edentulism are more than 65,000 depending on their incidence in maxillary and mandibular arches. (10) The primary objective of the classification is to facilitate the communication about the combination of missing teeth to edentulous ridges among students, dental practitioners and laboratory technicians.(11–13) Among the various methods of classification like Kennedy, Applegates, Avant, Neurohar, Eichner, ACP (American College of Prosthodontics) etc, Kennedy's classification is widely studied and clinically accepted by Dental Community.(2,13) As per Kennedy's classification, there are four main types of partially edentulous arches as Class I, Class II, Class III and Class IV. Kennedy's classification is widely accepted due to its advantages of immediate visualization and recognition of prosthesis support.(4,10) The patterns in the incidence of the various Classes of removable partial dentures should be reviewed periodically to serve as teaching guidelines.(14) There is an anticipated increase in the use of removable partial dentures (RPDs) due to the changing trends in dental treatment that encouraged retention of natural teeth.(15) Thus, edentulism has been regarded as a consequence of the success of various preventive and treatment modalities put in place by the health care delivery system. This has led to the appraisal of patterns of tooth loss in many different populations in several countries. (11). Partial edentulism is one of the widely studied topics in dentistry. The pattern of partial edentulism has been evaluated in many selected populations in different countries. Several studies have analyzeds the correlation between partial edentulism and its influencing factors like socio-economic parameters, age, gender, etc. (4,16–18) The need for periodic reviews of the trends in the incidence of the various classes of removable dentures has been advocated for the purpose of improving knowledge as well as assisting in addressing various problems that could arise as a result of partial edentulism. Our team has extensive knowledge and research experience that has translated into high quality publications(19–27),(28–33),(34–38) Hence the aim of the study is to analyse the age and type of partial edentulism in the lower arch.

MATERIALS AND METHODS

Study design and study setting
The present study was conducted in a university setting (Saveetha dental college and hospitals, Chennai, India). Thus the data available is of patients from the same geographic location and have similar ethnicity. The retrospective study was carried out with the help of digital case records of 299 patients who reported to the hospital. Ethical clearance to conduct this study was obtained from the Scientific Review Board of the hospital.

Sampling
Data of 299 patients were reviewed and then extracted. All patients with partial edentulism in the lower arch in the given duration of time period were evaluated. Only relevant data was included to minimize sampling bias. Simple

random sampling method was carried out. Cross verification of data for error was done by the presence of additional reviewers and by photographic evaluation. Incomplete data collection was excluded from the study.

Data collection

A single calibrated examiner evaluated the digital case records of patients who reported to Saveetha Dental College from June 2019 to june 2021. For the present study, inclusion criteria was data of patients with endodontic ally treated anterior teeth. Data obtained were age, gender and partial edentulism in lower arch. All obtained data were tabulated into Microsoft excel documents.

STATISTICAL ANALYSIS

The collected data was tabulated and analyzed with Statistical Package for Social Sciences for Windows, version 20.0 (SPSS Inc., Vancouver style) and results were obtained. Categorical variables were expressed in frequency and percentage. Chi square test was used to test association between categorical variables. Chi square tests were carried out using age and gender as independent variables and the type of partial edentulism as a dependent variable. The statistical analysis was done by Pearson chi square test. P value < 0.05 was considered statistically significant. Error graphs help to indicate estimated error or uncertainty to give a general sense of how precise a measurement is. This is done through the use of markers drawn over the original graph and its data points. Typically, error bars are used to display either the standard deviation, standard error, confidence intervals or the minimum and maximum values in a ranged dataset.

RESULTS

In our study, majority (58.1%) of the patients with partial edentulism in lower arch were between 41-60 years of age. (Figure1) Females showed slight predominance (59.8%) for partial edentulism in lower arch when compared to males (40.1%). (Figure 2)Kennedy's class I type of partial edentulism was found to be the highest (34.7%) among the patients. (Figure 3)On associating age with the type of partial edentulism, Kennedy's class I type was highest among all the age groups. The chi square value was found to be statistically significant (0.001). (Figure 4) Error graphs help to indicate estimated error or uncertainty to give a general sense of how precise a measurement is. This is done through the use of markers drawn over the original graph and its data points. Typically, error bars are used to display either the standard deviation, standard error, confidence intervals or the minimum and maximum values in a ranged dataset.

DISCUSSION

Partial edentulousness is a dental arch in which one or more but not all natural teeth are missing. Partial edentulism leads to several drawbacks to the subjects including clinical challenges and lifestyle compromises. Clinically, partial edentulism results in drifting and tilting of adjacent teeth, supra eruption of opposing teeth, altered speech, changes in facial appearance and tempero-mandibular disorders.(1,4)Generally, it occurs by caries, periodontal problems, traumatic injuries, impactions, supernumerary teeth, hypoplasia, over eruption neoplastic, cystic lesions, and extraction due to orthodontic reasons have also been reported.(1–3) Some studies have reported caries as the main causative agent for tooth loss. Also, studies have documented that age correlates positively with partial edentulism. (6,7)The primary purpose in using a classification for RPDs is to simplify the description of potential combinations of teeth to ridges.(39) In the present study, the Kennedy classification was preferred to fulfill this purpose. One of the principal advantages of the Kennedy classification is that it permits the immediate visualization of the partially edentulous arch, and enables a logical approach to the problems of design. A previous study reported a pattern of partial edentulism in the age group studied.(40) They found that the 4 and 5 decade of life had the highest prevalence of partial edentulism, similar findings were found in our study as well. There is an increase in percentage

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of class I & II in later stages as more teeth are extracted due to multiple causes in older age. Okoisor further established that the disease factors responsible for tooth loss was age related; with caries and periodontal diseases being the major causes of tooth mortality in children and adults respectively. In our study, the percentage of Kennedy's class I increased significantly in patients above 40 years of age. Zaigham AM et al., concluded that with an increase in age, there was an increase in Class I & Class II dental arch tendency and a decrease in Class III & Class IV.(4) Similarly Abdel Rahman HK et al., found that younger age group patients had more Class III and Class IV in both the arches and as age increases due to multiple teeth extraction, there was significant increase in Class I and Class II.(3) A previous study reported incidence of Kennedy's class III to be relatively high in younger age groups. It was found to be 49% in group I (20 – 29 years) and above 55% in group II (30 – 39 years). This may be because of early loss of first molar due to caries and afterwards the extension of the existing saddle due to further loss of teeth with increasing age. Kennedy's class IV was also higher in group I (20 – 29 years). One of the most common reasons is the trauma to maxillary central incisors at early childhood stage.(41) While in our study both Kennedy's class III and class IV were found to be high in older patients when compared to younger patients. This difference could be explained by the age distribution of the study population with more people in this study being in their 5th and 6th decade of life. The need for periodic reviews of the trends in the incidence of the various classes of removable dentures has been advocated for the purpose of improving knowledge as well as assisting in addressing various problems that could arise as a result of partial edentulism.

CONCLUSION

With an increase in age, there is an increase in the kennedys Class I & Class II type of partial edentulism in mandibular arch. Age had a significant relationship with the pattern of partial edentulism. Bilateral missing posterior teeth were significantly present higher in the middle age group of 41- 60 years.

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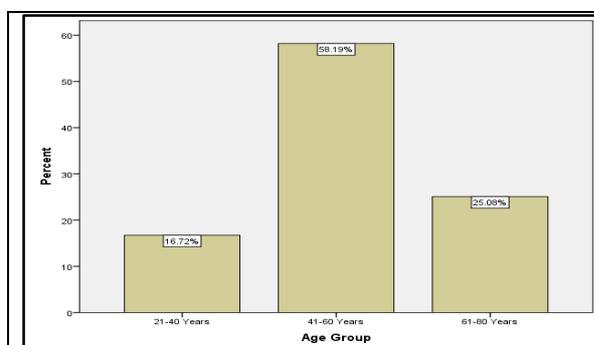
Figure1:This graph represents the distribution of patients based on age. X-axis denotes the age groups
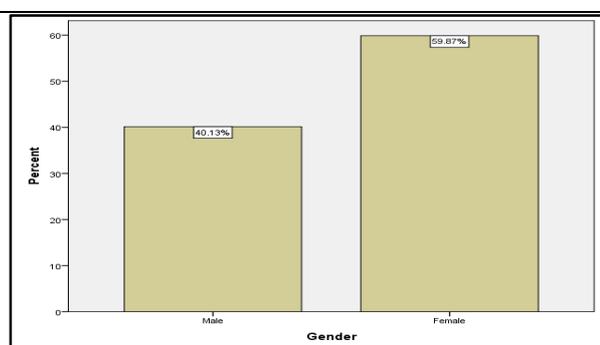
Figure2: This graph represents the distribution of patients based on gender. X-axis denotes the gender

and Y-axis denotes the percentage of patients.	and Y-axis denotes the percentage of patients.
Figure 3: This graph represents the distribution of patients based on the type of partial edentulism of the patients according to Kennedy's classification. X-axis denotes the type of partial edentulism and Y-axis denotes the percentage of patients.	**Figure 4:** This graph represents the association of age with the type of partial edentulism. X-axis denotes the various age groups and Y-axis denotes the number of patients with partial edentulism. The type of partial edentulism based on Kennedy's classification is denoted by separate colours. Kennedy's class I type was highest among all the age groups. The chi square value was found to be statistically significant (0.001).
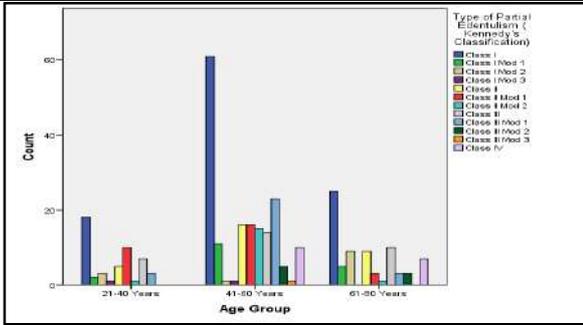
Figure 5: This graph represents the association of age with the type of partial edentulism. X-axis denotes the various age groups and Y-axis denotes the number of patients with partial edentulism. The type of partial edentulism based on Kennedy's classification is denoted by separate colours. Kennedy's class I type was highest among all the age groups. The chi square value was found to be statistically significant (0.001).

RESEARCH ARTICLE

ELNino based Rainfall Forecasting using Computational Algorithm

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ABSTRACT

Rainfall forecasting is one among the most demanding tasks carried over by the meteorologists and researchers throughout the World. Many classical, advanced stochastic and numerical models have been derived and implemented to forecast the rainfall of a certain region with much accuracy. This research work deals with the forecasting of rainfall by applying Artificial Neural Network (ANN) learning algorithms. The learning algorithm considered in this research article is Radial Basis Function (RBF). It uses the Gaussian function as the activation function. The Northeast monsoon rainfall in Chennai has been considered as a sample case study. The sea surface temperature anomalies were used as the predictor for forecasting. Based on the performance evaluation criterion results, Radial Basis Function algorithm performs better for forecasting rainfall in Chennai.

Keywords: Chennai, ElNino, Forecasting, MAPE, Radial Basis Function, Rainfall

INTRODUCTION

Nowadays researchers implement RBF Neural Networks in various fields like medical and engineering because of their learning speed and universal approximation. These networks replace Multi Layer Perceptron in forecasting weather parameters. The author [5] applied Artificial Neural Networks for forecasting Global solar radiation of certain Egyptian cities on a horizontal surface. [3] examined the approach of Artificial Neural Network by applying to the weather component, maximum temperature. The artificial neural networks were used to foresee the future dryland retorts to human and climate disturbances [1]. The authors established a novel approach to

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estimate the relationship between sand deposition in semi- arid grasslands of Nebraska Sandhills and external climatic conditions, land use, pressure and wildlife occurrence. Different artificial neural network models were examined [2] to identify the classification efficiency of Osteoporosis, an orthopedic disease. The authors forecasted this disease risk prediction using a set of clinical parameters by viewing the problem as a pattern classification problem.

Multilayer perceptions and probabilistic neural networks for risk factor prediction. It was concluded that probabilistic neural network gives better results than multilayer perceptron. [4] compared artificial neural network algorithms together with the conventional statistical evaluations and showed that artificial neural network gives better results than conventional statistical modeling by modeling, predicting and classifying the student academic performance. In this study [6], the authors proposed fourteen prediction models based on artificial neural networks to predict the COVID 19 outbreak. For this purpose, they have collected the confirmed cases of China, Japan, Singapore, Iran, Italy, South Africa and United States of America and the results revealed the importance of considering the maximum incubation period in predicting the outbreak. In the article [7], several classical statistical forecasting techniques together with the advanced ARIMA model were implemented and compared using the error measure RMSE for forecasting the COVID 19 for global data. The outcomes show that ARIMA model gives improved forecasting model than the other statistical techniques. With these literature surveys, it is evident that ANN plays a vital role in forecasting the weather parameters. Thus, this paper focuses in forecasting rainfall using the learning algorithm of ANN.

STUDY AREA AND MATERIALS

Chennai is the capital city of the state Tamil Nadu, India. Chennai is located at 13.04°N and 80.17°E on the southeast coast of India and in the northeast corner of Tamil Nadu. According to the report of Indian census during the year 2011, Chennai stands in the sixth position among most populous city and it is in the fourth position under the category, urban populous agglomeration in India.

Chennai features a tropical wet and dry climate. The weather experienced in Chennai is hot and humid for most of the year. The summer season begins in the month of March and spreads till October. The climate of Chennai is generally influenced by South Westerly winds from April to October whereas the North-easterly winds affect the climate during the remaining period of the year. Chennai receives the seasonal rainfall during the period October to December because of Northeast Monsoon winds. The highest rainfall of the city was 257cm in the year 2005. Chennai faces water supply shortage problem, which results in over-reliance on annual monsoon rains to reload its water reservoirs. The city's ground water levels have been reduced to very low levels. Hence understanding monsoon rainfall of Chennai helps in planning varied water resource projects in the city.

For the purpose of this research work, the dataset El Nino 3.4 (120°W-170°W and 5°S- 5°N) Sea Surface Temperature indices were obtained from National Oceanic and Atmospheric Administration, US for a period of 117 years (1901 – 2017). The Sea Surface Temperature anomalies are calculated by subtracting the long-term mean for a specific point in the ocean from the current value. Also, a dataset containing a total of 117 years (1901 – 2017) monthly rainfall totals of Chennai city were obtained from Indian Institute of Tropical Meteorology (IITM), Pune, India. From this, the Northeast Monsoon rainfall totals of every year were calculated by considering the total of monthly rainfall of October, November and December.

METHODOLOGY

The El Nino or Southern Oscillation is the outcome of the strong coupling and interaction between the Tropical Ocean and the atmosphere, which play a major role in the development of global climatic system. There exist complex and interesting inter- relationships between Sea Surface Temperature (SST) over the Pacific and Indian

Ocean basins and All India Summer Monsoon Rainfall. Most of the severe droughts in Tamilnadu have been associated with the El Nino events. But El Nino years have not always produced severe droughts. ElNino is characterized by unusually warm temperature in the tropical Pacific Ocean. During extreme events of the low or warm Southern Oscillation phase characteristic near-global patterns of anomalies are known to occur. They include positive departures of SST in the equatorial Pacific, torrential rains along the West coast of South America, drought in Australasia, failure of the Indian summer monsoon precipitation, abundant rain at the coast of equatorial East Africa, drought in the eastern South Africa, and deficient rainfall in Northeast Brazil and the Caribbean. The association of El- Nino years with below average rainfall which is 67% of the El-Nino years, is strong have been proved in Southern and Western Ghats of India, indicating a good relationship. Radial Basis Function (RBF) learning algorithms are often implemented in various medical and engineering domains because of their faster learning speed, more compact topology and universal approximation. These networks have been independently proposed by numerous researchers and are considered a famous replacement to the Multilayer Perceptron (MLP). It is a feed forward neural perceptron containing 3 different modules namely the input layer, the hidden layer and the output layer.

A parameter vector in the hidden module called centre exists in every neuron. By evaluating distance connecting the inputs of the network and the hidden module's centers, the outputs of the first module are determined. The outputs of the linear hidden layer namely the second module are the weighted forms of the returns of the first module.

The structure of a radial basis function learning network is depicted in Figure 2. The Euclidean distance connecting the centers and inputs are evaluated by each neuron which contains the basis function, which is an activation function. During the training period, the RBF network parameters are modulated such that the data used for training is made to fit the network model in best possible way where the Euclidean distance is generally taken to be the norm while the Gaussian function is assumed to be the radial basis function.

RESULTS AND DISCUSSION

The Northeast monsoon rainfall in Chennai is calculated by adding the rainfall amount received during the month October, November and December for all the years. For forecasting the next year northeast monsoon rainfall in Chennai, the previous year northeast monsoon rainfall and ElNino3.4 were considered to be the predictors. The radial basis function algorithm with one hidden layer is best suitable for weather forecasting. The dataset was splitted into training and testing sets. The training dataset consists of 100 years for the period 1901 – 2000 and the testing dataset consists of 17 years for the period 2001-2017. After training the dataset, the model is validated using the performance evaluation criteria. The performance evaluation criteria applied in this research work is Mean Absolute Percentage Error (MAPE). The error values obtained during the training and testing is tabulated below in Table 1.

CONCLUSION

Prediction of long-term rainfall is very important for those countries depending on Agro – based economy. In this article, Artificial Neural Network with Radial Basis Function have been implemented to develop the forecasting model and the results are compared using mean absolute percentage error measure. It is found that Radial Basis Function learning algorithm gives a high prediction yield. The fact that Artificial Neural Networks exhibit a comparable or even better performance than a conceptual model suggests that this approach could provide a useful tool in solving similar type of problems in time series analysis.

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Table 1: Error Measure obtained during the training

Error Measure	MAPE
Training	3.77343
Testing	3.01741

Fig.1.StudyArea, Chennai City
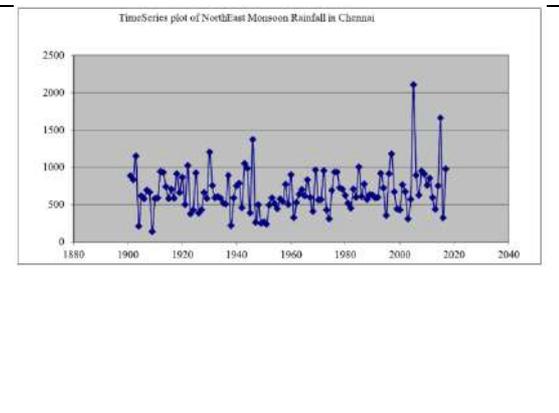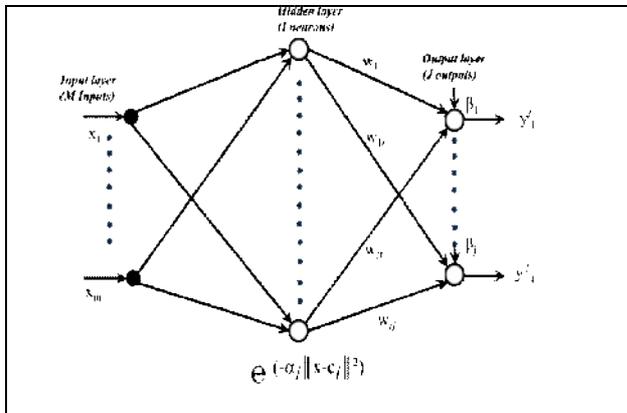
Fig.2. Architecture of Radial Basis Function	**Fig.3: Time series plot of Northeast monsoon rainfall in Chennai**
Fig.4: Timeseries plot of ElNino3.4	**Fig.5. Architecture of RBF while training**
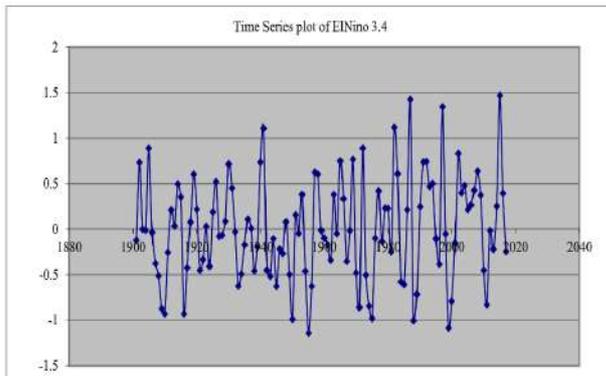
RESEARCH ARTICLE

A Comparative Study on Urinary Volatiles in Four Different Indigenous Cow Breeds (*Bos indicus*) of India during Pregnancy Period

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ABSTRACT

The Indigenous cow breed is an animal species that humans have domesticated for meat, agriculture, and dairy purposes. These native breeds are adapted to the soil, sanitary environment, and climate conditions. The present study aimed to compare the urinary biochemical and volatile profiles in four different species (Bargur, Ongole, Rathi, and Tharparkar) of pregnant female cow breeds (*Bos indicus*). The objective is to explore and document the volatile compounds present in the urine samples of four different species of pregnant female cow breeds. In the current study, urine of pregnant females from four different indigenous cow breeds, Bargur, Ongole, Rathi, and Tharparkar, were collected for analysis of volatile profiling. We have identified 52 volatile compounds. According to their molecular weight, 44.05 – 652 range compounds are volatile, and above 350 range compounds are semi-volatile in nature.

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The molecular formula consists of a minimum of two carbon atoms and a maximum of 38 carbon atoms. According to the carbon numbers, fewer carbon atoms have higher volatile properties, and a high number of carbon atoms have higher semi-volatile in nature. These chemical classes are all under alkane, alkyl, alcohol, acid, etc. The comparative analyses of four different indigenous breeds were different due to the metabolic process and their genetic makeup. Twelve biochemical analysis of pregnant female indigenous cow urine provided an effective way to check the health status of an animal.

Keywords: Volatile, Molecular weight, Carbon atoms, Native breed, Pregnant indigenous cow.

INTRODUCTION

India is the only country where most natural environmental conditions are found in one geographic region. Environmental factors influenced the evolution and adaptation of Indian cattle breeds. In the past, agriculture was successful, but hefty taxes hindered farmers from growing crops and making a living. *Bos indicus* have collected genes that provide thermo-tolerance at the physiological and cellular levels. Antibiotics and vaccines are useful. When an animal's immune system is impaired by genetics or the environment, antibacterial drugs don't kill all microorganisms (Prof. R. S. Chauhan, disease resistance in indigenous cows). Cow urine (gomutra) is referenced in Ayurvedic literature (Sushruta Samhita, Ashtanga Sangrah, and Bhav Prakash Nighantu) as a medicinal animal excretion having therapeutic benefits (Sushruta Samhita, AshtangaSangrah, and Bhav Prakash Nighantu). In Indian culture, the cow (Kamadhenu) is respected.

It is thought that there should be some differences in their metabolisms based on the physiology, genetic and microbial flora present in their gut. Consequently, it would be fascinating to look at how their metabolisms altered through time. It is easy to find the metabolic variation by analysing the urine. Urine samples taken from each of them should have produced the evidence needed to prove this. Urine is a non-invasive source of biological fluid that includes proteins, peptides, and metabolites, all of which are important for human health. Urine is a reflection of the physiological state of the organism as well as reproductive actions that are taking place within it. Hormones, volatiles, biomolecules, and proteins in urine may vary concentration during the estrous cycle. Oestrogen's influence on urine biochemistry is poorly understood. Urine analysis will identify numerous bovine illnesses. Because they lack visible symptoms, many of these illnesses go undetected and untreated. Cow urine is a cure-all for almost all ailments. Cow urine, one of the constituents of 'Panchagawya,' may heal a broad range of curable and incurable ailments, as ancient holy books indicate. Panchgawya is a combination of milk, curd, ghee, urine, and dung used as a fertiliser, insecticide, and pesticide. Cow urine is not only employed as a medicinal treatment, but also in agriculture and sericulture (Ipsita Mohanty *et al.,* 2014).

Cow's urine distillate includes bioactive chemicals that boost molecular activity and availability (antibiotic, antifungal, and anticancer drugs). Several volatile and non-volatile components in cow urine may have strong antibiotic activity (Shaw et al., 2007). Photo activated and purified cow urine removes germs. It became poisonous, killing drug-resistant bacterial species. Besides estrogen (Biddle et al., 2007), phosphorous (Bravo et al., 2003), nitrogen (Yan et al., 2007), chloride (Coppock *et al.,* 1979), potassium (Lebeda & Bus, 1997), and calcium (Van-Leeuwen *et al.,* 1976), urine proteins (Gabel *et al.,* 1986), and pheromones (Bi (Tauck & Berardinelli, 2007). India's agriculture and dairy industries rely heavily on cows (Jonker& Kohn, 2001). According to observations, forest-dwelling cows emit several beneficial herbal compounds in their urine (Ravi Kant Upadhyay et al., 2010).   Cows, wild oxen, and other animals' urine has been analyzed in various nations and is currently being done in India. Because urine is non-invasive compared to other biological fluids, there is interest in using it for diagnostics. The bovine urine proteome has received minimal attention as a biomarker source in disease and pregnancy, with only a few studies published (Shaked *et al.,* 2001, Simon et al., 2008). Although there is little information regarding indigenous breeds in India, their urine characteristics are unknown.

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Animals communicate for food, protection, and reproduction. Semiochemical communication can be intraspecific or interspecific. Allomones are interspecific semiochemicals favour the producer, whereas kairomones favour the recipient. Pheromones are semiochemicals used for intra-species communication. Chemical cues are important in mammalian reproduction. In animals, sex attractants such as urine, feces, and vaginal fluids, as well as exocrine glandular secretions, operate as sex attractants and aid in the finding and identification of mates (Aron, 1979; Balakrishnan&Alexander, 1985; Dominic 1991). Studies on pheromones have been done mostly on mice, rats, and rabbits but also on farm animals (Klemm et al., 1987; Signoret, 1991) and humans. (1983).Thebull can detect an estrus cow through olfactory communication.It has been claimed that urine and cervical mucus are likely candidates for cow pheromones (Preti, 1984), which help the bull in identifying the estrous period.

In order to anticipate ovulation in cows, it is necessary to observe the animals' estrous behaviour. Because this behaviour lasts just 12 to 21 hours (Schams *et al.,* 1977), it is difficult to determine if a woman is in estrus. However, it is possible that the capacity to identify estrus via sniffing the vaginal area and breathing urine chemicals is more accurate than the methods used by farmers and veterinarians to do so. None of the regular approaches for detecting estrous in cows is completely trustworthy or exclusive in its results. Despite the fact that there is no scientific proof for estrous detection, practically all male animals are capable of detecting estrus based on the scents of females (Kamatchi Rameshkumar *et al.,* 2008). The present study sought to determine the profile of volatiles in the urine of eight indigenous cow breeds and one exotic cow breed from India. In this study we have examined the four native breeds of India. Bargu breed cow, Ongole Breed cow, which are belongs to south India. Rathi and Tharparkar breed cows belongs to North India.

MATERIALS AND METHODS

Test animals
To test for health, four healthy pregnant indigenous (*Bos indicus*) cow breeds from a private farm in Trichy, including Ongole, Rathi and Tharparkar and Bargur Cattle breed have been reared at the Bargur Cattle Research Center in Bargur Hills, Andhiyur Taluk, Erode, India. For the experimentation, six animals from each breed were selected. All of the animals were kept / housed in a similar shed with adequate room, air circulation, and access to free water. All of the animals were treated in a comparable manner. Green fodder, dried straw, dried ground nut and sesame cake powder, maize, and other feed supplements were given to the experimental animals as a source of nutrition.

Sample collection
Before collecting urine from the cow, a mild massage was performed to the animal's stomach side. The urine samples were collected in glass containers, filtered using nylon mesh (16-120 m), and promptly stored at –20°C until they were analysed. Within a week of collecting the sample, the analysis was completed. It was necessary to obtain urine samples from the pregnant animals in this study. Urine samples of six animals in same breed was pooled and the samples pooled were used for further analysis.

Preparation of urine sample for GC-MS analysis
The urine samples were extracted with dichloromethane at a 1:1 ratio. In a nutshell, the vial containing urine and dichloromethane was shaken vigorously and vortexed for approximately 2–5 minutes. In order to achieve a clean separation, the vials were placed in the refrigerated for 5 to 10 minutes after that. The transparent solvent layer was then collected in a GC vial using a Pasteur pipette, and the results were utilised for volatile analysis using a GC-MS (Model: QP-5000 plus, Shimadzu, Japan) instrument (Achiraman and Archunan, 2006).

Gas Chromatography-Mass Spectrometry
The GC-MS analyses were made in QP-5000, (Schimadzu, Japan). Two μl of extract was injected into the GC-MS that contained 30 m glass capillary column with a film thickness of 0.25 μm (30 m X 0.2 mm i.d. coated with UCON HB 2000). The oven temperature program for each type of sample was unique, since individual samples were not

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resolved well at a single temperature programme. However, in any case, the initial temperature was 40°C for 5 minutes and the ramp temperature was differed. The area under each peak was used for quantitative calculations. The detection accuracy was about 1 ng/peak. The relative amount of each component was reported as the percent of the total ion current (TIC). The GC-MS was under the computer control at 70eV. Using ammonia as a reagent gas chemical ionization was performed. Identification of unknown compounds was made by probability based matching using the in-built library NIST 12 in the GC-MS.

RESULTS AND DISCUSSION

In this study, the Bargur pregnant urine volatile analysis revealed fifty nine volatiles (Figure: 1 and Table: 1). the value of this molecular weight range from 44 to 238 Da. Its carbon atoms have sizes ranging from C_2 to C_{16}, while its hydrogen atoms have sizes ranging from H_4 to H_{30}. Every chemical with a molecular weight lower than 350Da is considered a volatile compound, whereas every molecule with a molecular weight more than 350Da is considered a semi volatile compound. There are 51 organic chemicals and 8 inorganic compounds among the 59 volatile compounds. Inorganic chemicals like nitrogen and chloride were shown to be attached to volatile and semi-volatile molecules.

The retention time begins from 10.855 and continues until it reaches 33.812. Between these retention durations, we have identified 27 different pheromone compounds. All of these different group of chemicals such as alkanes, alkyls, alcohols, acids, ketones and etc. Some compounds like 4-Nonanone were found in plants. It is possible that they were derived from the cow's food. Further, five volatile compounds including Linalool, Phenol, 3-Methyl- (M-Cresol), Nonanol, Sebacic Acid, were unique to adult males possibly represent the adult males to the cow. According to Borg-Karlson et al (1996) linalool may act as both a sex and a food attractant. In field trials, a number of spring blooming plants release one or both of the linalool enantiomers, and the volatiles from their blooms attract males of C. cunicularius. Heath et al. (1992a) also stated that the pheromone was identified as a combination of linalool, p-cresol, and m-cresol based on examinations of hair pencil extracts of male cabbage loopers and volatiles released by males. In a flying tunnel, female cabbage looper moths were attracted to a mixture of linalool, p-cresol, and m-cresol, three chemicals isolated from male cabbage looper moth hair pencils (Heath et al. 1992a). Linalool may be found in the odour of honeysuckle blooms, which cabbage looper and other moths visit in quest of nectar (Pair 1994; Schlotzhauer et al. 1996).

The chirality of the major component, has an effect on female behaviour. In female, Nonanol is a sex pheromone component reported in caddis fly *M. angustata*, according to Francke and Schulz (2010). *T. infestans* produced Nonanal during courting and copula, which attracted male conspecifics. Another insect, *C. lectularius*, uses it as part of its aggregation pheromone. The notion of endocrine hormones regulating urine volatile excretion is well-established. In addition to studies on mice (Andreolini et al., 1987; Jemiolo et al., 1986; Achiraman and Archunan, 2006), voles (Boyer et al., 1988), and wolves (Raymer et al., 1986).Identification of these biochemical constituents in female urine will help to understand their functional role in physiology, diseases pathology, etc. Decanal (anti-fungal property), Hexanedioic acid (used to produce acidic tablets) are contain medicinal value .Pentanedioic acid it deters other males from approaching the pregnant cow (Krueger *et al.,* 2016).Figure:2 and Table: 2 showed the GC-MS profiles of Ongole breed cow. Ninteenvolatile compounds present in the Ongole breed cow. The molecular weight ranges of these compounds, from 44 to 338 Da. It has carbon atoms varying in size from 3 to 22, and hydrogen atoms ranging in size from 10 to 42. The 19 volatile substances include 16 organic chemicals and 3 inorganic chemicals. Starting at 4.885, the retention time will continue to increase all the way up to 40.165.

During retention time, it have been able to recognise 11pheromone compounds, including Oxalic Acid, phenol, Pentadecane, Propanoic Acid (Pseudoacetic Acid), and others. Alkanes, alkyls, alcohols, acids, ketones, and other

compounds fall within this category (Table: 5). Compounds like Oxalic acid and others are commonly found in plants animals and microbes. Phenol is a sex attractant pheromone (William *et al.,* 1975). The investigation exposed a total of 78 different volatile chemicals in south Indian breeds. They include a total of 38 pheromone compounds. Within these 38 pheromone compounds, Bargur breed cow have a total of 26 unique compounds, whereas Ongole breed cow have a total of 11 unique compounds. Hexanedioic Acid is a chemical that can only be present in South Indian breed cows and absent North Indian breed cows. Volatile analysis of Rathi breed cow urine contains 15 compounds in GC-MS analysis (Figure:3 and Table: 3). The chromatogram detected compounds with a molecular weight ranging between 84.14 to 340.6 Da. The molecular formula consists of minimum 4 number of carbon atoms to 22 carbon atoms. It contains hydrogen atoms from 4 to 44.The 15 volatile compounds include 13 organic chemicals and 2 inorganic chemicals. Inorganic compounds like sulphur and chloride were shown to be attached to volatile and semi-volatile molecules. There were 12 compounds which was also present in Tharparkar breed and consider as a common volatile for North Indian breed cows(Table: 5). However, there is a one compound namely 1-Hexanol, which was present in Rathi breed cow urine sample, and found absent in Tharparkar breed cow urine sample.The retention time starts from 4.63 and ends in 33.47.

In this timing we have got 12 pheromone compounds. During retention time, it have been able to recognise 12 pheromone compounds, including Styrene, Thiophene-D1, Oleic acid, Octadecanoic acid, Docosanoic acid and others which belongs to alkanes, alkyls, alcohols, ketones, and other compounds fall within this category.1-Hexanol is present only in Rathi breed cow. That may be a unique volatile compound for Rathi breed cow.It is claimed that 1-hexanol is a component of the smell of grass that has recently been mowed. Honey bees' Koschevnikov glands release alarm pheromones that are characterised by the presence of 1-hexanol. This alarm pheromone might not cause stinging, but it could assist encourage other nest members to participate in defensive activities (Boch *et al.,*1962). Figure:4 and Table: 4 explained the GC-MS analysis and presence of 21 compounds in urine of Tharparkar breed cow. Among these, the identified molecular weight of the compounds were ranging from 84 to 338.The carbon range of the compound between 4 to 21 whereas in hydrogen range of the compounds between 4 to 32. With respect to the study 17 organic compounds and 4 inorganic compounds were identified. Sulphur, nitrogen and chloride are the Inorganic compounds were shown to be attached to volatile and semi-volatile molecules. Ascorbic acid is the only one specific compound present in Tharparkar breed cow (Table: 5).Retention time of the chromatogram between 4.635 to 34.465. There are 11 pheromone compounds were recorded during the run. 9,12- Octadecadienoic Acid, (6R, 7R)-Bisabolone, 1-Decene, 1,14-Tetradecanediol and etc., are the pheromone compounds present in the Tharparkar cow's urine. The volatile compounds are belongs to alkanes, alkyls, alcohols. Ascorbic acid, often known as vitamin C, is the Tharparkar cow's distinctive compound. Vitamin C is another name for ascorbic acid. Pheromones are odorants composed of chemical molecules that are either volatile or semi-volatile (sometimes non-volatile). Animals emit pheromones in dung, urine, saliva, vaginal mucus, skin, and/or specific glands (Tirindelli et al., 2009).

These volatiles may express the uniqueness of the animals. As a result, we examined the urine volatile chemicals in relation to the stage of pregnancy in four breed cattle. We found changes in volatile profiles of all four breed cows under pregnant conditions, as expected. There is a possibility that certain volatile compounds are derived from the plant metabolites, because these volatile compounds were also identified from the insects that in habitatin the fodder plants that cow consumes. The release of some trail pheromones may be utilised as a straightforward method for locating their congestion.It was found that cow urine contains a number of potentially useful therapeutic chemicals. Cow's urine has been used as a medicine for centuries since ancient period and it was compared to the nectar. Several medicinal properties of cow's urine have been highlighted such as weight loss, reversal of certain cardiac and kidney problems, indigestion, stomach ache, edema, etc (Ramani et al.,2012). Cow urine is an important ingredient of panchgavya, a recipe made of cow products such as urine, milk, ghee, curd and dung. Urine analytes play an important role in diagnosis of several diseases and provides the clue about animal physiology.

Urine analysis of different cow and buffaloe breeds have carried out in India as well as in abroad, however, a comprehensive study on Indigenous breed was not conducted. Hence, the present study evaluated the urinary biochemical profile of eight indigenous breeds of India, but we present the data only for four cow breeds (Bargur, Ongole, Rathi and Tharparkar breed cow). The composition of the excretory products in cow vary according to various stages of reproductive phases, lactation, gestation, individual physiology, food ratio conversion, stress, hormonal interferences, etc. (Dominic, 1991). The metabolites observed in the urine are result of metabolism of food e.g, Beauchamp (1976) reported a simple diet change can change urine odour in mice and guinea pig (Ramesh Kumar et al., 2000). A significant role in the detection of many bovine diseases will be played by urine analysis. Many of these diseases may go unreported and untreated because they do not manifest themselves with obvious signs or symptoms.

CONCLUSION

The present study establishes presences of volatiles/semi-volatiles and biochemical constituents in urine of selected indigenous cow breeds of India. The chromatogram of each cow breed has unique volatile/semi volatile compounds. These metabolites appear to be indictors of animal physiology, pathological and reproductive status, also it serve as chemo attractants for conspecifics. These alterations in urine biochemical profile may be due to genetic makeup, individual physiology, food, symbiotic microbiota in gut and reproductive tract, etc. This is first attempt to document biochemical cues of Indigenous cow breeds in a comprehensive manner, however further study is warranted to reveal the role of microbes in pheromone production.

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Table: 1. List of volatile compounds present in Bargur cow

S.No	Rt	Area %	Compund Name	Molecular Formula	Molecular Weight
1.	10.855	0.5	4-Heptanone	$C_7H_{14}O$	114.19
2.	15.164	0.47	2,5-Octanedione	$C_8H_{14}O_2$	142.2
3.	15.688	0.4	3-Hexenol	$C_8H_{14}O_2$	142.2
4.	16.273	1	1-Hexanol, 2-Ethyl-	$C_8H_{18}O$	130.229
5.	17.18	0.55	4-Nonanone	$C_9H_{18}O$	142.24
6.	17.271	9.49	Phenol, 3-Methyl-	C_7H_8O	108.14
7.	17.753	0.89	Linalool	$C_{10}H_{18}O$	154.25
8.	17.826	1.61	Nonanal	$C_9H_{18}O$	142.24
9.	18.459	0.55	3-Nonen-2-One	$C_9H_{16}O$	140.22
10.	18.951	1.03	Phenol, 3-Ethyl-	$C_8H_{10}O$	122.16
11.	19.144	0.67	2,4,6,9-Dehydroadamantane	$C_{10}H_{12}$	132.2
12.	19.555	0.78	1,3-Cyclohexadiene-1-Carboxaldehyde, 2,6,6-Trimethyl-	$C_{10}H_{14}O$	150.22
13.	19.607	1.02	Decanal	$C_{10}H_{20}O$	156.26
14.	19.721	1.69	3-Nonen-5-One	$C_9H_{16}O$	140.22
15.	20.434	6.67	3-Propylphenol	$C_9H_{12}O$	136.19
16.	20.939	0.28	3-Cyclohexen-1-Ol, 1-Methyl-	$C_7H_{12}O$	112.17
17.	21.156	0.34	Ethanone	$C_7H_{12}O$	112.17
18.	21.584	0.54	2-Methyl-5-Hexen-3-Ol	$C_7H_{14}O$	114.19
19.	21.639	0.49	1-Undecene	$C_{12}H_{24}$	168.32
20.	22.095	0.22	1-Methoxydecane	$C_{11}H_{24}O$	172.31
21.	22.444	0.54	Oxiranecarboxamide,2-Ethyl-3-Propyl-	$C_8H_{15}NO_2$	157.21
22.	22.488	0.85	1-Hepten-4-Ol	$C_7H_{14}O$	114.19
23.	22.55	0.21	Pyridine, 4-(1-Pyrrolidinyl)-	$C_9H_{12}N_2$	148.2
24.	22.607	0.83	3-Cyanomethyl-2,3-Dehydroquinuclidine	$C_9H_{12}N_2$	148.2
25.	22.715	0.29	Cyclodecanol	$C_{10}H_{20}O$	156.26
26.	23.098	0.47	(-)-Trans-Myrtanylacetat		
27.	23.344	0.83	Trans-Z-.Alpha.-Bisabolene Epoxide	$C_{15}H_{24}O$	220.35

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28.	23.426	0.45	1-Undecanol	$C_{11}H_{24}O$	172.31
29.	24.308	0.34	Chloroacetic Acid, Nonyl Ester	$C_{11}H_{21}CLO_2$	220.73
30.	24.8	0.38	3,5,9-Undecatrien-2-One, 6,10-Dimethyl-	$C_{13}H_{20}O$	192.3
31.	25.267	0.29	2-Propanol, 1-(Isooctyloxy)-2-Methyl-	$C_{12}H_{26}O_2$	202.33
32.	25.5	3.63	Ethylene Brassylate	$C_{15}H_{26}O_4$	270.36
33.	25.81	0.82	1,11-Undecanediol	$C_{11}H_{24}O_2$	188.31
34.	25.883	0.57	2-Tridecenal	$C_{13}H_{24}O$	196.33
35.	26.006	1.23	Dodecadienol	$C_{15}H_{28}O$	224.38
36.	26.045	0.32	6-Tridecanol	$C_{17}H_{36}O$	256.5
37.	26.16	0.29	4-Methyladamantan-2-Ol	$C_{11}H_{18}O$	166.26
38.	26.622	0.22	Dodecyl Octyl Ether	$C_{20}H_{42}O$	298.5
39.	26.702	0.55	Quinoxalinedione	$C_9H_8N_2O_2$	176.17
40.	27.151	0.35	2,6,10-Dodecatrien-1-Ol, 3,7,11-Trimethyl-	$C_{15}H_{26}O$	222.37
41.	27.414	0.55	Palmitaldehyde, DiisopentylAcetal	$C_{26}H_{54}O_2$	398.7
42.	27.551	0.67	Cyclotridecanone	$C_{13}H_{24}O$	196.33
43.	28.096	2	2,5-Dimethyl-4-Hydroxy-3-Hexanone	$C_8H_{16}O_2$	144.21
44.	28.966	0.56	Hexadecenal	$C_{17}H_{32}O$	252.4
45.	29.245	0.28	1,5,9-Cyclododecanetriol	$C_{12}H_{24}O_3$	216.32
46.	30.213	0.38	9-Octadecenoic Acid	$C_{19}H_{36}O_2$	296.5
47.	30.971	1.29	Phenylpentanoic Acid	$C_{11}H_{12}O_3$	192.21
48.	31.01	0.39	Ethyl IsopropylaminoOximinoacetate	$C_7H_{14}N_2O_3$	174.2
49.	31.205	0.19	2,6-Dimethyl-2,4,6-Octatrienedial	$C_{10}H_{12}O_2$	164.2
50.	31.59	2.06	Dodecenol	$C_{14}H_{25}FO_2$	244.34
51.	31.959	0.44	Glycidyl Palmitate	$C_{19}H_{36}O_3$	312.5
52.	32.585	1.75	3-Pentanol	$C_7H_{16}O$	116.2
53.	32.68	0.38	1h-Pyrazole-5-Carboxylic Acid, 3-(1h-Indol-3-Yl)-, Hydrazide	$C_{12}H_{11}N_5O$	241.25
54.	32.797	0.45	Hexanedioic Acid	$C_{22}H_{42}O_4$	370.6
55.	33.206	2.26	Hexadecadienol	$C_{18}H_{32}O_2$	280.4
56.	33.321	2.94	Diethyl N-Hexadecylmalonate	$C_{23}H_{44}O_4$	384.6
57.	33.632	1.6	Pentadecadienol	$C_{15}H_{28}O$	224.38
58.	33.691	2.46	Undec-10-Ynoic Acid, Undec-2-En-1-Yl Ester	$C_{22}H_{38}O_2$	334.5
59.	33.812	0.19	Sebacic Acid	$C_{14}H_{24}CL_2O_4$	327.2

Table: 2. List of volatile compounds present in Ongole breed cattle.

S. NO	Rt	Area %	Common Name	Molecular Formula	Molecular Weight
1.	4.885	3.76	1,3-Dioxonane,4-Methynene-	$C_4H_6O_2$	86
2.	5.235	3.34	1,2,4,5-Tetrazine,1,4-Diethynhexahydro-	$C_6H_{16}N_4$	144
3.	6.125	3.14	OxalicAcid	$C_{12}H_{20}O_4$	228

4.	6.640	4.74	1-Decene	$C_{10}H_2O$	140
5.	7.465	1.87	1-Hexanol,2-Ethyl	$C_8H_{18}O$	130
6.	9.135	0.54	Pentadecane	$C_{15}H_{32}$	212
7.	33.470	1.1	(E)-13-DocosenoicAcid	$C_{22}H_{42}O_2$	338
8.	36.140	0.74	Propane	$C_3H_6Br_2$	200
9.	36.425	0.45	5-Decyne	$C_{10}H_{18}$	138
10.	36.535	0.64	4-Tert-Butylcyclohexyl Ethylphosphonofluoridate-	$C_{12}H_{24}FO_2P$	250
11.	36.620	1.05	1,1-Bis(2-Methyl-4-Hydroxy-5-Tert-Butylbhenyl-	$C_{26}H_{38}O_2$	382
12.	36.765	0.63	Hexane	$C_6H_{12}Br_2$	242
13.	38.075	3.95	Phenol	$C_{42}H_{63}O_3P$	646
14.	38.380	0.57	5-Acetamido-4,7-Dihydroxybenzofurzan-	$C_8H_7N_3O_4$	209
15.	38.500	0.65	AdipicAcid	$C_{26}H_{42}O_4$	418
16.	38.720	0.94	1-Piperidinocyclohexane Carbonitrile-	$C_{12}H_{20}N_2$	192
17.	39.320	0.68	Propolamine,N-[9-Borabicyclo[3.3.1]Non-9-Yl]-	$C_{11}H_{22}BN$	179
18.	39.980	0.66	FumaricAcid	$C_{28}H_{48}O_4$	448
19.	40.165	0.61	PropanoicAcid	$C_{15}H_{26}N_2O$	250

Table: 3. List of volatiles compounds present in Rathi breed cow

S.No	Retention Time	Area %	Compound Name	Molecular Formula	Molecular Weight
1.	4.636	2.4	Styrene	C_8H_8	104.15
2.	4.884	1.34	Thiophene-D1	C_4H_4S	84.14
3.	5.236	1.09	2-Ethoxyethyl 3-Methylbutanoate	$C_9H_{18}O_3$	174.24
4.	6.125	1.02	3,3-Diethoxy-1-Propyne	$C_7H_{12}O_2$	128.169
5.	6.613	1.52	1-Decene	$C_{10}H_{20}$	140.27
6.	7.467	0.74	1-Hexanol, 2-Ethyl-	$C_8H_{18}O$	130.229
7.	28.801	0.25	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	230.39
8.	29.328	10.24	9,12-Octadecadienoic Acid	$C_{18}H_{32}O_2$	280.4
9.	29.427	19.54	9-Octadecenoic Acid	$C_{18}H_{34}O_2$	282.5
10.	29.496	11.15	Oleic Acid	$C_{18}H_{34}O_2$	282.5
11.	29.715	1.13	(6r,7r)-Bisabolone	$C_{15}H_{24}O$	220.35
12.	29.773	2.71	Octadecanoic Acid	$C_{18}H_{36}O_2$	284.5
13.	31.672	0.31	Docosanoic Acid	$C_{22}H_{44}O_2$	340.6
14.	33.395	1.51	E,Z-1,3,12-Nonadecatriene	$C_{19}H_{34}$	262.5
15.	33.471	1.71	Oleoyl Chloride	$C_{18}H_{33}ClO$	300.9

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Table: 4. List of volatile compounds present in Tharparkar Breed Cow

S.No	Retention Time	Area %	Compound Name	Molecular Formula	Molecular Weight
1.	4.635	2.4	Styrene	C_8H_8	104
2.	4.885	1.34	Thiophene-D1	C_4H_3S	85
3.	5.235	1.09	2-Ethoxyethyl 3-Methylbutanoate	$C_9H_{18}O_3$	174
4.	6.125	1.02	3,3-Diethoxy-1-Propyne	$C_7H_{12}O_2$	128
5.	6.615	1.52	1-Decene	$C_{10}H_{20}$	140
6.	7.465	0.74	1-Hexanol, 2-Ethyl	$C_8H_{18}O$	130
7.	26.625	3.54	Ascorbic Acid	$C_{38}H_{68}O_8$	652
8.	28.800	0.25	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	230
9.	29.425	19.54	9-Octadecenoic Acid	$C_{18}H_{34}O_2$	282
10.	29.495	11.15	Oleic Acid	$C_{18}H_{34}O_2$	282
11.	29.715	1.13	(6R, 7R)-Bisabolone	$C_{15}H_{24}O$	220
12.	29.775	2.71	Octadecanoic Acid	$C_{18}H_{36}O_2$	284
13.	30.120	0.91	9,12- Octadecadienoic Acid	$C_{18}H_{32}O_2$	280
14.	31.020	0.3	Chloromethyl 2-Chlorododecanoate	$C_{13}H_{24}C_{12}O_2$	282
15.	31.670	0.31	Docosanoic Acid	$C_{22}H_{44}O_2$	340
16.	33.395	1.51	E,Z-1,3,12-Nonadecatriene	$C_{19}H_{34}$	262
17.	33.470	1.71	Oleoyl Chloride	$C_{18}H_{33}C_{1O}$	300
18.	33.580	0.25	Isoxazole-4-Carbonitrile, 5-Amino-3-Methyl	$C_5H_5N_3O$	123
19.	34.040	0.86	(R)-(-)-14-Methyl-8-Hexadecyn-1-O1	$C_{17}H_{32}O$	252
20.	34.115	1.51	GlycidylOleate	$C_{21}H_{38}O_3$	338
21.	34.465	0.24	Oxalic Acid	$C_{24}H_{46}O_4$	398

Table: 5. Comparative GC-MS volatile compounds present in specific south and north breed cows

S.No	RT	Area %	Molecular Formula	Molecular Weight	Compound Name	Bargur (South India)	Ongole (South India)	Rathi (North India)	Tharparkar (North India)
1.	10.855	0.5	$C_7H_{14}O$	114.19	4-Heptanone	✓	X	X	X
2.	15.164	0.47	$C_8H_{14}O_2$	142.2	2,5-Octanedione	✓	X	X	X
3.	15.688	0.4	$C_8H_{12}O$	100.16	3-Hexenol	✓	X	X	X
4.	17.18	0.55	$C_9H_{18}O$	142.24	4-Nonanone	✓	X	X	X
5.	17.271	9.49	C_7H_8O	108.14	Phenol, 3-Methyl-(M-Cresol)	✓	X	X	X
6.	17.753	0.89	$C_{10}H_{18}O$	154.25	Linalool	✓	X	X	X
7.	17.826	1.61	$C_9H_{18}O$	142.24	Nonanal	✓	X	X	X
8.	18.951	1.03	$C_8H_{10}O$	122.16	Phenol, 3-Ethyl-	✓	X	X	X
9.	19.607	1.02	$C_{10}H_{20}O$	156.26	Decanal	✓	X	X	X
10	20.434	6.67	$C_9H_{12}O$	136.19	3-Propylphenol	✓	X	X	X

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11	21.156	0.34	C_2H_4O	44.05	Ethanone	✓	X	X	X
12	21.639	0.49	$C_{11}H_{22}$	154.29	1-Undecene	✓	X	X	X
13	22.488	0.85	$C_7H_{14}O$	114.19	1-Heptenol	✓	X	X	X
14	23.426	0.45	$C_{11}H_{24}O$	172.31	1-Undecanol	✓	X	X	X
15	25.883	0.57	$C_{13}H_{24}O$	196.33	2-Tridecenal	✓	X	X	X
16	26.006	1.23	$C_{12}H_{20}O$	180.63	Dodecadienol	✓	X	X	X
17	26.045	0.32	$C_{13}H_{28}O$	200.36	6-Tridecanol	✓	X	X	X
18	26.702	0.55	$C_8H_4N_2O_2$	160.17	Quinoxalinedione	✓	X	X	X
19	27.151	0.35	$C_{12}H_{20}O$	218.37	Dodecatrienol	✓	X	X	X
20	28.966	0.56	$C_{16}H_{30}O$	238.4	Hexadecenal	✓	X	X	X
21	30.971	1.29	$C_{11}H_{14}O_2$	178.21	Phenylpentanoic Acid	✓	X	X	X
22	31.59	2.06	$C_{12}H_{24}O$	184.32	Dodecanol	✓	X	X	X
23	32.585	1.75	$C_5H_{12}O$	88.15	3-Pentanol	✓	X	X	X
24	32.797	0.45	$C_6H_{10}O4$	146.14	Hexanedioic Acid	✓	✓	X	X
25	33.206	2.26	$C_{16}H_{30}O$	238.34	Hexadecadienol	✓	X	X	X
26	33.632	1.6	$C_{15}H_{28}O$	224.38	Pentadecadienol	✓	X	X	X
27	33.812	0.19	$C_{10}H_{18}O_4$	202.25	Sebacic Acid	✓	X	X	X
28	6.125	3.14	$C_2H_2O_4$	90.30	OxalicAcid	X	✓	✓	✓
29	6.640	4.74	$C_{10}H_{20}$	140.27	1-Decene-	X	✓	X	X
30	7.465	1.87	$C_8H_{16}O_2$	144.12	1-Hexanol,2-Ethyl	X	✓	X	✓
31	9.135	0.54	$C_{15}H_{32}$	212.41	Pentadecane	X	✓	X	X
32	33.470	1.1	$C_{22}H_{42}O_2$	338	(E)-13-DocosenoicAcid (Brassidic Acid)	X	✓	X	X
33	36.140	0.74	C_3H_8	44.10	Propane	X	✓	X	X
34	36.425	0.45	$C_{10}H_{18}$	138	5-Decyne	X	✓	X	X
35	36.765	0.63	C_6H_{14}	86.18	Hexane	X	✓	X	X
36	38.075	3.95	C_6H_6O	94.11	Phenol	X	✓	X	X
37	39.980	0.66	$C_4H_4O_4$	116.07	FumaricAcid (Boletic Acid)	X	✓	X	X
38	40.165	0.61	$C_3H_6O_2$	74.08	PropanoicAcid (Pseudo acetic Acid)	X	✓	X	X
39	4.636	2.4	C_8H_8	104.15	Styrene	X	X	✓	✓
40	4.884	1.34	C_4H_4S	84.14	Thiophene-D1	X	X	✓	✓
41	6.125	1.02	$C_7H_{12}O_2$	128.169	3,3-Diethoxy-	X	X	✓	✓

					1-Propyne				
42	6.613	1.52	$C_{10}H_{20}$	140.27	1-Decene	X	X	✓	✓
43	7.467	0.74	$C_8H_{18}O$	130.229	1-Hexanol	X	X	✓	X
44	28.801	0.25	$C_{14}H_{30}O_2$	230.39	1,14-Tetradecanediol	X	X	✓	✓
45	29.328	10.24	$C_{18}H_{32}O_2$	280.4	9,12-Octadecadienoic Acid	X	X	✓	✓
46	29.427	19.54	$C_{18}H_{34}O_2$	282.5	9-Octadecenoic Acid	✓	X	✓	✓
47	29.496	11.15	$C_{18}H_{34}O_2$	282.5	Oleic Acid	X	X	✓	✓
48	29.715	1.13	$C_{15}H_{24}O$	220.35	(6r,7r)-Bisabolone	X	X	✓	✓
49	29.773	2.71	$C_{18}H_{36}O_2$	284.5	Octadecanoic Acid	X	X	✓	✓
50	31.672	0.31	$C_{22}H_{44}O_2$	340.6	Docosanoic Acid	X	X	✓	✓
51	33.395	1.51	$C_{19}H_{34}$	262.5	Nonadecatriene	X	X	✓	✓
52	26.625	3.54	$C_{38}H_{68}O_8$	652	Ascorbic Acid	X	X	X	✓
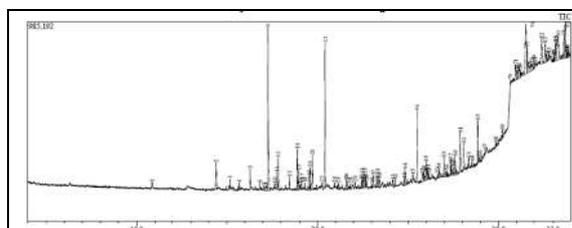
Fig. 1. GC-MS Chromatogram of Bargur breed cow
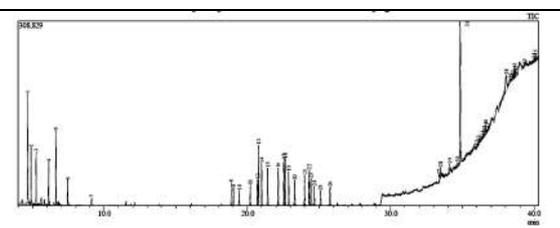
Fig. 2. GC – MS Chromatogramf Ongole breed cow
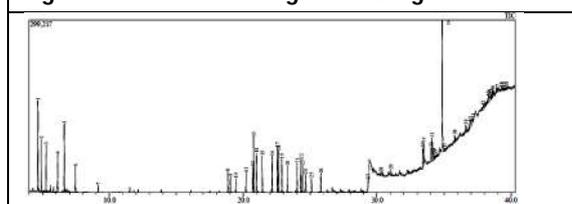
Fig. 3. GC – MS Chromatogram of Rathi breed cow
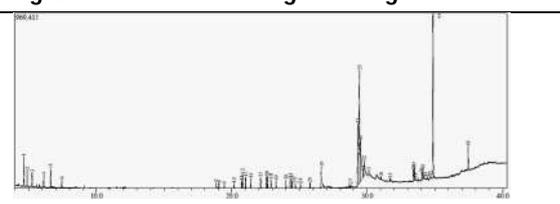
Fig. 4. GC – MS chromatography of Tharparkar Breed Cow

RESEARCH ARTICLE

Factors Influencing Learners' Gain of English Proficiency- A Statistical Approach

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ABSTRACT

This study aimed at determining the influencing factors that might have impact on the acquisition of English language. The article deals with the importance of English language to rural technical students in the present state of art and applying Taguchi technic to determine most influencing factors to address the problem. The subject of the study contains 300 students of Government Engineering College, Haveri. Studying in Civil, Mechanical, Computer Science, and Electronics and Communication belongs to different backgrounds, 18 influencing factors have been listed and this percentage of contribution is calculated using Regression analysis.

Keywords: Engineering students, Influencing factors, Taguchi, Regression analysis.

INTRODUCTION

The goal of education in the twenty-first century is to develop an integrated person with an integrated understanding of life, enabling one to deal with the ever-increasing complexity of existence. The language that is spoken the most over the globe is English. The language of communication with in modern world is English. Administrative and organisational communications are conducted primarily in English, with little emphasis placed on regional languages. To exchange knowledge, ideas, beliefs, and emotions, English is essential to the complete development of the person and society. A pupil should have a solid grasp of the language and also be able to articulate their thoughts clearly and fluidly. In multicultural and international businesses and communities, effective communication and teamwork across cultural, geographic, and linguistic boundaries are essential. For students to

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apply your knowledge and also be employable, they require communication skills. These abilities are the routes to improved communication. English language proficiency (EL) is recognised as being extremely significant in both learning and teaching and is one of the key elements of students' generic abilities. The competency of EL is being and has been improved through a variety of means. It is now a requirement for students taking professional courses to be proficient in EL. Numerous research on the subject have recently been conducted, with an emphasis on factors including gender, country, educational level, and form of instruction. However, issues like the student's or college's location, the type of education used in previous classes, the parents' qualifications, etc., have not been taken into account or discussed. There is a dearth of research on these variables and how they relate to students' EL proficiency, particularly among engineering students.

FACTORS INFLUENCING THE PROFIENCY

English is now widely spoken on a global scale and is used in many aspects of daily life. As a result, people of any age and nationalities have been learning English while socialising, studying abroad, finding employment, and other purposes. But while some people pick up English quickly, others have had trouble picking up the so-called lingua franca. Researchers have explored several elements that affect learners' English proficiency levels in studies that span the literature of English language teaching and learning. However, very few studies have attempted to combine all of these elements at once. In the meanwhile, a review of the influencing elements will increase the knowledge of English language learners, teachers, and researchers. It will also assist them in maximizing the positive factors and minimizing the bad ones. Several elements that affect applicants' results have been outlined below based on research done on the outcomes of students enrolled with in government engineering college Haveri. Medium of instructions

- Locality
- Parent's qualification
- Parent's occupation
- Gender
- Father's education
- Mother's education
- Parent's income
- Medium of study till 10th
- Mother tongue
- Mode of instruction
- Locality of the college
- Availability of advanced literature
- Internet facilities
- Class-strength
- Rank in eligibility
- Teaching/learning hours
- Teaching/learning mode (offline, online, blended, chalk and talk, use of ict tools, etc.)

On the basis of the aforementioned considerations, various groups of the students included in the study have been formed. Each element's results underwent a separate analysis, and an impact factor was awarded for each one. On a scale of 1 to 10, the impact factor's level has been graded. This 10-point evaluation is solely based on the pupils' final test scores.

TAGUCHI TECHNIQUE

The Taguchi approach is an effective instrument for creating high-caliber systems. The Taguchi technique to experimentation offers a systematic means to gather, analyse, and interpret data in order to meet the study's objectives [1,2]. When designing experiments, one can get the most information possible given the quantity of testing done by following these strategies. By adjusting the design parameters, Taguchi parameter design can maximise the performance characteristics and lessen the vulnerability of the system performance to a source of variation [3-5]. This is achieved by effectively applying data runs to a investigated variable combinations. This method is an effective

instrument for gathering data in such a controlled manner and for analyzing the impact of process variables on a given variable, which is one of the process variables' unknown functions. The choice of components is the most crucial step in the experiment strategy. To account for the impact of various factors on the goal value, the Taguchi technique generates a standard orthogonal array and specifies the experimentation strategy. Analysis on means and variance is used to examine the experimental data in order to determine the influence of various factors. This method can cut down on the tedious calculations and data collection involved in traditional data analysis techniques. This Taguchi technique was used in the current investigation to identify the important variables that will affect the student's competency. The number of components and their levels determine which orthogonal arrays are used in this method. The L27 orthogonal array having three factors as three levels has initially been taken into consideration in this work. For the selected factors, an analysis of variance (ANOVA) and a regression analysis have been conducted. The generalised regression equation would be generated after the relevant factor's contribution has been established.

Factors and their levels

The study's methodology, locality, and parent's educational level have been selected with three distinct levels out of all the aforementioned characteristics. The levels have indeed been defined on a 10-point scale based on student data from GEC Haveri as follows:

Medium of study

Kannada Medium	: 3
Kannada upto 7th std and then English	: 6
English Medium	: 8

Locality

Rural	: 2
Semi Urban	: 5
Urban	: 7

Parents Qualification

Illiterate/Below degree	: 4
Degree	: 6
Master degree and above	: 8

 The finalized table of factors and the levels has been shown in the table 1. Minitab, a programme for designing experiments, was used to analyse the data. The L27 orthogonal array is selected for data analysis and to have the highest level of conformance. Data with combinations of the parameters is accordance the with L27 orthogonal array have been gathered from the selected samples in order to understand the impact of study medium, location, and parental education. Three levels of each parameter have been selected. Table 2 displays the variables and findings for the L27 orthogonal array. With the use of an array table, which contains permutations and combinations of the factors being taken into consideration, the total number the samples analysis that must be performed is significantly decreased when using the design of experiments technique. The statistical tool MINITAB software is used to determine the contribution of each parameter. Each parameter's contribution is listed in Table 3 below, as well as the effect of each contribution is identified with a 95% confidence level. The analysis of variance (ANOVA) is used to determine which variables have a significant impact on the students' test results. The table provides details on how each aspect contributed to the procedure and how important they were to the student's outcome. The findings of a mean values of the selected parameters and their levels are shown in table 3 following the signal to noise (S/N) analysis. Additionally, the ranking has been made obvious. The data indicates that the parents' educational backgrounds come in second to the medium of instruction in terms of its impact on pupils' final test scores. The analysis of variance (ANOVA) was performed using the MINITAB software to ascertain how the selected parameters affected the outcome. Based on Table 4, it is clear that the study method has the biggest percentage impact on

students' results (38.4%), followed by the parents' educational attainment (29.69%) and location (25.41%). The findings indicate that pupils who may have arrived from the Kannada medium should receive a lot of attention.

Figure 1 displays main effect graphs for student results based on the influence of specific parameters. The Figure makes it abundantly evident that a student's method of study has a significant impact on their academic performance. This can be linked to the Kannada-speaking students' subpar comprehension and learning skills. In comparison to the medium of instruction and parents' educational backgrounds, the influence of locale has a negligible impact and makes up a smaller portion of the equation.

REGRESSION ANALYSIS

The medium of study, location, and parent's education are the factors that are independent and have an impact on the response variables. For this investigation, the generalised linear regression equation might be expressed as:

$$Y = A_0 + A_1M + A_2L + A_3P$$

The variables M, L, and P stand for the location, parent's education, and medium of instruction, respectively. A1, A2, and A3 are the relative coefficients for the independent variables M, L, and P.Using linear regressions, it was possible to determine the association between the parameters and the outcome based on the data collection and the ANOVA Tables 2 and 4. Following is the obtained correlation:

Given by the regression equation

Results = 24.0 + 3.07 Medium of Study + 2.29 Locality + 2.92 Parents Qualification

SUMMARY

There are numerous statistical methods for analysing how factors affect English language learning proficiency, however these methods need extensive data analysis. The aforementioned Taguchi technique can efficiently identify the most important aspect, on which care should be done to increase proficiency, and tackle any such problem with new parameters and their levels. Additionally, the regression equation can provide a generalised forecast of the outcomes within the specified level limitations. A same type of study can be done at several levels and with various variables. This method of designing experiments can account for any number of variables.

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Table 1. Taguchi design factors and their levels

Levels	Medium of Study	Locality	Parents Qualification
1	3	2	4
2	6	5	6
3	8	7	8

Table 2. L27 Orthogonal Array

Trial No	Medium of Study	Locality	Parent Qualification	Results In %
1	3	2	4	49
2	3	2	4	51
3	3	2	4	53
4	3	5	6	60
5	3	5	6	63
6	3	5	6	67
7	3	7	8	73
8	3	7	8	75
9	3	7	8	71
10	6	2	6	55
11	6	2	6	57
12	6	2	6	54
13	6	5	8	77
14	6	5	8	75
15	6	5	8	76
16	6	7	4	73
17	6	7	4	75
18	6	7	4	70
19	8	2	8	81
20	8	2	8	83
21	8	2	8	86
22	8	5	4	76
23	8	5	4	74
24	8	5	4	71
25	8	7	6	89
26	8	7	6	75
27	8	7	6	70

Table 3 Response Table for Signal to Noise Ratios Larger is better

Level	Medium of Study	Locality	Parents Qualification
1	62.44	63.22	65.78
2	68.00	71.00	65.56
3	78.33	74.56	77.44
Delta	15.89	11.33	11.89
Rank	1	3	2

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Table 4 Analysis of Variance for SN ratios

Source	DF	Seq SS	Adj SS	Adj MS	F	P	% Contribution
Medim of Study	2	6.226	6.226	3.1132	5.93	0.144	38.40
Locality	2	4.121	4.121	2.0606	3.92	0.203	25.41
Parents Qualification	2	4.814	4.814	2.4068	4.58	0.179	29.69
Residual Error	2	1.051	1.051	0.5254			
Total	8	16.212					

Figure 1. Main effect plots

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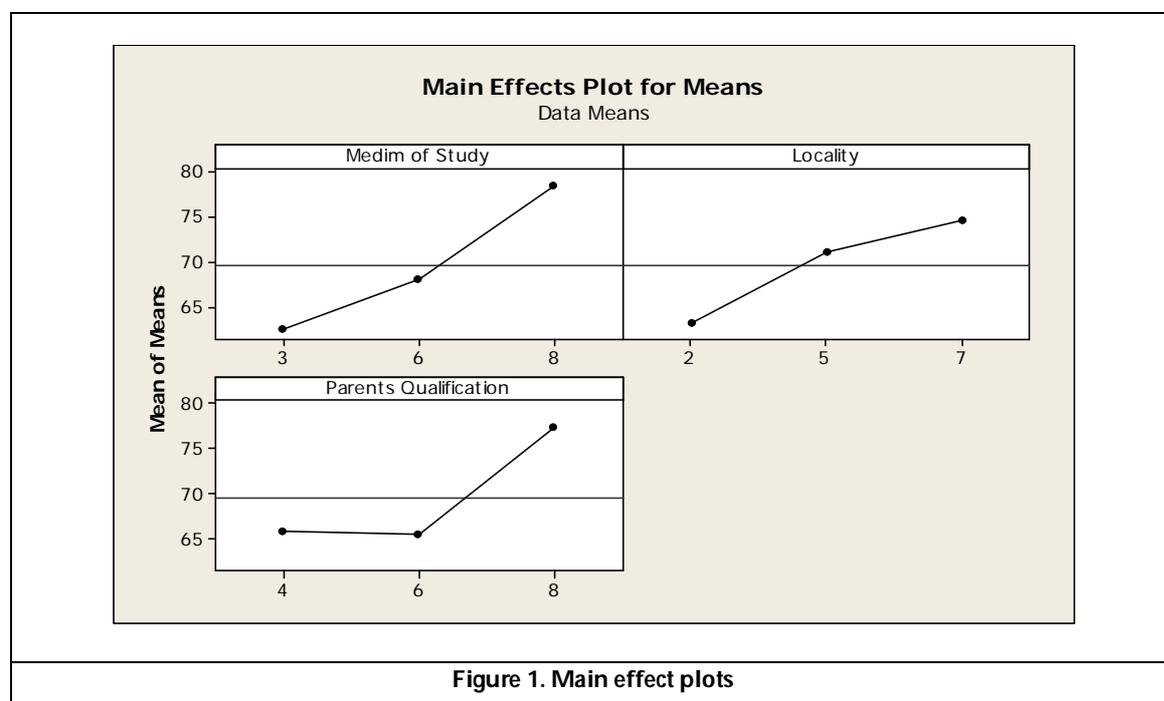
Capacity and Efficiency Improvement to Meet the Customer Demand using Lean Tools – A Case Study Approach

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ABSTRACT

Every day the Electrical accessories industry is optimizing their products to meet new needs and requirements of consumption. The underlying principle of minimization of waste for enhancing productivity has become profoundly influential since being developed into the lean construct. Either way, the basic idea is to bring production capacity, demand, and inventory into sync. It all depends on your product, demand, etc.,

Keywords: Electrical, industry, domain, technological.

INTRODUCTION

Every day the Electrical accessories industry is optimizing their products to meet new needs and requirements of consumption. Buyers of electrical accessories are craving for the best electrical product, durability, efficacy, quality, and most importantly price. The huge competition emanated from the business cycle three-principle patterns is tremendously substantial. Even though the Electrical accessories industry has evolved greatly during the previous century, it is still governed by business cycle fluctuations. Electricity companies are now an indispensable domain specialty. They perform multiple tasks related to the electric sector. By all means, the electric utility does not touch upon domestic use only. Nowadays electrical needs extend to vital parts of life such as education, health, tourism, technological centers etc.

Electrical accessories are shipped out to global market which is approx 40% of our cost of goods sold. Foreseeing the increase in demand (15 to 20%), and with lean tool implementation on existing line with minimal investment, customers are secured with highest quality and service level.

Problem statement: Under utilization of line capacity with respect to high customer demand and Minimal adaptation of Lean. Challenges faced in the Electrical accessories product line

- ❖ Capacity unutilized
- ❖ Low Industrial efficiency and line efficiency
- ❖ More Changeover with high Time
- ❖ Limited Elasticity & Flexibility
- ❖ Not compliance replenishment system
- ❖ High waste & Ergonomic issues
- ❖ Difficulty in management of product mix

This case study was done to focus to improve the line capacity and efficiency of Electrical accessories product line to meet customer orders on time.

ADOPTION OF LEAN TOOLS IN ELECTRICAL ACCESSORIES PRODUCTION LINE

Lean is considered an essential attribute of a successful manufacturing endeavor. The underlying principle of minimization of waste for enhancing productivity has become profoundly influential since being developed into the lean construct. The term "Lean" was coined in 1988 by John Krafcik, and defined in 1996 by James Womack and Daniel Jones to consist of five key principles; 'Precisely specify value by specific product, identify the value stream for each product, make value flow without interruptions, let customer pull value from the producer, and pursue perfection.' (Womack and Jones 1996 p10).

Value stream mapping

Specifying the value, desired by the customer, and identifying the value stream of the product that adds value and challenges all the wastes is the base line of Value stream mapping. Value stream mapping is the process of creating user-friendly format of the workflow using visualizations. Imagine that you have a business that builds a product for your customers. The Product development has different stages and steps involved in it. Each of these steps adds some value to the final product.

Value stream mapping is a great way to optimize the process cycle and make it more efficient. Value stream mapping helps to understand where there is waste in the product development system. This, in return, helps you plan on how to get the waste reduced or eliminated.

Current VSM Refer Figure-1 ,Current Value stream mapping output:
1. More internal movement
2. 5 point scheduling
3. Low line efficiency
4. High Changeover time
5. More rework
6. Capacity unutilized 70%

Reduced internal movement Electrical accessories product line
➢ Reduce Internal Movement by Feeder Re-layout– Spaghetti, LADM
Refer figure 2; Constraints are
1. Toggle printing far away from lock assy
2. Core assy internal movement is high

46062

3. Terminal subassy spread on existing layout
4. All subassembly finished storage away from finishing line

Actions taken
Part and subassembly travel distance reduced :
Toggle:24 metres
Screwing:4.8 metres
Riveting: 5.5 metres
Core assembly:23 metres

Eliminate multiple scheduling
➢ **Kanban implementation**

Eliminate multiple scheduling by Kanban systems Ball Kanban (Pull system of replenishment) Kanban- refer figure-4 is a visual method for controlling production as part of Just in Time (JIT) and Lean Manufacturing. As part of a pull system it controls what is produced, in what quantity, and when. It is a system of signals that is used through the value stream to pull product from customer demand back to raw materials. Its literal meaning is that of a flag or sign, when you see that number of balls you know that it is time to manufacture the next part. Kanban can take many forms but in most production facilities they will use Kanban cards or bins/or balls to control the process, although there are no limits to how you can control and design Kanban andits up to the organization.

In this case study, Ball Kanban refer figure -5 was been implemented to maintain the inventory and control the production and maintain the pull flow system, Just in time lean systems.

EPEI- Every part every interval EPEI is also known by the Japanese term Heijunka, which literally means "leveling." In this context, it refers to leveling production or inventory. Either way, the basic idea is to bring production capacity, demand, and inventory into sync. Break production into lots of whatever size is feasible given your capacity, then produce those lots in repeating cycles to meet demand and keep inventory from stagnating. The time for each lot to be produced is referred to as the interval, which can be anything from a single shift at the factory to an entire quarter. It all depends on your product, demand, etc.

EPEI Sheet Introduced for RCCB feeder Subassembly EPEI influences of Main KPI in this case study
Refer figure 6 and figure 7

Inventory level Milk run /Kanban implemented in Supplier place of manufacturing child partsin order to increase the delivery frequency and to match with the production batch size for this specific frequency.

Improved on time delivery and time to market
Made in to lower batch sizes and process made flexible.

Quality improvement
Scrap and rework reduction made process more reliable and save time and money

Change over time Minimization and improve line efficiency
Refer figure-8 -Pad printing machine change over reduced from 26 to 12.3 min Welding machine changeover reduced from 28 to 8 min

Variation reduction: Six sigma Welding capability improvement

➢ Fixture design has been changed

➢ Process parameters has been optimized

➢ Parts Critical quality dimensions cpk improved to 1.35(Refer minitab output from Figure 9)

Reduce rework

Rework reduced from 1000 ppm to 300 ppm by introducing Pokayoke in fixture(refer figure10 and camera detection in vision system (Figure 11) Benefits out of this case study(Refer Figure-12)

1. Capacity realized improved from 6900 to 9000 nos

2. Hourly rate improved by 25.7%

3. Industrial and line efficiency improved from 36% to 44%

4. Rework ppm reduced from 1000 ppm to 300 ppm

5. Increase in Customer service level from 92% to 100%

Learning's out of this Case study:

1. Cross functional team Gemba brainstorming session blown out more realistic ideas

2. Operator feedback and involvement extremely helpful during implementation

3. Team improved knowledge on VSM,EPEI,KANBAN & SMED concepts

4. Systematic approach with CFT team

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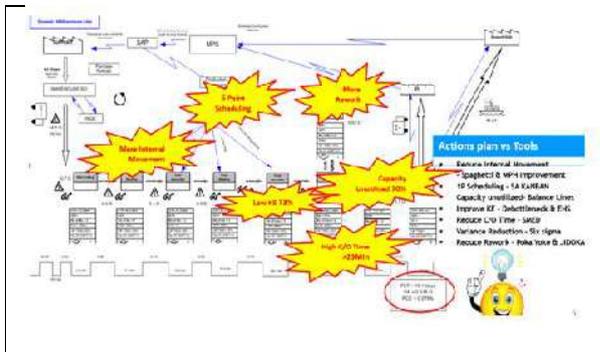
Figure 1. Current VSM
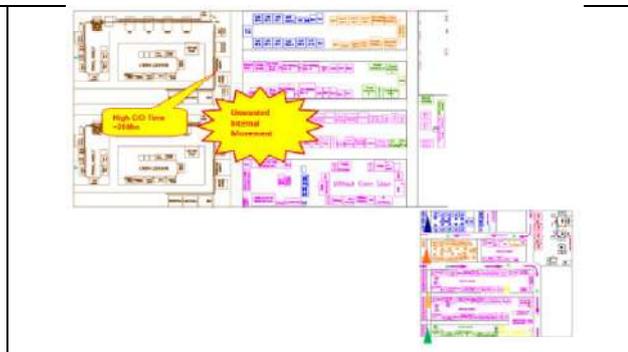
Figure 2. Electrical accessories product line

Figure 3. MPH Report

Figure 4. Eliminate multiple scheduling by Kanban systems

Figure 5. Ball Kanban(Pull system of replenishment)

Figure 6. EPEI influences of Main KPI in this case study

Figure 7. EPEI influences of Main KPI in this case study

Figure 8. Change over time Minimization and improve line efficiency
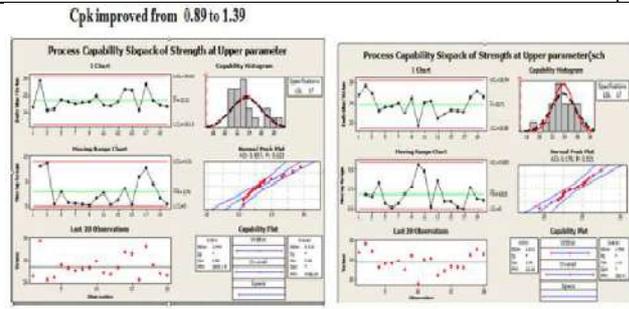
Figure 9. Cpk improved from 0.89 to 1.39

Figure 10. Rework reduced from 1000 ppm to 300 ppm
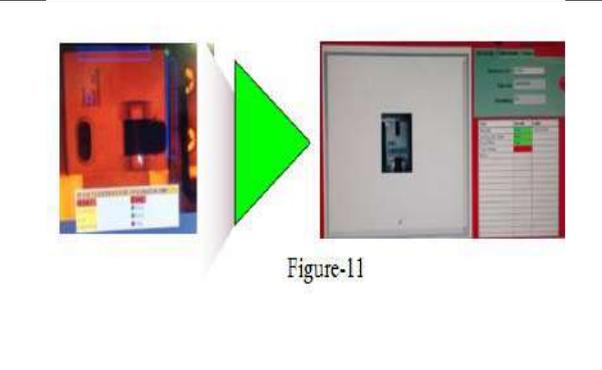
Figure 11. To Improve vision system by camera
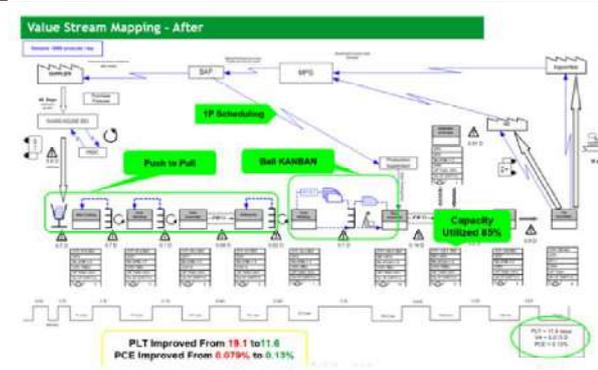
Figure 12. Value Stream Maping

RESEARCH ARTICLE

Studies on Integrated Nutrient Management and Multiple Harvesting of Coriander (*Coriandrum sativum* L.)

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ABSTRACT

Harvesting of good coriander crop with multiple cutting can increase profit to the growers. Fast growth after each cutting needs proper nutrient management with integrated manner minimizing health and environmental hazards. The present experiment was conducted at Babasaheb Bhimrao Ambedkar University, Lucknow, U.P., India to find out the effect of integrated nutrient management and multiple cuttings of coriander grown during *rabi* season. The study consisted of 15 treatment combinations with three replications and followed two factor randomized block design (Factor 1- Nutrient management and Factor 2- number of Cutting or harvesting. The observations were recorded for vegetative growth, leaf yield, flowering and leaf quality of Coriander cv. ACr-1 and treatment means were compared at 5% level of significance. The experimental finding revealed that application of urea with three cutting (N_1C_3) produced maximum green leaf yield followed by application of 50% urea with 50% KWM (N_3C_3). Whereas, N_1C_1 (urea with one cutting) followed by N_1C_3 (urea with three cutting) produced maximum seeds. Thus, it can be concluded that application of urea and three cuttings could be beneficial for maximum yield and better quality of coriander production and kitchen waste compost could be an alternative for future nutritional management.

Key words: Coriander, Cutting, Growth, INM, Quality, Yield

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INTRODUCTION

Among the primary nutrients, nitrogen has a considerable effect, not only on quantity of produce but on quality of produce also. It helps photosynthesis, respiration and protein synthesis. It imparts the dark green color of leaves, promotes vigorous vegetative growth and more efficient use of available input leads to higher productivity. Application of proper amount of nitrogen resulted in maximum plant height, number of leaves, number of primary and secondary branches, fresh weight of plant and leaf yield. Oil content and oil yield of coriander were improved by application of sulphur. Uptake of nitrogen, kitchen waste manure (KWM) by plants were also increased significantly with increase in nitrogen levels from 0 to 10 kg/ha. Coriander (*Coriandrum sativum* L.; family Umbelliferae) is one of the important spice crops whose edible leaves are produced within a short period and leaves are used for many purposes like in salad, coriander daal, chutney and seeds are used mostly as spice like coriander powder. It has very refreshing property and good for digestion. However, productivity of coriander is low as compared to actual yield potential due to incorrect application of agro-techniques particularly nutrient management. Now-a- days, there is increasing demand of green coriander leaves throughout the year and especially in off season. Nitrogen deficiency decreases leaf size and photosynthetic rate and consequently radiation interception and use efficiency [1]. But, judicial nutrient application encourages vegetative growth and yield of leaves and seeds of coriander [2, 3]. However, nutrient management through chemical mean is creating negative impact on human health, causing hazards to soil and environment. Thus, use of nutrients obtained from organic and natural sources is promoting for sustainable development. In this relation several cuttings of leaves at marketable size have been made to extend the duration of cropping, availability of leaves during off time as well as to get more crop from the some piece of land without repeated sowing of seeds which need more space, more time to grow and more cost. Due to multiple cutting of leaves nitrogenous fertilizer or nutrient management is very important to promote more vegetative growth. Therefore, the present investigation has been planned to produce coriander for its leaf production purpose mainly to fetch more income for the growers with the help of integrated nutrient management combined with kitchen waste manure (KWM) and multiple harvesting.

MATERIALS AND METHODS

The field experiment was conducted at Horticultural Research Farm of the Department of Horticulture, Babasaheb Bhimrao Ambedkar Univrsity, Lucknow –226025 (U.P.), India during Rabi season of 2017-18. Geographically Lucknow is situated at 26 0 50′ N latitude, 80 0 52′ E longitude and altitude of 123 meter above mean sea level (MSL). and has subtropical climate with an average annual rainfall of about 115 cm. The winters are severe and summers are dry and hot. The maximum temperature generally goes up to 43^0C in summer and minimum up to 3^0C in winters. Monsoon generally sets in during the third week of June and recedes by the end of September with heavy rainfall during monsoon season. Soil physical and chemical status of soil of experimental plot was texturally classified as sandy loam soil and slightly alkaline in nature. The decomposable waste materials indicated after complete composting, it was the dried under shed and compost was collected by sheaving. The NPK analysis was done and it showed that this compost has 625.81 kg/ha of nitrogen, 356.02 kg/ha of P_2O_5, and 6336.00 kg/ha of K_2O. There were 15 treatment combinations having five different levels of urea and kitchen waste manure (KWM) (N 1 - Urea 100%, N 2 – Urea 75% + KWM 25%, N 3 – Urea 50% + KWM 50%, N 4 – Urea 25% + KWM 75%, N 5 – KWM 100%) and three dates of leaf cutting (C_1- One cutting 30 Days after seed germination DAS), C_2- Two cutting (30 & 45 DAS), C_3- Three cutting (30, 45 & 60 DAS). The treatments were laid down with three replication and following two factor randomized block design. The observations were recorded for vegetative growth, leaf yield, flowering and leaf quality of Coriander cv. ACr-1. The observed data was analyzed statically [4] and significance study was done at 5 % probability level.

RESULTS AND DISCUSSION

The effect of nutrient and number of cutting has been showed that application of kitchen waste manure (KWM) recorded maximum (17.02 cm) height of plant (N_5) among the nutrient applied in the study at 30 days after sowing (DAS). In case of number of cutting, it was seen that second cutting (C_2) recorded the maximum height of plant (17.03 cm) followed by Three (C_3) cutting (16.74 cm) at 30 DAS. At 45 DAS, it was observed that two cutting (C_2) was attain highest plant height (17.03 cm) while, one cutting (C_1) gets minimum height (16.06 cm) of plant. KWM (N_5) also recorded maximum height of plant (17.04 cm) at 45 DAS. At 60 DAS, a N_5 (KWM 100%) also showed the maximum height of plant (16.93 cm) followed by N_2 (75% urea + 25% KWM) 16.68 cm. In the different cutting stages, C_2 was recorded maximum height of plant (16.92) whereas C_1 found minimum height of plant at 60 DAS.

The application of 75% KWM with 25% urea (N_4) produced the maximum number of leaves per plant (80.55). Whereas, in the number of cutting, the maximum number of leaves per plant (95.59) was observed with three cutting (C_3). The interaction effect showed a significant influence of nutrient and different cutting on number of leaves. It showed that the maximum number of leaves (106.99) per plant obtained from N_4C_3 (combination of 75% KWM and 25% urea with three cutting) followed by N_1C_3 (urea with three cutting). The minimum number of leaves (31.41) was found in N_5C_1 (KWM with one cutting). The application of nutrient produced the maximum width of plant canopy area (25.50 cm) among the nutrient applied, at 30 DAS. In case of number of cutting, it was seen that at single cutting (C_1) produced the highest plant canopy width (25.37 cm). The interaction of nutrient and multiple cuttings showed a significant effect on plant canopy. The clearly showed that application of urea (N_1) with three cutting (C_3) produced maximum leaves per plant (26.94 cm) followed by N_3C_1 (50% urea + 50% KWM with one cutting) (25.89 cm). The minimum canopy (24.00 cm) was observed in N_4C_2. At 45 DAS, it was seen that the application of urea (N1) produced the maximum plant canopy per plant (25.68 cm) among the nutrient applied. In case of number of cutting, it was showed that one cutting (C_1) produced the highest plant canopy per plant (25.34 cm).The interaction effect showed a significant effect of nutrient and number of cutting on plant canopy. It was clearly showed that application of 50% urea+ 50% KWM with one cutting (C_1) (N_1C_3) produced maximum canopy per plant (26.46 cm) followed by N_3C_1 (50% urea + 50% KWM with one cutting) (25.90 cm). The minimum canopy width (23.86 cm) was observed in N_2C_2. Similar result was also found by Kaswan *et al.* [5] and Lal *et al.* [6] when they experimented on coriander with use of vermicompost, FYM and compost mixtures. They reported that use of vermicompost along with recommended dose of fertilizer (RDF) 50kg/ha increased vegetative growth of palak and coriander.

Results showed that application of urea (N_1) produced the maximum fresh weight of leaves per plant (41.41 g), whereas, in case of number of cutting, it was seen that three cutting (C_3) produced the maximum fresh weight of leaves per plant (52.52 g). The interaction effect of nutrient and number of cutting clearly showed that application of urea with three cutting (N_1C_3) produced maximum green leaf yield per plant (62.29 g) followed by application of 50% urea with 50% kitchen waste manure (KWM) (N_3C_3). Tehlan and Thakral [7] found that different levels of nitrogen (30,60,90 and 120 kg N/ha) and leaf cutting (no cutting, one cutting at 45 days after sowing) and two cuttings at 45 and 60 days after sowing of coriander (*Coriandrum sativum* L.) have affected both green leaf yield and seed yield which was increased with the increase in N levels up to 90kg/ha. Similar finding was also made by Naik *et al.* [8].

Application of urea (N_1) also produced the maximum TSS (15.70 0 B) and three cutting (C_3) recorded the highest TSS of 15.24 0 B.. It was clearly showed that application of KWM with one cutting (N_5C_1) produced maximum TSS (16.06° B) followed by N_1C_1 (urea with one cutting) (16.04 $^\circ$ B) and minimum TSS (13.04 $^\circ$ B) was found in N_2C_1 (75% urea + 25% KWM). Two cutting (C_2) produced the highest number of flowers per plant (1139.06), application of 75% urea + 25 KWM (N_2) produced the maximum number of flower per plant (1160.42) and urea with one cutting (N_1C_1) and N_2C_1 (75% urea+25%KWM with one cutting) combination both provided highest number of flower per plant (1180.19). Similar pattern was also seen in case of seeds per plant when urea (N_1) produced the maximum number of seeds per plant (848.02) and 763.17 seeds in two cutting (C_2). While, interaction effect showed a significant effect

showing maximum seeds per plant (850.94) in N_1C_1 (urea with one cutting) followed by N_1C_3 (urea with three cutting) (848.72). The maximum green leaf yield per plant was reported under the treatment (N_1) and three time of cutting (C_3). But, after second cutting it reduced the green leaf yield because of decrease in vegetative part due to low moisture and less nutrient uptake and increase of temperature. Similar result was also found by Thakral [9] with three cutting. Sharangi [10] also reported similar pattern in cool season production.

CONCLUSION

The experimental finding of the present investigation revealed that among the nutrient application of kitchen waste manure (N_5) was found as the best treatment for growth, leaf yield and quality of coriander. In case of number of leaf cutting, it was seen that although three times cutting produced maximum leaf yield, but vegetative growth and leaf quality parameter were improved in case of two time cutting. Therefore, it may be concluded that combined treatment of KWM application along with three time leaf cutting may be good for more leaf yield but two cutting for quality improvement of coriander grown at Lucknow area.

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Table 1: Effect of multiple cuttings and various nutrition on plant height (cm) of coriander.

	30 DAS				45 DAS				60 DAS			
	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean
N_1	15.77	16.67	16.97	16.47	16.06	16.85	16.89	16.60	15.79	16.81	17.00	16.53
N_2	15.80	16.80	16.62	16.41	16.09	16.97	16.67	16.58	16.57	16.97	16.50	16.68
N_3	16.19	17.00	16.59	16.60	16.24	17.13	16.79	16.72	16.17	17.16	16.22	16.52
N_4	16.27	16.56	16.62	16.48	16.22	17.30	16.93	16.82	16.13	16.93	16.38	16.48
N_5	17.41	16.75	16.89	17.02	17.53	16.93	16.66	17.04	17.30	16.71	16.77	16.93

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Mean	16.29	16.76	16.74	16.59	16.43	17.03	16.79	16.75	16.39	16.92	16.58	16.63
SEm ±	N			22.32				14.38				15.77
	C			14.34				8.51				12.10
	NxC			8.02				6.20				7.95
CD (P≤0.05)	N			0.68				0.80				0.67
	C			0.52				0.62				0.52
	NxC			1.17				1.39				1.15

N_1- Urea (100%), N_2- Urea (75%) + Kitchen waste manure (25%), N_3- Urea (50%) + Kitchen waste manure (50%)
N_4- Urea (25%) + Kitchen waste manure (75%), N_5- Kitchen waste manure (100%),
C_1- One cutting (30 Days after seed germination DAS), C_2- Two cutting (30 & 45 DAS),
C_3- Three cutting (30, 45 & 60 DAS).

Table 2: Effect of multiple cuttings and various nutrition on number of leaves per plant of coriander.

		30 DAS				45 DAS				60 DAS		
	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean
N1	32.05	32.05	32.05	32.05	37.82	28.03	34.53	33.46	37.82	28.03	34.53	33.46
N2	31.55	31.03	32.94	31.84	38.14	34.58	24.94	32.56	38.14	34.58	24.94	32.56
N3	31.61	32.17	31.05	31.61	32.08	30.11	30.62	30.94	32.08	30.11	30.62	30.94
N4	31.50	31.72	32.17	31.79	27.85	35.72	37.41	33.66	27.85	35.72	37.41	33.66
N5	31.41	31.12	32.27	31.60	30.84	28.03	31.22	30.03	30.84	28.03	31.22	30.03
Mean	31.62	31.62	32.10	31.78	33.35	31.29	31.74	32.13	33.35	31.29	31.74	32.13
SEm ±	N			6.87				0.29				0.26
	C			3.77				0.22				0.20
	NxC			9.31				0.49				0.45
CD (P≤0.05)	N			0.83				0.83				0.76
	C			0.65				0.64				0.59
	NxC			1.44				1.45				1.31

Table 3: Effect of multiple cuttings and various nutrition on canopy spreading (cm) of coriander.

		East – West												North - South										
		30 DAS				45 DAS				60 DAS				30 DAS				45 DAS				60 DAS			
		C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
N_1		25.19	24.38	26.94	25.50	25.55	25.03	26.46	25.68	25.71	25.67	26.23	25.87	27.52	27.79	27.41	27.58	27.52	27.79	27.41	27.58	26.62	24.69	25.72	25.67
N_2		25.72	23.39	24.89	24.67	25.32	23.86	25.52	24.90	25.48	24.64	25.32	25.15	26.51	24.69	25.72	25.64	26.62	24.69	25.72	25.67	26.81	25.53	26.35	26.23
N_3		25.89	24.67	24.72	25.09	25.90	24.07	24.67	24.88	26.48	24.51	24.64	25.21	26.81	25.53	26.35	26.23	26.81	25.53	26.35	26.23	24.63	24.53	25.54	24.90
N_4		25.50	24.00	24.28	24.59	25.27	24.09	24.41	24.59	25.98	24.59	24.50	25.02	24.63	24.53	25.54	24.90	24.63	24.53	25.54	24.90	25.67	25.46	24.38	25.17
N_5		24.56	24.89	24.44	24.63	24.67	25.07	24.64	24.79	25.45	24.62	24.26	24.78	25.67	25.46	24.38	25.17	25.67	25.46	24.38	25.17	27.79	27.41	25.63	26.95
Mean		25.37	24.26	25.05	24.90	25.34	24.42	25.14	24.97	25.82	24.81	24.99	25.21	26.23	25.60	25.88	25.90	26.25	25.60	25.88	25.91	26.30	25.52	25.52	25.78
SEm ±	N			0.22				0.15				25.10				0.074				0.07				0.07
	C			0.17				0.12				8.65				0.058				0.06				0.06
	NxC			0.38				0.26				36.82				0.129				0.13				0.13
CD (P≤0.05)	N			0.64				0.44				0.38				0.168				0.17				0.17
	C			0.50				0.34				0.29				0.375				0.37				0.37
	NxC			1.11				1.11				0.66													

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Table 4: Effect of multiple cutting and various nutrition on TSS, number of flowers and number of seeds of coriander.

	TSS (°Brix)				Number of flowers/plant				Number of seeds/plant			
	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
N_1	16.04	15.04	16.01	15.70	1180.19	1173.67	1127.4	1160.42	850.94	844.40	848.72	848.02
N_2	13.04	14.05	15.06	14.05	1180.19	1173.67	1127.4	1160.42	782.57	759.35	752.77	764.8967
N_3	15.04	16.04	14.05	15.04	1143.44	1144.74	1144.4	1144.193	726.35	746.63	735.52	736.1667
N_4	14.02	15.03	16.03	15.03	1083.33	1084.39	1066.74	1078.153	771.60	758.36	769.62	766.53
N_5	16.06	14.04	15.05	15.05	1095.33	1118.86	1126.71	1113.633	684.40	671.39	666.78	674.19
Mean	14.84	14.84	15.24	14.97	1136.496	1139.066	1118.53	1131.364	763.17	756.03	754.68	757.96
SEm ± N				0.01				4.84				7.10
C				0.03				3.75				5.50
NxC				0.02				8.38				12.29
CD (P≤0.05) N				0.04				14.09				20.66
C				0.03				10.91				16.00
NxC				0.06				24.41				35.78

Table 5: Effect of multiple cutting and various nutrition on leaf yield per plant (g) of coriander.

	30 DAS				45 DAS				60 DAS			
	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
N_1	20.58	20.65	20.70	20.64	20.71	20.71	20.83	20.75	20.45	20.54	20.76	20.58
N_2	16.59	16.52	16.64	16.59	16.76	16.79	16.78	16.77	16.27	16.67	16.59	16.51
N_3	19.61	19.69	19.60	19.63	19.54	19.79	19.73	19.69	19.54	19.67	19.43	19.55
N_4	14.55	14.63	15.84	15.01	14.59	14.83	15.87	15.10	14.56	14.67	15.87	15.03
N_5	15.77	15.82	14.81	15.47	15.77	15.83	14.88	15.49	15.72	15.87	14.78	15.46
Mean	17.42	17.46	17.52	17.47	17.47	17.59	17.62	17.56	17.31	17.48	17.49	17.43
SEm ± N				0.05				0.06				0.02
C				0.04				0.05				0.01
NxC				0.09				0.10				0.03
CD (P≤0.05) N				0.15				0.10				0.04
C				0.11				0.14				0.02
NxC				0.25				0.13				0.03

RESEARCH ARTICLE

Aquatic and Marshy Angiospermic Plant Diversity of Gander Taal of Deoria District, Uttar Pradesh

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ABSTRACT

The wetlands ecosystem are aquatic, marshy, perennial, shallow, containing fresh water, and have been defined as multiple services provided on irrigation, agriculture field, fiber, water purification, climate regulation, flood control, domestic water supply and water for recreation. The aquatic ecosystem increases the productivity and help to balance the ecosystem. India is amongst rich variety of wetlands habitats. Wetlands are the most important ecosystems and very helpful of human life. The present studies on aquatic and marshy angiosperm diversity of Gander taal in Deoria District of Eastern Uttar Pradesh belonging to 25 Families comprising 43 Genera and 48 Species. Out of which 42 species belonging to Dicot, 5 species belonging to Monocot and one species is belonging to Pteridophytes. In the present study of Gander taal of Deoria district, some important aquatic and marshy angiospermic plants of wetland are observed like *Ageratum conyzoids, Alternanthera sessilis, Coldenia procubens, Commelina banghalensis, Cyperus rotundus, Dentella repens, Eclipta prostrata, Eichhornia crassipes, Ipomoea aquatica, Ludwigia adscendens, Ludwigia octovalvis, Marsilea minula, Nymphoides indica.*

Keywords: Aquatic and Marshy diversity of Gander Taal

INTRODUCTION

The wetlands are very useful water logged, productive and sometime unproductive natural water storage bodies as well as reservoirs on the earth. Wetlands also act as transition zone between permanently flooded and temporary area in the presence of hydromorphic soil and hydrophytic vegetation. The plants of water logging areas grow on a substrate that is at least periodically deficient of oxygen as a result of excessive water content. The submerged species serve as a food source for native fauna. For example: Marshy plants cover a large surface area in the wetland. Such plants which are found in marshy place, have small roots. The aquatic and marshy angiospermic plants are most important component of aquatic and wetland ecosystem. The aquatic ecosystems are important as they increase productivity and help to maintain the balance of ecosystems. The importance of aquatic diversity has been accepted fact throughout the world for sustainable life support system. However, these aquatic resources are still underutilization and given very less attention for scientific studies. Aquatic and marshy angiospermic plants comprise a perplexing assemblage of growth habits, often very flexible morphology. The wide phenotypic variations found in these plants are challenges for taxonomist in their identification. Compared to land plants, these plants exhibit numerous structural modifications which might have adaptive significance (Mishra and Narain, 2014).India has a major part of the landmass in the world, which shows the cultural diversity of the variety of geographical land area. India has a very rich variety of the vegetation. The hydrophytic plants of India were published by Biswas and Calder (1937). Later, Subramanyam (1962) published a volume on aquatic plants of India, followed by an enumeration of aquatic plants of India by Deb (1976). Cook (1996) published a volume on aquatic and wetland plants of India. Monographs have been prepared for only a few groups of aquatic plants by Subramanayam & Balakrishanan (1962), Subramanyam & Abraham (1968), Singh (1972), Subramanyam (1979), Kumar & Banerjee (1999), Kothari (2001). The plants found in wetlands of Uttar Pradesh were described by Sen (1959) Sahai & Sinha A.B. (1968) Srivastava et.al. (1987), Malaya & Singh (2004), and Saini (2010). In the present study, Gander Taal of Deoria District, Uttar Pradeshis surveyed on a brief account of wetland vegetation and enumerated with their diversity.

MATERIALS AND METHODS

STUDY AREA
The part of Eastern Uttar Pradesh has been considered as a structural entity on the topography, climate, soil, geography and socio-cultural profile. It is one of the rich diversity and very interesting region of wetlands. The present study is based on the exploration and study on aquatic and marshy angiospermic plants of Gander taal of Deoria district, Uttar Pradesh (Figure:1). The study has been carried out in the duration between second week of March and last week of June in the year 2021 at the Gander Taal in Deoria district, a Part of Eastern Uttar Pradesh (Figure:2). The survey on the aquatic and marshy wetlands of angiospermic plants of Deoria district carried out for the duration of four months during 2021. During this survey period, the plant species occurring in different aquatic, marshy bodies and water saturated areas of the Gander taal, were collected and photographs were also taken. The study area of Deoria district is surrounded by East in Bihar, west in Gorakhpur, north in Kushinagar and south in Mau. The lie between the parallels of 26° 24'18'' N latitude and 83° 46'33'' E longitude (Figure:3).

PLANT COLLECTION
The field survey of Gander taal of Deoria district was undertaken for four months to study the aquatic and marshy plants. The field observations like habit, habitat, flowering and fruiting seasons were also taken into consideration. Information's regarding medicinal or other uses was also collected. 4 - 6 plant specimens were collected from the moist areas and placed in the folds of newspaper. All morphological features observed in the specimen, were entered in the field notebook.

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PRESERVATION

The collected plant specimens dipped in $HgCl_2$ and alcohol mixed solution before pressing the blotting paper. The papers were changed in every alternate day. These blotters, when kept under pressure, remove moisture from the plants and the moisture is absorbed by the blotting sheets. The completely dried specimens were mounted on hand made Herbarium sheets with the help of modern synthetic glue. The specimens mounted provides data pertinent to field number, collection date, botanical name, family belongs, local name and uses in the form of the brief description.

IDENTIFICATION

The identification is the process by which a specimen is identified by the characters with the help of standard worldwide or Indian flora and other taxonomists, Bentham (1866), Hooker (1872-1897), Bailey (1949), Dassnayake & Fosberg (1991), Duthie (1903-1929), Fasset (1940), Raizada (1976), Cook(1996) and local flora viz. Mishra & Verma (1992), Verma et al., (1997), Singh et al., (2001) and others. The identification of specimen defined as description and illustrations compared with the genus and species keys and thereafter it was carefully compared with earlier identified plants of that species or variety as the case may be. Literature was consulted and collected from the library of DDU Gorakhpur University Gorakhpur, Department of Botany, DDU Gorakhpur University Gorakhpur and Department of Botany, St. Andrew's College Gorakhpur. The collected and preserved plant specimens were mounted on herbarium sheets. These sheets were labelled with the species names, botanical names, families and number of species. All collected plants were deposited in the herbarium, Department of Botany, St. Andrew's college Gorakhpur, Uttar Pradesh for future use.

RESULTS

The present study is based on the results of extensive survey and study on aquatic and marshy angiospermic plants of Gander taal of Deoria district of Uttar Pradesh. The aquatic and marshy plants, which have been included in the present studies on those plants, are normally found in nature growing in association with standing water. The level is at or above the surface of the soil. The plants may be floating, wetlands hydrophytes and partly submerged in the water. During the present study on maximum numbers of aquatic and marshy plants, diversity was observed during March to June and estimated inventory data. During the present study on aquatic and marshy angiosperms of Gander Taal, 48 species belonging to 43 genera of 25 families were identified. Out of which 42 species belonging to Dicot, 5 species belonging to Monocot and one species is belonging to Pteridophytes. The recorded plants have been enumerated with families, have been arranged according to Bentham & Hooker's (1862-1883) System of classification. However, genera within a family and species within a genus are arranged alphabetically. In the analysis, it was found that maximum number of plants belonging to family Asteraceae, which was followed by Malvaceae, Polygonaceae, Amaranthaceae, Solanaceae, Euphorbiaceae, Boraginaceae, Convolvulaceae, Onagraceae, Fabaceae, Commelinaceae, Cyperaceae, Primulaceae, Papaveraceae, Cannabaceae, Lamiaceae, Cucurbitaceae, Mazaceae,Verbinaceae, Ranunculaceae, Rubiaceae, Pontederiaceae, and lastly Marsiliaceae family. Among the dicot families, Asteraceae is the most dominant family with 7 species followed by 5 species of Malvaceae, 4 species of Polygonaceae, 4 species of Amaranthaceae, 3 species of Solanaceae, 2 species of Euphorbiaceae, 2 species of Convolvulaceae, 2 species of Boraginaceae, 2 species of Fabaceae, 2 species of Onagraceae, 1 species of Ranunculaceae, 1 species of Primulaceae, 1 species of Papaveraceae, 1 species of Cannabaceae, 1 species of Lamiaceae, 1 species of Cucurbitaceae, 1 species of Mazaceae, 1 species of Menyanthaceae, 1 species of Verbenaceae, and 1 species of Rubiaceae. Amongthe monocot familis, 2 species of Commelinaceae, 2 species of Cyperaceae and 1species of Pontederiaceae were recorded. One species of Marsiliaceae (the vascular cryptogams) was also recorded (Table: 1).

DISCUSSION

In the present study, the aquatic and marshy angiospermic plants have been classified into six morpho-ecologically life forms. The survey of 30 species have been recorded as wetlands hydrophytes, one species is free floating, one species are rooted hydrophytes, 9 species are rooted emergent hydrophytes, 2 species are amphibious hydrophytes and lastly 1 species are rooted submerged hydrophytes (Figure: 4).In the analysis, it was observed that largest number of plants species was herbs (80%) followed by 15% shrubs, 3% small trees and 2% amphibians plants.The plants collection with the analysis of maximum number show the flowering and fruiting period in almost throughout the year with 25% followed by the month of May with 16 % and April with 12%. Maximum plants show flowering and fruiting period in the month of May. After May a gradual decline is evident and it reaches to its lowest in the month of July to march with 2% of flowering. It can be concluded from the analysis that the best period for the collection of aquatic and marshy angiospermic plants is March to June month. An analysis of the life period of aquatic and marshy angiospermic plants revealed that 23 species (48 %) have been recorded as annual, 16 species (33%) as perennial, 8 species (17 %) have been found to be annual but sometimes perennial under favourable conditions and availability of water. 1 species (2%) were found to be annuals but sometimes as biennials under favourable conditions and availability of water (Figure: 5).The biological spectrum of Gander taal of Deoria district was studied as introduced by Raunkiær life form (1934). The biological spectrum is the ratio of life forms of the different species in terms of percentage. In the study, it was revealed that 75% plants are therophytes, 11% plants are hemicryptophytes, 6% chamaephytes, 4% errant vascular hydrophytes, 2% plants are phanerophytes and 2% plants are geophytes of this floristic community. The life forms of species grow under adverse climatic conditions were taken into account. The study revealed that highest number is recorded under the category of therophytes while lowest number is recorded under the category of geophytes (Figure: 6). As per IUCN red data list, 47 species are least concerned and 1 species is vulnerable.In this study, it was observed that some important aquatic and marshy angiospermic plants of wetlands are also found in Gander Taal of Deoria district. These are *Acmella ciliata, Ageratum conyzoids, Alternanthera sessilis, Amaranthus virdis, Ocimum tenuiflorum, Chenopodium album, Coldenia procumbens, Commelina benghalensis, Croton bonplantidianum, Cyperus rotundus, Cyperus scariosus, Dentella repens, Eclipta prostrata,, Eichhornia crassipes, Euphorbia hirta, Heliotropium indicum, Ipomoea aquatica, Lugwigia adscendens, Luwigia octovalvis, Marsilea minuta, Nymphoides indica, Persicaria glabra, Polygonum aviculare, Physalis minima, Sesbania bispinosa, Solanum nigrum, Urena lobata.* (Figure: 7).

ACKNOWLEDGMENT

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S.N.	Family	Name of plant species	Habit	Flowering and fruiting period	Life span	Raunkiaer Life form	IUCN Categories
**Table:1 : Aquatic and marshy angiosperms of dicot, monocot plants and fern**						

S.N.	Family	Name of plant species	Habit	Flowering and fruiting period	Life span	Raunkiaer Life form	IUCN Categories
1.	Ranunculaceae	*Ranunculus sceleratus* L.	H	June to September	A	Th	LC
2.	Papaveraceae	*Argemone mexicana* L.	H	January to May	A/B	Th	LC
3.	Malvaceae	*Corchorus tridens* L	H	Almost hroughout the year	A	Th	LC
4.	Malvaceae	*Melochia corchorifolia* L.	H	May to October	A	Th	LC
5.	Malvaceae	*Sida cordifolia* L.	H	September to December	P	Th	LC
6.	Malvaceae	*Urena lobata* L.	S	May to September	P	Th	LC
7.	Fabaceae	*Senna tora* (L.) Roxb.	S	October to February	A/P	Th	LC
8.	Fabaceae	*Sesbania bispinosa* (Jacq) W. Wight	S	April to June month	A	Ph	LC
9.	Onagraceae	*Ludwigiaadscendens*(L.) Hara.	H	Almost throughout the year	P	He	LC
10.	Onagraceae	*Ludwigia octovalvis* (Jacq) P.H. Raven	H	Almost throughout the year	P	He	LC
11.	Cucurbitaceae	*Cucumis engleri* (Gilg) Ghebret. &Thulin	H	April to November	A	Th	LC

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12.	Rubiaceae	*Dentellarepens* (L.) J.R.Forst. &G.Forst.	H	May to august	A	Th	LC
13.	Asteraceae	*Acemella ciliate* Kunth.	H	Almost throughout the year	A/P	Th	LC
14.	Asteraceae	*Ageratum conyzoides* L.	H	Almost throughout the year	A	Th	LC
15.	Asteraceae	*Caesulia axillaris* Roxb	H	January to May	A/P	Th	LC
16.	Asteraceae	*Eclipta prostrata* L.	H	March to July	A/P	He	LC
17.	Asteraceae	*Grangea maderaspatana* (L.)Poir.	H	Almost throughout the year	A/P	Th	LC
18.	Asteraceae	*Laphangium luteoalbum* (L.) Rchb	H	September to March	A	Th	LC
19.	Asteraceae	*Xanthium strumarium* L.	S	August to September	A	Ch	LC
20.	Primulaceae	*Anagallis arvensis* L.	H	January to April	A	Ch	LC
21	Menyanthaceae	*Nymphoides indica* Kuntz.	F/H	August to April	A/P	Ge	LC
22.	Boraginaceae	*Coldenia procumbens* L.	H	October to April	A	Th	LC
23.	Boraginaceae	*Heliotropium indicum* L.	H	Almost throughout the year	A/P	Th	LC
24.	Convolvulaceae	*Ipomoea aquatic* Forssk.	H	Almost throughout the year	P	Th	LC
25.	Convolvulaceae	*Ipomoea cardatotriloba* Dennst.	C	March to December	P	Th	LC
26.	Mazaceae	*Mazus pumilus* (Burm.f.) Streenis	H	May to October	A	Th	LC
27..	Solanaceae	*Nocotiana plumbiginifolia* Viv.	H	March to November	P	Th	LC
28.	Solanaceae	*Physalis minima* L.	H	August to November	A	Th	VU
29.	Solanaceae	*Solanum nigrum* L.	H	May to October	A	Th	LC
30.	Lamiaceae	*Ocimum tenuiflorum* L.	H	September to March	A/P	Th	LC
31.	Verbenaceae	*Phyla nodiflora* L. greene	H	May to October	A	Th	LC
32.	Amaranthaceae	*Achyranthes aspera* L.	H	September to April	P	Ch	LC
33.	Amaranthaceae	*Alternanthera sessilis* (L.) DC.	H	Almost throughout the year	P	Th	LC
34.	Amaranthaceae	*Amaranthus virdis* L.	H	July to May	A	Th	LC

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35.	Amaranthaceae	*Chenopodium album* L.	H	February to April	P	Th	LC
36.	Polygonaceae	*Persicaria glabra* (Willd.)M. Gomez	H	October to March	P	Th	LC
37.	Polygonaceae	*Persicaria maculosa* L.	H	June to October	A	Th	LC
38..	Polygonaceae	*Polygonum aviculare* L.	H	March to June	A	Th	LC
39.	Polygonaceae	*Rumex dentatus* L.	H	May to June	A	Th	LC
40.	Euphorbiaceae	*Croton bonplandianus* Baill.	H	Almost throughout the year	P	Th	LC
41.	Euphorbiaceae	*Euphorbia hirta* L	H	April to January	A	Th	LC
42.	Cannabaceae	*Cannabis sativa* L.	H	March to May	A	Th	LC
43.	Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms.	WH	September to May	P	EVH	LC
44.	Commelinaceae	*Commelina benghalensis* L.	H	June to October	A	He	LC
45.	Commelinaceae	*Commelin adiffusa* Burm.f.	H	October to July	A	He	LC
46.	Cyperaceae	*Cyperu srotundus* L.	H	April to November	P	He	LC
47.	Cyperaceae	*Cyperus scariosus* R.Br.	H	April to November	P	He	LC
48.	Marsileaceae	*Marsilea minula* L.	Am	………………	P	EVH	LC
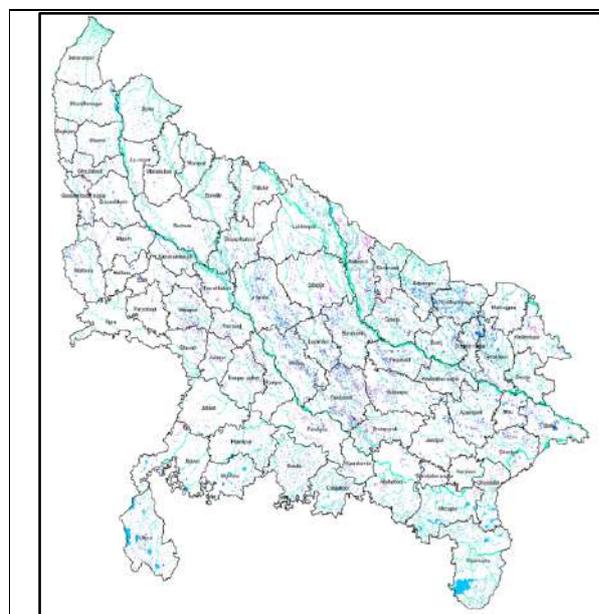
| **Figure 1: Map of Uttar Pradesh (MOEF2011)** | **Figure 2: Map of Deoria district (MOEF 2011)** |
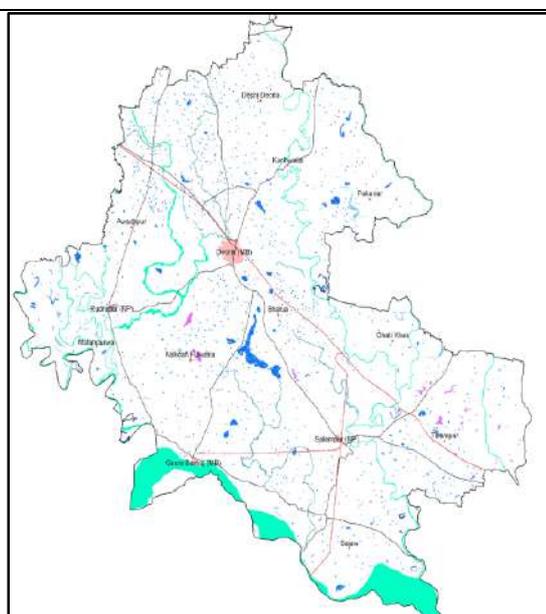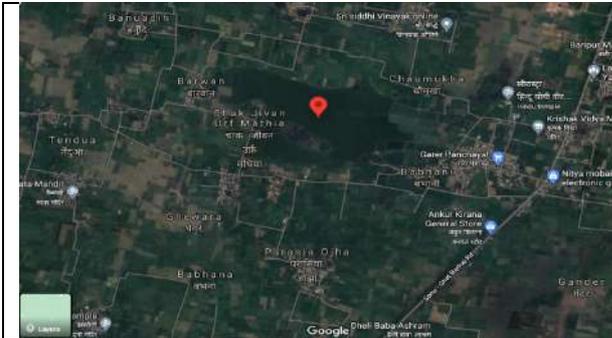
Figure 3: Satellite Map of Gander Taal, Deoria
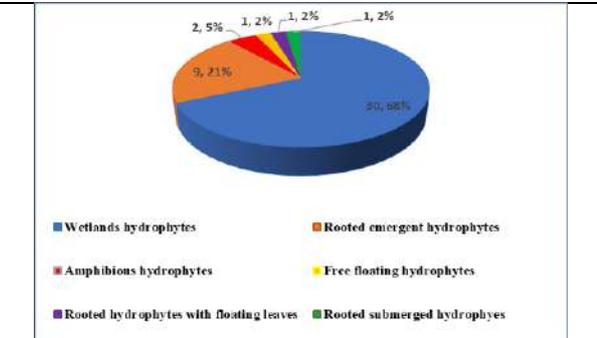
Figure 4: Morphological life forms of aquatic and marshy angiosperms
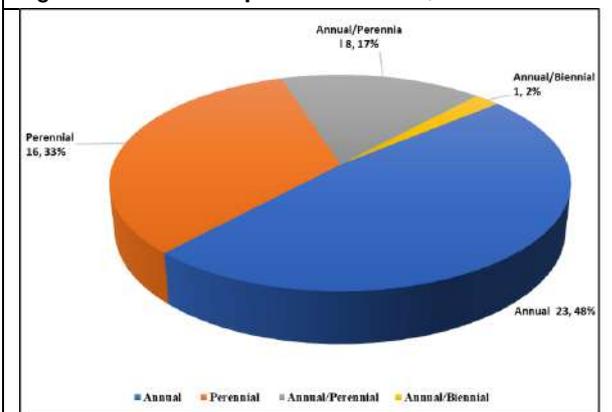
Figure 5: Life form of aquatic and marshy angiospermic plants
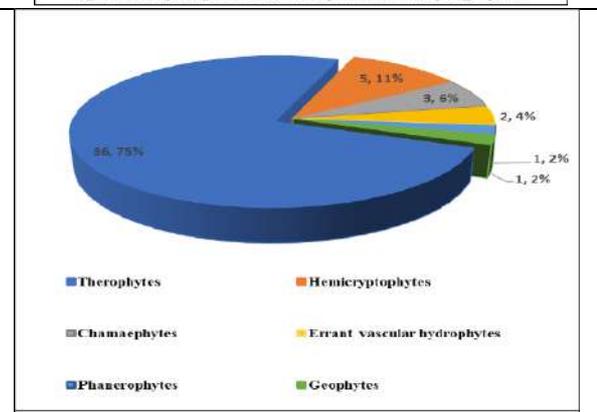
Figure 6 : Biological spectrum of Raunkiaer life forms

Acmella ciliata (Kunth).

Alternanthera sessilis (L.) DC

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Amaranthus virdis L.

Ocimum tenuiflorum L.

Chenopodium album L.

Coldenia procumbens L

Commelina benghalensis L.

Croton bonplandianum Bail

Cyperus rotundus L.

Cyperus scariosus R.Br.

Dentella repens L.

Figure 7: Aquatic and marshy angiospermic diversity of Gander taal in Deoria district

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Eclipta prostrate L.

Eichhornia crassipes Mart. Solms.

Euphorbia hirta L.

Heliotropium indicum L.

Ipomoea aquatic Forssk.

Ludwigia adscendens L

RESEARCH ARTICLE

Assess the Morbidity Status and Knowledge on Prevention of Common Health Problems among Sanitary Workers of Selected Commune, Karaikal- A Pilot Study Analysis

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ABSTRACT

The WHO described that 3.4 billion people (45%) of world population used a sanitation services in a safe manner and 2.4 billion people of global population utilized private sanitation services. As a result of inadequate water, sanitation, and hygiene 60% of total diarrhoeal deaths occurred every year from low and middle income industries. The major cause of death about 432000 is due to poor sanitation [1]. To identify the health problems of sanitary workers, to assess the knowledge regarding prevention of common health problems and to find the effectiveness of video teaching program me on prevention of common health problems. A quasi experimental study was carried out among 20sanitary workers (10 samples in experimental group and 10 samples in control group) from selected Commune at Karaikal District, Puducherry. Purposive sampling technique was used to select the samples. A semi structured interview schedule was used to collect the data from the samples. Among 20 sanitary workers (0% & 50%) were had adequate knowledge, (70% & 40%) were had moderate knowledge and (30% & 10%) were had inadequate knowledge in post test of control and experimental group respectively regarding prevention of health problems. Among 20sanitary workers (28.6% & 40%) were had respiratory problems, (60% & 40%) were had skin problems, (70% & 80%) were had eye problems, (30% & 10%) were had gastro intestinal problems, (40% & 40%) were had musculoskeletal problems, (60% & 50%) were had nervous problems like head ache in control and experimental group respectively. Many of the sanitary workers have lack of knowledge regarding health problems occurred by their nature of job. The sanitary workers are prone to get many occupational health hazards due to exposure to harmful toxins and pathogens. Health education was given to the sanitary workers to improve the knowledge on prevention of health problems. The findings revealed that there was significant difference between control and experimental group. It is interpreted that the teaching on prevention of health problems among sanitary workers was more effective in experimental group than the control group. The study concluded that the

knowledge and practice on prevention of health problems of the sanitary workers can be enriched by health education programmes.

Keywords: Knowledge, Video teaching, Sanitary workers, Health problems.

INTRODUCTION

In India the sanitation sectors are hazardous and many of the sectors are extremely hazardous. The working environment of sanitary workers put them to various risks such as exposure to sharp objects, chemicals, harmful pathogens etc., Prohibition of employment as manual scavengers and their rehabilitation act 2013 has banned manual scavengers.[2] Ministry of Housing and Urban affairs issued an advisory in 2019 to all cities or Urban Local bodies to systemize well equipped system of sanitation work to sage guard the sanitary workers. Swachh Survekshan 2021 scheme also was launched by Ministry of Housing and Urban Affairs Govt. of India to improve the safety of the sanitary workers. Unprotected way of working condition of sanitary workers may leads to severe illnesses to death [3]. The sanitary workers in developing countries are facing multiple health risks and health hazards including harmful diseases, drowning, suffocation etc. The report of the science and development network says better regulation and technology is needed to improve the working conditions of the low-grade labourers who are often among the most marginalised in society. The report states that 3.4 billion world's population have access to safely managed sanitation, where faeces are securely removed and treated away from households [4]. The majority of global households rely on informal and infrequent waste removal by workers who often lack basic safety equipment and face social stigma due to the unpleasantness of the task. The Sanitary workers are often exposed to harmful waste materials and there are in the risk of getting various infectious diseases. The following vaccines are suggested to the waste handlers to protect them variety of infectious diseases such as Hepatitis A vaccine, Hepatitis B vaccine, Typhoid vaccine and Tetanus toxoid vaccine. Also there is a need of Pre- placement examination and implementation of regular health check up. Pre placement examination includes family health history, occupational history and social history, complete physical examination, test for vision, hearing and communicable diseases. Periodic medical check is essential for sanitary workers because they are at the risk of getting communicable and non-communicable disease due to continuous exposure harmful waste [5].

OBJECTIVES OF THE STUDY

To identify the health problems of sanitary workers, to assess the knowledge regarding prevention of health problems and to assess the effectiveness of video teaching program me on prevention of health pronblems.

MATERIALS AND METHODS

In the present study quasi experimental design with control and experimental group was adopted. The pre test level of knowledge was assessed and video teaching was given on prevention of health problems. One of the Commune of Karaikal District was selected for the study. Totally 20 samples, 10 in control group and 10 in experimental group were selected for the present study with inclusion and exclusion criteria. Purposive sampling technique was used to select the samples for the present study. Informed consent was obtained from each samples. A semistructured interview schedule was used to collect the data from the samples. The instrument of the study consists of demographic variables and knowledge questionnaire. A health assessment proforma was used to identify the health problems of the sanitary workers. A video assisted teaching was given to the experimental group.

RESULT OF THE STUDY

The data collected from sanitary workers were analysed using descriptive statistical methods such as frequency, percentage, mean, standard deviation and inferential statistics. Among the study group majority of the samples 40%

& 70% were in the age of 31-50 years, All the samples 100% were Hindu. Maximum samples 30% & 50% were illiterate, majority of the samples 100% & 90% were married, 30% & 40% were had 5-10 years of working experience, 70% & 50% of the workers income was below Rs.15,000 /-. Among the study subjects, 30% & 20% of participants were had the habit of alcoholism 20% & 10% were smokers and 10% & 30% were tobacco chewers in control and experimental group respectively. Among 20 sanitary workers (28.6% & 40%) were had respiratory problems, (60% & 40%) were had skin problems, (70% & 80%) were had eye problems, (30% & 10%) were had gastro intestinal problems, (40% & 40%) were had musculoskeletal problems, (60% & 50%) were had nervous problems like head ache in control and experimental group respectively. In Control and experimental group, majority of them had inadequate knowledge (40% & 50%) in pre test and only one person had adequate knowledge in control group in pre test, whereas in post test 50% of samples had adequate knowledge in experimental group. It is interpreted that the video teaching on prevention of health problems was more effective among sanitary workers. Tab 3 reveals that, there was no significant difference in knowledge scores in pre test in total score as well as all the sub components) between control and experimental group (p>0.05). i.e. Mean Knowledge scores were higher in post test than pre-test with mean difference- 8.10 and Knowledge scores were higher in experimental group than control group which can be attributed to the effectiveness of intervention upon knowledge on Prevention of Health Problems among Sanitary Workers.

DISCUSSION

The present study reveals that among 20 sanitary workers (28.6% & 40%) were had respiratory problems, (60% & 40%) were had skin problems, (70% & 80%) were had eye problems, (30% & 10%) were had gastro intestinal problems, and (40% & 40%) were had musculoskeletal problems in control and experimental group respectively. In Control and experimental group, majority of them had inadequate knowledge (40% & 50%) in pre test and only one person had adequate knowledge in control group in pre test, whereas in post test 50% of samples had adequate knowledge in experimental group. It is interpreted that the video teaching on prevention of health problems was more effective among sanitary workers. This findings were supported by the study conducted by Anand Rajput et al (2020) which showed that the Pretest mean score of knowledge, attitude & practice was found 15.74±3.20 (mean ± SD) and Post-test mean score was increased up to 20.49 ±3.05 (mean ± SD) out of total score 28 and there was a highly significant difference between pre test and post test score (t = - 29.893, P=0.000)[6] and Ali Almasietal (2019)study showed that the women had adequate knowledge 79% & attitude 86% regarding solid waste management whereas the women 77% followed poor practices[7], Priyanka VPatil and R. K. Kamble (2017)study showed that musculoskeletal disorders (100%), respiratory problems (95%), dermatological problems (90%), headache (75%) and gastrointestinal problems (15%), allergies (100%), cough and cold (75%), hearing disorder (50%), malaria and typhoid fever (15%) and vomiting (10%) [8].

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Tab 1: Levels of Knowledge on Prevention of Health Problems among Sanitary Workers in Control and Experimental Group

	Control Group (n=10		Experimental Group (n=10)		(N=10)
Levels	**f**	**%**	**f**	**%**	
Pre Test					
Adequate (31-40)	1	10%	0	0%	
Moderately Adequate (20-30)	5	50%	4	40%	
Inadequate (0-19)	4	40%	6	60%	
Post Test					
Adequate (31-40)	0	0%	5	50%	
Moderately Adequate (20-30)	7	70%	4	40%	
Inadequate (0-19)	3	30%	1	10%	

Tab 2: Description of health problems of sanitary workers:

Health problems	Control Group (n=10)		Experimental Group (n=10)		Chi Square value	df	P value
	f	**%**	**f**	**%**			
Respiratory Problems							
Present	2	28.6%	4	40.0%	.235	1	.627
Absent	5	71.4%	6	60.0%			
Skin Problems							
Present	6	60.0%	4	40.0%	.800	1	.371
Absent	4	40.0%	6	60.0%			
Eye Problems							
Present	7	70.0%	8	80.0%	.267	1	.606
Absent	3	30.0%	2	20.0%			
Gastro Intestinal Problems							
Present	3	30.0%	1	10.0%	1.250	1	.264
Absent	7	70.0%	9	90.0%			
MusculoSkelatal Problems							
Present	4	40.0%	4	40.0%	.000	1	1.000
Absent	6	60.0%	6	60.0%			
Nervous Problems (Headache)							
Present	6	60.0%	5	50.0%	.202	1	.653
Absent	4	40.0%	5	50.0%			

Tab 3: Comparison of Knowledge Scores on Prevention of Health Problems among Sanitary Workers between Control and Experimental Group

Knowledge and Practice Scores	Control Group (n=10)		Experimental Group (n=10)		Mean Dif	Independent t Value	p value
	Mean	SD	Mean	SD			
Pre Test							
Sanitation and Waste	1.7000	1.15950	1.6000	1.17379	.10000	.192	.850
Health and Nutrition	2.3000	.48305	2.3000	.82327	.00000	.000	1.000
Health problems	11.7000	2.62679	8.9000	2.55821	2.80000	2.415	.027
PPE	5.4000	1.42984	5.2000	1.68655	.20000	.286	.778
Total Score	**21.1000**	**4.17532**	**18.0000**	**4.59468**	**3.10000**	**1.579**	**.132**
Post Test							
Sanitation and Waste	2.0000	1.05409	2.9000	1.19722	-1.784	-1.784	.091
Health and Nutrition	2.3000	.48305	3.6000	.51640	-5.814	-5.814	**.000**
Health problems	12.3000	2.31181	16.3000	3.43350	-3.056	-3.056	**.007**
PPE	5.6000	1.57762	6.5000	1.64992	-1.247	-1.247	.228
Total Score	**22.2000**	**3.76534**	**29.3000**	**5.41705**	**-3.403**	**-3.403**	**.003**

RESEARCH ARTICLE

Employee Perception on Training and Development Pogrammes Conducted in It / Ites Industries in Coimbatore

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ABSTRACT

Training and development play an important role in the achievement of any organizations' goals since it increases both effectiveness and efficiency not only for the organization but for the employees too. Similarly, it plays a critical process of improving the performance of employees in the organization. Employees are a major asset of any organization and their role cannot be underestimated. The IT sector has been playing an important role in the economic development of the country. Over a very short period of time, IT sector in India has achieved growth due to continuous support from the government. According to NASSCOM Indian ITES-BPO industry 2017 report, the year 2016-17 characterized a landmark year as aggregate revenue for the Indian IT-BPO sector is estimated to cross USD 101 billion. Continuing as the bastion for the sector, exports accounted for ~USD69 billion, growing by 16.3 percent over the last fiscal year. IT companies have attempted to apply many techniques in their training programmers to improve efforts to move towards a developed and satisfied state in their employees work and personal life. The aim of a research design is to provide an intended and prepared way of achieving the research objectives and to increase the validity and reliability. In all the cases, high degree of positive correlation existed between determinants of effective training and their performance. Moreover, it can also be concluded that in all the identified determinants that are enrichment of excellence, improved delegation of authority, personal growth thoughts of employees is sufficient enough to show their considerable importance in effective training.

Key words: organization, Employees, country, IT, positive,

INTRODUCTION

Training and development play an important role in the achievement of any organizations' goals since it increases both effectiveness and efficiency not only for the organization but for the employees too. Similarly, it plays a critical process of improving the performance of employees in the organization. Employees are a major asset of any organization and their role cannot be underestimated (Armstrong, 2011).Training and development is the field concerned with organizational activity aimed at bettering the performance of individuals and groups in organizational settings. It has been known by several names, including employee development, human resource development, and learning and development.

An organization which aspires to grow must be in tune with the changing needs of the society. Training becomes relevant in the context since it is only through training that the gap between performance of the organization and the felt need of a changing society can be neutralized. Training reduces the gap by increasing employees' knowledge, skill, ability and attitude. Employees are motivated to attend the training programmers by the management for self- development as well as organization development Employees realize the importance of training needs of training to achieve the organizational goals. Training and Development are terms which are sometimes used interchangeably. Development was seen as an activity associated with managers. In contrast training has a more immediate concern and has been associated with improving the knowledge and skill of non-managerial employees in the present job. In management education system, training system is a set of interdependent parts which together form a unitary whole that performs a well-defined function. It essentially has an input, a processing or transforming unit, an output and a feedback. Every organization needs the services of trained persons for performing the activities in a systematic way. The fast-changing technological development makes the knowledge of employees obsolete. They require constant training to cope with the needs of jobs. After selecting the employees, the next task of management is to give them proper training. Training makes a very important contribution to the development of the organization human resource and hence to the achievement of its aims and objectives. To achieve its purpose training needs to be effectively managed so that right training is given to the right people in the right form at the right time and at the right cost.

Training is a vital phase of management control. It helps in reducing accidents, eliminating wastages and increases the quality of work. The training programmers must focus on soft skill, such as interpersonal communication, team work, innovation and leadership. Most importantly the training has to be comprehensive, systematic and strategy with which the company is planning to fight the competition. In the future it is training that will act as between people, between strategy and between customers and the organization. The development of individual and teams through training is important for the achievement of long term goals of the organization. The existing favorable conditions and the rate at which it is expected to grow the BPO sector requires large number of trained and well-groomed employees. Any attempt which undermines the importance of training could adversely affect the quality of service provided and in turn becomes detrimental to the outsourcing business itself. Hence it is significant not only to identify the training needs of this sector but also the appropriate mode of training.

Indian IT Industry
The IT sector has been playing an important role in the economic development of the country. Over a very short period of time, IT sector in India has achieved growth due to continuous support from the government. Resultantly, the IT organizations spread throughout the Indiaand thereby are playing a dominant role in spurring development and growth of the economy. This chapter provides the origin and growth of IT sector in India along with their role in the changing scenario. It also includes an overview of the elect IT organizations under study.

Role of Software Industry in Developing the Indian Economy

The success of software industry in India was instrumental in driving the economy of thenation on to a rapid growth rate. As per the study of NASSCOM-Deloitte, the contribution of IT/ITES industry to the GDP of the country has soared up to a share of 5% in 2007 to 28% in 2018. Besides, this industry has also recorded revenue of over US $ 100 billion with a growth rate of 33% in the year 2108. The export of software also has grown up, which has been instrumental in the huge success of the Indian software companies as well as the industry. Software exports from India accounts for more than 65% of the total software revenue.

ITES-BPO Industry in India

According to NASSCOM Indian ITES-BPO industry 2017 report, the year 2016-17 characterized a landmark year as aggregate revenue for the Indian IT-BPO sector is estimated to cross USD 101 billion. Continuing as the bastion for the sector, exports accounted for ~USD 69 billion, growing by 16.3 percent over the last fiscal year. Despite challenges in the global market conditions, India sustained its growth trajectory. For FY2013, the export revenues are expected to grow by 11-14 per cent while the domestic revenues will grow by 13-16 percent. The IT-BPO sector continues to be one of the largest employers in the country directly employing ~2.8 million professionals, with over 230,000 jobs being added in FY2012.During this period, a large share of revenue (approximately 42%) and employment (approximately 45%) is estimated to be generated in the customer careservices such as Call Centers. (Source: NASSCOM, 2016- 17) India's fundamental advantages- abundant talent and cost- are sustainable over the long term. With a young demographic profile, where over 3.5 million graduates and postgraduates are added annually to the talent base, no other country offers a similar mix and scale of human resource. India enjoys a cost advantage of around 60-70% as compared to source markets. Additional productivity improvements and the development of tier 2/3 cities as future delivery centers, is expected to enhance India's cost competitiveness.

In the ITES sector, the Front-Line Employees (FLEs) are the principle interface between service providers and customers in many firms and play a significant role in influencing customer satisfaction. The role demands routine, scripted interactions that are continuously andautomatically distributed to them by the technology. These interactions occur under an extremeemotional demand of reconciling the need to maintain a friendly manner with customers with the strict job demands of meeting call centre quotas. Such conditions generate highly demanding, repetitive and stressful work roles as well as complex physiological, psychological and socio- cultural challenges for the employees.

REVIEW OF LITERATURE

1. Akilandeshwari and Chitra (2018) rejoice in their article that the document attempts to study the need for training programs in the Information Technology sector and analyzes employees' perception of various training programs organized by IT companies in the Chennai city. With the changing environment, technologies, trends, and weather conditions, Indian companies have started to think about the consequences of corporate training and people development as well as organizational growth. Their study concludes that training plays an important role in the development of organizations, improves performance and increases productivity, and ultimately puts companies in the best position to face the competition and stay on top for overa decade.

2. According to Kumar (2016) in his study, he states that the training and development offered byorganizations gives employees the feeling of a favorable work environment. This can have an impact on employee satisfaction with their work. It is relatively said that an employee seems tobe happy there will be less count in the error rate.

3. Kumar (2016) states that training and development help to strengthen weaknesses in professional skills; employees must elevate them to a higher level of skill and knowledge. Thisallows the employee to better understand their professional responsibilities

4. Kumar (2016) comments that the training and development programs increase the communication that exists within an organization and helps eliminate difficulties encountered by employees. Because

communication will always play an important role in the betterment of individuals and for the entire organization which relates to the growth of every other person involved in the business operations.

5. Edralin (2015) infers in his study that the training is a set of activities aimed at facilitating the learning of knowledge, attitudes, and skills among the employees of an organization to improve their current professional performance and their contribution to the achievement of organizational objectives. They concluded stating that it is only through the training and development for their employees they worked with confidence to make a desirable short term project.

6. Mpofu and Hlatywayo (2015) state that the training and development as an important factor that plays a strategic role in the success and effectiveness of any organization in the current global economy, which implies the use of innovative technologies and greater response to customer needs to make them the big competitor in the city.

7. Edralin (2015) argues that it is different from development which involves long-term planned efforts to improve the overall growth of human resources that will lead to the achievement of personal and organizational goals.

8. According to Kashi (2014), in his study, he states that training and development aim to strengthen the skills and abilities of employees by providing people and teams with the skills, knowledge, and abilities necessary to increase productivity, efficiency, improving skills, learning, and reducing employee waste, accidents and absenteeism. The study also said that it improves the development of the workforce and ensures the survival and growth of theorganization.

9. Edralin (2014) reveals that training and development, when structured, ensure that employees have a consistent experience and basic knowledge. It is a way to ensure that employees know the procedures by which things are done and know the organization's expectations of them.

10. .Learning and knowledge management according to the Talent Development Association (2014), knowledge and learning management is also the process of "capturing, distributing,and archiving intellectual capital to foster knowledge sharing and collaboration". Knowledge management supports employee learning in organizations by monitoring learning outcomes. Today, organizations virtually track employee learning through HRIS systems.

11. A study by Edralin (2014) infers that in his study the respondents had stated that the Training and development help develop employee creativity and solve problems. Once the creativity level is found to be increased they will always be in a positive mindset to perform the tasks. This may help them in achieving the target to a greater extend.

12. In the ATD model (2014), the training function was divided into separate areas of specialization, such as training design, learning impact assessment, management of learning programs, and knowledge management. Training is a holistic term that includes all of these elements and has existed as a vital human resource function over the years.

13. Jehanzeb & Bashir (2013) narrates that Organizational efficiency, improved productivity,sales, and overall profitability is what measures the effectiveness of training and development. Therefore, the comments stating that for an organization to maintain the value of its human resources, it must be aware of the job satisfaction of its employees and not only of the financial benefits of their work.

14. According to Elnaga and Imran (2013) in their study stated that a satisfied employee performs better in all the aspects of the business activities and is easier to motivate through anymotivating techniques and will demonstrate a commitment to achieve the organization's objectives within the given period frame.

15. A study by Jehanzeb and Bashir (2013) communicates that it is during the period when training and development programs are offered to employees, the competitive advantage and knowledge of this organization will expand. Microsoft Company and General Electric Company are good examples where this positive impact can be seen. These are the two companies that see training and development as investment opportunities and have continued to gain a competitive advantage in their respective sectors.

Statement of the Problem

IT companies have attempted to apply many techniques in their training programmers to improve efforts to move towards a developed and satisfied state in their employees work and personal life. The nature of the current problem is to handle the employees by the suitable training programmers. India is the leading off shoring destination for IT companies across the world. This study analyzed the effectiveness of training programmers provided during training given to IT employees for producing the results efficiently with satisfied state and to increase the organization growth.

Objectives

- To assess the demographic profile of the respondents and understand the training requirements of employees in IT & ITes Industries.
- To study and analyze the employee perception on training and development in IT/ITesIndustry.
- To examine the effectiveness of training and development on performance in IT/ITesIndustry.
- To understand how employees, perceive training imparted to them in IT & ITesIndustries.
- To offer implementable suggestions for enhancing the training given and improving employees' perception towards the level of satisfaction on the training conducted

Hypotheses of the study

For the empirical and statistical justification of the study, following hypotheses are framed:

- Demographic profile of the employee's significantly influences their perception towards essentials of training and development programmed.
- There exists significant association between nature of training attended by the employee's and their perception on reasons stated by the organization for conduct oftraining.
- There exists significant relationship between purpose of training and employee's level of perception towards post training benefits in general.
- There exists significant relationship between purpose of training and employee's level of perception towards post training benefits in particular.
- There exists significant relationship between purpose of training and employee's level of perception towards post training benefits to the organization.

RESEARCH METHODOLOGY

This study investigates the perception of employees about the training provided by theiremployers with special reference to IT sector. To ensure the accuracy of results it is therefore important to choose an appropriate research methodology.

RESEARCH DESIGN

The aim of a research design is to provide an intended and prepared way of achieving the research objectives and to increase the validity and reliability. The present study is a descriptive that seeks to explore the employees about the training provided by their employers and Satisfaction on training. This type of study requires a research that does an in-depth investigation and description of phenomena, and systematically classifies the attributes as accurately and precisely as possible. In order to attain the aim of the research design, it should address the serious questions including the unit of analysis and the method of data collection. These components are therefore discussed in the following section.

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Unit of Analysis

In this study, the unit of analysis is employees working in IT sector operating in Coimbatore city. Research method refers to make sure about the validity and reliability of a research and this research made use of quantitative method where it could come into view appropriate. In the following sub-section, the quantitative approach is briefly evaluated.

Research Approach

The study adopted quantitative approach. In the quantitative approach the data is found to be inthe quantity form and it could be classified into three factors like inferential, experimental and simulation. In the form of inferential it could form a database from which it could infer characteristics of the targeted population. In the experimental form the there is a feature of much control over the research environment, some of the variables are manipulated to observe the effect on the other variable. In the simulation aspect the construction of an artificial environment within which the relevant information and the data could be generated.

SAMPLING DESIGN

Population

The population includes employees from all levels who are working in IT sector operating in Coimbatore city.

Sample size

Samples are collected from employees from all levels who are working in IT sector operating in Coimbatore city in which the total sample size of the respondents are 337. 400 questionnaires were issued out of which 63 questionnaires were rejected due to its insufficiency and inaccuracy so therefore 337 respondents constituted the sample for the study.

Sampling techniques

Systematic random sampling method is adopted for collecting the primary data. The researcher asked the HR department of each IT company for list of employees with their employee ID numbers from all levels. Then the researcher randomly selected the employee ID numbers from the list and distributed the questioner for survey.

Data Collection

For data collection, the employees who were selected randomly from the list of employees given by the HR department of each IT company, were approached and they were briefed about the nature and purpose of the research that which is being done. The respondents were assured of the confidentiality of the results. After the willingness, the questionnaires were administered to them. A soft copy of the same research questionnaire was also provided to some of the respondents on request. The study used both primary data and secondary data.

- **Primary data:** The primary source of the study includes the perception of the respondents which was collected through administering the structured questionnaire.
- **Secondary data:** The secondary source of the data was collected from various journals, websites, books and magazines.

Measures Used

A structured questioner was used for collecting the survey data from employees of all levels who are working in IT sector operating in Coimbatore city. The questionnaire has five parts and they are such as:

- Personal profile
- Company Information/ Job Profile
- Employee's Perception towards Conduct of Training
- Employee's Level of Perception on the Effectiveness of Training

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- Employee's level of Satisfaction.

Cronbach's alpha Reliability Test
Statistically, reliability is defined as the percentage of the inconsistency in the responses to the survey that is the result of differences in the respondents. This implies that responses to a reliable survey will vary because respondents have different opinions, not because the questionnaire items are confusing or ambiguous. It could be estimated using stability orequivalence approaches.

Statistical Tools and Techniques
The statistical package for social sciences (SPSS-16th Edition) was used to conduct the analysis of the collected data. Various statistical techniques were employed to examine the data such as Percentage analysis, mean and standard deviation, Chi square, Analysis of Variance, Correlation Analysis and Regression analysis.

Percentage Analysis
Percentage Analysis is the method to represent raw structure of data as a percentage for betterunderstanding of collected data; this was done for the demographic factors of the respondents. **Cross-tabulation**
The cross-tabulation procedure forms two-way and three-way analysis. It provides measures of association for two-way and three-way tables.

Descriptive statistics
The descriptive statistics procedure displays the mean and standard deviation of the research.

Chi- square
Chi-square test is applied to test the goodness of fit, to verify the distribution of observed data with assumed theoretical distribution. It is used to test the influence of one factor over the other. It is a measure to study the divergence of actual and expected frequencies.

One-way Analysis of Variance
In statistics, analysis of variance (ANOVA) is a collection of statistical models, and their associate procedures, in which the observed variance in a particular variable is partitioned into components attributable to different sources of variation. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are all equal, and therefore generalizes t-test to more than two groups. Doing multiple two-sample t-tests would result in an increased chance of committing Type I error. For this reason, ANOVAs are useful in comparing two, three or more means.

Correlation
Correlation refers to any of a broad class of statistical relationships involving dependence. Correlation can refer to any departure of two or more random variables from independence, but technically it refers to any of several more specialized types of relationship between mean values. There are several correlation coefficients, often denoted or r, measuring the degree of correlation. The most common of these is the Pearson correlation coefficient, which is sensitive only to a linear relationship between two variables (which may exist even if one is a nonlinear function of the other). Other correlation coefficients have been developed to be more robust than the Pearson correlation – that is, more sensitive to nonlinear relationships.

ANALYSIS AND INTERPRETATION
Demographic Profile
This section of the study briefly outlines the demographic profile of the sample employees' working inIT industry in Coimbatore city.

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Interpretation

From the table 1 it has been inferred that, 16.3 per cent falls under the age category of below 25years, 40.9 per cent of the employees' in IT industries are aged between 26 -35 years. Followed by, 15.4 per cent of sample subjects' falls under the age category of 36 - 40 years, 12.2 per cent are under the age category of 41 - 45 years, 6.2 per cent falls under the age category of 46 - 50 years and 8.9 per cent falls under the age category of above years. Thus, it has been clearly identified that most of the employees' in IT industries are aged between 26 -35 years.

Interpretation

The table 2 indicates that out of 337 respondents surveyed, majority i.e., 61.7 percent of employees are male and the remaining 38.3 per cent of respondents are female. Hence it has been concluded that majority of the respondents are male.

Interpretation

The table 3 illustrates the work experience gained by the employees' working in IT industry inCoimbatore. Out of 337 employees' surveyed, 9.5 per cent of sample subjects' have 1-5 years' work experience, 29.7 per cent of respondents' have gained 6-10 years of work experience in IT, 27.3 per cent of respondents' have gained 11-15 years of work experience and 33.5 per cent of employees' have more than 15 years of work experience in IT. Thus it has been concluded that most of the respondents' have gained more than 15 years of work experience in IT.

From table 4 it can be inferred that out of 337 respondents, Conveying the Objectives and delivery issues of Training holds the first position which shows that the HR department are moreconcerned about the objectives and delivery issues. Infrastructure arrangements such as space, lighting, audio-visual arrangements is in second position where the respondents feels that HR department also takes care in providing basic facilities like space, lights and audio visual arrangementto conduct the training effectively. And Usage of Training aids : computer based training, role-plays, games, class room Training is in the third position which HR concentrates in providing computer aided trainings also. Conduct of Training, which hold fourth rank. Benefits of Training to the organization & employee are in fifth rank which shows that they have moderate knowledge in it. Interaction with trainer & Resource holds sixth position which clearly shows that they are slightly focused in it. Convenience of training to the employee which stands in seventh position. These are clearly indicated by its mean score where the mean score of Conveying the Objectives and deliveryissues of Training is 4.02 and Convenience of training to the employee mean score is 3.54.

From table 5 it can be inferred that out of 337 respondents, Production Improvement holds firstposition which shows that IT employees thinks that organizations are more focusing production improvement. Improvement in Quality is in second position where the respondent thinks that quality would be important next to production. And Meeting the Customer & Stakeholders Needs and Satisfaction are in the third position which shows that organisations are interested to gain satisfaction of customers and stakeholders. Direct Focus Objective Attainment, which hold fourth rank. Gain of Individual Employee Recognition of Organisational Goal is in fifth rank which shows that least preference. Effective Time utilization holds sixth position which clearly shows that they are less focused in it. Developing Team Work among employee which stands in seventh position. This shows that they are not much interested in it compared to other factors. Focus on Individual Employee skill development hold eighth position where the respondents thinks that the organisations do care about it in lesser consideration. These are clearly indicated by its mean score where the mean score of Production Improvement is 3.73 and the Focus on Individual Employee skill development mean score is 3.09

Suggestions

- Organizations can Identify the skills that employees need to do their jobs, identify gaps between where they are and what they need, and focus training on resolving the gaps.
- Company can consider doing follow-up mini-sessions a month or two after a training session, where participants can interact and talk about their experiences and challenges applying what was learned.
- Benchmark against the competition organization can be planned.

- Try to match different learning options to different learning styles.
- Company can plan Gasification and Give Prizes as Incentive to competing employees.
- Offer cross-departmental knowledge sharing to improve training and development; helping employees better understand what impact their day-to-day actions have in the company and in the different departments.
- Invest in personal development which includes Emotional Balance, Intellectual growth , Physical health.
- Design on boarding procedures and new-hire training that ensures employees will be knowledgeable, and focused on standards and customer satisfaction.

CONCLUSION

The new millennium will be one of knowledge and competition. This inevitably means that people as carriers of knowledge, will be the most important asset of the organization. IT Industries shall therefore Endeavour to make every employee's career an exciting one with adequate opportunities for personal development The present research thesis is an attempt to study the Effectiveness of Training and Development on Employee Performance and to analyze the Employee Perception of Training and Development in the IT/ITes industry.. The quest of the research was to find out the effectiveness of training and development on employee performance and associate the different variables with the employee performance. The present research is efforts to found out the key factor of effective training and employee performance and also give the suggestion to how boost the motivation of employees with effective training. Present study concluded that the responses of employees of IT industries on perception regarding determinants of effective training and their performance. In all the cases, high degree of positive correlation existed between determinants of effective training and their performance. Moreover, it can also be concluded that in all the identified determinants that are enrichment of excellence, improved delegation of authority, personal growth thoughts of employees is sufficient enough to show their considerable importance in effective training.

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Table 1: Age of the respondents

Age (in years)	Frequency	Percentage (in %)
Below 25	55	16.3
26 to 35	138	40.9
36 to 40	52	15.4
41 to 45	41	12.2
46 to 50	21	6.2
above 50	30	8.9

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Total	337	100

Table 2: Gender of the respondents

Gender	Frequency	Percentage (in %)
Male	208	61.7
Female	129	38.3
Total	337	100

Table 3: Work experience of the respondents

Work experience (in years)	Frequency	Percentage (in %)
1-5	32	9.5
6-10	100	29.7
11- 15	92	27.3
Above 15	113	33.5
Total	**337**	**100**

Table 4: level of Perception towards the training programmed conducted by the HRDepartment

Level of Perception towards the training programed conducted by your HR department	Mean	Rank
Conveying the Objectives and delivery issues of Training	4.02	I
Conduct of Training	3.68	IV
The convenience of training to the employee	3.54	VII
Infrastructure arrangements such as space, lighting, audio-visual arrangements, etc.	3.89	II
Usage of Training aids: computer-based training, role-plays, games, classroom Training	3.77	III
Benefits of Training to the organization & employee	3.62	V
Interaction with trainer & Resource	3.58	VI

Table 5:Perception towards the post- impact of training on the organization

Level of perception towards the post-impact of training on the organization	Mean	Rank
Direct Focus Objective Attainment	3.46	IV
Focus on Individual Employee skill development	3.09	VIII
Developing Team Work among employee	3.24	VII
Effective Time utilization	3.38	VI
Production Improvement	3.73	I
Improvement in Quality	3.68	II
Gain of Individual Employee Recognition of Organisational Goal	3.42	V
Meeting the Customer & Stakeholders Needs and Satisfaction	3.56	III

REVIEW ARTICLE

An Updated Review on Phytochemical Composition and Pharmacological Activities of *Prosopis glandulosa* Torr.: An Invasive Exotic Plant

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ABSTRACT

Prosopis species are commonly known as Honey mesquite in English and mezquitein in Spanish. These plants are widely used in traditional system of medicine for the treatment of several disorders such as bronchitis, dysentery, dermatitis, leprosy, lactation, toothache, rheumatism, relieving stomach pain, toothache, preventing miscarriage and treating snake bites. *Prosopis glandulosa* is one such plant which is used as a folk medicine in the treatment of asthma, breast cancer, boils, cough, eye inflammation, kidney stones and muscular pain. The presence of secondary metabolites namely alkaloids, flavonoids, terpenoids, proteins, amino acids, polyphenols, essential oils and glycosides present in various parts of plant highly attributes for the biogenic applications of the *Prosopis glandulosa*. The present review is mainly focused on the phyto chemistry and pharmacological applications of the *Prosopis glandulosa.*

Keywords: Biological activity; Cytotoxicity; Phytoconstituents; *Prosopi glandulosa*; *Prosopis juliflora*.

INTRODUCTION

Natural resources are a basic wealth that provides humanity with essential health needs and nearly 80% of the world population relies on plants for medicine. Secondary metabolites present in the medicinal plants are responsible for their therapeutic efficacy. The primary advantage of employing the plant as folk medicine is that it is highly

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trustworthy in the presence of phyto constituents, resulting in a synergistic effect [1,2] *Prosopis* species have been found all over Mexico, as well as in the northern United States. It thrives in watersheds with less than 150 mm of rainfall and on uplands with more than 750 inches of rain in acidic and alkaline settings.

It can be found at elevations of 1500 meters in areas with more than 200 winter days. Animals can be fed from the tree and it can also be used to make wood and charcoal. It also provides a good source of nutrition for bees. Cattle farmers in the United States who have had their ranges encroached upon by the organism and have established large-scale eradication programs regard it as a weed. Surprisingly, cattle are the primary source of plant growth in these ranges. [3]The various reviews were published by focussing on the growth, nutritional impacts, water loss measurement, nitrogen balancing, germination and some biological activities. The pharmacological aspects of *P. glandulosa* species were reviewed and published in 2019. [4]This review focuses particularly on the phyto chemistry and pharmacology of *P. glandulosa* species based on the literature indexed in Science Direct, Pub Med, Google Scholar, Embase, Research Gate, Springer and Google databases between 2011 and 2021. The keywords of this survey include *P. glandulosa*, *P. juliflora*, biological activity, phytochemical composition and pharmacology. Using these keywords individually and in combined form, the kinds of literature were collected and scrutinized based on the titles and abstracts of the research.

PROSOPIS GLANDULOSA TORREY

Synonyms
Prosopis juliflora var. glandulosa (Torrey)

Cockerell
*Prosopis juliflora*var. torreyana (Benson)
Prosopis juliflora var. constricta Sargent.

Common names
Honey mesquite (English), mezquite (Spanish).

Distribution
P.glandulosa has the broadest distribution of *Prosopis* species native to North America, with var. *glandulosa* found in Texas and north-east Mexico and var. torreyana located in the southwest United States, Baja California and Sonora. In eastern Texas and western Chihuahua, the variations overlap. *P.glandulosa* may have been naturalized in portions of North and Sahelian Africa, the Middle East and South Asia, despite not being well adapted to tropical climates. Large swaths of Australia and Southern Africa have been infected.[5]

ETHNOBOTANICAL USE
*P.glandulosa*is used in traditional medicine to treat dyspepsia, eczema, hernia and skin and umbilical disorders. In India, the gelatine/ jelly form of the whole plant of *P.glandulosa* is used in the treatment of diabetic Mellitus. In Guatemala, *P.juliflora* is used to treat gonorrhea.[6,5] In Pakistan, the leaves and gums of *P.juliflora* are used in the treatment of cough, muscular pain, eye inflammation, toothache, boils opening, breast cancer, kidney stones and asthma also used as an expectorant, pain killer and body tonic. In western Madhya Pradesh of India, the stem bark of *P.juliflora* is used in treating asthma. In Mexico and the southwestern United States, *P.glandulosa* Torrey var. glandulosa is used as a folk remedy in treating umbilical ailments, skin ailments, sore throat, dyspepsia, hernia and eruptions [3,9].

PHYTOCHEMISTRY
Secondary metabolites such as glucose, glycosides, terpenoids, alkaloids, polyphenols, flavonoids, proteins and amino acids, tannins, essential oil and other substances play a significant part in the bioactivity of *P.glandulosa* and

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P.juliflora species against various clinical situations. [10] The various phytochemicals of *P. glandulosa* and *P.juliflora* species are given in Table 1. The structures of various types of phyto constituents are also given in Figure 1-5.

PHARMACOLOGICAL ACTIVITIES

Due to the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, amino acids, polyphenols, proteins, essential oil, glucose and glycosides in the various parts including stem, root, leaves, inflorescence, legumes and seeds, *P. glandulosa* and *P. juliflora* species exhibit greater biological applications [12]. The various applications of the *P. glandulosa* and *P. juliflora* species are discussed as follows.

Anti-bacterial activity

The anti-bacterial activity of an indolizidine alkaloid namely 1, 6-juliprosopine isolated from the leaves of *Prosopis glandulosa*var. glandulosa was evaluated against *Staphylococcus aureus,* methicillin-resistant *S. aureus* and *Mycobacterium intracellulare*. The results showed that the indolizidine alkaloid showed greater activity towards *M. intracellulare* with The IC_{50} value of about 0.80 µg/ ml [9]. The antibacterial activity of an ethanolic extract of *P.glandulosa* leaves was investigated using the Disc diffusion method against *Staphylococcus epidermidis, Staphylococcus aureus*, *Shigella flexneri*, *Proteus vulgaris* and *Vibrio parahaemolyticus*. The ethanolic extract was suspected to have a moderate to significant inhibitory effect against *S.epidermidis*, *S.aureus*, *S. flexneri*, *P.vulgaris*, and *V. parahaemolyticus* with the MIC value of about 21.8 mm, 17.4 mm, 19.8 mm, 18.0 mm and 15.8 mm. The presence of phytochemicals such as flavonoids, terpenoids, and other natural phenolic compounds with free hydroxyl groups are responsible for the antibacterial action of the plant extract [10].

The acid value, iodine value, and saponification value of the seed oil of *P.glandulosa* and *P.juliflora* range from 4.99 to 9.59 percent. The total cholesterol content of *P.glandulosa* seed essential oil is greater (1680plus or minus 7.07mg/100g). The oils from the seeds included 53.7 to 68.46 % MUFA, 26.97-39.95 % SFA, and 3.7-6.95 % PUFA, respectively. The results indicated that all measured amounts of essential oils samples significantly suppressed the growth of *Escherichia coli, Klebsiella pneumonia* and *Streptococcus aureus*. Therefore, the seed oil of *P.glandulosa* and *P. juliflora* showed better antibacterial activity towards *E.coli, K.pneumonia* and *S.aureus*. [13]

Anti-fungal activity

The antifungal activity of prosopilosidine (PPD), a 2,3-dihydro-1H-indolizinium alkaloid derived from *P.glandulosa*, was tested against *Cryptococcus neoformans* in a mouse model of crypto coccosis. The result showed that PPD showed about 76 % of inhibitory activity towards *C. neoformans* at the dose of 0.0625 mg/kg as compared to 83 % with Amphotericin B (1.5 mg/kg). Furthermore, when delivered bid (twice a day) through an intraperitoneal route, PPD was found to be equally efficacious but less hazardous at 0.125 or 0.0625 mg/kg compared to Amphotericin B (1.5 mg/kg) [14]. The antifungal efficacy of *P.gladulosa* was evaluated against *Candida albicans* using acetone, methanol, petroleum ether and water extracts. *P.gladulosa* was found to have antifungal properties against six different strains of *C.albicans*. The results indicated that the aqueous extracts of *P.gladulosa* exhibit significant effects against *C.albicans*. [15]*P.glandulosa* extract was tested for antifungal activity against *Colletotrichum gloeosporoides, Fusarium oxysporum, Rhizopus oryzae* and *Rhizopus stolonifer*. The results indicated that the plant extract showed greater fungicidal activity towards *C. gloesporioides* and *F. oxysporum*, with 74.92 % and 64.82 % growth rate reductions but showed lesser activity towards *R.oryzae* and *R.stolonifer* [16].

Anti-cancer activity

In vitro anti-cancer potential of *P.juliflora* was tested against two prominent cancer cell lines, HePG2 (liver cancer) and MCF7 (breast cancer) using DPPH assay. The methanolic extract of *P.glandulosa* showed greater cytotoxic activity against the MCF7 cell line with the IC_{50} value of about 14.9 µg/ml.[6] The anticancer potential of *P.glandulosa* was determined against four human cancer cell lines, SKMEL (malignant melanoma), KB (epidermal carcinoma), BT-549 (epithelial carcinoma) and SK-OV-3 (ovarian cancer), as well as noncancerous cell lines, VERO and LLC-PK1. The result showed significant cytotoxic activities towards all the cancer cell lines. [17]

The antitumor activity of ethanol extract of *P.glandulosa* Torr. was evaluated against the Ehrlich as cites carcinoma (EAC) tumor model in Swiss albino mice in a dose-dependent manner. The activity was assessed using survival time, the average increase in body weight, hematological parameters and solid tumor volume. Oral administration of the ethanolic extract at the dose of 100, 200 and 400 mg/Kg, significantly increased the survival time and decreased the average body weight of the tumor-bearing mice. After 14 days of inoculation, the ethanolic extract was able to reverse the changes in the hematological parameters, protein and PCV consequent to tumor inoculation. Oral administration of the ethanolic extract was effective in reducing solid tumor mass development induced by EAC cells. [18] The cytotoxicity of *P.glandulosa* was evaluated on mouse pancreatic β-cells (β-TC3) and HepG2 (liver cancer) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium method as compared to Streptozotocin. The results showed that the extract showed a significant effect on both the cancer cell lines [19].

Cardio protective and anti-hypertensive effect

The cardio protective activity of *P.glandulosa* was studied. The result showed that the consumption of *P.glandulosa* significantly reduced infarct size after ischemia-reperfusion in DIO rats and CIRKO mice. The plant extract showed greater cardio protection activity by altering the survival pathway proteins PI-3-kinase/PKB/Akt. The anti-hypertensive activity of *P.glandulosa* was studied on HFD animal models. The results indicated that the *P.glandulosa* therapy both prevented and rectified the development of hypertension, which was evident in the reduction of water retention, and also decreases blood pressure in the HFD animal models [20].

Anti-diabetic effect

The anti-diabetic activity of *P.glandulosa* was evaluated on the male Wistar rat. The results showed that the *P.glandulosa* treatment resulted in a large increase in insulin levels and considerably decreasing blood glucose levels in a type 1 diabetic mouse. In addition, therapy with *P.glandulosa* resulted in an increase in small β-cells in the pancreas. When comparing treated to untreated Zucker rats, *P.glandulosa* therapy significantly lowered fasting glucose levels and improved IPGTT. *P.glandulosa* treatment improved basal and insulin-stimulated glucose absorption by cardiomyocytes generated from the selected group in the DIO insulin resistance paradigm. [21] In streptozotocin (STZ)-induced type 1 diabetes and diet-induced obesity (DIO) insulin-resistant rat models, a Western blot procedure was used to assess the expression of pancreatic growth factors Pdx-1 and Maf-A and also to identify the effect of *P. glandulosa* treatment. The results showed that the plant extract showed a reduction in the Maf-A and Pdx-1 expression in the pancreatic tissue and STZ mice in a type 1 diabetes paradigm [22].

Antimalarial activity

The antimalarial activity of *P.gladulosa* against *Plasmodium berghei* (NK-65 strain) infected mice was studied using Peter's 4-day suppression test. 2.107 parasitized red blood cells were collected from a highly infected donor mouse and administered intraperitoneally to male Swiss Webster mice. On different days (till day 28 post-infection), blood smears were made by clipping the tail end, stained with Giemsa, and examined under a microscope to determine parasitemia. The plant extract showed greater anti-malarial activity towards *P.berghei* infected mice. [17] A colorimetric technique is used to evaluate parasite lactate dehydrogenase (pLDH) activity which is used to determine the antimalarial activity *in vitro* against two *P. falciparum* clones (chloroquine-resistant) and Indochina W2. The selectivity indices (SI) were calculated by determining how poisonous materials were to mammalian cells (VERO; monkey kidney fibroblast). The results revealed a significant impact [17].

Anti-hyperlipidemic activity

The anti-hyperlipidemic effectiveness of an aqueous root extract of *P.glandulosa* on the development of experimental atherosclerosis in rabbits with hypercholesterolemia caused by a high-fat diet was investigated. In comparison to the normal control group, serum lipid values were considerably higher in the high cholesterol diet groups. The results indicated that both the thoracic and abdominal aortas of hypercholesterolemic rabbits developed athermanous plaques. When compared to high cholesterol diet rabbits, treatment with *P.glandulosa* root dramatically lowered total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, and very-low-density lipoprotein levels [23].

Anti-leishmanial activity

In THP-1 macrophage cultures, the antileishmanial activity of indolizidines alkaloid namely 1,6-juliprosopine which is obtained from the leaves extract was tested against *Leishmania donovani* promastigotes. The alkaloid showed significant anti-leishmanial activity towards *Leishmania donovani* [9]. The anti-leishmanial efficacy of *P.glandulosa* was tested against *Leishmania amazonensis* promastigotes. The result showed that the aqueous extract of *P.glandulosa* has higher anti-leishmanial efficacy against *Leishmania amazonensis* promastigotes [24].

Immunostimulant activity

RAW264.7 and THP-1 cells were used to test the immunomodulatory effects of the herbal extract IM-133N, which contained extracts of *P.glandulosa* Torr. The cells were incubated for 24 hours with IM-133N at doses ranging from 0 to 125 mg/ml and no cytotoxicity was observed. The results showed that the non-cytotoxic dosages of IM-133N efficiently upregulated iNOS, TNF, IL-6, IL-10, IL-8, and IFN- gene expression in both RAW264.7 and THP-1 cells. The results also showed that RAW264.7 or THP-1 cells produced more nitric oxide (NO) and tumor necrosis factor (TNF) in response to IM-133N. These findings showed that IM-133N stimulated NO production and promoted pro-inflammatory cytokine expression in monocytes and macrophages. [25]

Wound healing activity

The present therapeutic options for soft tissue injuries are ineffective and frequently result in delayed or partial muscle recovery. In a rat model, the efficacy of oral *P.glandulosa* medication on inflammation and regeneration in skeletal muscle after contusion injury was compared to standard diclofenac. Muscle repair was assessed using indicators of muscle satellite cell proliferation (ADAM12) and regeneration (Desmin). Although both chronic *P. glandulosa* and diclofenac treatment suppressed the neutrophil response to contusion injury, only chronic *P. glandulosa* treatment facilitated more effective muscle recovery (increased ADAM12 and Desmin expression), whereas diclofenac treatment inhibited repair despite effective neutrophil response inhibition. Results indicated that *P. glandulosa* treatment showed more effective muscle repair after contusion [26].

Hypersensitivity reactions

The pattern of immediate-type hypersensitivity reactions to aeroallergens in atopic asthmatic children was investigated in the city of Al-Khobar, in the Kingdom of Saudi Arabia's Eastern Province. The majority of asthmatic children in the study were found to be atopic with 86 % of the children testing positive for at least one allergenic extract. Furthermore, *P. glandulosa* was determined to be the most common sensitizer among the tree pollens we studied. The city of Al-Khobar has a great number of trees of various *Prosopis* species planted along the side roads. Also, a previous allergological investigation on the *Prosopis* family of trees in Saudi Arabia indicated that atopic persons in Al-Hofuf city, in the Eastern Province of Saudi Arabia, were sensitized to *P.juliflora* antigen at a rate of 11.11 %. When the population was examined for *P. glandulosa* species, it showed a 17.6% sensitization rate. [27] The phytochemicals present, pharmacological activity, types of extract used along with its method of determination of the *Prosopis* species is summarized in Table 2.

CONCLUSION

Prosopis is a vital healthful species that is used to treat various diseases. The presence of secondary metabolites namely juliprosinene, juliprosine, apigenin, luteolin-7-O-glucoside, coumarin, (-)-mesquitol, syringin, pheophytin A, pheophytin B, benzothiazole, dibutyl phthalate, procyanidin B_2, etc. playing a greater role in exhibiting significant activity. Particularly, the species *P. glandulosa* may have potential benefits as biocontrol agents for human pathogens. *P.juliflora* showed unique pharmacological actions against various diseases. However, further studies are needed to confirm their potential, determine their effect on human pathogens and identify the bioactive compounds in *P. glandulosa*.

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LIST OF ABBREVIATIONS
ADAM12-ADAM metallopeptidase domain 12
BT-549-Human Breast Cancer cell line
B-TC3-Murine Pancreatic Beta TC3 cells
DIO-Diet Induced Obesity
DMSO-Dimethyl Sulphoxide
DPPH-2,2-diphenyl-1-picryl-hydrazyl-hydrate
EAC-Ehrlich Ascites Carcinoma
HepG2-Human Liver Cancer cell line
IFN-Interferon
IL-10-Interleukin-10
IL-6-Interleukin-6
IL-8-Interleukin-8
iNOS- Inducible nitric oxide synthase
IPGTT-Intraperitoneal Glucose Tolerance Test
KB-Human Epithelial Carcinoma cell line
LLC-PK1-Pig Kidney Epithelial cell line
Maf-A-Macrophage activating factor-A

MCF-7-Human Breast Cancer cell line
MTT-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
MUFA-Mono Unsaturated Fatty Acids
Pdx-1-pancreatic and duodenal homeobox 1
pLDH- Parasite Lactate Dehydrogenase
PUFA-Poly Unsaturated Fatty Acids
RAW 264.7-Abelson leukemia virus-transformed cell line
SFA-Saturated Fatty Acids
SKMEL-Human Melanoma cell line
SK-OV-3-Human Ovarian Cancer cell line
THP-1-Acute MonocyticLeukemia
VERO-Monkey Kidney Fibroblast

Table 1. Phytochemical constituents present in *P.glandulosa* and *P.juliflora*

Phytochemical constituents	References
Alkaloids: N-methyl julifloridine, juliflorodine, prosoflorine, juliprosinene, juliprosine, secojuliprosopino, 2,4-Diphenyl-6,7,8,9 – tetrahydro-5H-cyclohepta (D) pyrimidine	[11]
Flavonoids: Apigenin, apiugenin 6,8 di cglycoside , crysoeriol 7-o-beta-D-glucoside , luteolin 7oglucoside , kaemferol 3-o-methyl ether ,isorhamnetin 3 –o-glucoside , isorhamnetin 3-o –rutinoside ,quercetin-3-o-lutinoside , (-)-mesquitol , ellagic acid.	[11]
Phenylpropanoids: (-)-Lariciresinol, Syringin	[11]
Tetrapyrroles: Pheophytin A and B	[11]
Others (alcohols, esters, diketones and alkanes): Fatty acids, Carbohydrates, Glycosides, Terpenoids, Proteins, Amino acids, Tannins, Coumarins, A-Tryptophan, Prosopidone, Benzothiazole, Diethyl phthalate, 4-isopropyl-1,6-dimethyl naphthalene, Diisobutyl phthalate, 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,8-diene-2,8-dione, Dibutyl phthalate, Mono (2-ethyl hexyl) phthalate, Procyanidin B₂.	[11]

Table. 2: Phytochemicals present, pharmacological activity, types of extract and method of determination of the *Prosopis*species

S.No	Part of the plant	Extract	Phytochemicals present	Activity	Method of determination	Re
1.	Leaves	Methanolic extract	Phenolic compounds	Anti-oxidant Anti-cancer	MTT-assay DPPH method	[6]
2.	Leaves	Ethanolic extract	Juliprosopine Juliprosine	Anti-bacterial Anti-leishmanial Anti-plasmodial Anti-fungal	NCLLS method used for antimicrobial activity Antiplasmodial activity determined by colorimetry method Anti-Leishmanialactivity measured by neutral red assay	[9]

3.	Leaves	Ethanolic extract	Flavonoids Terpenoids Phenolic compounds	Anti-microbial	Disc diffusion method	[10]
4.	Pods and flowers	Extracted oil		Anti-bacterial Anti-fungal Anti-microbial	Agar disc diffusion method	[13]
5.	Leaves	Ethanol	Prosopilosidine	*In vivo* Anti-Cryptococcal Activity *Invitro* anti-fungal	MTS assay & maximum tolerated dose method in female mice	[14]
6.	Leaves	Acetone Methanol Petroleum ether Water	Hydroxytryptamine Apigenin Isorhamnetin-3-diglucoside L-arabinose Quercetin	Antifungal activity	Agar wall disc diffusion method	[15]
7.	Leaves, flowers, And Branches	Aqueous extract	-	Anti-fungal	Disc diffusion method	[16]
8.	Leaves	Ethanol		Anti-tumor	Ehrlich ascites carcinoma (EAC) tumor model in Swiss albino mice	[18]
9.	Roots	Ethanol n-hexane Acetone Ethanol + water	-	Anti-diabetic	MTT assay on pancreatic B cells(B-TC3 cells)	[19]
10.	Pods	Powdered drug	-	Cardio protective activity Anti-Hypertensive	Western blot Pressure measured in DIO rats Infarct size measured in cardiac muscle	[20]
11.	Ground pods	-	-	Anti-diabetic	Alkaline phosphadasefuchsin method, Insulin sensitivity determination in cardiomyocytes obtained from DIO rats	[21]
12.	Dried and ground pods			Anti-Diabetic	Western blot protocol to determine the expression of	[22]

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					pancreatic growth factors: Pdx-1 and Maf-A Anti-diabetic activity	
13.	Leaves and branches	Aqueous extract	-	Anti-leishmanial	Disc diffusion method	[24]
14.	IM-133N herbal preparation containing *P. glanduosa*	Aqueous extract	Gallic acid Quercetin Apigenin Coumaric acid Luteolin Catechin Epicatechingallate	Immune-modulating activity	Measurement immunomodulatory effects using RAW264.7 and THP-1 cells	[25]
15.	Pods	Aqueous extract	-	Anti-inflammatory	Immunohistochemistry and Western blotting analysis.	[26]
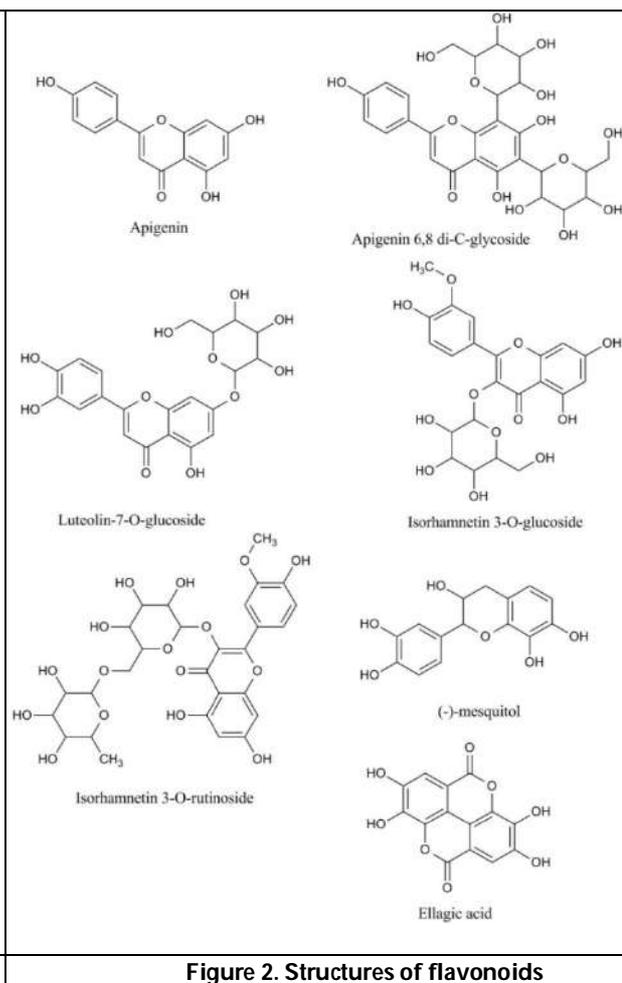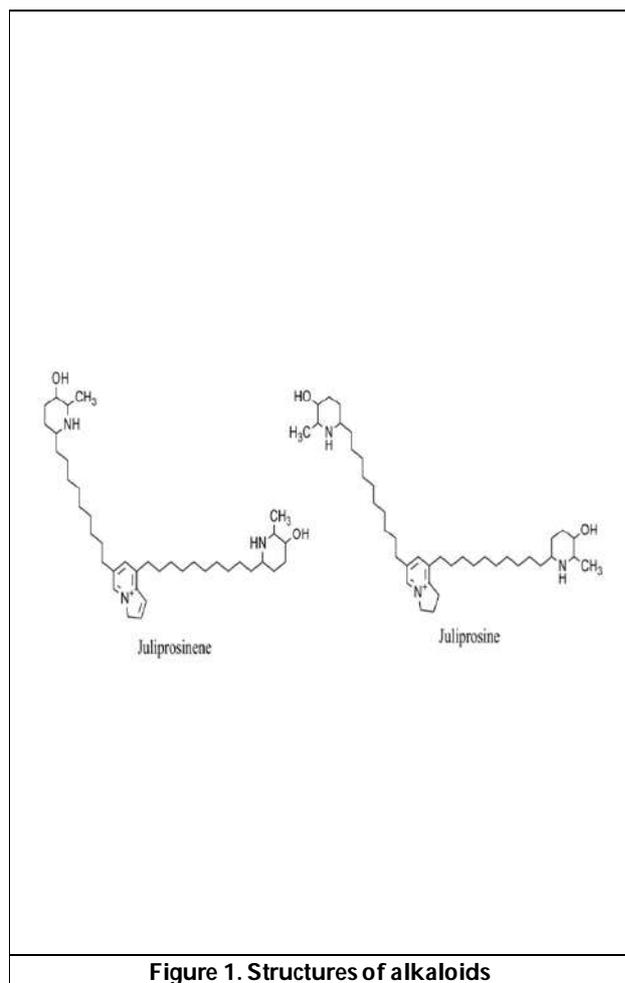
Figure 1. Structures of alkaloids

Figure 2. Structures of flavonoids

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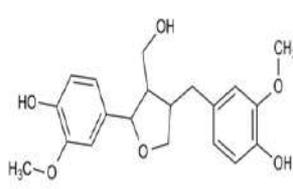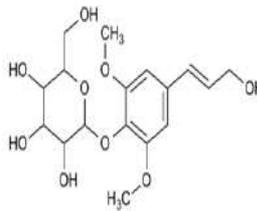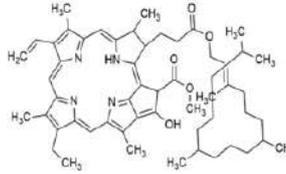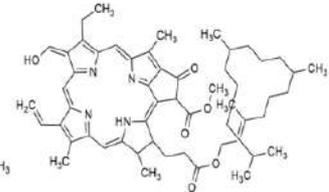
Figure 3. Structures of Phenylpropanoids

Figure 4. Structures of Tetrapyrroles
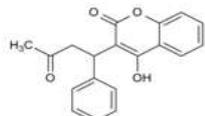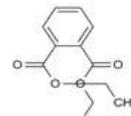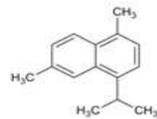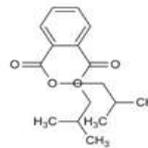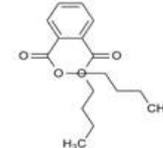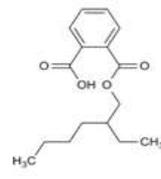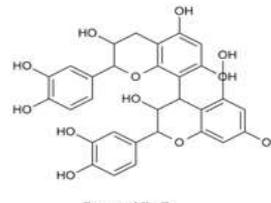
Figure 5. Structures of other compounds

RESEARCH ARTICLE

TLC Profiling and Phytochemical Evaluation of *Bergenia ligulata* Wall. and *Podophyllum hexandrum* Royle- An Endangered Medicinal Plant

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ABSTRACT

Podophyllum hexandrum (Berberidaceae) and *Bergenia ligulata* (Saxifragaceae) both are versatile medicinal plants used for centuries in herbal formulation for curing various ailments. In the present study, preliminary phytochemical investigation was done on these two species for the presence and absence of different bioactive compounds. The bioactive compounds which were present in methanol, acetone and water extract of both root and leaf of *Bergenia ligulata* are glycosides, phenolics, steroids, tannins, alkaloids, proteins, carbohydrates, however the extracts showed the negative result for terpenoids, flavonoids and saponins. In roots of *Podophyllum hexandrum* the compounds mostly in water, methanol and acetone extracts was glycosides, phenolics, steroids, tannins, alkaloid, saponins, flavonoids, and carbohydrates however extract showed the negative result for proteins and volatile oils in acetone and methanolic extracts only. Later the compounds were separated by using column chromatography and then fractions obtained from column chromatography were used for the TLC and the R_f values of each fraction were detected in TLC. The TLC analysis revealed the presence of bioactive compounds eluted on pre-coated TLC plates and is determined by calculating their R_f values.

Keywords: *Bergenia ligulata*,Column chromatography, phytochemicals, *Podophyllum hexandrum* and TLC.

INTRODUCTION

The relationship of human beings with their surrounding herbs and medicinal plants attracts its roots to the ancient civilizations [1]. Plants are a gift from nature to humans because they are the primary source of many bioactive chemicals used to produce modern and traditional drugs [2]. Approximately 35,000 to 70,000 plant species are used for medicines in the world and Around 80 percent of the world's population used medicinal plants to treat various problems such as Arthritis, Diabetes, Eye infection, Loss of hair, loose motion, Skin disease, Abortion, kidney stone, Cough, Hypertension, Stomach diseases, and Loss of alertness [3]. *Bergenia ligulata* Wall is amongst the high valued, endangered temperate medicinal herbs commonly called as 'Paashanbheda' in Indian systems of medicine and it belongs to the family Saxifragaceae [4]. It is a perennial herb that grows up to 50 cm tall and is found in the temperate Himalayan region (from Kashmir to Nepal). *Bergenia ligulata* is used as a cure for dysuria and stones in the kidney and ureter. Phytochemical analysis showed that *Bergenia ligulata* contains number of phytochemicals like Paashaanolactone, Afzelechin, Bergenin and Catechin [5]. *Podophyllum hexandrum* Royle (Divine drug) is an erect, glabrous, succulent herb 35-60cm tall with creeping, perennial, knotted rhizomes bearing numerous roots and is native to the Himalayan countries like Afghanistan, Pakistan, India, Nepaland Bhutan [3]. In Kashmir *Podophyllum hexandrum* has been employed in traditional system of medicine from ancient times and is locally known as Banwangun, since its red colored fruit (berry) is of the size of a small brinjal. Biological analysis of *Podophyllum hexandrum* revealed that it contains a number of biological compounds like Podophyllotoxin, epipodophyllotoxin, flavonoids such as quercetin-3-glycoside, podophyllotoxin and glycoside. The aim of the present study is to identify the Bioactive components in Methanol, Acetone and Water extracts of roots and leaves of *Bergenia ligulata* and *Podophyllum hexandrum* and the presence of different chemical compounds in methanolic extract of both the plants by using column chromatography and TLC.

MATERIALS AND METHODS

Collection and identification of plant materials
The healthy plants of *Bergenia ligulata* and *Podophyllum hexandrum* were collected from Gulmarg Himalayan region, Jammu and Kashmir, India from April to September. The authenticity of the plant was confirmed in Department of Botany Annamalai University Tamil Nadu, by referring the deposited specimen. The voucher number of the specimen is AUBOT-382 and AUBOT-383respectively. The fresh leaf and root parts of *Bergenia ligulata* and roots of *Podophyllum hexandrum* species was washed with tap water and then rinsed with distilled water, shade dried at room temperature and powdered.

Preparation of plant Extracts
The powdered plant samples (100 g/500 mL) were extracted successively with Acetone, Methanol and Water using Soxhlet apparatus at 55-85 °C for 72 hours in order to extract the compounds. Each extract was concentrated by evaporating solvents In rotary evaporator (Heidolph). The crude extracts were stored in amber vials and were placed at 4 °C for further use.

Preliminary phytochemical analysis
The extracts of roots and leafs of *Bergenia ligulata* and roots of *Podophyllum hexandrum* obtained were subjected to the preliminary phytochemical evaluation to identify the bioactive constituents present in the various alcoholic (methanol, acetone) and aqueous extracts by using standard protocols as described by [6,7,8,9, 10].

Testing for Alkaloids
Mayer's test
2 to 3 drops of Mayer's reagent (Mercuric chloride and potassium iodide in water) were added to the 1 ml of each plant extract. The appearance of a yellow color precipitate indicates the presence of Alkaloids.

Testing for Flavonoids
Sodium hydroxide test
To 2ml of each extract add 20% Sodium hydroxide (NaOH) yellow colour appears. Then add few drops of 70% HCl, yellow colour disappears. The disappearance and appearance indicates the presence of flavonoids.

Testing for saponins
Foam height test
10 ml of distilled water was added to the 1ml of each plant extract in a volumetric flask, shaken vigorously to froth and then left to stand for 10min. Measure froth up to 1ml indicates the presence of saponins.

Testing for Terpenoids
Salkowski Test
2ml of chloroform was added to 2ml of each plant extract, followed by 2 to 3 drops of concentrated sulphuric acid. The presence of terpenoids is indicated by deep red coloration.

Testing for Glycosides
Keller killani Test
To 1ml of each extract a few drops of Glacial acetic acid and ferric chloride and 2-3 drops of concentrated sulphuric acid were added. The appearance of Blue green colour indicates the presence of Glycosides.

Testing for Carbohydrates
Molisch test
To 2ml of each plant extract add 10 ml water, then add 2 drops of Ethanolic α- naphthol (20%) and add 2ml concentrated sulphuric acid. Appearance of reddish violet ring indicates the presence of carbohydrates.

Test for protein
Ninhydrin's test
To small quantity of extract solution 2 drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. Blue colour was developed which reveals the presence of proteins.

Test for Volatile oil
Spot test/ Residue test
Small amount of each plant extract was put on filter paper by means of capillary tube and visualize. Transparent with no yellow colour persist indicates the presence of volatile oils.

Test for Steroids
Liebermann Burchards Test
1ml of each plant extract were dissolved in 10 ml of chloroform and equal volume of concentrated H_2SO_4 was added by sides of the test tube. The presence of yellow green fluorescence indicates the positive test for steroids.

Test for Resin
Turbidity Test
Acetone and plant extract sample was mixed in 1:1 ratio and distilled water (2ml) was poured in mixture; turbidity was seen which specified the presence of resin.

Test for Tannins
Braymer's test
To 2ml of plant extract add 2ml of water and then add 2-3 drops of 5% ferric chloride. The formation of green precipitate indicates the presence of tannins.

Column Chromatographic Fractionation of Methanol Extract of *Bergenia Ligulata* and *Podophyllum hexandrum*

The bioactive components were isolated from methanolic extract of leaf and root of *Bergenia ligulata* and root of *Podophyllum hexandrum* plants using silica gel column chromatography A vertical glass column made of borosilicate material was employed for fractionation. The column was rinsed well with acetone and was thoroughly dried before packing. At the bottom of glass, a piece of wool is placed with the help of glass rod. Sea sand was added to the top of the glass wool upto the height of 1cm and was rinsed down using the solvents. Stopcock were closed and hexane was poured into the column upto 3/4th level. With packing material of 200g of silica gel (100-200 mesh size) was used. Slurry was prepared from silica and hexane and was poured from top of the column approximately 2/3rd of the column with simultaneous draining of the solvent to aid adequate packing of the column. Sea slurry sand was added to the top of the slurry to a depth of 1cm, and the sand particles were washed down with the solvent. 10gm of crude extract was dissolved in 5mL of methanol to form a slurry suspension which was then carefully poured to the top of the column without disturbing the surface. To prevent the drying of column solvent level 6cm from the extract was maintained. Gradient elution approach was used to separate fraction from methanol extract by using solvents ranging from low polarity to high polarity (i, e. Petroleum ether to methanol) in various ratios. The flow rate was adjusted to 1 ml/min and 10ml solvent was collected for each fraction.

Thin Layer Chromatographic Studies of *Podophyllum hexandrum* and *Bergenia Ligulata*

Methanolic extract of both *Podophyllum hexandrum* roots and *Bergnia ligulata* roots and leaves are subjected to TLC analysis. TLC separation was performed using aluminum sheets coated with silicagel 60 F 254 (MerckKGaA, Darmstadt, Germany). The fractions from column were collected separately and subjected to TLC to detect the presence of bioactive compounds. The separation of compounds occurs on the basis of polarity .0.1 to 1.0 µl of sample at distance of 1 cm from base with the help of glass capillaries were used to spot the sample. The solvents system taken in chamber was Ethyl acetate: Formic acid: Acetic acid and water (100:11:11:27)[11]. After presaturation with mobile phase for 20min for development. The run plates were dried and then used to detect the spot.

Detection of spots

All the dried plates were read under UV light (nm and nm) and movement of the solvents was marked on the TLC plates. The movement of the active compounds was expressed by its retention factor (RF), whose values were calculated for different samples using the following formula [12].

R_f = Distance travelled by the solute

Distance travelled by the solvent front on TLC plates

RESULTS AND DISCUSSION

Phytochemical analysis of three solvent extracts were carried out to detect the presence of phytochemical components of these two plant species such as Alkaloid, Flavonoid, Terpenoids, Glycosides, Steroids, Saponins, Resin, Tannin, Carbohydrates, Volatile oils and Proteins by using standard method. These tests indicate the presence of various phytochemical compounds which would be responsible for various medicinal properties of these plants. Phytochemicals such as alkaloids, flavonoids, terpenoids, glycosides etc. was need to discover and extended to novel therapeutically agents with improved efficiency. The results of phytochemical analysis are depicted in Table 1 and Table 2.

Yield of extract (%)

The crude extracts obtained after soxhlet extraction process was concentrated by permitting solvents to evaporate entirely under room temperature to obtain the actual yield of extraction. In phytochemical extraction, the percentage yield of extraction is essential for estimating the standard extraction efficiency for a particular plant,

different parts of the same plant or different solvents used. The yield of extracts obtained from the Rhizomes of the plants using water, acetone and methanol as solvents are depicted in the Table 3.

Percentage yield = Weight of the extract/ Weight of the dry plant material $\times$ 100

Column Chromatographic Fractions of *Bergenia ligulata* and *Podophyllum hexandrum*

In order to isolate compounds in the methanolic extract of both *Podophyllum hexandrum* roots and *Bergenia ligulata* roots and leaves column chromatography with solvent system petroleum ether: ethyl acetate (PE: EA) and chloroform: methanol (CH: ME) i.e., non polar to polar solvents was used.   The total number of fractions obtained after pooling of spots with same Rf value from TLC were as follows in case of column chromatography of *Bergenia ligulata* root .The elutes 1-25 of 10 ml respectively in solvent system PE:EA(100,9:1,8:2,7:3) formed fraction A; the elutes 26-59 aliquots of 10ml respectively in solvent system PE:EA(6:4,5:5,4:6,3:7,2:8) formed fraction B;the elutes 60 to 75 aliquots of 10ml respectively in solvent system PE:EA (1:9,0:100) formed fraction C ; the elutes 76 to 135 aliquots of 10ml respectively in solvent system CH:ME (100,9:1,8:2,7:3,6:4) formed fraction D;136 to 164 aliquots of 10 ml respectively in solvent system CH:ME (5:5,4:6,3:7) formed fraction E; 167 to 200 aliquots of 10 ml respectively in solvent system CH:ME (2:8,1:9,0:100) formed fraction F. The yields of the fractions and R$_f$ values of each fraction obtained are shown in Table IV, V and VI.

In case of *Bergenia ligulata* leaves the fractions obtained after pooling were as follows, elute1-36 aliquote of 10ml each in PE:EA (100,9:1,8:2, 7:3,6:4) formed fraction A; the elute 37-74 aliquote of 10ml each in PE:EA (5:5,4:6,3:7,2:8,1:9) formed fraction B: the elute 78-100 aliquote of 10ml each in PE:EA (100) formed fraction C; the aliquote 100-145 of 10ml each in CH:ME(100,9:1,8:2,7:3,6:4) formed fraction D; the 146-200 aliquote of 10ml each in CH:ME (5:5,4:6,3:7,2:8,1:9,100) formed fraction E.In case of *Podophyllum hexandrum* roots the aliquote 1-26 of 10ml respectively in PE :EA (100,9:1,8:2) formed fraction A ; the aliquote 21-56 of 10ml respectively in PE:EA (7:3,6:4,5:5) formed fraction B; the aliquote 56-74 of 10ml each in PE:EA (4:6,3:7,2:8) formed fraction C; the aliquote 75-103 (1:9,0:100) formed fraction D ,the aliquote 104-136 of 10ml respectively in CH:ME (100,9:1,8:2,7:3) formed fraction E; the aliquots 137-154 of 10ml respectively in CH:ME (6:4,5:5,4:6) formed fraction F; the aliquots 155-178 of 10 ml each in CH:ME (3:7,2:8) formed fraction G; the aliquote 179-200 of 10 ml each in CH:ME (1:9,0:100) formed fraction H .Totally 200 fractions were collected. Out of 200 fractions collected the solvent system was used with increased polarity. Different fractions obtained by using various solvent system also reflect an idea about their polarity by the use of the various solvent for column chromatographic studies which could be an important for the selection of the appropriate solvent system. The fractions acquired from the column chromatography are shown in table 4, 5 and 6.

TLC Analysis of *Bergenia ligulata* and *Podophyllum hexandrum*

The fractions obtained from silica gel column chromatography of methanolic extract of both *Bergenia ligulata* and *Podophyllum hexandrum* were tested for the detection of various spots using TLC.The Rf values obtained from thin layer chromatographic analysis of fractions collected from column chromatography are listed in the Table 4,5,6. The TLC result obtained from column fractions of methanolic extract of both *Podophyllum hexandrum* and *Bergenia ligulata* are shown as, In case of *Bergenia ligulata* root the TLC showed different spots which was visible under UV and the spots having the same Rf value were pooled into a single fraction. The fractions A showed Rf value 0.5,fraction B showed Rf value 0.23, fraction C showed Rf value 0.46, fraction D showed Rf value 0.55, fraction E showed Rf values 0.72, fraction F showed Rf value 0.66. In *Bergenia ligulata* leaf extract the total number of spots showing different Rf values was five, the Rf value of fraction A is 0.05, fraction B 0.4, the fraction C shows the Rf value 0.6, the fraction D shows the Rf value 0.65 and the fraction E shows the Rf value 0.7. In *Podophyllum hexandrum* roots the total number of spots showing different values were eight, the Rf value of fraction A is 0.05, the fraction B showed Rf value 0.8, the fraction C showed Rf value 0.15, the fraction D showed Rf value 0.17, the fraction E showed the Rf value 0.72, the fraction F showed Rf value 0.76, the fraction G showed Rf value 0.6, the fraction H showed the Rf value 0.2. Analysis of methanolic extract of both the plants by TLC showed the presence of various phytochemicals. Different phytochemicals in the plant extract have given different Rf values and these Rf values obtained from the bioactive compounds provide important evidence regarding their polarity and also the separation of these phytochemicals in

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separation process and the selection of appropriate solvent system. The TLC fingerprinting profile of methanolic extraction of *Bergenia ligulata* roots, leaves and *Podophyllum hexandrum* root under UV are shown in Figure 1 and under visible light are shown in Figure 2.

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Table1: Preliminary Phytochemical Analysis of *Bergenia ligulata* roots and Leaves

	Bergenia ligulata rhizome			*Bergenia ligulata* leaf		
Bioactive compounds	Acetone	Methanol	Water	Acetone	Methanol	Water
Steroids	+	++	-	+	-	-
Terpenoids	+++	+++	++	+++	++	++
Tannins	-	-	++	+++	+++	+++
Glycoside	+	++	-	++	++	-
Flavonoids	+++	+++	++	++	++	+++
Resin	+	++	-	+++	++	-
Alkaloids	-	++	-	+	++	-
Volatile oils	-	-	-	-	+	-
Carbohydrates	++	++	++	+	+	+
Protein	-	-	++	+++	+++	+++
Saponins	-	-	+++	-	-	+++

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Table 2: Preliminary Phytochemical Analysis of *Podophyllum hexandrum* Roots

Podophyllum hexandrum Rhizome			
Bioactive compounds	Methanol	Acetone	Water
Steroids	++	+	-
Terpenoids	+++	+++	+++
Tannins	++	++	+++
Glycoside	++	+	-
Flavonoids	+++	+++	+++
Resin	+++	+	-
Alkaloids	++	-	-
Volatile oils	-	-	+++
Carbohydrates	+	-	+
Protein	-	-	-
Saponins	-	-	+++

Table 3: The Percentage Yield in Different Solvents of *Podophyllum hexandrum* Roots and *Bergenia ligulata* Roots and Leaves.

Plant Name	Plant Part	Percentage Yield (%)		
		Acetone	Methanol	Water
Bergenia ligulata	Rhizome	48%	47%	48%
	Leaves	61%	49%	55%
Podophyllum hexandrum	Rhizome	25%	28%	16.66%

Table 4: Experimental Yield of *Bergenia ligulata* Root

Number of elutes (Aliquots of 10 ml each)	Solvent systems	Solvent ratio	Name of fractions	R_f value
1-25	Petroleum ether: Ethyl Acetate	100,9:1,8:2,7:3	Fraction A	0.44
26-59	Petroleum ether: Ethyl Acetate	6:4,5:5,4:6,3:7,2:8	Fraction B	0.23
60- 75	Petroleum ether: Ethyl Acetate	1:9,0:100	Fraction C	0.46
76-135	Chloroform: Methanol	100,9:1,8:2,7:3,6:4	Fraction D	0.55
136-164	Chloroform: Methanol	5:5,4:6,3:7	Fraction E	0.72,
165-200	Chloroform: Methanol	2:8,1:9,0:100	Fraction F	0.66

table 5: Experimental Yield of *Bergenia ligulata* Leaves

Number of elutes (Aliquots of 10 ml each	Solvent system	Solvent ratio	Name of fractions	Rf value
1-36	Petroleum Ether: Ethyl Acetate	100,9:1,8:2, 7:3,6:4	Fraction A	0.05
37-74	Petroleum Ether: Ethyl Acetate	5:5,4:6,3:7,2:8,1:9	Fraction B	0.4
75-100	Chloroform	100	Fraction C	0.6
101-145	Chloroform: Methanol	100,9:1,8:2,7:3,6:4	Fraction D	0.65
146-200	chloroform: Methanol	5:5,4:6,3:7,2:8,1:9,100	Fraction E	0.7

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Table 6: Experimental Yield of *Podophyllum hexandrum* Root

No. of elutes (Aliquotes of 10 ml each)	Solvent system	Solvent ratio	Name of fractions	Rf value
1-20	Petroleum ether: Ethyl acetate	100,9:1,8:2	Fraction A	0.05,
21- 56	Petroleum ether: Ethyl Acetate	7:3,6:4,5:5	Fraction B	0.8
57-74	Petroleum ether: Ethyl Acetate	4:6,3:7,2:8	Fraction C	0.15
75-103	Petroleum ether: Ethyl Acetate	1:9,0:100	Fraction D	0.17
104-136	chloroform: Methanol	100,9:1,8:2,7:3	Fraction E	0.72
137-154	Chloroform: Methanol	6:4,5:5,4:6	Fraction F	0.76
155-178	Chloroform: Methanol	3:7,2:8	Fraction G	0.6
179-200	Chloroform: Methanol	1:9,0:100	Fraction H	0.2

(a) *B. ligulata* roots	(b) *B.ligulata* leaves	(c) *P.hexandrum*
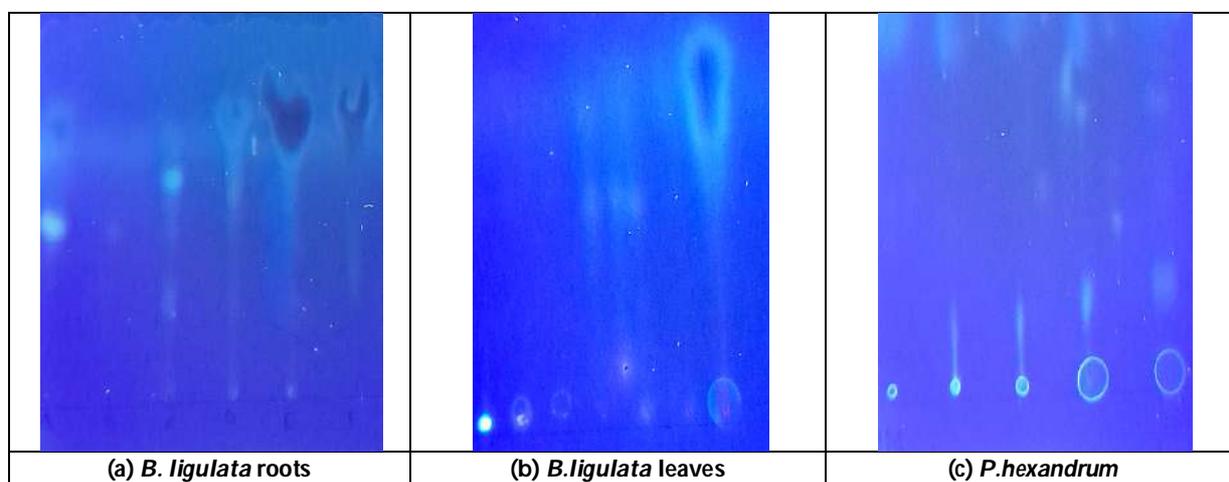
Figure 1. TLC fingerprinting under UV light

(a) B. ligulata roots	(b)*B. ligulata* leaves	*(c) P. hexandrum* roots
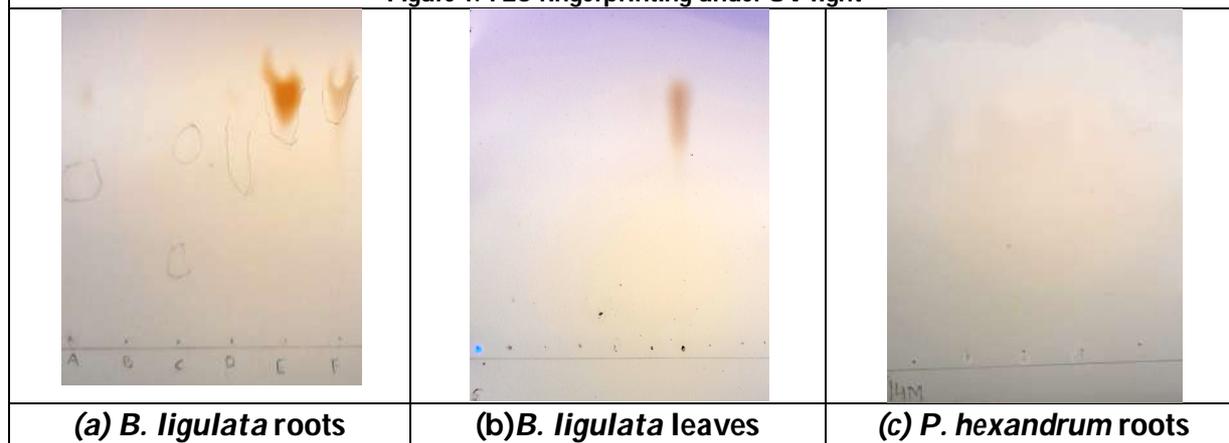
Figure 2. TLC Fingerprinting under Visible Light

RESEARCH ARTICLE

Two Stage Max-Min Algorithm for Solving Pentagonal fuzzy Transportation Problem

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ABSTRACT

Because of inherent ambiguity, fuzzy numbers and fuzzy values are commonly utilised in domains such as experimental sciences, artificial intelligence, and so on. This work uses pentagonal fuzzy numbers to discover the best solution to a special kind of optimization problem called a fuzzy transportation problem in a fuzzy environment. For fuzzy transportation problems, the values of cost, availability, and requirement are considered as pentagonal fuzzy numbers. In this two-stage cost-minimizing transportation problem, fuzzy pentagonal numbers are turned into crisp values using the range technique and then solved using the max-min method.

Keywords: Pentagonal Fuzzy Number, Two Stage Fuzzy Transportation, Range Technique – max-min Method, α -optimal solution.

