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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Growth of Tetrakis Thiourea Potassium Chloride as New Second Order Optical Material

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Received: 02 Feb 2021 Revised: 15 Feb 2021 Accepted: 25 Feb 2021

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ABSTRACT

A novel organometallic nonlinear optical (NLO) crystal, namely thiourea complex of tetrakis thiourea potassium chloride (TTPC), has been grown by slow evaporation solution growth technique. The harvested crystal is large in size. To our knowledge there is no report is available for the bulk size single crystal of TTPC. This material has a positive temperature coefficient and has been grown by slow evaporation solution growth technique. The grown crystal have been characterized by employing several techniques such as single crystal and powder X-ray diffraction, FTIR, UV-Vis-NIR spectra, thermo gravimetric analyses respectively. Etching studies have also been carried out in order to know the surface defects on the as grown specimen of TTPC. The relative second harmonic generation efficiency have been tested by using Nd: YAG laser as source.

Keywords: Solution growth technique, X-ray diffraction, FTIR, UV-Vis-NIR spectra, Thermal, Etching studies.

INTRODUCTION

Nonlinear optics (NLO) is playing a major role in the emerging photonic and optoelectronic technologies. New nonlinear optical frequency conversion materials have a significant impact on laser technology, optical communication and optical data storage [1]. The search for new frequency conversion materials over the past decade has led to the discovery of many organic NLO materials with high nonlinear susceptibilities [2,3]. However, their often in-adequate transparency, poor optical quality, lack of robustness, low laser damage threshold and inability to grow in larger size have impeded the use of single crystals of organic materials in practical device applications. Hence materials scientists focused their attention on novel materials in order to satisfy the present day technological requirements. In the recent past a new class materials have been developed i.e. semiorganic crystals. Semiorganic crystals have large nonlinearity, high resistance, too large induced damage, low angular sensitivity and good

mechanical hardness [4,5]. Recently metal complexes of thiourea analogs have been explored. Some of the potential thiourea complexes are zinc thiourea iodide (ZTI) [7], bisthiourea cadmium iodide (BTCI) [8], zinc thiourea sulphate (ZTS) [9,10]. These crystals have better nonlinear optical property than standard potassium dihydrogen phosphate (KDP)[11]. In the present study thiourea combined with potassium chloride to form a new semiorganic nonlinear optical material [12]. The present work deals with the growth, solubility, single crystal and powder X-ray diffraction, FTIR, UV-Vis-NIR spectra, thermal, etching studies of TTPC single crystals.

EXPERIMENTAL PROCEDURE

Solubility Measurement

Synthesis, solubility and growth TTPC salt was synthesized by dissolving thiourea (AR grade) and potassium chloride (AR grade) in the ratio 4:1 in double distilled water. TTPC crystals were grown form aqueous solution by slow evaporation method. TTPC was synthesized according to the following reaction:

$KCl + 4[CS(NH_2)_2] \rightarrow K[CS(NH_2)_2]_4Cl$

The synthesized TTPC salt was purified by successive recrystallization processes.

In order to grow bulk single crystals of TTPC, the solubility of the synthesized material have been determined at different temperatures from 30 to 55°C at the intervals of 5 K. The gravimetric method was adopted to determine solubility and this was carried out in a constant temperature bath. The solution was stirred continuously for 5 hours using an immersible teflon-coated magnetic paddle in a magnetic stirrer. From figure 1, it was found that TTPC has positive solubility-temperature gradient and solution growth technique can be followed for crystal growth. The saturation solution of TTPC was taken and the solution was filtered using whattman filter paper. The filtered solution was taken in the beaker and dried in dust free atmosphere with perforated cover. TTPC crystal of size 20 x 11 x 6 mm^3 has been harvested from the mother solution with a time span of 28 days. The harvested crystals are found to be transparent with the little hygroscopic nature and the grown crystal is shown in figure 2.

Characterization Techniques

Characterization The single crystal X-ray diffraction has been carried out using ENRAF NONIUS CAD4 diffractometer. PERKIN ELMER SPECTRUM RX1 Fourier Transform Infrared spectroscopy instrument was used to identify the various functional groups in the grown samples with a scan range of 400-4000 cm^{-1}. The optical properties of the crystals were examined between 200-1200 nm using LAMBDA-35 UV-Vis spectrometer. The NLO efficiency of the grown sample was measured by Kurtz powder [10] method using Nd:YAG laser as a source. Thermal analysis was carried out using STA409 PC thermal analyzer to study thermal behavior of the grown crystal. Etching study were carried out on the (100) plane of the TTPC crystal using water as an etchant.