INTRODUCTION

Transportation problem is used worldwide in solving certain real-world problems. A transportation problem plays a vital role in production industry and many other purposes. The transportation problem is a special type of Linear

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programming problem, which permit us to regulate the optimum shipping patterns between origins and destinations. The solution of the problem will allow us to determine the number of entities to be transported from a particular origin to a particular destination so that the cost obtained is minimum or the time taken is minimum or the profit obtained is maximum. In our day-to-day life situations, various decision-making problems such as fixing the cost of goods, profit for sellers, making decisions for real-life multi objective functions, etc. are looking for a solution by the transportation problem. In real-life problems, Zadeh [14] had introduced the uncertainty theory, which is very useful for copying a large amount of data. There are so many instants we need to find the maximum or minimum optimum solution for real-life problems. So, it is necessary to achieve this efficient solution with the minimum impact of the environment and communities. Ranking fuzzy number is a essential step in many mathematical models. The notions of fuzzy sets were first introduced by Zadeh [14]. Since its inception several ranking procedures have been developed. There onwards several authors presented various approaches for solving the FTP problems [1],[2],[4]. Some of these ranking approaches have been reviewed and compared by Bortolan and Degani [3]. Presently Chen and H Wang [5] reviewed the existing method for ranking fuzzy numbers and each approach has drawbacks in some aspects such as indiscrimination and finding not so easy to interpretate. Ranking regular fuzzy number were primary introduced by Jain [7] for decision making in fuzzy situations. Chan specified that in many situations it is not possible to confine the membership function to the general form and proposed the concept of generalized fuzzy numbers. Since then, remarkable efforts are made on the development of several methodologies. The development in ordering fuzzy numbers can even be found in [8],[9],[11],[12],[13]. Fuzzy numbers must be ordered before a decision is taken by a decision maker.

In a fuzzy transportation problem, the transportation expenditures, supply and demand quantities are fuzzy quantities. A new method of solving a fuzzy transportation problem based on the assumption that the decision-maker is uncertain about transportation cost was introduced by Amarpeet Kaur and Amit Kumar [16]. Liu and Kao Proposed a new fuzzy approach to the multi-objective transportation problem. Omaret.al [6] also suggested a parametric plan for solving the transportation problem under fuzziness. In 1976 Jain [7] had introduced a new method of ranking fuzzy numbers. In 1941, Hitchcock [17] initiated the fundamental transportation problem; still, the researchers recently focus on a lot of different methods that make a betterment of TP. Neetu Sharma [18] proposed a new way of solving TP, which is an alternative method for the North West Corner method. In 2014, a unique fuzzy number named fuzzy pentagonal number was introduced by T. Pathinathan and K. Ponnivalavan. In 2018, Ngastiti PTB, Surars B, sutimin [19] was initiated zero points and zero suffix method under Robust ranking for solving the fully fuzzy transportation problem.

In some situations, due to storage constraints, designations are unable to receive the quantity in excess of their minimum demand. After utilizing part of whole of this initial shipment, they are prepared to accept the excess quantity in the second stage. According to Sonia and Rita Malhotra [15], in such circumstances the product transported to the destination has two stages. Enough of the product is shipped in stage-I so that the minimum requirements of the destinations are satisfied and having done this the surplus quantities at the sources are shipped to the destinations according to cost consideration. In both stages the transportation of the product from sources to the destinations is done in parallel. Nagoor Gani A and Abdul Razak K [21] explained a two-stage fuzzy transportation problem in 2006. The goal is to keep the total transportation expenses in both stages as low as possible. Using pentagonal fuzzy numbers, Sathya Geetha S and Selvakumari K [20] developed a new strategy for handling fuzzy transportation problems in 2020. In this paper, we proposed a new approach, which is named as the recommended range method to solve the fuzzy transportation problem. The solving procedure is done in two stages.

It gives the minimum value for comparing all other existing methods such as the Northwest corner method, LCM method, and VAM method. Finally, an illustrative example is given for the best understanding of the given algorithm.

PRELIMINARIES

Fuzzy Concepts

L.A. Zadeh[14] advanced the fuzzy theory in 1965. The idea of fuzzy theory proposes a mathematical technique for dealing with imprecise concepts and problems that have many possible solutions. The notion of fuzzy mathematical programming on a general level was first proposed by Tanaka *et al* in the frame work of the fuzzy decision of Bellman and Zadeh[22].

Definition: Fuzzy Set

A fuzzy set A in R (real line) is defined as a set of ordered pairs

$$A = \left\{ x_0, \mu_A(x_0) / x_0 \in A, \mu_A(x_0) \to [0,1] \right\}$$ where $\mu_A(x_0)$ is said to be the membership function.

Definition: Fuzzy Number

A which is a fuzzy set on the real line R, must satisfy the following conditions.

(i) $\mu_A(x_0)$ is piecewise continuous

(ii) There exist at least one $x_0 \in R$ with $\mu_A(x_0) = 1$

(iii) A must be regular & convex

Definition: Triangular Fuzzy Number

A fuzzy number A is said to a triangular fuzzy number, which is named as (a_1, a_2, a_3) if its membership function $\mu_A(x)$ has the following characteristic.

$$\mu_A(x) = \begin{cases} \dfrac{x - a_1}{a_2 - a_1}, & if a_1 \le x < a_2 \\ 1, & if x = a_2 \\ \dfrac{a_3 - x}{a_3 - a_2}, & if a_2 < x \le a_3 \\ 0, & otherwise \end{cases}$$
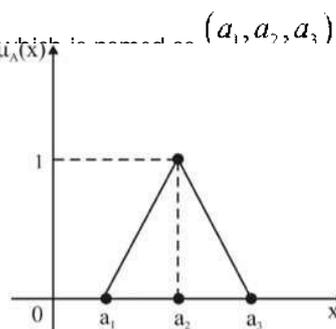
Definition: Trapezoidal Fuzzy Number

A fuzzy number A is a trapezoidal fuzzy number which is named as (a_1, a_2, a_3, a_4) where a1, a2, a3,a4 are real numbers whose membership function $\mu_A(x)$ is given by

$$\mu_A(x) = \begin{cases} 0, & if\, x < a_1 \\ \dfrac{x - a_1}{a_2 - a_1}, & if\, a_1 \le x \le a_2 \\ 1, & if\, a_2 \le x \le a_3 \\ \dfrac{a_4 - x}{a_4 - a_3}, & if\, a_3 \le x \le a_4 \\ 0, & if\, x > a_4 \end{cases}$$
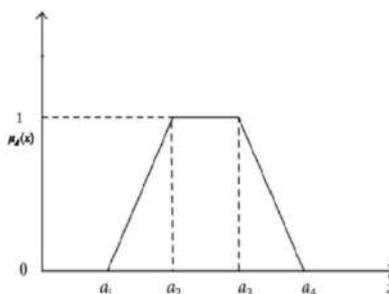
Definition: Pentagonal Fuzzy Number

A fuzzy number A on R is referred to as a pentagonal fuzzy number (PFN) or linear fuzzy number $(a_1, a_2, a_3, a_4, a_5)$, and its membership function $\mu_A(x)$ has the following characteristic

$$\mu_A(x) = \begin{cases} 0, & if\, x < a_1 \\ u_1\left(\dfrac{x - a_2}{a_3 - a_2}\right), & if\, a_1 \le x \le a_2 \\ 1, & if\, x = a_3 \\ 1 - (1 - u_2)\left(\dfrac{a_4 - x}{a_4 - a_3}\right), & if\, a_3 \le x \le a_4 \\ u_2\left(\dfrac{a_5 - x}{a_5 - a_4}\right), & if\, a_4 \le x \le a_5 \\ 0, & if\, x > a_5 \end{cases}$$
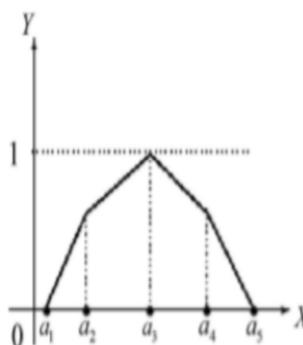
Definition

The α-level set of the fuzzy number $\overline{a}$ and $\overline{b}$ is defined as the ordinary set $L_\alpha(\overline{a}, \overline{b})$ for which the degree of their membership function exceeds the level $\alpha \in [0,1]$.

$$L_\alpha(\overline{a}, \overline{b}) = \left\{ a, b \in R^m \,/\, \mu_A(a_i, b_j) \ge \alpha, i = 1, 2, \dots m, j = 1, 2, \dots n \right\}$$

Arithmetic Operations

Let $\overline{A} = (a_1, b_1, c_1, d_1, e_1)$ and $\overline{B} = (a_2, b_2, c_2, d_2, e_2)$ are the two fuzzy numbers where $a_1 \le b_1 \le c_1 \le d_1 \le e_1$ and $a_2 \le b_2 \le c_2 \le d_2 \le e_2$ then the arithmetic operations are defined as

(i) Addition

$$\bar{A} + \bar{B} = \left(a_1 + a_2, b_1 + b_2, c_1 + c_2, d_1 + d_2, e_1 + e_2 \right)$$

(ii) Subtraction

$$\bar{A} - \bar{B} = \left(a_1 - e_2, b_1 - d_2, c_1 - c_2, d_1 - b_2, e_1 - a_2 \right)$$

(iii) Multiplication

$$\bar{A} * \bar{B} = \left(\frac{a_1}{5}\mu_\theta, \frac{b_1}{5}\mu_\theta, \frac{c_1}{5}\mu_\theta, \frac{d_1}{5}\mu_\theta, \frac{e_1}{5}\mu_\theta \right)$$ where $\mu_\theta = \left(a_2 + b_2 + c_2 + d_2 + e_2 \right)$

(iv) Division

$$\bar{A} \div \bar{B} = \left(\frac{5a_1}{\mu_\theta}, \frac{5b_1}{\mu_\theta}, \frac{5c_1}{\mu_\theta}, \frac{5d_1}{\mu_\theta}, \frac{5e_1}{\mu_\theta} \right) if\, \mu_\theta \neq 0$$ where $\mu_\theta = \left(a_2 + b_2 + c_2 + d_2 + e_2 \right)$

(V) Scalar Multiplication

$$k\bar{A} = \begin{cases} (ka,\ kb,\ kc,\ kd,\ ke)\, if\, k > 0 \\ (ke,\ kd,\ kc,\ kb,\ ka)\ if\, k < 0 \end{cases}$$

MATHEMATICAL FORMULATION OF FUZZY TRANSPORTATION PROBLEM

Consider a fuzzy transportation problem with m sources and n destinations with pentagonal fuzzy numbers. Let $a_i, (a_i \geq 0)$ be the fuzzy availability at source i and let $b_j, (b_j \geq 0)$ be the fuzzy requirement at destination j. Let c_{ij} be the fuzzy unit transportation cost from source i to destination j. Let x_{ij} denote the number of fuzzy units which has to be transported from source i to destination j. Then the problem is to find the feasible way of transporting the available amount at each source to satisfy the demand at each destination so that the total transportation cost is minimized. The mathematical formulation for the fuzzy transportation problem with pentagonal fuzzy numbers as factors in the case where total supply equals total demand is as follows:

$$Min\, z = \sum_{i=1}^{m} \sum_{j=1}^{n} c_{ij} x_{ij}$$

Subject to $\sum_{j=1}^{n} x_{ij} = a_i, i = 1, 2 ..... m$

$$\sum_{i=1}^{m} x_{ij} = b_j, j = 1, 2 ..... n.$$

$$\sum_{i=1}^{m} a_i = \sum_{j=1}^{n} b_j; i = 1, 2 ..... m; j = 1, 2 ..... n$$ and $x_{ij} \geq 0$.

Theoretical Development

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Let $\overline{b}_j$ be the minimum fuzzy requirement of a homogeneous product at the destination j and $\overline{a}_i$ the fuzzy availability of the same at source i. The Two stage Fuzzy Cost Minimization Transportation Problem (FCMTP) deals with supplying the destinations their minimum requirements in stage-I and the quantity $\sum_i \overline{a}_i - \sum_j \overline{b}_j$ is supplied to the destinations in stage-II, from the sources which have surplus quantity left over after the completion of stage-I. Mathematically stated, the stage-I problem is

$$\min_{x \in s_1} \left[c_1(x) \right] = \min_{x \in s_1} \tag{1}$$

Where the set s_1 is given by

$$S_1 = \begin{cases} \sum_{j=1}^{n} x_{ij} \le \overline{a}_i & i = 1, 2 .... m \\ \sum_{i=1}^{m} x_{ij} = \overline{b}_j & j = 1, 2 .... n \end{cases} \tag{2}$$

$x_{ij} \ge 0, \forall (i, j)$ corresponds to a feasible solution X= (x_{ij}) of the stage-I problem, the set $S_2 = \left\{ \overline{X} = \left(\overline{x}_{ij} \right) \right\}$ of feasible solutions of the stage-II problem is given by

$$S_2 = \begin{cases} \sum_{j=1}^{n} x_{ij} \le \overline{a}_i & i = 1, 2 .... m \\ \sum_{i=1}^{m} x_{ij} \ge \overline{b}_j & j = 1, 2 .... n \end{cases} \tag{3}$$

$\overline{x}_{ij} \ge 0, \forall (i, j)$ where $\overline{a}'_i$ is the quantity available at the ith source on completion so the stage-I, that is $\overline{a}'_i = \overline{a}_i - \sum j X_{ij}$ .Clearly $\sum i \overline{a}'_i = \sum i \overline{a}_i - \sum j \overline{b}_j$. Now, the stage-II problem would be mathematically formulated as:

$$\min_{\overline{X} \in S_2} \left[C_2 \left(\overline{X} \right) \right] = \min_{\overline{X} \in S_2} \left[\max_{|X|} \left(C_{ij} \left(\overline{X}_{ij} \right) \right) \right] \tag{4}$$

We focus at finding that feasible schedule $X = \left(x_{ij} \right)$ of the stage-I problem corresponding to which the optimal cost for stage-II is such that the sum of the shipment is the least. The Two-stage fuzzy cost minimizing Transportation problem can, therefore, be stated as,

$$\min_{X \in S_1}\left[C_1(X) + \left(\min_{\bar{X} \in S_2} C_2(\bar{X}) \right) \right] \tag{5}$$

The feasible solution of the problem (5) can be obtained. Further the problem (3) can be solved by solving the following fuzzy cost minimizing Transportation problem.

$$\min_{X \in S_2}\left[C(X') \right] = \min_{X' \in S_2}\left[\max\left(C_{ij}(X'_{ij}) \right) \right] \tag{6}$$

where S_2, the set of feasible solutions of (5), is defined as follows

$$S_2 = \begin{cases} \sum_{j=1}^{n} x'_{ij} = \bar{a}_i & i = 1, 2 \dots m \\ \sum_{i=1}^{m} x'_{ij} = \bar{b}_j & j = 1, 2 \dots n \end{cases} \tag{7}$$

$x'_{ij} \geq 0, \forall (i, j)$ where $\bar{a}_i$ and $\bar{b}_j$ represent fuzzy parameters involved in the constraints with their membership functions for $\mu_{\bar{A}}$ a certain degree α together with the concept of α level set of the fuzzy numbers $\bar{a}_i, \bar{b}_j$. Therefore, two stage FCMTP can be understood as non-fuzzy α -two stage FCMTP.

$$S = \begin{cases} \sum_{j=1}^{n} x_{ij} = \bar{a}_i & i = 1, 2 \dots m \\ \sum_{i=1}^{m} x_{ij} = \bar{b}_j & j = 1, 2 \dots n \end{cases} \tag{8}$$

$a_i, b_j \in L_\alpha\left(\bar{a}_i, \bar{b}_j \right)$, where $L_\alpha\left(\bar{a}_i, \bar{b}_j \right)$ are the α -level set of the fuzzy number $\bar{a}_i, \bar{b}_j$. Let $x\left(\bar{a}_i, \bar{b}_j \right)$ denote the constraint set of problem and supposed to be non-empty. On the basis of the α -level sets of the fuzzy numbers, we give the notion of α -optimal solution in the following definition.

Definition (α -optimal solution)

A point x· $\in X\left(\bar{a}_i, \bar{b}_j \right)$ is known to be α -optimal solution (α -two stage FCMTP), if and only if there does not exist another x, y $\in$ x(a, b), $a, b \in L_\alpha\left(\bar{a}_i, \bar{b}_j \right)$, such that $c_{ij}, x_{ij}, c_{ij}x_{ij}$ with strict inequality holding for at least one c_{ij} for corresponding values of parameters $\left(\bar{a}, \bar{b} \right)$ are called α -level optimal parameters. The problem (α -two stage FCMTP) can be re written in the following equivalent form (α -two stage FCMTP).

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$$S = \begin{cases} \sum_{j=1}^{n} x_{ij} = \overline{a}_i & i = 1, 2 \dots m \\ \sum_{i=1}^{m} x_{ij} = \overline{b}_j & j = 1, 2 \dots n \end{cases}$$

$$h_i^0 \le a_i \le H_i^0, h_j^0 \le b_j \le H_j^0$$

$$X_{ij} \ge 0 \forall i, j$$

(9)

It should be noted that the constraint $a_i, b_j \in L_\alpha\left(\overline{a}_i, \overline{b}_j\right)$ has been replaced by the constraint $h_i^0 \le a_i \le H_i^0$ and $h_j^0 \le b_j \le H_j^0$ where h_i^0, H_i^0 and h_j^0, H_j^0 are lower and upper bounds and a_i, b_j are constants. The parametric study of the problem (α'-two stage FCMTP) where h_i^0, H_i^0 and h_j^0, H_j^0 are assumed to be parameters rather than constants and (renamed h_i, H_i and h_j, H_j) can be understood as follows. Let X(h, H) denotes the decision space of problem (α'-two stage FCMTP) defined by

$$X(h, H) = \left(X_{ij}, a_i, b_j\right) \in R^{n(n+1)} \left| a_i - \sum_j X_{ij} \ge 0, \right.$$

$$b_j - \sum_i X_{ij} \ge 0, H_i - a_i \ge 0, H_j - b_j \ge 0,$$

$$a_i - h_i \ge 0, b_j - h_j \ge 0, X_{ij} \ge 0, i \in I, j \in J$$

(10)

RANGE TECHNIQUE

The range is defined as the difference between the maximum value and minimum value.

Range = Maximum amount – Minimum amount

MAX MIN METHOD- ALGORITHM

Step 1: Construct the transportation table and examine whether the total demand equals total supply then go to step 2.

Step 2: By using range technique, the fuzzy cost can be converted into crisp values to the given transportation problem.

Step 3: Find the difference between maximum and minimum of each row, and divide it by the number of columns of the cost matrix.

Step 4: Find the difference between maximum and minimum of each column, and divide by the number of rows of the cost matrix.

Step 5: We calculate the maximum of the resultant values and find the corresponding minimum cost value and do the allocation of that particular cell of the given matrix. Suppose if we have more than one maximum consequent value, We can select anyone.

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Step 6: Repeat procedures 1 to 5 until all the allocations are completed.

SOLUTION ALGORITHM

Step 1 : Construct the problem 4

Step 2 : Determine the points (a_1, a_2, a_3, a_4, a_5) and (b_1, b_2, b_3, b_4, b_5) for the fuzzy number in the formulation problem (Two stage FCMTP).

Step 3 : Convert the problem (α - two stage FCMTP) in the form of the problem (α' - two stage FCMTP)

Step 4 : Formulate the problem (α' - two stage FCMTP) in the parametric form.

Step 5 : Apply Range Technique to get the basic feasible solution.

Step 6 : Declare min (C_1+ C_2) as the optimal value of the objective function of the problem.

NUMERICAL EXAMPLE

Consider the Balanced fuzzy transportation problem. A Product is produced by four factories Factory1, Factory2, Factory3, Factory4. The product is supplied to four stores Store1, Store2, Store3, Store4. Production capacity of the four factories and the requirements of Demands are represented as fuzzy pentagonal numbers. Here Unit costs of fuzzy transportation are also represented as fuzzy pentagonal numbers as given below. Find the fuzzy transportation plan so that the total production and the transportation cost is minimum.

$$
\begin{array}{c}
 & Store1 & Store2 & Store3 & Store4 & Capacity \\
Factory1 & (2,4,6,8,9) & (3,5,7,8,9) & (2,4,5,6,7) & (3,4,6,7,12) & (28,29,30,31,32) \\
Factory2 & (0,2,5,6,8) & (4,5,6,8,11) & (2,3,5,7,11) & (1,5,6,9,11) & (24,25,27,28,29) \\
Factory3 & (1,2,3,4,5) & (2,3,4,6,8) & (4,5,6,8,9) & (6,7,8,9,13) & (37,38,40,41,42) \\
Factory4 & (3,5,6,7,8) & (1,5,6,7,8) & (2,7,8,9,10) & (3,3,4,5,9) & (48,49,50,51,52)
\end{array}
$$

$$Demand \quad (18,19,20,21,22) \quad (38,39,40,41,42) \quad (32,33,34,35,36) \quad (51,52,53,54,55)$$

Solution

The fuzzy pentagonal numbers are converted into a crisp value by using Range Technique in Two Stage Cost Minimizing Transportation Problem.

Consider α -level set to be α = 0.75, then we get $28.5 \le a_1 \le 31.5$, $24.5 \le a_2 \le 28.5$, $37.5 \le a_3 \le 41.5$, $48.5 \le a_4 \le 51.5$, $18.5 \le b_1 \le 21.5$, $38.5 \le b_2 \le 41.5$, $32.5 \le b_3 \le 35.5$, $51.5 \le b_4 \le 54.5$.

The α - optimal parameters are

$a_1 = 30, a_2 = 27, a_3 = 40, a_4 = 50$

$b_1 = 20, b_1 = 40, b_1 = 34, b_1 = 53$

Stage I

This is a balanced Transportation table. We take

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$$a_1 = 15, a_2 = 13, a_3 = 20, a_4 = 25$$
$$b_1 = 10, b_1 = 20, b_1 = 17, b_1 = 26$$

Apply the Max-Min Algorithm to find the maximum of the resultant values and find the corresponding minimum cost value and allocate the particular cost cell of the given matrix. If we have more than one maximum resultant benefits, we can select anyone.

The Transportation Cost
Z = 5∗15 + 7∗ 13 + 4 ∗ 10 + 6 ∗ 7 + 5 ∗ 2 + 7 ∗ 1+ 6 ∗ 25
Z = 415

Stage II
Now we take

$$a_1 = 15, a_2 = 14, a_3 = 20, a_4 = 25$$
$$b_1 = 10, b_1 = 20, b_1 = 17, b_1 = 27$$

The Transportation Cost
Z = 5∗15 + 7∗ 14 + 4 ∗ 10 + 6 ∗ 6 + 5 ∗ 2 + 7 ∗ 2+ 6 ∗ 25
Z = 423

∴ The Optimum Transportation cost for the given problem is given by
   Z = 415 + 423 = 838.

Comparison with Existing Methods
The comparison of the proposed method with the existing procedure based on the methodology provided by Sathya Geetha S and Selvakumari K is tabulated below, in which it is clearly shown that the proposed method provides the optimal results.

CONCLUSION

In this research, the proposed range strategy is used to solve the fuzzy transportation problem in two stages, which is relatively easy and also achieves the lowest transportation cost when compared to all other existing plans. Stage I entails exporting enough product to meet the destinations' minimum requirements, then transporting surplus amounts at the sources to the destinations based on cost considerations. At both phases, the product is carried in parallel from the source to the destination. The pentagonal fuzzy numbers are converted to crisp values using the Range approach, and then the Max-Min Algorithm is used to identify the best answer in two stages. This approach is able to overcome both storage and cost limits. In comparison to existing methods, the proposed method produces superior results.

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Table; 1 The fuzzy transportation problem is explicitly represented by the fuzzy transportation table:

	1	...	n	Supply
1	c_{11}	...	c_{1n}	a_1
$\vdots$	$\boxtimes$	...	$\boxtimes$	$\boxtimes$
m	c_{m1}	...	c_{mn}	a_m
Demand	b_1	...	b_n	

Table : 2 The fuzzy pentagonal numbers are converted into a crisp value by using Range Technique in Two Stage Cost Minimizing Transportation Problem.

	Store1	Store2	Store3	Store4	Capacity
Factory1	7	6	5	9	$(28,29,30,31,32)$
Factory2	8	7	9	10	$(24,25,27,28,29)$
Factory3	4	6	5	7	$(37,38,40,41,42)$
Factory4	5	7	8	6	$(48,49,50,51,52)$
Demand	$(18,19,20,21,22)$	$(38,39,40,41,42)$	$(32,33,34,35,36)$	$(51,52,53,54,55)$	

Table : 3 Apply the Max-Min Algorithm to find the maximum of the resultant values and find the corresponding minimum cost value and allocate the particular cost cell of the given matrix. If we have more than one maximum resultant benefits, we can select anyone.

	Store1	Store2	Store3	Store4	Capacity	$\dfrac{Max - Min}{4}$
Factory1	7	6	5	9	15	$\dfrac{4}{4}=1$
Factory2	8	7	9	10	13	$\dfrac{3}{4}=0.75$
Factory3	4 10	6	5	7	20	$\dfrac{3}{4}=0.75$
Factory4	5	7	8	6	25	$\dfrac{3}{4}=0.75$
Demand	10	20	17	26		
$\dfrac{Max - Min}{4}$	$\dfrac{4}{4}=1$	$\dfrac{1}{4}=0.25$	$\dfrac{4}{4}=1$	$\dfrac{4}{4}=1$		

Table: 4 Again, we find the maximum of the resultant values and then find the corresponding minimum cost value and allocate the particular cost cell of the given matrix. If we have more than one maximum resultant values, we can select anyone.

	Store1	Store2	Store3	Store4	Capacity	$\dfrac{Max - Min}{3}$
Factory1	7	6	5 15	9	15	$\dfrac{4}{3}=1.3$
Factory3	8	7	9	10	13	$\dfrac{3}{3}=1$

*Factory*3	4 ¹⁰	6	5	7	10	$\frac{2}{3} = 0.66$
*Factory*4	5	7	8	6	25	$\frac{2}{3} = 0.66$
Demand	-	20	17	26		
$\frac{Max - Min}{4}$	-	$\frac{1}{4} = 0.25$	$\frac{4}{4} = 1$	$\frac{4}{4} = 1$		

Table: 5 The same procedure will be repeated again and again until we reach the final allocation

	*Store*1	*Store*2	*Store*3	*Store*4	*Capacity*
*Factory*1	7	6	5 ¹⁵	9	15
*Factory*3	8	7 ¹³	9	10	13
*Factory*3	4 ¹⁰	6 ⁷	5 ²	7 ¹	20
*Factory*4	5	7	8	6 ²⁵	25
Demand	10	20	17	26	

Table: 6 Stage 2

	*Store*1	*Store*2	*Store*3	*Store*4	*Capacity*	$\frac{Max - Min}{4}$
*Factory*1	7	6	5	9	15	$\frac{4}{4} = 1$
*Factory*3	8	7	9	10	14	$\frac{3}{4} = 0.75$
*Factory*3	4 ¹⁰	6	5	7	20	$\frac{3}{4} = 0.75$
*Factory*4	5	7	8	6	25	$\frac{3}{4} = 0.75$
Demand	10	20	17	27		
$\frac{Max - Min}{4}$	$\frac{4}{4} = 1$	$\frac{1}{4} = 0.25$	$\frac{4}{4} = 1$	$\frac{4}{4} = 1$		

Table: 7 Repeat the procedure to get the final allocation.

	*Store*1	*Store*2	*Store*3	*Store*4	*Capacity*
*Factory*1	7	6	5 ¹⁵	9	15
*Factory*3	8	7 ¹⁴	9	10	14
*Factory*3	4 ¹⁰	6 ⁶	5 ²	7 ²	20

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*Factory*4	5	7	8	6	25	25
Demand	10	20	17	27		

Table: 8 The comparison of the proposed method with the existing procedure based on the methodology provided by Sathya Geetha S and Selvakumari K is tabulated below, in which it is clearly shown that the proposed method provides the optimal results.

Methods	Optimal solution
Zero suffix method	1,093
Northwest corner method	898
VAM method	886
LCM method	844
Proposed method	838

RESEARCH ARTICLE

A Study of English Writing Skills as Second Language of IX Standard Students of Rukminidevi Rungta Vidhyalay, Bharuch

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ABSTRACT

English has received the status of a Global Language as it is used as the First, Second, or Foreign language by most countries for intercommunication. To cater to the need of Lingua Franca, most of the Indian states have included it as a compulsory subject from primary education. In Gujarat, most of the technical courses and advanced studies are offered in the English language. However, it has been observed that many students face problems expressing their thoughts effectively while communicating in English. Inefficiency in English also leads professionals to lose career prospects. The present article studies the English Writing skills of the IX standard students of Rukmanidevi Rungta Vidhayalay, Bharuch, and proposes the suggestions to improve the English writing skills.

Keywords: Study, writing skills, second language, Intermediate level Learners

INTRODUCTION

The investigator is a teacher of Communication & Soft Skills at the undergraduate level. In academics, students' success mainly relies on their writing skills as a written examination is the primary tool of an assessment system. It was observed by the researcher that the students who learned English as a second language were struggling a lot in writing English. Upon interaction with these students, it was unveiled that there was a lack of exposure while they learned English during their primary education. Thus, the researcher got an objective to study the English writing skills of the intermediate level students studying in Gujarati medium. The statement framed for the research is: "A study of English Writing Skills as Second Language of IX Standard students of Rukminidevi Rungta Vidhyalay, Bharuch". The hypothesis framed was:

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1. There will be no significant difference between the close-ended and open-ended test items mean achievement score of the respondents to study Writing Skills.

Delimitation of the Study

The study will be delimited to only the IX standard students of Rukminidevi Rungta Vidhyalay, Bharuch studying in Gujarati Medium of Gandhinagar Secondary and Higher Secondary Education board, Gandhinagar.

REVIEW OF LITERATURE

To express well, one needs to have good writing skills. It is the predictor of academic success for students. This skill is much in demand at the workplace as one needs to draft emails and documents like reports, memorandum, proposals, applications, letters, notices, circulars, and minutes. To meet up the requirement for diversified written text, the need to produce quality writing has emerged. It needs a systematic way to learn. People usually write to express, pursue, or inform. A person may be very fluent in speaking but may not be able to write due to inadequate skills in writing. With mastery over writing skills, one can reach thousands of people at a time effectively. As writing is a productive skill, one must become very conscious about selecting language, words, structures, and patterns. Knowing the elements of proficient writing and observing how good writers write when their composition becomes successful is useful. M L Tickoo suggested points of a good writer as follows:

A Good Writer,
- thinks of the audience for their product.
- focuses on the primary purpose of writing.
- spends considerable time to plan.
- makes good use of reliable sources of knowledge.
- let his/her idea flow unhindered.
- follows a rough organization plan.
- seeks and makes use of feedback on the draft.
- gives much attention to choosing words.
- willingly (re)revises the writing to perfection.
- looks back at their product at long intervals.

Teaching Writing English as a Second Language. It is necessary to study the writing process and how it has been taught to the students. M L Tickoo, in his book 'Teaching & Learning English', has briefed the process as follow:
- The teacher sets a writing task.
- The Pupils write a composition and give it to a teacher.
- The teacher corrects its grammar and spelling, often using much red ink. (Alternative: The teacher writes a model composition for pupils to copy or asks them to do so based on one found in their grammar and composition book.)
- The pupils look at the teacher's corrections.
- They (may) hurriedly rewrite or make changes to satisfy the teacher.

Mechanics of writing

When a learner already knows one language and can efficiently write in that language, we do not need to worry about teaching writing as he may transfer the knowledge or sub-skills required for writing in a second language. This is especially useful when the script of the first and second language is the same or nearby. In this case, it will be very easy for a learner to master writing skills. However, when a learner wants to learn a second language, which has a very different script from the speech he acquires, one needs to start from the mechanics of writing skills. Handwriting & Spelling Handwriting & Spelling are the mechanics of writing. Each writer will have different

handwriting. Handwriting is marked by distinctiveness. Each letter has characteristics of its own. It will be helpful for learners if they learn to discriminate between shapes of alphabets.

Spelling

Spellings are an essential part of writing. The correct spelling comes through practice. Spellings can be learned through hearing the words repeatedly and seeing them in the printed form several times. Technique of learning spelling varies for learners. Some learn by listening while some by watching. Hence it is essential to provide the exposure needed by the learner. Remembering the words individually or writing them repeatedly (i.e. students are asked to write the spelling repeatedly for memorizing) may not help.

Punctuation

Punctuation is the one that may be taken very casually by the teachers and learners. However, it plays a very vital role in effective writing. When it comes to error-free writing correct mechanism of writing must be followed. Punctuation gives the correct meaning to the words. Lexical part only cannot communicate effectively. For syntax level, one has to rely on punctuations. Many times punctuation errors are overlooked during an assessment. Very few teachers consider it when it comes to examining writing skills.

Writing Process

The process of writing involves the following steps

Choosing a topic

The process of writing starts with choosing or getting a topic for writing. Before starting actual writing, one must have enough information about the subject. When liberty is given to the writer about selecting or choosing a topic, he needs to make a wise decision by choosing a topic on which he can write.

Brainstorming

Before starting writing, students can discuss the theme and sub-theme of writing with each other. They can discuss things in a group or pair. They can discuss ideas, knowledge about the assigned topic

Creating Outline

After brainstorming, the next stage is creating an outline of the main topic. Whatever has been discussed during the brainstorming activity, a writer can draft or write points for actual writing.

First Draft

A writer needs to put all the thoughts on paper related to the topic. He/she will try to have a bit of coherence while noting down the points in the draft.

Looping and clustering

The above technique can be included while writing the first draft. While writing, a writer can underline the main point. The second paragraph will be noted on the underline topics. The process goes on for the rest of the sections. This technique is called looping. It helps in getting coherence in writing. It also allows writers to move from general ideas to specific ideas. Clustering is related to getting the information about the main topic and gathering it point-wise.

Revising

Revision based on the feedback is very significant. The reader is the person who can tell a writer about where to improve. A writer may need to revise the article more than one time. However, it is for his/her benefit.

Publishing

This is the last step in the process of writing. After revising and finalizing, the draft will be ready to publish.

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Experiment

As the objective of the investigation is to study writing skills in English, the researcher adopted a survey method. The researcher designed questionnaire for survey which carried open and close-ended types of questions. The first questionnaire was drafted for students containing close-ended questions focusing on English Grammar and open-ended questions focusing on writing skills.

Population

The survey was conducted on 83 students of IX Standard studying in the school Rukminidevi Rungta Vidhyalaya, Bharuch.

Background of the school Rukminidevi Rungta Vidhyalaya, Bharuch

The school is located in the city area of Bharuch district, opposite to the railway station and the bus station. Hence, it attracts many students of the village area and city area of Bharuch district. It is a gran-in-aid school established before more than thirty-five years.

Procedure for designing Tool

All the exercises in the questionnaire were drafted by considering the IX standard syllabus of English subject offered in Gujarati medium schools affiliated with Gujarat Secondary and Higher Secondary Education Board, Gandhinagar and analyzing VII and VIII standards textbooks to expect the competency level.

Validity and reliability

The set of exercises was given to an expert and peers for review and validation. As per the comments provided by the experts, it was redrafted, which included the descriptive type of exercises as well. To check the reliability of the questionnaire, the researcher conducted a pilot survey on a group of 25 students studying in IX standard of Gujarat Secondary & Higher Secondary Education Board, Gandhinagar.

Data Analysis

Data collected from English Writing Test were analyzed statistically. Present research consisted of hypothesis, based on which analysis was carried out. Outcomes derived from analysis of hypotheses lead to the results of the research. Hypothesis of the present research is mentioned below: There will be no significant difference between the close-ended and open-ended test items mean achievement score of the respondents to study Writing Skills. The hypothesis is based on the close-ended and open-ended test items. There are 83 students on whom English Writing Test was implemented. The test was of 50 marks, from which test items of 25 marks were close-ended, and 25 marks were open-ended items. Close-ended questions are the type of questions that have specific answers. Examples of this type of question are: Fill in the blanks based on parts of speech or tenses, and grammar-related exercises. Open-ended questions are the questions that give liberty to the writer to express their though. This type of question does not have a specific answer, for example, Essay writing and Paragraph writing. Collected data from these two categories are analyzed to test this hypothesis. This analysis helped to know students' scores in a particular category.

Score Range of Close-ended and Open-ended questions

The above table is a range of scores received from the English writing Test. There are 83 students who appeared for the test. Analysis of the collected data indicated that there are 18 students who scored 0-5 marks for close-ended items and 67 students who scored 0-5 marks for open-ended test items. There are 52 and 11 students who scored 6-10 marks for close-ended and open-ended test items, respectively. There are 13 and 5 students who scored 11-15 marks for close-ended and open-ended test items respectively. But no student scored 16-25 marks for both closed-ended and open-ended test items.

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Findings
Data analysis led to major findings of the study. They are as follow:

The Students' competency in writing skills of English as a second language
The analyses of the open-ended and close-ended category shows that the Students perform better in close-ended questions in comparison to open-ended questions. The analysis says that the majority students struggle to write descriptive English. To overcome the same, following steps can be taken
- The textbook is based on the Communicative Language Teaching approach. The teachers shall be trained to teach through this approach.
- Communicative Language Teaching is a family of many approaches. Teachers shall be clear with the methodology of teaching.
- The school format does not allow to teach writing through this approach due to the limitation of time and resources.
- Teachers should encourage and motivate students to learn English writing skills by bringing awareness to the students about the need and importance of writing skills.
- Teachers shall understand the importance of rubrics. While assessing the writing, rubrics shall be set.

CONCLUSION

In the current era, English writing skills hold the status of the most needed skill. It is a key to entering the professional world of tomorrow. It has an important place in the school curriculum. The recommendations given in the study shall be useful to improve writing skills of the students.

ACKNOWLEDGMENT

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Table 1: Set of exercises

Ex. No.	Exercise	Reason
1	Choose the correct form of the verb from brackets.	The exercise was kept to check the knowledge of tense.
2	Fill in the blanks with the most appropriate word given in the brackets.	To check the knowledge of parts of speech.
3	Write an antonym for the below words.	To check the vocabulary level of the target group.
4	Frame questions using the words given in the brackets.	To check the knowledge of sentence structure.
5	Arrange the words in the correct sequence to draft meaningful sentences.	To check whether the students know of semantics or not.
6	Select the proper word from the brackets and use it in the appropriate place.	This exercise was taken from the textbook of standard IX. The purpose behind selecting this exercise was to know whether they are aware of the exercises given in the textbook or not.
7	Find out & underline spelling/grammar mistakes from the given sentences.	To know whether the students can identify incorrect writing or not.
8	Write an Essay on any one of the mentioned topics in 150 words.	To study free/creative writing.
9	Translate the paragraph in English.	To check understanding of both languages.
10	Describe the below picture in 150 words.	To study writing skills critically.

Table 2: Score Range of Close-ended and Open-ended questions

	Close-ended	Open-ended
0-5	18	67
6-10	52	11
11-15	13	5
16-20	00	00
21-25	00	00
Total	83	83
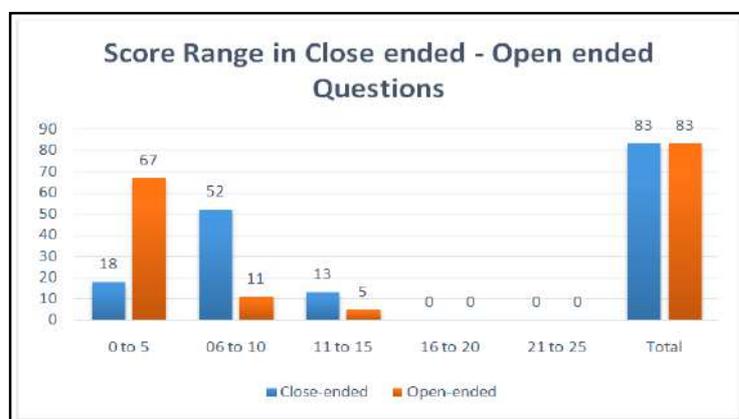
Graph 1. Score Range of Close-ended and Open-ended questions

RESEARCH ARTICLE

Impact of Sugar Mill Effluent on Germination and Biochemical Constituents of Blackgram (*Vigna mungo* L.) Variety CO Gg 912

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ABSTRACT

The present study was made to the effect of different concentrations of biologically treated sugar mill effluent on germination and biochemical constituents of *Vigna mungo* Co Gg 912. The physico-chemical analysis revealed that the sugar factory effluent was acidic in nature. The germination studies were conducted with various concentrations of biologically treated effluent (0, 10, 25, 50, 75 and 100%) and the data were assessed 15th day after germination. The percentage shoot length, root length, fresh and dry weight, leaf area increased up to optimum level of 10% and thereafter all the parameters drastically reduced. The photosynthetic pigment, carotenoid, protein and starch content increased up to 10% level and at higher concentrations reduced gradually. The amino acid and sugar content decreased up to 10% level and there after gradually increased. The accumulation of proline and glycinebetaine content increased with increasing concentration at extreme level of 100% sugar effluent.

Keywords: Sugar mill effluent, Germination, Glycinebetaine, Vigour index, Blackgram, Phytotoxicity.

INTRODUCTION

The sugar industry is a very important agro-based industry in India and discharged large amount of effluent into water bodies and cultivated land to create high pollution, which affects the plants and other living organisms (Vaithiyanathan *et al.,* 2017). Sugar production processing requires huge water for a number of steps and released almost equal quantity of effluent, which contain toxic materials. These recent studies have indicated that the effluent discharges from sugar consist a number of organic and heavy metal pollutant is dissolved or suspended from that can bring about changes in the physical chemical and physiological sphere of the biota. The effluent that is

generated from the sugar industry, if used directly for irrigation than it will disturb the soil fertility as well as affect the plant growth. The sugar mill effluent reduces the soil fertility use of industrial effluent and sewage sludge on agricultural land has become a common practice in India as a result of which these toxic metals can be transferred and concentrated into plant tissues from the soil. These metals have damaging effects on plants themselves and may become a health hazard to man an animal (Yadav *et al*, 2021;Ahamed *et al.,* 2018). The physico-chemical analysis of sugar mill revealed that it was blackish in colour and acidic in nature. The effluent containing large number of suspended solids, dissolved solids and total solids, which are responsible for high BOD and COD. Into that BOD and COD values and formed to be lower than the critical levels with neutral pH (Sangeetha *et al.,* 2018; Saifi and Singh, 2011, Selvakumar *et al.,* 2014) clearly indicated that sugar mill effluent extremely polluted the groundwater and made them unsuitable for human constitution. The inorganic elements such as chlorides, sulphates, ion, nitrate, ammonia and calcium were present 100 times more than the tolerant limits when compared to stranded specific for drainage water. Lower concentration of sugar mill effluent promoted the early seedling growth of *Vigna mungo* (Sivasakthi *et al.,* 2017), *Vigna radiata* (Baskaran *et al.,* 2009) and tannery effluent in *Solanum lycopersicum* (Venkataramani and Venkatesan, 2022). The lower concentration of sugar mill effluent increased the protein, chlorophyll, carotenoid and starch content of *Vigna mungo* (Elayaraj, 2014), *Vigna radiata* (Baskaran *et al.,* 2009) and tannery effluent in *Solanum lycopersicum* (Venkataramani and Venkatesan, 2022). The sugar mill effluent promoted the proline and glycinebetaine accumulation increased upto extreme level (Shafagat Ali *et al.,* 2020), *Oryza sativa* (Dubey *et al.,* 2018) *Peganum harmala* (Mahadavan *et al.,* 2016). The aim of this study to analysis the impact of sugar mill effluent impact on germination and biochemical studies to assessed.

MATERIALS AND METHODS

Effluent collections

The effluent samples were collected in plastic containers from the outlet of the East India Distilleries (EID Parry) sugar mill in Nellikuppam, Cuddalore taluk, Cuddalore district, Tamil Nadu, India. This sugar industry lies in latitude (13′05′.19.7′N) longitude (80′17′25.37′E). The effluent was brought to the Phytochemical Laboratory, Department of Botany, and stored in refrigerator at 4°C for further use.

Seed collection

Vigna mungo L. seeds were procured from Tamil Nadu Seed Research Institute, Aduthurai, Thanjavur district, Tamil Nadu.

Analysis of sugar mill effluent

The collected sugar mill effluent sample were analysed for their various physico-chemical properties in Phytochemical Laboratory, Department of Botany, Annamalai University as per the routine standard methods mentioned in APHA (2005).

Preparation of different concentration of effluent

The collected effluent sample from the outlet of sugar mill industry was treated as 100 per cent raw effluent. Different concentrations (0, 10, 25, 50, 75 and 100%) of sugar mill effluent were prepared freshly using distilled water whenever necessary. They were used for germination studies.

T_0 : Distilled water
T_1 : 10 ml effluent + 90 ml distilled water
T_2 : 25 ml effluent + 75 ml distilled water
T_3 : 50 ml effluent + 50 ml distilled water
T_4 : 75 ml effluent + 25 ml distilled water
T_5 : Raw effluent

Germination studies

The healthy and uniform sized black gram seeds were selected and surface sterilized with 0.1% $HgCl_2$ for 2 min and then thoroughly washed with tap water. Fifty seeds were placed equidistantly in plastic cups filled with 100 g sterilized soil [clay + sand (1:1)]. The seeds were irrigated with equal quantity of different concentrations of effluent and the seeds irrigated with distilled water were treated as control. Three replicates were maintained for each treatment including control. The germination percentage, shoot length, root length, seeding fresh weight and dry weight were taken and recorded on the 15th day seedlings. The values of seed vigour index, tolerance index and percentage of phytotoxicity were also calculated.

Germination percentage

The number of seeds germinated in each concentration was counted on the 15th day and the germination percentage was calculated using the following formula.

$$\text{Germination percentage} = \frac{\text{Number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

Shoot and root length (cm plant^{-1})

Five seedlings were taken from each treatment and their shoot length and root length were measured by using a scale and these values were expressed in cm plant^{-1}.

Fresh weight (g plant^{-1})

Five seedlings were collected from each treatment and their fresh weights were measured with the help of an electrical single pan balance.

Dry weight (g plant^{-1})

The same seedlings used for fresh weight were kept in hot air oven at 80°C for 24 h. Then, the seedlings were taken from the oven and kept in desiccators for some time. Their dry weights were taken using an electrical single pan balance.

Vigour index

Vigour Index of the seedlings was calculated using the formula proposed by Abdul-Baki and Anderson (1972).
Vigour index = Germination percentage × Length of seedlings

Tolerance index

Tolerance index of the seedling was calculated using formula proposed by Turner and Marshal (1972).

$$\text{Tolerance Index} = \frac{\text{Mean length of longest root in treatment}}{\text{Mean length of longest root in control}}$$

Percentage of phytotoxicity

The percentage of phytotoxicity of effluent was calculated using the formula proposed by Chou *et al.* (1978).

$$\text{Percentage of phytotoxicity} \; \frac{\text{Radicle length of control} - \text{Radicle length of test}}{\text{Radicle length of control}} \times 100$$

Chlorophyll (Arnon, 1949)

Five hundred mg of fresh leaf was ground in a mortar and pestle with 10 ml of 80 per cent acetone. The homogenate was centrifuged at 800 g for 15 minutes. The supernatant was saved. The pellets were extracted with

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10 ml of 80 percent acetone. The supernatant was saved and utilized for chlorophyll estimation. Absorbance was read at 645 and 663 nm in spectronic-20.

Chlorophyll 'a' (mg/1)   = $(0.0127) \times (O.D.663) - (0.00269) \times (O.D.645)$
Chlorophyll 'b' (mg/1)   = $(0.0229) \times (O.D.645) - (0.00468) \times (O.D.663)$
Total chlorophyll (mg/1) = $(0.0202) \times (O.D.645) + (0.00802) \times (O.D.663)$

Carotenoid
Carotenoid content was calculated by using the formula of Kirk and Allen (1965) and expressed in mg g^{-1} fresh weight. Carotenoid = $A. 480 + (0.114 \times A.663 - 0.638 \times A.645)$

Estimation of amino acid (Moore and Stein, 1948)
Extraction
Five hundred mg of plant materials were weighed and macerated with a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged for 10 min at 800 rpm. The supernatant was saved. The extract was used for the estimation of amino acid.

Estimation
One ml of the extract was pipette out into attest tube. A drop of methyl red indicator was added. The sample was neutralized with 1 ml of 0.1 N sodium hydroxide. To this, 1 ml of ninhydrin reagent was added and mixed thoroughly. The content of the test tube was heated for 20 min. in a boiling water bath. Five ml of the diluents solution was added and heated in water bath for 10 min. The tubes were cooled under the running water and the contents were mixed thoroughly. Blank was prepared without extract. The absorbance was read at 570 nm in a UV–spectrophotometer. The amino acid contents are expressed in mg g^{-1} fresh weight.

Estimation of protein (Lowry *et al.,* 1951)
Extraction
Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 20 per cent trichloro acetic acid. The homogenate was centrifuged for 15 min at 600 rpm. The supernatant was discarded. To the pellet, 5 ml, of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made upto 10 ml with 0.1 N NaOH. This extract was used for the estimation of protein.

Estimation
One ml, of the extract was taken in a 10 ml, test tube and 5 ml of 'reagent C' (Reagent A; 0.4 g of sodium hydroxide was dissolved in 100 ml of distilled water. To this solution, 2 g of sodium carbonate was added; Reagent B; One per cent of copper sulphate was mixed with equal volume of 2 per cent sodium potassium tartar ate; Reagent C; Fifty ml of reagent A and 1 ml of reagent B were taken and mixed and it was prepared freshly at the time of experiment) was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 ml of Folin-phenol reagent (One ml of folin-phenol reagent was diluted with 2 ml of distilled water) was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in the UV-spectrophotometer. The protein contents were expressed in mg g^{-1} fresh weight.

Estimation of sugars (Nelson, 1944)
Extraction
Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 80% ethanol. The homogenate was centrifuged for 10 min at 800 rpm. The supernatant was saved. Then, the ethanol is evaporated in a water bath at 50°C. The net content was made up to 20 ml with distilled water and the extract was used for the estimation of reducing sugar.

Estimation

One ml of extract was taken in a marked test tube. 1 ml of reagent 'C' (Reagent A: Twenty-five grams of anhydrous sodium carbonate, 25 g of sodium potassium tartar ate, 20 g of sodium bicarbonate and 200 g of anhydrous sodium sulphate were dissolved in 800 ml of distilled water and made up to 1000 ml. Then it was   filtered and stored in a glass stopper brown bottle; Reagent B: Fifteen per cent copper sulphate containing 1 or 2 drops of concentrated sulphuric acid. Reagent C: Fifty ml of reagent A and 1 ml of reagent B were mixed well and it was prepared freshly at the time of experiment) was added. Then, the mixture was heated foe 20 min at 100°C in a boiling water bath, cooled and 1 ml arsenomolybdate reagent (To 450 ml of distilled water, 25 g of ammonium molybdate, 21 ml of concentrated sulphuric acid were added and 3 g of sodium arsenate was dissolved in 25 ml of distilled water. The mixture was kept in a water bath at 37°C for 24 to 48 hrs the reagent was stored in a glass stopper brown bottle) was added. The solution was thoroughly mixed and diluted to 25 ml of distilled water. The sample was read in a UV-spectrophotometer at 520 nm. The sugar contents were expressed in mg g^{-1} fresh weight.

Preparation of reagents
Reagent A

Twenty-five g of anhydrous sodium carbonate, 25 g of Rochelle's salt (sodium potassium tartarate), 20 g of sodium bicarbonate and 200 g of anhydrous sodium sulphate were dissolved in 800 ml of distilled water and made up to 1000 ml. Then, it was filtered and stored in a glass stopper brown bottle.

Reagent B

Fifteen per cent copper sulphate containing one or two drops of concentrated sulphuric acid.

Reagent C

Fifty ml of reagent 'A' and 1 ml of reagent 'B' were mixed and it was prepared fresh at the time of experiment.

Arsenomolybdate reagent

To 450 ml of distilled water, 25 g of ammonium molybdate, 21 ml of concentrated sulphuric acid were added and 3 g of sodium arsenate was dissolved in 25 ml of distilled water. The mixture was kept in a water bath at 37°C for 24 hours

Estimation of starch

Five hundred mg of plant material was weighed and macerated in a pestle and mortar with 10 ml of 80% ethanol. The sample was centrifuged at 6000 rpm for 15 min the supernatant was removed and the pellets were extracted with 52% perchloric acid for 30 min at 0°C. The extract was centrifuged and supernatant was diluted upto 15 times. One ml of diluted sample was mixed with 2 ml of cold anthrone reagent in ice bath and it was boiled for 10 min at 100°C in a water bath. The content was cooled and the absorbance was read at 630 nm in a UV-spectrophotometer. The starch content was calculated by multiplying with 0.9 to the values obtained from the standard curve. The starch content was expressed in mg g^{-1} fresh weight basis.

Estimation of proline

Proline was extracted and estimated following the method of Bates *et al.* (1973).

Extraction

Five hundred milligrams of fresh plant material was homogenized in a mortar and pestle with 10 ml of 3% aqueous sulfosalicyclic acid. Then the homogenate was filtered through Whatmann No.1 filter paper. The residue was re-extracted and pooled and the filtrates were made upto 20 ml with aqueous sulfosalicylic acid and this extract was used for the estimation of proline.

Estimation

To 2 ml of proline extract, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added. The mixture was incubated for an hour at 100°C in a boiling water bath. Then the test tubes were transferred to an ice bath to terminate the reaction. Then 4 ml of toluene was added and mixed vigorously using a test tube stirrer for 20 seconds and the toluene containing the chromophore was separated from the aqueous phase with the help of a separating funnel and the absorbance was measured at 520 nm in a Spectrophotometer using a reagent blank. The proline content was determined from a standard curve with proline and the results were expressed in mg g^{-1} fresh weight.

Reagents

Acid-ninhydrin reagent

To 1.25 g of ninhydrin, 30 ml glacial acetic acid, 20 ml of 6 M phosphoric acid were added with agitation.

Estimation of glycinebetaine

The samples were extracted and estimated following the method of Grieve and Grattan (1983).

Extraction

Five hundred milligrams of finely ground dry plant sample was mechanically shaken with 20 ml of distilled water for 24 hours at 25°C. Time required for this step was determined by extracting the plant samples for 4, 8, 16, 24 and 48 h. The samples were then filtered through Whatmann No.1 filter paper and the filtrates were made up to 20 ml with deionized water and used for estimation immediately.

Estimation

One millilitre of the extract was diluted with 1 ml of 2 N H_2SO_4 and 0.5 ml of this acidified extract was cooled in ice water for 1 hour. Later 0.2 ml of cold potassium triiodide solution was added and mixed gently with a Vortex mixture and the tubes were stored at 4°C for 15 min and then centrifuged at 10,000 rpm for 15 min. The supernatant was aspirated with a fine tipped glass tube. The iodide crystals were dissolved in 9.0 ml of 1,2-dichloroethane with vigorous vortexing. After 2.5 hours the absorbance was measured at 365 nm in a spectrophotometer. Reference standard of glycinebetaine was prepared in 1 N H_2SO_4 and used for estimating the glycinebetaine content and the results were expressed in µg g^{-1} dry weight.

Preparation of reagent

Potassium triiodide reagent

15.7 g of iodine and 20 g of potassium iodide were dissolved in 100 ml of distilled water and gently stirred in a vortex mixture.

RESULTS

The results of the physico-chemical characterization of the sugar mill effluent is presented in Table 1. The effluent showed that it was blackish in colour acidic (pH 5.1) in nature containing high amount of total dissolved solids (1258). The electrical conductivity of the effluent was 1193 (Mm hos/cm) higher biological oxygen demand (3480 mg/l) and chemical oxygen demand (7880 mg/l). In addition to that, a amount of calcium (27.99 mg/l), magnesium (1.74 mg/l), sodium (0.426 ppm), sulphate (62 mg/l), lead (0.147 mg/l), iron (10.17 mg/l), zinc (0.26 mg/l), copper (0.08 mg/l) and potassium (4.255 mg/l) were also present in Table 1. The acidity, excessive hardeners total suspended solids, BOD, COD and high amount of salt of the sugar effluent reveal that effluent in highly toxic or polluted. These results agree with the previews results.

Germination and growth parameters

The highest seed germination percentage, plumule and radical length, fresh weight and dry weight, leaf area were reported in 10% of sugar mill effluent followed by control. The percentage of phytotoxicity values were negative in 10% concentration of sugar mill effluent treatment. At the same time, the germination percentage, plumule length,

radicle length was decreased gradually with increasing effluent concentration (Tables 2 and 3). The fresh weight and dry weight of blackgram in various concentrations of effluent are presented in Table 3. The result on the effect of sugar mill effluent to increased in fresh weight and dry weight (15.38; 35%) with increasing concentration of 10% effluent (12.32; 12.5%) and therefore the fresh and dry weight reduced (Table 3). The data on the effect of sugar mill effluent to increase the percentage of chlorophyll synthesis (30.8, 1.94 and 20.09) and starch content shoot and root (41.66; 46.42%) and protein (8.88; 19.33%) was higher at 10% level and thereafter both gradually increased. When compared to control (Figs 1-3). The percentage decreased the amino acid content of shoot and root (-19.38%; 22.22%) and sugar content of shoot and root (16.36; 44.82%) upto 10% effluent treatment and at higher concentration the both content increased gradually (Figs. 4 and 5). When compared to control. The percentage increased the accumulation of proline content shoot and root (62.42%; 36.57%) and glycinebetaine content of shoot and root (58.65% and 93.20) increased with increasing concentration upto 100% (Figs. 6 and 7) when compared to control.

DISCUSSION

It also contained more quantities of chloride, sulphate, silica, sodium, potassium, calcium *etc.* The similar findings were also reported in sugar mill effluent reported by Gupta *et al.* (2010), Sanjanisamual and Muthukkarupan *et al.* (2011), Sangeeta *et al.* (2018). The use of industrial wastewater for irrigation is a recent phenomenon, which has received attention of scientific community. It could be safely used for irrigation in dry areas with suitable dilution (Stomberg *et al.,* 1984, Somasekat *et al.,* 1984; Sahai and SrivastavaS, 1986; Raza and Vijayakumar, 1989; Manomani, 1992). Seed as well as the seedlings are extremely vulnerable to industrial effluents stress and the presence of polluting agents in the environment causes the deleterious effects on the germinating seeds and subsequently seedling growth. *Oryza sativa* (Sanjani and Muththukkarupan *et al.,* 2011) *Pistia stratiotes* (Vinod Kumar *et al.,* 2018) due to sugar mill effluent. Increase in germination percentage at lower concentration of the effluent undoubtedly indicates the stimulating effect of the diluted effluent to the physiologically active seeds by diluted effluent (Biradar *et al.,* 1989). It might be due to the fertilizing increased fertility of the soil in the presence of lower concentrations of the effluent. The high concentrations of the sugar mill effluent reduced the growth of seedlings. It may be due to the excess amount of minerals present in the effluent (Sivashakthi *et al.,* 2017). These observations are in conformity with previous reports made with sugar mill effluent on *Triticcum aestium* (Saurabh Saini and Shailja Pant, 2014; Zafar Iqbal khan *et al.,* 2018). Reduction in leaf area will lead to ultimate reduction in photosynthetic activity causing loss in food production and ultimately loss in yield. The reduction in effective tillers will certainly lead to reduction in total yield (Arindam, 1996; Arindam and Prasad, 1997). The increase in chlorophyll content at low concentration of industrial effluent may be due to the favorable effect of nitrogen and other elements such as calcium, sodium and chloride on the pigment system. The increase in starch content at low concentration could be attributed to the increased number of leaves, total leaf area and chlorophyll content of the plant.

The decrease in amino acid content at higher concentration could by attributed to the inhibitory effect of the effluent on protease activity. The increase in protein content of the plant treated with low concentration of the effluent could be due to rapid uptake of the nitrogen by plant (Srivastava *et al.,* 1988). There was increase in protein content at 10 per cent concentration content might be due to the increase in the concentration of various captions and anions present in the effluent and reduced rate of mineral absorption (Behera *et al.,* 1980). Similar trends were recorded in groundnut and paddy treated with sugar mill effluent *Arachis hypogaea* treated with fertilizer factory effluent and ragi treated with sugar mill effluent (Lakshmi and Sundaramoorthy, 2001). The increase in the amount of soluble and reducing sugars might be either due to inhibition in starch synthesis from hexose or stimulation of starch hydrolysis in (Agarwal and Agarwal, 1990). The increased amount of sugar content in low concentrations of effluent over control might be due to increase in chlorophyll content and increased rate of photosynthesis. It might also be attributed to greater absorption and assimilation of potassium and nitrogen, which play a vital role in carbohydrate synthesis (Tomar *et al.,* 1998). Proline increased in stem tolerance in plants through such mechanisms like osmo-regulation, protection of enzymes against denaturation, and stabilization of protein synthesis (Chun *et al.,* 2018). It has often been suggested that proline accumulation may constitute to osmotic adjustment at the cellular level and enzyme protection by stabilizing the

structure of macro molecules and organells. Increase in proline content may be either due to de novo synthesis or decreased degradation or at both (Ashraf and Foolad, 2007). Glycinebetaine greatly induced cold stress tolerance in plants through effective improvement in overall leaf water, sucrose and chlorophyll content, meanwhile, it also reduced the ROS and contents of abscisic acid as well (Cheng *et al.,* 2018). Glycinebetaine enhanced the growth the similar trends were conformity to other researchers *Triticum aestium* (Ahmed *et al.,* 2019) sugar beat (Kito et al, 2017), *Lens culinaris* (Molla *et al.,* 2014).

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Table 1. Physico - chemical properties of East India Distilleries (EID Parry) sugar mill effluent

S. No.	Parameters	Effluent
1	Colour	Blackish in colour
2	Odour	Decaying molasses small
3	pH	5.1
4	Electrical conductivity	1193 Mm hos/cm
5	Temperature	27′C
6	Total dissolved solids	1258
7	Biological oxygen demand (BOD)	3480 mg/l
8	Chemical Oxygen Demand (COD)	7880 mg/l
9	Calcium	27.99 mg/l
10	Magnesium	1.74 mg/l
11	Iron	10.17 mg/l
12	Lead	0.147 mg/l
13	Zinc	0.26 mg/l
14	Copper	0.08 mg/l
15	Potassium	4.255 mg/l
16	Sulphate	62 mg/l
17	Sodium	0.426 ppm

Table 2. Effect of biologically treated sugar mill effluent on seed germination percentage of *Vigna mungo* (Co Gg 912)

Treatment	No. of seeds sown	No. of seeds germinated	Germination percentage	Vigour index	Percentage of phytotoxicity	Tolerance index
T_0	20	19	95	1634	0.00	0.00
T_1	20	17	85	1768	-22.22	1.142
T_2	20	15	75	1230	+6.94	1.071
T_3	20	12	60	780	+29.16	1.000
T_4	20	9	45	564	+40.27	0.920
T_5	20	8	40	360	+52.77	0.780

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Table 3. Effect of biologically treated sugar mill effluent on growth parameters of *Vigna mungo* (Co Gg 912)

Treatment	Plant height (cm plant^{-1})		Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})		Leaf area (cm^2 plant^{-1})
	Shoot	Root	Shoot	Root	Shoot	Root	
T_0	14.4±0.289	2.8±0.173	0.73±0.017	0.16±0.012	0.13±0.017	0.041±0.005	1.469±0.017
T_1	17.6±0.346	3.2±0.115	0.82±0.012	0.18±0.017	0.15±0.013	0.054±0.007	2.383±0.015
T_2	13.4±0.577	3.0±0.231	0.66±0.023	0.15±0.014	0.10±0.017	0.044±0.005	2.262±0.013
T_3	10.2±0.231	2.8±0.115	0.58±0.017	0.14±0.013	0.07±0.013	0.036±0.003	1.581±0.010
T_4	8.6±0.346	2.6±0.173	0.52±0.012	0.13±0.010	0.04±0.011	0.020±0.002	1.544±0.013
T_5	6.8±0.346	2.2±0.058	0.40±0.012	0.11±0.012	0.02±0.010	0.009±0.001	0.894±0.028
C.D	1.159	0.482	0.05	0.041	0.034	0.014	0.059
C.V	5.421	9.676	4.478	15.759	22.066	23.764	1.935

Fig. 1: Effect of biologically treated sugar mill effluent on photosynthetic pigments (mg g^{-1} fr. wt.) of *Vigna mungo* (Co Gg 912)

Chl 'a' (C.D.=0.032**; C.V.=6.762*); Chl 'b' (C.D.=0.033**; C.V.=17.336*); Total chlorophyll (C.D.=0.039**; C.V.=5.822*) and Carotenoid (C.D.=0.039**; C.V.=14.537*) - *Significant at 1% level ** Not Significant

Fig. 2. Effect of biologically treated sugar mill effluent on starch content (mg g^{-1} fr. wt.) of *Vigna mungo* (Co Gg 912)

Shoot (C.D.=0.014**; C.V.=8.903*) Root (C.D.=0.012**; C.V.=24.975*) - *Significant at 1% level ** Not Significant

Fig. 3. Effect of biologically treated sugar mill effluent on protein content (mg g^{-1} fr. wt.) of *Vigna mungo* (Co Gg 912)

Shoot (C.D.=0.049**; C.V.=0.867**) Root (C.D.=0.052**;C.V.=4.443*) - *Significant at 1% level ** Not Significant

Fig. 4. Effect of biologically treated sugar mill effluent on amino acid content (mg g^{-1} fr. wt.) of *Vigna mungo* (Co Gg 912)

Shoot (C.D.= 0.044**; C.V.=0.761**) Root (C.D.=0.047**; C.V.= 4.604*) - *Significant at 1% level ** Not Significant

Fig. 5: Effect of biologically treated sugar mill effluent on reducing sugar content (mg g⁻¹ fr. wt.) of *Vigna mungo* (Co Gg 912)

Fig. 6. Effect of biologically treated sugar mill effluent on proline content (mg g⁻¹ fr. wt.) of *Vigna mungo* (Co Gg 912)

Fig. 7. Effect of biologically treated sugar mill effluent on glycinebetaine content (mg g⁻¹ fr. wt.) of *Vigna mungo* (Co Gg 912)

A Conceptual Frame Work with Regard to Corporate Governance in Top Ten Miniratna Status of Public Sector Enterprises in India

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ABSTRACT

Any organization's survival and growth is dependent on its corporate governance. It promotes and safeguards the interests of a corporate shareholders and stakeholders. Corporate Governance is not anything more than a set of ideas, novelty, creativeness, thinking having firm ethics, values, principles etc., which gives direction and outline to its people, employees and owners of companies. In Indian economy Public Sector Enterprises have to playing important role while independence. It creates basic sources like networks, employment generations, industrial purpose vehicles and other related infrastructural facilities. The current study is based as secondary sources of information, and the objective for this study has to examine the Conceptual Frame Work with Regard to Public Sector Enterprises in India. The research has to identify that the implementing corporate governance can only assist Central Public Sector Enterprises (CPSE) improve their performance in their particular industries. The right decision-making in the government agencies also paves the foundation for the sound Corporate Governance. While complying by the legislation, trading with simple majority shareholders, performance evaluates day-to-day activities of the organization.

Keywords: corporate shareholders, economy Public Sector Enterprises, sound Corporate Governance.

INTRODUCTION

The corporate governance refers to the set of strategy that influences manager decisions. Political, social, emotional, and evolutionary forces have all fueled the worldwide corporate governance discussion recently. Any organization's survival and growth is dependent on its corporate governance. It promotes and safeguards the interests of a

corporate shareholders and stakeholders. It is widely believed that excellent governance lowers an organization's capitals, which have been impact for its execution. Shareholder assurance is strengthened the effect of corporate governance rules.

OBJECTIVES OF THE STUDY

- Identify the Pinpoint of corporate governance enactment in India
- Identify the review of literature for the selected public sector enterprises
- Give profile of the selected public sector enterprises that govern corporate governance practices

SIGNIFICANCE OF THE STUDY

In various forms, corporate governance has existed since Vedic times. It has to take part in a significant task in the economics progress. Importance for the corporate governance cannot be contested from an innovative point of view. Good corporate governance has been the integral aspect of the most important firms in order to raise the company's long-term value. The most crucial elements of the corporate governance are integrity and ethics. The focus of this research is on the top ten companies under the Miniratna Status of Category – I Companies in the Central Public Sector Enterprises in India.

STATEMENT OF THE PROBLEM

Governance has become an inextricable aspect of today's business. In order to complete the corporate governance procedures, the degree of corporate governance framework associated to the Miniratna Status of Category – I Companies under the Central Public Sector Enterprises must be identified. Financial performance is a company's source of revenue and growth. The key issue addressed in this paper is the implementation of corporate governance mechanisms.

CORPORATE GOVERNANCE IN INDIA

Corporate Governance is not anything more than a set of ideas, novelty, creativeness, thinking having firm ethics, values, principles etc., which gives direction and outline to its people, employees and owners of companies and help them to flourish in global market Indian Corporate Bodies having adopted good corporate governance will reach themselves to a benchmark for rest of the world; it brings laurels as a way of appreciation. Corporate governance made of principles, values, and the management policies of a corporation which are inculcated and brought into practice. The significance of corporate governance lies in promoting and maintains reliability, clearness and accountability throughout the organization. (1)

CONCEPTUAL FRAME WORK

In Indian economy Public Sector Enterprises have to playing important role while independence. It creates basic sources like networks, employment generations, industrial purpose vehicles and other related infrastructural facilities and so on. Companies Act, 2013 and guidelines by SEBI has to provide the corporate governance framework to the PSEs in India. In the Ministry of Corporate Affairs (MCA) has to notify that the Companies Rules, 2014 for the management and administration, qualification and appointment of directors, board meetings and board powers and board accounts. The corporate governance framework rules were provided under Companies Act, 2013 for PSUs and all other companies. [3]

PRINCIPLES OF CORPORATE GOVERNANCE

Corporate governance has to extent certain key principles, which form the certain rules and policies for the corporate.

Transparency expose of the related information regarding corporate in timely and perfect manner is necessary. It helps stakeholder to identify their rights and day to day movement of the corporate.

Accountability makes positive for the liability of the person who takes decision for the interest of the others. Therefore persons like managers, chairmen, directors and other officers are supposed to be answerable to other stakeholders of the corporate.

Independence is essential for the top manager in glossy performance of the corporate. Board of Director should work without the interfering of any interested party in the corporate. [2]

REVIEW OF LITERATURE

Qazi (2017) [4] in his research paper identified that, SEBI has been recognized to have had a role in transparent and effective corporate governance. Several committees have been formed to strengthen the Markets transparency and integrity. He discovered that India has some of the best regulations in the world, but the issue is how corporations would monitor and follow these new laws in order to strengthen corporate governance. Karn Marwaha, (2017)[5] the goal of this research was to examine the legal laws pertaining to whistle blower protection for private employees. The researcher used primary and secondary data and read the Whistleblower Protection Act of 2011, the Official Secrets Act of 1923, and the Right to Information Act of 2005, among other things. He discovered that this Act simply offers a system for receiving the complaints and investigating claims of the public servant corruption or abuse of power. Qadorah (2018)[6], studied the effects of corporate governance procedures such as the frequency of meetings held and the independence of the directors on business performance in Jordan's increasing economy were explored. The data exposed in positive association connecting the board independence with the company performance, although there is no indication for the considerable relationship involving the board meeting regularity along with the firm performance.

Kumar (2020)[7], in his research impacted that the family businesses backed by affluent entrepreneurs were the main source of activity in the beginning industrialized India. Eventually, it has morphed towards the problem including security of both large and small type of members, creditors, customers, other outsiders as well as agency difficulties. Rajiah – Benett (2020)[8], their study focused the code outlined expected corporate practices in the expression of accounting openness with the declaration with the accordance accepted in the worldwide standards, as well as the importance of audit committees in guaranteeing CG compliance. Almaqtari et al (2020)[9], their research paper focused with the primary focus of significant corporate organizations in India being family-owned, it's important to examine the board's makeup and structure carefully. The question regarding the success of corporate governance is observing and the tremendous capability to acquire in the principal shareholders. Ararat et al (2020)[10], in their research, they observed with the intention of all the markets and enterprises around the world, the CG scenario in developing countries has evolved over time, with the accumulation of experience information leading to a greater the center of attention and formation of the major authority in the board. The researcher has revealed to facilitate not all Corporate Governance better designed to produce greatest potential results. Puni and Anlesinya (2020)[11], was examined in the research that discovered the director of internal as well as external considerable positive impact on firm performance, but that having an audit committee had a negative impact. The firm's financial performance improved when the frequency of board meetings was raised. Such observations provide some insight into the possibility of certain parts of Corporate Governance being followed in a developing country.

RESEARCH METHODOLOGY

The total of ten companies from Miniratna Status of Category – I Companies in the Central Public Sector Enterprises in India. The current study is based as secondary sources of information, and the objective for this study has to examine "A Conceptual Frame Work with Regard to Public Sector Enterprises in India".

Profile of Selected Companies

The companies were chosen mostly based on data availability and were classified as Public Sector Enterprises. The purpose of this research was to learn more about the Miniratna Status of Category I Companies in the Central Public Sector Enterprises. The following top ten Miniratna Status of Category I Companies in the Central Public Sector Enterprises were considered for research.

Airports Authority of India

The Airports Authority of India (AAI) was established by an Act of Parliament on April 1, 1995, when the National Airports Authority and the International Airports Authority of India merged. AAI oversees 137 airports, including 24 international airports (3 civil enclaves), ten custom airports (4 civil enclaves), and 103 domestic airports (23 Civil Enclaves). [12]

Antrix Corporation Limited

Antrix Corporation Limited (Antrix), was a wholly owned Government of India company under the control of administrative authority of the Department of Space (DOS) and it was enacted on September 28, 1992 (under the Companies Act, 1956). [13]

Balmer Lawrie & Co. Limited

Balmer Lawrie & Co. Ltd. has to began its corporate adventure as a Partnership Firm on 01.02.1867, in Kolkata, started by two Scotsmen, Stephen George Balmer and Alexander Lawrie. The company's turnover is Rs. 1,593 crores and a profit of Rs. 157 crores, now the company comes under a Miniratna - I Public Sector Enterprise under the Ministry of Petroleum and Natural Gas, Government of India. The company is now well-respected international diversified conglomerate with the presence of both the manufacturing and the service sectors. It has been four joint ventures and one subsidiary in India and overseas, viz., Steel Barrels, Industrial Greases and Specialty Lubricants, Corporate Travel and Logistics Services. The company has well-represented majority of the other businesses involved, such as chemicals and logistics infrastructure. [14]

Bharat Coking Coal Limited

Bharat Coking Coal Limited (BCCL) is the government-owned company that engages in coal mining and related activities. BCCL supplies about half of the incorporated steel sectors have to complete the prime coking coal demand. BCCL was incorporated in January 1972 and it operates 214 coking coal mines in Jharia and Raniganj coalfields, BCCL was taken over by the Indian government on 16th October 1971, and has assured the planned development of the country's limited coking coal resources. In 1st April 2020, the Company operates 36 coal mines, including 11 underground, 16 opencast, and 09 mixed mines. [15]

Bharat Dynamics Limited SSS

The headquarters of Bharat Dynamics Limited (BDL) in Hyderabad, and it was recognized on 16.07.1970 as a Public Sector Undertaking under the Ministry of Defense, Government of India, has to serve the Indian Armed Forces' manufacture stand for guided missile systems and supporting equipment. BDL has to operate four manufacturing services, three in Telangana (Hyderabad, Bhanur, and Ibrahimpatnam) and one in Andhra Pradesh (Visakhapatnam). BDL has to establish in a unit situated in Amravati, Maharashtra, as part of its enlargement plan has to meet the expanding demands of the Armed Forces. [16]

BEML Limited

BEML Limited (previously Bharat Earth Movers Limited) was incorporated on May 1964 has a public sector undertaking in Bangalore to produce rail coaches, spare parts and mining equipments. The company has to some extent disinvested and it controls 54 percent of the total stock, with the remaining 46 percent held by the public, financial institutions, foreign institutional investors, banks, and employees. BEML Limited, has a 'Schedule-A' company and it is critical to India's main sectors of defense, rail, power, mining, and infrastructure. The company began with modest sales of Rs. 5 crore in 1965 and has since grown to a turnover of more than Rs. 3,500 crore. [17]

Bharat Sanchar Nigam Limited

On 15th September 2000, Bharat Sanchar Nigam Limited (BSNL) was created. With effect from 1st October 2000, it took over the business, has to providing telecom services and network management from the former Central Government Departments of Telecom Services (DTS) and Telecom Operations (DTO) on a going concern basis. Except for Delhi and Mumbai, the firm provides telecom services throughout the country. BSNL is the Government of India owned Public Sector undertaking with a paid-up capital of Rs. 12,500 crores, consisting of Rs. 5,000 crores in equity and Rs. 7,500 crores in preference shares. Its entire revenue for the fiscal year 2020-21 was Rs.18, 595 crores. [18]

Braithwaite & Company Limited

Braithwaite was started in the year 1913 as the Indian subsidiary of Braithwaite & Co. Engineers Limited (UK) to handle structural steel manufacturing. From 1934, the Clive Works in Calcutta began producing wagons for Indian Railways. On 28th February 1930, the company was founded in Bengal as Braithwaite & Co (India) Ltd. On 11.01.2022, the Ministry of Railways granted Braithwaite & Co. Limited "MINIRATNA-I status"[19].

Bridge and Roof Company (India) Limited

Bridge and Roof Company (India) Limited was incorporated on 16th January 1920, and it began operations as a small structural fabrication shop in Howrah, West Bengal, India. It became a Public Sector Enterprise in the year 1972, under the Ministry of Petroleum and Natural Gas, and has been under the administrative supervision of the Ministry of Heavy Industries since 1987. Now it is fourth largest construction industry under CPSE. Bridge and Roof is honored to be one of India's famous tradition category of only 36 companies that have served for more than 102 years and have completed a hundred years journey[20].

Central Warehousing Corporation

The Central Warehousing Corporation (CWC) is a legislative entity constituted under the Warehousing Corporations Act, 1962. It is a Schedule A – Miniratna status under Central Public Sector Enterprise (CPSE). CWC has a total paid-up capital of Rs. 68.02 crore and the authorized capital of Rs. 100 crore. The Corporation's turnover for Financial Year 2020-21 was Rs. 2168.13 crore, up from Rs. 1727.63 crore the previous year. Food grain capacity utilisation averaged 49.92 lakh MT in Financial Year 2020-21, down from 52.22 lakh MT the previous year. A dividend of Rs. 131.47 crore has been paid to shareholders for the fiscal year 2020-21, calculated at 30% of profit after tax. [21]

CONCLUSION

There are issues with independence, non-executive director salaries; deliberate participation in low-productive activities, ministerial agendas interfering with board agendas, the audit committee's goodness and the audit committee's extended support, and compromising minority shareholder interests, among others. In such cases, implementing corporate governance can only assist Central Public Sector Enterprises (CPSE) improve their performance in their particular industries. The right decision-making in the government agencies also paves the foundation for the sound Corporate Governance. While complying by the legislation, trading with simple majority shareholders, performance evaluates day-to-day activities of the organization. It is not only the responsibility of top-level management; every employee should priorities ethical behavior and accepts it with a positive attitude.

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RESEARCH ARTICLE

Spatio-Tempopral Assessment of Primary Productivity of the Hot Water Spring Atri, Khorda, Odisha, India

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ABSTRACT

The primary producers are the first link in an ecosystem. They may be green plants or certain bacteria, are able to convert the inorganic matter into biomass by using solar radiation or chemical energy. The organic matter (biomass) formed due to assimilation and fixation of inorganic carbon and inorganic nutrient is known as Primary production. The total energy fixed in autotrophs is refferd as gross primary productivity. The amount of energy left after respiration of autotrophs is termed as net primary productivity. The net ecosystem productivity is the net amount of primary production after costs of respiration by plants, heterotrophs and decomposers. In this study, the GPP, NPP and CR are investigated from the hot spring Atri. Light and dark bottle methods are followed to determine the productivity. Water temperature was measured and the relation between Primary productivity is calculated. The data were expressed as Mean±SD.

Keywords: Hot spring. GPP, NPP, CR, Water temperature.

INTRODUCTION

The rate at which the solar energy is converted into organic substances by photoautotrophs (photosynthetic producers) and chemoautotrophs (chemosynthetic producers) is known as primary productivity. photoautotroph's obtain energy and nutrients from sunlight whereas chemoautotrophs obtain chemical energy through oxidation. Terrestrial and aquatic ecosystems have many similarities with their primary production but the fact is that in terrestrial system the exposure area to radiant solar energy is limited and restricted as the light cannot penetrate the

deeper strata. In aquatic bodies the light can penetrates into the deeper strata where harvesting is possible to meet the energy requirement. But same thing does not happen in hot springs due to high temperature and beyond the critical limits.

According to [10] the measurement of primary productivity not only gives information regarding the production of organic matters but also provides idea about the functional aspects of the ecosystem. The exact nature of the trophic levels and availability of energy for secondary production can be assessed by estimation of primary productivity. and the impact of climate change has a drastic effect on primary productivity of the ecosystem [3]. The gross primary productivity (GPP) is the total energy stored as organic compounds and food materials or the total carbon dioxide fixed by the photosynthetic organisms in the system. The respiration (R) is the amount of Co_2 lost from an organism or system from metabolic activity. The net primary productivity (NPP) is the net primary production after the costs of plants respiration. So NPP = GPP –R. The primary productivity also varies in water bodies of different zones of India. It is found that in tropical India, water bodies have more primary productivity in comparison to temperate zones [9].The primary productivity in both marine and fresh water bodies was investigated by [4],[5],[11],[1], [8], and [12]. A systematic study on primary productivity of the studied area and the relation between them was found scanty and so proper investigation was carried out to fulfil the objective of the work.

MATERIALS AND METHODS

Study site
Atri a small village in Khorda district of Odisha, India. It is famous for its perennial hot spring contains small doses of sulphur flavour when heated to 100°C. It is surrounded by Baghamari grampanchayat of Khorda and located between 20^0 10′ 42.6324″ N and 850 32′ 25.1304″E.

MATERIALS

BOD bottles (two light or transparent and one dark), Jute rope, burettes, reagents (Manganous sulphate solution, alkaline iodide, azide solution, sodium thiosulphate, concentrated sulphuric acid, starch indicator solution). The temperature of water was measured by mercury centigrade thermometer graduated up to 110°C in 0.1 graduations and considered to nearest 0.5°C.

METHODS

Primary productivity
In this study oxygen method by light and dark bottle technique [4] was followed. This method was considered advantageous because is comparatively simple and much more sensitive to low productivity. In the middle of each month samples were collected thrice in three bottles at 50 cm below the water surface. While collecting the sample the entry of air bubbles into the bottles were prevented by Winkler's fixative. Three bottles were named as 1 light bottle, 1 dark bottle and 1 control light bottle. Control light bottle was used for estimation of dissolved oxygen. The other light bottle and one dark bottle were suspended on to a raft and anchored at the depth from where the sample was drawn from the study site. After the end of incubation, the bottles were taken to the laboratory carefully and dissolved oxygen content was determined by Winkler's volumetric method. After getting the dissolved oxygen content, Gross primary productivity (GPP), Net primary productivity (NPP) and biotic respiration (CR) were calculated using the suitable formula.

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RESULT

The values of GPP, NPP, and CR are expressed in $g^c m^{-2} day^{-1}$ and temperature is in °C. The values are presented in form of mean and their standard deviation (Mean±SD) in table 1. The GPP, NPP and NPP/GPP are found to be highest in March in 2020 and 2021. The biotic respiration (CR) is found to be highest in February of both the years. The lowest GPP and NPP are seen in August of 2020 and September of 2021. In the year 2020 highest temperature recorded in month of May and lowest in November, but in the 2021 March showed the highest and lower range was maintained in other months. All the data are represented in table and figures. A comparative study on variation in primary productivity in the two years is presented (Fig.1. a,b) NPP/GPP (Fig.2) and range of water temperature in Figure 3 respectively. The primary productivity in the studied area is positively correlated with each other throughout the year. Correlation between GPP and NPP (Fig.4.a, b); GPP and CR (Fig.5. a,b); and NPP vs CR (Fig.6.a,b) in the year 2020 and 2021 respectively.

DISCUSSION

It was observed that the investigated data on primary productivity fluctuate throughout the year. The weather condition of site was observed and it affects the primary production which is similar to aquatic ecosystems studied by [7] and [8]. The minimum production of the hot spring in some specific months is due to cloudy rainy condition of the atmosphere corroborates with [6] [11]. High temperature also affects the productivity of the ecosystem is studied here   and coincides with [2] [13]. Our study shows a lower mean GPP in comparison to other ecosystems is due to increased water temperature, poor maintenance of reservoir/tank, decrease in photosynthesis inhibiting pollutant and long time stagnant condition. Therefore it is a major concern to look after the hot spring in order to incresae the primary production and to provide a healthy condition to the dependant hot spring organisms.

CONCLUSION

Our study revealed the primay production of the hot spring and the variation in different months throughout the studied years. Further elaborative investigation is suggested regarding the thermophilic organisms and their role towards primary production of the defined ecosytem.

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Table 1 Primary productivity of the hot spring Atri in different years.

Sl. No.	parameters	Unit	2020 Mean±SD	2021 Mean±SD
1	GPP	$g^c m^{-2} day^{-1}$	0.908±0.305	0.888±0.363
2	NPP	$g^c m^{-2} day^{-1}$	0.575±0.271	0.568±0.282
3	NPP/GPP	$g^c m^{-2} day^{-1}$	0.605±0.139	0.641±0.133
4	CR	$g^c m^{-2} day^{-1}$	0.341±0.109	0.331±0.114
5	Water temperature	°C	57.083±0.763	57.166±0.778
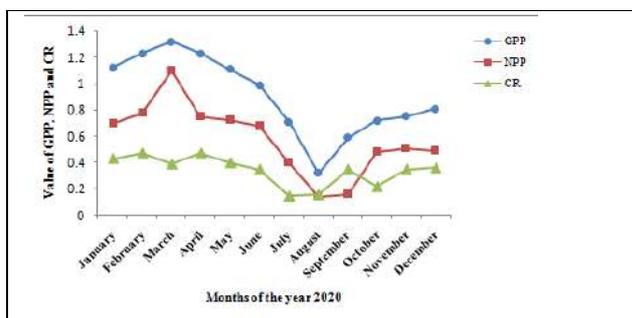
Fig.1.a Variation in GPP, NPP, and CR in the year 2020.	**Fig.1.b Variation in GPP, NPP, and CR in the year 2021.**
Fig.2 NPP/GPP in different months in the year 2020 and 2021.	**Fig.3 Variation in water temperature in different months in the year 2020 and 2021.**
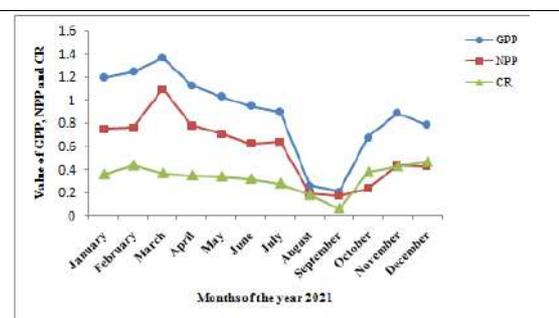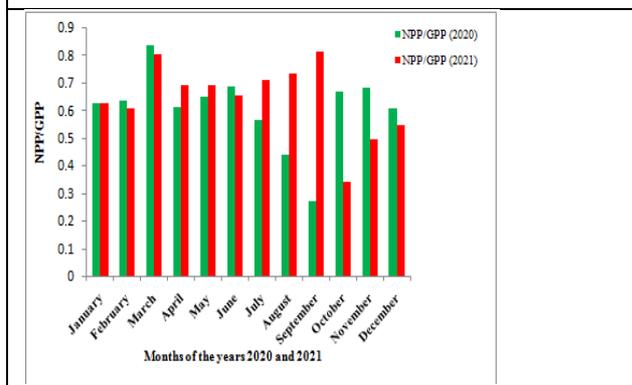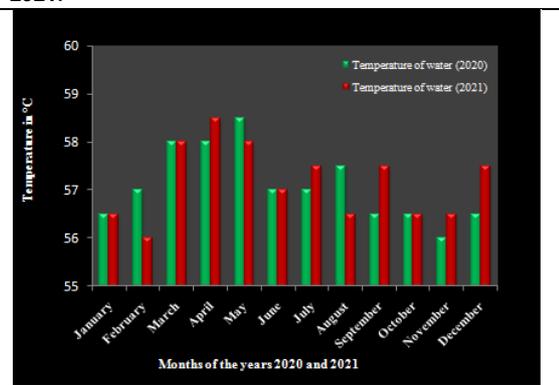
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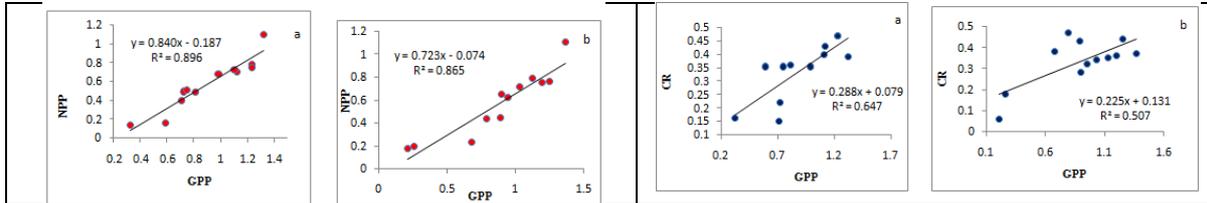
Fig.4 GPP vs NPP in the year (a) 2020 and (b) 2021. | **Fig.5 GPP vs CR in the year (a) 2020 and (b) 2021.**
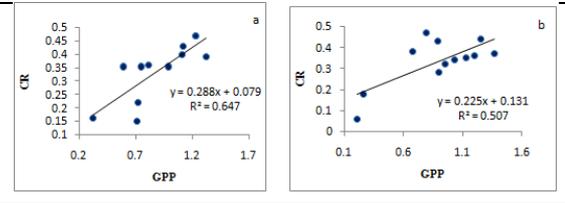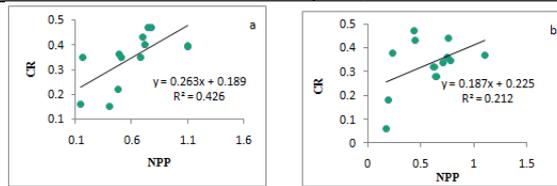
Fig.6 NPP vs CR in the year (a) 2020 and (b) 2021.

A Stochastic Model to Conclude Finest Conserve Inventory when the Gizmos are in Parallel

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ABSTRACT

Inventory control or stock control can be broadly defined as "the activity of checking a shop's stock.". It is the process of ensuring that the right amount of supply is available within a business. However, a more focused definition takes into account the more science-based, methodical practice of not only verifying a business' inventory but also maximizing the amount of profit from the least amount of inventory investment without affecting customer satisfaction. In this article the optimal conserve inventory between machines (Gizmos) in parallel is attempted. The output of first machine A is the input for second and third machines B and C. In between machines A and B , C and inventory is maintained. One of the efforts of attention in inventory control theory is the purpose of optimal volume of the buffer among the machines. The need for maintaining inventory arises in a number of situations in a manufacturing oriented inventory systems. The study reveals the optimum strategy for maintaining the inventory between machines. The basis for maintaining inventory machines is, due to ageing of machines and due to some outer reasons, the time taken for receiving the finest goods between parallel systems may affect the beginning of next machines. Here we study the optimal conserve inventory between machines in parallel. A generalized equation is derived when the number of parallel machines.

Keywords: stock, parallel system, conserve inventory, transmuted exponential distribution, order statistics.

INTRODUCTION

In Inventory control theory, the model to obtain optimal volume from the buffer stock is most important. The necessity for maintaining Inventory arises in several situations in a production based Inventory system viz, Hydro Electric system, Thermal power station etc., The Optimal conserve inventory betwixt the systems, namely, the system produces that input and the system that consumes the input obtained from the previous system is must. When the productions are in progress there is a break down at any stage it will spoil the entire system. Therefore storage points of inventory are created between adjacent stages so as to achieve a certain degree of independence in operating the stages. The recent applications in a production oriented inventory system viz., Hydro electric system, Thermal power station etc.,In the case of hydro – electric system, the input is the river-flow and the Dam is the conserve to hydro – electric station. The optimal conserve in this case is not exactly the conserve in the dam but it is only the problem of optimal discharge during the different periods of a planning horizon. The problem to obtain optimal conserve is solved. In this research work we judge a replica in which the system is conceptualized. We think '3' gizmos (machines). It is clear that the output of the first machine is the input for the second and third machine, and assumed that the utilization rate is stable. The basic model has been discussed by Hanssman [3] to find the optimal conserve inventory between two machines in series. Babu Krishnaraj R and Ramasamy K [2] have identified the model for Optimal Reserve Inventory between machines in series with Exponential distribution which is the case in parallel systems. Babu Krishnaraj R and Ramasamy K [1] is proved the result for the system of 'n' machines in parallel by the method of mathematical induction hypothesis. In that model, it is assumed that the unused time cost of second machine B is very high, so a conserve in between A and B is recommended. If ω is a random variable indicating unused time of B, it may be indicated that

$$\omega = 0 \; if \; \omega \le \frac{R}{c}$$

$$= \left(\omega - \frac{R}{c} \right), \;\; \omega > \frac{R}{c}$$

The Expected total cost due to Inventory holding cost and unused time of B per unit time is

$$E(C) \; = hR + \int_{\frac{R}{c}}^{\infty} \left(\omega - \frac{R}{c} \right) g(\omega) \; d\omega$$

Where $\dfrac{1}{\mu}$ shows the number of breakdowns of A per unit time.

To conclude the best possible R, we take $\dfrac{d\,E(C)}{dR} = 0$

$$\frac{dE(C)}{dR} = hR + \frac{k}{\mu} \int_{\frac{R}{c}}^{\infty} \left(\omega - \frac{R}{c} \right) g(\omega) \; d\omega = 0$$

By means of the Leibnitz rule the best possible resolution is obtained as $G\left(\dfrac{R}{c} \right) = 1 - \dfrac{c\mu h}{k}$

In this, the expression for optimal conserve 'R' is given, has a limitation that, it should be less than unity, otherwise the solution is not feasible. Hence the new improved solution with no restriction was obtained by Sathiyamoorthy. R andSachidhanandham .S (2007) as follows.

$$E(C) = hc \int_{0}^{\frac{R}{c}} \left(\frac{R}{c} - \omega \right) g(\omega) \, d\omega + \frac{k}{\mu} \int_{\frac{R}{c}}^{\infty} \left(\omega - \frac{R}{c} \right) g(\omega) \, d\omega$$

Using Lebinitz rule, we have $\quad G\left(\frac{R}{c} \right) = \frac{k}{c\mu h + k} < 1 \quad fo \ all \ k, \mu \ h.$

where

h - Inventory holding cost per unit / unit time for the conserve

k - Unused time rate per unit time for B

c - Constant expenditure rate of B

R – Conserve Inventory between A and B

ω = a continuous random variable denoting the revamp time or break down length of A and the p.d.f is g (.) and c.d.f G(.)

This solution is a modified edition of $G\left(\frac{R}{c} \right) = \frac{k}{c\mu h + k} < 1 \quad fo \ all \ k, \mu \ h.$

Model-I

In this article, 3 machines A,B and C are considered and the optimal values of the conserve inventory between A and B,C for the model discussed.

New Scripts

1. h = Inventory holding cost per unit

2. c - Constant expenditure rate

3. k_1, k_2 Indicates the unused time costs.

4. ω Indicates the repair time duration, which is a random variable.

5. U the random variable shows the inter arrival time between successive break down of A which is taken as Transmuted exponential distribution with parameter ψ.

6. R is the conserve inventory between A and B, C

The average level of inventory is $\int_{0}^{\frac{R}{c}} (R - c\omega) \, g(\omega) \, d\omega$ and assuming that the rate of consumption of B and C is

'c' per unit of time. Where $\dfrac{1}{\mu}$ denotes the breakdowns of A per unit time. Now assuming the revamp time subsequently first order statistic the optimal conserve inventory among A and B,C is obtained as

$$g\left(\omega_{(1)} \right) = n \left[1 - G(\omega) \right]^{n-1} g(\omega)$$

The expected total cost due to inventory holding cost and unused time per unit time is

$$E(C) = hc \int_{0}^{R/c} \left(\frac{R}{c} - \omega \right) g_{(1)}(\omega) \, d\omega + \frac{k_1}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) g_{(1)}(\omega) \, d\omega + \frac{k_2}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) g_{(1)}(\omega) \, d\omega$$

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To find optimal 'R', we find $\dfrac{dE\,(C)}{dR} = 0$, ie.,

$$= \frac{d}{dR}\left[hc\int_0^{R/c}\!\!\left(\frac{R}{c}-\omega\right)\; n[1-G(\omega)]^{n-1}\; g(\omega)\,d\omega + \frac{k_1}{\mu}\int_{R/c}^{\infty}\!\!\left(\omega-\frac{R}{c}\right)\; n[1-G(\omega)]^{n-1}\; g(\omega)\,d\omega \right.$$
$$\left. + \frac{k_2}{\mu}\int_{R/c}^{\infty}\!\!\left(\omega-\frac{R}{c}\right)\; n[1-G(\omega)]^{n-1}\; g(\omega)\,d\omega \right] = 0$$

$$= \frac{d}{dR}\left[nhc\int_0^{R/c}\!\!\left(\frac{R}{c}-\omega\right)[1-G(\omega)]^{n-1}\,g(\omega)\,d\omega + \frac{nk_1}{\mu}\int_{R/c}^{\infty}\!\!\left(\omega-\frac{R}{c}\right)[1-G(\omega)]^{n-1}(\omega)\,d\omega \right.$$
$$\left. + \frac{nk_2}{\mu}\int_{R/c}^{\infty}\!\!\left(\omega-\frac{R}{c}\right)[1-G(\omega)]^{n-1}\,g(\omega)\,d\omega \right] = 0$$

$$= nhc\,(I_1) + \frac{nk_1\,(I_2)}{\mu} + \frac{nk_2\,(I_3)}{\mu}$$

(1)

$$(I_1) = \frac{dE(C)}{dR} = 0 \Rightarrow \frac{d}{dR}\int_0^{R/c}\!\!\left(\frac{R}{c}-\omega\right)\; [1-G(\omega)]^{n-1}\; g(\omega)\,d\omega$$

By Leibnitz rule

$$= \frac{1}{c}\int_0^{R/c}[1-G(\omega)]^{n-1}\; g(\omega)\,d\omega$$

$$g(\omega) = 2\theta\psi e^{-2(\psi\omega)} + \psi e^{-(\psi\omega)} - \theta\psi e^{-(\psi\omega)} \quad G(\omega) = 1 + \theta e^{-(\psi\omega)} - e^{-(\psi\omega)} - \theta e^{-2(\psi\omega)};$$
$$1 - G(\omega) = \theta e^{-2(\psi\omega)} + e^{-(\psi\omega)} - \theta e^{-(\psi\omega)}$$

$$= \frac{1}{c}\int_0^{R/c}[\theta e^{-2(\psi\omega)} + e^{-(\psi\omega)} - \theta e^{-(\psi\omega)}]^{n-1}\; 2\theta\psi e^{-2(\psi\omega)} + \psi e^{-(\psi\omega)} - \theta\psi e^{-(\psi\omega)}\,d\omega$$

$$t = \theta e^{-2(\psi\omega)} + e^{-(\psi\omega)} - \theta e^{-(\psi\omega)}\,;\quad dt = 2\theta\psi e^{-2(\psi\omega)} + \psi e^{-(\psi\omega)} - \theta\psi e^{-(\psi\omega)}\,d\omega$$

$$= \frac{1}{c}\int_1^{\theta e^{-2\left(\frac{\omega R}{c}\right)}+e^{-\left(\frac{\omega R}{c}\right)}-\theta e^{-\left(\frac{\omega R}{c}\right)}} t^{n-1}(-dt)$$

$$= \frac{-1}{nc}\left[\left[\theta e^{-2\left(\frac{\omega R}{c}\right)} + e^{-\left(\frac{\omega R}{c}\right)} - \theta e^{-\left(\frac{\omega R}{c}\right)}\right]^n - 1\right]$$

(2)

$$(I_2) \Rightarrow \frac{dE(C)}{dR} = 0 \Rightarrow \int_{R/c}^{\infty}\!\!\left(\omega-\frac{R}{c}\right)\; [1-G(\omega)]^{n-1}\; g(\omega)\,d\omega$$

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$$-\frac{1}{c}\int_{R/c}^{\infty}[1-G(\omega)]^{n-1}\, 2\theta\psi e^{-2(\psi\omega)}+\psi e^{-(\psi\omega)}-\theta\psi e^{-(\psi\omega)}\, d\omega$$

$$=-\frac{1}{c}\int_{\theta e^{-2\left(\frac{\omega R}{c}\right)}+e^{-\left(\frac{\omega R}{c}\right)}-\theta e^{-\left(\frac{\omega R}{c}\right)}}^{0}[t]^{n-1}\,(-dt)$$

$$=\frac{1}{nc}\left[0-\left[\theta e^{-2\left(\frac{\psi R}{c}\right)}+e^{-\left(\frac{\psi R}{c}\right)}-\theta e^{-\left(\frac{\psi R}{c}\right)}\right]^{n}\right]$$

$$=\frac{-1}{nc}\left[\theta e^{-2\left(\frac{\psi R}{c}\right)}+e^{-\left(\frac{\psi R}{c}\right)}-\theta e^{-\left(\frac{\psi R}{c}\right)}\right]^{n}\tag{3}$$

$$(I_3)\Rightarrow\frac{dE(C)}{dR}=0\Rightarrow\int_{R/c}^{\infty}\left(\omega-\frac{R}{c}\right)\,[1-G(\omega)]^{n-1}\, g(\omega)\, d\omega$$

$$-\frac{1}{c}\int_{R/c}^{\infty}[1-G(\omega)]^{n-1}\, 2\theta\psi e^{-2(\psi\omega)}+\psi e^{-(\psi\omega)}-\theta\psi e^{-(\psi\omega)}\, d\omega$$

$$=-\frac{1}{c}\int_{\theta e^{-2\left(\frac{\omega R}{c}\right)}+e^{-\left(\frac{\omega R}{c}\right)}-\theta e^{-\left(\frac{\omega R}{c}\right)}}^{0}[t]^{n-1}\,(-dt)$$

$$=\frac{1}{c}\int_{R/c}^{\infty}[\theta e^{-2(\psi\omega)}+e^{-(\psi\omega)}-\theta e^{-(\psi\omega)}]^{n-1}\, g(\omega)\, d\omega$$

$$=\frac{1}{c}\left[\frac{t^{n}}{n}\right]_{\theta e^{-2\left(\frac{\psi R}{c}\right)}+e^{-\left(\frac{\psi R}{c}\right)}-\theta e^{-\left(\frac{\psi R}{c}\right)}}^{0}$$

$$=\frac{-1}{nc}\left[\theta e^{-2\left(\frac{\psi R}{c}\right)}+e^{-\left(\frac{\psi R}{c}\right)}-\theta e^{-\left(\frac{\psi R}{c}\right)}\right]^{n}\tag{4}$$

Substituting (2) & (3) ,(4) in (1), we get

$$=nhc\left(\frac{-1}{nc}\right)\left[\left[\theta e^{-2\left(\frac{\psi R}{c}\right)}+e^{-\left(\frac{\psi R}{c}\right)}-\theta e^{-\left(\frac{\psi R}{c}\right)}\right]^{n}-1\right]+\frac{nk_1}{\mu}\left(\frac{-1}{nc}\right)\left[\theta e^{-2\left(\frac{\psi R}{c}\right)}+e^{-\left(\frac{\psi R}{c}\right)}-\theta e^{-\left(\frac{\psi R}{c}\right)}\right]^{n}$$

$$+\frac{nk_2}{\mu}\left(\frac{-1}{nc}\right)\left[\theta e^{-2\left(\frac{\psi R}{c}\right)}+e^{-\left(\frac{\psi R}{c}\right)}-\theta e^{-\left(\frac{\psi R}{c}\right)}\right]^{n}=0$$

$$\left[\frac{h\mu c+k_1+k_2}{\mu c}\right]\left[\theta e^{-2\left(\frac{\psi R}{c}\right)}+e^{-\left(\frac{\psi R}{c}\right)}-\theta e^{-\left(\frac{\psi R}{c}\right)}\right]^{n}=h$$

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$$e^{-\left(\frac{\omega R}{c}\right)} = \frac{-(1-\theta) \pm \sqrt{(1-\theta)^2 + 4\theta\left[\frac{h\mu c}{h\mu c + k_1 + k_2}\right]^{\frac{1}{n}}}}{2\theta}$$

$$e^{-\left(\frac{\psi R}{c}\right)} = \frac{-(1-\theta) \pm \sqrt{(1-\theta)^2 + 4\theta\frac{[h\mu c]^{\frac{1}{n}}}{[h\mu c + k_1 + k_2]^{\frac{1}{n}}}}}{2\theta}$$

Taking the consideration for positive value

$$2\theta e^{-\left(\frac{\psi R}{c}\right)} + (1-\theta) = \sqrt{\frac{(1-\theta)^2[h\mu c + k_1 + k_2]^{\frac{1}{n}} + 4\theta[h\mu c]^{\frac{1}{n}}}{[h\mu c + k_1 + k_2]^{\frac{1}{n}}}}$$

$$\hat{R} = \frac{c}{\psi} \log\left[\frac{1}{2\theta}\left[\sqrt{\frac{(1-\theta)^2[h\mu c + k_1 + k_2]^{\frac{1}{n}} + 4\theta[h\mu c]^{\frac{1}{n}}}{[h\mu c + k_1 + k_2]^{\frac{1}{n}}}} - (1-\theta)\right]\right]^{-1}$$

Numerical Illustration of Model-I

The changes in R for the changes in the inventory holding cost h is given in the subsequent Table -1 and Figure -1
$c = 20, \psi = 0.5, k_1 = 100, k_2 = 75, \theta = 0.5, n = 20, \mu = 1.5$ are all predetermined.

The changes in $\hat{R}$ for the changes in k_1 is specified in the subsequent Table -2 and Figure-2
$h = 10; n = 20; k_2 = 75; c_1 = 20; \theta = 0.5, \gamma = 1.5; \psi = 0.5$ are all predetermined.

The changes in $\hat{R}$ for the changes in k_2 is specified in the subsequent Table-3 and Figure-3
$h = 10; n = 20; k_1 = 100; c_1 = 20; \theta = 0.5,; \psi = 0.5$ are all predetermined.

The changes in $\hat{R}$ for the changes in μ is specified in the subsequent Table- 4 and Figure-4
$h = 10; n = 20; k_1 = 100; k_2 = 75; c_1 = 20; \theta = 0.5,.5; \psi = 0.5$ are all predetermined.

Model-II

The revamp time subsequently nth order statistic the optimal conserve inventory among A and B,C is obtained as
follows $g_n(\omega) = n[G(\omega)]^{n-1} g(\omega)$
The expected total cost due to inventory holding cost and unused time per unit time is

$$E(C) = hc \int_0^{R/c} \left(\frac{R}{c} - \omega\right) g_{(n)}(\omega)\, d\omega + \frac{k_1}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c}\right) g_{(n)}(\omega)\, d\omega + \frac{k_2}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c}\right) g_{(n)}(\omega)\, d\omega$$

To find optimal 'R', we find $\frac{dE(C)}{dR} = 0$, ie.,

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$$\frac{dE(C)}{dR} = 0 \Rightarrow = hc \int_0^{R/c} \left(\frac{R}{c} - \omega \right) g_{(n)}(\omega)\, d\omega + \frac{k_1}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) g_{(n)}(\omega)\, d\omega + \frac{k_2}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) g_{(n)}(\omega)\, d\omega$$

$$\frac{dE(C)}{dR} = \frac{d}{dR} \begin{bmatrix} hc \int_0^{R/c} \left(\frac{R}{c} - \omega \right) \; n[G(\omega)]^{n-1}\; g(\omega)\, d\omega + \frac{k_1}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) \; n[G(\omega)]^{n-1}\; g(\omega)\, d\omega \\ + \frac{k_2}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) \; n[G(\omega)]^{n-1}\; g(\omega)\, d\omega \end{bmatrix} = 0$$

$$= \frac{d}{dR} \begin{bmatrix} nhc \int_0^{R/c} \left(\frac{R}{c} - \omega \right) \; [1 + \theta e^{-(\psi\omega)} - e^{-(\psi\omega)} - \theta e^{-2(\psi\omega)}]^{n-1}\; 2\theta\psi e^{-2(\psi\omega)} + \psi e^{-(\psi\omega)} - \theta\psi e^{-(\psi\omega)}\, d\omega \\ + \frac{nk_1}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) \; [1 + \theta e^{-(\psi\omega)} - e^{-(\psi\omega)} - \theta e^{-2(\psi\omega)})]^{n-1}\; 2\theta\psi e^{-2(\psi\omega)} + \psi e^{-(\psi\omega)} - \theta\psi e^{-(\psi\omega)}\, d\omega \\ + \frac{nk_2}{\mu} \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) \; [1 + \theta e^{-(\psi\omega)} - e^{-(\psi\omega)} - \theta e^{-2(\psi\omega)})]^{n-1}\; 2\theta\psi e^{-2(\psi\omega)} + \psi e^{-(\psi\omega)} - \theta\psi e^{-(\psi\omega)}\, d\omega \end{bmatrix} = 0$$

$$= nhc(I_4) + \frac{nk_1(I_5)}{\mu} + \frac{nk_2(I_6)}{\mu} \tag{5}$$

$$(I_4) = \int_0^{R/c} \left(\frac{R}{c} - \omega \right) [G(\omega)]^{n-1} g(\omega)\, d\omega$$

By Leibnitz rule

$$= \frac{1}{c} \int_0^{R/c} [G(\omega)]^{n-1}\; g(\omega)\, d\omega$$

$$= \frac{1}{c} \int_0^{R/c} [1 + \theta e^{-(\psi\omega)} - e^{-(\psi\omega)} - \theta e^{-2(\psi\omega)}]^{n-1}\; 2\theta\psi e^{-2(\psi\omega)} + \psi e^{-(\psi\omega)} - \theta\psi e^{-(\psi\omega)}\, d\omega$$

$$= \frac{1}{c} \int_0^{1 + \theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)}} [t]^{n-1}\, dt$$

$$= \frac{1}{nc} \left[1 + \theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)} \right]^n \tag{6}$$

$$(I_5) = \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) \; [G(\omega)]^{n-1}\; g(\omega)\, d\omega$$

By Leibnitz rule

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$$= \frac{-1}{c} \int_{R/c}^{\infty} [1 + \theta e^{-(\psi\omega)} - e^{-(\psi\omega)} - \theta e^{-2(\psi\omega)}]^{n-1} \, 2\theta\psi e^{-2(\psi\omega)} + \psi e^{-(\psi\omega)} - \theta\psi e^{-(\psi\omega)} \, d\omega$$

$$= \frac{-1}{c} \int_{1+\theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)}}^{1} [t]^{n-1} \, dt$$

$$= \frac{-1}{nc} + \frac{1}{nc} \left[1 + \theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)} \right]^{n} \tag{7}$$

$$(I_6) = \int_{R/c}^{\infty} \left(\omega - \frac{R}{c} \right) \, [G(\omega)]^{n-1} \, g(\omega) \, d\omega$$

By Leibnitz rule

$$= \frac{-1}{c} \int_{R/c}^{\infty} [1 + \theta e^{-(\psi\omega)} - e^{-(\psi\omega)} - \theta e^{-2(\psi\omega)}]^{n-1} \, 2\theta\psi e^{-2(\psi\omega)} + \psi e^{-(\psi\omega)} - \theta\psi e^{-(\psi\omega)} \, d\omega$$

$$= \frac{-1}{c} \int_{1+\theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)}}^{1} [t]^{n-1} \, dt$$

$$= \frac{-1}{nc} + \frac{1}{nc} \left[1 + \theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)} \right]^{n} \tag{8}$$

Substituting (6),(7),(8) in (5)

$$nhc \frac{1}{nc} \left[1 + \theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)} \right]^{n} + \frac{nk_1}{\mu} \left[\frac{-1}{nc} + \frac{1}{nc} \left[1 + \theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)} \right]^{n} \right]$$

$$+ \frac{nk_2}{\mu} \left[\frac{-1}{nc} + \frac{1}{nc} \left[1 + \theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)} \right]^{n} \right] = 0$$

$$\theta e^{-\left(\frac{\psi R}{c}\right)} - e^{-\left(\frac{\psi R}{c}\right)} - \theta e^{-2\left(\frac{\psi R}{c}\right)} = \left[\frac{k_1 + k_2}{k_1 + k_2 + h\mu c} \right]^{\frac{1}{n}} - 1$$

$$\left[\theta \left[e^{-\left(\frac{\psi R}{c}\right)} \right]^2 - (\theta - 1) e^{-\left(\frac{\psi R}{c}\right)} \right] - \left[1 - \left[\frac{k_1 + k_2}{k_1 + k_2 + h\mu c} \right]^{\frac{1}{n}} \right] = 0$$

$$e^{-\left(\frac{\psi R}{c}\right)} = \frac{(\theta - 1) \pm \sqrt{\dfrac{(\theta - 1)^2 [k_1 + k_2 + h\mu c]^{\frac{1}{n}} + 4\theta[k_1 + k_2 + h\mu c]^{\frac{1}{n}} - 4\theta[k_1 + k_2]^{\frac{1}{n}}}{[k_1 + k_2 + h\mu c]^{\frac{1}{n}}}}}{2\theta}$$

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$$\hat{R} = \frac{c}{\psi} \log \left[\frac{1}{2\theta} \left[\sqrt{ \frac{[(\theta-1)^2 + 4\theta][k_1 + k_2 + h\mu c]^{\frac{1}{n}} - 4\theta[k_1 + k_2]^{\frac{1}{n}}}{[k_1 + k_2 + h\mu c]^{\frac{1}{n}}} } + (\theta-1) \right] \right]^{-1}$$

Numerical Illustration of Model–II

The changes in $\hat{R}$ for the changes in the inventory holding cost h is given in the subsequent Table 5 and Figure 5 $c = 20, \psi = 0.5, k_1 = 100, k_2 = 75, \theta = 0.5, n = 20, \mu = 1.5$ are all predetermined

The changes in $\hat{R}$ for the changes in k_1 is specified in the subsequent Table-6 and Figure-6 $h = 10; n = 20; k_2 = 75; c_1 = 20; \theta = 0.5, \gamma = 1.5; \psi = 0.5$ are all predetermined.

The changes in $\hat{R}$ for the changes in k_2 is specified in the subsequent Table -7 and Figure-7 $h = 10; n = 20; k_1 = 100; c_1 = 20; \theta = 0.5, ; \psi = 0.5$ are all predetermined.

The changes in $\hat{R}$ for the changes in μ is specified in the subsequent Table-8 and Figure 4.8 $h = 10; n = 20; k_1 = 100; k_2 = 75; c_1 = 20; \theta = 0.5; \psi = 0.5$ are all predetermined.

CONCLUSION

In the above study, the optimal conserve inventory is obtained under Transmuted exponential distribution of functioning and stoppage times. The solution is anbetter version with no limitations.

(i) As the rate of 'h' specifically the inventory holding cost increases there is a decrease in the magnitude of the optimal inventory $\hat{R}$ equally in the case of the revamp time being first order statistic and also nth order statistic.

(ii) As the deficiency cost 'k' increases, the models propose that the optimal inventory range specifically $\hat{R}$ supposed to be superior. This is found to be accurate in equally the cases namely when the revamp time 'ω' follows first order statistic and also nth order statistic.

(iii) It may be prominent that as the value of ψ which is the parameter of exponential distribution for the duration of break down 'ω' increases, it implies that $E(\psi) = \frac{4 - \lambda}{2\gamma}$ decreases. Therefore the mean length of breakdown

is lesser and therefore the revamp will be carried out speedily. So a lesser inventory is adequate so that $\hat{R}$ decreases.

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Table-1 $\hat{R}$ **for the changes in the inventory holding cost h**

h	10	20	30	40	50	60	70	80	90	100
$\hat{R}$	0.2668	0.1487	0.1030	0.0790	0.0644	0.0539	0.0464	0.0411	0.0365	0.0328

Table-2 $\hat{R}$ **for the changes in** k_1

k_1	100	110	120	130	140	150	160	170	180	190
$\hat{R}$	0.2668	0.2787	0.2906	0.3025	0.3138	0.3252	0.3359	0.3467	0.3575	0.3677

Table- 3 $\hat{R}$ **for the changes in** k_2

k_2	75	80	85	90	95	100	105	110	115	120
$\hat{R}$	0.2668	0.2727	0.2787	0.2846	0.2906	0.2966	0.3025	0.3079	0.3138	0.3192

Table- 4 $\hat{R}$ **for the changes in** μ

k_2	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
$\hat{R}$	0.2668	0.2105	0.1740	0.1487	0.1293	0.1147	0.1030	0.0936	0.0860	0.0790

Table -5 $\hat{R}$ **for the changes in the inventory holding cost h**

h	10	20	30	40	50	60	70	80	90	100
$\hat{R}$	41.9542	35.8183	32.8586	31.0127	29.7042	28.7048	27.9154	27.2576	26.7038	26.2226

Table -6 $\hat{R}$ **for the changes in** k_1

k_1	100	110	120	130	140	150	160	170	180	190
$\hat{R}$	41.9542	42.5163	43.0387	43.5661	44.0647	44.5460	45.0227	45.4731	45.9156	46.3492

Table -7 $\hat{R}$ **for the changes in** k_2

k_2	75	80	85	90	95	100	105	110	115	120
$\hat{R}$	41.9452	42.2228	42.5163	42.7752	43.0387	43.3068	43.5614	43.8203	44.0647	44.3131

Table-8 $\hat{R}$ for the changes in μ

γ	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
$\hat{R}$	41.9452	39.2243	37.2832	35.8183	34.6379	33.6742	32.8586	32.1597	31.5502	31.0127

Figure-1 $\hat{R}$ for the changes in the inventory holding cost h

Figure- 2 $\hat{R}$ for the changes in k_1

Figure-3 $\hat{R}$ for the changes in k_2

Figure- 4 $\hat{R}$ for the changes in μ

Figure -5 $\hat{R}$ for the changes in the inventory holding cost h

Figure -6 $\hat{R}$ for the changes in k_1

Figure -7 $\hat{R}$ for the changes in k_2

Figure-8 $\hat{R}$ for the changes in μ

RESEARCH ARTICLE
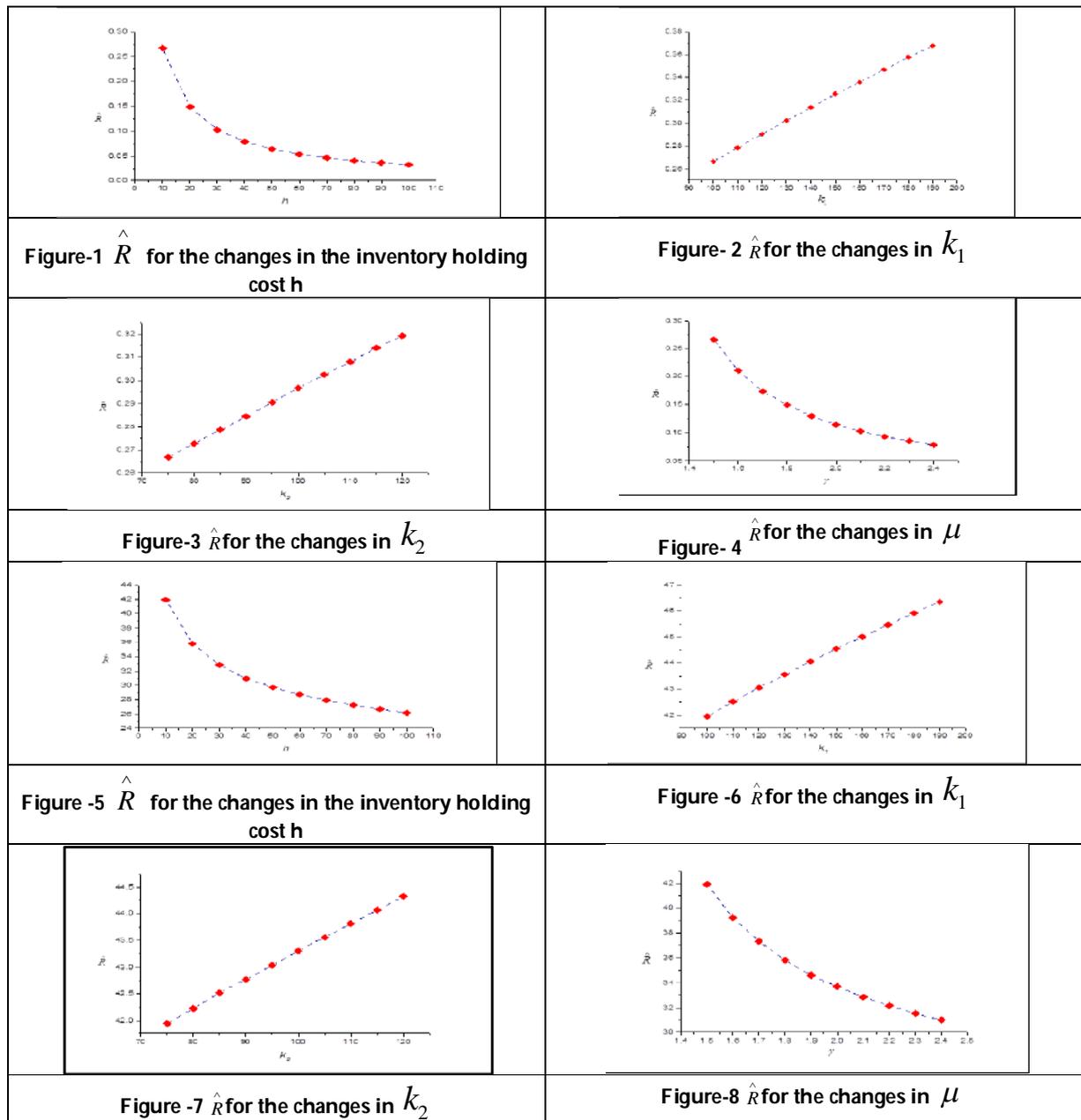
An Empirical Study on the Difference of Opinion Given by the Companies on csr Activity with Special Reference to Coimbatore Region.

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ABSTRACT

Corporate social responsibility is a strategy for businesses to give back to the society in which they thrive. The businesses legislation establishes minimal qualifying requirements and several parts of a company's CSR responsibility. A structures questionnaire was circulated among a sample of 67 respondents selected using probability sampling. Statistical tools such as Percentage analysis, Descriptive Statistics and One-way ANOVA Analysis were used for analysis. According to the study's results, the majority of businesses adhere to the same CSR templates, such as women empowerment, contributions, and scholarships, which enables them to easily meet their CSR quota. However, workers want businesses to make important contributions to society. Additionally, staff welcomed Covid-related events and donations to the relief fund. Participation of NGOs and the distribution of CSR monies to NGOs are also frequently considered as sustainable strategies. Employees assert that businesses, both legally and morally, has a duty to their employees. Employees also recognize that businesses that violate CSR regulations risk legal consequences. Employees also appreciate any assistance offered to smaller businesses since it benefits the company's brand, the economy, and society. Businesses must also be customer-focused, since the consumer is a critical component of the society that the business seeks to serve. Employees are also encouraged to participate in all of the company's public-oriented activities and initiatives.

INTRODUCTION

CSR is often portrayed as the process through which a firm balances economic, environmental, and social imperative while still achieving shareholder and stakeholder expectations.(Camilleri, 2017) In this context, it is crucial to differentiate between CSR, which may refer to a strategic business management initiative, and charity, sponsorship, or philanthropy(Porter & Kramer, 2002).A well-executed CSR strategy can result in a number of competitive advantages, including enhanced access to funding and markets(Cegliński & Wiśniewska, 2017), greater sales and profits(van Rekom et al., 2013), cost reductions(Perrini et al., 2011), improved productivity(Newman et al., 2020), an effective human resource base(Babcock, 2015), enhanced brand image and reputation(Mahmood & Bashir, 2020), customer loyalty(Islam et al., 2021), improved decision-making and risk management processes(Choudhary & Singh, 2012).

The majority of corporations are driven by moral convictions to embrace corporate social responsibility and doing so may result in a variety of benefits(Graafland, 2013). For instance, corporate social responsibility programmers may be a successful marketing technique, supporting a corporation in developing a good image with consumers, investors, and regulators(Hohnen, 2007). Additionally, CSR programmers have been shown to increase employee engagement and satisfaction—two crucial retention criteria. Such programmers may even attract people who have strongly held personal beliefs that fit with those of the organizations (Duarte Alonso et al., 2018). Finally, corporate social responsibility initiatives push business leaders to evaluate their recruiting and management practices(Frambo et al., 2021), their procurement of products or components(Hietbrink et al., 2010), and their value delivery to customers(Tekin et al., 2015). The bulk of the world's population resides in developing nations, each of which has its own unique set of social, political, and environmental challenges(Sherbinin et al., 2007). These nations are industrializing and are often characterized by insecure governments, increased unemployment, insufficient technical capability, uneven income distribution, unreliable water sources, and underutilized forces of production(United Nations, 2020). As a consequence of fast industrial growth, policies aimed at increasing foreign investment are followed, and investors are often eager to take advantage of fiscal advantages and inexpensive labour (Mcnally, 1999). While these techniques are economically rational, they have negative social and environmental consequences, including child labour, low or unpaid salaries, uneven career possibilities, occupational health and safety problems, and increased pollution(Tilt, 2016). These underlying factors that are unique to countries and regions influence the kind of activity undertaken by companies under CSR.

The companies act has under laid the foundational concepts on which the CSR of companies may be placed(Srivastava, 2021). Despite the fact that several facets of corporate social responsibility are allowed, most companies try to meet CSR requirements for the sake of it through minor philanthropic activities such as donations, scholarships etc.(Sanclemente-Téllez, 2017). This attitude towards CSR without taking real responsibility towards the society on which companies thrive corrupts the soul of the process. The current study attempts to trace these discrepancies through the opinion of company employees and hence arriving at the most commonly implemented CSR activities and the expectations of employees towards CSR.

REVIEW OF LITERATURE

Patil & Ramgouda, (2021)
Asserts that Corporate Social Responsibility has always been seen in India as a charitable endeavor. It is an activity that is carried out in accordance with Indian tradition but without purposeful action. At the moment, it has established itself as a yardstick for measuring business excellence. CSR was designed to meet a business's responsibility to society's needs, since business is an inherent element of society. All corporate enterprises have now recognized that business and society are inextricably linked rather than distinct. The purpose of this study paper is to determine the purpose of such CSR activities conducted by ITC Ltd. and HUL, as well as to assess both firms' CSR spending in various initiatives. The research is based on secondary data, and its primary purpose is to assess the

various kinds of CSR activities/projects conducted by both firms during the previous five years. These businesses have always placed a premium on contributing to the sustainable development of society and the environment, as well as on making the planet Earth a better place for future generations. The purpose of this research is to examine the evolution of CSR and to compare the CSR activities carried out by these two firms in India.

Amiri, (2019)

contemplates that Indian business firms have been adopting different corporate social responsibility (CSR) programmers for the benefit of society for many years. Corporate social responsibility has gained significance in the business sector in recent years from a variety of angles. Numerous Indian businesses have recognized the value of CSR efforts in marketing their brands. This research seeks to better understand organization's opinions of the CSR idea, the areas in which they have launched CSR practices, their CSR techniques, and the primary problems businesses encounter while implementing CSR activities. Throughout the research, it was shown that although individuals have varying interpretations of the CSR idea and have taken varying methods to CSR, enterprises in India face a number of problems. Additionally, the research examined the benefits of CSR and the primary objective of the firm in adopting CSR activities.

Poddar et al., (2019)

demonstrates the organic connection between the corporate social responsibility (CSR) activities undertaken by the Indian corporate sector and their alignment with the Sustainable Development Goals (SDGs) between 2014 and 2016, the period following the introduction of mandatory CSR under the Indian Companies Act. In this analysis, we highlight crucial areas related to SDG targets that the business sector has overlooked in terms of CSR efforts. We believe that further CSR expenditures should be made in climate change, biodiversity, sustainable consumption and production, marine life, and flora and fauna conservation. Sector research suggests that businesses operating in industries with a greater environmental footprint and effect are more concerned with CSR activities. The geographic study found that efforts should be made to improve corporate social responsibility spending in seven north-eastern states, Jammu & Kashmir, and Union Territories. This article advises that the system be re-evaluated in light of recent findings.

Ranjan & Tiwary, (2018)

writes that corporate social responsibility is critical in the age of globalization, not only for providing social services but also for popularizing the organization's brand. Additionally, it serves as a kind of advertising for industrial enterprises not just in India, but around the world. The research was undertaken at 10 reputable commercial and public sector organizations in the country to recognize the critical activities and effect of CSR. The research analyzed and assessed a variety of barriers and beneficial consequences. The data indicate that CSR benefits the organization's people resources, such as high morale, retention, quality output, and work satisfaction. As a result, CSR efforts should be integrated into all sectors in the nation, regardless of their financial capabilities. CSR also plays a role in risk management in a variety of ways. The competent authorities should conduct a thorough examination of the industrial organization's CSR efforts.

Tilt, (2016)

States that beyond the investigation of religious impact, it is important to examine sociocultural elements in various nations in order to comprehend CSR. Because perceptions of concepts such as CSR in Asia, the Middle East, and the Asian subcontinent are known to vary from those in the West, it is critical to understand how these differences might result in better (or worse) CSR results. The list of criteria that may be included is enormous, but some obviously significant ones include language, secularism, press freedom, access to information, value and attitude uniformity, and the presence of a national figurehead or personality. A longitudinal assessment of the economic growth process. Countries with rapidly developing economies, such as China and the Middle East; and countries with dramatically different historical economic contexts, such as Sri Lanka, where the need for development stems from conflict, provide rich contexts for examining how CSR is evolving alongside economic development.

OBJECTIVES OF THE STUDY

The study has been undertaken with the objective of study the different facets of Corporate social responsibility through the perspective of employees and to find the difference in opinion on CSR activities.

RESEARCH METHODOLOGY

Research Methodology supposedly solves the research problems. It may be understood as an art of scientific investigation. The methodology of the study would include conducting research with target audience as the employees of companies actively participating in CSR within the district of Coimbatore. This includes primary data collection by person administered survey for analysis and final interpretation of data.

Type of Research

It is descriptive type of research. Meaning that this research is to undergo research question, analysis of data and design.

Sources of Primary Data

The result of the project depends on the accuracy of the data. It is important that the data is appropriate within the researcher's bandwidth and context. Primary Data Collection Method was used for the study. This research includes primary data collected from employees of companies that involve in CSR activities. The study has used data collected from handing out questionnaires.

Type of Sampling

Since the population is countable, probability sampling has been taken. The simple random sampling technique has been followed by randomly selecting employees from different companies involving in corporate social responsibility.

No. of Sample Size: 67
Statistical tools used

Percentage analysis, Descriptive Statistics and One-way ANOVA Analysis.

ANALYSIS AND DISCUSSION

A. DEMOGRAPHIC FACTORS

The demographic variables are the core of any research as it represents the present conditions of the respondents.

INTERPRETATION

Table 1 represents the respondent company's number of years in business. According to 22.4 percent of the respondents, company has been in business for 1 to 5 years. 26.9 percent mentioned that the company has been in business for 6 to 10 years, 29.9 percent mentioned years of business as 11 to 15 years and 20.9 percent respondents were from companies that are above 15 years old in business. Nearly 80 percent of the companies are less than 15 years old.

INTERPRETATION

Table 2 records the nature of the company based on respondents' response. 26.9 percent of the respondents mentioned that their companies are private companies, 37.3 percent of the respondents were from public unlisted companies and 35.8 percent were from public listed companies.

INTERPRETATION

Table 3 records the criteria under which the respondent's company comes under CSR eligibility. 33.90 percent of the respondents mentioned that their company has a Net worth of rupees 500 crore or more. 30.51 percent responses mentioned that the company has a turnover of rupees 1000 crore or more. 35.59 percent of the responses mentioned a Net profit of rupees five crore or more. It must be noted that the above mentioned are the minimum eligibility criteria for a company to come under companies act for CSR.

INTERPRETATION

Table 4 displays the locality of the company. 28.4 percent of the respondents are from companies in urban region, 20.9 percent are from companies in sub-urban region, 26.9 percent are from rural region and 23.9 percent are from companies in semi-rural regions. As CSR opens opportunities to serve the regions around the company, the nature of the locality provides insight into the kind of contributions that can be done by the company.

INTERPRETATION

Table 5 records the data on percentage of average net profit allocated by the company towards CSR fund. 31.3 percent of the respondents mentioned that their company allocates less than 2 percent of average net profit as CSR fund. 29.9 percent mentioned that exactly 2% of average net profit is allocated for CSR and 38.8 percent of the respondents mentioned that more than 2 percent of average net profit is allocated for CSR.

INTERPRETATION

According totable 6 on the opinion of respondents on philanthropic responsibility of companies in CSR, the most popular opinions was 'Contributions to the COVID-19 relief fund help firms in meeting their CSR obligations and legislative requirements' with a mean score of 3.10 closely followed by the opinions 'Businesses might use non-governmental organizations (NGOs) for social work' and 'It is plausible to take measures against corporate social responsibility non-compliance' with a means core of 3.09. The least popular opinion was 'Businesses must provide scholarship programmes to encourage education' with a mean score of 2.94. It can be observed that COVID related relief measures are most expected by the respondents based on the current scenario. Also, companies cannot circumvent their philanthropic responsibility through providing scholarships alone.

INTERPRETATION

Table 7 records the respondent opinion on ethical responsibility of companies via CSR. The opinion 'EAP also includes fair labour policies' was the most popular among respondents with a mean score of 3.24 indicating that respondents are uncertain of fair labour policies within the company. The least popular notion was 'Apprenticeship programmes may assist local residents in obtaining employment with the organization' with a mean score of 2.94. This indicates that the employees are sceptical that such decisions may affect their employment.

INTERPRETATION

Table 8 records the respondent's opinion on legal responsibility of companies through the CSR framework. The opinion 'Larger firms must financially assist small and minority businesses' was the most popular notion among respondents with a mean score of 3.12. The least popular opinion was 'Purchasing goods from small businesses would help them grow.' With a mean score of 2.76. It can be observed that large companies must find ways of promoting smaller companies instead of only purchasing goods from them.

INTERPRETATION

Table 9 records the respondent's opinion on economic responsibility of companies under CSR. The most popular opinion 'Consumer protection measures contribute to the company's brand value' received a mean value of 3.33 closely followed by the notion 'Businesses may contribute to the development of good roadways' with a mean score of 3.21. The opinion 'Training programmes must be given to enable rural women to earn a living' received the least mean score of 2.91. It can be interpreted that the respondents expect a consumer oriented and public oriented CSR activity instead of travelling along off beaten paths such as women empowerment or scholarships. It must be

understood that respondents still support women empowerment but also expect better operations from the company.

INTERPRETATION

The null hypothesis "There is no significant relationship between years of incorporation and CSR responsibility "is rejected in the case of philanthropic, ethical, legal and economic responsibility of companies under CSR. Significant relationship was observed as the p-value was > 0.05 under all conditions tested. The difference was more significant within groups of same number of years of incorporation under philanthropic responsibility and economic responsibility. The mean difference shows that the difference was more pronounced between groups in the case of ethical responsibility and legal responsibility. Hence it can be concluded that there is a significant relationship between years of incorporation and CSR responsibility.

INTERPRETATION

The null hypothesis "There is no significant relationship between locality of the company and CSR responsibility" is rejected in the case of philanthropic, legal, and economic responsibility of companies under CSR. Significant relationship was observed as the p-value was > 0.05 under all conditions tested and accepted only in the case of ethical responsibility as p-value <0.05 under 5 percent level of significance. The difference was more significant within groups of same localities under philanthropic responsibility and legal responsibility. The mean difference shows that the difference was more pronounced between groups in the case of ethical responsibility and economic responsibility. Hence it can be concluded that there is a significant relationship between locality of the company and CSR responsibility in most cases.

FINDINGS OF THE STUDY

From the analysis data concerning philanthropic responsibility, it could be observed that most respondents were in favor of contributing towards COVID relief funds and projects as it is the need of the hour. Involvement of NGOs with companies in delivering the corporate responsibility towards the society is welcome by most. Also, the respondents welcome the fact that suitable countermeasures must be taken if the companies do not comply to CSR requirements. Also, employees believe that companies must contribute in a better way to the society rather than following the off-beaten track of women empowerment or scholarships even though they are not against them. Vis-à-vis the respondent opinion on ethical responsibility, employee expect a fair labour policy. This indicates that companies must also realize their responsibility towards their employees. Also, a cynic opinion towards employing local people is observed which might be an outcome of fear towards existing employee's job security. Under legal duty, it can be shown that workers believed that major corporations should find methods to promote smaller businesses rather than relying only on their purchases. Under economic responsibility of CSR respondents anticipate a consumer- and public-oriented CSR activity rather than deviating into familiar territory such as women empowerment or scholarship programmers. It is critical to understand that respondents continue to support women empowerment but also want the organization to operate on more efficient CSR contributions. Also, a significant relationship was noted between the maturity level of the company and its locality towards its CSR responsibilities.

CONCLUSIONS

Corporate social responsibility is a tool towards making companies contribute back to the society from which they are thriving. The companies act outlines minimum eligibility and different facets of responsibilities of companies under the CSR. From the findings of the study, it was seen that most companies follow the same templates such as women empowerment, donations and scholarships that help them achieve the CSR quota easily. But the employees expect companies to contribute to the society in a meaningful manner. Also, Covid related activities and contributions to the relief fund were most welcome by the employees. Involvement of NGOs and dispersal of CSR funds towards NGO activities is also widely accepted as sustainable measures. Employees claim that the companies have a responsibility towards its employees too both legally and ethically. Employees also accept the fact that

companies that do not comply to the CSR rules must face legal action. Employees also welcome any help extended to smaller companies as it contributes to the brand of the company, to the economy and the society. Companies must also be consumer oriented as the consumer is a vital part of the society which the company aims to serve. All public oriented activities and projects undertaken by the company are also welcome by the employees.

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Table 1: Number of Years In Business

No. of years in business	No. of Respondents	Percent
1 to 5 years	15	22.4
6 to 10 years	18	26.9
11 to 15 years	20	29.9
Above 15 years	14	20.9
Total	67	100.0

Source: Primary data

Table 2: Company Type

Company Type	No. of Respondents	Percent
Private	18	26.9
Public – Unlisted	25	37.3
Public- Listed	24	35.8
Total	67	100.0

Source: Primary data

Table 3: CSR Minimum Eligibility Criteria Is Satisfied By The Company According To Section 135, Companies Act 2013

Factor	No. of Respondents	Percent
Net worth of rupees 500 crore or more	40	33.90
Turnover of rupees 1000 crore or more	36	30.51
Net profit of rupees five crore or more	42	35.59
Total	118	100.0

Source: Primary data

Table 4: Nature of Locality of Company

Nature of locality of company	No. of Respondents	Percent
Urban	19	28.4
Sub-urban	14	20.9
rural	18	26.9
Semi-rural	16	23.9
Total	67	100.0

Source: Primary data

Table 5: Percentage of Average Net Profit Allocated By The Company For Csr Fund

Percentage Allocated	No. of Respondents	Percent
Less than 2%	21	31.3
Exactly 2%	20	29.9
More than 2%	26	38.8
Total	67	100.0

Source: Primary data

Table 6: Opinion on Philanthropic Responsibility of CSR

Factors	Mean	Std. Deviation
Businesses must assume social responsibilities in the process of reconstructing society.	2.99	1.33
Businesses might use non-governmental organizations (NGOs) for social work.	3.09	1.52
Businesses must contribute to society in the aftermath of natural catastrophes.	3.01	1.45
Contributions to the COVID-19 relief fund help firms in meeting their CSR obligations and legislative requirements.	3.10	1.45
Businesses must provide scholarship programmes to encourage education.	2.94	1.36
Businesses must support community health programmes.	2.99	1.42
Businesses must contribute to the construction of hospitals, schools, and other public facilities.	3.00	1.35
The Ministry of Corporate Affairs and the Government of India have made initiatives to address the needs of the society.	3.07	1.50
The government's efforts to promote corporate social responsibility are commendable.	3.06	1.35
It is plausible to take measures against corporate social responsibility non-compliance.	3.09	1.43

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It is plausible to take measures against corporate social responsibility non-compliance.	3.09	1.43
Valid N (listwise)		

Source: Primary data

Table 7: Opinion on Ethical Responsibility of CSR

Factors	Mean	Std. Deviation
Employee assistance programmes (EAP) must be included as part of corporate social responsibility (CSR).	3.00	1.52
Employees' family members must also be enrolled in the EAP.	2.96	1.52
EAP also includes fair labour policies.	3.24	1.38
Apprenticeship programmes may assist local residents in obtaining employment with the organization.	2.94	1.43
Women empowerment policies for employment must be implemented.	3.12	1.39
Avoiding child labour in any form is also a contribution to society.	3.03	1.49
Businesses must take responsibilities for pollution reduction.	3.13	1.31
Any kind of waste material must be dealt appropriately.	3.09	1.49
CSR must include policies aimed at ensuring the sustainable use of natural resources.	3.04	1.48
Businesses must take care not to jeopardize the surrounding eco-system.	3.09	1.49
Valid N (listwise)		

Source: Primary data

Table 8: Opinion on Legal Responsibility of CSR

Factors	Mean	Std. Deviation
Larger firms must financially assist small and minority businesses.	3.12	1.41
Purchasing goods from small businesses would help them grow.	2.76	1.46
Subcontracting with major enterprises might incentivize small businesses to develop new technology.	3.00	1.40

Collaboration on projects will assist small businesses in upskilling their employees.	3.00	1.39
Profitable businesses attract greater international investment.	3.07	1.32
Businesses with a stronger brand identity and reputation attract more overseas investment.	3.04	1.41
Valid N (listwise)		

Source: Primary data

Table 9: Opinion on Economic Responsibility of CSR

Factors	Mean	Std. Deviation
Consumer protection measures contribute to the company's brand value.	3.33	1.43
A company's products must guarantee that customers are not harmed.	3.10	1.49
Businesses that are receptive to customer input generate greater goodwill.	3.24	1.38
Businesses that practice CSR may acquire customer confidence.	3.28	1.45
Rural education initiatives must be implemented.	3.03	1.41
Companies may fund health-related institutions/camps.	3.12	1.33
Businesses may contribute to the development of good roadways.	3.21	1.43
Rural areas must be encouraged to participate in Clean India projects.	3.06	1.38
Training programmes must be given to enable rural women to earn a living.	2.91	1.33
Valid N (listwise)		

Source: Primary data

Table 10: Mean Score Difference between Years Incorporated And Csr Responsibilities

		N	Mean	Std. Deviation
Philanthropic Responsibility	1 to 5 years	15	31.20	4.16
	6 to 10 years	18	30.78	4.85
	11 to 15 years	20	29.55	3.47
	11 to 15 years	14	30.00	4.95
	Total	67	30.34	4.29
Ethical Responsibility	1 to 5 years	15	32.20	2.91
	6 to 10 years	18	29.50	6.00
	11 to 15 years	20	29.15	4.04
	11 to 15 years	14	32.50	3.82
	Total	67	30.63	4.58
Legal Responsibility	1 to 5 years	15	16.40	3.36
	6 to 10 years	18	18.17	3.13
	11 to 15 years	20	17.95	3.35
	11 to 15 years	14	19.57	3.18
	Total	67	18.00	3.35
Economic Responsibility	1 to 5 years	15	26.87	4.52
	6 to 10 years	18	28.67	4.54

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	11 to 15 years	20	28.30	6.39
	11 to 15 years	14	29.29	4.20
	Total	67	28.28	5.06

Table 11. ANOVA Analysis Between Years Incorporated and CSR Responsibilities

ANOVA						
CSR Responsibilities		Sum of Squares	df	Mean Square	F	Sig.
Philanthropic Responsibility	Between Groups	28.643	3	9.548	.506	.679
	Within Groups	1188.461	63	18.864		
	Total	1217.104	66			
Ethical Responsibility	Between Groups	152.722	3	50.907	2.605	.059
	Within Groups	1230.950	63	19.539		
	Total	1383.672	66			
Legal Responsibility	Between Groups	73.521	3	24.507	2.310	.085
	Within Groups	668.479	63	10.611		
	Total	742.000	66			
Economic Responsibility	Between Groups	46.821	3	15.607	.599	.618
	Within Groups	1640.790	63	26.044		
	Total	1687.612	66			

Table 12. Mean Score Difference Between Locality of the Company and CSR Responsibilities

CSR Responsibilities		N	Mean	Std. Deviation
Philanthropic Responsibility	Urban	19	31.16	4.60
	Sub-urban	14	29.64	4.34
	rural	18	30.33	3.66
	Semi-rural	16	30.00	4.77
	Total	67	30.34	4.29
Ethical Responsibility	Urban	19	28.37	4.51
	Sub-urban	14	31.29	5.43
	rural	18	32.78	3.84
	Semi-rural	16	30.31	3.61
	Total	67	30.63	4.58
Legal Responsibility	Urban	19	18.00	3.04
	Sub-urban	14	18.14	3.28
	rural	18	17.72	3.85
	Semi-rural	16	18.19	3.49
	Total	67	18.00	3.35
Economic Responsibility	Urban	19	26.89	5.16

	Sub-urban	14	28.07	5.72
	rural	18	28.11	4.43
	Semi-rural	16	30.31	4.80
	Total	67	28.28	5.06

Table 11. ANOVA Analysis Between Locality of the Company and CSR Responsibilities

ANOVA						
CSR Responsibilities		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Philanthropic Responsibility	Between Groups	21.364	3	7.121	.375	.771
	Within Groups	1195.741	63	18.980		
	Total	1217.104	66			
Ethical Responsibility	Between Groups	187.845	3	62.615	3.299	.026
	Within Groups	1195.827	63	18.981		
	Total	1383.672	66			
Legal Responsibility	Between Groups	2.237	3	.746	.064	.979
	Within Groups	739.763	63	11.742		
	Total	742.000	66			
Economic Responsibility	Between Groups	103.679	3	34.560	1.375	.259
	Within Groups	1583.933	63	25.142		
	Total	1687.612	66			

RESEARCH ARTICLE

Study of *Citrus* Fruits Waste Chemical Structure and Antimicrobial Activities of *Citrus limetta*

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ABSTRACT

Citrus fruits (CF) are among the most widely cultivated fruit crops throughout the world and their production is constantly increasing along with consumers' demand. Therefore, huge amounts of waste are annually generated through CF processing, causing high costs for their disposal, as well as environmental and human health damage, if inappropriately performed. The present study is carried out to evaluate the chemical structure of CF residues (i.e., seeds, exhausted peel, pressed pulp, secondary juice and leaves) and antimicrobial activity of different solvents extracts. Further experimental finding in the screening of bioactive compounds against various pathogens, Minimum Inhibitory Concentration (MIC) of extract and Qualitative analysis of phytochemicals on Citrus limetta has been made. Antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and zero or no antifungal activity against *Candida albicans*, *Trychophyton rubrum*, *Microsporum* and *Aspergillus niger*. Among all extracts Citrus limetta juice cold water has lowest MIC i.e.22.85 mg/ml in S. aureus and maximum in pomace column extract 42.42 mg/ml in E. coli but no MIC seen in seeds

Keywords: Citrus; waste; Antimicrobial activity; flavonoids; polyphenols; nutraceuticals; MIC, Citrus peel; phytochemicals

INTRODUCTION

Now as the urbanization is increasing day by day and use of drugs as medicine to cure diseases is establishing deep roots in society (which is having many side effects also), it becomes necessary to introduce naturally made medicines which can assure to have a cure without any side effects. In the last decades, the enhancement of life quality brought an unceasing growth of the worldwide population, leading to an excessive consumption of resources and, consequently, to a considerable production of waste. Therefore, a recycling/re-use perspective is necessary to allow the reduction of the extent of produced waste and related socio-economic costs [1]. *Citrus* fruits (CF) represent the most widely cultivated, processed and consumed fruits throughout the world thanks to their pleasant taste, and they have become relevant components of the diet also for their acknowledged beneficial properties, such as antioxidative, anti-inflammatory, anti-infective, anti-cancer and neuroprotective [4,5,6,7,8,9].Reviews suggest that flavonoids and phenolics were significantly greater in peel than the pulp, seeds and hence their fruit husk extracts shows antiproliferative activity against a panel of human oral, colon and prostate cancer cell lines. Citrus fruit contain high concentration of phenols, flavonoids, glycosides, hesperidins, hydrixycinnamates and its flavones analogue, diosmin etc. that all have exhibited anti- carcinogenic activity in various *in vitro* studies[2].

CITRUS FRUITS AND CITRUS WASTE

The genus *Citrus* L. belongs to the Rutaceae family, subfamily Aurantioideae [**11**]. The ultra structural study of fruit peel by means of scanning electron microscopy has clarified the schizolysigenous nature of the secretory tissues, where the essential oil is produced and stored [12]. Essential oil cavities occur characteristically also in the mesophyll of the leaves and in the petiole [12].Almost a fifth of the total *Citrus* cultivars are subjected to industrial processes [8], mainly for the production of their juices that represent the most consumed fruit juices worldwide, thus generating a large amount of processing waste (about 120 million tons per year) [10,15].However, this industrial process exploits only the 45% of the total fruit weight, whereas the rest, such as peel (flavedo; 27%), pulp (albedo and endocarp; 26%) and seeds (2%), constitutes a disposal rest [15].This waste is often thrown into landfills or rivers, causing environmental pollution or contamination of water, with depletion of dissolved oxygen levels. The high rate of pollution given by *Citrus* waste depends to its easy ferment ability, since it is abundant, chemically complex, biodegradable, thus requesting high oxygen demand. Generally, it has a low pH (3–4), with a high content of water (80–90%) and organic matter (95% of total residue) [16].Therefore, in order to reduce environment pollution and gain profit to industries, several alternatives were proposed for better management of CF waste, such as the production of fortified animal feeds, the use of fiber-rich components in confectionery products, the extraction of macro- and micro-nutrients, as well as the production of organic fertilizers, bio-fuels, enzymes and ethanol, employed in the food, pharmaceutical, and cosmetic industries [3,14,17].

COMPOSITION OF CITRUS WASTE

CF peel represents almost 50% of the wet fruit mass after juice extraction [**18**], and is particularly high in fragrant compounds, dietary fibers, pectin, natural pigments as well as polyphenols [19]. CF peel is mainly employed for the extraction of essential oils (EOs). Chemical composition of EOs consists of monoterpenes and sesquiterpenes compounds (i.e., hydrocarbons with two or three isoprene units in their structure) and oxygenated derivatives (i.e., alcohols, ketones, aldehydes and esters; Figure 1). Exhausted CF peels are a source of pectin and dietary fibers, which are also present in juice and pulp [21]. Pectin is a complex polysaccharide composed of D-galacturonic acid units linked together by α-1,4 glycosidic bonds, partially esterified with methanol or acetic acid (Figure 2). Flavonoids are a wide class of secondary metabolites, synthesized by plants to protect against ultraviolet radiation or pathogenic injuries. According to their structure (Figure 3), they are subdivided into six groups, which are flavones, flavanones, flavonols, isoflavones, anthocyanidins and flavanols [20,21]. *Citrus* flavonoids have been extensively studied for their anti-cancer [22], anti-inflammatory [23] and neuroprotective activities [24] Phenolic acids are also present in some amount [24] (Figure 4). They are divided into hydroxybenzoic (gallic, vanillic and syringic acids) and

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hydroxycinnamic acids (caffeic, ferulic, p-coumaric and sinapic acids), known to possess high levels of free radical scavenging activity [25].

MATERIALS AND METHODS

Sample collection& Extract preparations

The Sample *Citrus limon* was collected from local market of Ram University, Kanpur, UP. Used parts of sample were juice, peels and seeds. Antimicrobial activity of *Citrus limetta* peel extract perform at RRPG, Amethi, Awadh University, Ayodhya and Ram University, Kanpur, UP. Single colony from each of the cultured was added to 50ml of steriled liquid media & incubated at 37º C for bacteria 28º C for fungal pathogen for overnight. Media was removed by centrifuging the culture at 10,000 rpm for 5 min. and cells were resuspended in DW. Optical density of culture was maintained to 0.1 at 600 nm before use by diluting with steriled water.

Screening of bioactive compounds against various pathogens

The antimicrobial activity of *C. limetta* peel, juice, seeds and pomace extract was determined by agar well diffusion method against *S. aureus, P. aeruginosa* and *E. coli* [1]. About 20µl of each test bacterium was inoculated by pour plate method in sterile agar plate. After solidification of media wells were prepared by sterile borer and wells were filled by 30µl test sample, positive (Tetracycline if 50µg/ml) and negative control (autoclaved distilled water). Plates were incubated aerobically at 37 ^{0}C for 18 hours.

RESULTS AND DISCUSSION

The experiment was conducted on the collected samples and observation are summarized in different tabulated form (Table-1, 2 & 3)

CONCLUSIONS

Nowadays, a proper management of CF waste is crucial in order to enhance the sustainability of their cultivation. Indeed, a re-evaluation of CF by-products could reduce their accumulation in the environment, as well as allow the exploitation of their full potential. This because CF by-products are an economic and renewable source of high value-added compounds, hence researchers should follow a holistic approach to appreciate the whole CF, including its waste, employing better strategies for its recovery and valorization. This review provides scientific bases for the employment of CF by-products as nutraceuticals. To study the antimicrobial properties of such plant metabolites, solvent extraction procedure was adopted using various solvents and extraction conditions. Among all methods adopted, for several plant parts, yield was maximum for dried juice followed by seed and peel (Table 1). Plant extracts responded differently when screened against various pathogens, seeds extracts were found less active against pathogens in comparison to peel and juice (Table 2)

ACKNOWLEDGMENT

CONFLICT OF INTEREST

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Table-1: Qualitative analysis of phytochemicals

SN	Phytochemical constituents	Activity Performed	Results
1	**Test for reducing sugar**	Take 1 ml and 1gm of sample in a test tube and 10ml deionized water then add few drop of Fehling solution and heat at 40^0C in water bath. Brick red precipitate indicates positive result	+++
2	**Test for tannins**	2gm of aqueous extract in test tube add 2 drops of 5% ferric chloride if gives green color then test will be positive	++++
3	**Test for phlobatannins**	Take 10ml of aqueous extract and boil with few drop of 1% HCL. Deposition of red precipitation gives positive result	+
4	**Test for saponins**	Take of aqueous extract in test tube and add 5ml de-ionized distilled water and shake it vigorously allow it for few minutes if froth last for 15 minutes means presence of saponins. Olive oil can also be added for checking oil emulsion.	+++
5	**Test for terpenoids**	Take 5ml of aqueous extract add 2ml $CHCl_3$ followed by addition of 3ml conc. H_2SO_4 observe the reddish brown interface for presence of terpenoids.	++

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Table-2: Yields of different parts of *C. limetta* extracts

S. No.	Extracts	Yield (in g/100g of sample)		
		Peel	**Juice**	**Seed**
1	Methanol suspension	0.512	60.11	14.85
2	Methanol column	0.411	64.25	17.11
3	Methanol Soxhlet	2.30	72.85	10.96
4	Hot water extract	0.430	75.00	8.01
5	Cold water extract	0.39	41.10	15.62

Table-3: Antimicrobial activity of *Citrus limetta* peel extract

	Extracts (400mg/ml)	Zone of inhibition in mm		
		P. aeruginosa	*S. aureus*	*E. coli*
P E E L	Methanol + 70% Ethanol	0	0	0
	70% Ethanol	0	0	0
	80% Methanol +20% Ethyl acetate	0	0	0
	50%Methanol + 50% Ethyl acetate	0	0	0
	Hot water	10	12	12
	Cold water	14	12	15
	Tetyracycline	19	30	24
J U I C E	Methanol column	15	16	14
	Methanol suspension	16	16	14
	Methanol Soxhlet	14	14	18
	Hot water	1.8	1.6	1.7
	Cold water	1.8	1.9	1.6
S E E D	Tetracycline	21	30	26
	Methanol column	0	0	0
	Methanol suspension	0	0	0
	Methanol Soxhlet	0	0	0
	Hot water	0	0	0
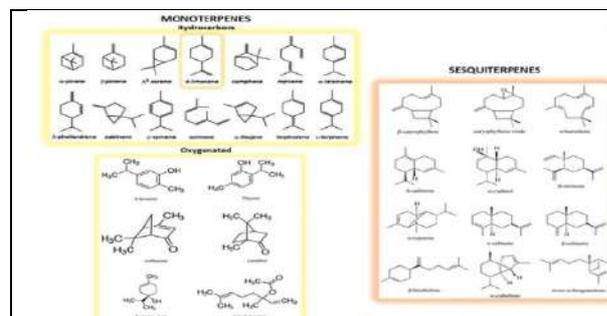
Figure 1. Structures of the main monoterpenes and sesquiterpenes present in CF peels.
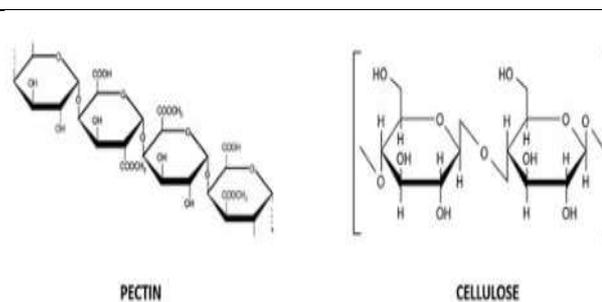
Figure 2. Chemical structure of pectin (D-galacturonic acid units linked together by α-1,4 glycosidic bonds; on the left) and cellulose (glucose units −300−3000 molecules- linked by a β-1,4 glycosidic bond; on the right), soluble and insoluble dietary fibers, respectively.

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Figure 3. Chemical structures of the main classes of flavonoids present in CF
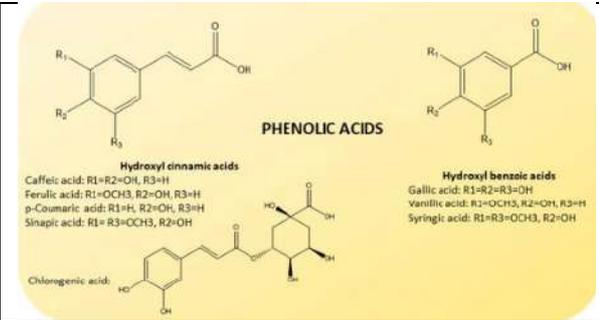
Figure 4. Chemical structure of phenolic acids present in CF juices.

RESEARCH ARTICLE

A New Modified DC-DC Converter for Output Voltage Regulation in DC Grid Wind System

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ABSTRACT

This paper proposed the Levy flight-based chicken swarm optimization (LFCSO) to follow the grid-joined wind framework with the highest power. In order to analyse the grid-associated wind framework, current, power, voltage, and irradiance characteristics are determined. Due to the low yield voltage of the source wind, a large advance up converter with high prosductivity is required when linked to the matrix power. The story of a large advance up converter based on swapped capacitors and inductors is told in this paper. The LFCSO algorithm with the adaptive neuro-fuzzy inference system is used to create control pulses for the transformer-coupled inductor DC–DC converter-less switching capacitor. The voltage addition in the DC–DC converter is increased when the switching capacitor-coupled inductor is used, and the wind power is maximised. To provide optimal results, the standard CSO method is upgraded with the help of Levy flight functions. The output of the suggested LFCSO algorithm is used as the input of the ANFIS technique to provide accurate and optimal results. Following that, the best findings are obtained, which serve as the system's pulses. Using the proposed technique, the working guideline is examined, and the voltage addition is calculated. From that point on, it forecasts the converter's exact maximum power based on its inputs. The wind system is tested and its properties are studied in different time instants under a variety of wind irradiance and partial shade conditions (PSCs). The suggested LFCSO with ANFIS method is implemented in Simulink/MATLAB, and the tracking performance is compared to that of traditional methods such the genetic algorithm (GA), perturb and observe (P&O) technique–Neuro-fuzzy controller (NFC), and fuzzy logic controller (FLC).

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Keywords: Transformer less switched capacitor coupled DC-DC converter voltage, current, ANFIS, Power, Wind grid connected, and optimization.

INTRODUCTION

The matrix-associated inverter is an important component of a wind-generating system. In the current lattice-associated wind system, the voltage source inverter with the current following limit is used. The output voltage, on the other hand, must be greater than the matrix voltage. The voltage source inverter becomes a buck inverter for DC-to-AC control shift in this manner. When the wind has a lattice voltage greater than the cluster voltage, an extra boost from a DC–DC converter is usually required to achieve the optimal AC yield. To make this a possibility, a highly effective low-effort item should be planned [1]. Only a few of the various converter plans are capable of providing high control with high efficiency. The primary mode of exchange Because of their numerous advantages, such as simple structure, high efficiency, high productivity, simple design, and basic control circuit, DC–DC converters such as buck, support, buck-help, Cuk, zeta, and forms have been used in various electronic applications. The additional power converter stage raises system costs and reduces efficiency. (a) Each inverter arm's upper and lower switch devices cannot be gated on at the same time. Surprisingly, a shoot through will completely destroy the electronics. (b) Dead time must be used, resulting in current yield contortion [2].

Convertibles have excellent execution and a high advance rate. Recently, several analysts have been striving to combine these types of converters into another form of combination converter in order to achieve a large advance up voltage gain. 7 Furthermore, basic switched-capacitor (SC) converters offer a wide range of applications due to the non-magnetic materials they use, as well as their small size and high power density [3]. Changing the turn proportions in a converter-based coupled-inductor can also result in a significant increase in gain voltage. However, the vitality stored in the spilled inductor causes a spike voltage on the basic switch, which causes the switch to fail [4]. The previously described problem of voltage source converters is overcome by a new transformer-less exchanging capacitor-coupled inductor DC–DC converter. A few research papers and lists have addressed the issue of low outputs and power losses in wind turbines, and have strongly recommended the use of an influence streamlining technique known as maximum power point tracking (MPPT) [5].

With the help of the DC–DC support converter, power losses are minimised. The transformer-less switching capacitor-coupled inductor DC–DC converter has numerous advantages in this application; it boosts wind current. Furthermore, the suggested converter exhibits the highest power in the wind to satisfy grid applications, requiring the MPPT to be concentrated. The majority of the most severe power point processes are based on the assumption that every phone in a similar module and total module in a similar string receives a comparable ordinance [6]. Perturb and observe (P&O) and gradual conductance are two widely used algorithms in commercial battery controllers and lattice-connected inverters that can perfectly follow the MPP under uniform lighting conditions. Existing approaches do not provide higher accuracy in partial shade conditions. Many optimization techniques developed by the researcher to address these challenges include the Ant Bee Colony Algorithm (ABC), Firefly Algorithm (FA), BAT algorithm, and others. Those techniques do not produce precise and optimum pulses. The disadvantages are mitigated by recently discovered algorithms [7].

Contribution of the research

- The research's main contribution is to look into the maximum grid power-associated wind system using a SC-coupled inductor DC–DC converter.
- The LFCSO with ANFIS algorithm is presented to improve the performance of the switched capacitor-coupled

inductor-based DC–DC converter.
- The wind system, which is connected to the grid, generates the most energy.
- Normal partial shading circumstances and irradiance are used to dissect the matrix-associated wind system.

The rest of the paper is organized as follows: the issue plan and the detailed clarification of the proposed method are introduced in Section. 3. Past to that, the ebband flow work research are displayed in Section. 2. The test outcomes and dialog are determined in Section. 4. Finally, Section. 5 finishes up the record. The remainder of the paper is organised as follows: Section 3 introduces the issue strategy and the comprehensive elaboration of the proposed method. Previous to that, Section 2 displays the ebb and flow work research. Section 4 determines the test results and dialogue. Finally, Section 5 brings the work to an end.

LITERATURE SURVEY

Various ways for analysing the features of matrix-associated wind are demonstrated, and some of them are reviewed here. [8] demonstrated a non-detached large advance up DC–DC converter with two inductors that is suitable for application generation. Their shown DC–DC structure gets shared information present with low swell by implementing an information parallel association, which also required a small capacitive channel at its information. In addition, by utilising double inductors in arrangement association at the yield organise, their topology can achieve high-gain voltage. The principle switches in their proposed converter are dynamic brace circuits with a common clamp capacitor [9]. [10] used a matrix-connected moveless wind inverter based on a DC–DC converter to maintain high efficiency even when the wind voltage falls below the peak estimation of lattice voltage. To minimise the evolution of fundamental mode spillage, a modulation approach was discussed. Furthermore, the eciencies of various transformer-less inverter topologies were explored and compared to the proposed topology. To confirm the investigation, point-by-point recreation experiments were done in Simulink/MATLAB.

In [11] described the main challenges in transformer-less topologies just as giving a survey on single-stage framework-associated wind systems, which were ordered into six gatherings depending on the quantity of switches required in the systems. The essential operational standards of diferent plans under the six classes of inverters were exhibited and thought about as far as spillage current, qualities, efciency, and weaknesses. Moreover, their proposed inverter structure and a pay procedure for wiping out spillage current have been discussed, followed by a neat report with the accessible ones. In [12] showed a wind system with a cascaded control scheme. It had two control loops, such as an external loop voltage controller and an inward circle current controller, for example. While the controls yield the flow of the inverter, the external circle voltage controller was used to ensure continual equalisation in the strong stream between the wind clusters and power lattice. In addition, the DC–DC support converter was programmed to maintain a constant voltage at the inverter's contribution. In this paper, a gradual conductance-based MPPT technique is used to obtain the equalisation power and focus on the biggest power. The present controller was created using a nonlinear versatile back venturing technique to command the grid's dynamic and responsive sections. The direction of these flows is directed toward the desired attributes, hence controlling the dynamic and receptive power transmitted into the network. According to the definition of control Lyapunov functions, the general strength of their framework was examined (CFLs).

In [13], all silicon carbide control semiconductors were used to create a 20 kHz, 20 kW high frequency (HF) most extreme power point following converter for a framework-associated wind supply. To improve the current transformation competence and power thickness, the researchers used SiC control MOSFETs on the small-voltage wind board side and silicon carbide Schottky diodes on the high-voltage DC yield. The next set of working standards, as well as its design circuit, are described in great detail. In terms of efficiency, the performed converter was compared to that of an old type IGBT and a mixture Si-IGBT rely on MPPT converter.

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The most extreme power point following is a significant approach to apply in order to obtain high exhibitions of a wind module with matrix associations and transform-less switching capacitor-coupled inductor DC–DC converter. P&O strategy, sliding mode control, particle swarm optimization GA, PSO, hill climb searching (HCS) control, FLC, incremental conductance (INC), and artificial neural networks are among the MPPT algorithms that have been investigated in writing (ANNs). ANN has a good presentation, but it has a few flaws, particularly in the rapid variation of climate conditions [14]. The P&O computation has a poor performance when there is an unanticipated difference in light variety. The FLC is well-known for its strength and remarkable resistance to disruptions.

The layout and modification of the FLC, on the other hand, necessitate a large number of prompts, a significant amount of computing time, and the use of a significant amount of memory. In reality, wind energy sources typically have a thin power thickness and are unable to maintain a constant yield voltage under extreme stacking conditions. The PSO system is based on the underlying part of the wind array, which takes an assembly rate under particular conditions. Although GA is one of the most effective methods for addressing optimization problems, it occasionally fails to handle particular variant optimization problems. It happens primarily as a result of inadequately developed wellness capacities, which result in terrible chromosome obstructs regardless of how lone wonderful chromosomal squares traverse [15]. All things considered, there's no absolute guarantee that a GA will find a global optimum. ABC necessitates a large number of iterations in order to optimise the answers, resulting in a higher computing cost. Artificial intelligence-based MPPT algorithms are desperately needed to solve these issues. The gain voltage in the DC–DC converter is not reduced in this proposed manner, and wind power is maximised [16]. The use of a switched capacitor-coupled inductor can result in a transformer-less switched capacitor-inductor DC–DC converter. The preceding part clarifies the portrayal of the described technique.

PROPOSED METHODOLOGY

The wind system is examined and integrated with the grid in this paper. An inverter is gathered with the use of the matrix to provide high control and maintain optimal operation. The output voltage of the wind must be enhanced with the help of a high advance up DC–DC converter for the best operation analysis. As a result, the voltage addition must be raised in order to increase wind intensity. A SC converter with a connected inductor can expand the voltage addition in this case. By charging the switched capacitors in parallel and releasing them in series while employing the voltage addition, the SC converter can be enlarged [17]. In any case, more switching capacitors are required when the voltage addition is increased.

Which are the elements that make up the topology. The voltage addition can be greatly increased by enveloping the inductor linked and changing the turn's percentage. The switched capacitor can be used to keep the energy stored in the spilled reactance while also increasing the voltage gain. As a result, no additional circuit snubber is required. As a result, a transformer-less high step-up DC–DC based on an exchanged capacitor and coupled inductor is proposed in this study. Normally, the P and O model is used to build and develop the control topology of MPPTs, however in this study, Levy flight-based chicken swarm optimization (LFCSO) is presented [18]. The network-associated wind framework, which is based on the suggested LFCSO algorithm, uses a transformer-less switching capacitor and a coupled inductor. Figure 1 shows the design of the grid-connected wind using the proposed method. It includes the wind array, grid, MPPT controller, and transformer-less most advance-up DC–DC converter, which is powered by an exchanged capacitor and connected inductor. The proposed converter-based grid-associated wind system's modelling and operation are detailed in the accompanying segment [19].

The wind system is employed to generate power in Figure 1 to meet the weight requirements on the framework side. Because of different circumstances such as PSC conditions, the wind cannot provide power continually. For the most part, the heap properties are used in the wind module activity. The optimal

adaptation happens only at one specific working point, which is defined as the most extreme power point, based on the load and internal resistance characteristics. When the temperature of the wind varies, the wind-generated power is insufficient to fulfil the weight need. To solve the problem, the controller in the DC–DC converter between the source and the load must be developed. Because changes in temperature and irradiance might affect the wind system, the MPPT controller must watch the most extreme power point in its linked curve whenever the irradiance and temperature vary [20]. The transformer-less huge advance DC–DC converter dependent on the switched capacitor and inductor linked is illustrated in this paper to build the presentation of the MPPT controller.

Modeling and operation of wind system

A general mathematical description of wind is offered in this section. The wind is considered an electrical device since it converts light energy to electricity. Wind boards provide a cost-effective source of energy. A potential contrast is formed when photons from daylight are retained and electrons from mole- cules are discharged on this semi-conductor board. This creates a flow stream in the material that ignores the potential contrast and consequently captures electricity [21]. Figure 1 depicts the primary use of a model for MPPT advances when using the proposed approach. A photocurrent, a diode, a resistor parallel communicating a current spillage, and a game plan resistor describing an internal protection from the current stream are all represented in the equal circuit of the general model. A wind's power and voltage are represented as follows:

$$I_c^{wind} = \frac{I_{ph}}{1-C^{-d}}\left[exp^{\frac{V_{oc}}{a}\left(\frac{1}{NKT}\right)} - 1\right] - \frac{V+R_s I}{R_{sh}} \qquad (1)$$

Wind models are expressed and controlled based on irradiation ranges. Each wind can operate at different irradiation levels between 600 W/m2 and 1000 W/m2 at any given time. The wind output power has been modified based on the irradiation, and it may be maintained constant by employing the right control signals generation for the DC–DC converter. The MPPT controller regulates the DC–DC converter's obligation cycle to maintain the wind operating point, which is usually at the MPP. The proposed MPPT-based controller is used to change the DC–DC converter's obligation cycle as specified by their inputs. The suggested DC–DC converter architecture and method of operation are described in detail in the next section [22].

Proposed SC and coupled inductor-based step-up DC–DC converter

The theoretical analysis of the converter and its various methods of activity are clarifed in this section. In this paper, we develop the high advance up DC–DC converter which is dependent on the switched capacitor and inductor. To increase the power of the converter, the coupled inductor is incited into the big advance up DC–DC converter depending on the switched capacitor [23]. To assimilate the vitality put away in the spillage reactance, the exchanged capacitor is prompted in the DC– DC converter. In this way, there is no compelling reason to include the snubber circuit into the plan of DC–DC converter. The suggested high advance up DC–DC converter is based on a switched capacitor and a linked inductor, which reduces switch voltage stress and switching loss. Furthermore, the illustrate converter's conduction loss is reduced thanks to the low on-state resistance MOSFET [24]. Figure 2 illustrates the circuit pictures of the illustrate converter. the circuit images of the illustrate converter are demonstrated in Figure. 2.(a),(b)

Analysis of the proposed DC–DC converter

The regulation of the yield current of switched capacitor con- verter, an inductor vitality stockpiling cell embraced to numerous switched capaci- tors, is illustrated in Figure. 3(a). After that, the switched capacitors C_1—C_4 can be directed. To achieve the big voltage gain, the switched capacitors are required to supply the weight. The connection of the switching capacitor depends upon four modules; one model is represented in Figure. 3(b). From Figure. 3(b), when switch Q is turned on, C_2 is in arrangement with the information voltage

and C_3 to deliver the load. The power increase of the big advanceup DC–DC converter is improved receiving the switched capacitor [25].

The controlled voltage is described, as well as the predicted voltage. Q is in the state of being turned off: The input voltage source, regulated voltage source, and inductor are all connected in an arrangement connection to charge C2. In contrast to the huge advance up DC–DC converter receiving SC, when the SC in the loop is used, the voltage of C2 is increased.

Voltage operation of converting

Three assumptions are introduced in this section to examine the voltage addition of the proposed converter, which are presented in the next section [26].

- All of the power gadgets on display are excellent.
- All of the capacitors are sufficiently large, and voltage swells are ignored.
- Because the spilling reactance is much lower than the polarizing inductance, mode 2 and mode 4 spans are shot, and mode 2 and mode 4 spans are discarded.

The proposed converter's power boost is mentioned in the study. In the study of Figure 3(b), the proposed converter and current converter are reported in Table 1. A DC–DC converter is included in the planned system to follow the most powerful wind. The high advance up DC–DC converter, which is dependent on the inductor coupled and exchanged capacitor, is designed here. The LFCSO-based ANFIS controller is used to achieve the best pulses. The proposed algorithm's approach is described in the following section.

RESULTS AND DISCUSSION

The proposed LFCSO technique, which is implemented in the MATLAB/Simulink [30] operating stage, is used to evaluate grid-connected wind. The proposed method is used to design the source said converter and grid side converter in this study. The proposed method, which is based on the current and voltage of the wind panel, is used to obtain the converter's optimal control pulse generation. The proposed technique brilliantly utilizes the voltage and current qualities as contributions to generate high wind power. The wind architecture has been thoroughly tested in both heap and grid operation environments. The sources of wind are classified as (a) constant condition and (b) ramp condition, respectively, for both of the aforesaid situations. In both loading circumstances, the wind is tested with the use of the proposed technique, which is compared to current techniques such as the P&O technique, FLC, and GA-NFC.

The wind irradiance is investigated in three scenarios, each with different load and grid operating parameters. The MPPT controller is constructed utilizing the proposed LFCSO approach, and the maximum power for a wind system is generated. The current and voltage control of the switched capacitor-inductor DC–DC converter circuit were predicted in order to achieve the best sinusoidal waveform [31]. This framework was reenacted in order to obtain proficiency with the wind-network-associated framework's activity. The proposed technique is compared to existing strategies such as the P&O procedure, FLC, and GA-NFC system. Initially, the panel irradiance, voltage, currents, and power are examined using the P & O approach, as shown in Figs. 4(a)–4(c) accordingly. Figure 5 depicts the basic method's power and administration under constant wind irradiance. Wind power coordinates their behavior for optimal administration, as demonstrated in Figs. 4(a) and 4(b), and the working for the time instants t 14 0–25 s is displayed. Using the proposed technique, the maximum tracking power is shown in the figure. At constant irradiance, the greatest power of the suggested model is shown at time t 14 0–25 s [32-35]. The greatest power point tracking is investigated for a variety of wind irradiances and time intervals. As a result, many methodologies are used to assess the voltage, power, and current performances. Figures 6 and 7 show the results of a comparison of the proposed and existing methodologies.

CONCLUSION

In this study, an LFCSO approach for tracking the wind system's maximum power is proposed. The signal control is intended for the transformer-less switching capacitor inductor DC–DC converter, and two inputs are given to the information of the illustrated algorithm. The CSO's performance is increased by utilizing a Levy combat function with a randomized parameter. The panel current and voltage are inputs to the proposed MPPT controller design. The proposed strategy's output is evaluated and defined as the control signal for achieving the highest extreme wind strength. The effect of partial shading is thoroughly examined in this work, with MATLAB/Simulink-based simulations. When the deviation over the wind array is narrow and the shade intensity is low, the effect is similar for all derived building types under altering irradiation conditions. The power consumed in the load was investigated under various shade patterns; it was discovered that the MPP of the heap, when the converter efficiency is taken into account, differs significantly from the wind of the MPP cluster. As a result, monitoring the converter input ability curve under PSCs would not ensure a high power exchange to the weight. The proposed approach was used to maximize the control pulses in order to raise the system's MPPT. The proposed strategy's ro bustness and dynamical exhibitions are evaluated and reported. The proposed strategy is contrasted, and current methods such as GA-NFC, P&O, and FLC strategies are proposed as alternatives. Furthermore, the proposed controller has been shown to be robust in the presence of the desired input wind irradiance changes. The proposed strategy's deviation rate of monitoring error performance is compared. It demonstrates that, when compared to other procedures, the proposed technique provides excellent results.

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Table 1. Comparison of proposed convertors with adopted converter

Property of Converter [27]	Proposed Topology [28]	Adopted Converter [29]
Voltage gain	$\dfrac{N_{sp} + 2}{1 - d_y}$	$\dfrac{2}{1 - d_y}$
Voltage stress Q, d_3	$\dfrac{V_o}{N_{sp} + 2}$	$\dfrac{V_o}{2}$
Voltage stress d_0, d_2	$\dfrac{(N_{sp} + 1)V_o}{N_{sp} + 2}$	$\dfrac{V_o}{2}$

Figure.1.Structure of the proposed method	**Fig. 2. Analysis of (a) circuit diagram for DC–DC converter depending on the SC and (b) equivalent diagram of the proposed converter.**

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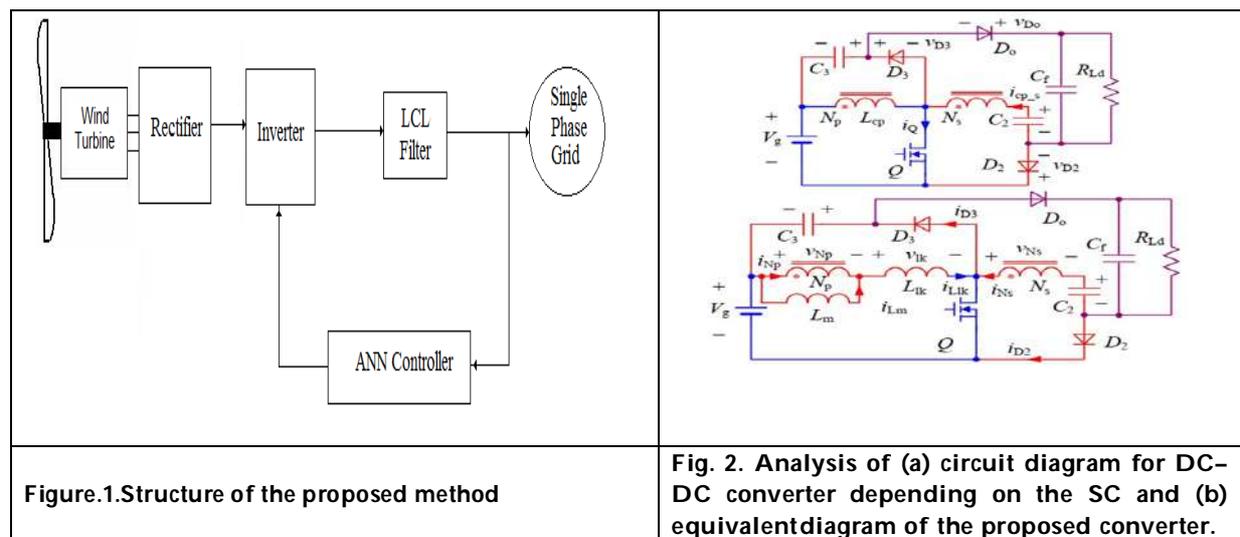
Balamurugan and Selvam
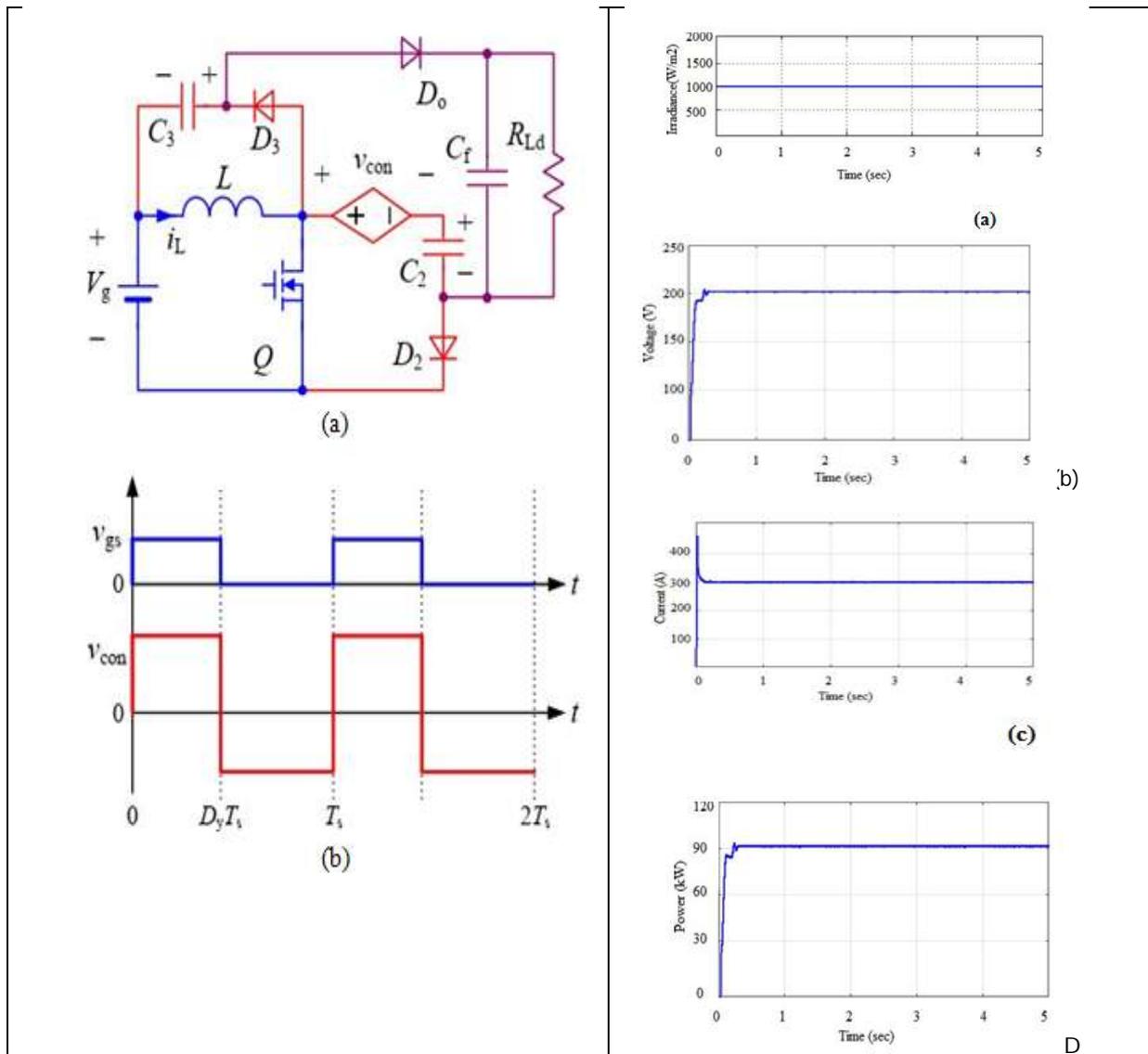
Figure. 3. Analysis of (a) improving the voltage increase by receiving a voltage source and (b) controlled voltage source of the voltage waveform.

Figure. 4. Performance of (a) irradiance, (b) panel voltage, (c) current, and (d) power using P& O technique.
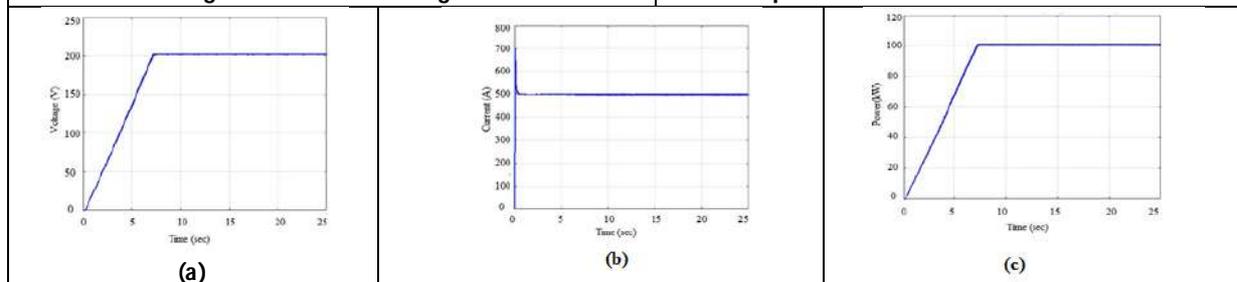
Figure. 5. Performance of (a) voltage, (b) current, and (c) power using proposed LFCSO technique.

Figure. 6. Comparison analysis of power using various methods.

Figure. 7. Investigation of power using (a) proposed technique and (b) FLC technique

RESEARCH ARTICLE

Reorienting Value System through Education: Vision NEP 2020

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ABSTRACT

Education is like a rope where the entire life surrounds it and carries to progress. Education means not only to do study and getting good marks. It means an individual becomes a good citizen. In the race of getting good marks and high rank, the students neglect their basic values like respect for elders/culture/traditions, honesty, truthfulness, cooperation, unity, non-violence, patience, brotherhood, etc. Values are deteriorating day by day to acquire high position, and status in society either by doing the wrong work such as cheating, stealing, revenge, hatred, jealousy, etc. The inculcation of Values through education is an integral part of the development of society as well as the nation. The cohesive working and the progress of the nation depend on it. The relevance of inculcation of the values is not a new aspect in the field of education. The children learn and absorb the values from their families, friends, books, school, society, culture, mass media, etc. Values enshrined through the Indian education system have evolved to a great extent from the ancient period to the modern technological era. As a citizen of India, the parents give more priority to how much the child learns values through education rather than the good academic marks. Through this paper, the researcher tried to revisit the inculcation of values through the ancient Indian education system to the present era.

Keywords: Education, Values, Indian Value-Oriented Content, Education System, Technology, Cohesive Working

INTRODUCTION

Education plays a significant role in the development of society as well as the nation. Only education changes the positive behavior of an individual. In the absence of Values, education is just like a flower without fragrance. Academic achievement of students like discipline, regularity, punctuality, honesty, truthfulness, achieving a high

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score, behavior, personality, etc also influence by how much the students have values. The importance of culture, beliefs, humanism, values, respect for others, teamwork, etc. cannot be replaced in the machine world. The aim of ancient Indian education was not only to the acquisition of Knowledge but also individual self-realization and liberation. Takshashila, Nalanda, Vikramshila, and Vallabhi, for example, were world-class institutions in ancient India that established the peak standards for transdisciplinary teaching and research and welcomed academics and students from all walks of life and from all over the world. Implementation of **National Policy of Education 2020**, this policy more emphasized on Indian Culture, Indian values and Educational Institutions should be like ancient famous universities Nalanda, Takshila. This new education policy will help to inculcate Indian Values with technological embedded which was missing in the education system for the last several years.

OBJECTIVES OF THE PAPER

To explore Value-Oriented Teaching-Learning Pedagogics in the Ancient Indian Education System.
To propose some suggestions to inculcate Indianized Essence in the present Education System.

ANCIENT PERIOD

Vedic period (before 600B.C)
In the Vedic period,the Gurukul education system was based on three steps of the learning process i.e. Sarvana(listening), Manana(reflecting), and Nididhyasana(meditation of the truth); educational ceremonies such as UpanayanaSanskar(students accepted the ritual by a guru) and Brahmacharya (where students bound in strict discipline); Vedic education laid stress on duties towards society and nation through deva runa(debt of almighty),pitruruna(debt of ancestors),rishi runa(debt of gurus) and manavaruna(debt of society) which helps to the inculcation of values through education to attain Moksha.

Buddhist period (600B.C.-1200A)
Gautama Buddha was the propounded Buddhist period. The objective of Buddhism was to attain liberation from all the sufferings of Nirvana and its aim of education is to achieve wisdom. The Buddhist education is based on 5 moral principles: not to kill a living being, not to steal from anyone, no use of alcoholic drugs, no use wrong speech which hurts anyone, and not having too much sensual pleasure; the threefold paths "Ethics(right action, right speech, right livelihood), Meditation(right mindfulness, right effort, right concentration) and wisdom(right view, right intention)".

Medieval period (1200A.D.-1800A.D.)
 In the medieval period, the aim of education focused on the Islamic and Mughal system and established schools, libraries, and different educational institutions that spread Islamic education and culture. Bismilla his the beginning stage of a child's educational ceremony. The students by heartily recitation of Quran as part of the curriculum through the activity inculcation of religious values (truth, love, brotherhood, peace, sacrifices). It more emphasizes practical education so that teacher-student relationship builds up.

BRITISH PERIOD TO POST-INDEPENDENCE PERIOD

Woods dispatch(1854):
In the wood dispatch more emphasized on moral education in the place of religious education but the British rule was unable to implement the principle of universalization of education.

Indian Education Commission/Hunter Commission (1882)
This commission recommended introducing moral textbooks based on natural religion and primary education should be life-oriented.

Narendra Deo Committee(1939)

This committee recommended that Hindi as a medium of instruction and co-curricular activities should be conducted in schools.

Religious Education Committee(1944)
In this committee, G. D. Burman suggested that time should be reserved for prayer in the school, acceptance of all religions-based curriculum, spiritual and moral education given to the students for the preservation of values in the whole life.

AFTER POST-INDEPENDENCE

Radhakrishnan Commission (1948-1949)
First education commission after the independence of India, this commission emphasized leadership growth, social efficiency, knowledge and wisdom development, a desire for higher life values, and cultural improvement and also stressed religious and moral instructions which help to develop a spirit of religious tolerance and secularism. Co-curricular activities like NCC and some other social schemes should be introduced in college which helps to promote the values.

Mudaliar commission(1952-1953)
A.Lakshmanaswami Mudaliar, the chairman of this commission suggested multipurpose schools which help to cater to the individual needs, taste, aptitude, and develop the democratic spirit; understanding the dignity of labor. This commission aims to strengthen the abilities of learners to happily live and become good citizens.

Kothari Commission (1964-1966)
Dr. D.S. Kothari, the chairman of this commission and its report entitled –"Education and National Development". It recommended that the education system put emphasized the development of fundamental, social, moral, and spiritual values.

NATIONAL POLICIES ON EDUCATION

National Policy Education (1968)
Indira Gandhi announced the first NPE; this policy inspired the teaching of the ancient Sanskrit language, which supports the transmission of India's culture, traditional values, and heritage.

National Policy Education (1986):
This policy was introduced by Prime Minister Rajiv Gandhi. This policy contains 12 parts and 157 paragraphs on different aspects of education and its 8th part i.e. "Reorienting the Content and Process of Education" more imbibed, cultivated, and nurtured the values through educational tools i.e. textbooks, libraries, language, sport, and physical education, population education, yoga, educational technology, etc. NPE 1986 special emphasized the removal of disparities and equalizing education opportunity.

National EducationPolicy (2020)
India's government has introduced National Education Policy 2020 envisions an education system deep-rooted in Indian culture that directly contributes to the transformation of India or Bharat. According to this Policy, our institutions' curriculum and pedagogy must instill in students strong esteem for the Fundamental Duties and Constitutional values. NEP 2020 should place a greater emphasis on instilling good values, discipline, care, and concern for one another, being helpful, learning to share, being friendly, and performing their responsibilities, as well as maintaining clean, simple hygiene. NEP 2020 gives a strong push to the use of technology in all aspects of education, which helps to instill values such as respect for teachers, the pursuit of truth through plagiarism, beauty through interactive e-content, tolerance, patience in listening, and speaking, and non-violence in discussion forums. During the pandemic, when the infectious corona virus disease started to spread to the whole world and

government decided to shut down or lock down the whole world. There was no option on how to continue the education. Then, educational institutions continue teaching-learning through online mode. The education system does not stop the inculcation of values in very crucial times. National education policy 2020 envisions the offline and online modes of education which support the inculcation of values to make INDIA A GLOBAL KNOWLEDGE SUPERPOWER. In this policy, the progress report card designed by states/UT under the guidance of PARAKH, NCERT, and SCERT includes cognitive, affective, and psychomotor domains which complete 360-degreecycle that supports happy livelihood life.

NEP 2020: A ROADMAP TO VALUE INCULCATION

NEP 2020envisions that the responsibility of teachers in nation-building is supreme and teachers are the key to bridging the gap between increasing materialism in education and a value-oriented learning environment. The NEP 2020 proposes that the following measures should be done in a phased manner to achieve the target of inculcating values of "Bhartiyata" in education:

TRAINING OF TEACHERS

PRE-SERVICE TEACHER TRAINING (PRESET)
Entrance to Pre-service teacher training programmers will be based on relevant subject and aptitude assessments run by the National Testing Agency, which will be standardized to account for the country's linguistic and cultural diversity to maintain uniform standards in teacher education. All B.Ed. training programmers will include pedagogy training for foundational stage, multi-level teaching, and evaluation, training provision for special education, educational technology use, and student-centered and cooperative learning.; these styles of pedagogy should be strong practicum training, and teacher training will be a priority; these pedagogical techniques should include a significant practical component, and teacher training will highlight the application of the Indian Constitution's Fundamental Duties (Article 51A) and other constitutional principles in classroom teaching at local schools. So that educators instill ideals in their students during their education, which will later reflect in their future teaching-learning.

IN-SERVICE TEACHER TRAINING (INSET)
For in-service teachers who want to improve their qualifications, and competency, the new education policy 2020 also helps to upgrade of knowledge of in-service teachers. The teacher should be also trained for adapting new technological pedagogical content knowledge .And the teacher should also be trained on how to maintain discipline, and respect the response of students during online teaching-learning. Training will include all teachers being aware of institutional requirements such as adequate and safe infrastructure, such as toilets, clean drinking water, clean and attractive spaces, electricity, computer gadgets, the facilities of internet, libraries, sports, and many other recreational resources. As per NEP 2020, Teachers will support Continuous Professional Development (CPD), Faculty Development Programs, Online Webinars, and Seminars to strengthen and update their teaching profession. Serving teachers must be familiar not only with technology but also with teaching and instructional design skills that will enable them to assist their students in constructivist thinking, experimentation, problem-solving, and learning linked to real-life situations; and teachers know how to use collaboration tools like moodle, google classroom and allow students.

CURRICULUM DEVELOPMENT WITH INDIAN VALUE-ORIENTED CONTENT
India is a multifaceted country. According to NEP2020, curriculum, pedagogy, and policy should be based on respect for gender, culture, and language diversity, as well as regard for the local context, always bearing in mind that education is a concurrent subject. The curriculum should be reflected Indian history, and students should be aware of the Indian value system; focus on the Indian language and have the cultural awareness of the Indian Knowledge system. India is the land where relationships have a prominent place such as Guru-Shishya, Mata-Pitta, Behan- Bhai,

Bharat-Bharatvasi, etc. So that curriculum should be framed as per Indian traditional values. The content should be reflected the localized Indian value orientation, which has evolved through millennia in ECCE. Both parents and ECCE institutions will benefit to inculcate the texture of Indian values from the NEP2020 curriculum framework. The content of textbooks should be tailored to the needs of the local community; it should address all aspects of religious, spiritual, psychological, economic, political, and environmental diversity. Books for kids at all levels will be generated, including through high-quality translation (where necessary utilizing technology) in all local and Indian languages, and extensively disseminated in schools and public libraries. To promote a reading culture across the country, public and school libraries will be greatly enlarged. There will also be the establishment of digital libraries. School libraries will be established - notably in villages - to serve the community outside of school hours, and book clubs may meet in public/school libraries to encourage and enable broad reading. High-quality textbooks in home languages/mother tongues will be made available, including in science. Early on, every effort will be taken to ensure that any gaps between the child's native language and the medium of instruction are addressed. Where possible, even if home language/mother-tongue textbook material is not available, the language of communication between teachers and students will remain mother-tongue. Every student in grades 6 through 8 will take a fun course that will give them an overview and hands-on experience of a variety of important vocational crafts and so on, as determined by states and local communities and mapped by local skilling needs. As part of the NCFSE 2020-21 framework, NCERT will produce a practice-based curriculum for grades 6-8. All basic human and constitutional values will be developed in all students. All curriculum and pedagogy will be redesigned from the foundational stage onwards to be deeply rooted in the Indian and local context and ethos in terms of culture, traditions, and so on – to ensure that education is maximally relatable, relevant, interesting, and effective for our students. The curricula's reduced substance and increased flexibility, as well as a renewed emphasis on productive rather than rote learning, must be matched by changes in textbooks. According to a prior study, textbooks should be written to ask important and engaging questions and to emphasize applications and problem-solving in order to assist students to gain self-confidence. (Jrall& Gupta;2020).

CO-CURRICULAR ACTIVITIES
Co-curricular activities play a vital role in the physical, mental, sociological development of students. According to NEP 2020, during Grades 6-8, all students will participate in a 10-day bagels session during which they will intern with local vocational experts such as painters, architects, carpenters, and artists. Similar internship opportunities to learn vocational courses may be provided to kids in Class VI through XII, even during the summer, these opportunities help to cater to the needs of learners. The past study revealed that young pupils must be taught about the environment and instilled with environmentally responsible conduct(Bakshi and Gupta;2022). Throughout the year, bagels days will be promoted for a variety of enrichment activities such as athletics, gardening, painting, and vocational arts and crafts. Another study explored the Indian vision for Education for Environmental Sustainability (EDS) which should be based on long-term sustainability, which stems from centuries of living in harmony with nature. Education for Environmental Sustainability (EESD) must be entrenched in all educational activities so that awareness leads to comprehension and understanding leads to action(Devi, Gupta, and Bakshi;2022). Children will be exposed to activities outside of school regularly through excursions to historical, cultural, and tourism sites, as well as meetings with local artists and craftsmen and visits to higher educational institutions in their village, Tehsil, District, and State. Eminent locals who may be engaged as master teachers to educate pupils about Indian localized professions, knowledge, attitude, and skills, such as local professional crafts. Kabaddi, Kho-Kho, Ludo, GilliDanda, Wrestling, cycling, and other healthy game tournaments should be arranged in every school to promote Indian culture.

Role of Teacher in Value Education
The teacher plays a vital role in the classroom to mould behavior of the students. Before a youngster, a teacher must set a high standard of moral behavior. Teaching and learning have a significant impact on a child's moral ideals. Teachers can instill numerous moral characteristics in pupils by organizing various curricular and co-curricular activities. Teachers may emphasize moral traits such as love, sacrifice, self-control, truthfulness, and uprightness in the teaching of various topics such as languages and social studies. Dramas, games, and sports present several

possibilities to appeal to students' moral sensibilities. The teacher is expected to serve not just as a facilitator for the acquisition of knowledge, but also as a value instiller and inner being modifier. The ancient Indian education system was centered on values. There is a need to assimilate ideas that will help us construct a better life, a better man, and a better character. In the sense that it aimed to realize the ultimate reality, harmonious growth, and spiritual well-being of an individual, education transcended all religions. The teacher-student connection was spiritual and heavenly. Individuals gained knowledge, efficiency, and meaning in life as a result of their intimate relationships. Education has recently devolved into the simple acquisition of information in cognitive learning areas for the sole aim of passing exams and gaining degrees. As a result, people with lopsided personalities are emerging. Man has become so narcissistic, materialistic, befuddled, frustrated, and perplexed that he has forgotten how to live with others. There is an overwhelming sense of emptiness, dreariness, and meaninglessness. Only the moral and spiritual basis on which we build our educational system can shape a child's personality and our country's future destiny. Instilling a sense of humanism in youngsters, as well as a profound concern for the well-being of others and the nation, can only be accomplished if we instill in them a strong sense of devotion to principles, which will restore people's pride in labor that offers order, security, and assured progress. Individuals' social, intellectual, emotional, spiritual, and other development is based on their values. Value Education is not a separate sphere of activity from other pursuits. Values are more important conceptions that are linked to fundamental human needs and societal demands. Values are acquired continuously in school through a variety of activities such as instruction, peer connections, co-curricular activities, and so on. As a result, education has a critical role in instilling essential ideals of humanism, socialism, and national integration in children, and it is difficult to work for both teachers and students. Teacher education is inextricably linked to society because it is a vital aspect of the educational system.

It is more than just an institution; its scope and purposes have expanded in recent years. The need of the hour is for competent, dedicated, and properly qualified teachers who can satisfy the needs of their students as well as society. In his capacity as chairperson of NCTE, Prof. J.S Raj put has the right to expect all-out excellence from teachers, as well as interactions that develop knowledge and ethical values among students and create an educational environment that fosters fraternity among fellow teachers and teacher educators. "When talking about teachers and the multiplier impacts of their labor," Rajput says, "one must instantly return to teacher training facilities." The number of teachers they generate has a direct relationship with the multiplier effects that will be seen in society." As a result, the teacher educator has a critical function to play. In the present scenario, teacher staught in the classroom using technology to make their lessons effective, but education is not possible without the presence of a teacher so both are important. The researchers revealed that at least one MOOC course should be completed. Online learning contributes helps to raising students' motivation and interest. (Jrall& Gupta;2021).The findings of the previous study revealed that E-content supports the teaching-learning process to be more interesting. The use of more computers simulation programs animation, flash movies, sound effects, text, videos, etc. creates and develops the interest of the students as well as motivates the teachers to develop E-content(Baliya&Jrall; 2020).

The following are some of the ways that values can be instilled in teacher trainees during the teaching and learning process in the classroom:

➢ In the classroom, basic human principles must be emphasized. Teacher educators should instill in teacher candidates the belief that a kid is born with values, and that it is up to the teacher to unearth them.

➢ The inculcation of values cannot be taught in separation; nevertheless, the teacher can provide opportunities for pupils to evaluate and reflect on values before acting on their thoughts. Teacher educators can engage students in active games in the classroom to instill values such as fairness, honesty, courage, and cooperation. Respect and love are best learned through interaction with peers who have a variety of cultural, ethnic, and personality features.

➢ Teacher educators should instill in teacher candidates a desire to understand human nature. The positive human values in a child can be brought out with loving attention and care.

➢ To foster a healthy school culture, all staff members must agree that value-based education is important to the school's mission.

➢   The key to motivating students to build value-based education is to celebrate national days.

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First and foremost, teacher educators must establish a level of quality and values that will be instilled in teacher trainees and the nation's youngsters. Various introspective and retrospective measurements must be taken from time to time to evaluate the quality of value education at all levels. The grassroots level of objective should be kept in mind during the curriculum development stage, i.e. inculcation of values through various subjects such as social sciences, language, mathematics, and so on. New textbooks should be rewritten in diverse languages with a local flavor and Indigenous content. Textbooks for pre-primary and primary grades will be based on moral value lessons, inquiry-based material, critical thinking, and continuity. finding of the study revealed that there is a significant difference in learning style of students with respect to the discipline of the study. So, the teacher should engage students in inquiry by providing opportunities to experiment, analyze information critically, make assumptions and solve problems both individually and in groups. The finding of the previous research study conducted on learning styles and academic performance of students pursuing pre-service teacher education programmers revealed that visual learning style is the most prominent learning style. It means that students learn when they visualize the things and the teachers should give the task of assignment on the basis of different teaching-learning styles like video-lecture, problem-solving, role-playing, drama, skit, poetry. Teachers must address to learning styles of students for fostering individual differences (Kiran & Devi; 2020). There to make real-life contacts. With so many changes, it's possible that it's time to improve and alter some areas of your business. The UNSECO DELLORS report should be helpful to reframe the new curriculum. The goal of "LEARNING TO LIVE TOGETHER" should be instilled in everyone. It should be included in the curriculum because it is so important in the early stages of development (Bakshi & Devi; 2021).

CONCLUSION

The following figure recapitalize the Key areas to be given due consideration for Value Inculcation in the Students. It can be concluded from the preceding discussion that the ancient Indian educational system preserved values that have evolved to a significant extent from ancient times to the modern technological era. During the Covid-19 epidemic, society, school, and the teacher must play a variety of roles because of the changing course of events. There is a need for Indian values which deteriorate somewhere after 34 years back New Education Policy 2020 more emphasis on Indian values. The most fundamental UNESCO pillar of education is "Learning to live together" (Dellor's report, 1996). It promotes teachers, teacher educators, education planners, and administrators to promote values such as peace, tolerance, human rights, democracy, justice, and equality in the classroom. This is a major need for attention and follow-up to improve and save the Indian quality educational system which is degraded by Macaulay, the British. Indian value-oriented content system must be articulated in a new context of the curriculum framework. Value education is a crucial component of social life, according to participating students, and it should be supplied to students in distance learning systems(Deveci, 2015). The current concept of education, "Schools come to community and community comes to school," is particularly effective in the Indian environment.

ACKNOWLEDGEMENTS

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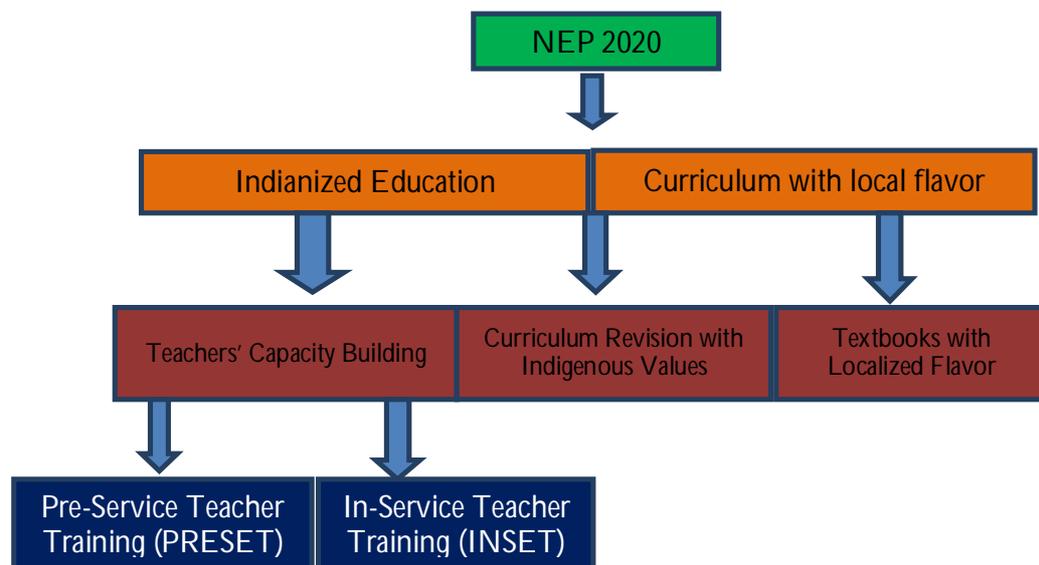
Figure 1:- NEP 2020: A ROADMAP TO VALUE INCULCATION
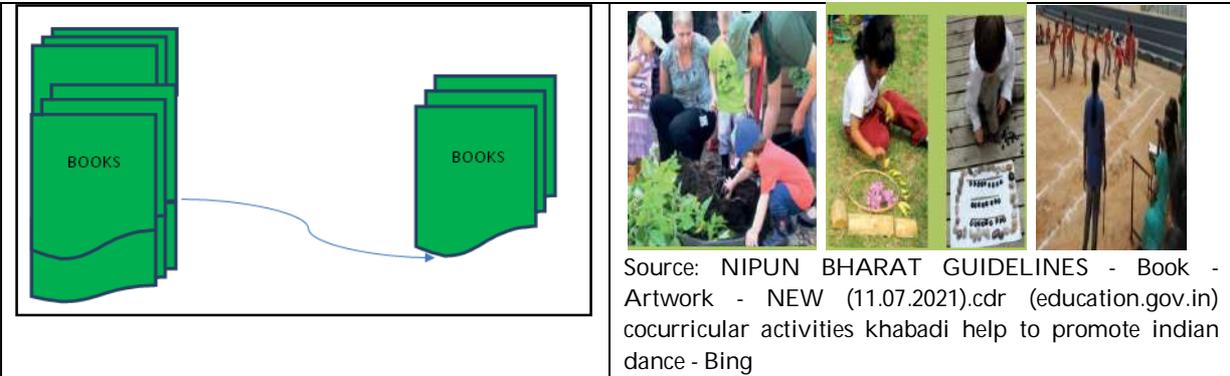
Figure 2: Reducing curriculum to the core essentials to minimize rote-learning.

Source: NIPUN BHARAT GUIDELINES - Book - Artwork - NEW (11.07.2021).cdr (education.gov.in) cocurricular activities khabadi help to promote indian dance - Bing

Figure 3 : Value of Co-curricular activities in direct and meaningful ways
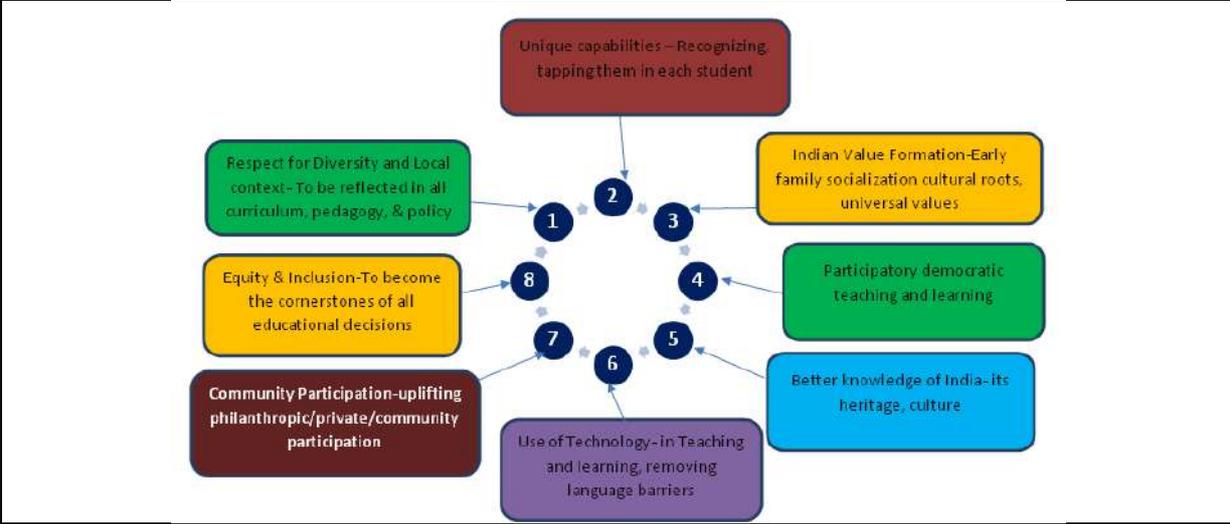
Figure4: Key Principles of National Education Policy, 2020

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RESEARCH ARTICLE

Open Innovation: Inclusion Criteria for Women Entrepreneurs

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ABSTRACT

Women entrepreneurs are predicted as successful with their strong potential capabilities. They are accounted and contributed for the economic development of the country. Yet gender disparities exist in women running businesses. There are many psychological factors differing men and women in taking risky business decisions. This paper attempts to observe the key psychological dimensions across demographic characters which determine the success of women entrepreneurs. The study was carried out step by step by understanding the demographic characters of the respondents, examining the psychological factors responsible for the success and finally analyzing significance of psychological dimensions across demographic characters. A structured questionnaire administered to 620 entrepreneurs who are running the business successfully for 5 years. Results conclude that need for achievement, risk taking, innovativeness and locus of control has significant influence on demographic characters like education, marital status and family type. The paper recommends women entrepreneurs to actively involve customers, external stakeholders and R&D consultancies to bring out the openness of innovation for SMEs strategically.

Keywords: risk taking, business decisions, creativity, openness

INTRODUCTION

Risk-bearing is a basic requirement for any business entrepreneur regardless of diversity in gender. It is one of the important characters which shows the capability of individual in facing hard situation. Business magnets often are very much planned risk takers who incessantly endeavour in spreading their business potentially (Mor et al., 2020).

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There are many reasons for the business risk like managing capital, technology adaption, infrastructure and legal policies (Budh et al,2020). Generally, women's entrepreneurial activity revolves around community, spiritual, social and mental factors for their achievement (Habib et al., 2005). As per GEM report (2014) nearly 34 percent are women entrepreneurs in India.In India, under the Micro, Small and Medium Enterprises Development (MSMED) Act, various state small industries development corporations, nationalized banks and nongovernmental organizations conduct various programs to promote potential women entrepreneurs with various assistances. MSME has separate division to look after the issues raised by women (Vijaykumar and Naresh, 2013). This fascinated many scholars to look into this particular division. However, a complete outcome of the investigation yet to be well-known. In-born factors of the entrepreneurs and gained knowledge from external substitute the entrepreneurs to effectively perform in the world. Because of the myth that society benefits from entrepreneurs' success, many scholars show their interest in identifying the relationship between the factors. (Rusu et al., 2012.Entrepreneurs defined success as earning a living or adequate income, while other entrepreneurs expressed their belief that success was customer satisfaction as well as respect. Zimmerer and Scarborough (2005) found that the growth of entrepreneurship arises in spite of risk and ambiguity in the business. Creativeness, looking after opportunity and employment are some of the factors for the growth of entrepreneurs in India. Walker and Brown (2004) described success using a financial categorization, such as profitability and growth. Whereas Ahmad and Seet (2009) referenced revenue, profit, or return on investment within the specific business. The intention of this study was not to define success; rather the study used the information shared by participants to expand the views of success and to further develop the concept of success .Locus towards achievement, is the main reason the growth of women entrepreneurs (McClelland, 1961). Zhang et al., (2009), clarified about the factors required for any business enterprises. Among personality theories of entrepreneurship, locus of control has received considerable attention. This locus of control enables different countries to adopt creative entrepreneurs which add value to the technological advancement across countries (Mueller and Thomas, 2001).For last few decades, entrepreneurial personality traits are under debate and few researchers discovered for dispositional factors for entrepreneurial behavior relates to personality traits, on the other hand, some researcher denies the relationship of entrepreneurial behavior and personality on their studies. Present study confined to women entrepreneurs in SME sector lot of studies focused on the performance of employees (Plotnikov et al., 2019) while this paper confines to the top level entrepreneur who are in decision making conditions.

Statement of the Problem
A large number of women today, dream of starting something of their own and wish to utilize their capabilities and be independent across world. But in Indian scenario, still there exists gender diversity. Women are still one step below in taking business decisions and in handling risky situations. This may be due to lack of exposure and fear of failure they hesitate to take bold decisions in their business. They face many challenges in running the business relating to various resources i.e., (financial, marketing, production, HRM, in receiving Govt. assistance) and work -life balance effecting the performance of the enterprise. Women entrepreneurs need to possess certain characteristics in order to convert their business venture into a successful one. There are many psychological factors differing men and women in taking risky business decisions. This raises questions like what may be the psychological factors responsible for women in taking business decisions? What are the factors accountable for women in taking risky situation? Few factors play a major role in carving the future success of an entrepreneur. They need to possess certain inherent psychological strength and motivation, they have to develop the skills required to run the business successfully, must be able to adjust to the socio-cultural challenges, and have sufficient resources and acumen in order to be successful in their enterprise.

Objective
This paper attempts to observe the key psychological dimensions across demographic characters which determine the success of women entrepreneurs

Specific Objective
- To get an insight about the descriptive characters of the selected entrepreneurs
- To understand the key psychological factors responsible for the success of women entrepreneurs
- To analyze the significance of psychological factors across demographic characters

Data Sources
Sample selection
- Samples are the women entrepreneurs in start-up stage and are running the business independently and successfully for equal to or more than 5 years. The data was first collected from women entrepreneurs who are registered with MSME, Karnataka. The sample were selected from 6 districts of Karnataka where the presence of MSME is imminent but the performance is still in the nascent stage.

Sampling Method
Convenience sampling was carried out to collect the data from the list of eligible entrepreneurs. The questionnaire was administered to the selected entrepreneurs through online or offline.

Analysis and interpretation
Table 1 depicts that there are a total of 620 respondents, out of which maximum number of women entrepreneurs (70 percent) are in the middle age group are registered with MSME, Karnataka. In case of educational qualification, all the selected entrepreneurs are literate and most of them are graduate. 31 percent are professionally qualified. It is observed from table that majority of the women (62 percent) has previous work experience but they informed that they do not have any entrepreneurial experience. It is interesting to note that most of them (49 percent) are from extended family.

Validity and reliability of the questionnaire
The overall reliability of the instrument of all the scale variables was $\alpha=0.753$ or 75.3%, which is above the recommended criteria of 0.7 (Cronbach, 1951) The reliability of all the constructs, the measurement model and relevant statistics are presented below: While analyzing the self-efficacy of psychological factors on women entrepreneurs, it was observed that though a large number of women disagree that they have the ability to achieve all the set goals, majority of the women agree that they are able to obtain the important outcomes and are better in most of the tasks that they perform when compared to others. A few more women entrepreneurs agrees that they perform effectively even when things are tough, they have the ability to overcome the challenges and believe that they can succeed in any work in which they set their mind up on. With respect to the locus of control, large number of women says that business success is a matter of luck. Though, women life is controlled by accidental happenings, many feel that their work or life controlled by powerful people (here powerful people means family persons or community elders in rural areas). Hence, they are not ready to plan wisely. Few women agrees that they are in control of their life. While analyzing the innovativeness as a personal psychological factor, most of women decided to be an entrepreneur because of the demand for skilled persons, society accepts creative thinking people and their ability of creativeness are valued more. At the same time, it was observed that there are many women agreed that they like to experiment with various ways of doing things and often surprise people with their novel ideas. When analyzing the risk-taking propensity as an important factor for determining entrepreneurial success it was seen that though there are a many women who agree that they have confidence in their ability to recover from the mistakes no matter how big and believe in trying new things. Majority of women agrees that they generally prefer stimulation to secure and taking risks make good sense only in the absence of acceptable alternatives. This shows that women entrepreneurs plan their future market in minimum acceptable limit and unlike other large sectors/ organizations they have no such structured format for the capitalization of fund or marketing their products or recruitment of employees. In case of need for achievement, majority of the entrepreneurs strongly agreed that they are ambitious. They want society recognition and require high status.

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Significance of psychological factors across demographic characters.
In order to explore the significance between groups and within groups, ANOVA was carried out between the psychological dimensions and the demographic factors like marital status, education qualification, risk propensity and type of family. ANOVA supports the analysis in finding the significance between groups and within groups. If the variance caused by interactions between different samples is much greater than the variance found inside values in a single group, that indicates the means aren't equal.

Psychological factors vs. Education qualification
H1a. Psychological dimensions significantly influence educational qualification in determining the entrepreneurial success.

Psychological factors vs. Marital Status
H1b. Psychological dimensions significantly influence marital status in determining the entrepreneurial success.

Psychological factors vs. Work experience
H1c. Psychological dimensions significantly influence work experience in determining the entrepreneurial success. Between Group Variation: The total variation between each group mean and the overall mean. Within-Group Variation: The total variation in the individual values in each group and their group mean.

Psychological factors vs. family type
H1d. Psychological dimensions significantly influence family type in determining the entrepreneurial success.
Results from Anova illustrates that, P value in educational qualification is less than 0.05 for locus of control and Need for achievement of the Psychological dimensions. Hence Ho is rejected an H1 is accepted. This implies that in spite of education qualification, these factors will not remain same for all entrepreneurs and they differ with entrepreneurs. Similarly in case Marital Status, P value is less than 0.05 in factors like locus of control, Risk -taking propensity and need for achievement. Hence here H0 is rejected and H1 is accepted. Marital status does not have greater impact for these factors. Also, it is evident that Locus of control, risk propensity and need for achievement does not depends upon the work experience. They differ with entrepreneurs' skills and creativeness. It is interesting to note that self-efficacy, innovativeness and risk propensity does not depend upon the nature of family type.

DISCUSSION

Small and medium sized enterprises are emerging rapidly and showing a potential economic development innovatively. The creative ideas drastically added many women in becoming potential entrepreneurs. Engaging innovation technologically has now become a new milestone in all sector (Hoffman et al. 1998) which now spreading in SMEs has become an important dimension for their women entrepreneur success. Industry competition and new product development added advantage to the adoption of technology oriented and for sustainability in the market. SMEs creative ideas varies from large companies (Lingyan et al 2021). Locus of control, risk propensity and achievement towards the goal enables the women entrepreneurs in gaining new innovative ideas with the available resources to be competitive in the market. Also, research studies (Spithoven et al, (2013) evidently discussed how SMEs gained potential insights through open innovation practices comparatively than large companies. Still a gap exists that SMEs especially women are omitted from the discussion on openness. Openness means knowledge gaining from external sources. This type of open innovation increase firms performance (Parida et al, 2012).Hence SMEs should concentric on open innovation process like various internal innovative gatherings, non- financial supports like training, knowledge sourcing and transformation rather than mergers and acquisitions which is a financial burden for SMEs. This will reduce the SMEs from risk propensity of the entrepreneur and progress their journey towards achievement of the goal. Finally, women entrepreneurs should concentrate on open innovation and for this to happen, family involvement is essential for providing the necessary support in all the organizational activities.

CONCLUSION

Technology search enables the entrepreneurs to obtain novel knowledge from external sources. For this the paper recommend women entrepreneurs to actively involve customers, external stakeholders and R&D consultancies to bring out the openness of innovation for SMEs strategically. The implication of this paper helps the women entrepreneurs to strategies their openness of innovation with external and internal capabilities to be more technologically competitive across various sector

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Table: 1 Demographic character of the respondents

Age	Frequency	Percent
<=30 years	130	21
30-35 years	166	26
35-40 years	165	27
40-45 years	159	26
Total	620	100
Educational Qualification		
Literate (HSC)	184	29
Undergraduate	203	33
Graduate	43	7
professionals	190	31
Total	620	100
Marital Status		
Married	308	50
Single	166	26
Divorcee	23	4
Widow	123	20
Total	620	100.0
Work Experience		
No	234	38
Yes	386	62
Total	620	100
Nature of Family		
Nuclear family	116	19
Extended Family	304	49
Single parent family	122	20
reconstituted family	78	12
Total	620	100

Reliability Statistics

Cronbach's Alpha	N of Items
.753	37

Table: 2 The reliability of all the constructs, the measurement model and relevant statistics are presented below:

Construct	N of items	Cronbach's Alpha
Personal factors	7	.725
Locus of control	7	.673
Innovation	7	.630
Risk taking propensity	7	.812
Need for achievement	5	.727
Success related factors	4	.736

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Table: 3 Psychological factors determining the success of women entrepreneurs

Dimensions	Factors	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
Self-Efficacy	Ability to achieve the set goals	0	**37.3**	28.5	19.4	14.8
	Confident in fulfilling difficult tasks.	12.9	17.7	**25.8**	20.5	23.1
	Able to obtain important outcomes	11.5	17.1	24	**32.1**	15.3
	Belief to succeed in the established work	11.1	17.1	23.7	**27.6**	20.5
	Ability to overcome challenges	14.5	18.7	22.7	**27.4**	16.6
	Better than others in most tasks	11.1	14.4	24.4	**30**	20.2
	Perform effectively in the tough situations	13.2	14.8	25.5	**28.5**	17.9
Locus of Control	Life is controlled by accidental happenings	10	25.3	25.5	**28.1**	11.1
	Wants are fulfilled because of luck	11	25.2	27.6	**26.5**	9.8
	Life is controlled by own actions	12.7	25.6	24.8	**25.5**	11.3
	Not wise to plan because of bad fortune affects	10	25.3	25.5	**28.1**	11.1
	Life events are controlled by powerful people	14.5	21.8	**26.5**	25	12.3
	Self-controlled life	11.9	26.3	23.4	**27.1**	11.3
	Business success is by luck	13.1	23.9	**29**	24	10
Innovativeness	Novel ideas surprise people	10	22.1	24.2	**28.1**	15.6
	Been asked for help with creative activities	11	20.6	26.3	**26.5**	15.6
	Mastering a skill than a new idea	11.9	23.1	21.8	**27.1**	16.1
	Work with original thinking	12.7	21	19.8	**33.5**	12.9
	Continue to do the new job as taught earlier	11.3	23.2	20.3	**32.9**	12.3
	Jobs with skill and practice over inventiveness	12.4	19.2	20.3	18.9	**29.2**
	Experiment with different ways of doing things	11.8	17.3	18.4	**29.4**	23.2
Risk Taking Propensity	Take risks only in the absence of acceptable alternatives	7.3	18.2	18.1	**32.1**	24.4

	Prefer stimulation to secure	6.9	19.5	20.8	**31.5**	21.3
	Ability to recover from mistakes	7.3	18.9	17.6	**29**	27.3
	Try new things without the fear for failure	6.8	19.4	17.7	**30.3**	25.8
	Believe that opportunity knocks once	8.5	20.5	20	**30**	21
	Handle losses and disappointments	5.3	18.7	21.6	**29.2**	25.2
	Concerned about the potential loses in decision making	5.8	16.6	20	**35.6**	21.9
Need for Achievement	Like to increase status and prestige	8.9	16.5	21.9	**29**	23.7
	Desire high earnings	13.7	17.9	**25.6**	25.5	17.3
	Achieve a higher position in the Society	11.8	17.3	22.1	**26.9**	21.9
	Have high ambition	10.5	19	18.5	**30.8**	21.1
	Recognition for the achievements	10.3	18.9	22.1	**27.1**	21.6

Table: 4 Psychological dimensions significantly influence educational qualification in determining the entrepreneurial success.

ANOVA						
		Sum of Squares	df	Mean Square	F	P
Personal factors (Self - Efficacy)	Between Groups	4.193	4	1.048	1.799	.127
	Within Groups	358.274	615	.583		
	Total	362.467	619			
Locus of Control	Between Groups	11.494	4	2.874	6.049	.000
	Within Groups	292.158	615	.475		
	Total	303.652	619			
Innovativeness	Between Groups	2.471	4	.618	1.276	.278
	Within Groups	297.853	615	.484		
	Total	300.324	619			
Risk – taking Propensity	Between Groups	2.942	4	.735	1.093	.359
	Within Groups	413.808	615	.673		
	Total	416.750	619			
Need for achievement	Between Groups	20.636	4	5.159	6.741	.000
	Within Groups	470.684	615	.765		
	Total	491.320	619			

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Table:5 Psychological dimensions significantly influence marital status in determining the entrepreneurial success.

ANOVA		Sum of Squares	df	Mean Square	F	P
Personal factors (Self - Efficacy)	Between Groups	4.626	3	1.542	2.654	.048
	Within Groups	357.841	616	.581		
	Total	362.467	619			
Locus of Control	Between Groups	5.033	3	1.678	3.461	.016
	Within Groups	298.619	616	.485		
	Total	303.652	619			
Innovativeness	Between Groups	3.045	3	1.015	2.103	.099
	Within Groups	297.279	616	.483		
	Total	300.324	619			
Risk – taking Propensity	Between Groups	24.240	3	8.080	12.681	.000
	Within Groups	392.510	616	.637		
	Total	416.750	619			
Need for achievement	Between Groups	20.205	3	6.735	8.806	.000
	Within Groups	471.115	616	.765		
	Total	491.320	619			

Table: 6 Psychological dimensions significantly influence work experience in determining the entrepreneurial success.

ANOVA		Sum of Squares	df	Mean Square	F	P
Personal factors (Self - Efficacy)	Between Groups	.470	2	.235	.401	.670
	Within Groups	361.997	617	.587		
	Total	362.467	619			
Locus of Control	Between Groups	3.377	2	1.688	3.469	.032
	Within Groups	300.275	617	.487		
	Total	303.652	619			

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Innovativeness	Between Groups	1.998	2	.999	2.067	.127
	Within Groups	298.325	617	.484		
	Total	300.324	619			
Risk – taking Propensity	Between Groups	12.493	2	6.247	9.534	.000
	Within Groups	404.256	617	.655		
	Total	416.750	619			
Need for achievement	Between Groups	5.606	2	2.803	3.561	.029
	Within Groups	485.714	617	.787		
	Total	491.320	619			

Table: 7 Psychological dimensions significantly influence family type in determining the entrepreneurial success.

ANOVA						
		Sum of Squares	df	Mean Square	F	P
Personal factors (Self - Efficacy)	Between Groups	8.702	3	2.901	5.051	.002
	Within Groups	353.765	616	.574		
	Total	362.467	619			
Locus of Control	Between Groups	1.623	3	.541	1.103	.347
	Within Groups	302.030	616	.490		
	Total	303.652	619			
Innovativeness	Between Groups	5.130	3	1.710	3.568	.014
	Within Groups	295.194	616	.479		
	Total	300.324	619			
Risk – taking Propensity	Between Groups	40.257	3	13.419	21.956	.000
	Within Groups	376.493	616	.611		
	Total	416.750	619			
Need for achievement	Between Groups	37.339	3	12.446	16.888	.060
	Within Groups	453.981	616	.737		
	Total	491.320	619			

RESEARCH ARTICLE

Data Interpretation through Pattern Matching in Big Data

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ABSTRACT

Frequent pattern mining is a field of data mining aimed at unsheathing frequent patterns in data in order to deduce knowledge that may help in decision making. A variety of algorithms for frequent pattern mining have been developed during the last two decades most of which have been found to be non-scalable for Big Data. For Big Data some scalable distributed algorithms have also been proposed. In this paper, we analyze such algorithms in detail. Initially conventional algorithms like Count Distribution are considered and move on to traverse other algorithms one by one and finally to algorithms basedon Map Reduce programming model.

Keywords: Frequent Pattern Mining, Distributed Systems,Big Data Analytics

INTRODUCTION

Data mining is the process of analyzing large sets of data for the purpose of generating new information or for the purpose of knowledge discovery. In general, data mining covers areas such as clustering, frequent pattern mining (FPM), classification and outlier detection [1, 2, 3]. FPM is a popular data mining method [ ] used to find attributes that frequently occur together. Some of its important applications are market basket analysis, stock market analysis, drug detection, etc. [5]. Several FPM algorithms have been proposed over the past decades such as Apriori [6], Eclat [7], FPGrowth [8], etc. [9, 10]. Identifying all frequent samples is a computationally complex and expensive task due to the large number of candidate samples. As a result, most algorithms become less efficient when it comes to the type of data that we are dealing with at the moment, Big Data. Social networking sites like Facebook produce more than 300 TB of data per day [11]. Amazon, Walmart and other giants record billions of transactions every year.

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Genome sequencing platforms can generate terabytes of data. To deal with the problem of huge data volume, researchers have proposed a number of parallel algorithms for the FPM problem. This article looks at some of the more recent classical and parallel FPM algorithms. Various parallel FPM algorithms have been discussed and analyzed in detail starting with classical algorithms and then moving on to modern algorithms.

Algorithms for Performing FrequentPattern Mining on A Distributed Environment

Several algorithms have been proposed for efficient frequent pattern mining on distributed systems, including the counting distribution, data distribution and candidate distribution algorithms of Agrawal and Shafer [12], Parallel FP growth algorithm. AI. [1], Single Pass Counting, Fixed Pass Count Combined Count, and Pass Count Combined Counting Algorithms by Lin et al. [15] and the distributed Eclat algorithm of Moens et al. AI [16]. Some of them are discussed here.

Count Distribution Algorithm
Notations

n-itemset	Itemset with n items
$Freq_n$	Set of frequent n-itemsets
$Cand_n$	Candidate itemset of size n
$Proc^i$	Processor i
DS^i	Dataset local to $Proc^i$
DSR^i	Dataset local to $Proc^i$ after re-partition
$Cand^i_n$	Candidate set of $Proc^i$ during pass n

The count distribution (CD) algorithm tries to minimize the interaction. It partitions the dataset horizontally into equal parts for different processors. Each processor automatically executes the Apriori algorithm and generates a hash tree on locally stored transactions. After each iteration, the total number of candidates is calculated as the sum of all numbers, across all processors. This algorithm has the limitation that each processor must keep the entire hash tree in its memory. The data distribution algorithm should be used if the entire hash tree cannot be stored in memory. This algorithm allows parallel computation to avoid any interaction. On the first pass, each processor generates its local candidate frequent set $Cand_i$. For the remaining passes, the algorithm is given below:

1. Every processor $Proc^i$ produces candidate set $Cand_n$ by making use of the frequent itemset $Freq_{k-l}$ that was produced in the previous iteration.

   2. $Proc^i$ performs a pass on its DS^i and to find local support counts for candidates in $Cand_n$

   3. $Proc^i$ synchronizes with all other processors and

   Pass 1:

This step is same as the step for n=1 in CD algorithm.
Pass n>1:

1. Processor $Proc_i$ produces candidate itemset $Cand_n$ from $Freq_{k-1}$ but keeps only a subset $Cand^i_k$ which has only $\underline{1}$

2. Processor $Proc_i$ produces candidate itemset $Cand_n$ from

   $Freq_{k-1}$ but keeps only a subset $Cand^i$ which has only 1 k

   N

   Item sets of $Cand_n$ Union of all subsets generated by every processor is $Cand_n$ and their intersection is a $\emptyset$.

Processor *Proc$_i$* calculates *Cand$_i$* using the local candidate set *Cand$_i$* after getting complete support count data. Again, the intersection of all *Cand$_i$* sets is ∅ and their union is *Freq$_k$.* broadcasts local *Cand$_n$* counts to develop global *Cand$_n$* counts.

3. Every processor *Proc^i* calculates *Freq$_k$* from *Cand$_n$.* using the global support count.

    If *Freq$_k$.* is a null set then the algorithm terminates, or else it continues. Every processor *Proc$_i$* will independently make this decision

4. All processors synchronize and exchange their local
   Cand$_i$ with other processors. Every processor has the complete$_k$*Freq$_k$* and can decide whether to continue the algorithm or to terminate it independently.

    Figure 1 shows the speedup of CD algorithm (on datasets D1456K.T15.14 and D1140K.T20.16) as the number of processors is increased

Data Distribution Algorithm
This algorithm uses memory of the system extra efficiently. The itemsets are partitioned and shared with processors. Each processor must find the count of its local subset of candidate itemsets and broadcast local data to remaining processors.Partitioning and the distribution of itemsets may affect performance of the algorithm, so it is desirable to have balanced load

Processors find the support count for local item sets in step 2 and broadcast or receive local data. Network congestion may become a problem in this setup so steps must be taken to prevent that.

Parallel Eclat
In Parallel Eclat (Par-Eclat), data is in vertical layout which is done by transforming the horizontal data into vertical itemset list. The two phases in the Parallel Eclat are given below

Initialization Phase
There are 3 sub-steps in the initialization step:

1. The support count for 2-itemsets are read after the preprocessing step has been done and all the frequent itemsets having count>= minimum support are introduced into *Freq2*.
   (*Freq$_k$* is the set of frequent k-itemsets)
2. Any of the clustering schemes between these two - the equivalence class or maximal hypergraph clique clustering is applied to *Freq2* to produce the set of possible maximal frequent itemsets which are then partitioned among all the processors to achieve load-balancing.
3. The database is repartitioned so that every processor obtains the tid-lists of all the 1-itemsets in the cluster appointed to it.

Asynchronous Phase
After the initialization phase, the tid-lists are accessible on local storage on every processor, so every processor can use.

its assigned maximal cluster to generate frequent itemsets without synchronizing with any other processor. A cluster is processed completely before the processor moves onto the subsequent cluster. Local database is examined

one time to complete this step. Therefore, there is less I/O overhead. Since a sublattice is induced by each cluster, either bottom-up traversal is used to produce all frequent itemsets or hybrid traversal is used to produce the maximal frequent itemsets, depending on the algorithm. Initially only tid-list for 1-itemset are available locally using which, the tid-lists for 2-itemsets clusters can be generated. These clusters are generally not big and so keeping the emerging tid-lists in memory creates no problem. 3-itemsets are produced by intersection of the tid-lists for 2-itemsets in the bottom-up approach. If the cardinality of the emerging tid-list >= minimum support the new itemset is added to *Freq3*. Then the resulting frequent 3- itemsets, *Freq3* are divided into sets of equivalence classes on the basis of common pre-fixes of size 2. Intersection of all pairs of 3-itemsets within an equivalence class is used to determine *Freq4*. The process can be repeated till all frequent itemsets are found. After finding *Freqk Freqk-1* is deleted. Memory space is thus needed for the itemsets in *Freqk-1* only within one maximal cluster. This algorithm is thus main memory space efficient. For top-down stage of the hybrid- traversal, memory needs to keep track of only the maximal element seen so far, along with the itemsets not yet seen.

Par-Eclat Algorithm

1. Create *Freq2* using 2-itemset support counts
2. Produce clusters from *Freq2* using Equivalence Classes
3. Assign clusters to processors
4. Scan local dataset part
5. Share appropriate tid-lists with remaining processors
6. Procure tid-lists from remaining processors *foreach* assigned cluster *C* find frequent itemsets using Bottom-Up traversal or Hybrid traversal

Single Pass Counting

It is the parallel implementation of Apriori algorithm using MapReduce. It makes Apriori efficient for mining association rules from massive datasets. The main step in Apriori algorithm is finding the frequency of candidate itemsets. This process of counting can be parallelized. In each step, the mapper program emits <x, 1> for every candidate x that is there in the transaction. The reducer collects the key-value pair emitted by the mapper and sums the value for each key, which gives the total frequency of the candidate in the database. The reducer outputs all the candidates which have enough support count. Single Pass Counting (SPC), at *k*-th pass of database scanning in a MapReduce phase, finds out the frequent *k*-itemsets.

Map(key, value = itemset in transaction t$_i$):

```
Input : Dataset D$_i$

foreach transaction tr$_i$ ∈ D$_i$ do

    foreach item j ∈ tr$_i$ do emit <j, 1>
    end

end
```

Reduce(key=item, value = count):

```
foreach key k do

    foreach value val in k do k.count+=val
    end
     if k.count>=min. supp. Count
```

        emit <k, k.count>

    end

  end

Phase-1 of SPC algorithm

In phase-2, the algorithm generates frequent 2-itemset using frequent itemsets *Freq1* which are located at the Distributed Cache in Hadoop

 Map(key, value = itemset in transaction t_i):

Input : Dataset D_i and *Freq$_{k-1}$*(k>=2).Fetch *Freq$_{k-1}$* from *DistributedCache.*
Build a hash tree for *Cand$_k$*=gen-apriori(*Freq$_{k-1}$*).foreach transaction tr$_i$ ∈ D$_i$ do
    Cand$_t$ = subset(*Cand$_k$*, tr$_i$)

    foreach item i ∈ *Cand$_t$* doemit <i, 1>
    end

  end

 Reduce(key=item, value = count):

foreach key *k* do

  foreach value *val* in *k* do

    k.count+=*val*end
  if k.count>=minimum_support_countemit <k, k.count>
  end

  end

Phase-2 of SPC algorithm

 Algorithm SPC:

1. Run Phase- 1 to find *Freq$_1$*

2. Run Phase-2 to find *Freq$_2$*
   forall *k*>2 and *Freq$_{k-1}$* ≠ *ϕ* Map function of phase-2 Reduce function of phase-2

  end

Distributed Eclat
Distributed Eclat (Dist-Eclat) algorithm is based on Eclat algorithm for mining frequent itemsets from large datasets in parallel using MapReduce. This algorithm focuses on speed. Dist-Eclat is quite fast but it requires huge main memory as it stores the complete dataset in memory in vertical format.

Dist-Eclat is 3-step method (figure 3). Each step can be distributed between mappers to maximise efficiency.
1. *Finding the Frequent Items*: In this step, the vertical database is partitioned into equal-sized sub-databases called shards and distributed among several mappers. Mappers extract the frequent singletons from their shard and

send them to reducer. All frequent items are gathered in the reduce phase.

2. *k-Frequent Itemsets Generation*: The set of frequent k- itemsets, *Freqk*, is generated. First, each mapper is assigned the combined form of local frequent item sets. Mappers find the frequent supersets of size k of the items using Eclat algorithm and running it up to level k. Then a reducer assigns the frequent itemsets to a new set of mappers. Distribution to mappers is performed by employing Round-Robin algorithm. *Subtree Mining*: This step uses Eclat algorithm to mine the prefix tree from the assigned subsets. Subtrees can be mined independently by mappers since sub-trees do not need information from other sub-trees

Figure 4 compares the runtime of Dist-Eclat with Parallel FP- Growth (PFP) [14] over del.icio.us dataset [17]. Minimum support threshold is denoted by the x-axis and the y-axis denotes the time taken by computation in seconds. Dist-Eclat is faster of the two algorithms

CONCLUSION

In this article, many distributed frequent pattern mining algorithms are experimented to test them and present a picture of their evolution over time. A comparison of their runtimes on different datasets is also presented. Two types of algorithms - those based on message passing and those based on the MapReduce approach are explored

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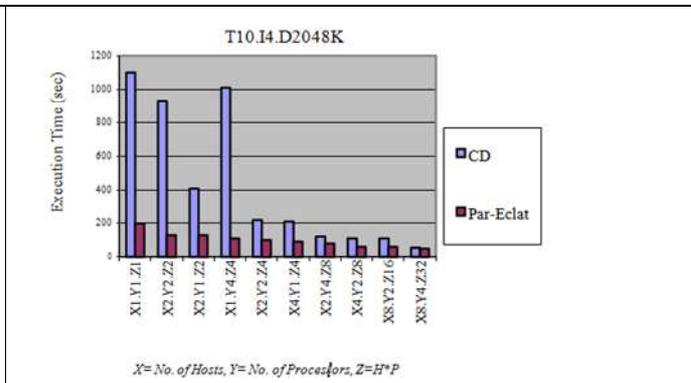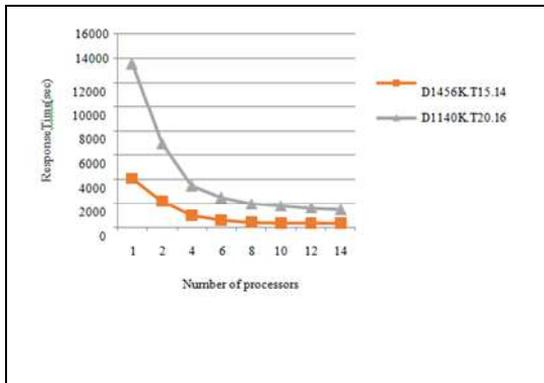
Figure 1: Speedup of Count Distribution Algorithm with increase in the number of processors

Figure 2: Comparison of Execution Time of Par-Eclat and Count Distribution
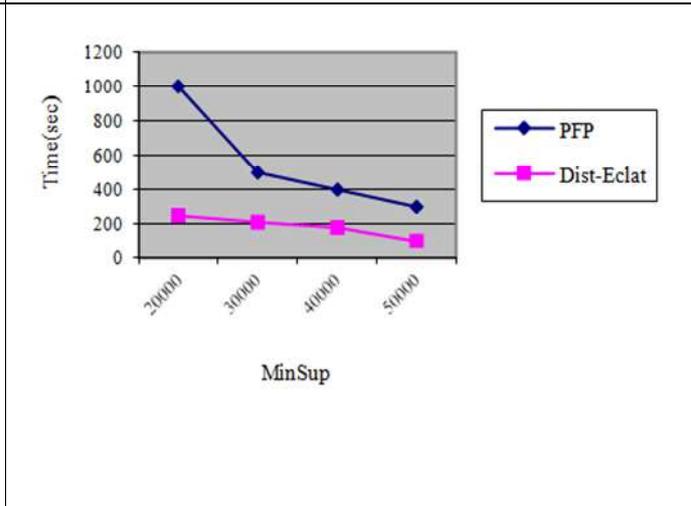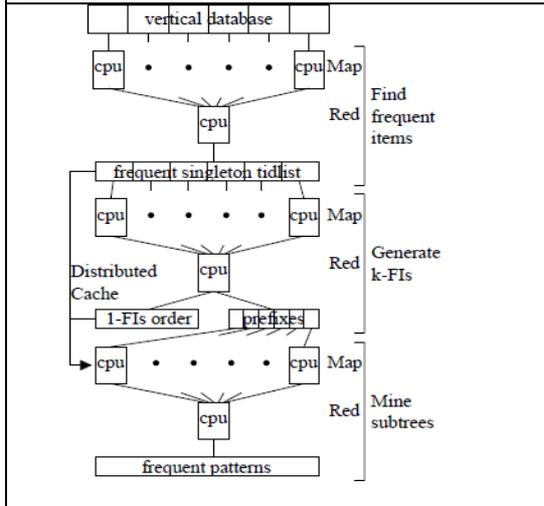
Figure 3: Eclat on Map Reduce Framework

Figure 4: Comparison of Computation Time of Dist-Eclat and PFP

RESEARCH ARTICLE

Stability Analyses in Rice Germplasm for Flowering Traits through Eberhart and Russell's Method and Gauch's Ammi Analysis

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ABSTRACT

Multi-seasonal trials are generally conducted in crop breeding program me to evaluate genotype(s) using various biometrical tools forms the main aim of the multi-seasons data analyses. The objective of the present inquiry was to cull out the stable genotypes with the aid of Eberhart and Russell's joint regression method and Gauch's AMMI analysis. The study included 70 rice genotypes evaluated over three consecutive seasons during 2020 and 2021. Based on the deviation of variance from error mean squares estimates, the ER method identified the genotypes *viz.*, G_3, G_{24} and G_{57} as stable genotypes for days to first flowering; G_{22}, G_{61} and G_{65} for height of the plant at first flowering and G_{14} alone for number of leaves at first flowering. AMMI analyses indicated that the genotype, season G×E effects were significant for all the three traits of interest. ANOVA revealed that the mean squares of the interaction principal components axes (IPCA1 and IPCA2) were found to be highly significant. Based on the ASI having lower mean performance over the grand mean revealed that the genotypes *viz.*, G_{51} and G_{66} recorded lowest ASI coupled with low mean for days to first flowering. The genotypes *viz.*, G_{13}, G_{22} and G_{61} for height of the plant at first flowering and the genotypes *viz.*, G_{33} and G_{57} for number of leaves at first flowering registered lowest ASV.

Keywords: Rice, Ebehart and Russell's joint regression, AMMI.

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INTRODUCTION

Rice is life for many of the Asians. About 90% of the world's rice is produced in Asia. Rice is the most important food crop for South Indians. India is the second large producer of rice in the world, with an average production of 172.580 million tonnes in 2019-'20 and average yield of 3878 kg ha^{-1} (GoI, 2020). The manifestation of any trait is the combined result of the genotype (G), the environment (E) and the interaction of genotype with the environment (G×E). It is imperative to inquire on the G×E interactions and stability, to evaluate the consistency of flowering in rice and develop genotypes that respond positively and consistently across seasons. The responses of the genotypes to varying seasons are not consistent, then the interplay of G×E interaction is evident.

G×E interactions are uneviled using univariate and multivariate analyses. Eberhart and Russell's (1966) model is an univariate method, in which the mean value of each genotype is regressed on the environmental index. It is extensively used, because of its implicit nature. But, this statistics are associated with stability and show little or no correlation with yield. Multivariate analysis of G×E interaction is an alternative but complementary method for evaluating stability. Gauch's (1992) AMMI model (Additive Main effect and Multiplicative Interaction) is a popular modification of ANOVA for deciphering the G×E interaction. This method brings out genotype and environment main effects and utilizes interaction principal components (IPCs), to define patterns in the G×E interaction or residual matrix, which explains a multiplicative model (Rumagosa and Fox, 1993). The AMMI model combines ANOVA from main effects of the genotype and environment with the principal component analysis of G×E interactions (Gauch and Zobel, 1996). AMMI model considered both *per se* performance and stability parameters simultaneously (Alwala *et al.,* 2010). Purchase (2000) developed the AMMI stability value (ASV) to quantify the genotype based on their stability. It is based on the first and second IPC scores for each genotype. This method is comparable with Eberhart and Russell's (1966) method.

MATERIALS AND METHODS

Seventy rice genotypes were evaluated for three consecutive seasons *viz.,* Navarai, *Kharif* and Navarai in the same location, during 2020 and 2021 (Table 1). The traits were conducted in Randomized Block Design (RBD) with three replications, in two row plots of 3 m length, with a spacing of 20 × 15 cm. Each plot consisted of 40 plants. Trials were conducted at Plant Breeding Farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu, India (Latitude 11°23'31.4"N; Longitude 79°42'53.09"E; MSL 5 m). Observations were recorded on days to first flowering, plant height at first flowering and number of leaves at first flowering. Linear regression were carried out for each of the 70 genotypes based on Eberhart and Russel's (1966) method. For genotypic performance across seasons, prediction assessment was conducted using AMMI method (Gabriel, 1978; Zobel *et al.,* 1998; Purchase *et al.,* 2000).

RESULTS AND DISCUSSION

Angiosperms are the most highly evolved and complex organisms with in the plant kingdom, flowering plants undergo alternation of generations. A vegetative shoot meristem enter to a flowering pathway by external stimuli such as temperature, and day length, apart from the age of the plant. Photosensitive plants require a specific day length to enter into reproductive phase. Bernier (1988) suggested that photoperiods are perceived by photo chrome in the leaves and that a flowering signal is transmitted to the shoot meristem, in the photosensitive plants. By contrast, photo insensitive plants do not require specific photoperiod for flowering. In photo insensitive plants, flowering occurs after the plant reaches a specific age, that is after a pre-set of number of leaf nodes have formed on the stem (Bernier, 1988, Singer and Daniel, 1986, Gebhardt and McDaniel, 1987). Drews and Goldbert (1989) opined

that the plants cannot be induced to flower, before this age. The present inquiry aims to find out the possible inter-play of age of the plant, number of leaves and height of the plant that are required for the rice plant to flower. The present investigation also aims to bring out the stable genotypes for flowering, using various stability models. The pooled analysis of variance results are presented in Table 2. It indicated that the season(s) and genotype (G) main effects were significant. There existed substantial variation among the genotypes as well as seasons. The percentage of variation explained by season and genotypes were comparable. The variation due to G×S interaction was considerable. Comparable percentage of 'S' main effects were previously obtained by Alwala *et al.* (2010) in maize trials, Baxevanos *et al.* (2008) in cotton trials.

The Eberhart and Russel regression analysis are presented in Tables 3-5. In the present study, out of 70 genotypes, only those which showed high mean (overall mean –2 SE) were considered for classification and characterization. These genotypes were classified into four groups according to the methodology followed by Mehra and Ramanujam (1979); Singh and Singh (1980) and Thirugnanakumar (1991).

Group	Mean	'b'	$\overline{S}^2 d$
I	Low mean	Around unity*	Around zero*
II	Low mean	Significantly deviating from unity	Around zero*
III	Low mean	Around unity*	Significantly deviating from zero
IV	Low mean	Around unity*	Significantly deviating from zero

*Deviation not significant

Population falling in group I will have average responsiveness and highly stable over seasons. Populations in group II will have above or below average responsiveness, that is well suited for stress or favourable seasons and will be stable in the respective seasons. Behaviour of the population falling in group III and IV cannot be predicted. The genotypes *viz.*, G_3, G_{24} and G_{57} were gathered in Group I, for days to first flowering and identified as stable genotypes. For plant height at first flowering, the genotypes *viz.*, G_{22}, G_{61} and G_{65} were selected as stable genotypes. Only one genotypes *viz.*, G_{14} alone was found as stable for number of leaves at first flowering.

Eberhart and Russel (1966) regression model has been widely used because of its explicit nature. This model subdivides the variability in the performance of any genotype into predictable (regression) and unpredictable (var-dev) components. Further, this model considers average response (regression) and stability (var-dev) of the genotypes. In the present investigation, the genotypes which fall in the Group I were inferred as stable over seasons. Accordingly, the genotypes *viz.*, G_{10}, G_{27}, G_{46}, G_{58}, G_{61} and G_{66} for days to first flowering; the genotypes *viz.*, G_{22}, G_{61} and G_{65} for plant height at first flowering and the genotype *viz.*, G_{14} for number of leaves at first flowering were culled out as stable genotypes, respectively. The mean number of days to first flowering was lowest in 42.11 (days) and was highest in 114.33 (days). Among the genotypes, G_{69} recorded the lowest days to first flowering followed by G_{65} and G_{38} genotypes. Variation for days to first flowering among the genotypes across the season indicated the presence of G×S interaction. For example, the genotype *viz.*, G_{21} performing best in S3 (33.33 days) among the genotypes but poor in S1 and S2 seasons.

The mean plant height at first flowering of the genotypes across season was 112.64 cm. The genotype G_{53} (73.67 cm) was having the lowest height followed by G_{65} (74.74 cm) and G_{33} (78.60 cm). The mean number of leaves at first flowering of the genotypes across seasons was 70.26 days. The genotype G_{47} (101.40) was having the highest number of leaves followed by G_{58} (100.44) and G_{14} (100.37). Genotype, seasons and GSI interactions were estimated by the additive main effect and multiplicative interaction (AMMI model). In the present inquiry, the ANOVA for all the six

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traits of interest were significant for genotype, season and GSI. GSI was inferred by changes in the relative performance of genotypes across seasons. The effects of genotype, followed by season and genotype by season interaction effects were responsible for variation. Because of high variation in genotypes may cause high genotypic variance. This has been documented from the findings of rice crop. In the present study, the results of ANOVA, the 70 genotypes revealed that the mean square of the first interaction principal component axes (IPCA1 and IPCA2) were fond to be highly significant (P<0.001). The variation could almost be contributed by the genotypic effects. The AMMI results showed that IPCA1 and IPCA2 explained that the interaction sum of squares of 100 per cent, indicating that the first two IPCA were sufficient to explain GSI in the traits of interest of rice genotypes.

AMMI analyses are presented in Tables 6 and 7. The relative magnitude of G, S and G × E interaction for the three traits of interest are presented in the form of ANOVA. Analysis of variance revealed that the genotype, season and G×S effects were significant for all the three traits of the present inquiry (P<0.01), except season effects for number of leaves at first flowering. Effects due to genotypes were found to be the main controlling source of variation for almost all the traits and its proportion ranged from 86.40 to 98.56 per cents for various traits. However, the contribution of genotypes and their interaction with season was less. For days to first flowering, season accounted for 0.64 per cent, genotypes 98.14 per cent and genotypes × season 1.22 per cent. For plant height at first flowering season accounted for 6.92 per cent, genotypes 88.76 per cent and G×S 4.33 per cent. For number of leaves at first flowering season accounted for 0.26 per cent, genotypes 78.44 per cent and G×S 21.30 per cent.

The mean number of days to first flowering was 63.52 days. The average number of days to first flowering was least in G_{69} and highest in G_{68}. The genotype G_{69} was having the least number of days to first flowering followed by G_{70}. The mean plant height at first flowering was least with the genotype G_{53} and highest with the genotype G_{26}. The mean plant height at first flowering was 112.64 cm. The mean number of leaves at first flowering was 70.26. It was least with the genotype G_{42}. It was highest with the genotype G_{47}. In the present study, the results of ANOVA, the 70 genotypes revealed that the mean squares of the interaction principal components axes (IPCA1 and IPCA 2) were found to be highly significant. The variation could be almost contributed by the genotypic effects (98.14 per cent for days to first flowering 88.76 per cent for plant height at first flowering and 78.44 per cent for number of leaves at first flowering) where as the G×S interaction effects contributed less (1.22 per cent for days to first flowering; 4.33 per cent for plant height at first flowering. Interestingly, the G×S interaction effect was high for number of leaves at first flowering (21.30 per cent).

The AMMI results also showed that IPCA1 and IPCA2 explained that the interaction sum squares of 100 per cent for all the traits of interest. The IPCA1 and IPCA2 accounted for 60.5 and 39.5 per cent, respectively and together contributed 100 per cent of the variability for days to first flowering of 70 genotypes tested with three seasons. The IPCA1 and IPCA2 accounted for 60.4 and 39.6 per cent respectively, for plant height at first flowering and 91.9 and 8.1 per cent respectively, for number of leaves at first flowering. The measuring of stability value quantitatively is called as AMMI stability value (ASV). The ranking of genotypes through AMMI model was considered to be the most appropriate single method of describing the stability of genotypes. They are furnished in Tables 11 to 13. Scores of IPCA1 and IPCA2 for each genotype, for the three traits and the corresponding ASV were calculated and their ranks were presented. Based on this, the lowest ASV having higher mean performance over the grand mean reveled that the genotypes *viz.,* G_{51} and G_{66} recorded the lowest ASI coupled with low mean for days to first flowering. The genotypes *viz.,* G_{13}, G_{22} and G_{61}, for plant height at first flowering registered lowest ASI coupled with low mean. The genotypes *viz.,* G_{33} and G_{57} for number of leaves at first flowering and the genotypes *viz.,* G_{14} for per day productivity registered lowest ASI coupled with high mean. The aforementioned genotypes were considered as the stable genotypes across all the seasons, where as the varieties with high ASI for the three traits of interest were considered to be suitable for the specific season, even though they recorded desirable mean values than the grand mean. The remaining genotypes were considered unsuitable to any season since they had a less average values whatever may be the ASI rank.

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The most powerful interpretive tool for the AMMI model is biplot analysis. Biplots are graphs to identify the inter-relationships between genotypes and seasons which are plotted on the same axes (Vargas and Crossa, 2000). There are two basic AMMI biplot, where the main effects of the traits of interest (genotype mean and environment mean) and IPCA1 scores for both genotypes and environments are plotted against each other. On the other hand, the second is AMMI where scores for IPCA1 and IPCA2 are plotted. The effect of each genotype and environment, IPCA1 *vs* the means (Figs. 1 to 3) and IPCA2 *vs* IPCA1 (Figs. 4 to 6) biplots are shown. In Fig. 1 to 6, the main effect (mean performance) was indicated by X-coordinate, the effects of interaction (IPCA1) were indicated by the Y-coordinate. For days to first flowering, the genotypes *viz.,* G_{15}, G_{17} and G_{11} had a low positive interaction with a higher main effect and made them the most preferable for selection. The genotype G_{69} had a low negative interaction, as evident from their low IPCA1 score. The genotypes plotted at the right hand side of the grand mean and near to PCA1=0. Line were found as G_9, G_2, G_{26} and G_{29}. The genotypes G_5 and G_{21} recoded desirable mean and large IPCA1 scores.

Many genotypes were stationed near the origin. Yet, some other genotypes were distant from the biplot origin. The stability of the genotypes as well as the extent of interaction effects of each genotype and environment, AMMI biplot were drawn using IPCA 1 and IPCA2 scores (Figs. 4 to 6). The genotypes *viz.,* G_{16}, G_{17} and G_{53} were found to be well away from the origin. Most of the genotypes were close to the origin. The genotypes *viz.,* G_{11}, G_{29} and G_{26} had a low positive interaction with a higher main effect for plant height at first flowering. The genotype *viz.,* G_{65} had a low negative interaction. The genotypes *viz.,* G_{11} and G_{69} were plotted at the right hand side of the grand mean and near to PCA1=0. The genotype *viz.,* G_{21} registered desirable mean with large PCA1 scores. The AMMI biplot drawn using ICPA1 and ICPA2 scores indicated that the genotypes *viz.,* G_{37} and G_{15} were found well away to the origin. Majority of the genotypes were closer to the origin. For number of leaves at first flowering, the genotypes *viz.,* G_{43} and G_{23} had a low positive interaction with a higher main effect. The genotypes *viz.,* G_2 had a low negative interaction. The genotypes *viz.,* G_4 and G_{23} were plotted at the right hand side of the grand mean and near to PCA1=0. The genotype *viz.,* G_{58}, recorded desirable mean with large PCA1 scores. The AMMI biplot drawn using ICPA1 and ICPA2 scores indicated that the genotype *viz.,* G_{37} was found away to the origin majority of the genotypes were clustered near to the origin.

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Table 1. Genotypes used in the present inquiry

Genotype No.	Name	Genotype No.	Name
G_1	Annanda	G_{36}	IC-0142508
G_2	Durga	G_{37}	IC-0123083
G_3	CR 1014	G_{38}	IC-0135529
G_4	Satyabhama	G_{39}	IC-0134873
G_5	CR dhan 204	G_{40}	IC-0207992
G_6	Phalguni	G_{41}	IC-0207955
G_7	CR dhan 203	G_{42}	IC-206447
G_8	CR dhan 305	G_{43}	IC-125757
G_9	CR dhan 601	G_{44}	IC-0514489
G_{10}	Kalinga III	G_{45}	IC-114312
G_{11}	Jalamani	G_{46}	IC-0627835
G_{12}	CR dhan 501	G_{47}	IC-0623213
G_{13}	CR dhan 101	G_{48}	IC-214312
G_{14}	CR dhan 202	G_{49}	IC-135191
G_{15}	CR dhan 310	G_{50}	IC-377869
G_{16}	CR dhan 408	G_{51}	IC-379136
G_{17}	CR dhan 307	G_{52}	IC-611162
G_{18}	CR dhan 303	G_{53}	IC-386231
G_{19}	Sumit	G_{54}	IC-ARC-11203
G_{20}	Tapaswini	G_{55}	IC-67725
G_{21}	Pooja	G_{56}	IC-264987
G_{22}	Vandana	G_{57}	IC-518987
G_{23}	Pyari	G_{58}	IC-ARC-7432
G_{24}	Improved Lalat	G_{59}	IC-ARC-10595
G_{25}	Gayatri	G_{60}	ADT 36
G_{26}	Samalei	G_{61}	ADT 37
G_{27}	Naveen	G_{62}	ADT 42
G_{28}	Anjali	G_{63}	ADT 43
G_{29}	Savala	G_{64}	ADT 45
G_{30}	CR dhan 701	G_{65}	ADT 48
G_{31}	Swarna Sub 1	G_{66}	ASD 16
G_{32}	IC-0098989	G_{67}	ADT 39
G_{33}	IC-0124198	G_{68}	CR 1009 (Sub 1)
G_{34}	IC-0135769	G_{69}	IC-0203398
G_{35}	IC-0123756	G_{70}	IC-0124570

Table 2. Stability parameters – X₁) Number of days to first flowering

Varieties	Mean $\overline{X}$	Regression 'b'	Deviation from regression $(\hat{Sd}^2)$
G_1	57.3333**	1.4969*	1.8765
G_2	75.7778	2.0069	10.154**
G_3	85.7778	1.0764	1.9787
G_4	72.4445**	1.3216**	0.081
G_5	81.2222	1.6512	36.1073**
G_6	61.7778**	0.4655**	-0.1829
G_7	61.7778**	0.5108	3.9101*
G_8	60.4444**	1.9673**	-0.1954
G_9	69.8889	3.3034**	9.6559**
G_{10}	50.6667**	0.6756	1.3657
G_{11}	106.5556	1.6819**	-0.177
G_{12}	107.0000	1.6061	35.1092**
G_{13}	67.0000	-0.6101	25.7092**
G_{14}	69.8889	0.571	6.8663**
G_{15}	61.6667	2.3627**	-0.0292
G_{16}	72.8889	-0.6654**	10.1182**
G_{17}	72.4444	2.2125**	0.5638
G_{18}	53.3333**	1.526	36.303**
G_{19}	55.1111**	0.0747	9.4712**
G_{20}	68.5556	0.7061	5.6008*
G_{21}	68.4444	6.941	265.6428**
G_{22}	60.7778**	-2.8673**	68.2685**
G_{23}	60.5556**	1.4117**	-0.0664
G_{24}	80.0000	0.746	1.6089
G_{25}	105.6667	0.9305	21.8209**
G_{26}	85.8889	0.5653	7.5272**
G_{27}	62.5556**	1.1916	2.8919
G_{28}	54.000**	0.9256	11.6741**
G_{29}	106.3333	0.7355	7.9055**
G_{30}	64.1111	0.8854	21.0447**
G_{31}	48.6667**	1.8019**	1.0266
G_{32}	52.7778**	0.2908	11.5248**
G_{33}	51.3333**	0.3908	8.7489**
G_{34}	53.4444**	0.4404**	0.126

Table 2 contd...

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Varieties	Mean $\overline{X}$	Regression 'b'	Deviation from regression $(\hat{Sd}^2)$
G$_{35}$	46.8889**	1.4618*	1.3847
G$_{36}$	55.4444**	1.5562	21.072**
G$_{37}$	47.6667**	-0.7962**	4.8262*
G$_{38}$	45.7778**	1.8322**	-0.0924
G$_{39}$	53.3333**	1.2615**	-0.1873
G$_{40}$	43.5556**	0.3008	9.7736**
G$_{41}$	57.7778**	3.3485**	10.1786**
G$_{42}$	47.5556**	1.1406	33.7179**
G$_{43}$	57.3333**	-0.7452	28.3179**
G$_{44}$	46.3333**	0.4459	6.7809**
G$_{45}$	56.5556**	2.1674**	0.4284
G$_{46}$	49.6667**	0.4758	3.2016
G$_{47}$	79.4445	0.8312	5.678**
G$_{48}$	53.7778**	1.812**	1.68
G$_{49}$	45.5556**	1.5217**	-0.0809
G$_{50}$	50.5556**	-0.51	21.4939**
G$_{51}$	49.3333**	0.5607**	0.288
G$_{52}$	56.3333**	0.6862**	8.4464**
G$_{53}$	48.3333**	0.8763	5.2816*
G$_{54}$	52.8889**	-0.0699**	3.2711
G$_{55}$	51.3333**	0.7962	4.8255*
G$_{56}$	103.4445	0.7511	5.2061*
G$_{57}$	91.1111	0.701	1.8386
G$_{58}$	46.7778**	0.4308	3.5157
G$_{59}$	54.2222**	0.7312	7.9621**
G$_{60}$	55.2222**	2.1124**	-0.0009
G$_{61}$	54.3333**	0.5559**	3.5784
G$_{62}$	59.2222**	3.0332	6.7625**
G$_{63}$	74.8889	0.9465	7.1262**
G$_{64}$	49.6667**	0.2557	10.3065**
G$_{65}$	45.5556**	0.5459	4.6822*
G$_{66}$	57.7778**	0.8361	1.1894
G$_{67}$	90.5555	0.6762	10.0801**
G$_{68}$	114.3333	0.6862	8.4444**
G$_{69}$	42.1111**	0.1799	6.363*
G$_{70}$	45.8889**	0.2748	15.6004**

Table 3. Stability parameters –X₂) Height of the plant at first flowering (cm)

Varieties	Mean $\overline{X}$	Regression 'b'	Deviation from regression $(\hat{Sd}^2)$
G_1	118.3833	1.892	243.707**
G_2	105.46**	0.3917*	5.3407*
G_3	127.17	0.6491	3.9171*
G_4	99.5778**	0.7265	54.3295**
G_5	142.7633	0.8417	23.1936**
G_6	104.7744**	0.6887	12.0162**
G_7	102.85**	0.3901	146.977**
G_8	116.5211	1.1299	-2.2289
G_9	122.8567	-0.2237	309.7824**
G_{10}	98.8767**	0.6839	16.359**
G_{11}	129.9144	0.7776	12.888**
G_{12}	140.5656	0.6033	1.4728
G_{13}	99.4467**	1.0977	6.4119*
G_{14}	112.7933	0.6168	12.3251**
G_{15}	114.7756	1.0487	13.1252**
G_{16}	110.8167	2.2267	740.1035**
G_{17}	117.2944	-1.1061**	-3.1634
G_{18}	108.7233	1.4595	518.1686**
G_{19}	124.1011	-0.3394	318.2013**
G_{20}	120.0344	0.6237	1.9535
G_{21}	128.6922	-0.3579	421.9006**
G_{22}	87.4544**	0.7762	-0.1833
G_{23}	115.4344	1.5925	84.6562**
G_{24}	118.1667	0.8654	38.4627**
G_{25}	118.6589	0.5288	125.0182**
G_{26}	161.3933	0.9549	101.1205**
G_{27}	105.7067**	0.5972**	-3.0248
G_{28}	99.6489**	0.8211	28.4145**
G_{29}	146.68	0.7522	17.0989**
G_{30}	113.4711	0.7103	3.9828*
G_{31}	125.3244	1.3863	112.8536**
G_{32}	105.91**	0.9573	58.0224**
G_{33}	78.6033**	0.894	50.095**
G_{34}	139.8989	0.8041	28.2772**
G_{35}	99.5911**	2.3184	646.8909**
G_{36}	83.7333**	1.1989	13.598**
G_{37}	93.9145**	0.5419	13.4156**
G_{38}	108.69	0.7309	24.2687**
G_{39}	119.6045	0.7594	20.255**
G_{40}	112.4478	0.8078**	-3.7822

G_{41}	125.5578	0.5546	27.5035**
G_{42}	114.3333	1.1785	225.7275**
G_{43}	126.3878	1.6438	84.974**
G_{44}	111.7922	0.4339	59.5079**
G_{45}	110.16	2.7051	583.359**
G_{46}	117.8689	1.1856	23.6658**
G_{47}	125.5211	0.7539	32.6561**
G_{48}	129.5178	1.7318	126.6212**
G_{49}	104.8556**	2.4891	539.8613**
G_{50}	113.8089	1.9106	115.3997**
G_{51}	115.9411	-0.2361	276.6136**
G_{52}	103.8744**	2.3891	348.706**
G_{53}	73.6689**	5.6124	4671.583**
G_{54}	105.2022**	3.4472	1247.595**
G_{55}	89.9311**	1.6755	155.1019**
G_{56}	113.4356	0.3529	120.6573**
G_{57}	119.5833	1.0632	56.6052**
G_{58}	114.7178	1.1467	-1.2489
G_{59}	94.8033**	0.6137	14.6286*
G_{60}	94.3211**	0.6076	26.5204*
G_{61}	102.2244**	0.7386	1.9501
G_{62}	93.2044**	0.4245	27.7869**
G_{63}	141.2722	0.6388	9.0604**
G_{64}	90.7378**	0.8613	41.2123**
G_{65}	74.7444**	0.63	1.2637
G_{66}	111.6856	0.8295	9.6967**
G_{67}	129.3678	1.9924	107.239**
G_{68}	116.6833	0.3423	9.0872**
G_{69}	125.2444	0.2595	55.379**
G_{70}	113.8822	0.2043	30.6288**

Table 4. Stability parameters –X_3) Number of leaves at first flowering

Varieties	Mean $\overline{X}$	Regression 'b'	Deviation from regression $(\hat{Sd}^2)$
G_1	95.5811**	5.4249**	2.9935
G_2	39.7556	0.1395	7.9614**
G_3	54.5933	0.113**	0.4909
G_4	87.7867**	0.4345	17.1773**
G_5	94.9189**	0.2443*	1.709
G_6	82.89**	-0.1446**	2.7839

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G_7	90.0867**	0.0585*	3.5422
G_8	57.3456	-2.0482**	5.144*
G_9	61.0078	-0.3542*	8.3426**
G_{10}	57.6144	0.0119**	1.5658
G_{11}	76.8833**	0.475	11.9246**
G_{12}	59.0856	0.2577	3.6748
G_{13}	58.4567	0.3406*	1.3651
G_{14}	100.3678**	0.7795	3.7422
G_{15}	81.58**	3.8035**	-0.4625
G_{16}	82.1033**	3.7277**	2.0927
G_{17}	59.0489	-2.1562**	4.8944*
G_{18}	86.4633**	4.1421**	1.848
G_{19}	71.6567**	3.7219**	-0.6417
G_{20}	96.0722**	0.2833*	2.7937
G_{21}	87.3778**	9.2176**	40.1616**
G_{22}	81.7433**	0.026*	3.3981
G_{23}	95.65**	6.2657**	-1.2435
G_{24}	95.9222**	0.4504	7.3495**
G_{25}	66.1556	0.2896*	1.6643
G_{26}	59.9	0.079**	0.7848
G_{27}	90.3178**	0.0232	6.6711*
G_{28}	51.3556	-0.0026**	1.2782
G_{29}	43.0222	0.089**	1.7319
G_{30}	62.6556	0.1317**	0.1323
G_{31}	58.0044	-1.1642*	16.448**
G_{32}	95.85**	0.1685*	3.2884
G_{33}	75.8055**	0.1268**	-0.0204
G_{34}	75.1856**	0.3672	8.5296**
G_{35}	72.4122**	4.1129**	-0.9923
G_{36}	58.8022	1.2703	96.2178**
G_{37}	59.2456	-2.1119	199.5695**
G_{38}	45.9511	0.4355	10.7426**
G_{39}	65.8244	0.4354	2.5041
G_{40}	61.2278	2.8581**	5.1924*
G_{41}	78.4511**	2.1757*	8.6288**
G_{42}	33.7044	0.1737*	2.2396
G_{43}	78.0356**	9.3595**	-1.8468
G_{44}	31.3689	-0.4923	102.566**
G_{45}	84.9578**	-3.7703**	8.3859**
G_{46}	89.8156**	0.2088	12.9741**
G_{47}	101.4**	0.0483	6.4*

G48	52.9833	-4.8649**	28.21**74
G49	54.08	-1.2135**	1.0193
G50	51.5333	5.1744**	7.4365**
G51	70.2878	3.2225**	3.2707
G52	70.6967	8.0907**	-1.377
G53	79.3111**	4.0943**	0.6147
G54	70.9611	3.9327**	-0.1596
G55	54.5722	-1.3952**	6.1921*
G56	81.8**	0.0045*	4.2691*
G57	60.8356	0.1871**	0.1688
G58	100.44**	5.1639	18776.35**
G59	74.9556**	0.3444	12.9673**
G60	76.2533**	-0.1674**	3.4309
G61	47.23	0.9316	11.629**
G62	55.5311	-4.4878**	28.3053**
G63	86.49**	0.1358	6.556*
G64	74.69**	0.2246	6.335*
G65	55.1833	0.2531*	2.0716
G66	39.8867	-1.7592**	2.7697
G67	72.2278**	0.1884	3.949*
G68	76.9067**	-0.257**	1.7389
G69	65.2422	0.9499	1.9567
G70	49.3733	1.2352	37.7227**

Table 5. Mean squares and per cent variation explained by genotype(G), season (S) and G×S interaction for various traits

Sources	df	Sum sq	Mean sq	'F' value	Pr (<F)	Explained percentage
X₁) Days to first flowering						
Env.	2	1351	675.50	77.42	5.193e-05**	0.64
Rep. (Env)	6	52	8.73	20.583	<2.2e-16***	-
Genotype	69	205881	2983.79	7038.177	<2.2e-16***	98.14
Env:Gen	138	2551	18.48	43.599	<2.2e-16***	1.22
Residuals	414	176	0.42			-
PC 1	70	1544.419	22.06313	52.04	0	60.5
PC 2	68	1006.335	14.799905	34.91	0	39.5
X₂) Plant height at first flowering						
Env.	2	16330	8165.1	63.077	9.359e-05***	6.92
Rep. (Env)	6	777	129.4	58.903	<2.2e-16***	-
Genotype	69	209595	3037.6	1382.222	<2.2e-16***	88.76
Env:Gen	138	10218	74.0	33.692	<2.2e-16***	4.33
Residuals	414	910	2.2			-

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PC 1	70	6174.929	88.21327	40.14	0	60.4
PC 2	68	4043.026	59.45627	27.05	0	39.6
X₃) Number of leaves at first flowering						
Env.	2	745	372.3	2.0949	0.2042	0.26
Rep. (Env)	6	1066	177.7	261.5947	<2e-16***	-
Genotype	69	227817	3301.7	4859.6486	<2e-16***	78.44
Env:Gen	138	61867	448.3	659.8568	<2e-16***	21.30
Residuals	414	281	0.7			-
PC 1	70	56872.589	812.46555	1195.84	0	91.9
PC 2	68	4994.662	73.45091	108.11	0	8.1

Significant Codes: 0.01 = ˙ ; 001 = ˙˙ ; 0 = ˙˙˙; 0.1 = 1 ; 0.05 = ′

Table 6. Mean, AMMI stability value and genotype selection index X₁) Number of days to first flowering

Genotype No.	Mean	IPCA 1	IPCA 2	ASI	RBSI
G₁	57.33	-0.23	0.49	3.56	66
G₂	75.78	0.51	0.15	4.09	59
G₃	85.78	-0.23	0.25	2.39	20
G₄	72.45	-0.08	0.26	1.77	26
G₅	81.22	0.95	-0.45	7.94	76
G₆	61.78	0.02	-0.33	2.10	40
G₇	61.78	-0.32	-0.01	2.51	49
G₈	60.44	-0.01	0.58	3.67	61
G₉	69.89	0.50	0.93	7.04	76
G₁₀	50.67	0.20	-0.37	2.76	72
G₁₁	106.55	0.02	0.39	2.43	17
G₁₂	107.00	0.94	-0.46	7.87	66
G₁₃	67.00	-0.81	-0.25	6.49	80
G₁₄	69.89	-0.42	0.11	3.35	51
G₁₅	61.67	0.07	0.75	4.74	72
G₁₆	72.89	-0.51	-0.54	5.24	69
G₁₇	72.44	0.14	0.60	3.91	58
G₁₈	53.33	0.96	-0.53	8.13	106
G₁₉	55.11	0.49	-0.98	7.23	106
G₂₀	68.56	-0.38	0.16	3.13	46
G₂₁	68.44	2.60	1.26	21.70	84
G₂₂	60.78	-1.32	-1.15	12.54	110
G₂₃	60.56	-0.06	0.29	1.89	38
G₂₄	80.00	-0.21	0.04	1.67	16
G₂₅	105.67	0.74	-0.69	7.21	66
G₂₆	85.89	0.44	-0.64	5.28	60
G₂₇	62.56	-0.28	0.36	3.11	49

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G_{28}	54.00	0.54	-0.52	5.35	94
G_{29}	106.33	0.45	-0.55	4.91	52
G_{30}	64.11	0.73	-0.71	7.19	83
G_{31}	48.67	0.18	0.32	2.45	69
G_{32}	52.78	-0.54	0.05	4.24	94
G_{33}	51.33	-0.47	0.05	3.71	87
G_{34}	53.44	0.09	-0.41	2.66	66
G_{35}	46.89	-0.20	0.45	3.20	86
G_{36}	55.45	0.73	-0.31	6.00	90
G_{37}	47.67	0.35	-1.37	9.05	134
G_{38}	45.78	-0.05	0.54	3.40	93
G_{39}	53.33	0.02	0.14	0.89	46
G_{40}	43.56	-0.50	0.02	3.90	109
G_{41}	57.78	0.52	0.95	7.17	88
G_{42}	47.56	0.92	-0.72	8.48	125
G_{43}	57.33	-0.85	-0.29	6.86	99
G_{44}	46.33	-0.42	0.04	3.27	91
G_{45}	56.55	0.13	0.58	3.80	68
G_{46}	49.67	-0.29	-0.06	2.31	65
G_{47}	79.44	-0.38	0.24	3.33	42
G_{48}	53.78	0.22	0.29	2.50	55
G_{49}	45.55	0.06	0.26	1.71	68
G_{50}	50.56	-0.74	-0.25	5.97	112
G_{51}	49.33	-0.11	-0.16	1.34	58
G_{52}	56.33	-0.47	0.22	3.88	76
G_{53}	48.33	-0.37	0.25	3.28	86
G_{54}	52.89	-0.30	-0.38	3.31	78
G_{55}	51.33	-0.35	0.19	3.00	72
G_{56}	103.44	-0.37	0.17	3.06	28
G_{57}	91.11	-0.23	0.02	1.77	13
G_{58}	46.78	-0.31	-0.07	2.42	77
G_{59}	54.22	-0.45	0.24	3.82	83
G_{60}	55.22	0.07	0.60	3.80	72
G_{61}	54.33	-0.31	0.01	2.40	59
G_{62}	59.22	0.42	0.84	6.22	82
G_{63}	74.89	-0.43	0.34	3.96	58
G_{64}	49.67	-0.51	0.01	4.00	100
G_{65}	45.56	-0.35	0.04	2.74	87
G_{66}	57.78	-0.19	0.07	1.51	38
G_{67}	90.56	-0.51	0.25	4.25	54
G_{68}	114.33	-0.47	0.22	3.88	40
G_{69}	42.11	0.40	-0.84	6.13	124
G_{70}	45.89	0.62	-0.98	7.84	130

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Table 7. Mean, AMMI stability value and genotype selection index X4) Height of the plant at first flowering (cm)

Genotype No.	Mean	IPCA 1	IPCA 2	ASI	RBSI
G_1	118.38	0.907	0.086	7.069	93
G_2	105.46	-0.261	0.611	4.348	84
G_3	127.17	-0.199	0.262	2.263	26
G_4	99.58	-0.408	-0.270	3.598	86
G_5	142.76	-0.273	-0.225	2.548	25
G_6	104.77	-0.247	0.101	2.020	58
G_7	102.85	-0.691	-0.191	5.502	96
G_8	116.52	0.085	-0.076	0.814	31
G_9	122.85	-1.064	0.220	8.390	68
G_{10}	98.88	-0.271	0.064	2.146	66
G_{11}	129.92	-0.234	-0.036	1.830	15
G_{12}	140.56	-0.186	0.370	2.739	30
G_{13}	99.45	0.168	0.138	1.569	58
G_{14}	112.79	-0.262	0.201	2.402	54
G_{15}	114.78	-0.181	-0.429	3.043	61
G_{16}	110.82	-1.023	-4.138	27.220	101
G_{17}	117.29	-0.455	2.959	18.946	92
G_{18}	108.72	1.151	1.327	12.238	116
G_{19}	124.10	-1.098	0.365	8.837	69
G_{20}	120.03	-0.186	0.332	2.541	43
G_{21}	128.69	-1.226	0.158	9.584	69
G_{22}	87.45	-0.133	0.155	1.425	67
G_{23}	115.43	0.554	-0.034	4.310	78
G_{24}	118.17	-0.329	-0.372	3.466	57
G_{25}	118.66	-0.620	-0.310	5.202	66
G_{26}	161.39	-0.484	-0.827	6.421	56
G_{27}	105.70	-0.122	0.500	3.288	78
G_{28}	99.65	-0.299	-0.237	2.760	79
G_{29}	146.68	-0.261	-0.042	2.050	13
G_{30}	113.47	-0.187	0.173	1.819	40
G_{31}	125.32	0.578	0.384	5.100	64
G_{32}	105.91	-0.373	-0.623	4.878	88
G_{33}	78.60	-0.362	-0.487	4.158	105
G_{34}	139.90	-0.302	-0.211	2.699	30
G_{35}	99.59	1.445	0.323	11.416	128
G_{36}	83.74	0.234	0.077	1.880	75
G_{37}	93.91	-0.284	0.298	2.892	89
G_{38}	108.69	-0.299	-0.074	2.373	59
G_{39}	119.60	-0.276	-0.081	2.202	36
G_{40}	112.45	-0.026	0.300	1.898	48
G_{41}	125.56	-0.348	0.153	2.873	41

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G₄₂	114.33	0.739	1.062	8.806	100
G₄₃	126.39	0.565	-0.106	4.443	57
G₄₄	111.79	-0.481	0.122	3.819	71
G₄₅	110.16	1.462	-0.345	11.574	116
G₄₆	117.87	-0.207	-0.724	4.826	69
G₄₇	125.52	-0.329	-0.172	2.778	41
G₄₈	129.52	0.675	-0.059	5.259	64
G₄₉	104.86	1.377	-0.114	10.728	120
G₅₀	113.81	0.687	-0.360	5.802	96
G₅₁	115.94	-1.022	0.321	8.194	77
G₅₂	103.87	1.146	-0.365	9.201	117
G₅₃	73.67	4.086	-0.686	32.058	140
G₅₄	105.20	2.126	-0.443	16.762	129
G₅₅	89.93	0.719	0.125	5.645	118
G₅₆	113.43	-0.646	-0.040	5.030	73
G₅₇	119.58	-0.348	-0.768	5.535	72
G₅₈	114.72	-0.046	-0.351	2.239	47
G₅₉	94.81	-0.276	0.182	2.427	77
G₆₀	94.32	-0.334	0.085	2.647	82
G₆₁	102.22	-0.164	0.166	1.648	53
G₆₂	93.21	-0.375	0.339	3.608	96
G₆₃	141.27	-0.238	0.207	2.266	20
G₆₄	90.74	-0.339	-0.385	3.576	96
G₆₅	74.74	-0.178	0.335	2.525	89
G₆₆	111.69	-0.204	-0.075	1.658	43
G₆₇	129.37	0.685	-0.511	6.221	70
G₆₈	116.68	-0.297	0.633	4.606	68
G₆₉	125.24	-0.504	0.396	4.641	54
G₇₀	113.88	-0.430	0.634	5.202	77

Table 8. Mean, AMMI stability value and genotype selection index X₆) Number of leaves at first flowering

Genotype No.	Mean	IPCA 1	IPCA 2	ASI	RBSI
G₁	95.62	0.040	-1.273	3.637	61
G₂	39.76	-0.239	0.227	2.384	102
G₃	54.59	-0.086	0.250	1.086	60
G₄	87.79	-0.355	0.130	3.426	72
G₅	94.92	-0.126	0.208	1.348	19
G₆	82.89	-0.142	0.319	1.637	41
G₇	90.09	-0.163	0.258	1.729	36
G₈	57.35	-0.140	0.868	2.807	100
G₉	61.01	-0.232	0.370	2.461	83
G₁₀	57.61	-0.117	0.276	1.365	61
G₁₁	76.88	-0.299	0.124	2.892	73
G₁₂	59.09	-0.171	0.200	1.738	71

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G_{13}	58.46	-0.119	0.181	1.255	56
G_{14}	100.37	-0.186	0.048	1.789	30
G_{15}	81.58	-0.020	-0.808	2.303	51
G_{16}	82.10	-0.230	-0.807	3.181	70
G_{17}	59.05	-0.133	0.900	2.856	98
G_{18}	86.46	-0.235	-0.928	3.469	68
G_{19}	71.66	-0.131	-0.796	2.588	69
G_{20}	96.07	-0.154	0.194	1.571	26
G_{21}	87.38	0.326	-2.334	7.331	73
G_{22}	81.74	-0.160	0.268	1.710	45
G_{23}	95.65	-0.151	-1.532	4.587	62
G_{24}	95.92	-0.239	0.137	2.320	41
G_{25}	66.15	-0.126	0.195	1.331	48
G_{26}	59.90	-0.095	0.259	1.171	52
G_{27}	90.32	-0.217	0.262	2.213	45
G_{28}	51.35	-0.108	0.281	1.308	67
G_{29}	43.02	-0.123	0.253	1.381	77
G_{30}	62.65	-0.072	0.246	0.983	46
G_{31}	58.00	-0.307	0.596	3.393	108
G_{32}	95.85	-0.161	0.226	1.672	29
G_{33}	75.81	-0.065	0.248	0.943	33
G_{34}	75.19	-0.253	0.160	2.471	72
G_{35}	72.41	-0.073	-0.902	2.658	68
G_{36}	58.80	-0.853	-0.162	8.188	113
G_{37}	59.25	1.273	1.031	12.548	121
G_{38}	45.95	-0.284	0.137	2.751	106
G_{39}	65.82	-0.151	0.150	1.509	57
G_{40}	61.23	-0.268	-0.561	3.025	93
G_{41}	78.45	-0.302	-0.367	3.080	77
G_{42}	33.70	-0.138	0.227	1.471	84
G_{43}	78.04	-0.213	-2.430	7.201	78
G_{44}	35.12	-0.147	0.214	1.534	86
G_{45}	84.96	-0.147	1.364	4.124	85
G_{46}	89.81	-0.305	0.200	2.976	63
G_{47}	101.40	-0.214	0.256	2.174	35
G_{48}	52.98	-0.317	1.662	5.618	129
G_{49}	54.08	-0.070	0.634	1.922	87
G_{50}	51.53	-0.367	-1.239	4.978	110
G_{51}	70.29	-0.242	-0.663	2.990	85
G_{52}	70.70	-0.153	-2.058	6.030	86
G_{53}	79.31	-0.199	-0.910	3.212	74
G_{54}	70.96	-0.165	-0.860	2.910	76
G_{55}	54.57	-0.174	0.676	2.542	97
G_{56}	81.80	-0.176	0.272	1.858	50
G_{57}	60.84	-0.075	0.230	0.972	46
G_{58}	100.44	11.506	-0.019	110.314	71

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G59	74.96	-0.308	0.161	2.990	85
G60	76.26	-0.155	0.324	1.752	57
G61	47.23	-0.308	-0.008	2.952	110
G62	55.53	-0.327	1.553	5.415	125
G63	86.49	-0.218	0.230	2.194	50
G64	74.69	-0.217	0.204	2.162	66
G65	55.18	-0.136	0.205	1.426	67
G66	39.89	-0.101	0.789	2.441	104
G67	72.23	-0.175	0.219	1.788	63
G68	76.91	-0.114	0.354	1.487	46
G69	65.24	-0.151	0.002	1.450	52
G70	49.37	-0.543	-0.120	5.216	123

Fig. 1. AMMI I biplot showing main effects and ICAP1 interaction effects of 70 rice genotypes and three seasons on X₁) Number of days to first flowering

Fig. 2. AMMI II biplot of first two principal components (IPCA 1 *vs* IPCA 2) of interaction effects X₁) Number of days to first flowering

Fig. 3. AMMI I biplot showing main effects and ICAP1 interaction effects of 70 rice genotypes and three seasons on X₄) Height of the plant at first flowering

Fig. 4. AMMI II biplot of first two principal components (IPCA 1 *vs* IPCA 2) of interaction effects X₄) Height of the plant at first flowering

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Fig. 5. AMMI I biplot showing main effects and ICAP1 interaction effects of 70 rice genotypes and three seasons on X$_6$) Number of leaves at first flowering.
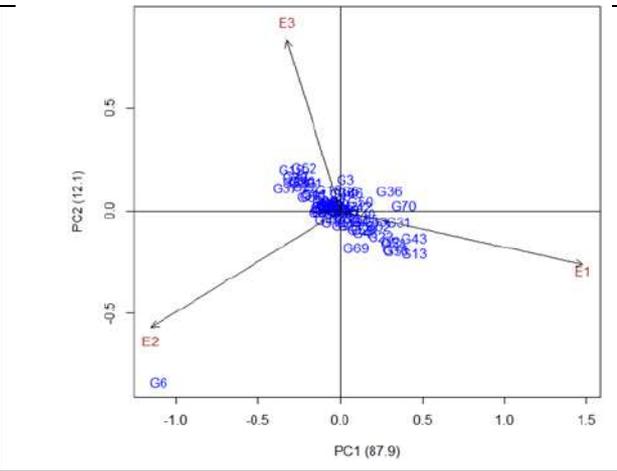
Fig. 6. AMMI II biplot of first two principal components (IPCA 1 *vs* IPCA 2) of interaction effects X$_6$) Number of leaves at first flowering

RESEARCH ARTICLE

Consumer Fast-Fashion - Oriented Impulse Buying with Reference To Chennai City

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ABSTRACT

Global fast fashion merchants have been infiltrating India's growing markets over the past few years, and Chennai is no exception. The impact of these determinants on fashion-oriented impulse purchases is then examined. This study examines both internal and external factors that influence the consumer's fashion-oriented impulse buying. In the empirical investigation, both qualitative and quantitative methods were used. It has been discovered that fast-fashion-oriented impulse purchases are influenced by emotion and hedonic value as well as fashion participation, sensory signals, and in-store promotion, in that order. These findings assist fast fashion retailers in better understanding the impulse purchasing habits of young consumers, enabling them to develop retailing mix strategies that will increase sales and, ultimately, sustainably expand their businesses.

Keywords: consumer rapid fashion, Chennai city, impulse purchase

INTRODUCTION

After being revolutionized in Western Europe at the beginning of the 1990s, fast fashion has grown popular in recent years, particularly in Chennai and other rising markets in India. The appeal of fast fashion can be attributed to its low price and appearance at fashion shows at the height of the most recent trend (Morgan & Birtwistle, 2009). Strong impulse buying, vertical integration of production in low-cost countries, as well as shifting consumer behavior, are all factors that have contributed to this new business model's outstanding growth in the fashion sector (Barnes & Lea-Greenwood, 2010; Morgan & Birtwistle, 2009). Most international fashion retailers, including Old Navy, UNIQLO, Zara, H&M, and others, have entered India's expanding market in recent years. In addition, Chennai has

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seen its per capita income rise, which has increased disposable income in the context of the economic recovery. This, combined with the city's youthful population, which has been particularly changing its behavior toward fast fashion goods, has turned Chennai into a hub for international fashion retailers. According to earlier research, purchasing fashionable clothing is crucial, and the majority of these purchases are made on a whim (Zhou & Wong, 2003). Therefore, by encouraging impulse buying when clients shop at their stores, fashion companies can greatly increase their revenues. Despite the fact that impulsive buying has been extensively studied in industrialized nations over the past 20 years, it has only recently emerged as a new subject of study, particularly in the fashion sector in India's rising markets, such as Chennai and other major cities (Nguyen, Jung, Lantz, & Loeb, 2003; Yu & Bastin, 2010). Fast fashion research is relatively new and primarily focuses on the perspective of the fast fashion producer in terms of the vertical supply chain while also incorporating a growing concern about corporate social responsibility as opposed to the perspective of the customer (Barnes & Greenwood, 2010). Fast fashion consumer behavior has only recently begun to take off in India in the 2010s (Hu & Shiau, 2015; Khare & Rakesh, 2010; Patil & Agadi, 2016). Research on the purchase habits of consumers of fashion is essentially nonexistent in Chennai. The goal of this study is to better understand the relationships between internal and external factors that may affect consumers who are the primary target market for the fast fashion industry while making fashion-related impulse purchases. The study assists fashion retailers effectively design the retailing-mix strategy to operate successfully in such a fiercely competitive fashion market as Chennai, which advances our understanding of the relationship between fashion-oriented impulse buy and its antecedents. This study also aims to make a theoretical contribution to the body of literature by re-examining and adding new knowledge to one of the internal factors, namely "hedonic value," which is defined as the pleasure shoppers experience while shopping in stores and which significantly influences consumers' impulse purchases. One characteristic of hedonic consumers is their propensity to make impulsive purchases when "strongly interacted and applauded" by their shopping companions, specifically other customers. According to the study, in a consumer setting, a crowded environment (with more lingering customers producing more interactions) increases the likelihood that an impulse purchase will be made because of "crowd effects," which have already been found to be unfavorable by prior studies, mostly in Western cultures. The essay is divided into four sections. The first section provides a quick overview of Chennai's fast fashion market. A brief literature review on fashion-related impulse buying in regard to its causes, which include both internal and external cues, is presented in the second section.

Literature Review

Impulse buying concept

Before generating interest in India, a sizable corpus of study on this topic was established in Western nations. Curiously, several Indian authors have recently evaluated the literature on impulse buying behaviour. Prior to 1987, an unplanned purchase that prioritised the product over the customers was referred to as an impulse buy. Later, by including its psychological components, such as excitement, pleasure, and an overwhelming want to buy, the definition of an impulsive purchase was changed from being a simplistic unplanned behaviour to a specialised conduct (D. W. Rook, 1987). Shoppers feel impulse consumption more so than the things (Rook & Hoch, 1985).

Fashion-oriented impulse buying

Fashion apparel is among the products that strongly encourage impulse buying. High consumer interest in fashion products during the 1990s allowed for the study of impulse buying behaviour (Han, 1991; Park, Burns, 2005; Predergast; Gutman, Mills, 1982; as referenced in Virvilait, Saladien, & Vinklyt, 2011). Beatty and Ferrell (1998) went on to say that a purchase that is primarily motivated by fashion is referred to as a "impulse purchase" because it was unplanned. It can also happen when a consumer decides to purchase fashion items right away without considering the repercussions in response to a number of internal and external marketing triggers (Rook, 1987).

Predictors of fashion-oriented impulse purchase

Typically, environmental and marketing-related aspects are considered external stimuli, whereas shopper-related factors are considered interior stimuli (Duarte, Raposo, & Ferraz, 2013; Youn & Faber, 2000). On the basis of the

model of impulse buying consumer behaviour, predictors of fashion-related impulse purchases were initially explored (Weinberg & Gottwald, 1982). Consumers are exposed to a particular environment and are impacted by a variety of internal and external stimuli when they are shopping in a store. As a result, impulse buying is solely influenced by stimuli (Rook & Fisher, 1995). The available empirical research on the influence of stimuli on impulse buy shows that both internal and exterior cues have the potential to directly or indirectly influence the Nanda (2013) study's impulse purchase (Aron, 2004; Park & Lennon, 2006).

External stimuli and the impact on impulse buying
Customers will react impulsively by buying or not buying when exposed to environmental stimuli such as retail environment, marketing-mix stimuli, demography, and socio-economic characteristics (Amanda & Brigitte, 2003; R. Virvilait et al., 2011; Youn & Faber, 2000). The link between exterior environmental inputs such climatic cues, in-store promotions, human variables, and social interactions and impulse purchase behaviour is a key focus of empirical studies (Yang et al., 2011; Kotler, 1973; Sharma, Sivakumaran, & Marshall, 2006).

Sensory cues, which include smell, sight, sound, and touch, are ambient signals that can have a significant impact on impulsive consumption (Mattila & Wirtz, 2001; Sharma et al., 2006; Sharma, Sivakumaran, & Marshall, 2010). The ambient environment elements that surround the customers and help to create a welcoming setting for shopping, including both concrete and abstract indications. Intangible factors include temperature, aroma, lighting, and music, whereas tangible factors include fixtures, store hygiene, displayed colours and items, and attractive decoration. These factors have a strong likelihood of influencing consumers' spontaneous purchases when they make purchasing decisions (Maymand & Ahmadinejad, 2011).

Visual presentations that transmit a brand's image or a company's value are referred to as visual merchandise (Lee & Yi, 2008). Visual cues serve to improve the store's reputation and increase sales in the context of clothes retailers (Bhatti & Latif, 2014; Khandai et al., 2012). The store's exterior, including its window displays and retail spaces, as well as its interior, which focuses on its signage, layout, presentation strategies, ambient conditions, point-of-purchase displays, floor merchandising, and brand name, are both used to examine the visual merchandising dimensions (Zhou & Wong, 2003). Salespeople play an important role to influence customer impulse purchase. Friendly, courteous, professional, knowledgeable and supportive staff who offer extraordinary customer service appear crucial to trigger impulse purchase dur-ing their store visit (Mattila & Wirtz, 2008; Peck & Childers, 2006; Ridgway et al., 2008). In-store promotion as part of marketing stimuli can amplify the magnitude of unplanned purchasing among consumers (Liao et al., 2009). Various promotion tools at the point of purchase including discounted offerings, attractive advertisement as well as special gifts, loyalty programs, etc. are found positively correlated with impulse buying behavior (Yang et al., 2011; e. a. Virvilaitė, 2011)

Social stimuli involve in the number, type and behavior of people that appear within the store including other buyers and salespeople (Robert, 2001), including crowded behavior, employee attitudes, and social interaction between consumers and salespeople that can stimulate impulse buying (Turley & Milliman, 2000). Among these social stimuli, perceived crowding was found the most influential factor of impulse purchase (Miyuri, 2017). Furthermore, according to Luo (2005), the presence and interaction of other shoppers within the store can trigger the impulse purchase.

Internal stimuli and the impact on impulse buying
The impulse buying's internal drivers focus directly on the individuals. Thus, considering the internal cues is a critical factor when examining impulse buying behavior (Kacen & Lee, 2002). There are three key internal consumers' characteristics such as emotional state, hedonic value and individual fashion involvement. Internal factors of impulsive purchase examine the internal motivation and characteristics of the buyers engaging in buying on impulse. Among internal cues, researchers usually highlight emotions, hedonic shopping value, product involvement and personal traits (Kacen & Lee, 2002; Tigert, Ring, & King, 1976). In addition, Agarwal (2015) proved that personal traits including demographic such as gender, income can trigger impulse purchase.

Fashion involvement

With regard to the buying behavior, involvement is described as an interaction between a consumer and a product or service and it involves the related activities when consumers make a purchase decision (Parker, Simmers, & Schaefer, 2014). Involvement is conceptualized as a multidimensional concept and measured by different scales (Warrington & Shim, 2000). In relation to fashion consumption, the involvement refers to the shoppers' interest with the purchase of clothing (Aron, 2004; Pornpitakpan, Yuan, & Han, 2017). Antecedents of fashion involvement include materialism, gender and age whereas consequences are composed of fashion subjective knowledge and consumers' confidence in decision making about fashion clothing (Aron, 2004) . In empirical studies, fashion involvement is measured by scales proposed by (Aron, 2004; Tigert et al., 1976). Specifically, the study shows a positive relationship between the level of fashion involvement and purchasing fashion goods which indicates that shoppers with higher level of fashion involvement are more likely to engage in fashion impulse buying (Aron, 2004; Park & Lennon, 2006)

Emotion

According to Wong and Bagozzi (2005) emotion is "the mental state that arises from the evaluation of events or thoughts of oneself". Both positive and negative emotion can affect the consumer purchase decision (Avinash & Chinmaya, 2009; Scheier & Carver, 1988). A consumer when shopping in the store with a positive mood may also affect his or her impulse buying. The emotional states of consumer such as pleasure, arousal are influenced by the environmental stimuli and this can lead to a positive approach toward purchasing (Donovan, Rossiter, Marcoolyn, & Nesdale, 1994). Interestingly, Verplanken and Herabadi (2001) revealed that impulsive buyers are more emotional than non-impulsive ones. As a result, consumers with positive emotion would spend more money when shopping and hence are more likely to make decisive decisions faster (Lee and Yi (2008). Therefore, consumer emotion can be an important determinant to predict impulse buying of the shoppers while engaging in buying fashion goods (Beatty & Ferrell, 1998; Cobb & Hoyer, 1986) *Hedonic value.* Consumer value can be categorized as utilitarian value and hedonic value that are considered by marketers as important factors to evaluate consumers' consumption experience to design effectively their marketing by a set of dimensions and items. Util-itarian consumers behave rationally while hedonic consumers seek for the sensation perceived from enjoyment from their shopping experience (Yu & Bastin, 2010). In this sense, the possession of a product is not even the main purpose to purchase (Babin, Darden, & Griffin, 1994). From the experiential perspective, hedonic consumption is defined as fantasy, emotional aspects of consumption, and multisensory experiences that are driven by benefit seeking when shopping such as the pleasure of using the product and aesthetic appeal such as sleekly design, appealing packaging, etc. (Geoff & Clive, 1998). In the empirical literature, hedonic shopping value is measured by elements such as novelty, fun, praises from others, and social interactions. Other variables suggested in the research work of Činjarević et al (2011) are environmental variables including surrounding characteristics and design. Most importantly, hedonic shopping value is found a predictor of impulse purchase (Piron, 1991; Ramanathan & Menon, 2006; D. W. Rook, 1987).

Proposed research model

Past research on impulse purchase in retail environment indicates that there is no unified attitude to factors affecting impulse purchase (Balaji & Babu, 2015). Therefore, in order to investigate factors affecting fashion-oriented impulse purchase among millennial consumers in a specific emerging market like Chennai, we propose our research model in Diagram 1. Based on the literature review analyzed in the previous session, five dimensions were indentified relating to External Stimuli(ES) named (1) Sensory cues(SC), (2) Visual merchandizing(VM), (3) Salespeople(SP), (4) In-store promotion(ISP), and (5) Social stimuli(SS) and 3 dimensions related to Internal Stimuli(IS) such as (1) Emotion(En), (2) Hedonic value (HV) and (3) Fashion involvement(FI). An additional dimension is incorporated to test the impact of gender and on Fashion-oriented Impulse Buying.

Hypothesis Formation

H₁: Sensory Cues positively impact on Fashion-oriented impulse buying
H₂: Social stimuli positively impact on FOIB
H₃: Visual merchandizing positively impacts on FOIB
H₄: In-store promotion positively impacts on FOIB

H$_5$: Salespeople positively impact on FOIB
H$_6$: Emotion positively impacts on FOIB
H$_7$: Hedonic value positively impacts on FOIB
H$_8$: Fashion involvement positively impacts on FOIB
H$_9$: There is a gender difference in terms of FOIB

RESEARCH METHODOLOGY

In the empirical literature, both unstructured and structured methods were applied to identify antecedents of impulse buying behavior. As such a research is inexistent in Chennai; we conducted a qualitative study to explore these external and internal factors contributing to fashion- oriented impulse behavior in the first step. The second step, we conducted a quantitative study to investigate the predictors of fashion-oriented impulse purchase.

Step 1: To identify factors that might influence fashion impulse purchase by conducting four focus groups in Chennai and at the same time to explore specific dimensions and items in connection with external and internal stimuli based on our literature review.
Step 2: Specific factors and items captured in the qualitative study will be added to design a structured questionnaire. The questionnaire is composed of three parts: the first part is related to socio-demographic characteristics of our surveyed sample. The second part deals with the consumer behavior toward fashion items and the last part focuses on fashion impulse purchase. To measure the importance of each variable, we use the five-point Likert scale.
Step 3: To run the factor analysis to extract factors and variables determining the antecedents of fashion impulse buying behavior and to test the relationship between external and internal factors and fashion-oriented impulse purchase.

Measurement scales were adapted from previous literature. In order to finalize the measurement scales to fit the proposed research model to the Chennai's context, we conducted four focus groups with 12 participants in each group, who are young college students, a potential target segment of fast fashion retailers in Chennai. This qualitative study explores the perception of all factors relating to fashion consumer behavior from Chennai's young consumer perspectives. Results from the four focus groups indicate that all of external and internal stimuli proposed in the research model were also explored. It is worth noting that internal cues such as emotion, hedonic motive, and in particular fashion involvement were shared with excitement among these young participants. Interestingly, the unique item contributing to the "salespeople" factor that could not be found out in previous research is about the gender importance raised by a number of participants. Female participants insisted on getting advices about women clothes from female salespeople.Based on the literature and the results of our qualitative study, we finalize our measurement scales exhibited in Table 1.

Sampling method and data collection
The survey was conducted during the period of June – November 2020, in Chennai city and its outskirts by online mode with 500 respondents with a convenience sample composed of young students and office workers, from 18-35-year old who represent the key target segment in Chennai. We received 322 usable questionnaires.

Research Findings
Descriptive Statistics Among 322 participants, 31.7% were male and 68.3% were female. Regarding the age, almost participants (88.8%) represent for Gen Y consumers who are from 18 to 25. College students occupy 73% followed by office workers with 19.6%. Income statistics show that 39.1% have income from Rs. 10,000 – Rs. 20,000; 39.8% from Rs. 20,001 – Rs. 30,000 and 21.1% earns above Rs.30,000. This is due to the fact that the study was conducted mostly with the majority of participants who are college students and they have quite low income (money earned from part-time jobs) or no income rather than the pocket money from their parents. Most of the participants (75%) reported that

they prefer to buy clothes in modern retailers such as supermarkets, shopping malls. This is due to the fact that young people prefer to shop at playful and fun environments where they can enjoy and experience the shopping trip, not just buying. A very important finding is that most of millennial consumers buy clothes on impulse with 94.7%

Extraction Method: Principal Component Analysis; Rotation Method: Varimax with Kaiser Normalization
The reliability analysis showed favorable results. All of factors exhibited in Table have a high degree of reliability in terms of Cronbach's Alpha (>0.7) (Hair et al., 2014). After factor reduction, 8 factors remain with 5 external stimuli (Sensory cues, Visual merchandizing, Salespeople, In-store promotion, Social stimuli) and Internal stimuli (Emotion, Hedonic value, Fashion involvement).

Impact of internal and external stimuli on fashion-oriented impulse purchase
The reliability of fashion-oriented impulse purchase gives good results with Cronbach's Alpha = 0.845.

Regression results
Findings of the multiple regressions showed in Table clearly indicate the three factors representing internal stimuli are statistically significant. Emotion and Hedonic value appear as the most important predictors of fashion-oriented impulse purchase with the coefficients of 0.304 and 0.299 respectively at the significance level of 5%. *Fashion involvement* seems less meaningful to affect impulse purchase among Chennai consumer compared with *Emotion* and Hedonic value as its coefficient is 0.151. Regarding external stimuli, among five factors, only Sensory cues and *In-store promotion* is statistically significant with the same coefficient of 0.169 while Visual merchandizing, Salespeople and Social stimuli are not statistically significant. These findings indicate that these two external stimuli (Sensory cues and In-store promotion) have a strong influence fashion-oriented impulse buying behavior among Chennai consumer when shopping for fashion products. The study shows another interesting factor which is gender that may have a strong effect in fashion-oriented impulse purchase. The relevant extant research works strongly prove that female consumers are likly to be more impulsive than male buyers. *T-test* is used in the study to determine gender differences related to fashion impulse purchase. *T-test* results confirm that there is a big difference in fashion impulse purchase among male and female Chennai consumers (Table). It can be seen that the Levene's Test for Equality of Variances has a Sig value of 0.048 <0.05. Thus, at 95% confidence level, the variances between two genders are not uniform. Also, the Equal variances not assumed with Sig = 0.068 <0.1 confirms that between gender groups (male and female), there is a difference in regard to the impulse purchase behavior toward fashion products.

In a nutshell, our results reflect the situation of the fast fashion landscape in Chennai. The fierce competition between global and local fast fashion retailers have, aggressively launched sales incentives and other promotional campaigns mainly at the points of purchase to attract patronage and increase sales through impulse purchase.

DISCUSSION

The research findings show that Emotion and Hedonic values appear as the most important predictors of fashion-oriented impulse purchase of Chennai consumers. This result concurs with the finding that highlighted variables that fashion retailers can use within the store and that can produce comfortable environment and thus can increase impulse purchase probability of the shoppers. In fact, when shoppers have good emotional feelings while entering the store, they are likely to have more pleasure and fun and this results in spending more time and money (Khandai et al., 2012; Lins et al., 2015). The empirical studies conducted by Chang, Eckman, and Yan (2011) revealed that in the Chennai context, young consumers having more positive emotional responses to the retail environment are more likely to buy more on impulse. Also, another important aspect to trigger impulsive consumption of young consumers is that retailers should find the ways to create a pleasing environment where shoppers can feel their important role or "the face" and thus can touch their hedonism that leads them to buy more impulsively. Regarding external stimuli, Sensory cues and In-store promotion are extremely crucial. This finding indicates that these two

external drivers have strong influence on fashion-oriented impulse buying behavior of Chennai consumers. They seem very close to case of Chennai, an emerging market having the largest young and middle-income consumers who tend to be impulsively attracted by in-store promotion activities when they buy fast fashion products. Furthermore, these young consumers nowadays are much more attracted by atmospherics or sensory cues that can lead to create excitement and pleasure while shopping. Our findings are consistent with previous research work results as In -store promotion and Sensory cues are important for fashion sellers to increase sales through impulse purchase at the point of purchase (Hultén, 2012; Mattila & Wirtz, 2001; Morrison et al., 2011; Nanda, 2013; Zhou & Wong, 2003).

CONCLUSION

Our research on factors determining impulse purchase behavior and their direct linkage to fast-fashion oriented impulse purchase among Chennai consumers come up with the two key findings. First, both external and internal factors that can stimulate impulse purchase behavior perceived by the Chennai consumers. Internal stimuli (Emotion and Hedonic value) appear the most powerful predictors of fashion-oriented impulse purchase. Meanwhile, external factors such as In-store promotion and Sensory cues are less influential in affecting impulse purchase. Findings in this research are meaningful for both global and local fashion brands targeting Chennai consumers. Specifically, fashion retailers should not only improve their in-store environment but also know how to contribute positive emotions, hedonic value and raise fashion involvement among young customers and thus can trigger their impulse purchase. This exploratory study results will help fashion retailers efficiently design retailing mix strategy in order to operate with more success in a highly competitive market.

Managerial Implications

The findings from our study reveal that in Chennai, a great number of consumers experience impulse purchase when shopping in fast fashion stores. Most importantly, their fashion-oriented impulse purchase is strongly influenced by both internal cues, in particular Emotion and Hedonic value and external cues such as Sensory cues and In-store promotion. This implies that fashion retailers need to better manage both internal and external stimuli that can trigger the buying impulsiveness among the youth segment. Specifically, our empirical tests indicate that managers should well take care of these key factors when designing a holistic marketing strategy. As internal stimuli, particularly Emotion and Hedonic value contribute significantly to fashion-oriented impulse purchase compared with external stimuli among young consumers in Chennai, fast fashion retailers need to concentrate their marketing efforts to enhance these internal factors. *Emotion* is revealed as the most influential factor on impulsive consumption of Chennai young consumers. This means that young consumers' impulse buying is emotionally driven, they first like and then buy fashion products. Therefore, fashion retailers should improve their stores' look and ambience in an attractive way in order to provide potential customers good feelings and thus can engage them to buy apparels on impulse. Along with positive emotion that fashion retailers should bring to their customers, Hedonic value becomes tremendously crucial in delivering the first impression and long-term relationship with the Chennai young buyers. This suggests that fashion retailers need not only make their shopping place enjoyable but also create unforgettable purchase experiments for customers during their store visit. Besides, similar to the two internal stimuli (Emotion and Hedonic value), Fashion Involvement also predicts impulse purchase among young consumers. This implies that fashion store managers need to elaborate effective integrated marketing communication strategies at their points of purchase to enhance stronger interest and consciousness in fashion trend and fashion goods among potential fashion consumers in Chennai.

Regarding external stimuli as antecedents of fashion impulse buying behavior, the two factors which are statistically significant are *In-store promotion* and *Sensory cues.* In fact, the former factor shows its attractiveness to Chennai consumers who have been increasingly responding to interesting different sales incentives in making their impulse buying decisions. Despite their income remains quite moderate, these young people tend become more and more fashionable and fashion clothing is considered as a significant part of their life. To afford fashion items, they are

excited about *In-store promotion* such as coupons, special discounts, and other loyalty programs. Therefore, fashion retail managers should consider designing the appealing in-store sales programs that can highly trigger unplanned purchase. The latter factor (Sensory cues) perceived extremely powerful in creating appealing atmospherics and enjoyable shopping experience for young customers. Therefore, fashion retailers targeting this segment should improve their store environment by carefully design fashionable and trendy items exposed to the lighting, scents, and colors. Last but not least, as there is a big difference of both genders toward fashion- oriented impulse purchase and for this reality, fashion retailers should invest wisely their differentiation strategy for the two distinct male and female segments in order to enhance their impulse purchase.

Research limitations and future research directions
First, the survey was conducted with a convenience sample with respondents representing the target market of fast fashion from 18-35 years of age and they are living in Chennai, with the highest income per capita and consumption level in the country. Although, our convenience sample is demonstrated adequate in this exploratory study, a larger sample covering in other larger could be examined in the future research. Second, our empirical study focuses on fashion-oriented impulse buying among the younger cohort of Chennai consumers who are mostly students from 18 to 25 years of age whom do not have high income and that might limit the perception on factors contributing to fashion impulse purchase. Therefore, the future study will expand to the older cohort of Gen Y who is from 25 to 35 with more independent life and higher income and more importantly having tremendous needs in fast-fashion goods consumption. Third, following a great number of authors such as Nanda (2013); Yu and Bastin (2010), this research investigates only the direct relationship between external and internal stimuli and fashion-oriented impulse purchase while ignoring the indirect relationships of each stimuli especially internal stimuli and fashion-oriented impulse purchase behavior. To understand deeply the complexity of the interaction of different factors getting involved in the impulse buying process and behavior, the future studies will examine these indirect relationships via moderators such as emotion or fashion involvement that are the most studied in the past few years (Aron, 2004; Park & Lennon, 2006).

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Based on the literature and the results of our qualitative study, we finalize our measurement scales exhibited in Table 1. Measurement instrument

Construct	Factors	Items	Source
External Stimuli	Sensory Cues	6	Mohan, Sivakumaran, and Sharma (2013)
	Visual Merchandizing	5	Zhou and Wong (2003)
	Sales People	5	Mohan et al. (2013)
	In-store promotions	6	Virvilaitė (2011); R. Virvilaitė et al. (2011) and Yang et al. (2011)
	Social stimuli	4	Turley and Milliman (2000); Miyuri (2017) and Luo (2005)
Internal Stimuli	Fashion involvement	6	Aron (2004); Tigert et al. (1976)
	Positive emotion	4	Amanda and Brigitte (2003)
	Hedonic value	5	Babin et al. (1994)
Impulse Buying	Fashion oriented impulse buying	6	Park and Lennon (2006)

Table 2: EFA Result

	Factor	Eigen values	Mean	Corrected Item-Total	Cronbach's
SENSORY CUES		8.991			0.845
Nice ambience	.754		3.74	.530	
Appealing color	.780		3.74	.691	
Appealing aroma	.822		3.60	.568	
Clean store	.636		3.77	.520	
Nice touching (materials)	.606		3.95	.590	
VISUALMERCHANDIZING		3.347			0.829
Good visualization of merchandise	.603		4.04	.463	
Product quality	.747		4.39	.350	
Variety of products	.662		4.05	.419	
Brand familiarity	.648		3.89	.378	
SALESPEOPLE		2.446			0.865
Employees' professional uniform	.502		3.59	.335	
Employees' attitude (friendly, courteous and helpful)	.635		3.62	.404	
Employees know fashion trend.	.547		3.68	.387	
Gender importance of sales people: female vendors	.775		3.75	.333	
IN-STORE PROMOTION		2.440			0.832
In-store ads	.628		3.66	.532	
Sales promotion	.894		3.69	.487	
Membership card	.810		3.73	.467	
Gifts	.793		3.80	.492	
Direct discount (specific events)	.664		3.75	.420	
Loyalty program	.728		3.82	.426	

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SOCIAL STIMULI					
Perceived crowding	.638	2.765	3.43	.477	0.856
Interaction with other shoppers	.873		3.46	.468	
Interaction with salespeople	.689		3.20	.383	
Social status	.665		3.47	.429	
EMOTION					
It's fun to buy something new.	.784	1.963	3.61	.516	0.877
Even if I don't spend money, I still feel excited about	.679		3.37	.461	
Shopping clothes is a pleasant activity for me.	.613		3.72	.447	
I feel a sense of personal satisfaction when I wear	.896		3.40	.474	
HEDONIC VALUE					
I like to observe others in the store.	.520	1.826	3.17	.455	0.759
When I feel depressed, going shopping outfits can get	.649		3.46	.518	
It seems that I explore a new world when I go shop-	.963		3.43	.501	
Shopping outfits makes me happy.	.672		3.58	.484	

Table: 3 Fashion Involvement

FAHSION INVOLVEMENT					
I am always keeping up with new trend, new fashion.	.766	1.336	3.36	.581	0.829
I keep my wardrobe up-to-date with the changing	.594		3.39	.585	
Fashionable, attractive styling is very important to	.761		3.52	.549	
Dressing well is an important part of my life.	.792		3.42	.567	
If I have to choose between the two, I normally dress	.502		3.19	.552	
I attach great importance to fashion clothing.	.943		3.29	.594	
I would say fashion clothing is central to my identity	.635		3.24	.530	

KMO = .855 - Bartlett's Test of Sphericity: x2= 7329.417, P= .000 - Total variance explained: 64.784%

Table: 4 Fashion Oriented Impulse Purchase

FASHION ORIENTED IMPULSE PURCHASE					
Most of the time, I buy clothes on impulse.	.692	3.023	3.70	.533	0.803
I enjoy purchasing new clothing that has just been	.644		3.94	.492	
"If I see it, I like it and I will buy it" describes me.	.770		3.62	.576	

I buy fashionable clothes on the spur of the moment,	.683		3.75	.559
When I go shopping for new clothes, I always over-	.735		3.54	.519
I normally buy other fashion pieces in addition to the	.727		3.49	.527

KMO =.854 - Bartlett's Test of Sphericity: x2= 494.112, P= .000 - Total variance explained: 50.379%

Table: 5 Regression results

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
	(Constant)	.186	.192		.970	.333		
	Visual merchandizing	.111	.041	.112	2.723	.007	.753	1.328
	Sensory cues	.154	.037	.169	4.195	.000	.786	1.273
	Salespeople	-.043	.029	-.057	-1.473	.142	.861	1.162
	In-store promotion	.147	.036	.169	4.118	.000	.756	1.324
	Social stimuli	-.015	.036	-.017	-.415	.678	.741	1.34 9
1	Emotions	.231	.031	.304	7.394	.000	.751	1.331
	Hedonic value	.246	.036	.299	6.784	.000	.656	1.525
	Fashion Involvement	.138	.039	.151	3.508	.001	.683	1.463

Table6: Relationship between gender and fashion-oriented impulse purchase

	Gender	N	Mean	Std. Deviation	Std. Error Mean
Fashion Oriented IP	Male	102	3.5654	.74431	.07370
	Female	220	3.7250	.68265	.04602

Table 7: Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Fashion oriented	Equal variances assumed	2.925	0.048	-1.897	320	.059	-.15964	.08418	-.32525	.00597
	Equal variances not assumed			-1.837	182.352	.068	-.15964	.08689	-.33108	.01179

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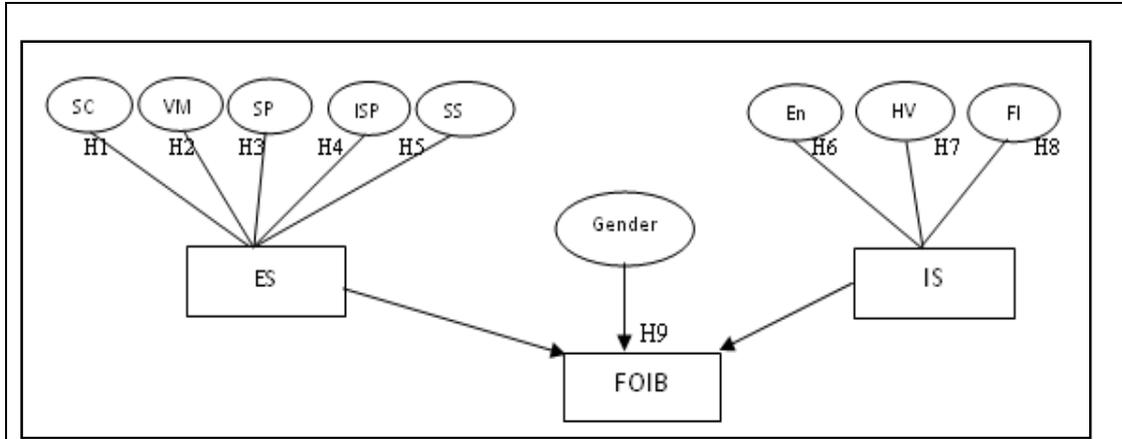
Fig 1: Proposed Research Model

RESEARCH ARTICLE

Investigations on *In-vitro* Anti-Inflammatory Activity of Ethanolic Extract of *Flacourtia indica*

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ABSTRACT

The present investigation was carried out to evaluate the anti-inflammatory property of *Flacourtia indica* aerial parts by two different methods, namely membrane stability assay and protein denaturation assay. Five different experimental concentrations (100, 200, 300, 400 and 500 µg/ml) were used in this study. Action is observed in a dose-dependent manner. In the protein denaturation method, 500 µg/ml of the selected extract showed maximum protection (78.06%) and the standard drug provided 83.14% protection. Similarly, in the membrane stability test, the extract selected at a concentration of 500 µg/ml showed maximum protection (77.09%) and the standard drug provided 92.05% protection. In addition, the hypotonic heat hemolysis test showed maximum protection (71.34%) at a concentration of 500 µg/ml and the standard drug provided 88.20% protection. We conclude that the ethanolic extract of *Flacourtia indica* shows potent anti-inflammatory activity at different concentrations compared with the standard drugs of Diclofenac sodium and aspirin. In addition, phytochemical analysis of *Flacourtia indica* revealed the presence of saponins, flavonoids, tannins, anthracene, phenols, amino acids and sugars. It is shown that these phytochemicals are responsible for maximum protection from protein denaturation, and membrane stability testing. Future work will focus on anti-inflammatory activity using in vivo models.

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INTRODUCTION

Inflammation is defined by pathologists as a normal protective response of living body cells to injury or injury, manifested as a limited and localized way of irritation, infection, and other adverse effects. Disease-causing agent. The living organism in an inflammatory state has many signs including rubor (red), calor (hot), pain (pain), tumor (swelling) in addition to lack of function [1]. Lysosomal enzymes released during inflammation induce a variety of disorders that lead to tissue damage by damaging macromolecules, and membrane lipid peroxidation is thought to be responsible for a number of disease conditions. such as heart attack, septic shock and rheumatoid arthritis, etc. This oxidative stress induces inflammatory cascades that damage cellular components.[2] The humoral and cellular mechanisms of inflammation are many and complex. They are involved in gene regulatory factors such as nuclear factor kappa B (NFκB) and signal transmitters synthesized by cells of the immune system such as cytokines and prostaglandins.[3] Many factors can cause out inflammatory process; Infectious agents, ischemia, antigen-antibody interactions, heat or physical shock.[4]Steroids, non-steroidal anti-inflammatory drugs (NSAIDs), and immune suppressants, commonly used to reduce symptoms inflammatory diseases, require long-term treatment, and their use is often associated with serious side effects. Such as gastrointestinal bleeding and peptic ulcer.[5]

Flacourtia indica (Burm.f.) commonly known as 'Baichi' or 'Katai'. It is an indigenous medicinal plant widely distributed in Bangladesh and India. Chufa tubers are daily ingredients of the diet of many people in North Africa and Spain[6] . Several therapeutically important natural compounds have been isolated (such as alkaloids, flavonoids, carbohydrates, tannins, saponins, and steroids) and they can serve as very potent and reliable drug candidate for treatment of various disorders like In Vitro Antioxidant Activity[7], Antimalarial Activity[8] , Anti cancer activity [9] diuretic activity[10], Antibacterial activity[11] , Anti-anxiety activity[12] ,antidyslipidemic activity[13], Hepatoprotective Activity[14].

EXPERIMENTAL PART

Plant material
Aerial parts of *Flacourtia indica* were collected during December 2021 from, Thirupathi hills, Andhra Pradesh, India. It was identified and authenticated by Prof.Dr. Madhavasetty, Department of Botany, University, Thirupathi, Andhra Pradesh, India. The voucher specimen was maintained in our laboratory for the future reference.

Preparation of extract: Aerial parts of *Flacourtia indica* was dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (500gm) of powder was subjected to continuous hot extraction in soxhlet apparatus using ethanol as solvent at a temperature range of 60-70°C. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The ethanolic extract of aerial parts of *Flacourtia indica*. Yield thick green semisolid residues (EEFI, 2.84% w/w). The extract were subjected to preliminary phytochemical screening and revealed the presence alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpeniods, and phenols .

Statistical analysis
Data were expressed as percentage (%) protection and mean ± SEM and were analyzed by one-way ANOVA followed by Dunnett's test for multiple comparisons using Graph pad prism version 5.03. Results were considered significant at p <0.05. (Prism graph pad 5.3 version).

METHODOLOGY

Assessment of In vitro anti-inflammatory activity
In vitro Evaluation of anti-inflammatory activity

1.Albumin denaturation inhibition: Anti-inflammatory activity of ethanolic root extracts of Senna singueana was studied according to the procedure of Mizushima et al. and Sakat et al. [15, 16] with some modifications. The inhibition of albumin denaturation was performed according to the protocol. The reaction mixture consisted of an equal volume of test extract of different concentrations (100–500 $\mu g/ml$) of bovine albumin. The pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extract was incubated at 37 °C for 20 min and then heated at 51 °C for 20 min. The absorbance is measured after cooling the sample to room temperature. The turbidity formed was measured at 660 nm using an ultraviolet (UV) visible spectrometer (Model: Shimadzu UV1800). The percentage inhibition of protein denaturation was calculated as follows

% inhibition = [Abs control -Abs sample]/Abs control×100

Membrane stabilization
Prepare a red blood cell (RB) suspension [16,17]. Blood was obtained from a healthy volunteer who had not taken nonsteroidal anti-inflammatory drugs for 2 weeks prior to the test. Blood samples were centrifuged at 3000 rpm for 10 min and washed 3 times with an equal amount of normal saline. Blood volume is measured and mixed at 10% v/v in normal saline

Heat-induced haemolysis
The activity was carried out according to the procedure of Sakat et al. and Shinde et al. [16,18] with some modifications. The reaction mixture (2 ml) consisted of 1 ml of test sample at various concentrations (100–500 $\mu g/ml$) and a 10% red blood cell suspension (1 ml). For control, only saline solution was added to the test tube. Aspirin as a reference was used. The reaction mixture was incubated in a water bath for 30 min at 56 °C. The reaction was stopped by cooling the reaction mixture under running water. The reaction mixture was then centrifuged at 2500 rpm for 5 min. The supernatant obtained was used to measure the absorbance at 560 nm. The experiment was performed three times. The rate of hemolysis was calculated as follows

%inhibition =[Abs control -Abs sample]/Abs control×100

Hypotonicity-induced haemolysis
Hemolysis due to hypotonicity was performed according to the protocol of Azeem et al. [19] with some modifications. Extracts of different concentrations (100–500 $\mu g/ml$), controls (diclofenac sodium 100 $\mu g/ml$) and controls were mixed separately with pH 7.0 phosphate buffer (1 ml), hyposaline (2 ml) and a suspension of RBC (0.5 ml). The reactions were incubated at 37 °C for 30 min. It was then centrifuged at 3000 rpm. The supernatant was transferred and the absorbance was performed at 560 nm. Percent haemolysis was calculated assuming 100% control. %protection = 100-[OD sample / OD control] ×100

RESULTS AND DISCUSSION

The percentage yield of ethanol extract of aerial parts of *Flacourtia indica* was found to be 2.84% w/w respectively.

Inhibition of Protein denaturation
Protein denaturation is known to cause inflammation. In thermal albumin denaturation, inhibition is very effective. Mechanism of anti-inflammatory activity investigated the ability of plant extracts to inhibit protein denaturation assay. The inhibitory efficacy of the sample is presented in Table 2. The maximum inhibition of protein denaturation for the sample and standard (diclofenac sodium) at 500 $\mu g/mL$ was found to be 78.06% and 83.14, respectively. %.

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The IC_{50} values of the sample and the standard were observed at 330 μg/mL and 360 μg/mL, respectively. From the above results, we found that the sample has less protein denaturation expression. Protein denaturation is a biochemical reaction that occurs during a chronic inflammatory response leading to loss of function in tissues. The ethanolic extract of *Flacourtia indica* (500 μg/mL) showed higher inhibition of protein denaturation compared with other concentrations [20].

Membrane Stabilization
Heat Induced Haemolysis
Human erythrocytes were used to study anti-inflammatory activity in vitro by membrane stabilization method HRBC. An investigation of the stability of the HRBC membrane was performed as it is similar to the lysosomal membrane. Due to the similarity of human erythrocytes to lysosome membranes, a possible effect of the ethanolic extract of *Flacourtia indica* might be inhibition of the release of lysosome content in neutrophils during inflammation. Hemolysis of the ethanolic extract of *Flacourtia indica* showed significant protection at 77.09% at 500 μg/mL (Table 3). Aspirin was used as standard drug with a maximum inhibition of 92.05% at 500 μg/mL and IC_{50} μg/mL. During lysosome stabilization, it is important to limit the inflammatory response. After restraining the inflammatory response, the release of the formed lysosomes is prevented. The ethanolic extract of *Flacourtia indica* showed significant protection in erythrocyte membranes against heat-induced hemolysis [20].

The phenomenon based on the protective effect in hypotonic saline induced in erythrocytes is well known and good for anti-inflammatory activity. Significant protection was shown at 71.34% at a concentration of 500 μg/mL in the ethanolic extract of *Flacourtia indica* and an IC_{50} of 400 μg/mL (Table 4). Diclofenac is used as the standard drug. It showed remarkably high protection at 88.80% at a concentration of 500 μg/mL and IC_{50} at a concentration of 410 μg/mL. (Table 4). [20].

CONCLUSION

The phytochemicals (polyphenol, alkaloid and flavonoid content) present in the ethanolic extract of *Flacourtia indica* aerial parts show potential in the formulation of anti-inflammatory drugs. EEFI inhibits various inflammatory parameters such as inhibition of albumin denaturation, proteinase activity, heat hemolysis, and hypotonicity. In vitro testing for the anti-inflammatory activity of ethanolic root extracts from the Senna singueana studies provided moderate activity. This study demonstrates that the ethanolic extract of *Flacourtia indica* has significant anti-inflammatory activity. Therefore, the sample can be suggested for the preparation of anti-inflammatory drugs. The current study gives rise to the idea that the sample could be used to discover a new anti-inflammatory drug. Further work is required to isolate, purify and characterize samples that can be used for future research.

CONFLICT OF INTEREST STATEMENT
We declare that we have no conflict of interest.

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Table 1: Summary of Phytochemical constituents

S.No	TEST	INFERENCE
1	Liebermann's test	Steroids absent
2	Salwoski test	Steroids absent
3	Schinoda test	Flavonoids present
4	Ferric chloride test	Tannins present
5	Dragandroff's test	Alkaloids absent
6	Brontanger's test	Anthraquinone absent
7	Kedde's test	Cardinolides absent
8	Legal's test	Cardinolides absent

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Table 2 : Inhibition of protein denaturation assay of Ethanolic extract of *Flacourtia indica*

S.NO	Concentration (µg/mL)	% Inhibition of standard * (Diclofenac sodium)	% Inhibition of sample* (EEFI)
1	100	37.90±0.26	35.11±0.38
2	200	50.26±0.46	48.20±0.53
3	300	59.85±0.54	54.79±0.25
4	400	69.05±0.65	65.90±0.48
5	500	83.14±0.44	78.06±0.51

(*Average value of 3 replicates)

Table 3 : Heat induced haemolysis of Ethanolic root extract of *Flacourtia indica*

S.NO	Concentration (µg/mL)	% Inhibition of standard * (Aspirin)	% Inhibition of sample* (EEFI)
1	100	64.90±0.34	47.19±0.37
2	200	83.23±0.33	63.35±0.45
3	300	86.59±0.36	66.83±0.48
4	400	90.64±0.48	73.83±0.43
5	500	92.05±0.38	77.09±0.33

(*Average value of 3 replicates)

Table 4 : Hypotonicity induced haemolysis of Ethanolic extract of *Flacourtia indica*

S.NO	Concentration (µg/mL)	% Inhibition of standard * (Diclofenac)	% Inhibition of sample* (EEFI)
1	100	38.75±0.55	18.50±0.46
2	200	51.99±0.27	30.00±0.54
3	300	71.41±0.45	50.94±0.52
4	400	74.98±0.46	58.74±0.45
5	500	88.20±0.36	71.34±0.38

(*Average value of 3 replicates)

RESEARCH ARTICLE

Assessment of Finger Pattern of Transgender with Comparison of Male, Female and Transwomen

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ABSTRACT

Fingerprint evidence is the most reliable and acceptable evidence to date. Fingerprints are obtained at the site of and in excavated things. Identification of the gender of fingerprints is an emerging field and many methods using the fingerprint physical features like the ridgecount and the ridge thickness have been used so far. Due to the tremendous potential of fingerprints as an effective method of identification, an attempt has been made in the present work to analyse their correlation with the gender of an individual transgender. This study investigated the fingerprint patterns of transgender people. There are 392 participants enrolled in this study; among them 31.1% (122) were females, 33.2% (130) were males and the remaining 35.7% (140) were transwomen. For analysis purposes males and females are combined together and they constituted 64.3% (252). To analyse the data SPSS (IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM Corp. Released 2019) is used. Significance level is fixed as 5% (α = 0.05).The observations in the study revealed that the fingerprint pattern proportions between all groups were not statistically significant.

Keywords: Fingerprint, Transgender, Chi-square test, Transwomen

INTRODUCTION

Dermatoglyphics refers to the branch of science in the study of the patterns of skin ridges (dermal ridges) present on the fingers, palm, toes and the soles of humans (Ramani et al., 2011). Wright et al (1972) has reported for the first time and are compared with dermatoglyphic findings in a normal population as well as with those of available parents of the infants. Ridge patterns exhibit many properties that reflect the biology of individuals. Ridge parameters such as fingerprint ridge count, ridge density, ridge thickness to valley thickness ratio, ridge width and fingerprint pattern

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types are used for gender determination. Variations in ridge parameters for males and females are found statistically (Kralík and Novotny, 2003; Cummins, 1941). Also, it is found that dermatoglyphic features differ statistically between the sexes, ethnic groups and age categories. It has been proved by various researchers that a fingerprint can be processed for sex determination (Nithin et al, 2011). Mansour et al (2014) has been discussed gender classification based on fingerprints using SVM. Gender classification was evaluated using dimensionality reduction techniques such as Principal Component Analysis (PCA) and Support Vector Machine (SVM). A dataset of 2600 persons of different ages and sex was collected as an internal database. Of the samples tested, 1250 samples of 1375 exactly identified male samples and 1085 samples of 1225 precisely identified female samples. Rijo et al (2013) have proposed a technique based on frequency domain analysis to estimate gender. They achieved an overall accuracy rate of 70%. Dongre and Jagade (2015) designed an efficient algorithm for the Fingerprint-based intelligent gender identification system, and after testing the samples, the performance efficiency of the proposed system was found to be 88% for females and 78 % for males. This method of gender identity will be helpful in short listing the suspects and victims from crime scenes and improve the performance of a system used for person recognition and human-computer interfaces. The main objective of the present study is to assess the fingerprint pattern of transgender women using a chi-square test.

METHODOLOGY

Simple descriptive statistics like mean, standard deviation and proportion in terms of percentage are calculated to analyse the data. The Normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results reveal that all variables follow Normal distribution. Therefore, to analyse the data, parametric methods are applied. To compare the mean values between groups, independent samples t-test is applied. To compare proportions between groups Chi-Square test is applied. If any expected cell frequency is less than five, Fisher's exact test is used. For variables which are statistically significant between groups, Fisher's linear discriminant functions were constructed to classify the groups. To analyse the data SPSS (IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM Corp. Released 2019) is used. Significance level is fixed as 5% ($\alpha = 0.05$).A total of 392 participants were enrolled in this study; among them 31.1% (122) were females, 33.2% (130) were males and the remaining 35.7% (140) were transwomen (Fig.1). For analysis purpose, males and females are combined together and they constituted 64.3% (252). The fingerprint samples were collected from the subjects residing in various parts of Tamil Nadu, India. The Digimizer scanner is used for sample collection. The fingerprint image is of 8-bit grey level with a size of 300 × 260 and a resolution of 500 dpi (Fig.2).

RESULTS AND DISCUSSION

The values show the prevalence of flexion crease in the right hand that the majority of the participants, 83.7% (328) have a single flexion crease in the right hand (Table 1 & 2). It was observed that it was more among males/females 84.5% (108), and a little less in transwomen 82.1% (115). The two-flexion crease was observed in 13.0% of the participant; among transwomen, it was 15.7% (22) and males/females with 11.5% (29). The three-flexion 3.6% (9), 2.1% (3), and 3.1% (12) were presented in the males/females, transwomen and overall, respectively. Only one male has a four-flexion crease in the right hand. The proportions between groups were not statistically significant (p > 0.05). The observations revealed the fingerprint pattern in the right-hand thumb finger. It was observed that just above half of the participants 55.1% (216) have ulnar loop patterns (Table 3). This ulnar loop pattern was more among male/females 62.3% (76) than transwomen 51.4% (72). The following predominant pattern was spiral whorl clockwise and was found with 17.3% (68) participants. This pattern was more prevalent among transwomen (20.7% (29) followed by males/females respectively with 15.5%) (39). The double-loop whorl has appeared in 7.7% (30) of participants with 8.6% (12) transwomen and males/females with 7.1% (18). Lateral pocketed ulnar loop whorl was observed in 6.6% (26) participants with transwomen 7.1% (10) and males/females with 6.3% (16). A concentric whorl pattern was observed in 5.4% (21) participants with transwomen 5.7% (8) and males/females with 5.2% (13). The other fingerprint loops were observed 3% or less in each pattern. These proportions between groups were not

statistically significant (p > 0.05). The observations revealed the fingerprint pattern in the right-hand index finger. It was observed that 44.6% (175) of the participants have an ulnar loop pattern (Table 4). This ulnar loop pattern was more among male / females 45.2% (114) and 43.6% (61) in transwomen. The next predominant pattern was spiral whorl clockwise, which was found with 16.1% (63) of participants. This pattern was more among males/females 17.1% (43) and 14.3% (20) among transwomen. Concentric whorl was appeared in 7.9% (31) of participants with 11.4% (16) transwomen and males/females with 6.0% (15). Plain Arch has observed in 6.1% (24) participants with 7.1% (10) transwomen and males/females with 5.6% (14). A transitional ulnar loop pattern was observed in 5.6% (22) participants with an almost similar proportion of transwomen with 5.7% (8), and males/females with 5.6% (14). Spiral whorl anti-clockwise pattern was appeared in 5.4% (21) of participants with 5.6% (14) males/females and 5.0% (7) transwomen. A radial loop was observed in 5.1% (20) of participants, 5.6% (14) of males/females, and 4.3% (6) of transwomen have this pattern. The other fingerprint loops were observed 3% or less in each pattern. These proportions between groups were not statistically significant (p > 0.05). The observations revealed the fingerprint pattern in the right-hand middle finger. It was observed that just above two-thirds of the participants, 70.7% (277) have ulnar loop patterns (Table 5). This ulnar loop pattern was more among males/females with 74.2% (187), and transwomen with 64.3% (90). The next predominant pattern was spiral whorl clockwise, which was found with 11.2% (44) of participants. This pattern was more among transwomen with 11.4% (16) and 11.1% (28) among males / females. Transitional ulnar loop pattern was observed in 4.8% (19) participants with transwomen 6.4% (9), males/females with 4.0% (10).

The concentric whorl has appeared in 3.6% (14) of participants with 7.9% (11) transwomen, followed by males/females with 1.2% (3). Plain Arch was observed in 3.1% (12) participants with 4.0% (10) males/females and transwomen with 1.4% (2). The other fingerprint loops were observed 3% or less in each pattern. These proportions between groups were statistically significant (p < 0.05). The observations revealed the fingerprint pattern in the right-hand ring finger. It was observed that one-third of the participants 33.2% (130) had ulnar loop pattern (Table 6). This ulnar loop pattern was more among males/females with 38.5% (97), followed transwomen with 23.6% (33). The next predominant pattern was concentric whorl and spiral whorl clockwise; both of them were found with 24.7% (97) and 24.5% (96) of participants, respectively. Concentric whorl was more among transwomen 27.9% (39) followed by males/females with 23.0% (58). Spiral whorl clockwise was more among transwomen with 28.6% (40) and males/females with 22.2% (56). The following predominant pattern was the transitional ulnar loop pattern, which was observed in 11.0% (43) participants with transwomen 11.4% (16) and males/females 10.7% (27). The other fingerprint loops were observed 3% or less in each pattern. These proportions between groups were not statistically significant (p > 0.05). The observations revealed the fingerprint pattern in the right-hand little finger. It was observed that two-thirds of the participants, 68.1% (267) have ulnar loop pattern (Table 7). This ulnar loop pattern was 70.2% (177) among males/females and 64.3% (90) in transwomen. The next predominant pattern was transitional ulnar loop pattern, it was found with 13.0% (51) of participants.

This pattern was almost similar in males/females with 13.1% (33) and transwomen with 12.9% (18). The Spiral whorl clockwise pattern was observed in 9.2% (36) of participants with transwomen 11.4% (16) followed by males/females with 7.9% (20). Concentric whorl was observed in 4.1% (16) of participants, it was more among transwomen 5.0% (7) followed by males/females with 3.6% (9). The other fingerprint loops were observed 3% or less in each pattern. These proportions between groups were not statistically significant (p > 0.05). The observations revealed the fingerprint pattern in the right-hand thumb finger. It was observed that closer to three-fifth of the participants 58.2% (228) have a loops pattern (Table 8). This loops pattern was high in males/females with 60.7% (153) and low among transwomen with 53.6% (75). The following predominant pattern was whorls pattern, it was found with 39.5% (155) of participants. This pattern was more among transwomen 45.0% (63) and in males/females it was 36.5% (92). The arches pattern was observed in only 2.3% (9) of participants, males/females 2.8% (7) and transwomen 1.4% (2). These proportions between groups were not statistically significant (p > 0.05 by Fisher's exact test). The observations revealed the fingerprint pattern in the right-hand index finger. It was found that 57.7% (226) of the participants have loops pattern (Table 9). This loops pattern was 58.7% (148) in males/females and 55.7% (78) in transwomen. The next predominant pattern was whorls pattern, it was found with 34.2% (134) of participants. This pattern was more

prevalent among transwomen (35.7% (50) followed by males/females with 33.3%) (84). The arches pattern was observed in 8.2% (32) of participants, transwomen 8.6% (12) and males/females 7.9% (20). These proportions between groups were not statistically significant (p > 0.05). The observations revealed the fingerprint pattern in the right-hand middle finger. It was observed that just above three-fourths of the participants, 76.3% (299) have loops pattern (Table 10) . This loops pattern was high in males/females with 78.6% (198) and transwomen with 72.1% (101). The following predominant pattern was whorls pattern, it was found with 19.9% (78) of participants. This pattern was more among transwomen 25.0% (35) followed by males/females with 17.1% (43). The arches pattern was observed in only 3.8% (15) of participants, males/females 4.4% (11) and transwomen 2.9% (4). These proportions between groups were not statistically significant (p > 0.05). The observations revealed the fingerprint pattern in the right-hand ring finger. It was observed that just above half of the participants 54.1% (212) have whorls pattern (Table 11). This whorls pattern was high in transwomen with 62.9% (88) followed by males/females with 49.2% (124). The following predominant pattern was loops pattern; it was found with 44.4% (174) of participants.

This pattern was more among males/females 49.6% (125) followed by transwomen with 35.0% (49). The arches pattern was observed in only 1.5% (6) of participants, transwomen 2.1% (3) followed by males/females 1.2% (3). These proportions between groups were statistically significant (p < 0.05 by Fisher's exact test). The observations revealed the fingerprint pattern in the little right-hand finger. It was found that four-fifth, 81.1% (318) of the participants have loops pattern (Table 12). This loops pattern was more in males/females with 83.3% (210) and transwomen with 77.1% (108). The following pattern was whorls pattern, it was found with 17.9% (70) of participants. This pattern was more prevalent among transwomen (21.4% (30) and less among males/females (15.9%) (40). The arches pattern was observed only in 4 participants, 2 males/females and transwomen each. These proportions between groups were not statistically significant (p > 0.05 by Fisher's exact test). The values revealed the fingerprint pattern in the left-hand thumb finger. It was observed that just above half of the participants 57.1% (224) have ulnar loop pattern (Table 13). This ulnar loop pattern was more among males/females with 59.5% (150), followed by transwomen with 52.9% (74). The following predominant pattern was spiral whorl anti-clockwise and it was found with 15.8% (62) of participants. This pattern was more among transwomen 17.9% (25) followed by males/females with 14.7% (37). Lateral pocketed ulnar loop whorl and double loop whorl were appeared in 6.6% (26) of participants. Lateral pocketed ulnar loop whorl was with 8.6% (12) among transwomen and 5.6% (14) among males / females. Double-loop whorl was with 9.3% (13) transwomen followed by males/females with 5.2% (13). Plain arch pattern was observed in 4.1% (16) participants with transwomen with 4.3% (6) and males/females with 4.0% (10). The other fingerprint loops were observed 3% or less in each pattern.

These proportions between groups were not statistically significant (p > 0.05). The observations revealed the fingerprint pattern in the left-hand index finger. It was observed that 43.4% (170) of the participants have ulnar loop pattern (Table 14). This ulnar loop pattern was more among transwomen 52.9% (74), followed by males/females with 38.1% (96). The next predominant pattern was spiral whorl anti-clockwise and it was found with 15.1% (59) of participants. This pattern was more among males/females with 17.1% (43) and transwomen 11.4% (16). Plain Arch was observed in 6.6% (26) participants with 7.9% (20) among males/females and transwomen with 4.3% (6). Concentric whorl was appeared in 6.4% (25) of participants with transwomen 7.1% (10) and males/females with 6.0% (15). Transitional ulnar loop pattern was observed in 5.9% (23) participants with males/females 6.3% (16), followed by transwomen 5.0% (7). Spiral whorl clockwise pattern was appeared in 5.1% (20) of participants with 5.2% (13) males/females and 5.0% (7) transwomen. Radial loop was observed in 4.8% (19) of participants, 5.2% (13) of males/females and 4.3% (6) of transwomen have this pattern. Tended arch was appeared in 3.6% (14) of participants with each of males/females and transwomen having 3.6% (9) 3.6% (5) respectively. The other fingerprint loops were observed 3% or less in each pattern. These proportions between groups were not statistically significant (p > 0.05). The observations revealed the fingerprint pattern in the left-hand middle finger. It was observed that below two-thirds of the participants 58.7% (230) have ulnar loop pattern (Table 15). This ulnar loop pattern was more among transwomen 60.0% (84), followed by males/females with 57.9% (146). The next predominant pattern was spiral whorl anti-clockwise and it was found with 14.3% (56) of participants. This pattern was 15.0% (21) among transwomen and 13.9% (35) among males / females. Plain Arch was observed in 7.1% (28) participants with 8.7% (22) mong

males/females and 4.3% (6) among transwomen. Transitional ulnar loop pattern was observed in 4.8% (19) participants with transwomen 6.4% (9) and males/females with 4.0% (10). Concentric whorl was appeared in 4.1% (16) of participants with 5.7% (8) transwomen followed by males/females with 3.2% (8). The other fingerprint loops were observed 3% or less in each pattern. These proportions between groups were not statistically significant (p > 0.05). The observations in the above table reveal the fingerprint pattern in the left-hand ring finger. It was observed that one-third of the participants 33.4% (131) have ulnar loop pattern (Table 16). This ulnar loop pattern was 36.1% (91) in males/females and 28.6% (40) in transwomen. The next predominant pattern was spiral whorl anti-clockwise with 21.7% (85) of participants. This pattern was more among transwomen 24.3% (34) followed by males/females with 20.2% (51). Concentric whorl was the next pattern with 18.6% (73) of the participants, it was more or less similar between males/females with 19.0% (48) and transwomen with 17.9% (25). The next predominant pattern was transitional ulnar loop pattern, which was observed in 14.8% (58) participants with transwomen 20.7% (29) and males/females with 11.5% (29).

The other fingerprint loops were observed 3% or less in each pattern. These proportions between groups were not statistically significant (p > 0.05). The observations reveal the fingerprintpattern in the right-hand little finger. It was observed that above two-thirds of the participants 69.4% (272) have ulnar loop pattern (Table 17). This ulnar loop pattern was almost similar in both groups, males/females with 69.4% (175) and transwomen with 69.3% (97). The following predominant pattern was transitional ulnar loop pattern, it was found with 11.5% (45) of participants. This pattern was also similar among both groups, transwomen 12.1% (17) followed by males/females with 11.1% (28). The Spiral whorl anti-clockwise pattern was observed in 8.4% (33) of participants with males/females 9.1% (23) followed by transwomen 7.1% (10). Concentric whorl was observed in 3.8% (15) of participants, it was more among transwomen 4.3% (6) followed by males/females with 3.6% (9). The other fingerprint loops were observed 3% or less in each pattern. These proportions between groups were not statistically significant (p > 0.05). The observations reveal the fingerprint pattern in the left-hand thumb finger. It was observed that closer to three-fifth of the participants 60.2% (236) have loops pattern (Table 18). This loops pattern was high among males/females with 63.1% (159) and transwomen with 55.0% (77). The following predominant pattern was whorls pattern, it was found with 35.7% (155) of participants. This pattern was more among transwomen 40.7% (57) than by males/females with 32.9% (83). The arches pattern was observed 4.1% (16) of participants, transwomen 4.3% (6), males/females 4.0% (10). These proportions between groups were not statistically significant (p > 0.05). The observations reveal the fingerprint pattern in the left-hand index finger. It was found that 55.9% (219) of the participants have loops pattern (Table 19). This loops pattern was high in transwomen with 62.1% (87), followed by males/females with 52.4% (132). The next predominant pattern was whorls pattern, it was found with 33.9% (133) of participants.

This pattern was 36.1% (91) among males/females and 30.0% (42) among transwomen. The arches pattern was observed in 10.2% (40) of participants, males/females 11.5% (29) and transwomen with 7.9% (11). These proportions between groups were not statistically significant (p > 0.05). The observations reveal the fingerprint pattern in the left-hand middle finger. It was observed that 64.8% (254) of the participants have loops pattern (Table 20). This loops pattern was high in transwomen with 67.9% (95) followed by males/females with 63.1% (159). The following predominant pattern was whorls pattern, it was found with 26.8% (105) of participants. This pattern was almost similar between transwomen 27.9% (39) and males/females 26.2% (66). The arches pattern was observed in 8.4% (33) of participants, males/females 10.7% (27) and transwomen 4.3% (6). These proportions between groups were not statistically significant (p > 0.05). The observations reveal the fingerprint pattern in the left-hand ring finger. It was observed that just below half of the participants 49.2% (193) have loops pattern (Table 21). This loops pattern was almost similar between transwomen 49.3% (69) and males/females with 49.2% (124). The following predominant pattern was whorls pattern; it was found with 48.7% (191) of participants. This pattern was also almost similar between males/females 48.8% (123) and transwomen 48.6% (68). The arches pattern was observed in only 2.0% (8) of participants, transwomen 2.1% (3) and males / females with 2.0% (5). These proportions between groups were not statistically significant (p > 0.05 by Fisher's exact test). The observations reveal the fingerprint pattern in the left-hand little finger. It was found that just above four-fifth, 81.6% (320) of the participants have loops pattern (Table 22). This loops pattern was almost similar between males/females with 81.7% (206) and transwomen with 81.4% (114). The

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following pattern was whorls pattern, it was found with 17.3% (68) of participants. This pattern was also similar between males/females 17.5% (44) and transwomen with 17.1% (24). The arches pattern was observed only in 4 participants, 2 transwomen and one male and female respectively. These proportions between groups were not statistically significant (p > 0.05 by Fisher's exact test).

CONCLUSIONS

In this paper we have verified samples of fingerprints from transgender and also from males and females. After feature extraction and comparison, we have found that the features of transgender fingerprints mostly tending towards male fingerprints characteristics.

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Table 1 Chi-square test to compare proportions between groups (RFC)

Right Flexion crease	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total n (%)	
1	213 (84.5)	115 (82.1)	328 (83.7)	
2	29 (11.5)	22 (15.7)	51 (13.0)	
3	9 (3.6)	3 (2.1)	12 (3.1)	0.542*
4	1 (.4)	0 (.0)	1 (.3)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

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Table 2 Chi-square test to compare proportions between groups (LFC)

Left Flexion Crease	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total n (%)	
1	213 (84.5)	115 (82.1)	328 (83.7)	
2	29 (11.5)	22 (15.7)	51 (13.0)	
3	9 (3.6)	3 (2.1)	12 (3.1)	0.542*
4	1 (.4)	0 (.0)	1 (.3)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 3 Fingerprint patterns in Right Thumb

Right Thumb	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total n (%)	
Plain Arch	7 (2.8)	2 (1.4)	9 (2.3)	
Ulnar Loop	144 (57.1)	72 (51.4)	216 (55.1)	
Transitional Ulnar Loop	8 (3.2)	3 (2.1)	11 (2.8)	
Transitional Radial Loop	1 (.4)	0 (.0)	1 (.3)	
Concentric Whorl	13 (5.2)	8 (5.7)	21 (5.4)	
Spiral Whorl Clockwise	39 (15.5)	29 (20.7)	68 (17.3)	
Spiral Whorl Anti-clockwise	2 (.8)	2 (1.4)	4 (1.0)	0.808*
Central Pocket Radial Whorl	0 (.0)	1 (.7)	1 (.3)	
Lateral Pocketed Ulnar Loop Whorl	16 (6.3)	10 (7.1)	26 (6.6)	
Lateral Pocketed Radial Loop Whorl	4 (1.6)	1 (.7)	5 (1.3)	
Double Loop Whorl	18 (7.1)	12 (8.6)	30 (7.7)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 4 Fingerprint patterns in Right index fingers

Right Index finger	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total n (%)	
Plain Arch	14 (5.6)	10 (7.1)	24 (6.1)	
Tented Arch	6 (2.4)	2 (1.4)	8 (2.0)	
Ulnar Loop	114 (45.2)	61 (43.6)	175 (44.6)	
Transitional Ulnar Loop	14 (5.6)	8 (5.7)	22 (5.6)	
Radial Loop	14 (5.6)	6 (4.3)	20 (5.1)	
Transitional Radial Loop	6 (2.4)	3 (2.1)	9 (2.3)	
Concentric Whorl	15 (6.0)	16 (11.4)	31 (7.9)	
Spiral Whorl Clockwise	43 (17.1)	20 (14.3)	63 (16.1)	0.885*
Spiral Whorl Anti-clockwise	14 (5.6)	7 (5.0)	21 (5.4)	
Central Pocket Radial Whorl	1 (.4)	1 (.7)	2 (.5)	
Lateral Pocketed Ulnar Loop Whorl	6 (2.4)	2 (1.4)	8 (2.0)	
Lateral Pocketed Radial Loop Whorl	1 (.4)	0 (.0)	1 (.3)	
Double Loop Whorl	2 (.8)	3 (2.1)	5 (1.3)	
Accidental Whorl	2 (.8)	1 (.7)	3 (.8)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

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Table 5 Fingerprint patterns in Right middle fingers

Right Middle Finger	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total n (%)	
Plain Arch	10 (4.0)	2 (1.4)	12 (3.1)	
Tented Arch	1 (.4)	2 (1.4)	3 (.8)	
Ulnar Loop	187 (74.2)	90 (64.3)	277 (70.7)	
Transitional Ulnar Loop	10 (4.0)	9 (6.4)	19 (4.8)	
Radial Loop	0 (.0)	1 (.7)	1 (.3)	
Transitional Radial Loop	1 (.4)	1 (.7)	2 (.5)	
Concentric Whorl	3 (1.2)	11 (7.9)	14 (3.6)	
Spiral Whorl Clockwise	28 (11.1)	16 (11.4)	44 (11.2)	**0.007***
Spiral Whorl Anti-clockwise	1 (.4)	3 (2.1)	4 (1.0)	
Central Pocket Ulnar Whorl	1 (.4)	1 (.7)	2 (.5)	
Central Pocket Radial Whorl	0 (.0)	1 (.7)	1 (.3)	
Lateral Pocketed Ulnar Loop Whorl	5 (2.0)	2 (1.4)	7 (1.8)	
Lateral Pocketed Radial Loop Whorl	3 (1.2)	0 (.0)	3 (.8)	
Double Loop Whorl	2 (.8)	1 (.7)	3 (.8)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 6. Fingerprint patterns in Right Ring fingers

Right Ring Finger	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Plain Arch	2 (.8)	3 (2.1)	5 (1.3)	
Tented Arch	1 (.4)	0 (.0)	1 (.3)	
Ulnar Loop	97 (38.5)	33 (23.6)	130 (33.2)	
Transitional Ulnar Loop	27 (10.7)	16 (11.4)	43 (11.0)	
Radial Loop	1 (.4)	0 (.0)	1 (.3)	
Concentric Whorl	58 (23.0)	39 (27.9)	97 (24.7)	
Spiral Whorl Clockwise	56 (22.2)	40 (28.6)	96 (24.5)	0.051*
Spiral Whorl Anti-clockwise	3 (1.2)	3 (2.1)	6 (1.5)	
Central Pocket Ulnar Whorl	2 (.8)	3 (2.1)	5 (1.3)	
Lateral Pocketed Ulnar Loop Whorl	5 (2.0)	1 (.7)	6 (1.5)	
Double Loop Whorl	0 (.0)	1 (.7)	1 (.3)	
Accidental Whorl	0 (.0)	1 (.7)	1 (.3)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 7. Fingerprint patterns in Right little fingers

Right Little Finger	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Plain Arch	1 (.4)	2 (1.4)	3 (.8)	
Tented Arch	1 (.4)	0 (.0)	1 (.3)	0.855*
Ulnar Loop	177 (70.2)	90 (64.3)	267 (68.1)	

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Transitional Ulnar Loop	33 (13.1)	18 (12.9)	51 (13.0)
Concentric Whorl	9 (3.6)	7 (5.0)	16 (4.1)
Spiral Whorl Clockwise	20 (7.9)	16 (11.4)	36 (9.2)
Spiral Whorl Anti-clockwise	1 (.4)	1 (.7)	2 (.5)
Central Pocket Ulnar Whorl	4 (1.6)	2 (1.4)	6 (1.5)
Lateral Pocketed Ulnar Loop Whorl	5 (2.0)	3 (2.1)	8 (2.0)
Double Loop Whorl	1 (.4)	1 (.7)	2 (.5)
Total	252 (100.0)	140 (100.0)	392 (100.0)

Table 8. Fingerprint patterns in Righthand fingers with generic patterns

Right Thumb	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	7 (2.8)	2 (1.4)	9 (2.3)	
Loops	153 (60.7)	75 (53.6)	228 (58.2)	0.212*
Whorls	92 (36.5)	63 (45.0)	155 (39.5)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 9. Fingerprint patterns in Right index fingers with generic patterns

Right Index finger	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	20 (7.9)	12 (8.6)	32 (8.2)	
Loops	148 (58.7)	78 (55.7)	226 (57.7)	0.845
Whorls	84 (33.3)	50 (35.7)	134 (34.2)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 10. Fingerprint patterns in Right middle fingers with generic patterns

Right Middle Finger	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	11 (4.4)	4 (2.9)	15 (3.8)	
Loops	198 (78.6)	101 (72.1)	299 (76.3)	0.144
Whorls	43 (17.1)	35 (25.0)	78 (19.9)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 11. Fingerprint patterns in Right Ring fingers with generic patterns

Right Ring Finger	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	3 (1.2)	3 (2.1)	6 (1.5)	
Loops	125 (49.6)	49 (35.0)	174 (44.4)	**0.012***
Whorls	124 (49.2)	88 (62.9)	212 (54.1)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

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Table 12. Fingerprint patterns in Right little fingers with generic patterns

Right Little Finger	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	2 (.8)	2 (1.4)	4 (1.0)	
Loops	210 (83.3)	108 (77.1)	318 (81.1)	0.305*
Whorls	40 (15.9)	30 (21.4)	70 (17.9)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 13. Fingerprint patterns in Left thumb fingers

Left Thumb	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Plain Arch	10 (4.0)	6 (4.3)	16 (4.1)	
Ulnar Loop	150 (59.5)	74 (52.9)	224 (57.1)	
Transitional Ulnar Loop	6 (2.4)	3 (2.1)	9 (2.3)	
Radial Loop	2 (.8)	0 (.0)	2 (.5)	
Transitional Radial Loop	1 (.4)	0 (.0)	1 (.3)	
Concentric Whorl	5 (2.0)	5 (3.6)	10 (2.6)	
Spiral Whorl Clockwise	5 (2.0)	2 (1.4)	7 (1.8)	
Spiral Whorl Anti-clockwise	37 (14.7)	25 (17.9)	62 (15.8)	0.386*
Central Pocket Ulnar Whorl	1 (.4)	0 (.0)	1 (.3)	
Central Pocket Radial Whorl	1 (.4)	0 (.0)	1 (.3)	
Lateral Pocketed Ulnar Loop Whorl	14 (5.6)	12 (8.6)	26 (6.6)	
Lateral Pocketed Radial Loop Whorl	7 (2.8)	0 (.0)	7 (1.8)	
Double Loop Whorl	13 (5.2)	13 (9.3)	26 (6.6)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 14. Fingerprint patterns in Left index fingers

Left Index Finger	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Plain Arch	20 (7.9)	6 (4.3)	26 (6.6)	
Tented Arch	9 (3.6)	5 (3.6)	14 (3.6)	
Ulnar Loop	96 (38.1)	74 (52.9)	170 (43.4)	
Transitional Ulnar Loop	16 (6.3)	7 (5.0)	23 (5.9)	
Radial Loop	13 (5.2)	6 (4.3)	19 (4.8)	
Transitional Radial Loop	7 (2.8)	0 (.0)	7 (1.8)	
Concentric Whorl	15 (6.0)	10 (7.1)	25 (6.4)	
Spiral Whorl Clockwise	13 (5.2)	7 (5.0)	20 (5.1)	0.248
Spiral Whorl Anti-clockwise	43 (17.1)	16 (11.4)	59 (15.1)	
Central Pocket Ulnar Whorl	3 (1.2)	0 (.0)	3 (.8)	
Central Pocket Radial Whorl	1 (.4)	1 (.7)	2 (.5)	
Lateral Pocketed Ulnar Loop Whorl	6 (2.4)	3 (2.1)	9 (2.3)	
Lateral Pocketed Radial Loop Whorl	7 (2.8)	2 (1.4)	9 (2.3)	
Double Loop Whorl	3 (1.2)	2 (1.4)	5 (1.3)	

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Accidental Whorl	0 (.0)	1 (.7)	1 (.3)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 15. Fingerprint patterns in Left middle fingers

Left Middle finger	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Plain Arch	22 (8.7)	6 (4.3)	28 (7.1)	
Tented Arch	5 (2.0)	0 (.0)	5 (1.3)	
Ulnar Loop	146 (57.9)	84 (60.0)	230 (58.7)	
Transitional Ulnar Loop	10 (4.0)	9 (6.4)	19 (4.8)	
Radial Loop	3 (1.2)	2 (1.4)	5 (1.3)	
Concentric Whorl	8 (3.2)	8 (5.7)	16 (4.1)	
Spiral Whorl Clockwise	6 (2.4)	1 (.7)	7 (1.8)	
Spiral Whorl Anti-clockwise	35 (13.9)	21 (15.0)	56 (14.3)	0.192*
Central Pocket Ulnar Whorl	3 (1.2)	1 (.7)	4 (1.0)	
Central Pocket Radial Whorl	0 (.0)	1 (.7)	1 (.3)	
Lateral Pocketed Ulnar Loop Whorl	5 (2.0)	5 (3.6)	10 (2.6)	
Lateral Pocketed Radial Loop Whorl	6 (2.4)	0 (.0)	6 (1.5)	
Double Loop Whorl	2 (.8)	2 (1.4)	4 (1.0)	
Accidental Whorl	1 (.4)	0 (.0)	1 (.3)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 16 Fingerprint patterns in Left middle fingers

Left Ring Finger	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Plain Arch	3 (1.2)	1 (.7)	4 (1.0)	
Tented Arch	2 (.8)	2 (1.4)	4 (1.0)	
Ulnar Loop	91 (36.1)	40 (28.6)	131 (33.4)	
Transitional Ulnar Loop	29 (11.5)	29 (20.7)	58 (14.8)	
Radial Loop	3 (1.2)	0 (.0)	3 (.8)	
Transitional Radial Loop	1 (.4)	0 (.0)	1 (.3)	
Concentric Whorl	48 (19.0)	25 (17.9)	73 (18.6)	
Spiral Whorl Clockwise	9 (3.6)	2 (1.4)	11 (2.8)	0.429*
Spiral Whorl Anti-clockwise	51 (20.2)	34 (24.3)	85 (21.7)	
Central Pocket Ulnar Whorl	7 (2.8)	3 (2.1)	10 (2.6)	
Central Pocket Radial Whorl	2 (.8)	0 (.0)	2 (.5)	
Lateral Pocketed Ulnar Loop Whorl	3 (1.2)	2 (1.4)	5 (1.3)	
Lateral Pocketed Radial Loop Whorl	2 (.8)	1 (.7)	3 (.8)	
Double Loop Whorl	1 (.4)	1 (.7)	2 (.5)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

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Table 17Fingerprint patterns in Left middle fingers

Left Little Finger	Gender			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Plain Arch	2 (.8)	1 (.7)	3 (.8)	
Tented Arch	0 (.0)	1 (.7)	1 (.3)	
Ulnar Loop	175 (69.4)	97 (69.3)	272 (69.4)	
Transitional Ulnar Loop	28 (11.1)	17 (12.1)	45 (11.5)	
Radial Loop	2 (.8)	0 (.0)	2 (.5)	
Transitional Radial Loop	1 (.4)	0 (.0)	1 (.3)	
Concentric Whorl	9 (3.6)	6 (4.3)	15 (3.8)	0.860*
Spiral Whorl Clockwise	5 (2.0)	2 (1.4)	7 (1.8)	
Spiral Whorl Anti-clockwise	23 (9.1)	10 (7.1)	33 (8.4)	
Central Pocket Ulnar Whorl	2 (.8)	4 (2.9)	6 (1.5)	
Lateral Pocketed Ulnar Loop Whorl	2 (.8)	0 (.0)	2 (.5)	
Lateral Pocketed Radial Loop Whorl	2 (.8)	1 (.7)	3 (.8)	
Double Loop Whorl	1 (.4)	1 (.7)	2 (.5)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 18. Fingerprint patterns in Left thumb fingers with generic patterns

Left Thumb	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	10 (4.0)	6 (4.3)	16 (4.1)	
Loops	159 (63.1)	77 (55.0)	236 (60.2)	0.283
Whorls	83 (32.9)	57 (40.7)	140 (35.7)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 19. Fingerprint patterns in Left index fingers with generic patterns

Left Index Finger	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	29 (11.5)	11 (7.9)	40 (10.2)	
Loops	132 (52.4)	87 (62.1)	219 (55.9)	0.157
Whorls	91 (36.1)	42 (30.0)	133 (33.9)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

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Table 20. Fingerprint patterns in Left middle fingers with generic patterns

Left Middle finger	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	27 (10.7)	6 (4.3)	33 (8.4)	
Loops	159 (63.1)	95 (67.9)	254 (64.8)	0.090
Whorls	66 (26.2)	39 (27.9)	105 (26.8)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 21. Fingerprint patterns in Left ring fingers with generic patterns

Left Ring Finger	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	5 (2.0)	3 (2.1)	8 (2.0)	
Loops	124 (49.2)	69 (49.3)	193 (49.2)	0.999*
Whorls	123 (48.8)	68 (48.6)	191 (48.7)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	

Table 22. Fingerprint patterns in Left ring fingers with generic patterns

Left Little Finger	Group			p-value
	Male / Female n (%)	Transwomen n (%)	Total N (%)	
Arches	2 (.8)	2 (1.4)	4 (1.0)	
Loops	206 (81.7)	114 (81.4)	320 (81.6)	0.839*
Whorls	44 (17.5)	24 (17.1)	68 (17.3)	
Total	252 (100.0)	140 (100.0)	392 (100.0)	
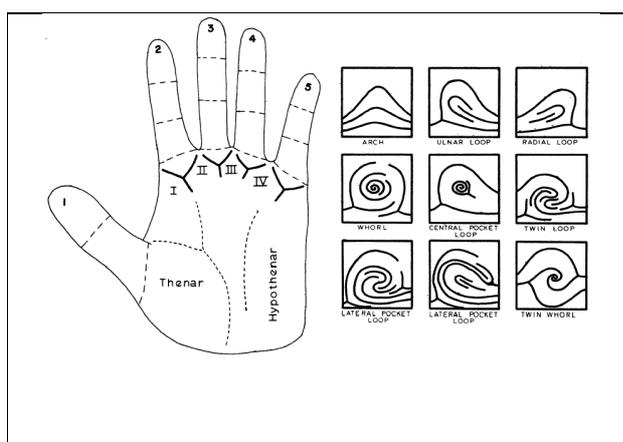
Figure 1 Areas of the hand and various dermatoglyphic pattern	**Figure 2 Digimizer 4.3.2, MedCalc Software**

RESEARCH ARTICLE

On Strong Generalized Derivations in Prime Near – Rings

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ABSTRACT

The concept of generalized derivations in near –rings is made stronger by defining a new concept strong generalized derivations in near – rings and possible analog of the previous results are investigated.

Keywords: Prime near-ring, 3-prime near ring and 2-torsion free near-ring.

INTRODUCTION

Let N be a (left) near – ring not necessarily with a multiplicative identity. Let Z be its multiplicative center. Define N to be 3 – prime if N is zero − symmetric and if for all a,b ∈ N \ {0}, aNb ≠{0}. Define N to be 2 torsion free if 2x = 0 implies x = 0. An additive endomorphism d of N is called a derivation of N if d(xy) = xd(y) + d(x)y, for all x, y ∈ N. An additive endomorphism F of N is called a generalized derivation associated with a derivation d of N if F(xy) = F(x)y +xd(y) for all x,y ∈ N. H.E.Bell [3] has proved the so many results.

Preliminaries
In this section we recall some well known results which we use there in the course of our proof.

Theorem 1.1[3]

Let N be a 3 – prime, 2 – torsion free near – rings. If N admits a non – zero generalized derivation f such that $f(N) \subseteq Z$, then N is a commutative ring.

Theorem 1.2[3]

Let N be a 3 –prime near ring and let f be a generalized derivation on N with associated derivation D. If $f^2 = 0$ then $D^2 = 0$. Moreover if N is 2 – torsion free then $D(Z) = \{0\}$.

Theorem 1.3[3]

Let N be a 3 – prime and 2 torsion free near – ring which admits a generalized derivation f withnon – zero associated derivation D such that f satisfies the additional condition $f(xy) = D(x) y + x f(y)$ for all $x,y \in N$. If $f(x)f(y) = f(y)f(x)$ for all $x,y \in N$, then N is a commutative ring.

In this paper we weaken the conditions of a generalized derivation and define a new concept "Strong generalized derivation" in a near – ring and investigate possible analogs of these results where generalized derivation is replaced by strong generalized derivation.

Definition 1.4[3]

Let N be a near – ring and f be an additive endomorphism of N and d be a derivation of N. An additive endomorphism F of N is said to be a strong generalized derivation associated with f and d if $F(xy) = f(x)y + xd(y)$ for all $x,y \in N$.

Remark 1.5[3]

Every generalized derivation is a strong generalized derivation.

Remark 1.6[3]

If N has multiplicative identity 1, then every strong generalized derivation is a generalized derivation. For, if F is a strong generalized derivation associated an additive endomorphism f of N and a derivation d of N, then $F(xy) = f(x)y + xd(y)$ for all $x,y \in N$.

Taking $y = 1$, we get $F(x) = f(x) + xd(1) \; \forall \; x \in N$. But $d(1) = 0$. Hence $F(x) = f(x) \; \forall \; x \in N$ and so F is a generalized derivation.

Theorem 2.1 [1]

If N is a 3 – prime and 2 – torsion free and D is a derivation such that $D^2 = 0$,then D = 0**Theorem 2.2 [1]**
If N is a 3 – prime, 2 –torsion free near – ring which admits a non – zero derivation D for which $D(N) \subseteq Z$, then N is a commutative ring.

Theorem 2.3 [1]

If N is a 3 – prime , 2 – torsion free near – ring admitting a non – zero derivation D such that $D(x)D(y) = D(y) D(x)$ for all $x,y \in N$, then N is a commutative ring.

Theorem 2.4 [1]

Let N be a 3 –prime near –ring

(i)   If $z \in Z \setminus \{0\}$, then z is not a zero divisor.
(ii)   If $Z \setminus \{0\}$ contains an element z such that $z + z \in Z$, then $(N, +)$ is abelian
(iii)   If D is a non – zero derivation and $x \in N$ is such that $xD(N) = \{0\}$ (or) $D(N)x = \{0\}$, then x=0

Theorem 2.5 [6]

If N is an arbitrary near – ring and D is a derivation on N. Then $D(xy) = D(x)y + xD(y)$ for all $x,y \in N$

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Theorem 2.6 [3]

Let N be an arbitrary near – ring and let f be a generalized derivation on N associated with a derivation D of N. Then (f(a)b +aD(b))c = f(a)bc + aD(b)c for all a, b, c ∈ N Theorem 2.7 .Let R be a prime near – ring. Let x,y ∈ R with $0 \neq x \in z$. If xy = 0 , then y = 0.

Proof

Now, xy = 0 ⇒ zxy = 0 ∀ z ∈ N

  ⇒ xzy = 0 ∀ z ∈ N (∵ ∀ x ∈ z)

Since R is prime, x = 0 or y = 0 , but x ≠ 0 so y = 0

MAIN RESULTS

Lemma 3.1

Let N be a 3 – prime near – ring (left) and F be a strong generalized derivation associated with an additive endomorphism f of N and a derivation d of N. If d(F(N)) = d(f(N)) = {0} then F(d(N)) = f(d(N)) = 0

Proof

By the hypothesis d(F(N)) = d(f(N)) = 0

(ie) d(F(x)) = 0 ∀ x ∈ N   ………………………………..(1)

and d(f(x)) = 0 ∀ x ∈ N   ………………………………..(2)

From (1) we get, d(F(xy)) = 0 ∀ x, y ∈ N   (ie) d(f(x)y +

xd(y)) = 0 ∀ x, y ∈N

(ie) d(f(x)y) + d(xd(y) = 0 ∀ x, y ∈ N

d(f(x))y + f(x) d(y) + d(x) d(y) + xd²(y) = 0 ∀ x, y ∈ N

Using (2) we get

$f(x) d(y) + d(x)d(y) + xd^2(y) = 0$ ∀ x, y ∈ N   ……………………………….(3)

Again applying d on (3) we get

$d (f(x)) d(y) + f(x)d^2(y) + d^2(x)d(y) + d(x)d^2(y) + d(x)d^2(y) + xd^3(y) = 0$ ∀ x, y ∈ N

Using (2) we get

$f(x) d^2(y) + d^2(x)d(y) + d(x) d^2(y) + d(x)d^2(y) + xd^3(y) = 0$ …………………………..(4)

Replacing y by d(y) in (3) we get

$f(x) d^2(y) + d(x) d^2(y) + xd^3(y) = 0$ ∀ x, y ∈ N   ………………………………..(5)

using (4) & (5) we get

$d^2(x)d(y) + d(x) d^2(y) = 0$ ∀ x, y ∈ N   ……………………………….(6)

Replacing x by d(x) in (3) we get

$f(d(x)) d(y) + d^2(x)d(y) + d(x)d^2(y) = 0$ ∀ x, y ∈ N   …………..………………..(7)

Using (6) and (7) we get f(d(x)) d(y)

= 0 ∀ x, y ∈ N

Then by Theorem 2.4 (iii) we have

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$f(d(x)) = 0 \,\forall\, x, y \in N$

(ie) $f(d(N)) = 0$

Remark 3.2
Taking $f = F$, we get Lemma 2.2 [3]

Theorem 3.3
Let N be a 3 – prime 2 – torsion free Near – ring. If N admits a non – zero strong generalized derivation F associated with a non – zero additive endomorphism f of N and a derivation d of N such that $F(N) \subseteq Z$ and $f(N) \subseteq Z$. Then N is a commutative ring.

Proof
Since $F \neq 0$ there exists $x \in N$ such that $0 \neq F(x) \in Z$. Since $F(x) + F(x) = F(x+x) \in Z$, $(N, +)$ is abelian by Theorem 2.4(ii) we shall now show that N is multiplicatively commutative.
Suppose that $d = 0$. Then $F(xy) = f(x)y$ for all $x, y \in N$
Since $F(N) \subseteq Z$ we get $f(x) y \in Z$ for all $x, y \in N$

So, $(f(x)y) w = w(f(x)y)$

  $= (w(f(x))y$

 $= f(x)wy \,\forall\, x, y, w \in N$ ($\because f(N) \subseteq Z$) This implies $f(x) (yw - wy) = 0$

$\forall\, x, y, w \in N$

Since $f \neq 0$, choosing $x \in N$ such that $f(x) \neq 0$ and invoking

Theorem 2.4

(i) we get $yw - wy = 0 \,\forall\, y, w \in N$

So, assume that $d \neq 0$. Let $c \in Z \setminus \{0\}$

Then $d(c) \in Z \setminus \{0\}$

Now $yf(x)c + yxd(c) = y(f(x)c + xd(c))$

$= yF(xc)$

$= F(xc)y \;\; (\because F(N) \subseteq Z)$

$= (f(x)c + xd(c))y$

$yf(x)c + yxd(c) = f(x)cy + xd(c)y \,\forall\, x, y \in N$ Since $c \in Z$ and $f(x) \in Z$,

we get

$yxd(c) = xy\, d(c)$ Since $d(c) \in Z$, we have

 $d(c)yx = d(c)xy$

$d(c) (xy - yx) = 0 \,\forall\, x, y \in N$ ……………………………(8)

we shall now that $d(c) \neq 0$ for $c \in Z$ Suppose $d(z)$

$= 0$

Since $F(N) \subseteq Z$ and $f(N) \subseteq Z$, we have $d(F(N)) = 0$ and $d(f(N)) = 0$. Then by

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Lemma 3.1.

F (d (N)) = 0 and f(d(N)) = 0

Now $F(d(x)y) = f(d(x))y + d(x)d(y) = d(x)d(y) \;\forall\; x, y \in$ N Since $F(N) \subseteq Z$

we get $d(x)\, d(y) \in Z \;\forall\; x, y \in$ N

Similarly $d(y)d(x) \in Z \;\forall\; x, y \in N$

If $d(y)d(x) = 0$, then $(d(x)d(y))^2 = d(x)d(y)\, d(x)d(y)$

$= d(x)0d(y)$

$(d(x)d(y))^2 = 0$ Since $d(x)\, d(y) \in\; Z$, we get $d(x)d(y) = 0$

Similarly, if $d(x)d(y) = 0$, then $d(y)d(x) = 0$

The only possibility is that $d(x)d(y)$ and $d(y)d(x)$ are non – zero central elements Now $d(x)d(x)d(y) =$

$d(x)\, (d(x)d(y))$

$= (d(x)\, d(y))d(x)$

$= d(x)d(y)d(x)$

$\Rightarrow d(x)(d(x)d(y) - d(y)d(x)) = 0 \;\;\forall\; x, y \in N$

$\Rightarrow\; d(x)d(y) - d(y)d(x) = 0 \;\;\forall\; x, y \in N$

$\Rightarrow\; d(x)d(y) = d(y)d(x) \;\;\forall\; x, y \in N$

So, by Theorem 2.3, N is Commutative

Remark 3.4
Taking f = F we get Theorem 1.1

Theorem 3.5
Let N be a 3 – prime near ring and let F be a strong generalized derivation associated with an additive endomorphism f of N and a derivation d of N . If $F^2 = f^2 = 0$, then $d^3 = 0$. More over if N is 2 – torsion free, then $d(z) = \{0\}$.

Proof
Now for x,y $\in$ N
$F^2(xy) = F(F(xy))$
$= F (f(x)y + xd(y))$
$= F(f(x)y) + F(xd(y))$
$= f^2(x)y + f(x)d(y) + f(x)d(y) + xd^2(y)$

By hypothesis $F^2 = f^2 = 0$, we get
$f(x)d(y) + f(x)d(y) + xd^2(y) = 0 \;\;\forall\; x, y \in N$ ……………………………(9)

Applying F on (1) we get
$F(f(x)d(y)) + F(f(x)d(y)) + F(\, xd^2(y)) = 0$

(ie) $f^2(x)d(y) + f(x)d^2(y) + f^2(x)d(y) + f(x)d^2(y) + f(x)d^2(y) + xd^3(y) = 0 \;\forall\; x, y \in N$

Applying $f^2 = 0$, we get

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$f(x)d^2(y)+f(x)d^2(y)+f(x)d^2(y)+xd^3(y)=0$ ……………………………(10)

Replacing y by d(y) in (1) we get

$f(x)d^2(y) + f(x)d^2(y) + xd^3(y) = 0 \; \forall \, x, y \in N$ …………………………………(11)

From (2) & (3) we get

$f(x)d^2(y) = 0 \; \forall \, x, y \in N$ …………….………………(12)

From (3) & (4) we get

$xd^3(y) = 0 \; \forall \, x, y \in N$ ……………………………..……….(13)

Since N is 3- prime $d^3(y) = 0 \; \forall \, y \in N$ (ie) $d^3 = 0$

Now , suppose that N is 2 – torsion free.

Assume that $d(z) \neq 0$. Then $d(z) \neq 0$ for some $z \in Z$ Let x,y $\in$ N such

that $xF(N) = xf(N) = 0$

Then $x \, F(yz) = 0 \; \forall \, y \in N$ $x(f(y)z + yd(z)) =$

$0 \; \forall \, y \in N$ $xf(y)z + xyd(z) = 0 \; \forall \, y \in N$

using $xf(N) = 0$ we get $xyd(z) = 0 \; \forall \, y \in N$

(ie) $xNd(z) = 0$

Since N is 3 – prime and $d(z) \neq 0$ we get $x = 0$ so from (4) we get $d^2(y) = 0$ for all $y \in N$

That is, $d^2 = 0$. Then by Theorem 2.1, $d = 0$.

This contradicts our assumption that $d(z) \neq \{0\}$ Hence $d(z) = \{0\}$

Remark 3.6

Taking f = F, we get Theorem 1.2

Theorem 3.7

Let N be a 3 – prime 2 – torsion free near ring which admits a strong generalized derivation associated with an additive endomorphism f of N and a derivation d of N such that $[F(x), F(y)] = [F(x), f(y)] = [f(x), f(y)] = 0$ for all x, y $\in$ N. Then N is commutative near ring.

Proof

Now $F(x) \, F(y) = F(y) \, F(x) \; \forall \, x, y \in N$ …………………...…………..(14)

Replacing y by yz we get

$F(x) \, F(yz) = F(yz) \, F(x) \; \forall \, x, y, z \in N$

$F(x) \, (f(y)z + yd(z)) = (f(y)z + yd(z))F(x)$

$F(x) \, f(y)z + F(x)yd(z) = f(y)z \, F(x) + yd(z))F(x) \; \forall \, x, y, z \in N$ Taking $0 \neq z \in Z$,

we get

$F(x) \, f(y)z + F(x)yd(z) = f(y) \, F(x)z + yF(x) \, d(z)$ using $F(x)f(y) =$

$f(y)F(x) \; \forall \, x, y \in N$, we get $F(x)yd(z) – yF(x)d(z) = 0$

(ie) $(F(x)y – yF(x)) \, d(z) = 0$

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$F(x)y - yF(x) = 0 \ \forall \ x, y \in N$

As $0 \ne d(z) \in Z$,

(ie) $F(N) \subseteq Z$

By hypothesis $F(x)f(y) = f(y)F(x) \ \forall \ x, y \in N$ Taking $x = xz$

we get

$F(xz)f(y) = f(y)F(xz) \ \forall \ x, y, z \in N$ $(f(x)z + xd(z))f(y)$

$= f(y) \ (f(x)z + xd(z))$

$f(x)z \ f(y) + xd(z)f(y) = f(y)f(x)z + f(y)xd(z)$

Let $0 \ne z \in Z$. Then $0 \ne d(z) \in Z$

Then $f(x)f(y)z + xf(y)d(z) = f(y)f(x)z + f(y)xd(z)$

Since $f(x)f(y) = f(y)f(x) \ \forall \ x, y \in N$

We get

$xf(y)d(z) - f(y)xd(z) = 0$

(ie) $(xf(y) - f(y)x)d(z) = 0$

As $0 \ne d(z) \in Z$, $xf(y) - f(y)x = 0 \ \forall \ x, y \in N$ (ie) $f(N) \subseteq Z$

By Theorem 3.3, N is a commutative ring.

Remark 3.8

Taking $f = F$, we get Theorem 1.3

In [3], H.E. Bell has proved Theorem 1.3. We give a short proof for Theorem 1.3 as below.

Proof of Theorem 1.3

By hypothesis $F(x)F(y) = F(y)F(x) \ \forall \ x, y \in N$ …………...………………(15)

Replacing y by yz we get

$F(x)F(yz) = F(yz)F(x) \ \forall \ x, y,z \in N$

$F(x)\{F(y)z + yd(z)\} = \{F(y)z + yd(z)\}F(x)$

$F(x)F(y)z + F(x) \ yd(z) = F(y)z \ F(x) + yd(z)F(x) \ \forall \ x, y,z \in N$

Taking $0 \ne z \in Z$. then $0 \ne d(z) \in Z$

$F(x)F(y)z + F(x) \ yd(z) = F(y) \ F(x)z + yF(x)d(z)$

using (1) we get

$(F(x)y - y \ F(x))d(z) = 0$

As $0 \ne d(z) \in Z$, we get

$F(x)y - y \ F(x) = 0 \ \forall \ x, y \in N$ (ie) $F(N)$

$\subseteq Z$

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Then by Theorem 1.1, N is a Commutative ring.

Theorem 3.9

Let R be a prime near – ring admitting a non – zero strong generalized derivation F associated with a non – zero additive map f : R →R and a non – zero derivation d of R satisfying either

(iv) $F([x,y]) = f([x,y]) = x^k [x^m,y]x^{\ell}$ (Or)

(v) $F([x,y]) = f([x,y]) = - x^k [x^m,y]x^{\ell}$

for all x,y ∈ R and for fixed integers k ≥ 0, ℓ ≥ 0 and m ≥ 1. Then R is Commutative.

Proof

By the hypothesis

$F([x,y]) = f([x,y]) = x^k [x^m,y]x^{\ell}$ ∀ x, y ∈ R   …………...…………..…………(16)

Replacing y by yx we get $F([x,yx]) = x^k [x^m,yx]x^{\ell}$

$F(y[x,x]+ [x,y]x) = x^k y [x^m,x]x^{\ell} + x^k [x^m,y]x^{\ell+1}$

(ie) $F([x,y]x) = x^k [x^m,y]x^{\ell+1}$ ∀ x, y ∈R $f([x,y])x +$

$[x,y]d(x) = x^k [x^m,y]x^{\ell+1}$

using (1) we get

$x^k [x^m,y]x^{\ell+1}+ [x,y]d(x) = x^k [x^m,y]x^{\ell+1}$

(ie) $[x,y] d(x) = 0$ ∀ x, y ∈ R   ……………………………(17)

Replacing y by zy we get

$[x,zy] d(x) = 0$ ∀ x,y,z ∈ R

$z[x,y]d(x) + [x,z] yd(x) = 0$ ∀ x,y,z ∈R

using (2) we get

$[x,z]y d(x) = 0$ ∀ x, y, z ∈ R

(ie) $[x,z]R d(x) = 0$ ∀ x, z ∈ R   …………………………(18)

Since R is prime either [x,z] = 0 or d(x) = 0

(ie) x∈Z(R) ∀ x ∈ R (or) d(x) = 0 ∀ x ∈ R If x ∈ Z(R), then xy =

yx ∀ y ∈ R

(ie) d(xy) = d(yx) ∀ y ∈ R

$d(x)y + x d(y) = d(y)x + yd(x)$

using x ∈ Z(R) we get

$d(x) y = y d(x)$ ∀ y ∈ R (ie) d(x)

∈ Z(R) ∀ x ∈R (ie) d(R) ⊆ Z(R)

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Then by Theorem 1.1, R is commutative.
Similar to the above steps.

Remark 3.10
The condition R is prime in Theorem 3.1 is necessary even in the case of arbitrary rings.

Theorem 3.11

Let R be a prime near-ring admitting a strong generalized derivation F associated with a non-zero additive map f:R→R and a non-zero derivation d of R satisfying

either (i) $D(x \circ y) = x^k (x^m \circ y)x^\ell = f(x \circ y)$

(or) (ii) $D(x \circ y) = - x^k (x^m \circ y)x^\ell = f(x \circ y)$, for all x,y ∈ R and for fixed integers k ≥ 0, ℓ ≥ 0 and m ≥ 1. Then R is Commutative.

Proof:

By the hypothesis

$D(x \circ y) = f(x \circ y) = x^k (x^m \circ y)x^\ell$ ∀ x, y ∈ R   ………………………………(19)

Replacing y by yx we get $D(x \circ yx) = x^k (x^m \circ yx)x^\ell$

Since $(x \circ yx) = (x \circ y)x$ ∀ x, y ∈ R

We have

$D((x \circ y)x) = x^k (x^m \circ y)x^{\ell+1}$

(ie) $f(x \circ y)x + (x \circ y)d(x) = x^k (x^m \circ y)x^{\ell+1}$

using (1) we get

$x^k (x^m \circ y)x^{\ell+1} + (x \circ y)d(x) = x^k (x^m \circ y)x^{\ell+1}$

(ie) $(x \circ y)d(x) = 0$ ∀ x, y ∈ R   …..………………………………(20)

Replacing y by zy we get $(x \circ zy)d(x) = 0$

∀ x, y, z ∈ R $(z(x \circ y) + [x,z]y) d(x) = 0$

using (2) we get

$[x,z]y \, d(x) = 0$ ∀ x, y, z ∈ R

(ie) $[x,z]R \, d(x) = 0$ ∀ x, z ∈ R

Since R is prime, either $[x,z] = 0 \, d(x) = 0$

(ie) x ∈ Z(R) (or) d(x) = 0 ∀ x ∈ R

If x ∈ Z(R), then xy = yx ∀ y ∈ R

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(ie) d(xy) = d(yx) ∀ y ∈ R

d(x)y + x d(y) = d(y)x + yd(x)

using x ∈ Z(R) we get

d(x) y = y d(x) ∀ y ∈ R   (ie) d(x)

∈ Z(R) ∀ x ∈R(ie) d(R) ⊆ Z(R)

Then by Theorem 1.1, R is commutative.

Similar to the above steps.

Remark 3.12
The condition R is prime in Theorem 3.3 is necessary even in the case of arbitrary rings.

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RESEARCH ARTICLE

Application of Block Chain Technology and Price Fluctuations in Cryptocurrency Market

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ABSTRACT

Crypto currency can be defined as money in digital or virtual form. It is powered by the technology called block chain to attain decentralization, immutability and transparency. In this article, we discuss about Cryptocurrency,block chain technology, the types of Crypto currency, the different exchanges to buy and sell crypto currencies, the price history analysis of Crypto currency for investment and practice.

Keywords: *Cryptocurrency, Block Chain, Bitcoin, Cardano, Solana, Litecoin, Ethereum, Doge coin, Shiba Inu*

INTRODUCTION

The term Cryptocurrency has become most common among technologists, business men and also among common people all over the world. It is a digital currency. Unlike physical currencies like coins and notes, they are not issued by a centralized authority. Anyone can create their own virtual currency. The transactions of these currencies happen virtually online and are verified and maintained by a decentralized system. There are no physical mode of transactions. They do not depend on the bank to send and receive payments. Cryptocurrency gained its name from the word cryptography which enables protecting and sending information in a secured or encrypted format over the network. Crypto currencies can be classified based on their usage. There are more than 5000 crypto currencies available and many are yet to gain their place in the market.

Statement of the Problem

➢ The recently proposed bill by the Indian government pursues to forbid all crypto currencies in India. The bill also proposed to create a structure for the formation of authorized virtual currency to be supplied by the Reserve Bank of India. The Union Budget 2022-2023 has suggested 1 percent tax deduction on payments towards digital currencies above Rs.10000 per year. Theft, hacks and money laundering may be the possible reasons for crypto ban in India. The price fluctuations in crypto currencies also exhibit risks and threats to the onlookers and Investors.ne has to face their own risks involved in investing and trading with crypto currencies.

OBJECTIVES OF THE STUDY
➢ To explore the usage of popular crypto currencies.
➢ To know the application of Block chain technology in the Crypto world.
➢ To study about the different Cryptocurrency exchanges.
➢ To analyze the price fluctuations of popular crypto currencies over the years.

REVIEW OF LITERATURE

Simanta Shekhar Sarmah (2018) in their article titled "Understanding Blockchain Technology". In this article they mentioned that Blockchain is a significant technical invention in 2018. Blockchain is a transparent money exchange system that had transformed the way a conducting business. Companies and tech giants have started investing significantly in the Blockchain market and it is expected to be net worth of more than 3 trillion dollars in the year 2023. It had become growing popular because of its undisputable security and ability to deliver broad solution to digital identity problems. It was a digital ledger in a peer-to-peer network. This paper described about a background on Blockchain technology, history, it's architecture, how it works, advantages and disadvantages and its application in different industries.

Danda B. Rawat, Vijay Chaudhary and Ronald Doku (2021) in their article titled "Blockchain Technology: Emerging Applications and Use Cases for Secure and Trustworthy Smart Systems". They stated that Blockchain, also known as a distributed ledger technology, stores different transactions/operations in a chain of blocks in a distributed manner without needing a trusted third-party. Blockchain was proven to be unchallengeable, which helps with integrity and accountability and, to some extent, confidentiality through a pair of public and private keys. Blockchain has been in the spotlight after successful boom of the Bitcoin. There have been efforts to leverage salient features of Blockchain for different applications and use cases. This paper presented a comprehensive survey of applications and use cases of Blockchain technology for making smart systems secure and trustworthy.

WajdeBaiod, Janet Light and Aniket Mahanti(2020) in their article titled "Blockchain Technology and its Applications Across Multiple Domains: A Technology Review". They described that Blockchain technology became an active area of research and a technological option for many businesses and industrial communities. With its distributed, decentralized, and trustless nature, block chain provided businesses with new opportunities and benefits through increased efficiency, reduced costs, enhanced integrity and transparency, better security, and improved traceability. Although block chain's largest applications have been in the finance and banking sector in this research identified experiments and proposed applications in different fields. This paper provided an overview of block chain technology; it brings together all the key design features, characteristics, and benefits of block chain that make it a superior and unique technology, and it presented the popular consensus protocols and taxonomy of block chain systems. Additionally, the paper surveys block chain-based applications across multiple domains such as in finance, insurance, supply chain management, energy, advertising and media, real estate and healthcare. It aimed at examine the industries' key issues, block chain solutions and use cases. The paper highlighted three broad limitations that

block chain technology presents: scalability, security and regulation and shows how these challenges could impact block chain application and adoption.

RESEARCH METHODOLOGY

➢ In this paper a descriptive research is done to systematically analyze crypto currencies and their price fluctuations.

➢ The analysis is done with the secondary data collected from finance.yahoo.com.

➢ Historical data of the price history of crypto currencies from their inception to January 2022 are taken for analysis.

➢ A few popular crypto currencies are chosen for analysis based on the articles " 10 important crypto currencies other than Bitcoin" by Aadamhayes in investopedia.com and "Top 10 Crypto currencies in February 2022" by Kat Tretina and John Schmidt in Forbes Advisor form Forbes.com

BLOCK CHAIN TECHNOLOGY

Blockchain is a decentralized, shared database technology originated in the early 1990s.It emerged as an alternative to the traditional client server network. They are a chain of blocks with distributed network architecture where each block contains electronic information and are linked to the preceding blocks. It enables a system to store and distribute information without being edited. When a fresh data comes in, a fresh block is created and the data is stored. It is then linked to the preceding block and thus making a chain of blocks. They help to store currencies in virtual wallets without any centralized authority like banks as an intermediary. They make the virtual currencies more secured by storing the virtual record of transactions and data in many nodes on a computer network. They are undeniable and distributable. The following diagram illustrates the working of a block chain. When a transaction is requested it is represented as a block and broadcasted to all the nodes in the network. The nodes in the network validate the transaction and after validation the block is added to the network and the transaction is completed.

Types of crypto currencies

There are more than 5000 crypto currencies available in the market. They are classified based on their utility as Defi, Metaverse, NFT and value tokens like Bitcoin, Ethereum, etc. Every Cryptocurrency has its own usage and description. The following are some of the commonly known crypto currencies.

Bitcoin

It was created in January 2009.It uses cryptography for security. They are virtual coins and their transactions are verified using Bitcoin mining. It is one of the most popular coin and also called as BTC. The name Satoshi Nakamoto is connected with this coin, but the identity of Bitcoin creator is unknown. Bitcoin is used for payments in online business and also purchased for investments since the value of this coin has risen exponentially. It is one the most used Cryptocurrency in the world and many people trust that Bitcoin will have a promising future and unavoidable in the international financial system. There are 21 million Bitcoin in total. It is calculated overall as technically optimistic for both short term and medium term investments. There are 21 million Bitcoin in total.

Ethereum

It is an open access to virtual currency which permits you to interchange money and create agreements without a centralized intermediary. It was released in 2015 and uses distributed computing technology. It is used in crowd funding, NFTs, decentralized exchanges, betting, games, global payments and prediction marketplaces and as collateral. It's more popular in the market .Recently twitter has added Ethereum to its virtual tipping feature.

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Litecoin

It is a peer to peer decentralized digital money which allows immediate payments to anyone in the earth. It was created in 2011.It   has a faster transaction speed comparatively than most other coins and hence one of the most transacted coins in the market. It is used for fund transfer and purchases. There are 84 million Litecoins are available in total.

Cardano

It was the first Cryptocurrency to be created based on peer reviewed studies and was created from evidence grounded techniques. It was founded in 2015. It is secured through multisignatures. It aims to give solutions to anomalies like increase in transaction fees, slowing down of network. It is used in healthcare, finance and farming. It aims to handle large number of transactions and is efficient. It also works on sustainability on the Cryptocurrency platforms. There are around 33 billion Cardano coins distributed and are actively used.

Solana

This is based on the native currency SOL and developed in the year 2018. It works in accomplishing great transaction speed. It is decentralized. It has a swiftly extended eco system and it is versatile. It has a circulating supply of 319,427,530 SOL coins. It is used in building smart contracts and trading. NFTs are also powered by Solana. It also supports building of various applications like games, social media and speculation. It uses a proof of stake mechanism to authenticate transactions.

Shiba Inu

It is a decentralized dog faced meme Cryptocurrency based on Ethereum developed in 2020.From then it has transformed from a fun currency to a decentralized eco system. It is a new wave of Cyptocurrency and used for trade. It has also launched a decentralized exchange called Shibaswap for swapping currencies. This currency has become popular in the recent times.

Dogecoin

It is a Cryptocurrency developed in 2013 and was initially promoted as a fun and friendly version of Bitcoin. It has a large active user community. It is mostly used in tipping service. This currency is used to reward content creators on various social media platforms like Reddit etc. There are many other Crypto currencies like Binance coin, Polkadot, stellar, Bitcoin cash, Tether, USD coin, Avalanche etc. One needs to check if it is a stable coin before buying. Also the risk factors of Cryptocurrency need to be considered.

Cryptocurrency exchanges

They are used to buy and sell crypto currencies. One needs to create and maintain an account with the exchange system. The exchanges can be classified as
➢ Centralized Cryptocurrency exchange
➢ Decentralized Cryptocurrency exchange

Centralized Cryptocurrency exchange

They are useful for beginners where direct exchange of currency like Rupees, Dollars, to Cryptocurrency can be done fast and with ease. Possible risks of hacking and money laundering are encountered in this type of exchanges. To minimize the risk of hackers stealing the assets, a personal wallet can be maintained.

Decentralized Cryptocurrency exchange

In Decentralized exchanges certified transactions take place. This increases accountability and the transactions are transparent. They are considered more secured than centralized Cryptocurrency exchanges. But direct conversion from other currency to Cryptocurrency is not possible here. One needs to have crypto currencies already to use this exchange. Hence it is considered less user friendly.

Centralized vs Decentralized Cryptocurrency exchange

A summary of the features of both centralized and decentralized exchanges are listed below.
. Cryptocurrency exchanges charge its clients for using them. The charges can be levied for withdrawals, trade and transfers. One has to consider the trading size, number of crypto coins available, user-friendliness, safety and trust worthiness of exchanges before choosing them

Price History Analysis of Crypto Currencies

Bitcoin

Fig 4 shows the price history of Bitcoin. The value of a single Bitcoin was $0.09 in 2010. In April 2013 it raised its peak to $29.60 and then fell back to $2.05 in November 2011. At the start of 2015 it reached $315.25.In 2017 the value sky rocketed from $1000 to $ 19,345.49. By the end of 2019 it fluctuated to $6,635.84.At the end of 2020 Bitcoin reached a price of $29.000.In 2021 its price hit its all-time high of $68,990.90 and at the end of 2021 it fell down to $49,243.29.The fluctuations in price of Bitcoin is said to be because of pandemic and the new variant of corona virus, Omicron

Ethereum

Fig 5 illustrates the price fluctuations in Ethereum. The value of Ethereum was just near to $0 in 2015.The price started to increase in 2017 and at the start of 2018 the price climbed to $1422.47.Then there was a decline in its price in 2019 and 2020.The price elevated in the end of 2021 and by the start of 2022 the price slightly declined to $3683.

Litecoin

Fig 6 represents the price history of Litecoin. The price was nearer to $0 up to 2017 and by the mid of 2017 it reaches a value of $50 .By the end of 2017, Litecoin's price was around $300.It suffered a crash in 2018 and again the price was fluctuating till 2019.But by the mid of 2021 it reached a new height around $346.Then it dipped and by the start of 2022 the price was as $143.

Cardano

The following fig 7 illustrates the price history of Cardano. There was a gradual increase in the price of Cardanoin the   year 2021.It reached its new height of around $2.7 and started declining after that. By the beginning of 2022 Cardano's price was around $1.3.

Solana

The price history of Solano is illustrated in the following fig 8. Solana was priced around $0 up to the mid of 2020.In 2021 The price of Solana started to increase gradually and by the end of 2021 the price was set to around $208.By the beginning of 2022 the price slightly declined to around $170.Solana is termed as Ethereum killer after its drastic price growth in the recent years.

Shiba Inu

Shiba Inu's price history since its inception is illustrated in the following fig 9. The coin's price was around $0 since 2020.By January 2022 Shiba Inu's price was $0.00047.

Dogecoin

The Price history of Dogecoin is illustrated in the fig 10. The price started to raise since the start of 2021.By April 2021 the value of Dogecoin was around $0.42.By the end of 2021 the price declined to $0.18 .

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CONCLUSION

The study concludes that the crypto market has a lot of opportunities to grow. It gives a high level of transparency on trade. They are more convenient to transact .The major risk involved in Cryptocurrency is its fluctuating price and security threats. As Cryptocurrency is not yet regulated by the government, one has to analyze the coin to be purchased, gain an in depth knowledge, know the potential risk involved in investing and trading. As Cryptocurrency is volatile, the investors and traders have to be updated regularly about the currencies and the Cryptocurrency exchanges. Usage of crypto currencies for investments and payments can be done by gaining a proper financial advice.

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Table 1: Features of centralized and Decentralized exchanges

Features	Centralized Exchange	De Centralized Exchange
Ease of Use	Easy and user friendly	Hard and less user friendly
Anonymity	No	Yes
Hack/ Server Down time	Yes	No
Features	Basic	Advanced
Control of Funds	Exchange	User
Liquidity	High	Low

Fig.1.Working of Block chain	**Fig.2.Centralized Exchanges**
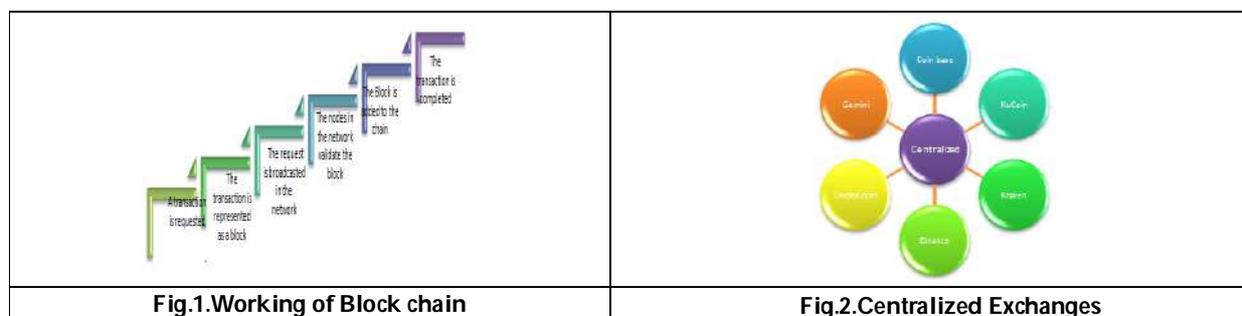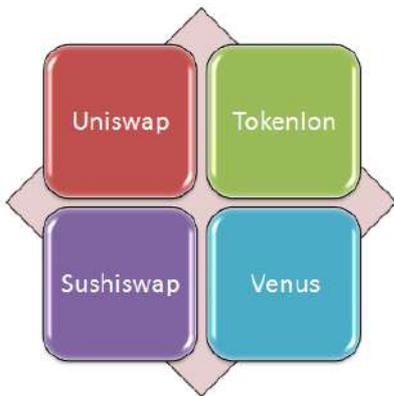
Fig.3. Decentralized Exchanges

Fig.4. Price history of Bitcoin ,secondary data obtained from finance.yahoo.com

Fig.5.Price history of Ethereum ,secondary data obtained from finance.yahoo.com

Fig.6. Price history of Litecoin, secondary data obtained from finance.yahoo.com

Fig.7. Price history of Cardano, secondary data obtained from finance.yahoo.com
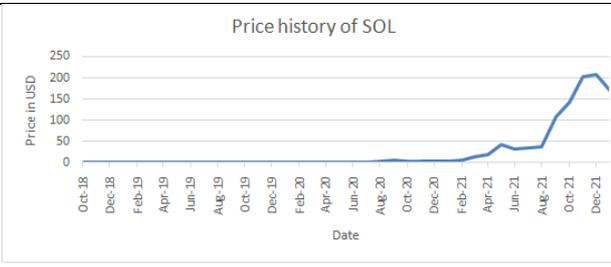
Fig.8. Price history of Solana, secondary data obtained from finance.yahoo.com
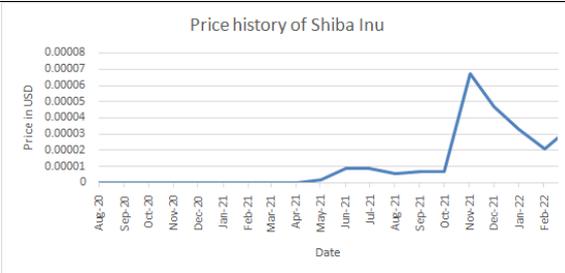
Fig.9. Price history of Shiba Inu, secondary data obtained from finance.yahoo.com

Fig.10. Price history of Dogecoin, secondary data obtained from finance.yahoo.com

RESEARCH ARTICLE

Effect of *Vernonia cinerea* Mediated AgNPs on Caspase-3-Induced Apoptosis in PANCSCs

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ABSTRACT

Pancreatic cancer is one of the most fatal tumors, with no present control over its lethality, due to late diagnosis and the lack of reliable therapeutic methods. Despite the experts' best efforts, the search for cancer medicines continues to be a maze. To combat this malignancy, efforts such as identifying novel therapy options and repurposing existing medications are ongoing. The current study utilizes the herbal plant *Vernonia cinerea* aqueous extract to test the anticancer potential of produced silver nanoparticles (AgNPs) on pancreatic malignant stem cell line. *V. cinerea* is a contemplative plant that has been used to treat abortion, cancer, and gastrointestinal disorders. The plant's toxicity was tested on mice, but the results were insufficient to draw any conclusions. This study demonstrated a simple green synthesis method for preparing silver nanoparticles as well as the superior anticancer activity of the plant *V. cinerea*. The appearance, morphology, properties, and elemental composition of synthesized AgNPs were examined by SEM, XRD, UV-VIS Spectrophotometer, FTIR, and EDX. Flow cytometric analysis was also

performed to determine the mechanism of AgNPs' anticancer efficacy. The results reveal that the AgNPs suppressed PANCSCs growth by inducing apoptosis (significantly higher apoptotic initiation when compared to control) via caspase-3 activation, and a cell cycle research was performed to confirm the cell cycle arrest that occurs in PANCSCs. As a result, the anticancer activity of AgNPs in PANCSCs has been demonstrated by the annual herb *V. cinerea*.

Keywords: Silver nanoparticles, Vernonia cinerea, PANCSCs, Anticancer activity, Apoptosis, and caspase-3 expression

INTRODUCTION

Cancer is the second most common cause of mortality worldwide, accounting for 9.6 million deaths in 2018. Cancer is responsible for around 1 in every 6 deaths worldwide. Cancer kills around 70% of people in low and middle-income nations. The advancements in technology and knowledge of neoplastic disease are benefiting opportunities to reduce the death rate from cancer through the creation of novel medications [1]. Cancer is caused by the change of normal cells into tumor cells in a multistage process that includes the progression from a precancerous lesion to a malignant tumor. The interplay of a person's genetic traits with physical (ultra violet radiation), chemical (tobacco and asbestos), and biological (virus and bacterium) factors causes these alterations [2]. Pancreatic cancer (PC) has risen to second position on the list of cancer-related deaths, with a relatively low 5-year survival rate [3], [4]. According to GLOBOCAN estimates for cancer incidence and mortality globally, PC cases are as high as 45900 in 185 countries, and deaths are nearly equal to incidence, i.e. 432000 in both men and women [5]. The search for effective therapy agents against PANCSCs leads to the investigation of numerous compounds that may have anticancer properties; one such group of therapeutic agents that has been thoroughly investigated for other cancers and has proven to be effective is natural products, particularly plant products [6]. The roots of plant-based remedies can be traced all the way back to the origins of many diseases [7], [8]. Plant-based medications are less expensive, highly effective, non-toxic, easy to get, and have few side effects [9], [10]. Medicinal plants are also used in the development of modern drugs that are effective against aggressive diseases [11], [12]. Docetaxel, paclitaxel, vinblastine, camptothecin, vincristine, and homoharringtonine are examples of anti-cancer drugs derived from plants that are used to treat cancers such as breast cancer, stomach cancer, prostate cancer, ovarian cancer, lung cancer, Hodgkin's lymphoma, non-lymphoma, Hodgkin's brain cancer, testicular cancer, bladder cancer, melanoma, and leukaemia [13].