RESULTS AND DISCUSSION

Powder X-ray Diffraction Analysis

Single crystal and powder X-ray diffraction analysis The single X-ray diffraction studies have been carried out to confirm the crystallinity and to calculate the lattice parameters of the grown sample. Using the tetragonal crystallographic equation, the lattice parameter values of TTPC crystals were calculated. Finally crushed powder crystal was subjected to powder X-ray diffraction analysis. The sample was scanned over the range 10 – 70 degree with a scan rate of 1°/min. The recorded X-ray pattern of TTPI is shown in figure 3. The crystal has retained its tetragonal structure with lattice parameters a = 20.1492 Å, b = 20.1492 Å, c = 8.3154 Å, V= 3375 A^3, $\alpha = \beta = \gamma = 90°$. This confirms that the TTPC single crystal system.

Fourier Transform Infrared Spectral Analysis

Fourier Transform Infrared analysis Infrared spectroscopy is used to identity the functional groups of the synthesized compounds. The recorded FTIR spectrum of TTPC is shown in figure 4. When it was compared with the spectra of pure thiourea [11] a few peaks were found to be slightly shifted. There are two possibilities by which the coordination of potassium with thiourea may occur either through nitrogen or through sulfur of thiourea. Most of the metals form complex through sulfur [12]. The study of the spectra of TTPC shows a shift in frequency, broad envelop positioned between 2683 and 3261 cm^{-1} corresponds to the symmetric and asymmetric stretching modes of NH_2 grouping thiourea. The bonds of thiourea were not shifted to lower frequencies on the formation of the potassium thiourea complex. This indicates that nitrogen to potassium bonds is not present in the coordination compounds. The absorption observed at 1464 and 1090 cm^{-1} in the present spectrum of TTPC corresponds to the 1470 and 1089 cm^{-1} absorption of thiourea respectively and can assigned to the N-C-N stretching vibration. The increase in the frequency can be attributed to the greater double bond character to the carbon to nitrogen bond on complex formation. From this spectroscopic investigation, the presence of all the fundamental functional groups of the grown sample was confirmed qualitatively. Comparison of vibration of thiourea and TTPC is given in table 1.

UV – Visible Spectral Analysis

Linear and nonlinear optical studies The UV-VIS-NIR transmission spectrum of TTPC is shown in figure 5. It is observed from the spectrum that TTPC has a wide optical transmission window (200 – 1100 nm). It has good transparency 65% and the lower cutoff wave length of the crystal is found to be 240 nm, and thus to ascertain the fact that the crystal can be used for laser applications. The second harmonic generation (SHG) conversion efficiency of TTPC was measured by Kurtz and Perry powder technique. The crystal was grounded into a homogenous powder of particles and densely packed between two transparent glass slides. A Q-switched Nd:YAG laser emitting a fundamental wavelength of 1064 nm (pulse edith 8ns) was allowed to strike on the sample cell normally. The SHG output 532 nm (green light) was finally detected by the photomultiplier tube. It is a potential material for frequency conversion.

Thermal Analysis

Thermal studies the thermo gravimetric analysis (TGA) of TTPC was carried out between room temperature (27°C) to 800°C at a heating rate of 20 °C per minute and the recorded spectrum is shown in figure 6. The experiment was performed in nitrogen atmosphere although the TG trace appears nearly straight up to 188.3°C. The differential thermal analysis (DTA) was also carried out in the same atmospheric condition (at a heating rate of 20 K per minute). A careful examination of DTA curve reveals that, a minor endothermic peaks around 183.3°C and 262.7°C, a steady decrease in weight observed (84.49%) up to 350°C. This may be due to the decomposition of the sample. At temperature above 350°C the final stage occurs, giving a total loss equal to 11.73%. The DTA trace indicates a strong endothermic starting at 183.3°C due to its melting of the crystal. Hence from the thermal studies it is concluded that the crystal can retain texture up to 183.3°C. It's application restricted up to 183.3°C only, which is higher that other semiorganic materials like CMTD (150°C), CMTG (100°C), (LACC) L-alanine cadmium chloride (110°C), (ATCC) triallylthiourea cadmium chloride (101°C), (ATCB) triallyl thiourea mercury chloride (133°C), (ATMB) allyl thiourea mercury bromide (125°C).