Nanotechnology is one of the most promising research areas in modern medical science. Based on specific characteristics such as shape and size, nanomaterial's exhibit improved properties that can be applied in both fundamental and applied research field [14]. on specific Gold and silver nanoparticles have emerged as a promising metal nanoparticles for numerous applications resembles bio-labeling, biosensors, biomedical science, imaging, and pathogens identification make them a valuable choice for many therapeutic and pharmacological applications [15], [16] Furthermore, these nanoparticles can be applied in cancer diagnostics and therapy, biological probes, brain implants, drug delivery, gene delivery, sterilization system, vaccine preparation, and improving electrical signaling in the heart. Over last few decades, there has been considerable interest in developing nanoparticles by various physiochemical and biological method. There are several chemical and physical techniques for the synthesis of silver nanoparticles has been reported, but the methodologies have several disadvantages including high energy consumption, utilization of toxic and highly reactive chemicals, which pose potential environmental and biological risks. Biological synthesis of metal nanoparticles using natural products is an alternative. The widely known natural products are plants bacteria, algae and fungi [16], [17], [18]. The vital reason to using plant extracts for silver nanoparticle synthesis is that they are easy extractable, safe, and nontoxic in most cases, have a broad variety of

metabolites that can aid within the reduction of silver ions, and are faster than microbes in the synthesis. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly and easily scaled up for large scale synthesis. The genus *Vernonia* (Asteraceae) are widely distributed in East and West Africa as well as in Indian subcontinents have long been used in traditional medicine to treat various types of diseases. *V. cinerea L*. (Asteraceae) is an annual herbaceous plant, distributed throughout India and grown as a weed plant [19]**.** It is commonly known as 'Sahadevi' in Sanskrit and Hindi, 'Little ironweed' in English. The plant which has contains numerous medicinal uses such as malaria, infertility, skin conditions and worms Various biological studies have confirmed the medicinal properties of this species notably, the analgesic in nature, antipyretic, anti-inflammatory, antimicrobial effects [20]. This species is also the source of cytotoxic and antiplasmodial. The most in-depth studies on the pharmacology of this plant has been carried out to evaluating the crude extract on the possible cytotoxic effect on cancer cells [21], [22]. These properties have created interest for studying the anticancer nature of *V. cinerea L*. with the help of in vitro studies. The present investigation deals with *V. cinerea L* whole plant mediated synthesis, characterization of AgNPs and its cell cycle arrest, caspase-3 expression studies and anti-proliferative effect on human pancreatic cancerous stem cells.

MATERIALS AND METHODS

Cell Line and Culture Medium
National Center for Cell Sciences in Pune, India, provided the pancreatic cancer stem cells. The cells were grown in DMEM high glucose medium supplemented with 10% FBS and incubated at 37°C in an incubator with a humidified environment of 5% CO_2 under standard conditions. After that, the cells were detached using trypsin-EDTA for 2 minutes before being rinsed in PBS for further analysis.

Collection and identification of Plant material
Whole plant of *Vernonia cinerea* were collected from the surrounding of Thondamuthur area (10.9899° N, 76.8409° E) Coimbatore, Tamil Nadu. India. It was verified and authenticated by Botanical Survey of India, Coimbatore, Tamil Nadu. The plant was washed with water many times to eliminate dust particles, then finely chopped and dried in the shade for 10 days. After that, the dried leaves were ground into a fine powder and employed in further research.

Preparation of Plant Extract
To obtain the aqueous leaf extract of *V. cinerea*, dried leaves were decocted (10% w/v, plant: water) for 15 minutes at a temperature of roughly 100°C (yield: 13.57 percent relative to dry plant). After vacuum filtration, the aqueous extract was freeze-dried and dissolved in PBS at concentrations suitable for biological experiments.

Phytochemical Screening
The qualitative phytochemical screening of *V. cinerea* extract standard procedures were followed to trace out the presence of active biomolecules i.e. alkaloids, flavonoids, phenols, saponins, proteins, steroids and tannins by Harborne, [23], Trease and Evans, [24] and Sofowara, [25].

Gas chromatography mass spectrometry (GC–MS) analysis
GC–MS analyses of *V. cinerea* plant extracts was carried out using the Perkin-Elmer Clarus 680 system (Perkin-Elmer, Inc. U.S.A) equipped with a fused silica column, packed with Elite-5MS) capillary column (30 m in length×250 μm in diameter×0.25 μm in thickness). Pure helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 mL/min. For GC– MS spectral detection, an electron ionization energy method was adopted with high ionization energy of 70 eV (electron Volts) with 0.2 s of scan time and fragments ranging from 40 to 600 m/z. The injection quantity of 1 μL was used (split ratio 10:1), and the injector temperature was maintained at 250 °C (constant). The column oven temperature was set at 50 °C for 3 min, raised at 10 °C per min up to 280 °C, and final temperature was increased to 300 °C for 10 min. The contents of phytochemicals present in the test samples were identified based on

comparison of their retention time (min), peak area, peak height and mass spectral patterns with those spectral database.

FTIR Spectroscopic Analysis of V. cinerea extract
The Fourier transform infrared spectrophotometer (FTIR) is one of the most powerful techniques for identifying chemical bonds (functional groups) in substances. For FTIR analysis, dried powders of distinct solvent extracts of each plant material were utilized. To make a translucent sample disc, 10 mg of dried extract powder was encapsulated in 100 mg of KBr pellet. Each plant specimen's powdered sample was placed into an FTIR Spectroscope (Shimadzu, IR Affinity1, Japan) with a scan range of 400 to 4000 cm-1 and a 4cm-1 resolution [26].

Biosynthesis and characterization of silver nanoparticles
The *V. cinerea* aqueous leaf extract was prepared adding 10 g of washed and finely cut leaves in a 300-ml Erlenmeyer flask filled with 100 ml of sterilized double-distilled water and then boiling the mixture for 5 min, before finally decanting it. The extract was filtered using Whatman filter paper no. 1, stored at −4 °C and tested within 5 days. The filtrate was treated with aqueous 1 mM AgNO$_3$ solution in an Erlenmeyer flask and incubated at room temperature. A brown-yellow solution indicated the formation of AgNPs, since aqueous silver ions were reduced by the *V. cinerea* extract generating stable AgNPs in water. Silver nitrate was purchased from the Precision Scientific Co. (Coimbatore, India). Green synthesis of AgNPs was confirmed by sampling the reaction mixture at regular intervals, and the absorption maxima was scanned by UV–vis, at the wavelength of 200– 800 nm in UV-3600 Shimadzu spectrophotometer at 1 nm resolution. Furthermore, the reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min; resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45 μm). An aliquot of this filtrate containing AgNPs was used for SEM, FTIR spectroscopy, XRD analysis, and EDX spectroscopy. The structure and composition of freeze-dried purified AgNPs was analyzed by using a 10-kV ultrahigh-resolution scanning electron microscope with 25 μl of sample sputter coated on copper stub, and the images of AgNPs were studied using a FEI QUANTA-200 SEM. The surface groups of the AgNP were qualitatively confirmed by FTIR spectroscopy [27], with spectra recorded by a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. EDX assays confirmed the presence of metals in analyzed samples [28].

Flow Cytometric Analysis
Flow cytometric was used to identify the effect on apoptosis, caspase-3 and cell cycle analysis by the silver nanoparticles synthesis from *V.cinerea*.

Detection of Apoptosis Activity
Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenance of tissue homeostasis. The apoptotic program is characterized by specific morphologic features, including loss of cell membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and inter nucleosomal cleavage of DNA. Briefly, the cells incubated with AgNPs for 48 h were collected and centrifuged at 1500 rpm for 5 min. The harvested cells are washed twice with PBS and decant PBS completely. Cells were followed by FITC Annexin V staining and incubated for half an hour in the dark. Then PI and 1X binding buffer was added and analysis by flow cytometry immediately after addition of PI.

Cell Cycle Analysis
This is a method for cell cycle analysis using Propidium Iodide (PI) that is, using the fluorescent nucleic acid dye PI to identify the proportion of cells that are in one of the three interphase stages of the cell cycle. Briefly, the cells were cultured in DMEM high glucose complete medium for 24 hours. The cells were treated with trypsin-EDTA solution thereafter, washed twice with cold PBS, and centrifuged. The pellet was resuspended in 50 ml cold PBS and 450 μl cold methanol for 1 hour at 4 °C. The cells were centrifuged at 1100 rpm for 5 minutes, pellet washed twice with cold PBS, suspended in 500 μL PBS, and incubated with 5 mL RNAse for 30 minutes. The cells were chilled over ice for 10 minutes and stained with propidium iodide for 1 hour and analyzed by flow cytometry.

Caspase-3 Expression Assay

Caspase-3 is one of the indicator for apoptosis in cells. Expression of caspase-3 was determined using flow cytometry as mentioned in the below mentioned protocol. First the pancreatic cancerous stem cells were seeded and processed as detailed in mitochondrial membrane depolarization assay. After trypsinization and washing with PBS, the cell pellet was incubated with 0.5mL BD Cytofix/Cytoperm solution. After 10 minutes, the cells were washed with 0.5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) containing 0.1% sodium azide. Next, 20μL anti–Caspase-3 antibody was added, and mixed thoroughly. The incubation was continued for 30 minutes in the dark at about 25°C, and subsequently washed with 1X PBS containing 0.1% sodium azide. At the end the cells were resuspended in 0.5mL PBS, mixed thoroughly and analyze by Flow Cytometry. If samples are not to be analyzed immediately, mix thoroughly just prior to analysis.

RESULT AND DISCUSSION

Phytochemical Screening of Vernonia cinerea

The phytochemical screening of Methanol and aqueous extracts of *V. cinerea* revealed the presence of certain phytocompounds which is summarized in (Table 1) Alkaloids, phenols, tannins, steroids, glycosides, flavonoids, carbohydrates and terpenoids were present in Methanolic extracts of *V. cinerea*. Alkaloids, phenols, saponins, flavonoids, carbohydrates and phlobtannins are the compounds that were found to be present in aqueous whole plant extracts. Our results compared with other researchers similarly, Haque et al., [29] reported that the phytochemical screening of the stem-bark and leaves extracts of *V. cinerea* confirmed the presence of triterpinoids, glycosides, steroids and ester. Moreover the phytochemical screening of methanol leaf extract of *V. amygdalina* shows the presence of the following constituents: Saponin, Alkaloid, Tannin, Phenol, Flavonoid, Glycoside and Terpenoid [30].

Gas chromatography-mass spectrometry (GC–MS) analysis of V. cinerea leaf extract

The GC–MS chromatogram of leaf extract of *V. cinerea* recorded a total of 27 peaks corresponding to the bioactive compounds that were recognized by relating their peak retention time, different peak areas (%) and mass spectral fragmentation patterns (Figure 1). Results of *V. cinerea* methanolic leaf extract identified 27 compounds (Table 2). Very recently, Ugbogu et al., [31] studied and reported the bioactive compounds extracted from same genus *Vernonia amygdalina* includes 6β,10β,14β trimethylheptadecan-15 α-olyl-15-O-β-D-glucopyranosyl-1,5 β olide, glucuronolactone, 11 α-hydroxyurs-5,12-dien-28-oic acid-3 α,25-olide, 10-geranilanyl-O-β-D-xyloside, 1-heneicosenol O-β-D-glucopyranoside, apigenin, luteolin (3´,4´,5,7tetrahydroxyflavone), vernolide, hydroxyvernolide, 3'-deoxyvernodalol , vernodalol, diterpene (ingenol-3-angelate), vernomygdin, 4-methylumbelliferone, cephantharin, cryptolepine, isocryptolepine, neocryptolepine, courmarins, vernolepin, and vernoniosides. Various *in vivo* and *in vitro* studies revealed that *V. amygdalina* and its bioactive components possess pharmacological activities such as antioxidant, anti-inflammatory, anticancer, antimicrobial, hepatoprotective, antidiarrheal, anti-diabetic, and neuroprotective activities.

FTIR Spectroscopic Analysis of V. cinerea extract

The FT-IR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio. The results of FT-IR analysis confirmed the presence of N-H, O-H, C=C, C-H, C-O and CH_3 functional groups (Figure 2). FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition. Similarly, the present FT-IR results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols on the basis of identification of functional groups of OH, CH and C=O which signifies the presence of these compounds in the methanol extracts of *V. amygdalina* [30].

Physico –chemical properties of synthesized AgNPs

The synthesized silver nanoparticles are always by UV–visible technique are the as shown in (Figure. 3). It shows the UV–vis spectra of synthesized silver nanoparticles at two different time interval. The colour change is due to the Surface Plasmon Resonance phenomenon. The sharp bands of silver nanoparticles were observed around 413nm, 415nm at 0hrs and 24hrs respectively. Previously, similar result has been reported in the synthesis of Au and AgNPs using the extracts of *Gloriosa superba* [32], *Tribulus terrestris* [33]. So we confirmed that *V. cinerea* whole plant extract has more potential to reduce Ag ions into Ag nanoparticles, which lead us for further research on synthesis of silver nanoparticles from *V. cinerea* whole plant extracts. The SEM result shows the surface morphology of AgNPs biosynthesized from *V. cinerea* whole plant extracts and it clearly indicated the irregular shapes in surface (Figure 4). Some smaller particles having spherical shapes as well as some other particles irregular shapes are observed. Further, it has been confirmed that with different sizes of nanoparticles is presented inside the used samples. The particle shape of plant-mediated AgNPs was mostly spherical and plated like projection and the size around 5–35 nm [34]. The energy dispersive spectrum of synthesized nanoparticles is shown in. Metallic silver nanoparticles considerably shows strong signal peak at 3keV which is due to surface Plasmon resonance (Figure 5) [35]. The presence of elements such as Ag, CI, Si, K, and O are shown in the graph. In this the synthesized nanoparticles show strong absorbance in the range 2.80-4keV.

The phase variety and grain size of synthesized Silver nanoparticles was determined by X-ray diffraction spectroscopy The diffraction peak values at 38.04° and 64.13° corresponds to lattice planes at (1 0 0) and (2 2 0) respectively of metallic silver. (Figure 6)The average grain size of the silver nanoparticles formed in the bio reduction process determined using Scherer's equation, where 'D' is the particle diameter size, 'K' is a constant, k is the wavelength of X-ray source (0.15406 nm), 'b' is the full width half maximum and 'h' is the diffraction angle. Similar appearance of diffraction patterns are observed with silver nanoparticles synthesized using plant extract [36], [37].FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles has showed the bands around 3490-3500 cm-1 corresponds to O-H stretching H-bonded alcohols and phenols. The peak found around 1500-1550 cm-1 showed a stretch for C-H bond, peak around 1450-1500 cm-1 showed the bond stretch for N-H. Whereas the stretch for Ag-NPs were found around 500-550 cm-1 (Figure 7). Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups [38]. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins might probably from the metal nanoparticles (i.e. capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium [39].

Cell Cycle Analysis of PANCSCs

Cell cycle distribution was investigated in this study by labelling silver nanoparticle-treated pancreatic malignant stem cells with propidium iodide and analyzing the percentages of G0/G1, S, and G2/M cells using flow cytometry. The G2/M population of pancreatic malignant stem cells treated with 63µg/ml silver nanoparticles was 38.07±1.7%, compared to 8.97±1.3 % in control cells. Furthermore, silver nanoparticle treatment reduced the proportion of cells in the G0/G1 phase of the cell cycle from 72.14±2.8% in control pancreatic malignant stem cells to 39.41±2.8% in treated pancreatic cancerous stem cells. (Figures 8) As a result, the current findings indicate that silver nanoparticles caused cell cycle arrest in the G2/M phase. Apoptosis was accompanied by a decrease in the percentage of cells in the G0/G1 phase and an increase in the percentage of cells in the G2/M phase, indicating cell cycle arrest at G2/M. Tumor initiation and progression have been associated to cell cycle dysregulation [40]. As a result, the cell cycle has emerged as a promising therapeutic target in the treatment of cancer. According to research, the majority of chemotherapeutic drugs elicit cell cycle arrest at the G0/G1 or G2/M stages, but cell cycle arrest at the S stage is uncommon [41].

Annexin V Apoptosis Detection Assay

The Annexin V FITC apoptosis detection kit was used to stain untreated and silver nanoparticle-treated (5 M) stem cells. The Annexin V FITC assay was used to collect data from the untreated and treated samples in a work list. After acquisition and analysis, the assay lab research reports were automatically prepared [42] [43]. Gating cells and identifying any changes in the scatter properties of the cells were done using FSC-H vs. SSC-H plots. The populations belonging to viable and non-apoptotic (AnnexinV–PI–), early (AnnexinV+PI–), and late (Annexin V+PI+) apoptotic cells are shown in Annexin V FITC-H vs. Propidium Iodide-H plots from gated cells (Figures 9). The test (treated stem cell) and control (untreated stem cell) had fare deviations of 27.47% and 99.43%, respectively, and the cells that reach necrotic death are 4.82% on the test and 0.05% on the control malignant stem cells. The presence of (Annexin V+PI–) in the cells that have achieved early apoptosis is a major impact factor in determining the role of silver nanoparticle synthesized are efficient, in that Annexin V has stained on the plasma membrane and the fluorescent of Annexin V has expressed in test 50.40%, which is higher than the control cells 0.45%. When compared to the control 0.07% [44] late (AnnexinV+PI+) apoptotic cells arranged high in the test 17.37%.

Analysis the Expression of Caspase-3

Caspase activity was measured in nanoparticle-treated pancreatic cancer stem cells using a fluorogenic caspase-3 substrate to directly address the role of caspase-3 in silver nanoparticle-induced apoptosis. The activation of caspase-3 by silver nanoparticles was concentration-dependent and before apoptosis, which is consistent with prior observations showing caspase activation is essential for DNA fragmentation [45]. When compared to untreated cells, the activation of caspase is highest in this study. The silver nanoparticles treated (test) activated at around 97.75%, while the untreated (control) activated at only 2.81% (Figure 10).

CONCLUSION

The study concluded the green synthesis of silver nanoparticles from the entire *V. cinerea* plant, as well as their anti-aging characteristics. Phytochemical analysis of the methanol and aqueous extracts of the dried plant sample of *V. cinerea* revealed the presence of Alkaloids, Phenols, Tannins, Steroids, Glycosides, Flavonoids, Carbohydrates, Terpenoids in the methanolic extract and Alkaloids, Phenols, Saponins, Flavonoids, Carbohydrates, and Phlobtannins in the a Silver nanoparticles were studied using a UV Vis spectrophotometer, and sharp bands of silver nanoparticles were found about 413nm and 415nm in 0 hours and 24 hours, respectively. The Silver nanoparticles were aggregated and clustered in structure to comply with the synthesis, as revealed by SEM analysis. The elemental composition of the EDX was determined using SEM and EDX: Ag, Cl, K and O. The findings of the FTIR analysis revealed the existence of the functional groups. The crystalline peaks seen in the Silver Nanoparticles were visible in the XRD patterns. It was also discovered that AgNPs from *V. cinerea* stimulate the activation of caspase-3, which leads to the induction of apoptosis in both sex trinsic and intrinsic ways, as evidenced by flow cytometry assays and cell cycle analysis to confirm the stage of programmed cell death. The study found that AgNPs could be successfully synthesized from the *V. cinerea* plant, and that the synthesized AgNPs could effectively inducer programmed cell death in pancreatic cancer stem cells.

CONFLICT OF INTEREST

The author (s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ACKNOWLEDGEMENT

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Table 1: Preliminary phytochemical analysis of *V. cinerea*

PHYTOCHEMICAL	METHANOL	WATER
Alkaloids	++	++
Phenols	++	++
Tannins	++	--
Saponins	--	++
Steroids	++	--
Glycosides	++	--
Flavonoids	++	++
Carbohydrates	++	++
Proteins	--	--
Phlobtannins	--	++
Terpenoids	++	--

Table 2: Compounds presented in GCMS analysis of *V. cinerea*

S. No	Compound Label	RT	Name	DB Formula	Hits (DB)
1	Methylenecyclopropanecarboxylic acid	3.562	Methylenecyclopropanecarboxylic acid	$C_5H_6O_2$	1
2	Cpd 4: D-Limonene	4.781	D-Limonene	$C_{10}H_{16}$	10
3	Cpd 6: Furyl hydroxymethyl ketone	5.223	Furyl hydroxymethyl ketone	$C_6H_6O_3$	2
4	Cpd 8: Triethyl phosphate	5.478	Triethyl phosphate	$C_6H_{15}O_4P$	4
5	Cpd 9: Maltol	5.566	Maltol	$C_6H_6O_3$	5
6	Cpd 13: Naphthalene	6.492	Naphthalene	$C_{10}H_8$	10
7	Cpd 18: trans-2-methyl-4-n-pentylthiane, S,Sdioxide	9.413	trans-2-methyl-4-n-pentylthiane, S,S-dioxide	$C_{11}H_{22}O_2S$	10
8	Cpd 23: Pentanoic acid, 5-hydroxy-, 2,4-di-tbutylphenyl esters	11.642	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenylesters	$C_{19}H_{30}O_3$	2
9	Cpd 24: Undecanoic acid, 10-methyl-, methyl ester	11.933	Undecanoic acid, 10-methyl-, methyl ester	$C_{13}H_{26}O_2$	3
10	Cpd 26: Benzene, (1-butylhexyl)-	12.176	Benzene, (1-butylhexyl)-	$C_{16}H_{26}$	2

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11	Cpd 27: Benzene, (1-propylheptyl)-	12.376	Benzene, (1-propylheptyl)-	$C_{16}H_{26}$	1
12	Cpd 29: Diethyl Phthalate	13.278	Diethyl Phthalate	$C_{12}H_{14}O_4$	6
13	Cpd 30: 1-Tetradecanol	13.375	1-Tetradecanol	$C_{14}H_{30}O$	10
14	Cpd 31: Hexadecane	13.528	Hexadecane	$C_{16}H_{34}$	1
15	Cpd 32: Benzene, (1-methylnonyl)-	13.561	Benzene, (1-methylnonyl)-	$C_{16}H_{26}$	2
16	Cpd 34: Benzene, (1-butylheptyl)-	14.19	Benzene, (1-butylheptyl)-	$C_{17}H_{28}$	1
17	Cpd 35: Benzophenone	14.247	Benzophenone	$C_{13}H_{10}O$	9
18	Cpd 36: Benzene, (1-propyloctyl)-	14.418	Benzene, (1-propyloctyl)-	$C_{17}H_{28}$	1
19	Cpd 37: Ar-tumerone	14.891	Ar-tumerone	$C_{15}H_{20}O$	1
20	Cpd 41: Benzene, (1-pentylheptyl)-	16.138	Benzene, (1-pentylheptyl)-	$C_{18}H_{30}$	1
21	Cpd 42: Benzene, (1-butyloctyl)-	16.233	Benzene, (1-butyloctyl)-	$C_{18}H_{30}$	1
22	Cpd 46: n-Pentadecanol	17.529	n-Pentadecanol	$C_{15}H_{32}O$	10
23	Cpd 49: 1,2-Benzenedicarboxylic acid, bis(2- methylpropyl) ester	19.066	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	10
24	Cpd 51: 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9- diene-2,8-dione	20.311	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	1
25	Cpd 52: Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	21.022	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	1
26	Cpd 54: n-Pentadecanol	23.129	n-Pentadecanol	$C_{15}H_{32}O$	5
27	Cpd 61: Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	35.983	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	1
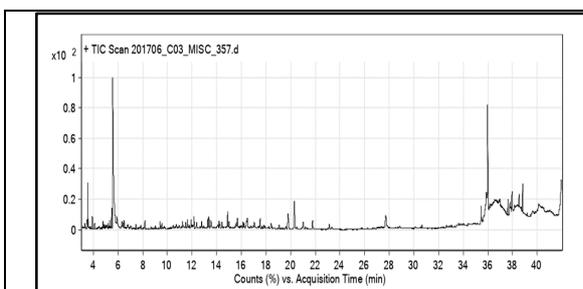
Figure 1: GCMS-mass spectroscopy of *V. cenerea* methanol extract

Figure 2: FTIR spectrum of *V. cenerea* methanol extract

Figure 3: UV- vis spectroscopy of *V. cenerea* synthesized AgNPs
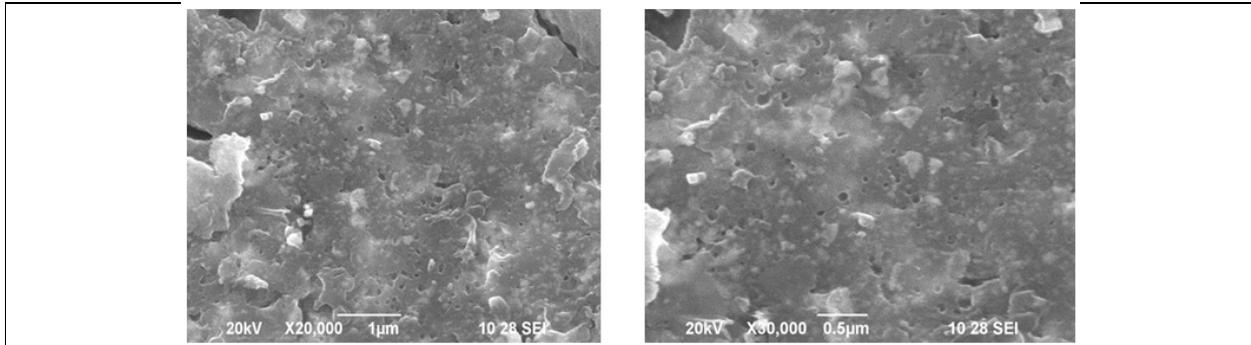
Figure 4: SEM analysis of *V. cenerea* synthesized AgNPs
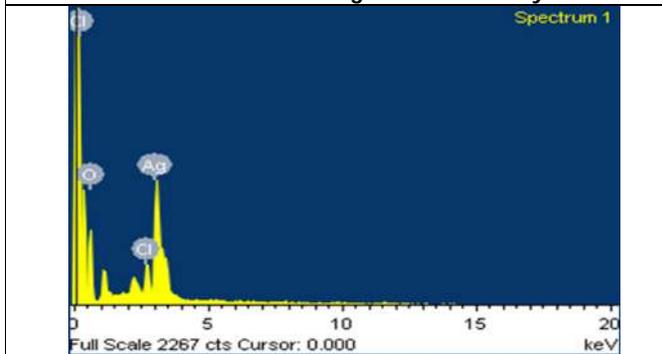
Figure 5: EDAX spectrum of *V. cenerea* synthesized AgNPs
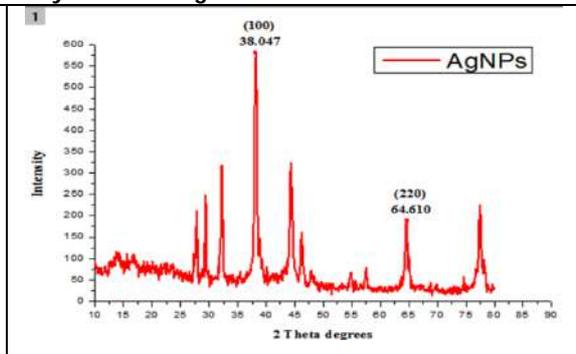
Figure 6: XRD pattern of *V. cenerea* synthesized AgNPs
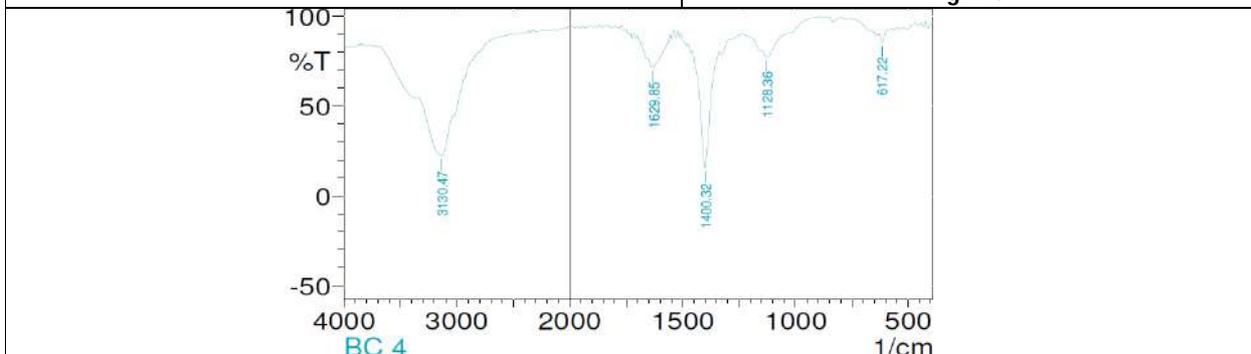
Figure 7: FTIR analysis of *V. cenerea* synthesized AgNPs
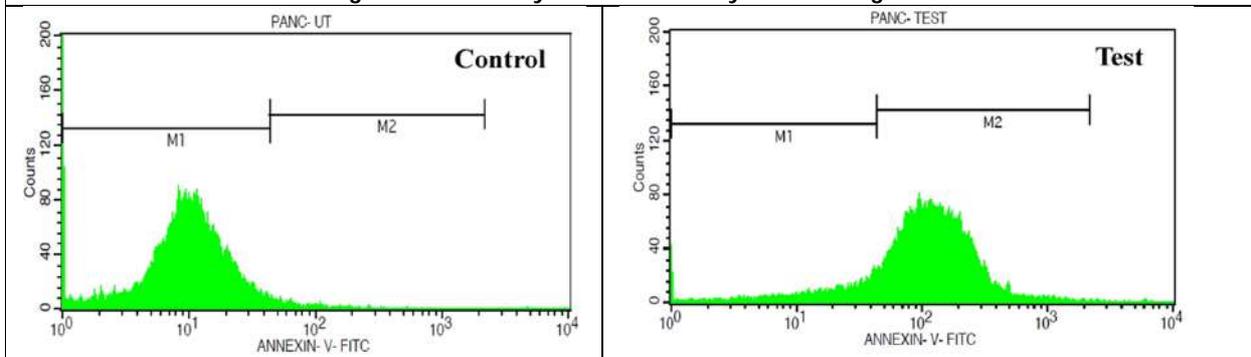
Figure 8: Cell Cycle Analysis of PANCSCs Cell Control Vs PANCSCs Treated With AgNps

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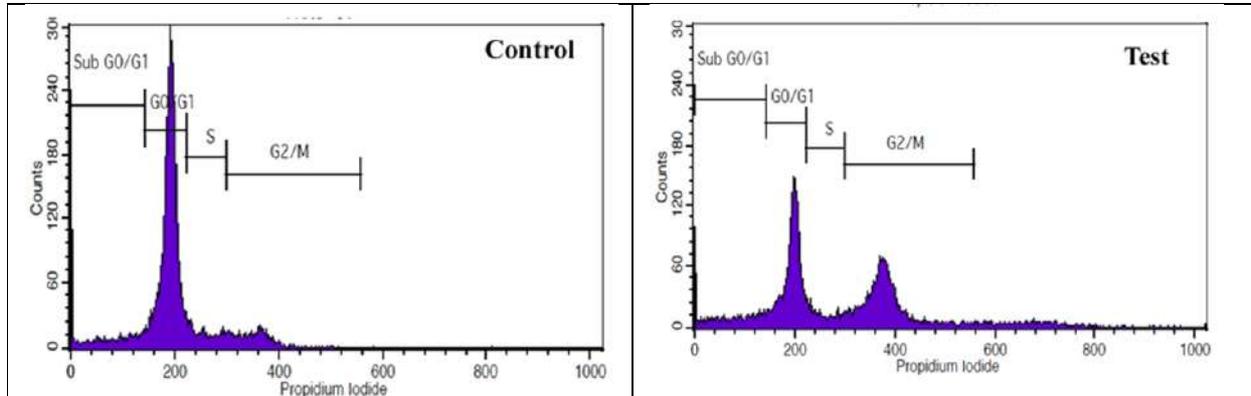
Figure 9: Apoptosis Annexin V vs. PI of PANCSCs Cell Control Apoptosis Annexin V vs. PI of PANCSCs Treated with AgNPs
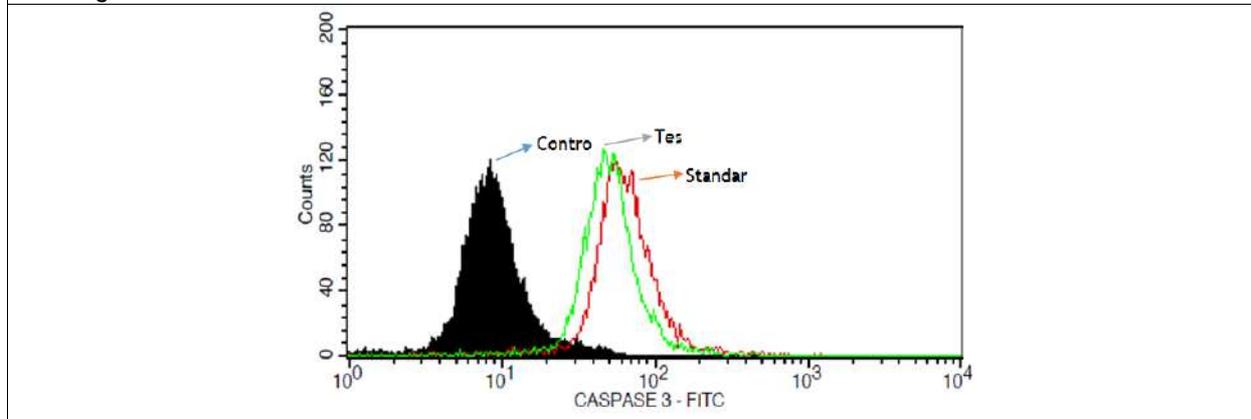
Figure 10: Overlay for the Caspase-3 Activity Along With Control, Test and Standard

RESEARCH ARTICLE

Hand Function and Hand Eye Coordination Compromise in Modified Radical Mastectomy – A Pilot Study

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ABSTRACT

Hand function assessment has been included as mandatory in physical examination of many neurological conditions, where the hand function is affected viciously, apart from other functions of the upper limb. All the subjects were assessed for a shoulder disability, hand function, and hand-eye coordination skills. SPADI scale was used to evaluate the subject's severity of pain and the associated disability among the samples. A correlation was performed individually between the shoulder disability scores and hand function and shoulder disability scores and hand eye coordination using a non-parametric analysis. The primary researcher was responsible for organizing the data. The demographic details of the subjects are provided in table It is also obvious from the study that duration from surgery also influenced the disability among the subjects.

Keywords: correlation, subjects, demographic, neurological

INTRODUCTION

Hand function assessment has been included as mandatory in physical examination of many neurological conditions, where the hand function is affected viciously, apart from other functions of the upper limb. A classic example of such a condition is hemiplegic following stroke [1]. Assessment of Hand Function helps in the intervention designing and also in the prognosis of stroke rehabilitation. When there is no direct impairment of the hand, like elbow and shoulder impairments, the HF examination is ignored. This is true in the case of most of the musculoskeletal conditions that affect the shoulder. But the fact is, proximal stability is a prerequisite for distal mobility, be it a gross motor activity or fine motor activity [2.] This is true in both the upper limb and lower limb where the proximal muscles significantly contribute to the distal muscle activity. While attempting to grasp any

object with hand, the resultant is simultaneous muscle contraction at the elbow and shoulder [3]. It is proved in the past that before the limb muscle contracted the trunk and proximal muscles contracted in an anticipatory manner to give a proper background for distal muscle activity [4]. This has strong physiological support as many neurons of the motor cortex have outputs that innervate a spinal motor neuron pool of both proximal muscles and intrinsic muscles of the hand. This claim was reinforced when it was proved by the Spike-triggered averaging of EMG activity which showed that neurons of the motor cortex have either facilitatory or inhibitory effects on the motor neuron pools of shoulder, elbow, wrist and hand muscles [5]. When the motor cortex is stimulated through a weak stimulus, a monosynaptic excitatory postsynaptic potential (EPSPs) is produced in the motor neuron of the distal and proximal muscles of both forelimb and the hind limb [6]. In most of the shoulder conditions that present with pain and functional limitations, there is always an associated impairment in muscles, skeleton, ligaments, cartilage, and nervous system, which results in musculoskeletal dysfunction. Despite a strong physiological and anatomical relationship between the proximal and distal musculatures being established scientifically, there exists no consideration for Hand Function assessment when assessing and treating painful shoulder. This study was conducted to analyze the HF (Hand Function) and hand-eye coordination (HEC) among subjects who underwent modified radical mastectomy for breast cancer.

METHODOLOGY

A total number of 12 patients were recruited for the study from 2 different specialty hospitals in Amreli namely Radhika General Hospital and Sri Shyam surgical Hospital. All the subjects who participated in the study were clearly explained about the study before participation and were requested to sign an informed consent. The subjects were recruited into the study if they fulfill the following criteria.

Inclusion criteria
o   Diagnosis of stage I – III cancer and operated with Modified Radical Mastectomy within last 12 month
o   Female age between 25 – 75 years

Exclusion Criteria
o   Subjects with prior h/o shoulder or neck surgery
o   Any neuromuscular conditions that can affect the functions of shoulder & neck functions
o   Patients having mental illness or having any legal issues
o   Patients who don't understand the Guajarati/Hindi/English language
o   Patients having cognitive defects that may interfere with the variables
o   The subjects who had metastasis and other complication that may dampen the improvement due to intervention.
o   Subjects with any other systemic conditions which were not controlled that may influence the results as a confounding variable as perceived by the primary researcher.

All the subjects were assessed for a shoulder disability, hand function, and hand-eye coordination skills. SPADI scale was used to evaluate the subject's severity of pain and the associated disability among the samples.[7] Apart from this shoulder evaluation, the Purdue pegboard task was administered to find the hand function/dexterity. The primary researcher performed the analysis of hand-eye coordination and hand function test with another physiotherapist (One assessor was responsible for measuring the time using a standard stopwatch, and the other was used to monitor the task for both HF and HEC tests.) where as a blinded assessor was used to assess the shoulder disability. There were three tasks performed by every subject three times with a gap of 60 seconds between each attempt.[8] The first subtask required the subject to place as many pegs as possible in 30 seconds in the target holes provided in the pegboard. This task was performed with both hands consecutively. For the first attempt hand was selected randomly. Task number two required the subjects to place as many pegs as possible using both hands, by placing pegs simultaneously. The third task required the subject to place the peg, then the washer and followed by the collar in a sequence prescribed by the guidelines. A battery of hand-eye coordination tests was administered to the patient

as follows. Drill 1 – Balloon tossing task -The subject had to bounce a balloon vertically up, back and forth with the affected hand, as long as possible, followed by the normal hand. The task had to be continued as long as possible and the subjects were not allowed to catch the balloon. The task was repeated three times on each hand. The best score for each hand was added and then used for statistical analysis. Drill 2 - Wall Ball Bounce task - The subjects stood 2M away from a wall and tossed a tennis ball onto the wall in a self-passed manner. The subject had to catch the ball on return with a single hand at first attempt without fumbling. The number of times the subject successfully performed the task in 60 seconds was measured. The test was performed with both hands. The task was performed only once and the score thus obtained for each hand was added and was used for statistical analysis.

Statistics

A correlation was performed individually between the shoulder disability scores and hand function and shoulder disability scores and hand eye coordination using a non-parametric analysis. A correlation was also drawn between the shoulder disability scores and duration following surgery, age of the subject.

RESULTS

The primary researcher was responsible for organizing the data. The demographic details of the subjects are provided in table 1. All of the participants were in the age group of 41-55 years with an average age of 47 years (2.1). 42% of the subjects had non dominant side being affected and 58% of the subjects were dominant hand affected. Most of the subjects were operated recently with an average duration of 4.3 (1.2) months. The correlation analysis of the age, side of affected and disability showed that there was no correlation with a p value of 0.076 and 0.761 respectively. Duration from surgery had a significant influence on the disability with a p value of 0.22 and R value of 0.651. In the analysis of the outcome measures pain scores and disability scores of SPADY scale were separately analyzed with the hand function and HEC scores for better understanding. Pain was having a negative significant correlation with hand function, HEC test 1 and HEC 2 with a p value of 0.002 (R value of -0.793), 0.006 (R value of -0.739) and 0.045 (R value of – 0.587) respectively. Disability was having a negative significant correlation with hand function and HEC test 1 with a p value of 0.004 (R value of -0.756), 0.007 (R value of -0.733) and 0.045 (R value of – 0.587) respectively. There was no correlation between HEC 2 test and disability with a p value of 0.076 and an R value of -0.530.

CONCLUSION

From the results of the study it is clearly proved that Shoulder pain and disability associated with radical mastectomy can influence the hand function and hand eye coordination inversely. It is also obvious from the study that duration from surgery also influenced the disability among the subjects.

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Table 1.Demographic data of the participants

Criteria		Numbers
Age	25 – 40 Years	3
	41 – 55 Years	6
	56 – 75 Years	3
Involved Side	Dominant Hand	7
	Non Dominant Hand	5
Duration of Ailment	1 – 3 Months	6
	4 – 6 Months	4
	7 – 12 Months	2

Correlations			Age	SPADY –Disability
Spearman's rho	Age	Correlation Coefficient	1.000	-.531
		Sig. (2-tailed)	.	.076
		N	12	12
	SPADY - Disability	Correlation Coefficient	-.531	1.000
		Sig. (2-tailed)	.076	.
		N	12	12

Correlations			SPADY - Disability	Side
Spearman's rho	SPADY - Disability	Correlation Coefficient	1.000	.098
		Sig. (2-tailed)	.	.761
		N	12	12
	Side	Correlation Coefficient	.098	1.000
		Sig. (2-tailed)	.761	.
		N	12	12

Correlations			SPADY - Disability	Duration
Spearman's rho	SPADY - Disability	Correlation Coefficient	1.000	-.651[*]
		Sig. (2-tailed)	.	.022
		N	12	12
	Duration	Correlation Coefficient	-.651[*]	1.000
		Sig. (2-tailed)	.022	.
		N	12	12
*. Correlation is significant at the 0.05 level (2-tailed).				

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Correlations			SPADY - Pain	Perdue pegboard
Spearman's rho	SPADY - Pain	Correlation Coefficient	1.000	-.793**
		Sig. (2-tailed)	.	.002
		N	12	12
	Perdue pegboard	Correlation Coefficient	-.793**	1.000
		Sig. (2-tailed)	.002	.
		N	12	12
**. Correlation is significant at the 0.01 level (2-tailed).				

Correlations			SPADY - Pain	HEC-1
Spearman's rho	SPADY - Pain	Correlation Coefficient	1.000	-.739**
		Sig. (2-tailed)	.	.006
		N	12	12
	HEC-1	Correlation Coefficient	-.739**	1.000
		Sig. (2-tailed)	.006	.
		N	12	12
**. Correlation is significant at the 0.01 level (2-tailed).				

Correlations			SPADY - Pain	HEC-2
Spearman's rho	SPADY - Pain	Correlation Coefficient	1.000	-.587*
		Sig. (2-tailed)	.	.045
		N	12	12
	HEC-2	Correlation Coefficient	-.587*	1.000
		Sig. (2-tailed)	.045	.
		N	12	12
*. Correlation is significant at the 0.05 level (2-tailed).				

Correlations			SPADY - Disability	Perdue pegboard
Spearman's rho	SPADY - Disability	Correlation Coefficient	1.000	-.756**
		Sig. (2-tailed)	.	.004
		N	12	12
	Perdue pegboard	Correlation Coefficient	-.756**	1.000
		Sig. (2-tailed)	.004	.
		N	12	12
**. Correlation is significant at the 0.01 level (2-tailed).				

Correlations			SPADY - Disability	HEC-1
Spearman's rho	SPADY – Disability	Correlation Coefficient	1.000	-.733**
		Sig. (2-tailed)	.	.007
		N	12	12
	HEC-1	Correlation Coefficient	-.733**	1.000

		Sig. (2-tailed)	.007	.
		N	12	12
. Correlation is significant at the 0.01 level (2-tailed).				

Correlations				
			SPADY - Disability	HEC-2
Spearman's rho	SPADY – Disability	Correlation Coefficient	1.000	-.530
		Sig. (2-tailed)	.	.076
		N	12	12
	HEC-2	Correlation Coefficient	-.530	1.000
		Sig. (2-tailed)	.076	.
		N	12	12

				SPADY		PERDUE	Hand Eye Co-ordination	
Sr No.	Age	Side Involved	Duration	Pain	Disability	PEG Board	Task 1	Task 2
1	1	2	1	31	42	62	14	34
2	3	1	2	18	28	78	18	37
3	3	2	1	20	27	79	19	39
4	1	1	1	32	44	60	14	33
5	2	1	2	19	21	80	17	36
6	2	2	1	29	48	60	12	33
7	2	1	1	30	38	69	14	34
8	2	2	3	21	25	77	18	38
9	3	1	3	21	27	75	18	35
10	1	2	1	27	32	71	15	36
11	2	1	2	22	28	58	13	30
12	2	1	2	28	40	72	16	38

Age	25-40	1
	41-55	2
	56-75	3
Duration	1-3 months	1
	4-6 months	2
	7-12 months	3
Side involved	Dominant	1
	Non dominant	2

REVIEW ARTICLE

Protagonistic Role of Gut Microbiota Nexus in Covid-19 Disease Severity and Management Strategies: A Concise Review

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ABSTRACT

The world has been imprecisely hit by the SARS-CoV-2 pandemic which increased the high mortality rate in double fold. A high range of beneficial microbial species harbors a gut micro biome harbors its strong effect in maintaining host immunity. During Covid infection the dysbiosis of such beneficial microbial species from the body drastically impairs the health by affecting the pulmonary or gastrointestinal system. However, the imbalance in microbial flora causes a change in the host immune system when opportunistic pathogens invade the host and subsequently colonize. Intake of the probiotics, despite other co-morbidities, maintains the body's internal physiology. Though the knowledge of gut microbiota relationship is limited with Covid-19. This review highlights the abundance or scarcity of beneficial microbial gut bacteria to Covid-19 severity and its management. Our findings indicated a significant alteration of gut microbiota composition in COVID-19 patients compared with healthy persons. This considerable extent of influence has proposed the gut microbiota as a potential diagnostic, prognostic, and therapeutic method for COVID-19.There is significant concrete evidence about this issue, and it is predicted to be increased in near future.

Keywords: SARS-CoV-2, Covid-19, Dysbiosis, Probiotics, ACE2 receptors.

INTRODUCTION

The viral diseases of the respiratory tract of human beings are one of the major cause of mortality in elderly and negotiated people worldwide. It has been assessed that a number of respiratory infections have occurred in the 21st century. Among the respiratory infections, Covid-19 has been one of the dreadful disease which causes infection in the upper respiratory tract but also influence the lower tract causing asthma, pneumonia, SARS or MERS(Singh et al., 2020). Since its inception in Nov 2019, the disease has affected nearly 188 countries around the globe(Singh et al., 2020).According to WHO, the world has with stands 383,509,779confirmed covid 19 cases as of 03 Feb, 2022 with a total of5,693,824 deaths worldwide. In India, the total cases confirmed withstand 41,803,318 as of 03Feb, 2022 with498,983 deaths(WHO, 2022).

SARS-CoV-2 is a β Corona virus that is solely responsible for the global pandemic due to novel Corona virus. This primarily infects the respiratory system ranging from causing symptoms such as fever, cough, pneumonia, and fatigue to hypoxia owing to acute respiratory syndrome(Kim, 2021).There have been cases of infected patients being asymptomatic or having mild symptoms while others develop severe respiratory syndrome(Donati Zeppa et al., 2020).The rate of mortality increases due to severe acute respiratory distress syndrome and systemic inflammatory response syndrome affecting the pulmonary area, leading to multi organ failure requiring a treatment in ICU in which complications may be fatal(Chattopadhyay & Shankar, 2021a).

A critical condition in Covid-19 disease leads to hypoxemia in which oxygen supplementation relatively declines and the patient has to be provided with mechanical ventilation and intensive hospitalization requiring a long time in ICU with intense stress for the healthcare professionals to counteract Covid. The mortality rate extends on the verge on being ventilated mechanically. Furthermore, several patients' health declines and multiple organ failure leads to prolonged hospitalization(Quahet al., 2020). During covid infection, the normal process of the body is disturbed in which nutrients absorption is inhibited in the gastrointestinal tract, causing gastroenteritis in infected individuals(Zhou et al., 2020). Certain diseases such as diarrhea, vomiting, and nausea also occur, which are thought to have created infection in the gastrointestinal tract site in different patients studied(Kim, 2021).

Due to the several changes in the host organism, such as hormonal changes, immunity disorder, variation in their genes, occurrence of disease, ageing, environmental factors, and nutritional folds, there has been a constant evolution of the microbiota. A healthy microbiota imparts sound physiological sustenance of the body. Sometimes the changes in the microbiota makeup can cause a life-threatening disorder or illness such as bowel inflammatory disease, causing problems in bacterial infection treatment(Ogunrinola et al., 2020). Due to the importance of gut microbiota in the maturation, modulation, and activation of the immune system and subsequent inflammatory processes, assessing the composition of gut microbiota in COVID-19 patients relative to healthy individuals may have diagnostic and/or prognostic significance. Additionally, therapeutic approaches influencing the microbial flora of the gut may expand the treatment options for COVID-19 patients and hasten their recovery.

Role of Gut microbiota nexus in human

The gastrointestinal tract of human possessing an enormous microbiota possesses the most dynamic components of the immune system. The gut, along with many commensal bacteria has an ample number of innate and adaptive immune cells, which plays an essential role in acquiring homeostasis in the body. The homeostasis is brought about by intestinal epithelial cells in colon isolating immune cells of the mucus membrane and the microbes. Several other cells and gut lymphoid tissue are associated in framing barrier against the invading pathogenic microbes (Vemuri et al., 2018).

The human microbiota harbors more than 100 trillion microbes in the human body particularly bacteria in the gastrointestinal gut (Thorn et al., 1978). It is hypothesized that the human gastrointestinal microbiota is regarded as an integral organ of the body (Wang et al., 2017). These microorganisms comprise a total of 150 times more genes

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than the total number of entire human genomes. The genomes of this microbiota represent the micro biome residing in a specific environment (Altveş et al., 2020). The microbiota has impacts on the physiology either in health or disease by enhancing or impairing the metabolic or immune functions of the body. Immune response develops due to the evolution of native microbiota and the immune system and in turn enhances the ability of the immune system to distinguish between the harmful and commensal microbes. The composition of gut microbiota stimulates the development of adaptive immunity in mammalian immune system. The changes of microbiota influenced by caesarean birth; diets are linked with the allergic rhinitis as a consequence of atypical immune function in children (Ogunrinola et al., 2020). Gut microbiota emphasizes the defense mechanism against harmful pathogen by conferring colonization in the epithelial cells competing for nutrition, producing metabolites as acetic acid and lactic acid in lowering the pH of the intestinal environment inhibiting pathogen growth and producing antimicrobial peptides such as bacteriocins, defensins offering resistance to the harmful pathogen invasion (Fong et al., 2020).

This disease is believed to be caused by disruptions in the gut micro biome. Difficulties resulting in gut dysbiosis can be understood as a combination of losing beneficial microbes, increasing potentially harmful microbes, and reducing bacterial diversity. The breakdown of epithelium and inflammation have been demonstrated to increase levels of angiotensin-converting enzyme 2 (ACE2). ACE2 is the target of SARS-CoV-2.In addition to increased gut permeability, pro-inflammatory bacterial products can leak out of the gut and circulate throughout the body, triggering immune responses. The gut micro biome is also known to be reduced in elderly and immune-compromised populations. Since many of these vulnerable patients had poorer clinical outcomes for COVID-19, this possibly supports the hypothesis that a compromised gut micro biome might negatively affect a patient's clinical outcome.

Host cell ACE2 receptor binding in Covid-19

Covid-19 infects the respiratory system which involves the entry of viral pathogen into the host in its respiratory tract owing to the binding of spike glycoprotein as ligand and cells expressing angiotensin-converting enzyme-2 as the usual receptor which is present in the respiratory and the gastrointestinal surface epithelial cells. When virus gets attached to the ACE2 receptor, the transmembrane serine protease type 2 which are present in the host cell cleave ACE2 and activates the spike protein on viral uptake, which in turn the virus enters inside the host cells, cleaves off the viral coating leading to release the viral RNA and reproduces in large numbers leading to host cell destruction and death(Kim, 2021).

The renin-angiotensin system encoding the regulatory enzyme ACE2 results in increased vulnerability causing intestinal inflammation due to damaged epithelial cells. The susceptibility in causing lung, cardiovascular and covid infections has its effect due to renin-angiotensin system. The ACE2 in gut cells is vital in controlling amino acid homeostasis, maintaining innate immunity, microbial gastrointestinal ecology and its absence may induce colitis. The changes in immunity of epithelial cells and gastrointestinal tract microbiota which are dependent on ACE2 can be controlled by inducing dietary amino acid tryptophan. Therefore inactivation of ACE2 can result in severe intestinal inflammation (Hashimoto et al., 2012). In Covid-19infection, the presence of gut microbiota modulates ACE2 receptor and regulates pro-inflammatory and immune signals (Segal et al., 2020).Covid-19 patients significantly decrease the number of CD4+ and CD8+ T cells. The gut microbes of patients suffering from Covid-19 secrete short-chain fatty acids such as butyrate and propionate, which control CD8+ T cell expression.
Gut Microbiota in Covid-19

The living microbes which enhances the microbial balance in the individuals host immune system are the probiotics such as Lactobacillus, Bifidobacterium, Bacteroides, and others (Khaled, 2021).If old aged individuals are supplied with dietary probiotics, *Bifidobacterium lactis* increases the function of tumoricidal natural killer cells. The probiotics such as species of phylum Firmicutes and Actinobacteria alleviate inflammation by regulating innate immunity responses, while some species are efficient in regulating the inflammatory response through Treg cells elevation. The intake of protein rich foods increases the gut microbes and helps reduces pathogenic microbiota. The probiotic species of *Bifidobacterium*or *Lactobacillus* helps to get rid of pathogens from the respiratory tract along with enhancing

the activity of APCs, T cells, and NK cells to release the antibodies in mucosa present in lung fluids. *Lactobacillus casei* phagocytose the macrophages present in the alveoli of the lungs and the higher expression of IgA, TNF-α, IFN helps in protection against infection by flu virus.

The potency of *Lactobacillus rhamnosus* and *L. paracasei*in human host increases vaccine efficacy in patients suffering from respiratory diseases. Individuals suffering from ARDS complications in Covid-19 can be improved with probiotics that regulate anti-inflammatory and pro-inflammatory cytokines. Species of family Ruminococcaceaea, *Faecalibacteriumprausnitzii* produces butyric acid, a short-chain fatty acid in the gastrointestinal tract which sustains integrity of the gastrointestinal barrier and possess anti-inflammatory activity by inhibiting NF-kB activity, by activating GPR41 and GPR43 and regulatory T cells. Along with probiotics, some prebiotics like galactosachharides, fructo-oligosaccharides, inulin and polydextrose enhance host immunity through gut microbiota alterations. These prebiotics decrease the cytokine IL-6 which is considered a factor in causing Covid-19 and increase the anti-inflammatory cytokine IL-10. Dietary intake of probiotics and prebiotics can prevent inflammation and severe conditions in Covid-19 patients again (Chattopadhyay & Shankar, 2021a).

Gut microbiota in older people infected with Covid-19 disease.
Across the globe, age is a factor for the susceptibility and death of patients due to Covid-19. Elderly individuals have clinical disorders or diseases such as diabetes mellitus, hypertension, obesity, or any other clinical severe conditions. People with such growing health concerns are on the verge of susceptibility to SARS-CoV-2 infection. Patients undergoing medications with drugs in treating hypertension, type 2 diabetes mellitus regulates the expression of the receptor ACE2, which facilitates SARS-CoV-2 infection(Wu & McGoogan, 2020).It is speculated that covid infection contributes to gut dysbiosis, causing inflammation and other clinical severe complications in patients.

Thus, the patients lack the higher quantity of bifidobacteria which are beneficial to the older individuals and as a result which enhances the increase of inflammatory bacteria in the body leading the people to be more vulnerable to covid infection. The increase in the mortality rate among the older patients with Covid-19 infection thus arises due to the variations occurred in the gut microbiota. The wide diversity of the microbes in the GI tract is relatively lower than that of the younger adults(Segal et al., 2020).A succession of changes in an infant's gut microbiota occurs due to the transition from breast-to-formula to weaning, together with the introduction of solid foods. Microbial succession in the GIT is also affected by a wide variety of external and internal hosts-related factors, despite the relative similarity of the gut microbiota in mothers and children.

In addition to the composition of the maternal microbiota, external factors include the type of food consumed and the amount of microbial load in the immediate environment. Dietary and temperature factors can also influence the succession of microbes(Mackie et al., 1999).The dysbiosis in the gut microbiota makes the gut barrier permeable to pass the pathogens and bind with the cells of the body. Due to the disruption of tight junctions between the cells of the gastrointestinal cells in Covid-19 patients develop leaky gut in which the patient succumbs to develop diarrhea, inflammation due to the excess secretions of IL-6 in plasma and fecal matter. This leak in the gut allows the SARS-CoV-2 disease to enter the blood stream and binds with the ACE2 receptors in the other parts of the body (Chattopadhyay & Shankar, 2021b).It is postulated that due to the disorder in gut microbiota in older people, the innate immune response is elicited, which develops chronic inflammation leading to unhealthy ageing(Segal et al., 2020).

Gut Microbiota in children
The rareness of morbidity and mortality in Covid-19 among infants is less than that of adults (Romano-Keeler et al., 2021). Gut microbiota in Covid-19 infection emphasizes a crucial role by identifying the risk of Kawasaki disease and multisystem inflammation syndrome in children with the use of micro biome-based profile(Yeoh et al., 2021). During Covid-19 infection in pregnancy, the rise in C-section for delivery has also been observed, affecting the fetus and the newborn ones due to transmission. The risk in C-section delivery affects the newborn ones in their initial life due to

the intestinal dysbiosis of microbiota in which colonization with a mixture of pathogenic microbes occurs in the skin compared to colonization of *Lactobacillus* by vaginal delivery. The increase in C-section significantly influences the micro biome of the newborn ones as C-section born infants have a lower diversity of microbiota compared with vaginally born infants, especially on Bacteroides colonization(Romano-Keeler et al., 2021). The inheritance of maternal microbiota in fetus helps in development of micro biome which occurs by placental exchange. Recent research has also found that microbiota is present in human placenta and has shown that it can be the route for maternal oral bacteria to be given to fetus like microbiota in the oral cavity(Aagaard et al., 2014). The microbial interaction in prenatal life is thus seen, but previous studies have not used techniques to quantify the number of bacterial cells and to differentiate accurately between DNA, dead cells, living cells and active cells that make it challenging to identify placental microbiota or pregnancy risk factors(Aagaard et al., 2014).It requires further research to validate the presence, amount, and effect of intrauterine microbiota on children's health. But the Covid-19 infected mother has the high rate of increase in swollen endothelial tissue of the endometrium and the impairment of functional capacity of the placenta due to altered blood flow to the intervillous space which may restrict the exchange in bacterial flora, which impacts the composition of microbial flora in newborn child (Jasmine & L., 2021).

Children are more susceptible to pediatric cases during Covid-19 but are usually not as severe as adults. The presence of antibodies in protecting against disease in childhood, lower rate of co-morbidities and several immunological responses are responsible for the less Covid-19 disease in children. In young children, the immune system is accustomed to facing new and novel challenges, however, in older individuals' memory plays a greater role. The thymus decreases its output of naive T cells and involutes at a rate of about 3% per year; cross-reactive antibodies to common-cold corona viruses are one possible explanation; another possibility is that constitutive differences in immune system states between young and old people are of importance. One example could be the skewing of T cells from T helper type 1 (TH1) toward more TH2 in young children (Saso & Kampmann, 2017). However, an increase in severe pediatric cases was reported demanding critical care but mortality rates remaining less than in adults(Marzano et al., 2021).Pediatric studies reveal the consequences of Covid-19 disease, which affects the health and development in infants and children, causing myopia, growth in obesity, atopic disorder, and mental health disorder .The initial steps in the prevention of Covid-19 disease transmission to the newborn child is to prevent skin-to-skin contact by separating and admitting the infant from Covid-19 infected mother into the Neonatal intensive care unit which interrupts the transfer of microbial flora into the oral and skin compromising gut and immune health. Other environmental factors such as homely exposure encountered in infant's use the surge of using solid surface disinfectants and anti-microbial hand sanitizers which reduces the development of microbial flora in the body(Romano-Keeler et al., 2021).

Restoration of Gut microbiota

The concept laid by Hippocrates in 431 BC which states "Let food be thy medicine" is the only way to prevent and maintain the homeostasis of the body when there is obscure way of therapeutic mechanism in an adverse condition (Rishi et al., 2020). As the gut microbiota of Covid-19 infected patients are altered, the restoration of gut microbiota can be modulated by the consumption of probiotics(Yu, 2021). Dysbiosis of microbiota in Covid-19 pandemic plays a significant role in health complications these are the sources of nutrition for maintaining the immune system in severe illness(Yu, 2021). The dysbiosis of microbiota post Covid-19 infection towards antagonistic health conditions can be reverted back by administering probiotic strains in maintaining gut microbiota homeostasis. The consumption of probiotics, prebiotics or rich dietary foods has the potential to stimulate the microbial flora to revive and restore the immune system, repair the damaged intestinal mucosa after infection, restore and maintain eubiosis of gut microbiota, gut intestinal enhancement, restriction of pathogenic microbes to adhere with epithelial tissue, dominance of opportunistic pathogens, antimicrobial stuff production, enhancement of mucosal surface immunity in the body which in turn drives the favorable health condition of the patients(Angurana & Bansal, 2020).

Consumption of prebiotics metabolizes preferentially by probiotic bacteria for growth (Gagliardi et al., 2018). Administration of probiotics and prebiotics, commonly termed synbiotics, confer complete health benefits. Consumption of multiple probiotic strains act synergistically and provide more efficacy than single-strain probiotics.

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The consumption of proper healthy diet prescribed by a nutritionist is a matter of enlightenment to the community of infected individuals. The enrichment of beneficial probiotics and eradicating the pathogenic microbe by consuming antipathogens as substitutes to antibiotics restores the gut microbiota (Rishi et al., 2020). Consumption of cheese increases *Bifidobacteria* which protects against pathogens and increases short-chain fatty acids (SCFA) production. The intake of fiber-rich diet increases microbiota diversity and SCFA production, which helps maintain type 2 diabetes and cardiovascular disease. Consumptions of polyphenols such as those found in tea, coffee, berries and vegetables such as olives, artichokes and asparagus increase intestinal barrier microbes such as *Bifidobacteria* and *Lactobacillus*, butyrate-producing microbes as *Faecalibacterium prausnitzii* and *Bacteroides vulgates* and *Akkermansia muciniphila*. These polyphenols result in decrease of metabolic syndrome and cardiovascular risk(Valdes et al., 2018).The diet's intake of iodine, Vitamin B, omega 3 fatty acids also modulates the gut microbiota. The dietary consumption which includes micronutrients such as zinc, folate, vitamin A and D influences the composition of gut microbiota This is good part but need more inputs and details (Gagliardi et al., 2018). Further research on probiotics and diet rich in favoring gut microbiota are the approaches to shed light on enhancing the rich diversity of essential microbes which drives health benefit (S. & A., 2021).

Future prospective
The current pandemic situation implies that immunity serves as a barrier between host and pathogen. Immunity is a continuous development process in the body that counter attacks the invading pathogen. The healthy state of the host body can be improvised by maintaining a strong immune system which requires adequate exposure of beneficial microbiota. The gut microbiota in human has a major role in maintaining the homeostasis of the body. The implications of Covid-19 infection in a variety of human organs with microbiota are still under investigation. The intake of dietary food products containing probiotics and prebiotics will keep the body rid of severe diseases and maintain the body's homeostasis. More study and research are the need to find the complications involved to understand the effects of Covid-19 and gut microbiota. Certainly, more research on the micro biome will undoubtedly boost the development of more preventive and therapeutic mechanisms.

CONCLUSION

Our research showed substantial alteration of gut microbiota composition in patients with COVID-19 compared to healthy persons. This considerable extent of impact has proposed the gut microbiota as a potential diagnostic, prognostic, and prospective therapeutic method for COVID-19.

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Table 1. List of Gut microbes associated in Covid-19 patients

Phylum	Species	Mode of action	REF
Bacteroidetes	*Alistipesonderdonkii*	Maintaining gut immune homeostasis by tryptophan-serotonin metabolism	(S. & A., 2021)
	Bacteroides dorei	Suppresses the expression of colonic ACE2	(Zuo et al., 2020)
	Bacteroides fragilis	Induces IL-10 production	(S. & A., 2021)
	Bacteroides stercoris	Produces short-chain fatty acid, metabolites are important in intestinal homeostasis	(Jyoti Chhibber-Goel, 2021)
	Bacteroides fragilis	Induces IL-10, Th1 cells production	(Rajput et al., 2021)
	Bifidobacterium lactis	Production of NK cells, leukocytes, CD4+ and CD25+ T cells, down-regulate allergic response	(S. & A., 2021)
	Bifidobacterium bifidum	Produces IFN-α, maintains immunogenic homeostasis	(Olaimat et al., 2020)
	Bifidobacterium breve	Down-regulation of inflammation through elevation of Treg cells, down-regulate allergic response	(S. & A., 2021)
Actinobacteria	*Bifidobacterium longum*	Alleviates inflammatory response by regulating innate immune	(Chattopadhyay & Shankar, 2021b)

		responses	
	Bifidobacterium adolescentis	Prevents activation of proinflammatory cytokine by inhibiting activity of NF-kB	(Ramos-Martínez et al., 2020)
	Bifidobacterium animalis sub sp. *lactis*	Suppress TNF-α and NF-b which causes inflammatory diseases	(Bozkurt & Quigley, 2020)
	Rothiamucilaginosa	Causes inflammation in lung	(Donati Zeppa et al., 2020)
	Collinsellaaerofaciens	Increasing the expression of proinflammatory cytokine IL-7 and Chemokines such as CXCL1 and CXCL5 reduces gut epithelial integrity	(Jyoti Chhibber-Goel, 2021)
	Enterococcus faecalis	Damage valves in adults	(Ramos-Martínez et al., 2020)
	Escherichia coli	Prevents action of myeloperoxidase of in gut inflammation	(Chattopadhyay & Shankar, 2021b)
	Klebsiella pneumonia	Enhances inflammation of epithelial cells by activating TLR4 and TLR2 and prevents host proteins action involved in immune homeostasis	(Chattopadhyay & Shankar, 2021b)
Enterococcus	*Lactobacillus acidophilus*	Produces IFN-α by monocytes	(Olaimat et al., 2020)
Proteobacteria	*Lactobacillus johnsonii*	Alleviates inflammatory response by regulating innate immune responses	(Chattopadhyay & Shankar, 2021b)
Firmicutes	*Lactobacillus plantarum*	Induces IL-10 production	(S. & A., 2021)
	Lactobacillus reuteri	Produces IFN- gamma, activates pro-inflammatory Th1 cells	(Olaimat et al., 2020)
	Lactobacillus paracasei	Increase TNF-α, IL-6, IL-8	(Olaimat et al., 2020)
	Lactobacillus rhamnosus	Down-regulation of inflammation through elevation of Treg cells	(Olaimat et al., 2020)
	Lachnospiraceae bacterium	Produces short-chain fatty acid, these metabolites are important in intestinal homeostasis	(Jyoti Chhibber-Goel, 2021)
	Roseburia intestinalis	Induced IL-10 production, anti-inflammatory cytokine IL-22	(Olaimat et al., 2020)
	Ruminococcus gnavus	Produces proinflammatory cytokines(IFN-γ, TNF-α) and activation of T helper cell response	(Jyoti Chhibber-Goel, 2021)
	Clostridium hathewayi	Up-regulate the ACE-2 colonic expression	(Zuo et al., 2020)
	Faecalibacteriumprausnitzii	Produces butyrate which possess anti-inflammatory properties	(Zuo et al., 2020)
	Akkermansia muciniphila	Induces IL-10 production	(S. & A., 2021)
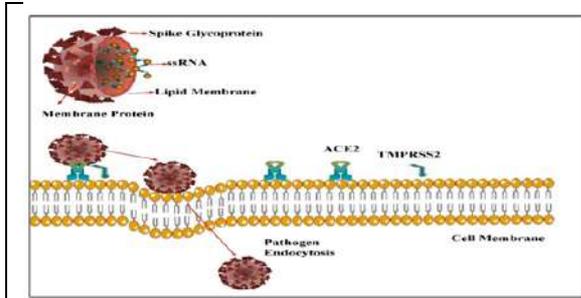
Fig 1: Covid-19 binds to ACE2 receptors in the epithelial cell membrane, Viral multiplication damages the epithelial tissues which in turn causes infection in the pulmonary system.
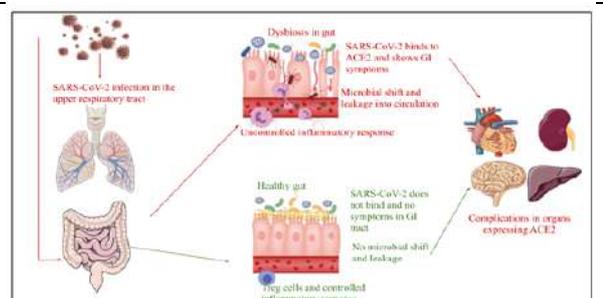
Fig 2: Infection due to Covid-19 binding with ACE2 due to microbial dysbiosis and the presence of healthy microbes showing its implication in the internal organs. Source: Drawn in Google drawing and some elements adopted from (Chattopadhyay & Shankar, 2021a).

RESEARCH ARTICLE

Impact of Antioxidants on *In vitro* Cultures of *Elaeocarpus ganitrus* Leaf Explants

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ABSTRACT

The present investigation deals with the effects of ascorbic acid, polyvinylpyrrolidone (PVP) and citric acid on the explants of *Elaeocarpus ganitrus*. The immature leaves were collected as explants and cultured on 1/2 and 3/4 strength MS and Anderson media. The antioxidants were taken in a range and several concentrations of ascorbic acid, PVP and citric acid were analysed. Out of various concentrations, some were observed to be helpful in minimizing browning of leaf explants, specifically when the antioxidants were taken in combination.

Keywords: Leaf tissue, ascorbic acid, citric acid and PVP

INTRODUCTION

Elaeocarpus is the largest genus of Elaeocarpaceae worldwide except from Africa and America. Most of the species are having economic importance due to several uses such as pharmaceutical, pharmacological even on religious ground [1]. It was observed that the fruit extract of *E. ganitrus* contains huge amount phenolic compounds [2]. From the available reports it was analyzed that several extracts from different parts of Rudraksh tree having vast therapeutic potential [3]. Study shown that seeds of *E. ganitrus* are rich source of tannins, carbohydrates, alkaloids, phytosterols,

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flavanoids and proteins [4]. On three clones of *Euclyptus urophyla* × *Euclyptus grandis* the effects of ascorbic acid and polyvinylpyrrolidone was evaluated [5]. The activated charcoal, polyvinylpyrrolidone and ascorbic acid were used in the studies related to micro propagation from the whole seedlings of *Sterculia urens* Roxb. well known as Indian Tragacanth important for its gum [6]. For the *in vitro* propagation of *Rhododendron maddeni*, PVP, ascorbic acid and citric acid were used in the medium [7]. From the shoot tips of *R.maddeni* Hook f. which were alginate encapsulated *in vitro* plant were regenerated [8]. In a study of micro propagation of *Strelitzia reginae* notable effect of plant growth regulators, antioxidants and wounding was observed on overall phenolic compound exudation. The antioxidants which were used during the investigation were activated charcoal and ascorbic acids. In various media both the antioxidants were separately mixed [15]. During *in vitro* propagation of *Persea Americana* Mill., the less browning and higher rate of survival were observed with 100mgL^{-1} ascorbic acid separately and in the mixture with 60mgL^{-1} ascorbic acid and 40 mgL^{-1} citric acid [16].

MATERIALS AND METHODS

The plant materials collected from 3-4 years old tree located inside SIET campus, Meerut (UP) India (Fig.1) which were further brought to the laboratory and washed gently (Fig.2). The explants were treated for 35min. with 1% bavistin (*w/v*) [14]. Within the laminar airflow the surface sterilization of explants were performed by using 70% ethanol for time period of 30 seconds and 0.1% (*w/v*) HgCl$_2$ for 4 min [14]. The explants were rinsed for 6 times each for 4 min. with sterile distilled water within the laminar hood [14]. The explants were cultured and maintained at 25±2°C under 16 h/8h environment under 60-70% humidity inside the culture room. The ½ and ¾ strength of MS and Anderson media were used [9], [10]. The ascorbic acid (AA) (20-160 mgL^{-1}), citric acid (CA) (5-20 mgL^{-1}) polyvinylpyrrolidone (PVP) (20-160mgL^{-1}) were used. For each medium pH was adjusted to 5.8±0.5 and 0.8% of agar was mixed in the respected medium. All the media were autoclaved for 20 min. at 121° C (at 15 psi). One way ANOVA was used for analysis of variance, and [*] mark used for denoting significant difference $P≤ 0.05$.

RESULTS AND DISCUSSIONS

The antioxidants in combination were found to be better for defending the leaf tissue from browning as compared to the media in which antioxidants were taken individually. Among the combined formulations of antioxidants, 140mgL^{-1} Ascorbic acid, PVP and 10mgL^{-1}citric acid was observed more responsive in ¾ and ½ MS (Fig.7, 8) & in ¾ and ½ Anderson (Fig.9, 10) medium, but it was also found that the other concentrations of antioxidants in combined form are capable of lowering down the browning effects. It was analyzed that without antioxidants leaf tissues in ¾ strength MS and Anderson medium turned brownish black (Fig.3). On the other hand in ¾ strength MS and Anderson medium, the combination of antioxidants better suited for the survival of the tissue from browning (Fig.4). Similarly, when ½ strength MS and Anderson medium were considered without antioxidants browning was observed in the *in vitro* cultures (Fig.5), whereas the conditions in which ½ strength MS and Anderson media consisting antioxidants in combined form, has shown the restriction of browning by antioxidants (Fig.6). The ascorbic acid and polyvinylpyrrolidone were used during micro propagation studies [11]. The ascorbic acid, citric acid, polyvinylpyrrolidone for the *in vitro* studies were used [12]. The explants materials were treated with antioxidants [13]. The combination of ascorbic acid150 mgL^{-1}, PVP150 mgL^{-1} and citric acid10mgL^{-1} were used in full strength MS, WPM and Anderson media fortified with growth regulators during the investigations related to callus initiation from the leaf tissue of *E.ganitrus* [14]. The antioxidants in combination of ascorbic acid and PVP in the concentration of 130 mgL^{-1} and citric acid 8 mgL^{-1} were used in the growth medium for nodal segments explants [17]. It was observed that the range of ascorbic acid and PVP from 100mgL^{-1} to 160mgL^{-1} along with citric acid is more responsive as compared to other concentrations. Several types of growth media of different strength containing concentrations of antioxidants and plant growth regulators may show differences in the results. In comparison of presented study with the earlier studies by the researchers, it was concluded that the response of antioxidants concentration can also show variations on the basis of explants type used during the *in vitro* tissue culture

experiments. Infact, it is commonly known that the collection of plant material as explants source from the trees growing in the natural environment within one geographical region to another may show variations in the outcome.

CONCLUSION

The presented study may be helpful for the plant growth media preparation by fortifying it with combination of antioxidants for maintenance of the *in vitro* cultures, containing explants of tree species (*E. ganitrus*) for tissue culture applications. The investigation may provide a way for further in-depth information regarding the use of antioxidants in the growth media for other explants materials of this species of Elaeocarpus and even for other species of the same genus.

Author Contributions

Rishi and Harinder Vishwakarma for experimental design, manuscript preparation, data observations and statistics. Ritesh Arya for manuscript corrections and plagiarism check. Dr. (Prof.) Amar P. Garg for conceptualization and monitoring

ACKNOWLEDGEMENTS

COMPETING INTERESTS
Exists none

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Fig.1 Tree of *E. ganitrus* from which leaves were collected for the investigation

Fig.2 *E. ganitrus* leaves
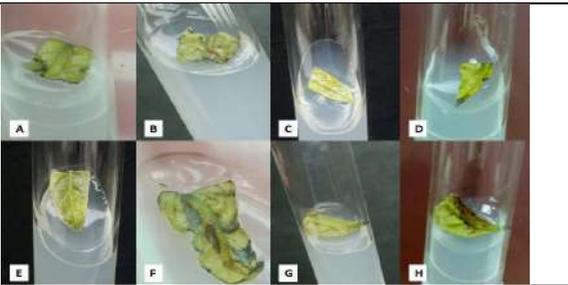
Fig. 3 Different Medium without Antioxidants to analyze the response on the leaf tissue of *E. ganitrus*. [A, B] ¾ strength MS medium [C, D] ¾ strength Anderson medium

Fig. 4 Different medium the antioxidants conc. in combination to analyze the response on the leaf tissue of *E. ganitrus*. [A-D] ¾ strength MS+ Ascorbic acid+ Citric acid+ PVP [E-H] ¾ strength Anderson+ Ascorbic acid + Citric acid + PVP
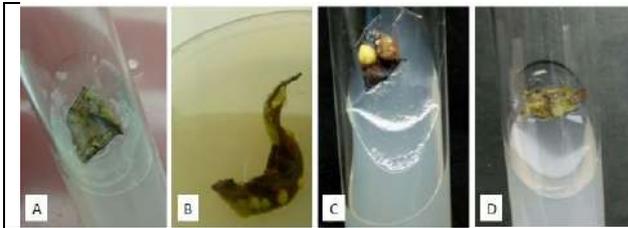
Fig. 5 Different medium without antioxidants to analyze the response on the leaf tissue of *E. ganitrus.* [A-B] ½ strength MS, [C-D] ½ strength Anderson
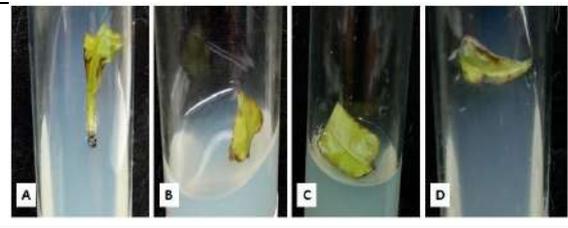
Fig. 6 Different medium and antioxidants conc. in combination to analyze the response on the leaf tissue of *E. ganitrus.* [A-B] ½ strength MS+ Ascorbic acid+ Citric acid+ PVP [C-D] ½ strength Anderson+ Ascorbic acid + Citric acid + PVP
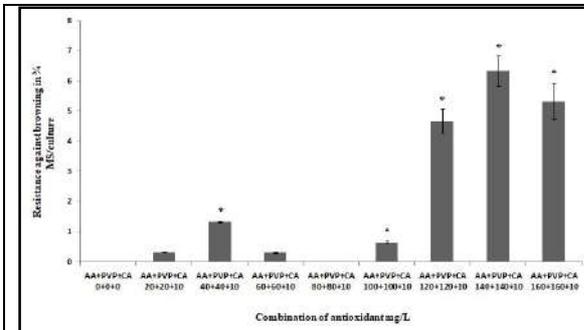
Fig. 7 The graph showing the combination of antioxidants [Ascorbic acid+ PVP+ Citric acid] response on the leaf tissue of *E. ganitrus* in ¾ MS medium.
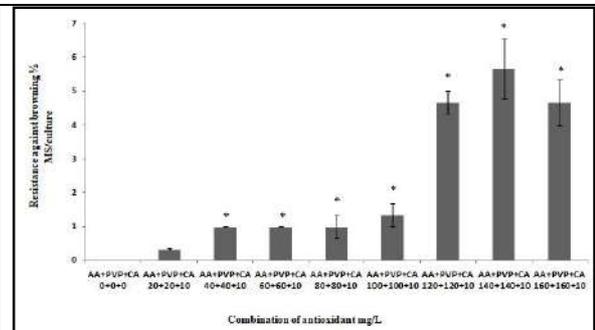
Fig. 8 The graph showing the combination of antioxidants [Ascorbic acid+ PVP+ Citric acid] response on the leaf tissue of *E. ganitrus* in ½ MS medium.
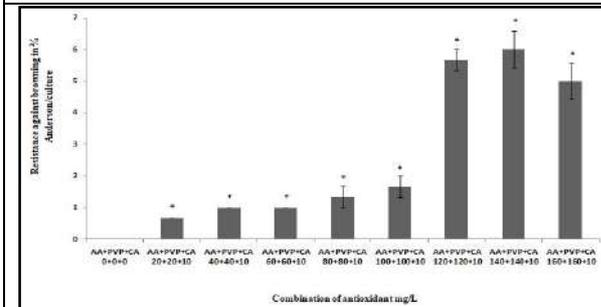
Fig. 9 The graph showing the combination of antioxidants [Ascorbic acid+ PVP+ Citric acid] response on the leaf tissue of *E. ganitrus* in ¾ Anderson medium.
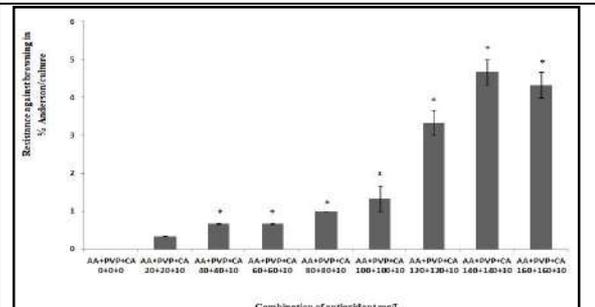
Fig. 10 The graph showing the combination of antioxidants [Ascorbic acid+ PVP+ Citric acid] response on the leaf tissue of *E. ganitrus* in ½ Anderson medium.

RESEARCH ARTICLE

Association of Endemic Orchid *Aerides crispa* Lindl on Invasive Species of *Agave americana* L.

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ABSTRACT

The genus *Aerides* is actually known as the "Child of the Air", it has strong growing monopodial epiphytes similar to the genus *Vanda.* Wild epiphytic tropical orchids usually grow on the surface of moss-curled tree barks, lichen-covered rocks, and well-dried tree branches due to the presence of organic matter, good aeration, and sufficient humidity. *Aerides crispa* is an endemic orchid of the Western Ghats, well adapted and associated with the invasive species of the *Agave americana* population by using the appropriate climatic conditions. This study resulted in the Gundada slopes of Koderi village in the Kundha reserve forest, showing a high population of *Aerides crispa* over the population of *Agave americana* associated with 15 other angiosperm plant species.

Keywords: endemic, invasive, *Aerides crispa, Agave americana,* Nilgiris, Western Ghats

INTRODUCTION

Orchidaceae is the second largest family of angiosperms [1]. The rarity and attractiveness of the orchid they saw as the 'Panda' in the plant kingdom [2]. The Western Ghats is one of the hot spots rich in biodiversity. It has more than 300 species of orchids. *Aerides crispa* from Orchidaceae is called "Curled *Aerides*" [3], [4]. *Aerides crispa* is endemic to

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the Western Ghats of India at altitudes of 800-1200 m [5]. It is one of the endemic epiphytic herbs, sometimes lithophytes', in places like rocks covered with lichen and mosses covered on tree branches in tropical forests. This species can grow through seed and also vegetatively via the stem part. *Agave americana* is called the Century plant, grows in different habitats and soil types, and tolerates high temperatures and extreme drought by the presence of a large, rhizome-like stem and succulent leaves. This species can disperse by bulbils, seeds, particularly vivipary, and rhizomes [6]. *Agave americana* is native to North America. Native populations of this species are noticed in the southern USA, North America, and Mexico [7], [8]. As an ornament, it was distributed all over the world. These species naturalize in countries across Africa, Europe, the Caribbean, Central, and South America, Oceania, and India [7]. It grows throughout coastal hedges, valleys, cliffs, sandy places, and roadsides in Portugal and Spain [9].

The roots of *Aerides crispa* gathered minerals and nutrients from the dry, moistened parts of *Agave americana.* Saprophytic orchids are called holomycotrophic orchids. They gathered minerals and nutrients from the medium through the presence of mycorrhizal fungi in the velamen roots [10]. Mycorrhizae are involved in the transfer of the nutrients to plants through a symbiotic relationship [11]. Invasive species *Agave americana* is used for various purposes, but in many places, the dispersal of this species is one of the causes of habitat loss. This documentation assists in the in-situ conservation of endemic orchids like *Aerides crispa* in the *Agave americana* Population.

METHODOLOGY

The study was conducted in the rocky slope of the *Agave americana* population in Koderi village, Gundada slopes of Coonoor (11.329148°N, 76.71828°E, Altitude 1885 m) during the year 2019 to 2021. To know prominence between *Aerides crispa* and *Agave americana* 5m X 5m quadrate were selected and Density was calculated, 10 Plots were recognized with a minimum spatial distance of 10m.

Relative Density was calculated by using the following formula, [12], [13].

$$\text{Relative density} = \frac{\text{Number of individuals of a species}}{\text{Number of individuals of all species}} \times 100$$

The collected plants were identified by various regional floras [14], [15], [16], [17], [18], [19], [20] and online databases [21], [22].

Endemic status: The current endemic status of *Aerides crispa* was collected from literature such as [3], [5], [17], [18], [21], [22], [23], [24], [25], [26], [27], [28], [29], [30] and online databases [17], [19], [31], [32].

Key characters of *Aerides crispa*
Epiphytic or lithophytes' herbs, Stem 15-50 cm height, .1-2 cm dia, older stem covered with persistent leaf sheaths of petioles. Leaves fleshy 10-20 x 2-3 cm, linear-oblong, unequally 2-lobed at the tip, sheathing at base, keeled along the midrib, deciduous above sheathing petiole. Inflorescence simple sometimes branched racemes. Peduncles, 10-20 cm long. Flowers aromatic with nectar, floral bracts minute, scaly. Flowers pinkish-white, 1.5-1.8 cm across. Sepals and petals 0.7-0.8 x 0.3-0.4 cm. Petals are slightly smaller. Lip 1.5-1.8 x 0.3-0.4 cm, lobed; side lobes very small, mid-lobe ovate-crenulate. Pedicel and ovary 0.8-1.2 cm long.

RESULTS AND DISCUSSION

The endemic status of *Aerides crispa* extended throughout the Western Ghats in the states of Tamil Nadu, Kerala, Karnataka, Goa, Gujarat, and Maharashtra. In the study site, ten populations of *Agave americana* and 17 plant species of angiosperms were identified each year from 2019-to 2021. The total number of individuals was sampled in

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different years *ie,* 2019 - 443, 2020 - 457, and 2021- 478. Table 1 shows during 2019 to 2021 *Agave americana* had the highest relative density. The next leading species was *Aerides crispa.* The relative density of *Aerides crispa* increases due to the relative density of substratum species *Agave americana.* This study shows that the adaptability of *Aerides crispa* with *Agave americana* and invasive species is not always harmful to endemic species. In this study site, velamen roots of *Aerides crispa* are lengthy (minimum 10 -18 cm in litho phyte, maximum 42-138 cm in epiphyte on *Agave americana*). The lengthy velamen roots surround the dry, moistened parts portion of leaf bases and inflorescence axis of *Agave americana.*

Agave americana is cultivated as a live fence in the Andes [33]. Spiny leaves of *Agave americana* protect *Aerides crispa* from other animal disturbances. *Agave americana* use to restore degraded areas, native flora, and fauna, mark lands, create terraces, protect fences, prevent erosion, maintain humidity, and act as an organic fertilizer for agro forestry systems [34]. The *Agave americana* can grow well on rocky slopes compared to other invasive species because it can tolerate dry conditions. The wet and dry parts of *Agave americana* serve as an ideal growing medium for *Aerides crispa.* Wind and habitat degradation pose threats to *Aerides crispa* [29]. In this study area, *Aerides crispa* handled habitat degradation by *Agave americana*, but it also serves as a growing medium for *Aerides crispa.*

CONCLUSION

Agave americana can grow in low to high altitude, effortlessly dispersed because people use it for different purposes. The natural spread of invasive species is difficult to control but can be maintained by utilization. This study states that *Agave americana* is suitable for the in-situ conservation of orchids such as *Aerides crispa* because moistened dry parts of *Agave americana* are nutritious. Invasive species are harmful to native plants but are not always harmful.

ACKNOWLEDGEMENT

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Table:1 Relative Density of plant species in the study area during the years 2019 to 2021

S.No	Binomial Name	2019	2020	2021
1.	*Agave americana* L.	12.64	12.69	15.48
2.	*Aerides crispa* Lindl.	10.61	10.94	13.39
3.	*Cymbopogon flexuosus* (Nees ex Steud.) W. Watson	9.48	10.07	9.62

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4.	*Themeda triandra* Forssk.	9.71	8.75	8.73
5.	*Lantana camara* L.	8.13	8.10	7.53
6.	*Osbeckia leschenaultiana*	8.35	8.53	7.53
7.	*Notonia grandiflora* Wall. ex DC.	6.32	6.35	6.07
8.	*Indigofera cassioides* DC.	5.87	5.69	5.44
9.	*Dodonaea viscosa* (L.) Jacq.	6.55	5.69	5.44
10.	*Ageratina adenophora* (Spreng.) R.M .King & H. Rob.	6.09	5.47	5.02
11.	*Ageratum conyzoides* Hieron.	3.61	3.94	3.77
12.	*Acacia mearnsii* De Wild.	2.93	3.50	3.35
13.	*Cyanotis arachnoidea* C.B. Clarke	2.71	3.50	2.72
14.	*Emilia sonchifolia* (L.) DC. ex DC.	2.93	2.84	2.51
15.	*Gymnosporia montana* (Roth) Benth.	1.81	1.75	1.67
16.	*Rubus ellipticus* Sm.	1.13	1.09	1.05
17.	*Ligustrum perrottetii* A.DC.	1.13	1.09	1.05
	Total	100	100	100
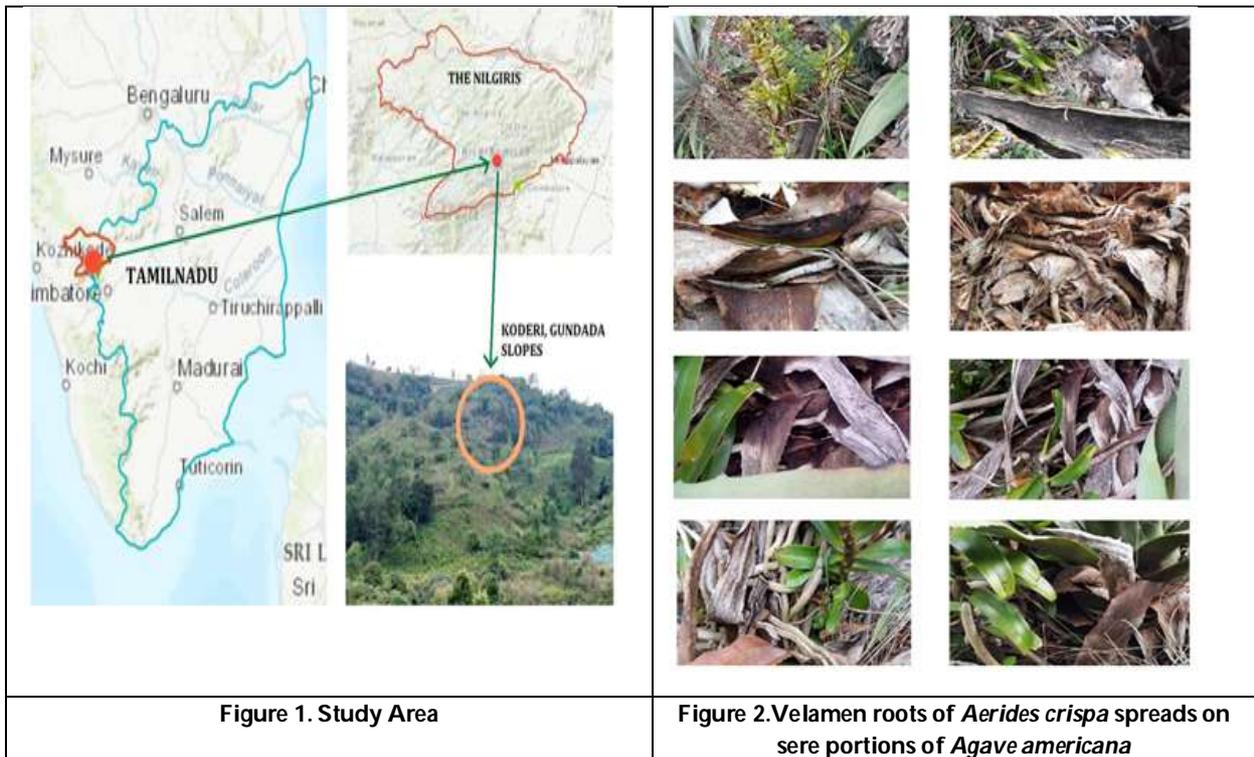
Figure 1. Study Area	**Figure 2.Velamen roots of *Aerides crispa* spreads on sere portions of *Agave americana***

RESEARCH ARTICLE

Different Machine Learning Techniques in Disease Prediction

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ABSTRACT

Machine Learning plays an important role in healthcare industry. The disease diagnosis is the major task in medical domain that are decided using different features. The expert knowledge is required to decide the disease and start the medication for patient. This system takes much time for disease prediction. The effective Machine learning methods are used to disease prediction with less processing time. The accuracy levels of these methods are high in diagnosis process. In this paper, we reviewed different machine learning techniques that are used for different disease classification. This work will be useful to find the effective methods for further processing.

Keywords: Machine Learning, Classification, Supervised Techniques, Unsupervised techniques, Logistic Regression, Decision Tree, K-Nearest Neighbour, Naïve Bayes, Support Vector Machine.

INTRODUCTION

The machine learning techniques are efficient in predicting and diagnosing disease using attributes. This method provides useful results because the machine learning models are enabling the systems which are learnt from experiences. The machine learning approaches supports various domain to handle the vast amount of data and extract the useful patterns from that. The system's with machine learning models can acquire the data for integrating knowledge using large observation power. This can be improved by tuning parameters in machine learning. This analysis is used for decision making that does not require any human control. The ability of this method is used for predicting the future occurrences with group of data. This can be done by applying different analytical methods on the collected data.

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Machine Learning in Disease Prediction

Machine learning method contains massive amount of methods that can be broadly classified into two types such as predictive models and descriptive models. In Healthcare industry, the patients details are collected and stored in different types. The medical tests are conducted based on collected data from human body. The test results are moved for expert judgment. The machine learning methods are used to reduce the processing time for disease diagnosis. This is used for predicting the disease based on the data values. The decision making with machine learning can be evaluated using different performance metrics such as accuracy, precision, recall, sensitivity and specificity. The machine learning algorithms are trained with different data values that are useful for classifying the data records into predefined groups.

Techniques in machine learning

The machine learning techniques are classified into three categories that are supervised machine learning techniques, unsupervised machine learning techniques and hybrid techniques. The supervised machine learning methods are analyzed the past data and trained with the values with class label. This learning knowledge is used in prediction using new values without class label. The unsupervised learning methods are used to predict and categorize the values into groups without any training process. The hybrid learning approach is used to combine the different machine learning methods to provide effective results.

CLASSIFICATION METHODS

The following classification methods are used in disease prediction such as
- Logistic Regression
- Support Vector Machine
- Decision Tree
- Random Forest
- Naïve Bayes
- K-Nearest Neigh bour
- Artificial Neural Network

Logistic Regression:

Logistic Regression is one of the classification methods which extends the ordinary regression method. This method is used for finding the probability which is used for categorize the new instances to a particular class. The probability values are denoted between 0 to 1. If the probability value is higher than the value 0.50, then the output indicates the class A. If the value is less than 0.50, it indicates the class B.

Support Vector Machine

Support vector Machine is used for both linear and Non-linear data classification. In this model, all the data values are arranged in n-dimensional feature vector which indicates n feature values. The hyper plane model is used to separate two different classes. This model provides effective results in classification where the classification errors are reduced. The support Vector Machine method selects the extreme vectors which are used to create the hyper plane.

Decision Tree

Decision tree is one of the prominent classification methods which use decision logic to classify the data. The tree shaped model with decision logic is used to categorize the data to produce the outcome for that record. The decision logic contains a condition. If the data satisfies the condition then it falls under true category. Otherwise it is comes under false category. These two categorizes are represented the branches of decision tree. This decision tree contains root node, decision nodes, branches and leaf nodes. The leaf node denotes the class for data.

Random Forest

The random forest is a famous classification method which ensembles more number of decision trees to improve the classification performances. To reduce the over fitting issues in decision tree, this random forest combines many decision trees. This method requires training process with maximum data including class label. This makes the model error-prone. In Random Forest, the new samples used to classify that can passed down in every decision tree to produce outcome. The forest method choose the maximum votes of each decision tree and produce the average of all decision tree results.

Naïve Bayes

This method is a classification technique based on Bayes Theorem. This provides the probability of an event which is calculated based on prior knowledge of event. This classifier focused a particular attribute in the class to find the appropriate class label.

K-Nearest Neighbour

This is one of the simple classification technique which is a simpler version Naïve bayes Method. But this method does not consider any probability value. This considers the 'k' Neighbour which are used to take vote from the Nearest Neighbors.

Artificial Neural network

This method consist a set of machine learning algorithms which are based on the neural function of human brain. This consist input layer, hidden layer and output layer process to find the output.

Related Study

Senthilkumar Mohan et al (SENTHILKUMAR MOHAN. 2019)presented the machine learning techniques for heart disease prediction. Different machine learning approached were analyzed in this work for heart disease prediction. They analyzed K-Nearest Neighbour method, Linear Regression method, Support Vector Machine method and Neural network methods. In this research work, they proposed hybrid random forest with Linear model (HRFLM0 method for effective heart disease classification. The results were compared with nine different machine learning approaches. The proposed method produced 88.4% accuracy which was higher than other machine learning models. Dhiraj Dahiwade et al (Dhiraj Dahiwade. 2019) proposed a general disease prediction system using the symptoms of various disease. The prediction was done using different machine learning approaches. They had used convolutional neural network model and K-Nearest Neighbour model for disease classification. The experimental results showed that the K-NN methods was slower than Convolutional Neural Network. The accuracy comparison showed that the K-NN method achieved 95% accuracy where the Convolutional neural Network method achieved 99% accuracy in disease prediction.

Min Chen et al (Min Chen. 2017)proposed the machine learning approach for disease prediction for Healthcare industry. They proposed the Convolutional Neural Network (CNN) based method with multi-model risk prediction system. They had used data which were collected from the hospital in china from the year 2013 to 2015. The total records were 20320848. They had implemented Machine learning and deep learning model for prediction. in this implementation the pre-processing methods were used to remove the noisy data. They proposed CNN based Unimodel disease risk prediction and CNN-based Multimodal Disease Risk prediction. The efficiency of these two methods were compared. The comparison results showed that the CNN-based Multimodal Disease Risk prediction achieved 94.8% accuracy which was higher than the CNN-based Unimodel Disease risk prediction. Tarigoppula V. S. Sriram et al (Tarigoppula V.S Sriram. 2015)presented the Intelligent Machine learning model for Parkinson Disease Prediction. for this research work, the dataset was collected from UCI repository. They had used different machine learning methods namely Bayes Net, naive Bayes, Logistic Regression, Simple Logistic, k-Star Model, AD Tree, J48 decision Tree, LMT and Random Forest. The accuracy of the model were presented in chart view to compare the efficiency of models. From the comparison results, the random forest method produced 90% accuracy which was higher that other models in Parkinson Disease Prediction.

K.Thenmozhi and P.Deepika (K. Thenmozhi . 2014) presented different decision Tree approach for Heart Disease Prediction. They analyzed various decision tree models like ID3 algorithm, C4.5 algorithm, C5.0 algorithm and J48 model. They also used GINI Index, Gain Ratio and Pruning Methods for improving the classification accuracy for heart disease prediction. They also used naive Bayes Model, Decision Tree and Neural Network for heart disease prediction using the Cleveland dataset. they highlighted that the Decision tree model achieved 99% accuracy using 15 attributes and 96% accuracy using 13 attributes. Kedar Pingale et al (Kedar Pingale. 2019) presented the disease prediction using machine learning approaches. They had used real time data which were collected from hospitals. The disease prediction system was implemented using K-Nearest Neighbour, Naive Bayes model and Logistic Regression. The collected dataset is divided into two sets for training and testing. The proposed model was trained with three different algorithms and test the data using three different methods. The accuracy of each model was used for comparison. The comparison results showed that Logistic Regression model produced high accuracy in disease prediction.

Gyanendra Chaubey et al (Gyanendra Chaubey. 2020) presented the Thyroid Disease Prediction with different machine learning approaches. They had used three different machine learning approaches like Decision Tree, K-Nearest Neighbour and Logistic regression models. In Decision Tree based approach, the entropy and information gain methods were used. The results analysis of three different methods were showed using accuracy and existing work results. They conclude their work that the Decision tree method achieved highest accuracy in existing work. But in this work, the K-NN method achieved good accuracy. Pooja rani et al (Pooja Rani. 2021) presented a decision support system for heart disease prediction. This prediction was done by using the machine learning approaches. They proposed hybrid feature selection algorithm to use feature selection in dataset. They combined Genetic Algorithm with recursive feature selection method. For pre-processing SMOTE (Synthetic Minority Oversampling Technique) and scalar methods were used. They focused data pre-processing techniques like Multivariate Imputation by chained Equations, feature selection process and SMOTE technique for clean the dataset. The classification models were used to classify the data. They used naive Bayes, Support Vector Machine, Logistic Regression, random Forest and Ada Boost methods for classification and prediction.

Apurv Garg et al (Apurv Garg. 2021) proposed the heart disease prediction model using machine learning methods. They focused the supervised classification models for producing prediction results using dataset. They used and compared two classification methods like K-Nearest Neighbour and Random Forest method. The Pandas library was used in experimental setup for implementing machine learning approach. They had compared the results of classifiers with two different approaches. The first comparison showed the performance of classifiers with all features and the second comparison showed the classifiers' performance with feature selection. In first comparison the Naive Bayes method achieved highest percentage that 85% accuracy in heart disease classification. In Second Comparison The AdaBoost method produced highest accuracy that 86.59% accuracy with feature selection.

Lambodar Jena et al (Lambodar Jena. 2020)proposed risk prediction of kidney disease with machine learning techniques. The classification model was designed to predict the kidney disease. The target class is focused and the classification methods were trained using training dataset. The feature selection methods were implemented to select the impactful features in dataset. the genetic search method was implemented for effective perdition of records to find the risk of kidney disease. The comparison of results using different machine learning classifiers showed that the genetic search increases the accuracy with less processing time. HarikumarPallathadka et al (Harikumar Pallathadka. 2021) proposed the usefulness of machine learning algorithms in management, healthcare and Agricultural domain. in this work, they highlighted that the machine learning applications are helpful to handle the complexities of real life. The techniques in machine learning is used in different domains to provide useful solutions for people. They discussed that the machine learning algorithms were used in artificial intelligence management like chatbots, image search, Handling Customer Data, Recommendation system, Inventory management, cyber security and portfolio management. The machine learning techniques are used in agriculture domain for plant disease prediction, crop production and continuous monitoring in fields. In management field, they highlighted that the machine learning techniques were used for sales growth, sales forecasting and inventory managements.

Ameer Sardar Kwekha-Rashid et al (Ameer Sardar Kwekha-Rashid. 2021)presented the analysis for Covid-19 using machine-learning applications. In this research work, the deep review analysis was done and the research articles were collected from Science Direct, Springer, Hindawi and MDPI journals. The machine learning methods were used to classify the records based on the class label. The comparative analysis showed that the supervised methods were more effective in COVID-19 prediction than the unsupervised methods in machine learning. There were seven different classification methods used for comparative analysis. Among that, the Logistic regression method produced highest accuracy in COVID-19 prediction. Ibrahim Mahmoodibrahim et al (Ibrahim Mahmood Ibrahim. 2021) proposed the role of machine learning methods in disease diagnosis using machine learning methods. In this work the overview of algorithms were presented. The impact of each machine learning methods and its classification process were explained. The models like Naive Bayes, Logistic Regression, Support Vector Machine, K-Nearest Neighbour, Decision Tree and Random Forest were reviewed for disease diagnosis. The comparison results highlighted that all the methods were produced good accuracy in disease classification but the Deep learning techniques were effective while handling the large amount of dataset.

P.Deepika and Dr.S. Sasikala(P Deepika 2020) proposed an enhanced model for cardiovascular disease prediction. The classification methods were used to classify the records which were collected from UCI repository. The article Swarm Optimization techniques were used for feature selection process. This method selected 7 attributes from the total dataset. The comparison results were analyzed using machine learning methods with feature selection using PSO and machine learning methods for classification without classification. The efficiency of machine learning methods were compared using Accuracy, Precision, Sensitivity, Specificity, recall and F1score. This work conclude that the classification methods were produced good accuracy by using PSO feature selection.

PankajChittora et al (Pankaj Chittora. 2021) proposed a machine learning perspective for Chronic kidney disorder. The collected dataset for this work contains 400 instances with 24 attributes. Feature selection methods were used in this work for selecting features. IBM SPSS tool was used for implementing machine learning methods for chronic kidney disorder. The machine learning methods like Artificial Neural Network, C5.0, Logistic Regression, CHAID, Linear Support Vector Machine, K-Nearest Neighbour and Random Forest methods were used. They also used McNemar's Statistical test for comparing two models. The experimental results showed that the Classifiers with SMOTE with Full Feature Selection achieved 98.86% accuracy.

FINDINGS AND DISCUSSION

The different machine learning methods were analyzed in this review. The different diseases were predicted with high accuracy using machine learning methods. From this literature analysis, it is clear that the feature selection methods were preferred to increase the accuracy and to reduce the processing time. The Decision Tree, Logistic Regression and K-Nearest neighbor Methods were achieved above 95% accuracy in disease prediction. The Convolutional Neural Network method in Deep Learning is also preferred when the large amount of dataset is used for prediction.

CONCLUSION

In this paper, the machine learning methods were reviewed for disease prediction. The performance of each classifier is compared with other machine learning techniques. The performance evaluation of each classifier is analyzed using Accuracy, precision, Recall, Sensitivity, Specificity, ROC curve, F1 Score, and AUC. From this review work, it is highlighted that the feature selection methods provided impactful results in disease prediction. The effective feature selection methods will be focused in further work for effective disease diagnosis.

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RESEARCH ARTICLE

Gastro Protective Activity of *Manilkara zapota* Bark Extract on Experimentally Induced Peptic Ulcer in Rats

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ABSTRACT

The anti-ulcer function of *Manilkara zapota* (Sapotaceae) bark MEMZ methanolic extract was investigated in Wistar rat pylorus ligation, indomethacin, and ethanol-induced ulcer models. The popular parameter calculated in the three models was the ulcer index. MEMZ developed substantial gastric lesion inhibition induced by Pylorus ligation-induced ulcer, indomethacin-induced ulcer& ethanol-induced gastric ulcer at doses of 300 mg/kg p.o. The extract (300 mg/kg) showed a substantial decrease in gastric volume, free acidity, and ulcer index (P<0.01) relative to the control index. This current study shows that in the three models, *Manilkara zapota* bark extract has potential anti-ulcer activity. These findings may also mean that methanolic extract has been discovered to have antiulcerogenic and ulcer healing properties, which may be due to its antisecretory action.

Keywords: *Manilkara zapota;* Pylorus ligation; indomethacin; Ethanol induced ulcer model; ulcer index.

INTRODUCTION

Peptic ulcer disease is a leading cause of death and morbidity around the world. Peptic ulcer is a digestive tract acid-induced lesion usually found in the stomach or proximal duodenum and characterized by denuded mucosa with a defect that stretches to the submucosa or muscularis propria [1]. Most peptic ulcer diseases have been thought to induce a hypersecretory acidic environment along with dietary causes or stress, but this understanding has changed

with the discovery of *Helicobacter pylori* infection and the widespread use of non-steroidal anti-inflammatory drugs (NSAID's) in the second half of the 20th century [2]. The approximate prevalence of peptic ulcer disease in the general population is 5-10% and incidence of 0·1–0·3% per annum [1-2].The pathophysiology of PUD indicates that this disease is due to a disturbance of the usual equilibrium between aggressive factors (gastric acid, pepsin and bile salts) and protective factors like prostaglandin (PG), mucosal blood flow, mucus and bicarbonate [3]. Other factors that lead to the formation of a peptic ulcer include spicy foods, fatty foods, caffeine-containing foods as well as depression and smoking enhance acid production in the stomach [4]. *Manilkara zapota L* (sapodilla) (synonyms: *Manilkara achras*, *Achras zapota*, and *Achras sapota*) is a tropical evergreen tree native to southern Mexico and Central America, but found in most of Brazil and India [5]. By the leaves of *M. zapota* is used to treat in Cough, cold, and diarrhea and also it has shown antioxidant and antimicrobial activity. Traditionally the bark extract also has been used to treat stomach issues, fever, and discomfort [6]. The object of the research is here to screen and evaluate the *M. zapota* bark extract for its anti-ulcer activities.

MATERIALS AND METHODS

Collection of plant material and identification
The extract of *Manilkara zapota* bark was collected from the Amrut Kesari Depot, Bengaluru in dried form. The plant material was identified and authenticated by Dr. Kuntal Das, Professor, department of Pharmacognosy from Krupanidhi College of Pharmacy, Bengaluru, with the herbarium reference number – CP-327/KCP/2018-19.

Preparation of extract
M. zapota bark were freed from foreign products by washing. The barks were then air-dried at a shedding temperature and dried at 40o C in an electric oven. The dried products of the plants were then powdered. Approximately 500 g of powdered content was soaked into a clean glass jar having 1500 ml 80% methanol. The jar containing *M. zapota* bark powder have been stored for 7 days by occasional shaking and stirring. Then a piece of clear, white cotton material filtered the entire mixture. It was then filtered through whiteman filter paper, concentrating at bath temperature not exceeding the 40°C with rotary evaporator, to produce gummy extract concentrate [6].

Phytochemical screening
The aqueous extract of *M. zapota* bark was subjected to quantitive analysis to investigate the presence of saponin, gums, reducing sugar, tannins, flavonoids, phenolic compounds, alkaloides, steroids, anthraquinone glycosides and amino acid. Also in the *M. zapota* plant bark extract, terpinodes (isoprenoides) were detected [7].

EXPERIMENTAL DESIGN

Experimental Animals
Animals were procured from the animal house of Krupanidhi College of Pharmacy, Bangalore. Male and female rats (n=72) of body weight 150-200gm were acclimatized in laboratory condition for 10 days prior to the experiment in a controlled temperature (25±4℃) and relative humidity (50-60)% with 12 hours light and dark cycle with food and water *ad libitum.*

Animal grouping
The rats will be grouped as follows:
Group 1: Normal/vehicle control
Group 2: Positive control (Ulcer induced)
Group 3: Test Dose (200 – 400 mg/kg) (Ulcer induced rats treated with *Manilkara zapota* bark extract) [8]
Group 4: Standard drug (Omeprazole 20 mg/kg-bodyweight) [8]

Antiulcerogenic activity
Ethanol induced ulcer model
In this study, rates were divided into 4 groups, each consisting of 6 nos. after 12 hours of fasting. Group 1 received normal diet; Group 2 represented the control group. Group 3 received *Manilkara zapota bark extract* orally at a dose 200 mg/kg body weight and Group 4 received Omeprazole orally at a dose of 20 mg/kg body weight for 3 days. On 3rd day after 60 minutes of extract and standard treatment to respective groups of animal'sethanol was administration orally at a dose of 1ml/ 200 g of body weight to respective groups of animals. Animals were sacrificed one hour after ethanol administration. The stomachs were removed, cut along the greater curvature, gastric juice was collected, after that washed with normal saline (0.9% Nacl) and the ulcer was scored. Gastric juice was used to determine the free acidity, total acidity and total protein [9].

Indomethacin induced ulcer model
Animals were pre-treated with Test and Standard (Omeprazole 20 mg/kg) for 15 days. After 24 hours of fasting, animals were orally treated with test and standard. One hour after the treatment, ulcer was induced by oral administration of Indomethacin at a dose of 100 mg/kg. After 4 hours, animals were anesthetized and euthanized by cervical dislocation, and stomachs were removed and opened along the greater curvature, gastric juice was collected, after that washed with normal saline and the ulcer was scored. Stomach content was used for the estimation of free acidity, total acidity and total protein content [10].

Pylorus ligation model
The animals were divided into four groups (n=6). Animals were pre-treated with test and standard (Omeprazole 20 mg/kg) for 15 days. The animals were kept fasted in an individual cage for 36 hours until they were pylorus ligated with water *ad libitum*. The mid-line incision below the xiphoid process exposed the abdomen under treatment of mild ether anaesthesia. The pyloric part of stomach was lifted out and ligated. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. After the pylorus ligation, therapies were performed intraduodenally. The rats were deprived of food and water for 6 hours of pylorus ligation, sacrificed with a high dose of anesthetic agent. The stomach was removed; cut along the greater curvature, stomach content was collected, washed with normal saline. The contents were measured, centrifuged for 10 minutes at 2000 rpm and evaluated pH level, SOD, and CAT [10].

Scoring of ulcer will be made as follows
Normal stomach............ (**0**)
Red coloration............... (**0.5**)
Spot ulcer................…….. (**1**)
Hemorrhagic streak....... (**1.5**)
Ulcers....................…..... (**2**)
Perforation................…… (**3**)
Mean ulcer score for each animal will be expressed as ulcer index.

The percentage of ulcer protection was determined as follows:-

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index - Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Determination of acidity

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1 \ mEq/L} \times 100$$

Statistical analysis

The values are represented as mean ± S.E.M and statistical significance between treated and control groups was analyzed using of one way ANOVA, followed by Dunnett's test where $P<0.05$ was considered statistically significant.

HISTOPATHOLOGICAL EVALUATION

The gastric tissue samples were fixed in neutral buffered formalin for 24 hours. Tissue sections from stomachs were histopathologically examined in order to study the *Manilkara zapota* bark extract for ulcerogenic or antiulcerogenic activity. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema and erosions using an arbitrary scale for the assessment of severity of these changes.

RESULTS AND DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms [11].To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis [12]. Pylorus ligation induced ulcer was used to study the effect of *Manilkara zapota* bark extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Albino rat model involves fasting of rats for 36 hours followed by ligation of pyloric end of the stomach. The ulcer index was determined 6 hours after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. The methanolic extract of *Manilkara zapota* bark and Omeprazole significantly decreased the total acidity, free acidity, acid volume, SOD and CAT. From this study it could be suggested that *Manilkara zapota* bark extract having an antisecretary effect. Its antiulcer activity is further supported by histopathological study which shows that *Manilkara zapota* bark extracts protect mucosal layer of stomach from ulceration and inflammation [13]. Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium. The *Manilkara zapota* bark extract protects against the lesions caused by ethanol induced gastric ulcer, and this antiulcer action might be attributed to both decreased stomach acid output and increased gastric cytoprotection.

Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection. Indomethacin (NSAID), induced ulcer model is also a well-established model to investigate ulcer protective agent. Indomethacin promotes the depletion of prostaglandin synthesis by inhibiting COX isoenzymes (COX 1 and COX 2). Prostaglandin stimulates mucus and bicarbonate secretion as well as mucosal blood flow which are defensive factors in ulcer formation and inhibit the secretion of hydrochloric acid [14]. Indomethacin induced suppression of prostaglandin synthesis in the intestinal mucosa results in gastric lesions in both rodents and humans. Administration of *Manilkara zapota* bark extract significantly reduced the gastric lesions compared to positive control group, which indicates gastro protective effect against indomethacin induced ulcers [15]. The results of the present study demonstrated that *Manilkara zapota* bark extract (200 mg/kg, 400 mg/kg) protected gastric mucosa and has ulcer-protective activity, as it is evident by the significant inhibition of ulcers in various ulcer models. *Manilkara zapota bark* extract (200 mg/kg, 400 mg/kg) produces a significant, dose dependent gastroprotective effect in ethanol, indomethacin induced ulcer model and antisecretory effect in pylorus ligation induced ulcer model. Thus, the

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methanolic extract of *Manilkara zapota* bark of the plant can be used as a new source of antiulcer drug in animals. Further experiments and detailed phytochemical analyses are needed to determine the phytoconstituents responsible for as well as the antiulcer mechanism involved.

ACKNOWLEDGEMENT

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Table 1: Effect of *Manilkara zapota* bark extracts and standard (Pantoprazole) on ulcer Index, free acidity, total acidity, total protein, acid volume, pH, in Ethanol induced ulcers in rats

SI. No	Treatment	No. of animals	Dose	Ulcer Index	Free Acidity (mEq/L)	Total Acidity (mEq/L)	Total Protein (µg/ml)	Acid Volume (ml)	pH
1	Normal (water)	6	2ml/kg	-	25.34± 0.08	71.16± 0.020	6.98±0.56	4.01±0.12	1.74±0.24
2	Control	6	1ml/kg	11.3± 0.42	49.3± 1.53	105.00± 3.13	10.45±0.83	8.01±0.42	2.1±0.09
3	Standard (Omeprazole)	6	20mg/kg	4.75± 0.48	19.8± 0.91	55.00± 0.95	9.20±0,03	1.84±0.06	3.2±0.35
4	Test (*M. Zapota* bark extract	6	300mg/kg	3.49± 0.11	31.1± 1.53	45±0.45	8.35±0.04	2.30±0.14	2.55±0.25

Values are expressed in terms of mean ± S.E.M. statistical analysis was carried by graph padInStat DTCG through one way ANOVA, followed by Dennett's t-test. *, $P < 0.05$; **, $P < 0.01$, *** $P < 0.001$ was considered as a significant.

Table 2: Effect of *Manilkara zapota* extracts and Standard (Pantoprazole) on pH, total acidity, SOD, Gastric wall mucus in Indomethacin induced ulcers in rats

SI. No.	Treatment	No. of Animals	Dose	pH	SOD (%Inhibition rate)	CAT (Units/mg)
1	Normal (water)	6	2ml/kg	1.74±0.24	55.12±2.4	33.2±2.7
2	Control	6	1ml/kg	2.74±0.14	33.43±1.8	16.8±1.2
3	Standard (Omeprazole)	6	20mg/kg	4.77±0.24	52.58±2.5	31.5±1.3
4	Test (*M. Zapota* bark extract	6	300mg/kg	3.6±0.19	41.18±1.9	26.9±1.6

Values are expressed in terms of mean ± S.E.M. statistical analysis was carried by GraphPad InStat DTCG through one way ANOVA, followed by Dunnett's t-test. *, $P < 0.05$; **, $P < 0.01$, *** $P < 0.001$ was considered as a significant.

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Table 3: Effect of *Manilkara zapota* bark extracts and Standard (Omeprazole) on pHSOD, CAT in Pylorus ligation induced ulcers in rats

Sl. No	Treatment	No. of Animals	Dose	pH	SOD (%Inhibition rate)	CAT (Units/mg)
1	Normal (water)	6	2ml/kg	1.74±0.24	55.12±2.4	33.2±2.7
2	Control	6	1ml/kg	2.74±0.14	33.43±1.8	16.8±1.2
3	Standard (Omeprazole)	6	20mg/kg	4.77±0.24	52.58±2.5	31.5±1.3
4	Test (M. Zapota bark extract	6	300mg/kg	3.6±0.19	41.18±1.9	26.9±1.6

N=6 animals in a group; *: Value significantly different at $p < 0.05$ vs. Normal, **: Value significantly different at $p < 0.01$ vs. Normal, ***: Value significantly different at $p < 0.001$ vs. Normal. †: Value significantly different at $p < 0.05$ vs. control, ††: Value significantly different at $p < 0.01$ vs. control, †††: Value significantly different at $p < 0.001$ vs. control. Data were analysed by using one- way ANOVA followed by Tukey multiple test.
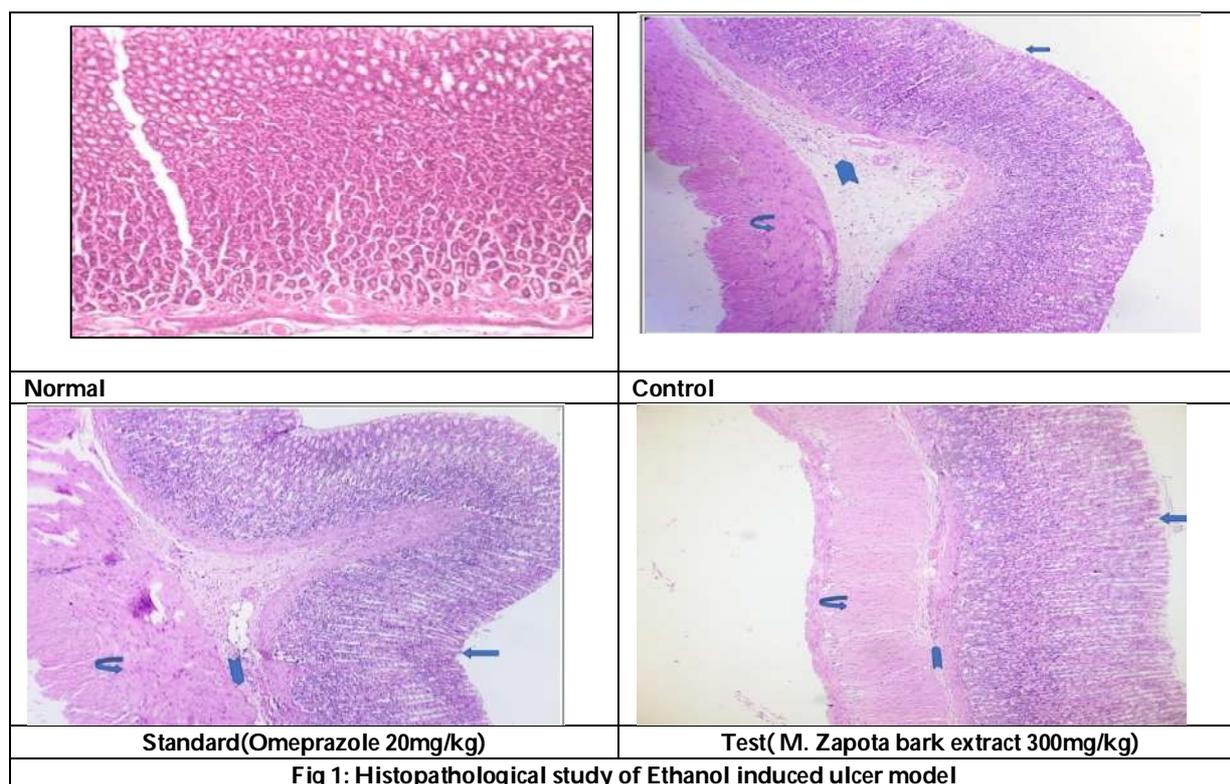
Normal	**Control**
Standard(Omeprazole 20mg/kg)	**Test(M. Zapota bark extract 300mg/kg)**
Fig 1: Histopathological study of Ethanol induced ulcer model	

Normal	Control

Standard (Omeprazole 20mg/kg)	Test (M. Zapota bark extract 300mg/kg)

Fig 2. Photographs showing of ulceration in Indomethacin induced ulcer in rats.

Normal	Control

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Standard (Omeprazole 20mg/kg)	**Test(M. Zapota bark extract 300mg/kg)**
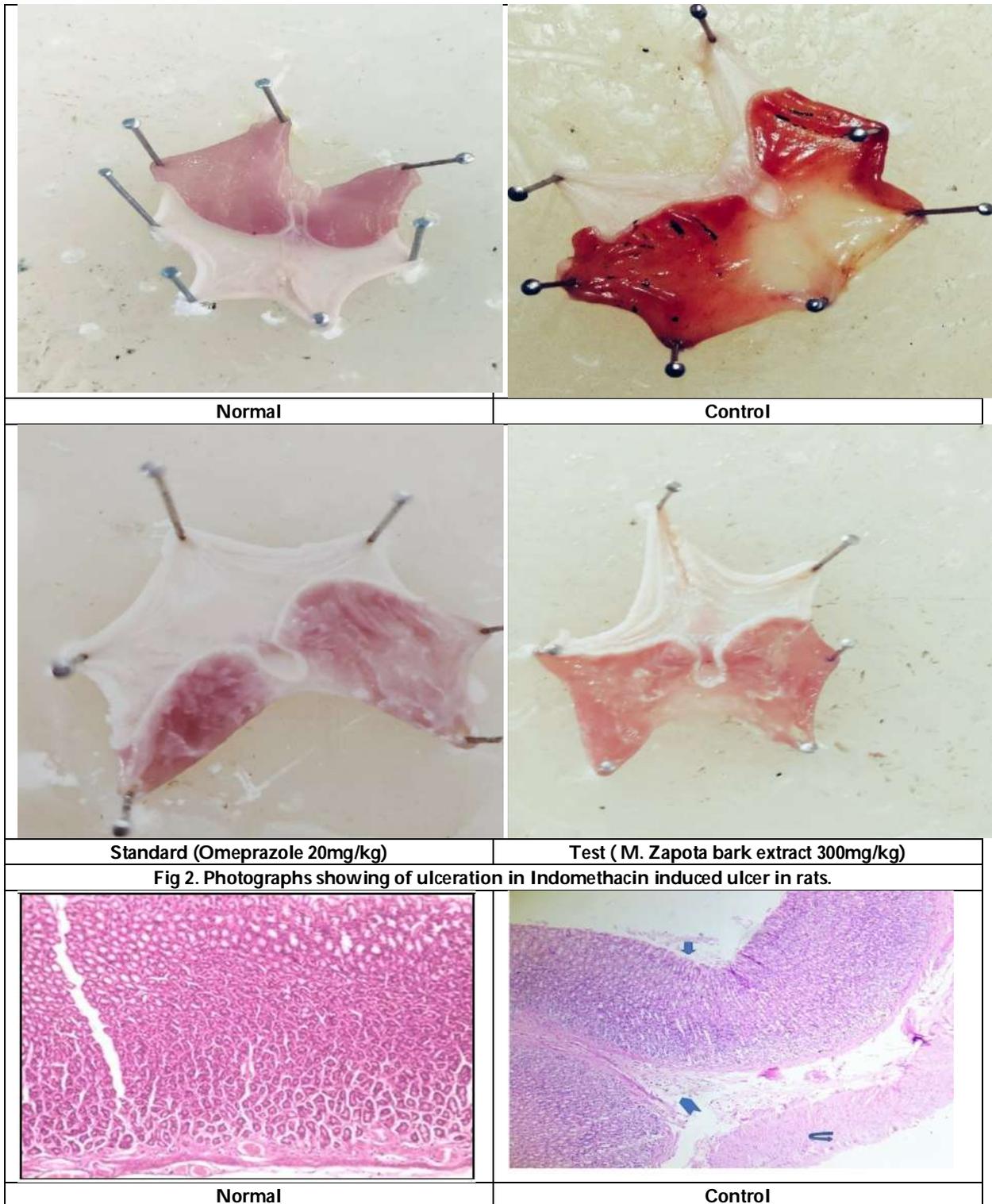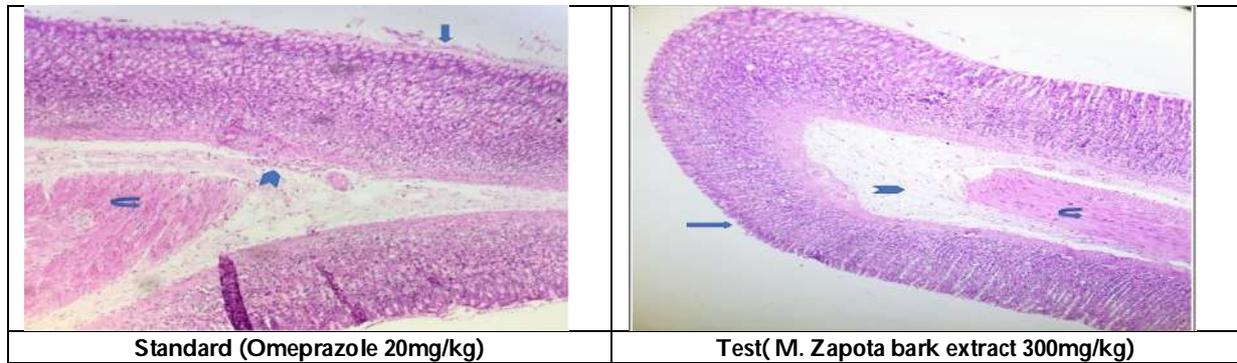
Fig 3. Histopathological study of Indomethacin induced ulcer model

Normal	**Control**
Standard (Omeprazole 20mg/kg)	**Test (300mg/kg)**
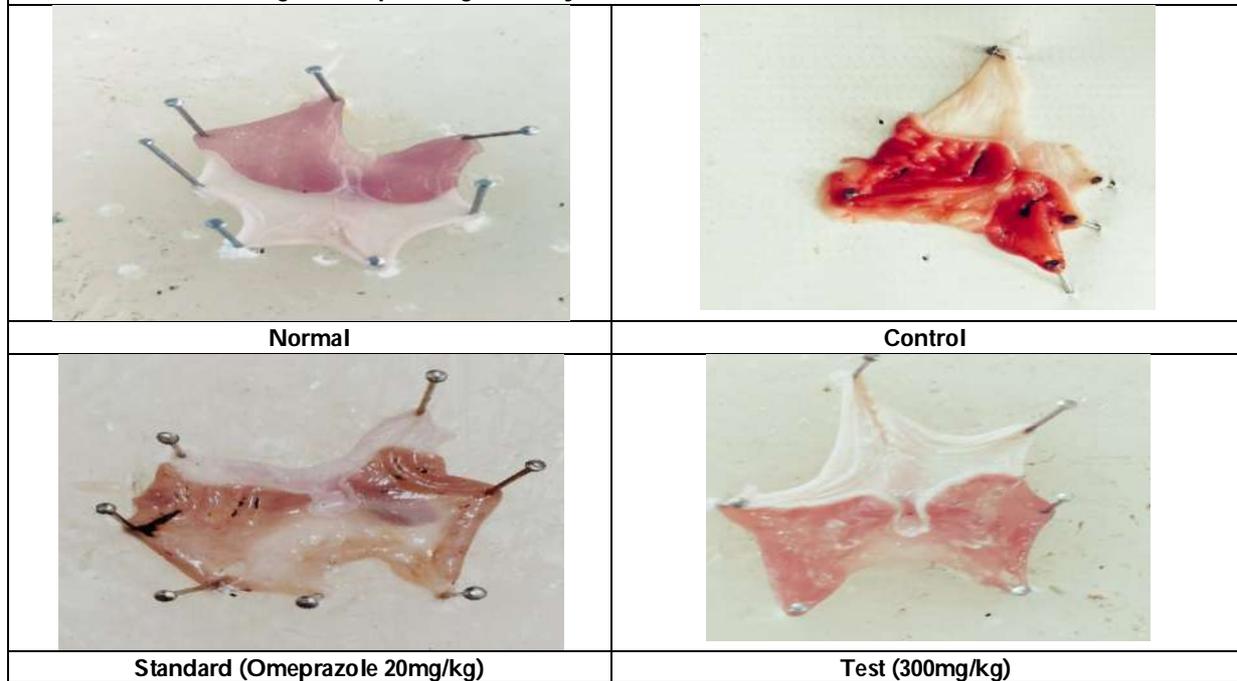
Fig 4. Photographs showing of ulceration in pylorus ligation induced ulcer in rats.

Normal	**Control**

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Standard	Test
Fig 5. Histopathological study of pylorus ligation induced ulcer model	

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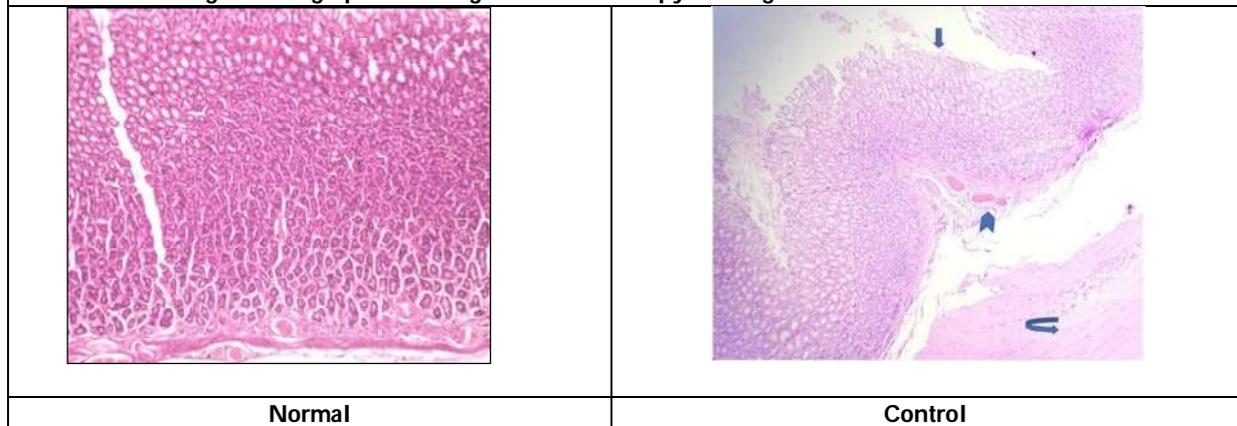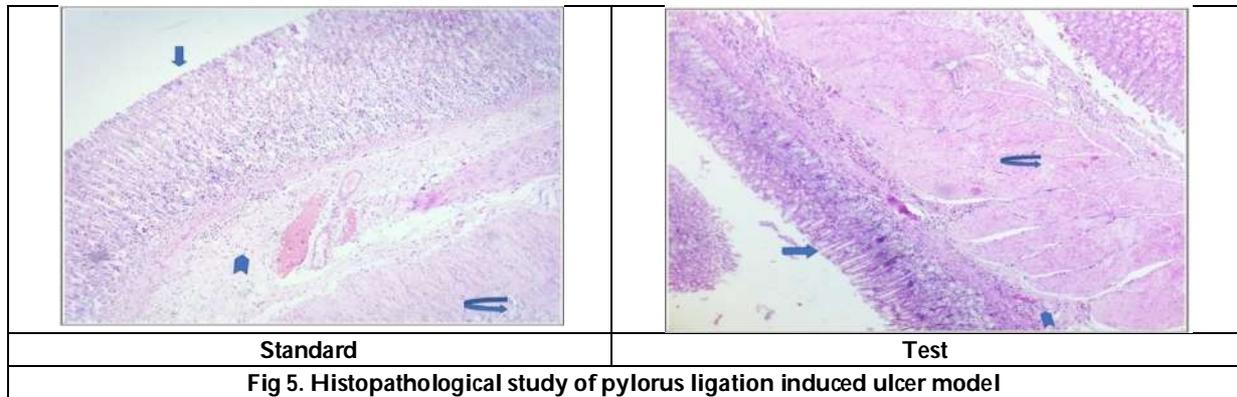
Transformation through E-Governance – A Maha Online Study for Maharashtra State

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ABSTRACT

Information communication and technology have been paving new ways for information transfer between various government agencies of national, state, local importance. [1].One of the remarkable application of ICT is e-governance. In India all states have introduced various citizen oriented e-gov projects for the citizens. The state of Maharashtra in India is leading ahead in introducing various citizen oriented e-governance projects for the utility of its users. It is a well known that such initiatives are designed for citizens and are critical in implementation but if implemented properly may result into improving state of art e-governance for the state. Hence, a critical look at these initiatives will help in assessing true resulting benefits to citizens and employees as well. In line with this a prominent e-governance initiative, Maha Online that covers the whole Maharashtra state, with as much as 389 services across 37 departments was studied. The descriptive study was conducted to gauge the service quality of Maha Online and citizens satisfaction was derived. Data was collected from five districts namely Ahmednagar, Pune, Satara, Sangli and Kolhapur consisting of sample of 750 respondents. The analysis showed that the citizens are partially satisfied with quality of e-governance service. The Flaws in the system are also presented. Paper is concluded with remedial measures to improve service quality and overall performance of MahaOnline.

Keywords: E-govenement, MahaOnline, aaplesarkar, , Maharashtra, service quality

INTRODUCTION

Electronic government is no longer just an option but an essential requirement for every country and every citizen. Successful role played by each stakeholder in rolling out the initiative and policies are at the core in making e-government a Success [2] . Some countries who have realized the importance of e-government initiatives have managed to successfully roll out their services. Such country includes United States, Republic of Korea, Singapore, France and Sweden etc. More than 200 countries are using websites to deliver e services to the citizens [3]. E-Government is a important avenue for developing countries like India to leap forward in their government's operations, provide infiltrate performance and reduce existing gaps in the system [4] . As far as India is concern, after comparing various e-gov projects implemented in India it is evident that the country is consistently progressing and implementing various e-government projects [3]. Government of Maharashtra has also come up with certificate program in e-governance for all its employees and CSC operators so that e-gov projects can be executed successfully. Some successful initiatives implemented includes Mahabhulekh, SARITA and Maha Rojgar to name a few. Given below is the brief background of Maha Online initiative.

MahaOnline
MahaOnline Ltd. is a joint venture between Government of Maharashtra and TATA Consultancy Services Ltd (TCS) formed in 2010. MahaOnline is now a apex body in implementing of e-Governance in Maharashtra. It provides support to various government departments in Maharashtra 2015,October] Retrieved through https://www.mahaonline.gov.in Molweb/1100 /about us . At present the functioning of all Maha e-Seva Kendra is monitored by MahaOnline. It ias also worth to mention that Maharashtra Government has passed the Maharashtra Right to Public Services Act 2015, on 21st August 2015 . 37 different category of services are provided such as Panchayat Raj, Revenue, Forest, Rural development etc. are provided online through portal www.aapalesarkar.mahaonline.gov.in. Government of Maharashtra has implemented "Maharashtra Right to Public Services Act 2015" to deliver e-services in transparent, efficient and timely manner to public. A Citizen has to make online application and submit all the required documents. Citizen then has to pay online fees through various payment gateways option enabled by the portal. An applicant after submitting the online application can also track the status of the application. The new act would upshot an action against the operator who will penalized for delays in providing the services [MRPSA,2015]. Now the system is 11 years old and has been matured. Since inception of the initiative, it took considerable time for the system to get established. The researcher wanted to perform midcourse evaluation highlighting the gaps. The researcher has also done certain noteworthy suggestions. Next section presents diagram for MahaOnline operations.

Time sequence diagram for application process flow for MahaOnline portal
The time sequence Fig.No. 1 illustrates sequence from the start of creation of a login through registration process to certificate delivery to citizen. This process is followed for almost 11 years. Citizen approach MahaOnline web portal with service request .First step is registration. A citizen has to register online on MahaOnline website. A citizen can register using either verifying UID option or uploading all proofs on the portal. Once the login is created then user can select the department, Agriculture, Home Department, Industries Energy and Labour Department, on selection of department user will get list of services of respective department for application. After selecting department, the list of certificates under it will be displayed. A citizen can select the required certificate and upload the necessary proofs to avail the benefit of online service delivery. Once the application is submitted and necessary proofs are attached then user can track the status of their application. A further fees is collected from applicant and token with expected date of delivery of certificate is communicated to the citizen. Citizen then is intimated through message and email regarding delivery of the e-certificate. Aaplesarkar portal also provides the facility to check and authenticate the e-certificate. As per "Maharashtra Right to Public Services Act 2015" lead time for certificate delivery is generally 7 days to 30 days. After 7 days when citizen can raise online appeal for non receipt of e-certificate . Thus system has now become more mature robust and time efficient.

- It may be noted that though MahaOanline started in 2010, and scale up to the expectations. The system is adding moiré services from various departments and plans to cover all services from various government departments under MahaOnline. In addition, no study has been conducted by the government or individuals to analyse if the existing system has matured and progressed successfully or are there any pitfalls exist in the current system so that rectifications can be done.
- Keeping this in mind, a pilot study in Ahmednagar district was carried out, to find out the progress of the MahaOnline, loopholes in the process (if any).
- It was reveled that problems exist in online service delivery where the targets are not met in terms of delivery time. Sometimes a certificate is not delivered in time and also has corrections in the certificate which further increases the lead time for service delivery. Staff at the backend are trained properly to execute online transaction. Also latest technologies such as internet and mobile systems are included in the existing system to bring in more ease of use and to study technology aspect. Therefore, a detailed study using a descriptive research based method is conducted. The next section discusses objectives and research methodology.

OBJECTIVES OF THE STUDY AND RESEARCH METHODOLOGY
Objectives
The main objective of research was to evaluate citizens satisfaction towards e-government project MahaOnline in five districts of Maharashtra. Secondary goal was to identify the loopholes in the current system.

Hypothesis
There is positive association between service quality and users satisfaction

H0: There is no positive association between service quality and users' satisfaction.
H1: There is positive association between service quality and users' satisfaction.

Service quality was defined by eight parameters .They are time bounded service, ease of use, performance and speed, connectivity, accuracy and service costs, transparency in service deliverance[7]. The loop holes in the system were found from primary data collected from the citizens.

Research Design
a) Sampling Method
A study is based on descriptive research which includes surveys and various data collection techniques. Sampling design used was Multistage Sampling method due to involvement of several districts and sub district and at E-Seva Kendra. At first stage, five districts within Maharashtra was screened namely Ahmednagar, Pune, Satara, Sangli and Kolhapur. At subsequent stage, two sub districts were selected from every district. At third stage, the citizens applying for e-service through Maha e-Seva Kendra were selected randomely. In all a sample of 750 was selected from all five districts. Table 1 shows districts and sub districts along with sample sizes.

Data Collection
Primary data was collected through two well-structured questionnaire. The questionnaire was designed for citizens . It consists of total 20 questions. Questions were used to judge services quality dimensions like timeliness, cost, transparency, ease of service delivery online, privacy , performance of the website, no. of days required to get the certificate etc. 5 questions were used to collect basic information of citizen like name, educational qualification and age etc whereas rest of the questions were targeted to collect information on overall online service delivery mechanism, complaints if any etc. Along with primary data secondary data was also gathered from various websites at international, national and state level governments' website, e-government research papers, e-governance policy. After data collection reliability of data was tested.

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Statistical tools used
Statistical software SPSS 20.0 was used for analysis and interpretation, Chi square test and factor analysis techniques were applied on data.

Reliability analysis
The reliability of the data was calculated through SPSS. For reliability analysis, chronbach's Alpha was calculated. Since value of alpha is greater than 0.70, the sample is said to be reliable.

ANALYSIS
A) Descriptive statistics
Following table provides descriptive statistics for the sets of variable in the study. Table 3.0 shows 68.4% of male mail citizens apply online through MahaOnline webportal whereas female percentage was 31.6%. Data related to age group shoes that users of age 18 to 25 are 40 %. Respondents in the age group 18 to 25 require nationality, income, domicile, non-creamy layer and caste certificate and rahivasi dakhala. As far as educational qualification is concerned it was found that 32 % respondents are HSC & graduates, 16 % respondents are below 10th, approx. 26 % respondents are post graduates. Users with PG and UG degree are large in number because they require different certificate like caste, nationality/domicile, on creamy layer etc to take benefit in the fees by government scholarship.

B) Analysis of citizens' satisfaction
Organizations are required to be built on three main pillars viz., processes, people and technology as shown in Fig. 2.In designing service delivery these three elements must be aligned to the society and citizens in it. System should start with processes of organizations (systems) then consider people who will operate it whether they have desired skills, knowledge, and attitude. The third element to consider is the technology, which support the processes and people. Citizen interact with people at Maha- e-Seva Kendra and apply online for certificate through use of technology for availing e-service .On the basis of this the study has highlighted the attributes of quality which are as follows:
- Timeliness: Timeliness time refers to the number of days required to deliver e- certificate
- Cooperation: Cooperation means assistance from employees of Maha e-seva Kendra.
- Ease of Use: Easiness refers to easiness of use of aaple sarkar portal in availing certificate.
- Performance & Speed: Speed refers to the performance of the website and ability to process request quickly.
- Accuracy: Accuracy is nothing but correctness of certificate.
- Connectivity: Connectivity refers to ability to connect to network or backend MahaOnline server.
- Service Cost: Service cost is nothing but the charges paid by user for online transaction to avail certificate.
- Transparency: Transparency refers to clearness of the procedure. Awareness of status of applications

Fig.3 shows level of satisfaction on 5 point likert scale for all quality parameters. It has been observed that majority of respondents are satisfied with services quality of SETU however they are dissatisfied about timeliness of services.

Timeliness
Citizens have reported that they get certificate on time. However it is significant to note that 40% respondents are satisfied with the timeliness of the service and 30% respondents are dissatisfied about the delays in getting the certificate.

Cooperation
53% citizens are satisfied with cooperation of staff at Mahae-Seva Kendra where citizen visit and make online application. whereas 34% citizens are dissatisfied.

Ease of Use
*Many c*itizens also directly apply online for service delivery. Almost 59 % respondents feel that webportal Aaple sarkar is easy to use and navigate. Demo videos are displayed on the website regarding how to register and apply for certificate thus make their task of application easy. However 25 % respondents also feel that it is difficult to use the web portal.

Performance & Speed

Along with ease of using the website it is also important to be fast and meet consumer expectations. As per 55% or the respondents performance and speed of the website is extremely good.

Accuracy

Percentage of satisfied users for accuracy is more. Certificates delivered to citizens are accurate however 28% respondents also complains about errors in the certificate which takes time for further correction.

Connectivity

Connectivity to the server is extremely important factor for speeding up of delivery of services. It is also significant to note that 55% respondents are satisfied with network connectivity, 13% are neutral and 32 % report about connectivity issues which need to be looked upon and rectified.

Service Cost

MahaOnline charges minimal fees than prescribed by government .

Transparency

As far as transparency is concered 57 % respondents are satisfied with transparancy maintained by MahaOnline which shows that respondents get infornation about what is the status of their application.

Citizen Satisfaction study

In general majority of respondents are pleased with online service deilivery mechanism and this percentage is 57 % which is quite significant. Percentage of respondents who are neither satisfied nor dissatisfied is 12.8 and 27% are dissatisfied about the service delivery.

C) Hypothesis testing

Cross tabulation Test

Chi square test was used along with cross tab feature to figure out association between service quality and citizen satisfaction. The level of significance used was 5%. Timeliness, Ease of Use, Performance & Speed, cooperation, accuracy, connectivity, service cost and transparency are evaluated to measure citizen satisfaction. Table no.4 depicts chi square significance is 0.000 at 5% level. Hence, the null hypothesis is rejected (H0) and alternative hypothesis is accepted (H1). It must be remember here that values 1 to 5 are given to "highly dissatisfied, dissatisfied, neither satisfied nor satisfied, satisfied and highly satisfied" using the software SPSS.

D) Factor Analysis

This technique is used when database had multiple factors. It is used predominenetly used to eliminate and reduce number of factors. Stage1 is to verify if the data is proper and correct for technique of factor analysis. Kaiser Meyer Olkin Test (KMO) and Bartlett's Test resulted into value of 0.840 and 0.000 respectively indicated importance.

Total Variance Explained

The technique of principal component analysis was also used. Table No.5 shows rotated component matrix for factors. It is much evident from the following table that 1st component relates for 44.02% of the variance, then 2nd component relates to 18.47% of the variance whereas 3rd factor related to 17.12 % of the variance. It is relveled that the cumulative % of variance explained by the first 3 factors is 75%.

Component Matrix

The component matrix shows the association of each factor with every other factor. Factor loadings basically measure the key determinant. Factors can be clustered together as follows.

Factor 1- Ease of Use, Performance & Speed, Connectivity (Technology attributes)

Factor 2- Service Cost , Transparency, Accuracy (Quality attributes)

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Factor 3-   Timeliness & Cooperation (staff attributes)

It is clear that components Ease of Use, Performance & Speed, Connectivity are heavily loaded on factor I. Service Cost, Transparency; Accuracy is loaded on factor 2, whereas component Timeliness & cooperation is loaded on factor 3. It is significant to mention here those same outcomes are also reveled from Diagram 3 for level of satisfaction where Ease of Use, Performance & Speed, Connectivity are viewed as three satisfactory factors which can be further improved. It also shows that users are satisfied with Service Cost , Transparency, Accuracy which is loaded on factor 2. In nutshell we can say that Technology attributes and quality attributes are influencing users' satisfaction. Thus all three factors shall be enhanced for better service delivery. And further to ensure more citizen satisfaction.

SUMMARY OF FINDINGS

The objective of the paper was to evaluate quality of services and citizen satisfaction and identify any loopholes in the system. Even though the system is operational and mature there exist certain loopholes in the system. Our Descriptive study gives guidelines for mid course evaluation of MahaOnline. It also offers noteworthy insights into how service quality is reflected in the minds of citizens. Findings from the study (section 4.2) revealed that the in general citizens are satisfied with services, online delivery of the services and overall operational aspects of website. Results of chi square test reflects that p value for all factors was <0.05 thus H1 hypothesis is accepted i.e. There is significant positive association between service quality and customer satisfaction. Findings of factor analysis can be summerised as given below.

- KMO and Bartlett's test was significant with a value of 0.840 and 0.000 respectively.
- Three extracted components accounts for 80.52% of total variance.
- Exploratory factor analysis with varimax rotation yielded 3 factors based on eigen value cut off of 1.

SUGGESTIONS

- Technical problems need to be addressed on priority:- The problems related to technology can solved by ensuring proper connectivity so that user from not only urban but rural area also should be able to use portal smoothly.
- System should be robust and must reduce the downtime of the server and thus speeding up the delivery of the services.
- Training must be given to staff for back end processing of applications which causes unnecessary delays in service delivery.
- Though aaplesarkar mobile app has been launched by government it is not functional and frequently gives errors. Thus it is necessary to develop fully functional mobile app for online services delivery
- Ther quality control department must be try to improve accuracy of the service as it introduces unnecessary delays.
- Since MahaOnline is spanning   over full state and also has much larger functional scope so more and more services from all government departments must be integrated in the current system.

CONCLUSION

In this research paper, MahaOnline which is one of the mature and important schemes of e-Government project implemented across all states in Maharashtra is studied extensively. An in depth analytical study of the system from the questionnaire data was carried out using SPSS. The findings and the remedial measures to revamp them are also suggested. Suggestions given in study can be useful for government employees, centre operators for improving e service delivery.

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Table 1: Districts with sample sizes

District	Sub-District	Sample size	Total sample size per district
Pune	Haveli	78	150
	Baramati	72	
Satara	Satara	82	150
	Karad	68	
Sangli	Tasgaon	80	150
	Vita	70	
Ahmednagar	Rahuri	95	150
	Nagar	55	
Kolhapur	Karveer	75	150
	Ajara	75	
	Total	750	750

Table 2: Reliability Analysis

District	Sample Size	No of Items	Chronbach's Alpha
Pune	150	20	0.950
Satara	150	20	0.887
Sangli	150	20	0.809
Ahmednagar	150	20	0.786
Kolhapur	150	20	0.912

(Source: Data Analysis performed with IBM SPSS 20.0)

Table 3: Demographic Characteristics Of Citizens

No.	Name	Type	Frequency	Percentage
1	Gender	Male	513	68.4
		Female	237	31.6
2	Age group	18-25	300	40.0
		26-35	163	21.7
		36-45	168	22.4
		46-55	68	9.1
		More than 55	51	6.8
3	Education	Up to 10th	119	15.9
		HSC & Graduation	240	32.0

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		Post graduation	196	26.1
		Don't know read write	95	12.7

(Source: Primary Data from Questionnaire 1)

Table 4: Cross Tab with Chi square values for service quality attributes

No	Cross Tab Parameters	Chi-Square Value	Df	Sig	Remark
1	Timeliness	323.805	16	0.000	P value < 0.05
2	Cooperation	265.160	16	0.000	P value < 0.05
3	Ease of Use	341.570	16	0.000	P value < 0.05
4	Performance & Speed	290.812	16	0.000	P value < 0.05
5	Accuracy	316.618	16	0.000	P value < 0.05
6	Connectivity	364.286	16	0.000	P value < 0.05
7	Service Cost	308.224	16	0.000	P value < 0.05
8	Transparency	325.111	16	0.000	P value < 0.05

(Source: Data Analysis with SPSS 20.0)

Table 5: Component Matrix

Rotated Component Matrix[a]			
	Component		
	1	2	3
Timeliness		.656	
Cooperation		.609	
Ease of Use	.843		
Performance & Speed	.724	.631	
Accuracy		.688	.600
Connectivity	.825		
Service Cost		.752	
Transparency	.625	.703	
Citizen satisfaction	.657		
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.			
a. Rotation converged in 6 iterations.			

Source: Data Analysis with SPSS 20.0)
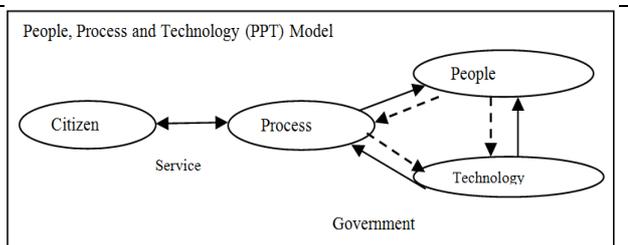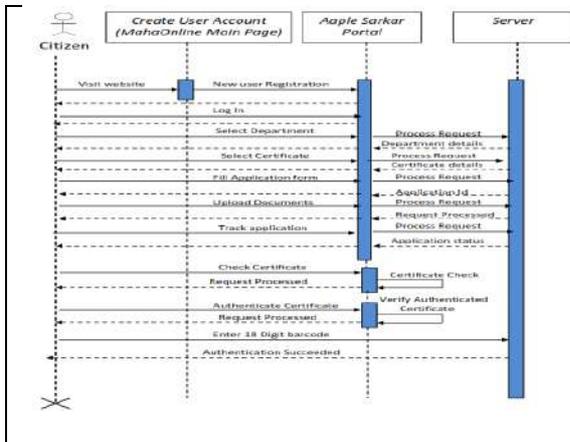
Figure 1: Time Sequence Diagram for operations flow for MahaOnline portal (Source: Primary & Secondary Data)

Figure 2 :PPT Model (Source:Peppard and Rowland Philip,2002)
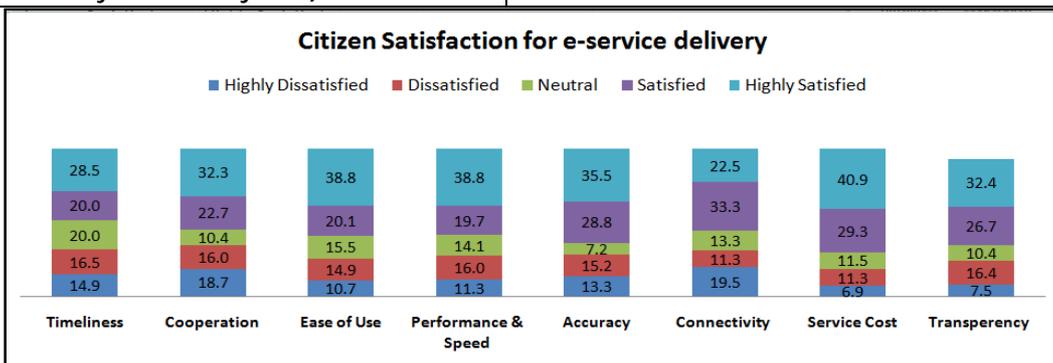
Figure 3: Citizen Satisfaction for e-service delivery (Source: Primary Data from survey)

RESEARCH ARTICLE

Stock Market Prediction using Long Short Term Model (LSTM)

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ABSTRACT

Stock market forecasting has piqued the interest of scholars, owing to the potential monetary gain from trading stocks in the future, as well as the need to comprehend the information concealed in stock market data. For stock price prediction and stock price movement prediction, many machine learning algorithms and statistical models have been presented. We looked into a variety of machine learning methodologies, such as regression and stock market prediction algorithms. We describe the rise in stock market prediction methods and models, compare these models to determine the accuracy of stock market value predictions for any particular firm, and determine the advantages and disadvantages of these specific models by learning numerous strategies. The LSTM model is being used to forecast future stock values. Many investors want to know how the stock market will perform in the future. In this case, an excellent stock market prediction system can assist investors and traders by providing information such as the future value of specific stocks.

Keywords: Stock market forecasting, Deep learning, Recurrent neural networks, Long short term memory, Back propagation..

INTRODUCTION

For an investor it's a risk phenomenon where they invest their saving and hope it brings back the return in higher yield. If the evaluation of the same increases, then the stock evaluation and its price increases causing the financial gain to both the parties. The stock market is made up of a large number of investors and traders who purchase and sell stocks, causing prices to rise and fall. The principles of supply and demand drive stock prices, and the purpose of stock purchases is to make money and a big profit by purchasing stocks in firms whose perceived worth is predicted to rise in Indian society, it is even considered a secondary business, with people considering it to be a sign of good fortune. The stock market varies a lot, and there are a lot of complex financial indicators. However, as technology progresses, the possibilities of consistently profiting to the rise in the stock market, parallelly it aids experts in locating most indicators in order to make a better prediction. Stock market prediction has been one of the most difficult goals of Machine Learning (ML) research field. Because the stock market is nonlinear, this research attempts to go much beyond the standard machine learning research, which primarily focuses on creating seemingly intelligent computers. The rate of fluctuation can be influenced by equity, interest rates, security options, warrants, mergers, and ownership of large financial businesses or companies. Even so, no one can dependably predict the stock market's trajectory. As a result, Deep Learning prediction necessitates an iterative process of knowledge discovery and system improvement, which may be accomplished using a variety of models such as RNN. The stock market is a promising financial venture that has the potential to generate significant wealth as well as profit. The stock market, on the other hand, is a high-risk investment due to its volatility.

Because of the potential financial reward, it has long been a hot topic in the fields of technology, finance, and mathematics. The stock market is a very important investment outlet since a large amount of money is bought and exchanged there. Furthermore, stock market prediction entails demonstrating whether the stock market can be accurately predicted or not. With the vast number of fast computers and vast amounts of information available on the Internet, stock markets are more accessible from anywhere on the internet. Gated units are a type of unit that avoids the problem of vanishing or exploding gradients. LSTMs are one of the most used cells in RNN implementation. They have been used to solve a range of sequence modelling problems in different application areas, including video, audio, natural language processing, time-series modelling, and geo-spatial modelling, due to their effectiveness. Deep learning stock analysis will be advantageous for new investors who want to invest in the stock market based on the numerous criteria that the program considers. It comprises daily tasks such as the computation of the Sensex and the past value of a stock. For trading in equities, debt instruments, and derivatives, the exchange provides an efficient and liquid market. Based on the company's stock valuation, this software will analyze future stock prices.

RELATED WORK

Forecasting stock returns has become a popular topic of study over the previous few decades. The researchers intended to identify a straight line between stock returns and macroeconomic variables used as input for majority of the time. Many literatures have emerged in the aftermath of the finding of nonlinearity in stock market index returns. The majority of them demanded that the nonlinear model be described prior to the stock returns could be calculated. The calculation has been completed. However, ANN was created because stock market returns are noisy, uncertain and nonlinear. Has proven to be a more effective means of representing the basic link between both the price of a stock as well as its performances. Several additional statistical techniques in terms of determining determinant factors. Different sets of input factors have been used to forecast stock returns in the literature. To anticipate the the same set of statistics on stock returns, alternative input variables are used. Others explored the incorporation of heterogeneous market information and macroeconomic variables. Some authors employed data from a single time series as their input, while others used data from many time series. Some researchers went so far as to pre-process these data sets past sending them to ANN for forecasting

Kim, Kyoung-jae, Han, Ingoo. (2000). Algorithms with genetic components use feature discretization in ANN to forecast stock price indexes. This study proposes a genetic algorithms (GAs) technique to artificial neural network feature discretization and connection weight determination in order to anticipate the stock price index (ANNs). For the approach of training the network, feature subset selection, and topology optimization, prior studies proposed numerous architectures of ANN and GA. Furthermore, in the vast majority of these studies, GA is used just to enhance the learning algorithm. GA is utilised in this work not only to enhance the learning algorithm, but also to minimise the complexity of the feature space. GA optimises both the layer connection weights and the feature representation thresholds at the same time. The genetically evolved weights help to overcome the gradient descent algorithm's well-known drawbacks. Furthermore, globally found feature representation minimises the feature space's dimensionality and eliminates irrelevant elements. The GA method to the feature discretizes the model outperformed each other two traditional models in experiments.

Min and Lee [2] employed the machine learning to predict insolvency. Among the approaches they investigated were the Support Vector Machine, discriminant function analysis, binary logistic regression, and 3 fully integrated back-propagation neural networks. When Lee used support vector machines to forecast a company's credit rating, the data showed that support vector machines beat other strategies. They employed a variety of financial indicators and assessments to obtain a 60 percent accuracy rate, including the interest cover ratio, normal income to total assets, Net profit to stakeholders' equity, total liabilities ratio, and others are some examples. The use of neural networks to predict business credit ratings was also investigated, with accuracy ranging from 75% to 80% in the US and Taiwan markets, respectively.

In a study [3], Tsai and Wang used ensemble learning, which consists of decision trees and artificial neural networks, to try to forecast stock values. They used Taiwanese stock market data to generate a dataset that included fundamental, technical, and macroeconomic indicators. Decision Tree + Artificial Neural Network Performance F-score performance was 77 percent when trained using Taiwan stock exchange data. A single approach disclosed the F-score, which led to a 67% boost in performance. Guresen et al [4]. He considers ANN to be one of the best approaches for modelling the stock market since it lacks conventional formulae and can be quickly adjusted to market movements. ANN can learn by doing and use what they've learnt to build interpolations and extrapolations. When a ANN is employed to solve a problem, it goes through a learning phase in which it collects patterns and builds a customised representation of the problem (Braga, Carvalho, Ludermir, 2007). White created the first ANN-based stock price prediction model (1988). The author created a feed forward network to discover unknown regularities in stock price swings. The goal was to verify Fama's (1970) an efficient market hypothesis, which asserts that stock prices follow a random walk, by looking at IBM stock's daily returns. Despite the fact that he could not produce good prediction results, the study demonstrated the possibility for such analysis. Since then, a large number of academics have been actively involved in the development of predictive models that might be employed in the stock market with certainty in the future. Tsibouris and Zeidenberg [5] (1995) tested the efficient market hypothesis by assessing six New York Stock Exchange shares and finding a competence to anticipate prices based on the prior price series. Other studies have demonstrated that ANN outperforms statistical approaches like the regression model and the ARIMA technique in terms of prediction performance (Kolarik, Rudorfer, 1992; Refenes, 1995; Refenes, Zapranis, 1996).

PROPOSED METHOD

This research aims to forecast the stock's value based on its prior performance and tendencies. It necessitates historical stock market data, as the study focuses on deep learning approaches. As a result, a reliable source with relevant and sufficient data for the forecast is required.

Algorithm : Stock prediction using LSTM Input: Historical stock price
Output: Prediction for the stock price based on stock price variation
1. Read the stock prices data into data frame and print the data frame

2. Because many organisations estimate their profit/loss using close values, eliminating all rows except close in a data frame is a good idea.

3. As LSTM are sensitive to the scale of data so we use minmax scaler

4. Divide the data into 65 percent training and remaining for test

5. Divide the training in x-train,y-train and testing by x-test, y-test by using time steps[100]

6. Created a Stacked LSTM model

7. Adding Adam optimization and the loss as a mean squared error to the LSTM

8. Predict the train and test data, as well as the RMSE, then visualise the results.

9. Next predict the future 30 days stock price and plot the graph.

The training system is where we practice and adapt our model to the parameters, while test data are used exclusively to determine the model's performance. For modelling, the output of training data is available, while test data are not visible, for which predictions need to be made .To minimize loss, we divide the dataset into a ratio of 65:25 i.e., 65 percentage as training data, remaining 35 percentage as testing data and standardize the values for the data set.

Data Preprocessing

This model utilises a normalisation approach to normalize the features in the 0 to 1 range. Avoiding distorting the statistical disparities between values, the data is normalised onto a comparable scale. Time stamping is used to predict the value X_{T+1} in a data set from $[X_0, X_1, X_2,..., X_T]$ in the setting of a true date-stream. For the 61st observation, we discover the predicted data using the data from the previous 60 observations. This one is handy for identifying valuable data trends.

LSTM

LSTMs were created with the goal of preventing long-term dependence issues. They don't have to think about recalling information for lengthy periods of time; it's as if it's second nature to them! For LSTM, the horizontal axis that goes through the graph's top, the cell state is critical. The cell state of the conveyor belt is comparable. It runs straight down the whole chain after only a few minutes of linear exchanges. It is simple for data to pass unmodified along it. The LSTM has the ability to delete and add data about cell states that are controlled by gate structures in real time. Gates are a type of device that allows for the selective transfer of data. They are made up of a neural sigmoid net layer plus point-specific propagation. The sigmoid layer produces numbers between 0 and 1, indicating how much each portion should pass. "Nothing must be allowed to pass," zero value implies, but a one value says that" all must be allowed to pass!" All the gates in the LSTM safeguard and regulate the cell state

1) Optimization of Gradient descent algorithm in LSTM: : The stochastic gradient descent algorithm is introduced. When the model decides on the gradient descent direction, it does so based on the BPTT algorithm's formula. can also be created by picking at random with a specified probability. Not only do we save money, but we also save the environment. improve the speed of iteration and the number of iterations likelihood of reaching or jumping out of the ideal spot When there is a local optimum, it is called the local optimal. In order to achieve The step size of iterations is also established in a similar manner. Furthermore, the error function would fall to a very tiny value in the latter stages of iterations, and the adjustment of the because the weight value is obtained by deriving the error function using t, every weight change is small and training is nearly steady. We introduce the function of iterative step size and error to address this problem

Where loss n is the n th training's error, Ln is the n-th iteration's step length. The training impact is unaffected by alpha adjustment since the mistake in the prior training procedure is usually bigger than 1. When the loss is minimal, alpha has a lot of control on the step size range. This will solve the problem of option values updating slowly in later iterations.

To construct the LSTM we need to import a few Keras modules
1. The neural network is initialised in a sequential manner.
2. Dense lets you add your model to a densely connected neural network layer.
3. LSTM is often used to add a layer of Long Short Term Memory.
4. Drop out by adding drop-out layers for reducing overfitting.

Activation Function : This is used to determine the output of a neural network and to map the value among 0 and 1. Transfer Function is another name for it. Based on the non-character of stock prices, the activation function utilised in this model is the RELU and optimizer is adam. When stacked layers are taken into account, it is also incredibly efficient. $R(x) = max(0, z)$

Adam Optimizer: Adam optimizer is employed because it uses momentum to calculate gradient rather than typical gradient-finding methods such as Stochastic Gradient Descent. It is efficient and takes minimal memory. Adaptive learning is used to discover the optimum result faster than previous approaches.

To give an optimization procedure, Adam features the best characteristics of both the AdaGrad and RMSProp approaches for noisy issues with sparse gradients. As a result, ADAM is the best optimizer for neural network training. Adam Optimizer inherits the strengths or excellent qualities of the aforementioned two systems andimproves on them to give a more optimized gradient descent. Using the Momentum and Root Mean Square Propagation formulas:

Parameters Used: Because mp and vp were both initialised as 0 (using the methods described above), they have a propensity to be 'biased towards 0', as both 1 2 = 1

This Optimizer addresses the issue by calculating 'bias-corrected' mp and vp. This is also made to keep the weights in control until gradually approach the global minimum, which helps to minimise major oscillations.

After each cycle, we instinctively adjust to the gradient descent so that the process stays controlled and impartial, thus the name Adam. We now employ the bias-corrected weight parameters (m hat)p and (v hat)p instead of the regular weight parameters mp and vp. We get the following results when we plug them into our general formula.
Performance: Adam optimizer improves on preceding models' attributes to give far higher performance than previously used models, outperforming them by a huge margin in terms of giving an optimised gradient descent. In terms of training cost (minimal) and performance, Adam Optimizer exceeds the majority of the optimizer by a considerable margin (high).

Dropout layer: Layer with a dropout In this method, We just eliminate part of the neural network's neurons at random to lower the likelihood of over fitting and also the model's processing cost. It aids in drawing emphasis to more powerful features while also reducing the weight age of common features.

Mean Square Error: It's the total of the squared differences between the actual and expected or estimated values. By obtaining mean square error we can tell the accuracy of output value and know efficiency that our model was working on provided data.

RESULTS

A. Original vs Predicted Data
We have predicted the taken data using LSTM and there is only very very slight difference between original and predicted data.

B. Prediction for future 30 days
We will predict future 30 days by taking last 100 days data The Last 100 days of the test data is taken as temporary input and we will add every output of future days as input to it. This condition will go on until it complete 30 times. At last, we will get 130 days of input and last 30 days are the future one's.

Datasets That Have Been Used:
This dataset covers all of the information for each financial company, including the opening and closing prices, the highest and lowest prices, the number of shares, and the growth or reduction in stock prices. the datasets we have taken are
Apple Company (AAPL)- 27-05-2016 to 04-01-2022
Oracle Company(ORCL)- 08-02-2015 to 07-02-2021
IBM Company(IBM)- 08-02-2015 to 07-02-2021
Google Company(GOOGL)-08-02-2015 to 07-02-2021

CONCLUSION

Long Short-Term Memory (LSTM) outperforms other models such as decision trees, regression, and artificial neural networks (ANN) in terms of prediction accuracy. Our algorithm can track the evolution of opening prices for both assets, according to the testing results. This point of view analysis may be combined with the LSTM to increase weight training and accuracy. On the Yahoo financial dataset, two approaches were used in this paper: LSTM and Regression. Both strategies have shown an increase in the accuracy of forecasts, resulting in good outcomes. The use of the recently released machine learning algorithms to stock prediction has shown encouraging results, paving the way for their implementation in successful exchange systems. It has led to the conclusion that utilising machine learning techniques, it is feasible to anticipate the stock market with greater accuracy and efficiency. The popularity of stock market trading is increasing all the time, forcing specialists to seek out new methods for predicting the future. As a consequence, precision is achieved. The LSTM algorithm is more exact than the ARIMA approach. The LSTM's accuracy is on par with that of other future prediction systems. When compared to other prediction algorithms, LSTM is extremely accurate.

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Table 1: Accuracy Rate of Existing Methods

S.No	Existing Method	Accuracy
1	Artificial Neural Network	78
2	Support Vector Machine	85
3	Genetic Algorithms	92.8
4	Feed Forward Neural Network	93.4
5	Decision Tree	91.7
6	LSTM(Proposed Method)	95.4
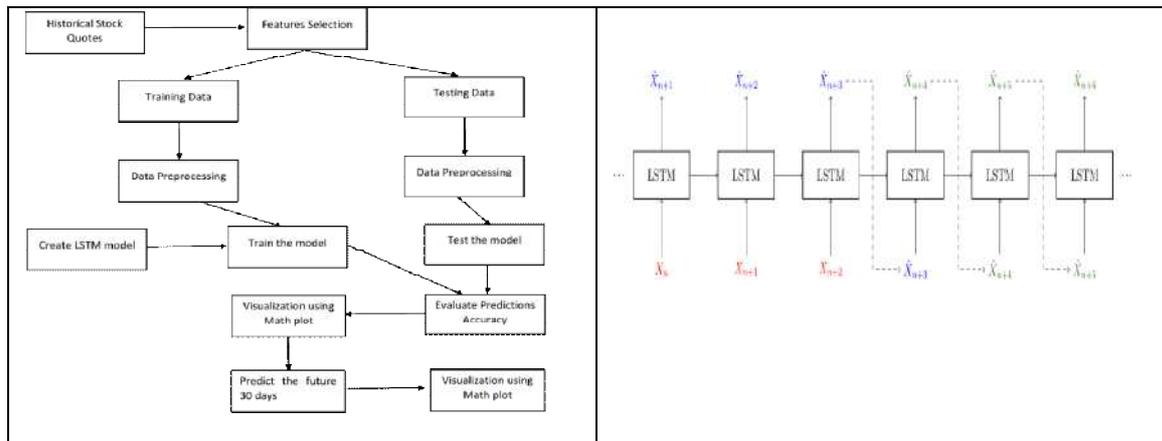
Figure 1:Activation function

Figure 2:Time Stamping
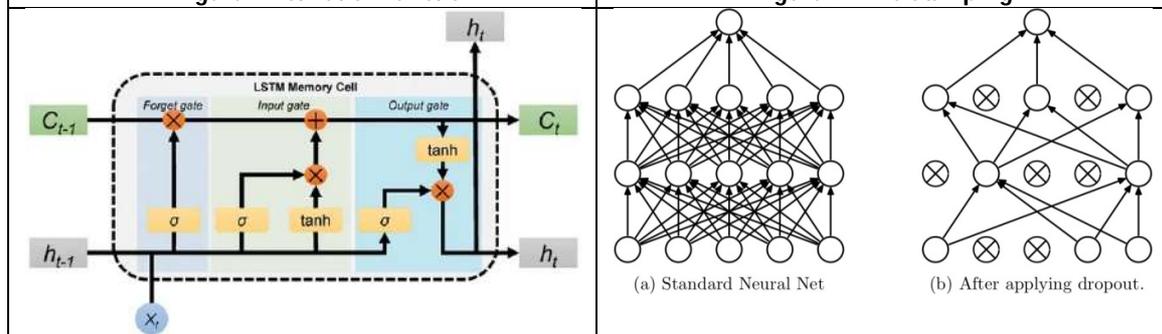
Figure 3: LSTM

Figure 4: Droputlayer
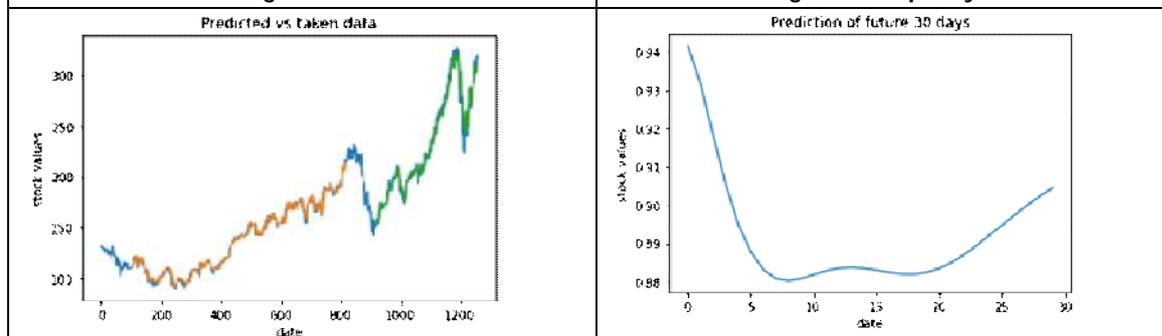
Figure 5: Output of takend at

Figure 6: Stock prices forn ext 30 days

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RESEARCH ARTICLE

Effect of Janda's Approach Versus Core Stability Exercise in Chronic Non-Specific Low Back Pain – A Comparative Study

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ABSTRACT

Non-specific low back pain is the pain that is not because of the determined known pathology. It is typically associated with pain, soreness and/or stiffness in the lower back region and functional disability in the patients. Aim of the study is to compare the effect of janda's approach and core stability exercise in chronic non-specific low back pain patients. Comparative study was conducted between 2 groups. 40 subjects were selected according to inclusion and exclusion criteria and divided into 2 groups. Group A was the janda's approach group and group B was the core stability exercise group. Both the groups perform exercise for 4 weeks. Before and after the end of the 4th week NPRS and MODI scale were taken. The data was entered and analyzed by using SPSS software version 25. Unpaired t-test was used and p-value is 0.000 so, statistically proven that there was significant difference between GROUP A AND GROUP B in patients with non-specific low back pain. This study concluded that janda's approach and core stability exercise both are the effective treatment for chronic non-specific low back pain but janda's approach is more effective than core stability exercise in reducing pain and improving function.

Keywords: Non-specific low back pain, lower crossed syndrome, janda's approach, core stability exercise

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INTRODUCTION

Low back pain (LBP) is the most common musculoskeletal condition and the most frequent cause of disability in adult population [1,2]. Low back pain is described as a pain or discomfort situated below the margin of the 12th rib and above the inferior gluteal fold, with or without leg pain [2]. Low back pain is divided into three group by Waddell which is known as 'diagnostic triage'. Three categories of low back pain are

• Specific spinal pathology
• Nerve root pain or radicular pain
• Nonspecific low back pain[2]

Infection, structural deformity, osteoporosis, fracture, inflammatory disorder like ankylosing spondylitis, radicular syndrome, cauda equina syndrome type of recognizable known pathology is not a reason for non-specific low back pain [2,9]. Non-specific low back pain is mainly occur due to muscular imbalance. ''S" shaped posture of the lower back identified by tight hip flexors and back muscles paired with weak abdominal muscle and gluteus Maxim us muscle is defined as lower crossed syndrome (LCS) and it is also known as pelvic crossed syndrome [4]. Tightness of the thoracolumbar extensors on the dorsal side crosses with tightness of the iliopsoas and rectus femoris along with that weakness of the deep abdominal muscle ventrally crosses with weakness of the gluteus Maxim us and medius are seen in lower crossed syndrome [4].

Based on the phylogenetic development Janda identified two categories of muscle. (Janda, 1987) [8,10].
Functionally, muscles are often classified as "tonic" or "phasic" which means flexors or extensors muscles. Muscles which are vulnerable to tightness or shortness are tonic system muscles and muscles which are prone to weakness or inhibition are phasic system muscles that is noted by Janda.[2]

According to the Janda's Approach, the treatment should be arranged into three stages:[5]
1. Normalization of the peripheral structures: Postural and ergonomic education and correcting the biomechanics of a peripheral joint helps in restoring proper posture alignment.
2. Restoration of muscle balance: In order to attempt the strength of a weakened muscle one should first addressed restoring normal muscle length.
3. Facilitation of afferent system and sensory motor training: improvement in movement coordination and improvement in ideal mechanical loading of biological structures is done by the facilitation of afferent system and sensory motor training [5].

The Janda's approach is effective treatment for correcting lumbar lordo sis, for increasing abdominal and gluteal muscle strength as well as decreasing tightness of hip flexors and back extensors muscles [5]. For non-specific low back pain core strengthening exercise (CSE) are the universal physiotherapy treatment.[3]these exercise helps in stabilizing the spine and train muscle activity pattern without unessential overloading the tissue [3]. In chronic low back pain condition core stability exercise are very effective treatment in short-term pain reduction and it will improve physical function in patients of low back pain.[6]Different core stability exercise are widely used for the treatment in patients with chronic low back pain [13]. The core stability exercise are used to strengthen the spinal muscles to improve their ability in maintaining the neutral spine by help of the abdominal, back, neck and shoulder girdle muscles as stabilizers rather than movers.

MATERIALS AND METHODS

RESEARCH METHOD
Study design: - Comparative Study
Source of Data:- Sainath physiotherapy clinic, Bopal, Ahmedabad

Sampling Method:-Simple Random Sampling
Sample Size:-40 low back pain patients
Study Duration:-4 weeks (3 session/week)

INCLUSION CRITERIA
- Age group between 25-45 years
- Patients with chronic non- specific low back pain diagnosed by orthopedic doctor.
- Male and female both
- Subjects who are willing to participate in my study.
- Patient with VAS grade 2 to 7.

EXCLUSION CRITERIA
- Back pain with trauma or any injury.
- Any neurological symptoms like prolapsed intervertebral disc (PIVD), radiculopathy.
- Any systemic pathological condition like rheumatoid arthritis, ankylosing spondylitis.
- History of any recent abdominal or back surgeries.
- Any congenital condition of lumbar spine.
- Pregnancy.
- Subjects who are on regular fitness program.

MATERIALS USED IN THE STUDY
Low mat, Pencil, Consent form, Paper, Pen

OUTCOME MEASURE
- Numeric Pain Rating Scale [NPRS]
- Modified Oswestry low back pain Disability questionnaire [MODI]

PROCEDURE
40 subjects were selected from sainath physiotherapy clinic whose age is between 25-45 years. Subjects were selected according to inclusion criteria remain subject were excluded according to the exclusion criteria of the study. The subject were divided into two equal groups; GROUP A and GROUP B by simple random sampling. Group A is Janda's approach group and group B is core stability exercise group. The total treatment session is for 4 weeks, 3 session/week. Pre and post treatment measure were taken by NPRS and modified Oswestry disability questionnaire of each subject in both the groups.

GROUP A (N=20) JANDA'S APPROACH
This group will receive JANDA'S APPROACH session for 4 weeks / three time in a week

1. Stretching exercise for erector spinae & back extensors muscle.
2. Stretching for iliopsoas and rectus femoris muscle.
3. Strengthening exercise for abdominal muscle.
4. Strengthening of gluteal Maxim us muscle.
- Stretching exercise performed actively 30 second hold 3 repetitions.
- Strengthening exercise performed by 10 second hold 10 repetitions. [1]

GROUP B (N=20):- CORE STABILITY EXERCISE
This group will receive core stability exercise for 4 weeks.

1. Curl up
2. Bridging

3. Side plank
4. Front plank
5. Pelvic floor exercise
6. Side lying with abduction
7. Straight leg raise
8. Tandem standing with perturbation in form of rapid arm movements
9. Diaphragmatic strengthening exercises
- 1st week – 6 reps
- 2nd week – 9 reps
- 3rd week – 12 reps
- 4th week – 15 reps with 5-10 sec hold.

RESULTS

Total 40 patients were selected of non-specific low back pain according to inclusion criteria and others were excluded according to exclusion criteria of the study. These 40 patients were randomly divided into 2 groups. 20 subjects in GROUP A in which janda's approach were given and 20 subjects in GROUP B in which core stability exercise were given. The data was entered and analyzed by using SPSS (statistical package for social science) software version 25.

In group A and group B, NPRS A PRE-POST is 4.4 and MODI A PRE-POST is 38.9   .
In group A and group B, NPRS B PRE-POST is 2.5 and MODI B PRE-POST is 30.35   .
So, group A has a significant effect than group B.

All of the above were shown between two group comparisons. Unpaired t-test was used and p- value is 0.000 so, statistically proven that there was significant difference between GROUP A (JANDA'S APPROACH GROUP) AND GROUP B (CORE STABILITY EXERCISE GROUP) in patients with non-specific low back pain. But, group A (JANDA'S APPROACH GROUP) is more effective to reduce pain and improve function than group B (CORE STABILTY EXERCISE GROUP). Hence alternative hypothesis is in favor of JANDA'S APPROACH (H1) is accepted and null hypothesis (H0) of no difference is rejected.

DISCUSSION

The present study was designed to compare the effect of janda's approach (stretching and strengthening exercise) and core stability exercise on patients with chronic non-specific low back pain. In this study 40 patients were selected whose age is between 25-45 years and according to other inclusion and exclusion criteria. Patients were randomly divided into 2 groups. GROUP A received JANDA'S APPROACH and GROUP B received core stability exercise for 4 weeks. Both the groups were assessed before and after the treatment to determine the reduction in pain and functional limitation by Numeric pain rating scale and modified Oswestry disability questionnaire. At the end of 4 weeks, the patients in both the group showed reduction in pain improvement in function. But janda's approach group showed statically significant improvement than core stability group. Thus, the study result shows that janda's approach (stretching and strengthening exercise) is more effective than core stability exercise on outcome of pain (NPRS) and function (MODI).

Darshana G Hirani investigate the effect of core stability exercise versus posterior pelvic tilt exercises on chronic low back pain. In which 30 patients were divided into 2 groups. Group A received core stability exercise and Group B received posterior pelvic tilt. NPRS and RMDS were taken as a outcome measure. They concluded that core stability exercise are more effective on chronic low back pain. So, core stability is also an effective treatment for low back pain.

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The findings of this study are similar to Sapna Nandlal Tank. Who did study on effect of janda's approach on pain and function in patients with non-specific low back pain- an interventional study [2]. In that study due to 3 dropouts 31 patients were divided into 2 groups; GROUP A was the control group and GROUP B was the janda's approach group. GROUP A was given hot pack -20 min, prone press up, core stability exercise and ergonomics for 2 weeks. GROUP B was given conventional physiotherapy plus stretching and strengthening exercise (janda's approach) for 2 weeks. Pain intensity and improvement in functions were measured by NPRS (numeric pain rating scale) and MODI (modified Oswestry disability questionnaire). The result concluded that stretching and strengthening exercise (JANDA'S APPROACH) along with conventional treatment is more effective than control group ($p < 0.05$).

Non-specific low back pain mainly occurs due to muscle imbalance. In which abdominal and gluteal muscle weakness is seen. Due to abdominal muscle weakness anterior pelvic tilt occurs and lordo tic curvature increases. Along with that tightness of the rectus femoris, iliopsoas and back extensors muscles occurs. In janda's approach stretching exercise for back extensors muscles, iliopsoas and rectus femoris muscle is given. Along with that strengthening of abdominal muscles and gluteal muscles is given. So, janda's approach is helpful in correcting muscle imbalance by strengthening of the abdominal and gluteal muscles and by stretching of tight muscles. It also corrects anterior pelvic tilt and lumbar lordo sis by reducing muscle imbalance. Therefore, reduction in pain and improvement in function is seen. So, janda's approach is more effective in reducing pain and improvement in function in patients with non-specific low back pain compare to core stability exercise as it corrects muscle imbalance.

CONCLUSION

This study concluded that janda's approach and core stability exercise both are the effective treatment for chronic non-specific low back pain but janda's approach is more effective than core stability exercise in reducing pain and improving function.

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Table 1. Comparison of Between Groups

OUTCOME	MEAN DF± SD	UNPAIRED T-TEST	p- VALUE
NPRS GROUP A (JANDA'S APPROACH)	4.400±0.754	-7.002	0.000
NPRS GROUP B (CORE STABILTY EXERCISE)	2.500±0.761		
MODI GROUP A(JANDA'S APPROACH)	38.900±7.799	-6.083	0.000
MODI GROUP B(CORE STABILTY EXERCISE)	30.350±11.784		
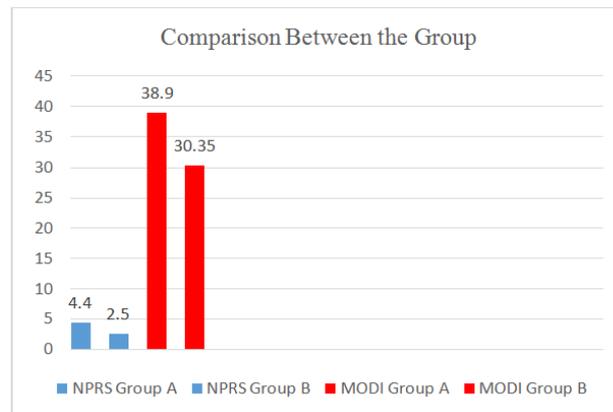
Graph 1. Comparison of Between Groups

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RESEARCH ARTICLE

Phytochemical Composition and *In vitro* Antioxidant Activity of Aerial Part of Aqueous Extract of *Stachytarpheta jamaicensis* (Verbenaceae)

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ABSTRACT

Medicinal plants are used by 80% of the world population as the only available medicines, especially in the embryonic country. *Stachytarpheta jamaicensis* (*S.jamaicensis*) is widely known for its high medicinal importance in traditional and folk medicinal systems in various countries. The objective of the present study was designed to evaluate the fluorescent characters, presence of phytochemical composition, quantify the total phenolic, alkaloids and flavonoid contents of aqueous extract of aerial part of *S.jamaicensis* and its *in vitro* antioxidant potential against free radicals. The *S.jamaicensis* was powdered and tested for fluorescence analysis, followed byextracted with aqueous and screened by qualitative and quantitative phytochemicals analysis. The *in vitro* antioxidant potential of the extract was assessed by using 1, 1-diphenyl-2-picryl-hydrazyl(DPPH), hydroxyl radical and superoxide radicals as free radical scavengers by using the spectrophotometric method. During the preliminary phytochemical analysis of this species exhibited the presence of phytochemical compounds like flavonoids, alkaloids, tannins, triterpenoids, polyphenols and steroidsas major phytochemical groups while saponins and glycosides were not detected. Alkaloids content of 12.78± 0.17 mg/100 gm, flavonoids and total phenolics content of 29.32 ±0.16 mg RE/ gm and 33.67 ±0.15 mg GAE/ gm respectively. The *in vitro* antioxidant activity of the species, of *S. jamaicensis* aerial part extract required for 50% inhibition of DPPH radical scavenging effect (IC$_{50}$) was recorded as 172.87μg/mL,hydroxyl radical scavenging activity was recorded as 178.58 μg/mL and superoxide radical scavenging activity was recorded as 232.79μg/mL. These findings clearly demonstrated that the aerial part of the aqueous extract of *S.jamaicensis* possesses different bioactive

compounds and has the potential to be a powerful antioxidant and appears to be an alternative to synthetic antioxidants and this justifies its therapeutic usage.

Keywords: *Stachytarpheta jamaicensis*, antioxidants, superoxide radicals, phytochemicals.

INTRODUCTION

Natural sources of bioactive compounds found in medicinal plants provide therapeutic benefits and affordable treatments against a wide range of ailments. Globally, medicinal plants have been used to treat diseases instead of chemically synthesized drugs. Global interest in medicinal plants has been growing as they contain a great deal of cultural knowledge and provide natural products that are vital to health security for millions of people. Medicinal plants are widely used and efficient, leading studies to extensively demonstrate their secondary metabolites, including their effect on various medicinal properties such as algesic, antimicrobial, antioxidant, antihypertensive, anti-nociceptive, antidiarrheal and anti-inflammatory [1]. Due to the potential benefits of herbal medicine in the treatment of various chronic and infectious diseases, phytochemical processing of antioxidant properties has been on the rise recently [2]. A free radical is an unstable molecule with unpaired electrons, electrically charged and highly reactive, causing damage to proteins, carbohydrates, lipids, and even nucleic acids and are commonly known as reactive oxygen species (ROS). In addition to being produced by the body's normal metabolism, they can also be induced by various environmental factors and chemicals. Free radicals include superoxide anion, hydrogen peroxide, hydroxyl and nitric oxide radicals. Oxidative stress occurs when the body produces more free radicals. These species are neutralized by antioxidant enzymes. There are numerous physiological disorders viz. cancer, Parkinson's disease, Alzheimer's disease, myocardial infarction and diabetes linked to an imbalance between the body's capacity to generate ROS and its ability to fight them with antioxidants [3].

Therefore, maintaining the balance between free radicals and antioxidants is crucial to health and well-being. Therefore, stability between free radicals and antioxidants is vital for proper physiological function. Polyphenols, which are found abundantly in medicinal plants, have been found to be good sources of natural antioxidant compounds, and they are more often superior to fruits and vegetables in terms of antioxidant activity. The consumption of these plant products protects the body from a number of health conditions caused by free radical-mediated cell damage [4]. There are many secondary metabolites present in plants that serve as sources of antioxidants and reduce oxidative stress by scavenging free radicals [5]. *Stachytarpheta jamaicensis* (L.) Vahl belongs to the Verbenaceae family and also known as Brazilian tea, verbena cimarrona, rooter comb, or blue porter weed [6].It is a wild species of herbaceous plant that grows in the warm tropical regions of America, as well as in the subtropical forests of Africa, Europe, Asia and Oceania. The flowers of *S.jamaicensis* are usually a mixture of bluish and pinkish colours, but could also occur with a purple or deep blue colour.

The leaves are opposite, green in colour and have a smooth surface. The petioles are unique to this species of *S.jamaicensis* because of the distinct apex and petal regions [7]. The essential medicinal herb *S.jamaicensis* has a critical function in a wide range of traditional and folk medicines, in particular the conventional ones. Historically, it is used to treat respiratory conditions and allergies, as well as treat coughs, colds, fever, constipation, digestive complications and dysentery and to promote menstruation among many other uses. It has also been shown to contain numerous phytochemical constituents, which are essential to health and wellness. A number of illnesses are treated with leaves, stems, and roots of this plant. We may be able to improve traditional-ethnic drugs from *S.jamaicensis* in the near future as the pharmaceutical industry seeks to grow new drugs from herbal sources. In order to improve and introduce recent products into the community in order to give them greater economic and therapeutic potential, systematic studies and development work must be carried out [8].With the aim of this study, we hope to investigate phytoconstituents and antioxidant capacity of *S.jamaicensis* aerial extracts using several *in vitro* methods.

MATERIALS AND METHODS

Collection of plant material

The plant material used for this research became developed, mature, fresh and healthful aerial part of *S.jamaicensis* collected from in and around the region of Dharmapuri district of Tamil Nadu, India. The collected plant was recognized by Dr. P. Radha, Research Officer (Botany), Central Council for Research in Siddha, Govt of India, Mettur Dam, Salem, Tamil Nadu, India. The plant was washed with distilled water and dried at an environmental temperature (27-37ºC day time) for two weeks within side the absence of sunlight. After drying, the aerial part was pulverized to obtain a fine powder and packed in airtight bottles and saved at room temperature awaiting extraction.

Fluorescence analysis

The development of an identification system for powdered vegetable drugs based on the fluorescence of the drugs. In order to determine the fluorescence properties of dried powdered aerial parts of S.jamaicensis, different chemicals were applied. It is common for substances that are not fluorescent to be converted into fluorescent derivatives or decomposition products by interacting with different reagents. They had been subjected to fluorescence evaluation in daylight and in short UV- light (254 nm) and long UV- light (365 nm).

Processing of plant

The powder was extracted in distilled water with the use of a Soxhlet apparatus for 6 hours. This extract was focused on a rotary evaporator at 65◦C. To evaporate the final solvent, the extract was kept in an oven at a temperature of 40-50ºC for 8 hrs. The concentrated extract of the aerial part of *S.jamaicensis* was stored in an airtight closed box and stored at 4°C waiting for use in phytochemicals evaluation and *in vitro* bioassay.

Phytochemical screening

Bioactive non-nutrient plant compounds screening of the above extract was carried out through the use of the usual protocolsfor the presence of alkaloids, flavonoids, saponins, tannins, glycosides, proteins, terpenoids, steroids, carbohydrates and polysterols. Detection of those phytochemicals was based on visual observation following colour change or formation of a precipitate after the addition of specific reagents. Furthermore, the amount of some essential phytochemicals like alkaloids and flavonoids were performed based on the method [9,10] respectively. Total phenolics was estimated through the method [11] andtotal carbohydrate was determined by the method [12].

Evaluation of *in vitro* antioxidant scavenging assays of the studied plant extract

The aqueous extract of aerial part of *S.jamaicensis* was used for the free radical scavenging ability *in vitro* by different chemical assays: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay[13], hydroxyl radical scavenging assay[14] and superoxide radical scavenging assay[15]. All the experiments were performed in triplicates and average values were taken.

DPPH radical scavenging activity

The DPPH is a stable; nitrogen cantered free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH (1, 1- diphenyl-2-picryl-hydrazine). The degree of change in colour from purple to yellow can be used as a measure of the scavenging potential of antioxidants in extracts [16].

Prepared 0.1 mM solution of DPPH in methanol freshly and 1 ml of this solution was added to 3 ml of the solution of all extracts in methanol at different concentrations (60, 120, 180,240 and 300µg/ml). The mixtures were shaken vigorously and allowed to stand for 30 min at 27C in dark at room temperature. The control was prepared as above without any extract, and methanol was used for the baseline correction. Then the absorbances were measured at

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517nm using a UV-VIS spectrophotometer (Genesys 10s UV: Thermo electron corporation). Tannic acid was used as a standard. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity. Tannic acid was used as a standard compound (positive control) with concentrations 2-10µg/ml. The capability of scavenging the DPPH radical was calculated by using the following formula. DPPH Scavenging effect (%) = (Control OD-sample OD)/Control OD) x 100

Hydroxyl radical (·OH) scavenging activity

Stock solution of EDTA (1 mM), $FeCl_3$ (10nM), ascorbic acid (1mM), H_2O_2 (10mM) and deoxyribose, (10nM), were prepared in distilled deionised water. The assay was performed by adding 0.1 ml EDTA. 0.01 ml of $FeCl_3$, 0.1 ml H_2O_2, 0.36 ml of deoxyribose, 1 ml of the extracts of different concentrations 60, 120,180,240 and 300 µ/ml) dissolved in the distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 0.1 ml of ascorbicacidinsequence.Themixturewasthenincubatedat37°Cfor1hr.1mlportionofthe incubated mixture was mixed with 1ml of 1 ml of 10% TCA and 1 ml of 0.5% TBA in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen was measured spectroscopic at 532 nm. Mannitol was used as the reference compound (positive control) with concentrations 10-50µg/ml. The hydroxyl reference compound (positive control) with concentrations 10-50µg/ml. The radical scavenging activity of the extracts was reported as % inhibition of deoxyribose degradation and was calculated by using the following equation.
Hydroxyl radical scavenging effect (%) = (control OD – sample OD)/ control OD) x 100

Superoxide radical scavenging activity

The superoxide anion radicals were generated in 3 ml 0f Tris - HCl buffer (16mM, pH 8.0), containing 0.5 ml of nitro blue tetrazolium (NBT) (0.3 mM), 0.5 ml NADH (0.936 mM)solution, 1 mL extracts different concentration (60, 120, 180, 240 and 300 µg/ml) 0.5 ml tris- HCl buffer (16 mM, pH 8.0), The reaction was started by adding 0.5ml phenazine metho- sulphate (PMS) solution (0.12mM) to the mixture incubated at 25°C for 5 min and the absorbance was measured at 560nm against a blank sample, ascorbic acid. Quercetion was used as the reference compound (positive control) with concentrations 10-50 µg/mL. The percentage inhibition was calculated by using the following equation,
Superoxide radical scavenging effect (%) = (control OD – sample OD)/ control OD) ×100

Statistical analysis

The values of DPPH radical, hydroxyl radical and superoxide radical scavenging activity of the aqueous extract of aerial part of *S.jamaicensis* are expressed as mean±standard deviation of the response of three replicates per sample.

RESULTS AND DISCUSSION

The development of new therapeutic agents depends greatly on the development of bioactive non-nutrient compounds derived from plants.

Fluorescence analysis

Observations were carried out under UV light (254 nm and 365 nm) and visible light on powdered aerial parts of *S.jamaicensis* after remedying them with the various chemicals and the interpretations were summarizedin Table 1.According to the findings of the present study, the aerial part of the plant powder had a wide variety of fluorescent colours such as green, pale green, fluorescent green, brown and black in response to different chemical treatments. We also observed drab olive, ink blue and black charcoal under varying light condition. A plant substance is said to fluoresce when exposed to ultraviolet light, which indicates diverse nutritionally beneficial molecules are present. As a crude drug is assessed qualitatively in this way, it is an important parameter of pharmacognosy, as well as a means of detecting reliable models and noteworthy adulterants [17].Fluorescence studies help in the detection of drugs that are almost complicated to discriminate. These drugs show a correlation between their chemical components and

their fluorescent behavior under a variety of conditions. Analyzing the sample for adulteration provides information as to whether adulteration occurred, and therefore is an analytical tool used to diagnose adulteration [18].

Phytochemical screening in aqueous extract of *S.jamaicensis*

The therapeutic propensity of the plants can be assessed by performing initial qualitative screening to ensure the presence of phytochemicals. It is the initial step in the discovery of new lead compounds and different types of natural products in herbal medicine. In addition, it may be used to search for bioactive agents that can be used to synthesize very useful drugs [19]. Plant extractions can be carried out using aqueous solutions due to their low toxicity. As listed in Tables 2 and 3, different phytochemical constituents were quantified and analyzed by using standard protocols in an aqueous extract of *S.jamaicensis* aerial part. The present study evidenced that a wide range of biologically dynamic ingredients like alkaloids, flavonoids, tannin, proteins, triterpenoids and steroids were present in aqueous extract (Table 2). There are a number of phytochemical compounds that are involved in the medicinal properties of this plant. However, in our study, the aqueous extract not exhibited the presence of saponins, coumarin and glycosides but are rather due to low concentrations of these compounds. Compounds belonging to the respective groups have been reported to impart various medicinal characteristics to the plants. Tannins are the polyphenolic compounds obtained from plants, have tremendous activity against diarrhea, hemorrhage, virus and hemorrhoids, bacteria, fungi and parasites and also impart anti-cancer and cytotoxic activity [20]. Presence of these compounds justifies the therapeutic potential of *S.jamaicensi*. Hence *S.jamaicensi* is used for the treatment of alarge number of infectious diseases. The flavonoids and total phenolic compounds in the sample were expressed as rutin equivalents and gallic acid equivalents respectively. As shown in Table 3, total phenolic contents obtained were 33.67 ± 0.15 mg GAE/g and total flavonoid contents obtained were 29.32 ± 0.16 mg RE/ g of the aqueous extract of *S.jamaicensis*. A phytochemical is defined as a non-nutritional compound produced by plants as a form of self-defense against pests, microbes, and environmental stressors. Phenolic compounds are the key phytochemicals and have been touted as accounting for most of the free radical scavenging activity of plants or plant products[21].The development of natural antioxidant compounds from plants has generated a lot of interest among scientists. According to several studies, total phenolic content is strongly related to highly effective free radical scavengers in terms of high antioxidant activity and redox properties [22] and therefore, reduce the risk of developing certain degenerative diseases [23].These compounds possess diverse biological activities, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities [24]. Phytochemical analysis suggests that the plant may serve as a potential source of antioxidants, since it contains phenolic compounds [25]. Therefore, it is expected that this species will have many medicinal applications. In the current work, alkaloids, flavonoids and phenols were present in considerable quantity in the plant extract. Fruits, vegetables and medicinal plants produce these secondary metabolites in large amounts, which contribute significantly to their antioxidant activity.A wide array of microorganisms *in vitro* have been shown to be susceptible to the antimicrobial effects of flavonoids, which are hydroxylated phenolic compounds synthesized by plants in response to microbial infection [26]. They possess anti-allergic, anti-inflammatory, antiviral, anti-cancer and antioxidant activities. They probably produce their activity by forming complexes with extracellular and soluble proteins and with bacteria's cell walls. The antioxidant properties of phenolic compounds are well demonstrated *in vitro* and *in vivo* in association with their ability to prevent oxidative damage to lipids and lipoproteins [27]. A number of plants have been screened to determine whether they contain compounds that are therapeutic in nature and have been identified as having a therapeutic role [28].

Antioxidant activity

An aqueous extract of the aerial part of *S.jamaicensis* was screened for antioxidant activity against several free radicals by measuring the levels of various radical scavengers, including DPPH radical scavengers, hydroxyl radical scavengers and superoxide radical scavengers. The results are reported in Tables 4,5 and 6 in the form of percentage inhibition (n=3) as well as IC_{50} value. Hydroxyl radical is a potent reactive species that causes severe pathogeneses to cell membrane phospholipids and reacts with polyunsaturated fatty acids. It is a very toxic and short-lived free radical which *initiates* chain reaction that damages cellular integrity [29].The results for hydroxyl radical scavenging activity of aqueous extract of aerial part of S.jamaicensis with compared to *manifold* are reported in Table 5. The

extract exhibited dose-dependent scavenging potential. However, the efficacy was found to be lower than that of *manifold*. The IC$_{50}$ values are defined as the concentrations required to quench 50% of free radicals in the reaction mixture under the assay conditions, and lower IC$_{50}$ values indicate greater antiradical activity. By plotting the corresponding extract concentration as the scavenging effect, it was calculated graphically in the linear range using a calibration curve[30]. The DPPH is one of the stable free radicals with a purple color powder that loses its chromophore after reacting with proton donors such as phenols to turn into a yellow pigment (diphenyl-picrythydrazine). Reduction in the color was measured by spectrophotometer (λmax 517). The change in colour extent depends upon scavenging capabilities of antioxidant crude extract [31]. Using water extract of the aerial part of S.jamaicensis, we found that the free radical scavenging activity increased with increasing doses of DPPH radical scavenging activity with an IC$_{50}$ value of 172.87g/mL. However, the DPPH radical scavenging effect of tannic acid was higher than the extract of *S.jamaicensis.* The extract exhibited high antioxidant activity against a variety of free radicals, and during the experiment, the concentration of the extract influenced the degree of antioxidant activity, confirming that antioxidant activity depends on the extract concentration. Antioxidant capacity of a sample can be attributed mainly to the presence of three major groups of polyphenolic compounds (phenolic compounds, flavonoids and tannins). Polyphenolic compounds are responsible for most of a sample's antioxidant capacity [32]. Among them, phenolic compounds are the largest group of polyphenols that accounted for the antioxidant activity of plants [33].In this study, it was shown that the antioxidant potential of the aqueous extract of *S.jamaicensis* aerial part may be attributable to the presence of a number of phytochemical compounds that act as free radical scavengers in the body and helps protect the body against the damaging effects of free radicals, which can be used as potent sources of natural antioxidants. With all these results, we can conclude that *S. jamaicensis* can be used as a source of safe and natural antioxidant compounds.

CONCLUSION

S.jamaicensis aqueous extract is rich in phytochemicals with proven antioxidant activities. The phytochemical analysis conducted on the aerial parts of aqueous extract revealed the presence of pharmacologically active phytochemicals. The overall therapeutic activities might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that aerial parts of the investigated plant could be a potential source of natural antioxidant that could have importance as therapeutic agents in preventing or slowing the progress of aging and age-associated oxidative stress-related degenerative disease. Consumable foods should contain natural products, which have multi-therapeutic phytochemicals that help to restore the balance between the natural antioxidants, free radical scavenging and enhance body defense against disease. In the future, the compound which is responsible for pharmacological activities will be purified and used as a commercial drug. All of the results obtained above suggested that *S.jamaicansis* could be used to produce safe and natural antioxidant compounds that have a potential role in treating oxidative stress-related diseases.

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Table 1: Fluorescence Analysis of Aerial Part of *S.jamaicensis* Powder

S. No.	Experiments	Visible day light	UV- light	
			Short wave length (254nm)	Long wave length (365nm)
1	Powder as such	Brown	Green	Black
2	Powder+ 1N NaOH	Green	Fluorescent Green	Dark Green
3	Powder+ Conc. HCl	Brown	Light Green	Ink Blue
4	Powder+ Conc. H_2SO_4	Dark Brown	Cadmium Green	Black
5	Powder+ 50% HNO_3	Coffee Brown	Olive drab	Charcoal Black
6	Powder+ Acetic acid	Green	Dark Green	Dark Black
7	Powder+ Fecl$_3$	Light Green	Dark Green	Dark Black
8	Powder+ Petroleum Ether	Green	Green	Charcoal Black
9	Powder+ Acetone	Brown	Dark Green	Black
10	Powder+ Chloroform	Brown	Light Black	Charcoal Black
11	Powder +Ethanol	Dark Green	Light Green	Black
12	Powder+ Methanol	Brown	Fluorescence Green	Dark Black
13	Powder+ Ammonia	Light Green	Green	Charcoal Black
14	Powder+ 40% NaOH+ 10% Lead acetate	Brown	Fluorescence Green	Black
15	Powder+ 1N HCl	Brown	Light Green	Ink Blue

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Table 2: Preliminary Qualitative Phytochemical Analysis of Aqueous Extract of Aerial Part of *S.jamaicensis*

S.No.	Phytochemicals	Results
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	-
4	Tannins	+
5	Glycosides	-
6	Proteins	+
7	Terpenoids	+
8	Steroids	+
9	Polyphenols	+
10	Carbohydrates	+
11	Coumarin	-

Note: +: Present, -: Not present

Table *3*: Quantitative Phytochemical Analysis of Aqueous Extract of Aerial Part of *S.jamaicensis*

S.No.	Phytochemicals	Results
1	Alkaloids (mg/ 100 gm)	12.78±0.17
2	Total flavonoid contents (mg RE/ gm of extract)	29.32±0.16
3	Total phenolic contents (mg GAE/ gm of extract)	33.67±0.15
4	Total carbohydrates (mg glucose/ gm of extract)	25.89±0.12

Values are mean of three independent analyses of the extract ± standard deviation (n = 3)
RE-Rutin equivalents; GAE – Gallic acid equivalents

Table 4: DPPH Radical Scavenging Activity of Varying Concentrations of Aqueous Extract of Aerial Part of *S.jamaicensis*

Sample extract dose (µl/mL)	Percentage of Inhibition	Standard Tannic acid	
		Concentration (µg/mL)	Percentage of Inhibition
100	35.34±0.17	2	20.12±0.22
200	55.14±0.22	4	39.01±0.15
300	74.06±0.03	6	50.15±0.33
400	82.58±0.11	8	55.45±0.42
500	90.72±0.31	10	66.72±0.35
IC_{50} (µg/mL)	172.87		6.676

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Table 5: Hydroxyl Radical Scavenging Activity of Varying Concentrations of Aqueous Extract of Aerial Part of *S.jamaicensis*

Sample extract dose (µl/mL)	Percentage of Inhibition	Standard mannitol	
		Concentration (µg/mL)	Percentage of Inhibition
100	32.42±0.25	10	15.22±0.11
200	51.15±0.11	20	25.45±0.05
300	85.12±0.08	30	40.12±0.36
400	102.17±0.14	40	54.11±0.15
500	145.36±0.37	50	72.01±0.48
IC$_{50}$ (µg/ml)	**178.58**		**34.244**

Values are mean of three independent analyses of the extract ± standard deviation (n = 3)

Table 6: Superoxide radical scavenging activity of varying concentrations of aqueous extract of aerial part of *S.jamaicensis*

Sample extract dose (µl/mL)	Percentage of Inhibition	Standard Quercetin	
		Concentration (µg/mL)	Percentage of Inhibition
100	20.24±0.14	10	14.15±0.21
200	42.14±0.44	20	28.47±0.47
300	69.22±0.23	30	40.22±0.44
400	84.58±0.01	40	65.10±0.16
500	105.12±0.15	50	84.01±0.45
IC$_{50}$ (µg/mL)	**232.79**		**24.658**

Values are mean of three independent analyses of the extract ± standard deviation (n = 3)

RESEARCH ARTICLE

On-line Customers Buying Behaviour Prediction using XGBoost Algorithms in Python

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ABSTRACT

The trend of online shopping is increasing day by day. The seller firms like amazon, Flipkart, etc. are continuously collecting the shopping data and doing research on it to improve their business or return on investment (ROI). Research is carried out on this data using high computing and machine learning techniques like supervised and unsupervised learning methods. The prediction of customer buying behaviour is a challenging task because customer behaviour is depending on many factors and it very dynamic in nature. Any activity performed by customer on e-commerce website is known as its behaviour. As a case study a dataset related to Diwali purchase is used and predict whether customer will purchase in next Diwali or not. In this dataset we have different type customers like Male and Female customers, married and unmarried customers, customers having one, two or three children's, etc. Under ensemble learning method we used XG Boost algorithms to predict the customer behaviour. Python is used to implement the XG Boost algorithms on Diwali customers buying data. Customer behaviour is a very important activity of any business. Therefore, to improve the ROI any firm needs to analyse customer behaviour and act accordingly. For success of any business customers are the key elements.

Keywords: Customer's Behaviour, ROI, Ensemble Learning, XG Boost, Python, Machine Learning.

INTRODUCTION

Broadly customer behaviour can be categories as online and offline customer behaviour. Offline shopping depends on physical movement, rush hours, nature of shopkeeper, communication skills, physical examinations of products, distance between shop and customers, mode of travels, etc., but these factors does not have any effect on online shopping. Therefore, online shopping is easier and more convenient than offline shopping. E-commerce websites giving the more facilities to the customers like read the reviews about any product, secure digital payment, buy from anywhere and at any time, cart the product for future purchase, home delivery, easy way to return of the product, etc., so customers are being attracted towards online shopping.

The act of a person during the buy of products or services is known as customer buying behaviour. These acts are regularly and continuously stored in electronic form and known as customers buying behaviour data. In this study we are using customer buying data of a retail company. The data is related to Diwali festival which is a biggest festival of India and peoples do more purchase on Diwali. This secondary data contains more than 45 columns and more than 5 lakhs records. The data contains gender, marital status, number of childes, age, location, etc. of customers. After data pre processing of data XG Boost algorithms applied on this data and make some predictions. Ensemble learning is learning from individual models, which are combined and final model is prepared. Ensemble learning gives good result because it minimizes the biasness. Ensemble learning is divided into two categories known as bagging and boosting. In bagging different bags of data (with replacement) is created from actual data and separate model is applied on each bag of data. In this process learning happens from different angles of data. Decision tree and random forest algorithms are the examples of bagging techniques. On the other hands ADA Boost and XG Boost algorithms are the examples of boosting technique. In bagging technique learning process is parallel, all individual models have equal weight age in final model and all individual trees are fully grown tree. In boosting technique learning process is sequential, all individual models do not have equal weight age in final model and all individual trees are not fully grown tree. XG Boost (Extreme Gradient Boost) is an implementation of Gradient Boosting algorithms. It is fast and giving good result with more data. XG Boost parameters can be divided into three types known as general parameters, booster parameters and task parameters. Tuning of XG Boost parameters is an important task because result is depending on the values of theses parameters.

OBJECTIVE OF THE STUDY

The objective of this study is to analyse the customer buying data and predict the customer behaviour using XG Boost algorithms. This secondary data is related to Diwali festival. Peoples do more buying in this festival and we predict whether peoples will buy in next Diwali or not. Using python, machine learning tools are applied on this data and predicts the customers buying behaviour. To get the improved results we must tune the parameters of XG Boost algorithms.

RESEARCH METHODS

Scikit-learn is a popular, commercially usable open-source machine learning package which is written in python, C & C++. It is used in supervised and unsupervised learning. The main functionality of this tools/library is clustering, classification, regression. It is built on NumPy and SciPy. The XG Boost algorithms with followings parameters are used.

```
XGBClassifier(base_score=None, booster=None, colsample_bylevel=None,
colsample_bynode=None, colsample_bytree=None, enable_categorical=False,  gamma=None,
gpu_id=None, importance_type=None, interaction_constraints=None,
learning_rate=None,max_delta_step=None, max_depth=None, min_child_weight=None,
missing=nan, monotone_constraints=None, n_estimators=100, n_jobs=None,
num_parallel_tree=None, predictor=None,  random_state=None, reg_alpha=None,
```