Differential Scanning Calorimetric

Differential scanning calorimetric (DSC) study was performed using DSC200 PC in the temperature range 20-400°C at a heating rate of 20 K/min in the nitrogen atmosphere and the spectrum is shown in figure 7. 6.800mg sample was used for the present analysis. From this measurement, we found that the crystal is stable up to its melting point (183.3°C).

Etching Studies

The crystals with defects may destroy the mechanical and electrical properties, which affect the usefulness of the crystals. The nonlinear optical properties such as SHG efficiency, damage threshold etc, depend on the crystalline perfection. Etching is one of the selective tools to identify the defects in the as grown crystals. When the crystal is dissolved in a solvent, the reversal of the growth taking place by giving the well defined etches pits. The etching time depends on the solubility of the crystalline material in different solvents. In the present work, water is used as an etchant and etching is done on (100) face of TTPC crystals. For the etching time of 10s, etch pits are produced on (100) face of the TTPC crystal and is shown in figure 8. It may due to the vacancy of the atoms in the some areas of the surface of the specimen.

CONCLUSION

Single crystals of TTPC were grown by the low temperature solution growth technique. Solubility of TTPC has been found out for different temperatures and TTPC crystals were grown by slow evaporation technique because of its positive temperature coefficient. The crystal structure of TTPC was confirmed from powder XRD analysis. The presence of various functional groups was identified from FTIR analysis. The optical behavior assessed by UV-visible measurement. From Kurtz and Perry technique the powder SHG efficiency of TTPC was found to be higher than that of KDP. The thermal analysis has revealed the thermal stability of the crystal. From the etching studies well defined etch pits were observed on the surface of the specimen. It may due to vacancy of atoms on the surface of the specimen.

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Table 1 Comparisons of IR band frequencies (cm^{-1}) thiourea and TTPC.

Thiourea	BTCC	ZTS	BTCA	BTCF	PTC	TTPC	Assignments	
740	719	717	725	720	716	730	C = S	Stretching
1089	1106	1126	1110	1111	1097	1090	N-C-N	Stretching
1417	1393	1404	1413	1373	1404	1428	C = S	Stretching
1470	1495	1515	1494	1510	1484	1464	N-C-N	Stretching
1627	1623	1633	1627	1635	1620	1588	NH_2	Bending

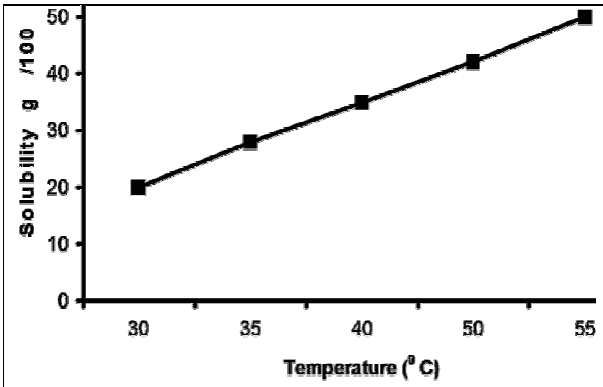

Fig. 1. Solubility curve of TTPC.

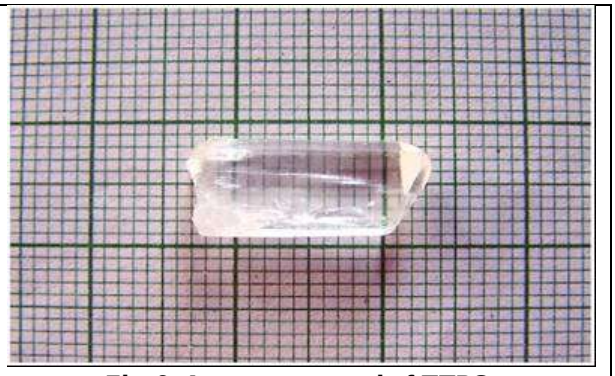

Fig. 2. As-grown crystal of TTPC.