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```
reg_lambda=None, scale_pos_weight=None,  subsample=None, tree_method=None,
validate_parameters=None, verbosity=None)
```

The research methods may be divided into many steps like data collection, handling null and missing values, conversions of data, adding and removing attributes, data splitting, model selection and deployment, interpretation of results.

Data Collection
The data is searched on internet and download the suitable data. This secondary data is related to Diwali festival purchase, which contains more than 45 columns/attributes and more than 5 lakhs records. Each record is a transaction performed by a customer. The data attributes contains gender, marital status, number of childes, age, location, etc. of customers

Description of Attributes
Table 1 contains four columns namely attributes names, data types of attributes, meaning of attributes, meaning of binary data. This table 1 describe the dataset.

Data Pre-Processing
The secondary data may contain many attributes which is of no use, null values in the dataset, abnormal values of attributes known as outliers. During data pre-processing we deals with whole dataset and make it suitable to our model to get better results. Inter Quartile Range (IQR) Outlier Treatment done and outliers are removed from the attributes: NO_OF_CHILDREN, LTD_QUANTITY, LTD_AMOUNT, FTD_QUANTITY, FTD_AMOUNT, TOTAL_TRANSACTIONS_OVERALL, ADGBT_OVERALL, TOTAL_TRANSACTION_AMOUNT_OVERALL, TOTAL_QUANTITY_OVERALL, TOTAL_DISCOUNT_OVERALL, AVERAGE_TRANSACTION_AMOUNT_ OVERALL, AVERAGE_QUANTITY_OVERALL, AVERAGE_DISCOUNT_OVERALL, PREMIUM_PRODUCT, TOTAL_OCASSION_PRODUCT, RECENCY, DIWALI_TRANSACTIONS, DIWALI_AMOUNT.

MODEL PREPARATION
Machine learning algorithms needs numerical data not string type data. All the needed attributes having categorical data are converted to numerical data by creating dummy attributes. Further gender, marital status and number of childes are combined and make a new attributes *gmc*. Where g stands gender, m stands marital status, c stands having child or not. The gmc attributes contains 16 different categorical values. Gender contains three types of values, N stands for Null value for gender, M stands for Male, F stands Female. Marital status also contains three types of values, N Stands for Null value for marital status, S stands for single (unmarried), D stands for double (married). C contains two types of values, Y stands for yes (have child), N stands for no (not have child). The gmc contains values like NNN', 'MDN', 'FNN', 'MNN', 'FDN', 'MSN', 'FSN', 'NSN', 'FDY','NDN', 'MDY', 'FNY', 'FSY', 'NDY', 'MNY', 'NNY', 'NSY', 'MSY'. All these values with their percentage are displayed in fig 1.

The *train_test_split* method is used to split dataset into two parts train and test having training test ratio 80 and 20% respectively. Model learn the patterns from training dataset and test it on testing dataset.

X_train,X_test,y_train,y_test = train_test_split(features_final,Response, test_size = 0.20)

Independent variables training dataset: (423724, 90)
Dependent variable training (y): (423724,)
Independent variables testing dataset: (105932, 90)
Dependent variable testing (y): (105932,)

After splitting the dataset into training and testing datasets the model is trained with the following parameters. base_score=0.5, booster='gbtree', colsample_bylevel=1, colsample_bynode=1, colsample_bytree=1, gamma=0,

gpu_id=-1, importance_type='gain', interaction_constraints='', learning_rate=0.3, max_delta_step=0, max_depth=6, min_child_weight=1, missing=nan, monotone_constraints='()', n_estimators=100, n_jobs=16, num_parallel_tree=1, random_state=0, reg_alpha=0, reg_lambda=1, scale_pos_weight=1, subsample=1,tree_method='exact', validate_parameters=1, verbosity=None.

Fig2 shows the 30 attributes with their F score which play important role in prediction of next buying using XG Boost model and the accuracy is more than 90%. Table 5 is Confusion Metrics containing four types of values True Positive (TP), True Negative (TN), False Positive (FP) and False Negative (FN). As per confusion metrics TP = 156, TN = 95309, FP = 198 and FN = 10269. Using these values Precision, Recall, F1 score and Accuracy are calculated as:

Precession
Precision is defined as out of all positive predictions what percentage is true positive and its value lies between 0 & 1.
Precision = TP / (TP+FP)
Precision = 156 / (156+198) =156/354 = 0.440677

Recall
Recall is defined as out of all positive predictions what percentage is predicted positive.
Recall = TP / (TP+FN)
Recall = 156/ (156+10269) = 156 / 10425 = 0.014964

F1 Score
F1 score is the harmonic mean of precision and recall. F1 score use both false positive(FP) and false negative (FN) values.
F1 score = 2*(Precision * Recall) / (Precision + Recall)
F1 score = 2 * (0.440677 * 0.014964) / (0.440677 + 0.014964)
F1 score = 0.013188 / 0.455641 = 0.028943

Accuracy
Accuracy represents the correctly classified data over total data. It is good for balanced data and not good for imbalanced data.
Accuracy = (TP + TN) / (TP+TN+FP+FN)
Accuracy = (95309 + 156) / (95309 +156 + 198 +10269)   = 95465 / 105932
Accuracy = 0.901191, which is more than 90%.

RESULT

Dataset having more than 5 lakhs records is used to predict the next purchase on the Diwali festival based on past purchased data. The dataset was having null values and outliers which was treated well using proper methods, to get the better results. The whole dataset divided into 80% training and 20% testing dataset using train_test_split function. The responder's attributes are taken as dependent variables and others are taken as independent varibles. XG Boost model is applied on dataset with the parameters.

base_score=0.5, booster='gbtree',colsample_bylevel=1,colsample_bynode=1, colsample_bytree=1, gamma=0, gpu_id=-1, importance_type='gain', interaction_constraints='', learning_rate=0.3, max_delta_step=0, max_depth=6, min_child_weight=1, missing=nan, monotone_constraints='()',n_estimators=100,n_jobs=16,num_parallel_tree=1,random_state=0, reg_alpha=0, reg_lambda=1, scale_pos_weight=1, subsample=1, tree_method='exact', validate_parameters=1, verbosity=None.

Using these parameter values XG Boost model produced output as Accuracy: 0.9011913302873541,   Precision: 0.4406779661016949, Recall: 0.014964028776978418 and fbeta: 0.8362072893810687. Table 4 is the classification report

showing precision, recall, f1-score, support, accuracy, macro and weighted average. The XG Boost model giving more than 90% accuracy by using above mentioned parameter values. This accuracy is good and can be improved by changing the parameter values. The study shows that small change in learning_rate parameter value has great impact accuracy.

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Table 1: -Details of data attributes

Column Names	Data Types	Meaning	Data Values
New_ID	int64	Customer ID	
GENDER	object	Gender of the Customer	
MARITAL_STATUS	object	Marital Status of the Customer	
NO_OF_CHILDREN	int64	No of children of the Customer	
CHILD1GENDER	object	Gender of Child 1	
CHILD2GENDER	object	Gender of Child 2	
CHILD3GENDER	object	Gender of Child 3	
LTD_OCCASION	int64	Was last transaction on Occasion	0: NO, 1: YES
LTD_OFFER_APPLIED	int64	Was an offer applied on last transaction	0: NO, 1: YES
LTD_QUANTITY	int64	Quantity purchased on last transaction	
LTD_AMOUNT	int64	Amount paid on last transaction	
FTD_OCCASION	int64	Was first transaction on occasion	0: NO, 1: YES
FTD_QUANTITY	int64	Was an offer applied on first transaction	0: NO, 1: YES
FTD_OFFER_APPLIED	int64	Quantity purchased on First transaction	
FTD_AMOUNT	int64	Amount paid on First transaction	
TOTAL_TRANSACTIONS_OVERALL	int64	Total transactions till date	
CUSTOMER_LEVEL_OVERALL	object	Customer level depending on transactions	
ENGAGEMENT_DAYS_OVERALL	int64	Total distinct days customer transacted	
ADGBT_OVERALL	int64	Average days gap between customers transactions	
TOTAL_TRANSACTIO	int64	Total amount paid by customer till date	

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N_AMOUNT_OVERALL			
TOTAL_QUANTITY_OVERALL	int64	Total Quantity purchased by customer till date	
TOTAL_DISCOUNT_OVERALL	int64	Total Discount availed by customer till date	
AVERAGE_TRANSACTION_AMOUNT_OVERALL	int64	Total Amount / Total Transactions	
AVERAGE_QUANTITY_OVERALL	int64	Total Quantity / Total Transactions	
AVERAGE_DISCOUNT_OVERALL	int64	Total Discount / Total Transactions	
TOTAL_BIRTHDAY_PRODUCT	int64	Total Products purchased by customer on the birthday	
PREMIUM_PRODUCT	int64	Total premium product purchased by the customer	
TOTAL_ANNIVERSARY_PRODUCT	int64	Total Products purchased by customer on the Anniversary	
TOTAL_OCASSION_PRODUCT	float64	Total Products purchased by customer on the occasions	
FAV_DAY	int64	Favorite day of the week of customer to make a purchase	
FAV_MONTH	int64	Favorite Month of the year of customer to make a purchase	
TIERNAME	object	Tier name of customer belongs to	
AGE	float64	Age of the Customer	
CITY	object	City of the customer	
STATE	object	State of the customer	
SPOUSEAGE	float64	Spouse age of the customer	
RECENCY	int64	Days since last transaction	
INACTIVITY_RATIO	int64	Days been inactive	
TENURE	int64	Days since first transaction	
DOB_DAYS_LEFT	float64	Days left for Customer's Birthday	
ANNIVERSARY_DAYS_LEFT	float64	Days left for Customer's Anniversary	
SPOUSE_DOB_DAYS_LEFT	float64	Days left for Customer's Spouse Birthday	
DIWALI_TRANSACTIONS	int64	Total Diwali Transactions	
DIWALI_AMOUNT	int64	Total Diwali Amount	
DIWALI_DISCOUNT	int64	Total Discount availed by customer till date	
PAST_DIWALI_PURCHASER	int64	Has customer bought anything in last Diwali's	
RESPONDERS	int64	Responded or not in Diwali 2017	0: NO, 1: YES

Table 2: - Null values display in percentages

Missing Value Column Name	No. of Missing Values	% of Missing Values
GENDER	86882	16.403477
MARITAL_STATUS	175064	33.052396
CHILD1GENDER	389077	73.458433
CHILD2GENDER	439493	82.977064
CHILD3GENDER	508891	96.079531
TOTAL_OCASSION_PRODUCT	251157	47.418891
CITY	107223	20.243894
STATE	136070	25.690259
DOB_DAYS_LEFT	113396	21.409368
ANNIVERSARY_DAYS_LEFT	277691	52.428557
SPOUSE_DOB_DAYS_LEFT	309811	58.492871

Table 3: - Outliers detection

Data Field Name	count	min	Max	Mean	std	25%	50%	75%
NO_OF_CHILDREN	529656	0	3	0.5	0.86	0.00	0	1
LTD_QUANTITY	529656	1	363	1.6	1.80	1.00	1	2
LTD_AMOUNT	529656	0	12198617	76085.9	120851.40	17659.00	39202	91915
FTD_QUANTITY	529656	1	500	2.1	2.79	1.00	1	2
FTD_AMOUNT	529656	0	18144500	79253.7	129840.76	16882.00	38660.5	93056.25
TOTAL_TRANSACTIONS_OVERALL	529656	2	119	2.8	1.59	2.00	2	3
ADGBT_OVERALL	529656	1	726	174.3	137.84	64.00	145	265
TOTAL_TRANSACTION_AMOUNT_OVERALL	529656	480	30343117	228117.5	348996.72	62779.75	131983	269214.5
TOTAL_QUANTITY_OVERALL	529656	2	704	5.2	5.75	2.00	4	6
TOTAL_DISCOUNT_OVERALL	529656	0	5465307	12217.9	45108.07	0.00	2268	8329
AVERAGE_TRANSACTION_AMOUNT_OVERALL	529656	240	15171558	77022.6	93651.83	26578.00	51355.5	95698
AVERAGE_QUANTITY_OVERALL	529656	1	251	1.6	1.50	1.00	1	2
AVERAGE_DISCOUNT_OVERALL	529656	0	1785534	4014.2	13772.56	0.00	882	3033
TOTAL_BIRTHDAY_PRODUCT	529656	0	112	0.3	1.00	0.00	0	0
PREMIUM_PRODUCT	529656	0	237	3.5	2.86	2.00	3	4
TOTAL_ANNIVERSARY_PRODUCT	529656	0	45	0.3	0.91	0.00	0	0
TOTAL_OCASSION_PRODUCT	529656	0	264	1.3	2.01	0.00	1	2
RECENCY	529656	0	728	216.5	166.95	88.00	171	320
DIWALI_TRANSACTIONS	529656	0	10	0.4	0.66	0.00	0	1

| DIWALI_AMOUNT | 529656 | 0 | 20303713 | 29533.5 | 107058.40 | 0.00 | 0 | 13783 |
| DIWALI_DISCOUNT | 529656 | 0 | 2572002 | 1048.7 | 11435.91 | 0.00 | 0 | 0 |

Table 4:- Classification Report

	Precision	Recall	f1-score	Support
0	0.90	1.00	0.95	95507
1	0.44	0.01	0.03	10425
Accuracy			0.90	105932
Macro avg	0.67	0.51	0.49	105932
Weighted avg	0.86	0.90	0.86	105932

Table 5:- Confusion Metrics

RESPONDERS	Predicted NO	Predicted YES
Actual NO	95309	198
Actual YES	10269	156
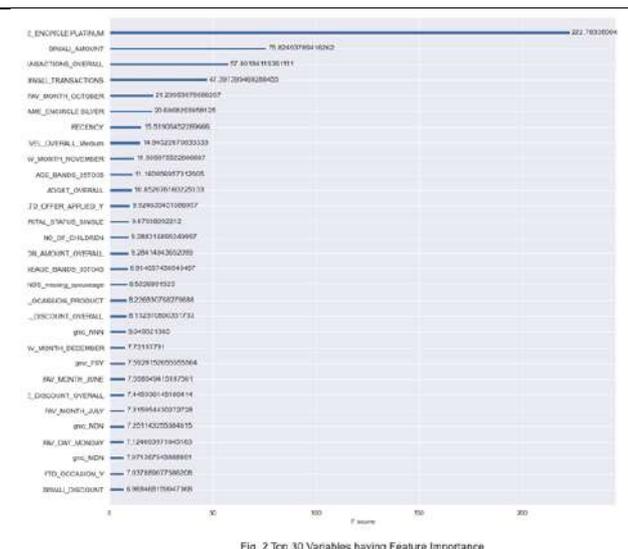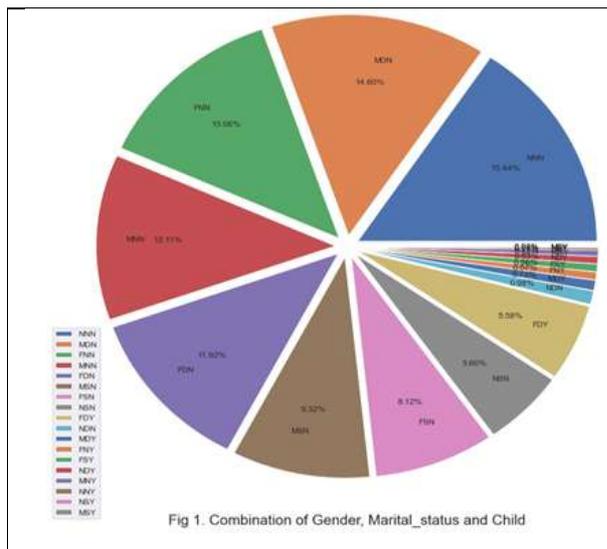
Fig 1. Combination of Gender, Marital_status and Child

Fig. 2 Top 30 Variables having Feature Importance

RESEARCH ARTICLE

Integrated Plant Nutrient System on the Soil Fertility and Performance of Greengram in Coastal Soil

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ABSTRACT

A field experiment was conducted in a farmer's field at Mandabam coastal village, near Chidambaram, Cuddalore District of Tamilnadu during January –March 2019. To find out the influence of integrated plant nutrient system on the soil fertility and performance of greengram in coastal soil. Texturally, the experimental soil was sandy loam with initial soil characteristics (0-15 cm layer) of the experimental site were, pH–8.37 and EC–4.05 dSm^{-1}. The soil registered low organic carbon status of 2.31 g kg^{-1}, 131.47 kg ha^{-1} of alkaline KMnO$_4$–N; 9.28 kg ha^{-1} of Olsen–P and 154.87 kg ha^{-1} of NH$_4$OAc-K, respectively. The available Zn (DTPA extractable Zn) content (0.71mg kg^{-1}) was also low in soil. The various INM treatments imposed in the study included T$_1$–Control (100% NPK/ RDF alone), T$_2$–100% NPK + FYM @ 12.5 t ha^{-1}, T$_3$ –100% NPK + Composted coirpith (CCP) @ 12.5 t ha^{-1}, T$_4$ –100% NPK + FYM @ 12.5 t ha^{-1} + Rhizobium @ 2.0 kg ha^{-1}(BF), T$_5$–100% NPK + CCP @ 12.5 t ha^{-1}+ Rhizobium (BF), T$_6$–100% NPK + FYM @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}, T$_7$–100% NPK + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1} , T$_8$–100% NPK + ZnEFYM @ 6.25 t ha^{-1} + BF + Pink Pigmented Facultative Methylotrophs (PPFM) @ 1.0 % Foliar spray and T$_9$ –100% NPK + ZnECCP @ 6.25 t ha^{-1} + BF + PPFM @ 1.0 % Foliar spray twice at pre flowering stage and flowering stage. The study included the above treatments which were arranged in a Randomized Block Design (RBD) with three replications, using greengram var. ADT 5. The results of the study indicated that the combined application of 100% recommended dose of NPK + BF + PPFM @ 1.0 % Foliar spray

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twice along with Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha⁻¹ was significantly superior in increasing the soil properties, yield and nutrients uptake by greengram.

Keywords: INM, Coastal Saline Soil, Greengram Yield, Nutrient Availability and Microbial Population.

INTRODUCTION

Pulses are the important sources of proteins, vitamins and minerals and are popularly known as "Poor man's meat" and "rich man's vegetables" contribute significantly to the nutritional security of the country. Among the pulses, greengram (or *Vigna radiata* L. is the third important pulse crop after chickpea and red gram, cultivated throughout India for its multipurpose uses as vegetables, pulse, fodder and green manure crop. The seeds are more palatable, nutritive, digestible and non-flatulent than other pulses grown in country. It occupies as good position due to its high seed protein content (25%), ability to store the soil fertility through symbiotic nitrogen fixation. It is grown usually as rainfed as well as rice fallow and being a photo-sensitive and short-duration crop, can also be grown during summer season. Greengram accounts for 10-12% of total pulse production in the country. In Tamilnadu, the area under greengram is 1.8 L ha, with production of 1.2 L t and productivity of 642 kg ha⁻¹ (Saravanan *et al.*, 2013). The area under salt affected soils in India and Tamilnadu are 6.74 m ha and 3.68 m ha. Productivity of most of the crops in salt affected soil is low due to salt stress. Greengram is important pulse crop that is grown in saline soil. However, the productivity of greengram in coastal saline soil is very low, because of less availability and uptake of nutrients and poor organic matter (Fageria *et al.*, 2011). Hence, use of amendments and application of farm yard manure and other organics reduces the effects of salt stress. Improper use of inorganic fertilizer depletes the soil fertility and productivity besides the reduction of nutritional quality of pulses. Thus the integration of inorganic fertilizer and organic manures resulted in better growth, yield and nutrient uptake. Being leguminous crop, greengram has ability to fix atmospheric nitrogen in soil but it is adversely affected in coastal salt affected soils. Therefore application of bio-fertilizer is needed to ensure nodulation and nitrogen fixation (Kalaiyarasi *et al.*, 2019). In this context, the productivity of this crop is very low because of their cultivation on marginal and sub marginal lands of low soil fertility where little attention is paid to adequate fertilization.

In addition, the coastal areas have much lower NPK use, compared to inland areas. The nutrient imbalances created by continuous use of chemical fertilizers, particularly nitrogenous fertilizer alone or combined with sub-optimal rates or total omission of other nutrients is the primary cause of unsustainable greengram yields in coastal areas. There is a strong consensus among scientists that incorporation of organic manure is essential to sustain soil health and crop production. Recycling of organic wastes in coastal soils have multidimensional effect in improving all the soil related constraints. Now a day's nutrient enrichment/fortified with organic manures especially micronutrients (Zn, Fe and B) is one of major approach for meeting out this great challenges for increasing higher pulse production (Arulrajasekaran, 2019). In this context, considering the inherent poor soil fertility, poor yield and economic condition of the farmers, it is an imperative need that the enrichment of locally available organic wastes will be thought of, for achieving sustainable yield at minimum cost. Coastal areas in particular are endowed with a variety of natural resources and a wide variety of organic wastes which are mean to contain plant nutrients and several growth principles and enzymes which can sustain soil health and crop production. Organic matter plays an important role in the improvement of soil physical properties such as the promotion of soil aggregation, improved permeability and moisture holding capacity. With regard to chemical properties, organic matter often accounts for at least half the cation exchange and buffering capacity of the soil. With regard to biological properties soil, organic matter increases the population of beneficial soil microorganisms. Hence, in the present investigation was conducted to study the effect of integrated nutrient management practices on the soil properties, yield and nutrient uptake by greengran in coastal saline soil.

MATERIALS AND METHODS

A field experiment was carried out in a farmer's field during January – March, 2019 at Mandabam coastal village, to find out the effect of integrated nutrient management on the soil properties, yield and nutrient uptake by greengram in coastal saline soil. The various INM treatments included were, T_1–Control (100% NPK/ RDF alone), T_2–100% NPK + FYM @ 12.5 t ha^{-1}, T_3–100% NPK + Composted coirpith (CCP) @ 12.5 t ha^{-1}, T_4–100% NPK + FYM @ 12.5 t ha^{-1} + Rhizobium @ 2.0 kg ha^{-1}(BF), T_5–100% NPK + CCP @ 12.5 t ha^{-1}+ Rhizobium (BF), T_6–100% NPK + FYM @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}, T_7–100% NPK + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1} , T_8–100% NPK + ZnEFYM @ 6.25 t ha^{-1} + BF + Pink Pigmented Facultative Methylotrophs (PPFM) @ 1.0 % Foliar spray and T_9 –100% NPK + ZnECCP @ 6.25 t ha^{-1} + BF + PPFM @ 1.0 % Foliar spray twice at pre flowering stage and flowering stage. The experimental plots were arranged in a Randomized Block Design (RBD), with three replications, using greengram variety ADT 5. The experimental soil had sandy loam texture with pH- 8.37; EC- 4.05 dSm^{-1}; organic carbon- 2.31 g kg^{-1}, and DTPA-Zn 0.71 mg kg^{-1}. The alkaline KMnO$_4$–N; Olsen-P and NH$_4$OAc-K, were low, low and medium status, respectively. Calculated amount of inorganic fertilizer doses of Nitrogen (25kgNha^{-1}), Phosphorus (50 kg P$_2$O$_5$ ha^{-1}) and Potassium (25 kg K$_2$O ha^{-1}) were applied through urea, DAP and muriate of potash, respectively. Half of the N and entire P$_2$O$_5$ and K$_2$O were applied as basal and the remaining half dose of N was applied in two splits at flowering and pod formation stage. Required quantities of different organics *viz.*, Farm yard manure and composted coirpith as per the treatment schedule were incorporated into the soil. Enriched organic manures like zinc enriched FYM (ZnEFYM) and zinc enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} were applied basally and well incorporated into the soil as per the treatment schedule. Required quantities of ZnSO$_4$ were also applied through soil as per the treatment schedule. Foliar spray of Pink Pigmented Facultative Methylotrophs (PPFM) @ 2.0 per cent twice at Pre Flowering Stage (PFS) and at Flowering Stage (FS) was applied as per the treatment. The biofertilizer namely *Rhizobium* @ 2.0 kg ha^{-1} were incorporated as per the treatment schedule. The soil samples were collected at flowering stage (FS), pod formation (PFS) and harvest stages (HS) and analyzed for major (N, P and K) and micronutrients (Zn) status of soil. The soil samples were also analysed microbial population (Subba Rao, 1995). The plant samples were collected at critical stages of greengram viz., flowering, pod formation and at harvest stages and analyzed for the concentration of nutrients like N, P, K and Zn were estimated using the standard procedure as outlined by Jackson (1973) and uptake were calculated. At harvest stage, seed and haulm yield were also recorded.

RESULTS AND DISCUSSION

PHYSICO-CHEMICAL PROPERTIES OF THE SOIL (Table 1)
The influence of various INM treatments, application of recommended dose of fertilizer (RDF) + biofertilizer along with organics or Zn-enriched organics and foliar spray of PPFM in altering the pH, EC and organic carbon content of the soil at different critical stages of greengram was significant.

pH (Soil reaction)
The effect due to different INM treatments, proved its worthiness in reducing the soil pH at all the growth states of greengram. The various INM treatments exerted a significant role in reducing the pH of the soil at all the growth stages viz., flowering, pod formation and at harvest stage. The treatment recommended dose of NPK alone recorded a pH of8.46at flowering, 8.44 at pod formation and 8.42 at harvest stage, respectively. Though both the organics studied were useful in reducing the soil pH. Among the various INM treatments, application of Zn enriched composted coirpith (ZnECCP) @ 6.25t ha^{-1} + BF (Rhizobium) through soil application and foliar spray of PPFM @ 1.0% twice (T_9) brought out significant reduction of soil pH to a greater extend at all the growth stages. At harvest, this treatment recorded the lowest pH of 7.82. This was followed by the next best treatment T_8, the application of Zn enriched FYM (ZnEFYM) @ 6.25t ha^{-1}+ BF (Rhizobium) through soil application and foliar spray of PPFM @ 1.0%registered a pH of 7.89at harvest stage. This was followed by the treatments arranged in the descending order like T_7> T_6> T_5> T_4> T_3 and T_2. These treatments were also statistically significant. The control recorded the highest pH (8.42) of the soil.

Electrical Conductivity (EC)

The influence of various IPNS treatments through inorganic NPK fertilizer, biofertilizer along with Zn enriched organics in reducing the EC of soil was significant. Both the organic sources (FYM and CCP) exerted favourable influence in significantly reducing the EC of soil. The lowest EC was recorded with treatment T_9, the application of Zn enriched composted coirpith @ 6.25t ha^{-1}(ZnECCP) + Rhizobium @ 2 kg ha^{-1} along with recommended dose of NPK and foliar spray of PPFM @ 1.0% twice which registered 3.87, 3.45 and 3.02 dS m^{-1} at FS, PFS and at harvest stage, respectively. This was followed by T_8 the application of Zn enriched FYM @ 6.25t ha^{-1} (ZnEFYM) + Rhizobium @ 2 kg ha^{-1} along with recommended dose of NPK and foliar spray of PPFM @ 1.0% twice which recorded an EC value of 3.88, 3.69 and 3.32 dS m^{-1} at the same critical stages, respectively. This was followed by the treatments T_7, application of RDF + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1} and T_6, application of RDF +FYM@ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}which recorded the EC value of 3.41 and 3.45 dS m^{-1} at harvest stage, respectively. This was followed by the treatments arranged in the descending order likeT_7 > T_6> T_5> T_4>T_3 and T_2. These treatments were also statistically significant. The highest EC was recorded in control (4.06 dS m^{-1}).In the present investigation, the various INM treatments favoured for a greater reduction of pH and EC. The effect being much pronounced with the application of 100% NPK + *Rhizobium* + Zn enriched composted coirpith through soil and foliar spray of PPFM. At the harvest stage, the pH was reduced to 7.82 and EC was reduced to 3.02dS m^{-1} as compared to 8.42 and 4.06dS m^{-1} in 100% NPK treatment. The reduction in salinity and pH with different INM treatments might be due to the organic acids and complexes produced by the incorporation of organics. Several earlier reports were available for the beneficial influence of the organics in reducing the pH and EC (Chalwade *et al.,* 2006 and Kodeeswaran, 2015). The decrease in soil pH may be attributed to the higher production of CO$_2$ and organic acid during the decomposition of organics and bio-resources. Whereas, observed the lowering of EC and alleviation of salt effects due to the application of phytomass and FYM/CCP amendments in salt affected soils. These results are conformity with Akbari *et al.* (2010).

Organic Carbon

The coastal sandy soil showed poor organic carbon status. A profound effect due to different INM treatments in increasing the OC status of soil was well evidenced in the present study. The influence ofZn enriched organic manures in enhancing the organic carbon content of soil was significant. Application of recommended dose fertilizer (RDF) or NPK alone recorded the lowest organic carbon content of 2.23 g kg^{-1} at harvest stage. All the organic manure applied treatments from T_2 to T_9 significantly increased the organic carbon content of soil. Of all the treatments, a significantly higher organic carbon content was recorded in T_9, application of RDF + ZnECCP @ 6.25 t ha^{-1}+ BF along with foliar application of PPFM @ 1.0% (3.25 g kg^{-1}). This was followed by the next best treatment T_8, application of RDF + ZnEFYM @ 6.25 t ha^{-1} + BF along with foliar application of PPFM @ 1.0% which recorded a organic carbon content of 3.16 g kg^{-1} at harvest stage. This was followed by the treatment T_7, the application of RDF + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1} and treatment T_6, the application of RDF + FYM @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}CCP @ 12.5 t ha^{-1} which recorded an organic carbon status of 3.01 and 2.82 g kg^{-1}at harvest stage, respectively. This was followed by the treatments arranged in the descending order as T_5>T_4> $T_3$and T_2. The lowest organic carbon content was recorded in control (without organics/RDF alone).Zinc enriched organic manuring with FYM and composted coirpith was reported to increase the status of organic carbon and total nitrogen (Kumar *et al.,* 2000). The effect due to organic amendments as a component of IPNS treatment in increasing the organic carbon content in soil would be expected due to higher content of organic matter present in them as reported by Kumawat *et al.,* 2013. The increased biological activity will hasten the decomposition process of the added organic manures and resettling of component organic carbon into the soil. Further, the increased organic carbon content of post harvest soil with application of various INM treatments in pulses was reported by Sharma and Abraham, (2010).

Available Major Nutrients (Table 2)

''Nutrients availability in soil (Table 2)'' for the next sub heading paragraph of alkaline KMnO$_4$ –N, olsen P, etc,

Alkaline KMnO$_4$-N

The availability of nitrogen in coastal saline soils are very low due to poor crop residues and microbial activity and leaching of nutrients associated with poor structure and low use efficiency of applied nutrients. The influence of

various INM treatments in significantly increasing the availability of nitrogen in soil was well evidenced. Among the various INM treatments, application of recommended dose of fertilizer (RDF) + BF (Rhizobium) @ 2 kg ha^{-1} along with Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} through soil and foliar spray of PPFM @ 1.0% twice (T$_9$) recorded the highest alkaline KMnO$_4$ –N content of 157.74, 148.64 and 139.53 mg kg^{-1} at FS, PFS and harvest stage, respectively. This was followed by the treatment T$_8$, which received RDF + BF + ZnEFYM @ 6.25   t ha^{-1} + PPFM @ 1.0% through foliar application. This treatment recorded149.71, 141.09 and 132.51 mg kg^{-1} of alkaline KMnO$_4$–N at flowering, pod formation and harvest stage, respectively. The treatments T$_7$ (RDF + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}) and T$_6$ (RDF + FYM @ 12.5 t ha^{-1} + BF +ZnSO$_4$ @ 25 kg ha^{-1}) which recorded a lowest alkaline KMnO$_4$ –N content   as compared to above said treatments. This was followed by the treatments arranged in the descending order like T$_5$> T$_4$> T$_3$ and T$_2$. At all the growth stages, control registered the lowest alkaline KMnO$_4$-N content in the soil.The betterment increase in available nitrogen in soil due to incorporation of organic materials might be also attributed to the enhanced multiplication of microbes by the incorporation of enriched organics and composted organics or crop residues for the conversion of organically bound N to inorganic form. Further, the favorable soil conditions under zinc enriched composted coirpith might have helped in the mineralization of soil N leading to the build up of higher available N reported by Selvi (2002).

Olsen-P

The effect due to the application of INM treatments in enhancing the available P content of soil was significant at all the critical stages like flowering, pod formation and at harvest stages of green gram. The treatment 100 per cent recommended dose of fertilizer (RDF) + BF + ZnECCP @ 6.25 t ha^{-1} through soil and foliar application of PPFM @1.0 per cent twice(T$_9$)recorded the highest available P content of 14.93 kg ha^{-1} at FS, 12.79 kg ha^{-1} at PFS and 10.75 kg ha^{-1}at harvest stage, respectively. This was followed by the next best treatment registered with T$_8$ which received RDF + BF + ZnEFYM @ 6.25 t ha^{-1}along with foliar application of PPFM@ 1.0% twice. This was followed by the treatments T$_7$, application of RDF + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1} and treatment T$_6$, application of RDF +FYM @ 12.5 t ha^{-1} + BF along with ZnSO$_4$ @ 25 kg ha^{-1}which recorded a Olsen-P content of 10.17, 9.55 and 8.96 kg ha^{-1} at harvest stage, respectively. This was followed by the treatments arranged in the descending order viz., T$_5$> T$_4$> T$_3$ and T$_2$. These treatments were also statistically significant with each other. The control treatment T$_1$, recorded the lowest Olsen-P content of 5.92 kg ha^{-1} at harvest. Larger build up in available P with organics may be attributed to the influence of organic manure in increasing the labile P in soil through complexation of cations like Ca^{2+} and Mg^{2+} which are mainly responsible for the fixation of phosphorus in soil. Further, the increased phosphatase activity with INM treatment might have played a signifying release of more organic bound P as reported by Chalwade *et al*. (2006). Incorporation of FYM and composted coirpith along with inorganic fertilizers and biofertilizer increase the availability of P and this is attributable to reduction in fixation of water soluble P, increased mineralization of organic P due to microbial action and enhanced mobility of P. These results are in conformity with the findings of Ramalakshmi *et al.* (2014).

NH$_4$OAc-K

The positive influence of various IPNS treatments through inorganic fertilizers, biofertilizer along with Zn enriched organics was also significantly increased the NH$_4$OAc-K content of the soil in the present investigation. Of all the treatments, application of recommended dose of NPK + BF + Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} by soil along with foliar spray of PPFM @ 1.0 per cent twice (T$_9$) registered the highest NH$_4$OAc-K content of 179.45, 174.38 and 168.31 kg ha^{-1} at FS, PFS and at the harvest stages, respectively. This was followed by the treatment (T$_8$) which received RDF + BF + Zn enriched FYM (ZnEFYM) @ 6.25 t ha^{-1}through soil and foliar spray of PPFM @ 1.0% recorded a value of K availability at flowering (170.43kg ha^{-1}), at pod formation(165.53 kg ha^{-1}) and at harvest (159.95 kg ha^{-1}) stage, respectively. This was followed by the treatment T$_7$,(RDF + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}) and treatment T$_6$,(RDF + FYM @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}) which recorded a lowest NH$_4$OAc-K (151.51 and 143.04 kg ha^{-1})content at harvest stage as compared to above said treatments. This was followed by the treatments arranged in the descending order as T$_5$>T$_4$>T$_3$ andT$_2$. These treatments were also statistically significant. The control treatment recorded the lowest soil K availability at all the critical stages of greengram. The addition of organics along with recommended dose of NPK fertilizers reduced the K fixation and release of K due to the interaction of organic

matter with clay besides the direct addition of potassium to available pool of the soil contributed for increased K availability. Organics also minimized the leaching loss of K by retaining K ions on exchange sites of the decomposition products. Similar results were also reported by (Kumawat *et al.* 2013). Earlier findings of Senthilvalavan and Ravichandran (2016) also reported increased availability of potassium in rice-pulse cropping system of soil with INM practices.

DTPA-Zn

Coastal soil is known for the restricted availability of zinc in soil due to poor organic matter, high leaching, prevailing high pH and salinity. The profound influence of various INM treatments in increasing in DTPA–Zn content of soil was well established in present study. The availability of DTPA-Zn content in the soil significantly increased at all the growth stages of green gram with the application of different INM treatments. The highest available zinc status at flowering (2.34 mg kg^{-1}),pod formation (1.68 mg kg^{-1}) and at harvest stage (1.09 mg kg^{-1}) was recorded with the combined application of 100 per cent recommended dose of NPK + BF @ 2 kg ha^{-1} + ZnECCP @ 6.25 t ha^{-1} through soil and foliar spray of PPFM @ 1.0% twice at pre flowering and flowering stage (T$_9$). This was followed by the treatment which received RDF + BF + ZnEFYM @ 6.25 t ha^{-1} through soil along with foliar spray of PPFM @ 1.0% twice (T$_8$) which recorded 1.03 mg kg^{-1} of DTPA-Zn content of soil at harvest stage. This treatment was followed by recommended NPK + BF + CCP @ 12.5 t ha^{-1} along with ZnSO$_4$ @ 25 kg ha^{-1}(T$_7$) and treatment T$_6$ which received NPK + BF + FYM @ 12.5 t ha^{-1} along with ZnSO$_4$ @ 25 kg ha^{-1}(T$_6$) which recorded a DTPA-Zinc content (0.92 and 0.84 mg kg^{-1}) of soil at harvest stage. This was followed by the treatments significantly arranged in the descending order as T$_5$ > T$_4$> T$_3$ and T$_2$. The control treatment (RDF alone) registered the lowest DTPA-Zn availability of 0.47 mg kg^{-1} at harvest stage.

The highest DTPA-Zn content was recorded with the treatment RDF + Rhizobium along with ZnECCP application. The increased use efficiency of applied micronutrient fertilizer and their availability with the addition of micronutrients along with organics in complexing and mobilizing property might have increased the DTPA-Zinc content of the soil. Earlier findings of Sridevi *et al.* (2010) support the present findings. In general, most of the saline soils, including the coastal region are well supplied with micronutrients with exception of Zn. The deficiency of micronutrients is induced in saline soils due to the reduction of sulphate salts to sulphides and subsequent precipitation of the micronutrients (Subba Rao *et al.*, 1994). The increased zinc availability might be attributed to the direct addition of these nutrients by fertilizer and enriched organic manures, which maintain maximum available Zn and other micronutrients status in post harvest soil. Further the complexation of micronutrients with applied organics might have mobilized and increased the availability of Zn in soil. These findings are accordance with Patel *et al.* (2016).

Green gram yield (Table 2)

The green gram responded well for the integrated plant nutrients application. The significant influence of recommended NPK, biofertilizer along with Zn enriched organics in increasing the grain and haulm yield of green gram was well documented in the present study. The yield realized under the nutrient poverished coastal saline soil, the highest seed yield (982 kg ha^{-1}) and haulm yield (2079 kg ha^{-1}) was recorded with combined application of recommended dose of fertilizer (RDF) + *Rhizobium* @ 2 kg ha^{-1} + ZnECCP @ 6.25 t ha^{-1} through soil along with foliar spray of PPFM @ 1.0 per cent twice at pre flowering and flowering stage (T$_9$). This was followed by the treatments T$_8$, (RDF + BF + ZnEFYM @ 6.25 t ha^{-1} through soil application and foliar application of PPFM @ 1.0%), T$_7$ (RDF + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}) and T$_6$ (RDF + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}) which recorded the seed (929,875 and 824 kg ha^{-1}) and haulm (1972,1858 and 1721kg ha^{-1}) yield of green gram, respectively. This was followed by the application of organics and biofertilizer alone or without micronutrient treatments T$_5$, (RDF + CCP @ 12.5 t ha^{-1} + BF), T$_4$ (RDF+FYM @ 12.5 t ha^{-1} + BF), T$_3$ (RDF + CCP @ 12.5 t ha^{-1}) and T$_2$ (RDF +FYM @ 12.5 t ha^{-1}) which recorded the lowest seed and haulm yield as compared to above said INM treatments (organic, inorganic and BF). Among the various IPNS treatments, the treatment (T$_9$), 100% recommended dose of NPK + Zn enriched composted coirpith @ 6.25 t ha^{-1} along with Rhizobium @ 2 kg ha^{-1} and foliar spray of PPFM @ 1.0% twice recorded a seed and haulm yield of 982 and 2079 kg ha^{-1} which was 42.36 and 46.94 per cent increase over control or 100 per cent NPK

alone (without micronutrients, BF and organics). The control treatment T_1, 100 per cent NPK alone recorded a lower seed (566 kg ha^{-1}) and haulm (1103 kg ha^{-1}) yield of greengram, respectively.

The overall improvement in yield attributing characters of tomato was obtained with the application of recommended dose of NPK + Rhizobium + Zn enriched composted coirpith @ 6.25 t ha^{-1} through soil application and foliar application of PPFM @ 1.0 per centtwice at pre flowering and flowering stage. Application of NPK + biofertilizer and Zn enriched organic manures helped in the slow and steady rate of nutrient release into soil solution to match the absorption pattern of green gram thereby increased the yield. Further, the favourable effect of Zn and NPK nutrients on seed and haulm yield was also could be attributed to their effect in maintaining soil available nutrients in balanced proportions Singaravel *et al.* (2016). Foliar application of pink pigmented facultative methylotorphs (PPFM) at pre flowering and flowering stages of crop growth were effectively absorbed in the plant system and translocated into sink which resulted in more number of pods plant-1 and more number of seeds pod^{-1}. Further, increased in photosynthesis during growth stages might be contributed for greater assimilates supply to the pods which resulting in better seed setting and also betterment of higher seed yield of green gram. PPFMs excrete auxins and cytokinins, plant growth hormones that influence more number of flowering, pod filling and play critical roles in a plant's response to water/ saline stress condition. The results are in conformity with Sivakumar *et al.* (2017). Under present study, the overall improvement in increased growth and yield attributes in terms of number of pods plant^{-1}, number of seeds pod^{-1} and 100 seed weight resulted in increased yield of green gram with INM application. A plethora of evidences are available for the beneficial effect of INM on green gram with earlier reported by Marimuthu and Surendran, (2015).

Microbial Population of the Soil (table 3)

A microbial population of soil microorganisms *viz.,* bacteria, fungi and actinomycetes was significantly increased with various INM treatments was well evidenced. An increase in the microbial population upto pod formation stage and a decline thereafter was noticed (at harvest stage). Among the various INM treatments, the combined application of recommended dose of NPK +BF along with Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} through soil application and foliar spray of PPFM@ 1.0% twice (T_9) recorded the highest population of bacteria (22.18$\times 10^6$), fungi (16.53$\times 10^5$) and actinomycetes(10.48 $\times 10^4$) at pod formation stage. This was followed by the treatment T_8 (application of RDF /100% NPK + BF + ZnEFYM @ 6.25 t ha^{-1}through soil along with 1.0% foliar spray of PPFM) which registered the population of bacteria (21.02$\times 10^6$), fungi (15.68 $\times 10^5$)and actinomycetes (9.94 $\times 10^4$) at pod formation stage, respectively. This was followed by the treatment T_7 (RDF + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}) and treatment T_6 (RDF + CCP @ 12.5 t ha^{-1} + BF + ZnSO$_4$ @ 25 kg ha^{-1}) which recorded the microbial population like bacteria (19.87$\times 10^6$and 18.68 $\times 10^6$), fungi (14.79$\times 10^5$ and13.88 $\times 10^5$) and actinomycetes (9.37$\times 10^4$and 8.78 $\times 10^4$) at pod formation stage, respectively. The treatments T_5 (RDF + ZnSO$_4$ @ 25 kg ha^{-1} + BF + CCP @ 12.5 t ha^{-1}), treatment T_4 (RDF + ZnSO$_4$ @ 25 kg ha^{-1} + BF + FYM @ 12.5 t ha^{-1}), treatment T_3 (RDF + ZnSO$_4$ @ 25 kg ha^{-1} + BF) and treatment T_2 (RDF + ZnSO$_4$ @ 25 kg ha^{-1}) which recorded the lowest microbial population count noticed at all the growth stages as compared to above said INM treatments. The lowest microbial population of soil was noticed with application of NPK alone as compared to various INM treatments. In the overall improvement of microbial counts in soil microorganisms with application of organics along with micronutrients may be due to better soil biological environment of coastal saline soil (Prasad, 2008 and Aminum Nahar *et al.* (2013). Further, the availability of readily mineralized C and N and improvement in the physico-chemical properties of the soil due to the application of organics might have increased the microbial population of the soil. These results are in parity with the results reported by Abdullahi *et al.* (2013). Major Nutrients Uptake (table 4)

Major nutrients (NPK) uptake (Table 4)

The NPK uptake of greengram at all the critical stages of crop growth and in seed and haulm was significantly increased with the various INM treatments. Integrated application of recommended NPK and Zn enriched organics along with biofertilizer through soil and foliar spray of PPFM significantly increased the uptake of major nutrients by greengram. Among the various INM treatments evaluated, the treatment T_9 (RDF + Rhizobium @ 2 kg ha^{-1} + Zn enriched CCP @ 6.25 t ha^{-1} through soil and foliar spray of PPFM @ 1.0 %) registered the highest N (33.48 and 39.31kg ha^{-1}) P (6.58 and 5.41kg ha^{-1}) and K(9.65 and 15.01 kg ha^{-1}) by seed and haulm, respectively. This was followed by T_8,

application of RDF + BF + Zn enriched FYM @ 6.25 t ha^{-1} through soil and foliar spray of PPFM @ 1.0 per cent, T_7 application of RDF + CCP @ 12.5 t ha^{-1} + BF along with ZnSO$_4$ @ 25 kg ha^{-1} and T_6 application of RDF + FYM @ 12.5 t ha^{-1} + BF along with + ZnSO$_4$ @ 25 kg ha^{-1} which recorded a lowest NPK uptake as compared to above said IPNS treatments. This was followed by the treatments arranged in the descending order like $T_5 > T_4 > T_3$ and T_2 (without BF and micronutrients). These treatments were also statistically significant. The lowest NPK uptake was registered in control (100% NPK alone).The overall improvement of NPK uptake with application of NPK, biofertilizer and Zn enriched organic manures increased the absorption power of the soil for cations and anions, particularly nitrogen and phosphate. These ions are released gradually during entire growing period of the crop which might have increased concentration as well as improved the plant growth and accumulation of greater biomass which helped to increase the uptake. In line with the present study Patel *et al.* (2016) also reported similar results. Further added Zn enriched organics improved the organic carbon content of soil through decomposition which helped in the release of organically bounded both macro and micronutrients in soil. These findings are in agreement with Ashwani *et al.* (2016).

Zinc use Efficiency (Table 5)

In coastal saline soil, the applied zinc is prone for leaching. The inherent soil properties like poor organic matter, low CEC and high pH leads the deficiency and poor zinc use efficiency (ZUE). The profound influence of various INM treatments in increasing in DTPA–Zn content and the use efficiency of applied Zn was well established in present study. The application of ZnSO$_4$ as inorganic fertilizer applied treatments (T_6 and T_7) recorded the lowest Zn use efficiency *viz.*, Agronomic efficiency and apparent zinc recovery by recording 12.28 and 15.19 kg kg^{-1} and 0.41and 0.60 per cent respectively. In the first hand, the fortified CCP and FYM application exerted profound influence in increasing the zinc use efficiency as compared to ZnSO$_4$ application. The zinc use efficiency in terms of agronomic efficiency and apparent zinc recovery increased to a range of 12.28 to 19.81 kg ha^{-1} and 0.41 to 0.70 per cent respectively with micronutrient namely Zn enriched manure application(FYM and CCP). However the treatment, T_9 – application of 100% recommended dose of fertilizer (RDF) + zinc enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1}, accounted for the highest zinc use efficiency by recording 19.81kg ha^{-1}and apparent Zn recovery of 0.70.This was followed by the treatment T_8, which received RDF+ zinc enriched FYM (Zn EFYM) @ 6.25 t ha^{-1} recorded a zinc use efficiency of 17.28kg ha^{-1} and apparent Zn recovery of 0.61, respectively. The present investigation clearly brought out more efficacious nature of Zn enriched organic manure in increasing the zinc use efficiency by the way of recording significantly higher agronomic efficiency and apparent Zn recovery. Among the INM treatments, T_9, NPK + *Rhizobium* + Zn enriched composted coirpith and PPFM foliar spray was associated with increased Zn agronomic efficiency (19.81kg ha^{-1}) and apparent zinc recovery (0.70%), respectively. This might be due to the various reason explained earlier DTPA-Zn content in soil viz., i) Reduction in soil pH and EC (salinity) ii) Reduced for precipitation and fixation of micronutrients in soil. iii) By forming soluble organic complexes etc. Similar finding was earlier reported by Singh *et al.,* (2002) and Shivay and Prasad, (2012).

Zinc uptake (Table 5)

The effect due to the integrated application of RDF + Biofertilizer along with Zn enriched organic manures and PPFM foliar spray had significant influence on zinc uptake by green gram at all the critical stages viz., flowering, pod formation and at harvest stage in grain and haulm. Among the various INM treatments, the highest Zn uptake by seed (19.16 g ha^{-1}) and haulm (38.06 g ha^{-1}) was recorded with the application of RDF + BF + ZnECCP @ 6.25 t ha^{-1} and foliar spray of PPFM @ 1.0 per cent twice (T_9). This was followed by application of 100% NPK (RDF) + BF + ZnEFYM @ 6.25 t ha^{-1} through soil and foliar application of PPFM @ 1.0 per cent (T_8), application of 100% NPK + CCP @ 12.5 t ha^{-1} + BF along with ZnSO$_4$ @ 25 kg ha^{-1} (T_7) and application of 100% NPK + FYM @ 12.5 t ha^{-1} + BF along with ZnSO$_4$ @ 25 kg ha^{-1} (T_6) which recorded a Zn uptake of 31.96, 30.18 and 28.37 g ha^{-1} by seed and 64.71, 61.12 and 57.48 g ha^{-1} by haulm, respectively. This was followed by the treatments which received organics and biofertilizer or organics alone along with recommended NPK (without Zn) applied treatments. The treatment T_5, (100% NPK + CCP @ 12.5 t ha^{-1} + BF) and T_4, (100% NPK + FYM @ 12.5 t ha^{-1} + BF) recorded a lowest Zn uptake of green gram as compared to above said treatments (micronutrients, Zn enriched organics and PPFM foliar spray). This was followed by the treatments T_3 and T_2. These two treatments were also statistically significant. The control (100% NPK alone)

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treatment recorded the lowest Zn uptake at all the critical stages of greengram.In the present investigation, application of different INM treatments recorded the highest Zn uptake. The treatment receiving 100% recommended dose of NPK + Rhizobium @ 2.0 kg ha^{-1}along with zinc enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1}through soil and foliar spray of PPFM @ 1.0% twice registered the highest Zn uptake. This might be attributed to increase total dry matter production, growth and yield components of green gram. Further, improvement in the availability and higher absorption by green gram resulted in higher uptake of these nutrients. The increased uptake of micronutrient with the Zn has been well documented by Regar and Yadav, (2017).

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Table 1. Effect of integrated nutrient management practices on the physico-chemical properties of coastal soil

Treatments	pH			EC (dS m^{-1})			OC (g kg^{-1})		
	FS	PFS	HS	FS	PFS	HS	FS	PFS	HS
T_1	8.46	8.44	8.42	4.02	4.04	4.06	2.11	2.14	2.23
T_2	8.41	8.38	8.35	4.00	4.03	3.95	2.20	2.23	2.35
T_3	8.37	8.33	8.28	3.97	4.02	3.86	2.27	2.31	2.45
T_4	8.29	8.26	8.21	3.95	3.97	3.78	2.33	2.42	2.53
T_5	8.23	8.21	8.13	3.93	3.91	3.63	2.40	2.55	2.74
T_6	8.19	8.15	8.04	3.92	3.88	3.45	2.45	2.70	2.82
T_7	8.11	8.08	7.96	3.90	3.83	3.41	2.51	2.83	3.01
T_8	8.08	8.01	7.89	3.88	3.69	3.32	2.59	2.92	3.16
T_9	8.03	7.95	7.82	3.87	3.45	3.02	2.64	3.01	3.25
SE_D	0.01	0.02	0.03	0.004	0.006	0.007	0.01	0.03	0.04
CD (p=0.05)	0.03	0.05	0.06	0.010	0.013	0.015	0.02	0.07	0.08

Table 2. Effect of integrated nutrient management practices on the nutrients availability in soil and yield of greengram

Treatments	Alkaline KMnO$_4$-N (kg ha^{-1})			Olsen-P (kg ha^{-1})			NH$_4$OAc-K (kg ha^{-1})			DTPA-Zn (mg kg^{-1})			Yield (kg ha^{-1})	
	FS	PFS	HS	FS	PFS	HS	FS	PFS	*HS*	FS	PFS	HS	Seed	Haulm
T_1	93.29	87.88	82.94	8.97	7.30	5.92	107.07	103.78	100.91	1.17	0.66	0.47	566	1103
T_2	101.41	95.46	90.07	9.76	8.03	6.48	116.19	112.62	109.39	1.32	0.82	0.56	619	1236
T_3	109.37	103.12	97.12	10.57	8.68	7.12	125.23	121.37	117.76	1.48	0.97	0.62	672	1345

T_4	117.32	110.71	104.0	11.32	9.33	7.67	134.30	130.21	126.13	1.63	1.12	0.69	721	1460
T_5	125.49	118.24	111.35	12.20	10.08	8.34	143.38	138.97	134.59	1.76	1.24	0.75	773	1603
T_6	133.66	125.88	118.41	12.44	10.73	8.96	152.37	147.82	143.04	1.91	1.35	0.84	824	1721
T_7	141.72	133.55	125.43	13.39	11.46	9.55	161.36	156.70	151.51	2.05	1.46	0.92	875	1858
T_8	149.71	141.09	132.51	14.06	12.13	10.17	170.43	165.53	159.95	2.21	1.58	1.03	929	1972
T_9	157.74	148.64	139.53	14.93	12.79	10.75	179.45	174.38	168.31	2.34	1.68	1.09	982	2079
SE_D	3.66	3.46	3.23	0.34	0.28	0.25	4.18	4.07	3.88	0.05	0.03	0.02	22.47	47.54
CD (p=0.05)	7.84	7.41	6.96	0.73	0.62	0.53	8.94	8.70	8.32	0.11	0.08	0.05	48.10	101.75

Table 3. Effect of integrated nutrient management practices on the microbial population of coastal soil

Treatments	Bacteria (x 10^6/g soil)			Fungi (x 10^5/ g soil)			Actinomycetes (x 10^4/g soil)		
	FS	PFS	HS	FS	PFS	HS	FS	PFS	HS
T_1	15.19	12.65	10.32	11.04	9.41	8.24	6.72	6.01	5.44
T_2	16.48	13.86	11.38	11.99	10.35	9.01	7.31	6.54	6.05
T_3	17.75	15.02	12.45	13.03	11.24	9.73	7.85	7.10	6.58
T_4	18.99	16.14	13.40	14.02	12.10	10.55	8.47	7.62	7.14
T_5	20.26	17.49	14.48	15.01	13.04	11.32	9.11	8.23	7.67
T_6	21.53	18.68	15.46	15.96	13.88	12.14	9.76	8.78	8.19
T_7	22.81	19.87	16.55	16.55	14.79	12.96	10.39	9.37	8.75
T_8	24.07	21.02	17.51	17.51	15.68	13.72	10.97	9.94	9.24
T_9	25.31	22.18	18.45	18.49	16.53	14.47	11.53	10.48	9.76
SE_D	0.55	0.51	0.42	0.43	0.37	0.33	0.25	0.23	0.22
CD (p=0.05)	1.19	1.09	0.90	0.92	0.81	0.71	0.53	0.51	0.47

Table 4. Effect of integrated nutrient management on the major nutrients uptake by green gram

Treatments	N- uptake				P- uptake				K-uptake			
	FS	PFS	HS		FS	PFS	HS		FS	PFS	HS	
			Seed	Haulm			Seed	Haulm			Seed	Haulm
T_1	17.68	22.35	19.60	22.17	3.25	3.89	3.28	2.49	3.57	5.83	5.26	8.33
T_2	19.34	24.58	21.71	24.59	3.64	4.32	3.73	2.93	3.94	6.41	5.97	9.21
T_3	20.92	26.60	23.36	26.65	4.02	4.69	4.07	3.41	4.30	6.92	6.52	9.99
T_4	22.53	28.54	24.64	28.76	4.38	5.13	4.63	3.76	4.63	7.48	7.06	10.83
T_5	24.16	30.75	26.58	31.00	4.73	5.59	4.99	4.14	4.99	8.09	7.54	11.71
T_6	25.72	32.72	28.32	33.04	5.09	5.95	5.46	4.49	5.31	8.63	8.12	12.57
T_7	27.24	34.86	30.01	35.18	5.42	6.43	5.81	4.78	5.66	9.24	8.66	13.36
T_8	28.78	36.99	31.76	37.27	5.76	6.89	6.22	5.12	6.02	9.78	9.15	14.18
T_9	30.35	38.94	33.48	39.31	6.71	7.31	6.58	5.41	6.37	10.35	9.65	15.01
SE_D	0.69	0.87	0.76	0.83	0.14	0.16	0.15	0.12	0.14	0.23	0.21	0.35
CD (p=0.05)	1.49	1.88	1.63	1.78	0.29	0.35	0.32	0.26	0.31	0.50	0.47	0.75

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Table 5. Effect of integrated nutrient management on the zinc use efficiency and uptake by greengram

Treatments	Zinc use efficiency		Zinc uptake (g ha^{-1})			
	Agronomic efficiency	Apparent Zn recovery	FS	PFS	HS	
					Seed	Haulm
T_1	-	-	17.66	37.95	19.16	38.06
T_2	-	-	19.39	41.80	21.15	42.30
T_3	-	-	21.01	45.29	23.01	46.43
T_4	7.38	0.26	22.58	48.72	24.73	49.95
T_5	10.33	0.35	24.23	52.20	26.68	53.86
T_6	12.28	0.41	25.75	55.76	28.37	57.48
T_7	15.19	0.60	27.36	59.22	30.18	61.12
T_8	17.28	0.61	28.94	62.58	31.96	64.71
T_9	19.81	0.70	30.48	65.53	33.72	68.19
SE$_D$	0.38	0.01	0.70	1.51	0.78	1.58
CD (p=0.05)	0.82	0.03	1.50	3.24	1.68	3.39

RESEARCH ARTICLE

Utilizing Digital Tools: an Approach towards Blended Learning

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ABSTRACT

During the COVID-19 pandemic, social distancing paved a way to the entire education system to use digital technologies to promote and continue impartation of knowledge. This allowed a more effective process of blended learning and teaching. The paper describes a blended learning research by teachers in UK. It reviews and reflects representative blended learning analysis in further education sector, by considering the effect of blended learning on teacher's performance while rendering lesson to students for boosting their confidence in learning. Various digital tools are identified to support teaching in blended learning for one student as well as a cluster of learners. In terms of research methodology, the research is descriptive research using convenient sampling and data is gathered by the method of structured questionnaire to analyse research data of 50 teaching staff. It illustrates the initial stage of a continuing study that is being taken into consideration. The objective is to utilise individual learner targets while designing a blended learning model for the development of particular abilities. The study examines the value of group programmes that use blended learning techniques and time-bound blended learning sessions. It outlines the planning considerations that must be made when creating blended learning sessions, identifying and gaining access to the learning environments, delivery strategies, and resources needed to support a mixed approach. The findings are based on a survey which ran to gather data with the help of structured questionnaire about the adoption of planning and delivering a blended learning programme in UK. The survey findings, which are included in the study, assess a variety of tutor-developed digital tools made for use in blended learning and in the classroom. Finally, we recommend further research to validate the paradigm, which we hope will encourage improvements in blended learning to accelerate in a specific context, able to be real time.

Keywords: Digital tools, Blended Learning, Blended Learning Environments of blended learning and Blended learning approaches.

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INTRODUCTION

What is Blended Learning (BL)?
A commonly used term in applied linguistics is blended learning (BL), which is the process of using a combination of in-person and online instruction during the educational process, Eryilmaz, (2015). The Traditional Language Approach (TLA) is characterized by meetings in person and full organizational control over the process, according to Khan and Jumani (2012). Makik, (2015) illustrated how specific teaching objectives are met using teaching strategies and techniques. Modern times are known as the technological age. In the diverse minds of learners, BL does not apply the same rule. BL is all about discovering novel concepts that offer several opportunity to combine different learning methods. As explained by Marsh (2012), BL means a combination of different learning environments; the meaning of the term varies depending upon the context. By using BL, instructors and students can obtain and explain more effectively. Modern methods and techniques are demanded for educating students today. The use of various blended learning tools in teaching learning process has increased the opportunity for learning to take place outside of the classroom through distance learning as well as inside the classroom through face-to-face encounter. (F M Hawi1 & P Sudira2,2019).

According to this definition, Traditional Language Approach (TLA) is referred to as a learning process that requires learners and instructors to be physically present at the same time. It is mainly the chalk and talk method that has been used for centuries in imparting education. In his definition of blended learning (2020), Mishra referred to it is a synthesis of various delivery strategies, educational paradigms, and learning styles, according to Graham (2006). A hybrid approach also includes the deliberate integration of classroom instruction with online instruction (Garrison & Kanuka, 2004). Described by Allen and Seaman (2009) as a Blended learning is a model of learning that mixes online and face-to-face instruction. In the past two decades, blended learning (sometimes called hybrid education) has gained popularity across the globe as a result of the distance-learning movement and online education. Due to the combination of pedagogy and technology at that time, the definitions of the term have changed since then (Friesen 2012). A great solution to this problem is the combination of face-to-face and synchronous, or asynchronous, online components, which can be creatively used to link formal and informal learning. It is often limited by teachers' digital capabilities. The characteristics of the 'blend' will also depend on the institution's culture and learner's need. As they struggle with the development of digital technologies, the sophistication of online courses (like MOOCs), and the rising expectations of students for flexible and differentiated learning, the majority of educators are turning to blended approaches, such as flipped learning and self-blended learning. The usage of various blended learning methods in the teaching and learning process has increased the opportunity for learning that takes place outside of a classroom through distant learning as well as inside a classroom through face-to-face encounter. (F M Hawi1 & P Sudira2, 2019).

Blended learning Environments
It is the setting for learning that combines online and in-person learning activities. This educational setting makes use of both online and in-person learning activities. This setting mixes in-person education with computer-mediated learning. It's a setting for learning that blends education delivered face-to-face and through computers. Using a combination of ways to teach integrating classroom instruction Students can pick the time, speed, and place of their learning through online courses. Combining in-person instruction with online communication. It is a setting for learning where education is both face-to-face and computer-mediated. Students in this programme have some control over time, place, path, and pace as well as a physically safe environment away from home. They can also learn in part through online learning. Its instruction is computer-mediated and face-to-face.

Learning environments can be merged to
Provide more learning spaces and opportunities; Support course administration tasks (such as communication, submission of assessments, marking, and feedback);Increasing the quantity and quality of resources and information available to pupils;-Increase opportunities for student interaction and collaboration to motivate and engage them.

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Approaches to blended Learning
Numerous digital technology tools are accessible on platforms that are both patented and open source. Creating mixed learning strategies can appear to be a challenging task. Finding the best strategy to fulfil your students' needs can be difficult, especially when instructors are being pushed to do more with less.(Developing blended learning approaches, July 2020)

Some of the Digital Mostly preferred by the learners as well as teachers
Mentimeter
Create interactive presentations & meetings, whether you are remote, hybrid or onsite. Prepare - Create interactive presentations with the easy-to-use online editor. Ask questions Live polls, quizzes, word clouds, Q&As, and more. Using their smartphones, your audience can interact with the presentation by answering questions. Visualize their responses in real-time to create an enjoyable, interactive experience. Following up - Once your Mentimeter presentation is complete, you can share and export your results.

A teacher who uses it said "Short and simple to use. I made a few presentations utilising the word cloud and scale/slide tool. Several options for use with students. The idea of utilising the scale to demonstrate confidence in a subject before and after learning appeals to me. I enjoyed this tool a much. I have discussed it with a few of my co-workers. When we return, the classroom will be a terrific place to use it, but I've already started using it online. I appreciate that it is anonymous so that learners won't be as anxious.

Poll everywhere
Utilize live polling, surveys, Q & As, quizzes, dialogues, and other interactive tools to keep attendees interested during distant meetings and in hybrid workspaces. By displaying real-time employee feedback, you can assess engagement. Utilize a navigation and folder structure that are completely adaptable to meet the specific workflows of your team to organise your content. Intelligent apps You may poll people while you're on the go by downloading the Poll Everywhere app for PowerPoint. You can quickly add polls to already-created Keynote and Google Slides presentations.

A teacher who uses it said "I really like the different options with this resource".

Nearpod
Use formative evaluations and dynamic media features to visualise and promote student knowledge to improve learning outcomes. Improve student learning by putting engaging lessons, materials, and content all in one location. delivering individualized, enhancing, or additional help to students wherever they are learning in accordance with their needs (physical classroom, remote, hybrid). Interactive slides: Based on information gathered about a student's comprehension, create interactive classes with slides. Interactive videos - Involve students in interactive video lessons that review ideas and include built-in comprehension questions. Thousands of customised, ready-to-use classes from top teachers. Gamification and activities - For deeper learning and engagement, incorporate gamification and activities like Time to Climb, Matching Pairs, Draw It, and NEW Drag & Drop into Nearpod. This enables, you can modify your educational experience to suit your needs. Choose from a huge selection of activities spanning all grades and subjects. Whatever your preferred learning method—classroom, online, or hybrid—you'll feel comfortable. You can use the same lesson no matter what device type or availability you have. A teacher commented.

I found this the most useful tool for gathering responses from learners. There are lots of different ways of collecting responses, for example open-ended questions, drawing on the whiteboard, collaborate, adding Flipgrid. You can also upload PPs which it will convert into a presentation. I think a lot of the tools that are available have many of the same facilities, and it might be important to restrict yourself to a few, so that both you and your learners become fluent in using them. The danger in using too many different one is that how to use the technology becomes the focus of the session, rather than the teaching itself".

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Classroom screen

Engage your students and support classroom activities by using Class room screen's intuitive tools. Has a lot of standard classroom features? There's a timer, a text box, a stop watch, a writing pad, a QR code and more.

"You can login with a Google account. Some useful features in this app. I like: the QR code that students can scan to link to another URL; different background patterns for drawing- would need to be an interactive whiteboard; for maths teachers, in type text there is the option to type x squared with the floating 2. It is aimed more at face to face teaching and managing a class, but perhaps could be used through something like Zoom or Meet to share the screen".

YO Teach

Teacher-created and student-moderated chat rooms run through this backchannel web application. There's no charge and it's easy to use. QR codes and links are available for students to use.

Whiteboard.fi

Assignments - Students in your room can now receive assignments from you. In this way, the student can load and deal with the assignments at their own pace, like the push. You receive an overview of all the submissions once they have completed an assignment. Assignments can be used for assigning homework to students when using permanent rooms. Even if you as a teacher are not present will be able to access your permanent room, load assignments, and submit them. There are many new possibilities opened up by this. As well as incorporating the new Tools for learning maths.

"I love this. I can differentiate subtly as well but sending different questions to different students. Students actually interact and it works on all devices. I am considering paying for a subscription to get the feedback options. One issue is that with the free version, you have to put all the resources on new each day, but I just screen shot after the first session and then copy and paste. Another is that it does require a decent internet connection, so some of my groups really struggle".

Online stickies boards

When your collaborators cannot meet and think together in the same room, what happens when sticky notes provide an easy way to brainstorm? With an infinite canvas, anyone can generate and share ideas from a computer, tablet, or smartphone.

I've used Linoit quite a it's excellent for revision and it's great that the students interact with it. I like this for sharing comments on work that the students have done in a common way so that they can look and consider and then feedback to me.

QR code generator

Typically composed of a black and white pixel pattern, QR Codes are two-dimensional versions of barcodes. As part of Toyota's auto production, Denso Wave, a subsidiary of the Japanese company, developed them to mark components in order to accelerate logistics processes.

"You could create a live treasure hunt for students. Took me a little white to get the links to work as I wanted but has some great potential".

Other tools are Video resources such as Screencasting, Questioning/ quizzing, Flashcards + mindmaps
Literature review

The start of 2020 marked the beginning of closures in numerous countries worldwide including UK due to the COVID-19 pandemic. It is important to discuss how to fight the pandemic by discussing the methods of learning. While experiencing these unfamiliar periods, teachers adapt to better serve students by adapting to new teaching and learning circumstances. As stated by Diab and Elgahsh (2020), E-learning has become a crucial improvement to the educational process especially in this period in which education utilizes technology to the maximum. In the

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realm of e-learning, the fields of online learning, online training, and technology-enabled teaching are combined. When a teacher and student meet directly at the same location and time, the learning takes place face to face. Session times are synchronous in-person instruction. Face-to-face sessions do not require communications technologies (Caner, 2012). In accordance with research (Graham, 2006), blended learning is being recommended for three main reasons:

1) Enhancing the efficiency of education.
2) Greater convenience and access.
3) Cost-effectiveness improvements.

This facilitates instructors in creating mobile apps to organize and teach students multi-tasking. According to Alebaikan and Troudi, (2010), BL would make it easier to communicate with students and teachers, thus ensuring more operative learning. Aslam, (2015) points out that the future of Pakistan lies with the kids of today, yet Pakistan's educational practises squander that potential. To bond gaps in teaching modernly, some attentive steps are required. Blended learning methods can enable the colleges to prepare students for the workplace. The ease approach to English language skill can be learned through blended learning. It is confirmed by Jordan (2004) that combines in-person training with online learning increases the chances for community involvement, thus strengthening its cohesiveness. It provides an opportunity for students to engage with their professors and with each other. The use of BL in higher education is expanding for a variety of reasons (Bliuc, Goodyear & Ellis, 2007; Dziuban, Hartman, & Moskal, 2004; Garrison & Vaughan, 2008; Graham, 2006; Oh & Park, 2009; Osguthorpe & Graham, 2003; Shea, 2007).Academic learning could be improved by integrating asynchronous learning networks with on-campus courses (Hiltz & Turoff, 2005), which could eventually result in blending all classes (Masie, 2006; Massy, 2006; Ross & Gage, 2006). With modern technologies increasingly being invested in education, blended learning succeeded in combining traditional and online education should be combined in a dynamic, flexible environment that is advantageous to both professors and students (Halverson & Graham, 2019). Goals and Research Questions for the Current Study: The goal of the current study is to examine blended learning implementation from the perspective of the instructors who used the technologies to deliver the lectures. Along with student characteristics and blended learning design elements, it also concentrated on knowledge construction, performance, and intrinsic motivation and how these connect to each other in a blended learning environment.

Objective of the research

The following are the review criteria for the current study:

I. The purpose of the current study is to examine how blended learning has been implemented from the perspective of the teachers who employed the technologies for lesson delivery.
II. Find out how important is Blended Learning and
III. Analyze the Concept, Need, Challenge and Trends of Blended Learning.

Research Issues

1.How have college teachers found the face-to-face component of blended learning to be in terms of their experiences and opinions?
2.How have college teachers used the online component of blended learning to learn, and what are their experiences and perspectives on it?
3. From a teacher's perspective, what are the technical issues with blended learning implementation?

Objectivity of the Study

The study's conclusions will improve teacher-training programmes, computer-assisted content learning, and the study of blended learning. Additionally, by conducting this study, it will be possible to give recommendations to programme directors and administrators about how to effectively support and equip teachers for instruction in a blended language learning context. This is a vital addition because it is anticipated that the trend of converting college courses to a blended learning model will continue and greatly grow over the next few years. This study may also make a contribution to the conceptual framework for assessing the effectiveness and student views of a blended

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learning environment. The study's final possible contribution focuses on how little, if any, prior research has looked into how teacher behaviour and practise may affect students' impressions of a mixed language learning setting.

Research Methodology: The following resource methodology was used for the investigation's objectives;

Research Method: Descriptive

Type of Sampling: Convenience sampling

Sample size: 53Teacher of Education (FE) i.e. post +14 (students above 14 years of age) UK

Type of data collection: Use of a structured questionnaire for a survey.

Source of Data Collection: A systematic questionnaire was used to gather primary data, and published sources such as journals were used to gather secondary data.

Study context

The setting of this study's investigation is to explore the experiences of teaching staff in Further Education(FE) i.e. post +14 ((students above 14 years of age) while using various digital tools for blended learning during COVID-19 in UK. We chose this setting for a number of reasons. The researcher's experience in the FE sector, in particular, gave them a unique perspective on this setting, including the difficulties teachers confront as well as the wants and needs of students. Secondly, there is abundance use of blended learning via various digital tools by FE teachers. The efficacy of blended learning is the subject of this study. First goal is to discover whether blended learning process using various digital tools is able to satisfy the demands of the educators as they establish a learning environment for their pupils. The study's second objective is to assess the teachers' opinions of the blended learning environment's effectiveness. Finding out how the third and final objective is how the usage of blended learning will lead to specific skill development in teachers.

Data Analysis
Sample breakup:
Interpretation: The sample for the research comprised mostly academic staff which is $8 in numbers out of 53.
Interpretation: Most of the teacher who were a part of this ongoing research were full time Teachers
Interpretation: From the above analysis its very much clear that blended learning makes the teacher confident when thy are imparting lesson using digital tools which is the view of nearly 32 of the respondents.
Interpretation: The above analysis sheds light how blended learning is useful in achieving the outcomes in term one, shows that it able to meet its purpose. which is indicated by 37 of the respondents.
Interpretation: The above analysis indicates that Engagement Of Students, Wellbeing and progress of the students is evident due to Blended learning which is indicated by 131,117 and 137 of the respondents .
Interpretation: There is substantial change in the wellbeing of the teachers which is evident from above analysis as indicated by 117 of the Respondents. More specifically there is a change in professional development of the teachers due to blended learning in usage.

Hypothesis Testing

Ho: Blended level does not Increases the confident and development of teachers.
H1: Blended level Increases the confident and development of teachers.

Hypothesis: The Blended level Increases the confident and development of teachers.

Testing statistically: sign binomial test

Variables and measurement

On a five point scale, respondents were asked to comment on the following claims: (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree).

a. Change in the teaching aspects increases my work load
b. Change in the teaching aspects increases Professional development
c. Changes in teaching practices increases engagement

Cut point: Using the CUT POINT option with the value "3" in IMB SPSS 21, the original 5-point scale was changed to a 2-point scale. As a result, the newly developed categories were:

>3: In agreement = 3: Disagree

Test ratio: The test ratio was set at 0.5. Since more than 50% of replies to one category show that this category is more popular.

Thus, P = 0.5

H0: P 0.5 (responses indicate "AGREEMENT" in a greater-than-or-equal-to-50 percent of cases).
H1: P 0.5 (less than 50% of responses are "AGREED" responses).
Significant level: = 0.05

Interpretation

a. A change in the teaching aspects makes my workload more demanding

Test proportion: 0.5, Observed proportion: 0.28, and p = 0.000

Therefore, fewer than 50% of respondents concur that changing certain components of my teaching will raise my workload.

b. Improvements in teaching methods lead to more professional growth

Test proportion: 0.5, observed proportion: 0.32, and p = 0.000

c. Therefore, fewer than 50% of respondents concur that changing teaching methods will lead to greater professional development.

d. Changes in teaching practices increases engagement

Test proportion: 0.5, Observed proportion: 0.30, and p = 0.000

a. As a result, fewer than 50% of respondents concur that changing teaching methods promotes engagement.

The hypothesis that a blended level increases instructors' confidence and professional growth is proven because for all three variables the observed proportion (AGREEMENT) is less than 50% and the p value is less than 0.05.

Blended learning research findings

Students are engaged more in the learning process. Also their access has been increased with special reference to time and physical location. There is high Level of learning and high student's faculty. Different types of online methods are used resulting in faculty and student satisfaction. Teacher require much of time in preparing to physical classes. Blended learning hardly enhances the research contribution of the teachers. Technical skills like usage of Mentimeter, Kahoot, Yacapaca, Maths Everywhere and GoConqr is more required by teachers in order to cope with the changes to their teaching practice. Blended Learning has affected both teachers and students in a positive way.

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11. Journal of International Education Research – Fourth Quarter 2015 Volume 11, Number 4 Copyright by author(s); CC-BY 253 The Clute Institute The Effect Of Blended Learning Approach On Fifth Grade Students' Academic Achievement In My Beautiful Language Textbook And The Development Of Their Verbal Creative Thinking In Saudi Arabia Feras Mohammed Al-Madani, Northern Border University, Saudi Arabia

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Table 1: Sample breakup

Role in the College		
Sr.No.	Response	Frequency
1	A member of academic staff	48
2	Clinician	5
Total		53

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Table :2 Role status		
Sr.No.	**Response**	**Frequency**
1	Full-time	51
2	Part-time	2
Total		53

Table : 3. Overall impression of blended learning		
Sr.No.	**Response**	**Frequency**
1	Confident to deliver my teaching	12
2	Somewhat confident	20
3	Stressed	7
4	Excited	8
5	Ups and Downs	6
Total		53

Table 4. To achieve the learning outcomes for your teaching in Term 1		
Sr.No.	**Response**	**Frequency**
1	Not at all	9
2	A little	7
3	Somewhat	4
4	Mostly	13
5	Completely	20
Total		53

Table: 5 Change in your teaching practice impacted these aspects

Sr.No.	**Change in your teaching practice impacted these aspects**	**Rating Total**
1	Engagement Of Students	131
2	Wellbeing of Students	117
3	Progress of Student	137
4	Formative assessment	109
5	Summative assessment	107

Table: 6 Change in your teaching practice impacted these aspects

Sr.No.	**Change in your teaching practice impacted these aspects**	**Rating Total**
1	Wellbeing of Yours	117
2	Workload of Yours	104
3	Research of Yours	96
4	Engagement of Yours	103
5	Professional development of Yours	139

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Table 7. Binomial Test

<table>
<tr><th colspan="6">Binomial Test</th></tr>
<tr><th></th><th>Category</th><th>N</th><th>Observed Prop.</th><th>Test Prop.</th><th>Exact Sig. (2-tailed)</th></tr>
<tr><td>Change in the teaching aspects increases my work load</td><td>Group 1   <= 3</td><td>47</td><td>0.72</td><td>0.50</td><td>0.000</td></tr>
<tr><td></td><td>Group 2   > 3</td><td>6</td><td>0.28</td><td></td><td></td></tr>
<tr><td></td><td>Total</td><td>53</td><td>1.00</td><td></td><td></td></tr>
<tr><td>Changes in teaching practices increases professional development</td><td>Group 1   <= 3</td><td>45</td><td>0.68</td><td>0.50</td><td>0.000</td></tr>
<tr><td></td><td>Group 2   > 3</td><td>8</td><td>0.32</td><td></td><td></td></tr>
<tr><td></td><td>Total</td><td>53</td><td>1.00</td><td></td><td></td></tr>
<tr><td>Changes in teaching practices increases engagement</td><td>Group 1   <= 3</td><td>44</td><td>0.70</td><td>0.50</td><td>0.000</td></tr>
<tr><td></td><td>Group 2   > 3</td><td>9</td><td>0.30</td><td></td><td></td></tr>
<tr><td></td><td>Total</td><td>53</td><td>1.00</td><td></td><td></td></tr>
</table>
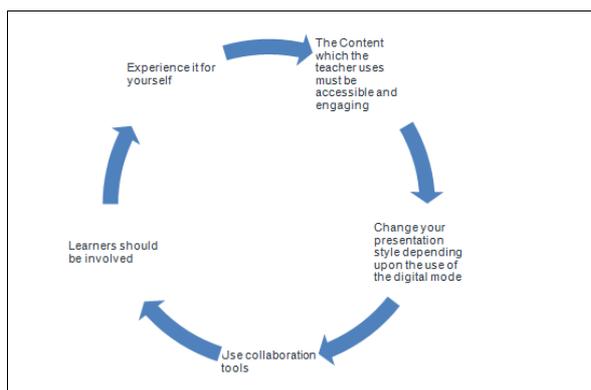
Fig.1. Some of the Digital Mostly preferred by the learners as well as teachers
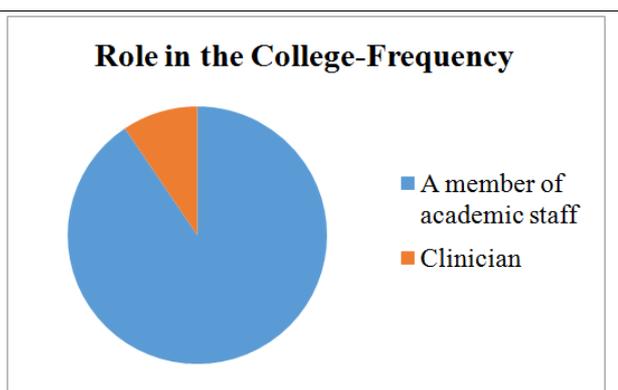
Fig.2. Role in the College-Frequency
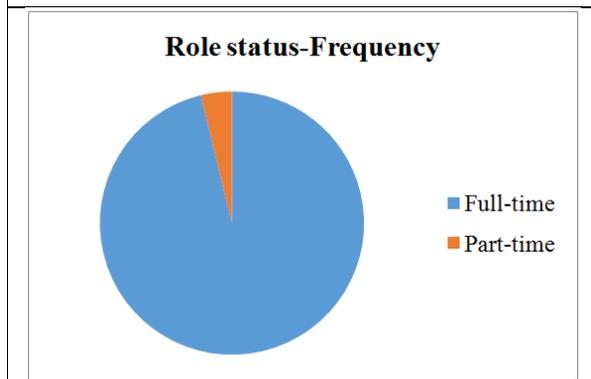
Fig.3. Role status-Frequency
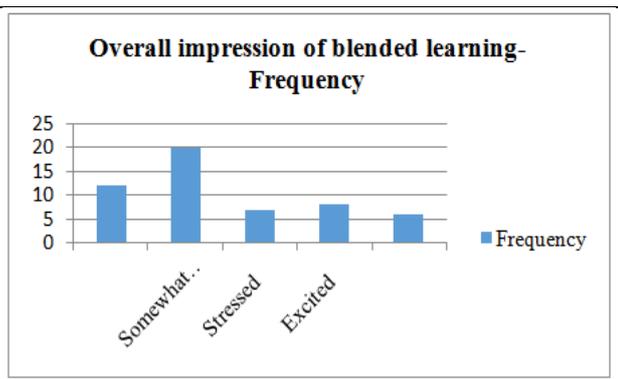
Fig.4. Overall impression of blended learning- Frequency

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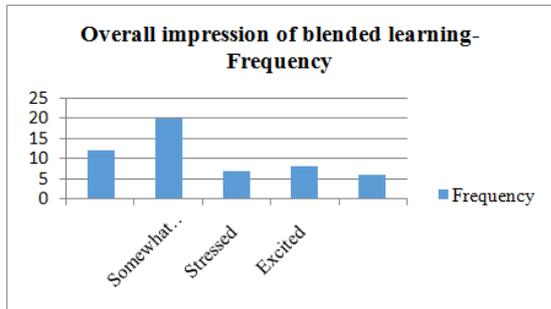
Fig.5. Overall impression of blended learning- Frequency
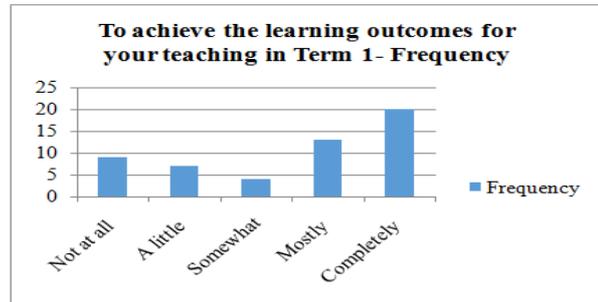
Fig.6. To achieve the learning outcomes for your teaching in Term 1- Frequency
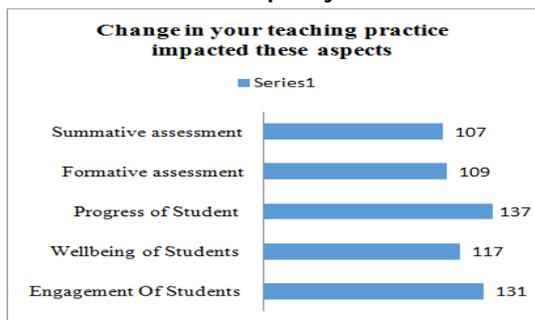
Fig. 7. Change in your teaching practice impacted these aspects
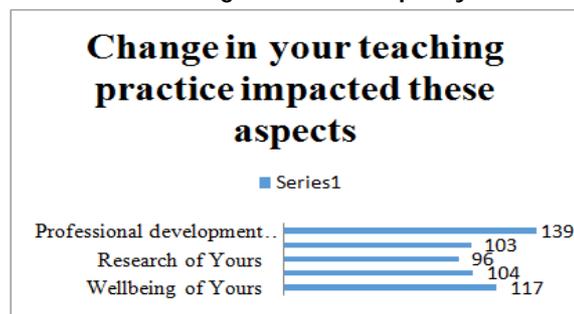
Fig.8. Change in your teaching practice impacted these aspects

RESEARCH ARTICLE

Egyptian Vulture, Woolly-Necked Stork, Indian Black Ibis and Black Kite Nesting on Mobile Towers in and Around Udaipur District, Rajasthan

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ABSTRACT

Egyptian vulture (*Neophron percnopterus*), Woolly-necked stork (*Ciconia episcopus*), Indian black ibis (*Pseudibis papillosa*) and Black kite (*Milvus migrans*) nesting on mobile tower is an example of rapid adaptation of bird's to anthropogenic and urban development activities. The cutting of large and old trees, habitat loss due to deforestation, expansion of urbanization, industrialization and mining of rocks leads to a lack of appropriate and suitable natural nesting or roosting sites for birds. Alteration in the natural environment and loss of habitats is responsible for birds nesting on anthropogenic-made structures like mobile towers, electric poles etc.

Keywords: Mobile tower, Nesting, Birds, Urbanization, Habitat, Trees

INTRODUCTION

Environmental changes resulting due to anthropogenic activities such as urbanizations, industrialization, establishment of new colonies and roads, deforestation, habitat fragmentation and human disturbance have a significant impact on the diversity and abundance of birds (Blair, 1996; Sauvajot *et al.,* 1998; Fernandez-Juricic, 2000; Fernandez-Juricic and Tellera, 2000;Mckinney, 2006; Bhattacharya and Roy, 2013; Yu and Guo, 2013; Bhattacharya *et*

al., 2013).The favourable habitat is continuously declining due to changes in the composition of vegetation and also affected by modification in the environment. In the last decade, the rapid growth of communication structures has increased anthropogenic interference with nature, resulting in an adverse impact on physical, biological and ecological systems (Gottlieb *et al.,* 2011). Electromagnetic radiation may also be responsible for changes in bird composition due to changes in nesting and breeding strength (Chamberlain *et al.,* 2009; Bhattacharya and Roy, 2013). Almost 241 bird species died due to tower collisions and radiation (Reynolds, 2001).

Mouritsen and Ritz (2005) estimated that approximately 6.8 million birds died due to collision with towers every year. Electromagnetic frequency has been shown negative and harmful impact on chick development, disrupting a variety of functions like brain development, cell and membrane development, enhancing the rate of cellular apoptosis, blood vessel rupturing and alteration in the blood brain barrier (Kalantari *et al.,* 2015). Rapid expansion of urbanization increasing the density of buildings, highways, flyovers, electric transmission lines and communication towers, which negatively impacts natural habitats like forests, wetlands, uncultivated areas, mountain ranges and agricultural land. These structures are utilized by birds and mammals as shelters, roosting and nesting sites (Mainwaring MC, 2015). and On the other hands, mobile tower, electric power lines, buildings and road-vehicles transport increase the risk of wildlife survival (Morelli *et al.,* 2014; Mainwaring MC, 2015).

Bird species have become more reliant on power lines and mobile towers for perching, roosting and nesting in urban areas (Infante and Peris, 2003; Anderson and Hohne, 2007). Electromagnetic radiation has a greater impact on birds than it does on humans because birds have a thin skull and feathers that can act as a dielectric receptor for microwave radiation. Birds also act as biological indicators for low-intensity electromagnetic radiation (Liboff and Jenrow, 2000). Nest construction is a taxonomically ubiquitous behaviour in insects, fishes, reptiles, birds and mammals all builds nests to lay eggs and rearing of hatchlings (Hansell MH,2000). The selection of nesting sites usually depends upon the habitat characteristic features of the surrounding species, which may change their nesting preference depending on the natural habitat in which they live (Castillo-Gomez and Moreno-Rueda, 2011). In the present study, we discuss the mobile tower nesting of Egyptian vulture (*Neophron percnopterus*), woolly-necked stork (*Ciconia episcopus*), Indian black ibis (*Pseudibis papillosa*) and black kite (*Milvus migrans*) in and around Udaipur district, Rajasthan.

MATERIAL AND METHODS

The Present study was carried out in various parts of Udaipur district including rural and urban area. Nikon 8X40 Binocular was used for behavioral observation and taking photograph with the help of Nikon P 1000 SLR Camera. Regular observation was taken of Egyptian vulture, Woolly-necked stork, Indian Black ibis and Black kite. Photographs were taken without disturbing birds during the study.

RESULT AND DISCUSSION

Nest construction is an essential and vital part of the life cycle and taxonomically significant among birds, mammals, reptiles and even in insects (Hansell, MH 2000). Selection of nesting sites and nesting material is a crucial and remarkable factor for determining breeding success among animal groups, including avian taxa (Coulson JC, 1968; Mc Crimmon DA, 1978; Ryder PL and Ryder JP, 1981; Rendell and Robertson, 1989; Martin TE, 1991; Tuomenpuro, 1991). The Egyptian vulture is a globally endangered species and its populations have continuously declined in most parts of the world (Botha *et al.,* 2017). The Egyptian vulture is a small scavenger and distributed throughout the Palaearctic, Afro-tropical and western Indo-Himalayan geographical regions (Ferguson-lees *et al.,* 2001). Usually, Egyptian vultures are found in high rocky plains, savannahs, semi-desert grassland and croplands in wintering sites. During the breeding season, they are generally seen in mountain terrains, hilly areas, woodlands, plateaus, cultivated areas and near anthropogenic settlements (Ferguson-Lee and Christie, 2001; Cramp and Simmons, 1980;

Sara and Divittorio, 2003; Arkumarev *et al.,*2014).Usually, Egyptian vulture construct nest in hidden and camouflaged caves, rock cliffs, tall trees, ancient and historical places like forts and temples. Besides all this places, we observed an Egyptian vulture nesting on the mobile tower. It was continuous for last two years, 2020 and 2021, on the same mobile tower (Figure 1, 2).The nesting of Egyptian vultures was located at the edge of Lotus pond, near Kailashpuri Village, Udaipur district on mobile tower was 24°44'34N 73°42'59''E. Male and female both participate in the collection of nesting material, nest construction and nest rearranging.

The woolly-necked stork is a large-sized wading bird and distributed in the Indian subcontinent. They prefer a wide variety of habitats, including flooded grassland, irrigated cropland, cultivated areas, near the lack and artificial water bodies like dams and canals and also inhabit deep forest areas (Ali and Ripley, 1978). According to Birdlife International (2021), this species is categorized into vulnerable categories and their population is continuously declining due to habitat loss and destruction of natural habitat. The nesting season of the woolly-necked stork may vary geographically too; The nesting season from July to September in southern India and December to March in northern India (Ali and Ripley, 1978). Generally, they construct nests on tall and large trees like *Bombax ceiba*, *Ficus benghalensis, Ficus religiosa,Dalbergia sissoo* and *Tamarinducs indica* (Ali and Ripley, 1978; Hume, 1980). Woolly-necked storks nesting was observed on rock cliffs in the Chambal river valley by Rahmani and Singh (1996) and Vyas and Tomar (2007).

We observed nest of woolly-necked stork on a mobile tower in Bhatevear village, Udaipur district, Rajasthan, 24°37'03''N 73°58'36''E. During observation, we found one adult sitting inside the nest, rearranging twigs and branches (Figures 3, 4). At the same time, other adults were flying near the mobile tower. Inside the nest, eggs and hatchlings were not sighted due to height of mobile tower.

The black ibis is a common bird of South Asia, with populations found all around India, Pakistan, Nepal and Bangladesh (Ali and Ripley, 1983, Rasmussen and Anderton, 2012). The red-napped black ibis is a medium-sized wading bird species and belongs to order Pelecaniformes and family Threskiornithidae (Hancock *et al.,* 2001; Sibley and Ahlquist, 1972; Eisenmann *et al.,*1984; IUCN, 2016). According to IUCN red list category, the red-napped black ibis was categorized as the least concerned species (IUCN, 2016). Red-napped black ibis roost and breed near the wetland and usually nest on canopy of trees and bushes such as *Acacia*, *Prosopis* and *Ficus* (Balakrishnan and Thomas, 2004; Balakrishnan and Thomas, 2004; Senma and Acharya, 2009). The red-napped black ibis, on the other hand, does not roost or nest in colonies, prefer to build a single nest on a tree (Senma and Acharya, 2009).Black ibis is solitary nesters and usually construct nests on tall trees. Their ecology were studied by (Soni *et al.,* 2010; Chavda, 1997; Soni *et al.,* 2010). Large trees such as *Azadirachta indica* and *Ficus religiosa* are choice of places to build black ibis nests (Soni *et al.* 2010). According to Ali and Ripley (1983), black ibis frequently used the abandoned nests of crows, kites and vultures. Mohamed Samsoor Ali *et al.,* (2013) observed black ibis nesting on power transmission line pylons in the Samakhiyali area of Gujarat state.

During the field visit, we found Indian black ibis (*Pseudibis papillosa*) nesting on mobile towers at Sanjay Park, Fathesagar Road, Udaipur district, Rajasthan at 24º35′44′′N and 73º39′53′′E(Figure5, 6). During observation, we found one nestling was present in the nest and an adult black ibis preened to nestling (Figure 7). Nestling neck movements were also observed inside nest(Figure 8).The nest was made mainly of leaves and twigs. This area consists of a variety of plant species, mainly *Azadirachta india*, *Acacia nilotica*, *Mangifera indica*, *Eucalyptus*, *Butea monosperma, Ficus religiosa* and *Prosopis juliflora*. During observations, we noticed that one individual always stays in the nest while the other individual go off to find food. At the time of the movement, black kites were also flying and foraging in this area. When the black kite flies over the nest, the adult black ibis begins to make noise and stands inside the nest, while the other individual flies away from the nest on *Eucalyptus* tree(Figure 9, 10).

Black kite (Milvus migrans) is a medium-sized raptor and belongs to family Accipitridae and order Accipitriiformes; found in Africa, Asia, Australia and Eurasia (Ferguson-Lees and Christie, 2001; Birdlife International, 2021). The

black kite is a resident raptor species in India and presence of a larger population in urban areas makes it a least concerned species according to IUCN status (Mahabal and Bastawade, 1987; Naoroji, 2006; Bird Life International, 2009). It is an opportunistic predator and scavenge that can be sustained in a wide variety of habitats, its diet is highly diverse and also influenced by geographical locations (Sergio *et al.,* 2003; Cortes-Avizanda *et al.,* 2011; Panuccio *et al.,* 2013; Tanferna *et al.,* 2013). Black kite breeding ecology has been extensively studied throughout the world, particularly in Japan and Europe (Haneda and Koizumi, 1965; Koga *et al.,* 1989; Fiuynski and Wend land, 1968; Meybure, 1969; Desai and Malhotra, 1979; Fiuynski, 1981). Various factors are responsible for nest-site selection in black kites.

Usually they prefer nesting on tall trees and are also influenced by local vegetation compositions and some time they also construct nests on mobile towers and electric pylons (Kumar *et al.,* 2014; Bakhtin, 2015). Large-scale changes in ecosystems, such as human settlement, vanishing of natural and forest habitats, and deforestation, may become limiting factors for selection of black kite nesting sites (Boeker and Ray, 1971; Scott, 1985; Hunt *et al.,* 1999). On April 18, 2022, black kite nest were observed on a mobile phone tower near Vidhya Bhawan Society, Fatehpura, Udaipur, Rajasthan,24°36′36″ N and 73°41′66″ E. During observation, one black kite adult remained in the nest (Figure 11). At the same mobile phone tower one deserted nest were observed. It is may be of black kite and house crow (Figure 12). One adult sitting in an egg-incubating position and other adult fly around mobile tower looking for food and occasionally sitting away from the nest(Figure 13). Approximately 25-30 black kites were roosting on different mobile tower in the study area (Figure 14). The nesting of these birds on mobile towers indicates the rapid expansion of urbanization and the lack of suitable nesting and roosting sites in the study area.

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Figure 1- Egyptian vulture nesting on mobile tower in Udaipur district	**Figure 2- Overview of mobile tower and Egyptian vulture nest**
Figure 3- Woolly necked stork rearranging nest on mobile tower	**Figure 4- Overview of mobile tower and woolly necked stork nest in Udaipur**

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Figure 5- Mobile tower nesting of Indian black ibis in urban area of Udaipur district

Figure 6-Overview and habitat around of mobile tower nesting of Indian black ibis

Figure 7- Indian black ibis adult individual preen to nestling

Figure 8- Indian black ibis adult and nestling in mobile tower nest

Figure 9- Indian black ibis standing in nest

Figure 10- Indian black ibis sitting on *Eucalyptus* tree near by nesting tower

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Figure 11- Black kite nesting on mobile tower in urban area of Udaipur district

Figure 12- Deserted nest on mobile tower in urban area of Udaipur district

Figure 13- Black kite adult individual sitting away from nest

Figure 14- Black kite roosts on mobile tower in urban area of Udaipur district

RESEARCH ARTICLE

Comparative Study of Inspiratory Muscle Training versus Sandbag Breathing on Respiratory Parameters in Older Individuals

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ABSTRACT

Aging human adapts varying bodily functions over the time naturally. Respiratory system also gets affected by aging by reducing muscle strength of diaphragm mainly. There is two interventions that is giving effective result for respiratory function that are In spiratory muscle training by threshold device and Sandbag breathing. Need for study is to compare this both treatment. 30 subjects were chosen by using inclusion and exclusion criteria with the age of 60-75 years. Pre and post FEV1, FVC, MVV, FEV1/FVC was checked. In spiratory muscle training was given by using threshold loaded and sandbag breathing. Whole procedure was given three days per week for four weeks. Intervention shows significant result in both the group except for FEV1/FVC in In spiratory muscle training group. For between group results suggest more significant in Sandbag breathing. Both the treatment is useful and gives positive effects for respiratory muscle strengthening. Among them sandbag breathing is more effective.

Keywords: In spiratory muscle training, Sandbag breathing, Spirometry, Aging, Respiratory muscle strengthening.

INTRODUCTION

Aging human adapts varying bodily functions over the time naturally [1]. It affects in various system in our body so overall health gets affected due to age. It mainly affects muscle strength. With aging one might struggle or have difficulties to achieve simple routine task which were simple but with deteriorating muscle strength feel quite

challenging [2]. One of the most vulnerable system in a human body is respiratory system which goes through different types of structural, physiological and immunological changes with age [3]. Some physiological changes which is decrease in muscle fiber type 2 and respiratory systems get affected by aging as there is impaired muscular integrity. This decrease in type 2 muscle fibers decreases the elasticity of lung compliance and chest wall compliance there for overall reduce in total respiratory system compliance. The studies have shown that there is roughly 25% of decrease in elastic strength of diaphragm in individuals with older age [4]. From the age of 65 and onwards there is a continuous and consistent decline in the strength of respiratory muscles. (Enright at al. 1994) [5].

As being a loco motor muscle the diaphragm can respond to certain load applying methods on it, as diaphragm is also a skeletal muscle like all the other muscles which functions like one too.[6] In spiratory muscle training could potentially help generate some pressure and thus increasing the resistance to muscle fatigue and also helping its functionality with a higher capacity of blood flow redistribution and higher lung air volume [7]. The most effective, efficient and almost risk free and safe method of training old aged patients to help them improve their lung capacity and functions by using device is known as In spiratory muscle training.[8]Training with threshold Loaded device not only helps with respiratory functions and strength of in spiratory muscles but some studies even have noted and suggested that overall greater cardiopulmonary fitness achieved by the better utilization of respiratory muscles.[9]

Gaining back some of the in spiratory muscle strength helps the individual in physically demanding tasks as maximum oxygen consumption increases and muscle fatigue decreases. IMT helps to minimize the risk and Damage comes with the ageing [10]. Being a conventional way of breathing we all are familiar with the concept of abdominal breathing with inhaling cause the abdomen to bulge and exhaling cause the abdomen to retract[11]. Intra-abdominal pressure (IAP) breathing which is characterized by the gradually increasing pressure in the abdomen while inhaling and exhaling which eventually strengthens the abdominal muscles [12]. Patient who have lack of respiratory muscle strength just by virtue of being elderly or are not aware of it for them this sandbag breathing technique is vital tool to achieve a higher diaphragm strength and a general moral booster as well as improved deep breaths. All this because sandbag provides a necessary resisting weight for in spiratory muscles [13].

Different changes that happens due to age is in respiratory mechanics and physiological capacity that decreases. This includes decrease in ratio of forced expiratory volume in a second and forced vital capacity as well as volume of air that remains in the lung after forced expiration that is RV also increases with that functional residual capacity also increases [14]. To assess the patient's respiratory functions, we can use spirometry. There are two types of lung volume is there one is dynamic and other is static. Forced expiratory volume in one second (FEV1), Forced vital capacity (FVC), and FEV1/FVC Ratio are all examples of dynamic lung volumes. Static Lung Volumes: Total lung capacity, vital capacity, Residual Volume and Functional residual volume[15].This volume varies depending upon Height and weight in other term body size [15].

MATERIALS AND METHODOLOGY

30 subjects were taken from sainath hospital bopal-Ahmedabad by using simple random sampling for this experimental study of 4 weeks duration.

Inclusion criteria: Age: 60-75 years, asymptomatic subjects related to breathing of either gender, FEV1/FVC ratio 70-75 % in range, Participants who agreed to take part in the research were included in the study, Non-smokers.
Exclusion criteria: Chronic lung disease like asthma, obstructive or restrictive pulmonary disease, diabetes (Allet et al. 2008), heart disease that prevents physical activity, beta blocker medication, vertigo in the last 6 months, musculoskeletal disorders, Spinal surgery, ribs fracture and gastric problems, Participating in any rehabilitation training protocol, If subject is not understanding any of the information.

Procedure of outcome measure

FEV1, FVC, MVV and FEV1/FVC was checked using spirometry

Spirometry: Doctors use this standard test to check lungs functioning how well it is working. Main concept of this test is by checking airflow in and out into lungs.

Subjects should be in sitting position on chair. Nose clip is placed in nose so by it nostrils remains closed. Examiner also place breathing mask around the mouth. Then ask patient to take a deep breath in and hold it for some period and then exhale as much as they can by breathing mask. [16]And Ask patient to repeat the same for at least three times so we can get accurate result. There might be chances that patients can't understand the task so give them time to understand. Ask patient to feel free if they want to perform it again and take highest value among them as your final result.

Procedure of intervention

Group A: In spiratory muscle training (Photograph 1, 2)

15 subjects will be taken in one group checked for FEV1, FVC, FEV1/FVC ratio and MVV before and after in spiratory muscle training. Subjects were advised not to take food before and after the exercise[17] and they were asked to be in sitting position on chair with back supported 90-degree angle of torso and thighs in comfortable position. Nose clip was used to prevent any type of leakage of air that decrease the effort expended [18]. Mouthpiece of IMT threshold device was given in mouth and participants were asked to take deep breath through mouth as much as they can. 30 consecutive dynamic breaths twice a day with 5 minutes of interval in between was performed. Load on device was set at 50% of MIP for individuals. That way the resistance was improved. Participants were instructed to begin breathing from residual volume and to increase the in spiratory volume with each repetition. Sessions was set three days per week which is alternative for four weeks [19].

Group B: Sand bag breathing (Photograph 3)

15 subjects will be taken in one group checked for FEV1, FVC, FEV1/FVC ratio and MVV before and after sandbag breathing. Subjects were advised not to take food before and after the exercise [17] and they were lie on back with little with or without support of pillow under head and neck with hands aside resting on plinth. Start with relaxed breathing for few repetition and after that According to the patients' 50 percent of 1RM, a one-kilogram sand bag was placed over the upper abdomen in the diaphragmatic region. And further go for progression. Patient is asked to do inspiration, hold for 2 seconds and expiration.[20]Exercises performed for 10 repetitions /set. 4 sets/session, 5 minutes of interval was given to them between two sessions.[20]Sessions was set three days per week which is alternative for four weeks.

RESULT

Statistical analysis was performed using SPSS version 25. Total thirty subjects out of thirty-seven completed the four weeks of training protocol. In this study group A Received In spiratory muscle training through threshold device (n=15) and group B received Sand bag breathing (n=15). Both the groups were similar in age, gender and pulmonary function. The study Analysis was done to evaluate between group interventions (IMT Versus Sandbag breathing), Pre-post intervention. Within group effects were explored using paired t-test and between group effects were explored by using independent t-test. In the result data shows value of mean, standard deviation (SD), t value and degree of freedom (df). Statistical significance was set at $p<0.05$. From total 30 subjects there were 12 females and 18 males in study they were equally divided in both the groups. By using independent sample test results for FEV1, FVC, MVV and FEV1/FVC shows significant improvement in Sandbag breathing as p value is <0.05 (Graph 1,2,3,4)

As shown in table 2 within group result by using paired t test shows significant results in FEV1,FVC and MVV in both the groups except FEV1/FVC as its p value is >0.05.

DISCUSSION

This study shows comparison of in spiratory muscle training by using threshold device versus sandbag breathing on pulmonary functions like FEV1, FVC, MVV and FEV1/FVC how it gives effects on parameters in 30 older individuals. One study that helps to understand our study result is done by Francesco V Ferraro et al. by using respiratory muscle training in older they stated that the desired improvement would be to seen in spirometry measurement rather than physiological changes following some IMT are most probably refer to task-learning effect, which is in accordance to Mills et al. (2015).So we can see improvement in our study might be due to both task learning effects and by neural activation [21].

The above result can be elaborated further by Eastwood PR et al. by giving reason for the same that simple neural conditioning which results from repeated task, which creates learning effect and phenomena that helps gather some respiratory muscle strength by increasing and thus bettering of the neuromuscular recruitment pattern [22]. A study carried and reported by Feriani et al. 19,Did study for aged women with metabolic syndrome and he saw the positive results with only 7 days training with IMT device in that spring is set as a resistance. This study is easily replicable with desired group of elderly people and an attached report of similar study on principle was performed by me with using that threshold loading device for respiratory muscle training. On the other hand our second group received sandbag breathing which mainly works to activate Diaphragm this gain support by Lee (16), observed some improvements in pulmonary function and muscle strength in adults with forward head posture by meticulously understanding the contraction of diaphragm.

More significant result of sandbag breathing on respiratory parameters get support from Gross D et al in which he did study on various breathing exercise on tetra plegic patients by using EMG activity that suggest that group A of tetra plegic patients during Maximum voluntary breathing we found that Abdominal weight maximum voluntary (AWMV) breathing ensued an increase in EMG activity which indicates that AWMV Breathing trains diaphragm. While In spiratory resistance maximum Voluntary breathing (IRMV) breathing ensued an increase in EMG which serves the purpose of providing more training to sternoclei do mastoid. These observations might help us understand the important steps to learn and preserve the respiratory muscle strength [23]. Hodges PW et al. says that many elderly patient or patient with some sort of respiratory illness are already self-trained to use costal respiration method and using accessory breathing muscles instead of diaphragm. So this brings us to the conclusion that the subject themselves have to learn to identify the proper contraction of the diaphragm so they can perform the exercises with most discipline [24].

CONCLUSION

From this entire contain of result and discussion, we conclude that both the groups are significantly effective in improving pulmonary function in elderly individuals. As p value for all the data is <0.05 which shows significant improvement. On other hand Sandbag breathing is more significantly effective for improving respiratory function among two.

Clinical implication
Physiotherapist can include this as a routine program for geriatric population. This breathing technique can be used for any patients with respiratory condition as well as in normal asymptomatic individual. It Works on abdominal muscle also so we can treat patients with back pain also. Sandbag breathing is affordable and easy to perform so can advice at home also

Limitation
Long term Follow up is lacking

Future recommendation

Can check effects on EMG activity, Can see effects on functional capacity and MIP, Compare the protocol with and without sandbag, Can add control group, Can check on diseased population

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Table 1: independent sample test for between group results

	Independent Sample test			
	T	df	Sig.(2 tailed)	Mean difference
FEV1 Post	-3.951	28	0.000	-0.512
FVC post	-3.416	28	0.002	-0.419
MVV post	-2.230	28	0.034	-10.800
FEV1/FVC post	-2.764	28	0.010	-0.05808

Table 2: Paired sample test of within group results

Group			Mean	Standard deviation	T	Sig (two tailed)
IMT	Pair 1	FEV1 Pre FEV1 Post	-0.16333	0.13526	-4.679	0.000
	Pair 2	FVC pre FVC post	-0.16867	0.14422	-4.530	0.000
	Pair 3	MVV pre MVV post	-11.933	7.750	-5.963	0.000
	Pair 4	FEV1/FVC pre FEV1/FVC post	-0.0098533	0.0528123	0.723	0.482
Sandbag breathing	Pair 1	FEV1 Pre FEV1 Post	-0.59533	0.09650	-23.893	0.000
	Pair 2	FVC pre FVC post	-0.53600	0.12460	-16.660	0.000
	Pair 3	MVV pre MVV post	-21.133	5.579	-14.671	0.000
	Pair 4	FEV1/FVC pre FEV1/FVC post	-0.0478400	0.0572044	-3.239	0.006
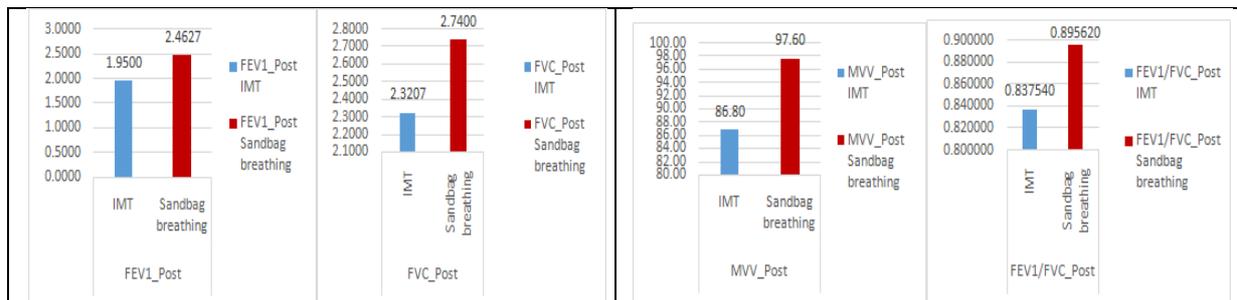
Graph 1 & 2: Post Mean of IMT and Sandbag breathing for FEV1 and FVC

Graph 3 & 4: Post Mean of IMT and Sandbag breathing for MVV and FEV1/FVC

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Figure 1. IMT with threshold device

Figure 2. IMT threshold device

Figure 3. Sandbag breathing

RESEARCH ARTICLE

Synthesis, Characterization and Antimicrobial Screening of Some Newly Synthesized Isoxazoles Derivatives

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ABSTRACT

The isoxazole is one of the mostly studied heterocyclic moiety found in most of the natural products capable to show variety of biological activities. Also, it is considered as an important pharmacophore possessing a wide range of pharmacological activities and thus showing applicability in the field of medicinal chemistry. The substituted isoxazoles have been reported to exhibit broad range of biological activities. Considering its applicability in various filed we have synthesized some analogues of 3-(4-subtitutedphenyl)-5-substitutedisoxazoles form 1-(4-substitutedphenyl)-3-substitutedchalcones and screened them for their antimicrobial efficacy.

Keywords: Chlorophenyl chalcone, methoxyphenyl chalcone and substituted isoxazoles etc.

INTRODUCTION

The heterocyclic compounds such as isoxazoles and their derivatives are considered as one of the important classes of heterocyclic compound as they possess broad spectrum of biological properties. Modification in their structures has offered a high degree of diversity that has proved to behelpful for the development of new biologically active agents with improved efficiency. In the past few decades, heterocycles play a significant role in the field of agriculture, medicine and industries. The isoxazole skeleton, a crucial type of nitrogen-oxygen containing heterocycles, has been used in many pesticides and drug design because of their insecticidal [1-3], herbicidal [4-6],

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fungicidal [7], antiviral [8-10] and anticancer activities [11]. Recently, Yu *et al.* [12] reported a series of 3,4,5-trisubstituted isoxazoles possessing insecticidal properties. Sun et al. [13] reported some analogues of isoxazole containing benzoylurea moiety capable for showing insecticidal properties. Raed A. Al-Qawasmeh *et.al.*[14] reported indole substituted isozaxazole possessing antimicrobial and anticancer properties. Ahmad M. Eid et.al.[15] reported novel isoxazole-amide derivatives possessing anticancer and antioxidant activity. Literature survey reveals their importance in the field of heterocyclic synthesis, a significant amount of effort has been taken to develop more efficient biologically active agent by using these nuclei [16-18]. Considering the above finding, we have aimed to synthesized some isoxazole analogues and test them for their antimicrobial efficacy.

Experimental

The structure of all the newly synthesized compounds was characterized on the basis IR, NMR and Mass spectroscopy. The IR spectra were recorded on Perkin-Elmer spectrophotometer. ^{1}H NMR spectra were recorded on Bruker Avance-II 400 NMR spectrophotometer in DMSO using TMS as an internal standard.

Preparation of 1-(4-chlorophenyl)-3-(4-methoxyphenyl) chalcone(1a)

To a cooled solution of NaOH (40%) and ethanol, 4-chloroacetophenone (0.01 M)was added followed by addition of substituted 4-methoxy benzaldehyde (0.01 M), the reaction mixture was stirred for 2-3 hours till the mixture become viscous and then the mixture was kept overnight in a refrigerator. The product, thus separated, was filtered and washed with cold water. Then it was crystallized in rectified spirit to yield the compound 1a.

Mol. Formula $C_{16}H_{13}ClO_2$(1a): White amorphous solid,yield 79%, **Elemental analysis (%)**: **C** 70.41/70.46; **H** 40.78/40.80; **Cl** 12.95/13.00; **O** 11.71/11.73. **IR (KBr cm^{-1})** 1654 (=C-C=O), 1566 (Ar ring), 1440 (Ar C=C), 1332 (C-O), 1262 (C-OCH$_3$),686(C-Cl).**ESI-MS** [M+H]$^+$ Calculated for $C_{16}H_{14}ClO_2$: m/z 273.06, found 273.03, **^{1}H-NMR (500 MHz, DMSO)** δ 8.14 (d*, J* = 7.9 Hz, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.81 (d, *J* = 15.6 Hz,1H), 7.72 (d, *J* = 15.5 Hz, 1H), 7.63 (d, *J* = 7.9 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 3.82 (s, 3H).

Preparation of 1-(4-chlorophenyl)-3-(2-chlorophenyl) chalcone(1b)

To a cooled solution of NaOH (40%) and ethanol, 4-chloroacetophenone (0.01 M)was added followed by addition of substituted 2-chlorobenzaldehyde(0.01 M),the reaction mixture was stirred for 2-3 hours till the mixture become viscous and then the mixture was kept overnight in a refrigerator. The product, thus separated, was filtered and washed with cold water. Then it was crystallized in rectified spirit to yield the compound 1b.

Mol. Formula $C_{15}H_{10}Cl_2O$ (1b): Pale yellow crystalline Solid, yield 78% ,**Elemental analysis (%)**: **C** 65.00/65.01 ; **H** 3.62/3.64 ; **Cl** 25.55/25.58; **O** 5.73/5.77, **IR (KBr cm^{-1})** 1685 (=C-C=O), 1560 (Ar ring), 1440 (Ar C=C), 1332 (C-O), 627 (C-Cl)**, ESI-MS**[M+H]$^+$ Calculated for $C_{15}H_{11}Cl_2O$: *m/z* 277.01, found 276.97, **^{1}H-NMR (500 MHz, DMSO)** δ 8.19 (m, 3H), 8.06 (d, *J* = 15.5 Hz, 1H), 7.96 (d, *J* = 15.7 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.52 – 7.44 (m, 2H).

Preparation of 1-(4-nitrophenyl)-3-(4-chlorophenyl) chalcone(1c)

To a cooled solution of NaOH (40%)and ethanol, 4-nitroacetophenone (0.01 M) was added followed by addition of substituted 4-chlorobenzaldehyde (0.01 M), the reaction mixture was stirred for 2-3 hours till the mixture become viscous and then the mixture was kept overnight in a refrigerator. The product, thus separated, was filtered and washed with cold water. Then it was crystallized from rectified spirit to yield the compound 1c.

Mol. Formula $C_{15}H_{10}ClNO_3$(1c): Yellow amorphous Solid, yield 80%,**Elemental analysis (%)**: **C** 62.60/62.62 ; **H** 3.47/3.50 ; **Cl** 12.30/12.32**N**4.85/4.87;**O** 16.66/16.68; **IR (KBr cm^{-1})** 1664 (=C-C=O), 1616 (Ar ring), 1438 (Ar C=C), 1319 (C-O); **ESI-MS**[M+H]$^+$ Calculated for $C_{15}H_{11}ClNO_3$: *m/z* 288.04; **^{1}H-NMR (500 MHz, DMSO)** δ 8.38 (d, *J* = 8.3 Hz, 2H), 8.34 (d, *J* = 8.5 Hz, 2H), 7.97 – 7.90 (m, 3H), 7.81 (d, *J* = 15.5 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 2H).

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Preparation of 1-(4-hydroxyphenyl)-3-(2-chlorophenyl) chalcone(1d)

To a cooled solution of NaOH (40%) and ethanol, 4-hydroxyacetophenone (0.01 M) was added followed by addition of substituted 2-chlorobenzaldehyde (0.01 M), the reaction mixture was stirred for 2-3 hours till the mixture become viscous and then the mixture was kept overnight in a refrigerator. The product, thus separated, was filtered and washed with cold water. Then it was crystallized from rectified spirit to yield the compound 1d.

Molecular Formula $C_{15}H_{11}ClO_2$ (1d) : Cream amorphous solid, yield 74% , **Elemental analysis** (%): **C** 69.62/69.64 ; **H** 4.27/4.29 ; **Cl** 13.67/13.70; **O** 12.34/12.37; **IR (KBr cm^{-1})** 3209 (OH), 1651 (=C-C=O), 1616 (Ar ring), 1438 (Ar C=C), 1317 (C-O); **ESI-MS**[M+H]$^+$ Calculated for $C_{15}H_{12}ClO_2$: *m/z* 259.05, found 259.01; **^{1}H-NMR (500 MHz, DMSO)** δ 10.69 (s, 1H), 8.18 (d, *J* = 6.9 Hz, 1H), 8.09 (d, *J* = 8.2 Hz, 2H), 8.00 (d, *J* = 15.5 Hz, 1H), 7.95 (d, *J* = 15.6 Hz, 1H), 7.56 (d, *J* = 7.1 Hz, 1H), 7.51 – 7.42 (m, 2H), 6.93 (d, *J* = 8.2 Hz, 2H).

Preparation of 1-(4-chlorophenyl)-3-(4-chlorophenyl) chalcone(1e)

To a cooled solution of NaOH (40%) and ethanol, 4-chloroacetophenone (0.01 M) was added followed by addition of substituted 4-chlorobenzaldehyde (0.01 M), the reaction mixture was stirred for 2-3 hours till the mixture become viscous and then the mixture was kept overnight in a refrigerator. The product, thus separated, was filtered and washed with cold water. Then it was crystallized from rectified spiritto yield the compound 1e.

Mol. Formula $C_{15}H_{10}Cl_2O$ (1f): White amorphous solid, yield 81%, **Elemental analysis (%): C** 65.00/65.01; **H** 3.61/3.64; **Cl** 25.55/25.58; **O** 5.74/5.77.

Preparation of 1-(4-hydroxyphenyl)-3-(4-chlorophenyl) chalcone(1f)

To a cooled solution of NaOH (40%) and ethanol, 4-hydroxyacetophenone (0.01 M) was added followed by addition of 4-chlorobenzaldehyde(0.01 M), the reaction mixture was stirred for 2-3 hours till the mixture become viscous and then the mixture was kept overnight in a refrigerator. The product, thus separated, was filtered and washed with cold water. Then it was crystallized in rectified spirit to yield the compound 1f.

Mol. Formula $C_{15}H_{11}ClO_2$ (1f): White amorphous solid, yield 81%, **Elemental analysis (%): C** 69.61/69.64; **H** 4.26/4.29; **Cl** 13.67/13.70; **O** 12.35/12.37.

Preparation of 1-(4-chlorophenyl)-2,3-dibromo-3-(4-methoxyphenyl) chalcone(2a)

To Tetrabutylammonium tribromide (TBATB),1-(4-chlorophenyl)-3-(4-methoxyphenyl) chalcone (0.01 M) and Water (0.5ml) were added, the whole mixture was mixed thoroughly and left at room temperature with occasional grinding for 15 min. Upon completion of the reaction, 20 ml of water was added to the reaction mixture and stirred for 10 min. The reaction mixture was filtered , washed with water and air dried to afford the compound 2a.

Molecular Formula $C_{16}H_{13}Br_2ClO_2$ (2a) : Cream amorphous solid, yield 76% , **Elemental analysis** (%): **C** 44.40/44.43 ; **H** 3.01/3.03 ; **Br** 36.93/36.95 ;**Cl** 8.18/8.20; **O** 7.38/7.40; **IR (KBr cm^{-1})**1685 (Br-C-C=O), 1608 (Ar ring).**ESI-MS**[M-Br+H]$^+$ Calculated for $C_{16}H_{13}BrClO_2$: *m/z* 354.06, found 353.63, [M-2Br+H]+ Calculated for $C_{16}H_{13}ClO_2$: m/z 272.73, found 273.88, [M-2Br-NO2+H]+ Calculated for $C_{15}H_{10}ClO$: m/z 242.70, found 242.99**^{1}H-NMR (500 MHz, DMSO)** δ 8.28 (d, *J* = 8.4 Hz, 2H), 8.75-8.65 (m, 4H), 6.95 (d, *J* = 8.1 Hz, 2H), 6.70 (d, *J* = 11.2 Hz, 1H), 5.78 (d, *J* = 11.4 Hz, 1H).

Preparation of 1-(4-chlorophenyl)-2,3-dibromo-3-(2-chlorophenyl) chalcone(2b)

To Tetrabutylammonium tribromide (TBATB, 1-(4-chlorophenyl)-3-(2-chlorophenyl) chalcone (0.01 M) and Water (0.5ml) were added, the whole mixture was mixed thoroughly and left at room temperature with occasional grinding for 15 min. Upon completion of the reaction, 20 ml of water was added to the reaction mixture and stirred for 10 min. The reaction mixture was filtered, washed with water and air dried to afford the compound 2b.

Molecular Formula $C_{15}H_{10}Br_2Cl_2O$ (2b) : White amorphous solid, yield 78% , **Elemental analysis** (%): **C** 41.22/41.23 ; **H** 2.29/2.31 ; **Br** 36.55/36.57 ; **Cl** 16.21/16.23; **O** 3.65/3.66.

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Preparation of 1-(4-nitrophenyl)-2,3-dibromo-3-(4-chlorophenyl) chalcone(2c)

To Tetrabutylammonium tribromide (TBATB), 1-(4-nitrophenyl)-3-(4-chlorophenyl) Chalcone (0.01 M) and Water (0.5ml) were added, the whole mixture was mixed thoroughly and left at room temperature with occasional grinding for 15 min. Upon completion of the reaction, 20 ml of water was added to the reaction mixture and stirred for 10 min. The reaction mixture was filtered, washed with water and air dried to afford the compound 2c.

Molecular Formula $C_{15}H_{10}Br_2ClNO_3$ (2c) : Cream amorphous solid, yield 76%, **Elemental analysis** (%): C 40.25/40.26 ; **H** 2.23/2.25 ; **Br** 35.70/35.71 ; **Cl** 7.90/7.92 ; **N** 3.11/3.13 ; **O** 10.71/10.73; **IR (KBr cm^{-1})** 3084 (C-H arom.), 1691 (Br-C-C=O), 1608 (Ar ring), 1288 (Ar N-O) **ESI-MS**[M-Br+H]$^+$ Calculated for $C_{15}H_{11}BrClNO_3$: *m/z* 366.96, found 366.2, [M-2Br-OMe+H]+ Calculated for $C_{15}H_{10}ClO$: m/z 242.70, found 242.99 **^{1}H-NMR (500 MHz, DMSO)** δ 8.52 (d, *J* = 8.4 Hz, 2H), 8.46 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.53 (d, *J* = 8.1 Hz, 2H), 6.79 (d, *J* = 11.2 Hz, 1H), 5.83 (d, *J* = 12.3 Hz, 1H).

Preparation of 1-(4-hydroxyphenyl)-2,3-dibromo-3-(2-chlorophenyl) chalcone(2d)

To Tetrabutylammonium tribromide (TBATB), 1-(4-hydroxyphenyl)-3-(2-chlorophenyl) chalcone (0.01 M) and Water (0.5ml) were added, the whole mixture was mixed thoroughly and left at room temperature with occasional grinding for 15 min. Upon completion of the reaction, 20 ml of water was added to the reaction mixture and stirred for 10 min. The reaction mixture was filtered, washed with water and air dried to afford the compound 2d.

Molecular Formula $C_{15}H_{11}Br_2ClO_2$ (2d) : Greyish amorphous solid, yield 80% , **Elemental analysis** (%): **C** 43.03/43.05 ; **H** 2.63/2.65 ; **Br** 38.17/38.19 ; **Cl** 8.46/8.47; **O** 7.64/7.65.

Preparation of 1-(4-chlorophenyl)-2,3-dibromo-3-(4-chlorophenyl) chalcone(2e)

To Tetrabutylammonium tribromide (TBATB), 1-(4-chlorophenyl)-3-(4-chlorophenyl) chalcone (0.01 M) and Water (0.5ml) were added, the whole mixture was mixed thoroughly and left at room temperature with occasional grinding for 15 min. Upon completion of the reaction, 20 ml of water was added to the reaction mixture and stirred for 10 min. The reaction mixture was filtered, washed with water and air dried to afford the compound 2e.

MolecularFormula$C_{15}H_{10}Br_2Cl_2O$ (2e): White amorphous solid, yield 79%, Elemental **analysis** (%): **C** 41.21/41.23; **H** 2.29/2.31; **Br** 36.55/36.57; **Cl** 16.22/16.23; **O** 3.65/3.66. **IR (KBr cm^{-1})** 3084 (C-H arom.), 1685 (=C-C=O), 1608 (Ar ring). **ESI-MS**[M-2Br+H] $^+$Calculated for $C_{15}H_{11}Cl_2O$: *m/z* 277.01, found 277.79, [M-2Br-Cl+H] + Calculated for $C_{15}H_{10}ClO$: m/z 242.70, found 242.95.**^{1}H-NMR (500 MHz, DMSO)** δ 8.30 (d, *J* = 7.8 Hz, 2H), 7.85 (d, *J* = 7.7 Hz, 2H), 7.73 (d, *J* = 7.8 Hz, 2H), 7.52 (d, *J* = 7.7 Hz, 2H), 6.67 (d, *J* = 11.4 Hz, 1H), 5.79 (d, *J* = 12.9 Hz, 1H).

Preparation of 1-(4-hydroxyphenyl)-2,3-dibromo-3-(4-chlorophenyl) chalcone(2f)

To Tetrabutylammonium tribromide (TBATB), 1-(4-hydroxyphenyl)-3-(4-chlorophenyl) chalcone (0.01 M) and Water (0.5ml) were added, the whole mixture was mixed thoroughly and left at room temperature with occasional grinding for 15 min. Upon completion of the reaction, 20 ml of water was added to the reaction mixture and stirred for 10 min. The reaction mixture was filtered, washed with water and air dried to afford the compound 2f.

Molecular Formula $C_{15}H_{11}Br_2ClO_2$ (2f) : White amorphous solid, yield 78%, **Elemental analysis** (%): **C** 43.02/43.05 ; **H** 2.63/2.65 ; **Br** 38.16/38.19 ; **Cl** 8.45/8.47; **O** 7.63/7.65. **IR (KBr cm^{-1})** 3377 (OH), 1647 (=C-C=O).**ESI-MS**[M-2Br-OH+H]$^+$ Calculated for $C_{15}H_{10}ClO$: *m/z* 242.70, found 242.96.**^{1}H-NMR (500 MHz, DMSO)** δ 10.92 (s, 1H), 8.16 (d, *J* = 8.0 Hz, 2H), 7.83 (d, *J* = 11.7 Hz, 2H), 7.51 (d, *J* = 7.8 Hz, 2H), 6.96 (d, *J* = 7.9 Hz, 2H), 6.56 (d, *J* = 11.3 Hz, 1H), 5.76 (d, *J* = 11.2 Hz, 1H).

Preparation of 3-(4-chlorophenyl)-5-(4-methoxyphenyl) Isoxazole(3a)

A mixture of 1-(4-chlorophenyl)-2,3-dibromo-3-(4-methoxyphenyl) chalcone, hydroxylamine hydrochloride and ethanol were refluxed for 30 minutes. Then aqueous solution of potassium hydroxide (30%) was added to it and refluxed for 4 hours. It was then allowed to stand for 30 minutes at room temperature was then cooled and acidified

with dilute HCl. The resulting solid was washed with water, dried and crystallized from absolute alcohol to afford the compound 3a.

Molecular Formula C$_{16}$H$_{12}$ClNO$_2$(3a): Brownish Crystalline Solid,yield 73%, **Elemental analysis (%):** **C** 67.23/67.26; **H** 4.20/4.23; **Cl** 12.37/12.41 **N**4.87/4.90;**O**11.17/11.20

IR (KBr cm^{-1}) 1605 (C=N), 1584 (C=C), 1407 (N-O).**ESI-MS**[M+H] $^+$Calculated for C$_{16}$H$_{13}$ClNO$_2$: *m/z* 286.73, found 286.88. ^{1}**H-NMR (500 MHz, DMSO)** δ 7.92 (d, *J* = 7.3 Hz, 2H), 7.85 (d, *J* = 7.3 Hz, 2H), 7.64 (d, *J* = 7.0 Hz, 2H), 7.56 (s, *J* = 5.8 Hz, 1H), 7.10 (d, *J* = 8.3 Hz, 2H), 3.83 (s, 3H).

Preparation of 3-(4-chlorophenyl)-5-(2-chlorophenyl) Isoxazole(3b)

A mixture of 1-(4-chlorophenyl)-2,3-dibromo-3-(2-chlorophenyl) chalcone, hydroxylamine hydrochloride and ethanol were refluxed for 30 minutes. Then aqueous solution of potassium hydroxide (30%) was added to it and refluxed for 4 hours. It was then allowed to stand for 30 minutes at room temperature was then cooled and acidified with dilute HCl. The resulting solid was washed with water, dried and crystallized from absolute alcohol to afford the compound 3b.

MolecularFormulaC$_{15}$H$_9$Cl$_2$NO(3b) : Brown amorphous Solid, yield 71%**, Elemental analysis (%) : C** 62.06/62.09 ; **H** 3.11/3.13 ; **Cl** 24.41/24.44 ; **N**4.80/4.83;**O**5.48/5.51.

IR (KBr cm^{-1}) 1608 (C=N), 1582 (C=C), 1409 (N-O).**ESI-MS**[M+H]$^+$ Calculated for C$_{15}$H$_{10}$Cl$_2$NO: *m/z* 291.15, found 290.85. ^{1}**H-NMR (500 MHz, DMSO)** δ 7.98 (d, *J* = 7.9 Hz, 2H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.66 (m, 3H), 7.55 (m, 2H), 7.49 (s, 1H).

Preparation of 3-(4-nitrophenyl)-5-(4-chlorophenyl)Isoxazole(3c)

A mixture of 1-(4-nitrophenyl)-2,3-dibromo-3-(4-chlorophenyl)Chalcone, hydroxylamine hydrochloride and ethanol was refluxed for 30 minutes. Then aqueous solution of potassium hydroxide (30%) was added to it and refluxed for 4 hours. It was then allowed to stand for 30 minutes at room temperature was then cooled and acidified with dilute HCl. The resulting solid was washed with water, dried and crystallized from absolute alcohol to afford the compound 3c.

Molecular Formula C$_{15}$H$_9$ClN$_2$O$_3$(3c) : Brown amorphous Solid, yield 74% **, Elemental analysis (%) : C** 59.88/59.91 ; **H** 3.00/3.02 ; **Cl** 11.77/11.79 ;**N**9.29/9.32;**O**15.93/15.96

IR (KBr cm^{-1}) 1605 (C=N), 1588 (C=C), 1408 (N-O).**ESI-MS**[M+H]$^+$Calculated for C$_{15}$H$_{10}$ClN$_2$O$_3$: *m/z* 301.70, found 302.01. ^{1}**H-NMR (500 MHz, DMSO)** δ 8.41 (d, *J* = 8.7 Hz, 2H), 8.16 (t, *J* = 8.2 Hz, 2H), 7.95 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 6.9 Hz, 1H), 7.63 (t, *J* = 7.9 Hz, 2H).

Preparation of 3-(4-hydroxyphenyl)-5-(2-chlorophenyl) Isoxazole(3d)

A mixture of 1-(4-hydroxyphenyl)-2,3-dibromo-3-(2-chlorophenyl)chalcone, hydroxylamine hydrochloride and ethanol was refluxed for 30 minutes. Then aqueous solution of potassium hydroxide (30%) was added to it and refluxed for 4 hours. It was then allowed to stand for 30 minutes at room temperature was then cooled and acidified with dilute HCl. The resulting solid was washed with water, dried and crystallized from absolute alcohol to afford the compound 3d.

Molecular Formula C$_{15}$H$_{10}$Cl NO$_2$(3d) : White amorphous Solid, yield 68% **, Elemental analysis (%) : C** 66.29/66.31 ; **H** 3.68/3.71 ; **Cl** 13.01/13.05 ; **N**5.13/5.16;**O**11.75/11.78.

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Preparation of 3-(4-chlorophenyl)-5-(4-chlorophenyl)Isoxazole(3e)

A mixture of 1-(4-chlorophenyl)-2,3-dibromo-3-(4-chlorophenyl)chalcone, hydroxylamine hydrochloride and ethanol was refluxed for 30 minutes. Then aqueous solution of potassium hydroxide (30%) was added to it and refluxed for 4 hours. It was then allowed to stand for 30 minutes at room temperature was then cooled and acidified with dilute HCl. The resulting solid was washed with water, dried and crystallized from absolute alcohol to afford the compound 3e.

MolecularFormulaC$_{15}$H$_9$Cl$_2$NO(3e) : YellowCrystalline Solid, yield 74%**, Elemental analysis (%) : C** 62.06/62.09 **; H** 3.11/3.13 **; Cl** 24.41/24.44 **; N**4.80/4.83;**O**5.48/5.51

Preparation of 3-(4-hydroxyphenyl)-5-(4-chlorophenyl) Isoxazole(3f)

A mixture of 1-(4-hydroxyphenyl)-2,3-dibromo-3-(4-chlorophenyl) chalcone, hydroxylamine hydrochloride and ethanol was refluxed for 30 minutes. Then aqueous solution of potassium hydroxide (30%) was added to it and refluxed for 4 hours. It was then allowed to stand for 30 minutes at room temperature was then cooled and acidified with dilute HCl. The resulting solid was washed with water, dried and crystallized from absolute alcohol to afford the compound 3f.

Molecular Formula C$_{15}$H$_{10}$ClNO$_2$(3f) : Whitish amorphous Solid, yield 74% **, Elemental analysis (%) : C** 66.29/66.31 **; H** 3.68/3.71 **; Cl** 13.03/13.05 **; N** 5.15/5.16 **; O** 11.75/11.78.

IR (KBr cm^{-1})3475 (OH), 3116 (Ar-H), 1600 (C=N), 1588 (C=C), 1406 (N-O). **ESI-MS**[M+H]$^+$Calculated for C$_{15}$H$_{11}$ClNO$_2$: *m/z* 272.70, found 272.83.**^{1}H-NMR (500 MHz, DMSO)** δ 10.32 (br s, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.74 (d, *J* = 9.0 Hz, 2H), 7.64 (d, *J* = 8.6 Hz, 2H), 7.36 (s, 1H), 6.93 (t, *J* = 9.0 Hz, 2H).

Antimicrobial Screening

The antimicrobial activities of compounds 1a,1b,1c,1d, 2a,2c,2e,2f, 3a,3b,3c,3f have been assayed at the concentration of 125μg/disc against some pathogens *viz.* bacteria *Staphylococcus aureus, Enterococci, Escherichia coli, Pseudomonas aeruginosa* and some fungi *viz. Candida albicans, Aspergillus niger.* The efficacy of titled compounds is given in following table.

RESULT AND DISCUSSION

The results of antimicrobial screening indicate that the titled compound shows good to moderate antimicrobial activity against tested bacteria *viz. Staphylococcus aureus, Enterococci, Escherichia coli, Pseudomonas aeruginosa* some fungi *viz. Candida albicans, Aspergillus niger.* The newly synthesized titled compounds are capable to cramp the growth of bacterial and fungal pathogens.

ACKNOWLEDGEMENT

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Table 1. Preparation of 3-(4-hydroxyphenyl)-5-(4-chlorophenyl)Isoxazole(3f)

Sr.No.	Compounds	R_1	R_2	R_3	R_4
1.	1a, 2a, 3a	-H	-H	-OCH$_3$	-Cl
2.	1b, 2b, 3b	-Cl	-H	-H	-Cl
3.	1c, 2c, 3c	-H	-H	-Cl	-NO$_2$
4.	1d, 2d, 3d	-Cl	-H	-H	-OH
5.	1e, 2e, 3f	-H	-H	-Cl	-Cl
6.	1f, 2f ,3f	-H	-H	-Cl	-OH

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Table 2. Antimicrobial Screening

Compound	Zone of inhibition (mm)					
	Bacterial pathogens				Fungal pathogens	
	Staphylococcus aureus	*Enterococci*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
1a	12	18	15	20	12	10
1b	10	16	10	17	14	11
1c	12	19	20	11	13	11
1d	13	14	14	18	10	10
2a	16	17	10	10	12	15
2c	13	11	10	12	12	14
2e	13	10	17	10	17	13
2f	15	24	20	13	15	16
3a	24	24	20	23	20	17
3b	10	23	16	25	10	16
3c	16	21	10	25	18	15
3f	13	10	19	22	17	19
Gentamicin	08	08	08	08	--	--
Cabendizum	--	--	--	--	08	08

Scheme 1. Preparation of 3-(4-hydroxyphenyl)-5-(4-chlorophenyl)Isoxazole(3f)

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RESEARCH ARTICLE
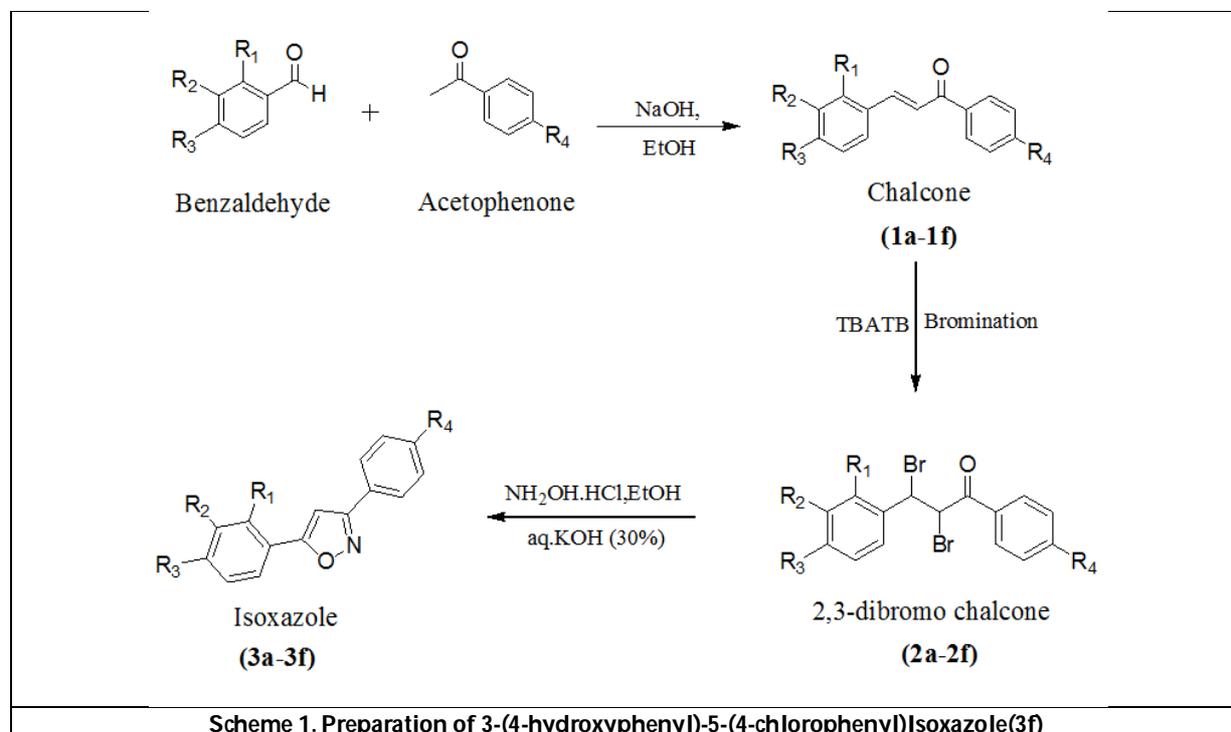
Preliminary Analysis and Applications of Bio-Enzymes from Fermented Vegetable and Fruit Peel Wastes

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ABSTRACT

Bio-enzymes can be produced by fermenting vegetable and fruit peels along with jaggery and water in the ratio 1(Jaggery): 3(peel waste): 10(water) in an airtight plastic container. In which jaggery acts as a carbon source or energy for the micro-organisms to grow. The enzymatic and antimicrobial efficacy of bio-enzyme acquired from vegetable and fruit peel wastes of *Carica papaya* (Papaya), *Ananas comosus* (Pineapple), *Lagenaria siceraria* (Bottle gourd) and *Luffa acutangular* (Ridge gourd) were analyzed. Analysis on enzymatic activity for Amylase by Starch Hydrolysis, Cellulase by Carboxymethylcellulose (CMC) Hydrolysis, Protease by Gelatin and Lipase by Tween 20 were performed. Antimicrobial activity for the bio-enzyme was analyzed with *Escherichia coli* by incorporating well diffusion method and a good antimicrobial potential was exhibited by the crude extracts of Pineapple and Ridge gourd. This method is a concept of zero-waste economy in which bio-enzymes and biomass are produced.

Keywords: bio-enzyme, peel wastes, zero-waste economy, bio-mass, antimicrobial, Well diffusion method, fermentation, Bottle gourd, Ridge gourd, Pineapple, Papaya.

INTRODUCTION

In India, nearly 21 tons of vegetable wastes are disposed yearly. In India, the total waste generation is growing at a rate of 1.33%, per capita waste generation is 450 grams per day. In today's scenario, people do not seem to care much about the environment, so they pollute the air, water, and soil. Wastes from vegetables and fruits are generated which makes the majority of soil and water pollution. The greenhouse gas emitted by this waste is also a cause of pollution. Proper measures in the need of time must be taken so that we can preserve our nature's gift. Enzymes are used to produce environmental eco-friendly products. An organic solution which is produced by fresh vegetable and fruit peel along with jaggery and water with the aid of microorganisms like yeast and bacteria produces the enzyme bio- cleaners [16]. The organic solution is comprised of organic acids, mineral salts, and can be used to compose, decompose, catalyse, and transform [11]. The Bio cleaner uses beneficial bacteria to break down wastes, soils, and bad odours by producing enzymes and they break the molecules into smaller ones. The bio-enzyme containing cleaning properties are highly effective, safe and have high specificity. They are also environmentally friendly and has cheaper preparation methods [12]. So, the enzyme bio-cleaners are referred as 'green chemicals' and are used as a cleaning aid for household and for various applications in industrial fields [8][16]. Also, these bio-enzymes have potential antioxidant and antimicrobial properties [4]. The remaining biomass obtained can be used as an organic manure to the plants by farmers and can be used by people in the urban areas for kitchen gardens to produce pest-free and healthy plants [17]. Because of the minimal effort and simple accessibility, this method of producing the bio-enzyme can be done in every household. This is not used for human consumption. The main objective is to analyse and investigate the enzymatic activity of the bio-enzyme obtained from vegetable and fruit peels and explore the efficiency of the obtained biomass as bio-manure. This study helps to reduce the accumulation of peel waste and create a sustainable environment by using bio-cleaners and bio-manure.

MATERIALS AND METHODS

Bio Enzyme Preparation
A clean plastic container was used for fermentation process. Appropriate quantity of water and jaggery was added as it acts as a nutritive medium for the growth of microbes. Peels of vegetables and fruits were taken, they were cleaned and dried properly to prevent contamination. The bio-enzyme was prepared by taking the ratio of 1 part of jaggery, 3 parts of vegetable and fruit peel wastes and 10 parts of water thereby in the total ratio of 1:3:10. The lid is closed firmly and kept it under room temperature in a shady place [1]. Due to fermentation, Carbon dioxide gas being formed. So, the container lid was periodically opened to release carbon dioxide gas, for up to 30 days. And for the next 2 months, leave the container airtight and undisturbed. The date of preparation, changes that takes place in the color of the liquid and the formation of biomass were recorded. The Figure.1 depicts the method carried out to produce bio-enzyme [9][15].

Filtration and Separation of Biomass and Enzyme concoction
After 90 days, a clear liquid was formed, which contains the bio-enzyme concoction, and the biomass was formed. Filter the liquid containing the bio-enzyme concoction and segregate the biomass. The bio-enzyme concoction was taken for the analysis of enzymatic and antimicrobial efficacy of the enzyme were obtained [2].

Biomass drying and use as manure
After segregating and drying the biomass was used as an organic manure to the plants, on using this for plants, it results in the growth of healthier plants, which are resistant to pest and parasites [7].

Lyophilization
Lyophilization includes preliminary freezing of the filtrate in a controlled manner to handle the crystallized ice structure [6]. In primary drying, the frozen filtrate was kept in vacuum, sublimation process takes place so that the unbound water can be removed. Since the sample was dried by performing lyophilization process, a fine stability

between vacuum and temperature is important. Thus, a batch with no effect on the efficacy of the product was produced after drying [6]. In order to attain this, the product underwent freeze drying conditions for 6 hours.

Starch hydrolysis for amylase enzyme activity

Nutrient Agar 50ml with 1% starch was prepared and 20ml of the same was poured on a sterile plate. After which 4mm size wells were bored and 50μl of crude extract was loaded into the wells, they were incubated for 24 hours at 37°C. The iodine solution (indicator) was poured into the plates for proper visualization of hydrolysis activity by the enzyme amylase which was seen as a region of clearance over the dark blue color of iodine solution around the wells [10].

Carboxymethyl cellulose (CMC) hydrolysis for cellulose enzyme activity

Nutrient Agar 50ml with 1 % Carboxyl Methyl Cellulose was prepared and 20ml of the same was poured on a sterile plate. By using sterile cork borer (4mm size) wells were bored and 50μl of crude extract was added. The plates were kept for incubation at 37°C for 24 hours. After that, 0.3% Congo red solution was added to the enzyme plate as an indicator. Congo red was washed with water and after 10 minutes the plate was flooded with 1 N NaCl as de-staining solution after which the regions of clearance which indicated the presence of the enzyme activity was noticeable [3].

Protease enzyme activity

Nutrient Agar 50ml with 1% Gelatin was prepared and 20ml of the same was poured on the petri plate. 4mm size wells were bored using a cork borer and 50μl of crude extract was poured into the wells. The plates were kept for incubation at 37°C for 24 hours. After incubation, acidic Mercuric chloride was added as an indicator to determine the presence of protease enzyme [13].

Lipase enzyme activity

Nutrient Agar 50ml with 1% Tween 20 was prepared and 20ml of the same was poured on the petri plate. 4mm size wells were bored using a cork borer and 50μl of crude extract was poured into the wells. The plates were kept for incubation at 37°C for 24 hours. Lipase activity will be seen by the presence of precipitation around the wells [5].

Antimicrobial well diffusion assay

100 ml Nutrient Agar was prepared and 20 ml of the same was poured in sterile plates. The plates were allowed to set, then 100μl *Escherichia coli* culture was inoculated and spread plate was prepared. Spread plate was incubated for 4 hours. Kanamycin was used as positive control in the plates with concentration of 10μg/ml. By using sterile cork borer (4mm size) wells were bored and 50μl of crude extract was added (Table 2.9.1). The plates were incubated at 37°C for 24 hours. After incubation, zone of inhibition was observed to determine antimicrobial activity of the crude extract [14].

RESULTS AND DISCUSSION

Fermentation and separation of crude extract

The bio-enzyme was prepared by taking the ratio of 1 part of jeggery, 3 parts of vegetable and fruit peel wastes (Ridge gourd, Bottle gourd, Pineapple and Papaya peels separately) and 10 parts of water in the ratio of 1:3:10. The vegetable and fruit waste included the peels of Ridge gourd, Bottle gourd, Pineapple and Papaya. All these components were mixed in the above-mentioned ratio and were allowed to ferment in an air-tight plastic container for 3 months (Figure 2). In the first month, the gases produced were removed every 24 hours without disturbing the container. In the following 2 months, the bio-enzyme in the container was left undisturbed to ferment further. At the end of the third month, the clear liquid containing bio-enzyme concoction and biomass was filtered (Figure 3). The bio-enzyme was lyophilized to increase the shelf life of the product (Figure 4). Further the bio-enzyme concoction

was analyzed for enzymatic and antimicrobial activity. Whereas the biomass was segregated, dried, and used as a bio-manure.

Screening for amylase enzyme activity

Amylase enzyme breaks down starch molecules into glucose subunits. In order to analyze the results for starch hydrolysis test, iodine solution was poured on the agar plate. Iodine reacts with the starch to form a dark blue complex. Thus, the hydrolysis of starch will lead to the clearance of dark blue color (Figure 5). The amylase enzyme activity was observed in Bottle gourd, Pineapple and Papaya.

Screening for cellulose enzyme activity

Carboxyl Methyl cellulose was broken down into β-glucose or smaller polysaccharide subunits by the cellulose enzyme. Congo red solution was added to indicate the zones where the enzyme activity occurred. Congo red was washed away and 1N NaCl was flooded in the plates to visualize the zones more clearly. Presence of cellulose enzyme was found in Bottle gourd, Ridge gourd, Pineapple and Papaya as appearance of clear zone was observed after flooding the plates with 1N NaCl solution (Figure 6).

Screening for protease enzyme activity

Protease enzyme activity is determined when protease reacts with gelatin. Protease breaks down peptide bond present in gelatin into smaller units. In order to interpret the enzyme activity, acidic mercuric chloride was prepared and added to the plate which will lead to the formation of clear zone around the well. But for Ridge gourd, Bottle gourd, Pineapple and Papaya protease enzyme activity was not observed.

Screening for lipase enzyme activity

Lipase enzyme reacts with Tween20 and gets precipitated around the wells if there is presence of lipase enzyme activity in the crude extract. But for Ridge gourd, Pineapple, Bottle gourd and Papaya lipase enzyme activity was not observed.

Screening for antimicrobial activity by well diffusion method

Anti-microbial activity of the bio-enzyme was analyzed using *Escherichia coli* cells. The cells were cultured and spread over the plate and then the positive control Kanamycin (10μg) and the crude extract was loaded into the wells. Resistance for the growth of *Escherichia coli* cells was observed when the inhibition zones diffused across the well (Figure 7) The diameter of the zone was measured and compared with the control to find out the extent of inhibition of the crude extract. Zone of inhibition for Kanamycin, Ridge gourd and Pineapple was 2.9cm, 2.5cm and 1.5 cm in diameter, respectively. Antimicrobial activity for Bottle Gourd and Papaya was not observed.

Applications of crude extract and biomass

The crude extract after filtration was diluted in a ratio 1: 10 where 1 part of the crude extract was added to 10 parts of water and used as a pesticide or insecticide for the plants. The plants which were initially affected with white molds appeared healthier after spraying the diluted crude extract (Figure 9 (a)). It serves as an efficient alternative for the harmful synthetic pesticides. The residues collected were used as an organic manure for the plants. Compared to the normal growth rate, the growth of the plants with the manure seemed to be fast (Figure 8,9,10,11 (b)(c)(d)). For the farmers, usage of organic manure serves as the one which is cheaper compared to the synthetic fertilizers which they use usually. Therefore, bio-enzymes act as natural fertilizers and pesticides. They enrich the soil and contribute to the health and growth of the plants. Adding to the beneficiaries, people in urban areas can collect vegetable and fruit peels going to the garbage and prepare this bio-enzyme out of it and use for various activities like mopping floors, disinfecting the tiles and tabletops, as laundry softener, stain remover, cleaning clothing and footwear. This bio-enzyme can also be used as bio-manures and natural pesticide in the kitchen garden or terrace garden.

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CONCLUSION

Organic solution without synthetic chemicals using household natural fermentation process of native microbes can be set up in every household kitchen in plastic containers. For plants, using bio enzyme helps in the uniform growth of plants, since it comprises of various growth factors. This creates a positive impact on maintaining our environment. As various enzyme activities were analyzed in the crude extract such as the presence of amylase in Bottle gourd, Pineapple and Papaya, cellulose in Bottle gourd, Ridge gourd, Papaya and Pineapple, these enzymes can be applied in agricultural and textile industries. Also, the presence of antimicrobial activity in Ridge gourd and Pineapple crude extract for *Escherichia coli* extends its application as disinfectants in various industries. The usage of organic manure serves as the one which is cheaper compared to synthetic fertilizers. Also, the harmful effects created by the usage of the chemical fertilizers is avoided and people will get an awareness on the organic manure. Also, the soil pollution of the farmland is avoided. Therefore, bio-enzyme acts as natural fertilizers and pesticides. They enrich the soil and contribute to the health and growth of the plants. Once the method is standardized based on the type of vegetable and fruit peels used, type of enzymes obtained, kind of microbial growth that dominates, the application of the enzymes will be much more beneficial. Bio-enzymes have the capacity to rejuvenate contaminated water. Bio-enzymes obtained from the crude extract can be stored for a longer period by lyophilizing the crude extract. As lyophilization is a low temperature dehydration process, it is performed to enhance the shelf life and easy transportation of the powdered filtrate. Bio-enzyme cleaner is the most inexpensive and versatile too. It is purely safe and ecofriendly. Therefore, the bio-cleaners are produced using renewable resources and regarded as a key factor for sustainability.

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Table 1 This table gives the composition to perform antimicrobial activity by well diffusion method.

COMPONENTS	VOLUME
Nutrient Agar	20ml
E. coli	100µl (for preparing spread plate)
Kanamycin	25µl
Crude extract	25µl
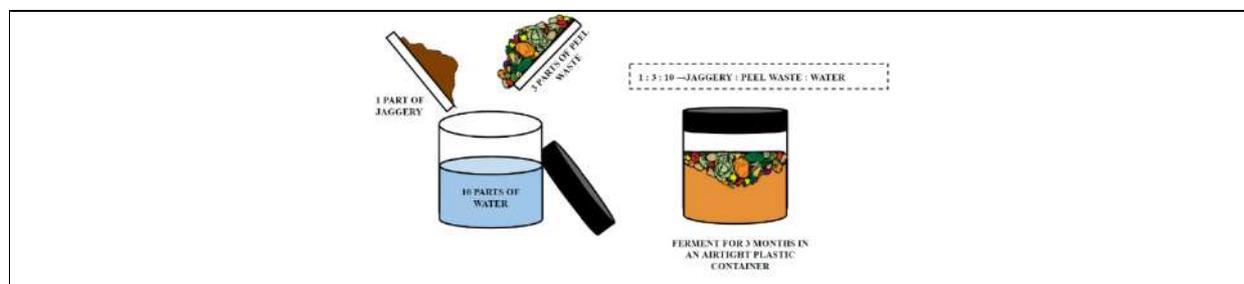
Figure 1. Graphical depiction of the products used for fermentation process
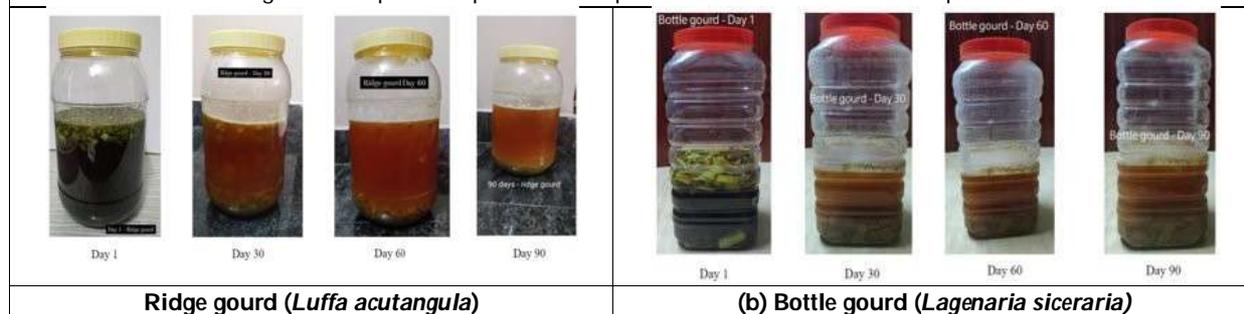
Ridge gourd (*Luffa acutangula*)	**(b) Bottle gourd (*Lagenaria siceraria*)**

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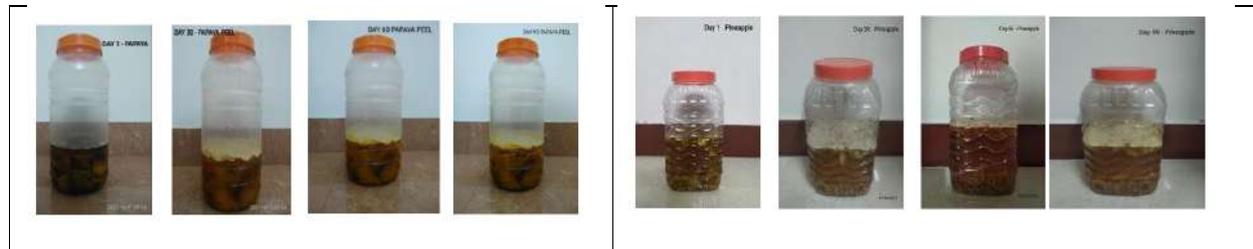
(c) Papaya (*Carica papaya*)　　　　**(d)** Pineapple (*Ananas comosus*)

Figure 2. Fermentation process of (a) Ridge gourd (*Luffa acutangula*) and (b) Bottle gourd (*Lagenaria siceraria*) (c) Papaya (*Carica papaya*) (d) Pineapple (*Ananas comosus*)

Figure 3. Separation of Filtrate and Biomass from bio-enzyme concoction
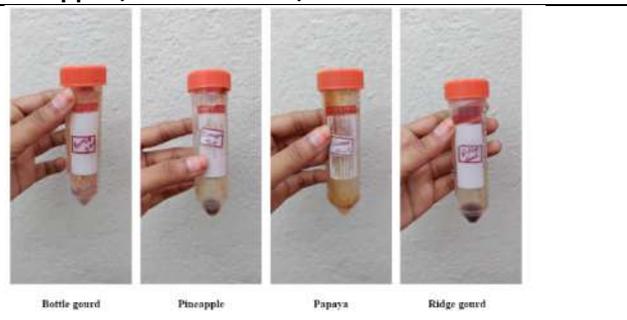
Figure 4. Lyophilized product of the bio-enzyme crude extract
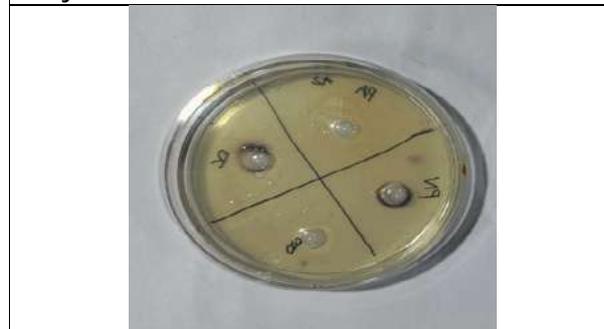
Figure 5. Amylase activity of crude extract of Pineapple, Papaya and Bottle gourd
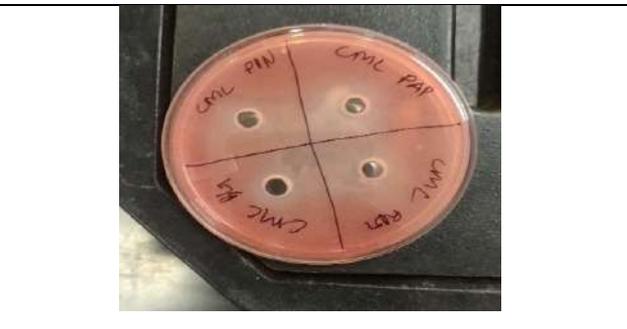
Figure 6. Cellulase activity of the crude extracts

a

b
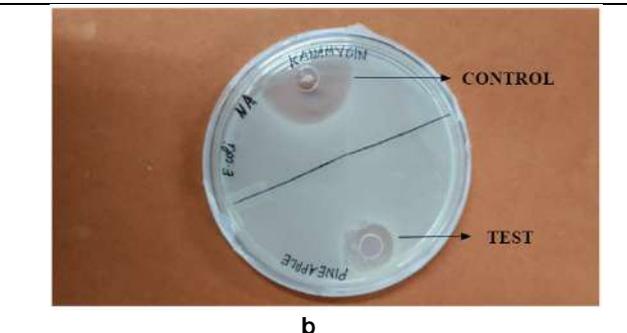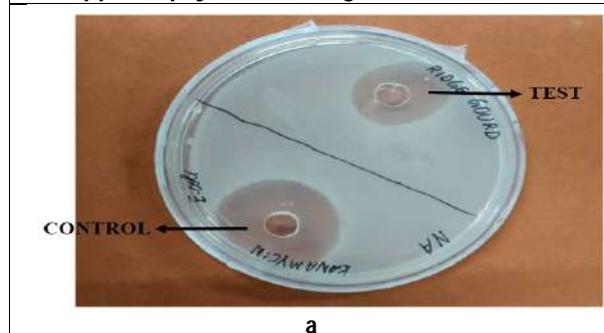
Figure 7. Observation of zone of inhibition due to antimicrobial activity (a) Ridge gourd and (b) Pineapple.

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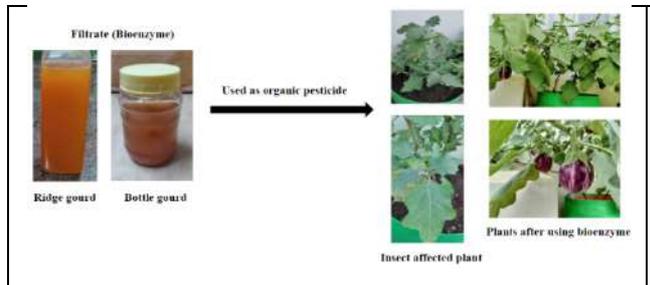
Figure 8 (a) Bio pesticidal activity of the filtrate	**Figure 9 (b) Residue (Ridge gourd and Bottle gourd) used as fertilizer for plant growth**
Figure 10 (c) Residue (Papaya) used as fertilizer for plant growth	**Figure 11 (d) Residue (Pineapple) used as fertilizer for plant growth**
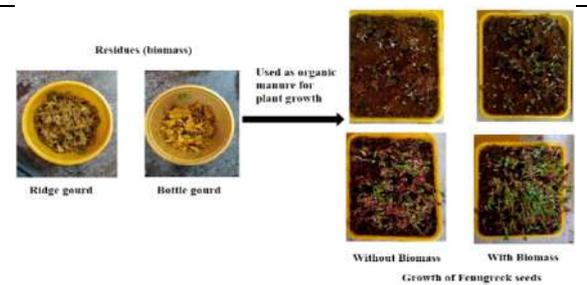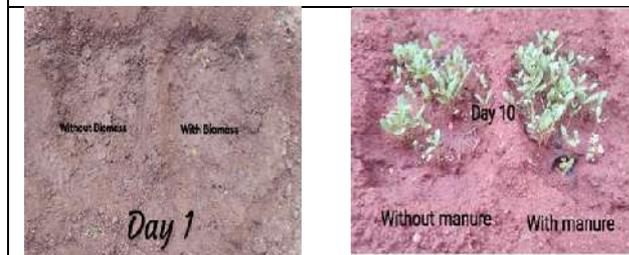
RESEARCH ARTICLE

Effect of Yogic Practices on Selected Body Mass Index and Systolic Blood Pressure among Middle Aged Women Suffering with Hypothyroidism

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ABSTRACT

The motive of the random organization experimental have a look at become to discover the impact of yogic practices on Body Mass Index and Systolic Blood Pressure amongst center middle-aged women struggling with hypothyroidism. It became hypothesized that there could be great variations on decided on physiological variables inclusive of Body Mass Index and Systolic Blood Pressure because of yogic practices amongst center hypothyroidism middle aged women than the control group. For the motive of the have a look at, 30 middle aged hypothyroidism women had been decided on randomly through the use of random organization sampling layout from Chennai, among the age organization of 35 to 45 years and that they had been divided into companies A and B having 15 topics each. Pretest become performed for the 2 Groups (A and B) on the chosen structured variables earlier than the begin of the program. Group A become given yogic practices; Group B (Control Group) didn`t acquire any particular treatment, but in active rest. After the experimental length of eight weeks, the 2 Groups (A and B) had been retested once more at the identical decided on structured variables as posttest. Analysis of co-variance (ANCOVA) become used to discover the great variations among experimental group and the control group. proved that the Experimental group confirmed great variations than the control group The effects of the have a look at proved that yogic practices reduced each the chosen physiological variables inclusive of Body mass index and systolic blood stress amongst center middle-aged women suffering with hypothyroidism. The hypothesis become established at 0.05 level of confidence Hence, it become

concluded that yogic practices are useful for middle aged women suffering with Hypothyroidism to keep wholesome Body Mass Index and Systolic Blood Pressure.

Keywords: Yogic practices, Blood Pressure, Systolic blood pressure, Body mass index (BMI), Middle age.

INTRODUCTION

Hypothyroidism (underactive thyroid) is a condition in which your thyroid gland doesn`t produce enough of certain crucial hormones. Hypothyroidism may not cause noticeable symptoms in the early stages. Over time, untreated hypothyroidism can cause a number of health problems, such as obesity, joint pain, infertility and heart disease. Hypothyroidism is common. It affects women more frequently than men and usually at around middle age. Hypothyroidism affects 1 in 1,000 men and 18 in 1,000 women. Asymptomatic hypothyroidism is much more common, affecting about 28 out of 1,000 men and 75 out of 1,000 women.

Causes of hypothyroidism, an autoimmune disease. Surgical resection of part or all of the thyroid radiation therapy. Congenital hypothyroidism (hypothyroidism in which a baby is born). Thyroiditis. dosage. Too much or too little iodine. Damage to the pituitary gland. The thyroid gland is rich in sympathetic and parasympathetic nerves. Some studies suggest that sympathetic stimulation or infusion of epinephrine or norepinephrine may increase secretion of thyroid hormone. (Goodman, H. Maurice,2003) The thyroid hormones control the metabolism of cells, which is their speed of activity. If there is too little hormone, the body cells work too slowly; too much results in them working too fast. Thyroid hormones regulate the rate of oxygen consumption. This metabolic effect affects the utilization of sugar, protein and fat, which are the main components of food.

Thyroid hormones have similar effects and affect the proper functioning of all cells in the body, especially in certain tissues and functions. For example, the physical and mental development of a baby growing in utero depends on whether the mother has the right amount of thyroid hormone until the 12th week of pregnancy, until her thyroid gland begins to function. In children, too few hormones will slow growth, and too many hormones will cause the child to grow faster than normal. Yoga practice balances the endocrine system and is suitable for hypothyroidism.

OBJECTIVES OF THE STUDY
The purpose of this study was to investigate whether yoga practice in middle-aged women with hypothyroidism made a significant difference in the selected physiological variables.

PURPOSE OF THE STUDY
The purpose of the study was to find out the effect of yogic practices on Body Mass Index (BMI) and blood pressure (Systolic) among middle aged hypothyroidism women.

HYPOTHESIS
It was hypothesized that there would be significant differences on Body Mass Index (BMI) and blood pressure (Systolic) among middle aged hypothyroidism women due to yogic practices than the control group.

DELIMITATIONS
The subjects were middle aged hypothyroidism women only.
The study was delimited to middle aged women from Chennai city only
Age of subjects was ranged from 35 to 45 years only.
Independent variable was yogic practices only.
The dependent variables were restricted to Body Mass Index (BMI) and blood pressure (Systolic) only.

REVIEW OF RELATTED LITERATURE

Kumari, N Suchetha et. al., (2011) investigated a study on "Effect of yoga therapy on body mass index and oxidative status." Forty obese male and female were selected as subjects. The changes in body weight, body mass index, blood sugar, MDA level and total antioxidant status was estimated before and after one month of yoga therapy. The data was analyzed using paired't' test at 0.05 level of significance. There was a significant decline in the body weight, body mass index, fasting blood sugar and post prandial blood sugar, MDA and significant increase in total antioxidant level after yoga when compared to that before the yoga therapy. Yoga therapy is beneficial in maintaining better health by regulating body mass index, oxidative status by improving the psychological functions of the body and helpful to overcome the complications of obesity.

Latha and KV Kalliappan (1991**)** conducted a study on Yoga, Pranayama, Thermal Biofeedback Techniques in the Management of Stress and High Blood Pressure. The sample of the study consisted of 14 essential hypertensive patients. Seven of these patients underwent training in yoga and thermal biofeedback technique for a period of six months. Other seven patients served as a comparison group. Results showed a significant reduction in the systolic blood pressure during treatment phases. Moderate reduction in the diastolic pressure was noticed, only when the thermal feedback was introduced. This also corresponded to significant reduction in the intake of anti-hypertensive drugs. Training in yoga and thermal biofeedback procedures were not effective in altering the perceptions associated with stressful experiences.

MATERIALS AND METHODS

To achieve the purpose of the study, 30 hypothyroidism women were selected randomly for the study from Chennai, between the age group of 35 to 45 years and they are equally divided into two groups I and II with 15 subjects in each group. Preliminary test was taken for the two groups (I and II) on the selected dependent variable before the start of the training program. Group I was given yogic practices for 60 minutes six days for a total period of eight weeks. Group II (control group) was permitted to undergo their routine and normal life style during the course of experiment without any specific training. After eight weeks, the two groups were rested again on the same selected dependent variable, the selected physiological variables such as Body Mass Index (BMI) and blood pressure(Systolic). Analysis of co-variance (ANCOVA) was used to find out the significant differences between experimental groups and the control group. The test of significance was fixed at 0.05 level of confidence.

YOGIC PRACTICES

1.Loosening the joints.
2.Surya Namaskar
3.Asanas
 • Navasana
 • Paschimottanasana
 • Noukasana
 • Ardhamatsyendrasana
 • Pawan mukatasana
 • Janusirsasana
 • Salabasana
 • Sarvangasana
 • Matsyasana
 • Ardhahalasana
 • Bhujangasana

- Savasana
- Pranayama
- Anulomvilom
- Kapalapathi
- Ujjai
- Yoga Nidra

RESULTS AND DISCUSSIONS

The data pertaining to the variable collected from the groups before and after the training period were statistically analyzed by using analysis of covariance (ANCOVA) to determine the significant difference and the hypothesis was tested at 0.05 level of confidence. The obtained F-ratio value for the BMI (Body Mass Index) and Systolic blood pressure were greater than the table value, indicating that there was a significant difference among the post test and adjusted posttest means of the yogic practice group than the control group on physiological variables.

DISCUSSION ON HYPOTHESIS

It was hypothesized that there would be significant differences on selected Physiological variables such as BMI (Body mass index) and Systolic blood pressure due to yogic practices among middle aged women suffering with Hypothyroidism than the control group. The results proved that there were significant differences on Body mass index(BMI) and blood pressure(Systolic)(both decreased)due to yogic practices than the control group among middle aged women suffering with Hypothyroidism.

CONCLUSION

It is concluded that yogic practices decreased Body mass index (BMI) and blood pressure(systolic) significantly among middle aged women suffering with hypothyroidism.

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Table I. Analysis of Co-variance (ANCOVA) for the Pre, Post and Adjusted Post Test Mean Values for Yoga Group and Control Group on BMI (Kg/M2)

Test	Experimental Group – I (yogic practices)	Control group	Source of variance	df	Sum of square	Mean square	F
Pre-test mean	24.63	25.54	Between	1	0.69	0.344	0.11
			Within	28	262.47	3.02	
Post-test mean	22.49	24.96	Between	1	362.40	181.20	53.69*
			Within	28	293.60	3.37	

Adjusted mean	22.79	25.14	Between	1	360.72	180.36	53.22*
			Within	27	291.466	3.39	

*Significant at 0.05 level of confidence. (Table F-ratio at 0.05 level of confidence for 1 & 28 (df) =4.21, 1 & 27 (df) =4.22

Table 2. Computation of Mean and Analysis of Covariance of Systolic Blood Pressure of Experimental And Control Group (mm/hg)

Test	Experimental Group (Yogic Practices)	Control group	Source of variance	Df	Sum of square	Mean square	F
Pre-test mean	141.1667	141.87	Between	1	24.16	12.078	0.88
			Within	28	1193.00	13.71	
Post-test mean	126.5	140.63	Between	1	3275.02	1637.51	125.12*
			Within	28	1138.63	13.09	
Adjusted mean	126.58	140.63	Between	1	3250.66	1625.33	124.78*
			Within	27	1120.223	13.03	

* Significant at 0.05 level of confidence. (The table value required for significance at 0.05 with df 1and 28 and 1 and 27 are 4.10 and 4.21 respectively)
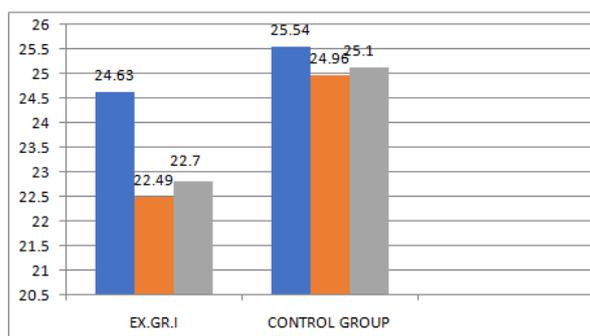
Figure 1. Bar Diagram Showing the Pre, Post and Adjusted Post Mean Values of Yoga Group and Control Group on BMI (Kg/m2)
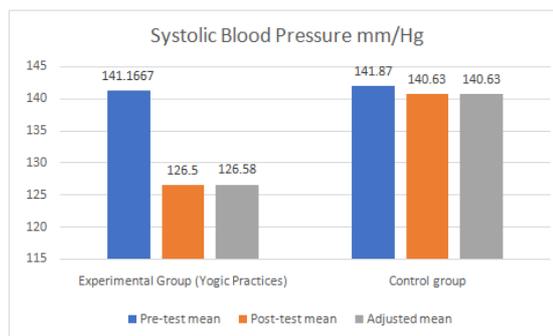
Figure 2. Bar Diagram Showing Adjusted Post-Test Values Of Control Group And Experimental Group On Systolic Blood Pressure

RESEARCH ARTICLE

In silico docking of Isorhamnetin on Molecular Proteins of Protein Kinase B Pathway

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ABSTRACT

Protein kinases (PK) or AKT are intracellular enzymes and are of 3 types, namely Protein Kinase A (PKA), Protein Kinase B (PKB) and Protein Kinase C (PKC). Isorhamnetin has cardioprotective, neuroprotective, anti-cancer, anti- apoptotic, anti-inflammatory, anti-oxidative and antidiabetic action. A pilot insilico docking studies of isorhamnetin with PKB conducted by us showed significant binding energy of -8.69 kcal/mol. Also studies show that flavonoids can modulateAKT/PKB and multiple interconnected pathways. Hence, the aim of the present study was to evaluate the insilico activity of isorhamnetin on 24 molecular proteins of protein kinase B pathway using Autodock 4.2 software. In our insilico study, isorhamnetin exhibited good binding energy for 22 molecular proteins of Protein KinaseB pathway namely Bax (-9.42 kcal/mol), WNK1 (-9.38 kcal/mol), Myt1 (-9.34 kcal/mol), GSK-3 (-8.86 kcal/mol), PKB (-8.69 kcal/mol), p27(-8.17 kcal/mol), NF-κB (-8.06 kcal/mol), p53 (-7.93 kcal/mol), AKT3 / PKB-γ (-7.75 kcal/mol), PKC-δ (-7.72 kcal/mol), MDM2 (-7.66 kcal/mol), caspase-9 (-7.54 kcal/mol), Raf (-7.41 kcal/mol), PI3K (-7.16 kcal/mol), eNOS (-7.09 kcal/mol), AKT1 / PKB-α (-6.80 kcal/mol), mTOR / S6K1 (-6.69 kcal/mol), FOXO4 (-6.31 kcal/mol), PLK1 (-6.24 kcal/mol), Bcl2 (-5.87 kcal/mol), FOXO1 (-5.78 kcal/mol) and PDK1 (-5.31 kcal/mol).In addition PDK1, FOXO1 andBcl2 exhibited favourable inhibition constant of 129.19 µM, 57.55 µM and 49.43µM, good binding energy of -5.31 kcal/mol, -5.78 kcal/mol and -5.87 kcal/mol and the number of hydrogen bonds formed were 2,2and 1 respectively. Of this PDK1

had higher inhibition constant (129.19 µM) and good binding energy (-5.31 kcal/mol) respectively and two molecular proteins (MAPKAP- K2 and IRS1) exhibit little binding energy.

Keywords: Molecular docking, Protein Kinase, Isorhamnetin, PDK1.

INTRODUCTION

Protein kinase (PK) plays important role in regulation of metabolism, cell survival, motility and cell-cycle progression [1]. There are 3 types of protein kinases as PKA, PKB and PKC. PKB/AKT is a serine/threonine kinase and consists of PKB-α (AKT1), PKB-β (AKT2) and PKB-γ (AKT3). Many hormones, growth factors and extracellular matrix components act as ligand for PKB/AKT. The binding of ligand activates phosphoinositide 3-kinase (PI3K). PI3K generates phosphatidylinositol-3,4,5-trisphosphate (PIP3) which leads to translocation of PKB/AKT to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase-1 (PDK1) [2]. It plays pivotal role in neuroprotection, platelet aggregation and mammary gland involution. Dysregulation of PKB causes cancer, CVS, CNS, inflammation and diabetes as well as schizophrenia [1].

Isorhamnetin is O-methylated flavonol, a metabolite of quercetin [3]. It is naturally present in various plants such as *Hippophae rhamnoides, Ginkgo biloba, Entomoscelis american, Solidago canadensis, Brassica juncea* and exhibits anti-microbial, anti-oxidant, anti-cancer, anti- inflammatory, anti-obesity, anti-diabetic, anti-artherosclerosis, anti-tuberculotic, anti-osteoporosis, anti-hypoxia, anti-viral, anti-vitiligo, anti-hyperuricemia, cardio protection, neuro protection, lung protection and hepatoprotective [4]. A pilot insilico docking studies of isorhamnetin with PKB conducted by us showed significant binding energy of -8.69 kcal/mol. Also studies show that flavonoids can modulate AKT/PKB and multiple interconnected pathways. Hence, the aim of the present study was to evaluate the insilico activity of isorhamnetin on molecular proteins of protein kinase B pathway.

MATERIALS AND METHODS

The structure of the isorhamnetin (PubChem ID: 5281654) was downloaded from NCBI PubChem compound database. The ligand was prepared by Marvin Sketch and it is converting into PDB format using OpenBabel-2.3.1. 24 molecular proteins of PKB pathway namely Protein kinase B (PKB), serine / Threonine kinase 1 (AKT1/PkB-α), serine / Threonine kinase 3 (AKT3/PKB-γ), B-Cell lymphoma 2 (Bcl2), mammalian target of rapamycin (mTOR), Glycogen synthase kinase 3 (GSK3), Protein kinase C delta type (PKC-δ), Phosphoinositide 3-kinase (PI3K), 3-phosphoinositide-dependent protein kinase-1 (PDK1), Mitogen-Activated Protein Kinase-Activated Protein Kinase 2(MAPKAP-K2), Bcl2 associated X (BAX), cysteine-dependent aspartate directed proteases(caspase-9), Rapidly Accelerated Fibrosarcoma (Raf), Cyclin-dependent kinase inhibitor (p27), mouse double minute 2 (MDM2), Myelin transcription factor 1(Myt1), Nuclear Factor of kappa light polypeptide gene enhancer in B- cells inhibitor, alpha (IκBα), Nuclear Factor kappa B(NF-κB), Tumor protein (P53), Forkhead transcription factors 1(FOXO1), Forkhead transcription factors 4(FOXO4), Polo-like kinase 1 (PLK1), insulin receptor substrate-1 (IRS1), lysine deficient protein kinase 1(WNK1), endothelial nitric oxide synthase (eNOS) were selected and imported from RCSB PDB with their respective PDB ID. The proteins ID were PKB(4O75), AKT1 / PKB-α(1UNR), AKT3 / PKB-γ(2X18), Bcl2(6VO4), mTOR(5WBH), GSK-3(1J1B), PKC-δ(1YRK), PI3K(3I5R), PDK1(1w1G), MAPKAP-K2(3S4E), Bax(4S0O), caspase-9(6E27), Raf(4XV9), p27(6ATH), MDM2(3LBL), Myt1(3P1A), NF-κB(5E69), p53(3LHO), FOXO1(6QZS), FOXO4(3L2C), PLK1(5NJE), IRS1(5U1M), WNK1(4PWN) and eNOS(6CIE).The heteroatoms of these proteins were removed and saved in the PDB format. Isorhamnetin was docked with the above 24 molecular proteins of the Protein Kinase B pathway using Autodock 4.2 (Version 1.5.6) [5].

The interaction between isorhamnetin and molecular protein of PKB were predicted using Autodock 4.2 software. In this, the preparation of receptor was done by adding all hydrogen atoms (polar only) to the macromolecule to correct the calculation of partial atomic charges. Gasteiger charges were calculated for each of the atom in the protein receptor molecule in Autodock 4.2. Grid parameter of size 60*60*60 Å was created with 0.375 Å spacing followed by picking an atom and centering the geometric centre of the targeted protein to run the autogrid file. The docking parameters for the lamarckian genetic algorithm were as follows: population size of 150 individuals, mutation rate of 0.02, crossing over of 0.8 and 25 docking runs. The ligand was docked with proteins using Autodock 4.2 (Version 1.5.6) software. The binding energy, inhibition constant, number of hydrogen bonds and active residues interaction were assessed [6-9].

RESULTS

In our insilico study, isorhamnetin exhibited good binding energy for 22 molecular proteins of Protein KinaseB pathway namely Bax (-9.42 kcal/mol), WNK1 (-9.38 kcal/mol), Myt1 (-9.34 kcal/mol), GSK-3 (-8.86 kcal/mol), PKB (-8.69 kcal/mol), p27(-8.17 kcal/mol),   NF-κB (-8.06 kcal/mol), p53 (-7.93 kcal/mol), AKT3 / PKB-γ (-7.75 kcal/mol), PKC-δ (-7.72 kcal/mol), MDM2 (-7.66 kcal/mol), caspase-9 (-7.54 kcal/mol), Raf (-7.41 kcal/mol), PI3K (-7.16 kcal/mol), eNOS (-7.09 kcal/mol), AKT1 / PKB-α (-6.80 kcal/mol), mTOR / S6K1 (-6.69 kcal/mol), FOXO4 (-6.31 kcal/mol), PLK1 (-6.24 kcal/mol), Bcl2 (-5.87 kcal/mol), FOXO1 (-5.78 kcal/mol) and PDK1 (-5.31 kcal/mol).   In addition PDK1 (1W1G), FOXO1 (6QZS) andBcl2 (6VO4) exhibited favourable inhibition constant (Table 1)129.19 µM, 57.55 µM and 49.43µM, good binding energy of -5.31 kcal/mol, -5.78 kcal/mol and -5.87 kcal/mol and the number of hydrogen bonds formed were 2,2and 1 respectively. Of this PDK1 had higher inhibition constant (129.19 µM) and good binding energy (-5.31 kcal/mol) respectively and two molecular proteins (MAPKAP- K2 and IRS1) exhibit little binding energy.

DISCUSSION

AKT is activated by growth factors and its activation leads to downstream of PI3K stimulation which translocates PKB from cytoplasm to plasma membrane. PI3K is activated on phosphorylation of receptor tyrosine kinases by growth factors, activation of G-protein coupled receptors and activation of integrin signalling. Feedback mechanism of AKT signaling stimulates multiple mitogenic signaling pathways such as RAS, phosphoribulokinase, PLC-γ and PKC pathway.PI3K-AKT and RAS-ERK pathways inhibit one another and also coordinate together for key cellular growth and proliferation. The substrates of AKT are AMPK, GSK3, FOXO and mTOR. PKB is a complex pathway and its aberration leads to developmental anomalies, cancer, cardiovascular disease, type 2 diabetes, neurological, inflammatory and autoimmune diseases [1,10]. Thus, pharmacological targeting of this pathway will pave way to the treatment of multitude of diseases but till now no successful commercial treatment is possible due to drug adverse effects. Phytoconstituents have the advantage of holistic approach in preventing and curing disease with minimal side effects since they act on multiple molecular proteins thus synergizing their action.

Bax is implicated in cancer [11] and autoimmune disease[12] and docking of isorhamnetin with Bax (Table 1) showed binding energy of -9.42 kcal/mol, 0.12 µM inhibition constant and 1 hydrogen bond. WNK1 maintains ion homeostasis via Ca^{2+} dependent interaction with synaptotagmin and hence it regulates the blood pressure and circadian rhythm. Its aberration leads to monogenic hypertension I and II, cancer and peripheral neuropathy[13,14]. Our docking study report shows that isorhamnetin with WNK1 (Table 1) resulted with binding energy of -9.38 kcal/mol, 0.13 µM inhibition constant and 3 hydrogen bonds. Studies show that isorhamnetin has anti-hypertensive activity by two ways, vasodilatory and decrease of calcium load leading to anti-hypertensive activity. Our drug is exhibiting good binding energy and hence targeting WNK1 can throw light in the treatment of these diseases. Myt1 plays pivotal role in the development of nervous system and its disturbance leads to demyelinating birth defects and cancer [15]. From our study it was found that isorhamnetin with Myt1(Table 1) exhibited the binding energy of -9.34 kcal/mol, 0.14 µM inhibition constant and 3 hydrogen bonds.

Docking of isorhamnetin with Bax, WNK1 and Myt1(Table 1) yielded highest binding energy of above -9 kcal/mol (-9.42 kcal/mol, -9.38 kcal/mol and -9.34 kcal/mol) respectively with nearly 3 hydrogen bond formation. This reflects that isorhamnetin may prove to be efficient in disease associated with Bax (cancer and autoimmune disease), WNK1 (regulationof blood pressure and circadian rhythm) and Myt1 (demyelinating disease and cancer). GSK-3 is involved psychiatric, neurological diseases, inflammatory diseases, diabetes and cancer [16]. From our insilico study, docking of isorhamnetin with GSK-3 (Table 1) showed the binding energy of -8.86 kcal/mol, 0.32 µM inhibition constant and 2 hydrogen bonds. PKB has been implicated in various diseases such as cancer, diabetes, bipolar disorder, Alzheimer disease, Parkinson disease and brain ischemia. Docking of isorhamnetin with PKB (Table 1) exhibited binding energy of -8.69 kcal/mol, 0.43µM inhibition constant and 2 hydrogen bonds. Our docking of isorhamnetin with p27 (Table 1) resulted with the binding energy of -8.17 kcal/mol, 1.02 µM inhibition constant and 2 hydrogen bonds. p27 inhibits cell cycle progression at G1 and is involved in cancer and hyperparathyroidism. NF-κB has been involved in number of autoimmune diseases such as rheumatoid arthritis, type 1 diabetes, multiple sclerosis, systemic lupus erythematosus and inflammatory bowel disease [17-19]. Docking of NF-κB (Table 1) with isorhamnetin showed the binding energy of -8.06 kcal/mol, 1.24 µM inhibition constant and 2 hydrogen bonds. The results are consistent with previous studies showing thatisorhamnetin can inhibit the downstream inflammatory factors expression and the inflammatory response through the negative regulation of NF-κB pathway [4].

Docking of isorhamnetin with GSK-3, PKB, p27 and NF-κB (Table 1) yielded highest binding energy of above -8 kcal/mol (-8.86 kcal/mol, -8.69 kcal/mol, -8.17 kcal/mol and -8.06 kcal/mol) respectively with nearly 2 hydrogen bond formation. This reflects that isorhamnetin may prove to be efficient in disease associated with GSK-3 (CNS disorder, cancer and diabetes), PKB(cancer, CNS disorder and diabetes), p27 (cancer)and NF-κB(cancer, autoimmune disease, inflammatory disease and diabetes). p53 moderates cell-cycle arrest, gene stability and apoptosis and its aberration leads to neurodegenerative disease (Alzheimer's and Parkinson disease), ischemic stroke and cancer [20]. Docking of isorhamnetin with p53 (Table 1) gave binding energy of -7.93 kcal/mol, 1.53 µM inhibition constant and 1 hydrogen bond. AKT3/PKB-γ has been implicated in neuronal protection is involved in post-natal brain development. The disease associated with AKT3/PKB-γ are autoimmune encephalomyelitis, demyelination and axonal injury [21]. AKT3/PKB-γ (Table 1) on docking with isorhamnetin showed the binding energy of -7.75 kcal/mol, 2.09 µM inhibition constant and 2 hydrogen bonds and isorhamnetin may prove as promising therapy. PKC-δ is involved in autoimmune diseases, diabetes, Parkinson's disease and cancer. Docking of isorhamnetin with PKC-δ (Table 1)showed the binding energy of - 7.72kcal/mol, 2.18 µM inhibition constant and 3 hydrogen bonds. MDM2 has been implicated in cancer [22], autoimmune disease, neurodegenerative diseases, cardio vascular disease, nephropathy, dementia, diabetes, inflammation, obesity and sterility [23]. Docking of MDM2 (Table 1) with isorhamnetin showed the binding energy of -7.66 kcal/mol, 2.42 µM inhibition constant and 3 hydrogen bonds.

caspase-9, an initiator of intrinsic apoptosis which on deregulation causes neurodegeneration, atherosclerosis and autoimmune disease [24]. caspase-9 (Table 1) on docking with isorhamnetin showed the binding energy of -7.54 kcal/mol, 3.0 µM inhibition constant and 1 hydrogen bond. Raf has multiple functions such as cell cycle regulation, cell survival and apoptosis. Disease associated with Raf protein are rheumatoid arthritis [25], allergic inflammation, ischemic stroke [26] and cancer. Docking of Raf (Table 1) with isorhamnetin exhibited the binding energy of -7.41 kcal/mol, 3.7 µM inhibition constant and 1 hydrogen bond. Deregulation of PI3K signaling is considered as hallmark of cancer [27], diabetes, neurological diseases like Alzheimer's and Parkinson's and cardiovascular diseases [28]. Docking of PI3K (Table 1) with isorhamnetin exhibited the binding energy of -7.16 kcal/mol, 5.64 µM inhibition constant and 2 hydrogen bonds. eNOS exhibits vasodilation, vasoprotective, blood pressure regulation and anti-atherosclerotic effect [29]. Docking of isorhamnetin with eNOS (Table 1) showed the binding energy of -7.09 kcal/mol, 6.37 µM inhibition constant and 2 hydrogen bonds. This is similar to previous studies that the vasodilative mechanism of isorhamnetin may be related to endothelial NO/GC/cGMP pathway and cyclooxygenase pathway and production of PGI2, thus exerting vasodilator effect. Isorhamnetin might decrease the levels of Ca2+ which is the mechanism for its hypotensive effects [4].

Docking of isorhamnetin with p53, AKT3, PKC-δ, MDM2, caspase-9, Raf, PI3K and eNOS (Table 1) yielded significant binding energy of above -7 kcal/mol (-7.93 kcal/mol, -7.75 kcal/mol, -7.72 kcal/mol, -7.66 kcal/mol, -7.54 kcal/mol, -7.41 kcal/mol, -7.16 kcal/mol and -7.09 kcal/mol) respectively and nearly 2 hydrogen bond formation. This reflects that isorhamnetin may prove to be efficient in disease associated with p53 (cancer and CNS disorder), AKT3 (CNS disorder), PKC-δ (cancer, CNS disorder, autoimmune disease, CVS disorder and diabetes), MDM2 (cancer, CNS disorder, autoimmune disease and diabetes),caspase-9 (cancer, CNS disorder and autoimmune disease), Raf (cancer, CVS disorder and autoimmune disease), PI3K (cancer, CVS disorder, autoimmune disease and diabetes) and eNOS (CVS).

Variation in AKT1 is associated with disease such as schizophrenia, bipolar disorder, type 2 diabetes, cancer and Parkinson disease[30,31]. Isorhamnetin on docking with AKT1 (Table 1) resulted in the binding energy of -6.80 kcal/mol, 10.43 µM inhibition constant and 2 hydrogen bonds. Previous studies also show that isorhamnetin exerts antitumor effect in cancer by regulating AKT signal transduction pathways. mTOR is implicated in physiological function of protein synthesis, cell size, energy metabolism, lipid metabolism, autophagy and lysosome biogenesis[32]. On docking with isorhamnetin and mTOR or S6K1(Table 1), the binding energy of -6.69 kcal/mol, 12.47 µM inhibition constant and 4 hydrogen bonds.   Deregulation of mTOR signaling is associated with Alzheimer's disease, Huntington disease, Parkinson disease, tuberous sclerosis, cortical dysplasia[33], cancer, epilepsy, diabetes and aging[34,35]. Forkhead box O4 (FOXO4) is the subfamily of transcription factors of FOXO. They directly express the target genes and involved in the mechanism of longevity, stress tolerance, apoptosis, DNA damage repair and proliferation[36]. This protein associated with rhabdomyosarcoma[37], post menopausal osteoporosis and male late out hypogonadism. Docking of isorhamnetin with FOXO4 (Table 1) exhibited the binding energy of -6.31 kcal/mol, 23.57 µM inhibition constant and 2 hydrogen bonds.PLK1 functions are centrosome maturation, checkpoint recovery, spindle assembly, cytokinesis and apoptosis. Deregulation of PLK1 causes graft versus host disease and neurological disorders including Alzheimer's disease and Huntington's disease[38]. Docking of PLK1 (Table 1) with isorhamnetin showed the binding energy of -6.24 kcal/mol, 26.47 µM inhibition constant and 2 hydrogen bonds.

Docking of isorhamnetin with AKT1, mTOR, FOXO4 and PLK1 (Table 1) yielded acceptable binding energy of above -6 kcal/mol (-6.80 kcal/mol, -6.69 kcal/mol, -6.31 kcal/mol and -6.24 kcal/mol) respectively and nearly 2 hydrogen bond formation. However, further studies are required to evaluate the role of isorhamnetin in diabetes, CNS disorder and cancer by AKT1, mTOR, FOXO4 and PLK1. Bcl2, an apoptotic factor, is implicated in cancer[39], stroke and neurodegenerative diseases[40]. Bcl2(Table 1) on docking with isorhamnetin exhibited the binding energy of -5.87 kcal/mol, 49.43 µM inhibition constant and 1 hydrogen bond. FOXO is involved in cancer, Alzheimer's disease, Parkinson disease and atherosclerosis. Docking of FOXO1 (Table 1) with isorhamnetin showed the binding energy of -5.78 kcal/mol, 57.55 µM inhibition constant and 2 hydrogen bonds. PDK1 plays a key role in signaling pathways and is responsible for cancer [41], prion disease and Alzheimer's disease [42]. Docking of PDK1 (Table 1) with isorhamnetin exhibited the binding energy of -5.31 kcal/mol, 129.19 µM inhibition constant and 2 hydrogen bonds.

Docking of isorhamnetin with Bcl2, FOXO1 and PDK1 (Table 1) yielded significant binding energy of above -5 kcal/mol (-5.87 kcal/mol, -5.78 kcal/mol and -5.31 kcal/mol) respectively and nearly 2 hydrogen bond formation. This gives us prospective hope for addressing Bcl2, FOXO1 and PDK1 for targeting cancer and CNS disorder. Previous preclinical studies of isorhamnetin shows that it acts by modulating AKT, PI3K, NF-κB, Nrf2, iNOS, caspase 3 and 8, FasL, PKC-ε, Bcl2, Bax, caspase, cytochrome-C and PPAR-γ. Based on our insilico study, we would like to establish that it acts by modulating BAD, WNK1, Myt1, p27, AKT3, PKC-δ, MDM2, caspase-9, PLK1 and PDK1. Studies shows that isorhamnetin exhibit anti-alzheimer activity by exhibiting anti cholinesterase, increase cholinergic synapses, neuroplasticity and antioxidant activity. Isorhamnetin potentiates the nerve growth factor-induced neurite outgrowth and hence a potential medicine for neurodegenerative disorders [4]. Hence from our study we would like to propose that isorhamnetin has neuroprotective and anti-demyelinating properties owing to the modulation of Myt1, AKT3, AKT1, PKC-δ, MDM2, FOXO1, FOXO4 and PLK1 molecular proteins.Studies show that isorhamnetin displays antioxidant properties by activating PI3K/AKT /mTOR signal transduction pathway and Nrf2 / ARE signal

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transduction pathway to inhibit apoptosis[4]. It inhibits both intrinsic and extrinsic apoptotic pathway via FAS/FASL, Bcl2 and Bax[43], cytosolic release of cytochrome c, activation of caspases and modulation of peroxisome proliferator-activated receptor γ activation pathway[44,45]. Studies shows that isorhamnetin exhibit anti-cancer activity by attenuation of cell survival, cell cycle, telomere activity, inflammation, angiogenesis, invasion, apoptosis, proliferation, necrosis[46], antioxidant and genotoxic activity[47]. It acts on cell cycle by upregulating Cdk inhibitors namely p21 and p27[48] which further decreases the expression of wee1 and cyclin B1. It also cause reactive oxygen species-dependent cell cycle arrest at the G1, S, G2/M phase [4]. Being a flavonoid it modulates the phosphorylation of AKT and PDK1 followed by reduced phosphorylation of downstream survival factors (BAD, GSK-3β, mTOR, IκBα). It also decreases the levels of angiogenic factor (VEGF-A) and inflammatory enzymes (COX-2, iNOS). It prevents metastasis by decreasing the matrix metalloproteinase and PPAR-γactivity[45,49]. Our study highlighted that isorhamnetin owes its anticancer properties by regulation of AKT1, PDK1, PKC-δ, FOXO1 and FOXO4 molecular proteins of PKB pathway.

AKT signaling pathway plays important role in insulin signaling and resistance by promoting glucose uptake, glycolysis, ATP production which inhibits AMPK activity. Isorhamnetin activates the JAK2/ STAT pathway and promotes glucose uptake by increasing GLUT4 translocation to maintain glucose homeostasis [50] and also inhibits aldose reductase and sorbitol accumulation in diabetes [51]. Isorhamnetin downregulates p47phox and free radical production in blood vessels [52] and exerts cardioprotective activity through SIRT1 and inhibiting TGF-β/Smad signaling pathway [4]. By activation of PI3K/AKT and HO-1 pathway, it confers anti-atherosclerotic activity[53].Till date studies shows that INH exerts vasodilative and hypotensive effect by endothelial NO/ GC/cGMP pathway, cyclooxygenase pathway and decrease Ca2+levels [4]. However we would like to highlight that isorhamnetin may interact with WNK1 protein also to maintain blood pressure and circadian rhythm. Isorhamnetin downregulates NF-κB signal transduction pathway and c-Jun nuclear translocation inhibits iNOS expression and NO production and activates Nrf2 pathway[54] by increasing antioxidative defense system and reducing the inflammatory signaling pathways [55]. Our study shows that isorhamnetin exhibit anti inflammatory properties not only because of NF-κB and Nrf2 pathway but also by modulating AKT3, MDM2, PLK1 molecular proteins of AKT pathway.

Docking of PDK1 with isorhamnetin exhibited the binding energy of -5.31 kcal/mol, 129.19 μM inhibition constant and 2 hydrogen bonds. Thus this is the first study to show that isorhamnetin has good PDK1 inhibition. However further invitro and invivo studies are necessary to establish the role of isorhamnetin as a PDK1 inhibitor in cancer and Alzheimer disease.

CONCLUSION

Our *In silico* docking study shows that isorhamnetin can modulate vast majority of protein of PKB pathway. Previous preclinical studies of isorhamnetin shows that it acts by modulating AKT, PI3K, NF-κB, Nrf2, iNOS, caspase 9, FasL, PKC-ϵ, Bcl2, Bax, caspase, cytochrome-C and PPAR-γ. But based on our insilico study, we would like to add on that in addition to the above proteins, isorhamnetinalso acts by modulating WNK1, Myt1, AKT3, PKC-δ, MDM2, PLK1 and PDK1 of the AKT pathway.

ACKNOWLEDGEMENT

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Table 1. Docking score of isorhamnetin with various molecular proteins of protein kinase b pathway using autodock 4.2.

SL. NO.	PROTEIN	PDB ID	BINDING ENERGY (kcal/mol)	INHIBITION CONSTANT (µM)	NUMBER OF HYDROGEN BONDS	PROTEIN RESIDUES
1.	Bax	4S0O	-9.42	0.12	1	4s0o:A:ALA42:HN
2.	WNK1	4PWN	-9.38	0.13	3	4pwn:A:LEU369:HN 4pwn:A:MET304:HN 4pwn:A:GLY370:HN
3.	Myt1	3P1A	-9.34	0.14	3	3p1a:A:ASP251:HN 3p1a:A:CYS190:HN 3p1a:A:HIS161:HE2
4.	GSK-3	1J1B	-8.86	0.32	2	1j1b:B:ILE728:O 1j1b:A:ILE228:O
5.	PKB	4O75	-8.69	0.43	2	4o75:A:ASN140:HD21 4o75:A:GLN85:HE22
6.	p27	6ATH	-8.17	1.02	2	6ath:A:ARG122:HE 6ath:A:ARG157:HN

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7.	NF-κ B	5E69	-8.06	1.24	2	5e69:D:DG40:H21 5e69:D:DG39:H22
8.	p53	3LHO	-7.93	1.53	1	3lh0:A:SER1503:HN
9.	AKT3 / PKB-γ	2X18	-7.75	2.09	2	2x18:G:TRP22:HN 2x18:G:HIS88:HE2
10.	PKC-δ	1YRK	-7.72	2.18	3	1yrk:B:VAL11:HN 1yrk:B:ALA13:HN 1yrk:B:GLN8:HE22
11.	MDM2	3LBL	-7.66	2.42	3	3lbl:C:ASP46:HN 3lbl:C:ARG29:HE 3lbl:C:THR47:HN
12.	caspase-9	6E27	-7.54	3.0	1	6e27:C:ARG35:HH22
13.	Raf	4XV9	-7.41	3.7	1	4xv9:A:ARG575:HH12
14.	PI3K	3I5R	-7.16	5.64	2	3i5r:A:ASN57:HN 3i5r:B:LYS3:HZ3
15.	eNOS	6CIE	-7.09	6.37	2	6cie:C:ARG262:HE 6cie:C:ASN374:HD22
16.	AKT1 / PKB-α	1UNR	-6.80	10.43	2	1unr:A:ARG86:HH21 1unr:A:ASN53:HD21
17.	mTOR / S6K1	5WBH	-6.69	12.47	4	5wbh:D:ARG2076:HE 5wbh:C:ARG2110:HH11 5wbh:A:LYS2090:HZ1 5wbh:D:ARG2076:HH21
18.	FOXO4	3L2C	-6.31	23.57	2	3l2c:A:ALA96:HN 3l2c:A:GLY145:HN
19.	PLK1	5NJE	-6.24	26.47	2	5nje:A:PHE409:HN 5nje:A:ASN527:HD21
20.	Bcl2	6VO4	-5.87	49.43	1	6vo4:A:SER43:OG
21.	FOXO1	6QZS	-5.78	57.55	2	6qzs:B:ASP97:OD1 6qzs: B:ASP97:O
22.	PDK1	1w1G	-5.31	129.19	2	1w1g:A:SER421:O 1w1g:A:GLN442:OE1
23.	IRS1	5U1M	-4.88	263.09	1	5u1m:A:ILE211:O
24.	MAPKAP-K2	3S4E	-3.93	1320	1	3s4e:A:LYS70:HZ3

RESEARCH ARTICLE

X-Ray Diffraction Studies for the Determination of Crystallite Size of ZnO Nanoparticles: Scherrer Formula and Williamson-Hall Plot Approach

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ABSTRACT

The analysis of the XRD pattern is considered as a simple and effective method for estimating the crystallite size and the corresponding lattice parameter. X-ray diffraction patterns of ZnO nanoparticles (NPs) were analyzed using the Scherrer formula and the Williamson–Hall method for the determination of the crystallite size. The XRD pattern confirmed the presence of the crystalline hexagonal wurtzite structure of ZnO NPs. The average crystallite size was found 30.8 and 52.9 nm by the Scherrer formula and W-H approach respectively. ZnO NPs were also characterized using the FESEM technique for the confirmation of the formation of ZnO NPs.

Keywords: ZnO nanoparticles, XRD, Scherrer formula, Williamson–Hall method, FESEM.

INTRODUCTION

ZnO, n-type semiconductor from group II-VI has a wide direct bandgap 3.37 eV, high electron mobility (210 cm^2 V^{-1} S^{-1}) and large excitonic binding energy (60 meV) which is one of the most attractive materials for various applications due to its notable structural and optical properties [1-2]. Many products contain ZnO as an additive, including adhesives, cement, glass, paints, ceramics, plastics, batteries, pigments, fire retardants, ferrites, first-aid tapes, rubber, Lubricants and food (as a source of Zn nutrient) [3-5]. Many synthetic methods have been used to

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synthesize ZnO nanostructures, according to published reports, including chemical vapor deposition (CVD), physical vapor deposition (PVD), electrode position, RF magnetron sputtering, spray pyrolysis, reactive evaporation, Sol-gel auto combustion and co-precipitation method have been used to synthesize the ZnO Nanostructures [6-16]. The co-precipitation method has been a relatively facile and cost-effective approach due to its good crystallinity, low-cost equipment, low reaction temperature, high efficiency and simple process. XRD analysis is an important tool for the estimation of the crystallite size and lattice parameters. Different methods such as Scherrer formula, Williamson–Hall and Halder–Wagner procedures are applied for determining the crystallite size and the corresponding strain [17-20].

In the present work ZnO nanoparticles (NPs) were synthesized using a simple co-precipitation method. From X-ray diffraction patterns using Scherrer and Williamson–Hall methods, the crystallite size and corresponding parameters could be determined. The surface morphology of prepared material was studied using the FESEM technique.

MATERIALS AND METHODS

Synthesis and Characterization of ZnO NPs
The ZnO NPs were prepared using a simple co-precipitation method. In the present experiment, required quantity of zinc acetate dihydrate (Loba A.R. Grade) was dissolved in ethanol and stirred at 70 ℃ on a magnetic stirrer leading to the formation of a homogeneous solution. At room temperature keep the prepared solution for 24 hours for nucleation afterward annealed at 500 ℃ for 3 hours in a muffle furnace. The prepared ZnO powder was crushed for about 1 hour in a mortar and pestle. The very fine ZnO NPs were obtained. To determine the grain size, crystallinity and structural parameters of ZnO NPs, XRD analysis was done using Bruker D2 phaser using filtered Cu Kα radiation (1.54056 Å) in the 2θ range 20–80°. The surface morphology of the ZnO sample was studied using FESEM (Zeiss Ultra, 55 FE-SEM).

RESULTS AND DISCUSSION

XRD analysis of ZnO NPs
The structural analysis and determination of crystalline parameters are done using the X-ray diffraction (XRD) technique. The XRD analysis of the ZnO NPs arrays grown by the co-precipitation method is shown in Fig. 1. The remarkable diffraction peaks at 2θ positions 31.98°, 34.63°, 36.47°, 47.73°, 56.79°, 63.04°, 66.56°, 68.11°, 69.27°, 72.73° and 77.12° correspond to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) planes. The obtained diffraction patterns are refers to the hexagonal wurtzite structure of ZnO NPs that was coordinated with JCPDS file No. 01-079-0205 (Inset of Fig.1). Furthermore, the XRD patterns revealed no additional impurity peaks, confirming the purity of the ZnO phase.

Determination of crystallite size using Scherrer formula
The Scherrer relation was used to estimate the average crystallite size [17].

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

where D represents the crystallite size, K is the shape factor (0.9), λ is the of X-ray wavelength of Cu-Kα line (1.54056 Å), β is the Full width at half maximum (FWHM) of the peaks, θ is the diffraction angle in radians. The average crystallite size of ZnO NPs is 30.8 nm. Detailed calculations of all values are given in Table 1.

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Determination of crystallite size using Williamson-Hall method
The average crystallite size of ZnO NPs was also estimated using Williamson-Hall equation [19].

$$\beta \cos\theta = \frac{0.9\lambda}{D} + 4\varepsilon \sin\theta \quad (2)$$

The crystallite size (*D*) values can be estimated from the intercept of a graph of $\beta(\cos\theta)$ against $4(\sin\theta)$, whereas residual strain (ε) is the slope of the graph (Fig. 2). The average crystallite size of the ZnO NPs is 52.9 nm. The detailed calculations of crystallite size are given in Table 2. The observed value through the W-H method is greater than the Scherrer formula due to the consideration of the microstrain of ZnO NPs.

Morphological studies
The morphology, surface roughness and size of the ZnO NPs were studied with FESEM technique. Fig. 3 displays the plane view of the FESEM image of ZnO NPs at 200 nm which indicates stepped nanorods with hexagonal nanostructures distributed randomly in all directions. This is agreed with the XRD data.

CONCLUSION

In the present work, ZnO NPs have been effectively synthesized using a simple co-precipitation method. The hexagonal wurtzite structure of the ZnO lattice was confirmed by XRD analysis. FESEM study shows the porous and uniform morphology of pure ZnO NPs which is decent for various applications. The average crystallite size of ZnO NPs was estimated using Scherrer and Williamson–Hall approaches. The average values of the crystallite size were 30.8 and 52.9 nm by Scherrer and Williamson–Hall method respectively. The observed value through the W-H method greater is due to consideration of the microstrain of ZnO NPs.

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Table 1: The characteristics of the ZnO NPs for Scherrer relation.

(hkl)	FWHM [°2θ]	d-spacing [Å] (d= n. λ/2sinθ)	2θ	θ	θ in radian	sinθ in radian	cosθ in radian	(β) radian FWHM	D nm D=K λ / [βcos(θ)]
(1 0 0)	0.23	2.795	31.98	15.99	0.27	0.27	0.96	0.00415	36.2
(0 0 2)	0.20	2.587	34.63	17.31	0.30	0.29	0.95	0.00359	42.1
(1 0 1)	0.24	2.461	36.47	18.23	0.31	0.31	0.94	0.00426	35.7
(1 0 2)	0.27	1.903	47.73	23.86	0.41	0.40	0.91	0.00485	32.6
(1 1 0)	0.31	1.619	56.59	28.39	0.49	0.47	0.87	0.00549	29.9
(1 0 3)	0.32	1.473	63.04	31.52	0.55	0.52	0.85	0.00572	29.6
(2 0 0)	0.38	1.403	66.56	33.28	0.58	0.54	0.83	0.00676	25.6
(1 1 2)	0.36	1.375	68.11	34.05	0.59	0.56	0.82	0.00637	27.4
(2 0 1)	0.38	1.355	69.27	34.63	0.60	0.56	0.82	0.00675	26.0
(0 0 4)	0.36	1.298	72.73	36.36	0.63	0.59	0.80	0.00645	27.8
(2 0 2)	0.40	1.235	77.12	38.56	0.67	0.62	0.78	0.00699	26.4
Average									**30.8**

Table 2: The characteristics of the ZnO NPs for Williamson-Hall equation.

(hkl)	2θ	βcosθ	4sinθ	Intercept (c)	Slope (m)	Microstrain (ε)	D (nm) D = K λ /c
(1 0 0)	31.98	0.003984	1.08				
(0 0 2)	34.63	0.003411	1.16				
(1 0 1)	36.47	0.004004	1.24				
(1 0 2)	47.73	0.004414	1.60				
(1 1 0)	56.59	0.004776	1.88				
(1 0 3)	63.04	0.004862	2.08	0.00262	0.00118	1.18 x 10⁻³	52.9
(2 0 0)	66.56	0.005611	2.16				
(1 1 2)	68.11	0.005223	2.24				
(2 0 1)	69.27	0.005535	2.24				
(0 0 4)	72.73	0.005161	2.36				
(2 0 2)	77.12	0.005452	2.48				
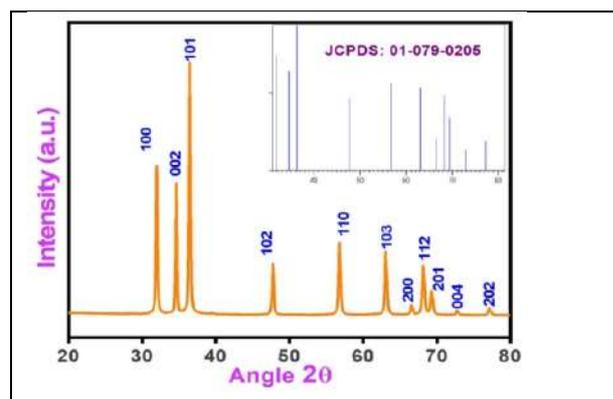
Fig. 1: XRD pattern of ZnO NPs. Inset shows diffraction peaks of ZnO NPs of JCPDS card number 01-079-0205.
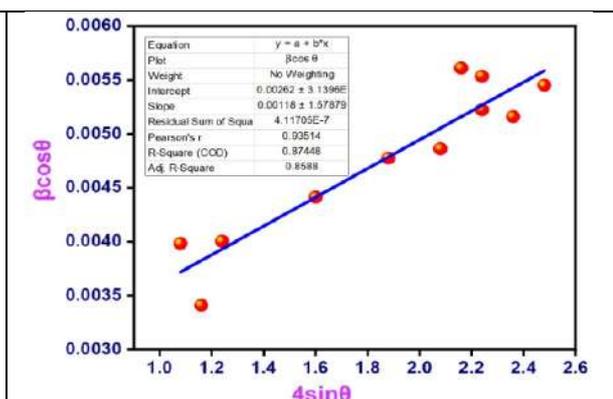
Fig. 2: Plot of β(cosθ) against 4(sinθ) of W-H analysis for ZnO NPs.
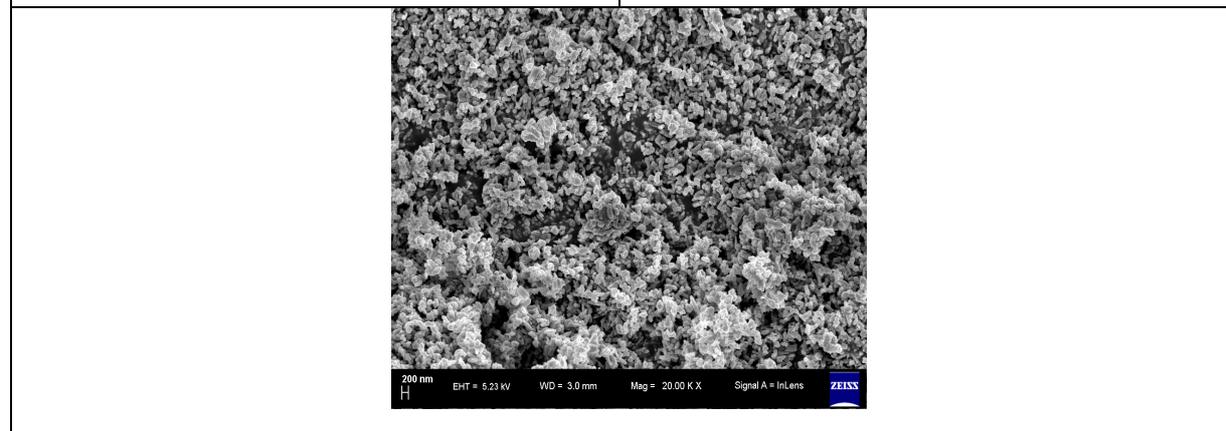
Fig. 3: FESEM image of ZnO NPs at 200 nm.

RESEARCH ARTICLE

Influence of Winery Effluent on Growth and Biochemical Constituents of Three Halophytic Species

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ABSTRACT

The present study deals with the effect of different concentrations of winery effluent (0, 10, 25, 50, 75 and 100) on growth and biochemical constituents of three halophytic species; *Suaeda maritima, Sesuvium portulacastrum* and *Ipomoea pes-caprae* were made to assessed. The growth parameter such as shoot, root length, fresh and dry weight increased up to 25% winery effluent in *Suaeda maritima* and *Sesuvium portulacastrum* and the optimum level of 10% level in *Ipomoea pes-caprae and* thereafter drastically reduced the growth parameter. All the three halophytic plants survived up to 100% level. The chlorophyll pigment, protein and starch content increased up to increasing level of 25% in *S. maritima, S. portulacastrum* and 10% in *Ipomoea pes-caprae* and thereafter the content reduced gradually. The amino acid and sugar content decreased up to optimum level of 25% in *S. maritima* and *S. portulacastrum* and 10% in *Ipomoea pes-caprae* and thereafter gradually increased the amino acid and sugar content. The proline content increased with increasing concentrations of 100% winery effluent for all the three halophytic species.

Keywords: Winery effluent, fresh weight, proline, amino acid, photosynthetic pigment.

INTRODUCTION

In recent decades, the continuous growth of the world population and the quest for material goods has generated a massive expansion in industrial and agricultural production. Demand for food is quickly rising and will continue to rise with increases global population. Since the 1960s, intensive irrigation and massive use of chemical fertilizers and

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pesticides has allowed an increase in food production obtained from the same amount of land. However, extensive use of mineral fertilizers and pesticides has caused serious contamination of soil and water, decreasing the quality water and land for human purposes (Melo *et al.*, 2012). Furthermore, several environmental pollution problems have been observed incites and urban areas with concentrated population, large energy consumption and high waste production, traffic emissions and industrial activity (Luo *et al.*, 2012). The continuous pollution of soil, air and water has a direct adverse impact on ecosystems, cultural heritage, and human health (Phalan *et al.*, 2011).

Phytoremediation relies on the use of plants and their associated rhizospheres to degrade, stabilize and/or remove soil contaminants and it is a technology that has been of greatest interest in recent years. This environmentally friendly and low cost technology can be used to decontaminate soils, water and sediments containing organic compounds and/or metals (Kramer, 2010). There is a great interest in applying phytoremediation to agriculture as well as to military and industrial fields that present contamination problem (Pignattelli *et al.*, 2012; Testiati *et al.*, 2013). Polluted waters that can be phytoremediated include sewage and municipal wastewater, agricultural runoff/drainage water, industrial wastewater, landfill leach ate and mine drainage. For phyto extraction, plants should possess the ability to grow quickly, to produce large amounts of biomass, to be easily harvested and to accumulate a variety of metals in their harvestable parts (shoots). In phyto stabilization, plants should have the capacity to retain contaminants in the roots and to produce large amounts of biomass (Andreazza *et al.*, 2011). As yet, no plant is known to fulfill all of these criteria. Limitations on plant growth in sites heavily contaminated with multiple compounds (both organic and inorganic) compromised the efficiency of phytoremediation (Sirguey and Ouvrard, 2013). Recent progress in plant physiology, biochemical and molecular fields provides a strong scientific basis for some strategies for achieving this goal (Tan *et al.*, 2013). To elucidate the phytoremediation of winery effluent heavy metals using three halophytic species at its growth and biochemical analysis were assessed on monthly interval.

MATERIALS AND METHODS

Plant material
Three species of fast growing salt marsh halophyte herbs like *Suaeda maritima* L. *Sesuvium portulacastrum* L and *Ipomoea percaprae* Sweet were selected for the characterization and screening for phytoremediation of heavy metals from winery effluents with special reference for morphological studies. The experimental site was located at Botanical Garden, Annamalai University, Cuddalore district, Tamil Nadu, India.

Winery effluent collection
The Raw effluent was collected from winery effluent industry Udhamapalayam, Theni Districts. In clean plastic container stored effluent and kept it 4°C for further studies.

Pot culture experiment
The experiment was conducted in an open-air area with nature light, temperature and humidity. Sand + Red soil (3:1 ration) free from pebbles and stones were filled in polythene bags. The seedling/cutting from the selected species of similar size were transplanted from the nursery bed and planted at the polythene bags. The experiment comprised of the following 5 set of treatments with five replicated and average values are reported. Plants were watered for every 15 days, depending on the evaporative demand. Plants were harvested for experimental purpose at intervals of 30, 60, 90 and 120 days. During each and every sampling day, samples were randomly collected, washed thoroughly with tap water followed by distilled water and used for experimental purpose.

S. No.	Treatment	Method
1	Control	Without any treatment (plants are irrigated with tap water only)
2	Winery effluent treatment	10, 25, 50, 75 and 100% of winery effluent was treated 250 mL for 4 times with an alternative 5 days intervals

Photosynthetic pigments

Chlorophyll was estimated according to (Arnon, 1949). Tissue for chlorophyll estimation was washed five times with deionized water and blotted on tissue paper. Plant tissue homogenized in 80% acetone and incubated in the dark for 6 h. (0.1 g) was homogenate was centrifuged at 10000 rpm for 10 minutes. The supernatant obtained was read at 645 and 665 nm in spectrophotometer-20

Estimation of carotenoids

The same chlorophyll extract was measured at 480 nm a in spectrophotometer to estimate the carotenoid content using the method of Kirk and Allen (1965).

Estimation of protein

Fresh tissue weighing 0.5 g was macerated in 20 per cent trichloroacetic acid using mortar and pestle. The homogenate was then centrifuged at 600 rpm for 30 minutes and the supernatant was discarded. The supernatant was saved for the estimation of protein by using the method of Lowry *et al.* (1951). The absorbance was read at 660 nm in a spectrophotometer against an appropriate blank. Bovin serum albumin was used as the standard.

Estimation of amino acids

Free amino acid was measured by the method prescribed by of Moore and Stein (1948) also called as ninhydrin method and is widely used. Absorbance was recorded at 570 nm using Spectrophotometer (Spectrascan) UV 2700). Leucine was used as the standard.

Estimation proline

Proline was extracted from the leaves and estimated by the method of (Bates (1973). Homogenates of the leaf samples were prepared in 3% sulphosalicylic acid. Pink colour was developed by a reaction with glacial acid and ninhydrin. The colour was separated in toluenr layer and intensity of the colour was measurement at 529 nm spectrophotometrically.

Estimation of sugar and starch

The estimation of sugar content by the methods of Nelson (1944) and the starch content followed by the method of Dubois *et al.* (1956).

RESULTS AND DISCUSSION

The present study indicated after 120 days of halophytes plants cultivated in winery effluent it shows maximum growth was observed in op timulated 25% of in *Suaeda maritima* and *Sesuvium portulacastrum* and 10% in *Ipomoea pes-caprae* (Figs. 1a-d) All the three halophytes species could survive up to 100% winery effluent. The above optimum level of 25 and 10% drastically reduced the growth rate. The growth parameter such as shoot and root length, fresh and dry weight increased up to optimum level of 25% in *Suaeda maritima* (44.5, 12.5 cm pant[-1] and 104.5, 43.25 g plant[-1] *Sesuvium portulacastrum.* (70.2, 12.6 cm plant[-1] and 160.5, 69.58, g plant[-1] and 10% in *Ipomoae pes-caprae* (137.0, 56.0 cm plant[-1] and 190.4, 64.50 g plant[-1]) the above optimum level drastically reduced the growth rate.

Halophytes show high potential to tolerate and accumulate metals in their tissues by triggering mechanisms for toxic metals detoxification with adequate compartmentation in the vacuole or in the cell wall and peptide detoxification with phytochelatins (Sousa *et al.,* 2008). The increase in biomass in excess of these metals might be due to high protein formation, resulting in the induction of photosynthesis, as well as increasing carbohydrate translocation. The reduction in fresh weight and dry weight due to heavy metal treatment may be attributed to the decreased metabolic rate and reduce transport from the cotyledons, at the same time it may also due to the higher rate of leakage in the membrane permeability (Venkatesan 2020; Sathya and Venkatesan, 2022). The photosynthetic pigment such as chlorophyll 'a' chlorophyll 'b' and total chlorophyll content increased up to optimum level of 25% in *Suaeda maritima*

(1.154, 0.486 and 1.648 mg g^{-1} fr. wt.) and *Sesuvium portulacastrum* (0.733, 0.406 and 1.139 mg g^{-1} fr. wt.) and 10% in *Ipomoae pes-caprae* (0.470, 0.366 and 0.836 mg g^{-1} fr. wt.) and thereafter reduced the pigment content (Fig. 2). Chlorophyll 'a' and 'b' and carotenoid are the main photosynthetic pigments and they play important role in photosynthesis. The changes in the amount of pigment were evaluated as the changes in photosynthesis. Changes of pigment content under salt stress are used as parameter for selection of tolerance and sensitive cultivars in crop plants (Eryilmaz, 2007). Thamayanthi and Sharavanan (2011) reported the phytoremediation of cadmium on growth and pigment contents of zinnia plants. Effect of mercury and cadmium on pigment content of pigeonpea (*Cajanus cajan*) reported by Aruna and Mohanty (2014).

The amino acid and sugar content of leaf, stem and root up to optimum level of 25% in *Suaeda maritima* (0.767, 0.305, 0.279 and 0.799, 0.410, 0.255 mg g^{-1} fr. wt.) and *Sesuvium portulacastrum* (0.807, 0.667, 0.210 and 0.811, 0.4810, 0.245 mg g^{-1} fr. wt.) and 10% in *Ipomoae pes-caprae* (0.776, 0.691, 0.400 and 0.814, 0.510, 0.341 mg g^{-1} fr. wt.) and above optimum level gradually increased the amino acid and sugar content up to extreme level of 100% (Figs. 3 and 4). The protein and starch content increased up to optimum level of 25% in *Suaeda maritima* (3.944, 2.445, 2.006 and 3.201, 2.007, 1.970 mg g^{-1} fr. wt.) *Sesuvium portulacastrum* (3.992, 2.446, 2.001 and 3.910, 3.226, 2.669 mg g^{-1} fr. wt.) and 10% *Ipomoe pes-caprae* (5.44, 3.104, 2.44 and 3.668, 2.644, 2.30 mg g^{-1} fr. wt.) and there after reduced drastically (Figs. 5 and 6).

Protein and amino acids are regarded to play a significant role in metal chelation, by which heavy metal detoxification and tolerance in plants take place (Hall, 2002). Proteins are hydrolyzed by proteases to releases to release amino acids for osmotic adjustment during NaCl stress in *Bruguiera parviflora* (Parida *et al.,* 2004). Similar trend of increase in protein content under saline conditions was observed in *Suaeda maritima* by Flowers and Hall (1978). In general, the protein content increased with increasing concentration up to an optimal level. Beyond the optimum level, the protein content deceased in *Ipomoea pes-caprae* (Venkatesan and Chellapan, 1998), *Ceriops roxburghiana* (Rajesh *et al.,* 1999), *Heleochola setulosa* (Joshi *et al.,* 2002), *Leptochloa fusca* (As hour and Mekki, 2006) and *Aster tripolium* (Geissler *et al.,* 2009).

However, reduction in protein and amino acid content was reported by influence of several heavy metals with some plants such as, Nagoor and Nyas (1999) in *Triticum aestivam*, Gardea-Torresdey *et al.* (2004) in *Convolvulus arvensis*, Romero-Puertas *et al.* (2007) in pea, Dinakar *et al.* (2008) in *Arachis hypogaea*, John *et al.* (2009) in *Brassica juncea* and Hamid *et al.* (2010) in *Phaseolus vulgaris*.

The proline content increased with increasing concentration up to extreme level of 100% of winery effluent (Fig. 7) of *Suaeda maritima* (0.340, 0.299 and 0.210 mg g^{-1} fr. wt.), *Sesuvium portulacastrum* (0.355, 0.310 and 0.224 mg g^{-1} fr. wt.) and *Ipomoea pes-caprae* (0.255, 0.204 and 0.150 mg g^{-1} fr. wt.). Proline is also believed to function as compatible solute in balancing cytoplasm and vacuolar water potentials (Ali *et al.,* 1998), to protect various enzymes in the cytoplasm (Lin and Kao, 1996). Proline the most common osmolyte, has been reported to accumulate in many salt marsh halophytes in parallel with increased external salinity and is considered a reliable biochemical marker of salt stress.

A marked increase in proline content was observed in present study, plants treated with up to 90% of winery effluents after 120 days of cultivation period when compared to control plants. The increase in proline content due to different stress, common observation. The accumulation of proline in plants is a general response to same abiotic stress (Jaleel and Azooz, 2009). But it is the fact that metal stress also increases the proline content, which has been meagerly reported in the literature. It may be argued that proline accumulation helps to conserve nitrogenous compounds and protect the plant against heavy metal stress. These results also support the view that proline acts as a membrane stabilizing agent under stress conditions (Poschenrieder and Barcelo, 2004; Manivasagaperumal *et al.,* 2011).

The considerable accumulation of proline as a result heavy metals stress has been reported by John *et al.* (2009) and Pant *et al.* (2011). This would be evident from the study of Zengin and Munzuroglu (2005) in cadmium; Backor *et al.* (2004) in copper; Wang *et al.* 2009) in mercury; Azooz *et al.* (2011) in zinc and lead Azooz *et al.* (2012) in copper and lead.

The increase in proline content in NaCl treated studies are in accordance with several studies that proline content progressively increased with increasing concentration of NaCl in *Ipomoea pes-caprae* (Venkatesan and Chellappan, 1998), Kochia prostrate (Karimi *et al.,* 2005), *Suaeda salsa* and *Limonium bicolor* (Liu *et al.,* 2008), *Avicennia marina* (Patel *et al.,* 2010), *Cynometra iripa* (Desai and Chavan 2011) and *Sesuvium portulacastrum* (Lokhande *et al.,* 2012). Proline function as an osmolyte, radical scavenger and cellular redox-potential buffer. Up-regulation of proline is often encountered in plants under heavy metal stress (Sharma and Dietz, 2009). Proline has significant beneficial functions under metal stress by three major actions, namely metal binding, antioxidant defense, and signaling. A large body of data suggested that proline accumulates in response to Cd, Cu and other heavy metals (Rastgoo and Alemzadeh, 2011 and Rastgoo *et al.,* 2014).

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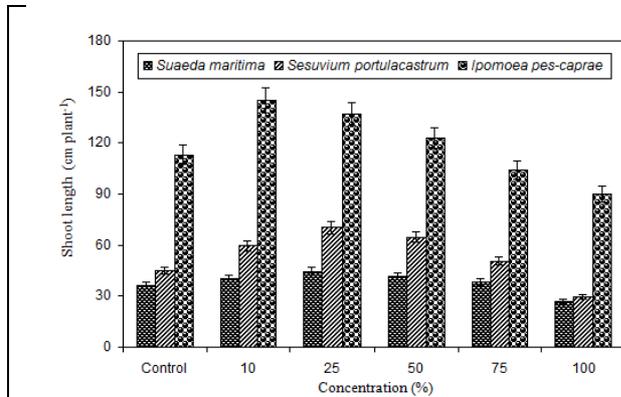
Fig. 1a. Effect of different concentrations of winery effluent on shoot length of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae* on 120th day after treatment
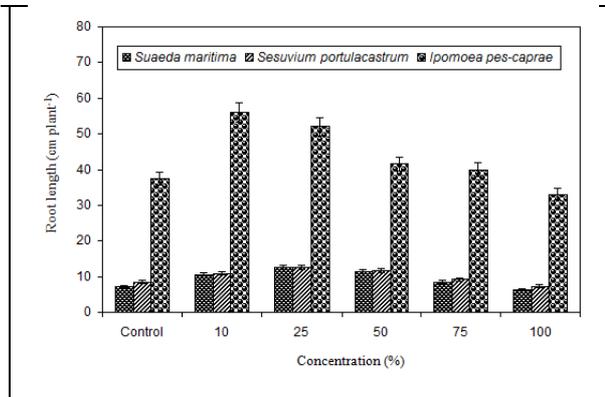
Fig. 1b. Effect of different concentrations of winery effluent on root length of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae* on 120th day after treatment
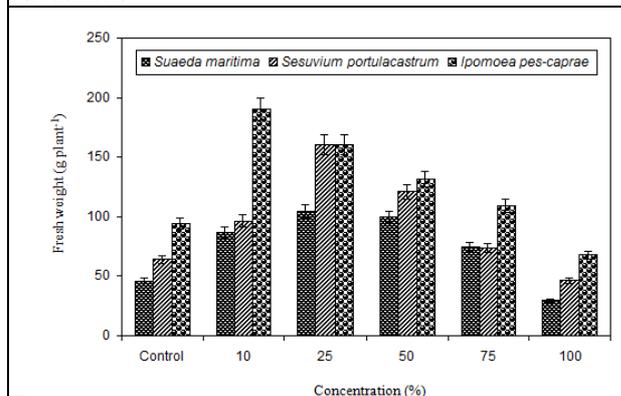
Fig. 1c. Effect of different concentrations of winery effluent on fresh weight of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae* on 120th day after treatment
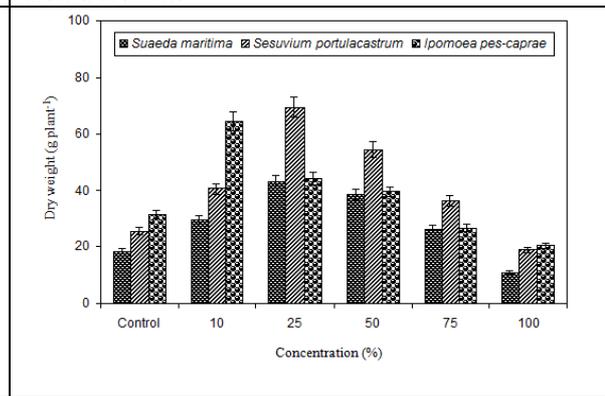
Fig. 1d. Effect of different concentrations of winery effluent on dry weight of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae* on 120th day after treatment
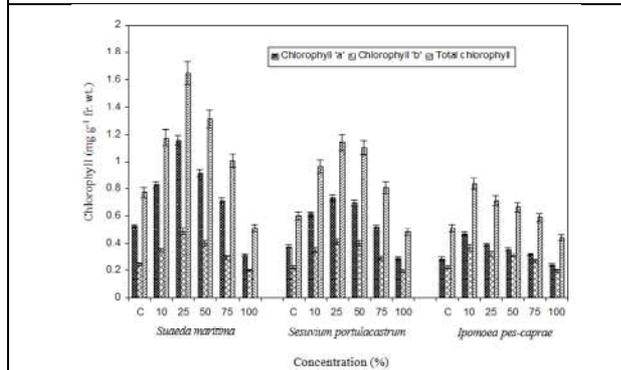
Fig. 2. Effect of different concentration of winery effluent on chlorophyll content (mg g⁻¹ fr. wt.) of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae*
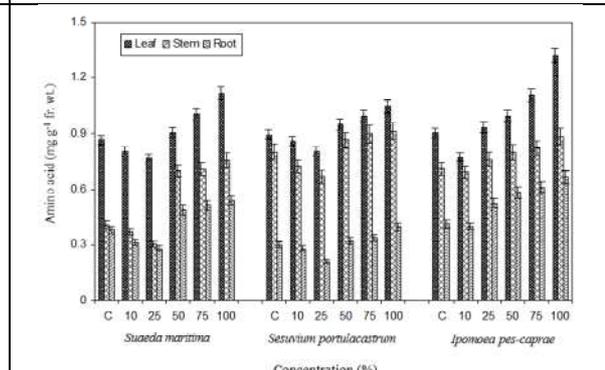
Fig. 3. Effect of different concentration of winery effluent on amino acid content (mg g⁻¹ fr. wt.) of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes caprae*
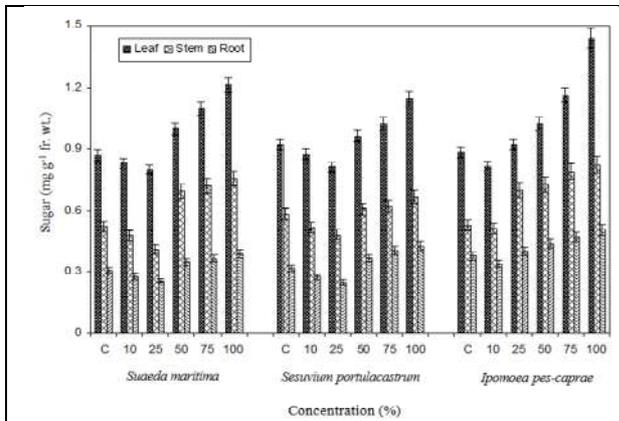
Fig. 4. Effect of different concentration of winery effluent on sugar content (mg g⁻¹ fr. wt.) of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae*
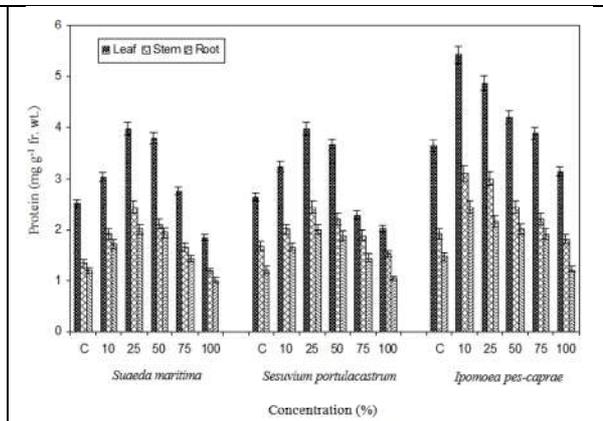
Fig. 5. Effect of different concentration of winery effluent on protein content (mg g⁻¹ fr. wt.) of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae*
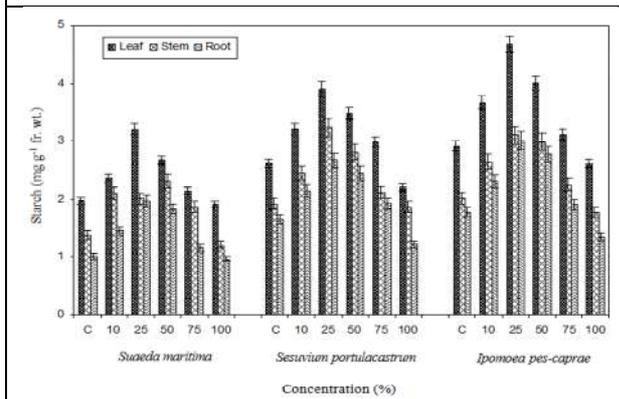
Fig. 6. Effect of different concentration of winery effluent on starch content (mg g⁻¹ fr. wt.) of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae*
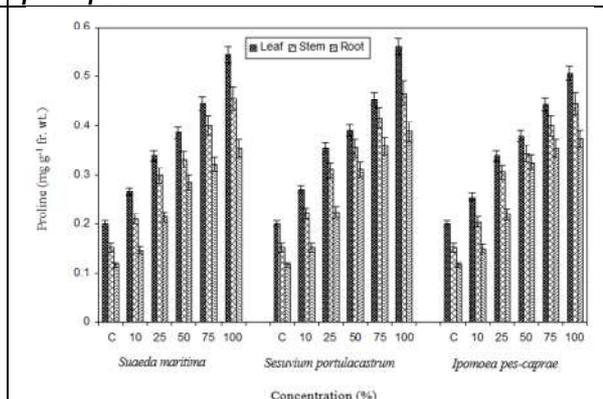
Fig. 7. Effect of different concentration of winery effluent on proline content (mg g⁻¹ fr. wt.) of *Suaeda maritima*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae*

RESEARCH ARTICLE

Studies on Antioxidative Properties of *Nyctanthes arbor-tristis* – A Possible Therapeutic Repository of Natural Antioxidants

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ABSTRACT

This study was undertaken to explore the antioxidant properties of various parts of *Nyctanthes arbor-tristis* L. (NA). The plant parts studied included leaves, stem, flowers and fruits. The ethanolic extracts were tested for their free radical quenching potential and their reduction potential. The presence of antioxidant enzyme in various plant parts was studied in aqueous extracts. Results showed that the ethanolic extracts of stem and flowers of *Nyctanthes arbor-tristis* demonstrated higher radical scavenging activity at majority of the concentrations used when compared to leaf and fruit extracts. The stem and flower extracts also showed higher reduction potential when compared to that of leaves and fruits. Among the several antioxidant enzymes studied ascorbate peroxidase was present in highest concentrations in various parts of *N. arbor* followed by catalase which was followed by polyphenol oxidase, peroxidase and superoxide dismutase in the extracts.

Keywords: *Nyctanthes arbor-tristis*; free radicals; antioxidants; antioxidant enzymes; radical scavenging activity, Pearson's correlation

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INTRODUCTION

Antioxidants are low concentrated substances, which slow down or inhibit the generation of free radicals [1]. As a result of normal aerobic cellular metabolic processes, reactive oxygen species (ROS) and reactive nitrogen species are generated. Free radicals are responsible for various pathological processes like aging, DNA damage, cancer, strokes, cardiac and central nervous system (CNS) diseases etc.[2][3]. The body protects itself from free radicals by endogenous antioxidants like glutathione, uric acid and antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase [4]. Medicinal plants are the source of wide variety of chemical constituents which possess free radical scavenging activities. These are popularly known as phytochemicals. Different categories of phytochemicals reported in plants include phenolic (flavonoids, tannins etc.), nitrogen compounds (alkaloids, betalains etc.), carotenoids, vitamins and other secondary metabolites found to have antioxidant activity [5].

Nyctanthes arbor-tristis L. (Oleaceae) (NA), commonly known as night-flowering jasmine or 'Harshingar', belongs to the Oleaceae family. It is used in various medicinal systems such as Ayurveda, Siddha, and Unani systems[6]. The whole plant as well as its parts has been used for the treatment of sciatica, arthritis, malaria, enlargement of spleen and as blood purifier [6]. Leaves of *N. arbor* have been reported to possess numerous pharmacological activities such as anti-allergic, anti-inflammatory, analgesic, antipyretic, antimalarial, leishmanicidal, anthelmintic activities. The bark of this plant is used to treat the bronchitis and snakebite[7]. Seeds, leaves and flowers exhibit hepatoprotective, immunostimulant, antiviral, antifungal, antileishmanial properties[8]. The present study was conducted to investigate the possibility of scavenging ROS and the presence of antioxidant enzymes in extracts from leaf, flower, stem, and fruits of *Nyctanthes arbor-tristis* Linn (NA).

MATERIAL AND METHODS

MATERIALS
Chemicals and reagents
All the reagents and chemicals used in the present study were of analytical grade with high purity.

Plant collection and authentication
Fresh leaves, stem, flowers and fruits were collected in the early morning (collection of flowers and fruits were in their peak seasonal period) from Jolva village of South Gujarat zone (Lat, Long 21.1719896, 73.0003919). The collected plant materials were botanically authenticated (Voucher No-VNSGU/2019/09/TC-01) by Dr. Farzin M. Parabia (Botanist and Associate professor) from Department of Biosciences, VNSGU, Surat, Gujarat, India.

METHODS
Preparation of extract for non-enzymatic antioxidant assays
Fresh plant material i.e., fruits, flowers, stem and leaves were washed thoroughly with water, oven dried & blended to fine powder. The samples thus obtained were subjected to modified Soxhlet extraction with ethanol[9]. Extracts were concentrated using rotary vacuum evaporator and left-over solvent was evaporated to dryness. By dissolving the known quantity of ethanol, Stock solution of plant extracts were prepared.

Non-enzymatic antioxidant assays
Three different assays were carried out viz ABTS-2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging assay, FRAP-ferric reducing antioxidant power assay and CUPRAC-cupric ions reducing assay. Ethanolic extracts of different parts such as leaf, stem, flower and fruit of NA were compared for their radical scavenging activity (RSA) and reducing property.

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a)   ABTS-2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging assay

This radical scavenging assay was performed according to Re et al., 1999 method with few changes [10]. By taking equal volume (10 ml) of 7 mM ABTS$^{.}$ solution with 2.4 mM potassium persulfate solution, stock solution of ABTS$^{.}$ was prepared and then left this mixture in dark for 16 hours at room temperature. To obtain an absorbance of (0.70±0.02) at 734 nm, 1 ml of ABTS solution was diluted with distilled water. Various concentrations (0.02 – 0.2 mg/ml) were prepared for different plant extracts. Aliquots of plant extracts were mixed with 2 ml of ABTS$^{.}$ and the ABTS$^{.}$ scavenging effect of antioxidants was measured at 734 nm against blank. The comparison of ABTS$^{.*}$ scavenging capacity of the extracts was done with a standard viz., butylated hydroxytoluene (BHT). The declining in the intensity of blue color of ABTS$^{.*}$to colorless solution recommend the scavenging potential of different plant extracts. The inhibition of ABTS$^{.}$ radical cation scavenging activity percentage was calculated using following formula:

ABTS$^{.}$ radical scavenging activity (%)=A_c–A_t/A_c×100   Eq. (1)
Where, A_c=absorbance of the control and
          A_t=absorbance of test sample.
          IC_{50} value of each extract was determined from their graph to determine their effective concentrations.

FRAP-Ferric Reducing Antioxidant Power

Total ROS quenching potential of the different ethanolic extracts was compared using ferric reducing antioxidant power assay as per the method described by Benzie and Strain, 1996 with a slight modification[11]. 1 ml of ethanolic extract (1mg/ml) was mixed with 2.5 ml volume of 0.2mM PBS (pH 7.4) and 1% $K_3[Fe(CN)_6]$ respectively. This reaction mixture was incubated at 50°C for 20 min. To this, addition of 2.5 ml of 10% TCA (trichloroacetic acid) was done and then centrifuged at 3000 rpm for 10 min. 2.5 ml of distilled water and 0.5 ml of 1% ferric chloride was added. The measurement of absorbance was done at 700 nm against blank prepared (1 ml of ethanol instead of extracts in the reaction mixture). In this assay, Ascorbic acid was used as a standard. Increase in absorbance at 700 nm shows measure of antioxidant activity. Results of FRAP assay expressed in mg of ascorbic acid equivalents (mg AAE)/gm dry weight of extract.

Cupric reducing antioxidant capacity (CUPRAC)

Reducing ability of Cupric ions present in ethanolic extracts of NA leaves, stem, flowers and fruits was determined by the method of Apak *et al.,* 2008[12].1 ml of 1mg/ml extracts were mixed with 1ml 0.01M CuCl2.2H2O, 1 ml 7.5 mM Neocuproine reagent, 1 ml 1 M ammonium acetate. Resulting reaction mixture was incubated for 30 minutes at room temperature. The absorbance of reaction mixture was measured at 450 nm against blank. Gallic acid was used as standard. The increase in absorbance at 450 nm indicated the reducing capacity of the extracts. Results of CUPRAC assays were expressed in mg of gallic acid equivalents (mg GAE)/gm dry weight of extract.

Extract Preparation for Antioxidant Enzymatic assays

Fresh plant tissues (2gm) were weighed, washed and dried on blotting sheet. Plant materials were immediately transferred on ice to prevent from proteolytic activity. 5 ml of extraction medium (0.1 M phosphate buffer, pH 7.5 containing 0.5 mM EDTA and 2% polyvinylpyrrolidone) was used to homogenize the tissue in mortar and pestle at 4°C. Resulting homogenates were centrifuged at 10,000rpm and at 4°C for 30 minutes. The supernatants were used for the assay of antioxidant enzymes.

Enzymatic Antioxidant assays
SOD-Superoxide dismutase

Superoxide dismutase was determined by the method of Dhindsa *et al.,* 1981 [13].3 ml of reaction mixture was prepared containing 100 µl 1.5 M sodium carbonate, 200 µl 0.2 M L-methionine, 100 µl 2.25 mM nitrobluetertazolium, 100 µl 3 mM EDTA, 1.5 ml 0.1 M potassium phosphate buffer and 1 ml distilled water. 100 µl of crude enzyme extract was added to the reaction mixture and control contained 100 µl potassium-phosphate buffer. The reaction was started by addition of 100µl of riboflavin and placing the tubes containing reaction mixture

to the white light source of 15 W for 15 min. The reaction was terminated by switching off the light and placing the tubes in dark. Absorbance was recorded at 560 nm using Schimadzu (UV-1800) spectrophotometer and enzyme activity calculated. One unit (1U) of SOD activity was interpreted as amount of enzyme required to cause 50 % inhibition of nitrobluetertazolium. Specific activity was recorded as U mg^{-1} of protein.

CAT-Catalase

Catalase activity was determined according to the method represented by Hugo Aebi, 1984[14]. The reaction mixture contained 3960 µl of phosphate buffer (pH 7.4), 20 µl of enzyme extract and 20 µl 30 % H_2O_2. In control H_2O_2 was replaced by water. Absorbance of test reaction was recorded for 5 min at every 30 sec. intervals at 240 nm. Catalase enzyme specific activity was calculated using following formula and result was expressed as U mg^{-1} of protein.

CAT specific activity = ([Test – Control] ×Total reaction volume×10^3)/(Σ × Δt × mg of protein)   Eq. (2)

Where, Σ = 39.4 mol^{-1}cm^{-1} at 240 nm, Δt = 30 sec.

APX-Ascorbate peroxidase

Ascorbate peroxidase activity was estimated As per the protocol described byNakano and Asada, 1981[15].The test reaction mixture contained 2.898 ml phosphate buffer (pH 7.4), 0.6 µl EDTA, 1.5 µl ascorbate, 50 µl 30% H_2O_2 and 50 µl of crude enzyme extract. The control was prepared without crude enzyme extract. Absorbance was recorded at 290 nm for 5 min at every 30 sec intervals. Ascorbate peroxidase specific activity was calculated using the formula given below and result was expressed as U mg^{-1} of protein.

APX specific activity = ([Test – Control] ×Total reaction volume ×103)/ (Σ × Δt × mg of protein)   Eq. (3)

Where, Σ = 2.8 mol-1cm-1 at 290 nm and Δt = 30 sec.

POX-Peroxidase

Peroxidase activity was measured by an increase in absorption due to oxidation of pyragallol by H_2O_2 reaction driven by peroxidase according to the method described by Nagavani and Rao., 2010 with few modifications [16]. The test reaction mixture contained 3 ml pyragallol solution and 200 µl crude extract whereas control was prepared without enzyme extract. The reaction was started by adding 15 µl 30 % H_2O_2. Increase in absorbance was recorded at 470 nm for 5 min at every 30 sec intervals. Peroxidase specific activity was calculated using given formula and result was expressed as U mg-1.

POX specific activity = [Test – Control] × Total reaction volume×103/ (Σ × Δt × mg of protein)   Eq. (4)

Where, Σ = 22.6 mol-1m-1 at 470 nm and Δt = 30 sec.

GR-Glutathione Reductase

GR assay performed with few modifications according to the method described byMannervik, 1985 [17].3 ml of reaction mixture contained 1.5 ml 0.1 M potassium phosphate buffer, 100 µl 30 mM oxidized glutathione, 350 µl 0.8 mM NADPH, 300 µl 1% W/V BSA and 100 µl crude enzyme extract. The decrease in absorbance of test recorded after adding enzyme extract at 340 nm for 5 min at every 30 sec intervals. Control was prepared without crude enzyme extract. Glutathione reductase activity determined by following formula and result was expressed as U mg^{-1}.
GR specific activity = ([Test – Control] × Total reaction volume ×103)/ (Σ × Volume of enzyme extract (0.1 ml) × mg of protein)   Eq. (5)

Where, Σ = 6.22 mol-1cm-1 at 340 nm

PPO-Polyphenol oxidase (PPO)

Polyphenol oxidase activity estimated using catechol as substrate using Zauberman et al., 1991 method with few modifications [18]. Test reaction mixture contained 1.5 ml 0.1 M phosphate buffer and 0.2 ml crude enzyme extract. Reaction was started by adding 10 µl 40 mM catechol solution and increase in absorbance was recorded using spectrophotometer at 412 nm for 5 minutes at an interval of 30 seconds. One unit (U) of enzyme activity was defined as 0.001 change in absorbance of 0.001 per minute. PPO activity calculated using following formula and result was expressed as U mg-1 of protein.

PPO activity = [Abs. of Test/min – Abs. of Control/min]×Total reaction volume/(0.001)   Eq. (6)

Determination of protein

Protein concentrations were found using method of Lowry *et al.* 1951 where bovine serum albumin (BSA) was taken as the standard [20].

Statistical Analysis

To verify the statistical significance, all the experiments were conducted in triplicates. Results were expressed as mean ± standard deviation (SD) using Excel. The values were considered significant at $*p<0.05$ compared to standard using one-way ANOVA followed by Tukey's post hoc analysis. Normal distribution was checked via Shapiro Wink's test and as it was found to be normally distributed. Linear correlation between total phenolic, flavonoid, alkaloid contents and different antioxidant assays was calculated using Pearson's correlation method. This correlation was established with the help of SPSS 20.0 version. Rank analysis was performed for antioxidant enzymes using excel. The p values <0.05 were considered significant.

RESULTS

Estimation of Non-enzymatic Antioxidant activity

The calibration curve for ABTS assay showed the linearity of BHT is in the range of 25 – 200 µg/mlwith a correlation coefficient (R^2) of 0.99 (Fig. 1A). The IC_{50}value is that concentration of extracts that inhibits 50% of the free radical. Fig. 1B shows the free radical scavenging activity (IC_{50}) in ethanolic extract from different Stem and flower extracts of NA exhibited higher RSA at majority of the concentrations used when compared to leaf and fruit extracts. The reducing power of a potential of various extracts were measured by FRAP assay which reduces the Fe^{3+} (ferric ion) to the Fe^{2+} (ferrous ion) resulting in a deep blue complex. The calibration curve showed the linearity of standard ascorbic acid in the range of 0.1 – 1.0 mg/mlwith a correlation coefficient (R^2) of 0.99 (Fig. 2A). The highest antioxidant potential was exhibited by stem extract > flower extract > leaf extract > fruit extract as shown in Fig. 2B. The FRAP activity of various plant extracts were significantly lower ($p < 0.05$) when compared to standard viz., ascorbic acid.

The calibration curve showed the linearity of standard gallic acid in the range of 0.1–1.0 mg/mlwith a correlation coefficient (R^2) of 0.99 (Fig. 3A). The ability to reduce cupric ions was significantly higher ($p > 0.001$) in flower extract followed by leaf, stem and fruits extracts when compared to that of gallic acid which was used as a standard (Fig. 3B).

Antioxidant enzymes present in extracts from different parts of Nyctanthes arbor-tristis

Rank analysis of the antioxidant enzymes studied placed ascorbate peroxidase and catalase in 1st and 2nd rank respectively which indicates that the presence of these two enzymes in maximum amounts in different parts of the plant. These were followed by polyphenol oxidase, peroxidase, glutathione reductase and superoxide dismutase. Ascorbate peroxidase was highest in leaves > stem > flowers > fruits. Catalase activity was highest in flower extracts followed by leaves > stem > fruits. Polyphenol oxidase was found highest in flowers> stem > leaves > fruit.

Peroxidase activity found highest in stem > leaves > fruits > flower. Highest glutathione reductase activity was detected in leaves followed by stem > flowers > fruits. Superoxide dismutase was detected only in flowers and stem (Fig. 4).

Correlation between major secondary metabolites and antioxidants

In order to establish correlation (r) between secondary metabolites and antioxidants of *N. arbor*, Pearson's correlation analysis was done. The correlation coefficient varied among different antioxidant assays. Comparatively very high linear positive relationship (highlighted values) observed between the CUPRAC activity with phytochemicals. In this study negligible correlations were observed for secondary metabolites with Ferric ion reducing power assay (FRAP) whereas moderate negative correlation observed for plant phytochemicals with ABTS assay (Table 2). These data suggest that cupric ion reducing capacity was because of phytochemicals present in *N. arbor* extracts.

DISCUSSION

Plants and plant parts comprises diverse group of compounds like polyphenols (phenolic acids, flavonoids, lignans, anthocyanins and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin C and E) which act as natural antioxidants [21][22].The polyphenols and carotenoids are reported to have antibacterial, antiviral, anti-aging, anti-inflammatory, and anticancer properties[23][22]. Therefore, phytochemical analysis of naturally occurring compounds in plants may help to identify these plants as potential sources of natural antioxidants. Phytochemical analysis of plants also contributes to the development of antioxidant drugs[24]. Synthetic phenolic antioxidants like butylated hydroxyanisole (BHA), are commonly used antioxidants[25]but due to its carcinogenic and environmental effects made an alarming concern in its use[26]. In the present study extracts from the leaves, stem, flowers and fruits of *Nyctanthes arbor-tristis* (NA) were screened for their free radical scavenging activity and the presence of antioxidant enzymes. The antioxidant activities of different parts of *Nyctanthes arbor-tristis* is were compared to that of BHA which was used as a standard antioxidant. All the parts of the plants studied viz., leaves, stem, flowers and fruit exhibited free radical scavenging activity. The free radical scavenging activity varied in different parts.

Three antioxidant assays were used to evaluate the extracts of Nyctanthes arbor-tristis is viz., ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant power (CUPRAC), which measured the reducing power of the extracts while the (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid (ABTS) assay measured the free radical quenching capacity of the extracts. ABTS.+assay is used as an index antioxidant activity[27]. Stem and flower extracts of Nyctanthes arbor-tristis exhibited the highest RSA. Similar results have been reported in studies with Grewia carpinifolia[28]. The results of FRAP assay were similar to that of ABTS assay. FRAP assay was performed to measure reducing potential of an antioxidant present in the plant extracts. Antioxidants react with a ferric tripyridyltriazine (Fe3+-TPTZ) complex to create a colored due to ferrous tripyridyltriazine (Fe2+-TPTZ)[11]. Generally, the reducing properties are attributed to antioxidant compounds which exert their action by spliting of reactive free radical chain via a hydrogen atom donation[29]. The flower and the stem extracts showed higher reducing capacity in the CUPRAC assay. The flowers of NA have been shown to exert sedative effects on rats which have been attributed to their antioxidant properties[30]. The antioxidant activity of the extracts of NA may be attributed to their phenolic content. Our previous study has demonstrated the existance of phenols, flavonoids and alkaloids in the leaf, stem, flower and fruit extracts of NA[31]. Flower and stem extracts were reported to possess higher phenols, flavonoids and alkaloids which explains their higher reducing capacity (as observed by FRAP and CUPRAC assay) observed in this study. The antioxidant power of phenolics is because of its reducing property which make them hydrogen donors and singlet oxygen quenchers[32]. A positive correlation was noticed between the phytochemicals and CUPRAC activity of the ethanolic extracts (Table 2). The oxygen radical scavenging potential in the leaf extract has been attributed to the presence of highly branched polysaccharide containing esterified phenolic acids[33].

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The antioxidant enzymes viz., SOD, CAT, glutathione peroxidase and glutathione reductase are the front line of defense against the free radicals. They inhibit the generation of free radicals by dissipating the reactive oxygen species[4]. Peroxidases are ubiquitously distributed antioxidant enzymes that catalyze oxidation of numerous electron donor substrates concomitant with the breakdown of hydrogen peroxide. The non-animal plant peroxidases (class III peroxidase) are involved in various crucial physiological processes of plant such as growth and development throughout their life cycle[34]. The peroxidase activity was highest in the stem and leaves and lowest in the flowers. The high peroxidase activity in the leaves may attribute to increased generation of O_2^- during photosynthesis[35].

In this study ascorbate peroxidase and catalase were the two antioxidant enzymes found in higher concentration than other enzymes in various extracts. Catalases and ascorbate peroxidases dissipate H_2O_2 and thereby protecting the plants from its deleterious effects. Catalase is found in peroxisomes and does not need a reductant to break down H_2O_2. The affinity of APX is higher for H_2O_2 and reduces it to H_2O using ascorbate as an electron donor. It is present in the cytosol, chloroplasts, mitochondria, peroxisomes and apoplastic space. They help the plant is stress adaptation[36]. The highest APX activity in the leaf extracts of NA may help to breakdown the reactive oxygen species (ROS) generated during photosynthesis. Glutathione reductase (GR) is a flavo-protein oxido-reductase which reduces oxidized glutathione (GSSG) to its reduced form (GSH). This enzyme recruits NADPH as a reductant. Leaves of NA exhibited the highest GR activity which may be due to its localization in the chloroplast.

Almost all the parts of NA expressed free radical scavenging activity and the presence antioxidant enzymes. These results agree with the results of earlier studies of Khanapur *et al.,* 2014 [37].The enzyme polyphenol oxidase followed APX and CAT in its RANK distribution, which is copper holding enzyme in plants. It is also known as a responsible factor for the enzymatic browning of fruits. However, it has also been reported to possess antioxidant property which depends on its phenolic content[38][39]. SOD was the enzyme which was detected in least amount. In this study SOD was detected only in the stem and flower extracts of NA. Plants are known to have three is forms of SOD viz. copper/zinc, manganese and iron SOD[40]. SODs bring about the dissipation of super oxide anion to molecular oxygen and H_2O_2. The absence of detectable SODs in the leaves and fruits may be attributed either to their inhibition by superoxide anions[41]generated in the chloroplasts or interference by peroxidases [42]. It can be said that *Nyctanthes arbor-tristis* parts exhibited free radical quenching potential and significant antioxidant enzyme activities which could be attributed to their phenolic content. Therefore, this plant may be a potential candidate for the natural antioxidant which may be used in ameliorating diseases involving free radicals.

CONCLUSION

Nyctanthes arbor-tristis is one of the medicinal plants with antioxidant properties and antioxidant enzymes which makes it a potential candidate for natural antioxidants.

ACKNOWLEDGEMENTS

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Table 1: Comprehensive analysis of Antioxidant enzyme activities of *N. arbor*.

Enzymatic Assay	Leaf	Stem	Flower	Fruit
SOD	-	1.515±0.035	3.025±0.586	-
CAT	97.835±0.049	75.355±3.684	115.425±2.877	.47±1.03263
POX	16±0.509	16.305±0.346	5.41±0.014	13.605±0.007
APX	309.94±1.781	82.03±0.169	56.665±5.621	89.115±0.601
GR	10.115±0.049	9.82±0.311	0.865±0.091	0.74±0.509
PPO	21.33±2.828	22.625±4.094	26.475±2.085	5.56±2.149

Table 2: Simple Linear regression analysis of secondary metabolites with antioxidant assays

Correlations		TPC	TFC	TAC	ABTS IC_{50} values	FRAP values	CUPRAC values
TPC	Pearson Correlation	1	.679	.880	-.350	-.143	.908*
	Sig. (2-tailed)		.321	.120	.650	.857	.042
	N	4	4	4	4	4	4
TFC	Pearson Correlation	.679	1	.910	-.621	.300	.914*
	Sig. (2-tailed)	.321		.090	.379	.700	.046
	N	4	4	4	4	4	4
TAC	Pearson Correlation	.880	.910	1	-.694	.279	.948*
	Sig. (2-tailed)	.120	.090		.306	.721	.042
	N	4	4	4	4	4	4

*$P < 0.05$, TPC-Total phenolics content, TFC-Total flavonoids content, TAC-Total alkaloids content

Figure 1A: Standard graph for ABTS assay using BHT as a control	Figure 1B: The graphical comparison between IC_{50} values of different extracts of *N. arbor* compared to control (BHT). *$p<0.05$ and **$p<0.01$ with respect to standard BHT using One-way ANOVA test. ns- non-significant.

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Figure 2A: Standard graph for FRAP assay using Ascorbic acid as a control

Figure 2B: The graphical representation of Ferric ion reducing power assay of crude ethanolic and alkaloid extracts of *N. arbor* parts

Figure 3A: Standard graph for CUPRAC assay using Gallic acid as a control

Figure 3B: The graphical representation of Cupric ion reducing capacity assay of crude ethanolic and alkaloid extracts of *N. arbor* parts

Fig. 4: Graphical representation of different antioxidant enzymes in different extracts of *N. arbor* parts.

RESEARCH ARTICLE

Opportunities and Satisfaction Level of Studies in Aquaculture and Related Subjects

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ABSTRACT

The survey study was focused on student's response on subject matter, opportunity and scope of studies in Aquaculture and allied subjects. Aquaculture is fast growing sector and contributes large portion of country's revenue. It is a largest raised area for the job seekers with the degree and skill in aquaculture and allied subjects. The survey was carried out online through structured Google form. Among the total participated respondents 96% were from Department of Aquatic Biology, Veer Narmad South Gujarat University, Surat, Gujarat, India. Other 4% respondent were from other institutes. Among all the respondents involved in the survey 56.3% were male and 48.7% were females. Majority of the respondents revealed that they were aware about the scope and employment opportunities of the program they are enrolled with. Respondents showed overall satisfaction with their course content. The respondents suggested their opinions for progression of the existing course content to compete with advancing Aquaculture sector. The study can be useful for all those who are concerned with aquaculture and allied subjects. The survey is also noteworthy for its focus on results and retention of students enrolled in respective programs. The data and suggestions provided by the respondents would be useful to plan future actions regarding course content in this field. The obtained conclusion from the survey may provide information about actions that can be taken to maintain high level of satisfaction and improve student learning.

Keywords: Aquaculture, Study, Student, Teaching, Learning

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INTRODUCTION

The process of teaching and learning is the education. It is important to gain knowledge and skill through the process of education and the success depends on the extent of quality provided during process of education. The concept of quality in context of education implies multifarious aspects [1].Student outcomes, student retention, attrition, and graduate rates are some of the key measures of the quality and overall effectiveness of the higher educational institution[2,3]. Content that is reflected in relevant curricula and materials for the acquisition of basic skills, especially in the areas of literacy, numeracy and skills for life [4]. Mazumder explored the comparison and contrast the quality of higher education in public and private universities of Bangladesh, a study was conducted, to evaluate student satisfaction in institutions [5]. The administration of any program is always concerned with the successful running of it. Delivery with quality is prime focus for the ultimate success of any program[6].Studies have shown student satisfaction to have a positive impact on student motivation, student retention, recruiting efforts and fundraising[7,8].The purpose of this study was to identify aspects of the educational experience that are associated with students' overall expression of satisfaction and determining which features of the student experience are most closely related to satisfaction. The respondents during the survey rated many aspects of their educational experience, from skill development and personal growth to the quality of curriculum and job opportunities. Survey asked students to make an overall statement about their satisfaction with their studies. The study may provide information about actions that can be taken to maintain high levels of satisfaction and improve student learning.

METHODOLOGY

Survey was carried out using structured digital questionnaire through Google form to collect the relevant information from the students who had passed out and from those who are undertaking the course related to Aquaculture science. The responses received were processed component wise to evaluate the feedbacks received to draw the conclusions.

RESULTS AND DISCUSSION

 Among the total participated respondents 96% were from Department of Aquatic Biology, Veer Narmada South Gujarat University, Surat, Gujarat. Other 4% respondent were from other institutes. Among all the respondents involved in the survey 56.3% were male and 48.7% were females(Fig.1).95.8% revealed that they were aware about the future scope of their studied field whereas 4.2% agreed that they were not aware about the scope of their courses(Fig.2).The respondents revealed that the reasons for opting the degree in Aquatic biology was to explore the potential in aquaculture field, work as a researcher in aquatic science, engage in culture of aquatic species as well as they also showed interest to work as a technician in aqua farms.79.2% of the respondents replied that they were knowing about the demand of aquatic biologist in Aquaculture sector and willing to take career in this sector in different areas(Fig.3 &fig.4) . With respect to total respondents, 95.8% respondents felt that adequate number of hands on training /skill development program is required for strong candidature in this sector(Fig.5).52.1% of the respondents availed some types of skill development training in their studied field so as to overcome difficulties during their career period(Fig.6) . The training undertaken by the respondents were Viz,., technical aspect of grow out culture of *L. Vannamei*, training of aquaculture farming at farm sites, training at shrimp culture grow out ponds, training in processing plants, in sea food industry , training regarding Quality Control& Quality Auditor .Some of them had attended Aqua Farmer Awareness Program.89.6% of the surveyed respondents agreed that their subject matter knowledge can uplift aquaculture sector during divesting situation like climate change and other vulnerability(Fig.7).89.4 % of the respondents who took their education as Aquatic Biologist were satisfied with their existing course and rest 14.6% replied various reasons for dissatisfaction. The reasons were not as much of practical knowledge, lacking numbers of field visits and educational tours so as to increase their academic exposures. They revealed that practical's should be field oriented and exposed to advanced technology applied in the aquaculture

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sector. Sinha indicated that in competitive market its very important to retain good employees, that contribute towards the attainment of Organizational goal and customer satisfaction as well [9].

The respondents suggested their views for upgrading the existing course content to compete with advancing Aquaculture sector. The suggestions can be enumerated as:

- Students need to get more practical knowledge. It makes feel more positive and curious when they see the things and process what they learned in theories.
- Syllabus should be equivalent to Aquaculture sector of ICAR
- More practical aspect than theories
- Need intensive field work
- Internship period should maximum of three to four months or one crop of farming
- Training facilities regarding aquaculture and farm should be included at institution.
- Entrepreneurs and startup should be discussed
- Institute must review of their pass out batches students, to incorporate their experiences
- Increase in number of campus placement
- Placement cell can work actively for the guidance for employment
- A Good Mentorship and Proper Funding for research

CONCLUSIONS

Analysis of the survey data revealed that majority of the students showed highly positive attitude towards course content of the studies in Aquaculture and allied subjects. They revealed that skill development training and field-oriented syllabus is essential for strong candidature in this sector for holding satisfied career. Respondents exposed that farm-oriented practical are essentials for correlating theories with practical concepts.

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Fig.1 Gender composition of respondents

Fig.2:Awarness about scope of course

Fig.3: Awarness about employment opportunityin Aquaculture sector

Fig.4: Area of interest as career option

Fig.5:Prerequisite of training and skill

Fig.6:Respondent's with training

Fig.7: Application of the studied course

Fig. 8:Overall satisfaction of the subject content

RESEARCH ARTICLE
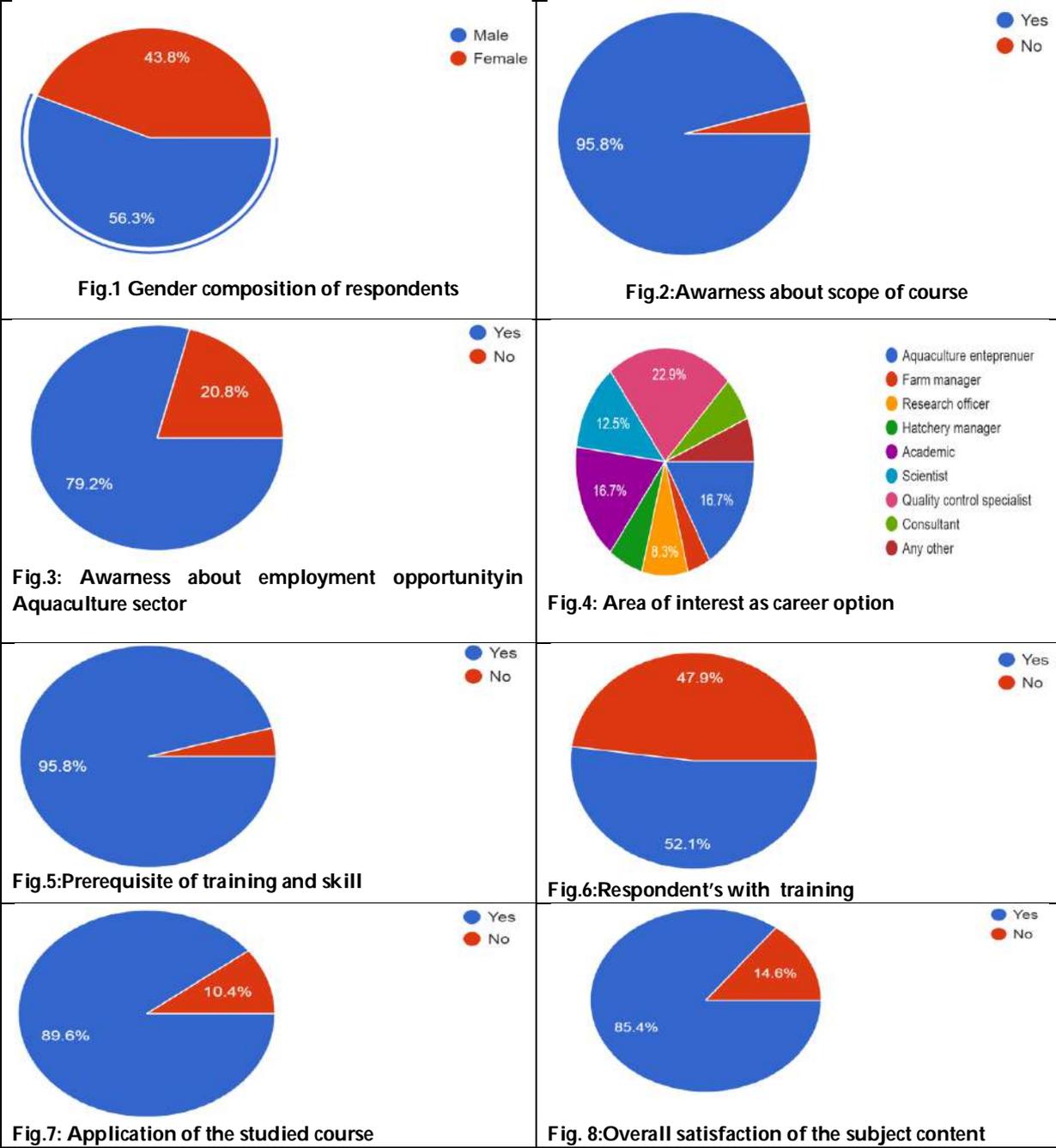
A Framework for eHealth Care Based on Medical IoT

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ABSTRACT

There has been a lot of research into healthcare services (HS) and technological breakthroughs during the last ten years. In recent times due to covid pandemic people are afraid to visit hospitals. They are expecting virtual help to address their issues. This can be taken as highest priority. Patient safety has improved, prices of expenditure towards health care have decreased, health services have become more accessible, and operational efficiency in the healthcare business has grown.The present article provides an up-to- date assessment of IoT (HIoT)-based technologies' prospective healthcare applications. From the viewpoints of enabling technology, HS, and applications, this article evaluates the evolution of the use of the HIoT in tackling different healthcare concerns. Future issues and concerns with HIoT systems are also explored. Finally, the present research aims to give a comprehensive source of information on the numerous areas of application of HIoT by giving a thorough source of information on the many industries in which it is used.

Keywords: HIOT, WMSN, IOMT

INTRODUCTION

The benefits of today's rapidly increasing technology and innovation must be embraced to enhance access to great health care services for individuals in local health institutions. Health care delivery is the administration and delivery of health services to access a wide range of prevention and therapeutic services, according to individual needs, across time and at different levels of the health system. Quality health care services are essential for increasing everyone's quality of life, gaining timely access to the health-care system, preventing diseases and disabilities, diagnosing and treating health issues, and minimizing deaths caused by negligence.[1][2] Health-care service availability, resources, timeliness, and personnel are all aspects to consider. This article focuses on patient healthcare, which includes the use of IoT and e-Health technologies to improve the health and well-being of patients. The Internet of Things (IoT) has arisen as one of the most recent innovations in information and communication technology, and it has had a big impact when paired with health services, particularly e-Health. IoT technology connects the Internet to a variety of products in our daily lives, such as sensors and operating systems (medical devices, household appliances, and so on), and this has revolutionized the way we interact with our surroundings, thanks to their communication and processing capabilities. Various access networks, powered by technologies like RFID, wireless sensor networks, embedded, real-time, and semantic web services, are expected to connect billions of sensors and actuators to the Internet. IoT is used in smart cities, home surveillance and automation, health care, productivity, energy and resources, smart grid, smart transportation system, and traffic management.

The implementation of this new technology, IoT, in health care services brings comfort to patients and doctors because of its multiple systems, such as real-time monitoring, patient information management system, and health management system. Medical equipment, such as portable devices, may be linked to IoT technology for remote monitoring, real- time monitoring, and e Health consultations. Everyone and everything is expected to be able to connect at any time and in any place thanks to the Internet of Things. To create this connection, any service from any pipeline, route, or network must be utilized. The Internet of Things (IoT) Connectivity Concept is what this is called. According to the IEEE IoT community, "a self-organized and adaptive system that includes sensory and cognitive networks with the goal of linking" all "things, including everyday and industrial goods, in such a way that they become smart, organized, and communicative." This research is based on the Internet of Things (IoT) terminology and understanding of the IoT Connectivity Concept. The focus here is on the integration of IoT technology with e-Health solutions, especially how IoT technology (RFID) is linked with Electronic Medical Record (EMR) patient information so that each patient may make his or her own conclusion. An electronic medical record (EMR) used to maintain track of a patient's health information is known as a patient EMR. Each patient will be given a Radio Frequency Identification (RFID) tag in order to have access to their medical data stored on the health facility's website.

Users' system development to fulfill the following objectives: Improving the collecting of patient medical data that had never been examined before and delivering it to patients in a care environment where they had never been before. Improving IoT-driven initiatives to save costs and enhance health by expanding access and quality of treatment. Develop and deploy embedded technologies for all IoT- enabled health systems, such as: Patient data sensors; microcontrollers that process, analyze, and send data wirelessly; and microprocessors that allow rich user interaction. Creating healthcare-specific gateways that evaluate and send sensor data to the cloud on a regular basis. Creating queries that may be used to organize table data in a descending and incremental way.

Health care is changing from hub-based to personal e Health as IoT-enabled technology enables for quicker and safer preventative treatment, cheaper overall costs, enhanced patient practice, and greater stability. Efficient e Health IoT systems may be tailored to each user, offering them highly flexible access to rich medical data and allowing them to make efficient healthcare choices while being observed surreptitiously. If this notion is to realize its full potential, the development of wireless medical nerve networks (WMSNs), which are at its centre, is a key challenge. The usage of WMSNs in future ioT-based e Health systems is an important problem covered in this book. In order to manage such difficulties, which WMSN must meet specific demands and restrictions, such as size limits, manufacturing costs, and

resistance to environmentalconditions inherent in maritime locations, which vary from cost and efficiency to security and quality of service. The book focuses both the composition and exploitation of elements to illustrate this. Impact of neurosurgery networks in smart cities, portable patient surveillance technology testing, wireless overview of medical programmes, Internetsecurity issues at WMSN and e Health, smart hospital roomsand automated systems, neuroscience skills on smart cloud networks, smart intelligence programmes , and clever programmes and services for the blind are just a few topics to consider.

In today's world of health care, everyone wants things to be easy, economical and the best degree of comfort supplied by technology. The Internet of Things (Io MT) is agood aspect of making grownups more current in this sense.The appropriateness and usefulness of the Internet of Things (IoT) in the healthcare business is underlined solely in this research. In addition, it is being examined if Io MT is a collection of sensing devices from diverse businesses. During the hearing and transmission, Io MT offers an online iew of all medical instruments (Io E) to gather and enjoy a considerable quantity of data. The communication of clothes not only adds complexity, but also increases energy consumption, which is another key hurdle to managing the medical world properly. Energy awareness approaches are apromising solution to reduce energy loss and enable efficient data transmission for health care systems.

Current specialists present an in-depth study report on the life expectancy of the IoT gadget, which estimates that it will grow by 2020. It is possible to handle many differentnetworks owing to sophisticated and autonomous sensor self-driving behaviour. There is a considerable requirement for energy management and battery life of the whole systemin these sorts of networks. Various review studies have underlined the new position of Io MT and numerous associated sectors as the backbone of the healthcare business, and their expanding relevance. Excellent energy saving at best during the transmission of essential medical data to Io MT is a significant focus point that still needs a lotof effort. In this respect, by investigating the internal mechanism of the neurological system, this research focuses on the present demands of patients, clinicians, and the Io MT system as a whole.

In addition, a few essential aspects from consumers and the medical market feature are considered in order to properly regulate and monitor battery life and health of tinydevices. How to supply the most demanding energy. demands in the Io MT system, for example, some of the most challenging challenges. What percentage of device battery life will be saved? Is the Io MT power model able to fulfil market demand adequately? What is the link betweenthe suggested algorithm to conserve energy, framework, and the demands of the common citizen in the field of inexpensive, low-cost medical care? In this situation, all of these fundamental difficulties may be overcome by embracing flexible trends and flexible procedures. Other targeted industries will be regarded as creating economic and adaptable solutions for this. Healthcare is a new platform to inspire the technical team to establish trendsand energy efficiency. The Internet is important to the change of the complete Io MT ecosystem. For the simplicity of numerous users, smart wearable devices unite entire industries in the medical business. By modifying the worldwide medical scenario, the authors build new data management systems.

MOTIVATION AND LITERATURE SURVEY
It is a web-based patient care system that keeps track ofa patient's health record from the moment we log into the network until patients are at the top of the network, delivering verified health data. It allows people to keep their medical records and give their doctors access to them as needed. Physicians who do not have access to patient data should be able to do so. In the meantime, they will need to use patient authorization and provide basic information to validate themselves. This allows them to transfer their health care information to any computer with an Internet connection.

It works in the same sense, and doctors are asked to help manage their medical procedures. This involves keeping all of their patients' information online, as well as managing accounts and invoices. They may also issue a Health Card that identifies certain important information such as blood group, diet or drug resistance, and important medical history situations that may be helpful in an emergency and provide as many resources as needed by local

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health authorities. in an emergency and provides as many resources as needed by local health authorities. Virtual monitor which is not involved physical contact is now feasible in the current situation like covid, healthcare business due to Internet of Things (IoT) - enableddevices that can keep patients safe and healthy while enabling clinicians to deliver high-quality treatment. Patientengagement and satisfaction rise as contact with the doctor becomes simpler and more efficient. Furthermore, remotelymonitoring the patient's health helps to shorten hospital stays and avoid recurrence. The Internet of Things has a substantial influence on cutting health-care costs and improving treatment outcomes.

The Internet of Things (IoT) is undeniably changing the healthcare business by rethinking the location of devices and human communication in healthcare delivery solutions. Patients, families, doctors, hospitals, and insurance companies all benefit from IoT applications in health care. Patients and Internet of Things - Patients reach individual attention with wearable gadgets like straps and other wireless devices such as blood pressure and cuffs to monitor heart rate, gl u comers, and more. These gadgets can be set up to remind you of things like calorie counting, exercise, fixed times, blood pressure changes, and much more. This has a profound effect on single people and their families. The alarm system delivers messages to family members and health workers concerned when normal human activities are disrupted or altered. Doo Doctors - Using clothing and other IOT-enabled home surveillance technology, doctors can better track the health of their patients. They can track whether patients adhere to their treatment plans, and whether they need emergency medicalcare or not. IoT allows healthcare providers to be vigilant and diligent in their interactions with patients. Data from IoT machines may assist physicians in determining the method of eye care for their patients and achieving desired out comes. IoT Hospitals - In addition to monitoring patients'health, IoT machines are beneficial in a variety of hospital settings.

Wheelchairs, defibrillators, nebulizers, oxygen pumps, and other monitoring devices are all monitored in real time using sensory IoT devices. Referral of medical personnel tovarious locations may also be monitored in real time. The spread of disease is a major problem for hospital patients. Powerful IoT hygiene monitoring equipment helps prevent infection in patients. Asset management, such as drug development control and environmental monitoring, such as refrigeration temperature monitoring and humidity control, is also aided by IoT devices. Internet of Things and Health Insurance Companies - Health insurers have many other options when it comes to smart devices connected to the IoT. Insurance companies can use the data obtained by healthcare devices to record and process claims. By using this data, they will be able to detect fraud claims and see opportunities to writedown.

IoT devices promote transparency between insurers andcustomers in transaction, pricing, claim management, and risk assessment. As a result of the data-driven options adopted by the IoT across all operating processes, customers will have a fair view of the basic concept behindall the choices made and consider the results. This vision was created to assist in the management of many of the responsibilities of small public and private hospitals. It also helps with payment and account management data. Other non-medical information is provided as requested by hospitals. Anyone who wants a health card can get it at a lower price.

Common users of the program are guests and doctors. Personal information (name, age, gender, user id, and password) and (weight, blood group, allergy, diagnosis, treatment, and medication) are uploaded and reviewed online and available anywhere in the world. This approach has also been used to develop telehealth skills. Patients in rural areas will benefit from Online Medical Advice as theywill avoid traveling to remote areas. Health advantages There are several advantages of using eHealth. As a result, the Dutch government is pushing healthcare providers to provide more eHealth services.

The following are some of the advantages of eHealth
You will save time
Telehealth has the potential to save time. Patients, for example, may schedule an appointment with their online care provider. They also don't have to leave their homes if they schedule an online consultation (via a video link, for example).

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Becoming aware of your own health

People may have a better understanding of their health by using the digital personal health care facility. They may share all or part of their data with a health care practitioner or informal caregiver if they want to avoid having to disclose their medical history again. This enables the health care professional to work more effectively, decide suitable therapy faster, and prevent errors. Patients get greater control over their health when their knowledge of their condition improves.

Minimal administrative overhead

Doctors have little documents and may communicate information with their colleagues securely and conveniently.

METHODOLOGY

Proposed IoMT based Framework

First, In this part, we propose a framework for the IoMT system based on remote health care, which contains three primary layers: layer 1, medical device data collecting, layer 2 device integration (layer 1), and specialised IoMT protocols in layer 2 by hearing and sending data. Remote health care monitoring using effective medical equipment and methods is covered. A database of data gathered to advise and assist medical hospitals, physicians, and nurses is also connected with the third layer. Additionally, such information may be saved and utilized in   future   illness analyses.

Proposed Energy efficient on-off Algorithm for IoMT

To preserve power during data transmission to the IoMT system, we propose ON-OFF (EEOOA) energy- saving algorithm (Fig.2). The suggested EEOOA operating policy is based on sensory devices' functioning and sleep intervals. More energy will be conserved or less energy will be absorbed in this manner. As a result, new energy-saving technologies are needed to handle significant power dissipation during the data transmission phase. It's also challenging to maintain and monitor the IoMT's complexity as the number of sensory devices in health care grows. Furthermore, EEOO causes hearing loss and reduced transmission capacity while passing data from the patient's side to the medical field. In order to manage the magnetic field, Eq. (1) offers a power model for effective data transfer in the IoMT system, in which we merely consider the components of the sensor and transmission.

It's also worth noting that neurons use less energy than muscles. E is the total of the output power of all the sensor nodes S, which is a mix of sensory and transmission power, as shown in eq. (1). The pieces to be moved are indicated by b, while the distance between nodes I and j is indicated by d.

$$E = \sum_{i=1} \left(El_{sen_i}(b) + E_{t\hat{x}_i}(b, d_{ij}) \right) \qquad (1)$$

Because EEOOA is based on the duty cycle mechanism, it uses the notion of the transceivers ON and OFF time. When the transceiver's radio is turned off, energy is saved, and energy is somewhat drained during the ON period, i.e., the radio's active time, as illustrated in the flowchart and pseudocode of the EEOOA in Fig.3.

RESULTS AND DISCUSSION

This section examines energy dissipation during data transmission in the IoMT by concentrating on the sensing and transmission phases. Because the detecting element uses less energy, it is not worthy of significant concern; nevertheless, the transmission component uses around half of the total power, which is a big problem that should be addressed by delivering the unique EEOOA. The energy model in eq. was created to improve the IoMT and all sensor   devices   (1). Because   the   energy-intensive transmission component degrades the most often utilized

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aspect of medical treatment. As a consequence, introducing EEOOA to control the energy of the IoMT system is crucial.

CONCLUSION

In order to fulfil the expectations of patients and physicians who have championed EEOOA in order to preserve the developing potential of the entire community, the transfer of Energy Efficient data to IoMT is a critical requirement of today's medical market. This work gives a two-fold contribution. First, the power model offers EEOOA during data transmission to IoMT, and it is emphasised in the transfer component's power output. Second, IoMT provides a three-layer novel data transport paradigm. Furthermore, simulated data demonstrate that EEOOA performs better than earlier methodologies. We hope to employ our recommended EEOOA utilising Bluetooth low energy (BLE) technology in the near future

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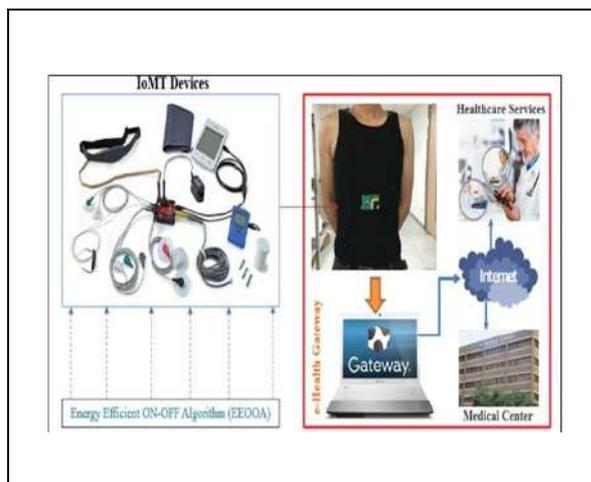
Fig.1 Proposed IoMT based framework for eHealthcare using EEOO Algorithm
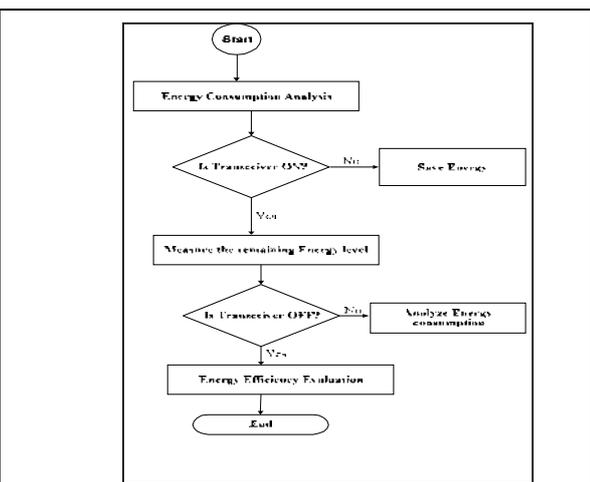
Fig. 2. Flowchart of proposed EEOOA

Fig. 3. Pseudocode of proposed

Fig. 3. Pseudocode of proposed EEOOA
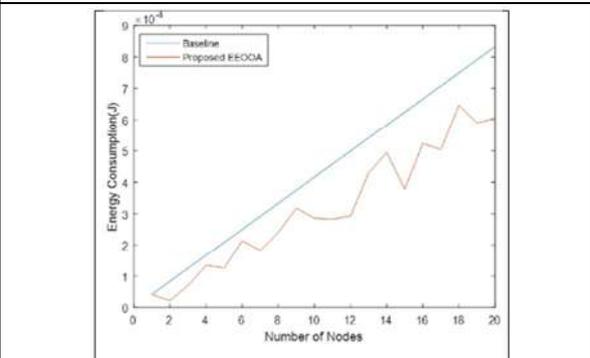
Fig. 4. Energy consumption analysis of the Baseline and EEOOA

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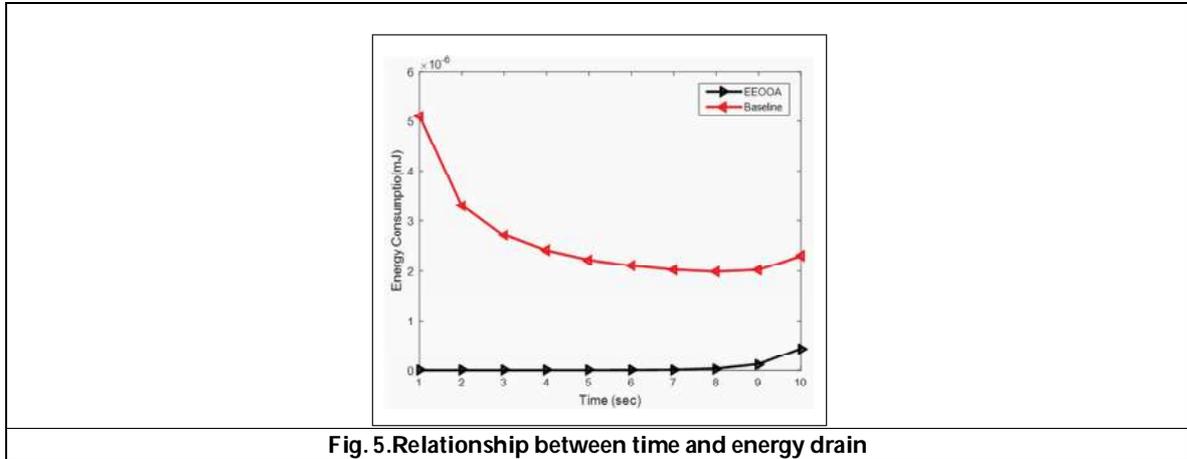
Fig. 5.Relationship between time and energy drain

RESEARCH ARTICLE

Microfinance and Bank Linkage Programme: Growth and State in India

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ABSTRACT

Despite the development of rural banking system and rural lending by banks, even in the post-nationalization, a larger number of rural poor continued to remain out of the formal banking system. SHG and Bank linkage programmed has emerged as a tool to reach the rural people. SHG and Bank linkage programmed is one of the models of microfinance in India apart from microfinance institutions. This study focuses on analyzing growth of SHG and Bank linkage programmed in terms of savings, loan disbursement and non-performing assets.

Keywords: Microfinance, Bank, Self-help groups, Non-performing assets, India.

INTRODUCTION

Small Help Group (SHG) and Bank linkage programmed has been one of the prominent models exist in India other than Microfinance Institutions (MFIs). SHG and Bank linkage programmed connects members of the group with formal financial service providers. SHG and Bank linkage programmed aimed at enhancing banking service penetration and deepening credit. SHG and Bank linkage programmed model has got wide acceptance among rural poor as it rescues them from the clutches of moneylenders and pawnbrokers. Women and rural poor participate largely in SHG and Bank linkage programmed. Government of India and various state governments in India have been supporting the SHG and Bank linkage programmed extensively. As a result, SHG and Bank linkage programmed has grown leaps and bounds in India and reached many milestones. As of 31st March 2021, there are about 112.23 lakh members having the total savings of Rs 37,477.61 crore. This study focuses on progress of SHG and Bank linkage programmed and its state in India.

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REVIEW OF LITERATURE

India's 70% of the population lived in rural side and only 30% of rural population had access to bank deposits (Lakshmanan, 2008). Microfinance and Bank linkage programmed enhanced women empowerment (Aruna and Jyothirmayi, 2011). Microfinance and Bank linkage programmed reduces poverty level of participants when compared to non-participants of Microfinance and Bank linkage programmed (Bansal, 2010).Microfinance and Bank linkage programmed increased income level of its beneficiaries (Vansiya, 2015). Strong empirical evidence exists for the relationship between financial performance and outreach (Ngo, 2015).

Objectives of the study
Primary objectives of this study are given below.
- To analyze the progress of SHG-Bank linkage programmed in India in terms of savings
- To analyze the progress of SHG-Bank linkage programmed in India in terms of loan disbursed.
- To analyze Non-performing Assets (NPAs) under SHG-Bank linkage programmed

Progress of SHG and Bank linkage programmed in India
The SHG and Bank linkage programmed model of microfinance has emerged as a successful microfinance model at the global level. As of 31st March 2021, the SHG and Bank linkage programmed model serves 13.87 crore families and provides social, economic, and financial empowerment to the households(NABARD, 2022). Table 1 reveals the progress of SHG-Bank Linkage Programmed from 2018-19 to 2020-21. Number of SHGs has increased by 12.8%. Number of women SHGs has spiked by 13.9%. However, amount of loans disbursed to SHGs has slightly down by 0.4%. At the same time , amount of loan outstanding with SHGs has increased by 18.5%. Figure 1 states the progress of SHG- Bank linkage programmed in India in recent years (2018-19 to 2020-21) in terms of number of SHGs with savings linkage, amount of savings outstanding, number of SHGs that availed loans during the year, amount of loans provided, number of SHGs with loan outstanding, and amount of loan outstanding. Number of SHGs with savings, amount of savings outstanding, loans given to SHGs, and amount of loan outstanding have increased.

Non-performing Assets (NPAs) are those assets of banks wherein principal or interest or both have not been paid by the loan takers for a period of 90 days. NPAs are critical issue for commercial banks as they affect profitability, and reputation of the commercial banks. In SHG and Bank linkage programmed, the banks provide loans to the members of SHGs without any collateral at an affordable rate interest. It is essential to check whether the SHGs that availed loan from the commercial banks pay back regularly or not. Further, regular repayment of loans availed by the SHGs will motivate the banks to advance further loan to the SHGs and regular repayment will construct positive mindset about SHGs among the bank personnel and the public. Table 2 presents NPAs against SHGs in the banks as of 31st March 2021. Table 2 reveals that there is a 4.73% NPAs against SHGs on an average. Co-operative banks have more NPAs (5.55%) against SHGs followed by commercial banks (3.99%). Interestingly, commercial banks have more NPAs against SHGs than Regional Rural Banks. In total, there are Rs 488921.34 worth of NPAs against SHGs. Level of NPAs against SHGs is a point to be noted. 4.73% NPA is a factor is to be considered with a lot of seriousness. Government of India and the concerned financial intermediaries should take necessary actions to recover those NPAs.

CONCLUSION

This study aimed at analyzing progress of SHG and Bank linkage programmed in India as SHG and Bank linkage programmed is one of the successful microfinance models at the global level Further, large number of women participate in SHG and Bank linkage programmed as it empowers them at social, economic, and financial level. The study data reveals that SHG and Bank linkage programmed has been progressing well steadily both in terms of number and value. However, there is a factor that needs attention is NPAs of banks against SHGs. On an average

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4.73% NPAs exist against SHGs. It is to be dealt rationally and loan advancing mechanism is to be modified in such a way to decrease NPAs.

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Table 1: Progress of SHG-Bank Linkage Programme

Particulars	2018-19		2019-2020		2020-21	
	SHGs Number	Amount	SHGs Number	Amount	SHGs Number	Amount
Total SHGs	100.14	23324.4	102.43	26152.0	112.23	37477.6
All women SHGs	85.31	20473.5	88.32	23320.5	97.25	32686.0
Loans given to SHGs	26.98	58317.6	31.46	77659.3	28.87	58070.6
Loans outstanding	50.77	87098.1	56.77	108075	57.8	103289

Table 2: Non-performing Assets of Banks against SHGs

Name of the intermediary	Non-performing Assets of Banks against SHGs		
	Total loan outstanding	Amount of NPAs	% of NPA to total loan outstanding
Commercial Banks	5978569.70	303385.52	5.07
Regional Rural Banks	3592322.25	143460.38	3.99
Co-operative Banks	758078.88	42075.44	5.55
Total	10328970.83	488921.34	4.73
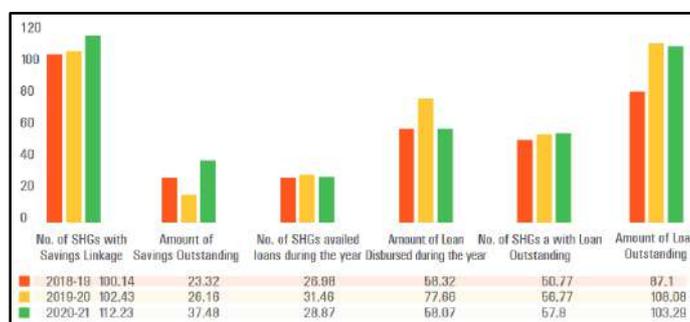
Source: Status of Microfinance in India, 2021
Figure 1: Progress of SHG-Bank Linkage Programme

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RESEARCH ARTICLE

Effect of Pilates Exercise Along with Conventional Therapy and Conventional Therapy Alone in College Going Students with Text Neck Syndrome: A Comparative Study

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ABSTRACT

According to a study college going students, have maximum usage of mobile phones. Text neck is a condition where a person has their head flexed in a forward position and is bent down looking at their phones for prolonged periods of time in turn leading to repetitive stress injury or overuse syndrome. The present study aimed to assess the comparison of the effectiveness of Pilates exercise along with conventional therapy and only conventional therapy in Text neck syndrome.30 subjects for the study will be selected from Ahmadabad Physiotherapy College with age group of 18-26 years. The subjects were divided into 2 groups, Intervention group and control group. The total treatment will be for 4 weeks and 3 days/week. Treatment time would be 60 minutes/day. The data was analyzed by using SPSS version 25, Significance tests for difference in means was done using paired t-tests. As shown by the results both groups have significant effect on ROM and VAS but when comparing both groups, group A has more effect on ROM whereas both the groups are equally effective on VAS. According to the results we can conclude that both the groups, are effective in decreasing neck flexion range of motion and neck pain but group A was more effective.

Keywords: Text neck syndrome, Neck pain, VAS, ROM, Goniometry.

INTRODUCTION

Smartphone's are one of the most popular device nowadays that cause potential risk of musculoskeletal problems due to its over and prolonged use [1]. According to a study college going students, generally in their 20's have maximum usage of mobile phones [3][9]. Many harmful postures are undertaken while using mobile phones. It was found that mobile phone users detected with musculoskeletal problem majorly had flexed neck posture while they used their mobile phones, as stated by a study in Thailand [3][9] .Some neck pain troubles such as neck pain with unknown cause, job induced neck ache, tension headache, postural neck ache are resulted by variations in the deep cervical flexors activation[5]. Because of a fixed forward posture many muscles get weaken like deep cervical flexors, scapular retractors and lower trapezius muscles. Pectoralis major and neck extensors become shorten and because of tired upper trapezius muscle generally pain is being reported due to overwork. According to a study while using smaller devices as compare to computer desktops exhaustion and tension in shoulder and neck muscles occurs faster because with mobile phones, iPad the user needs to constantly bend down, the capacity to work reduces and adopts more slumped position, (by Shim and Zhu) [7]. The neck pain and damage resulted from looking down at the mobile phones, electronic gadgets or other wireless devices very often and for prolonged period of time describes the term 'Text neck' [4]. Around 8-10 hours per day is spent by excessive mobile phone users while 3 hours per day by normal mobile phone users [4]. Most commonly soreness and neck pain is caused by Text neck syndrome. Additionally, bending head forward towards your smart phones for more time scale can lead to upper back ache plus extensive upper back muscles spasm, even shoulder muscle spasm could possibly occur due to shoulder ache and tightness [2]. A US chiropractor named Dr. Dean L. Fishman coined the term "Text Neck". Text neck describes a condition where a person has their head flexed in a forward position and is bent down looking at their phones or other electronic gadgets for prolonged periods of time in turn leading to repetitive stress injury or overuse syndrome. When one's ears are aligned with one's centre of the shoulders in an erect position, the weight exerted by the muscles of neck by an average head is around 10-12 lbs. About 27 pounds of force on the neck is assumed to be placed while tilting head forwards to 15 degree, increasingly 24 pounds at 30 degree, 49 pounds at 45 degree and 60 pounds at 60 degree [2][3][4].

FIGURE 1.1 HOW TEXTING AFFECTS SPINE

Smartphone's have smaller screens compared to other electronic gadgets hence more neck flexion is required by the users, resulting more work by the shoulder muscles even; concluding that in more neck flexed position increased muscle work is needed for stabilizing the neck [3].At different neck flexion angles difference was seen in neck muscle activity; the Cervical Erector Spine (CES) muscle activity increased while the Upper Trapezius (UT) muscle activity decreased when neck flexion angles increased. The activity of both Upper Trapezius muscles and Cervical Erector Spine were stated as less at 0-15 degree of neck flexion angle. Hence, neck flexion angles between 0-15 degrees should be adopted by Smartphone users [3]. According to some studies it is indicated that our human psychology is affected negatively by prolonged use of mobile phones. Excessive mobile phone users face isolation, sleep disorders, loneliness, and depression [4]. The Pilates program consists of a physical activity program aimed at achieving harmony between body and mind and was developed during the First World War by Joseph Hubertus Pilates[5]. If attention is paid on upper-limb strength exercises, scapula-thoracic and cervical exercises and stretching then it can be a great relief in neck pain and these elements are also a part of Pilate's program as shown by a study [8]. In the Pilates protocol generally global muscles are targeted in the exercise session but in case of paying special focus for particular muscle or area in relation to underlying disease and hence can be a beneficial treatment strategy in population suffering from neck ache[6]. The present study aimed to assess the comparison of the effectiveness of Pilates exercise along with conventional therapy and only conventional therapy for college going students with Text Neck Syndrome, to decrease increased neck flexion angle and neck pain while using mobile phones with goniometry.

MATERIALS AND METHODOLOGY

The source of data was taken from Ahmedabad Physiotherapy College, and the study design was comparative study. Materials used are universal goniometer, mat, chair, Smartphone, paper, pen, consent form. 30 students were selected on basis of following inclusion criteria: (1.) with the age group of 18-26 with and without neck pain, (2.) minimum 3 months experience of using their phone, (3.) spending 4-5 hours on phone, (4.) both boys and girls, (5.) keeping neck flexed more than 15 degree while using phone. Participants who were having history of any injury, fall on outstretched hand within last one year or suffering from conditions like Rheumatoid Arthritis, Osteoarthritis, cervical pathologies etc. were excluded. The subjects will be divided into 2 equal groups by random sampling method. Group A namely Intervention group and Group B namely Control group. The total treatment will be for 4 weeks and 3 days/week. Treatment time would be 60 minutes/day. Pre and post treatment measure will be taken with Visual Analogue Scale for pain measurement. For cervical range of motion pre and post measurement will be taken by the Universal Goniometer.

PROCEDURE: Group A
 Intervention group
 were given Pilates exercise plus conventional therapy for 4 weeks, 3 times/ week. Following are the exercises included in Pilates protocol: Hip twist level 1(fig 2.1), Double leg stretch level 1(fig 2.2), Double leg stretch level 2(fig 2.3), One leg stretch level 1(fig 2.4), Clam level 1(fig 2.5), Shoulder bridge level 1(fig 2.6) Scissors level 1(fig 2.7), Arm opening level 1(fig 2.8), Breast stroke Prep level 1(fig 2.9), Breast stroke prep level 2(fig 2.10).

Group B: Control group
 This group will receive only conventional therapy for 4 weeks, 3 times a week.
-Neck and scapula focused exercise:
Cervical stretching, Craniocervical flexion, Cervical retraction exercise, Scapular retraction, Lateral pull down, Push up plus exercise.

Outcome Measures:
1. VAS [13]
 2. Universal Goniometer [14]

RESULTS

Total 30 patients were taken with Text Neck Syndrome and randomly divided into 2 groups, 15 candidates in Group A- Interventional group and 15 candidates in Group B- Control group. The data was taken from Ahmadabad Physiotherapy College and analyzed by using SPSS (statistical package for social sciences) software version 25. Significance tests for difference in means was done using paired t-tests.

TABLE 3.1: TABLE SHOWS WITHIN GROUP COMPARISION OF ROM AND VAS IN GROUP A.
TABLE 3.2: TABLE SHOWS WITHIN GROUP COMPARISION OF ROM AND VAS IN GROUP B.
TABLE 3.3: TABLE SHOWS BETWEEN GROUP COMPARISION OF GROUP A AND GROUP B
GRAPH 3.1: GRAPH SHOWS GROUP A PRE AND POST MEAN
GRAPH 3.2: GRAPH SHOWS GROUP B PRE AND POST MEAN
GRAPH 3.3: GRAPH SHOWS GROUP A AND GROUP B COMPARISION OF ROM AND VAS

As shown by the results for group a (intervention group) and group b (control group) both have significant effect on rom and vas but when comparing both group a and group b, intervention group has more effect on rom than control

group whereas both the groups are equally effective on vas. Hence alternative hypothesis in favor of Pilates exercise program and conventional therapy (H₁) is accepted and null hypothesis (H0)of no difference is rejected.

DISCUSSION

In this research, candidates used their mobile phones daily and utilized more than 4-5 hours on their mobile devices. Constant looking down can also result in variation in the original curve of the cervical spine also might resume more often neck flexion posture and multiply the levels of stress on the cervical spine [10]. The present study is based on the variations of the neck flexion angle and neck ache during prolonged usage of mobile phones. In this study attention is paid on the cervical flexion angle, which varies from person to person at the time of using their smart phones. The hours of mobile usage were also taken into consideration. When any person continues to be in a particular position for prolonged duration then due to that static posture blood flow can be shunted and muscles would get less nutrients and hence adapt neck pain which can get prominent [11] . Hence this study has also included and focused on students having neck pain along with looking for variations in neck flexion angle while mobile device usage. According to a study done by Hae- Jung Lee et al, (2016) in Journal of Korean Physical Therapy, they aimed to investigate Range of Motion, neck posture, muscle endurance and self- report of pain and disability in Smartphone users. 78 university students with age criteria of 18-30 years were taken. They concluded that functioning in daily activities should be evaluated as preventive measure from developing more severe neck pain [12]. Hence in this literature, importance is given to neck pain and neck flexion angle of the participants having Text Neck Syndrome and the age group selected is 18-26 as they are more addicted to their phones.

Participants having both neck pain and no pain in neck were taken. According to the outcome measures for pain that is VAS after 4 weeks of Pilates and conventional therapy protocol, the average value of neck pain pre VAS 2.6 reduced to post VAS as 1.6. When the head is in flexed position or when person suffers from text neck than the deep cervical flexors namely long us colli and long us capitis which run down the front of the cervical spine and help in flexing the neck forward are lengthened while the deep neck extensors namely semispinalis cervicis and capitis and multifidus becomes shortened. While the muscles present at upper back are elongated and chest muscles become less elongated. Pilates exercise has shown miraculous benefits for back ache but less studies are done for Text neck. Pilates when done with proper mechanism under proper surveillance can be very helpful in Text Neck. Basically Pilate's protocol strengthens the core muscles for stabilization, which with time helps to relax and reduce neck tension and hence helping in reducing neck pain and because of good stability to muscles reducing the neck flexion angle. Pilates also benefits in neck disorders because it has a correlation with breathing properly. Proper breathing is essential to open the joint space in neck and help lengthen the neck muscles to reduce neck trouble and opt good posture. When for prolonged time the neck is flexed then due to that posture some muscles work too hard while others get weakened, so with Pilates gradually the strength of weakened muscles get increased and also can aid in decreasing the increased neck flexion angle. Also the Pilates protocol works on deep postural muscles including the neck. When the spine is strengthened then it creates an upward posture and will make the Text Neck posture uncomfortable to get habituated by one's body. Thus Pilates helps to reduce the neck stress and hence keeping the posture balanced properly. Pilate's exercises is chosen carefully for this study keeping in mind about the beginners that they can do comfortably without any need for specialized area or equipment.

CONCLUSION

According to the results we can conclude that both the groups, group A - intervention group and group- B control group are effective in decreasing neck flexion range of motion and neck pain but group A was more effective when compared with group B. Hence Pilates exercise program can be a very useful adjunct with routine conventional therapy in patients with Text Neck Syndrome. The present study states that Pilates exercise program along with conventional therapy when compared with conventional therapy alone is more beneficial in Text Neck syndrome

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patients. Neck pain and reduced Range of Motion are the major symptoms of Text Neck Syndrome hence Pilates clinically can be an effective adjunct to a management program in patients also suffering from neck pain. Similar study can be done not only in college going students but also in people who have more desk job, prolonged sitting work or more usage of mobile phones. Even people above 20 years have extensive use of mobile phones hence similar study could be carried out in that age group, even a comparison between different age groups can be carried out. In the present study the sample size is limited. The treatment duration could also be longer. Long-term follow up after the study should be taken.

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TABLE 1: TABLE SHOWS WITHIN GROUP COMPARISION OF ROM AND VAS IN GROUP A.

OUTCOME	PRE MEAN	POST MEAN
ROM	35	28
VAS	2.68	1.63

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TABLE .2: TABLE SHOWS WITHIN GROUP COMPARISION OF ROM AND VAS IN GROUP B.

OUTCOME	PRE MEAN	POST MEAN
ROM	34.53	32.93
VAS	1.9	1.5

TABLE 3: TABLE SHOWS BETWEEN GROUP COMPARISION OF GROUP A AND GROUP B

OUTCOME	GROUP	MEAN ± SD	UNPAIRED –T TEST	P VALUE
ROM	GROUP A	28± 3.9	3.57	< 0.05
	GROUP B	32± 3.6		
VAS	GROUP A	1.6± 1.3	0.052	> 0.05
	GROUP B	1.5± 1.4		

Fig.1 Hip Twist

Fig.2 Double Leg Stretch Level 1

Fig. 3 Double Leg Stretch

Fig. 4 One Leg Stretch Level

Fig. 5 Clam Level 1

Fig.6 Shoulder Bridge Level 1

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Fig. 7 scissors level 1

Fig. 8 arm opening level 1

Fig. 9. Breast Stroke Prep Level 1

Fig.10 Breast Stroke Prep Level 2
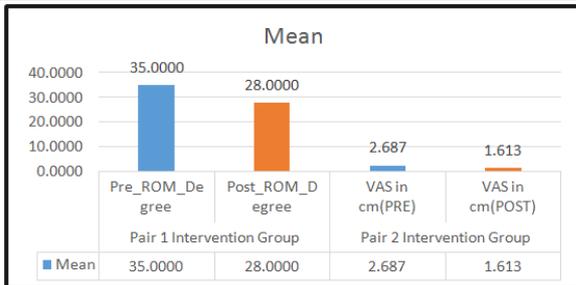
Graph 1: graph shows group a pre and post mean
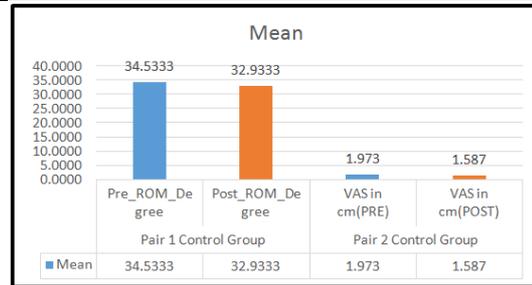
Graph 2: graph shows group b pre and post mean
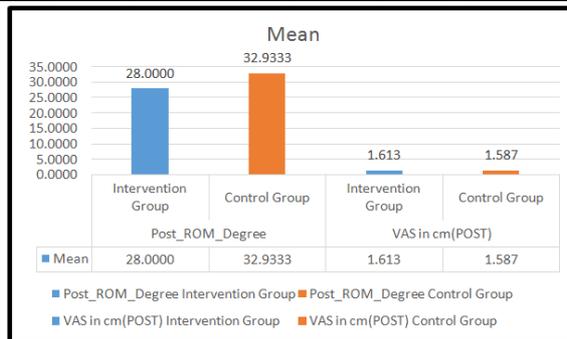
Graph .3: Graph Shows Group A And Group B Comparison Of Rom And Vas

RESEARCH ARTICLE

Home Automation using Node MCU with Power Failure Detection

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ABSTRACT

In the current technological revolution, automation in the area of industry and homes using Internet of Things (IoT) is the major dominant tool in the wo Internet of Things (IoT). Because of its wider range of benefits and user friendly approach makes customer's job easily and effectively. An IoT based automation system explains to the monitoring and maintenance of all house hold appliances and resources from our hands without the assistance of human power and other equipment. This research paper analyzes the various home applications strategies and their operating by remote control mode without involving the support of human power and other equipment. These processes play a very important role in society for all people and especially for people with physical disabilities. In this paper also indicatesa wide range of home mechanization methods with a standard harp known as Arduino and types of LAN based networks like blue tooth, Wi-Fi and network friendly.

Keywords: system explains, strategies and, Wi-Fi and network friendly, technological revolution,

INTRODUCTION

Generally, home automation refers to the process of controlling various home appliances remotely. There are various frameworks that help implement this type of system. IoT devices are capable of being monitored remotely over the internet. Domestic automation or demotics refers the constructing automation for domestic applications and it is called as smart home as well as smart residence. This system influences the domestic attributes inclusive of illumination level of lights, climate conditions, amusement parks and house hold equipment. It is also able to include home protection which included to get permission to manage and alarm structures. When these are linked with the

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internet, domestic gadgets are a vital constituent of internet of things which is also called as IOT. Typically, this device connects managed gadgets to a critical clever home hub which is known as gateway. The consumer interface for manage of the machine uses either wall-established terminals, pill or desktop computers, a cellular telephone software, or an internet interface that could additionally be on hand off-site via the internet. At the same time as there are numerous competing providers, there are growing efforts towards the open source structures. But, there are problems with the modern- day kingdom of domestic automation which included a lack of standardized security measures and deprecation of older gadgets without backwards compatibility.

MECHANISM

The most popular in home automation is Node MCU (ESP8266) which is cost efficient and its Wi-Fi capabilities and Arduino IDE support makes it easier for IOT applications and it is also a open source application as anybody can access it. To control or monitor the status using Wi-Fi shield for our home appliances and also receive notification by triggering some actions which reduces the cost. The Wi-Fi shield requires connection to the internet from a wireless based system and internet connection. It demonstrates via local network where no internet access is needed. ESP 8266 and the relays are the heart of our project. As they play a main role in the working.

PROPOSED METHOD

This paper is about home automation using Node MCU. The appliances of the house can be switched ON/OFF without any physical contact and anywhere from the globe. In our project we are using local network to control them. The client device (i.e) smart phone or laptop and the Node MCU must be connected through same local network. The web application is loaded in the client device and from the client device, the appliances are switched ON/OFF. This sends signal to Node MCU and it triggers the relay which acts as a switch and ON/OFF the appliances. Here any number of appliances can be connected. 230V Ac supply is connected to a 12V voltage regulator and then this 12V is connected to a DC-DC connector which gives 3.3V as the input voltage for Node MCU is 3.3V. Relay is given a constant voltage of 5V through an adaptor. It is also possible to use batteries instead of an adapter. A fuse is added for safety measures. When there a sudden raise or lower current, fuse prevents the appliances from damage. We have also attached four LED lights to indicate the power supply and status of the Wi-Fi hotspot and the appliances. The voltmeter reading is shown in the hardware project. Here we are adding a bulb and a socket but by using this mechanism any number of devices can be added.

OBJECTIVE AND BLOCK DIAGRAM DESCRIPTION

This paper describes the IoT base control mechanisms using Node MCU. The appliances of the house can be controlled (ON/OFF) without any physical contact and anywhere from the globe. In this automation process, Internet based network is involved to control different home appliances. The block diagram of the proposed system is shown in Figure.1. This block diagram shows the working concept of the complete project. The component Node MCU unit is basically a microcontroller or the central governing unit of the system. The consumer practices the mobile application controls based on different features and properties of the appliances. The role of mobile command involves in user side as a voice or switching mode and drives the signal to the unit of Node MCU. Wifi network is needed in this operation

HARDWARE WORKING

The client device (i.e) smart phone or laptop and the Node MCU must be connected through same local network. The web application is loaded in the client device and from the client device, the appliances are switched ON/OFF. This sends signal to Node MCU and it triggers the relay which acts as a switch and ON/OFF the appliances. Here any number of appliances can be connected. 230V Ac supply is connected to a 12V voltage regulator and then this 12V is connected to a DC-DCconnector which gives 3.3V as the input voltage for Node MCU is 3.3V. Relay is given a constant voltage of 5V through an adaptor. It is also possible to use batteries instead of an adapter. A fuse is added for safety measures. When there a sudden raise or lower current, fuse prevents the appliances from damage. We have also attached four LED lights to indicate the power supply and status of the Wi-Fi hotspot and the appliances.

The voltmeter reading is shown in the hardware project. Here we are adding a bulb and a socket but by using this mechanism any number of devices can be added.

SOFTWAREWORKING
To prevent our home automation from hacking we have added a password login page that sends the wrong message with the wrong password. In a web application, there is a red energy index where gray is weak and where red is strong. Light switches and sockets turn green by turning on and turning gray off. It also displays a notice if there is a power failure and any burglar movement in the house. Web application coding is done with HTML and CSS and jQuery

PROPERTIES OF PROTOTYPE MODEL
The properties of developed model are:
• It creates a wireless based remote switching circuits system of all household equipments
• It uses Wi-Fi to establish wireless control, for the range of 150 feet circular space.
• Radio buttons are used as a ON/OFF command switches from user's smart phone.
• Security and controllable operation can be possible for all the appliances
• Integrating design has helped all types of appliances and extend their power range.
• Smart phone itself provides display over various results of all the parameters and their working status

POWER FAILURE DETECTION
Power failure detection is added by showing notification in the web applicationthrough the client's device. When no one at house we receive notification if any movement occurs in our house by adding PIR sensor. This sensor detects the heat released by human or animals thus sends notification. The project is also insulated in an acrylic case.

BURGLAR MOTION   DETECTION
A PIR sensor is added to our project. When this PIR sensor is ON, we get notification and an alarming sound when any living object enter into an empty house. This may help us to know if there unfamiliar or any burglar in the uninhabited house. Passive Infra-Red sensor can sense when a warm body passes through. Thus, we can call the nearby police to check our house from remote location. The alarming system can alert the neighbors to safe their belongings and to capture the burglar as well.

RESULTS

The achieved outcome of this project are
• The appliances in our house can be controlled or monitored from our remote location.
• If there is any power failure detection that is notified in the web application for our knowledge.
• If there is any burglar movement in our house it is notified in the web application by using PIR sensor.
• In order to prevent the house from being hacked we are adding a login page in which only the people who knows the password can access it.

CONCLUSION

There are never-ending opportunities are available to produce further revolution in home automation process. From this paper it is observed that the home automation system be able to made effectively and less operative cost which reduces overall cost of the environment. This system helps the operation of household appliances in various ranges from safety lamps, televisions. air conditioning system, lighting system and safety monitoring around the compound. By combining information and communication technology (ICT) with renewable energy systems such as solar power or wind power, households can make independent decisions about whether to conserve or use

something, leading to positive and positive environmental impacts. electricity bills for consumers using the system. When implementing this researcher propose to use sensory data about consumer activity within the home to anticipate consumer needs and to balance that with energy consumption.

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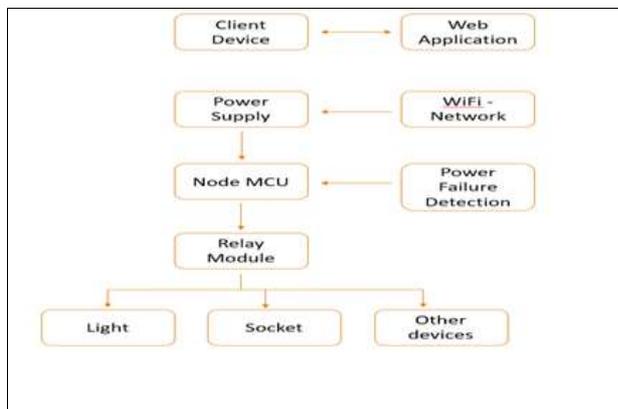
Figure.1 Block diagram of proposed model	**Figure.2 Hardware circuit of proposed model**
Figure 3. Testing of lights by proposed model	**Figure 4. Testing of Sockets working by proposed model**
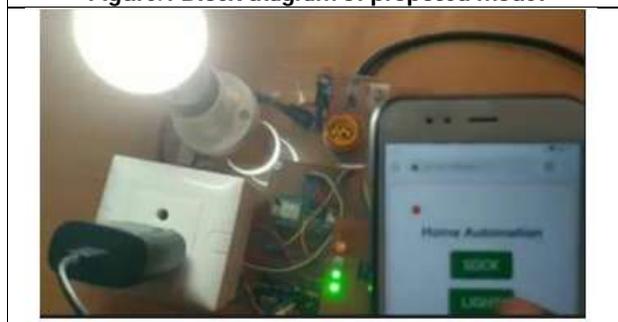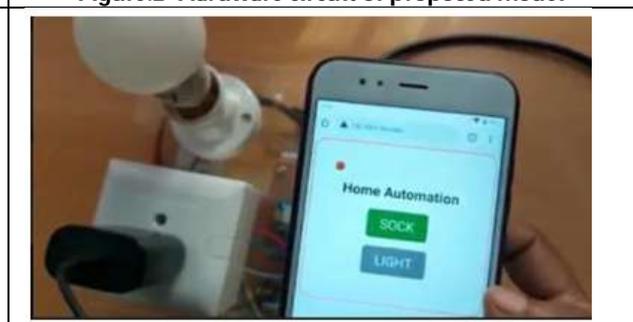
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Figure 5. Testing of PIR sensor in proposed model
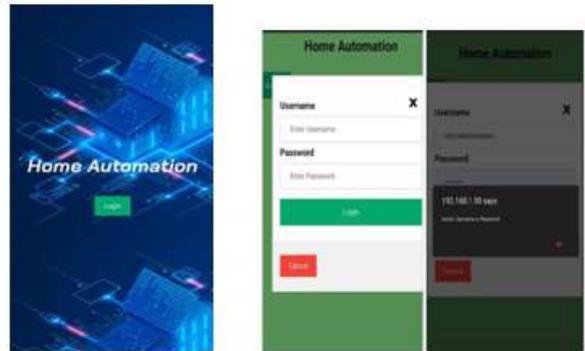
Figure 6 Testing of software Login page
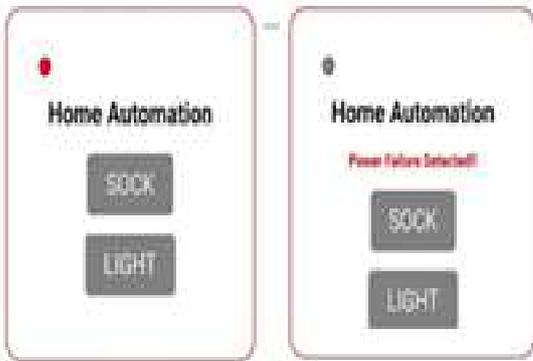
Figure 7. Power Failure Detection

Figure 8. Burglar Motion Detection

RESEARCH ARTICLE

Entropy for <100>+<111>Tunnelling Model

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ABSTRACT

We explore the many body localization features of single-particle mobility edges in fermionic systems. Random disorder and Anderson models with totally incompatible lattices are examined. Many body localizations are examined while comparing interaction and internal entropy and assessing the scalability of particle number fluctuations in subsystems. Each crystalline structure deviates from the regular atomic composition prescribed by the symmetry and structure of the unit cell in question in some way. Imperfections are these aberrations from the idealized crystal structure. Because thermodynamic rules are used to describe the formation of crystals, all of which have certain defects, translational symmetry inside a crystal is compatible with them. At a finite temperature, each a rise in the number of defects enhances entropy, this reduces the amount of free energy. The crystal has a definite concentration of imperfection with in equilibrium state. The amount of a given sort of imperfection is linked to the type of crystal lattice, its binding energy of a lattice, as well as the geometry of the defect itself. Entropy is a measure of a system's disturbance; the higher the entropy, more and more disorder there must be. The moments inside a magnetic field would be slightly organized, reducing the system's entropy. This region is interesting in terms of understanding the contribution of conduction electrons to the heat capacity of metals because of the low heat capacity associated to solid lattice vibrations with temperatures below 1^0K. The goal of this research paper is to determine the formulation of entropy for the <100> + <111> tunneling model. We originally devised the defect addition towards the specific heat for said <100> + <111> tunneling model for this purpose.

Keywords: Tunnelling Model, Specific Heat, Entropy

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INTRODUCTION

It is a well-known truth that unblemished crystals do not exist. Every crystalline structure has some type of deviation. The atomic arrangement determines the symmetry and structure of every unit cell.As we all know, the Helmholtz free energy is used to describe the creation of crystals. Given that these equations are used to explain the creation of crystals - all of which possess defects - we might well be curious about how a crystal's translational symmetry conforms to thermodynamic rules.

$$F = U - TS \quad\quad\quad\quad \text{.. (I)}$$

"U" refers for internal energy, and "S" stands for entropy, and ought toexistminimal within the condition of specific temperature, equilibrium is reached.

$$S = K_B \ln W \quad\quad\quad\quad \text{.. (II)}$$

Where "W" is the measure of probable means of distribution of system components.

Theory

The entropy will be zero since there can only be one way (W=1) to organize atoms in a certain way at various places in a perfect crystal. A fault in a unit cell's construction, on the other hand, causes the unit cell to seem distinct from others. There are as many ways to arrange the flaws as there are spots in the unit cell in this example, and the entropy is calculated by using the following:

$$S = K_B \ln N \quad\quad\quad\quad \text{... (III)}$$

Here "N" is the total number of sites in a single unit cell. The above equation expresses the defect's contribution to the crystal's entropy. As a result, each increase in defect concentration increases entropy, lowering the free energy at a constant temperature. The crystal has a fixed concentration of imperfection in the equilibrium state. We just explored one form of defect in the preceding example, but as a natural outcome of the intrinsic disorder connected with the limited temperature, any type of imperfection (imaginable) could be present; even if some of them are very small in quantity. At a certain temperature, any increase in defect concentration increases entropy, lowering free energy. Crystal defects affect colour, conductivity of pure semiconductors, elasticity, and crystal strength [1]. Entropy is a measure of how disordered a system appears to be; the higher the entropy, the more disorganization appears to exist. Moments in a magnetic field will become more structured, reducing entropy in the system. If indeed the magnetic field may be withdrawn without affecting the spin system's entropy. The spin system's order will appear to be at in the dearth of a field, a lesser temperature than a particular degree of order. Mostly from the system involving lattice vibrations can entropy flow into the spin system whenever the specimen gets demagnetized adiabatically. As the temperature drops, more moments align, lowering entropy [2]. The entropy of said lattice vibrations is mostlyminorat the temperature of interest; therefore the entropy of the spin systems would exist basically stable throughout adiabatic demagnetization of the sampling. Magnetic cooling is still a one-time process, not a cyclic one [3].Because of the low heat capacity related by means of solid lattice vibrations at temperatures at and below1^0K; this region is interesting in terms of determining the effort of transmission electrons towards the heat capacity of metals. A lot of heat capacity observations of regulator superconducting metals have been conducted in the temperature range 1^0K to 4^0K, which is accessible using liquid helium techniques, and yet no observations in the adiabatic demagnetization region had been made until recently. For the first time, the adiabatic demagnetization zone has been discovered. Because of the weak heat capacity associated with solid lattice vibrations at temperatures around and below 1^0K, this area is critical in evaluating the contribution of conduction electrons to metal heat capacity [4]. And Goodman [5] has reported comparable measurements on the super conducting condition.

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The regular state heat capacity " C_n" is commonly assumed to become the total of an electronic as well as a lattice heat capacity, that appear to be equivalence with the first and third powers of temperature, respectively for sufficiently low temperatures [6].

$$C_n = \gamma \text{T} + \frac{12}{5}\pi^4 \text{ R}\left(\frac{T}{\theta}\right) \qquad \text{.. (IV)}$$

Where "R" is stand for universal gas constant "T" stands on behalf ofthe temperature, θ is Debye vibrations of the lattice at a particular temperature, γ is a constant proportional to the density of states at the Fermi surface and may be influenced by electron correlations [6] and electron phonon interactions [7].A superconductor's electronic heat capacity is designed to give insight into the nature of something resembling the superconducting state; particularly, its temperature dependence should be connected to the energy gap, as current theories imply. [8]. The resolve of Bardeen, Cooper, and Schrieffer [9] yields estimated electronic superconducting state heat capacity "C_{es}" that is an exponential function of temperature for temperatures considerably under the critical temperature "T_c"

$$\frac{C_{es}}{\gamma T_c} = \text{a Exp}\left(\frac{-bT_c}{T}\right) \qquad \text{.. (V)}$$

Measurements at temperatures below 1^0K are of particular interest as a test of this relation.The lattice heat capacity at the transition temperature for "Sn", "In", "Tl", and "Pb" with transitions at $3.7^0 3.4^0$, 2.4^0, and 7.2^0K is 45 percent, 77 percent, 83 percent, and 94 percent of respective total heat capacities Some soft superconductors together with lower transition temperatures, on the other hand, have tiny lattice heat capacities; the equivalent fraction for "Al", "Zn", "Cd" with transition temperatures of 1.2^0K, 0.8^0K, and 0.5^0K are 1%, 3%, and 3%, respectively. is 45 percent, 77 percent, 83 percent, and 94 percent of respective total heat capacities.

CALCULATION

At very low temperatures, specific heat in ionic crystals changes proportional to T^3. However, even modest concentrations of paraelectric impurity have a significant influence on the ionic crystals' specific heat. At low temperatures, the Temperature variation indicates araisein specific heat. If the presence of impurity causes the states to be separated into energy levels the maximum contribution of impurity to specific heat at a given temperature ($\frac{\Delta}{K}$). Schottky anomaly is the name given to such peaks in specific heat. This oddity, which can be seen in most impurity systems, may be explained using the single multiplet tunnelling model [10, 11].The contribution of impurities to the specific heat for the<111> and <110> + <111>tunnelling model have already developed by the Raj Kumar et al s [12, 13]. In this paper, we confine ourselves to determining the expression for the entropy of <100> + <111> tunnelling model. The average energy to "N" impurity per unit volume at temperature "T" can be computed by dividing the ground state tunnelling multiple into "P" levels.

$$E = \frac{N\sum_{I=1}^{P} g_i\, E_i \text{Exp}\left(\frac{-E_i}{kT}\right)}{\sum_{I=1}^{P} g_i\, \text{Exp}\left(\frac{-E_i}{kT}\right)} \qquad \text{... (V)}$$

Where "g_i" denotes the level's degeneracy,"E_i" denotes the energy divide between the first and i^{th} levels.

Specific Heat for<100> + <111> model

Raj Kumar et al. have already discovered the contribution of impurities to the specific heat for <110> + <111>tunneling models [12], inspired from above I have calculated the impurity contribution for <100> + <111>tunneling model

$$C_v = 6Nky^2\left[\frac{e^{-y}+5e^{-2y}+7e^{-3y}+4e^{-4y}+2e^{-5y}}{(1+3e^{-y}+6e^{-2y}+3e^{-3y})^2}\right] \qquad \text{.. (VI)}$$

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On the expanding the exponential and Neglecting its higher terms we get the following simplified forms as:

$$C_v = 6Nky^2 \left[\frac{(1-Y) + 5(1-2Y) + 7(1-3Y) + 4(1-4y) + 2(1-5y)}{[1 + 3(1-Y) + 6(1-2Y) + 3(1-3Y)]^2} \right]$$

$C_v = 6Nky^2 \left[\frac{(19-58y)}{(13-24y)^2} \right]$ ………………………………… (VII)

or

$C_v = \frac{Nk}{96} (19 - 58y) \left[1 + \left(-\frac{13}{24y} \right) \right]^{-2}$ …………………...........………….............................………..… (VIII)

Now on Expanding using Binomial Theorem and Neglecting Higher terms, we get

$$C_v = \frac{Nk}{96} (19 - 58y) \left[1 + \frac{13}{12y} \right]$$

Replacing y $= \frac{E}{kT}$

$C_v = \frac{Nk}{96} \left(19 - \frac{58E}{kT} \right) \left(1 + \frac{13kT}{12E} \right)$ ……………………………...…… (IX)

Using concepts of Kinetic theory, the mean energy of one molecules

E $= \frac{3}{2}$ kT

$$C_v = \frac{Nk}{96} \left(19 - \frac{58 \times \frac{3kT}{2}}{kT} \right) \left(1 + \frac{13kT}{12 \times \frac{3kT}{2}} \right)$$

$C_v = \frac{Nk}{96} (19 - 87)(1 + \frac{13}{18})$

$C_v = \frac{Nk}{96} (-68) \left(\frac{31}{18} \right)$

$C_v = Nk(-1.22)$

$C_v = -1.22$Nk ……………………………………..…........…….. (X)

Entropy for <100> + <111>tunneling model

Since the entropy [11] equation is written as

S $= \int \frac{1}{T} C_v dT$ …………………………...………………..…..... (XI)

Hence putting the value of C_v from equation (X) in the equation (XI), we get the entropy formula for the<100> + <111>tunneling model.

$S = \int \frac{1}{T} (-1.22Nk) dT$

$S = (-1.22Nk) \int \frac{1}{T} dT$

$S = (-1.22Nk) \log_e T$ …………………………………..…..... (XII)

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The Variation of Entropy with temperature for the model <100> + <111>when Electric field applied along <100> is shown below using equation (XII) impurities likes $RbCl:Ag^+$ are shown below in Figure.1

RESULT AND DISCUSSION

Equation (XII) for entropy is the result of the current theoretical investigation for the <100> + <111> tunneling model. This finding can be used in future theoretical research to explain aberrant outcomes from a variety of experimentally and theoretically available systems. From Figure 1,we can deduce that the entropy of <100>+<111> tunnelling model increases as the temperature rises. It may also be used to explain tautomerization observed in single prophycene molecules like Cu (111) as well as Au (111) surfaces utilizing a combination of scanning tunnelling and low temperatures. Scanning Tunneling Microscopy (STM) and density functional theory (DFT) experiments [14, 15] .

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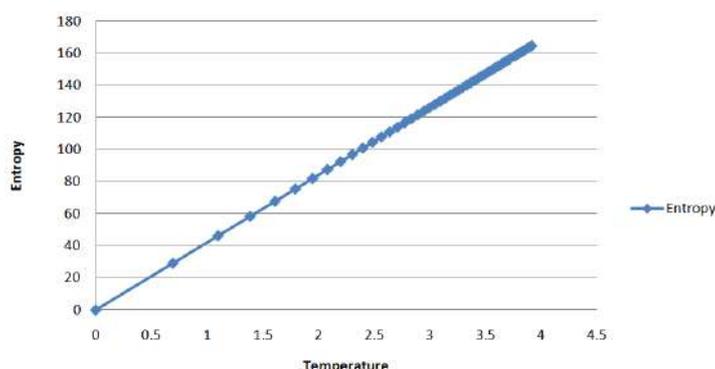
Figure: 1 The Variation of Entropy with temperature for the model <100> + <111>when Electric field applied along <100> is shown below using equation (XII) impurities likes $RbCl:Ag^+$ are shown below in Figure.1

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RESEARCH ARTICLE

Lanthanide (III) Complexes with Tridentate Schiff Base Ligand, Antioxidant and Antimicrobial Studies

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ABSTRACT

The tridentate N3-type Schiff base 2,6-di(pyridin-2-yl)-4-(thiophen-2-yl)pyridine was synthesized from the condensation reaction of 2-acetyl pyridine and thiophene -2-carbaldehyde with ammonia using one pot methodology. This hybrid ligand was used for synthesis of lanthanide complexes (La, Sm, and Eu) as novel potential biological agents. The lanthanide complexes were characterized on the basis of elemental, FT-IR, Uv-Visible, Mass spectrometry as well as molar conductivity. The complexes were screened for in vitro antibacterial and antifungal activity against the multidrug resistant pathogens, such as *Escherichia coli, Staphylococcus aureu*s and *Aspergillus niger.* The antimicrobial results revealed that Sm^{+3} complex has a good potency against different gram positive and gram negative bacteria in comparison with other complexes. All lanthanide complexes showed a moderate antioxidant activity with IC_{50}. The DPPH· radical scavenging effects of the Schiff base ligand and its Ln (III) complexes were screened. The Ln (III) complexes were significantly more efficient in quenching DPPH· than the free Schiff base ligand.

Keywords: Terpyridine, One pot method, Lanthanides, Antioxidant studies, Anti-microbial activity.

INTRODUCTION

Terpyridine or tpy, discovery in the early 1930 s by Morgan and Burstall [1, 2], terpyridine, (tpy) has piqued people's curiosity. Since this ground breaking study, a slew of derivatives have been created by grafting various substituents onto the terpyridine core utilizing a variety of synthetic methods [3- 6]. Terpyridine molecules have three nitrogen

atoms, making them capable of chelating a wide spectrum of ions from the main group, transition metals, and even lanthanides. These molecules have found uses in a variety of sectors, including photovoltaic materials [7,], Nano materials [8], biomarkers [9], medicinal chemistry [10–12], and catalysts [13]. Terpyridines and their derivative complexes are also commonly used in supramolecular chemistry. Lanthanide coordination chemistry has been one of the most studied topics of chemistry in the last decade [14-16]. The fascinating structures [17-18] and prospective applications in a wide range of fields, such as diagnostic tools in biology [19], catalysis [20], luminescence [21], and magnetism [22], pique chemists' interest in these areas of research. The acyclic ligand's flexibility and many coordination sites allow for a variety of structures with lanthanide ions [23-24].This sort of ligand can be used to make a variety of coordination molecules. The literature contains several reports on its antibacterial [25], antifungal [26], anticancer [27], and depressive [28] properties. Because of the foregoing, the goal of the study is to prepare, characterize, and assess the biological activity of lanthanide complexes. From the condensation reaction of 2-acetyl pyridine and thiophene -2-carbaldehyde with ammonia, we can make tridentate Schiff base 2, 6-di(pyridin-2-yl)-4-(thiophen-2-yl)pyridine. Various approaches were used to establish the structure of the ligand and its lanthanide complexes (La, Sm, and Eu), including elemental analysis, molar conductivity, UV–visible, NMR, and infrared (IR) spectroscopies. The biological activities of the free ligand and its lanthanide complexes were further studied utilising agar well diffusion and minimum inhibitory concentration (MIC) methods to assess their antibacterial activity against diverse harmful bacterial strains. In addition, using the 2,2 diphenyl1picrylhydrazyl (DPPH) test method, the antioxidant properties of the free ligand and its complexes were assessed in terms of their radical scavenging capability.

EXPERIMENTAL

Materials and reagents [Ln (NO$_3$)$_3$(H$_2$O)$_6$] (Ln = La, Sm,), [Eu(NO$_3$)$_3$(H$_2$O)$_5$], thiophene -2-carbaldehyde , 2-acetyl pyridine and ammonia were purchased from Sigma Aldrich Chemical Company. All other solvents and reagents were of analytical grade are used without further purification.

Synthesis of Schiff base ligand L2
To a solution of 2-acetylpyridine (5 ml; 41 mmol) in ethanol (100 mL) are added thiophene -2-carbaldehyde (2.5 ml; 20 mmol), potassium hydroxide pellets (0.5 g; 67 mmol) and 25% aqueous ammonia (60 mL) using one-pot methodology. The reaction mixture was stirred at room temperature for 24 h. The solid was then filtered on a glass-sintered funnel and washed with ice-cold 50% ethanol until washings were colourless. The product was dried under vacuum. The ligand was obtained as a brown solid. An analytical sample was obtained by recrystallization in ethanol. Molecular formulae of ligand C$_{19}$H$_{13}$N$_3$S. ^{1}HNMR (400MHz, CDCl$_3$) δ: 7.25-7.17(t,1H,J=8.57), 7.54-7.46(m,2H,J=7.8),7.79-7.74(d,1H,J=8.5),7.94-7.93(d,1H,J=6.1),8.05-7.97(m,2H,J=8.1),8.62-8.60(d,4H,J=1.7),8.74-8.71(d,2HJ=5.1). ^{13}CNMR (100MHz, CDCl$_3$) δ: 116.52, 121.48, 125.23, 127.34, 129.17, 129.76, 138.07, 141.10, 143.39, 149.92, 155.21, 156.37. The synthesis of ligand L2 shown in scheme1.

Synthesis of lanthanide complexes
Lanthanide complexes were prepared by refluxing and stirring 1:2 ratio of metal [La (NO$_3$)$_3$(H$_2$O)$_6$], (0.5 g, 0.1 mmol) and ligand (1g, 0.3 mmol) on a water bath for 8 h. After cooling to room temperature the precipitate was collected then washed with methanol for several times and dried in vacuum at room temperature All other Sm and Eu complexes were prepared in a similar manner.

RESULTS AND DISCUSSION

Characterization of Schiff base ligand L2
The ligand L2 structure is identified by elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectral studies. The synthetic route for the ligand L2 is presented in Scheme 1. The results of elemental analysis with molecular formula and the

percentage yield of the ligand L2 are presented in Table 1. Mass spectrum of ligand L2 as shown in Figure 1. ^{1}HNMR spectrum of ligand in Figure 2 and ^{13}CNMR spectrum of ligand as shown Figure 3.

Characterization of lanthanide complexes

The isolated solid complexes were characterized by elemental analysis, Mass, IR, molar conductance, UV–visible spectroscopy. All these complexes are stable in air at room temperature, non-hygroscopic, insoluble in water and most organic solvents and soluble in DMF and DMSO solvents. The yields, molar conductivity values and elemental analysis of Ln(III) complexes and ligand L2 are summarized in Table 1. The stoichiometry of the complexes derived from elemental analysis correspond to the general formula $[Ln(L2)_2(NO_2)x,]$. These propositions are also in accord with molar conductivity measurements, IR, and UV–Visible presented in the following sections. The Mass spectrum of $[Sm(L1)_2(NO_3)_2]$ complex as shown in Figure 4.

Molar conductivity measurements

The molar conductivities of the Ln (III) complexes in DMF solutions at 25 °C were measured and tabulated in Table 1. The molar conductivity values are in the range reported for 1:1 electrolytes reveals that the all complexes are non-electrolytes ($\Lambda_m = 29.27 – 37.28 \ \Omega^{-1} \ cm^2 \ mol^{-1}$) [29].

Infrared spectroscopy

The important infrared frequencies along with the tentative assignment of the ligand L2, and its respective Ln(III) complexes are presented in Table 2. The IR spectra of the La complexes displayed the ligand characteristic bands with the appropriate shifts due to complex formation and the infrared of the other Ln(III) complexes displays the same manner. It was found that the C=N of the azomethine group occurs at 1642 cm^{-1} in the free Schiff base. After complexation, these bands are shifted to lower wave numbers indicates a double bond character of the imine bonds and a coordination of the azomethine nitrogen atoms to the Ln(III) ion [30 ,31] This coordination was further supported by the appearance of a medium intensity band around 454 to 440 cm^{-1} assigned to (Ln–N) vibration. This coordination was further supported by the appearance of a medium intensity band around 471 to 464 cm^{-1} assigned to (Ln–O) vibration. The infrared spectrum of La (III) complex displays several intense bands at 1477 cm[-1](v_1), 1029 cm[-1](v_2), 817 cm[-1](v_3) and 1286 cm^{-1} (v_4) and they are assigned to the coordinate nitrate ion (C_2V), these nitrate group can acts as bidentate coordination. The frequency separation ($\Delta v = v_1 - v_4$) between the asymmetric and symmetric stretching of this group can be mode to distinction between these binding states. The difference between is approximately 191 which can be suggested that the coordinate NO_3^- cm[-1] ions in the complexes are bidentate coordination.

UV–Visible spectroscopy of complexes

UV–Vis absorption spectra of the lanthanide complexes were carried out in DMF solvent at room temperature. The UV–Vis spectra values of the maximum absorption wavelength (λ max), frequency of Ln (III) complexes {Ln = La, Sm, and Eu } and their assignment are listed in Table 3. [32- 33]. The Electronic spectrum of $[Sm(L2)_2(NO_3)_2]$complex as shown in the Figure 5. Tentative general structure for $[Ln (L1)2(NO_3)_2]$complexes as shown in the Figure 6.

Antimicrobial activity

The MIC of the ligand L2 and its Ln(III) complexes against different types of Gram-negative ,Gram-positive bacteria and Fugal activities were determined and tabulated in Table 4. DMSO was used as a negative control and CEFADROXIL used as positive standards for antibacterial. FLUCONAZOLE was used as a reference for antifungal studies. These compounds exhibit moderate to strong antimicrobial activity. Comparatively a better activity is found for the bacteria rather than the fungi. The Sm(III) complex exhibits a higher activity than the other metal complexes towards fungal species. The Sm(III) complex shows a good activity, especially against the Gram-negative and Gram positive bacteria such as *E. coli* and *S.aureus*. The antimicrobial activity of the complexes is greater than those of the free ligand, this indicates that the complexation to metal enhances the activity of the ligand. This is explained on the basis of Overtone's concept and chelation theory. [34, 35].

Antioxidant activity

The antioxidant activity of the ligand **L2** and its Ln(III) complexes have attracted increasing interests and been substantially investigated [36]. Figure 7. Shows the plots of DPPH• free radical scavenging activity % for the ligand **L2** and its Ln(III) complexes. It is obvious that the scavenging activity increases with increasing sample concentration in the range tested. As shown in Table 5. The free radical scavenging activity of ligand L and its lanthanide complexes was determined by their ability to bleach DPPH radical, which has been previously reported. This assay provides information on the reactivity of a compound with a stable free radical. Ln (III) complexes are significantly more efficient in quenching DPPH radical than free ligand L. The antioxidant activity of the Schiff base is due to hydrogen- or electron-donating tendency to DPPH• so as to produce a stable diamagnetic molecule. Molecules with greater ability to donate electrons or hydrogen to DPPH• have a higher antioxidant activity. The interaction of ligand L with the positively charged Ln (III) increases the electron density drawn from the nitrogen and oxygen atoms, which makes the N— H or O— H bonds more polarized. Among the examined lanthanide complexes, the Sm (III) complex has the highest activity. It can quench the DPPH radical more efficiently than the other lanthanide ions. In them, complex Sm (III) exhibited excellent antioxidant activity compare with Eu (III) and La (III) complexes found to have IC50 value and (%) inhibition. Addition of Ln complex to DPPH· Radical as shown in the Figure 8.

CONCLUSION

In this work the tridentate Schiff base ligand **L2** and its Ln(III) complexes are synthesized and characterized. Analytical and spectral data reveal that the ligand **L2** coordinates to the central Ln(III) ion [La(III), Sm(III) and Eu(III)] with coordination number 10. The antimicrobial activity results show that the synthesized Ln(III) complexes possessed good antibacterial activity higher than that of the corresponding ligand **L2**. The antioxidant activity of the Ln(III) complexes on DPPH• is concentration dependent and higher than that of the free ligand L2. The $[Sm(L2)_2(NO_3)_2]$ complex show both anti-microbial and anti-oxidant higher than compared with $[La(L2)_2(NO_3)_2]$ and $[Eu(L2)_2(NO_3)_2]$.

ACKNOWLEDGEMENTS

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Table 1: Analytical and Physical Characterization Data for Ligand L2 and Its Metal Complexes

Compound	Molecular Weight Found (Calculated)	Melting point (^{0}C)	Colour Yield (%)	Elemental analysis Found (calculated)				Molar conductivity Ω^{-1} cm^2 mol^{-1}
				C(%)	H(%)	N(%)	Ln(%)	
Ligand L2	315.39 (315.28)	142-144	Brown (60.45)	72.34 (72.37)	4.15 (4.15)	13.32 (13.32)	-	-
[La(L2)$_2$(NO$_3$)$_2$]	893.06 (893)	189-191	Yellow (72.86)	51.09 (51.10)	2.93 (2.93)	12.54 (12.54)	15.56 (15.56)	37.28
[Sm(L2)$_2$(NO$_3$)$_2$]	904.36 (904.5)	210-212	Brown (73.73)	50.46 (50.45)	2.89 (2.89)	12.38 (12.38)	16.58 (16.61)	32.25
[Eu(L2)$_2$(NO$_3$)$_2$]	906.04 (906)	221-223	Orange (78.43)	50.36 (50.36)	2.89 (2.89)	12.36 (12.36)	16.77 (16.77)	29.27

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Table 2: Infra-Red Spectral Data for Ligand L2 and Their Lanthanide Complexes

Compound	v(C=N)	v(NO₃⁻)					v(M-O)	v(M-N)
		υ_1	υ_2	υ_3	υ_4	υ_1- υ_4		
Ligand L2	1642	-	-	-	-	-	-	-
[La(L2)₂(NO₃)₂]	1591	1477	1029	817	1286	191	471	440
[Sm(L2)₂(NO₃)₂]	1601	1468	1045	824	1263	205	468	449
[Eu(L2)₂(NO₃)₂]	1615	1471	1052	838	1281	190	464	454

Table 3: Electronic Spectral Data for Lanthanide Complexes

Compound	Wavelength λ max (nm)	Frequency (cm⁻¹)	Band assignments
[La(L2)₂(NO₃)₂]	289	34,602	$\pi \rightarrow \pi^*$ transition
	330	30,303	$\pi \rightarrow \pi^*$ transition
	355	28,169	$\pi \rightarrow \pi^*$ transition
	369	27,100	$\pi \rightarrow \pi^*$ transition
[Sm(L2)₂(NO₃)₂]	283	35,335	$\pi \rightarrow \pi^*$ transition
	325	30,769	$\pi \rightarrow \pi^*$ transition
	351	28,490	$\pi \rightarrow \pi^*$ transition
	365	27,397	$\pi \rightarrow \pi^*$ transition
[Eu(L2)₂(NO₃)₂]	290	34,482	$\pi \rightarrow \pi^*$ transition
	334	29,940	$\pi \rightarrow \pi^*$ transition
	348	28,735	$\pi \rightarrow \pi^*$ transition
	370	27,027	$\pi \rightarrow \pi^*$ transition

Table 4: Antimicrobial Activity of Schiff Base Ligand (L2) and Its Lanthanide Complexes at Various Pathogens

Tested compounds	Gram(-) bacteria	Gram(+) bacteria	Fungal species
	E.coli	*S.aureus*	*A.niger*
Ligand L2	-	-	
[La(L2)₂(NO₃)₂]	++	+	+
[Sm(L2)₂(NO₃)₂]	++	++	++
[Eu(L2)₂(NO₃)₂]	++	+	+
DMSO (- ve control)	-	-	-
FLUCONAZOLE (+ ve control)			+++
CEFADROXIL (+ ve control)	+++	+++	

NOTE: Inhibition zone :0 mm(-)inactive; 1-5 nm(+)= less active; 6-10 mm(++) moderately active; 10-15 mm(+++) highly active;

Table 5: Scavenging Effect of Schiff Base Ligand (L2) and Its Lanthanide Complexes on DPPH Free Radical At Various Concentrations

Compound	DPPH scavenging activity (%)				IC_{50}
	25μM	50μM	75μM	100μM	
Ligand L2	36.42	56.13	67.42	85.63	-
[La(L2)₂(NO₃)₂]	27.13	38.42	47.11	56.38	76.18
[Sm(L2)₂(NO₃)₂]	29.13	40.26	51.32	64.45	56.52
[Eu(L2)₂(NO₃)₂]	33.03	48.19	59.21	72.33	65.07
Ascorbic acid	32.42	43.36	56.67	69.43	41.12

Scheme 1: synthesis of Ligand L2
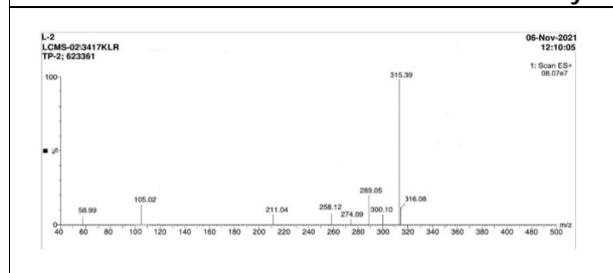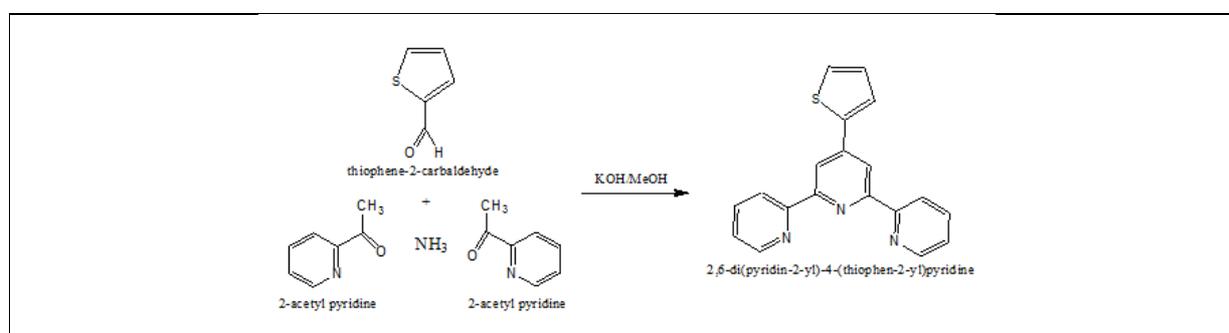
Figure1: Mass spectrum of Ligand L2
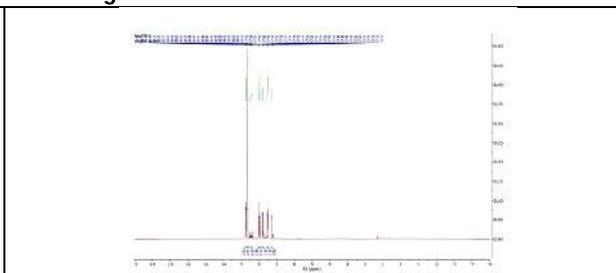
Figure 2: ^{1}HNMR spectrum of Ligand L2
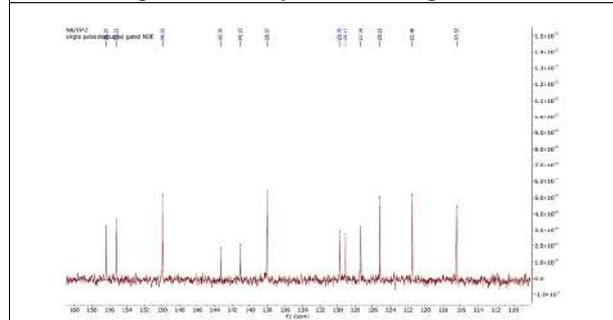
Figure 3: ^{13}C NMR spectrum of Ligand L2
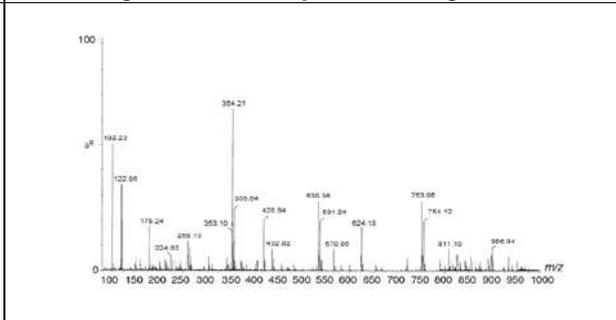
Figure 4: Mass spectrum of [Eu(L2)₂(NO₃)₂]complex.

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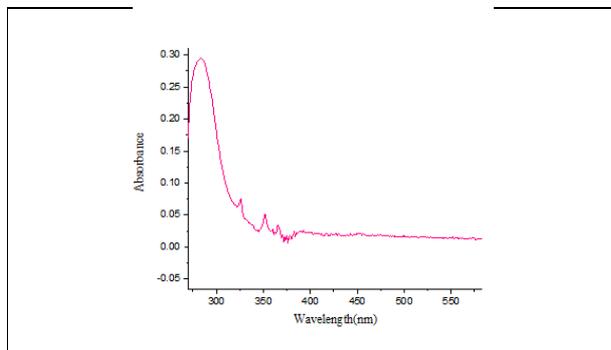
Figure 5: Electronic spectrum of [Sm(L2)$_2$(NO$_3$)$_2$] complex
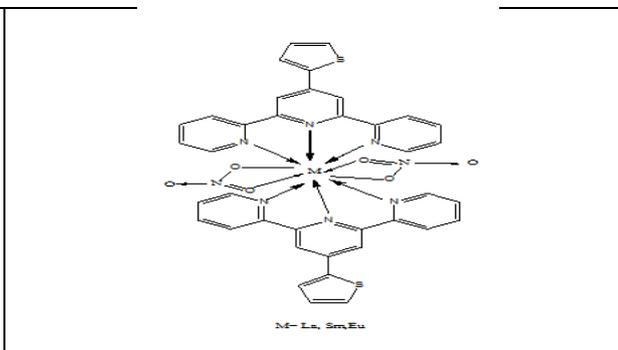
Figure 6: Schematic representation of lanthanide metal [M(L2)$_2$(NO$_3$)$_2$]complex
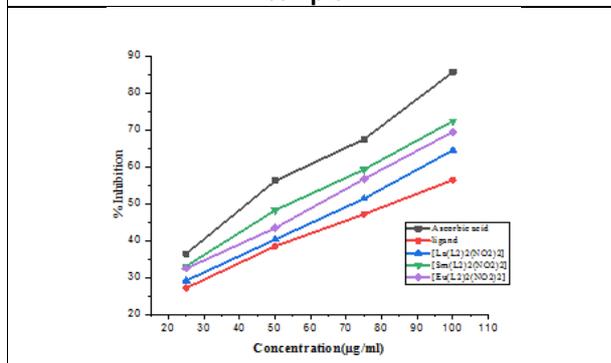
Figure 7: Antioxidant activity of the ligand and the lanthanide complexes
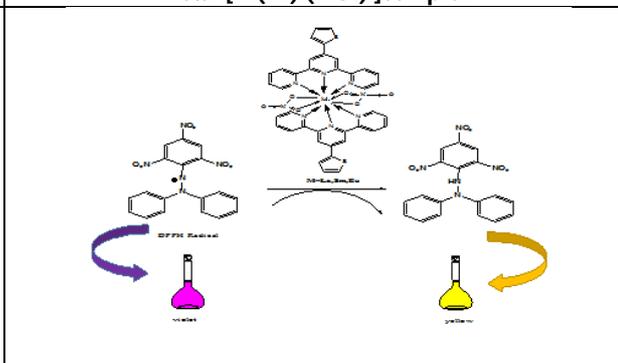
Figure 8: Addition of Ln complex to DPPH. Radical

RESEARCH ARTICLE

Blink and Neck Based Gesture Controlled System to Assist Physically Challenged People

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ABSTRACT

The wellbeing administration area has been ceaselessly attempting to further develop the help given to the physically challenged individuals needing versatility help. In this day and age, human-machine cooperation is becoming far and wide. In this way, with the presentation of new advancements the hole among machines and people is being decreased to facilitate the way of life. This is a Real-time observing framework, by which people interface with the machine through movements of the neck. This is a massive guide for individuals for whom versatility is an incredible thing. This paper manages the plan and execution of an accelerometer-based neck movement-controlled framework remotely utilizing a little low-cost, 3-pivot accelerometer. An alarm system based on blink counts for essential need fulfillment is also incorporated. This framework is intended for incapacitated individuals who generally rely upon others in their routine particularly in getting starting with one spot then onto the next. This framework will help the physically challenged people to make them self-subordinate with the end goal of the movement for which these individuals are reliant on others for the vast majority of the occasions. Additionally, real-time health care monitoring, report analysis, and live location tracking are stored for a The subsequent interval of time is utilizedin case of emergencies.

Keywords: Accelerometer, Ultrasonic sensing, oxygen level monitoring, pulse rate sensing, Temperature measuring, GPS tracking, Blink count sensing, Report generating

INTRODUCTION

The difficult issue looked at by quadriplegics and incapacitated individuals are their requirement for autonomous mobility. This wheelchair is exceptionally intended for those patients who can't move their appendages except their heads. They need outside help to play out their everyday exercises. The fundamental goal of this undertaking is to give a mechanized framework for disabled persons to manage the wheel turn which is dependent on the neck movement of a genuinely tested individual. This wheelchair is worked by distinguishing the movement of the neck also giving such incapacitated patients, a specific level of autonomy and opportunity in their movement. To work with these individuals for their autonomous movement, the accelerometer is fitted on the individual neck. In light of the neck movements, the accelerometer will drive the wheel fitted o the wheelchair. The wheel seat can be driven in any of the four bearings and it can likewise be constrained by utilizing android. The robotized wheelchair depends on a basic electronic control framework and the mechanical game plan that is constrained by a Controller. We utilized an accelerometer one of the parts of the inertial estimation unit (IMU).

Accelerometer distinguishes the movements of the neck and conveys messages to the miniature regulator which is node MCU. The accelerometer is utilized to gauge the movements of the neck and convert them to a simplesignal by utilizing encoder and decoder conventions and sending them to other associated gadgets by utilizing an Retransmitted and an RF recipient. A microcontroller is utilized to break down the encoded flags and make an interpretation of them into helpful orders. A Node MCU microcontroller is utilized to straightforwardly associate the gadgets to perform completely in the remote control. The Temperature and the Heartbeat sensors continually measure the boundaries which are additionally shown on the application.

The plan we proposed is financially at a low cost and straightforward. No particular adjustment is needed before use. The extra attribute of a wheelchair in the equipment configuration is that it can be worked in 2 modes for example either physically or by utilizing the patient neck. By utilizing an android application, the movement count is of four ways. The Ultrasonic sensor assists the user by identifying the objects so that the user will stay away from objects until the client is capable to deal with the work. Temperature, oxygen sensor, eye blink sensor, and Heartbeat sensors continually measure the boundaries of the patient and if it passes its boundary esteems then the buzzer rises with their location with the help of GPS.

PROPOSED METHODOLOGY

The capacity of the accelerometer is to measure the level of shifting of the neck developments. The upsides of the estimations are simple factors and are pretty much futile now. These simple factors then, at that point, get moved to the Node MCU microcontroller, is modified to perceive the bunch of simple factors through a surge of simple qualities. Those are get via accelerometer and relegate explicit capacities as per its programming. Block diagram of the proposal is depicted in the Fig 1. The Artificial Intelligence (AI) of controller substructure begins determining watch for simple elements meets its predetermined values and which it transfers the capacity, results in LOW or HIGH, in the decision and control section. If the microcontroller does not detect a match from the received value, the factors are discarded, and the microcontroller continues to obtain more current estimation codes from the accelerometer. This process will continue as far as a correct is discovered. When a correct variable is found, the microcontroller authorizes the allot capacity to it and move the data in advanced structure elected handed on in digital design to be encipher by the encoder Integrated chip and further transfers at a specific frequency (in this study it is 433MHz). The process is now broken. Temperature, oxygen, eye blink, heartbeat, and ultrasonic sensors are associated with a similar Node MCU controller board. It ceaselessly screens the patient's temperature and heartbeat. Fig 2. Depicts the architecture chart of the monitoring system.

Assuming that the qualities in the sensor passed their boundary values then the inbuilt buzzer in the framework starts buzzing and it alerts the relatives by a notification message. The ultrasonic sensor is utilized to keep away from the obstacles like edges or flights of stairs in the path of the wheelchair. The proposed framework comprises of NODE MCU, heartbeat sensor, eye blink sensor, oxygen sensor, temperature sensor, ultrasonic sensor, Voltage controller, Android application, Motor driver (L293DIC), DC motor, accelerometer ESP8266 module. This block diagram clarifies the connection of the proposed neck movement-based wheelchair to help physically challenged individuals. The observing framework constantly checks and records the conditions of being of the physically challenged individuals and sends the information to the android application which is an application utilizing NODE MCU controller. The associated sensors send information to the NODE MCU. The NODE MCU interface with the web through gesture wi-fi and send the information received from those associated sensors to the Internet.

The accelerometer consists of the x, y, and z-axis, where x, y, z axis are connected to A0,A1 and A2 port of Arduino respectively.L293DIC consists of four inputs IN1,IN2, IN3, IN4 and four outputs OUT1, OUT2, OUT3, and OUT4. Where it is connected to pins 8, 9, 10, and11 the output of the L293DIC pins OUT1,2,3,4istied the Wheels of the chair. The ultrasonic sensor consists of a trig pin and echo pin. The echo pin is tied to the A0 pin of the Arduino board and the trig pin is tied to A1 pin of the Arduino board. The heart beat sensorisattache The heartbeat sensor is attached to the A2 pin of the Arduino Board and the Temperature sensor is attached to the A3 pin of the Arduino board. The oxygen sensor isattached to the A4 of the Arduino board and the GPS module is attached to the A5 pin of the Arduino. For display of the report, the display module is attached to the 0 and 1 stpin of the Arduino board.

WORKING PRINCIPLE
The Accelerometer put on the patient's neck estimated the tilt Produced by the neck movement. The patient was made to shift his/her head in the right, left, front, or in reverse course. The tilt compares to the Analog voltage (values). ADC is used to convert the analog signal into digital values in which digital Correspond to the amplitude. 5. When we switch on the power supply, the accelerometer will show some value then the value is been noted and get stored in the Node MCU board. Typically, the range of the accelerometer is from 300 to 400. For each direction, it will have some limit values. The obtained digital signal (value) is sent to the motor driving IC (L293D). The source code for Node MCU has been written in the embedded c language. Based on the source code the Node MCU drives the motor driver (L239D) which helps in driving the DC motor simultaneously. The input voltage is 12v 2 Amps and changing over into 5V 2Amps with the help of 7805 Voltage regulator step-down transformer has been utilized to venture down the voltage from (230V) to Required Voltage (12V battery). A bridge rectifier has been utilized to change over an alternating current input into a direct current output (2Amps).

A filter circuit has been utilized to dispose of the exceptional high frequencies(distortion) that are being there on ac input lines.7805 voltage regulator has been used to maintain the fluctuations, is a famous voltage regulator integrated circuit I(IC) and to control the voltage in there commended range that can be tolerated by the electrical equipment using that voltage. The patient is checked by utilizing the Heartbeat sensor and Temperature sensor etc... Ifany, Abnormality is found in the health condition, assuming heartbeat or Temperature is low or high, it will be shown or notified on the Android application. If heartbeat (more than 72bpm) and temperature (more than 37 degree Celsius or 99.5 F) is varied message is sent. Obstacle detection is automatically done with the help of an ultrasonic sensor.

SOFTWARE IMPLEMENTATION
In this project, we are using Proteus software for implementation purposes. We used Potentiometer for varying the values of sensors and a motor driver IC is used to drive the wheels of a wheelchair. We used a buzzer to indicate how the patient's health condition like whether it is normal or abnormal. If it is abnormal then the buzzer starts an alarm.

OUTPUT

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As an output, the motor starts running while varying the values of the potentiometer. If the potentiometer rises above a certain value, then it starts buzzing on. LED is used to indicate the power supply. The output of the simulation is shown in figure

CONCLUSION

This undertaking is created utilizing NODE MCU, Accelerometer, Motor driver IC, Temperature, Heartbeat, and Ultrasonic sensors effectively. This framework is intended for incapacitated individuals who typically rely upon others in their day-to-day routine particularly in getting starting with one spot then onto the next. This framework will help the impeded and paralyzed people groups to make them self-subordinate with the end goal of development for which these individuals are reliant upon others the majority of the occasions. An individual with debilitated legs and arms can utilize this wheel seat proficiently in case he can move his neck. The android application component would bring more accommodation for the debilitated individuals over motor control and temperature and oxygen and heartbeat, blink analyzing and GPS is continually estimated and during crises message is shipped off the relatives.

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Fig 1. Overall Block Diagram	Fig 2. Monitoring System
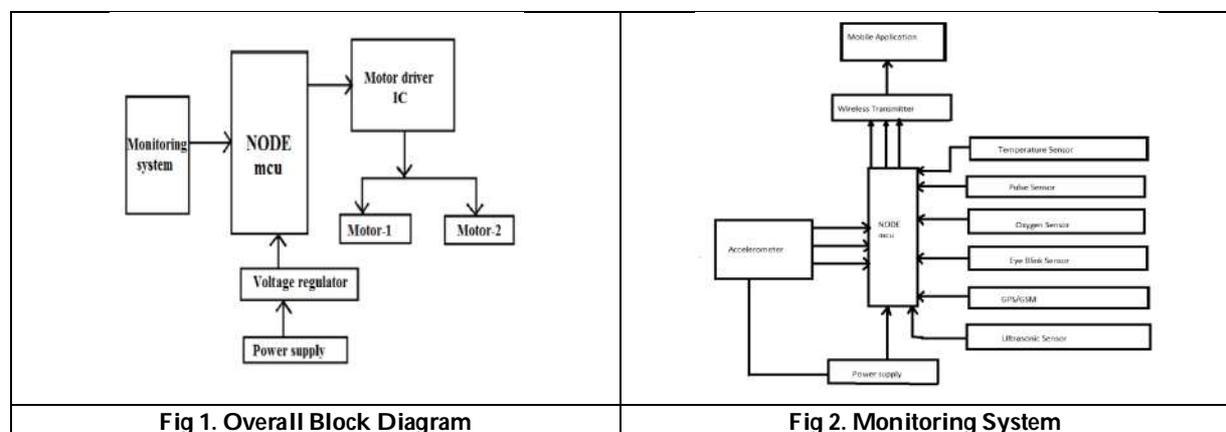
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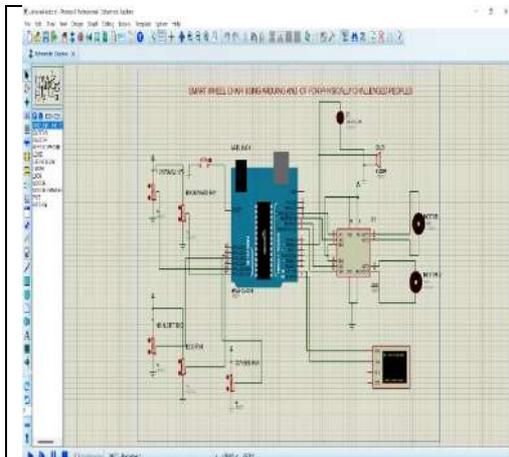
Fig. 3. Proteus Implementation.	**Fig.4. Output**

RESEARCH ARTICLE
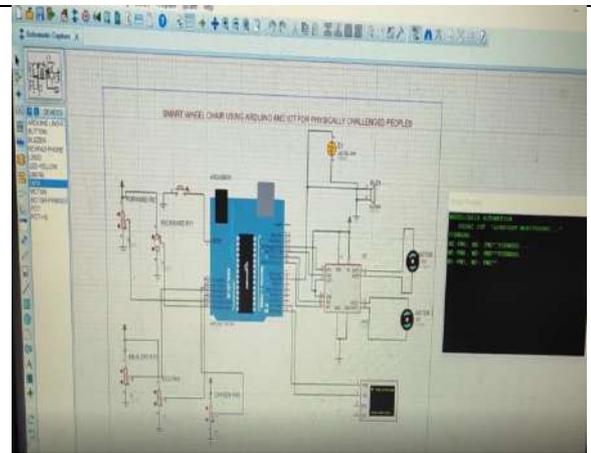
Real Time Driver Drowsiness Detection System using Multi-Task CNN

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ABSTRACT

The face, being a vital aspect of the body, communicates a great deal. When a driver is tired, his or her facial expressions, such as blinking frequency, are different from what they are in a normal state. In this paper, we propose a system that uses video images to detect the drivers' fatigue status, such as the duration of eye closure, without requiring them to wear any devices. The system introduces a new face-tracking algorithm to improve tracking accuracy due to the shortcomings of previous algorithms. the system also devised a new facial region detection method based on 68 key points. then the system uses these facial regions to assess the drivers' condition.By combining the features of the eyes, the system can alert the driver using a fatigue warning. The experimental results showed that achieved around 92% accuracy.

Keywords - Drowsiness, Distraction, Eye detection, Eye Tracking, Face Detection, Convolutional Neural Networks,Deep learning

INTRODUCTION

One of the leading causes of car accidents and deaths is drowsy driving. As a result, detecting and indicating driver fatigue is a hot topic of research. The bulk of available approaches are car-based, behaviorally -based, or physiologically-based. Some approaches are invasive and distract the driver, while others demand the use of pricey sensors and data processing. As a result of the current investigation, a low-cost, real-time drowsiness detection system with adequate accuracy has been developed. Drowsiness is detected using adaptive thresholding developed

on the basis of the values of facial landmarks on the detected face. In the proposed system, a webcam records the video, and image processing techniques are used to detect the driver's face in each frame. Face tracking and facial key point detection are used in a novel system for assessing a driver's level of weariness. To track the driver's face using CNN. (Convolution Neural Network), and then to recognize facial areas utilizing facial key points. The eyes will then be detected, and if they are closed, the alert system will appear. Transportation demands a more rapid expansion of car parks. The automobile is now a necessary form of mobility for most people. In 2017, 97 million automobiles were sold globally, representing a 0.3 percent increase over 2016 [1]. In 2018, the total number of vehicles in use was estimated to be more than 1 billion [2]. Although the vehicle has altered people's lifestyles and made daily tasks easier, it is also linked to a number of negative consequences, such as traffic accidents. According to data published by the National Highway Traffic Safety Administration [3], there were 7,277,000 traffic accidents in the United States in 2016, with 37,461 deaths and 3,144,000 injuries. Fatigued driving was responsible for roughly 20% to 30% of the traffic accidents in these studies.

As a result, fatigued driving poses a significant, hidden risk intraffic accidents. The fatigue-driving-detection system has become a prominent research area in recent years. Subjective and objective detection methods are the two types of detection procedures. A driver must participate in the subjective detection method, which is related to the driver's subjective views through procedures such as self-questioning, and is used to estimate the vehicles being driven by weary drivers, supporting the drivers in planning their schedules accordingly.The objective detection approach, on the other hand, does not require drivers' feedback because it analyses the driver's physiological state and driving-behavior parameters in realtime [4]. The information gathered is used to determine the driver's level of weariness. In addition, objective detection is divided into two categories: contact and non-contact. Non-contact is less expensive and more convenient than contact because the system does not require computer vision technology or a sophisticated camera, allowing the gadget tobe used in more cars

LITERATURE SURVEY

Vehicle-based, behavioral based, and physiological-basedtechniques are all used to identify drowsy driving. In car-based detection, several parameters are there. Driver drowsiness is defined as the detection of any abnormal change in the values of the metrics. To identify tiredness, the visual behavior of the driver is examined, such as eye blinking, eye closing, yawn, head bending, and so on. Both vehicle-based and behavioral-based assessments are non-intrusive because the former does not require any sensors on the drivers, while the latter simply requires a basic camera to detect the traits. Physiological signals such as ECG, EOG, EEG, heartbeat, pulse rate, and others are monitored in physiological-based methods, and drowsiness or weariness is recognized using these metrics. Because the sensors are linked to the driver, the physiological based detection is an intrusive measurement that will disturb the driver [17].

In recent years, with the rapid development of deep learning methods, particularly in the field of picture classification and recognition, the use of a convolutional neural network (CNN) to train the extraction of image features has gotten a lot of press. The CNN can learn the most effective deep aspects of photos automatically. When compared to other vision-based methods, CNN can learn picture features while avoiding the flaws in standard artificial feature extraction. At the moment the use of CNN in drowsiness detection is mostly focused on detecting the state of the human face and eyes. As a result, the facial drowsy expression are trained and mastered utilizing computer vision technology paired with video processing and CNN to realize the driver's drowsy expression [14].

Drowsiness can be caused by a variety of conditions, according to Melissa and her co-authors. Drowsiness is one of the factors that contribute to car accidents. The system was created to identify the driver's tiredness [23]. The level of distraction, blinking, and yawns can all indicate tiredness. As aresult, the camera has a range for recording the area. Researchers face a significant hurdle in detecting drowsiness. Researchers rely heavily on sleepiness symptoms in both manual and automatic approaches to predict a drowsy driver. Manual approaches, on the other hand, are extremely difficult and unreliable in terms of preventing traffic accidents. The human perspective of the problem is the foundation of manual approaches

PROPOSED ARCHITECTURE

The Proposed architecture is shown as in figure 1. The proposed model is build based on convolutional neural network. In the proposed system, a webcam captures the video of the driver's face. System will evaluate the driver's level of fatigue based on face tracking and facial key point detection. Using the facial key points CNN (Convolution Neural Network) will monitor the eyes of the driver by comparing the datasets. This system will reduce the false alarm and perfectly detects the eyes regions using facial key points. This system can be done in free of cost

DATASET DETAILS

There are many types of data sets like raw data set, image dataset, audio data set etc. This project comes under the Image dataset. Four variant of eye images are collected like open right eye, closed right eye, open left eye and closed left eye. In each variant more 3000 images are there. The system dataset has been collected from KAGGLE. The datasets for open right eyes, open left eyes, closed right eyes, and closed left eyes are collected in this system. Each of the datasets shown above contains thousands of photos. Each image in the opened right eye is permuted with each image in the opened left eye. Each image in the closed right eye is permuted with each image in the closed left eye. All of the permuted photos will be saved in two pickle files, one for open eyes and one for closed eyes

PREPROCESSING

Pre-processing refers to the transformations applied to our data before feeding it to the algorithm. Data Preprocessing is a technique that is used to convert the raw data into a clean data set. In other words, whenever the data is gathered from different sources it is collected in raw format which is not feasible for the analysis

CNN ALGORITHM

Convolutional neural network is mostly used for image recognition. CNN is a Deep Learning algorithm which can take in an input image, assign importance (learnable weights and biases) to various aspects/objects in the image and be able to differentiate one from the other.

ALGORITHM IMPLEMENTATION

Implementing the CNN (Convolutional neural network)Algorithm. Then train the dataset multiple time for getting a accurate detection. The goal behind CNN is to monitor the human eyes by comparing the datasets the angle of the eye to compensate for the CNN's limitations regarding eye closure recognition. After the CNN validates that the driver's eye is open, we use the angle of the eye to validate the result. A blink is the process of the eye closing and opening

TESTCASES

The system is perfectly detecting the eye region by using facial key points. The system will alert person in the form of alarm .the test cases mentioned above passed successfully. No defects encountered.

DROWSINESS DETECTION

Drowsy detecting technology can detect the type of the driver's drowsiness and exhaustion. The system consists of a camera with an alarm mounted in front of the driver and inside the vehicle. For the purpose of detecting tiredness, the camera takes video of the driver's facial expressions. The learned data sets will be compared to the continuous video frames collected by the camera. The trained data sets are made up of a variety of images with open and closed right and left eyes, as well as a mouth. An alarm will be triggered if the continuous video frames acquired by the camera match the visuals in the trained data sets. The alert signifies that the driver is tired or likely to fall asleep, i.e., it denotes drowsiness. The driver and other co-passengers in the vehicle are alerted, and the alarm will only turn off when the driver's facial expressions return to normal. By detecting driver drowsiness, the drowsiness detection prevents accidents and assures safe driving. In the above image 5 the system is monitoring the eye of the man who is sitting in front of the camera. If the eye of the driver was closure more than a few second the system will start give the alert message. In the above image the eye of the man who is sitting in front of the camera was closed more than a 5

to 10 seconds, The System alerting a man by using the fatigue alarm.

CONCLUSION

The accuracy level was improved using a CNN (convolutional neural network) approach. The proposed strategy has been successfully proved to be effective in drastically enhancing theaccuracies of other current approaches, with the proposed system presently achieving 92 percent accuracy utilizing the CNN algorithm. The data has been well-trained, and the system can easily anticipate the outcome. The detection of drowsiness is a safety function. A safety feature that can aid inthe prevention of accidents caused by drivers who have fallen asleep. The device works well even when drivers are wearing glasses and in low-light situations. During monitoring, the system can determine whether the eyes are open or closed, with a high level of precision, allowing the system to accurately forecast the driver's face and fatigue status. The alarm beeps to inform the driver when the eyes are closed for approximately 10 seconds. Many accidents will be avoided asa result of this, and the driver's and vehicle's live will be preserved. Only the most expensive luxury cars have a system for driver safety and vehicle security. Driver safety can also be provided in regular cars using sleepiness detecting systems.

ACKNOWLEDGEMENTS

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Fig. 1 Example of Driver Drowsiness	**Fig. 2 Architecture Diagram**

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Fig.3 Dataset Images

Name of the Test:	Eye Detection
Item Tested:	Eye
Sample Input:	Driver's face
Expected output:	Eye Detection
Actual output:	Same as expected output
Remarks:	Successful

Fig.4 Test case 1

Name of the Test:	System Alert
Item Tested:	Alarm
Sample Input:	Drivers face in drowsy condition
Expected output:	Alert in form of alarm
Actual output:	Same as expected output
Remarks:	Successful

Fig. 5 Test case 2

Fig. 6. Monitoring Eye

Fig.7. Drowsiness Detection

RESEARCH ARTICLE

Testicular Response to Exogenous Gonadotropins in the Albino Rats Treated with Tramadol

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ABSTRACT

The present study on effect of exogenous administration of gonadotropins after the effect of tramadol determined the recovery level in testicular activities like gravimetric changes, histological and biochemical analysis in male albino rats. Five groups of healthy adult male albino rats having six rats in each group were taken, group II received 3mg tramadol/100g body weight for 21 days in 0.2 ml saline, group III and IV of animals initially treated with tramadol and to know the recovery state administered for effected rats 5 IU human chorionic gonadotropin (hCG) and 5 IU primordial pregnant mare's serum gonadotropin (PMSG) in 0.2 ml saline for next 7 days i.e., from day 21 to day 28 respectively. Group V rats administered with 3mg tramadol for 21 days along with IU hCG + PMSG in 0.2 ml saline for next 7 days in intraperitoneally to know the synergistic action of the drug every day between 10 to 11am and group I maintained as control. After the experimental period the rats were sacrificed and for the gravimetric, histological and biochemical analysis of testis were carried out. The administration of gonadotropins to induce tramadol suppressed gonadal activity and increased the body weight, testicular weight and accessory organs weight and also noted increase in the sperm count. The micrometric measurements like the diameter of testis, seminiferous tubules also increased in the tramadol treated rats with the inducing gonadotropins. Diameter of epididymis and epithelial cell height of epididymis highly significant increase with hCG + PMSG administration. Combination of gonadotropins is more effective to retain the animal physiology in a normal state.

Keywords: Gonadotropins (hCG and PMSG), Tramadol, Testis, Gravimetric, Biochemical, Accessary organs.

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INTRODUCTION

Tramadol serve as an opioid analgesic drug that has a dual mechanism of pain-relieving and sedative nature with the reuptake of serotonin and nor epinephrine inhibitor as agonist [1, 2]. Currently, opioid drugs are more familiar and widely prescribing in the medical world [3]. Zhou et al., [4] reported the use of tramadol as analgesic treatment for the cancer or chronic neuropathic pain and to solve the issues of post-operation. However, it is well recognized as a therapeutic agent but also reported many side effects on various physiological systems. The opioid drug has been exhibited organ specific toxicity in chronic condition as a result hepatotoxicity and nephrotoxicity were more common to damage the liver and kidney respectively [4]. The neurotoxicity has been proven by the tramadol treatment in prolonged and continuous administration resulted in weight loss of rats' brain [3]. Various findings described that long-term of tramadol administration weakens the memory function in rodent models by activation of μ-opioid receptors [5,6]. Whereas, chronic administration of tramadol induce histopathological complications, increasing cell death in rat cerebral cortex with oxidative stress and other abnormalities [7]. In reproductive studies, tramadol used as sexual stimulants in the absence of erectile dysfunctions to urge the sexual drives and the sexual desire to achieve long-lasting and harder erection, it is also noticed higher coital frequency and the willingness to delay in ejaculation [8]. Previous studies were revealed on tramadol can be used as erotic medications to treat the erectile dysfunction and premature ejaculation with consideration of drug abuse associated with various degrees of discernible side effects [9, 10].

In medical issues tramadol is prescribed for treatment of various pain in level of moderate to severe intensity [11]. The reported side effects of tramadol include headache, dizziness, somnolence, nausea, constipation, sweating, pruritus and central nervous system stimulation [11]. Tramadol has low plasma protein binding (20%) and wide tissue distribution. It is eliminated primarily through the liver (metabolism to O-desmethyl tramadol and N-desmethyl tramadol) and partially through the kidney (up to 30% of dose). O-desmethyl tramadol has a considerably greater affinity for opioid receptors than the parent drug and is a more powerful analgesic. The half-lives of the active ingredients range from 4.5 to 9.5 h, and total plasma elimination of tramadol is reasonably high (600 ml/min). The interaction potential of tramadol is considered negligible because it does not influence the disposition of other drugs [12]. Chronic use of tramadol has been reported to adversely affect the male reproductive tissues. For example, Safarinejad et al., [13] demonstrated severe reproductive toxicity, like increased sperm DNA damage mediated by testicular oxidative damage. Men consuming therapeutic opioids several times daily for treatment of chronic pain frequently develop erectile dysfunction, decreased libido, fatigue and depression [14].

Over intake of Tramadol concentration may lead to accumulation of toxic metabolites, increase the risk of drug drug interactions and reduced elimination level from the body to induce chronic level of toxicity [15]. The hypothalamus regulates the release of pituitary gonadotropins i.e., follicular stimulating hormone (FSH) and luteinizing hormone (LH) through the neural signals causing release of gonadotropins releasing hormone (GnRH) [16]. According to several investigators CNS influencing drugs inhibit the release of FSH and LH from the pituitary acting through hypothalamus, blocking the neural stimulus to the gonadotropin releasing hormone [17, 18]. It is well established that both the gonadotropins are essential for the testicular function, and are required for the completion of steroido genesis and spermatogenesis [19, 20]. Therefore the present investigation is taken up to test the efficacy of exogenous gonadotropins (hCG and PMSG) on the maintenance of testicular functions and their spermatogenesis process in tramadol affected albino rats.

MATERIALS AND METHODS

Healthy male albino rats of Wistar strain from inbred colony, weighing 150 - 180gm, of 60 - 90 days old were maintained at room temperature of 20 ± 28 ℃ with lighting schedule of 12 h light and 12 h darkness. They were maintained in individual cages and divided in groups each containing six animals and fed with balanced diet as described by CFTRI (Central Food and Technological Research Institute) Mysore, Karnataka, India and water ad

libitum. The acclimatization of the animals lasted for 7 days before tramadol administration for the experiments. It was carried out in accordance with ethical regulations for the animal care and use of laboratory animals. The study parameters like gravimetry of testis and accessory organs and histological, histometrical, biochemical analysis of testis by selected dose of tramadol through intraperitoneal as well as exogenous gonadotropins to study the recovery of organ functions.

Drugs
Tramadol (Trambax OD) generic is an opioid analgesic), 100mg tablets, were purchased commercial product of Ranbaxy Laboratories, India from local drug houses.

Experimental Groups

The animals were divided into following groups

Group-I: Received 0.2 ml saline/100 g body weight i.p. for 21 days and served as control.

Group-II: Received 3 mg tramadol/100 g body weight i.p. for 21 days in 0.2 ml saline through intraperitoneal route.

Group-III: The rats treated with 3 mg tramadol for 21 days and received 5 IU PMSG in 0.2 ml saline for next 7 days i.e., from day 21 to day 28 respectively

Group-IV: The rats treated with 3 mg tramadol for 21 days, received 5 IU hCG in 0.2 ml saline for next 7 days i.e., from day 21 to day 28 respectively.

Group-V: The rats treated with 3 mg tramadol for 21 days, received 5 IU PMSG + hCG in 0.2 ml saline for next 7 days i.e., from day 21 to day 28 respectively

All the animals were sacrificed by cervical dislocation after 24 h of the last injection. The testis were dissected out immediately and separated from adherent tissue, weighed up to the nearest mg on electronic balance to determine gravimetry. Organs from one side of each animal were fixed in Bouin's fluid for histological studies. They were embedded in paraffin, sectioned at 5 μ, stained with Ehrlich hematoxylin and Eosin. The micrometric measurements like diameter of testis, its epithelial cell height were made from randomly chosen 20 sections appearing round at cross sections from each group using ocular and stage micrometers [21]. Spermatogenic elements count was made from randomly chosen 20 round cross section taken from the middle part of the testis [22]. Organs from other side were used for biochemical estimations of the protein content [23],total cholesterol [24] and glycogen [25] of testicular tissues were estimated.

Statistical Analysis
All the values were statistically analysed by Student's-'*t*' test using SPSS (19.0.1.) [26].
Data are expressed as the Mean + S.E. Statistical significance was set at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS

Gravimetric and micrometric changes of the testis, tramadol treatment has showed significant reduction in the weight of testis and accessory organs. Administration of exogenous gonadotropins (hCG, PMSG, hCG + PMSG) for 7 days to the tramadol treated rats has showed significant increase with gonadotropin administration (Figue 1-4).
Impact of tramadol adversely affected on biochemical parameters of testis, after that 7 days treatment of exogenous gonadotropins reversed the inhibitory effect of tramadol on testicular function in albino rats. Cholesterol, the precursor for sex steroid hormone is decreased, protein and glycogen increased due to exogenous gonadotropin

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treatment, in comparison with tramadol treated and controls but it is significant, with PMSG and hCG + PMSG treatment (Figure 5-8).

Histopathological parameters were disturbed due to the treatment of tramadol in testis, after 7 days treatment of exogenous gonadotropins increased the number of spermatogonia, it is highly significant, only with PMSG treatment and it shows the reversible nature of drugs administration. The number of spermatocytes and spermatids were increased significantly, with PMSG and hCG + PMSG, though hCG treatment has caused increase in the spermatocytes and spermatids and it was non-significant (Figure 8-10).

DISCUSSION

Use of opioid significantly increased in the last two decade, primarily easy access may resulted by medical prescription to increase the level of opioid use. Physicians are also made such drug as common administration as analgesics for various medical issues. Many scientific studies were evidenced the effect of opioids on the endocrine system, however, a systematic research work in in vivo level not found maximum on endocrine system of opioids to correlate the effect on humans and animals. In mammals use of opioids derivatives in endogenously inhibit the function of LH-RH and their release, to reuptake all the inhibited function revered by the antagonist naloxone administration [24, 27].

The opioid administration influences CNS and elevates the secretions to release of pituitary gonadotropins via hypthalamo- hypophyseal system also partially supported increase the secretion of prolactin hormone. In current study shows the inhibitory action of Tramadol administration for 21 days in male albino rats and caused reduction in the weight of testis, regression in spermatogenesis and sperm count, which may be due to the non-availability of pituitary FSH, further administered 7 days of hCG and PMSG to retain their normal physiological function and found significant action with the synergy along with tramadol administration. The increasing levels of cholesterol and decrease in the weights of accessory organs may be due to non-availability of LH for steroidogenesis which has resulted in reduction of circulating androgens, hCG administered alone or in combination with PMSG might have stimulated the steroidogenesis in the Leydig cells [28-31] as a result of which the cholesterol may utilized by the steroidogenesis process. PMSG being more in the treatment to induce FSH and supported the spermatogenesis as a result, the spermatogenic elements are increased after PMSG treatment in the tramadol treated rats [32,33]. However, the administration of combined dose of gonadotropins (PMSG + hCG) is more effective as already stated in the synergistic action, because PMSG acts like FSH and hCG like that of LH and both are essential gonadotropins for normal spermatogenesis and steroidogenesis process in rats. Increase in the weight of accessory organs indicates the production of androgens in the testis and it is noticed by the changes in the histological and morphological studies after the treatment of tramadol to reversible action of drug and exhibited as such as control rat physiology.

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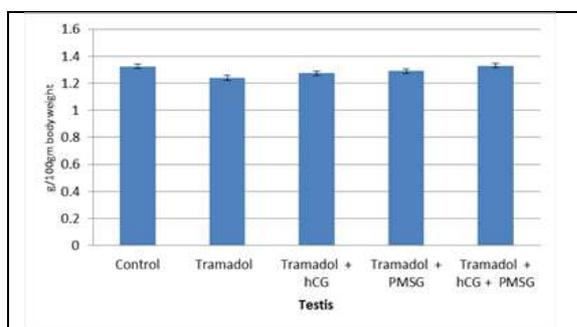
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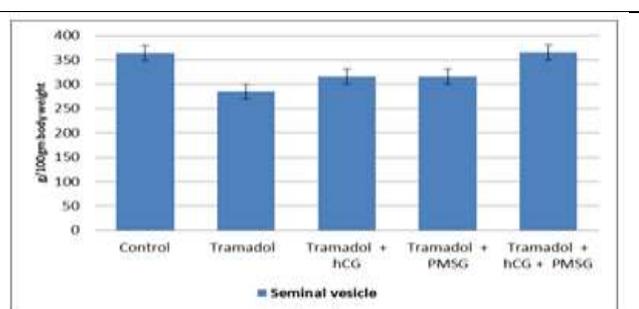
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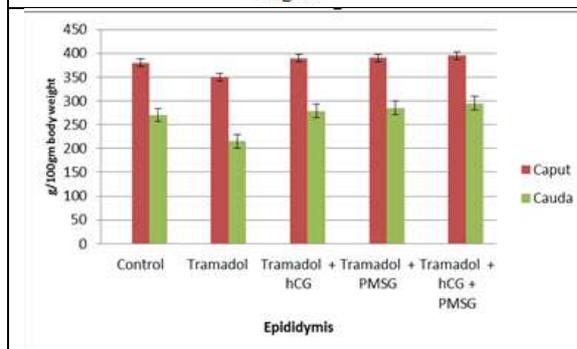
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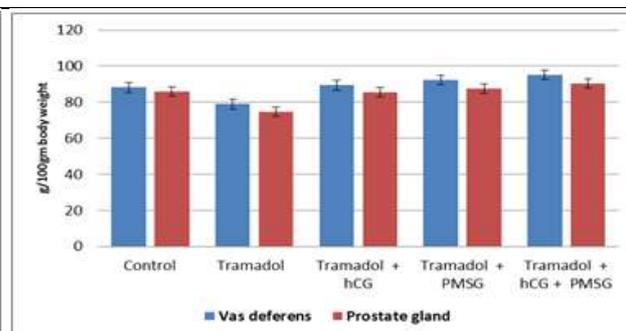
Fig. 4.

Six animals were maintained in each group. M±SE = Mean ± Standard error.

Figure 1-4: Effect of Exogenous Gonadotropins on the Weight of Testis and Accessory Reproductive Organs in Tramadol treated Albino Rats
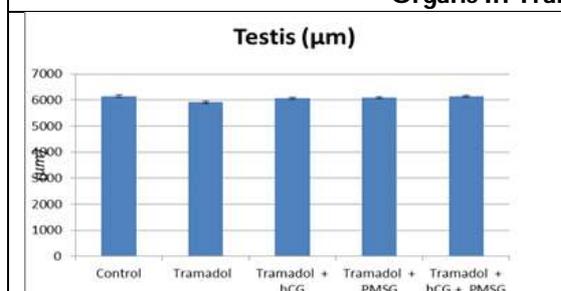
Fig. 5.
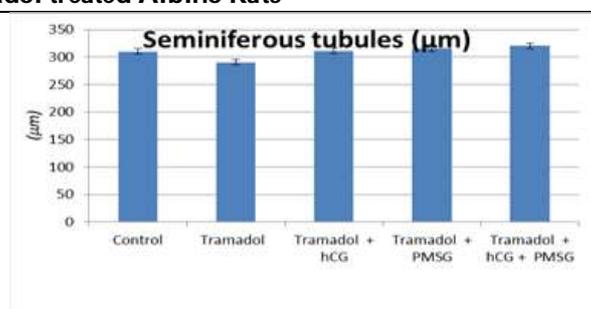
Fig. 6.

Somanath Reddy C. Patil and Preeti
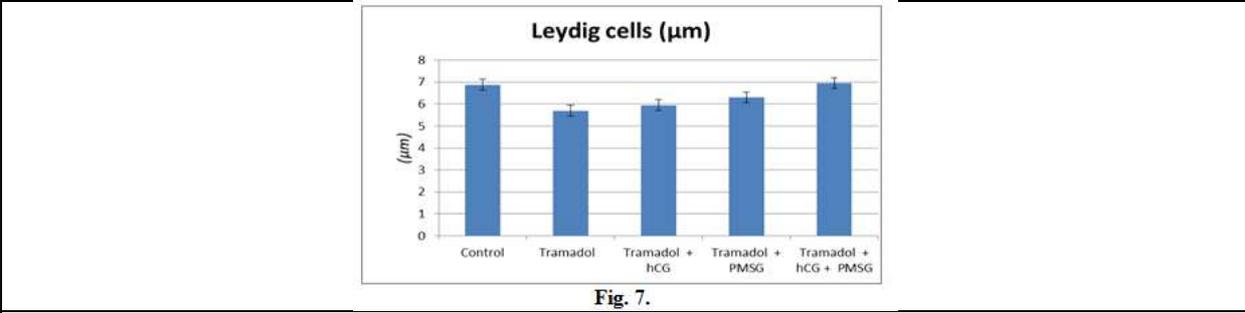
Fig. 7.

Six animals were maintained in each group. M±SE = Mean ± Standard error.

**Figure 5-7: Effect of Exogenous Gonadotropins on Biochemical Changes of Testis in Tramadol treated Albino Rats.**
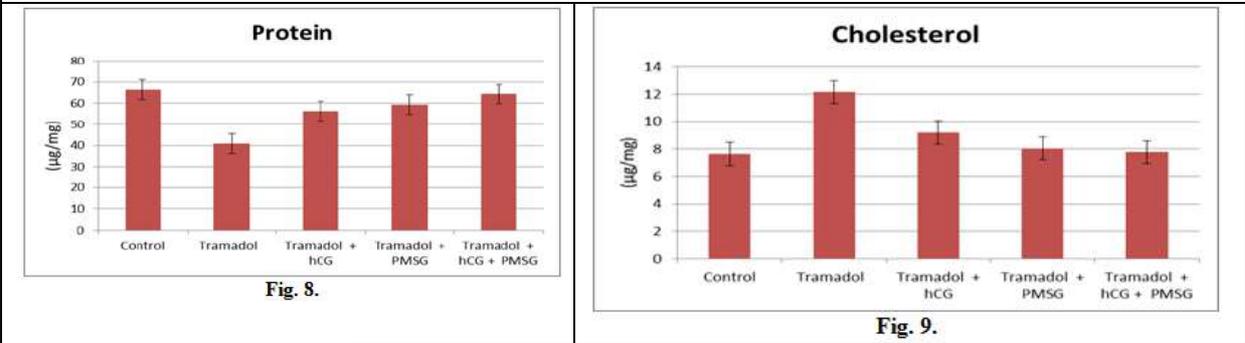

Fig. 8.

Fig. 9.
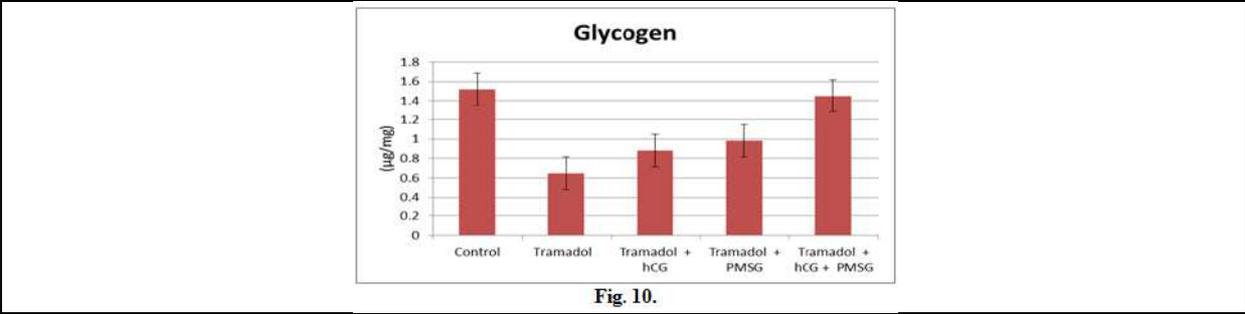
Fig. 10.

Six animals were maintained in each group. M±SE = Mean ± Standard error.

Figure 8-10: Effect of Exogenous Gonadotropins on Histometric Changes of Testis in Tramadol treated Albino Rats.

RESEARCH ARTICLE

Influence of Different Concentrations of Methanolic Extract of *Areca catechu* L. on *Drosophila melanogaster* Developmental Stages

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ABSTRACT

Areca nut (betel nut) is commonly used as a masticatory substance. Chemically it is rich in tannins, polyphenols and alkaloids. Arecoline, a component of the areca nut, is one of the most potent psychoactive substances known to mankind. Crude extract of *Areca catechu* L is prepared using soxhlet extractor and dried in a hot air oven to obtain the crystals of the extract, which is used in the current study. Published data indicates its addictive nature and involvement in various disorders including cancer. Developmental stages in *Drosophila melanogaster* are studied by exposing them to different concentrations of methanolic extract of *Areca catechu* L. The time taken for the overall growth and development of the flies are recorded and analyzed using statistical tools. Analyses revealed that the flies treated with methanolic extract showed variations in their morphology, egg laying behavior and circadian rhythm as compared to the control group. This analysis indicates significant differences between the control and test groups, and similar results could be identified in higher organisms treated with methanolic extract of *Areca catechu* L. This further strengthens the published data indicating the addictiveness and the negative impact that areca nut can have on higher organisms such as humans.

Keywords: *Areca catechu L*, *Drosophila melanogaster*, Eclosion, Development, Circadian rhythm.

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INTRODUCTION

Areca catechu Linn, commonly known as Betel palm or Betel nut, is grown in India, Malaysia, and other south asian countries [1,2]. It is an economically important seed crop in these countries. Areca nut consist of carbohydrates, fats, fibers, polyphenols including tannins and flavonoids. They are used as masticatory substances either alone or in combination with betel leaves. They are also useful as industrial and medicinal products. Arecoline is the major chemical constituent of *Areca catechu* [3,4]. It is the fourth most psychoactive substance after Alcohol, Caffeine and Nicotine [5]. There are also reports of oral cancer, oral sub mucous fibrosis, Type-2 diabetes in people habituated with Areca nut use [6,7,8]. Arecoline ($C_8H_{13}NO_2$) is a tetrahydropyridine that functions as a muscarinic acetylcholine receptor agonist. [8].

Drosophila melanogaster is widely used as a model organism in the field of biological research [9]. Drosophila cultures are easy to maintain, breed, mutate and different stages of development can be observed in a short period of time owing to their short life span. Genetically, human beings and fruit-flies share about 61% of genes responsible for diseases[10]. About 50% of the Drosophila protein sequences have mammalian analogues, in conjunction to this homology, effects of many diseases, defects and abnormalities that can be seen in Drosophila can be correlated to the similar ones that are seen in human beings. Historically Drosophila is a very good model organism to study many human diseases. Fruit Flies display similar physiology and regulatory organs to that of mammals. There are a number of genes that have been identified that affect the physiology and are conserved in mammals[11].

In the current study *Drosophila melanogaster* was treated with Methanolic extract of *Areca catechu* L in three different concentrations (0.1%, 0.5% and 1%). The purpose of this work is to study the morphology and developmental pattern in *Drosophila melanogaster*. Flies grown in the standard wheat cream agar media and the flies grown in media containing different concentrations of the Methanolic extract are studied and the results are documented and analyzed.

MATERIALS AND METHODS

Preparation of Areca nut Extract
The *Areca catechu* (Thirthahalli variety) was procured from Sringeri, Karnataka. Various extracts of the seeds were prepared using the soxhlet apparatus. Out of all the extracts prepared methanolic extract was preferred as the results were promising [12]. The soxhlet extraction method was carried out for 15 cycles, once the solvent in the thimble turned colorless, the reaction was stopped and the sample was placed in a hot air oven. After three days, the sample was taken out and the crystals of *Areca catechu* L were collected and stored [13].

Drosophila Stock and culturing
Drosophila melanogaster, Oregon K strain was obtained from Drosophila Stock Center, Mysore University. The flies were subcultured using the standard Wheat cream agar media. The composition of the media is as follows -
For 1000 ml of Water
Rava 100g, Jaggery 100g, Agar-Agar 10g, and Propionic acid 7.5 ml [14].

Isolating Virgin flies
Male and female *Drosophila melanogaster* flies were allowed to mate in the bottles containing wheat cream agar media. After hatching the eggs, the flies were transferred to new culture bottles. The bottles with eggs were monitored for the eclosion of flies. The development occurs in 10-12 days under controlled environmental conditions. Virgin flies were isolated by anesthetizing them using Diethyl Ether [14]

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Egg-laying Behavior

Methanolic extract of *Areca catechu* was used to treat *Drosophila melanogaster* flies in different concentrations ranging from 0.1% to 1% and the results were recorded and tabulated. The egg-laying rhythm in the flies was studied by observing them under the stereomicroscope [15, 16, 17].

Larval Growth

Larvae hatched from the eggs were observed for any changes in the morphology and development pattern. Three instars of larvae were observed for any changes in their size, shape, and development time. The results were recorded and tabulated [18].

Pupation and emergence of adult flies

Once the larvae entered the pupal stage, they were continuously monitored for emergence. The time taken for eclosion was recorded and a comparison was made between the control and test groups. The process of eclosion was studied under the controlled conditions of 12 hrs light and dark environment. The variability was kept constant and only the compound used in this work brought the variability [19,20]

Time Taken to Complete the Life Cycle

The *Drosophila melanogaster* flies were cultured and maintained in Wheat cream agar Media. Once the Virgin female flies were isolated, They were used for the experimental setup. The flies were grown in normal media and media containing different concentrations of methanolic extract of areca nut. The time taken by Drosophila flies to complete the life cycle was monitored throughout the experiment and for three generations. The data were recorded and tabulated [20].

RESULTS

Egg laying Behavior

Methanolic extract of *Areca catechu* was used to treat Drosophila melanogaster flies in different concentrations ranging from 0.1% to 1% and the results were recorded and tabulated.

Larval Growth

Larvae feeding on the media containing different concentrations of Methanolic extracts of *Areca catechu* showed variation in their growth.

Pupation and emergence of adult flies

Table 3, Table 4 , Table 5 figure 3 and figure 4

DISCUSSION

Drosophila melanogaster flies have shown to be an excellent model for studying a wide range of human disorders. Many researches have been published by scientists all around the world, and they believe that eating Areca nuts causes a variety of ailments, including cancer. Areca nut is a psychoactive stimulant that has been utilized for thousands of years by humanity. In current society, it has both traditional and aesthetic value. Chemically Areca nut is made up of Alkaloids, flavonoids, triterpenes, tannins, fatty acids etc., Alkaloids include Arecoline, Guvacine, Arecaidine, Guvacoline, etc.,[23]. According to vedic literature, areca nut has been used as a therapeutic substance, and users have claimed greater well-being and stamina. It is said to have aphrodisiac effects as well. Thus according to reports, using Areca nuts causes feelings of well-being, euphoria, decreased hunger, salivation, and enhanced alertness. There are reports suggesting that prolonged use of areca nut results in adverse effects on human health. Products containing areca nut, even without tobacco, are associated with increased risk of developing oral cancer []. Areca nut extract containing are coline is shown to inhibit growth and protein synthesis in cultured human periodontal fibroblasts.

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This paper focuses on the development of Drosophila melanogaster when treated with methanolic extract of Areca catechu. *Areca catechu* was procured from Sringeri Taluk of Chikmagalur district, Karnataka, India. Concentrations ranging from 0.1% to 1% were used to treat Drosophila flies and the results were recorded. The flies treated with different concentrations of the methanolic extract when compared to the ones grown in standard wheat cream agar media, showed variation in the egg laying behavior, Larval development and eclosion behavior. A total of three generations of flies were observed and the data was compiled and studied. Virgin female flies were allowed to mate with male flies, and they began producing eggs on day two and continued for the next 4-5 days. When the test groups were compared to the control group, this behavior was seen in all of them. The Circadian clock regulates Drosophila egg laying behavior [24,25,26]. Under normal circumstances, rhythmic egg laying involves a sequence of actions that culminate in the deposition of eggs. The egg laying ability of flies treated with different dosages of Methanolic extract of Areca catechu L was significantly lowered in the current study conducted in our laboratory [27,28]. The time taken for the egg laying was also recorded and it showed variation between control and the test groups. The experiment conducted on Swiss albino mice showed that administration of Areca nut extracts resulted in the death of the pregnant dams with the increase in dose.

The unprocessed areca nut and aspirin shown to cause more toxicity in animals. Death frequency of the fetuses of pregnant dams increased as the dosage of aspirin or either of the unprocessed and processed areca nut extracts [6]. Prolonged use of areca nut directly or in combination with tobacco or other products, has resulted in deterioration of the oral health of the users [7,8]. The experiment used three generations of flies, and the student t-Test verified that there was a significant variation in the amount of eggs laid by flies in each generation [25]. The larval growth was also affected by the methanolic extract of Areca nut when used in different concentrations. With highest concentration, there was a significant reduction in the total number of larvae. The timing of egg hatching into larva was also shown to be affected. Three generations of larvae were studied, there was a gradual decline in the larval number. These results were very similar with pupal growth and emergence of adults as well[25,26]. The external conditions like temperature and Humidity were maintained constant during the study period. The *Drosophila melanogaster* has a circadian rhythm of 12 hours of light and 12 hours of darkness. This cycle is maintained under normal circumstances. Early in the morning, adult flies emerge from the pupa. The behavior of flies grown on media with different concentrations of Areca nut extract, on the other hand, fluctuated. The normal cycle of adult flies emerging from pupa was observed to be disturbed.

CONCLUSION

Under normal circumstances, *Drosophila melanogaster* takes about 12 days to complete its life cycle. However, in this study, we observed that treating *Drosophila* with methanolic extract of areca nut disrupted their circadian rhythm and delayed their life cycle. Further, these treatments significantly affected their growth and development. These findings are consistent with previous research that suggests that long-term use of areca nut causes a variety of ailments, including cancer. The outcome of this study could form the basis for future applications of areca nut in medicine.

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Table 1. F0, F1 and F2 generation

	Control	Test 1%	Test 0.5%	Test 0.1%
F1	123	76	101	121
F2	140	95	119	129
F3	145	101	122	136

Table 2. F0, F1 and F2 generation

	Control	Test 1%	Test 0.5%	Test 0.1%
F1	115	65	88	101
F2	110	58	82	111
F3	115	61	84	115

Table 3(a) F0 Generation

	Control	Test 1%	Test 0.5%	Test 0.1%
Pupa	**108**	**52**	**66**	**99**
Adult	**89**	**28**	**62**	**82**

Table 3(b). F1 Generation

	Control	Test 1%	Test 0.5%	Test 0.1%
Pupa	**83**	**49**	**65**	**91**
Adult (F2 Generation)	**85**	**29**	**43**	**70**

Table 3(c). F2 Generation

	Control	Test 1%	Test 0.5%	Test 0.1%
Pupa	**89**	**54**	**71**	**105**
Adult (F3 Generation)	**82**	**35**	**49**	**77**

Table 4. F1 Generation

Time Duration to complete Life Cycle (F1)				
F1 Generation in Days	**Egg**	**Larva**	**Pupa**	**Adult**
Control				
Bottle 1	**2**	**7**	**3**	**12**
Bottle 2	**2.5**	**8**	**3**	**13.5**
Bottle 3	**2**	**8**	**4**	**14**
F1 Generation in Days	**Egg**	**Larva**	**Pupa**	**Adult**
0.1 %				
Bottle 1	3	7	3	13
Bottle 2	2	8	3	13
Bottle 3	3	8	3	14

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F1 Generation in Days	Egg	Larva	Pupa	Adult
0.5 %				
Bottle 1	3	8	4	15
Bottle 2	2.5	8	3	13.5
Bottle 3	2.5	9	3	14.5
F1 Generation in Days	**Egg**	**Larva**	**Pupa**	**Adult**
1.0 %				
Bottle 1	3.5	9	3	15.5
Bottle 2	4	10	3.5	17.5
Bottle 3	4	10	3	17

Table 5. F2 Generation

Time Duration to complete Life Cycle (F2)				
F2 Generation in Days	**Egg**	**Larva**	**Pupa**	**Adult**
Control				
Bottle 1	2.5	8	3	13.5
Bottle 2	2	8	3	13
Bottle 3	2	7	3	12
F2 Generation in Days	**Egg**	**Larva**	**Pupa**	**Adult**
0.1 %				
Bottle 1	2.5	8	3	13.5
Bottle 2	3	7.5	4	14.5
Bottle 3	3	7	4	14
F2 Generation in Days	**Egg**	**Larva**	**Pupa**	**Adult**
0.5 %				
Bottle 1	3	8	3	14
Bottle 2	3.5	7	4	14.5
Bottle 3	3	9	3	15
F2 Generation in Days	**Egg**	**Larva**	**Pupa**	**Adult**
1.0 %				
Bottle 1	5	8.5	4	17.5
Bottle 2	4	8	4	16
Bottle 3	4	8	5	17

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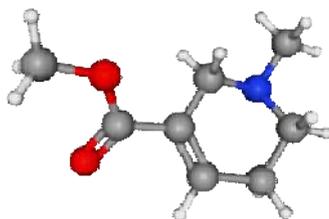
Fig,, 1 3D image of Arecoline (Pubchem)
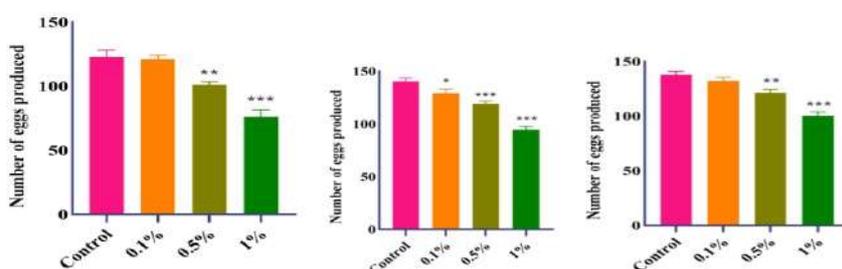
(: *p<0.05; **: p<0.01; ***: p<0.001; Student's t-test)

Figure 1. Number of eggs produced by *Drosophila melanogaster* when treated with different concentrations of *Areca catechu* extract. a. F0 generation, b. F1 Generation and c. F2 generation.
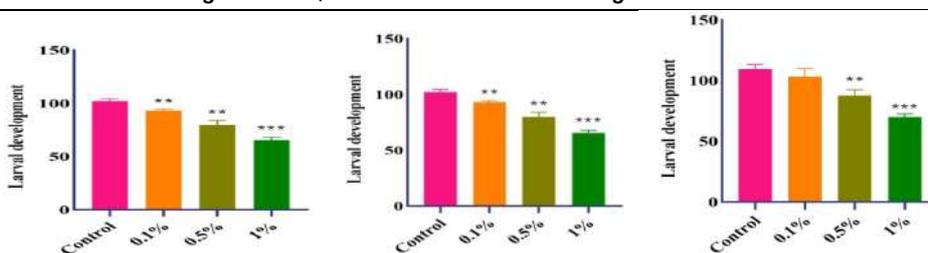

(: *p<0.05; **: p<0.01; ***: p<0.001; Student's t-test)

Figure 2. Larval development in *Drosophila melanogaster* when treated with different concentrations of *Areca catechu* extract. a. F0 generation, b. F1 Generation and c. F2 generation.
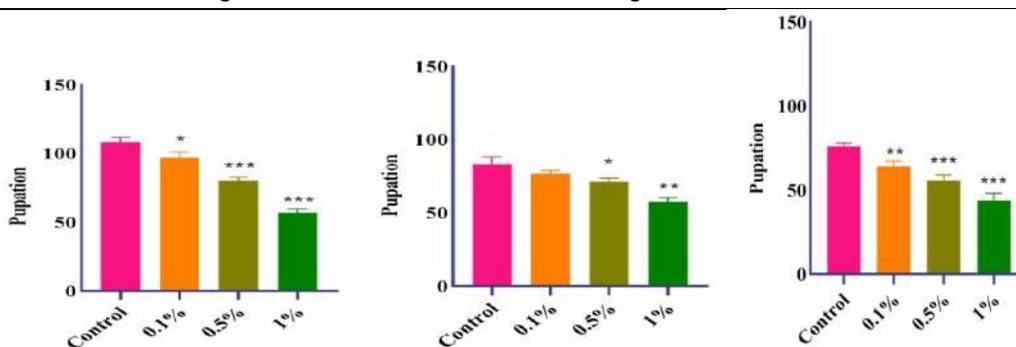
(: *p<0.05; **: p<0.01; ***: p<0.001; Student's t-test)

Figure 3. Pupation in *Drosophila melanogaster* when treated with different concentrations of *Areca catechu* extract. a. F0 generation, b. F1 Generation and c. F2 generation.
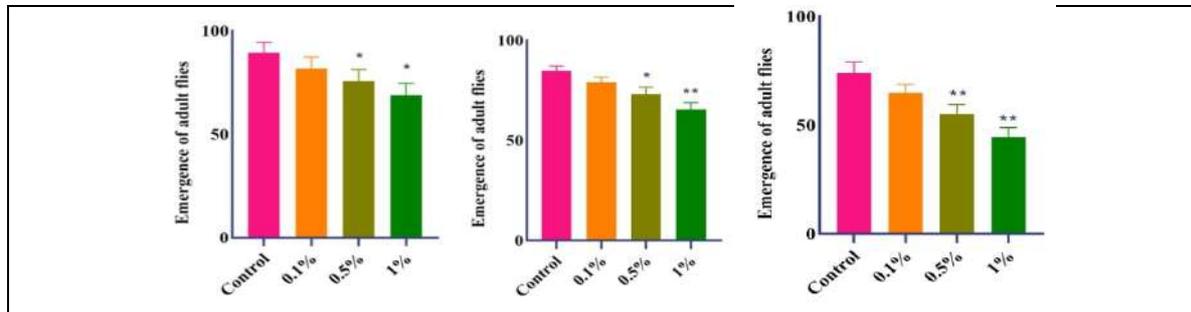
Figure 4. Emergence of Adult flies in *Drosophila melanogaster* when treated with different concentrations of *Areca catechu* extract. a. F1 generation, b. F2 Generation and c. F3 generation.(:*p<0.05; **: p<0.01; ***: p<0.001; Student' st- test)Time Taken to complete the Life Cycle F1 Generation

(a)   (b)   (c)   (d)
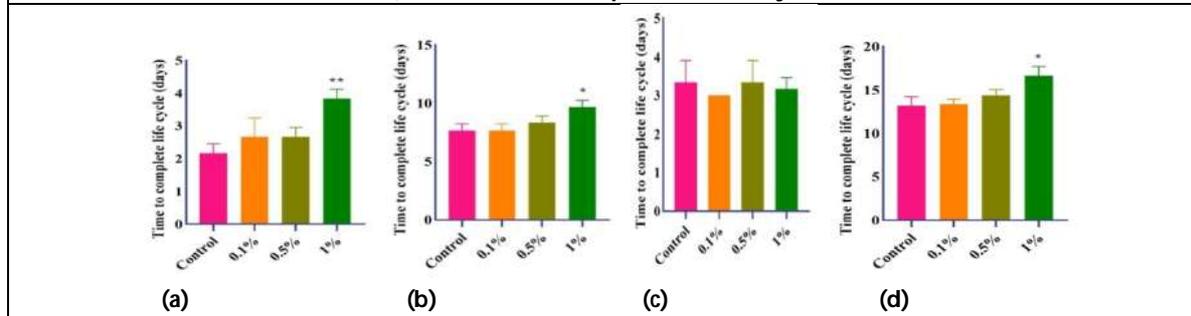
(: *p<0.05; **: p<0.01; ***: p<0.001; Student's t-test)

Fig. 5. Time taken to complete the Life Cycle in *Drosophila melanogaster* when treated with different concentrations of *Areca catechu* extract. a. Egg, b. Larva and c. Pupa and d. Adult.

(a)   (b)   (c)   (d)
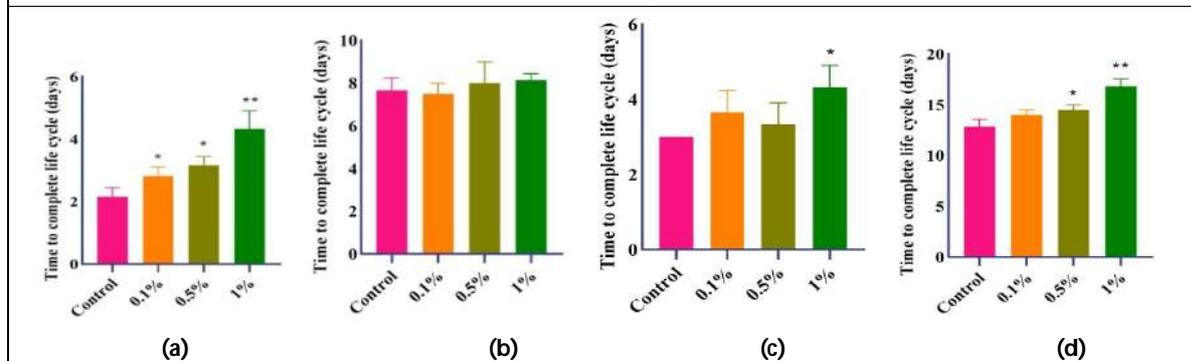
(: *p<0.05; **: p<0.01; ***: p<0.001; Student's t-test)

Fig. 6. Time taken to complete the Life Cycle in *Drosophila melanogaster* when treated with different concentrations of *Areca catechu* extract. a. Egg, b. Larva and c. Pupa and d. Adult

RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles (AgNPs) using *Mimusops elengi*: Ovicidal, Larvicidal, Adulticidal Activities and Smoke Toxicity Effect against Dengue Vector, *Aedes aegypti*

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ABSTRACT

Mosquitoes spread deadly diseases to humans, killing millions of people every year. It may be cause of nuisance biters and especially known as vectors and transmit several pathogens and viruses in humans and animals. Insecticides derived from synthesized natural products have been a top priority in this field. In the present study a novel method of plant-mediated synthesis of silver nanoparticles (AgNPs) using an inexpensive plant extract of *Mimusops elengi*, acting as a reducing and capping agent. AgNPs were characterized by UV–vis spectrophotometry, FTIR, SEM with EDX, XRD, zeta potential and particle size analysis. In mosquitocidal assays, LC_{50} of *M. elengi* leaf extract against *Aedes aegypti* (*Ae. aegypti*) larvae to pupae were 267.439 ppm (larva I) to 507.841 ppm (pupa) and the LC_{50} of *M. elengi*-synthesized AgNPs were 22.393 ppm (Larva I) to 41.658 ppm (pupa), respectively. Both the *M. elengi* extract and AgNPs reduced longevity and fecundity of *Ae. aegypti* adults. Aduldicidal activity of both extract and AgNPs were tested against *Ae. aegypti* adults, the LC_{50} values of *M. elengi* extract were 370.548 and AgNPs were 26.942. Smoke toxicity experiments conducted against *Ae. aegypti* adults showed that *M. elengi* leaf-based coils evoked mortality rates comparable to the permethrin-based positive control (60 and 66 %), respectively. In ovicidal experiments, egg hatchability was reduced by 100% after treatment with 40 ppm of AgNPs and 500 ppm of *M. elengi* extract. Overall, our results highlighted that *M. elengi* could be useful and alternative to develop bio-formulated mosquitocidal properties against vector control strategies.

Keywords: *Mimusops elengi*, AgNPs, SEM, XRD, FTIR, Mosquitocidal activity.

INTRODUCTION

Mosquitoes transmit several debilitating diseases in humans like malaria, yellow fever; chikungunya, dengue fever, zika virus, lymphatic filariasis, and Japanese encephalitis. Mosquitoes can also induce allergic reactions in people, including localized skin reactions and systemic reactions like angioedema [1]. Dengue fever has spread rapidly in urban and semi-urban areas, posing a significant global public health threat. Dengue infects around half a billion individuals, resulting in half a million hospitalizations and 24,000 deaths [2]. Dengue fever is on the rise in India, and in the last decade, it has spread to over 128 countries, putting 3.97 billion people at risk [3]. The number of dengue cases reported to WHO increased over 8 fold over the last two decades, from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Reported deaths between the year 2000 and 2015 increased from 960 to 4032, affecting mostly younger age group. The total number of cases seemingly decreased during years 2020 and 2021, as well as for reported deaths.

The environment and non-targeted consequences organisms that live in water plants have received a lot of attention and safer sources of extracts or phytochemicals and mosquito repellents [4]. For their antioxidant, antimutagenic, and anticancer properties, phenolics or polyphenols, including flavonoids, have gotten a lot of attention [5]. The genus *Mimusops* is native to the tropical parts of Africa and Asia. *Mimusops elengi* L. is an evergreen tree 30 feet tall, with a greyish brown fissured bark, wavy and dull green leaves, oblong berry fruit and creamy fragrant flowers. It is distributed in tropical and subtropical regions [6]. Taraxerone, taraxerol, betulinic acid and spina sterol, sodium salt of betulinic acid and urosolic acid, Fatty acid esters of al phaspinasterol was isolated from the bark [7], Fruit and seed of *Mimusops elengi* showed presence of Quercitol, ursolic acid, dihydro quercetine, quercetine, β - d glycosides of β sitosterol, alphaspinasterol after Saponification [8], Hentriacontane, carotene and lupeol from the leaves, heartwood and roots were isolated [9]. *M. elengi* has many medicinal properties via, ant helmintic Activity, anti-anxiety activity, antihyperlipidemic, antiulcer, anti-inflammatory, Antioxidant, antiurolithiatic, antimicrobial, Cytotoxic, Antidiabetic, Wound healing. These phytochemicals study may be having a mosquitocidal potential activity [10]

Nanotechnology is a rapidly developing branch of science that incorporates many disciplines such as biology, chemistry, physics, food, medicine, electronics, aerospace, and medicine and it examines the design, manufacture, assembly, and characterization of materials that are smaller than 1–100 nm in size [11]. Plant-mediated bio-synthesis (green synthesis) of nanoparticles has several advantages over chemical and physical approaches, including cost, simplicity, and the absence of high pressure, energy, or temperature, as well as the use of highly hazardous compounds [12]. Plant-based products have been proposed as a viable and environmentally benign method for the production of nanoparticles [13]. Polyphenols, flavonoids, triterpenes, tannins, glycosides, vitamins, proteins, and steroids are examples of plant phytochemicals that can be utilized to reduce metal ions to nanoparticles in a single process, ensuring good control over size distribution and crystallinity [14]. A rising variety of plants have been successfully investigated for efficient and quick extracellular synthesis of nanoparticles with high insecticidal capabilities against medical and veterinary pests in this scenario [15].

In the present study, we analyzed the plant-mediated synthesis of silver nanoparticles (AgNPs) using *M. elengi* as a reducing and capping agent. The AgNPs were characterized by UV–vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), zeta potential and particle size analysis. We also investigated the mosquitocidal properties of the *M. elengi* extract and *M. elengi* plant mediated-synthesized AgNPs against young instars of the dengue vector, *Ae. aegypti*. Moreover, synthesized AgNPs of *M. elengi* on ovicidal, adult longevity/fecundity and adulticidal activity of *Ae. aegypti* were evaluated. In addition, we checked the smoke toxicity effect of herbal coils prepared using leaves of *M. elengi* on the *Ae. aegypti* adults were studied.

MATERIALS AND METHODS

Collection and Preparation of Plant Extracts

M. elengi leaves were obtained from Institute of Forest Genetics and Tree Breeding, Coimbatore. The freshly harvested leaves were properly washed in running tap water and rinsed in distilled water before being cut into little bits, shade-dried at room temperature for 10 days, powdered with a laboratory blender, and stored in a 1000 mL airtight bottle. The dried powder of bark and fruit were extracted with methanol using Soxhlet equipment to prepare the extracts. The solvents were concentrated using a rotatory evaporator at reduced pressure after efficient extraction. The crude extracts were weighed, and the yield was calculated as a percentage. One gram of plant residue was diluted in 100 mL of acetone (fixative agent to separate aqueous impurities that affect the chemical composition of plant crude extract) and used as a 1% stock solution. Experimental concentrations were made from this stock solution.

Ae. aegypti rearing

The *Ae. aegypti* eggs were collected from National Centre for Disease Control (NCDC), Mettupalayam (Tamil Nadu, India). Eggs were transferred to laboratory conditions [27±2 °C, 75–85 percent relative humidity, 14:10(L/D) photoperiod] and placed in 18×13×4 cm plastic containers containing 500 ml tap water, where they would hatch. Daily, the larvae were fed a 3:1 (w/w) mixture of dog biscuits (Pedigree, USA) and hydrolyzed yeast (Sigma-Aldrich, Germany). Larvae and pupae were collected, transferred to 500 mL dechlorinated water in glass beakers, and tested in following tests [16].

Maintenance of pupae and adults

With the use of a dipper, the pupae were gathered from the culture trays and transferred to plastic containers (1×2×1×2 cm) containing 500 mL of water. For adult emergence, the plastic jars were kept in a 90×90×90-cm mosquito cage. Mosquito larvae were kept at 27±2°C, 75–85 percent relative humidity, and a 14:10 (light/dark) photoperiod. A 10 % sugar solution was provided for a period of 3 days before blood feeding.

Blood feeding of adult mosquitoes

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days, to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Biosynthesis and characterization of silver nanoparticles

The *M. elengi* aqueous leaf extract was prepared by adding 10 g of washed and finely cut leaves in a 300-ml Erlenmeyer flask filled with 100 ml of sterilized double-distilled water and then boiling the mixture for 5 min, before finally decanting it. The extract was filtered using Whatman filter paper no. 1, stored at −4°C and tested within 5 days. The filtrate was treated with aqueous 1 mM $AgNO_3$ solution in an Erlenmeyer flask and incubated at room temperature. A brown-yellow solution indicated the formation of AgNPs, since aqueous silver ions were reduced by the *M. elengi* extract generating stable AgNPs in water. Silver nitrate was purchased from the Precision Scientific Company (Coimbatore, India).

Green synthesis of AgNPs were confirmed by sampling the reaction mixture at regular intervals, and the absorption maxima were scanned by UV–vis spectrophotometer, at the wavelength of 200–800 nm in UV-3600 Shimadzu spectrophotometer at 1 nm resolution. Furthermore, the reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min; resulting pellets were dissolved in deionized water and filtered through Millipore filter paper (0.45 μm). An aliquot of this filtrate containing AgNPs were used for SEM, FTIR spectroscopy, XRD analysis, and EDX spectroscopy. The structure and composition of freeze-dried purified AgNPs were analyzed by using a 10-kV ultrahigh-resolution scanning electron microscope with 25 μl of sample sputter coated on copper stub and the images of AgNPs were studied using a FEI QUANTA-200 SEM. The surface groups of the AgNPs were qualitatively

confirmed by FTIR spectroscopy [17], with spectra recorded by a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. EDX assays confirmed the presence of metals in analyzed samples [18]. The size of AgNPs was determined by using the particle analyzer Malveern Zetasizer nanosizer. AgNPs size was analyzed measuring the size-dependent fluctuation of scattering of laser light on AgNPs [16].

Larvicidal and pupicidal toxicity in laboratory conditions

Twenty-five *Ae. aegypti* larvae (I, II, III, or IV instar) or pupae were placed for 24 hrs in a glass beaker filled with 250 mL of dechlorinated water plus the desired concentration of *M. elengi* methanol extract (100, 200, 300, 400, and 500 ppm) and *M. elengi* synthesized AgNPs (10, 20, 30, 40 and 50 ppm). Larval food (0.5 mg) was provided for each tested concentration [19]. Each concentration was replicated five times against all instars. Control mosquitoes were exposed for 24 hrs to the corresponding concentration of the solvent. Percentage mortality was calculated as follows: Percentage mortality = (number of dead individuals / number of treated individuals) × 100

Ovicidal activity

Following Su and Mulla [20], in ovicidal activity experiments, *Ae. aegypti* eggs were collected from entomology laboratory placing ovitraps (i.e. Petri dishes, diameter 60 mm, lined with filter paper and containing 100 ml of water) inside each cage. Ovitraps were stored in the cages for 2 days from the blood meal of females. The eggs laid on filter paper lining were examined using a photomicroscope (Leica ES2, Germany). Then, the eggs were placed in a cage with six glass cups (diameter: 6 cm). Five of them were filled with water plus the *M. elengi* methanol extract doses as follows: 100, 150, 200, 250 and 300 ppm (extract) and *M. elengi* synthesized AgNPs (10, 20, 30, 40 and 50 ppm). The control cup was filled with distilled water. 100 eggs were placed in each cup. Five replicates were done for each dosage. After the treatment, the eggs from each concentration were transferred to distilled water cups for hatching assessment after counting the eggs under microscope. The percent egg mortality was calculated on the basis of non-hatchability of eggs with unopened opercula. The hatch rates were assessed 48 hrs post-treatment using the following formula [16]:

Egg mortality % = (number of hatched larvae / total number of eggs) × 100

Adulticidal bioassay

Sugar-fed adult female mosquitoes (5 to 6 days old) were used. The *M. elengi* extract or synthesized AgNPs were diluted with acetone to make different concentrations. The diluted plant extracts were impregnated on filter papers (140×120 mm). A blank paper consisting of only ethanol was used as control. The papers were left to dry at room temperature to evaporate off the ethanol overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring 125×44 mm following the method in WHO (1981) [21]. One tube served to expose the mosquitoes to the plant extract and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 16-mesh-size wire screen. Sucrose-fed and blood-starved mosquitoes (20) were released into the tube and the mortality effects of the extracts were observed every 10 min for 3 h exposure period. At the end of 1, 2 and 3 h exposure periods, the mosquitoes were placed in the holding tube. A Cotton pad soaked in 10 % sugar solution with vitamin B complex was placed in the tube during the holding period of 24 hrs. Mortality of the mosquitoes was recorded after 24 hrs. The above procedure was carried out in triplicate for plant extract and synthesized AgNPs of each concentration [22].

Adult longevity and fecundity of *Aedes aegypti*

Adult longevity and fecundity experiments were carried out following Suresh et al. [23]. For this assay, in each replicate 20 adults (10 h-old males and females) of *Ae. aegypti* were exposed to *M. elengi* methanol extract doses as follows: 100, 200, 300, 400 and 500 ppm (extract) and *M. elengi* synthesized AgNPs (10, 20, 30, 40 and 50 ppm), applied on Whatman filter paper No.1 (size 12 × 15 cm²) lining a glass holding tube (diameter 30 mm, length 60 mm). Control was DDH$_2$O treated filter paper. For all replicates the exposure time was 60 min. Afterwards, *Ae. aegypti* were transferred to chiffon cages (30 × 30 cm) and fed ad libitum with 10 % (w/v) glucose. Eggs were collected daily

from female *Ae. aegypti* after 3 days of blood meal [24]. Mean fecundity was determined by dividing the no of eggs in ovitraps the number of mating females (n = 20). Mortality was noticed every day and the average lifespan of all adult mosquitoes was determined.

Smoke toxicity assays against *Ae. aegypti*

Leaves of *M. elengi* were used to prepare herbal coils for smoke toxicity assays against *Ae. aegypti*. Coils were prepared as described by Rajaganesh et al. [16], using 4 g of powdered leaves, 2 g of sawdust (binding material), and 2 g of coconut shell charcoal powder (burning material). The three materials were mixed with distilled water forming a semi-solid paste. Mosquito coils (0.6-cm thickness) were prepared from the semi-solid paste and then dried in the shade. Negative control coils were prepared following the same method, without adding *M. elengi*. Positive control was commercial permethrin-based coils [25]. Following Murugan et al. [18], experiments were conducted in a glass chamber measuring 140 cm× 120 cm×60 cm. A door measuring 60 cm×30 cm was situated at the front of the chamber. In each test, 100 adult female mosquitoes (age 5 days old, blood-starved for 3 days) were released into the chamber and were provided with a 10 % (w/v) sucrose solution. An immobilized pigeon with a shaven belly was tied inside the tightly closed chamber. Each pigeon was used only once. The experiment was repeated five times on five separate days for each treatment (i.e., *M. elengi* based coil, positive and negative controls). All mosquitoes were exposed to the vapor of burning coils for 1 h. After each experiment, the numbers of fed and unfed (alive and dead) mosquitoes were counted. The protection provided by the smoke from the plant samples against biting *Ae. aegypti* was calculated in terms of percentage of unfed mosquitoes due to treatment:

[(Number of unfed mosquitoes in treatment–Number of unfed mosquito in negative control=Number of treated mosquitoes) × 100]

Data analysis

SPSS software package 16.0 version was used for all analyses. Mosquito toxicity data from laboratory assays were transformed into arcsine/proportion values and then analyzed using a two-way ANOVA with two factors (i.e., dosage and mosquito instar). Means were separated by Tukey's HSD test. Furthermore, mosquito mortality data from laboratory assays were analyzed by probit analysis, calculating LC_{50} and LC_{90} following the method by Finney [26]. Ovicidal data were transformed into arcsine $\sqrt{}$proportion values and analyzed by ANOVA with two factors (i.e. dose and species). Means were separated using Tukey's HSD test (P < 0.05).

RESULTS AND DISCUSSION

Characterization of biosynthesized silver nanoparticles

The colour of the *M. elengi* leaf extract changed from yellowish to brown when it was combined with silver nitrate aqueous solution due to the reduction of Ag^+ ions to Ag^0 nanoparticles (Fig. 1). We discovered a considerable shift in peak maxima in UV–vis experiments. After 240 minutes, the maximum absorption was at 440 nm, with 20.10 a.u. The synthesis of AgNPs in the *Phyllanthus emblica* solution was confirmed by the results of UV-visible spectrophotometers, which exhibited a spectrum of surface plasmon resonance (SRP) ranging from 430 to 436 nm of absorption band [27]. The colour change over time could be connected to changes in nanoparticle concentration and size. As a result, absorbance peaks can be utilized to estimate particle size and stability. The absorbance maximum of smaller AgNPs is approximately 420 nm, which increases with particle size and decreases when the particle size goes beyond Nano dimensions.

The diffraction pattern of AgNPs synthesized by *M. elengi* conformed to the lattices of crystalline AgNPs, according to XRD analysis (Fig. 2). Based on the face centered crystalline structure of AgNPs, the intense peaks matched to (110), (102), (004), (200), (210), (220), and (102) Bragg reflection. The XRD pattern revealed that the AgNPs produced by *M. elengi* were crystalline in form. AgNPs synthesized from *Allium fistulosum* had 2h ranges of 37.48°, 47.78°, 55.74° and 68.87° which corresponds to (111), (112), (020) and (220), respectively [28].

The FTIR spectrum of the aqueous suspension containing AgNPs biosynthesized using *M. elengi* leaf extract is shown in Fig 3. 3379, 2936, 2824, 2537, 2035, 1650, 1452, 1022, and 645 cm1 were the main transmittance peaks. These peaks also suggest that carbonyl groups from amino acid residues may act as capping agents, preventing AgNP aggregation and thereby stabilizing the medium [29]. The AgNPs biosynthesized with *M. elengi* leaf extract were predominantly spherical in shape, with sizes ranging from 35 to 65 nm, according to SEM (Fig. 4). These capped AgNPs were poly dispersed and persistent in solution for at least 8 weeks, according to our findings. Ankanna et al. [30] found that biogenic silver nanoparticles made from *Boswellia ovalifoliolata* stem bark were well-dispersed and had a mean size of 30–40 nm, which matched our findings.

The EDX pattern revealed the chemical composition of AgNPs produced by *M. elengi* (Fig. 5). It demonstrates the existence of pure silver and other components, indicating that AgNPs are biosynthesized. The strong optical absorption band peak at 3 keV is typical of metallic silver nanocrystallites absorption range [31]. Furthermore, the faint signals are most likely attributable to X-ray emission from *M. elengi* leaf extract proteins/enzymes. The particle size distribution analysis for our *M. elengi*-synthesized AgNPs was evaluated using dynamic light scattering and was consistent with SEM analysis (Fig. 6a). The particle diameter distribution indicated a range of around 30 to 120 nm, with an average particle size of 70 nm (Fig. 6b). Dwivedi and Gopal [32], recently reported that silver and gold nanoparticles generated by *Chenopodium album* were stable over a wide pH range due to their strong zeta potential.

Larvicidal and pupicidal toxicity against *Ae. aegypti* in laboratory

The leaf extract of *M. elengi* showed great toxicity against *Ae. aegypti* larvae and pupae in experimental circumstances. A dose-dependent impact was discovered, which is consistent with prior findings with other plant extracts [33] 267.439 ppm (larva I), 316.785 ppm (II), 358.599 ppm (III), 457.516 ppm (IV), and 507.841 ppm (pupa) were the LC_{50} values for *M. elengi* extract (Table 1). The presence of botanical compounds previously identified by GC/MS analysis [34], which primarily affect the midgut epithelium and secondarily affect the gastric caeca and malpighian tubules in mosquito larvae, may be linked to the larvicidal activity of *M. elengi* leaf extract [35]. AgNPs produced by *M. elengi* were extremely toxic to *Ae. aegypti* larvae and pupae, with LC_{50} values of 22.393 ppm (larva I), 24.178 ppm (II), 29.944 ppm (III), 35.684 ppm (IV), and 41.658 ppm (pupae) (Table 1). A rising number of green-synthesized AgNPs have recently been found to have comparable larvicidal and pupicidal toxicity against various mosquito vectors [36]. Abutaha et al. [37], reported the *Cinnamomum burmanni* extract caused mosquito larval mortality with LC_{50} and LC_{90} values of 184.28 and 263.92 mg/mL against *Cx. pipiens*.

Ovicidal activity

In ovicidal tests, egg hatchability of *Ae. aegypti* was reduced following treatment with *M. elengi* extract, and *M. elengi* produced AgNPs caused 100 percent death after treatment with 500 ppm and 40 ppm, respectively, while control eggs hatched 100 percent (Table 2). To our knowledge, there have been few attempts to shed light on the ovicidal capabilities of plant extracts and manufactured AgNPs. Shehata et al. [38], found that the hatchability of those eggs was reduced from 94.9 % in the control to 59.4 % and 34.8 % at 25 and 50 ppm *Pulicaria jaubertii* extract concentrations, respectively. Recently, Thelma and Balasubramanian, [39] highlighted the ovicidal activity of silver nanoparticles synthesized by *Bacillus marisflavi* against the *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi*, the LC_{50} and LC_{90} values for the ovicidal activity were 13.96 ppm and 63.31 ppm, 24.54 ppm and 69.93 ppm, 29.14 ppm and 65.84 ppm, respectively.

Adulticidal activity

The table 3 shows the adulticidal activity of *M. elengi* aqueous extract and synthesized AgNPs against the adult of *Ae. aegypti*. The adult perished after displaying restless activity and aberrant wagging at increasing concentrations. The mortality rates were proportionate to the concentration. The LC_{50} (LC_{90}) values for *M. elengi* leaf extract and synthesized AgNPs were 37.548 (760.872) ppm and 26.942 (61.364) ppm, respectively. Similarly, Ejeta et al. [40] highlighted the highest adulticidal activity was observed in methanol extract of *Azadirachta indica* with 75% adult mortality at 300 ppm and lowest LC_{50} of 106.65 ppm and LC_{90} of 1,293 ppm. The lowest larvicidal and adulticidal activity was observed in methanol extracts of *Ocimum lamiifolium* with 63.35% larval mortality and leaf extract

of *Moringa olifeira* with 50% adult mortality at 300 ppm, respectively. This result is lower compared to the finding of Messebo et al. [41], where ethanol leaf extract of *Ocimum lamiifolium* showed 90% mortality against adult *An. arabiensis* after 1 h exposure 0.25(v/v) in other parts of Ethiopia.

Impact on mosquito longevity and fecundity
Adult longevity and fecundity of *Ae. aegypti* were reduced after a single treatment with *M. elengi* extract and *M. elengi*-synthesized AgNPs (Table 4). The longevity were lowered to 9.6 days following treatment with 500 ppm *M. elengi* aqueous extract and 5.4 days after treatment with 50 ppm *M. elengi* synthesized AgNPs, as compared to 25.4 days in the control group. The female fecundity were also greatly reduced after treatment with *M. elengi* extract and *M. elengi*-synthesized AgNPs; 145 eggs were recorded in the control, while eggs recorded in treatments were 155.2 (100 ppm), 123.8 (200 ppm), 103.4 (300 ppm), 89.2 (400 ppm), and 74.2 (500 ppm) of *M. elengi* extract and synthesized AgNPs were eggs at 146.4 (10 (Table 4). Arjunan et al. [42] found a reduction in adult longevity in *An. stephensi* after treatment with AgNPs produced using *Annona squamosa* (4.2 in male and 11.7 in female at 10 ppm).

Smoke toxicity of *M. elengi* -based coils against *Ae. aegypti*
The table 5 shows the findings of smoke toxicity tests against *Ae. aegypti* biting activity conducted with *M. elengi*-plant based coils. The percentages mean of unfed mosquitoes after treatment with the leaf-based coil were 60% respectively. The positive control had a slightly higher mortality rate. The percentages of unfed mosquitoes after a single treatment with the leaf-, stem, and root-based coils made using *Phyllanthus niruri* were 58, 40, and 61 %, respectively, in agreement with our experiments [23]. Very recently, Wendimu and Tekalign, [43] highlighted the mean smoke repellency index of smoke from burning *Azadirachta indica, Eucalyptus camaldulensis* and *Ocimum forskolin* repellency index (R) of *Ae. aegypti* and *An. arabiensis* by direct burning.. The mean repellency index (R) of the smoke against *An. arabiensis* was 93.27% and against *Ae. aegypti was* 93.26%. Mosquito adults exposed to *M. elengi* smoke oviposited fewer eggs in our investigation (data not shown), confirming our hypothesis that some plant-borne chemicals can limit mosquito fecundity.

CONCLUSION

In this study, to describe a simple, easy, and environmentally acceptable method for the synthesis of AgNPs that uses *M. elengi* leaf extract as a reducing and capping/stabilizing agent. The quick and low-cost synthesis of AgNPs was validated by UV–Vis spectroscopy, XRD analysis, SEM, EDX spectroscopy, DLS, Zeta and FTIR spectroscopy. The results contribute to the body of knowledge concerning the efficacy of AgNPs synthesized from *M. elengi* leaf extract against dengue vector, *Ae. aegypti*. Moreover, at low doses, the plant-made AgNPs were effective larvicidal/pupicidal, ovicidal and adulticidal, lifespan and fecundity chemicals. This study provides to demonstrate the practical application of *M. elengi* leaf extract and *M. elengi*-synthesized AgNPs, the focus on nano-formulate useful for the socio-economic, eco-friendly approach for vector control program me.

ACKNOWLEDGEMENTS

INTEREST CONFLICT
The authors declare no conflict of interest.

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Table 1: Larvicidal and pupicidal toxicity of *M. elengi* leaves extract and synthesized AgNPs against dengue vector, *Aedes aegypti*

Treatment	Target	LC_{50} (LC_{90})	95% Confidence Limit LC_{50} (LC_{90})		Regression equation	x^2 $(d.f. = 4)$
			LCL	UCL		
M. elengi leaf extract	I instar	267.439 (495.346)	244.565 (458.530)	289.295 (544.757)	$y = -1.504 + 0.006x$	5.252 *n.s*
	II instar	316.785 (601.522)	290.406 (547.370)	344.117 (679.205)	$y = -1.426 + 0.005x$	1.171 *n.s*
	III instar	358.599 (684.199)	328.690 (612.194)	393.092 (793.724)	$y = -1.411 + 0.004x$	1.418 *n.s*
	IV instar	457.516 (895.214)	409.584 (760.296)	531.226 (1137.700)	$y = -1.340 + 0.003x$	0.763 *n.s*
	Pupa	507.841 (984.194)	447.881 (818.478)	610.721 (1302.689)	$y = -1.366 + 0.003x$	0.528 *n.s*
M. elengi synthesized AgNPs	I instar	22.393 (43.804)	12.403 (35.496)	29.224 (66.532)	$y = -1.340 + 0.060x$	11.206 *n.s*
	II instar	24.178 (47.768)	14.123 (38.562)	31.450 (74.126)	$y = -1.313 + 0.054x$	10.714 *n.s*
	III instar	29.944 (62.283)	26.946 (55.943)	32.948 (71.787)	$y = -1.187 + 0.040x$	1.726 *n.s*
	IV instar	35.684 (74.489)	32.187 (65.126)	39.851 (89.836)	$y = -1.178 + 0.033x$	1.306 *n.s*
	Pupa	41.658 (86.956)	37.230 (73.791)	48.056 (110.706)	$y = -1.179 + 0.028x$	0.941 *n.s*

LC_{50} = lethal concentration that kills 50% of the exposed organisms

LC_{90} = lethal concentration that kills 90% of the exposed organisms

LCL = Lower Confidence Limit

UCL = Upper Confidence Limit

χ^2 = chi-square; n.s. = not significant ($\alpha = 0.05$)

Table 2: Ovicidal activity of *M. elengi* leaf extract and synthesized AgNPs against dengue vector, *edes aegypti*

Treatment	Percentage of egg hatchability					
	Concentrations (ppm)					
M. elengi leaf extract	Control	100	200	300	400	500
	98.6±1.14	52.2±1.78	33.6±1.14	18.8±1.92	8.2±1.30	NH
M. elengi synthesized AgNPs	Control	10	20	30	40	50
	97.8±0.83	41.2±1.30	19.4±1.14	8.6±1.81	NH	NH

Values were means±SD of five replicates.

NH-No hatchability (100% mortality)

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Table 3. Aduldicidal activity of *M. elengi* leaf extract and synthesized AgNPs against dengue vector, *Aedes aegypti*

Treatment	Concentration (ppm)	Mortality (%) (mean±SD)	LC$_{50}$ (LCL-UCL)	LC$_{90}$ (LCL-UCL)	x^2
M. elengi leaf extract	Control 100 200 300 400 500	0.0 ± 0.0 19.2 ± 2.04 28.2±1.92 41.2± 1.30 52.8± 1.92 67.2 ± 1.92	370.548 (334.926-414.565)	760.872 (663.964-920.729)	0.106n.s
M. elengi synthesized AgNPs	Control 10 20 30 40 50	0.0 ± 0.0 27.2± 1.30 39.2±1.30 53.4± 1.14 69.4± 2.30 80.6 ± 1.51	26.942 (23.564-30.051)	61.364 (54.795-71.434)	0.126n.s

Table 4. Fecundity and longevity of *M. elengi* leaf extract and synthesized AgNPs against dengue vector, *Aedes aegypti*

Treatment	Treatment (ppm)	Adult longevity (days)		Fecundity (Nos.)
		Male	**Female**	
M. elengi leaf extract	**Control**	25.4 ± 1.14	34.8 ± 0.83	178.2 ± 3.70
	100	21.2 ± 1.48	31.6 ± 1.51	155.2 ± 3.11
	200	16.6 ± 1.14	24.4 ± 1.14	123.8 ± 2.28
	300	13.8 ± 1.48	21.2 ± 1.48	103.4 ± 2.07
	400	11.2 ± 1.64	15.0 ± 1.22	89.2 ± 2.16
	500	9.6 ± 1.14	13.2 ± 1.64	74.2 ± 2.86
M. elengi synthesized AgNPs	**Control**	25.2 ± 1.30	35.2 ± 0.83	181.2 ± 2.16
	10	19.4 ± 1.14	27.6 ± 2.30	146.4 ± 2.40
	20	13.4 ± 1.51	20.4 ± 1.14	115.0 ± 2.91
	30	10.8 ± 1.92	16.8 ± 1.92	86.6 ± 1.67
	40	8.2 ± 1.30	10.0 ± 1.58	70.8 ± 2.28
	50	5.4 ± 1.14	8.6 ± 1.14	56.8 ± 1.92

Table 5. Smoke toxicity effect of *M. elengi* leaf powder against dengue vector, *Aedes aegypti*

Treatment	No. of mosquitoes used	Fed mosquitoes	Unfed mosquitoes		Total	% unfed over control 1
			Alive	**Dead**		
M. elengi leaf based coil	100	16	24	60	84	60
Control I	100	76	24	0	24	0
Control II	100	10	44	46	90	66

The values are mean of triplicate value observed during the experiment

Control I*= Negative control – blank without plant material

Control II*= Positive control – Mortein coil.

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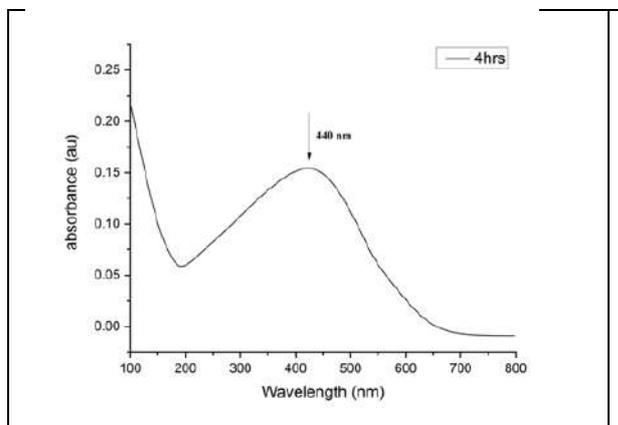
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Fig 1: UV-visualization of the absorption spectrum of *M. elengi*-synthesized AgNPs after 4 h from the reaction	**Fig 2: X-ray diffraction pattern of *M. elengi*-synthesized AgNPs**
Fig 3: Fourier transform infrared (FTIR) spectra of vacuum-dried *M. elengi*-synthesized AgNPs	**Fig 4: Scanning electron microscopy (SEM) micrograph showing the morphological characteristics of AgNPs synthesized using the *M. elengi* leaf extract**
Fig 5: Energy dispersive X-ray (EDX) spectrum of AgNPs synthesized using the leaf extract of *M. elengi.*	**Fig 6: Particle size analysis of AgNPs synthesized using the leaf extract of *M. elengi***
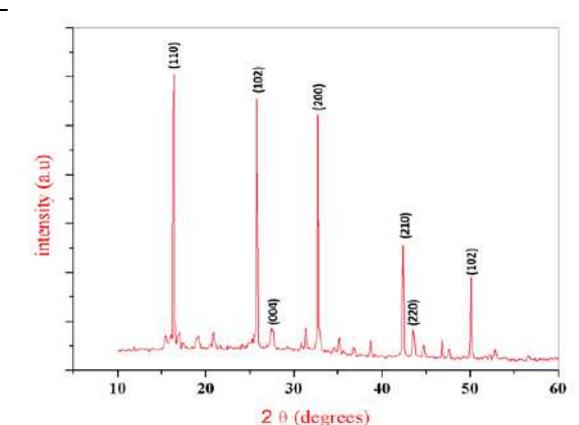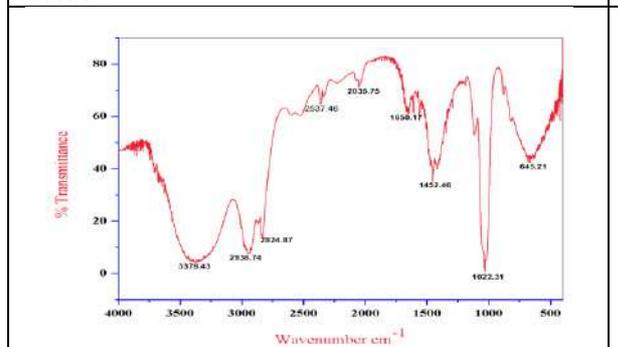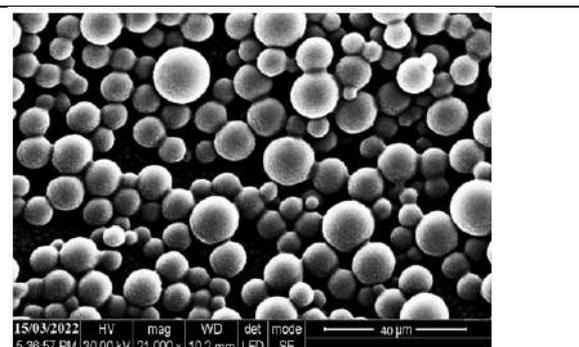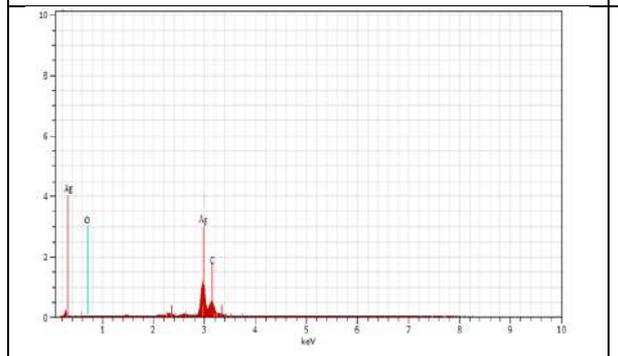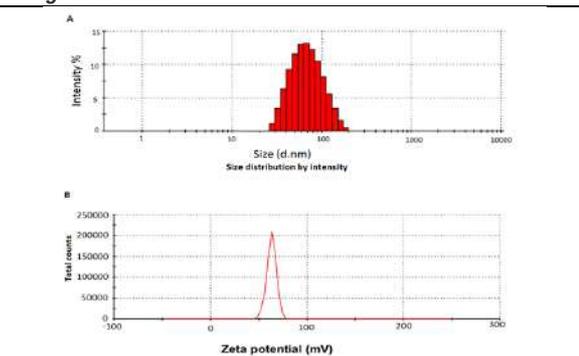
RESEARCH ARTICLE

Effect of Patellar Kinesio Taping Along with Strengthening Exercise in Osteoarthritis with Pre – Dominant Patellofemoral Joint Involvement on Parameters Like PFJES and Q-Angle

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ABSTRACT

To evaluate the effect of patellar Kinesio taping along with strengthening exercise in treating osteoarthritis with pre dominant patellofemoral joint involvement on parameters like PFJES and Q-Angle. 40 Subjects (13 males and 27 females) aged at least or more than forty years of age are included into the study. 40 subjects are divided into two groups 20 each according to the simple random sampling. Group A is taken as the control group. Group B is taken as an experimental group.Group A considering control group is given only strengthening exercise whereas group B experimental is given Kinesio taping along with strengthening exercise. Protocol is provided for 5 days week for 4 weeks where in experimental group Kinesio tape is provided in form if I shaped band for patellar taping and y shaped tape for quadriceps muscle once in a week for 4 week. Later effect was observed outcomes measures PFJES and Q-Angle. The result showed significant results in both the groups when compared within the groups but when seen the result of unpaired t test no significant difference is seen in the values of PFJES scores and values of Q- angle showed almost the same results in both experimental as well as control group. There has been no change seen in the Q- angle in both control as well as experimental group whereas almost same effect observed in the values of the PFJES scores in control as well as experimental group. Thus Kinesio tape has no significant effect on both the outcome measures chosen.

Keywords: Patellofemoral joint, PFJES, Q-Angle, Kinesio tape, strengthening exercise.

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INTRODUCTION

Noticeable   in almost 70% of adults having complaint knee pain , and there are many prevalence studies that support that patellofemoral joint osteoarthritis is more common than tibiofemoral joint involvement .In early course of knee OA disease patellofemoral joint involvement is observed first compared to the other compartment [1] and is observed in 55% of people aged under 50 years [2]. Shearing forces acting between the patella and femoral condyle due to incorrect alignment or malalignment of patella can develop patellofemoral arthralgia in the longer run. Patellar tendon acts as pulley and provides the leverage thus making knee extension efficient. Another major role of patella is that it holds the quadriceps tendon away from axis of movement in the last 30 degrees of knee extension. With this patella also manages as a guide for patellar tendon quadriceps, reduces shear forces in quadriceps mechanism, manages capsular stress in knee, serves as bony guard for cartilage of femoral condyles and enhances aesthetic appearance of knee [3]. The mediolateral translation that patella undergoes during knee joint movement is referred to as patellar shift .The inability of patella to move sideways or in up and down direction required for the easy and smooth movement of the joint can be one of the reason for the decrease in the ROM of the knee joint thus creating imbalance in the patellofemoral joint.

The net effect of pull of the quadriceps and patellar ligament is commonly assessed clinically using the Q (quadriceps) angle. The q- angle is formed between a line connecting the anterior superior iliac spine to the midpoint of patella and line connecting the tibial tubercle and the mid- point of patella .The normal range for Q angle is considered between 10 to 15 degrees. The Q angle of 20 or more is considered as abnormal, creating excessive lateral forces patella that may predispose the patella to pathological changes.[4] the range of normal Q- angle varies in literature, and there is controversy whether a wider pelvic anatomy in women contributes a greater Q- angle [5]. Q- Angle will be measured while participants will be in standing position, the knees exposed in full extension, the patella directed forward in the sagittal plane, and foot in neutral position will be measured using universal goniometer . The angle will be recorded in degrees [6]. Reliability of Q angle ICC= 0.72-0.83 when patient is assessed in standing position [7].

In subjects lacking the muscular flexibility restoration of the tight muscles such as IT band, hamstring and quadriceps has been proved beneficial .Excessive lateral pressure syndrome with a tight lateral retinaculum and tight IT band often responds dramatically to iliotibial band stretching and low load, long duration stretching of lateral retinaculum.[5] Increased stress in lateral retinaculum or decreased strength in Vast us medial is oblique can cause patella to tilt externally, thus increasing compression laterally and reducing it medially. There is some indication that the patellar connections to IT band may cause great lateral pull on patella when the IT band is stiff [8]. Function has been assessed by patellofemoral joint evaluation scale (PFJES) (0-100 points). Excellent results equals 90 to 100 , good points equals 80-89 points , fair equals 60-79 points, and poor equals less than 60 points [9]. Reference of PFJES is "Eleven year follow- up of patellofemoral pain syndrome" karlsson j et al published in 1996 [9]. Kinesio taping one of the methods of therapeutic taping was given by kenzokase in 1990 and due to its great results has become very popular in treating the sports injuries.

Kinesio tape has the characteristics where it being made of cloth with acrylic glue made in a wavy pattern making it good for ventilation allowing the skin to breathe, it sticks to the skin according the contour of the structure where it has been taped and has the minimum chances of the skin allergies. The flexible patterns moves with the skin and permits normal movement. It is water and sweat resistant so it can be kept on to shower and bathe. It has shown good results in decreasing the pain, reducing the inflammation, muscle ailment where the compulsion of mobility / function is present. Kinesio tape can be continuously used foe 3 to 7 days, and keep the therapeutic effects for daily usage [10]. According to the science of kinesiology Kinesiological taping is designed on the theory that muscles in the body are in charge of all the movements occurring in the body and also having the control of other components like circulation of blood and body temperature.

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There are different types of taping available for therapeutic purpose and one of them is rigid taping also known as McConnell taping introduced by Jenny McConnell in 1984 an Australian physiotherapist widely used for PFPS. It is used for patellar tracking but it does not have the properties like Kinesio tape also possess less amount of flexibility and cannot be worn for a longer duration of time thus Kinesio taping has been used for the experimental group rather than the McConnell taping. Thus the present study has been conducted to see the effect of Kinesio taping along with strengthening exercises in treating osteoarthritis patients with pre-dominant patellofemoral joint involvement.

MATERIALS AND METHODOLOGY

● SOURCE OF DATA: Sainath hospital
● STUDY DESIGN: An experimental study
● SAMPLE SIZE : 40
● SAMPLE DESIGN: Simple Random Sampling
● DURATION OF STUDY: 1 year

MATERIALS USED

● Plinth
● Goniometer
● Consent form
● Pen
● Pencil
● Kinesio tape
● Scissors
● Weight cuffs for strengthening

METHODOLOGY

● Subjects aged at least or more than 40 years of age are included into the study.
● Both gender are included.
● Subjects are thoroughly explained about the exercise protocol and research being carried out, written consent is taken after getting ethical permission.
● Subjects having knee osteoarthritis with pre-dominant patellofemoral joint involvement are taken.
● Subjects fulfilling all inclusion criteria and having evidence of lateral patellofemoral osteophytes on weight bearing radiographs are taken into the study.
● 40 subjects are divided into two groups 20 each according to the simple random sampling.
● Group A is taken as the control group.
● Group B is taken as an experimental group.
● Group A considering control group is given only strengthening exercise whereas group B experimental is given Kinesio taping along with strengthening exercise.

TREATMENT DURATION
o Exercises are performed once a day, 5 days a week for 4 weeks.
o Kinesio Taping is provided once a week as the effect of Kinesio tape lasts for 3 to 7 days in experimental group. And same exercises were provided in both the groups .Exercises provided are listed below and type of taping is described as well.
  Ankle pumps. 20 rep
  Static quadriceps exercise (10 maximal isometric quadriceps contraction)

☐ Last degree knee extension. 10 rep

☐ High sitting knee extension. 10 rep

☐ Vast us medial is muscle contraction in sitting position squeezing a rolled up towel between the knees along with gluteal contraction.10 rep

☐ Hip abductor strengthening in side-lying first actively later (after 1 week) according to progressive resisted exercise protocol according to patient's repetition maximum . 10 rep.

☐ Straight leg raising first actively later (after 1 week) according to progressive resisted exercise protocol according to patient's repetition maximum . 10 rep

☐ Hip external rotator strengthening.10 rep

☐ Muscles undergoing tightness will be stretched.

☐ Exercises were performed once a day, 5 days a week for 4 weeks.

☐ Patellar Kinesio taping with enough force (50 – 75% tension) to medially shift patella for mechanical correction.

☐ Taping is given once a week for 4 weeks along with strengthening exercises, while patellar position has been corrected with the help of Kinesio tape and forces work correctly to maintain the proper knee biomechanics.

☐ Taping is given in form of y- shaped on the quadriceps muscle which is one of the muscle facilitating technique for more muscle fibres recruitment. As well as I – shaped tape is provided for patellar correction.

RESULTS AND DISCUSSION

This study involved 40 subjects aged forty years and above, matching the inclusion and exclusion criteria. These subjects were randomly divided into 2 groups control group (exercise only) and experimental group (exercise + taping). The parametric test was used in statistical analysis using SPSS version 25. Demographic values were compared within and between the groups using paired (independent T - test) and unpaired T- test respectively. The result showed significant results in both the groups when compared within the groups but when seen the result of unpaired t test no significant difference is seen in the values of PFJES scores and values of Q- angle showed almost the same results in both experimental as well as control group. Knee pain and functional limitations related to patellofemoral joint osteoarthritis is very common, which in majority can be treated in primary rather than secondary care. Kinesis taping along with knee strengthening exercises is recommended treatment for osteoarthritis, but for pre dominant patellofemoral joint osteoarthritis, but there are limited RCTs based evidence to support the recommendation.

According to the article " Q-angle in patellofemoral pain relationship with dynamic knee valgus , hip abductor torque , pain and function ." the Q- angle is a well- known used outcome measure for checking knee problems especially with patellofemoral pain syndrome . the more the q-angle larger lateralization force on the patella , which in turn increases retro patellar pressure between the lateral facet of patella and lateral femoral condyle . And this continuous pressure on long turn cause PFPS over a long period of time and even causes degeneration of joint cartilage of patella. Hubert's and Haynes reported that 10 % increase in q- angle increases the stress by 45%. Q-angle not only affects the static alignment of lower limb in frontal plane , but also the lever arm distance between the canters of hip and knee joint alters the capacity of hip abductor muscles to greater torque [9].

Thus hip abductor strengthening has provided significant improvement in functional scale (PFJES) as given in both control and experimental group. Even after providing the strengthening of the muscles there has not been enough strength acquired to keep the patella in position due to many reasons like hypotrophy of the muscles which is age related lesser motor unit recruitment also age related. Other than that Kinesio tape tends to have flexible material which provides greater mobility rather than stability and in spite of providing the patellar Kinesio tape for correction of patella it unable to keep the joint in alignment as knee is a major weight bearing joint which could be the reason of having no change being observed in Q-angle of both control as well as the experimental group.

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Patellofemoral joint evaluation scale (PFJES) is a widely used scale for knowing the functional status of the patient with the patellofemoral osteoarthritis as all the components are designed for the major impairments observed in the patellofemoral joint arthritis which has a score between 0- 100 , where higher the score lesser the disability which is also observed after providing the intervention that there is increase in the score after providing the intervention in both the groups when observed within the groups. As there is increase in the muscle strength after the strengthening and reduction in the pain after taping is the reason behind increase in the PFJES score thus increasing the functional capacity of patient and thus it being beneficial. But when compared between the groups it is observed that the improvement in the PFJES scores is same in both the control as well as experimental group and no additional effect of taping has been observed in improving the PFJES scores which can be due to the reasons like the greater amount of the arthritic changes in the joint or Kinesio tape unable to hold the patella in the correct position there by not correcting the biomechanical aspect and not treating the muscular imbalance created.

According to the article by Van Tiggelen *et al* "Effect on bracing on prevention of anterior knee pain – A randomized prospective study." Suggested that patients who does not have any symptoms the data are limiting and conflicting, with patellar taping noted to be ineffective or detrimental therefore the pre rehabilitation does not support use of patellar taping until supported by addition research. Thus Kinesio tape is not being effective on outcome measures like PFJES scores and Q-angle due to the following reasons discussed above [11].

CONCLUSION

The study was conducted to see the effect of patellar Kinesio taping along with strengthening exercise in treating osteoarthritis with pre – dominant patellofemoral joint involvement on the parameters like Q- angle and PFJES (patellofemoral joint evaluation scale) for checking the functional ability of the individual. There has been no change seen in the Q- angle in both control as well as experimental group whereas almost same effect observed in the values of the PFJES scores in control as well as experimental group. Thus Kinesio tape has no significant effect on both the outcome measures chosen.

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Table 1. Outcome Measures

OUTCOME MEASURES	EXPERIMENTAL GROUP MEAN(SD)	CONTROL GROUP MEAN (SD)	t value	P value
PFJES	82.20 (13.46)	75.80 (12.27)	-1.571	0.125
Q- ANGLE	21.50 (0.65)	21.50 (0.65)	0.846	0.402

Abbreviations: PFJES – patellofemoral joint evaluation scale Q- Angle – Quadriceps angle
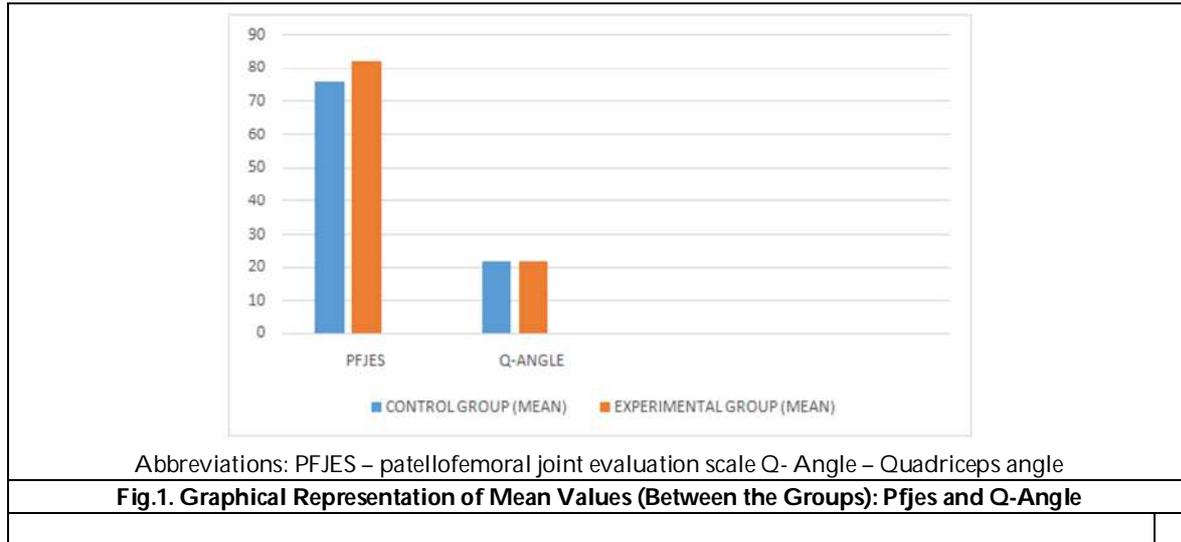
Fig.1. Graphical Representation of Mean Values (Between the Groups): Pfjes and Q-Angle

Impact of Orphanage on Child Personality Development and Psychological Well Being among Children in Selected Orphanage Home at Karaikal District

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ABSTRACT

Children are the future of our country. Wellbeing of children is essential for country's development as they constitute to the future human resource of the country. The emotional and behavioral problems are more among orphans and other vulnerable children because they are exposed to abuse, exploitation, neglect, lack of love and care of parents. The research approach used for this study was quantitative approach. The research design for this study is non experimental descriptive research design. Sample size was 100 orphan children. Purposive sampling technique was used for the study. The collected data was analyzed by using both descriptive & inferential statistics. The study result found that out of 100 samples the majority of orphan children had 63 (63 %) had average personality development, 27 (27%) had low personality development and 71 (71%) had average psychological well being, 29 (29%) had poor psychological well being. There was a significant association found between the personality development and psychological wellbeing with sex. The main conclusion drawn from this study was orphanage home have the major impact in personality development and psychological wellbeing among orphan children.

Keywords: Impact, orphanage, children, personality development, psychological well being.

INTRODUCTION

The orphanage home is one of the numerous social institution that have emerged over the year to carter for some of the social needs of orphan associated with problems. Children who are deprived of the parental care and a secure family environment often become vulnerable to a host of psychological problems and psychiatric disorders. These children are usually reared in institutional homes set up by the government or private agencies, which provide some semblance of order into their lives. In these homes also, the problems of overcrowding, inadequate personal attention, poor academic environment, and frequent moves may affect the psychological health of these children. The term "orphan" refers to those children and adolescents who have lost both the parents and the term "vulnerable" refers to those in institutional homes who were abandoned by their parents or those who had run away from home and had no contact with their families. At times, the term "children" is used to refer to both "children" and "adolescents." Orphans are far away from their families, they live in an untreated life. You can give them a good way to live, train them in future life, good luck to them and also help to develop the mindset andto guide them all the problems in an orphanage is the best institute. UNICEF and global partners define an orphan as a child under 18 years of age who has lost one or both parents to any cause of death. There were nearly 140 million orphans globally in 2015, including 61 million in Asia, 52 million in Africa, 10 million in Latin America and the Caribbean, and 7.3 million in Eastern Europe and Central Asia. This large figure represents not only children who have lost both parents, but also those who have lost a father but have a surviving mother or have lost their mother but have a surviving father. Personality development is the relatively enduring pattern of the thoughts, feelings, and behaviors that distinguish   individuals from each other. The dominant viewpoint in personality psychology indicates that personality emerges early and continues to develop across one's lifespan. Adult personality traits are believed to have a basis in infant temperament, meaning that individual differences in disposition and behavior appear early in life, potentially before language of conscious self-representation develop. When a child loses either of his parents or both, he faces so many economic and mental issues down the line. For example, one child dies every three seconds as a result of poverty, hunger or similar causes, which makes an average of 10,000 children mortalities every day. Most of them are orphans and abandoned children.

STATEMENT OF THE PROBLEM

Impact of orphanage on child personality development and psychological well being among children in selected orphanage home at Karaikal district.

OBJECTIVES

- To assess the personality development among children   in orphanage home.
- To assess the psychological well-being among children   in orphanage home.
- To find the impact of personality development & psychological wellbeing among children in orphanage home.
- To assess the relationship between personality development & psychological well being among children in orphanage home.
- To determine the association between personality development   among children   in orphanage home with their selected demographic variables.
- To determine the association between psychological well being   among children   in orphanage home with their selected demographic variables.

MATERIALS AND METHODDS

RESEARCH APPROACH
Research approach used for this study was quantitative approach.

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RESEARCH DESIGN
The research design is connected with overall framework conducting the study. The research design used for this study was non experimental descriptive research design.

SETTING OF THE STUDY
The study will be conducted in selected orphanage home at Karaikal.

POPULATION
Population isall elements (individuals, object or substance) that meet the certain criteria for inclusion in a study. The study population will be all the children in selected orphanage home.

SAMPLE AND SAMPLING TECHNIQUE
SAMPLE
Sample is a subset of the population selected for a particular study and members if the samples are study subjects. The sample of the study comprises of children in selected orphanage home at karaikal district.

SAMPLE SIZE
The sample size consists of 100 children in selected orphanage home during the data collection period.

SAMPLING TECHNIQUE
Sampling is the process of obtaining information about on entire population by examining only a part of it.
A purposive sampling technique is used for the study. Concerned center authorities were approached and consent obtained from the children and guardian. The participants for the study were collected on the basis of selection criteria.

SELECTION CRITERIA
The samples are selected based on the following:

INCLUSIVE CRITERIA

- children who are residing in orphanage home.
- male and female children between the age of 13-18 yrs.
- who are able to read and speak Tamil / English

EXCLUSIVE CRITERIA
- children with severe mental health problems and chronic physical disorder
- children who are not willing to participate in the study.
- single parent child

Data collection process
Part I
Demographic variables: It consists of demographic variables of orphan such as age , sex, reason for being institute, admitted age in home, years of stay in the orphanage home, admitted age in orphanage home, education status, reason for not attending school, medical services available.

Part II
EZ Yale Personality Questionnaire:
A personality assessment questionnaire is a self report questionnaire designed to elicit respondents perceptions of themselves with respect to six personality and behavioural dispositions such as Communications skills, emotional responsiveness, self-esteem, self confidence, self - adequacy, dependence.

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Carol D. Ryff, psychological wellbeing scale

The Psychological Wellbeing (PWB) Scale measures six aspects of wellbeing and happiness: autonomy, environmental mastery, personal growth, positive relations with others, purpose in life, and self –acceptance.

LIMITATIONS AND RESEARCH NEEDED

1.The study is limited to orphan children.

2.The study is limited to 100 samples.

The study recommendations are the following

A similar study may be conducted on larger samples at different areas for generalize findings.

An experimental study can be undertaken with control group.

A study can be undertaken to assess the effectiveness of counseling towards promotion positive personality development and psychological wellbeing among children.

A comparative study can be conducted by comparing the personality development & psychological wellbeing among orphan children & biological children.

DISCUSSION

The findings of the study revealed that there is a significant impact on personality development & psychological wellbeing of orphan children based on sex. Sandeep Kaur et al, (2017), reported that out of 120 orphan children, 109 (91%) reported various types of psychological problems. Most of (94.17%) of the orphan children had moderate psychological well-being. Age of orphan children, period of stay in orphanage home and BMI had significant impact on physical health problems and psychological well-being.

CONCLUSION

The present study results revealed that orphanage homes have a significant impact on child's personality development and psychological wellbeing. Though social welfare services and training of professional social workers can succeed out making their contribution towards to improving the orphan children personality development and promote their healthy lifestyle.

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Table 1: Impact of Personality Development & Psychological Wellbieng Among Orphan Children

S.NO	STATISTICS	VALUE	'P' VALUE
1	**Chi square**	103. 273	
2	**Cox &snell R square**	0.088	<0.01
3	**Nagelkerke R square**	0.130	

Table 2: Association between Chi –Square Results of Demographic Variables of Personality Development among Orphan Children.

S.No	Demographic Variables	chi-square value	Df	P Value	Results
1	Age	.006	1	.939	NS
2	Sex	3.714	1	.054	SIG.
3	Reason for being in institute	.562	1	.454	NS
4	Years of stay in the orphanage home	.063	1	.802	NS
5	Admitted age in orphanage home	.178	1	.673	NS
6	Are you going to school for getting education at present	0.238	1	0.618	NS
7	Reason for not attending school	1.237	1	0.293	NS
8	Frequency of meals provided by the orphanage home per day	.524	1	.469	NS
9	Do you have guardian	1.070	1	.301	NS
10	Medical services available	.442	1	.506	NS

Table 3: association between chi –square results of demographic variables of psychological wellbeing among orphan children.

| S.No	Demographic variables	chi-square value	df	'P' Value	Results
1	Age	1.929	1	0.165	NS
2	Sex	9.194	1	0.002	SIG.
3	Reason for being in institute	1.477	1	0.224	NS
4	Years of stay in the orphanage home	1.477	1	0.224	NS
5	Admitted age in orphanage home	0.999	1	0.318	NS
6	Are you going to school for getting education at present	0.121	1	0.684	NS
7	Reason for not attending school	0.928	1	0.153	NS
8	Frequency of meals provided by the orphanage home per day	1.018	1	0.313	NS
9	Do you have guardian	0.103	1	0.748	NS
10	Medical services available	0.009	1	0.926	NS

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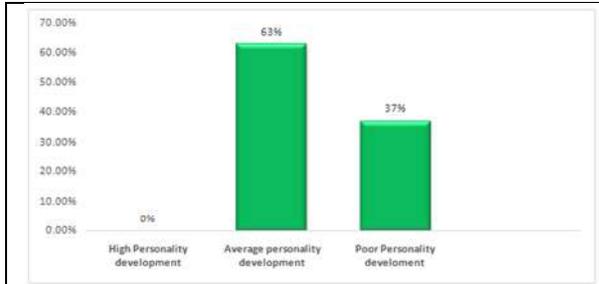
| Fig:1 Data on Level of Personality Development Among Children In Orphanage Home	Fig:2 Data On Level of Psychological Wellbeing Among Children In Orphanage Home
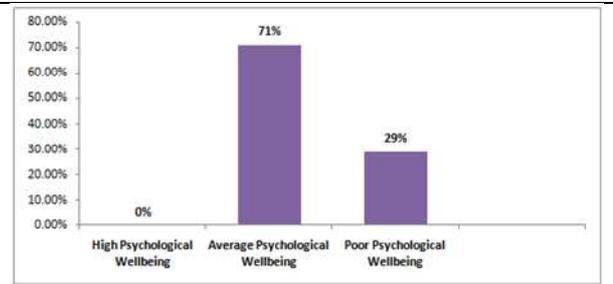
RESEARCH ARTICLE

Isolation of Microalga Strains from Fresh Water Habitat and Evaluation of Lipid Content Profiles for Biodiesel Production

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ABSTRACT

Microalgae can be an alternative option to produce fuel because of their versatility as biomass source. In the present research work chlorella and *Spirulina* sp were isolated from the fresh water habitat. For the production by assessing biomass productivity and lipid content, these two microalgae species were evaluated. Among these two microalgae chlorella sp showed the highest biomass productivity of 1.32 g dwt L^{-1}. While *Spirulina* sp showed the lowest biomass productivity of 1.20g dwt L^{-1}. Lipid productivity of chlorella sp was highest (0.44g L^{-1}) compared to *Spirulina* (0.37g L^{-1}) Biomass and lipid content of microalgae in autotrophic growth condition also studies highest biomass, lipid content was recorded. In 45th days by chlorella followed by *Spirulina*.

Keywords: Microalgae, Biomass, chlorella sp, lipid content

INTRODUCTION

Microalgae are biomass feed stock that has found remarkable application in biodiesel production. Microalgae have a complicated composition that necessitates the use of specialised harvesting technologies. They are made up of a diverse array of photosynthetic, heterotrophic organisms from various phylogenetic groupings and taxonomic divisions. They are tiny unicellular organisms that use sunlight as an energy source and CO_2 as a carbon source to produce biomass with better yields than photosynthetic plants (S.Kimanet.,al.,2016). They are distributed world, inhabiting predominantly fresh and seawater ecosystems. Due to the high lipid content in their cells, microalgae may be utilized to generate biofuel as an alternative energy source, with the added benefit of using less land than

terrestrial plants (Xin, L.et. al., 2010). Microalgae can produce 58,700 l/ha of oil for biofuel, according to a few studies, which is more than other crops like jatropha and canola, which produce 1892 L/ha and 1190l/ha, respectively. (Singh. J et. al.,2010).The high lipid content of microalgae biomass is a key element in its biofuel potential. Yielded 22% lipids from their biomass. (Zhang. Q et. al.,2014) . Biodiesel can be made from edible oil seed crop like sunflower, palm, rapeseed, soybean, coconut, and other, which are referred to as first-generation biodiesel feedstock. However, using such feedstock's for biodiesel production has caused issues because it disrupts the global food supply and security balance. In recent years, non-edible seed crops such as jatropha, karanja, and Mahua, as well as waste cooking oil, grease, and animal fats, have gained prominence as second-generation feedstock's for biodiesel synthesis. On the other hand, these second-generation feedstock's are inadequate to fully replace present transportation needs. Micoalgalhas has lately gotten a lot of interest as a third-generation feedstock (Nauthiyal P et. al., 2014). With their biotechnological abilities, oleaginous species have been suggested as possible sources of oil for biofuels, such as substitutes for gasoline, kerosene, and diesel. Because they are both renewable and carbon neutral, they are important for both the environment and the economy (R.Slade.et. al., 2009). Biofuels are good for the environment, cutting down on greenhouse gas emissions, growing businesses in the area, improving social structure, agriculture, and securing supplies (Reijnders L., et. al., 2006). Biodiesel will help to create a balance between agricultural, economic development, and environmental protection (Meher LC., et. al., 2006).

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF MICROALGAE

Water samples for microalgae isolation were collected aseptically from areas in a freshwater pond near Annamalai University that seemed to be undergoing algal growth. Ten milliliters of water were transferred to a 500 milliliter conical flask holding 400 milliliters of sterilized BBM medium, then incubated for three weeks on a rotational shaker at 27°C and 150rpm under continuous illumination with white fluorescent light at intensities of of 40 lmolm^{-2}s^{-1}. Every two days, the flasks were checked for algal growth using an optical microscope, and serial dilutions in zarrouk's media were produced from flasks that showed signs of growth. By inoculating 50ml of culture solution onto petri plates containing zarrouk's medium that had been toughened with 1.5 percent (w/v) of bacteriological agar, subcultures were produced. Additional 50ml aliquots of the same dilution were inserted into the wells of 93 microliter plates containing 400ml zarrouk's medium, and the results were recorded. Each of the initial flasks was subjected to the same set of treatments. For two weeks, the culture petri plates were incubated at 27 degrees Celsius under constant light. The consistency of the culture was shown by repeated plating and close scrutiny under a microscope (John DM and colleagues, 2003).

BIOMASS, LIPID CONTENT AND LIPID PRODUCTIVITY

The total lipid was extracted from the biomass using a process developed by Bligh and Dyer that was significantly modified. A 50mL microalgae culture was collected by centrifuging it at 4000rpm for 15 minutes. Then it was re-dissolved in 1 mL of pure water. It was necessary to sonicate the sample for 30 minutes at maximum power after it had been combined with 1.25ml chloroform and 2.5ml of methanol. Immediately after sonication, the tube was incubated overnight at 27°C at 100rpm. Then 1.25ml of chloroform was added of the extraction mixture on next day and the sonication again for 30min. After separation of chloroform and aqueous methanol layer. 1.25ml water was added and then centrifuged at 4000rpm for 10min .The second extraction was performed by 2.5ml chloroform. After gently removing chloroform layer. From the bottom and voterxing. The chloroform portion were collected and washed with 5ml 5% Nacl solution and evaporated at 50°c in an oven to dryness. Gravimetrically, the weight of the crude lipid recovered from each sample was determined. The biomass concentration (mg/L) is expressed as the dry weight of the microalga biomass.

RESULTS AND DISCUSSION

ISOLATION AND IDENTIFICATION OF THE MICROALGAE

Outlying the microalgae was philologically and microbial. The conclusion of the experiment was the microalgae are different shape viz., (Fig: 1) spherical and filamentous of the microalgae were recognized using the morphological characterization, the micro algae isolated from pond water was identified are *chlorella* and *Spirulina* sp and the details insert in Table: 1The results revealed that the chlorella, *Spirulina* sp was isolated sample of where as identified in green, colonial, unicellular microalgae (payanada, et. al., 2010). From fresh water ponds Chidambaram TamilNadu, India. (Shankar et. al., 2013) has reported to have isolated similar microalgae from this study have been implicated in biodiesel production. Dayananda et al. (2010) Reported isolated and identified chlorellasp for its growth and lipid oil content, where cultured samples of the green, colonial, unicellular microalgae. By morphological inspection under a microscope based on cell morphologies, the two isolated micro algal cultures were identified as the genera *Spirulina* and chlorella. (Chinnasamy S. et. al., 2010).

BIOMASS, LIPID CONTENT AND LIPID PRODUCTIVITY

This two algal species was evaluated the production of lipid by biofuel making and lipid production in 250 ml flask by using Growing cultures 21 days after incubation beyond same condition. *Chlorella sp* point out the more biofuel production at 1.32g dwt L^{-1} like wise *Spirulina* sp less amount of biomass production 1.20g dwt L^{-1} compare the both microalgal species tested (Table -2) Lipid content data for various algae species is easily available and has been published dependably in the literature. (Griffiths. MJ et. al.,2009). The ability to induce substantial accumulations of lipids in many distinct microalgae species may result in a high oil output from a variety of microalgae. In previous studies, it was found that total lipid concentrations of 30% to 50 % of dry biomass weight were common. Some microalgae had total lipid concentrations above 90 % because they grew in different conditions(Mata TM.et., al., 2010).

BIOMASS AND LIPID CONTENT OF GROWTH CONDITION IN AUTOTROPHIC CONDITION

These studies showed that, autotrophic condition to analyze the biomass concentration of microalgae strains were 3.3, 5.8, 9.3g/L and lipid content of these strains are 0.5g , 1.3g and 2.9g for *Spirulina sp*, 4.3, 6.5 and 10.2g/l and lipid content of these strains are 1.0, 2.1 and 4.2g/l for chlorella sp. This result is similar to the findings for *chlorella vulgaris* (Liang Y., et al 2009) and *Chlorella protothecoides* (Heredia – Arroyo T.et., al 2010),

CONCLUSION

Algae has a great ability to fix Co_2, thus it is an on interesting method for the removal of gases exited from power plants and can be used to reduce greenhouse gases with higher biofuel yield. production of microalgae biomass and con Almost all of these advantages are due to the fact that these plants have evolved over billions of years to create and store energy in the form of oil, and they do it more efficiently than any other natural or artificial process. So microalgae biomass are essential as preferring biofuel. Microalgae were isolated, identified, cultured, and collected from freshwater as a result of this research. The majority of the isolated microalgae have a high biodiesel production potential.

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Table: 1Microalgae observation at microscope

S.NO	Algae name	Morphology character
1.	Chlorella sp.	Spherical in shape
2.	*Spirulina* sp.	Filamentous shape

Table: 2 Biomass lipid content and lipid productivity

S.no	Microalgal strain	Biomass productivity (g dwt L^{-1})	Lipid productivity (gL^{-1})	Lipid content (% biomass)
1.	Chlorella sp.	1.32	0.44	20
2.	*Spirulina* sp.	1.20	0.37	18.7

Table: 3Different growth rates of microalgae under autotrophic condition

S.No	Microalgae	15th day		30th day		45th day	
		Biomass (g/L)	Lipid (g/L)	Biomass (g/L)	Lipid (g/L)	Biomass (g/L)	Lipid (g/L)
1.	*Spirulina sp*	3.3±0.4[b]	0.5±0.1[b]	5.8±0.2[b]	1.3±0.2[b]	9.3±0.2[b]	2.9±0.3[b]
2.	*Chlorella sp*	4.3±0.5[a]	1.0±0.3[a]	6.5±0.1[a]	2.1±0.1[a]	10.2±0.4[a]	4.2±0.4[a]

| *Spirulina sp* | *Chlorella sp* |

Fig: 1: Microscopic observation of *Spirulina* sp and chlorella sp

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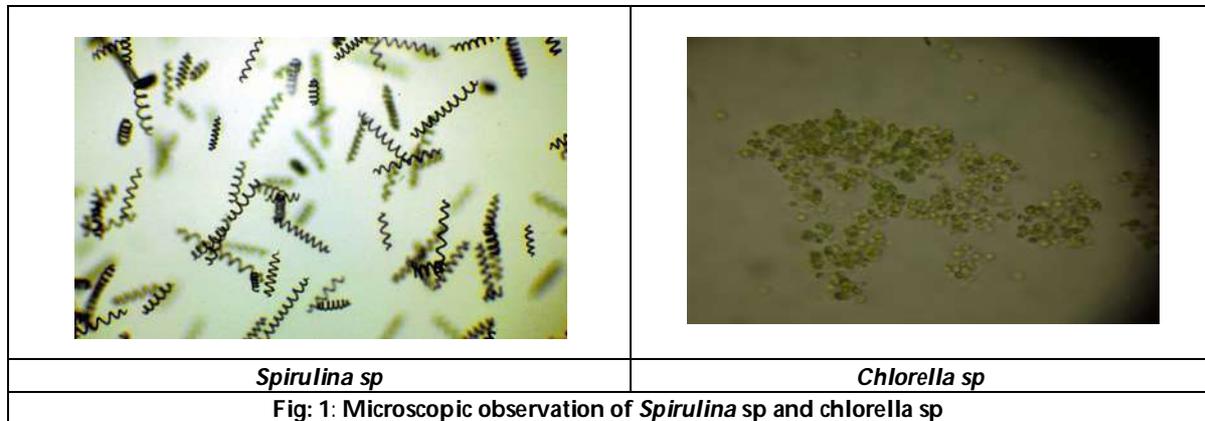
Regulation of the Intracellular ROS Level is Critical for the Antiproliferative Effect of *Terminalia bellirica* in the Hepatocellular Carcinoma Cell Line -HepG2

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ABSTRACT

Terminally Billerica, an antioxidant falconoid, has been known that it can induce the cell cycle arrest and apoptosis of hepatocellular carcinoma (HCC) cells by the stabilization or induction of p53. Here, we found that TB reduced the proliferation of HepG2 cells significantly. Interestingly, TB down-regulated the intracellular ROS level in HepG2 cells. Functional study using saran showed that the proliferation of HepG2 cells was still regulated by TB in the absence of p53. Furthermore, we confirmed the effect of TB on HepG2 cells by H_2O_2 supplementation. This study demonstrates that the ant proliferative effect of TB on HCC cells can be mediated by reducing intracellular ROS, which is independent of p53 expression.

Keywords: TB, Reactive Oxygen Species, HepG2, Ant proliferative, Apoptosis

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common types of cancer and the second leading cause of cancer related deaths in the world (1). HCC is characterized by genetic changes affecting multiple signal TB cascades, resulting in the uncontrolled growth of hepatocytes (2). Because of the heterogeneous etiology of HCC, only some HCC patients (about 30%) can be treated with appropriate treatment modalities such as liver resection,

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transplantation, or local ablation (3). To date, only one targeted chemotherapy agent sorafenib (Nexavar, Bayer), has been approved for treating HCC patients (4). However, not all HCC patients show a positive response to sorafenib (5). Therefore, it is vital to identify a molecular target specific for HCC and to develop suitable chemotherapy agents. The activation of several signal TB pathways has been implicated in the pathogenesis of HCC, such as reactive oxygen species (ROS), which can trigger the oxidative damage of bimolecular. Indeed, one of the main features of cancer cells is a persistent pro-oxidative state that leads to intrinsic oxidative stress (6,7). ROS participate in carcinogenesis from initiation to malignant conversion, by mutation of the proto-ontogenesis and tumor suppressor genes and subsequent activation of the signal transduction pathways (8, 9).Therefore, numerous studies have suggested that antioxidants that can quench intracellular ROS would be helpful for reducing cancer risk (9,10). Flavonoids are polyphenolic plant pigments that are ubiquitous to plant cells. These are antioxidants and free radical scavengers protecting against the oxidative reactions taking place in the body. The average daily intake of these secondary metabolites from natural sources for humans is 1—2 g per day [8]. Research on flavonoids has gained significant importance as they act as functional compounds with the potential to serve as derivatives of new drugs. Also, beneficial effects of falconoid-rich natural products have been established [9]. Supplementing processed foods with flavonoids is being practiced to warrant sufficient daily intake to enhance the immune system [4].

One such important plant widely used in the treatment of various ailments and a rich source of flavonoids and other bioactive compounds is Triphala Roxby. Throb. *(T. Billerica)* is a deciduous medicinal tree that is seen mostly in the moist valleys, lower hills of India and in other parts of South East and Central Asia. It belongs to the Combretaceae family and is a rich source of bioactive constituents [5]. Various parts of the tree are used for different therapeutic purposes. How- ever, the dry fruits of TBpossesses maximum health benefits [6]. Traditionally it is one of the principal constituents of the Ayurvedic formulation Triphala, where it is used as an expectorant [8]. Ethanol extract of TBWA's determined to be effective against several pathogenic organisms like P. vulgarism, E. coli, B. subtitles and S. aurous [9]. Separation and purification of vital bioactive constituents from the dried fruits of TB will open new inroads in the pharmaceutical, chemical, food and processing industries. Furthermore, TB can induce the cell cycle arrest and apoptosis of HCC cells by the stabilization or induction of p53 (2, 6). p53, a tumor suppressor protein involved in cancer prevention, can regulate the cell cycle, apoptosis, and DNA repair (7). Accordingly, several compound that up regulate p53 functions have been reported to inhibit the proliferation of cancer cells by inducing cell cycle arrest or apoptosis (18). Despite the significant roles of ROS and p53 in cancer survival, there are few reports showing their relationship in the ant proliferative effect of TB on HCC. In this study, the ant proliferative effect of TB was investigated using two different HCC cell TBs, HepG2. TB reduced the proliferation of HepG2 cells. Interestingly, it was found that TB down-regulated the intracellular ROS level of HepG2 cells. In addition, the expression of p53 was up-regulated by TB. However, functional studies using small-interfering RNA (saran) showed that TB was still able to regulate the proliferation of HepG2 cells in the absence of p53. Moreover, the ant proliferative effect of TB on HepG2 cells was reduced by H2O2 supplementation. Overall, the present study demonstrates that TB can regulate the proliferation of HCC cells by reducing intracellular ROS, which is independent of p53 expression, and suggests that TB is useful for HCC treatment as an antioxidant.

MATERIALS AND METHODS

TB (91950), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 4.6 diamidino-2-phenyTBdole (DAPI), 5,5′,6,6′-tetrachloro-1, methanol, dithiotrheitol (DTT), 2′, 7′–dichloro fluoresce in (DCF), phosphate buffered TB (PBS), and rib nuclease (Raze) were purchased from Sigma Aldrich, company St.Louis, USA antioxidant assay kit was purchased from Cayman Chemical Company Ann Arbor, USA. All other chemicals and reagents used were of analytical grade and obtained either from Sigma Aldrich or Hymenia laboratories, Mumbai, India. Primary antibodies against HepG2 cells were transected with saran against the p53 gene (sip53, forward; 50-ACUCCAGUGGUAAUCUACTT-30 and reverse; 50- UAGAUUACCACUGGAGUCTT-30) using Lipofactor-2000 (Aptabio, Korea).

Cell TB

Human hepatocellular carcinoma cell TB Hip G2 was obtained from National Centre for Cell Science (NCCS), Pane, India. The cells were cultured in Dulbecco's Modified Eagles Medium/nutrient mixture F-12 Ham (DME-F12) medium and Roswell Park Memorial Institute-1640 (RPMI-1640) medium supplemented with10% fetal bovine serum (FBS) and 1% pencil TB/streptomycin (100U/ml pencil TB and 100µg/ml streptomycin) at 37°C in a humidified incubator with 5% CO2. Cells were subculture before each experiment and were grown to 60–70% confluence before treatment with TB. Cells were treated with TB for 24 and 48h respectively. All set of experiments were carried out within 5 additional passages.

MTT assay

The cytotoxic effects of TB on HepG2 were determined by MTT assay. In brief, cells (1×105 cells/well) grown in 96-well micro liter plates were incubated for 24,and 48h with different concentrations of TB (24,40µM). Following the incubation, medium was discarded and 20µl of MTT (5mg/ml) was added to each well and incubated for 3h at 37°C. Later, the Formosan crystals formed were solubilized with diethyl sulfoxide (DMSO) and the color developed was quantified using a micro liter plate reader (Bio-Rad) at a measuring wavelength 570 nm and a reference wavelength 630nm. Cytotoxicity is expressed as percentage of viable cells over the control cells.

AO/EB staining assay

The characteristic apoptotic morphological changes in TB treated and untreated HepG2 cells were assessed using AO/EB staining. In brief, HepG2 cells were grown in 6-well plates (5000cells/ well) and incubated with TB for 24 and 48h, respectively. Then, the cells were washed with ice-cold phosphate buffered saline TB (PBS) and fixed with 6% glutaraldehyde solution for 30 min. The fixed cells were then washed once with PBS and stained with AO/EB (1mg/ml in PBS) for 15 min. The nuclear changes and chromatin condensation in the cells were visualized through a fluorescent microscope (Carl Zeiss, Jena, Germany).

Measurement of the Intracellular ROS

Level the intracellular generation of ROS was analyzed with the probe dichlorofluorescein dictate (H2DCFDA, Sigma-Aldrich, MO), a membrane-per meant fluorescent probe that is widely used to monitor intracellular ROS production. After treatment with TB, cells were incubated with 10 mM H2DCFDA at 37⁰C for 30 min in the dark. Subsequently, the cells were harvested, washed three times with PBS, and DCF fluorescence distribution was measured using Guava Easy Cite (Millipore, MA).

Biochemical assessment of antioxidant enzymes and lipid per oxidation

Control and TB treated cells in T75 flask were trypsinized and washed with PBS. The harvested cells were suspended in 10µM dithiothreitol, 130mM KCl, 50mM PBS and vortexes . Later, the cells were centrifuged at 10,000rpm at 4°C for 10 mines and the resulting supernatant was used for biochemical estimations. The levels of lipid per oxidation byproducts, thiobarbituric acid reactive substances (TBARS) in HepG2 were determined by the method of Niihau's and Samuelsson (1968). The activities of enzyme and nonenzymic antioxidants such as superoxide dismutase (SOD), catalane(CAT),glutathione(GSH)andglutathioneperoxides(Gap)wereassayed by the method of Kaka et al. (1984), Sinhala (1972), Elman (1959) and Rotrucketal (1973) respectively.

Mass Spectrometry/Gas chromatography

Mass spectrometry (GC–MS) The representative extract obtained using TB were dried and reconstituted in ethanol for GC—MS analysis. GC—MS analysis was carried out using a gas chromatography (PerkinElmer Claus 500) with a 250 lm diameter and 30 m long Elite-5 capillary column (5% phenyl/95% dimethylpolysiloxane) equipped with a mass selective detector in the electron ionization mode. About 2L of the sample was injected for the analysis. Helium was used as the carrier gas and supplied at the flow rate of 1 mL/min, with a split ratio of 1:10. The oven temperature was increased from 60 °C to 150 °C at the ramp rate of 6 °C/min and held at 150 °C for 2 min. This was followed by

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temperature increase to 280 °C at the rate of 4 °C/min and held at the same temperature (280 °C) for 5 min. The injector was maintained at 280 °C. The mass spectra were recorded at 70 eV over a mass-to-charge ratio range of 40—450 am. The chemical compositions were identified by direct comparison of their mass spectral pattern in NIST 2005 mass spectral library.

Scanning Electron Microscopy
The surface morphology of the feed material separated after TB were investigated using a field emission scanning electron microscope (JSM 6701F, JEOL, Japan). The samples were sprinkled on the carbon pasted stub, following which a thin layer of gold was sputter coated to render the surface conductive.

Statistical Analysis
The measurements were expressed as mean ± standard deviation (SD) for each experiment. All analyses were performed in triplicates and each experiment was repeated at least two times. Student t-test was used to determine whether the differences between the experimental and control groups were statistically significant. P values less than 0.05 were considered statistically significant ($P < 0.05$; $P < 0.01$; $P < 0.001$).

RESULTS

Analysis of Triphala isolated from TB extract using Fourier Transform- Infra Red (FT-IR) spectroscopy
The FT-IR spectra of pure isolated Triphala showed typical hydroxyl stretching frequencies due to phenol and carboxyl acid groups in the region 3220-3390 cm-1, overlapped with aromatic C-H stretch at 3020 cm-1 (Fig3). The characteristic bands of COOH is elucidated with the presence of band near 1700 cm-1 due to carbonyl C=O stretch and a band near 1450 cm-1 appears due to C-O single bond. The C=C double bonds of aromatic appear at 1620 cm-1. The spectral feature in the range 1240 – 1330 cm-1 was attributed to the phenol stretch. The weaker bands in the range 1020-694 cm-1 are for the out of plane CH bending of aromatic ring. The reported framework bands clearly coincide with earlier report suggested for Triphala with the extract of TB on HepG2 Hydrogen bonding has a significant influence on the peak shape and intensity, causing peak broadening and shifts in absorption to lower frequencies. The CH stretching bands of alkenes and aromatic C-H stretch vibrations are clearly seen at 2973 to 1447 cm–1. Almost all the carbonyl C=O stretching bands are strong and occur at 1870 to 1550 cm– 1, whereas ketenes, aldehydes, carboxylic acids, amides and esters showed IR absorption at 1709 to 1610 cm–1. Conjugation, ring size, hydrogen bonding, satiric and electronic effects often result in significant shifts in absorption frequencies. C-N bending alkaloids have absorption bands at 1447-1206 cm1.

Growth inhibitory effect of TB
The effect of TB on growth inhibition of HepG2 cells were evaluated by MTT assay as shown in Fig.4. Notably, TB exhibited growth inhibitory effects against HepG2 cells with an IC50 value 24 μM at 24 h treatment and 40 μM at 48 h treatment. Further, the mode and mechanism of TB induced apoptotic cell death on HepG2 cells was further explored.

Apoptotic cell death on AO/EB on TB in HepG2
The result were as follows The control (untreated cells) hepG2 cell TB appears green fluorescence indicates that the live HepG2 cell TB take up the stain Alcidine orange and showed the green fluorescence.

Effect of ROS Level in TB
Effect of TB on the proliferation and ROS level of HepG2 cells the effect of TB on cellular proliferation was first investigated HCC cell TB, HepG2. In comparison with control cells (treated with the vehicle [DMSO] alone), the proliferation of HepG2 cells was down-regulated by TB (Fig.6). Cell proliferation was observed until 2 days after TB

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treatment by microscopy. The proliferation and morphology of HepG2 cells were affected by TB after 48 h. The ant proliferative effect of TB was also confirmed in other HCC cell TB, PLC/PRF-5. The variation in the ROS level in HepG2 cells after TB treatment was measured using H2DCFDA as a probe for detecting intracellular ROS. The number of fluorescence positive cells in the M2 region was reduced to half after treatment with TB, indicating that TB reduced the intracellular ROS level in HepG2 cells. On the other hand, the cell number in the fluorescence-negative region was increased up to two-fold. These results demonstrated that TB can regulate the ROS level in HepG2 cells, after TB treatment. TB increased the expression of p53 and HO-1 but decreased that of cyc TB A and CHK1 The expression of p53 and HO1 was increased but that of CHK1 was decreased in response to the increase in TB up to 100 mM. The relative analysis also showed the significant change of protein expression. These results indicated that TB can regulate the proliferation and antioxidant activity of HepG2 cells by increasing the expression of p53 and HO-1.

Effect of TB on lipid per oxidation and antioxidant enzymes

Treatment of HepG2 cells with TB showed a significant increase in the levels of TBARS and a significant decrease in the activities of enzyme and non-enzyme antioxidants such as TBARS, SOD and CAT as compared to the control untreated cells (Fig. 7 (a) and (b)), reveal TB the anti-oxidant role of TB in HepG2 cells.

Gas Chromatography in TB on HepG2 cell TB

The GC- MS was performed on Joel, ACCU TOF GCV. The GC coupled with high-resolution mass spectrometer (HRMS) and with library search uses EI ionization mode. About 20 µl samples were injected at 240°C (Injector temperature) in the column (30 m x0.32 mm ID) with carrier gas as helium. GC oven temperature programmed started at 80° C for 2 min followed to final temperature of 280° C. The sample analyzed at constant flow of 2 ml / min. The mass range analyzed at m/z [10 - 600] with ionization detected, recorded using Flame Ionization Detector (FID). The complete Data acquisition was performed by NIST library search. GC MS confirm the presence of rhodopin and octadecanoic acid as the active principles in eliciting anti-inflammatory along with anti-cancer properties on HepG2 cancer cell TB on the extract of TB.

Scanning Electron Microscopy

The scanning electron microscopy technique is a powerful method for investigation of surface structures of herbal medicines namely leaves, fruits, pollen grains and seeds. The particle morphology of the extract of TB and therein purified sample Triphala was investigated using scanning electron microscopy (Fig.9). SEM not only produces images that are analogous to those from an optical microscope, but it also can produce images whose contrast is based on compositional variations of specimens. The SEM images of TB and purified Triphala with different morphologies for sample is compared at 1, 5 and 10µm, respectively. The SEM image of crude shows an irregular morphology with a mixture of uneven crystals with no obvious inter crystal TB (Fig.9 (a, b and c)). The presence of a distinct rod like morphology was observed for purified sample, with twisted, TB of fibrils.

DISCUSSION

The seven hallmarks of cancer include explicative immortality, cell death resistance, and evasion of growth suppressors, inflammation, angiogenesis, invasion and metastasis. Among these, inflammation is considered to be an important factor for tumor promotion and progression in precancerous cells via triggering the expression of genes that prompts cell proliferation and survival (11).The present study was carried out to characterize the bioactive constituents present in the herbal extract of TB using FT-IR analysis. TB was selected for the current investigation to detect the functional group of sample. The FT-IR analysis reported the presence of phenols, alcohols, amines and carboxylic acid. The electron-microscopic examination of the cultured cells showed changes frequently found in liver cells after exposure to xenobiotics. The morphologic difference between "programmed cell death", i.e. apoptosis, and necrosis, continues to be debated. Ultra structural changes have been described as characteristic for both forms of damage, but it has been indicated that the changes seen in apoptosis only represent the earlier stage of cells

undergoing necrosis (Gradually 1997). In parallel with other alterations (viability, enzymatic leakage and ROS production) Hip G2 cells showed changes described in apoptosis, such as bleb formation and reduction/disappearance of microvillus on the cell surface, chromatin clumping and imagination adjacent or along the internal nuclear envelope. Mitochondria and endoplasmic reticulum may also show changes, but these have been observed long before the emergence of the concept of apoptosis and were interpreted as early changes preceding the stage of necrosis. In aerobic cells, ROS generation is known to be a crucial factor. The level of ROS in the cells under normal cellular circumstances is well balanced by the antioxidants such as SOD, CAT, and GSH but upon cellular injury this balance is disrupted altering the cellular defense mechanism. Flavonoids especially TB are well-known to impart beneficial effects in the antioxidant defense mechanism of various hematoma cells *via* inhibiting and/or suppressing the factors that promote cellular oxidative stress (12). However, in this study the pro-oxidant nature of TB (Falconoid) increased the ROS generation, stimulated oxidative stress by increasing lipid per oxidation levels, thus enhancing apoptosis.

The electron-microscopic examination of the cultured cells showed changes frequently found in liver cells after exposure to xenobiotics. The morphologic difference between "programmed cell death", i.e. apoptosis, and necrosis, continues to be debated. Ultra structural changes have been described as characteristic for both forms of damage, but it has been indicated that the changes seen in apoptosis only represent the earlier stage of cells undergoing encoring parallel with other alterations (viability, enzymatic leakage and ROS production) Hep G2 cells showed changes described in apoptosis, such as bleb formation and reduction/disappearance of microvillus on the cell surface, chromatin clumping and imagination adjacent or along the internal nuclear envelope. Mitochondria and endoplasmic reticulum may also show changes, but these have been observed long before the emergence of the concept of apoptosis and were interpreted as early changes preceding the stage of necrosis.

Drug resistance is a major obstacle that limits the potency of cancer chemotherapy. Transient drug resistance may occur due to an imbalance in the intracellular redo balance or *via* mutations in the functional genes or the tumor microenvironment also imparts influence on the uptake, metabolism and distribution of drug from a cancer cell (13). Increased GSH levels, over expression of anti-apoptotic proteins (ex. Bcl2, Bcl-xL), lack of functional p53 and decreased expression of pro-apoptotic proteins (ex. Bax) contributes to drug resistance during chemotherapy (14). Based on the present findings and our previous *in vivo* study (12 weeks) explaining the antineoplastic efficacy of TB, only a small fraction of non-dividing hematomas survived TB treatment. Furthermore, TB has significantly decreased the GSH activity, expressions of Bcl2, mutated p53 and cell cycle regulators and unregulated the expression of Bax. Numerous studies have pointed out the role of extracellular matrix (ECM) components including collagen in both drug resistance at cellular level and tissue mediated drug resistance. Collagen deposition in cancer tissues can increase drug resistance by inhibiting the drug penetration in the tissues and in parallel can also increase apoptotic resistance (15). Interestingly our previous *in vivo* result has shown that during HCC, TB inhibits collagen deposition in the liver tissues. Also a recent study by (16), reported the potency of TB promote the cytotoxic effect of 5-Fluorouracil (5-FU), a common chemotherapeutic drug for colorectal, gastric and liver cancer in cubical practice, in 5-FU resistant SGC7901/5-FU cells. Overall, these findings provide evidence that HCC cells may not exert drug resistance during long-term TB treatment

CONCLUSION

Taken together, our results revealed that TB inhibited cell viability, cell migration and induced apoptosis in HepG2 cells in a time-dependent manner. Besides, TB exerted pro-oxidant effect by decreasing oxidative stress *via* triggering the ROS TB. The anti-inflammatory activity of TB was potentiated through inhibiting NF-κB pathway and its downstream targets that contributes to anti-apoptosis. Thus, TB effectively induced apoptosis, along with suppression of ROS, proposing that the anti-cancer activity exerted by TB is associated with the simultaneous regulation of the two molecular pathways, NF-κB and Nrf2. TB might be considered as an attractive anti-cancer agent for the management of HCC.

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| **Figure : 1 Terminalia bellirica fruit** | **Figure : 2 Chemical structure of Terminalia bellirica** |
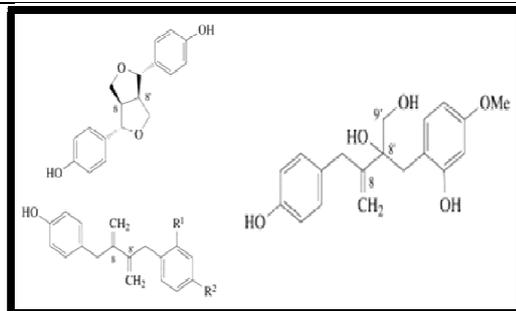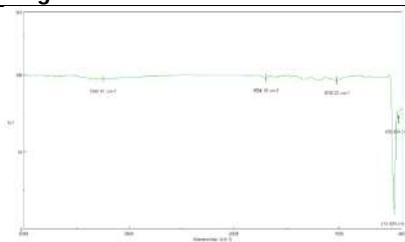
Fig :3(a) FT-IR provides valuable information regarding the identification of compounds or functional groups present in extracts of TB .Shows the FT-IR analysis of the TB. The appearance of strong absorption bands in the region of 3339 to 3332 cm–1 usually comes from stretching vibrations of the O-H and N-H stretching frequencies.
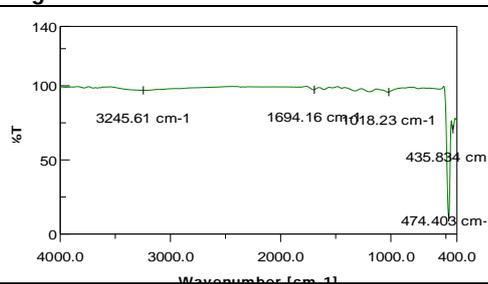
Fig: 3(b) Aromatic compounds contain delocalized p electrons from the resonance-stabilized double bonds, showing skeletal vibrations (including C-C stretchings within the ring) in the region between 1650 to 1400 cm–1 and weak combination and overtone bands in the region between 2000 to 1650 cm–1. This result shows that TB contains various compounds such as aldehydes, ketones carboxylic acids, amides, esters and alkaloids
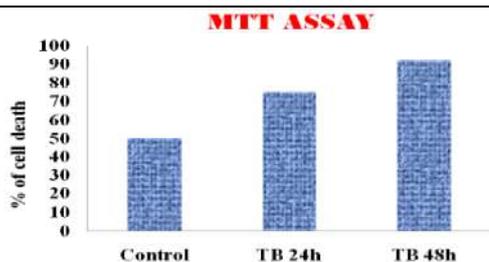

Fig : 4 Analysis of cell viability in TB treated HepG2 cells by MTT assay. HepG2 cells grown in 96-well plates were treated with or without the indicated concentrations of TB for 24 and, 48 h. Following the incubation period, cell viability was assessed by MTT assay. All results are expressed as the mean of three independent experiments. Values are given as means ± SD of three independent experiments in each group. $p < 0.05$; statistically significant when compared to the control value.
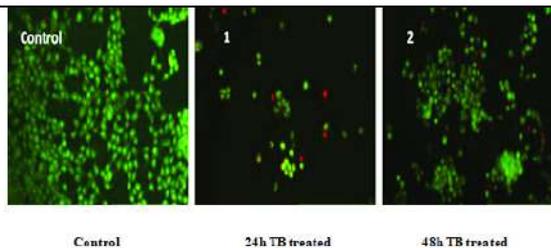
Fig: 5(a, b and c) The test sample TB treated for 24h, 48h on HepG2 cell TB appears green fluorescence indicates that the cell death happened and it take up the Ethidium bromide stain at 800μgm/ml concentration. The morphological characters of cell such as chromatin condensation and the loss of nuclear envelope confirm that the cell death occurred by means of apoptosis. All results are expressed as the mean of three independent experiments. Values are given as means ± SD of three independent experiments in each group. $p < 0.05$; statistically significant when compared to the control value.

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Fig : 6 Bar chart representing fluorescence intensity of cells in each group. Data are presented as the means ± SD of three independent experiments in each group. Significant difference between treatment and control groups is indicated $p < 0.05$ (*).

(Fig.6). The expression of p53 and HO-1 was upregulated by TB after 12, 24 h and 48h, respectively. However, the expression of cyc was not affected by TB. The relative analysis comparing each protein with β-actin showed the statistical significance. The variation in protein expression was also verified in HepG2 cells treated with various concentrations of TB.

Fig: 7 (a) and (b) Effect of TB on the levels of enzymatic antioxidant in HepG2 cell of control, 24h TB treated and 48h TB treated. Activity is expressed as 50% of inhibition of antioxidant for SOD μmoles of hydrogen peroxide decomposed/min/mg protein for catalase; μmoles of glutathione oxidized/min/mg protein for GPx; units/min/mg protein for TBARS; μmoles of GSH conjugate formed/min/mg protein for protein for GR. Values are given as means ± SD of three independent experiments in each group. a,b $p < 0.05$; statistically significant when compared to the control value.

Fig :8 Values are given as means ± SD of three independent experiments in each group. $p < 0.05$; statistically significant when compared to the control value.

(Fig. 9 (a, b and c)). At a similar magnification of 10 μm, the observed features of purified Triphala coincide with the SEM images of original Triphala which was obtained through recrystallization using the batch supercritical anti-solvent process. In conclusion, the present study shows the preliminary qualitative analysis of TB in non polar and polar solvents in SEM images on HepG2 cell TB.

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Figure : 10. Method

RESEARCH ARTICLE

Platinum-Based Regimens on Triple Negative Breast Cancer

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ABSTRACT

Breast cancer is the most frequent cancer among women in the world and it remains a leading cause of cancer death in women globally. Among BCs, triple-negative breast cancer is the most aggressive, and for its histochemical and molecular characteristics is also the one whose therapeutic opportunities are most limited. The current review updates the evidence on platinum-containing regimens for women with a specific breast cancer subtype of triple-negative metastatic breast cancer (mTNBC). Analysis of data indicated that platinum-containing regimens may have provided a small survival benefit to mTNBC patients (HR 0.85, 95% CI 0.73 to 1.00; 958 women; moderate-quality evidence) with no evidence of heterogeneity (P = 0.41; I^2 = 1%). Data of treatment comparisons showed that platinum regimens may improve survival benefits when compared to non-platinum regimens.

Keywords: chemotherapy, platinum, triple-negative breast cancer, regimens.

INTRODUCTION

Breast cancer is the most common cancer diagnosed in women, accounting for more than 1 in 10 new cancer diagnoses each year. It is the second most common cause of death from cancer among women in the world. Anatomically, the breast has milk-producing glands in front of the chest wall. They lie on the pectoralis major muscle, and some ligaments support the breast and attach it to the chest wall. Fifteen to 20 lobes are circularly arranged to form the breast. The fat that covers the lobes determines the breast size and shape. Each lobe is formed

by lobules containing the glands responsible for milk production in response to hormone stimulation. Breast cancer always evolves silently. Most patients discover their disease during their routine screening. Others may present with an accidentally discovered breast lump, change of breast shape or size, or nipple discharge. However, mastalgia is not uncommon. Physical examination, imaging, especially mammography, and tissue biopsy must be done to diagnose breast cancer. The survival rate improves with early diagnosis. The tumor tends to spread lymphatically and hematologically, leading to distant metastasis and poor prognosis. This explains and emphasizes the importance of breast cancer screening programs.

Triple-negative Breast Cancer -overview

Triple-negative breast cancer(TNBC) accounts for about 10-15% of all breast cancers. The term triple-negative breast cancer refers to the fact that the cancer cells do have estrogen or progesterone receptors (ER or PR) and also don't make any or too much of the protein called HER2. (The result is "negative" in all three tests performed on the cells). These cancers tend to be more common in women who are younger than 40, are black, or have a BRCA1 mutation.Triple-negative breast cancer differs from other types of invasive breast cancer in that it grows and spreads faster, has limited treatment options, and has a worse prognosis(outcome).

Diagnosis

Once a diagnosis of breast cancer has been made through imaging tests and a biopsy, the cancer cells will be checked for certain characteristics. If the cells do not have receptors for estrogen or progesterone and do not make too much of the HER2nprotein, it is considered triple-negative breast cancer.

Triple-Negative Breast Cancer Survival Rates

Triple-negative breast cancer is considered aggressive cancer because it grows quickly, is more likely to have spread by the time it is found and is more likely to come back after treatment than other types of breast cancer. The prognosis is usually not as good as it is for other types of breast cancer. Survival rates provide an idea of the percentage of people with the same type and stage of cancer who are alive for a certain amount of time (usually 5 years) after diagnosis. These rates can't tell you how long you will live, but they can help you better understand how likely your treatment is to work.

Triple Negative Breast Cancer Treatment

Triple-negative breast cancer has fewer treatment options than other types of invasive breast cancer. This is because the cancer cells do not have the estrogen or progesterone receptors or enough of the HER2 protein to make hormone therapy or targeted drugs effective .If cancer has not spread to distant sites, surgery is an option. Chemotherapy may be given first to shrink a large tumor followed by surgery. Also, chemotherapy may be given after surgery to lower the chance that cancer will come back. Radiation may also be an option depending on certain characteristics of thetumor. Because hormone therapy and HER2 drugs are not options for women with triple-negative breast cancer, chemotherapy is often used. In cases where cancer has spread to other parts of the body (stage IV), other treatments that may be considered include PARP inhibitors, platinum-based chemotherapy, or immunotherapy.

Literature Review

In [1], the addition of platinum to neoadjuvant chemotherapy based on anthracyclines, cyclophosphamide, taxanes, and fluorouracil, could improve PCR rates in patients with TNBC. For women with mTNBC, there was moderate-quality evidence of a small survival benefit in reducing the number of breast cancer recurrences and may appear to cause tumors to shrink when compared to the chemotherapy without using platinum. An Assessment of toxicity showed that women receiving platinum-containing regimens experienced higher rates of grade 3 and 4 nausea/vomiting and anemia than women receiving non- platinum regimens anemia and vomiting compared to treatment without platinum.

In [2], the administration of CBDCA to PDX-bearing mice induced increased levels of tumor cell necrosis and reduced tumor size. Treatment with CBDCA and anti-PD-1 antibodies reduced TNBC tumor volumes and slightly

improved survival rates. More importantly, immunochemotherapy with CBDCA and anti-PD-1 antibodies before surgery improves the outcome of a secondary tumor after surgery via increasing the number of tumor-specific CD8+ T cells in the tumor microenvironment of murine TNBC.

In [3], The probability of PCR was higher in the platinum-based chemotherapy group versus the control group (RR = 1.45, 95%CI 1.28–1.64); however, no impact on long-term outcome was observed. Neoadjuvant treatment regimens containing platinum resulted in a non-significant increase in the risk of febrile neutropenia and in a significant increase in the risk of serious adverse events, G3–G4 anemia and G3–G4 thrombocytopenia: 11.3% versus 0.8%, RR = 15.66

(95%CI 6.38–38.44). Further follow-up might clarify the long-term impact of platinum- containing regimens. In [4], Compared with ACT, the overall survival (OS) of NACT was poor (HR = 1.59; 95% CI = 1.25–2.02; P = 0.0001), and there was no significant difference in disease-free survival (DFS) between the two treatments (HR = 0.85; 95% CI =0.54–1.34; P = 0.49). NACT with pCR significantly improved the OS (HR = 0.53; 95% CI = 0.29–0.98; P = 0.04) and DFS (HR = 0.52; 95% CI = 0.29–0.94; P = 0.03), while the OS (HR = 1.18; 95% CI = 1.09–1.28; P < 0.0001) and DFS (HR = 2.36; 95% CI = 1.42–3.89; P = 0.0008) of patients with residual disease (RD) following NACT were worse compared to those receiving ACT.

In {5}, the meta-analysis showed that neoadjuvant and adjuvant chemotherapy combined with capecitabine significantly improved both DFS and OS in early-stage TNBC patients with tolerable AEs. There were benefits to DFS in the groups with the addition of capecitabine, adjuvant chemotherapy, and lymph node positivity. In [6], the addition of bevacizumab to CT was beneficial for TNBC patients, and olaparib had a great effect on PFS and ORR, especially for those with BRCA mutations. When combined with CT, targeted agents including iniparib, sorafenib, cetuximab, and ipatasertib may have better efficacies for treating TNBC. In [7], the development of various nanomaterials and nanotechnology had allowed the improvement of cancer biomarkers area with high precision and sensibility which was not the case some years ago.

In [8], P-, B-, PPi-, and Za-containing regimens achieved higher PCR than standard chemotherapeutic agents. BP-containing regimens had a better PCR than B-containing regimens. In indirect comparisons, Za-, BP-, P-, and B-containing regimens were the top four strategies with the highest probability for PCR. Benefit-risk analysis showed that B-containing regimens had the highest acceptability of being the best treatment for better PCR achievement with fewer SAEs. The addition of P, B, BP, PPi, and Za to standard chemotherapeutic agents enhanced the PCR, but a balance between efficacy and safety should be carefully considered. B- containing regimens might be the best choice for neoadjuvant chemotherapy due to their better efficacy and tolerability. In [9], the addition of platinum-based agents improved PCR compared with neoadjuvant therapy based on anthracyclines, cyclophosphamide, taxanes, and fluorouracil (49.1% vs. 35.9%; OR: 1.87, 95% CI: 1.23–2.86). Hematological adverse events were similar in both groups, except for more thrombocytopenia in the platinum-based group (OR: 7.96, 95% CI: 3.18–19.93).

In [10], Platinum-based chemotherapy showed PCR benefit of 40%vs27% (OR1.75,95% CI 1.46– 2.62< 0.0001) in the neo-adjuvant setting. Subgroup analysis showed increased pCR rates (44.6%vs27.8%) with the platinum plus taxane regimen (< 0.0001). In metastatic TNBC, three RCTs were analyzed (N = 531), and platinum treatment did not show a PFS advantage (HR1.16,95%CI 0.90– 1.49,p = 0.24).

METHODOLOGY

Data collection

We obtained relevant studies published in 2018 and identified further potentially relevant studies from previous trial reports, systematic reviews, and meta-analyses. An extensive literature search of the PubMed, Embase and Cochrane databases was performed from their inception to 2 February 2020, with no language restriction, using

the following MeSH terms and/or text words: 'breast neoplasm', 'breast cancer', 'breast carcinoma', 'breast tumor', 'mammary cancer', 'breast malignant tumor', 'neoadjuvant therapy, 'neoadjuvant treatment', 'neoadjuvant therapy, 'cisplatin', 'carboplatin', 'platinum', and 'Platidiam'.

Data extraction
A standardized data extraction form was used to extract relevant information from each study. For each eligible study, the following information was extracted: the first author's name, publication year, patients' countries, patients' ages, study design, number of participations, tumor characteristics, chemotherapy regimen, and follow-up results.

Statistical Analysis
Data was collected in Microsoft office, an Excel Spreadsheet, and statistical analysis was performed using software version 10. The baseline characteristics have been summarized using the descriptive statistics method(number of patients(n), mean, standard deviation, median) response to the chemotherapy with platinum regimen was helped to find mortality and adverse events of the patients have been summarized using this method.

RESULT

Relevant records were identified in the electronic databases. Of these, 82 potentially eligible studies were reviewed with full text. However, although the study by Enriquez et al. is a single-armed trial, it was controlled using historical patient data; this study was therefore included in our analysis. Altogether, we determined whether the patient's data from age 30 to 50 years and also determined whether the patients were diagnosed with Breast Cancer or normal.

Patient characteristics
14 female patients from age 30 to 50 were included in this study; all of the patients belong to Breast cancer. Summary demography and baseline data are provided in table 1.

Platinum compared to non-platinum regimens for metastatic triple-negative breast cancer: OS, PFS, and OTRR

Platinum compared to non-platinum chemotherapy regimens for women with metastatic triple-negative breast cancer:					
Patient or population: women with metastatic triple-negative breast cancer (mTNBC)					
Setting: hospital					
Intervention: platinum					
Comparison: non-platinum chemotherapy regimens					
Outcomes	**Anticipated absolute effects* (95% CI)**		**Relativ e effect (95% CI)**	**No. of participants (treatment-comparisons)**	**Quality of the evidence (GRADE)**	**Comments**
	The risk with non-platinum chemotherapy regimens	**The risk with platinum containing regimens**				
Overall survival (OS)	1-year risk of death		HR 0.85 (0.73 to 1.00)	958 (6)	▢▢▢▢ MODERAT E3	Heterogenei ty: Chi² = 5.05, df = 5 (P = 0.41); I² 1%
	510per 1,0001	455 per 1,000 (406 to				

	510)2				
	The 2-year risk of death				
	711per 1,0001	652 per 1,000 (596 to 711)2				
Progression-free survival/time to progression (PFS/TTP)	The 1-year risk of progression or death		HR 0.77 (0.68 to 0.88)	1077 (8)	⬜⬜⬜⬜ VERY LOW 4 56	Heterogeneity: Chi² = 34.78, df =7 (P <0.0001); I² 80%
	936per 1,0001	880 per 1,000 (846 to 911)2				
	The 2-year risk of progression or death					
	970per 1,0001	933 per 1,000 (908 to 954)2				
Objective tumor response rate (OTRR) (assessable participants)	368per 1,0007	515 per 1,000 (449 to 585)	RR 1.40 (1.22 to 1.59)	1205 (10)	⬜⬜⬜⬜ LOW 45	Heterogeneity: Chi² = 21.44, df = 9 (P = 0.01); I² 58%

GRADE Working Group grades of evidence

High quality (⬜⬜⬜⬜): We are very confident that the true effect lies close to that of the estimate of the effect

Moderate quality (⬜⬜⬜⬜)**:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low quality (⬜⬜⬜ ⬜)**:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low quality (⬜⬜ ⬜ ⬜ or ⬜ ⬜ ⬜ ⬜)**:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Each ⬜symbol represents a downgrading of the quality of evidence one level from the highest level of 'high quality (⬜⬜⬜⬜).'

1Estimated from the average of non-platinum group Kaplan-Meier probabilities from the 3 highest weighted treatment-comparisons in Analysis 1.1.

2Estimated as 1000*(1-S(t)HR) where S(t) is the estimated probability of survival for non- platinum participants and HR is the pooled hazard ratio (Guyatt 1998)

3Downgraded quality of evidence one level for 'serious imprecision' because the confidence interval for the pooled estimate is wide and crosses or nearly crosses unity.

4Downgraded quality of evidence one level for 'serious indirectness' because this outcome is a surrogate endpoint of questionable validity for assessing the more important

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outcome of OS in the context of metastatic breast cancer (Burzykowski 2008). 5Downgraded quality of evidence one level for 'serious inconsistency' because there was substantial evidence of heterogeneity.

6Downgraded quality of evidence one level for suspected publication bias (forest plot asymmetry).

7Estimated from all 10 mTNBC treatment-comparisons in the review with OTRR results.

Platinum-containing regimens and toxicity profile

Platinum compared to non-platinum chemotherapy regimens for treatment related death, nausea/vomiting, nephrotoxicity, anaemia, hair loss, leukopaenia **and treatment discontinuation due to adverse event**

Patient or population: women with metastatic triple-negative breast cancer (mTNBC)
Setting: hospital
Intervention: platinum
Comparison: non-platinum chemotherapy regimens

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No. of participant s (treatment - compariso ns)	Quality of the evidence (GRADE)	Comments
	Risk with non-platinu m chemothera py regimens	Risk with platinu m containing regimen s				
Treatment-related death (safety population)	5 per 1000 1	5per 1,000 (1 to 23)	(RR 1.06, 95% CI 0.24 to 4.61)	843 (5)	⬚⬚⬚⬚ LOW 3	
Nausea/vomiting* grade 3 or 4 (safety population)	15 per 1,000 1	72 per 1,000 (29to 177)	(RR 4.77, 95%CI 1.93 to 11.81)	655 (3)	⬚⬚⬚⬚ LOW 3	

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and
its 95% CI).

CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality (⬚⬚⬚⬚):
We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate quality (⬚⬚⬚⬚): We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that
it is substantially different.

Low quality (⬚⬚⬚ ⬚): Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.

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Very low quality (☐☐ ☐ ☐ or ☐ ☐ ☐ ☐)**:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

Each ☐ symbol represents a downgrading of the quality of evidence one level from the highest level of 'high quality (☐☐☐☐).'

1Estimated from all treatment-comparisons contributing data for pooling for this outcome (including treatment-comparisons with non-estimable eGects due to no events in
either arm).

2Downgraded quality of evidence one level for 'serious imprecision' because the confidence interval for the pooled estimate is wide.

3Downgraded quality of evidence two levels for 'very serious imprecision' because the confidence interval for the pooled estimate is very wide.

4Downgraded quality of evidence one level for 'serious inconsistency' because there was evidence of heterogeneity across studies (P < 0.05)

*data on vomiting was included if data on nausea/vomiting was reported separately

Interference

While analyzing the patient's data we can understand that platinum based regimens are more effective than the non-platinum based regimens. The probability of pCR and survival rate was higher in the platinum-based chemotherapy.This potential benefit needs to be weighed against the risks of toxic side effects from platinum-based regimens such as nausea, vomiting, and aneamia. But these side effects are quite reasonable when compared to the non-platinum regimens..

RESULT AND DISCUSSION

In conclusion, for women with TNBC, there was an evidence of survival benefit from platinum- based regimens compared to non-platinum regimens. This review found that for women with TNBC, a chemotherapy containing platinum reduces the number of breast cancer recurrences appears to cause tumours to shrink. This review found that the patients receiving chemotherapy containing platinum may increase the chance of severe nausea, anemia and vomiting compared to treatment without platinum. The use of platinum-based regimens for TNBC patients given the excess toxicity associated with such regimens. Nonetheless, some women and clinicians may consider platinum-based regimens worth trying given that nausea and vomiting can be manageable with modern antiemetic, and that carboplatin can be a less toxic alternative tocisplatin.

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Table 1. Summary demography and baseline data

Parameters	Values
Total number of patients (N)	14
The severity of the patients (%)	12.2%
Sex(N)	1
Female	4
Age	
Tricenarian (30-39)	5
Quadragenarian (40-49)	8
Quinquagenarian (50-59)	1
ER Status (positive)	9
ER Status (negative)	5
PR Status (positive)	8
PR Status (negative)	6
HER2 Final Status (positive)	1
HER2 Final Status (negative)	13
A tumor (T1)	3
A tumor (T2)	6
A tumor (T3)	4
Metastasis-Coded (positive)	0
Metastasis-Coded (negative)	14

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RESEARCH ARTICLE

Alleviating NaCl Stress by Improving Growth and Yield in *Arachis hypogaea* L. by Exogenous Application of Brassinolide and Paclobutrazol

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ABSTRACT

Salinity is a major a biotic stress limiting growth and productivity of plants in many areas of the world due to increasing use of poor quality of water for irrigation and soil salinization. Peanut (*Arachis hypogaea* L.) is one of the most important legume crops grown worldwide as a source of edible oil and vegetable protein. Arid and semi-arid regions constitute more than half of the peanut production area and 15% of these regions are salt-affected. In this following study the effects of NaCl, NaCl+Brassinolide, NaCl+ Paclobutrazol, Brassinolide (BL) and Paclobutrazol (PBZ) treatment were investigated in Growth parameters such as Dry weight, Fresh weight, Root length, Stem length, Total leaf area, Membrane stability index, Electrolytic leakage index, Relative water content and photosynthetic pigments contents. The plants of BL and PBZ shows exemplary recuperation under salinity. Improvements have been sighted morphologically as well as in cellular activities. Where stem length, leaf area, membrane stability index, electrolyte leakage, relative water content greatly recovered by BL, at the same time root length, fresh weight, dry weight and photosynthetic pigments shows remarkable recovery by PBZ under salinity.

Keywords: Salt stress, Brassinolide (BL), Paclobutrazol (PBZ), Growth, Pigment Composition

INTRODUCTION

Environmentalstressesseverelyaffectthegrowthanddevelopmentinplants.Increased soil salinization is the leading cause of crop productivity loss worldwide [1] and water scarcity [2]. Increased salinity disrupts nutrient balance, carbon metabolism, plant development, and photosynthesis, resulting in physiological drought in plant cells[3]; [4];[5].Plants under stress produce primary and secondary metabolites that act as antioxidants and osmoprotectants to protect against reactive oxygen species (ROS). [6], [7] without hampering the normal functioning of cells [8].

These osmolytes, such as amino acids, soluble sugars, proline, and protein, help crop plants maintain turgor pressure under stress at various stages of their lives [9].Photosynthesis is highly sensitive to adverse environmental conditions [10]. The saline stress induced ROS accumulation causes PSII photo- inhibition and/or photo-damage [11] which lead oxidative damage of cellular components. The antioxidant machineries help to mitigate the oxidative damages cause by excess ROS [12]. Peanut (*Arachis hypogaea* L.) is an important leguminous crop and cultivated in arid and semi-arid regions [13] and also a major oilseed crop in India. However, the crop is susceptible to salinity, and productivity is severely affected [14]. The application of BL and PBZ to peanut (*Arachis hypogaea* L.)in this study results in reduced salinity stress and improved plant growth and yield.

MATERIALS AND METHODS

Plant material and Experimental Design
Peanut (*Arachis hypogaea* L.) seeds (VRI-2 variety) were purchased from Tamilnadu Agricultural University, India. The present study was conducted at the Botanical Garden, Department of Botany, Annamalai University, TN. The geographic coordinates of the experimental area was located at11°23'23.1"N/ 79°43'05.3"E. Healthy Seeds were surface sterilized with 0.2% mercuric chloride (HgCl$_2$) solution for 2 minutes (min) and washed extensively with sterile double distilled water(ddH$_2$O). VRI-2 cultivar had 60 pots divided into six groups. Each group, with ten replicates, were given mixed fertilizer once with manure. The soil composition was red soil: Sand: Farm yard manure in the ratio of 1:1:1. Plants were treated in Control, NaCl (100mM), NaCl (100mM) +BL (3μM), NaCl (100mM) +PBZ (68.06μM), BL (3μM)and PBZ (68.06μM).To maintain particular salinity level in pots, the soil samples from each pot were checked with Electrical Conductivity Meter in regular interval. Plants were harvested for morphological, physiological and chlorophyll pigment analysis at 30, 40 days and 50 days after sowing.

Root length
Root length was recorded by measuring below the point of root-shoot transition to fibrous root and the lengths of lateral roots were taken as total root length. The root lengths were expressed in cm plant[-1].

Stem length
The length between stem tip and point of the root stem transition region was taken as stem length. The stem lengths are expressed in cm plant[-1].

Total leaf Area
The total leaf area was measured using LICOR Photo Electric Area Meter (Model LI-3100, Lincoln, USA) and expressed in cm² plant[-1].

Fresh weight
The plant roots and shoots were washed with the tap water and blotted gently and tissue paper. The root and shoot fresh weights were taken by using an electronic balance (Model - XK3190-A7M) and the values were recorded and expressed in gm plant[-1].

Dry weight

After taking fresh weight, the plants were dried at 60°C in a hot air oven for 48 hours. After drying, the weights were measured and the material was kept in same oven until the constant dry weight obtained. The values were expressed in gm plant^{-1}.

Electrolyte leakage

The total inorganic ions leaked out in the leaves were estimated by the method of Sullivan, 1979. Leaf discs 20 in number were taken in a boiling test tube of 10 ml deionized water and electrical conductivity (ECa) was measured. The content was heated at 45°C and 55°C for 30 min each in a water bath and electrical conductivity (ECb) was measured. Later the content was again boiled at 100 °C for 10 min and electrical conductivity (ECc) recorded. The electrolyte leakage was calculated by using the formula:
Electrolytic leakage (EL) was calculated as,

$$Electrolyte\ leakage\ (\%) = \frac{EC_b - EC_a}{EC_C} \times 100$$

Membrane Stability Index

MSI was determined according to the method of Sullivan (1972). For this purpose, a fully expanded young leaf (5th leaf) was selected from each genotype, treatment and replication. Twenty pieces (1 cm diameter) cut from these leaves were submerged into distilled water contained in test tubes. The tubes were kept at 10°C in a cooled incubator for 24 h, followed by warming at 25° C and measuring the electrical conductivity N(C1) of the contents. The leaf samples were then heated by autoclaving for 15 min and electrical conductivity of the medium measured again (C2). Cellular injury was determined by using the equation:

$$1 - [\frac{1 - \frac{T1}{T2}}{1 - \frac{C1}{C2}}] \times 100$$

(Where T and C refer to treatment and control, respectively, and 1 and 2 refer to conductivity one and two.)

Relative water content

For determination of RWC, fresh leaves were detached from each treatment, replicate, and genotype and weighed immediately to record fresh weight (FW), followed by dipping half of their portion in distilled water for 12 h. The leaves were blotted to wipe off excess water, weighed to record fully turgid weight (TW), and subject to oven drying at 70°C for 24 h to record the dry weight (DW). The RWC were determined by the equation proposes by Turner (1986).

$$RWC\ (\%) = \frac{[FW-DW]}{[TW-DW]} \times 100$$

Chlorophyll pigment contents

Chlorophyll analysis was done on fresh leaves. The method for estimation of pigments was used that of Arnon (1949). Fresh leaf sample (500 mg) was grinded in Ten mL of 80% acetone and chlorophyll was extracted. The absorbance of the extract was deciphered in a spectrophotometer at 645, 680 and 663 nm against 80% acetone blank.

RESULT AND DISCUSSION

Root length

The root length of NaCl treated plants have all time reduction in root length compared toother treatments (Fig.1). NaCl+PBZ treated plants shows improved root length than the NaCl treated plants. Similarly, NaCl+BL treated

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plants also shows the higher root lengths than NaCl treated plants. But when the plants treated with PBZ the root length increases than of Control, but lower than the BL plants.The root length was significantly affected by salinity and treatment. Root length was decreased by increasing salt stress [15].It's reported that the root length and dry mass reduced due to salinity in wheat plant [16].Increasing salinity imposed a negative effect on root growth including: root area, root volume, main root length, total root length in purslane *Portulaca oleracea* L. [17]. The root length was significantly affected was decreased by salinity in sunflower [15]. BLameliorate the salt stress on growth in wheat *Triticum aestivum* L. [18].

Application of BL alleviated salinity injury of potato *Solanum tuberosum* L. by enhancing the root/shoot ratio, shoot length, shoot weight, root weight, and biomass [19]. In *Arabidopsis thaliana*, it is indicated that BL promote cell elongation at the meristematic zone of the root [20]. PBZ increased the root length and dry weight as a result of increased root growth and root system expansion. This may influence the ability of treated plants to make contact with water [21]. Root-bleeding sap is the indicator of root pressure; therefore, the improved root bleeding sap is attributed to higher root growth and root vigor in response to the paclobutrazol application in *Zea mays* L. [22]. It's observed that a higher root activity in rice and wheat treated with PBZ. Thus, the application of paclobutrazol may improve plant performance under stressful condition through stimulating root activity of the plant [23].

Stem length
The stem length of NaCl-treated plants is reduced than other treatment (Fig. 2). Plants treated with NaCl+BL had increased stem length than those treated with saline. Similarly, plants treated with NaCl+PBZ have longer stem lengths than plants treated with NaCl. Plants treated with PBZ, on the other hand, have increased stem length than 'Control' plants, but are a bit shorter than BL plants.The growth of cells is the most important process that is affected by saline stress it causes secondary drought and it eventually leads to the decrease in the growth of cells leads to decrease in the plant height. The Results showed that the plants' stem length was significantly affected by BL, PBZ. The increasing the concentrations of NaCl developed a decline in the lengths of the plants, this study done on moth bean *Vigna aconitifolia* L. [24], on radish plant *Raphanus sativus* L. [25], cowpea *Vigna unguiculata* L. [26], and on black gram *Vigna mungo* L. [27]. It is also reported that wheat facing salinity stress exhibited reduced stem length [28]. Brassinolide could facilitate biological yield and yield-related traits because of its ability to detoxify salinity stress induced by NaCl, through the modification of various gas exchange parameters in chickpea *Cicer arietinum* L.[29] andvegetable mustard*Brassica juncea* L. [30].Reduction in vegetative growth due to PBZ inhibition effect was reported in Peach to increase the distribution of photo assimilates towards reproductive growth which encouraged the formation offlower buds and hence induced the flowering [31].

Total Leaf Area
The PBZ treated plants are having higher total leaf area compare to 'Control' and BL treated plants (Fig. 3). The leaf area of NaCl+PBZ and NaCl+BL have the higher leaf area respectively compare to the NaCl treatments. Another typical response to salt stress is a reduction in total leaf area. Indeed, decreased leaf growth is the earliest response of glycophytes exposed to salt stress [32].The observed reduction in the leaf area may be considered as an avoidance mechanism, which minimises water loss by transpiration when the stomata are closed [33]; [34]. A significant increase was recorded in plant height, leaf area, number of spikes/plant, number of seeds/spike, length of spike/plant, test weight, grain yield after BL treatment in wheat *Triticum aestivum* L.[35]. The salt tolerance improvement in the water spinach *Ipomoea aquatica* L. using paclobutrazol application has been recently proved [36].

Fresh weight and Dry weight
The Fresh weight and Dry weight decrease under saline treatment but the plant get recovered when it get treated with BL and PBZ (Fig. 4 and Fig. 5). The plants of Control show high F.W and D.W compared to other treatments plants of NaCl+BL and NaCl+PBZ. Salt severely affected the growth and resulted significant reduction in shoot length, fresh weight and dry weight in peanut *Arachis hypogaea* L. [37]. Cyclin dependant kinase activity reduced under salt stress conditions which increased the duration of cell division and decreased the number of cell divisions per unit time, ultimately reducing the growth of leaves and the plant [38].Brassinolide application alleviated the

suppression of rice *Oryza sativa* L. seedling growth caused by salinity as a result of increasing shoot length, and seedling fresh and dry weight [39]; [40]; [41]. The plant height, fresh weight and dry weight are highly affected by the PBZ treatment in wheat *Triticum* aestivum L. [42].

Membrane Stability Index (MSI)

The plants under salt stress show high reduction in MSI than Control plants (Fig. 6). NaCl+BL and NaCl+PBZ treated plants show reduced MSI than NaCl treatment alone. In addition to it, plants of BL and PBZ show high MSI, it means the plants cellular membranes are in good condition. Membrane stability index (MSI) decreased under salt stress in all the tested pea genotypes at all NaCl treatments but maximum reduction was noted. Since, membranes damage increased with increase in salinity level so MSI can be considered as very significant tool for evaluating the salt tolerance [43]. BL application reduced electrolyte leakage and improved membrane stability index (MSI) in common bean *Phaseolus vulgaris* L. plants grown under salt stress suggesting the positive role of BL in salt stress management in plants [44]. However, under NaCl stress, application of PBZ improved MSI in rice *Oryza sativa* L. [45].

Electrolyte leakage determination (EL)

The EL value increased in plants with NaCl treated plants (Fig. 7). Addition to it has been seen that the lowest EL values recorded at BL next to PBZ. The NaCl+BL and NaCl+PBZ plants shows comparatively high EL values than the control plants. Electrolyte leakage is an indicator of membrane injury. Plasma membranes are the primary site of ion-specific salt injury [46]. Therefore, electrolyte leakage from plasma membranes is reported as one of the most important selection criteria for identification of salt-tolerant plants canola *Brassica napus*L. [47]. Electrolyte leakage was sharply induced by salt stress, and exogenous BL can decrease electrolyte leakage in tea crab apple *Malus hupehensis* L. [48]. Exogenous BL treatment decreased electrolyte leakage contents and enhanced the leaf relative water content under salt stress condition in ray grass *Lolium perenne*L. [49]. ThePBZ treated-plants had lower levels of electrolyte leakage in siam tulip *Curcuma alismatifolia* Gagnep. [50].

Relative water content (RWC)

The RWC values of NaCl treated plants is low than other treatments (Fig. 8). The plants of NaCl+ BL and NaCl+ PBZ shows slightly reduction than the Control plants. The RWC values are recorded higher in BL plants next to PBZ plants. Relative water content significantly decreased with the increasing salinity in rice *Oryza sativa* L. [51]. Similar results were also reported in wheat *Triticum aestivum* L. [52], pepper *Capsicum annuum* L. [53]. RWC was also reduced and water deficit increased in roots and leaves of the seedlings of milky iris *Iris lacteal* Pall. under NaCl stress [54]. BL was also reported to ameliorate the adverse effects of salt on relative water content in salt-stressed false wheat grass *Leymus chinensis* Trin. [55] and strawberries [56]. Protection of PBZ treated plants from salinity was associated with smaller leaf for loosing less water which improved RWC in salt-stressed plants of wheat *Triticum aestivum*L. [57].

Chlorophyll pigment contents

The chlorophyll-a, chlorophyll-b and total chlorophyll values are high in the plants of PBZ treatment (Fig. 9, Fig. 10 and Fig. 11). BLs treated plants have less pigment contents than of PBZ treated plants. NaCl treated plants have all time lower chlorophyll pigments than others. The plants of control have higher pigments compared to NaCl+ BLs and NaCl+ PBZ treated plants.Salinity decreased all photosynthetic pigments in sorghum *Sorghum bicolor* L. [58]. The content of chlorophylls showed a significant response to BL treatment under salinity in potato *Solanum tuberosum* L. [59].It also showed that plants treated with paclobutrazol synthesized more cytokinin, which in turn enhanced chloroplast differentiation and chlorophyll biosynthesis, and prevented chlorophyll degradation [60]. Chlorophyll concentration per unit leaf area was enhanced by PBZ due to a great concentration of chlorophyll in a much smaller leaf area and synthesized more cytokinin which in turn enhanced chloroplast differentiation and chlorophyll biosynthesis and prevented chlorophyll degradation in common garden peony *Paeonia lactiflora* Pall. [61]. PBZ significantly increased chlorophyll content and photosynthetic rates of rape *Brassica napus* L.[62].

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Carotenoid pigment content

The carotenoid pigments are abundant in the plants of the PBZ next to the BLs plant (Fig. 12). Plants treated with NaCl+BL and NaCl+PBZ have larger levels of carotenoid pigment than plants treated with NaCl. The carotenoid pigment content in control plants is greater than in NaCl treated plants. Carotenoids are important antioxidants that protect the photosynthetic machinery from damaging environmental influences and are also precursors to important vitamins [63]. Carotenoids protect plasma-membrane lipids from further oxidation and improve stress tolerance in the eared watermoss *Salvinia auriculata* Aubl. plant by scavenging directly active oxygen species generated during stress [64]. Improvement of total carotenoids in green microalgae *Chlorella vulgaris* Beijerinck by exogenous application of 28-homoBL [65]. PBZ improved chlorophyll and carotenoid contents in response to salinity in sweet sorghum *Sorghum bicolor* (L.) Moench [66].

Yield

The plants under salt stress show high reduction in yield attributes (such as: No. of seeds/pod, No. of pods/plant, 100-seeds weight and yield/plant) than Control plants (Fig. 13). NaCl+BL and NaCl+PBZ treated plants show reduction in yield attributes than NaCl plants. In addition to it, plants of BL and PBZ show increased yield attributes than control plants. Under high salinity stress conditions, the plant displayed lower grain weight, and fewer spikelets per panicle, all of which contributed to a decrease in harvest index and grain yield of rice *Oryza sativa* L. [67]. From fig. 13, the plants of NaCl treatment shows higher reduction in yield attributes than other plants. Salt stress has recently been identified as the most harmful environmental stress, resulting in a significant reduction in agricultural productivity and quality [68]. The use of BL in crops has revealed a significant protective role against salt stress, which can have a wide range of negative effects on crop biomass and yield in cucumber *Cucumis sativus* L. [69]. Under saline conditions, paclobutrazol soil drenching improved ionic relations, gaseous exchange characteristics, oxidative defense system, and yield in Quinoa *Chenopodium quinoa* willd [70].

CONCLUSION

Peanut is a major leguminous crop grown in arid and semi-arid locations where adverse circumstances like high salinity predominate and have a detrimental impact on productivity. Photosynthetic reactions are affected by salinity and osmotic stress. Overall, the plants respond well to the exogenous application of BL and PBZ. Plants exposed to BL and PBZ showed substantial improvements in morphological parameters as well as photosynthetic pigments when under saline stress. However, the molecular mechanism involved in function of stress protection remains to be explored.

ACKNOWLEDGEMENT

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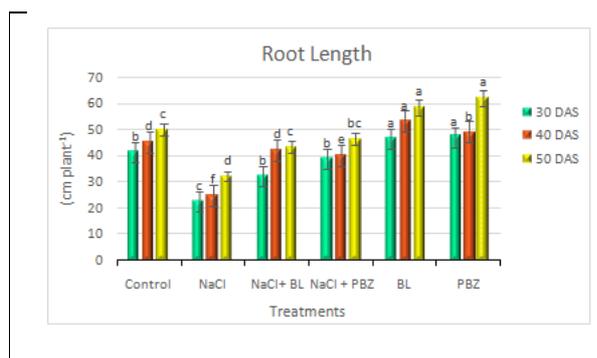
Fig. 1. Effect of exogenous application of BL and PBZ on root length of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.
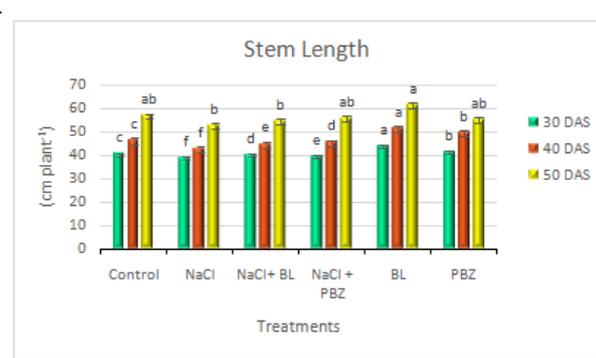
Fig. 2. Effect of exogenous application of BL and PBZ on stem length of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.
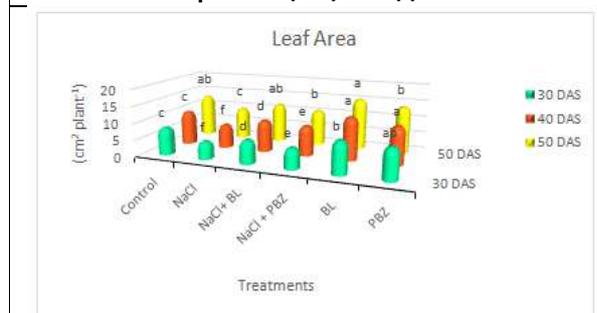

Fig. 3. Effect of exogenous application of BL and PBZ on leaf area of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.


Fig. 4. Effect of exogenous application of BL and PBZ on fresh weight of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

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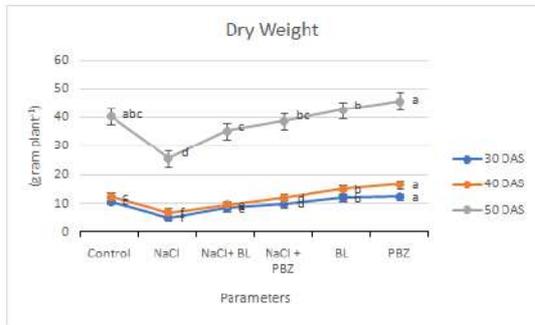
Fig. 5. Effect of exogenous application of BL and PBZ on dry weight of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error**.**
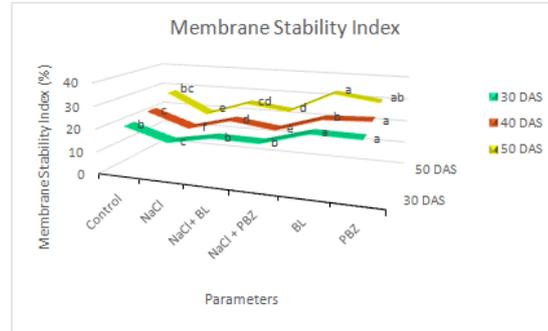

Fig. 6. Effect of exogenous application of BL and PBZ on membrane stability index of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.
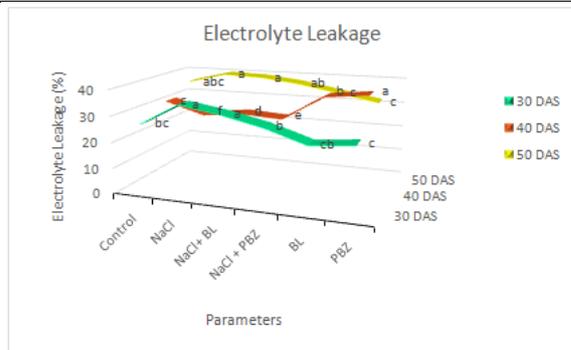
Fig. 7. Effect of exogenous application of BL and PBZ on electrolyte leakage of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.
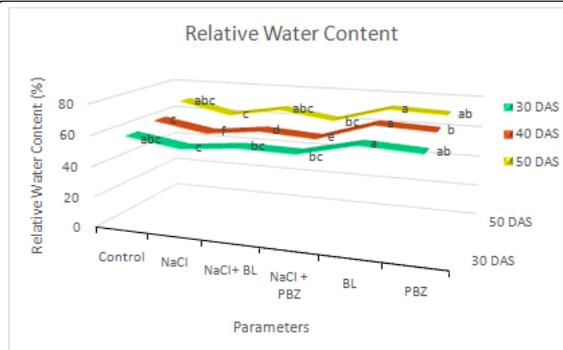
Fig. 8. Effect of exogenous application of BL and PBZ on relative water content of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.
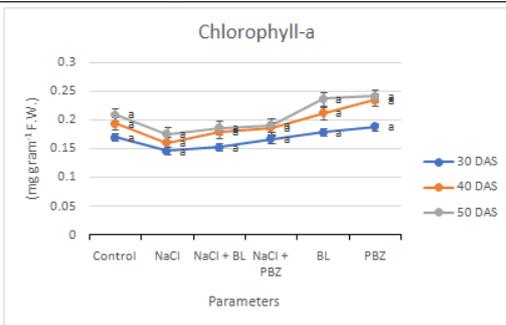
Fig. 9. Effect of exogenous application of BL and PBZ on chlorophyll-a of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.
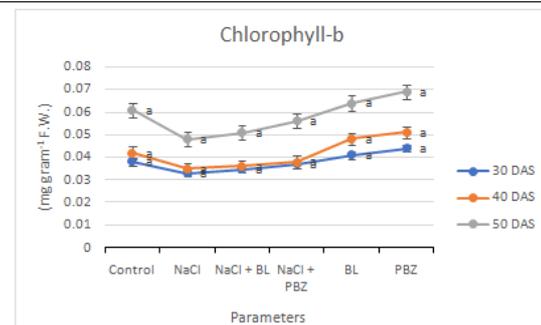

Fig. 10. Effect of exogenous application of BL and PBZ on chlorophyll-b of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

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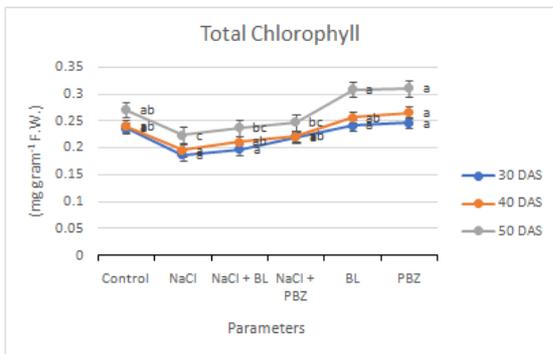
Fig. 11. Effect of exogenous application of BL and PBZ on total chlorophyll of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.
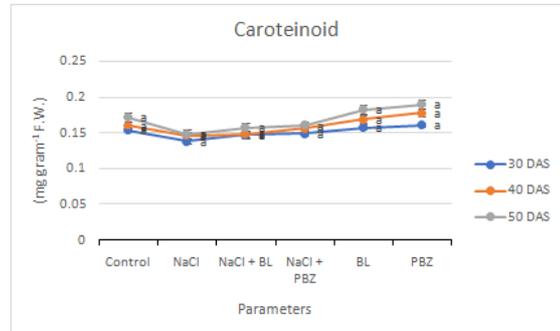

Fig. 12. Effect of exogenous application of BL and PBZ on carotenoid of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.
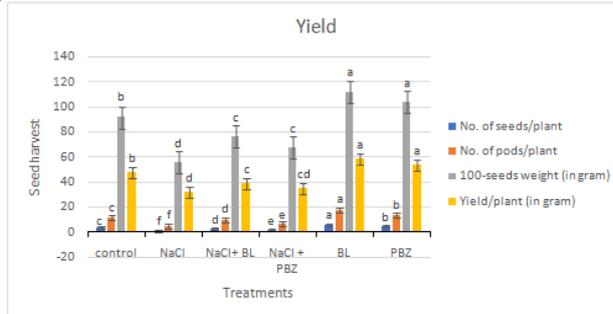

Fig. 13. Effect of exogenous application of BL and PBZ on yield attributes of peanut (VRI-2 variety) under 150mM NaCl stress. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

RESEARCH ARTICLE

Effect of Global Postural Re-Education on Work Related Shoulder Pain Amongst Blue - Collar Workers – An Interventional Study

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ABSTRACT

Work-related musculoskeletal disorders are also known as repetitive strain syndrome or overuse injuries. 51% of the cleaners categorized as the blue-collar workers are likely to suffer from work related shoulder pain due to repetitive movements. The purpose of this study was to investigate the effect of global postural re-education on work-related shoulder pain amongst cleaners. Total 30 hospital cleaners aged 20-40 years were enrolled in this study. Out of which, 15 subjects included in the experimental group underwent Global Postural Re-education and 15 subjects comprising the control group underwent self-stretching exercises for twice a week. Evaluation was carried out using Constant-Murley Score and NPRS before and after 4 weeks of protocol. There was more significant increase in Constant-Murley Score and reduction in NPRS in experimental group compared to the control group. As per the results obtained, Global Postural Re-education is proven more effective compared to self-stretching exercises in work related shoulder pain amongst blue-collar workers.

Keywords: Work related shoulder pain, Blue-collar workers, Global Postural Re-education, Stretching exercises, Cleaners.

INTRODUCTION

Musculoskeletal disorders refer to any abnormal relationships of bones and tissues, tendons, ligaments and muscles of the body [1]. Work-related musculoskeletal disorders, also known as repetitive strain syndrome or overuse

injuries, have been known for a remarkable proportion of work injuries and workers' compensation claims in Western industrialized nations since 1980s. Discomforts like soreness, pain, sprains and strains when the exposure contributing to injury is bodily reaction, reaching, repetitions, bending, twisting or overexertion. The risk factors for musculotendinous injuries resulting from carrying out forceful or repetitive tasks include overstretch ischemia, compression, overexertion, friction etc [2]. The early middle ages (21-30 years) and the late youth (31-40 years) were likely to suffer from musculoskeletal disorders [3]. Shoulder disorders are classified according to: i) Anatomical localization ii) Pathological process iii) Mechanism iv) Etiology. Amongst them, etiology related disorders include work-related shoulder pain and repetitive strain syndrome [4]. Work-related factors are found to be the leading reasons in the incidence of shoulder pain [5].

Among all the blue-collar workers, cleaners are more likely to carry out repetitive movement of shoulder leading to work-related shoulder pain. Recent studies plotted muscular engagement and postures of cleaners. According to reports, cleaners have high amount of repetitive shoulder movement for a major portion of their working time which are found to be catastrophic in terms of shoulder load [6]. Musculoskeletal disorders associated to these professions have an impact on 90% of the population; with the prevalence of shoulder pain being the highest which is 41.1% [7]. Evidence suggested that for a considerable amount of time i.e. about 43% of the time spent in cleaning work, the cleaners were found to be working with arms above the shoulder level resulting in work-related shoulder pain [8]. In order to reduce the severity and/or occurrence of the overuse injuries, emphasis must be laid on increasing the flexibility by carrying out stretching programs. The available range of motion around a specific joint is known as versatility [9]. Alteration of the viscoelastic properties by modifying the length of the muscle tendon units is termed as flexibility with regard to the function. Viscoelasticity refers to the ability of the tissue to deform or alter its length on application of the load applied over particular time and to regain its original size on removal of the force just like stretching and liberating an elastic band [10]].

Philippe-Emmanuel So chard developed a physical therapy method known as Global Postural Re-education. This technique is emphasized on the muscle chains forming the muscular systems, which may undergo shortening as a result of previously mentioned physiological, behavioral and psychosocial factors. The goal of this technique is based on the stretching mechanism by elongating the shortened muscles using the viscoelastic properties of the tissue and hence, enhancing the contraction of the antagonists avoiding postural asymmetry [11]. Global Postural Re-education is based on stretching the anti-gravity muscles as a whole. The muscles connected by a particular muscle chain is stretched for about 15-20 minutes [12]. This technique demonstrates two muscle chains i.e. the anterior muscle chain and the posterior muscle chain as well as other secondary chains.

This study is carried out to compare the effect of Global Postural Re-education technique developed by So chard et al. and self-stretching exercises at workplace according to the Hess and Hecker criteria on work-related shoulder pain among cleaners [12,13]. Given the clinical manifestations of work related shoulder pain, such as pain, decreased range of motion, the is a rationale that the hypothesis that Global Postural Re-education can benefit the cleaners with work related shoulder pain. The cleaners tend to undergo shoulder pain due to repetitive movement of shoulder and arm for prolong period of time. Also, there is lack of evidence suggesting exercises for the same in blue-collar workers. However, it is necessary to suggest an exercise protocol in order to reduce the prevalence of work-related shoulder pain among cleaners. Hence, the aim of this study is to investigate the effect of global postural re-education on work-related shoulder pain amongst cleaners.

MATERIALS AND METHODS

The study was conducted at Sainath Hospital and Bopal ICU and Trauma Centre. It was approved by the Institutional Ethics Committee for Human Research – Sainath Hospital, Ahmadabad (IECHR-SAINATH HOSPITAL). This Experimental Study consisted of 30 hospital cleaners. The inclusion criteria were both male and female aged 20-40 years, those having work related shoulder pain for >1 month with NPRS>=3. Those having Any

surgical history within last year any orthopedic conditions like spondylosis, osteoarthritis etc., history of systemic inflammatory conditions such as rheumatic arthritis, trauma, fracture or dislocation, neurological/cardiovascular conditions, pathological findings like tumor, infection etc. congenital abnormalities, psychiatric illness were excluded from the study. The subjects were divided into 2 groups using simple random sampling method with 15 subjects in each group. The work related shoulder pain was confirmed using Cornell Musculoskeletal Discomfort Questionnaire (shoulder region). All the subjects were assessed using Constant-Murley Score(ICC= 0.80-0.96) and Numerical Pain Rating Scale before and after the study (ICC = 0.27 to 0.84). The experimental group was given Global Postural Re-education. Before entering the exercise program, the subjects were instructed to breathe freely without holding the breath during the exercise and were explained to exercise safely within the range that did not cause pain during exercise. The exercise program included stretching of anterior muscle chain and posterior muscle chain [12].

To stretch the anterior muscle chain (Figure 1)
- The subject was in supine-lying position with head, waist and pelvis well-aligned and arms abducted to 30 degrees with palms facing upwards. The hip joint was abducted and externally rotated such that both the soles of feet are in contact with each other. The ankle joint was kept at 90 degrees. After 10 minutes, the arms were abducted up to 140 degrees and the knees were extended gradually. Total duration was 20 minutes.

To stretch the posterior muscle chain (Figure 2)
- The subject was in supine lying position with both the legs hanged with a sling connected to ceiling or supported at the wall. The hip joint was bent at 90 degrees with knees slightly flexed and both the arms were abducted to 30 degrees. After 10 minutes, head, waist and pelvis were kept aligned while gradually extending the knees and abducting the arms. Total duration was 20 minutes.

The control group was given general stretching exercises of neck and shoulder to perform during their work. The exercises included chin tucks, cervical flexion, extension, rotation and side flexion. Other exercises included shoulder shrugs, protraction and retraction of shoulders, cross-chest stretch, palm press and reaching for the sky pose. The subjects were asked to hold these positions for 15-20 seconds and repeat for 2-3 times [14]. One of the outcome measures used was the Constant-Murley Score is a 100-point scale comprising of number of individual parameters related to shoulder region including two subjective and two objective categories. The scale is categorized into 4 subscales related to a) Pain (15 points) b) Activities of Daily Living (20 points) c) Range of Motion i.e. flexion, abduction, internal and external rotation (40 points) and d) Strength (25 points) [15]. The other measure used in this study is the Numerical Pain Rating Scale. It is a 11-point scale with 0 = 'No Pain' and 10 = 'Worst Pain' [16]. The data was analyzed by Shapiro-Wilk test, which showed no normal distribution. Therefore, as per the statistics, non-parametric tests i.e. Wilcox on Signed Ranked Test for within group analysis and Mann Whitney U test for between group analysis were applied in the present study. The results were analyzed using SPSS (Statistical Package for Social Sciences) Version 20.0.

RESULTS

The mean age and standard deviation of the subjects in Group A was 25.4 and 6.72 respectively, whereas that of the subjects in Group B was 26.8 and 6.12 respectively. As shown in table 1., within group analysis was carried out using the Wilcox on Signed Ranks Test. The pre mean of CMS was 62.4 with the SD of 6.89; whereas the post mean after 4 weeks of treatment was found to be 78.53 with the SD of 6.11 in Group A. The obtained Z-value was -3.421. This finding suggested that there was significant difference in Constant-Murley Score before and after the intervention in Group A ($p<0.05$). Whereas, the pre mean of CMS was 65.80 with the SD of 9.93 and the post mean were 70.93 with the SD of 9.3 following the treatment in Group B. The Z-value obtained was -3.426. The results showed significant difference before and after the treatment in Group B ($p<0.05$).

The pre mean of NPRS was 4.37 with the SD of 0.765; whereas the post mean after 4 weeks of treatment was found to be 2.70 with the SD of 0.988 in Group A. The obtained Z-value was -4.607. This finding suggested that there was significant difference in Constant-Murley Score before and after the intervention in Group A (p<0.05). Whereas, the pre mean of NPRS was 4.07 with the SD of 0.799 and the post mean was 3.07 with the SD of 1.033 following the treatment in Group B. The Z-value obtained was -3.217. The results showed significant difference before and after the treatment in Group B (p<0.05). Table .2 shows the between group analysis which was carried out using Mann Whitney U test. In Group A, mean difference of CMS is 16.13 and that of NPRS is 5.13. Whereas, in Group B, the mean difference of CMS is 5.13 and that of NPRS is 1.07. This suggests that both the groups showed significant improvement but Group A showed better improvement as compared to Group B.

DISCUSSION

The core purpose of the present study was to compare the effect of Global Postural Re-education and segmental self-stretching exercises on work related shoulder pain amongst blue-collar workers. It is known that about 41.1% of the blue-collar workers suffer from shoulder pain because of their work style [7]. Identification of workloads in different repetitive movements leading to work-related shoulder pain among cleaners is essential as it may restrict them to concentrate at work. Global Postural Re-education technique is based on stretching of the entire anterior and posterior muscle chain of the body by providing different positions. In the present study, a specific exercise protocol is designed for the hospital cleaners having work related shoulder pain as per the Cornell Musculoskeletal Discomfort Questionnaire. In a study conducted by A Leclerc, J-F Chastang *et al*, they suggested that activities performed repetitively for a particular number of years result in high incidence of shoulder pain. There he found significant changes in work behavior related to shoulder pain [17]. In previous research by Caroline Mitchell, Elaine Hay *et al*, they found that diverse occupations requiring repetitive movement of the upper limbs are linked with more risk of shoulder disorders. They also mentioned that poorer prognosis results with increasing age [4]. Another study conducted by Jose Luis Pimentel do Rosario *et al.* showed that stretching exercises must be provided to prevent poor postures resulting in pain at work and to enhance muscular flexibility [18].

During a particular repetitive motor task, the amplitude of variability was seen to increase in a study conducted by Pascal Madeleine *et al* [19]. Evidence also suggested that the Global Postural Re-education program of 4 weeks focusing on stretching the entire anterior and posterior muscle chain of the body shows more significant improvement compared to traditional physiotherapy exercises in chronic nonspecific neck pain [20]. As per the previous researches, the Global Postural Re-education program leads the patients to involve actively by performing postures causing realignment of joints and stretching the shortened muscles [21]. Also the previous studies have shown the correlation between stretching exercises and pain reduction. Stretching exercises are found to show greater improvement in pain reduction by increasing flexibility and encouraging correct position in work related musculoskeletal disorders [22]. Jonsson *et al.* suggested that shoulder muscles carry the static load during the cleaning task which showed about twice the level of 2%-5% maximum voluntary contraction which is beyond the threshold limit given for 8 hours working day. Also the peak load levels of cleaners were found to be 54% maximum voluntary contraction [8,23]. Evidence suggested that Global Postural Re-education had significantly greater improvement on decreasing pain and improving quality of life on comparison with segmental stretching among those having scapular dyskinesis associated with neck pain as it focused on stretching of the muscle chains along with postural correction [24].

Given the data analysis and the results obtained, the alternate hypothesis that there is significant effect of Global Postural Re-education on work related shoulder pain amongst blue-collar workers has been accepted and null hypothesis has been rejected. These results are consistent with those obtained by other studies which compared this technique with the segmental stretching to achieve pain reduction. The reason that Global Postural Re-education focuses on stretching of the entire muscle chain along with stabilizing the whole spine; hence improving overall posture; whereas segmental stretching allows compensatory movements to occur in other body parts which may

cause insufficient load on other muscle groups also support the results. As the Global Postural Re-education engages the muscles in eccentric contraction while providing the stretch, it is found to be more efficient in comparison to segmental self-stretching which focuses on mere passive stretch [18].

CONCLUSION

This study demonstrated that Global Postural Re-education program showed significant improvement in decreasing pain, improving the functional ability and range of motion on work related shoulder pain amongst blue-collar workers. The protocol showed decreased NPRS score and increased Constant-Murley Score at the end of 4 weeks. Therapists should attempt to identify the relation of heavy muscle loading due to repetitive movements with increasing pain and decreasing functional capacity and must support the patients to overcome postural barriers in order to optimize the treatment outcome. Therefore, Global Postural Re-education protocol provided to the hospital cleaners proved to be significantly efficient for work related shoulder pain.

Limitations and future scope of the study

The limitations of this study are small sample size, only hospital cleaners amongst the blue-collar workers were included in the study, postural changes were not considered and mid-term and long-term follow-up was not taken. This study can be further conducted using large sample size, including all the workers associated with the blue-collar jobs. Further researches can be conducted to determine the maintenance of long-term benefits of the Global Postural Re-education program and focus on comparing Global Postural Re-education with other treatment protocol.

ACKNOWLEDGEMENT

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Table 1.Subjects Characteristics

Age	Mean ± SD	Gender	Percentage
Group A	25.4 ± 6.72	Male	53.3%
Group B	26.8 ± 6.12	Female	46.7%

Table 2. Within Group Analysis of CMS and NPRS

Group	Outcomes	Pre mean ± SD	Post mean ± SD	z-value	P value
Group A	CMS	62.40 ± 6.895	78.53 ± 6.116	-3.421	<0.01
	NPRS	4.37 ± 0.765	2.70 ± 0.988	-4.607	
Group B	CMS	65.80 ± 9.93	70.93 ± 9.30	-3.426	<0.01
	NPRS	4.07 ± 0.799	3.07 ± 1.033	-3.217	

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Table 3.Between Group Analysis of CMS and NPRS

OUTCOME	GROUP	MEAN ± SD	UNPAIRED T-TEST	P value
CMS	Group A	16.13 ± 7.36	-3.421	<0.01
	Group B	5.13 ± 2.56	-3.426	
NPRS	Group A	2.33 ± 0.724	-4.607	
	Group B	1.07 ± 0.704	-3.217	

Figure 1. GPR to stretch the anterior muscle chain

Figure 2. GPR to stretch the posterior muscle chain

RESEARCH ARTICLE

Current Physiotherapy Practice for Chronic Ankle Instability: Challenges and Future Directions

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ABSTRACT

Chronic ankle instability (CAI) is Persistent residual symptoms of ankle sprain for more than 6 weeks along with Repetitive bouts of ankle sprain and the feeling of ankle "giving away". The treatment program mostly follows the conservative line of treatment. Physiotherapy management for CAI focuses on the impairments caused due to CAI and prevention of long- term complications of the same. Hence the purpose of this study is to review the current practices and techniques of physiotherapy in CAI, its challenges and future direction. Articles published on an online electronic database were included in these studies. The current study included 26 articles that include different physiotherapy intervention in CAI. The reviewed studies provided evidences that denotes about the challenges faced in CAI which is mostly emphasizes on only one domain of treatment and the future directions to the studies regarding CAI and construction of a patient centric structured protocol for the early prognosis with long term effects of the treatment.

Keywords: chronic ankle instability, physiotherapy, rehabilitation, ankle sprain.

INTRODUCTION

Chronic ankle instability (CAI) is Persistent residual symptoms of ankle sprain for more than 6 weeks along with Repetitive bouts of ankle sprain and the feeling of ankle "giving away". Daily activities like Walking and running can be affected [1]. The anterior talofibular ligament (ATFL) is the weakest ligament in ankle and carries very low load because of which it is most commonly injured in an ankle sprain. The calcaneofibular ligament (CFL) is stronger

and larger and runs obliquely in compared to ATFL. The posterior talofibular ligament is thick and strong and because of that it is rarely injured. Ankle sprains typically involve injury to the lateral ligaments because of hyper supination a combination of inversion, plantar flexion, and internal rotation of the not fully loaded rear foot in relation to the tibia [2]. It usually occurs in physically active individuals of any Age. 70% of individuals with acute ankle sprain develops chronic ankle instability. About 93% of the patient with acute ankle sprain ignore the condition and takes icing and rest. Avoidance of proper intervention is not taken by these patients therefore they develop CAI. The impairments connected with chronic ankle instability can be ligamentous laxity, proprioceptive deficit and balance deficit. Perceived ankle instability was common with almost a quarter of healthy adults. Research says that chronic ankle instability is 2.6 times higher in females then in males and increased body mass index (BMI) are at higher risk of ankle sprains as BMI has a direct effect on the ankle joint because of abnormally high joint loading [3].

Chronic ankle instability is of two types functional instability and mechanical instability. Functional instability depends on the patient generated reports or complaints that could be accompanied by clinical laxity while mechanical instability can be identified by physical examination and is commonly associated to the pathological joint laxity [1]. In CAI, due to recurrent ankle sprain there is a disruption in the connective tissue as well as mechanoreceptors present in the ligaments and muscles. The damage to the sensory receptors was believed to create proprioceptive deficits and prevent the central nervous system's accurate perception of where the ankle joint was in space.[2] This deficit, consequently, would lead to an increased incidence of the ankle giving way into hyper supination because there was inadequate peroneal muscle response i.e. muscle weakness and this both impairments eventually lead to balance deficits.

Clinicians are met with many issues that need to be addressed after lateral ankle sprains, including muscle weakness, postural-control deficits, decreased range of motion, and the frequent occurrence of reinjury. clinicians are challenged to find approaches that will improve ankle stability and prevent reinjury. Patients looking for physical therapy to treat an ankle sprain are usually worried about pain, swelling and then eventual recovery of the ankle joint stability. Conventional physical therapy techniques such as balance, proprioceptive training, taping, electrotherapy, thermotherapy, etc., has shown good results in decreasing the oedema and impairments associated with CAI. Current interventions are commonly used are balance training and proprioceptive training which has been proved to be an effective modality in the rehabilitation and prevention of recurrent sprains in those with CAI however, there is limited evidence of its effectiveness. Evidences strongly recommend using ankle taping as a powerful proprioceptive training. Ankle taping helps in adjusting the abnormal excessive movements, improves the firing rate of cutaneous mechanoreceptors which helps in better proprioceptive feedback and better balance control and ankle function. Strength training is thought to support muscular gains during initial weeks because it enriches neural factors. Therefore, strength training is seen to improve proprioception and balance deficits.

There is a lot of literature present that evaluate treatment strategies for CAI. This issue is further taken ahead by the probability that these reviews vary in quality and scope (with several reviews for one prevention/ treatment type) and their inclusion of papers with a high degree of overlap in the injury target (acute ankle sprain/CAI). Thus, there is a need that this evidence should be put in a systematic manner to ascertain evidence-based recommendations for the treatment of acute ankle sprain and CAI. The aim of this paper was to provide a systematic review of evaluating treatment strategies for CAI. A secondary aim was to identify the current gaps in the literature for researchers, and identify the challenges and provide with future direction. The aim of this paper is to provide a systematic overview of different kinds of interventions and evaluating treatment strategies for CAI. A tributary aim is to identify the challenges faced by the clinicians while treating CAI and future directions.

METHODOLOGY

Search Strategy

We performed a systemic review study on the current physiotherapy practices for chronic ankle instabiliy and discuss the evidence-based studies, challenges and future scopes. For the purpose of the review, an electronic search for relevant articles using PUBMED, MEDLINE, Pedro, Research Gate, Google Scholar and CINHAL databases up to April 2022 was done wherein Mesh search terms and free words like "chronic ankle instability", 'Physical Therapy", "Physiotherapy Treatment", "acute treatment", "Ankle exercise", "Strengthening Exercises", "balance Training" "sensorimotor training"were used. In addition to the electronic search, articles were searched manually for relevant studies. Articles were selected based on authors expertise, self- knowledge and reflective practice.

Study Selection

Studies which focused on physiotherapy as one of the choices of treatment in individuals with chronic ankle instability. The studies included individuals diagnosed with chronic ankle instability.

Data Extraction

All steps in the selection and extraction processes were assessed independently by two reviewers. The titles and abstracts of the retrieved references were screened. Full texts of relevant publications were reviewed and were included if they met the inclusion criteria

REVIEW OF LITERATURE

982 citations identified from literature search of electronic databases

456 articles were excluded on the basis of abstract screening

526 potentially relevant articles were selected focusing on the treatment options in Juvenile Rheumatoid Arthritis

513 articles were excluded on the basis of other treatment options / year of publication

26 articles were selected which were potentially relevant for this review

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RESULT

The current study included 26 articles that fulfilled the inclusion criteria suggesting that different intervention strategies have a major role in treating the clinical manifestations in individuals with chronic ankle instability. A study done by M. Spencer Cain, which is based on Four-Week Ankle-Rehabilitation Programs in Adolescent Athletes with Chronic Ankle Instability here they gave 12 intervention sessions for 6 weeks period. 4 rehab groups were made. Group 1 was given resistance training to the ankle muscles with the use of theraband Group 2 was given ankle platform board intervention Group 3 was given combination therapy of both resistance training and ankle platform board intervention Group 4 was given no treatment. result showed That balance training group had significant improvements in the FADI and the FADI Sport scores, in the magnitude and the variability of TTB measures with eyes closed, and in reach distances with the poster medial and the poster lateral directions of the SEBT. There was a significant group time interaction for the FADI (P = 0.03) and the FADI Sport (P = 0.009) scores.

P < 0.05 for pre-test to post-tests comparisons within the balance training group. P < 0.05 for between-groups comparisons at post-test. Conclusion of this study was Each rehabilitation group performed better than the control group and Group differences compared with the control group were supported by small to large effect sizes. Limitation of this study was the PRO questionnaire used in this study is valid for adults but evidence is less about the validation of this questionnaire in adolescents [4]. Emily A. Hall, carried out a strength training intervention on the deficits faced by the patients with chronic ankle instability wherein 'group 1'was given resistance band exercises and on the other hand 'group 2' was given slow reversible PNF techniques. Control group was given no treatment. The resistance-band protocol group improved in strength (dorsiflexion, inversion, and eversion) and on the visual analog scale (P <.05) the proprioceptive neuromuscular facilitation group improved in strength (inversion and eversion) and on the visual analog scale (P <.05) as well. The RBP and PNF groups improved from pretest to posttest (P <.05). No difference was identified in the CON group (P < .05) [5].

Rafael Sierra-Guzma´n, Fernando Jime´nez-Diaz, Carlos Ram´ırez, Paula Esteban, Javier Abia´n-Vice´, did a study on Whole-Body–Vibration Training and Balance in Recreational Athletes with Chronic Ankle samples were randomly assigned to a vibration (V), no vibration (NV), or control group. The V and NV groups performed unilateral balance training on a BOSU 3 times weekly for 6 weeks. The V group trained on a vibration platform, and the NV group trained on the floor. They observed no differences among the 3 groups for any of the 3 measurements (P >.05). They observed no differences (P < .05) among the 3 groups. Body-composition variables showed No differences among groups before or after the training (P <.05). We also observed no group-by-time interaction effect for any of the variables (P < .05) [6].

Roel De Riddera, Tine Willemsa, Jos Vanrenterghemb, Ruth Verrelsta, Cedric De Blaisera, and Philip Roosen carried a study on Taping Benefits Ankle Joint Landing Kinematics in Individuals with Chronic Ankle Instability. The aim of their study was to evaluate whether the use of taping changes ankle joint kinematics during a sagittal and frontal plane landing task in subjects with CAI. 28 participants with CAI performed a forward and side jump landing task in a non-taped and taped condition. The taping procedure existed of a double 'figure of 6' and a medial heel lock. 3D ankle joint kinematics were registered. Statistical Parametric Mapping was used to assess taping effect on mean ankle joint angles and angular velocity over the landing phase. For both the forward and side jump, a less plantar flexed and a less inverted position of the ankle joint was found in the preparatory phase till around touch down in the taped condition (p<0.05). Taping is capable of altering ankle joint kinematics prior to touch down, placing the ankle joint in a in a less vulnerable position at touchdown [7].

Patrick O. McKeon and Erik A. Wikstrom performed a study on Sensory-Targeted Ankle Rehabilitation Strategies for Chronic Ankle Instability. Four groups were made, ankle joint mobilization, plantar massage, and triceps surae stretching were given in each group and one was the control group. Joint mobilization resulted in the most meaningful improvements in weight-bearing dorsiflexion whereas plantar massage had the most meaningful effect

on single limb balance. Stretching the triceps offers benefit as well, but these benefits may be maximized potentially in combination with the other STARS. While STARS would not typically not used in isolation, this study provides initial evidence that the comparative effectiveness can be used to systematically target sensory pathways that may be advantageous in the rehabilitation of CAI. Future studies are needed to determine the extent of benefit of STARS in combination as well as their synergistic effects when combined with other interventions [8]. From the above articles it is stated that the treatment regime given for CAI is beneficial even though it focuses on only one domain, the time duration differs but still provides the same effect.

DISCUSSION

This systematic review highlights the current evidence on the use of physiotherapy in the treatment of chronic ankle instability, including the benefits, drawbacks, and future directions. There is consistent evidence that physiotherapy treatment can assist such people improve their range of motion, reduce discomfort, increase muscle strength, balance, functional capability, and quality of life.

Current evidences also state that ankle taping has been proven effective in powerful proprioceptive training for improving proprioception and balance in patients with CAI. Ankle taping helps in adjusting the abnormal excessive movements that occur during sports such as excessive ankle plantar flexion or excessive varus stress. Also, ankle taping improves the firing rate of coetaneous mechanoreceptors which helps in better proprioceptive feedback and better balance control and ankle functions. Motaz Alawna, Ayman A. Mohamed carried this study to see the long-term effects of taping in which both taping and bandaging significantly increased vertical jump immediately after one session, after 2 weeks and 2 months. The improved vertical jump in the present study might occur because external supports could help individuals with CAI to compensate for their impaired neuromuscular control by afferent feedback mechanisms and increase mechanical stability [9]. Roel De Riddera , Tine Willemsa did a study to find out taping benefits in ankle landing kinematics this study showed that taping is capable of altering ankle joint kinematics prior to touch down, placing the ankle joint in a in a less vulnerable position at touchdown [7].

Studies were done on bracing and foot orthosis in CAI. People with ankle instability in particular seem to benefit from a semi-rigid ankle brace, which allows them to keep decreased ankle inversion angles. Lower ankle inversion angles and velocities with a semi-rigid brace may explain reduced injury incidences with brace application. ankle bracing had significant effects on joint kinematics and kinetics as well as on peroneal activation before and after ground contact [10]. Foot orthoses have been shown to have a positive influence on subjects who have recently experienced an ankle sprain and on subjects with chronic ankle instability. There is evidence that foot orthoses can influence multiple levels of neuromuscular control of the ankle. Improvements in somatosensory feedback and reduced muscular load seem to be the most viable mechanisms by which foot or thoses may positively affect patients with chronic ankle instability [11].

Strength intervention studies focuses on strength aspect of CAI ie. Strengthening of the ankle dorsiflexors, ankle plantar flexors, ankle invertors and electors. Emily A. Hall did a study for a period of 6 weeks on comparing the effect of resistance tube strengthening protocol and PNF strength protocol. Both protocols showed clinical benefits in strength and perceived instability. T W Kaminsk did a study on effects of strength and proprioception on evertors and invertors of foot which showed no significant changes in the strength of the foot muscles. Strength protocols has also shown mild impact on improvement in balance and proprioception too [5,12].

Balance intervention studies has been done with different durations ie Jay Hertel did a e 4-wk progressive balance-training program which included hop to stabilization, hop to stabilization and reach, hop to stabilization box drill, progressive single-limb stance balance activities with eyes open, and progressive single-limb stance activities with eyes closed which showed improved dynamic stabilization after landing whereas [4] D. Cruz-Diaz, R. Lomas-Vega, M. C. Osuna-Pérez, F. H. Contreras, A. Martínez-Amat did a 6-week exercise therapy program comprising of 7 tasks

performed with different training material who also showed improved dynamic postural control, both the study used same outcome measures to see the improvements[13]. One study was also done on balance using STAR treatment for a duration of 2 weeks and showed increased probability of treatment success [8]. Studies done by Zeinab shiravi and sanam tavakoli on cognitive task in CAI patients which include dual task performance. Study by Zeinab Shiravi showed decreased postural sway during the dual task performance but the study done by Sanam Tavakoli showed that cognitive load may contribute to the increased risk of repeated ankle sprain [14,15]. Overall, the outcomes of this study show that while there is data on the usefulness of physiotherapy in chronic ankle instability, there is a lack of evidence on structured exercise programs that address all of the limitations that a patient with CAI faces. Lack of methodical planning of the protocol, the duration of therapy sessions to be offered, and the overall treatment schedule.

Challenges

1. There are Studies done on balance which focuses on single leg task training, or training on different surfaces and its progression for proprioception and balance but it does not involve evaluating motor strategies which basically act as natural protective strategies which maintain the equilibrium and prevent falls. Studies also have not stated that how motor strategies may change or adapt the gait pattern in chronic ankle instability.
2. Gait training after all the rehabilitation in CAI does not focus on backward walking which can also be considered as a study for intervention. Backward walking (BW) is an activity with additional complexity compared to regular forward walking (FW). Kinematic and kinetic studies demonstrated greater range of ankle dorsiflexion, reduced plantar flexion, and more even plantar pressure distribution. BW requires greater muscle activity and increases activation of the sensor motor control system due to altered or absent visual feedback.
3. Most of the studies quantified only the immediate effects of the intervention on a task after the training but, the retention of the program remains unknown, and it is unclear if the protocol program shows improvements in a broader range of tasks.
4. Little consideration has been given to the role of the distal and proximal tibiofibular joints or the effect of hypo mobility at any of the joints of the ankle complex. Both hyper mobility and hypo mobility can alter the rotational axis of a joint, leading to aberrant joint mechanics. Joint hypo mobility can be physiologic or arthrokinematics (accessory motions) in nature Hypo mobility at any joint in the lower extremity kinetic chain can challenge the motor-control mechanisms of an individual and lead to joint instability. Alteration in the arthrokinematics of the joint, produce limitations of the accessory movements of roll and glide between the joint surfaces.

These challenges mentioned above are the ones that are not been taken care of and require attention for the better outcomes in the protocols given.

Future directions

1. While most of the current literature excludes individuals who have engaged in recent rehabilitation out of a desire to eliminate a potential confounding variable, in the real-world patients may engage in multiple rehabilitation attempts in sequence if they are not satisfied with their outcomes. Future research should test the effect of their intervention training in individuals who have had recent rehabilitation, but potentially not achieved the results they desire.
2. In future importance to repeated interventions over longer periods are necessary to evaluate the clinical use of the interventions.
3. The choice of exercises, as well as the total training volume during the intervention, should both be addressed. Adding self-mobilization as a warm-up protocol in other sports could be beneficial when designing rehabilitation programs for patients with CAI.
4. In Strength-Training Protocols with Chronic Ankle Instability. Future investigators should evaluate the effectiveness between local (single-joint) and global (multi-joint) by focusing not only on the ankle but on the entire lower extremity. That would determine whether more deficits are improved while saving the clinician time and energy.

5. Long-term follow-up testing would identify the lasting effects of each rehabilitation protocol. Calculating effect sizes in the statistical analyses will help determine whether rehabilitation protocols are clinically relevant.
6. Further evidence showed that ice can be applied before ankle rehabilitation without adversely affecting dynamic neuromuscular control. Investigation in patients with acute ankle sprains is warranted to assess the clinical applicability of these interventions.

Although CAI is a multifactorial condition, the purpose of research is to better understand how it becomes chronic and to limit future injury, and this systemic review study help us give a better picture of how well we are accomplishing this goal.

CONCLUSION

The above reviewed studies provided literature about different interventions of physiotherapy as a choice of treatment in chronic ankle instability. We concluded that the treatment protocol of physiotherapy intervention usually focuses on only one domain which might be either balance, strength or proprioception and only one technique like taping, mobilisation, balance board exercises etc which anyways showed improvement in the condition but the time varies. However, one should also pay attention to the age, intensity of work and daily activities done by the patient. So, after taking all these factors into consideration from the above review studies we can determine that this systematic review provides a summary of different physiotherapy techniques in healthy individuals and there is a need for a Physical therapy intervention which should be a centric approach or structured treatment in Chronic ankle instability.

ACKNOWLEDGEMENT

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Table 1.Current physiotherapy research Studies summarizing Physiotherapy as a treatment in chronic ankle instability.

Study title	Name of the author and year	Intervention	Conclusion
Sensory-Targeted Ankle Rehabilitation Strategies for Chronic Ankle Instability [8]	Patrick O. McKeon1 and Erik A. Wikstrom2 2016	6 treatment sessions over a period of 2 weeks and each session lasted for 5 minutes sensory-targeted ankle rehabilitation strategy includes joint mobilisation, plantar massage and stretching.	STARS treatment offers exclusive contributions to the rehabilitation outcomes in CAI
Four-Week Ankle-Rehabilitation Programs in Adolescent Athletes with Chronic Ankle Instability [16]	M. Spencer Cain, Rebecca J. Ban, Yu-Ping Chen, Mark D. Geil, Benjamin M. Goerger, Shelley W. Linens, 2020	12 intervention sessions for 6 weeks, here 4 rehab groups were made. 1.Resistance training 2. ankle platform board intervention 3. combination 4. control group with no treatment	Each rehabilitation group performed better than the control group Group differences compared with the control group were supported by small to large effect sizes
Effects of 6 Weeks of Balance Training on Chronic Ankle	D. Cruz-Diaz, R. Lomas-Vega, M. C. Osuna-Pérez, F. H. Contreras, A.	The 6-week exercise therapy program comprised of single limb	The 6-week multi-station balance training program improves the subjective

Instability in Athletes: A Randomized Controlled Trial [13]	Martínez-Amat 2015	stance and double limb stance exercises performed with different training material	feeling of instability, improved balance in patients with CAI but no change in pain.
Manipulative Therapy Plus Ankle Therapeutic Exercises for Adolescent Baseball Players with Chronic Ankle Instability [19]	Ho-Jin Shin, Sung-Hyeon Kim, Han Jo Jung, Hwi-young Cho, and Suk-Chan Hahm 2020	The intervention group received HVLA plus resistance exercise twice a week for 4 weeks, while the control group received resistance exercise alone	significant improvement was seen in pain intensity, range of motion before and after the intervention but no such significant change was seen in balance ability.
A Randomized Controlled Trial Comparing Rehabilitation Efficacy in Chronic Ankle Instability	Cynthia J. Wright1, Shelley W. Linens2, and Mary Spencer Cain2	3 sessions per week for over 4 weeks time 1. exercises on wobble board 2. exercises with resistance tube	Comparative efficacy of a 4-week intervention of either WB or RT exercises. Showed significant improvement in symptoms reduction and enhanced performance therefore, it is useful to use either of the intervention to reduce symptoms and improve performance.
Effects of joint mobilization on chronic ankle instability: a randomized controlled trial [23]	David Cruz-Díaz, Rafael Lomas Vega, Maria Catalina Osuna-Pérez, Fidel Hita Contreras & Antonio Martínez-Amat	Movement with mobilisation was given Two sets of 10 repetitions, separated by a 2-min rest, were performed as described by Mulligan	The application of the Weight bearing MWM joint mobilization seems to be effective in the treatment for DFROM, dynamic postural control, and in self-reported instability.
Strength-Training Protocols to Improve Deficits in Participants with Chronic Ankle Instability: A Randomized Controlled Trial [5]	Emily A. Hall, Carrie L. Docherty, Janet Simon, Jackie J. Kingma, Joanne C. Klossner,	6-week protocol Group 1 – rubber resistance band strength training Group 2 -slow reversal PNF technique, which involves a concentric contraction of the antagonist muscle, followed by a concentric contraction of the agonist muscle Group 3 – control group no treatment	Isometric ankle strength increased after both rehabilitation protocols. Neither rehabilitation protocol had a clinical effect on dynamic balance or functional performance Using the VAS, patients in both rehabilitation protocols noted improvements in perceived ankle instability.
People with chronic ankle instability benefit from brace application in highly dynamic change of direction movements [10]	Patrick Fuerst1, Albert Gollhofer, Markus Wenning2 and Dominic Gehring 2021	3 groups were formed 1. control group 2. CAI with mechanical instability 3. CAI with functional instability.	maximum ankle inversion angles and velocities can be reduced during injury-relevant movements with a change of direction by the application of a semi-rigid brace.

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Short-Foot Exercise Promotes Quantitative Somatosensory Function in Ankle Instability: A Randomized Controlled Trial [21]	Eunsang Lee Juchul Cho Seungwon Lee	Intervention 1.Short-foot exercise program 2.Proprioceptive sensory exercise	SFE training significantly improves proprioception and dynamic balance in patients with CAI who have experienced recurrent
Short-term and long-term effects of ankle joint taping and bandaging on balance, proprioception and vertical jump among volleyball players with chronic ankle instability [9]	Motaz Alawna, PhD, MSc, PT, Ayman A. Mohamed, PhD, MSc, PT	3 groups were made of intervention 1. Ankle taping 2. Ankle placebo taping 3. Ankle bandaging	The current study indicated that ankle taping and bandaging immediately improve vertical jump Thus, both tapping and bandaging should be prolonged to produce an improvement in ankle joint proprioception, vertical jump, and balance among CAI.
Balance Training for Persons with Functionally Unstable Ankles [17]	Susan Rozzi, Scott M. lephart, Sterner, Lori Kuligowski,	A unilateral, multilevel, static and dynamic balance training program 3 times a week for 4 weeks. Subjects from the experimental group trained only the involved limb, and the nonimpaired group trained a randomly selected limb.	This study suggests that balance training is an effective means of improving joint proprioception and single-leg standing ability in subjects with unstable and nonimpaired ankles.
Effect of cognitive task on postural control of the patients with chronic ankle instability during single and double leg standing [14]	Zeinab Shiravi, Saeed Talebian Moghadam, Mohammad Reza Hadian, GholamrezaOlyaei, 2016	chronic ankle instability patients randomly performed single and double leg standing in isolation or concurrently with a digit-backward cognitive task.	The findings confirm the effect of a concurrent digit-backwards memory task on single leg standing balance in chronic ankle instability patients.
Comparison of 3 Preventive Methods to Reduce the Recurrence of Ankle Inversion Sprains in Male Soccer Players [18]	Farshid Mohammadi,	The subjects were individually and randomly assigned to 4 study groups: group 1 the proprioceptive program, group the strength program, group 3 used orthoses, and group 4 was the control group.	Proprioceptive training is an effective intervention to reduce the rate of ankle sprains among male soccer players who suffered ankle sprain.

The effect of dual tasking on foot kinematics in people with functional ankle instability [15]	SanamTavakolia, Saeed Forghanya, b, Christopher Nester	21 physically active participants with FAI and 19 matched healthy controls completed trials of normal walking (single task) and normal walking while performing a cognitive task (dual task).	Participants with FAI demonstrated different ankle movement patterns and increased movement variability during a dual task condition. Cognitive load may increase risk of ankle instability in these people
Sport injury prevention in individuals with chronic ankle instability: Fascial Manipulation versus control group: a randomized controlled trial [20]	Simone Brandolini, Giacomo Lugaresi, Antonio Santagata, Andrea Ermolao, Marco Zaccaria, Aurélie Marie Marchand, Antonio Stecco	Three groups followed a specific training program. The control group followed normal training protocols and received standard medical care. The study group received an additional three FM treatment sessions.	The 6 months outcomes in the study group showed statistically significant improvements. The 1-year follow-up reported the absence of any reported trauma in the study group. FM was effective in improving ROM, and symptomatology in footballers with CAI. FM intervention was effective in preventing injury in the study sample.
Effects of Foot Orthoses on Patients with Chronic Ankle Instability [11]	Douglas H. Richie	This article reviews the current research relevant to the use of foot orthoses in patients with chronic ankle instability and clarifies the suggested benefits and the shortcomings of these investigations.	Foot orthoses have been shown to have a positive influence on subjects who have recently experienced an ankle sprain
Hop-Stabilization Training and Landing Biomechanics in Athletes With Chronic Ankle Instability: A Randomized Controlled Trial [22]	Mohammad Karimi Zadeh Ardakani, Erik A. Wikstrom, Hooman Minoonejad, Reza Rajabi; Ali Sharifnezhad,	A 6-week supervised hop-stabilization training program that consisted of 18 training sessions	The 6-week hop-stabilization training program altered jump-landing biomechanics in male collegiate basketball players with CAI.
Neuromuscular Training for Chronic Ankle Instability [24]	Chung-Wei Christine Lin, Eamonn Delahunt, Enda King	Neuromuscular training can lead to small, short-term improvements in function compared with no training. the training effect allowed him to improve function and return to sporting activities.	The results of this review provide stronger evidence than individual studies that neuromuscular training can improve short-term functional outcomes in people with chronic ankle instability

Twelve-week biomechanical ankle platform system training on postural stability and ankle proprioception in subjects with unilateral functional ankle instability [25]	Alex J.Y. Lee a, Wei-Hsiu Lin	A 12-week training program and a progression test for controlling the platform in certain directions and advancing to next training level was given	These improvements in postural stability appear to reflect improved neuromuscular ability along with enhanced functional joint stability, as ankle proprioception also demonstrated the same positive improvements after training
Muscle Reaction Time During a Simulated Lateral Ankle Sprain After Wet-Ice Application or Cold-Water Immersion [26]	Peter K. Thain, Christopher M. Bleakley, Andrew C. S. Mitchell	A total of 54 physically active individuals were taken group 1- Wet-ice application, group 2 - cold-water immersion, or an untreated control condition applied to the ankle for 10 minutes	Ten minutes of joint cooling did not adversely affect muscle reaction time or muscle amplitude in response to a simulated lateral ankle sprain. These findings suggested that athletes can safely return to sporting activity immediately after icing.
Ankle-Joint Self-Mobilization and Cross Fit Training in Patients with Chronic Ankle Instability: A Randomized Controlled Trial [27]	David Cruz-Diaz, Fidel Hita-Contreras, Antonio Mart´ınez-Amat, Agustin Aibar-Almaza´n, Kyung-Min Kim,	Participants in the self-mobilization plus Cross Fit group and the Cross Fit training-alone group pursued a Cross Fit training program twice a week for 12 weeks. The self-mobilization plus Cross Fit group performed an ankle self mobilization protocol before their Cross Fit training, and the control group received no intervention	Ankle-joint self-mobilization and Cross Fit training were effective in improving ankle DFROM, dynamic postural control and self-reported instability in patients with CAI.
Predicting Balance Improvements Following STARS Treatments in Chronic Ankle Instability Participants [28]	Erik A Wikstrom, and Patrick O McKeon,	three treatment groups (plantar massage, ankle joint mobilization, calf stretching) that received 6, 5-minute treatment sessions over a 2-week period.	Self-reported functional deficits, worse single limb balance, and number of previous ankle sprains are important determining if chronic ankle instability participants will have an increased probability of treatment success
Effect of strength and proprioception training on eversion to inversion strength ratios in subjects with unilateral functional ankle instability [12]	T W Kaminski, B D Buckley, M E Powers, T J Hubbard, C Ortiz	four treatment groups: strength training, proprioception training, strength + proprioception training and control	Six weeks of strength and proprioception training (either alone or combined) had no effect on isokinetic measures of strength.

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Effects of Tai Chi on the neuromuscular function of the patients with functional ankle instability: a study protocol for a randomized controlled trial [30]	Huiru Tang, Min Mao, Daniel T. P. Fong, Qipeng Song, Yan Chen, Zhipeng Zhou, Cui Zhang, Jiangna Wang, Xuewen Tian and Wei Sun	The participants in the simplified Tai Chi exercise program (STCEP) group will receive a 12-week Tai Chi training. The participants in the control group will receive a low-intensity exercise program and health education for 12 weeks.	This study attempts to estimate the effect of Tai Chi intervention on the neuromuscular function of individuals with CAI.
Whole-Body Vibration Training and Balance in Recreational Athletes with Chronic Ankle Instability [6]	Rafael Sierra-Guzma´n, Fernando Jime´nez-Diaz, Carlos Ram´ırez, Paula Esteban, Javier Abia´n-Vice´n,	The VIB and NVIB groups performed unilateral balance training on a BOSU 3 times weekly for 6 weeks. The VIB group trained on a vibration platform, and the NVIB group trained on the floor.	Only the VIB group showed improvements on the Biodex Balance System, whereas the VIB and NVIB groups displayed better performance on the SEBT.
Taping Benefits Ankle Joint Landing Kinematics in Individuals with Chronic Ankle Instability [7]	Roel De Riddera, Tine Willemsa, Jos Vanrenterghemb, Ruth Verrelsta, Cedric De Blaisera, and Philip Roosen	28 participants with CAI performed a forward and side jump landing task in a non-taped and taped condition. The taping procedure existed of a double 'figure of 6' and a medial heel lock.	Taping is capable of altering ankle joint kinematics prior to touch down, placing the ankle joint in a in a less vulnerable position at touch down.

RESEARCH ARTICLE

Investigation on High Voltage Polymeric Outdoor Insulators to Prevent from Flashover

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ABSTRACT

Many factors have contributed to the widespread use of silicone rubber outdoor insulators in distribution networks, including their light weight, ease of installation and transportation, high mechanical strength-to-weight ratio, resistance to vandalism, attractive appearance, and superior insulation performance, among others. It is the topic of this inquiry to look at silicone rubber insulators that have been in use for a substantial length of time. Because of its great efficacy and efficiency, partial discharge is the predominant way of testing high-voltage transmission and distribution systems. Using aged polymeric insulation, it was determined to undertake laboratory testing under alternating current voltage, variable humidity conditions, and varying amounts of NaCl. The experiment was carried out with the help of NaCl and a piece of aged polymer insulation. It took 100 days to get the temperature of the heating chamber back to where it had been before the accident. Whenever this form of treatment was applied in a therapeutic setting, it was meant to imitate the events that a patient may encounter in the real world. A PD monitoring system is required in order to obtain the PD waveforms and the phase-resolved patterns required by the PD monitoring system. We investigate the pulse properties of PD pulses in both the temporal and frequency domains in order to compare the pulse characteristics of PD pulses in both the temporal and frequency domains. This allows us to compare the pulse characteristics of PD pulses in both the temporal and frequency domains. Following an in-depth review and analysis of the data collected thus far, the findings reveal that outdoor polymeric insulators lose their efficacy with time and that partial discharge pattern analysis can identify silicone rubber insulator flashovers. In addition, the data imply that outdoor polymeric insulators lose their efficiency over time due to exposure to the elements.

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INTRODUCTION

When it comes to insulating characteristics, both porcelain and glass — two of the oldest and most cost- effective materials available — have advantages and disadvantages to take into consideration. Polymeric insulators have surpassed ceramic insulators in recent years, owing to their low weight and mobility, as well as their capacity to withstand vandalism [1–2]. Other criteria to consider are their strong mechanical strength-to-weight ratio and their attractiveness on a visual level. Material ageing is inevitable for polymeric insulators installed in tropical or coastal industrial or coastal settings due to severe environmental conditions present throughout the year. Salt and other airborne particles lower the surface insulating strength of dry band surfaces. resulting in an increase in the intensity of the electromagnetic field generated. It is possible for partial discharge (PD) to cause degradation of polymeric insulators over time if the insulator is exposed to this. While it is possible to prevent flashover by utilizing an insulator surface, doing so is both time-consuming and expensive.

It is possible for a flashover to develop when equipment is exposed to moist or unclean conditions, which causes leakage currents to build. The electrical surface resistance of hydrophobic polymers is lowered as a result of water absorption and contamination, which both occur over time and as a result of interaction with the surrounding environment. Leakage currents [3] can be used to determine the amount of pollution present. Sarathi and colleagues [4] and other researchers [5] devised a multi-resolution decomposition technique for analysing leakage current signals that may be used to leakage current signals. If you're seeking for a quick and easy approach to determine the condition of a polymeric outdoor pollution layer's polymer layer, partial discharge detection is your best option (PDD). As a result, by examining the impacts of polymer dispersion (PD) on polymeric insulation, it is feasible to forecast how the insulation would perform in tropical pollution conditions.

It is the purpose of this study to put old silicone rubber insulators through their paces in the laboratory under a range of pollution and relative humidity conditions. When utilizing a partial discharge monitoring device that is capable of collecting waveforms as well as phase-resolved patterns of PD, it is feasible to get partial discharge signals. We explore the characteristics of PD pulses in both the temporal and frequency domains with the goal of drawing comparisons between virgin and aged polymeric insulators. After considering the evidence, it is possible to infer that the performance of an outdoor polymeric insulator degrades with age and that the flashover of silicone rubber insulators may be detected using partial discharge pattern analysis.

Experimental setup

This research makes use of polymeric insulators that have been aged both intentionally and naturally. More than five years after they were provided to clients by the Electricity Board, natural silicone rubber insulators are still in excellent condition. [6, with a leakage distance of three millimetres and a core diameter of seventy millimetres] was used for the contamination testing on an 11 kV polymeric insulator with three millimetres of leakage distance and a core diameter of seventy millimetres Figure 1 depicts an image of the polymeric insulator that was used in this investigation, as well as a sketch of the substance. An aqueous solution of sodium chloride (Nacl) was sprayed onto the surface of the polymeric insulator to simulate the salty contamination seen in coastal locations. For the purpose of maintaining the required relative humidity level in the fog chamber, four Ultrasonic Nebulizers were used. The relative humidity of the fog chamber was measured using a hydrothermal device that was mounted on the wall of the room.

In Fig. 2, you can see the experimental setup that was used in this study. During the whole testing procedure, the test insulator was positioned vertically in the fog chamber to ensure proper air circulation. An 11-kV rms at 50 Hz test was used to determine the voltage and frequency of the system under consideration. We put it through its paces using the clean fog method specified in IEC 60507 standards. We cleaned the insulator with isopropyl alcohol and

distilled water before to conducting the tests. This procedure was carried out in order to ensure that there were no potentially dangerous compounds in the substance.

To identify partial discharges, a 100 MS/s sampling rate and an input sensitivity of less than one millivolt were utilised in conjunction with a sensitivity of less than one millivolt [7,8]. The waveform characteristics of PD pulses were investigated in order to establish whether the waveform might be utilised to assess pollution intensity. This occurred because the insulator was not connected to the coupling capacitor at the time of the occurrence. During the experiment, pulses from the insulator's ground connection were picked up by the high frequency transformer and propagated all the way around the insulator.

The following test conditions were used in the laboratory to conduct the experiments.
(i)   Isolator composed of virgin silicone rubber with a clean surface that is both dry and damp.
(ii) The third type of insulator has varying amounts of pollutants (10g/liter and 30g/liter) and relative humidity levels (RH) at different temperatures (e.g., 75 percent , 85 percent , and 95 percent).
(iii) Figure 3 depicts the use of an aged insulator at changing pollution levels (10g/liter and 30g/liter) and at varying relative humidity levels (75 percent) under various environmental conditions.

RESULTS AND DISCUSSION

Test Results with 10g/l pollution

In this study, an un aged silicone rubber specimen was subjected to a non-polluting test with an applied voltage of 11kVrms and a relative humidity level of20-40 percent and a relative humidity level of 20-40 percent, respectively, in order to determine its durability. The same virgin insulator was then put to the test in a fog chamber with a relative humidity of 100 percent and no pollution was utilised to produce the fog in order to determine its effectiveness. During the two clean tests, there were no substantial discharges of any type to be seen. Following that, pollution tests were performed on both the un aged and aged specimens from the fog chamber. Both silicone rubber insulator specimens were initially coated with pollutants at a concentration of 10 g/l. In order to investigate the dry band arcing, samples are maintained at a variety of relative humidity levels. The specimen was kept in a fog chamber with a relative humidity of 60% for the duration of the first step of testing. Following that, the relative humidity is progressively increased to 95 percent of the fog chamber.

Figure 3 displays the typical un aged silicone rubber insulator phase resolved partial discharge patterns for 10g/l pollution with varying relative humidity values ranging from 75 percent to 95 percent in the presence of varied relative humidity values. Figure 4 displays typical phase-resolved partial discharge patterns of naturally aged silicone rubber insulator at 75 to 95 percent humidity when polluted with 10g/l of a toxic substance. When compared to dry, clean surfaces, the amplitude of PD increases considerably with increasing relative humidity, as seen by the PRPD patterns. It is necessary for the conductor to have a very large potential gradient along its surface in order to create an arc. [9,10]

Test Results with 30g/l pollution

When the amount of fog within the chamber was increased from 70% to 100%, the silicone rubber insulators were treated with a sodium chloride solution with a salinity of 30 gram/liter, which increased the life of the insulators. Figures 5 and 6 demonstrate that when exposed to different relative humidity levels, un aged and aged insulators exhibit PRPD patterns that are quite similar to one another. With increasing relative humidity, the size of the PD is shown to increase in magnitude. Increased leakage current in polymeric insulators is caused by the formation of droplets on the surface of the insulator when relative humidity is high (see Figures 11 and 12). In light of this comparison, it can be concluded that the magnitude of pollutant concentration (PD) in 30 g/l increased with time, and the pattern density expanded over the water column. Increasing surface conductivity, according to this theory, leads to the formation of additional leakage routes, which results in the growth of leakage current [13,14].

When a poisonous bright spot passes through the ground electrode, it generates a considerable temperature increase and chemical change, which results in erosion and loss of Hydrophobicity [4], which extinguishes the arc burning. Consequently, in its un aged form, the surface of the specimen is very hydrophobic. The Hydrophobicity of the material may increase with time as a result of contamination and the high electric field of 11 kV applied to the sample. PD amplitude increases dramatically with relative humidity, as seen in the prior trials, and its magnitude increases significantly with increasing relative humidity.

PD Pulse comparison

The typical PD pulses of virgin and aged silicone rubber insulators are displayed in Figure 7 at varied degrees of surface contamination. When compared to the un aged instances, the pulses created by the aged polymeric insulators have a significantly longer duration than those produced by the un aged ones. After being subjected to highly contaminated surroundings, the PD pulses take on the appearance of being practically continuous in nature. Pulse durations and dominant frequency components can differ greatly between aged polymeric insulators and fresh polymers, as might the frequency components themselves. A greater understanding of flashover may be gained by noticing that the size and length of PD pulses both grow significantly in an aged insulator, which serves as a clear signal.

From the above test results, the following points are observed

(i) Because their resistance has diminished with age, older insulators have longer pulse durations.
(ii) It has been shown that the size of the PD pulses increases in both the positive and negative phases of aged insulators
(iii) Aging results in a large number of PD pulses.
(iv) The PRPD pattern is visibly distributed in older samples as pollution levels increase, which is consistent with previous findings.
(v) The presence of polymeric insulator flashover may be detected simply from the PD data.

CONCLUSION

It was decided to investigate the partial discharge patterns and PD pulses of silicone rubber insulators that were both unaged and aged in this study. Using various levels of relative humidity and pollutant contamination, tests are carried out in accordance with IEC 60507 and IEC 60508 specifications. The temporal domain characteristics of PD pulses are found to be influenced by the severity of the pollutant in the silicone rubber insulator. When old silicone rubber insulation deteriorates, the degree of PD activity increases, making it more active than new silicone rubber insulation in the long run. It is possible to employ partial discharge patterns on polymeric insulators exposed to pollution to detect flashover in a short period of time by detecting partial discharge patterns.

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Figure 1. shows the schematic circuit of the experimental setup

Fig. 2 Experimental setup

Fig. 3. PRPD pattern results of unaged silicone rubber insulator at 10g/l pollution (a) RH is 75% (b) RH is 85% (c) RH is 95%
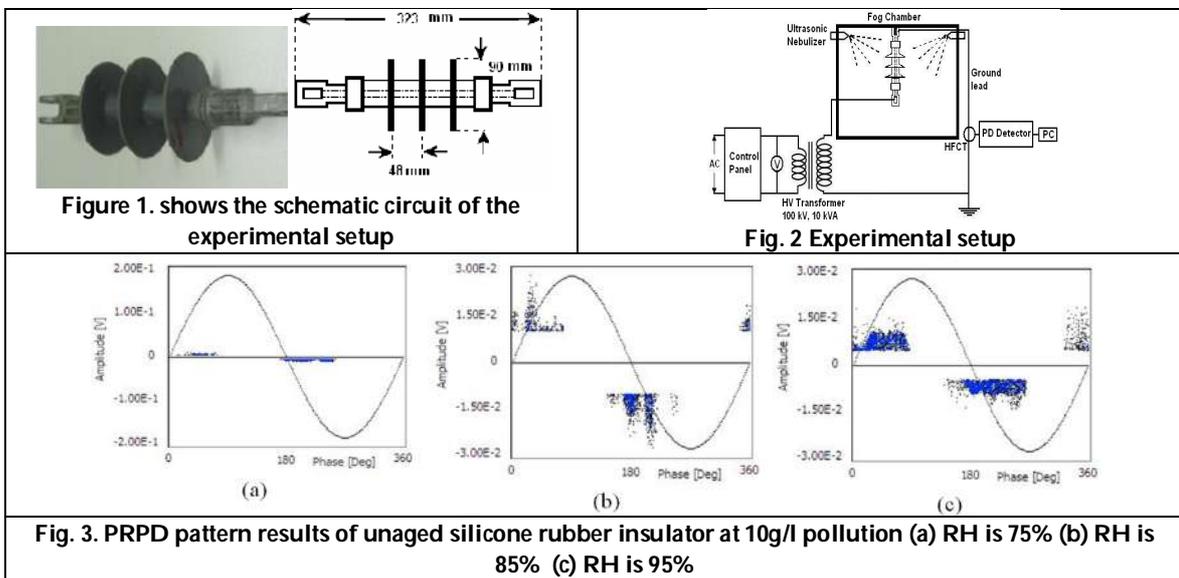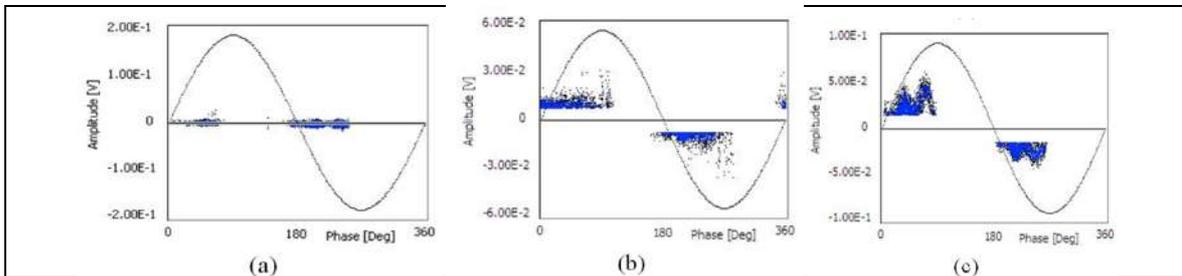
Fig. 4. PRPD pattern results of aged silicone rubber insulator at 10g/l pollution (a) RH is 75% (b) RH is 85% (c) RH is 95%
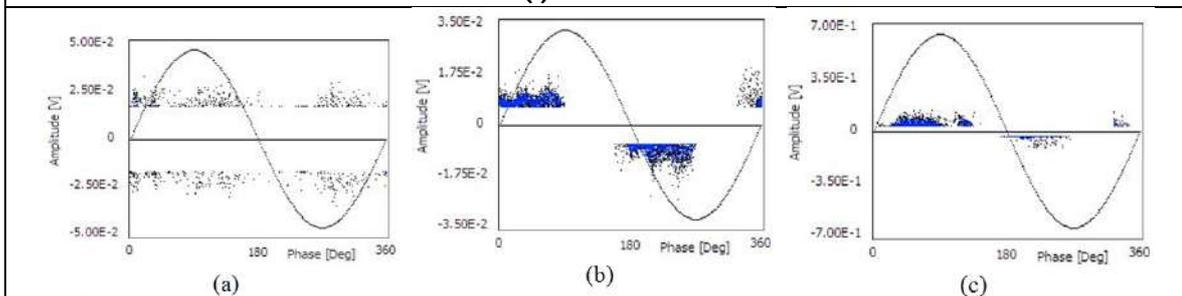

Fig. 5. PRPD pattern results of unaged silicone rubber insulator at 30g/l pollution (a) RH is 75% (b) RH is 85% (c) RH is 95%
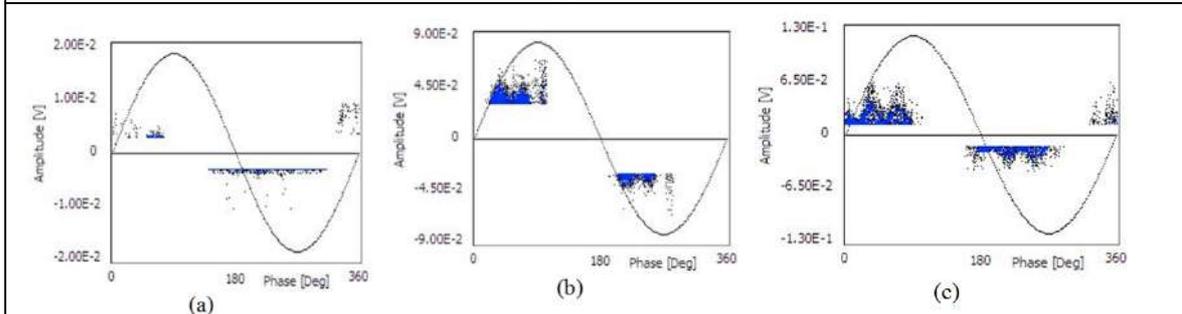

Fig. 6. PRPD pattern results of aged silicone rubber insulator at 30g/l pollution (a) RH is 75% (b) RH is 85% (c) RH is 95%
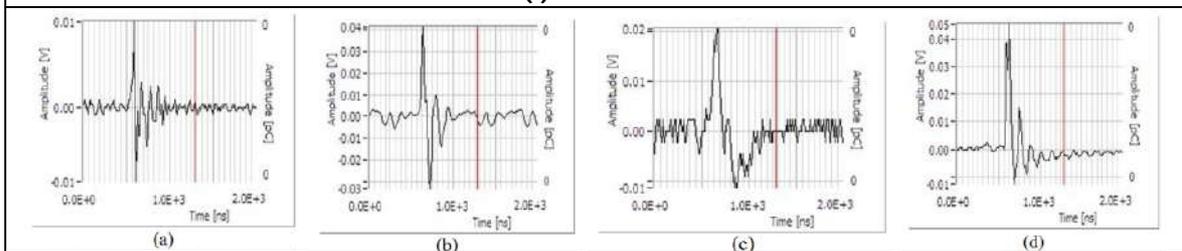

Fig. 7 PD Pulse results (a) unaged 10g/l (b) aged 10g/l (c) unaged 30g/l (d) aged 30g/l

RESEARCH ARTICLE

Perception and Realization of IPR among the Dental Practitioners: A Questionnaire Study

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ABSTRACT

Intellectual Property Rights refers to the legal rights granted with the aim to protect the creation/ innovation of the intellect irrespective of educational qualifications and implies to all subjects .The main aim is to benefit the intellects (engineers, lawyers, scientists, researchers, medical and dental professionals etc.) who are actual credit takers of the tech-scientific innovations. In order to survey the dental professionals about IPR, questionnaire method was used and 100 dental professionals participated in the survey .In current situation of globalization, IPR is the important point in worldwide trade practices and livelihood across the world. These rights enhance the innovative surroundings by giving identification and economic payback to creator or inventor whereas the lack of IPR understanding and its fruitless execution may hinder the economic, technical and societal developments of nation. Hence propagation of IPR knowledge and its suitable execution is paramount necessity for any nation.

Keywords: Intellectual property rights, perception, dental practitioners, copyrights, trademarks, patents.

INTRODUCTION

Intellectual property right signifies the legal rights conferred to the innovator or originator to shield his innovation or origination for a specified period. Intellectual property is an extensive conception that cover different kinds of legally acknowledged rights originating from some kind of intellectual innovation, or that are otherwise associated to ideas [1]. The name Intellectual property is linked to human brain applied for innovations [2,3]. It upgrades advancement in science and technology ,arts and culture, traditional knowledge [1]. On account of modernity, it is utmost significant to be ahead in innovations and creativeness to contend the stiff competitions in equipment and deal. India

is well acknowledged for its scholarly skills in the fields of software engineering, missile technology, Moon or Jupiter mission and other technological areas. However, India lags in generation of IPR resources in terms of registered patents, industrial design, trademarks, etc. It is very distressing circumstance for strategy makers as well as for the population as a whole. The growth of any society unswervingly depends on IPR and it's policy frame work. Lack of IPR understanding resulted in the death of inventions, increased risk of encroachment, economic defeat and rejection of an intellectual era in the country. Thus, there is a ominous need for dissemination of IPR information so as to improve original inventions and developments in the ground of research and technology.

Numerous hard work with regard to facts of human resources, era, power, art, funds, etc are mandatory to discover or build something new. The eventual initiative by which invention or creation took place is an insubstantial property of the person, who took effort for the creation or foundation. Therefore, as per law, legal rights or monopoly rights are given to originator or innovator to gather the economic profit on their invention or origination. The Intellectual property rights (IPR) are territorial human rights by which holder can advertise, purchase or authorize his Intellectual Property (IP) similar to physical property.

Even though one has to register IPR at legal authority in some acceptable or appreciable outline to state their profit. Each kind of IPR gives extraordinary rights to its inventor and or creator to maintain and produce economic profit which further motivates skill and societal developments. On the basis of type of invention and creation of human intelligence and their applications the intellectual property rights are classified as follows: i) Patents, ii) Trademarks, iii) Industrial designs, iv) layout design of semiconductor integrated circuit, v) geographic indications of source, vi) Copyright and related rights [4]. The intellectual property rights (IPR) are insubstantial in nature and gives exclusive rights to inventor or creator for their precious invention or creation. In current situation of globalization , IPR is the important point in worldwide trade practices and livelihood across the world. These rights enhance the innovative surroundings by giving identification and economic payback to creator or inventor whereas the lack of IPR understanding and its fruitless execution may hinder the economic, technical and societal developments of nation. Hence propagation of IPR knowledge and its suitable execution is paramount necessity for any nation.

History

The laws and administrative process associated with IPR have their origin in Europe. The movement of granting patents began in the fourteenth century. The first familiar copyrights emerged in Italy. In the year 1847, Copyright law entered through an enactment during the East India Company's regime   India. In the year 1856, George Alfred DePenning have made the first application for a patent in India and India's statutory Trademarks law dates back to 1860.[1] The history of IPR is much difficult and appealing; it was in 500 BCE, when a Sybaris, Greek state, made it to citizens to patent for year the "New Refinement in Luxury". Another facet of IPR says they were formed for political and religious hostility and protection. 1n 1556, the Stationers, monopoly it in England to curb the ability of Protestant Reformation movement by placing the whole printing industry under this company. The Government and Church wanted to hinder spreading of ideas.

In actual it was in 1883, under Paris Convention, inventors had given safety even if their innovations were being used in other countries. Subsequently, WTO defined Intellectual property rights as the rights given to persons over the creations of their minds. Such rights usually give the creator an exclusive right over the use of his/her creation for a specified period. Intellectual property is the only way to have deep insight of nationals of any country. In today's global village concept, IPR comes with other aspects i.e. educational, commercial, and economic growth and development to any country. In 2017, US filed for 59624 patents, China for 48882 patents and Japan 48208 patents Indian is not standing anywhere among 10 too. So, it's a matter of much distress for India like big education infrastructure with huge researchers to stand up and investigate the situation. As we know the knowledge plays a key role in development in all aspect of improvement of any country, therefore, this paper has taken knowledge about IPR [5].

Illustration of Types of Intellectual Properties [6,7]

Initially, only patent , trademarks and industrial designs were protected as Industrial property but now the term " Intellectual Property" has a much broader meaning .IPR enhances technology advancement in the following ways. Common types of IPR include [8].

1. Patents
2. Trademarks
3. Copyrights and related rights
4. Designs

Patents [8]

A patent is an exclusive right given for an innovation ,which is an outcome or a method that gives a new route of doing something , or provides a new practical solution to an issue. It gives care for the innovation to the owner of the patent. The protection is granted for a limited duration i.e for 20 years. Patent protection means that the innovation cannot be made ,used, distributed or sold in the market without patent owner's consent.

Trademarks [9]

This shields signs, symbols, logos, words or sounds that differentiates your products and services from those of your contenders. It supports customers recognize and acquire a product or service because it's creation and trait , designated by it's distinctive trademark ,meets their demands. The initial term of registration is for 10 years , then it may be renewed sporadically.

Copyrights [8,10]

The form of works covered by copyright includes those of performing artists in their performances, novels, newspaper, paintings, musical compositions .Copyright provides preservation for the project itself. Copyright lasts for a given period after which the work is supposed to enter the public field.

Designs

The term of industrial design rights differ from country to country from 10 to 25 years. The protection for design is for 10 years [4]. This specified period can be increased further for 5 years. This shields designs such as drawings or computer models [9]. The objective of this research paper is to evaluate the awareness and knowledge of dental practitioners about Intellectual property right (IPR).

Aim

The aim of this study was to evaluate the awareness and knowledge of dental practitioners about Intellectual Property Right (IPR).

Objectives of the study

1.To discuss in detail about the level of awareness regarding IPR.
2.To examine the attitude regarding IPR awareness among Dental practitioners.
3.To find out the role of IPR in research field.

Research Methodology:

For the purpose of collection of data , questionnaire method was used. According to the objective of research a self-administered questionnaire was prepared and was circulated to 100 Dental Professionals who have given consent to participate in the study. It is to find out whether they are aware about IPR or not.
Following are the questions which were asked

Data Analysis and Interpretation

The above figure shows that 93.7 % of the dental practiotioners are aware about the full form of IPR and others having no idea about Intellectual property right. The above figure shows that 62.2 % of dental practitioners have been heard about the term IPR and the remaining 37.8% of them are un ware about this term. The above figure shows that only 13 % of the Dental practionerrs have attended the workshop/seminar and conference and remaining 87% of them haven't yet attended. The above figure shows that 54.4% of them came to know about IPR from their colleagues and remaining 16.2 % from university authority,14.7% from newspaper/journal and from their own interest. The above figure shows that only 8.3 % of the Dental practitioners says that IPR exist in their University and 22.2% of them says no and the remaining 69.4 % of Dental practioners don't know whether IPR exist in their University or not. The above figure shows that 67.3% of Dental practitioners says that IPR deals with Patent, copyright and trademark , 12.1% says that IPR deals with copyrights and trademark, 9.3% says Patent and other 11.2% have no idea about any of the above.

The above figure shows that 76.9 % are unaware about the courses on IPR and 14.8% of Dental practitioner says that the IPR is under UGC and remaining dental practitioner says UGC, NPTEL, Swayam. The above figure shows that only 8.3 % of Dental practitioners have applied for patent and 91.7% haven't yet applied for any patent. The above figure shows that 56.7 % of Dental practitioner says that IPR are applicable both nationally and internationally ,31.7% says that it depends on applicants preference ,7.7% of them says it is applicable only nationally and remaining 3.9 % says IPR is applicable only internationally. The above figure shows that 53.7 % of Dental practitioner got motivated , 13% of them says they will work in this direction ,14.8% practitioners by compulsion and 18.5 % didn't got motivated.

DISCUSSION

The research paper was carried out to evaluate the awareness and knowledge of dental practitioners about Intellectual Property Right (IPR).The objective was to discuss in detail about the level of awareness regarding IPR and to examine the attitude regarding IPR awareness among Dental practitioners and also to find out the role of IPR in research field. In this present study, awareness of IPR is very less in our dental profession. In cognition based economy, intellectual property rights are very much needed for advanced societal growth. The IPR is primary urgency to be a part of local as well as gobal competitive trade as without proclamation of IPR awareness and execution, generating the innovative domain is really unworkable. It is necessary to incorporate IPR in basic educational structure and elevate IPR registration by supporting the innovators and creators .India is having all the resources in terms of available basic material ,inexpensive labour, innovative and creative devoted manpower [4].
In this present study, when the dental practitioners were questioned that for what do the IPR stand for, most of the dental practitioners exclaimed that IPR stands for Intellectual Property rights.93.7 % of them were aware about this and remaining dental practitioners stated Indian property rule, Indian property right and Intelligent Property right ,which shows that few are still not aware about the IPR. The another query was that , have they ever heard the term IPR before and we came to know that that 62.2 % of dental practitioners have been heard about the term IPR and the remaining 37.8% of them were unware.

On the other side the concept and knowledge of intellectual property rights were moderate among the Malaysian undergraduate students ,in a study done by Hway Boon Ong et al. Also a study was done on 647 stakeholders in Chennai by Dr. D Gnasekaranetal in 2014 to evaluate the knowledge of IPR ,it was concluded that the stakeholders had fair understanding of intellectual property rights. As reported by Sulekha et al , a study which was conducted on 50 scholars of kurukshetra university , the male scholars were 54% , 46% , it was concluded that they were not aware of the intellectual property rights.11

When investigated about attended workshop /seminar/conference on IPR, according to this present study we came to know that only 13 % of the Dental practioners have attended the workshop/seminar and conference and

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remaining 87% of them haven't yet attended. The another interrogation was about the existence of IPR in the University, as per the response only 8.3 % of the Dental practitioners says that IPR exist in their University and 22.2% of them says no and the remaining 69.4 % of Dental practioners don't know whether IPR exist in their University or not. In this study, it was also stated that 76.9 % are unaware about the courses on IPR and 14.8% of Dental practitioner says that the IPR is under UGC and remaining dental practitioner says UGC ,NPTEL, Swayam. When the dental practitioners were asked that have they ever applied for any patent , we came to know that only 8.3 % of Dental practitioners have applied for patent and 91.7% haven't yet applied for any patent. In a study done by Dr. Sufiya Ahmed, 2 Prashant Kumar Varun shows that 47% respondent were aware that intellectual property rights are the part of human right while 40% respondents are unaware about it. 50% research scholars are aware about copyright and patent while 23% of research scholars are having no idea about intellectual property right [12].

According to our study ,56.7 % of Dental practitioner says that IPR are applicable both nationally and internationally , 31.7% says that it depends on applicants preference ,7.7% of them says it is applicable only nationally and remaining 3.9 % says IPR is applicable only internationally. The last query was to know that after participating in this survey, have they got motivated about IPR. So as per our study 53.7 % of Dental practitioner got motivated ,13% of them says they will work in this direction ,14.8% practitioners by compulsion and 18.5 % didn't got motivated. On the other side in a study done by Deeksheetha et al ,half of the dentists had encouraging viewpoint while the other half had a pessimistic point of view towards intellectual property rights. The Male dentists had a more positive attitude towards intellectual property rights in the female dentists and the dentist with 5 to 10 years of experience and a better attitude towards intellectual property rights than their senior and junior counterparts no previous research studies were done to assist the attitude of IPR [11].

The limitations of this present study are the limited sample size. This can be improved by carrying a widespread study population within the state and outside the state to obtain more responses. Henceforth, more similar studies need to be done in a pan India level among dental practitioners and medical practitioners as a multi-centred approach instead of a single centred approach. The study of intellectual property rights must be encouraged in all the institutions of higher learning. Execution of intellectual property rights in research careers, especially in UG and PG levels, should be done incorporated with the additional training in IPR for a better career in the field of research and development. Workshops and further training should be organized in universities and schools accompanied with the provision of practical knowledge on plagiarism and on the usage of copyright material. Moreover, the students and the professionals should be educated about the repercussions due to the negligence of the laws of Intellectual property rights [11].

CONCLUSION

According to this study, the level of awareness regarding the intellectual property right among the Dental practitioners is very less. The large number of respondent are not aware of intellectual property rights .However some of the Dental Practioners have little knowledge of intellectual property rights [5]. A proper knowledge about IPR is very useful in future dentistry because daily we are using new technology, new equipments in our practice and Institutions. Therefore we should be aware of our rights and should protect our rights and respect others rights.

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Question 1: IPR Stands for?	**Question 2: Have you ever heard the term IPR before?**
Question 3:Ever attended any workshop /seminar/conference on IPR ?	**Question 4: From where did you come to know about IPR ?**
Question 5: Whether IPR cell exist in your University ?	**Question 6:IPR cell deals with?**
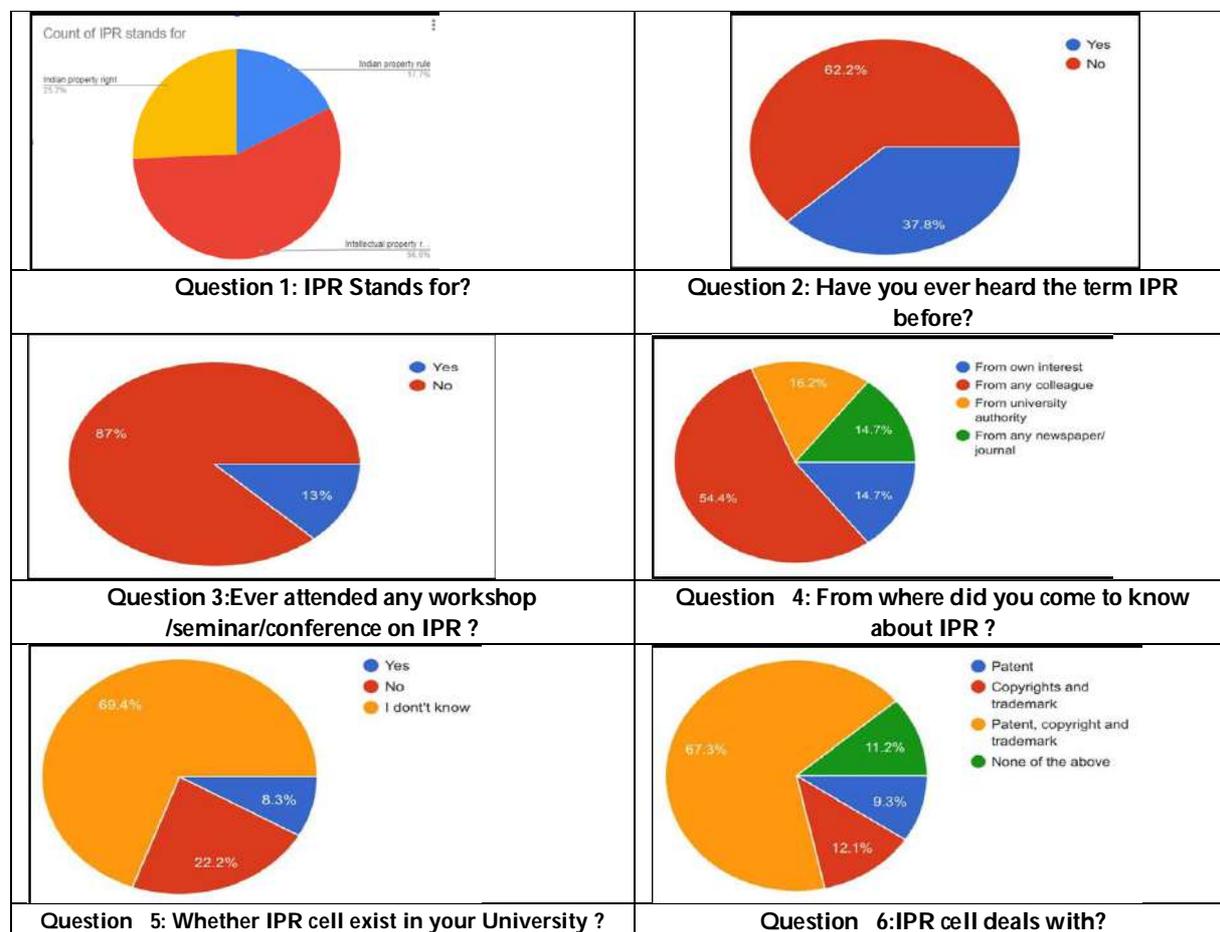
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Question 7: Awareness about any of the courses on IPR by any organization	**Question 8:Have anyone ever applied for any patent?**
Question 9:IPR are applicable for?	**Question 10: After participating in this survey, have you got motivated about IPR ?**

RESEARCH ARTICLE

Classifying Periodontal Diseases: the Path Taken Till Now

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ABSTRACT

This article discusses the past, present and possible future classification of periodontal diseases. It outlines the reasons for using a classification system from a clinical perspective and provides a critical appraisal of the latest classification. The major changes introduced in the 1999 system are discussed alongside the rationale behind the recommended nomenclature. The aim was to review and update the 1999 classification with regard to the diseases and conditions, and to develop case definitions and diagnostic considerations.

Keywords: Classification, Periodontal disease, Gingivitis, Periodontitis, Peri-implantitis

INTRODUCTION

Why do we need a classification system?

Classification systems of diseases have arisen because it allows the clinicians to develop structures which can be used to identify diseases in relation to aetiology, pathogenesis and treatment. Once a disease has been diagnosed and classified, the aetiology of the condition and appropriate evidence-based treatment is suggested to the clinician [1]. Common systems of classification also allow effective communication between health care professionals using a common language. A classification, however, should not be regarded as a permanent structure. It must be adaptable to change and evolve with the development of new knowledge [2]. Classification systems are used for most diseases and help clinicians to design appropriate therapeutic strategies, based on evidence from appropriately conducted clinical trials. Periodontal diseases are no exception. Classification of periodontal disease helps in the development of frameworks to study the aetiology, pathogenesis and treatment of diseases; in addition it provides the international healthcare community with a way of communicating in a common language [3]. In order to obtain more knowledge

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about the causation of periodontal diseases the various forms of the disease have to be classified. Classification also helps us to formulate the treatment plan, predict the outcome of treatment as well as to educate the patient about the disease [4].

Disease classification: essentialist or nominalist? That is the question!

There are two schools of thought- the Essentialists and the Nominalists.

The essentialists, bases itself on the idea that the diagnosis is the name of the disease that caused the patients signs and symptoms: a periodontal diagnosis is a clinicians best guess as to what condition or disease the patient has. In the essentialist view, the number of disease categories in a disease classification seems to result from a cross-classification of current thinking with new knowledge, and can lead to quite a few diagnostic labels.

The nominalists call for the recognition of the fact that if we are to obtain more knowledge about the causation of periodontitis we need to firmly establish criteria for defining a periodontitis case for research. A disease classification based on nominalist principles describes, for each disease in the classification, a set of criteria that are fulfilled by all persons who have the disease but are not fulfilled by persons who do not have the disease or have other diseases. Effectively, this constitutes a screening examination, in which the oral cavity is thoroughly examined for the presence of oral lesions in need of treatment, whether affecting the dental hard tissues, the periodontium or other oral tissues. Thereby, the dental / periodontal diagnostic process is one of disease detection, rather than a classical deductive differential diagnostic process such as that facing the physician trying to explain the patients complaints [5].

HISTORICAL PERSPECTIVE

Already some 4000 years ago, the Egyptians and the Chinese described periodontal diseases as inflammatory conditions, and Hippocrates (460-335 B.C.) discussed the etiology and pathogenesis of different forms, including the situation when "the gums were bleeding or rotten"[6] The early English contribution to the understanding and management of periodontal diseases was made by John Hunter, a physiologist and surgeon of broad intellectual and scientific interests, widely known for his 1771 work on *The natural history of the human teeth*. In another major work from 1802, *Apractical treatise on the diseases* of *the teeth*, Hunter proposed a classification of the periodontal diseases that identified inflammatory processes in the gingiva as important factors in the dissolution of the alveolar bone. Riggs' in his article in *Dental Cosmos* in 1882 categorized the diseases of the gingiva and the bone on calculus deposits and other foreign bodies that made the tooth surface rough as "Pyorrhea alveolar is''. Miller gave the concept that periodontal disease was a multifactorial disease resulting from a nonspecific infection and a disturbance of normal host-parasite relationships. He provided no evidence to substantiate the theory; nevertheless, his work was a significant step toward the conceptualization of the relationship between a pathogenic microflora and periodontal disease.

Gottlieb suggested that [7,8] following the completion of active tooth eruption and in the absence of disease, the entire length of the epithelium from the gingival margin to the cementoenamel junction was structurally attached to the tooth surface. Under normal conditions no gingival crevice existed between the gingiva and the tooth surface, and a break in the continuous integument would, according to this school of thought, result in permanent damage to the area.

Waerhaug challenged this concept by Gottlieb [9]. In experimental studies, he demonstrated that, if they were severed by probing or other clinical manipulations (such as scaling or tooth cleaning), the tissues involved would not be permanently compromised. Rather, the normal microscopic den to-gingival relationship would be re-established, provided that there was no bacterial infection introduced the term epithelial cuff to emphasize the dynamic nature of the normal relationship between gingiva and the tooth surface. Twenty years later, Schroeder & Listgarten [10] described the sub-microscopic structures of the relationship between the tooth and the junctional epithelium.

Admittedly, details of the natural history of these diseases are yet unresolved and there are still voids in knowledge of the microbiology, immunology and tissue responses during periodontal health and disease. In 1966, the first World Workshop of Periodontics was held, where Ramfjord et al focused on seven areas related to the periodontium and the periodontal diseases: anatomy and physiology, epidemiology, etiology, pathology, prevention, treatment, and occlusion. The extensively documented original "blue book" report of the 1966 World Workshop (Ramfjord et al., 1966) remains a vital scientific and historic document in the annals of periodontology. The success of that first workshop is also apparent by its defining role in establishing the topics and format for subsequent World Workshops held in 1977, 1989, and 1996 [11].

DOMINANT PARADIGMS IN THE HISTORICAL DEVELOPMENT OF CLASSIFICATION SYSTEM
The development and evolution of classification systems for periodontal diseases have been largely influenced by paradigms that reflect the understanding of the nature of periodontal diseases during a given historical period. Over time, thoughts that guided the classification of periodontal diseases can be placed into three dominant paradigms:
1.Clinical features of the diseases (1870–1920),
2.Concepts of classical pathology (1920–1970),
3.Infectious etiology of the diseases (1970–present).

Classification systems in the modern era represent a blend of all three paradigms since there is a certain amount of validity to some of the earliest thoughts about the nature of periodontal diseases. As classification systems have evolved, newer thoughts about periodontal diseases have been superimposed on a matrix of older ideas that are still considered to be valid. Many people appear to believe that classification systems are rigid and fixed entities that should not be changed. In fact, classification systems should be viewed as dynamic works-in-progress that need to be periodically modified based on current thinking and new knowledge. Unfortunately, it seems that once people learn and accept a given classification, no matter how flawed it may be, they are extremely reluctant to accept revisions to their favourite system of nomenclature.

CLINICAL CHARACTERISTICS PARADIGM (1870-1920)
For the period from approximately 1870 to 1920 very little was known about the etiology and pathogenesis of periodontal diseases. Accordingly, the diseases were classified almost entirely on the basis of their clinical characteristics supplemented by unsubstantiated theories about their cause. Many authors in this era had put forward their difference of opinion for the etiological role of either local factors or systemic or that in some cases both local and systemic factors, in the causation of the disease. In the late 1800s and early 1900s clinicians used case descriptions and their personal interpretation of what they saw clinically as the primary basis for classifying periodontal diseases.

C.G Davis in 1879 gave as classification of periodontal disease and believed that there were three distinct forms of destructive periodontal disease [12].

Gingival recession with minimal or no inflammation.
Periodontal destruction
Davis apparently believed that calculus exerted mechanical pressure on the gingiva causing the alveolar bone to resorb because of lack of nutrition.

'Riggs' Disease' loss of alveolus without loss of gum.

Short comings of C.G Davis classification
1.No emphasis was given on age of onset of diseases and rate of progression
2.Inadequate or unclear classification criteria.
3.Little or no scientific evidence was used to support the opinions of the clinicians of that time.
4.Periodontitis which can be due to systemic diseases is not considered.

CLASSICAL PATHOLOGY PARADIGM (1920-1970)

As the field of periodontology began to mature scientifically in the first half of the 20th century, many clinical scholars in both Europe and North America began to develop, and argue about, nomenclature and classification systems for periodontal diseases. What emerged from this debate was the concept that there were at least two forms of destructive periodontal disease inflammatory and non-inflammatory ('degenerative' or 'dystrophic').

Gottlieb Classification of Periodontal Disease(1920) [13]

Gottlieb is generally considered to be the first author who clearly distinguished various forms of periodontal disease.

In the 1920's he classified periodontal disease into four types
1.Schmutz Pyorrhea
2.Alveolar atrophy or diffuse atrophy
3.Paradental-Pyorrhea
4.Occlusal trauma

Schmutz Pyorrhea

was thought to be the result of the accumulation of deposits on the teeth and was characterized by inflammation, shallow pockets, and resorption of the alveolar crest.

Alveolar atrophy or diffuse atrophy

was described as a non inflammatory disease exhibiting loosening of teeth, elongation, and wandering of teeth in individuals who were generally free of carious lesions and dental deposits. In this disease, manifesting pockets are formed only in later stages.

Paradental-Pyorrhea

was characterized by irregularly distributed pockets varying from shallow to extremely deep. This form of disease may have started as Schmutz-Pyorrhea or as diffuse atrophy.

Occlusal trauma,

A form of physical overload which was believed to result in resorption of the alveolar bone and loosening of teeth.

The first classification scheme to be accepted by the American Academy of Periodontology (AAP) was that of Orban in 1942.(Table 1)

INFECTION/HOST RESPONSE PARADIGM (1970 TO PRESENT)

Soon after the 1876 publication of Robert Koch [15] in which he provided experimental proof of the germ theory of disease, some dentists began to suggest that periodontal diseases might be caused by bacteria. W.D. Miller [15] in particular, was an early proponent of the infectious nature of periodontal diseases. He took into consideration in every case of pyorrhea alveolaris: (1) predisposing circumstances, (2) local irritation, (3) bacteria. He was, however, an early advocate of the 'Infection/Host Response Paradigm'. From 1965 to 1968 that the Infection/Host Response Paradigm began to move in the direction of becoming the dominant paradigm [16]. The next major discovery in periodontal microbiology was the preliminary demonstration in 1976–1977 of microbial specificity at sites with periodontosis. This finding, coupled with the demonstration in 1977–1979 that neutrophils from patients with juvenile periodontitis (periodontosis) had defective chemo tactic and phagocytic activities marked the beginning of the dominance of the Infection/Host Response paradigm.

The next major landmark in the classification of periodontal diseases emerged from the 1989 World Workshop in Clinical Periodontics. This classification, although soundly based in the Infection/Host Response paradigm, depended heavily on the age of the affected patients and the rates of progression.

At the time the classification was proposed it was recognized that, 'Overlap exists among categories and cases exist that do not clearly fit into any single category' [17]. In addition, it was acknowledged that considerable 'heterogeneity' existed within the Refractory Periodontitis category since, 'it includes patients who are unresponsive to any treatment provided whatever the thoroughness or frequency – as well as patients with recurrent disease at few or many sites. Different forms of periodontitis proposed in the classification shared many microbiologic and host response features, which suggested extensive overlap and heterogeneity among the categories.

The rate at which periodontitis progresses is highly variable and depends on such factors as:
• innate and acquired host susceptibility.
• composition and quantity of the sub gingival flora .
• the nature of genetically determined host–bacterial interactions

The final major problem with the 1989 classification was its arbitrary and heavy reliance on age of the affected patients or age of onset of the disease.

American Academy of Periodontology(AAP)1977-1989 (Table 2.)

American Academy of Periodontology (1986) [18].
1.Adult periodontitis
2.Juvenile periodontitis Pre-pubertal Localized juvenile periodontitis Generalized juvenile periodontitis
3.Necrotizing ulcerative gingivo-periodontitis
4.Refractory periodontitis

World Workshop in Clinical Periodontitis (1989)[19]

I. Adult periodontitis
II. Early onset periodontitis
a. Prepubertal periodontitis - Generalized / Localized
b. Juvenile periodontitis -Generalized /Localized
c. Rapidly progressive periodontitis
III. Periodontitis associated with systemic disease Down Syndrome, Diabetes type I,Papillon-Lefevre Syndrome ,AIDS ,Other diseases
IV. Necrotizing ulcerative periodontitis (NUP)
V. Refractory periodontitis

In 1993 , Ranney proposed four categories, Elimination of refractory periodontitis and periodontitis associated with systemic diseases.
I. Adult periodontitis
II. Early onset periodontitis
III. Necrotizing ulcerative Periodontitis
IV. Periodontal Abscess

New classification system was proposed by the "1999 International Workshop for a Classification of Periodontal Diseases and Conditions" in (Table 3) to correct some of the shortcoming associated with the previous system that had been in use since 1989.
• It did not include a gingivitis or gingival disease category.
• The periodontitis categories had non-validated age dependent criteria.
• There was extensive crossover in rates of progression of the different categories of periodontitis. 'Rapidly progressive periodontitis' was a heterogeneous category.
• There was extensive overlap in the clinical characteristics of the different categories of periodontitis.

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• 'Refractory periodontitis' and "Pre-pubertal periodontitis" was a heterogeneous category.

CONCLUSION

With the present knowledge we have aimed to classify periodontal disease in a simpler format, which is beneficial to the periodontal fraternity, general dental practitioners and the patient. To quantify or grade the progression of periodontal disease does not come under the scope or ambit of this classification system. The extent of disease is a clinical presentation at a particular stage of the disease. The sole discretion in selecting the treatment plan rests with the clinician based on clinical picture, radiological features, associated risk elements and the patient's systemic condition.

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Table 1. Orban classification of Periodontal Disease (1942) [14]

ATROPHY	HYPERTROPHY	TRAUMATISM
PERIODONTAL ATROPHY	**GINGIVAL HYPERTROPHY**	**PERIODONTAL TRAUMATISM**
1. Local trauma (toothbrush) 2. Presenile 3. Senile 4. Disuse 5. Following inflammation 6. Idiopathic	1. Chronic irritation (see inflammation) 2. Drug action (Dilantin sodium) 3. Idiopathic (Gingivoma, Elephantiasis, Fibromatosis)	Occlusal Trauma
Simultaneous atrophy of gingival margin and alveolar bone. Recession of gingiva, exposure of cementum surface. No inflammation; no pockets. In disuse, atrophy of periodontium sets in with osteoporosis in the supporting bone.	In most idiopathic cases overgrowth of fibrous connective tissue of gingiva (fibromatosis, elephantiasis gingivae). Accumulation of inflammatory elements in cases of inflammatory origin. In dilantin sodium cases also extensive epithelial growth.	Compression of periodontal membrane. Thrombosis, necrosis, bone resorption on side of pressure. Stress on fibers of tension side. Widening of periodontal membrane as the result of functional adaptation. If trauma acts in axis of tooth, fiber attachment becomes stronger and bone more dense.
Exposure of root surface on some or all teeth. Thermal sensitiveness. Gingiva mostly atrophic, thin, pale. In disuse, extrusion of the tooth occurs without gingival recession.	Overgrowth of gingiva often covering entire crown of teeth. If inflammation present, bleeding; if missing, gingiva tough, not bleeding. Sometimes localized to papillae, other times generalized.	Cusp interference leads to loosening of teeth due to widening of periodontal space. Sensitiveness to percussion, sometimes to thermal changes. If trauma is in one direction, tooth may move out of occlusion.

Pathology	INFLAMMATION		DEGENERATION
Nomenclature	Gingivitis	Periodontitis	
Etiology	**LOCAL** Calculus Food Impaction Irritating restoration Drug action etc.	**SYSTEMIC** Pregnancy Diabetes Other endocrine dysfunctions Tuberculosis Syphilis Nutritional disturbances Drug action allergy	1, Systemic disturbances a. Diabetes b. endocrine dys- functions c. Blood dyscrasias d. Nutritional dis- turbances e. Nervous disorders f. Infectious diseases (acute & chronic) 2. Hereditary 3. Idiopathic
Pathology	Acute or chronic inflammation localized at gingival margin a. Edematous b. Serous c. Purulent d. Ulcerative (Vincent's) e. Hemorrhagic f. Desquamative g.	Chronic inflammation of gingiva progressing into bone causing resorption of supporting structure. Acute abscess formation in deep pockets. Acute inflammation	Degeneration of the connective tissue elements in the periodontal membrane. Bone resorption. Widening of the periodontal space, migration of epithelial attachment. Hypertrophy of epithelial rests.

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	Hypertrophic h. Etc.	close to wall of pocket, migration of leukocytes through wall of pocket.	Pocket formation.
Clinical Picture	Manifold according to pathology. Swelling and bleeding of gingivae. Change of color from pale pink to red or bluish red. Pocket formation slight.	Deep pockets on all or single teeth depending on the case. In acute abscess formation extensive pain. In Simplex cases much calculus. In Complex cases little calculus, if any.	Migration of teeth. Formation of diastemas. Loosening of teeth. Often caries immunity. As a rule attacks young girls and older men. Pocket formation a late symptom (Periodontitis complex). There are cases where all teeth are attacked and cases where only one or some teeth are affected.

Table 2.American Academy of Periodontology(AAP)1977-1989

1977	1986	1989

I. Juvenile Periodontitis
II. Chronic marginal periodontitis
	I. Juvenile Periodontitis
	A. Prepubertal
	B. Localized juvenile periodontitis
	C. Generalized juvenile periodontitis
	II. Adult Periodontitis
	III. Necrotizing Ulcerative Gingivo-Periodontitis
	IV. Refractory Periodontitis
		I.Early-OnsetPeriodontitis
		A.Prepubertal periodontitis
		  1. Localized
		  2. Generalized
		B. Juvenile periodontitis
		C. Rapidly progressive periodontitis
		II. Adult Periodontitis
		III. Necrotizing Ulcerative Periodontitis
		IV. Refractory Periodontitis V. Periodontitis Associated with Systemic Disease

Table 3. 1999 International Workshop for a Classification of Periodontal Diseases and Conditions

I. Gingival disease	

A. Dental plaque-induced gingival diseases
1. Gingivitis associated with dental plaque only
i. Without other local contributing factors
 ii. With local contributing factors
2. Gingival diseases modified by systemic factors
i. Associated with the endocrine system
  1. Puberty-associated gingivitis
  2. Menstrual cycle-associated gingivitis
  3. Pregnancy-associated
   a. Gingivitis
   b. Pyogenic granuloma
  4. Diabetes mellitus-associated gingivitis
 ii. Associated with blood dyscrasias
	4. Gingival lesions of genetic origin
	i. Hereditary gingival fibromatosis
	  ii. Other
	5. Gingival manifestations of systemic conditions
	i. Mucocutaneous disorders
	   a. Lichen planus
	   b. Pemphigoid
	   c. Pemphigus vulgaris
	   d. Erythema multiforme
	   e. Lupus erythematosus
	   f. Drug-induced
	   g. Other

1. Leukemia-associated gingivitis
   2. Other
 3. Gingival diseases modified by medications
i. Drug-influenced gingival diseases
   1. Drug-influenced gingival enlargements
   2. Drug-influenced gingivitis
     a. Oral contraceptive-associated gingivitis
     b. Other
 4. Gingival diseases modified by malnutrition
i. Ascorbic acid-deficiency gingivitis
  ii. Other
B. Non-plaque induced gingival lesions
 1. Gingival diseases of specific bacterial origin
i. Neisseria gonorrhea-associated lesions
  ii. Treponema pallidum-associated lesions
  iii. Streptococcal species-associated lesions
  iv. Other
 2. Gingival diseases of viral origin
i. Herpes virus infections
    Primary herpetic gingivostomatitis
    Recurrent oral herpes
    Varicella-zoster infections
  ii. Other
 3. Gingival diseases of fungal origin
i. Candida-species infections
    Generalized gingival candidiasis
  ii. Linear gingival erythema
  iii. Histoplasmosis
  iv. Other
	ii. Allergic reaction
	   1. Dental restorative materials
	    a. Mercury
	    b. Nickel
	    c. Acrylic
	    d. Other
	   2. Reactions attributable to
	    a. Toothpastes/dentifrices
	    b. Mouth rinses/mouthwashes
	    c. Chewing gum additives
	    d. Foods and additives
	   3. Other
	 6. Traumatic lesions (factitious, iatrogenic, accidental)
	i. Chemical injury
	  ii. Physical injury
	  iii. Thermal injury
	 7. Foreign body reactions
	 8. Not otherwise specified

II. Chronic periodontitis
    A. Localized
    B. Generalized
III. Aggressive periodontitis:
    A. Localized
    B. Generalized
IV. Periodontitis as a manifestation of systemic diseases:
 A. Associated with hematological   disorders
 1. Acquired neutropenia
 2. Leukemias
 3. Other
 B. Associated with genetic disorders
 1. Familial and cyclic neutropenia
 2. Down syndrome
 3. Leukocyte adhesion deficiency syndromes
 4. Papillon-Lefevre syndrome
 5. Chediak-Higashi syndrome
 6. Histocytosis syndrome
	VII. Developmental or acquired deformities and conditions:
	A. Localized tooth-related factors that modify or predispose to plaque induced gingival diseases/ periodontitis
	 1. Tooth anatomic factors
	 2. Dental restorations/appliances
	 3. Root fractures
	 4. Cervical root resorption and cemental tear
	B. Mucogingival deformities and conditions around teeth
	 1. Gingival/soft tissue recession
	   a. Facial or lingual surfaces
	   b. Interproximal (papillary)
	 2. Lack of keratinized gingiva
	 3. Decreased vestibular depth
	 4. Aberrant frenum/muscle position
	 5. Gingival excess
	   a. Pseudo pocket

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7. Glycogen storage disease
8. Infantile genetic agranulocytosis
9. Cohen syndrome
10. Ehlers-Danlos syndrome (Types IV and AD)
11. Hypophosphatasia
12. Other
C. Not otherwise specific
IV. Necrotizing periodontal diseases:
  A. NUGs
  B. NUP
V. Abscesses of the periodontium:
  A. Gingival abscess
  B. Periodontal abscess
  C. Pericoronal abscess
VI. Periodontitis associated with endodontic lesions:
A.Combined periodontal-endodontic lesion
	b. Inconsistent gingival margin
	c. Excessive gingival display
	d. Gingival enlargement
	6. Abnormal color
	C. Mucogingival deformities and conditions on edentulous ridges
	1. Vertical and/or horizontal ridge deficiency
	2. Lack of gingival/keratinized tissue
	3. Gingival/soft tissue enlargement
	4. Aberrant frenum/muscle position
	5. Decreased vestibular depth
	6. Abnormal colour
	D. Occlusal trauma
	1. Primary occlusal trauma
	2. Secondary occlusal trauma

Table 4. A new classification scheme for periodontal and peri- implant diseases and conditions – Introduction and key changes from the 1999 classification

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Table 5. Periodontal Health Table

Table 6. Forms of Periodontitis

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Table 7. Periodontal Manifestations of Systemic Diseases and Developmental and Acquired Conditions

Table 8. Peri-Implant Diseases and Conditions

Figure 1. Disease Classification

I. Adult Periodontitis

II. Early Onset Periodontitis

    A. Prepubertal Periodontitis

        1. Generalized

        2. Localized

    B. Juvenile Periodontitis

        1. Generalized

        2. Localized

    C. Rapidly Progressive Periodontitis

III. Periodontitis Associated with Systemic Disease

IV. Necrotizing Ulcerative Periodontitis

V. Refractory Periodontitis

Figure 2. Infection/Host Response Paradigm

REVIEW ARTICLE

Heat Shock Proteins: An Ubiquitary Role in Medical and Dental Sciences

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ABSTRACT

Heat shock proteins are a specific type of protein that occurs due to alterations in ecologic conditions, resulting in the synthesis of such proteins. Recent research is focused at several levels, classified from basic molecular biology approaches to therapeutic applications. Many functional roles for HSPs are known, but the mechanisms for these several functions are not entirely understood. This article shows the ubiquitary role of heat shock proteins: as immunomodulant, molecular chaperons, fungal morphogenesis and antifungal therapy, regulatory role in neurodevelopment, and as biomarkers and periodontal vaccines in periodontal disease.

Keywords: Heat shock proteins, Universal role, Immunomodulant, Antifungal, Salivary biomarkers, Periodontal vaccine

INTRODUCTION

Heat shock proteins (Hsps) are evolutionarily preserved molecular chaperones with essential roles in cell development and survival. Based on comparable molecular mass, they can be generally classified into two families. First, the small, ATP-independent Hsps, and secondly, the large, ATP-dependent Hsps [1]. These proteins are ubiquitous, occurring in all organisms from bacteria and yeast to humans. HSPs come in a variety of forms and are categorized into families based on their molecular weights. There are substantial facts that HSPs play important physiological roles in normal conditions and situations involving both systemic and cellular stress [2]. Heat shock proteins are a specific type of protein that occurs due to alterations in ecological conditions, resulting in the synthesis of such proteins [3]. These proteins are generated by cells in response to environmental stimuli such as high

temperature, mechanical stress, and infection, and participate in vital physiological processes in the cell such as folding, assembly, and translocation of polypeptides across membranes .[4]There is the occurrence of both cytotoxic and tissue damage of some of the Microbial Hsps which in turn may result in immune responses .[3]Recent research is focused at several levels, classified from basic molecular biology approaches to therapeutic applications. Many functional roles for HSPs are known, but the mechanisms for these several functions are not entirely understood.[2]For the past couple of years, an understanding of the universal roles of the major heat shock proteins has begun to evolve. This article shows the ubiquitary role of heat shock proteins: as immunomodulant, molecular chaperons, fungal morphogenesis and antifungal therapy, regulatory role in neurodevelopment, and as biomarkers and periodontal vaccines in periodontal disease.

History

The heat shock response was identified by Ferruccio Ritossa in 1962.[5] He visualized the presence of a specific group of proteins in the fruit fly Drosophila Melanogaster as a response to high temperature and then termed it Heat shock proteins .[6]

What Are Heat Shock Proteins and Their Classification?

Heat shock proteins are ubiquitous proteins that are seen in every existing active bacteria, fungi, and virus[7]They are immensely preserved proteins with large homogeneity between bacteria and mammalians.[8] They are categorized according to their molecular weight, size, structure, and function. HSP families consist of[8,9]

Ubiquitary role of Heat Shock Proteins

As Immunomodulant
Functions
The Heat shock proteins can be identified by Tregs and are immunogenic. The proteins are seen in whole-cell and they express themselves at the time of the inflammatory process. To reach a lasting immunotolerance, Tregs intend to be a promising goal. The treg activation occurs during the recognition and binding of their specific antigens.[8]Inflammatory arthritis can be controlled by immunization with Hsps, in which they activate Tregs. Hsps can be involved in the modulation of the host and also in the development of autoimmune conditions. The Hsps also can process and present antigens. [10,11]

Conclusion By Studies: [12-19]

i.   In a study done by Bakthisaran R et alon heat shock proteins concluded that Protein kinase B activation can be controlled by Hsp 27.
ii. Wu Y et al concluded that Th1 and Th17 can be stimulated against streptococcus pneumonia by Hsp40
iii. Cui J et al concluded that in pneumococcal infection, the Hsp40 induces the production of the pro-inflammatory cytokine, which activates the signal pathways P13k and JNk resulting in IL-6 secretion.
iv. Calderwood et al concluded that- In Type I diabetes mellitus, Hsp 60 induces both anti-inflammatory and proinflammatory cytokines
v.   Juwono J et al concluded that-In Type 2 diabetes mellitus, there is the release of proinflammatory cytokines when Hsp60 interacts with TLR 2 and TLR 4
vi. Borges T.J et al estimated that-In Chronic inflammatory disease,Hsp70 stimulates the anti-inflammatory cytokine production.
vii. Gross C et al analyzed that -Hsp70 acts as an extracellular confined recognition spot for NK cells in Cancer. Interface through TKD motif with NK cells results in cytolytic outbreak intermediated by NK cells.

viii.   Mbofung R.M et al evaluated that –Hsp90 endorses the killing of melanoma cells by implication in T cell-mediated anti-humor response in cancer.

As Molecular Chaperons
Functions
Protein folding and the assembly of multimeric structures in vivo is not a completely spontaneous process but is promoted by proteins known as molecular chaperones.

Hsp70s and Chaperonins or Cpn60s [20]
The 70-kDa family of Hsps (Hsp7Os) and the 60-kDa family of Hsps (termed chaperonins or Cpn6Os) together form the two major groups of molecular chaperones. Hsp7Os are found inside the mitochondria and the endoplasmic reticulum (ER) plays an important role in the translocation of proteins from the cytosol into those organelles by binding during the early stages of translocation. Cpn6Os, found in both eukaryotes and prokaryotes, have been shown both in vivo and in vitro to bind unfolded proteins, preventing their aggregation and facilitating folding. Although Hsp7Os and Cpn6Os are the best characterized, evidence implicates other proteins, including Hsp9O, proline isomerizes, and disulfide isomerizes, in protein folding. In addition, HsplO4, an Hsp synthesized only during times of stress, has been postulated to be involved in similar processes.

Hsp 60 [21,22,23]
Hsp6O facilitates protein folding and assembly in an ATP-dependent manner by involving directly with the unfolded protein. Although Hsp6O functions in the folding and assembly of proteins whosetarget is the matrix, its role in the translocation of protein passing from the matrix into the inner membrane space remains to be concluded.

Hsp9O [24]
Hsp9O is a substantial protein, localized primarily in the cytoplasm. The function of Hsp90 was to deliver a regulatory function, blocking activation by steric interference and maintaining the unfolded conformation of these proteins until they were properly localized. It now appears that Hsp9O is also vital for their functional activation, perhaps for folding into a potentially active conformation. Hsp70 is also found in the Hsp90-receptor complex and may play a major role in receptor biogenesis.

Hsp104 [25]
The Hsps discussed thus far be included in processes, such as protein biogenesis, which are necessary under normal growth conditions. However, without HsplO4, cells have difficulty surviving under less than ideal conditions such as high temperatures. Without HsplO4, yeast cells are defective for this induced thermo tolerance. In addition, the expression of HsplO4 is essential for the naturally high thermo tolerance of stationary phase cells and spores, as well as for tolerance to ethanol.

CONCLUSION BY STUDIES [26,27]

-Parsell et al.do not indicate that HsplO4 is a protease, but, rather, they suggest that it may manage a protease or, by some other mechanism, be involved in avoiding or resolving aggregation of vital cellular structures during times of stress. Thus, as the Hsps discussed above, the picture that came into existence for the HsplO4 function seems to be one of mediating protein-protein interactions. Surprisingly, the defect in induced thermo tolerance in HsplO4 cells can be partially submerged by over expression of the Hsp7O Scalp, considering that the functions of these two proteins are related.

As Antifungal Therapy and In Fungal Morphogenesis
Functions
A critical factor and an important role being played by stress in the functional characteristics of fungi. Researches revealed that heat shock proteins such as 90,70,21 and 40 play a crucial part in functional variations, stress alteration, and antifungal resistance.[28,29]

CONCLUSION BY STUDIES

A study done in a fumigatus in a genome-wide gene expression in response to Amphotericin B,ensued in hsp8 down regulation. [39] Studies on S. cerevisiae also revealed that usage of the slight quantity of Guanidinium hydrochloride with yeast discontinues the action of Hsp104 by carrying in together the M-domain of Hsp104, henceforth halting the Hsp104-Hsp70 interface, causing the inhibition of stress tolerance of yeast [40]. Existing research reveals that five different fungal infections can be vaccinated through heat-killed yeast [41]. Various groups of fungi such as Coccidioides posadasii,C Albicans) when inoculated in mice model, different Hsps are upregulated i.e hsps such as 70,40,90,60.[42]

As a Regulatory Role In Neurodevelopment [1]
Functions
In cell survival and growth, Hsps are a huge family of developmental preserved molecule chaperons with essential roles. Hyperthermia and Hypoxia are various stressors that prompt the countenance of Hsps,which in succession, interconnect with co-chaperones to control cell development and existence.Angiogenesis,Neurite outgrowth, and cell migration are all emerging confirmation that Hsps may endorse or impede neurodevelopment.

As Salivary biomarkers and Periodontal vaccines
Salivary Biomarkers
Salivary protein ranges from 0.5 to 3 mg/dl. Heat shock proteins 60,70,90 are seen in saliva due to passive transport from blood serum. The Source of heat shock protein in the saliva is largely from the salivary glands, mucosal cells, periodontal tissues either from exudate or direct blood contamination, and other sources from the bloodstream.[43] The various immunological and cytoprotective action of salivary HSP 70 is due to the defense action of these proteins by protecting the mucosal and periodontium within unspecific defensive alert system. Their presence show pronounced immune functions in poor oral health .[44] Surface defense against toxins is one of the most important and major cytoprotective roles of these proteins, thus decreasing cell apoptosis and necrotic liability.

Functions
Hsp70 plays an important role in defense function on the tooth surface, by binding and agglutinating the microbes via salivary complexes, thus the presence of these HSPs enhance the protection against heat stress and alters Ph. providing cytoprotective effect and thus aiding in defense.[45] Thus the goal of these chaperons is to regulate the response to any detrimental factors, including temperature, radiation, hypoxia, and toxins, and to prevent misfolding of proteins.

CONCLUSION BY STUDIES

Research showed a significant increase in HSP 70 in individuals with poor oral health which may be attributed to its role in stressful conditions. The extracellular role of HSP 70 explains its cytoprotective property in events of pathological and physiological stress. It maintains pulpal health, and periodontal health, aiding in the repair of irritated dental hard and soft tissues and reverting the inflammatory conditions [45]. 1.A study conducted by Pileggi and Graham (2009) explains the expression of HSP 70 in 24 hours of pulpal injury as an early response to pulpal trauma and thus can be a method for measuring the degree of trauma in dental pulp .[46]

2.Ramya Netravathy et al. (2016) showed that Circulating HSP 60 levels may play a role in representing the systemic inflammatory state produced by periodontal disease but salivary HSP 60 may not be used as a surrogate to determine systemic inflammation .[47]

Periodontal Vaccine

History of Periodontal Vaccines

In the early 20th century, three periodontal vaccines were employed [48,49,50] pure cultures of streptococcus and another organism, autogenously vaccines, and stock vaccines eg. Vancott's vaccine, and Inava endocarp vaccine.

Functions

Periodontal vaccination is indicated in patients with severe periodontal disease with loss of bone and teeth, inflammation, and association with oral bacterial infection below the gum line, and exacerbated diabetes and cardiovascular disease. Heat shock protein (HSP) shares a high sequence homology among periodontopathic bacteria [51], which can be utilized as a vaccine to cross-protect against multiple pathogenic species.A vaccine incorporating the poly-reactive monoclonal antibody recognized peptide number 19 of 37 synthetic peptides spanning the whole molecule of P. gingivalis HSP60 might be useful in multifactorial diseases such as atherosclerosis and diabetes supporting the role of molecular mimicry in the periodontal-atherosclerosis link.[52]

CONCLUSION BY STUDIES

o   Ebersole, et al. (1991) [53]
•   Animal used-Primate
•   Vaccine Administered-Whole cell antigen of P. gingivalis and P. intermedia
•   Result of study-Two-fold increase in serum Ig G, Ig M, and IgA antibodies that were highly specific for these immunogens.
o   Person G (2005) [54]
•   Primate model study
•   Vaccine administered-P. gingivalis
•   Decreased rate and severity of bone loss
o   Puth S, et al. (2019) [55]
•   *In vitro* Study
•   Vaccine administered-Divalent mucosal vaccine consisting of a mixture of FlaB-to and Hgp44-FlaB fusion proteins targeting virulence factors of Fusobacteriumnucleatum and Porphyromonas gingival is respectively.Result of study-Antisera inhibited F. nucleatum mediated biofilm formation, coaggregation of P. gingivalis and Treponema denticola, and P. gingival is-host cell interactions.

CONCLUSION

This review has strived to reveal the ubiquity role of HSP. In recent years, various studies have narrated the characterization of Hsps produced by oral micro-organisms. Like Hsps involved in other diseases, these stress-induced proteins may contribute to the pathogenic process of oral infections.
There is an accumulation of evidence to advocate that Hsps play a universal role, however, the exact roles of Hsps remain largely unexplored.

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Table 1: Ubiquitary role of Heat Shock Proteins

hsp family	Molecular weight (kda)	Cellular location	Functions
sHSP	12-43	Mitochondria	Stop gathering other proteins by assembling protein "garbage" act as dustmen of cells
HSP40	40	Cytosol	They function in specific pairs with Hsp70 to promote protein folding, protein transport, and degradation.
HSP60	60	Mitochondria	The endorsement of protein folding and refolding
HSP70	68	Cytosol/Nucleus	The endorsement of protein folding and refolding
HSP90	90	Cytosol	Sustain substrate proteins and manage their active or inactive state, stopping other proteins' aggregation.
HSP100	100	Cytosol	Disaggregation of proteins

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Table 2: Depiction of heat-shock protein in fungi created based on weight, location, and their roles.

Hsps	Molecular Wt.	Cellular Location	Cellular Functions
Hsp90 [30,31]	90	Cys, ER, N	Folding and preservation of client proteins, involved in transcriptional and posttranscriptional processes and activation of signal transducers
Hsp70 [32,33,34]	68	CDS, N, ER, R, M	Early folding of nascent polypeptide and ATPase activity
Hsp21 [35,36]	20–30	ER	Alteration of Fungi in ecological stress and pathogenicity
Hsp40 [37,38]	40	CDS, M, ER	Cell physiology and cofactor of Hsp70

RESEARCH ARTICLE

Effect of patellar Kinesio Taping Along with Strengthening Exercise in Treating Osteoarthritis with Pre – Dominant Patellofemoral Joint Involvement on Parameters Like NPRS and 1 RM

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ABSTRACT

To evaluate the effect of patellar Kinesio taping along with strengthening exercise in treating osteoarthritis with pre dominant patellofemoral joint involvement on parameters like NPRS and 1RM.
40 Subjects (13 males and 27 females) aged at least or more than forty years of age are included into the study. 40 subjects are divided into two groups 20 each according to the simple random sampling. Group A is taken as the control group. Group B is taken as an experimental group. Group A considering control group is given only strengthening exercise whereas group B experimental is given Kinesio taping along with strengthening exercise. Protocol is provided for 5 days week for 4 weeks where in experimental group Kinesio tape is provided in form if I shaped band for patellar taping and y shaped tape for quadriceps muscle once in a week for 4 week. Later effect was observed outcomes measures NPRS and 1RM. The result showed significant results in both the groups when compared within the groups but when seen the result of unpaired t test significant difference is seen in the values of NPRS. The values of 1RM showed almost the same results in both experimental as well as control group thus not significant.
Both the control as well as experimental group showed significant results after protocol was provided. Additional effects of taping was observed only on the NPRS thus reducing the pain and was not significant on other outcome measure like 1RM.

Keywords: Patellofemoral joint,NPRS, 1RM, Kinesio tape, strengthening exercise.

INTRODUCTION

To practice evidence based practice there is need of good evidence and constant touch with all the new researches that are coming out associated with the concerned topic. In older individuals one of the very familiar reasons of knee pain is observed in the knee   because of the age related arthritic changes observed in the knee joint, thus it becoming usual complains in the family practice. One of the pseudonymous category of arthritis is said to be patellofemoral joint osteoarthritis. Since PFJ involvement has major role in symptoms rather than TFJ involvement PFJ OA can detrimentally affect wellbeing of an individual by making them economically less productive and functionally dependent on others.

Signs observed because of the arthritis are pain in the knee anteriorly and posteriorly crepitus, instability, knee with a restricted range of motion and swelling [1]. Subjects with patellofemoral pain syndrome are at higher risk of developing patellofemoral osteoarthritis in later life if correct diagnosis is not made and proper treatment not administered. These authors found 22% of 118 patients with patellofemoral arthritis had retro patellar knee pain as adolescent [2]. Pain has been considered as the major complaint where it also plays an important role in the functional dependency thus one of the outcome is selected as numeric pain evaluation scale (NPRS) which helps in finding out the effect on the protocol in the terms of the pain assessment. Pain was assessed a numeric pain rating scale (NPRS) reliability ICC = 0.67.To look for the muscle power increase after strengthening protocol the out- come measure used will be repetition maximum. "the use of progressive resistance exercise for the restoration of muscle power and volume after injury was first described by de Lorme in 1945. The poundage is determined by testing repetition maximum. Lifting of weight may involve either static or dynamic muscle work according to the circumstances and the movement is slow and controlled".

The maximum weight which can be lifted only once through a prescribed range is called one repetition maximum and the maximum weight which can be lifted ten times at natural speed without rest between lifts is the 10 repetition maximum [3]. Test retest reliability of 1RM ICC = 0.64 – 0.99 [4].

Muscle function
Knee extensor muscle function
The only muscle in the front of the thigh and major muscle for knee extension is the rectus femoris, Vastus medialis, Vastus lateralis and Vastus intermediaries together making the quadriceps .If the foot is being fixated then closed chain will be created and then hamstring and sole us muscle will help in knee extension by pulling tibia posteriorly [5].

Closed chain function When in stance phase of gait and in standing position knee forms closed chain. Following the theory of reverse muscle pull knee extensors controls the flexion of the knee and allows the extension movement to be stable .When in standing position the knee is locked and quadriceps does not need to work when gravity line falls anteriorly to axis of motion. At this time tension in hamstring and gastrocnemius tendons support the posterior capsule [5].

Knee flexor muscle function
Hamstring is the major knee flexor and also influences the rotation of tibia on femur .Due to the knee flexor being a muscle having attachment at the two joints hip and knee it works more efficiently when they are together lengthened during the hip is in flexion position .During closed chain weight bearing activities hamstring muscle can assist with knee extension by pulling on tibia [5]. Thus keeping into mind all the biomechanical components acting over the knee   joint strengthening exercise protocol has been made which may result in reducing the pain, increasing the strength, reacting on quadriceps angle and in making a patient functionally more independent. Thus exercises involving quadriceps muscles , hip abductors muscles , external rotator of hip muscle strengthening and stretching

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of muscles going in tightness and correction of biomechanical components is included. And 1RM of the three muscles (quadriceps, hip abductors and hip external rotators) is taken as well.

Kinesio Taping

Kinesio taping one of the methods of therapeutic taping was given by kenzokase in 1990 and due to its great results has become very popular in treating the sports injuries .The property of tape of it being flexible works on the VMO and VL muscle imbalance. Kinesio taping method gives the therapist the liberty to treat the cause of any symptom by taping it in different manner thus working on the different structures involved. By taping for knee it works on correcting the patellar position, improving the proprioception and increasing the firing rate in the Vastus medialis muscle. There are many studies which say that there is observed increase in the local blood circulation and lymphatic circulation due to the specific properties of Kinesio tape they also tend to be waterproof and have highly ventilated material which allows the skin to breathe [6].

MATERIALS AND METHODOLOGY

● SOURCE OF DATA: Sainath hospital
● STUDY DESIGN: An experimental study
● SAMPLE SIZE : 40
● SAMPLE DESIGN: Simple Random Sampling
● DURATION OF STUDY: 1 year

MATERIALS USED

● Plinth
● Goniometer
● Consent form
● Pen
● Pencil
● Kinesio tape
● Scissors
● Weight cuffs for strengthening

METHODOLOGY

● Subjects aged at least or more than 40 years of age are included into the study.
● Both gender are included.
● Subjects are thoroughly explained about the exercise protocol and research being carried out, written consent is taken after getting ethical permission.
● Subjects having knee osteoarthritis with pre-dominant patellofemoral joint involvement are taken.
● Subjects fulfilling all inclusion criteria and having evidence of lateral patellofemoral osteophytes on weight bearing radiographs are taken into the study.
● 40 subjects are divided into two groups 20 each according to the simple random sampling.
● Group A is taken as the control group.
● Group B is taken as an experimental group.
● Group A considering control group is given only strengthening exercise whereas group B experimental is given Kinesio taping along with strengthening exercise.

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TREATMENT DURATION
o Exercises are performed once a day, 5 days a week for 4 weeks.
o Kinesio Taping is provided once a week as the effect of Kinesio tape lasts for 3 to 7 days in experimental group. And same exercises were provided in both the groups. Exercises provided are listed below and type of taping is described as well.
- Ankle pumps. 20 rep
- Static quadriceps exercise (10 maximal isometric quadriceps contraction)
- Last degree knee extension. 10 rep
- High sitting knee extension. 10 rep
- Vast us medialis muscle contraction in sitting position squeezing a rolled up towel between the knees along with gluteal contraction.10 rep
- Hip abductor strengthening in side-lying first actively later (after 1 week) according to progressive resisted exercise protocol according to patient's repetition maximum. 10 rep.
- Straight leg raising first actively later (after 1 week) according to progressive resisted exercise protocol according to patient's repetition maximum. 10 rep
- Hip external rotator strengthening.10 rep
- Muscles undergoing tightness will be stretched.
- Exercises were performed once a day, 5 days a week for 4 weeks.
- Patellar Kinesio taping with enough force (50 – 75% tension) to medially shift patella for mechanical correction.
- Taping is given once a week for 4 weeks along with strengthening exercises, while patellar position has been corrected with the help of Kinesio tape and forces work correctly to maintain the proper knee biomechanics.
- Taping is given in form of y- shaped on the quadriceps muscle which is one of the muscle facilitating technique for more muscle fibers recruitment. As well as I – shaped tape is provided for patellar correction.

RESULTS AND DISCUSSION

This study involved 40 subjects aged forty years and above, matching the inclusion and exclusion criteria. These subjects were randomly divided into 2 groups control group (exercise only) and experimental group (exercise + taping). The parametric test was used in statistical analysis using SPSS version 25. Demographic values were compared within and between the groups using paired (independent T - test) and unpaired T- test respectively. The result showed significant results in both the groups when compared within the groups but when seen the result of unpaired t test significant difference is seen in the values of NPRS. The values of 1RM showed almost the same results in both experimental as well as control group.

The knee joint is divided into two components patellofemoral joint and tibiofemoral joint. The two compartments are anatomically and functionally distinct and patellofemoral joint involvement by OA is more prevalent than tibiofemoral involvement, particularly in women with knee pain [7]. The purpose of the study is to evaluate the effect of patellar Kinesio taping along with strengthening exercise in treating osteoarthritis with pre dominant patellofemoral joint involvement on parameters like NPRS for pain evaluation, 1RM to check the muscular strength. The result goes in favor of reducing the pain because of the Kinesiological tape due to the following mechanism. Potential skin lifting effect of Kinesiological tape Kinesio tape has a specific pattern (wavy) in nature this design of the tape gives it property through which it creates convolutions which lifts the skin as well as fascia thus creating the sub dermal vacuum dilating the blood vessels and lymph vessels which in turn increases the blood circulations as well as the promoting fluid flow.

Kinesio tape is among very few therapeutic tape that does not restrict the mobility and also tends to have property of water resistance. The tape has 5 major functions.

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● Skin: relieve pain and improve proprioception.
● Circulatory / lymph: removes stagnation of the lymphatic fluid. It enhances natural fluid flow between the layers of the tissue.
● Fascia: return homeostasis to the facial matrix.
● Muscle: restore or promote normal muscle function.
● Joint: improve joint biomechanics and alignment.
● It's an intervention used to stimulate the sensory system [8].

Strengthening protocol was provided to both the groups control as well as experimental and was given to the major knee muscles getting involved like quadriceps, hip abductors, hip external rotators and later progressive resisted exercise was provided according to the RM thus giving significant effect and increase in the strength of the muscles thus improving the functional capability which was administered through the functional scale patellofemoral joint evaluation scale. Quadriceps strengthening and particularly the Vast us medial is oblique is emphasized because it's believed its weakness may predispose for muscle imbalance and concomitant patellar stress [9]. According to the article by Mirzabeigi E *et al* "Isolation of the Vast us medial is oblique muscle during exercise." stated that Vast us medial is training is very necessary to improve VL/VMO onset timing differences. Without the use of electrical stimulation it is difficult to isolate Vast us medial is muscle however it is believed that VMO activity is greater with hip in external rotation." Thus strengthening of the muscles in both the groups has shown significant results as protocol was designed on basis of strengthening the muscles like quadriceps , hip external rotators and hip abductors [10].

- The result goes in favour of the strengthening with reference from the article by Thiago r. stanos *et al* "Effectiveness of hip muscle strengthening in patellofemoral pain patients: a systemic review said that the participants where both hip and knee muscles were trained presented with less pain ascending descending stairs [11].

CONCLUSION

The study was conducted to see the effect of patellar Kinesio taping along with strengthening exercise in treating osteoarthritis with pre – dominant patellofemoral joint involvement on the parameters like NPRS for pain evaluation, 1RM for checking the muscular strength. Both the control as well as experimental group showed significant results after protocol was provided. Additional effects of taping was observed only on the NPRS thus reducing the pain and was not significant on other outcome measure like 1RM.

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Table 1. Comparision between the Groups
Table for Unpaired T-Test

OUTCOME MEASURES	EXPERIMENTAL GROUP MEAN(SD)	CONTROL GROUP MEAN (SD)	t value	P value
NPRS	2.60 (1.09)	3.80 (1.15)	3.376	0.002
1RM (QUADS)	3.42 (1.10)	3.52 (1.27)	0.266	0.792
1RM (HIP ABD)	2.95 (0.98)	3.02 (0.91)	0.250	0.804
1RM (HIP ER)	2.87 (0.94)	2.85 (0.84)	-0.088	0.930

Abbreviations: 1RM – Repetition maximum

Quads – Quadriceps

HIP ABD – Hip abductors

HIP ER – Hip external rotators.
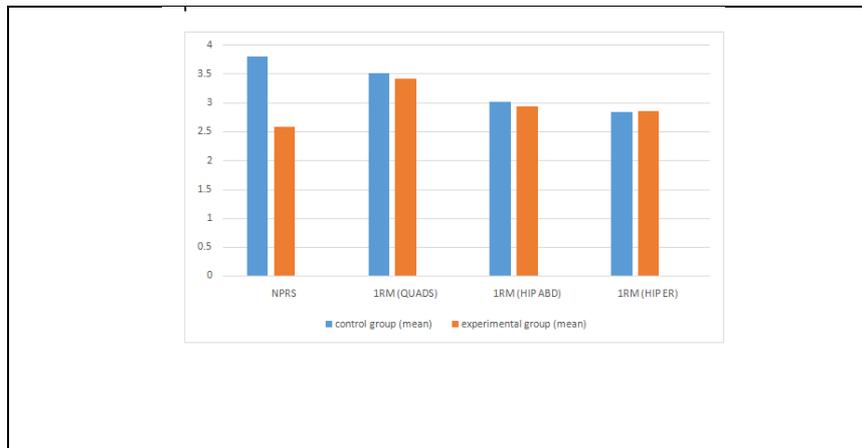
Fig. 3.Graphical Representation of Mean Values (Between The Group) : NPRS and 1RM (QUADS, HIPABD, HIP ER)	**Fig. 2. I Shaped for Patellar taping Y shaped for quadriceps**

RESEARCH ARTICLE

Biochemical Studies on the *Cissus quadrangularis* Plant Extract Treated Fish *Oreochromis mossambicus Peters*

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ABSTRACT

Cissus quadrangularis L. is an Indian medicinal plant that belongs to the vitaceae family. The purpose of this study was to see how *Cissus quadrangularis* affected the biochemical changes in Oreochromis mossambicus, a freshwater fish. To determine the impact of *Cissus quadrangularis* plant extract on total protein, calcium, and phosphorous levels in *Oreochromis mossambicus* tissues (gill, liver, and muscle), samples were taken at 7, 14, and 21 days. *Cissus quadrangularis* affected fish were compared to control fish. According to the findings of this study, *Cissus quadrangularis* is favorable to fish growth and development.

Keywords: *Cissus quadrangularis, Oreochromis mossambicus,* Tissues, protein calcium, and phosphorous.

INTRODUCTION

Ayurveda and Ayurvedic remedies are based on Indian medicinal plants. When used properly and in accordance with the fundamental principles, they create miraculous results. Their duty is not limited to only curing diseases; they also make use of the human body. As a result, Ayurvedic medicines are appropriately referred to as "life elixirs." From ancient times to the present, Ayurvedic herbs have played an important part in Ayurvedic medicine. Humans have relied on nature for their basic requirements, such as food, housing, clothing, transportation, fertilizers, flavors and scents, and medicines, throughout history [1]. Plants with medicinal properties have long been a primary source of therapeutic substances for the relief and complete treatment of many human ailments. Medicinal plants are widely used by all parts of the population in India, either directly as folk cures, in various indigenous systems of medicine, or indirectly in the pharmaceutical manufacturing of contemporary

pharmaceuticals. The industry based on medicinal plants is increasing at a pace of 7.15 percent each year. India, as a rich natural resource reservoir, has enormous potential to grab global markets for medicinal and aromatic plants and their derivatives [2].

In India alone, more than 200 distinct plants are employed for medicinal formulations for both internal and exterior usage, according to national health specialists [3]. *Cissus quadrangularis* L. is a member of the vitaceae family and an indigenous medicinal plant of India; it is known as "asthisnghara" in Sanskrit, which means "to strengthen the bones." This plant has been used safely by countries. One that heals bones and joints, provides pain relief without side effects, aids in the healing of overuse injuries, aids in the resolution of gastrointestinal issues such as ulcers or acid reflux, is high in antioxidants and vitamins, is a real plant that has been used and recorded in ancient Ayurvedic texts, and has been proven by modern medicine to be completely safe. *Cissus quadrangularis* L. plant extract is similar to several other similar products. A large number of phytonutrients work together synergistically to provide an effect that is considerably bigger than the sum of their parts. Tendril shoots and young leaves are utilized in a variety of dishes. Scurvy is reported to be cured by drinking the plant's juice. Vitamin C, carotene, and anabolic steroid compounds are abundant in the plant.

As an asthelmintic, antibacterial, digestive tonic, analgesic, and cure for scurvy and asthma, the plant has been utilized for centuries. Hormone replacement medication in postmenopausal women has been found in numerous studies to increase the risk of breast cancer and heart disease, and many women are exploring for alternatives to oestrogen to help prevent osteoporosis. Despite the fact that there appears to be no published studies proving that *Cissus* enhances bone density in osteoporosis or helps prevent the condition, the herb's ability to expedite fracture repair suggests that it may also increase bone density. *Cissus* is also high in the antioxidants vitamin C and beta-carotene, in addition to the above-mentioned qualities. In addition to calcium oxalate, *Cissus quadrangularis* contained 479mg of ascorbic acid and 267 units of carotene per 100g of freshly made paste [4]. *Cissus* also has qualities that are comparable to aspirin or anti-inflammatory medications like ibuprofen on a mg basis.

Constituents of *Cissus guadrangularis* are one of the ingredients in the Ayurvedic preparation "Laksha Gogglu," which has been shown to be highly effective in relieving pain, reducing swelling, and promoting the healing process of simple fractures as well as allied disorders associated with fractures. The mechanism through which Cissus obtains its analgesic and anti-inflammatory effects is not well understood. It may work centrally, but it prevents arachidonic acid from being converted to inflammatory prostaglandins. It is one of the most extensively used components in Ayurvedic medicine for the treatment of piles, anorexia, indigestion, chronic ulcers, asthma, wounds, and fracture healing. The herb is used to cure haemorrhoids, anorexia, indigestion, and asthma in traditional medicine [5]. In many parts of the world, traditional medicine relies on the usage of a broad array of plant species, including Africa.

Phototherapy continues to serve a significant role in the treatment of diseases, particularly among the poor. *Cissus quadrangularis* Linn (Vitaceae) is a plant that thrives in Africa's Savannah areas and is native to India and Malaysia (Cameroon, Mali, Mauritania, Senegal, Somalia and Chad*). C. quadrangularis* is utilized in the treatment of Sickle Cell Anemia, Syphilis, and Gonorrhea in sahelian locations. As an analgesic used for fractures, colds, aches, malaria, abscess, and asthma [6]. The plant is also utilized in the treatment of epilepsy in Cameroon (Personal communications). Chemical analysis has revealed that *C. quadrangularis* contains sterols, steroids, tannins, flavonoids, carotenes, ascorbic acid, and linoleic acid [7]. Biologically active chemicals derived from natural sources have always piqued the curiosity of infectious disease researchers. In India, common people utilize *Cissus quadrangularis* to speed up the healing of fractures. *Cissus quadrangularis* has a high concentration of ascorbic acid, carotene, phytosterol substances, and calcium, according to phytochemical tests [8]. Shirwaikar et al. [9] investigated the antiosteoporotic activity of *Cissus quadrangularis* Linn. Ethanol extract on ovariectomized rats. Medicinal plants have long been employed in various traditional systems because to their immunity to a variety of ailments [10]. For thousands of years and in many parts of the world, medicinal plants have been utilized as traditional therapies for a variety of human ailments. More than 30% of all plant species have been used for medical reasons at one time or another. Herbal goods are now associated with safety, in contrast to synthetics, which are seen to be harmful to

humans and the environment. Medical plants are crucial in the treatment of diseases in impoverished nations with limited resources. Herbal medicine is founded on the idea that plants contain natural compounds that can help people stay healthy and get better [11, 12]. Alkaloids, flavonoids, tannins, and phenolic chemicals are the most important physiologically active elements of plants.

Magnoliopsida *Cissus quadrangularis* is an old medicinal plant endemic to Ceylon and India's hotter regions. It has been utilized by common people in India to aid in the healing of fractures. It was used as a general tonic and analgesic in ancient Ayurvedic writings, with specific bone fracture mending effects. This review covers the botanical description, phytochemistry, Osteoblastogenesis activity, and molecular healing pathways in osteoporosis [13]. Metal contaminant remediation is a new discipline in the broad field of environmental bio-ago-technology. Plant-based medicines have traditionally played an important role in traditional medicine around the world. The growing popularity of herbal medications in recent years, owing to their fewer adverse effects when compared to synthetic treatments and antibiotics, has underlined the need of medicinal plant conservation and growth. A plant-based diet has numerous health benefits. Many life-threatening disease processes can be prevented, slowed, or even reversed using it [14]. On the basis of its general hardiness, disease resistance, high yield, potential, and capacity to grow on a wide range of natural and cheap artificial diets, *Oreochromis mossambicus* has a lot of advantages as a culture species [43]. It can also survive low oxygen levels, overcrowding, severe environmental circumstances, and a wide range of salinities while still producing highly satisfactory flesh [15].

Tilapias are the second most frequently farmed fresh water fish in the world, trailing only carps [16]. Biochemical studies of fish tissue are of great interest because of their specificity in connection to the fish's dietary values and the assessment of their physiological needs at various stages of life. Proteins are mostly absorbed by fish in their muscles. Fish protein has a strong biological and growth-promoting value due to its comparatively high digestion. Fish tissue is of particular importance because of its specificity in connection to the fish's dietary values and for the assessment of their physiological needs at various stages of life. Proteins are mostly absorbed by fish in their muscles. Fish protein has a strong biological and growth-promoting value due to its comparatively high digestion. In terms of nutrition, fish is a high-protein, high-quality fat and micronutrient-dense food [17]. Total protein, calcium, and phosphorus constituent biochemical analysis varies substantially between animals and individuals, depending on age, sex, environment, and season [17, 18].

Kor (1995) claims that fish biochemical composition is directly linked to feed intake, migratory, and sexual changes during spawning. Calcium and phosphorus are required for proper bone formation, with more of both minerals needed during childhood and adolescence to avoid rickets and osteomalacia [19] [20]. Free amino acids could also be used as a precursor for energy generation and the synthesis of necessary proteins in response to stress. The analysis of protein and total amino acid content can be used as a diagnostic tool to evaluate the cell's physiological phase. The most important and plentiful biochemical ingredient in an animal's body is protein. Proteins play a crucial role in all biological processes [21]. Amino acids and protein are essential nutrients. Protein aids in the detoxification of toxicants that enter the animal body by assisting in the creation of micros anal detoxifying enzymes [22]. The *Cissus quadrangularis* plant extract was examined in biochemical investigations on *Oreochromis mossambicus* (gill, liver, and muscle). The acclimatization periods were during 7, 14, and 21 days of treatment.

MATERIALS AND METHODS

Procurement and rearing of experimental fishes,
Oreochromis mossambicus commonly called African Mouth breeder is widely distributed in the freshwater of India. Even though the fish O. *mossambicus* is a freshwater fish but they can able withstand estuarine environment. The fish *Oreochromis mossambicus* ranging in lengt 10-18 ± 1.5 cms in total length and 40 –70 ± 6.25 grams in weight were collected using hand nets from Gadilam river, Devnampattinam village, Cuddalore, Tamil Nadu, India The collected fishes without any least disturbance were transported in polythene bags filled half with water and were stocked up

in 500 litre capacity tank having dechlorinated tap water. The fishes were acclimatized for 15 days. This mode of transit proved successful, since there was no mortality in all consignments throughout the course of this study. The fishes were fed twice daily with prepared fish feed pellets.

Collection of Cissus *quadrangularis*

The plant *Cissus quadrangularis* was freshly collected from the rural area nearer to Periyapet village, Cuddalore district sufficient quantity in a sterile polythene cover and transported to the laboratory. Then chopped to pieces and crushed to paste with required amount of water. The paste weighed to 200grams and packed in polythene cover stored in the refrigerator for further use.

Preparation of Fish Feed,

For the present study, two different types of feeds were prepared following Hardy's Square Method [23]. Control feed - Feed 1(Table.1). *Cissus quadrangularis* plant extract mixed feed - Feed 2. The control feed was a standard based diet. It was prepared as a mixture of Rice bran (6.30%), Tapioca flour (21.30%), Groundnut oil cake (13.50%), Wheat flour (15.60%), Corn flour (15.20%), Rice pori (4.20%), Soya meal (22.70%), Vitamin and mineral mix (1.10%). The proximate composition of all the dried, powdered ingredients was analyzed according to AOAC procedures (1990). Feed pellets were prepared by following the methods of Bindu and Sobha [24]. Weighed portions of finely powdered components were added to a large mixing bowl and carefully combined. In a pressure cooker, the dough was cooked stem to tail for 30 minutes. The dough was allowed to cool before being enriched with a 1% vitamin and mineral mix nd palletized with a hand pelletizer. The pellets were extruded and dried in a hot air oven at 60 degrees Celsius overnight. Standard methodologies were used to conduct a proximate analysis of diets (AOAC, 1990) [25].

Plant extracts preparation,

Four different polarities of solvents were used to extract the plant material. Starting with Hexane (Polarity index 0), 50ml Chloroform (Polarity index 4.1), 50ml Ethyl Acetate (Polarity index 4.4), and 50ml Methanol (Polarity index 5.1) were added, followed by 250ml distilled water (Fig.1). One plant material (100g) was extracted consecutively for 24 hours on a shaker at 200 rpm with a 1:1:1 solvent to dry weight ratio. After that, the extracts were filtered through a Whatman filter paper, and the filtrate was collected in glass beakers. For best effectiveness, the plant material was re-extracted numerous times. The filtrates were vacuum-dried and the solvents recovered were used for extraction in a Rotor Vapor Aspirator. The extracts were kept in vacuum desiccators to dry out even more. They were then kept at 4°C in sealed containers for future analysis.

Experimental Procedure

Oreochromis mossambicus weighing 40-55gms were divided in to 3 groups and stocked at random into 3 different concrete tanks each tank was assigned a specific type of feed. The following experimental groups were conducted in the freshwater fish *Oreochromis mossambicus* for the period of 7, 14 and 21 days.

Group I	Fish exposed t tap water and fed with feed -I (Control feed1)
Group II	Fish exposed to tap water and fed with feed 2 (*Cissus quadrangularis* extract mixed for 7 days
Group III	Fish exposed to tap water and fed with feed 2 (*Cissus quadrangularis* extract mixed for 14 days
Group IV	Fish exposed to tap water and fed with feed 2 (*Cissus quadrangularis* extract mixed for 21 days

Experimental design,

The experiment was design to elucidate the effect of the *Cissus quadrangularis* plant extract on the tissues(gills, liver and muscles) of fish *Oreochromis mossambicus* in their biochemical changes in normal and plant extract treated 7, 14 and 21 days of treatment. LC 50 values are not calculated because the plant extract is not a toxic material to living beings but promotes the physiological efficiency in various ways of the organisms. *Cissus quadrangularis* aqueous plant extracts for the fish *Oreochromis mossambicus* (batch of 10 fishes) after 96 hours in semi static system was 500ppm to see the after the completion of 21 days.

The fishes were active throughout the period of study. The fish were transported to the lab and acclimatized for a fortnight in a fiber fish tank before being utilized in the experiment. By rinsing the fish tanks with potassium permanganate solution, the fungal infection was held at bay. The fish were disinfected with a 0.1 percent potassium permanganate solution and kept in well-aerated tap water for three weeks. Stress, bodily harm, and mortality are all put to the test. Individuals who were damaged, terribly diseased, abnormal, or deceased were dumped. To limit the cumulative effects of animal excreta in the test trough, feeding was stopped two days before to the start of the trials. For 7, 14, and 21 days, the fish were exposed to plant extract and a control. A control group was kept in the same conditions as the experimental group. Every day, the *Cissus quadrangularis* plant extract with water and regular water was replaced. The fish in both the experimental and control groups were slaughtered after 7, 14, and 21 days of exposure. The fish remained active throughout the research time.

Biochemical studies
Estimation of Tissue Protein
The protein content of the tissues was calculated using the Lowry et al. method [26]. The tissues (200mg gills, liver, and muscles) were separated, and a 20% homogenate was centrifuged for 15 minutes at 3,000 rpm. The supernatant was discarded, and the residue was suspended in 1.0 mL sodium hydroxide solution at 0.1 N. 4 ml copper carbonate solution was added to 0.5 ml of this solution (equal to 10 mg of tissue) in a clean test tube. By lateral shaking, the ingredients were combined, and 0.4ml of folin phenol (1:1 dilution) reagent was added. For 3 minutes, the properly combined components were maintained at room temperature. In a UV visible spectrophotometer, the colour generated was measured at 600 nm against a reagent blank (Jusco Model-650). The standard graph was made with bovine serum albumin (Sigma Chemical Co.). The tissue protein content was measured in mg/dg wet weight of tissues.

Estimation of Calcium
Estimation of calcium was done with Flame photometer model PFP7 as described by Bibby scientific method. The tissues of normal and plant extract treated (200 mg of gills, liver and muscles) were isolated and 20% homogenate was centrifuged at 3,000 rpm for 15 minutes. 5 ml of the homogenate was added with 5 ml of 0.1M oxalic acid and 95ml of 0.1M ammonium oxalate. The mixture was aspirated in the flame photometer. The intensity was noted for every sample with absorbance. The same method was adopted the known standard solution for the preparation of calcium standard curve. The quantity of calcium for the samples calculated from the calcium standard curve.

Estimation of Phosphorus
Estimation phosphorus was carried out by adopting molybdate method [27]. The tissues of normal and plant extract treated (200 mgs of gills, liver and muscles) were isolated and 20% homogenate was centrifuged at 3,000 rpm for 15 minutes. Equal volume of ammonium molybdate and the homogenate was taken and made as a monoreagent. This monoreagent was stable for 8 weeks. So it was stored at 2-8°C. Monoreagent was prepared for the tissues of normal and plant extract treated gills, liver and muscles of Oreochromis mossambicus. Monoreagent was added with 1 ml of sulphuric acid. Pink to light brown colour was developed in the solution depends upon the amount of phosphorus present in the samples. The intensity of colour was read out in the UV visible spectrophotometer (Jusco Model-650) with 340 nm wave length against the reagent blank and the Normal samples. The amount of phosphorus (mg/dg) was calculated by using the following formula

$$\text{Amount of phosphorus mg/dg} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of standard}} \times 5$$

Statistical Analysis
The values are expressed as mean SE. Data were statistically analyzed by Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT) [28] which was applied to find out significant difference between various treatment means and control means for the observed parameters.

RESULTS

Oreochromis mossambicus was treated with the plant extract of *Cissus quadrangularis* to study about the changes in the biochemical aspects of gills, liver and muscles. Experiments and results were conducted and recorded respectively.

Biochemical changes
Total Protein
The quantity of total protein was determined in the normal and plant extract treated gill, liver and muscle of *Oreochromis mossambicus* and tabulated (Table.2). The level of total protein shows that the muscle contains more than the liver in the normal and plant extract treated fishes. There is a gradual increase of total protein noticed in all these tissues from normal to plant extract treated fishes (Fig. 2)

Calcium
The quantity of calcium was determined in the normal and plant extract treated gill, liver and muscle of *Oreochromis mossambicus* and tabulated (Table. 3). Higher level of calcium was noticed in the normal gill (3.5 $\pm$ 0.12 mgs/dg) and gradually increased in the gills of plant extract treated fishes. The accumulation of more calcium (6.1 $\pm$ 0.07 mgs/dg) was observed in the muscles of plant extract treated fishes. Overall the quantity of calcium shows progressively increased in the gill, liver and muscles from normal to plant extracted treated fishes (Fig. 3)

Phosphorus
The quantity of phosphorus was estimated in the normal and plant extract treated gills, liver and muscles of *Oreochromis mossambicus* and tabulated (Table. 4). Phosphorus level shows fluctuations in the liver and gradually decreased in the plant extract treated fishes. The accumulation of more phosphorus (3.6 $\pm$ 0.07 mgs/dg) was observed in the gills of plant extract treated fishes. Accretion of phosphorus was noticed in the normal gills and muscles to gills and muscles of plant extracted treated fishes (Fig. 4)

DISCUSSION

Tilapia (*Oreochromis mossambicus Peters*) is a salt-tolerant, mouth-brooding cichlid native to Africa with enormous aquaculture potential. *Cissus quadrangularis L.* belongs to the vitaceae family and is a native medicinal plant of India. It is known in Sanskrit as "asthisnghara," which means "to strengthen the bones." The goal of this study was to see how the *Cissus quadrangularis* plant extract affected the biochemical changes in the tissues (gills, liver, and muscles) of the fish *Oreochromis mossambicus* in normal, 7, 14, and 21 days of therapy.

Total protein
When compared to normal fish total protein content in the gills, liver, and muscles was found to be higher in *C. quadrangularis* plant extract treated (21 days) fish. The osmolarity of the blood and renal impairments are shown by the quantitative determination of total serum protein, which indicates the liver's capability for protein synthesis and denotes the osmolarity of the blood. As a result, serum protein could be used as a biomarker to detect any harmful alterations in fish. Citarasu (2010)[29] stated that the active principles of *C. quadrangularis* may be responsible for the increase in protein content in the gills, liver, and muscles of *O. mossamibicus* using *P. niruri* extracts with fish.

Calcium
Calcium is important for bone formation and fish is known to be a good source of this mineral, especially small fish[30,31,32].Several studies reveals about the calcium deposition, accumulation and physiological action in the fishes [33,34,35,36,37]. Minerals such as Ca and P are closely related to metabolism especially in bone formation and the maintenance of acid-base equilibrium in fish. Almost the entire store of calcium (99%) and most of the phosphorus (80%) in the body of are in the form of bones, teeth and scales. The remaining small portions are widely

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distributed throughout the organ and tissues. Moreover, it has been established that the fish derived inorganic elements such as Ca and P from the surrounding water as well as from diets. Some minerals are easily absorbed by fish from environmental water [38].

In the present study, the values of calcium in the gills of the normal, *C. quadrangularis* plant extract treated 7, 14 and 21 days *O. mossamibicus* were 3.5 ± 0.12, 3.7 ± 0.19, 4.3 ± 0.11 and 5.8 ± 0.14 mg/dg respectively. The values of calcium in the liver of the normal, *C. quadrangularis* plant extract treated 7, 14 and 21 days *O. mossamibicus* were 3.4 ± 0.21, 3.6 ± 0.18, 3.8 ± 0.13 and 4.2 ± 0.22 mg/dg respectively. The values of calcium in the muscles of the normal, *C. quadrangularis* plant extract treated 7, 14 and 21 days *O. mossamibicus* were 3.1 ± 0.06, 4.1 ± 0.04, 5.3 ± 0.05 and 6.1 ± 0.07 mg/dg respectively. Thilsted et al. reported Ca content of 1.06 to 1.26% in 5 minor carps [39]. Ogino and Takeda found that Ca content of rainbow trout in different experimental group was 0.81-0.82% which is similar to the values obtained in the present study [40]. The result shows that the plant extract treated gills; liver and muscles gradual accumulation calcium were due to the effect of plant extract. Accumulation of calcium was more in the muscles compared to gills and liver. This may be clearly implies that the accumulated calcium in the muscles used muscular contraction later passed to bones. It is obvious that the calcium level in muscles and gills.

Phosphorus

The phosphorus content in the present study ranged between 2.0 ± 0.01 and 3.6 ± 0.07 mg/dg in gills, 2.1 ± 0.11 and 1.7 ± 0.08 mg/dg in liver, 1.9 ± 0.06 and 3.3 ± 0.10 mg/dg in muscles. The highest value in gills (3.6 ± 0.07 mg/dg) was obtained in 21 days plant extracted treated *O. mossamibicus.* CSIR reported phosphorus content of 1.20% in sharpunti, Puntius sarana [41]. Yone and Toshima reported that phosphorus content of vertebrae of carp fish was 1.90 ± 0.06% which is more or less similar to the values obtained with *O.mossamibicus* in the present study [42]. Moreover the phosphate content in the liver of plant extracted treated *O.mossamibicus* was decreased from the normal. It may be due to release of phosphate by the active principle of *Cissus quadrangularis.*

CONCLUSIONS

*Cissus quadrangularis b*elongs to the family vitaceae and is an indigenous medicinal plant of India. *Oreochromis mossambicus Peters,* commonly called as tilapia is a salt-tolerant fish. The total protein content in the gills, liver and muscles were noticed that increased level in *C. quadrangularis* plant extract treated fishes. The plant extract treated gills; liver and muscles gradual accumulation calcium were due to the effect of plant extract. Accumulation of calcium was more in the muscles compared to gills and liver. This may be clearly implies that the accumulated calcium in the muscles used muscular contraction later passed to bones. It is obvious that the calcium level in muscles and gills. The phosphate content in the liver of plant extracted treated *O.mossamibicus* was decreased from the normal. It may be due to release of phosphate by the active principle of *Cissusquadrangularis*. From these biochemical findings it is concluded that the plant extract promotes the growth of the fish *O.mossamibicus.*

ACKNOWLEDGEMENT

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval
This article does not contain any studies with human volunteers or animals being involved by any of the authors.

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Informed consent

None

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Table: 1 Proportion of ingredients and proximate composition of the feed percentage composition (g).

Feed ingredients	Quantity (100 g)	Quantity (200 g)
Groundnut oil cake	13.500	27.00
Tapioca flour	21.300	42.60
Wheat flour	15.600	31.200
Corn flour	15.200	30.400
Rice bran	6.300	12.600
Soya Meal	22.700	45.400
Rice Pori	4.200	8.400
Vitamins/ minerals (Riboflavinin micrograms)	1.100	2.200

Table: 2 Quantity of total protein (mgs/dg) in the gills, liver and muscles of normal and plant extract treated fish *Oreochromis mossambicus*

Tissues	Normal	7 days	14 days	21 days
GILL	63.0 $\pm$ 2.27	63.4 $\pm$ 2.32	64.2 $\pm$ 2.11	65.8 $\pm$ 2.21
LIVER	43.4 $\pm$ 1.09	43.6 $\pm$ 0.10	43.8 $\pm$ 1.08	44.2 $\pm$ 1.06
MUSCLE	83.6 $\pm$ 2.31	84.0 $\pm$ 2.42	85.6 $\pm$ 2.12	86.4 $\pm$ 2.22

Table: 3 Quantity of calcium (mgs/dg) in the gills, liver and muscles of normal and plant extract treated fish *Oreochromis mossambicus*

Tissues	Normal	7 days	14 days	21 days
GILL	3.5 $\pm$ 0.12	3.7 $\pm$ 0.19	4.3 $\pm$ 0.11	5.8 $\pm$ 0.14
LIVER	3.4 $\pm$ 0.21	3.6 $\pm$ 0.18	3.8 $\pm$ 0.13	4.2 $\pm$ 0.22
MUSCLE	3.1 $\pm$ 0.06	4.1 $\pm$ 0.04	5.3 $\pm$ 0.05	6.1 $\pm$ 0.07

Table: 4 Quantity of phosphorus (mgs/dg) in the gills, liver and muscles of normal and plant extract treated fish *Oreochromis mossambicus*

Tissues	Normal	7 days	14 days	21 days
GILL	2.0 $\pm$ 0.01	2.3 $\pm$ 0.04	2.8 $\pm$ 0.06	3.6 $\pm$ 0.07
LIVER	2.1 $\pm$ 0.11	2.0 $\pm$ 0.08	1.8 $\pm$ 0.11	1.7 $\pm$ 0.08
MUSCLE	1.9 $\pm$ 0.06	2.4 $\pm$ 0.02	3.1 $\pm$ 0.04	3.3 $\pm$ 0.10

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Fig.1. Schematic diagram of Plant extracts preparation of fish food.	**Fig.2. Quantity fluctuations of total protein in the gills, liver and muscles of normal and plant extract treated fish *Oreochromis mossambicus***
Fig.3. Quantity fluctuations of calcium (mgs/dg) in the gills, liver and muscles of normal and plant extract treated fish *Oreochromis mossambicus*	**Fig: 4 Quantity fluctuations of phosphorus (mgs/dg) in the gills, liver and muscles of normal and plant extract treated fish Oreochromis mossambicus**
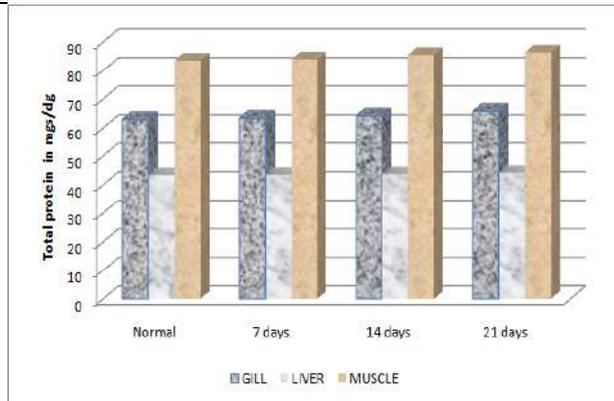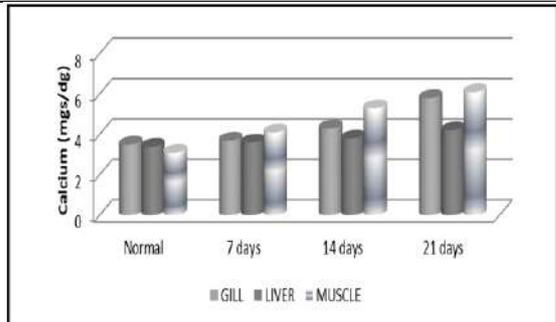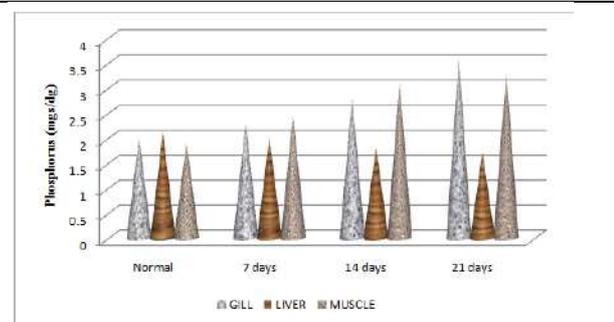
REVIEW ARTICLE

Characterization of High and Low Molecular Weight Glutenin Subunits in Wheat using DNA Markers: An Overview

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ABSTRACT

Grains are an important element of human diet, consumed by foremost world's population, and grains provide significant amounts of protein and calories to help maintain normal body function. While rice and maize dominate the world's grain production, wheat is an important human crop, accounting for about 20% of our energy (calorie) needs and 25% of our dietary protein. Wheat is utilized to produce different consumable products which include bread, cookies, macaroni, pasta, spaghetti, cakes, pizza, chapatti, etc. However, the grade of product is correlated with protein content. Embryonic wheat proteins are categorized into gluten and non-gluten proteins. These proteins are sub-categorized into four different groups which include albumin, globulin, gliad in and gluten in . Considering solubility of protein in acid or alcohol, it is categorized into gluten in which is a polymeric protein which is further subdivided into HMW (high molecular weight gluten in) and LMW (low molecular weight gluten in) on basis of molecular weight distribution, and gliadin which is a monomeric protein and is further subdivided into $\alpha, \beta, \gamma, \omega$ gliadin on the basis of electrophoretic mobility. Non-gluten protein includes albumin and Globulin. Molecular markers are molecules that are utilized to screen characteristic of repetitive source. In-plant breeding, three types of molecular markers are being used viz biochemical, morphological, and DNA molecular markers each of them is having its role in plant breeding. Different researchers use different markers to identify the quality characteristics of different wheat varieties.

Keywords: Wheat, Protein, Grain hardness, HMW-GS, LMW-GS, Molecular markers, SSR, SNP.

INTRODUCTION

Wheat is found in cereals, which are a key part of the human diet consumed by the majority of the world's population. Cereals contain a considerable amount of proteins and calories that aid in the maintenance of normal body processes [1]. Wheat accounts for 20% of human energy needs (calories) and 25% of the protein diet of humans. Wheat belongs to Gramineae family, first observed in Middle East in the Levant and the Ethiopian Highlands, but currently it is cultivated all around the human population [2]. Wheat was first cultivated around ten thousand years ago, in the neolithic revolution phase, human life style has been changed from gathering food by hunting to active farming and agriculture. Early varieties that were cultivated were diploid and tetraploid, and their genetic analysis revealed they were originated from South-Eastern Turkey [2, 3].

Wheat is a self-pollinating polypoid crop with high nutritional content that is used as a cereal. Wheat is considered as an essential source of energy, also contributes to the major protein and fiber content in human [4]. Wheat accounts for around 0.2 percent of the entire calorific intake of the world's people, demonstrating how significant wheat is in the food materials and baking shops or pastry shops, industries [5].Wheat quality improvement has been a significant task, and amount and composition of endosperm protein in wheat grains are strongly related to this property[6]. Wheat flour has a unique flour composition and viscoelastic properties that set it apart from other plants used in food production [7]. Wheat is regularly used around the world to make a variety of food products like noodles, pasta, bread, chapatti, cookies, and cakes [7,8]. Wheat-based foods have recently become more popular as a result of changing lifestyles and a growth in the urban population. As a result, the quality of the wheat end product has become critical. The end product's quality is an important and complex aspect of bread wheat [8]. Wheat's milling and dough qualities are two factors that decide if it is suited for a certain product. The balance and interplay of different floor components have an impact on proteins, carbohydrates, lipids, and water and affects wheat quality. Wheat quality refers to how good one product is as compared to similar products. This depends on various parameters like physical and chemical characteristics [9]. Physical characteristics are involved in influencing wheat grain quality and chemical influence in protein content.

Major factors that affect end product wheat quality are texture, starch, lipids, dietary fibres, seed storage proteins, and gluten in [10]. The texture of the grain is one of the most essential aspects that are involved in determining the milling and baking property of the wheat, as well as the finished product's application. Based on seed texture, wheat can be divided into four categories: hard, medium-hard, medium soft and soft [9]. Different milling qualities affect the texture of wheat end-product quality. Grains having a soft feel are easier to grind, resulting in flour with more intact starch granules. On the other hand, hard textured grains take longer to mill and produce flour with more damaged starch granules [11]. The hard-texture of wheat flour is mostly used for baking yeast-based breads. Soft flour wheat is preferred for baking cookies because it has less damaged starch, which gives the final product a crunchier texture. Major carbohydrate content of wheat grain is dominated by starch which contributes 65-75% of total carbohydrate in dry grain. Wheat starch's rheological qualities have an impact on dough properties.

Amyloplast deposit starch in granules during endosperm stage [12, 13]. On the basis of granule size granules are categorized in: A, B, and C type. Size of A-type granules are larger than B type and are deposited early in the seed development process [14]. C-type granules are the smallest and are deposited later in the seed development process. The ratio of amylose to amyl pectin varies between three types of granules, and their distribution influences the final product's quality [15]. Lipids make up roughly 1-2 percent of the overall weight of the floor. Interaction of lipids with starch and protein in granules affect the final product quality [16]. Lipid quantity present on the surface of starch granules as free fatty acids is related to grain texture, lipid content is less in hard wheat as compared to others [16, 17]. Polar and non-polar lipids can be distinguished as the polar fractions, which mostly consist of glycolipids and phospholipids, have been linked to beneficial effects on dough baking quality, whereas non polar lipids, which primarily consist of triglycerides, have been linked to negative effects on dough baking quality [18, 19]. In terms of both quality and nutrition, dietary fibres constitute a significant component of wheat. While dietary fibres provide

many health benefits to consumers, they also have an impact on wheat processing quality. Cell-wall consists of polysaccharides, such as arabinoxylan signified by (AX) otherwise as (1- 3) (1-4)—D-glucan, which make up the majority of wheat flour as dietary fibres. Both soluble and insoluble forms of arabinoxylan (AX) and (1-3)(1-4) D-glucan have been reported to alter bread quality and starch gluten separation. AX affects the flour's ability to absorb water as well as the dough's development time [2, 20]. D-Glucan, on the other hand, is linked to increased water absorption, increased viscosity, and decreased loaf volume.

The storage proteins in wheat seeds, as well as the texture, are the most essential components that influence the ultimate product's utilization [21]. Wheat seed storage proteins give dough viscoelasticity and extensibility, allows production of variety of wheat-based items including bread, pasta, noodles, cakes, and pastries. In embryos, two different forms of proteins are discovered namely gluten and non-gluten proteins. Gluten is a protein found in considerable amounts in the endosperm. Glutens consist of85% of the total bread wheat grain proteins [22]. These two embryonic wheat proteins were one of the first proteins to be isolated and studied in humans. Biologically it acts as a source of micronutrients carbon, nitrogen, and energy for seed germination and growth [23].The newly identified unique gluten protein whose structure is similar to gliadin has an antifungal activity in addition to its storage function. The first comprehensive study was conducted by Osborne in 1907.Osborne has developed a cereal seed protein classification system based on sequential extraction and solubility differences. Albumin iswater-solublehowever, globulin is water insoluble but saline soluble, gliadin is soluble in organic compounds like 70% to 90% ethanol, and glutenin is soluble in dilute acids and alkalis[7, 24].

Being such an important food crop the biggest challenge has been related to wheat quality, attempts have been made to increase wheat quality and grain yields, and to enhance grains quality for end products in order to feed increasing population; wheat quality is considered as one of the major areas of wheat breeding programs [7].One of the major advancements in the field of wheat crop breeding has been development of molecular markers; which includes biochemical, morphological markers and DNA markers. Though morphological and biochemical markers have wide application, it is very limited in number and less accurate as it is prone to change with environment. This creates a gap area which is filled by development of DNA based markers. DNA markers has gained interest because it offers precise and high throughput results for alleles before breeding, and helps in developing novel varieties that contains desirable traits. This review mainly focuses on the gluten protein and DNA based molecular markers used for enhancing wheat quality traits. Laterally with brief of gluten protein and its types.

Morphological and Biochemical Structure of Gluten Protein
Wheat embryonic proteins are classified into two types i.e., gluten and non-gluten proteins. On the basis of solubility in water, saline solution, and ethanol wheat seed proteins are categorized as albumin, globulin, and gluten [25]. They make up roughly 8-15 percent of total flour weight. Based on solubility in acid or alcohol solutions gluten are classified into gluten in and gliadin. Glutenin is a polymeric protein, it is further subdivided into HMW (high molecular weight) and LMW (low molecular weight) based on molecular weight distribution; and glia din is a monomeric protein, and is further subdivided into α, γ, ω gliadin based on the order of mobility on electrophoresis (Figure 1). While non-gluten protein includes albumin and globulin [26]. Glutenins and gliadins are the gluten proteins that are the most important determinants of baking quality among these fractions [27]. Gliadins and gluten ins are both involved in the synthesis of gluten polymer and responsible for end product quality. Gluten proteinsviscoelastic characteristics allow the dough to retain CO_2 during the baking process; as a result, the end products have a distinct texture [28]. Glutenins are polymeric proteins that give dough viscoelastic qualities, whereas gliadins are monomeric proteins that give dough extensibility.

There are 3classes of gliadin that can be structurally distinguishedi.e., α, γ, and ω. The distinction among those classes is primarily based on therange of cysteine residues present in chain. α includes 6 cysteine residues, γ includes 8 whereas ω lack cysteine residues[29]. The cysteine residue is exceedingly conserved and participates in disulfide formation. Structurally gliadin have domains of variable sizes. N terminalincludesamino acids ranging from 5-14 residues and the repetitive area includes 100 residues which can be structured in repetitive sequences of 1 or more

than one motif. Glutamine, proline and hydrophobic amino acids are major constituents of these motifs along with minor constituent phenylalanine or tyrosine. However, C terminal comprises of non-repetitive un its whichinclude polyglutamine and precise lysine or arginine which consist of sulphur-containing amino acids [30, 31]. Secondary structure of α and γ gliadin in cludesβ turns and α helixes in the repetitive area and non-repetitive area respectively that effects a compact globular shape. But in the case of ω gliadin, it includes β- sheets how ever few α helixes also present [30, 29]. Thereafter it's been shared, α gliadin has a compact and irregularshape, however γ gliadin has prolonged spiral tertiary structure. In the case of ω gliadin it adapts a stiff coil in place of a compact shape [30, 32]. The glutenin fraction is made up of polymers containing the HMWGS (high molecular weight glutenin subunit),LMWGS (low molecular weight glutenin subunit). SDS-PAGE can easily discriminate between glutenin subunits which differs in molecular weight. Interchain disulfide bonds helps in stabilization of bulky glutenin polymers [33]. Molecular weight of HMWGS is 70,000-90,000Da, while the molecular weight of LMWGS is 30,000-45,000Da[34]. Both HMWGS and LMWGS are used in the production of complex gluten polymers and have a significant impact on processing quality.

Subunits of glutenin may beacquiredvia means of treating gluten in with disulphide bond decreasing agent[30, 35]. The primary and secondary shape of LMW is like the α, γ gliadin as the LMW subunit additionally incorporate inter-chain double sulphide bonds via way of means of which they're forming glutenin polymers. LMW ischaracterized into B, C, and D classes on the basis of mobility on SDS page, and sub-classes of glutenin subunits can also be regarded as LMW-s, LMW- m, and LMW-l [30, 29]. HMW has subunits one with more molecular weight which is X-type and other low molecular weight Y-type(83000-88000,67000-74000 respectively). In durum wheat, the HMW glutenin incorporate4 to 6 subunits, however, in a few cases; there are frequently four due to a few gene silencing mechanisms. HMWGS isn'tfundamentalparticipants in deciding gluten elasticity, however, are a key issue in bread making [30].

X and YHMWGS have three region shaving N, C terminal. The length and the period of those domain vary as C-terminal areaincludeforty-two amino acids whereas N-terminal domain contains approx. 81-100 amino acids, however the central region contains 600 to 850 amino acids. Ends of HMWGS contains cysteine residues and N and C-terminal have secondary shape, particularly including α helix, however the central region is rich in glutamine, proline, and glycine, overall, the central region is designed in such a way it produces super secondary shape. HMWGS adopts an inflexible rod-like confirmation with the assistance of pertinent repetitive areas [30, 3]. In1985, Graceland proposed since LMWGS contains shape-enhancing disulphide bond and an accompanying lateral bond, explains that the lower backbone of the molecule is the simplest due to the presence of HMWGS. HMWGS and LMWGS were sequentially polymerized in lab conditions, which has tendency to form linear polymer[37]confirming cross-linking between HMWGS and LMWGS [30, 38]. According to the Gravel's version, and gradual reduction of glutenin macro polymers, and some immuno-cytochemical studies of GS localization, a branched version of the glutenin macropolymer was proposed and HMWGS occurs as a chain that forms a core structure which is attached with LMWGS [39,40]. The glutenin backbone contains the HMWGS chain and has a branch of LMWGS, which is consistent with Gravel, another version with backbone or branch containing LMWGS and HMWGS, respectively, has been described [30, 40]. Considering the charge ratio of HMWGS and LMWGS (about 1: 2) and the molecular weight ratio of subunits, the branching model is also subtle. Therefore, the basic molecular unit of the glutenin polymer may contain 6 HMWGS linked by interchain disulphide bonds and about 30 LMWGS. Major glutenin molecule is composed of abovethan 10 units [30, 37]. An important factor in determining polymer stability is intermoleculardisulphidebonds, but hydrogen bonding between HMWGS and within HMWGS are also significant [41, 42].

According to various studies, the HMWGS protein is an important determinant of gluten and has elastic properties that induce the synthesis of larger glutenin polymers, making it an important ingredient in bread preparation. The Glu1 loci GluA1, B1, and D1 are present on the chromosomes 1A, 1B, and 1D, and responsible for synthesis of these proteins. Each locus is linked to two genes which codes for subunits x and y HMW glutenin, respectively. Molecular weight of x subunit is comparativelymore than Y subunit. According to National Institute of Human Genome, Glu1A

has 3 allelic genotypes, Glu1B has 11 allelic genotypes, and Glu1D has 6 allelic genotypes [43]. The HMW glutenin subunit has 6 genes, but most hexaploid wheat varieties have only 35 HMW glutenin subunits for gene suppression. The HMW glutenin subunit is represented on the chromosome1A by both of the genome [44, 7]. 47-60% variability in bread quality can be explained by composition of HMW glutenin subunit only [45]. For large sample of wheat varieties, quality of pan-baked bread compared with the composition of the HMWGS which is further compared againstquality of each allele at the Glu1 locus. The GluD1 gene has the greatest effect on baking quality. Several research groups have discovered DNA markers for the HMW glutenin subunit in wheat [7].

Low molecular weight glutenin (LMWGS) has higher molecular weightas compared subunits of HMWGS and is prevalently found in the endosperm which after digestion carries nutrients to the embryo during seed germination [46]. LMWGS is regulated by genes present on short arms of chromosomes at distal end i.e., 1A, 1B, and 1D. GliA1, GliB1, and GliD1 are the three loci at the distal ends of at 1AS, 1BS, and 1DS. Other gliadins are encoded by three complex loci (GliA2, GliB2, and GliD2) on group 6 chromosomes. LMWGS have three N-terminal variants LMW-i, LMW-m and LMW-corresponding to amino acid is oleucine, methionine and serine residues, respectively [43]. With certain gliadin and LMW glutenin alleles, an essential relationship with dough extensibility was found [7]. Genetic control and its effect on gluten proteins and baking quality, especially in the case of LMWGS (as important as HMWGS in terms of baking quality)is still unknown [47]. Gliadin is a family of proteins which shares homologous sequences as gluten in.

DNA Markers for screening of HMW and LMW-GS in wheat

Molecular markers are molecules that identify the characteristics of repetitive sources. In-plant breeding, three types of molecular markers is used: biochemical, morphological, and DNA molecular markers, each of which plays a unique role in plant breeding. The most commonly used type of molecular marker is the DNA basic molecular marker and selected on the basis of sensitivity of PCR [7, 48, 49]. The first PCR-based DNA marker used for wheat polymorphism analysis was reported by Dovidio in 1990. PCR markers have the advantage of being able to recognize small sequence differences between alleles, which are sufficient for specific PCR markers development [7, 48, 50, 5]. Locus-specific primers were designed by Dovidio and Aderson, which was used to identifyIDy10, IDx5 genes. A PCR primer pair was developed for continuous amplification of the Y-type gene; these primers are effective in amplifying genes corresponding to diploid and tetraploid progenitor cell types [52]. Six dominant marker actions and applications were engrossed in amplifying the entire cryptographic region of the seven HMWGS genes (Ax2 *, Ax1, Axnull, Dx5, Dy10, Dx2, and Dy12) [53]. Various markers have been developed which targets completely different gluten alleles and markers for the GluB1 allele based primarily on the sequence diversity of the Bx-short gene[7, 54].In 2012 co-dominant DNA marker was discovered for Bx6 subunit that affects wheat bread quality.Zhang in2006 developed primers sequence to aim the Y-type gene sequence of HMW glutenin which is located at GluB1 locus and intended to DNA sequence mutation in the promoter region, ultimately captured PCR events to identify HMW bases by subunits [55]. Ragupathy in 2008,successfully develop the subunit marker of the glutenin gene. GluB1al crystal code isover-expressed Bx7 subunit and acts as a DNA marker [56]. Liu in 2008, developed an effective collection of three co-dominant markers suitable for the peak power in marker assisted selection of the HMW glutenin subunitwhich is coded at GluA1 and GluD1 loci [50]. Abdelin in2008 uses DNA markers to screen newly developed Saudi Arabian wheat varieties for the presence of the HMWGS gene. They utilized four versions of the Saudi Arabian line and two other versions (namely KSU 102, KSU 103, KSU 105, KSU 106, Yecora Rojo, and Westbrot) [57]. Ax2 * is used for the A genome, Bx7 and By8 are used for the B genome, and Dx2 / DDx5 and Dy10 / Dy12 are used for the D genome. After using the marker, Glu allele A1 had KUS103 and KUS106 varieties and Ax2 * did not exist, but GluD1 had Dy12,and as subunit KUS103 KUS105 contained Ax2 *, Dx5, Dy10. Rojo and western bread, all five genes were present. Espi in 2012, GluB1 locus damaging ASMC marker, 224 Chinese wheat varieties and other superior strains [58], 273 CIMMYT strains and other superior strains [59], and other 718 varieties of wheat and superior strains from 20 varied nation. 142 bread wheat varieties of varied nations are HMWGS [7, 60] (Table 1).

Cloning and sequencing of 100 collection tags of LMWGS-specific gene families, sub genes, and pseudo genes have already been done from many wheat varieties [7, 61, 62, 63]. Haiin2005 obtained 69 identified LMWGS gene

sequences from Gen Bank and clubbed into9 groups to generate incredibly conserved putative amino alkanoic acid sequences in the N-terminal region and nine analogs sustained. They tried to create a primer set specifically for the LMWGS foundation at the mounted site [64]. Ikeda in 2006, together created 10 laboratory accuracy markers that are consistent with the disclosed Ester collection [62]. LMW gluten inaccurate PCR markers were developed on the basis of polymorphic LMW genes to differentiate wheat varieties; microsatellite markers used for mapping hexagonal wheat [65].

Alleles specific accurate PCR markers sustain single nucleotide polymorphisms (SNPs) within the γ-gliadin gene Gli1 and then the exact alleles are associated with the GluA3, GluB3, and GluD3 LMW glutenin loci, respectively [7, 66]. Six LMWGS genes are characterized at the GluD3 locus in frame 1D, seven STS markers are assembled, and the corresponding sequence of wheat varieties contains five GluD3 alleles a, b, c, d, e. increase amplification [63]. Zhao [63] developed GluD3 and GluB3 genes-based STS markers. In addition, GluA3 and GluB3 LMWGS are thought to have major effect on processing of Triticum aestivum buyer's products. Completed by designing accurate 7-output and 10-output PCR primers targeting the LMSGS gene on the representative loci GLUA3 [67] and GluB3 [68]. Using a mixed approach i.e., PCR with conserved primers and high-resolution capillary electrophoresis, researchers developed substitute marker machine designed for determining the LMWGS gene of bread wheat family [69]. The marker machine is designed especially for high flip-out scoring of LMWGS gene to deck bread that works well in wheat breeding programs, 273 CIMMYT wheat varieties [7, 59], 182 Indian wheat varieties [7, 70] and 718 wheat verities including an increasing line has already been characterized. The use of accurate markers for LMWGS genes / allelic genes has been completed (Table 1).

In 2009, Dan Liang worked on 273 CMMVT bread wheat varieties and used gene-specific markers for the HMWGS and LMWGS genes to characterize quality-related genetic traits [71]. Of the 142 varieties, 90 varieties showed positive results for the Ax2 * locus and contained the 675 b p Bx17 subunit amplified using the marker Bx FP, 527 bp By8 specific primer ZSBY8F5R. Bx17 indicates the By9 subunit and was generated using the markers ZSBY9aF1 / R3. Bx20 was detected using the 800bp marker MAR. Translocations were also observed in the allele GluB3j.Also, the marker ZSBy8F5 / R5 separates By8 and By8 * [71]. Through this study, they know that the presence of translocations adversely affects quality parameters, but the Glu1 and Glu3 loci and their combinations are involved in viscoelastic properties and the combination of several alleles and it was concluded that it compensates for the adverse effects associated with translocation. Yildirim in 2011 [72], studied the wheat landrace of γ-gliadin and LMWG glutenin protein associated to pasta cooking and analyzed γ-gliadin and LMWG glutenin using DNA and protein markers together. This study used 13 SSRs linked to the GluB1 locus, 1 STS, and 2 GAG markers (Table 1). Gamma-gliadin and LMWG glutenin varieties were isolated using APAGE and SDS-PAGE techniques.

They worked on 28 races, 17 of which had γ-gliadin 45 and LMW2 glutenin protein. As a result, this study of marker use was able to effectively use DNA markers in conjunction with protein markers to identify the quality of wheat in the QTL or genetic region. Islam [2] used four SSR markers to study genetic diversity among 12 wheat genotypes (Table 1). 10out of the 12 genotypes detected to have an average of 22.5 alleles per locus, and similar observations were made by Salem et al. As reported in 2008, 27 alleles were found per locus with 3.2 alleles on average. Ali et al., 2013 [73], worked on 117 Pakistani wheat genotypes along with control varieties. They used 14 DNA markers to check presence of genes that control quality characteristics of Pakistani wheat varieties. The four DNA markers used were (Table 1), ω selectin, GluA3d, GluB3, and GluD1d. GluD1d produces 450bp fragments in 98% varieties. The fragment is associated with HMWGS encoded by GluD1d and has a beneficial effect on gluten starch. The GluA3d marker was 448bp and was present in 18% of varieties. It encodes the LMWG subunit and significant for the quality of the dough. GLUB3 is 636 bp, present in 65% of cultivars, associated with the GluB3 allele and non-1B-1R translocation, and GluB3 is LMWGS. The remaining 35% of varieties did not show the GLUB3 allele. 41 varieties show 636 bp and 1076 bp GluB3, ω select in. Chaudhary in 2016 [5], sought to confirm5% gliadin effect on selected soil nearby wheat varieties with different sweltering performances. The varieties they took differ in both physicochemical and HMWGS compositions. In their research, protein content was found to be major player for quality and quantity for end-product.

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All varieties have different protein contents and they also have different SDS sedimentation levels and gluten in/gliadin ratios. It can be seen that some cultivars have high protein content but low SDS sedimentation values, while other cultivars have high sedimentation and low protein content. This indicates that the dry strength property is related to the amount and type of HMWGS, which has also been observed in previous studies. Lama in 2018 [74], worked on 26 varieties that used biochemical and molecular markers (Table 1) to characterize the baking quality of Bangladeshi wheat varieties and disinfect the presence of whole grain proteins and genes corresponding to HMWGS by SDS PAGE and PCR. In 26 varieties, they received protein content in the range of 12-14%, suitable for making bread. The allele GluA1 allele Ax2 * that was present at her locus was frequently found, and four different alleles are present at the GluB1 locus (Bx7, Bx7 + By8, By7 + By9, and Bx17 + By18). 16 SNP markers for detecting GluA3 and GluB3 loci using the Bangladeshi varieties Dx5 + Dy10 and AX2 *, Bx7 + By8, Dx5 + Dy10.GluA3b, GLUA3e are easy to detect, but GLUA3e, GluA3d, GluA3f are not easy to detect, but the alleles GluB3 loci Glub3d, GluB3h, and GluB3i are the worst, respectively, on SDS-PAGE. The LMWGS band GluB3h was detected in the allele GLUB3 c or i. To identify GluA3, they used 7 SNP markers and 19 STP markers for the GluB3 allele. The fragments they saw after amplification were the same size as previously identified [67, 68].

CONCLUSION

Wheat grants an important influence on the nutrient supply on human eating regimen it additionally presents uncooked fabric to diverse industries to make distinct varieties of meals materials [78]. As wheat is having visco-elastic belongings that makes it greater appropriate for meals merchandise, however, the best cease product is decided with the aid of using different factors like lipid, protein0, starch, water, etc [79]. Among them, maximum essential is a protein that decides dough best of cease merchandise of wheat. Wheat proteins are categorized into Gluten and non-gluten proteins and their characteristics are to offer carbon-nitrogen supply for seed germination [23]. These are in addition divided into HMW-GS and LMW-GS on basis of electrophoretic mobility [26]. Extensive research had been carried out to recognize the mechanism at the back of the gluten on wheat and cease product best. The HMW-GS glutenin percentage has the very best effect on dough best modern studies have made clean-to-go-looking gluten genes for wheat best improvement. Based on ton studies in the wheat genomic era, new HMW-GS and LMW-GS were recognized from wheat relatives. Different biotechnological processes have been carried out in breeding for coming near applicable developments decreasing the time to provide cultivars with advanced characteristics [80].

DNA markers play a critical position in breeding programs. PCR method is used for easy developments that require a small quantity of DNA and assist in screening a huge wide variety of varieties. We are capable of cast-off alleles which gave us the pleasant evaluation to discover and display a huge wide variety of markers at a totally low cost. The improvement with in side the DNA base marker machine has revolutionized the rural science, but positive demanding situations are nevertheless there like infection in PCR response and identity of 3 alleles in un married response isn't always a great deal sturdy as SDS-PAGE. The wide variety of DNA markers probably to be had is extraordinarily high, with, for example, numerous million SNPs having been determined via genome sequencing tasks in humans[48]. In conclusion, the DNA markers are used for searching the best varieties of wheat and has great importance in wheat breeding programs for screening of wheat verities that are then useful to mankind. These verities are considered as best because of the complex nature and they have impact on the surroundings because of their overall performance.

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Table 1. Molecular markers overview table

Locus	Allele	Primer Sequence	Reference
Glu-A1	Ax2*	F:ATGACTAAGCGGTTGGTTCTT R:ACCTTGCTCCCCTTGTCTTT	[54]
	Ax1	F:ATGACTAAGCGGTTGGTTCTT R:ACCTTGCTCCCCTTGTCCTG	[75]
	Axnull	F:ACGTTCCCCTACAGGTACTA R:TATCACTGGCTAGCCGACAA	[75]
Glu-B1	BX7	F:ATGGCTAAGCGCCTGGTCCT R:TGCCTGGTCGACAATGCGTCGCTG	[75]
	Bx6	F:CGCAACAGCCAGGACAATT R:AGAGTTCTATCACTGCCTGGT	[54]
	Bx17	F:CGCAACAGCCAGGACAATT R:AGAGTTCTATCACTGCCTGGT	[54]
	Bx7	F:CGCAACAGCCAGGACAATT R:AGAGTTCTATCACTGCCTGGT	[54]
	Bx7^{OE}	F:CCTTGTCTTGTTTGTTGCC R:CCACTTCCAAGGTGGGACTA	[54]
Glu-D1	DX2	F:GCCTAGCAACCTTCACAATC R:GAAACCTGCTGCGGACAAG	[75]

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	DX5	F:GCCTAGCAACCTTCACAATC R:GAAACCTGCTGCGGACAAG	[75]
	DY10	F:GTTGGCCGGTCGGCTGCCATG R:TGGAGAAGTTGGATAGTACC	[70]
	DY12	F:GTTGGCCGGTCGGCTGCCATG R:TGGAGAAGTTGGATAGTACC	[70]
Glu-A3	GluA3d	F: TTCAGATGCAGCCAAACAA R:TGGGGTTGGGAGACACATA	[76]
	GluA3e	F:AAACAGAATTATTAAAGCCGG R:GGCACAGACGAGGAAGGTT	[76]
	GluA3f	F:AAACAGAATTATTAAAGCCGG R:GCTGCTGCTGCTGTGTAAA	[76]
	GluA3g	F:AAACAGAATTATTAAAGCCGG R:AAACAACGGTGATCCAACTAA	[76]
	GluA3ac	F:AAACAGAATTATTAAAGCCGG R:GTGGCTGTTGTGAAAACGA	[76]
ω-secalin		F:ACCTTCCTCATCTTTGTCCT R:CCGATGCCTATACCACTACT	[77]
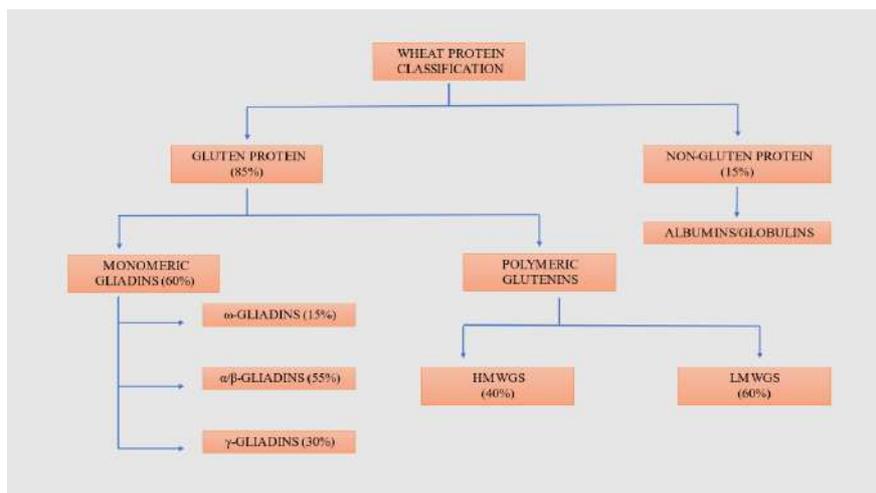
Figure 1: Classification of wheat protein

RESEARCH ARTICLE

Analysis of Prevalence and Associated Factors of Female Athlete Triad (FAT) Syndrome among Sedentary College Students in Chennai – A Single Centered Study

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ABSTRACT

To analyze the prevalence and associated factors of the female athlete triad (low energy availability, menstrual dysfunction, and low bone mineral density) among sedentary college students in chennai. To screen the female college students using the Low Energy Availability in Female-Questionnaire for the female athlete triad syndrome. This is a single-centred prospective cross sectional study conductedat Faculty of Physiotherapy, Meenakshi Academy of Higher Education and Research. One hundred and eighty seven female students participated in this study. Each participants completed the LEAF-Q questionnaires assessing the injuries, gastro-intestinal function and the menstrual function status. 40 students (21%) among 187 had chances or probability of developing FATSyndrome. Among the 187 students 12 students (6 %) were 17 years old, 37 students (20 %) were 18 year old, 37 students (20 %) were 19 year old, 67 students (35 %) were 20 year old and 28 students (15 %) were 21 year old , 4 students (2%) were 22 year old and 2 students (1 %) of syudents were 23 year old. Among the 40 high risk students 3 students (7%) were 17 years old, 8 students (20%) were 18 years old,11 students (28%) were 19 years old, 15 students (38%) were 20 years old and 3 students (7%) were 21 years old.Out of these 40 students11 of students (28%) had low body mass index and 14 (37%) had high body mass index.Out of 187 students , 36 (19.25%) have answered to the injury component , 150 (80.21%) have answered to the gastro-intestinal component and 118 (63.10%) have answered to the mentural function component. In this study

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21% (40) of the sedentary students have higher chances of developing high risk FAT syndrome. So Implementing an early education programme in primary school with an emphasis on learning about poor energy availability, menstrual abnormalities, low BMD, and calcium requirements has the potential to prevent several components of the triad.

Keywords: female athlete triad syndrome (FAT), female students, low-energy availability (LEA),low bone mineral density, menstural dysfunction, low energy availabilty questionnare (LEAF-Q).

INTRODUCTION

The female athlete triad is a syndrome that includes low energy availability (burning more calories than one consumes), menstrual dysfunction, and low bone mineral density, though all three components are not required [1].According to American college of sports medicine the Female Athlete Triad is a syndrome that affects physically active females and girls which includes the interconnected components such as disordered eating, amenorrhea, and osteoporosis. The main cause for developing the Triad is the pressure placed on young women to achieve or maintain an unrealistically low body weight and are at more risk. Female Athlete Triad may occur either alone or in combination and can impair physical performance and cause morbidity and mortality [2]. According to Sundgot Borgen long periods of dieting, weight oscillations, coaching changes, injury, and casual weight comments made by coaches, parents, and friends were the most common causes cited by athletes for the onset of disordered eating [5]. Low energy availability (with or without disordered eating), functional hypothalamic amenorrhea, and osteoporosis at the extreme end of the range are all included in this complete definition. Low energy availability is also emphasised as a common mechanistic issue that underpins all of the components [2,4].

Many professionals, physical therapists, and coaches are not aware of it and, as a result, do not screen for it. Educating about the female athlete triad syndrome helps to prevent, recognise, treat, and reduce its risks. At the preparticipation physical screening and/or annual health screening test, as well as whenever an athlete presents with any of the Triad's clinical symptoms, athletes should be evaluated for the Triad. Early identification and intervention must be provided for patients with any component of the female athlete triad to avoid long-term health consequences [1,2,3]. Thyroid function and prolactin levels have not been studied in any prevalent research to see if comorbid medical problems play a role in menstrual disruption. Furthermore, few research have looked at reproductive hormones to see if gynecologic pathology is a cause. Finally, many of the prevalence studies have focused on elite endurance athletes , there are only few studies comparing spectrum of athletes to a comparison group of non-athletes [6,7]. When compared to athletes, sedentary teenage girls have a lower BMD [8-11].

Components
Low energy availability
Energy Availability (EA) is calculated by deducting daily food energy intake from daily exercise energy expenditure, which is then adjusted for fat-free mass (FFM). In female adults, optimal Energy Availability is 45 kcal/kg FFM per day, but it could be much greater in adolescents who are still growing and developing. With or without the presence of disordered eating/eating disorder, the spectrum of Energy Availability varies from ideal to inadequate. The American academy of pediatrics defines the low energy availability as when the caloric intake is insufficient to compensate for exercise-related energy expenditure, the triad occurs, resulting in negative impacts on reproductive, bone, and cardiovascular wellness [2,4]. The researchers discovered that when the number of triad-related risk factors grew, the probability of bone stress injuries increased as well [12].

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Menstural dysfunction

Anovulation and luteal dysfunction to oligomenorrhea and amenorrhea (primary or secondary) are among the menstrual abnormalities linked to the triad [13]. Functional hypothalamic amenorrhea is a term used to describe amenorrhea caused by insufficient Energy Availability."Functional" is a term which refers to the reduced healthy reproductive endocrine axis due to a lack of energy [2]. According to a research, Sedentary teenagers had a 21 % rate of monthly irregularity compared to 54 % for teenage athletes [14]. Primary amenorrhea in athletes ranges from 1.2 percent to 6%, secondary amenorrhea from 5.3% to 30%, and oligomenorrhea from 5.4% to 18% when studying particular types of menstrual irregularity [15-20]. In addition to elevated cholesterol and impaired endothelium function, oligomenorrhea and amenorrhea have been linked to cardiovascular risk factors [21,22].

Low bone mineral density

In 259 femaleadolescents and young adult athletes who participated in competitive or recreational exercise, a recent multisite prospective study discovered the significance of single and multiple triad-related risk variables forstress injuries of the bone [12]. EA restriction has been proven to produce oestrogen suppression, increased bone resorption, and bone formation suppression [23]. The levels of bone formation and resorption markers in amenorrheic teenage athletes are significantly lower than those in non-endurance runners, indicating a state of overall decreased bone turn-over (24). According to Barrack et al, adolescent endurance runners have a greater frequency of low BMD 40% than ball or power sport athletes 10%. These findings point to a possible suppression of bone accumulation in adolescent runners, though other factors , such as genetically determined bone accrual patterns, rate of maturation, specific type of current and previous physical activity, and EA and menstrual differences were commonly found between non-endurance and endurance athletes [25].

The Low Energy Availability (LEA) in Females Questionnaire (LEAF-Q) is a validated questionnare in female endurance athletes to determine the risk of the female athlete triad (triad). Participants were divided into two groups based on their LEAF-Q scores (greater than or equal to 8). Low bone mineral density with 100% sensitivity, 95% confidence interval [15.8%, 100%] and menstrual dysfunction with 80.0% sensitivity, 95% confidence interval [28.4% , 99.5%] were identified by the injury and menstrual function subscale scores, respectively [26]. In order to appropriately diagnose current Energy Availability and/or reproductive function and/or bone health, the 25-item LEAF-Q showed an appropriate sensitivity (78%) and specificity (90%). When screening female athletes at risk for the Triad, the LEAF-Q is a useful tool in proving the existing Disorderd eating behavioural screening devices for early detection and intervention [27].

According to American college of Cardiology, to be defined as an active athlete, they must be trained in sports with the goal of improving their performance/results (all of these criteria must be met). They must be competing in sports on a regular basis. They must be enrolled in a local, regional, or national sport federation and be officially registered. They must make sport training and competition as their primary activity or personal interest, committing many hours per day, all or most days, to these activities, which must take precedence over other sorts of professional or leisure activities. Many researches and studies have been done on the prevalence of Female Athlete Triad syndrome in various colleges, places and also on various athletic fields. But this study finds the prevelence and its associated factors of female athlete triad syndrome among sedentary college students in chennai. The need for this study is to find out whether sedentary students are at high risk for the Female Athlete Triad Syndrome.

METHODOLOGY

This is a cross- sectional study performed on 187 female students of FACULTY OF PHYSIOTHERAPY, MAHER. Thestudy population were the college students.The students who were absent on that day were excluded from the study. The study was carried out with 187 sedentary students who did not participate in any athletic events. The studywas performed and carried on normal individuals within the purview of ethical circumference. Inclusion criteria for the study were the Female students between the age of 17-23 years. Exclusion criteria included the

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students who have not yet started menstruating, known metabolic disease, pregnancy, history of thyroid disorder, pituitary tumor, or cardiovascular disease and on the medication for any conditions related to gastro intestinal function and fractures. The screening for the study was done usig the LOW ENERGY AVAILABILITY IN FEMALE – QUESTIONNAIRE (LEAF-Q). The subjects were explained about the questionnaire and were instructed to fill accordingly.187 students filled the LEAF- Questionnaire and were scored using the LEAF-Q scoring.

The questionnaire consists of the 3 factors that includes the scoring of i). injuries, ii). gastro intestinal function and iii). menstrual function and use of contraception. The questionnaire also had the self-administered questions such as the participants demographic data, height,weight, training hours, past medical history, personal history. The injury component describes the student's recent fall and fracture giving the details regarding the bone health. The gastro-intestinal component describes student's digestive and bowel function. The menstural component decribes the usage of oral contraceptives and their menstural function such as the flow during the mentural period and the duration of the menstural cycle, which is also corelated to the activities, number of days between periods, missed or skipped periods and also the history of any hormonal diseases such as thyroid disease.The total score of≥ 8 is considered to be at risk for the factors that contribute to the female athlete triad syndrome. The students who have the score equal to (or) greater than 8 will be further referred to the physician for further screening and treatment.

RESULTS

40 students (21%) among 187 had chances or probability of developing FAT Syndrome (figure.1). Among the 187students 12 students (6 %) were 17 years old, 37 students (20 %) were 18 year old, 37students (20 %) were 19 year old, 67 students (35 %) were 20 year old and 28 students (15 %) were 21 year old, 4 students (2%) were 22 year old and 2 students (1 %) of students were 23 year old (figure.2.). Among the 40 high risk students 3 students (7%) were 17 years old, 8 students (20%) were 18 years old,11 students (28%) were 19 years old, 15 students (38%) were 20 years old and 3 students (7%) were 21 years old(figure 2.1).Out of these 40 students 27% (11) of students had low body mass index and 35% (14) had high body mass index(figure.3).Out of 187 students , 36 (19.25%) have answered to the injury component , 150 (80.21%) have answered to the gastro-intestinal component and 118 (63.10%) have answered to the menstrual function component.

DISCUSSION

Thompson and Gabriel (2004) stated that the athletic population was found to be at risk of the female athlete triad with 37 athletes and 18 non-athletes in their research. Female athletes were shown to be at risk for disordered eating, osteoporosis, and menstrual cycle disorders with 24.5 %, 24 %, and 25.5 %, respectively. Such as also Non-athletes were shown to be at risk for disordered eating, osteoporosis, and menstrual cycle dysfunction in 21 %, 18 %, and 6 %, respectively. Anne Z. Hoch et al.., (2009) stated that Low energy availability was seen in the same proportion of athletes (36%) and also in sedentary persons (39%) (P = 0.74). Menstrual irregularities was found in athletes (54%) and in sedentary students (21%) (P , 0.001). Low bone mineral density was found in 16 percent of athletes and 30 percent of sedentary subjects using dual-energy x-ray absorptiometry (P = 0.03). One or more components of the triangle are present in a significant number of high school athletes (78%) and a surprising number of sedentary students (65%).In this study about 21% (40) of students have higher probability of developing the Female Atheltic Triad syndrome. With the age ditribution between 17-23 , 3 out of 12 were under 17- years, 8 out of 37 were under 18- years , 11 out of 38 were under 19- years, 15 out of 66 were under 20-years and 3 out of 28 were under 21- years. Out of 40 students 11 had low body mass index and 14 had higher body mass index. Among the 187 students ; 36 have answered for th einjury component, 150 have answered for the gastro intestinal function component and 118 have answered for menstural function component.

CONCLUSION

The presence of significant incidence of triad features in sedentary students, early intervention and education about the Female Athlete Triad syndrome among the students, teachers, Physiotherapists, physical educators and trainers has the ability to prevent severity of the triad components, improving health and preventing long-term consequences**.** More researches should be conducted in the future to assess and to find the awareness about female athlete triad syndrome among Physiotherapists, physical educators, and also among teachers. More research should be conducted in the future to assess and to find the awareness about female athlete triad syndrome in college students. The study can be recommended for male students, excluding the menstruation factor. The study can also be done on the larger sample size and can be done on the multi-centred study setting.

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| **Figure.1 High risk female atheletic triad syndrome among students** | **Figure.2 Normal age distribution of the students** |
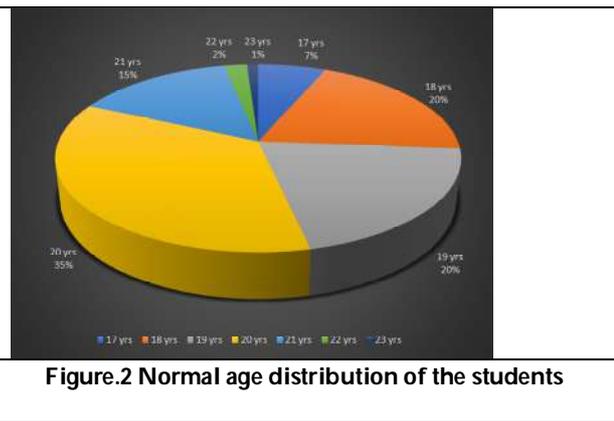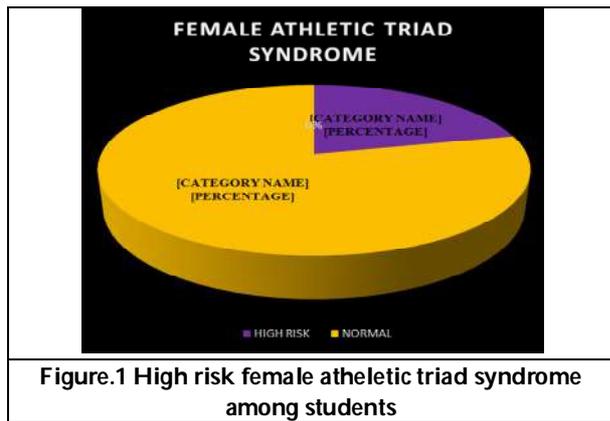
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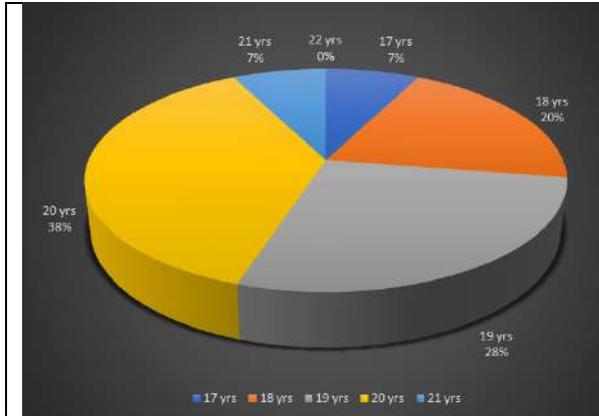
| Figure 2.1 Age distribution of high risk fat | Figure.3 Association of BMI and high risk fat |
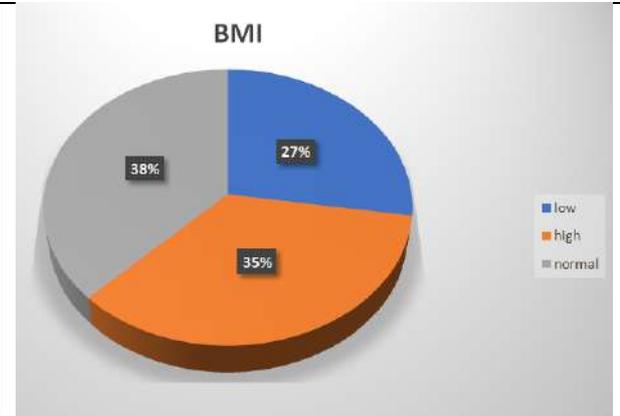
RESEARCH ARTICLE

Effect of Muscle Energy Technique and Eccentric Exercise on Calf Muscle Flexibility in Younger Adults: A Pilot Study

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ABSTRACT

Muscle stiffness in the lower limb and the consequential decrease in the joint flexibility are considered to be major etiological factors in the calf muscle dysfunction. Calf muscles (plantar flexors) are prone to tightness because of prolong periods of sitting in modern sedentary lifestyle. This tightness is associated with a decrease in ankle dorsiflexion as well as many disorders such as shin splints, achillis tendinitis, plantar fasciitis, and muscle and joint sprains. Reduced ankle dorsiflexion range of motion (DFROM) can affect gait and physical activity and is associated with falls. The study intended to ascertain the effectiveness of a Muscle energy technique and Eccentric exercise to improve the flexibility of calf muscle. 20 subjects were divided into two groups. Group-A received muscle energy technique (n=10) and Group-B trained with eccentric exercise(n=10). Both groups received training of 5 sessions per week for 5 weeks. Treatment outcomes were assessed by static ankle flexibility test and ankle dorsiflexion range of motion. Result was statistically inspected using t-test by SPSS version 25. After 5 weeks of training period, both the group showed significant improvement for calf muscle flexibility but Group-B had more improved scores than Group-A. This study concluded that Eccentric exercise was found more effective in improving the flexibility of calf muscle than Muscle energy technique.

Keywords: Eccentric exercise, MET, calf flexibility, static ankle flexibility test, ankle dorsiflexion ROM, younger adults.

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INTRODUCTION

Muscle stiffness of the lower extremities and the consequential decrease in joint flexibility is considered to be major etiological factors in musculoskeletal injuries [1]. Calf muscle tightness (i.e., decreased flexibility or increased stiffness) is associated with a decrease in ankle dorsiflexion as well as many disorders such as shin splints, Achilles tendonitis, plantar fasciitis, and muscle and joint sprains [2]. The calf muscle is composed of the gastronomies and the sole us muscle that attach to the strong Achilles tendon [3]. It plays an important role in postural control and gait. Reduced ankle dorsiflexion range of motion (DFROM) can affect gait and physical activity and is associated with falls [4]. Nowadays, a common sedentary lifestyle includes office work, driving automobiles, using public transportation, and screen time. In modern Western societies, those behaviors can sum up to a sitting time of 8.4–9.3 hours per day [5,6]. Recent research related to the corona virus disease 2019 (COVID-19) indicates that sedentary behavior time has further increased since the beginning of the pandemic, particularly in people who are now working from home [7]. Those prolonged periods of sitting are considered as an independent risk factor for health, including increased muscle stiffness [8], fatigue, discomfort [9].

Flexibility is the ability to move a single joint or series of joints smoothly and easily through an unrestricted pain-free range of motion [10]. Having good flexibility allows a person to increase his physical performance, allowing the joint the ability to move further with less energy. Flexibility increases blood supply and nutrients to joint structures, which in turn increases circulation, leading to greater elasticity of surrounding tissues, which reduces muscle tension, decreases the risk of injury [11]. Flexibility exercises help to increase the length of the musculotendinous unit. The terms "flexibility exercise" and "stretching exercise" are frequently interchanged. Flexibility training has been shown to improve joint range of motion and prevent exercise-induced muscle injury [12].

Muscle energy technique (MET) is a manual technique developed by osteopaths that are now used in many different manual therapy professions [13]. Muscle energy technique is a system of manual therapy for the treatment of movement impairments that combines the precision of passive mobilization with the effectiveness, safety, and specificity of reeducation therapists and therapeutic exercise [14]. The therapist localizes and controls the procedures, while the patient provides corrective forces and energies for the treatment as instructed by the therapist, MET focuses on joint range of motion limitation, and uses light to moderate force muscular contraction precisely controlled to affect a specific joint, to restore normal joint motion [15,16]. The eccentric training has been described by Fahlström *et al* (2003). Eccentric training refers to muscular action in which muscles lengthen in a controlled manner and also lengthen the muscle-tendon unit [17,18] and previous studies have indicated that eccentric training could increase sporting or functional performance [19].

There are so many techniques are in practice to improve muscle or soft tissue flexibility and many studies have been carried out to find the effective technique which can be used for soft tissue manipulation. But there is a lack of evidence that comparing muscle energy technique and eccentric exercise to increase the flexibility of calf muscle in a younger population. The purpose of the study is to compare the effectiveness of muscle energy technique and eccentric exercise on calf muscle flexibility in younger adults.

METHODS AND MATERIALS

A pilot study was conducted at Ahmadabad Physiotherapy College. 20 subjects with calf muscle tightness were taken in the study according to inclusion criteria. They were divided into 2 groups 10 in each group. Group A treated with muscle energy technique and Group B was trained with eccentric exercise. The inclusion criteria are (a) Age group 20-25 years (b) Both male and female (c) BMI 23-25. Exclusion criteria are (a) History of recent injury in and around ankle joint (b) History of acute/chronic pain in lower limb (c) skin diseases (d) History of cardiovascular and respiratory problems (e) Joint instability (f) Hyper or hypo mobile joint (g) Age below 20 years and above 25 years (h) BMI below 23 and above 25.

Outcome measures of this study are the Static ankle flexibility test and Ankle dorsiflexion range of motion. Static ankle flexibility test is mostly used for calf muscle tightness and Ankle DFROM is mostly used for a range of ankle joints. Static ankle flexibility test is done in standing position, facing towards to the wall with feet flat on the ground and toes touching the wall. The subject has to lean onto the wall with the hands, chin, and chest touching the wall. After that subject is asked to slide slowly his feet away from the wall as far as possible keeping the feet flat on the ground, also the body and knees are fully extended and the chest in contact with the wall. At the maximum possible position of the above description, the distance between the toe and the wall is measured. This test was performed at least 3 times and the best distance is recorded. Ankle dorsiflexion range of motion measurements (DFROM) done in supine lying with fully extended knees. Goniometry's proximal axis at the head of the fibula to the lateral malleolus and the distal axis at the base to the head of the 5th metatarsal. The examiner secured the tibia and fibula of the lower extremity using 10cm-wide straps to prevent knee motion. Ankle was maintained in neutral position during the measurements and subjects were instructed not to do active assistance, the subjects flexed the calf muscles as far as possible. After that examiner pushed back with sufficient strength to encounter notable tension in the calf muscle. Each measurement was repeated 3 times.

Group A was treated with muscle energy technique for the calf muscle. The subject was supine with feet extending over the edge of the table, with the knee flexed over a rolled towel for soleus and the knee straight for gastrocnemius. Ask subject to do dorsiflexion till feel the restriction, after that the subject was asked to do plantar flexion and therapist apply resistance, during this movement subject have to do inhale deep breath. This contraction was held for 7-10 seconds together with a held breath. On slow-release and do exhalation, the ankle was dorsiflexion slightly and painlessly beyond the new barrier, with the subject's assistance (and the tissues were held in slight stretch for at least 10 seconds to allow a slow lengthening of tissues). The repetition of this technique is done for 5 times in each session/day for 5 days for 5 weeks. Group B was trained with eccentric exercise. The interventions start from upright body position, and standing with the whole bodyweight on the anterior half-part of the foot, with the ankle joint in plantar flexion lifted by the non-intervention leg. The intervention leg is in full dorsiflexion and returned to its neutral position with the assistance of the other leg. Loaded the calf muscle of the intervention leg only eccentrically, without concentric loading. All subjects have to do eccentric exercise training till they didn't feel any discomfort. This training was done with 15 repetitions over 50 sec in three sets (3×15 repetitions) for 5 days/week for 5 weeks.

RESULT

A pilot study was done with 20 younger adults aged between 20-25 years. These subjects were randomly divided into 2 groups and intervention was given in the form of muscle energy technique and eccentric exercise. The parametric test was used in statistical analysis because the distribution was normal. Paired t-test was used to see the pre and post-treatment effects. Unpaired t-test was used for to do comparison between the both groups. Table 1 shows the mean age of both groups. Table 2 shows paired t-test of Group A and Group B pre and post mean of fall outcome measures. Table 3 shows unpaired t-test between Group A and Group B. Result showed significant improvement in all the outcome measures in Group B as p-value being.

DISCUSSION

Calf muscle tightness is occurring due to prolonged sitting in this modern lifestyle [20]. This can lead to many muscle injuries of the calf muscle. In the present study, I investigated that short-term training can improve the range of the ankle joint and improve the flexibility of the calf muscle in the younger population. J.K.Dhamelia and Mr.P.B.Balamurugan had done a study on "muscle energy technique over static stretching on the flexibility of the calf muscle in younger adults." And they stated that there is a significant improvement in flexibility of calf muscle after a MET program and the subject presented a higher performance on static ankle flexibility. In this present study MET of the calf muscle can improve the flexibility of the calf muscle in younger adults after 5 weeks of intervention.

Hee-jin Jang et al had done a study on "Comparison of the Duration of Maintained Calf Muscle Flexibility after Static Stretching, Eccentric Training on Stable Surface and Unstable Surfaces in Young Adults with Calf Muscle Tightness." They concluded that eccentric training helps to lengthening of the muscle-tendon unit which will increase sarcomeres in the series and also increase the muscle fibers. In this present study, there is a significant improvement in ankle range of motion and flexibility of the calf muscle in group B who were trained with eccentric exercise. The result showed that range of motion and flexibility of calf muscle improved after eccentric exercise training (Group B) because there are more number of repetitions and it also lengthen the musculotendinous unit compared to MET.

CONCLUSION

This study concluded that there was a significant improvement in ankle range of motion and flexibility of the calf muscle in younger adults. Both the techniques are effective but group examination showed that eccentric exercise (Group B) is better than MET (Group A).

FUTURE OF THE STUDY
we can conduct a study in a larger population, we can conduct this study in the different age groups to improve the flexibility of calf muscle.

CONFLICT OF INTEREST
Nil

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Table 1 shows mean age of both groups

AGE	MEAN	SD
GROUP A	23.9	0.87
GROUP B	24	0.81

Table 2 showed paired t test analysis of both groups

	SAF PRE	SAF POST	ADROM RT PRE	ADROM RT POST	ADROM LT PRE	ADROM LT POST
GROUP A	25.61	27.43	13.2	15.6	13.6	15.4
GROUP B	26.16	30.42	13.7	18.9	13.6	18.9

Table 3 shows comparison of both groups

	SAF	ADROM RT	ADROM LT
DIFFERENCE GROUP A	1.82	2.4	1.8
DIFFERENCE GROUP B	4.26	5.2	5.3

Graph 1. group a outcomes pre and post	Graph 2. group b outcomes pre and post
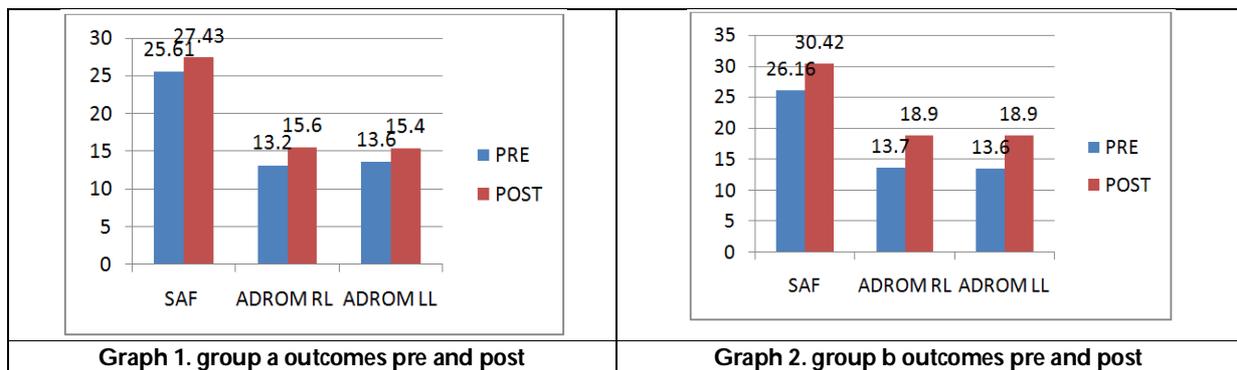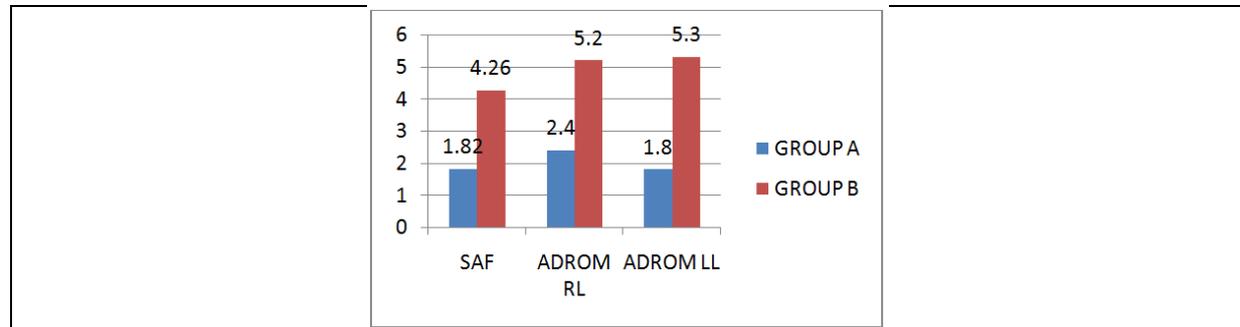
Graph 3. Comparison of Both Groups

REVIEW ARTICLE

Review Study on Genetic Variability, Character Association and Path Coefficient Analysis of Okra

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ABSTRACT

Okra is a worldwide known vegetable crop with numerous health benefits. It belongs to the Malvaceae family and is believed to originate from Ethiopia. Plant breeding is one of the main areas in the agriculture sector to develop the different crops with desirable traits. The main aim of most plant breeding programs is to increase the yielding ability of the crop plants, after a thorough understanding of the magnitude of genetic variability, character association, and path analysis. So, it is only possible if we know the work which has been done in that particular field. Therefore the literature has been reviewed for the better formulation of breeding plans.

Keywords: okra, genetic variability, genetic advance, correlation, path coefficient.

INTRODUCTION

Okra is among the oldest grown crops in the world. Its botanical name is *Abelmoschus esculentus (L.) Moench*. It is worldwide known by the different names such as Gumbo, Quingumbo, Quaio, ladyfinger, Bhendi in India, Gombo in French, and Bamiah in Arabic etc. Okra belongs to the Malvaceae. The crop of okra grows in spring-summer and rainy season and The best region for okra growth is considered to be as warm, subtropical and tropical around the world (1). According to the various literatures, India is believed to be the origin place of Okra but there is strong evidence, which shows the crop flourished before that time duration in Ethiopia. The Okra is not only used as an edible source but it is having many other uses also (2). In some regions, apart from the pod consumption, the okra

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leaves and shoots can also be consumed, when they are tender. Okra crop is having so many uses in terms of its nutritional values and its medicinal effects and very less prone for the diseases and pest attack so there arises a need to improve the crop. For improving, hybridization techniques can be used. For producing the hybrids it's important to know the basic parameters related to the germination, flowering, fruit and quality of the crop. Ariyo, 1990, contributes that the genetic variability studies are very beneficial for the breeders as it helps them to choose the best variety to expand the production of the crop. Plant breeders are the most responsible people in the agriculture sector. The main responsibility of the plant breeder is to develop the desirable traits in different crops which are free from insects and pathogens and are drought resistant. Their other responsibilities include to select the most appropriate plant among the plant population so that they can get the maximum yield out of that crop after the utilization of minimal resources. For all these things they should know the various disciplines such as seed science, soil science, crop physiology, entomology, genetics, and the new technologies to get the desirable produce.

The plant breeders analyze the scientific data and draw the critical conclusion for selecting the best genotype/varieties which are evaluated in laboratory and field trials(3). The main aim of most plant breeding programs is to increase the yielding ability of the crop plants, after a thorough understanding of the magnitude of genetic variability, character association, and path analysis. So, it is only possible if we know the work which has been done in that particular field. In okra, various researches have been conducted and there is huge literature available but they are more specific to some of the characters which are commonly studied by all. The literature has been reviewed under the following headings

1. Performance of genotypes and genetic variability, heritability, and genetic advance
2. Character association and path analysis

Performance of genotypes and genetic variability, heritability, and genetic advance (Table1)

N. K. Singh et al., 2020 (4) conducted a study during the summer season involving 59 genotypes of okra in the region of Tarai (Uttarakhand). The findings also suggest that the values for PCV were much higher than GCV, which indicated the environmental effect on the characters. Rana et al., 2020 (5) assessed the genetic variability, correlation, and path analysis for yield and yield contributing characters in seventeen genotypes of okra during the year 2018. The result indicated that for all the characters, the PCV value was higher than the GCV value with a narrow difference indicating little influence of environment on the expression of characters. Alake et al, 2019 (6) investigated the 20 okra landraces (2 years) sourced from five agroecological core zones of okra production in Nigeria.

Improvement in the production of the crop is the ultimate goal of any breeding program. Yield is dependent on the characters and the environmental factors. According to Burton, 1952, GCV with heritability provide the most reliable information on the amount of genetic advances to enhance the yield". This investigation provided the suggestion that the heterosis breeding could be effectively used to improve k and mg. Kumari et al., 2019 (7) experimented to estimate the extent of genetic variability for yield in thirty-one hybrids of okra. High heritability and high genetic advance were recorded for yield. Massucato et al., 2019 (8) evaluated the genetic diversity among okra landraces in brazil.

The genetic parameters confirmed the existence of variability among accessions, and high accuracy and heritability indices were found for the traits related to fruit and plant height. Saleem et al., 2018 (9) conducted a genetic variation study among the twenty-four okra genotypes for yield and various quality characteristics. In this study the result shows high heritability for all the traits except fruit girth and protein content. This study also revealed two varieties of Irka as the potent local genotype in terms of yield. Priyanka et al., 2018 (10)analyzed the 29 genotypes of okra and the results revealed that there was a high magnitude of genetic variability and a high degree of transmission of the majority of the growth and yield associated traits. This indicated the involvement of additive gene action and more chances of fixing by selection to improve such traits. Bello & Aminu, 2017 (11)experimented with the level of genetic divergence and heritability of eight characters in the 2015 and 2016 dry seasons using irrigation in okra. The results

implied that different genetic constitutions and preponderance of additive effects governed these characters, thus presenting a significant opportunity for selection. Beiger et al., 2017 (12) research confirmed the presence of sufficient variability in the experimental materials. Rani et al., 2017 (13)evaluated fourteen genotypes of okra for yield and various yield attributing traits. The investigation suggest that the influence of the environment on their expression. Muluken et al., 2016 (14) conducted experimental research on 23 local and two exotic varieties of Ethiopian okra. Results revealed the presence of significant differences among genotypes for all traits except for. Both heritability and genetic advance values were high for yield related traits. B. Chandra mouli , 2016 (15) studied thirty okra genotypes for yield and yield attributing traits. The results suggest direct selection based on these traits in genetically diverse materials could be effective for the desired improvement. Shiva ramegowda et al., 2016 (16)evaluated thirty-six okra germplasm for yield and yield-related traits and found that the germplasm studied possessed sufficient variability for all the traits and suggests that environmental influence is minimal for the traits studied. Olayiwola et al., 2015 (17) researched a total of ten okra accessions (2 years). The finding of the study shows there was a large degree of variability among genotypes for all traits.

Osawaru et al., 2014 (18) studied the twenty-five agro morphological characters. This study suggests that the variability observed in this study for all traits assessed was indicative of the differences in the genetic make-up of the cultivars. Ibrahim et al., 2013 (19) investigated the genetic behavior of some biological and economic traits of 14 okra populations. The results showed high significant variations among populations for all the studied traits. Koundinya et al., 2013 (20) study supported the fact environment did not much influence the estimates of genetic performance. Nwangburuka et al., 2012 (21) results suggesting the effect of additive genes.

Character association and path analysis

Yield is considered to be dependent upon several of its component traits. In this case, character association is beneficial for plant breeders to select the best variety for cultivation. Even though the relative yielding ability of a variety is subjected to large fluctuations depending on the environmental conditions, improving several sub-traits, which influence yield, can attain genetic improvement in component characters. The importance of correlation study in a selection program is appreciable when highly heritable characters are associated with yield, which had low heritability. The study of simple correlation does not provide an exact picture of the relative importance of the direct and indirect influence of each of the component characters on the desired character. So this can be overcome by the path coefficient analysis technique by further partitioning the correlation coefficient into direct and indirect effects. Investigations on the correlation and path analysis in okra are briefly reviewed (Table 2 and Table 3).

Rynjah s, et al., 2020 (22) conducted a correlation and path coefficient analysis study in thirty-six okra genotypes for fourteen. The study suggested improving the yield selection should be based on fruit girth, fruit length, fruits per plant, plant height, fruit weight, and short flowering period. Ashraf et al., 2020 (23)conducted a complete block design experiment in three replications of selected genotypes of okra, and the result was based on correlation and path analysis. The results indicated that different genotypes varied significantly regarding all the studied characters. The fruits per plant and fruit weight of different genotypes had a high degree of significant positive association with fruit yield and a high positive direct. Mohammed et al., 2020 (24) researched thirty-six okra genotypes, The result of the study shows that the genotypic correlation was positive, and significant for fruit yield per hectare with stem diameter, plant height, leaf length, leaf width, peduncle length, fruit length, fresh fruit mass, hundred seed mass, seed yield per hectare, and ash content.

Path coefficient analysis indicated fresh fruit mass, and seed yield per hectare had positive direct effects on fruit yield per hectare at phenotypic and genotypic levels. R janarthanan et al., 2020 (25) research on 250 randomly tagged plants in f2. The correlation coefficient analysis revealed the importance of the number of fruits per plant, plant height at final harvest, number of primary branches at final harvest, and Internodal length in exercising selection from segregating population as they were found to exert positive and significant association with yield. Rathava et al., 2019 (26) undertook their study on 31 genotypes of okra to determine the nature of association among different

yield attributes, and their direct, and indirect contribution toward yield. The findings revealed that the fruit yield per plant has shown a positive and highly significant correlation with plant height, fruits per plant, average fruit length, and fiber content at both genotypic and phenotypic levels. Path coefficient analysis revealed that the number of fruit per plant had maximum direct contribution towards fruit yield followed by average fruit weight, average fruit diameter, and plant height. Shehal rathod, 2019 (27) experimented during Kharif-2016. In this study correlation and path coefficient analysis of 12 quantitative characters. In the f2 population of both crosses the fruit yield per plant exhibited a positive and highly significant correlation with the number of fruits per plant, plant height at final harvest, Intermodal length, number of branches per plant, and fruit length. Shuirkar et al., 2018 (28) studied the association, and path analysis of characters of okra genotypes, and the results were as follows, plant height, the days to 50% flowering, and the fresh fruit weight was recorded as positive, and significant correlation with length of the internodes. Neeraj Singh et al, 2017 (29) researched eighty genotypes of okra and results study suggested adapting the selection process to have further improvement in okra planting.

Pithiya et al., 2017 (30) conducted a study on twenty-eight germplasm lines of okra with the purpose to improve yield. The result concluded that the plant height and 100-seed weight showed a positive and significant correlation with yield. Path coefficient analysis on various yield contributing characters revealed that the number of fruits per plant, number of seeds per fruit, plant height, fruit length, 100-seed weight, and number of branches showed a direct positive effect on yield. Aminu et al., 2016 (31) result finding denotes that the genotypic coefficient of variation was higher than the phenotypic variation for the entire yield-contributing characters. Path coefficient analysis showed that the number of pods per plant exhibited a positive and direct influence on the pod yield and the indirect influence of other yield components through this character also contributed mainly towards pod yield. Sundaram, 2015 (32) finding suggests that the number of fruits and leaf area index is having a direct effect on the yield which indicates the importance of adapting the selection process in plant breeding.

S. Kumar et al., 2015 (33) revealed that the total number of fruits per plant, and total yield per plant not only had a positive significant association with marketable pod yield per plant Prajna et al., 2015 (34) studied forty-five okra genotypes in a randomized complete block design with two replications. Fruit yield per plant showed a highly significant negative correlation with average fruit weight. Saryam et al., 2015 (35) conducted the field experiment of correlation, and path analysis in 60 genotypes of okra for fruit yield and this indicated that close interrelationship between genotypic, and phenotypic correlation co-efficient. Abd Allah, 2015 (36) estimated the direct, and indirect effects of some characteristics related to fruit, and seed production in five okra genotypes during the 2013 and 2014 summer seasons. The results indicated a positive direct effect on the number of branches per plant with yield. This indicates a true relationship, and direct selection through this trait may be effective for improving the seed yield of okra. Hence, these traits may be used simultaneously with other characters.

Umesh, et al., 2014 (37) investigated fifty-two okra for ten traits and finding indicated that selecting the plant with the length of internodes, number of fruit per plant, the height of the plant, number of first fruiting nodes, and length of fruit might help improve yield per plant in okra for formulating the desired plant with a high yield with superior marketable character. M. T. Reddy et al., 2012 (38) researched 100 germplasm lines of okra. Correlation and path coefficient analyses revealed that fruit weight, the total number of fruits per plant, and the number of marketable fruits per plant not only had a positive significant association with marketable pod yield per plant but also had a positively high direct effect on marketable pod yield per plant. Divya Balakrishnan et al., 2010 (39) researched fifteen genotypes of okra, and study suggests that selection of genotypes with short growth habits, short flowering periods, and short fruit length will help to minimize the shoot and fruit borer infestation.

ACKNOWLEDGEMENT

Hemant and Harmeet Singh Janeja

Conflicts of Interest / Competing Interest

- The authors have no relevant financial or non-financial interests to disclose.
- The authors have no conflicts of interest to declare that are relevant to the content of this article. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.
- The authors have no financial or proprietary interests in any material discussed in this article.

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Table 1: Review of literature for Performance of Genotypes and Genetic Variability, Heritability and Genetic Advance Mean

CHARACTER	MEAN	GCV	PCV	H²	GA	GA%	REFERENCE
Plant height	92.32	17.8	21.69	67.4	19.97	30.09	Singh et al., 2020
	57.76	10.26	10.36	98.06	12.09	20.93	Rana et al., 2020
	54.04	63.82	78.03	81.8	-	27.54	Alake, 2019
	149.021	6.59	8.71	0.57	9.85	10.29	Kumari et al., 2019
	73.28	22.56	23.55	91.73	44.51	-	Rathod et al., 2019
	138.87	13.79	9.15	44.1	17.39	12.52	Priyanka et al., 2018
	103.6	10.45	10.89	92	21.39	20.65	Badiger et al., 2017
	70.9	76.39	80.88	0.94	17.5	-	Sharma & Prasad, 2015
	50.94	14.09	15.3	83.93	-	26.6	Akotkar PK, De DK, 2010
Number of Branches	2.06	15.52	21.74	50.9	0.55	22.81	Singh et al., 2020
	6.74	5.08	9.01	31.78	0.39	5.9	Rana et al., 2020
	0.3003	13.39	19.58	0.47	0.38	18.85	Kumari et al., 2019
	2.18	48.34	58.66	67.9	-	82.05	Rathod et al., 2019
	2.05	26.24	16.815	41.1	0.455	22.192	Priyanka et al., 2018
	3.32	1.61	8.47	33.3	0.11	5.8	Badiger et al., 2017
	2.92	24.8	29.9	48.41	20.2	7.4	Bello & Aminu, 2017
	3.41	0.26	0.32	0.8	0.94		Sharma & Prasad, 2015
	2.43	6.88	11.96	33.1	-	8.15	Akotkar PK, De DK, 2010
Internodal Length	6.34	14.27	15.87	80.9	1.44	26.43	Singh et al., 2020
	3.66	17.27	18.51	87.07	1.21	33.21	Rana et al., 2020
	-	7.14	16.83	0.18	0.67	6.24	Kumari et al., 2019
	3.77	19.31	36.73	27.64	-	20.92	Rathod et al., 2019
	5.4	8.59	5.39	39.4	0.37	6.97	Priyanka et al., 2018
	4.69	16.88	23.47	51.7	1.17	25.01	Badiger et al., 2017
	5.66	23.96	25.6	0.88	2.61	46.11	Rani et al., 2017
	7.83	14.89	18.3	66.2	1.95	24.9	Karri & Acharyya, 2012
	2.68	13.89	16.06	74.75	-	24.74	Akotkar PK, De DK, 2010

Number of nodes per plant	-	11.61	14.89	0.61	2.51	18.66	Kumari et al., 2019
	22.68	17.57	8.38	22.8	1.87	8.24	Priyanka et al., 2018
	18.51	7.27	10.61	0.47	1.88	9.72	Rani et al., 2017
	8.4	11.14	15.08	54.64		16.97	Akotkar PK, De DK, 2010
	8.3	6.88	16.95	16.5	0.72	5.75	AdeOluwa, O.O.et al, 2011
	20.26	16.13	14.32	78.83	5.31	26.19	B. Chandramouli, et al, 2016
Leaf area	98.01	11.82	11.83	99.7	23.83	24.31	Rana et al., 2020
	156.72	7.96	8.9	80	0.15	14.67	Anil Kumar Pradhani, 2018
	313.02	16.62	16.49	98.35	105.43	33.68	B. Chandramouli, et al, 2016
	344.8	3.52	3.88	82.2	22.68	6.58	Saleem et al., 2018
Leaf area Index	0.87	7.96	8.9	80	27.21	14.67	Anil Kumar Pradhani, 2018
Number of leaves per plant	11.98	7.71	11.1	48.26	1.32	11.04	Rana et al., 2020
	21.49	6.05	6.11	98.2	3.08	12.37	Anil Kumar Pradhani, 2018
	13.08	5.94	13.59	19.1	0.67	5.12	Karri & Acharyya, 2012
	28.2	14.7	15.32	90.1	8.07	28.63	Saleem et al., 2018
Stem Girth	17.91	13.24	15.83	70	3.66	22.82	Singh et al., 2020
	20.89	3.89	8.64	20.3	0.87	3.61	Anil Kumar Pradhani, 2018
	1.9	4.88	8.47	33.3	0.11	5.8	Badiger et al., 2017
	31.65	12.22	15.44	62.2	6..30	19.91	Muluken et al., 2016
	-	9.18	10.56	75.6	3.59	16.44	Saleem et al., 2018
	1.11	7.28	6.47	79.16	0.13	11.86	Koundinya et al., 2013
Length of petioles	21.32	12.16	9.54	61.54	3.27	15.41	B. Chandramouli, et al, 2016
Node at first flower	4.83	4.95	3.51	50.2	0.24	5.12	Priyanka et al., 2018
	3.44	12.31	17.77	48	0.6	17.56	Badiger et al., 2017
	4.63	12.96	16.59	0.61	0.96	20.73	Rani et al., 2017
	4.42	4.5	6.9	42.5	0.27	6.1	Karri & Acharyya, 2012
	5.23	19.46	18.16	0.87	1.81	34.89	T. Reddy et., 2012
	4.73	13.23	7.86	35.35	0.46	9.63	B. Chandramouli, et al, 2016
DFF	61.26	77.97	82	95.09*	-	28.95	Alake, 2019
	-	1.53	2.37	0.41	0.7	2.02	Kumari et al., 2019
	47.91	9.38	11.06	71.96	-	16.4	Rathod et al., 2019
	71.8	10.52	11.31	79.07	-	18.42	MAHESWARI, 2019
	39.36	1.87	9.96	3.5	0.28	0.72	Badiger et al., 2017

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	41	8.04	7.82	94.58	-	15.67	Shivaramegowda et al., 2016
	49.83	3	5.06	35.2	-	3.67	Swati Barche et al., 2014
	72	4.71	-	5.15	0.83	-	Ibrahim et al., 2013
	46.28	4.35	5.18	70.6	3.48	7.51	Karri & Acharyya, 2012
Days to 50 per cent flowering	48.08	4.24	4.76	79.2	3.58	7.77	Singh et al., 2020
	55.66	4.28	4.61	86.33	4.56	8.2	Rana et al., 2020
	-	0.64	1.2	0.28	0.32	0.7	Kumari et al., 2019
	47.63	5.3	4.66	77.4	4.02	8.45	Priyanka et al., 2018
	40.67	0.19	1.05	0.18	0.38	-	Sharma & Prasad, 2015
	50.42	4.01	4.78	70.5	3.5	6.94	Karri & Acharyya, 2012
	46.4	7.95	7.43	0.87	6.63	14.28	T. Reddy et., 2012
	70.04	13.96	15.84	77.69	17.76	25.35	Muluken et al., 2016
Total number of fruits per plant	16.99	29.29	32.04	83.6	6.97	55.16	Singh et al., 2020
	16.56	11.93	12.62	89.48	3.85	23.26	Rana et al., 2020
	10.17	0.96	1.68	57.1	-	14.98	Alake, 2019
	-	14.12	20.71	0.47	2.32	19.84	Kumari et al., 2019
	11.69	34.78	39.06	79.29	-	63.79	Rathod et al., 2019
	28.4	14.6	9.27	40.3	3.44	12.12	Priyanka et al., 2018
	18.16	17.89	20.06	79.5	5.97	32.85	Badiger et al., 2017
	7.86	3.37	3.6	0.94	3.66	-	Sharma & Prasad, 2015
	3.1	15.34	21.49	51	-	22.58	Akotkar PK, De DK, 2010
Total fruit per plant	204.55	38.15	40.55	88.5	111.01	73.94	(Singh et al., 2020)
	187.34	14.61	14.62	99.91	56.37	30.09	(Rana et al., 2020)
	99.19	1.34	27.75	4.82	-	0.53	(Alake, 2019)
	-	16.36	16.68	0.96	91.7	33.03	(Kumari et al., 2019)
	28.4	14.6	9.27	40.3	3.44	12.12	(Maheswari, 2019)
	397.7	15.45	10.25	44	55.72	14.01	(Priyanka et al., 2018)
	140.87	11.43	19.62	33.9	19.31	13.71	(Badiger et al., 2017)
	45.62	18.68	24.17	59.71	-	29.73	(Akotkar PK, De DK, 2010)
	197.83	29.26	31.19	0.88	11.91	6.02	(Rani et al., 2017)
Average fruit weight	11.95	11.31	13.74	67.7	2.24	19.16	(Singh et al., 2020)
	11.26	17.41	18.19	91.56	3.86	34.32	(Rana et al., 2020)
	-	7.56	10.81	0.49	1.94	10.88	(Kumari et al., 2019)
	14.88	9.9	11.2	78.24	-	63.79	(Rathod et al., 2019)
	17.5	5.57	3.96	50.5	1.01	5.8	(Priyanka et al., 2018)

	18.95	9.36	14.47	41.8	2.36	12.47	(Badiger et al., 2017)
	11.38	1.88	2.35	0.8	2.52		(Sharma & Prasad, 2015)
	14.87	11.17	13.75	65.94		18.69	(Akotkar PK, De DK, 2010)
	20.04	7.94	10.91	0.53	2.38	11.88	(Rani et al., 2017)
Fruit length	13.38	6.76	12.14	31.1	0.73	7.76	Singh et al., 2020
	10.04	5.68	8.51	44.64	0.78	7.83	(Rana et al., 2020)
	9.79	1.09	1.28	85.06*	-	20.27	(Alake, 2019)
	-	5.55	9.88	0.32	0.93	6.42	(Kumari et al., 2019)
	13.3	6.13	9.43	42.33	-	8.22	Rathod et al., 2019
	14.82	5.86	5.41	85	1.52	10.27	Priyanka et al., 2018
	14.49	7.71	14.05	30.1	1.26	8.72	Badiger et al., 2017
	11.45	1.37	1.55	0.88	2.26	-	Sharma & Prasad, 2015
	12.36	7.45	10.41	51.22	-	10.98	Akotkar PK, De DK, 2010
Fruit Diameter	1.47	8.82	12.83	47.3	1.65	12.5	Singh et al., 2020
	1.43	7.32	9.57	58.58	0.16	11.55	Rana et al., 2020
	5.16	0.22	0.84	26.28	-	9.63	Alake, 2019
	-	5.26	6.14	0.73	0.16	9.3	Kumari et al., 2019
	1.47	0.68	5.27	1.67	-	0.18	Rathod et al., 2019
	1.69	3.08	1.47	23	0.02	1.46	Priyanka et al., 2018
	2.03	7.51	11.84	40.2	0.2	9.8	Badiger et al., 2017
	1.57	0.01	0.02	0.51	0.16	-	(Sharma & Prasad, 2015)
	1.33	7.77	9.74	63.7	-	12.78	(Akotkar PK, De DK, 2010)
Number of seed per fruit	54.9	10.47	11.9	77.4	10.5	18.98	Singh et al., 2020
	92.37	64.12	133.49	48.03	-	12.37	Alake, 2019
	-	6.46	11.22	0.33	4.6	7.65	Kumari et al., 2019
	49.55	7.99	10.71	55.6	6.08	12.27	Karri & Acharyya, 2012
	46.08	23.8	24.2	96.7	32.45	48.2	Anil Kumar Pradhani, 2018
Seed per fruit	4.66	28.39	28.61	87.57	2.7	58.04	K, 2016
	88.77	21.73	19.59	81.35	-	32.31	AdeOluwa, O.O.et al, 2011
Hundred seed or test weight	5.73	0.15	0.26	57.96*	-	10.36	Alake, 2019
	3.9	7.26	13.4	29.34	-	8.1	Rathod et al., 2019
	4.23	44.5	46.01	93.5	3.75	88.66	K, 2016
	15.86	2.45	15.86	2.38	18.42	-	Rani et al., 2017
	5.49	12.06	11.83	96.12	1.31	-	AdeOluwa, O.O.et al, 2011
Number of ridges on the	5.14	8	8.57	87.19	-	15.39	Akotkar PK, De DK, 2010
	5.2	3.15	8.78	12.9	0.12	2.32	Badiger et al., 2017

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fruit surface	5.18	8.42	8.92	89.1	0.85	16.4	Karri & Acharyya, 2012
	5	18.1	18.34	97.5	1.98	36.81	Anil Kumar Pradhani, 2018
	8.97	14.19	3.78	7.1	0.19	-	AdeOluwa, O.O.et al, 2011
	7.64	10.34	11.16	85.89	1.51	19.75	Muluken et al., 2016
Fruit shelf life	2.33	18.38	25.38	52.5	1.18	27.44	Anil Kumar Pradhani, 2018
Chlorophyll content	8.72	21.4	21.9	95.1	3.75	43.03	Salonki, 2018
Moisture Content	-	2.99	4.92	37	2.93	3.75	Salonki, 2018
	82.54	5.7	5.8	96.4	20	24.23	K, 2016
Phenol Content	1.61	18.86	19.5	93.54	0.6	37.59	K, 2016

Table 2: Review of literature of correlation coefficient for different characters in okra

CH		DFF	DF	PH	SG	IL	PL	NLP	FFN	NNP	NPB
DFF		-	-	0.62	-	-	-	-0.06	-	-	-0.02
	G	1.000	-	-0.216*	-	-	-	-	-	-	-0.387**
	P	1.000		-0.183							-0.113
	G	-	-	-	-	-	-	-	-	-	-
	P										
DF	G		1.000	-0.55**	-	-	-	-	-	-	-
	P		1.000	-0.53**							
	G	0.0265	-	-	-	-	-	-	0.2067 0.2019*	-	-
	P	0.2132									
PH	G	-	-	1.000	-	-	-	-	-	-	-
	P			1.000							
		0.13	-	-	-	-	-	0.47	-	-	-0.05
	G	0.2044	0.3048			0.4587			0.0247		0.0891
	P	0.0887	0.1882*			0.2871**			0.0151		0.0597
	G	-	-	1.000	-	-	-	-	-	-	-
	P			1.000							
	G	0.0597	-	-	-	-	-	-	-	-	-
	P	0.0891									
	G	-	-	0.2996	-	0.4604	0.5176	-	-	-0.3205	-0.2892
	P			0.153*		0.2183**	0.2928***			-0.1629*	-0.1157
SG	G	0.033	-	-	1.000	-	-	-0.5	-	0.262*	-0.273*
	P	0.059		0.319** -0.115	1.000			0.24		0.194	-0.029
	G	-	-	-	-	-0.0422	0.2424	-	-	0.0451	0.3387
	P					-0.0235	0.2231**			0.0568	0.2860***
IL	G	0.1396	0.4546	-	-	-	-	-	0.0618	-	-
	P	0.1093	0.4420*						0.0628		
	G	-	-	-	-	-	0.0021	-	-	-0.1026	-
	P						0.0014			-0.1029	
PL	G	-	-	-	-	-	-	-	-	-0.0886	-
	P									-0.0852	
NLP	G	0.373**		-0.253*				1.000			-0.207
	P	0.213		0.012				1.000			-0.089
FFN	G	0.1724	-	-	-	-	-	-	-	-	
	P	0.1420									
NNP	G	0.151 0		-0.052	-	-	-	0.403	-	1.000	-0.084
	P	0.128		-0.006				0.138		1.000	-0.117
	G	-	-	-	-	-	-	-	-	-	-
	P										
NPB	G		0.26	0.21	-	-	-	-	-	-	1.000
	P		0.27	0.22							1.000
		-0.02		-0.05				0.33			

	G/P	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
	G	-	-	0.447**	-	-	-	-	-	-	1.000
	P			0.245*							1.000
	G	-0.3976	-0.1518			-0.1887			-0.3512		-
	P	-0.2186*	-0.1228			0.1673*			-0.314*		
	G	-	-	-	-	-0.2884	-0.1195			0.4297	-
	P					-0.2254**	-0.0847			0.3926***	
	G		0.14	0.60*					-	-	–0.50**
	P		0.11	0.59*	-	-	-	-			–0.47**
		0.04		0.42	-	-	-	0.1	-	-	-0.14
	G			0.471*	-	-	-	-	-	-	0.145
	P			0.317**							0.103
	G	-	-	-	-	-	-	-	-	-	-
	P										
	G	-	0.11	0.18	-	-	-	-	-	-	–0.13
	P		0.13	0.17							–0.10
	G			-0.173	-	-	-	-	-	-	-0.216*
	P	-		-0.109							-0.113
	G	-	0	0.52**	-	-	-	-	-	-	0.19
	P		0.47**	0.48**							0.2
	G	-		0.257	-	-	-	-	-	-	0.184
	P			0.067							0.052
	G	-	-	-	-	-	-	-	-	-	-
	P										
YPP	G		–0.51**	0.61**	-	-	-	-	-		0.32*
	P	-	–0.43**	0.50**						-	0.29*
		0	-	0	-	-	-	0.48	-	-	0.11
	G	-			0.1856	-0.0282	0.1668			-0.3431	0.0289
	P				0.1505*	-0.0485	0.1519*			-0.3070***	0.0208
AFW	G		0.52**	0.29			-				0.18
	P	-	0.48**	0.25	-	-		-	-	-	0.1
		-0.31		0.07	-	-	-	0.24	-	-	0.13
	G	-	-	-	-	-	-	-	-	-	-
	P										
SPF		-0.21	-	0.15	-	-	-	0.19	-		0.18
	G		-	1.00	-	-	-	-	-	-	-
	P			1.00							
HSW		0.17		0.4				0.24			-0.15
	G	-	-	-	-	-	-	-	-	-	-
	P										

CH		FD	FL	YPP	AFW	SPF	SYF	HSW	REFERENCE
		-	-0.04	-0.09	-0.12	0.17	-0.21	0.17	K.U.Ahamed et al.,2015
DFF	G/P	0.214	-0.245*	-0.264*	-0.750* / -0.442***		-0.442*	-0.156 / 0.061	Pithiya PH, et al., 2017
	G/P	-	0.0639 / -0.0529	0.1319 / 0.0876	-0.0762 / -0.0788	-0.0669 / -0.0524	-	-	Shuirkar et al.,2018
DF	G/P	-	-	-	-	-	-	-	Aminu et al., 2016
	G/P	-	0.2783 / 0.1943*	0.001 / 0.0012	0.2944 / 0.2809**	0.2626 / 0.2525*	-	-	Shuirkar et al.,2018
PH	G/P	-	-	-	-	-	-	-	
		-	0.42	0.53	0.51	0.07	0.15	0.40**	K.U.Ahamed et al.,2015
	G/P		0.1435 / 0.0897	0.0730 / 0.0522	0.2267 / 0.1350*`	0.5317 / 0.3387**			
	G/P	-						-0.026 / 0.227*	Pithiya PH, et al., 2017
	G/P	-	-	-	-	-	-	-	Shuirkar et al.,2018

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Trait									Reference
	G		0.4379	-	-	-	-	-	Saryam et al., 2015
	P		0.2675***	-	-	-	-	-	
SG	G	-0.238*	-0.357*	-0.078	-0.338**		0.208	-0.546**	Pithiya PH, et al., 2017
	P	-0.069	-0.153*	0.037	-0.051		0.063	-0.269*	
	G	-	0.1097	-	-	-	-	-	Saryam et al., 2015
	P	-	0.0866	-	-	-	-	-	
IL	G	-	0.291	-0.1094	0.6563	0.5211	-	-	Shuirkar et al.,2018
	P	-	0.1753*	-0.1104	0.6475**	0.5021**	-	-	
	G	-	-0.0295	-	-	-	-	-	Saryam et al., 2015
	P	-	-0.382	-	-	-	-	-	
PL	G	-	0.2407	-	-	-	-	-	Saryam et al., 2015
	P	-	0.2303**	-	-	-	-	-	
NLP	G	0.17	-0.064	-0.432**	-0.607**		-0.793**	0.158 –	Pithiya PH, et al., 2017
	P	0.155	-0.099	-0.136	-0.188		-0.318**	-0.416**	
FFN	G	-	0.3149	-0.1120	0.4263	0.1757	-	-	Shuirkar et al.,2018
	P	-	0.1915*	-0.1139	0.4222**	0.1708	-	-	
NNP	G	-0.227*	0.195	0.193	-0.28		0.193	-0.266*	Pithiya PH, et al., 2017
	P	-0.115	0.183	0.105	-0.022		0.142	0.263*	
	G	-	-0.2943	-	-	-	-	-	Saryam et al., 2015
	P	-	-0.2741***	-	-	-	-	-	
NPB	G	-	-	-	-	-	-	-	Aminu et al., 2016
	P	-	-	-	-	-	-	-	
		-	0.18	0.14	0.11	-0.22	0.13	-0.15	K.U.Ahamed et al.,2015
	G	-	-	-	-	-	-	-0.235*	Pithiya PH, et al., 2017
	P	-	-	-	-	-	-	-0.040	
	G	-	-0.3031	-0.1143	-0.1948	-0.1391	-	-	Shuirkar et al.,2018
	P	-	-0.1665*	-0.1039	-0.1847	-0.1263	-	-	
	G	-	-0.3402	-	-	-	-	-	Saryam et al., 2015
	P	-	-0.2771***	-	-	-	-	-	
	G	-	1.000	0.18	-	-	-	-	Aminu et al., 2016
	P	-	1.000	0.19	-	-	-	-	
		-	-	0.54	0.57	0.29	0.36	0.28*	K.U.Ahamed et al.,2015
	G	-	1.000	0.195	0.391**		0.122	0.049	Pithiya PH, et al., 2017
	P		1.000	0.136	0.266*		0.084	0.241*	
	G	-	-	0.1611	0.4784	0.5539	-	-	Shuirkar et al.,2018
	P	-	-	0.0897	0.2732**	0.3107**	-	-	
	G	1.000	-0.22	-0.30	-	-	-		Aminu et al., 2016
	P	1.000	-0.19	-0.27	-	-	-		
	G	1.000	-	-0.243*	-	-	-0.039	.210	Pithiya PH, et al., 2017
	P	1.000	-	-0.114	-	-	-0.000	-0.035	
	G	-	-	-0.30	-	-	-	-	Aminu et al., 2016
	P	-	-	0.27	-	-	-	-	
	G	-	-	1.00	-	-	-	-0.016	Pithiya PH, et al., 2017
	P	-	-	1.00	-	-	-	-0.347**	
	G	-	-	-	-0.0585	0.0363	-	-	Shuirkar et al.,2018
	P	-	-	-	-0.0585	0.0393	-	-	
YPP	G	0.25	0.72**	0.79**	1.000	0.75**	-	-	Aminu et al., 2016
	P	0.2	0.63**	0.65**	1.000	0.61**	-	-	
		-	-0.57	0.99**	-	0.44	-	0.44**	K.U.Ahamed et al.,2015
	G	-	0.3924	-	-	-	-	-	Saryam et al., 2015
	P	-	0.3496***	-	-	-	-	-	
AFW	G	0.85**	0.53**	0.1	-	1	-	-	Aminu et al., 2016
	P	0.78**	0.51**	0.07		1	-	-	
		-	0.29	0.33	0.44	0.52	-	-0.01	K.U.Ahamed et al.,2015
	G	-	-	-		0.5837	-	-	Shuirkar et al.,2018
	P	-	-	-		0.5633*	-	-	
SPF		-	0.36	0.32	0.36	0.52	-	-0.52	K.U.Ahamed et al.,2015
	G	-	-	-	-	-	-	0.383**	Pithiya PH, et al., 2017
	P	-	-	-	-	-	-	-0.287*	
HSW		0.28	0.44	0.44	-0.01	-			K.U.Ahamed et al.,2015
	G	-	-	-	-	-	-	1.00	Pithiya PH, et al., 2017
	P	-	-	-	-	-	-	1.00	

Where CH: Character, DFF: DFF, DF: Days to 50% Flowering, PH: Plant Height, SG: Stem Girth, IL: Internodal Length, PL: Petiole Length, NLP: Number of leaves per plant, FFN: First Fruiting Node, NNP: Number of Nodes per Plant, FD: Fruit Diameter, FL: Fruit Length, AFW: Average Fruit Weight, SPF: Seeds Per Fruit, HSW: Hundred Seed Weight, NBP: Number of Branches Per plant, SYP: Seed per Plant, YPP: Per Plant

Table 3: Review of literature on path coefficient analysis for different characters in okra

CH	DF	PH (cm)	FFN	FL	FD (cm)	FPP	SPF	HSW (g)	YPP (g)	Reference
PH	0.130	-	-	0.420	-	0.530	0.150	0.400	0.510	(Ahamed et al., 2015)
	-0.050	**0.094**	-0.020	0.030	0.005	0.070		-	0.060	(Reddy et al., 2013)
	0.019	**-0.010**	0.000	0.000	0.019	0.358	0.000	-	0.530	(Das et al.,2012)
	0.028	**0.003**	-0.038	0.003	-0.021	0.256	-0.123	-	0.314	(Das et al.,2012)
FD	0.007	-0.003	-0.005	0.005	**-0.061**	-0.008		-	-0.013	(Reddy et al., 2013)
	0.041	-0.005	-0.014	-0.005	**0.035**	0.576	-0.011	-	0.599	(Das et al.,2012)
	0.079	0.001	-0.175	0.003	**-0.408**	0.573	-0.173	-	0.759	(Das et al.,2012)
DF	-	0.130	-	-0.040	-	-0.090	-0.210	0.170	-0.120	(Ahamed et al., 2015)
	-0.160	0.090	0.000	0.430	-0.010	0.100		-	0.006	(Reddy et al., 2013)
	-0.070	0.003	0.017	0.005	-0.024	-0.656	0.010	-	-0.788	(Das et al.,2012)
	-0.090	-0.001	0.200	-0.002	0.035	-0.639	0.138	-	-0.811	(Das et al.,2012)
FPP	-0.090	0.530	-	0.540	-	-	0.320	0.440	0.990	(Ahamed et al., 2015)
	0.342	0.392	-0.190	0.186	0.064	**0.497**		-	0.444	(Reddy et al., 2013)
	0.528	-0.004	-0.017	-0.004	0.023	**0.864**	-0.011	-	0.962	(Das et al.,2012)
	0.073	0.001	-0.213	0.002	-0.029	**0.787**	-0.133	-	0.908	(Das et al.,2012)
FPP	-0.040	0.420	-	-	-	0.540	0.360	0.280	0.570	(Ahamed et al., 2015)
	0.002	-0.002	0.001	**-0.006**	0.000	-0.002	-	-	-0.003	(Reddy et al., 2013)
	0.041	-0.006	-0.009	**-0.007**	0.023	0.492	-0.010	-	0.508	(Das et al.,2012)
	0.051	0.001	-0.110	**0.005**	-0.027	0.367	-0.143	-	0.436	(Das et al.,2012)
SPF	-0.210	0.150	-	0.360	-	0.320	-	-0.210	0.360	(Ahamed et al., 2015)
	0.054	-0.007	-0.014	-0.004	0.029	0.676	**-0.013**	**-**	0.800	(Das et al.,2012)
	0.066	0.001	-0.156	-0.189	-0.037	0.556	**-0.189**	**-**	0.963	(Das et al.,2012)
HSW	0.170	0.400	-	0.280	-	0.440	-0.210	-	0.440	(Ahamed et al., 2015)
FFN	-0.470	0.310	**-1.140**	0.220	-0.080	0.450	-	-	0.390	(Reddy et al., 2013)
	-0.050	0.000	**0.022**	0.003	-0.022	-0.682	0.008	-	-0.788	(Das et al.,2012)
	-0.076	0.000	**0.238**	-0.002	0.030	-0.703	0.124	-	-0.811	(Das et al.,2012)
YPP (g)	0.000	0.510	-	0.570	-	0.990	0.360	0.440	-	(Ahamed et al., 2015)
	0.610	-0.620	0.310	-0.510	-0.190	-0.830	-	-	**-0.930**	(Reddy et al., 2013)

Where, CH: Character, PH: Plant Height, DF: Days to 50% Flowering, FL: Fruit Length, FD: Fruit Diameter, SPF: Number of Seed per Fruit, HSW: HSW, NPP: First Flowering Node and YPP: Yield per Plant