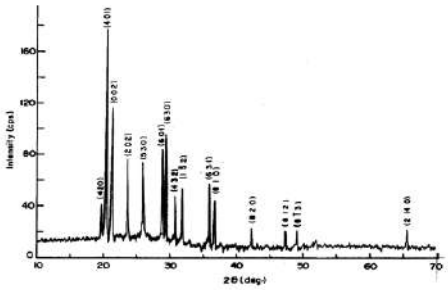

Fig. 3. Powder XRD pattern of TTPC.

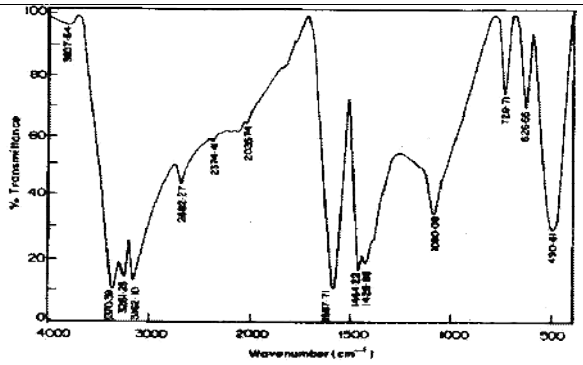

Fig. 4. FTIR spectrum of TTPC

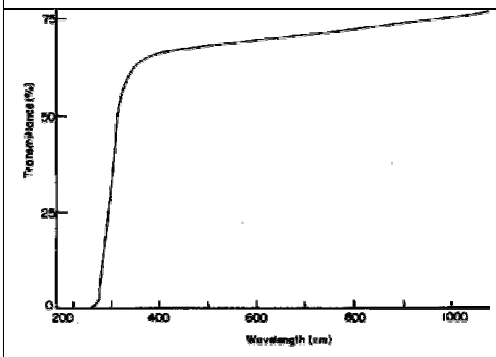

Fig. 5. Optical transmission spectrum of TTPC crystal.

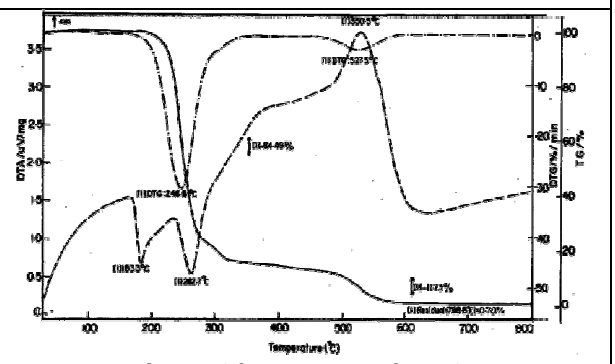

Fig. 6. TG/DTA curves of TTPC.

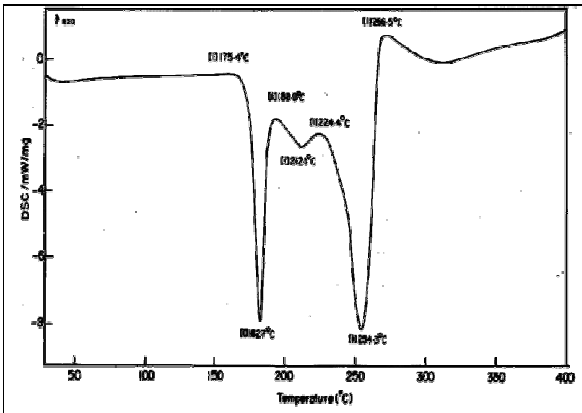

Fig. 7. DSC curve of TTPC.

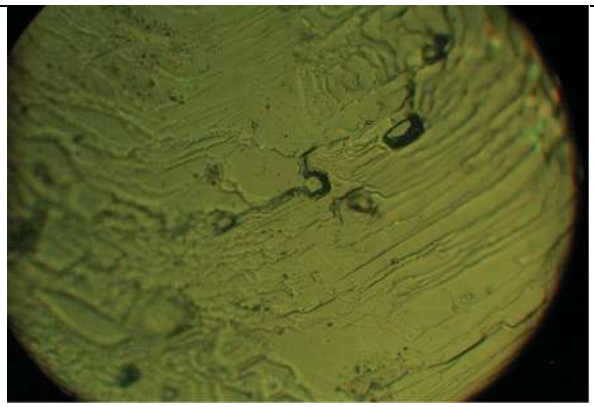

Fig. 8 Observed etch pits of as grown crystal of TTPC.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Anthropometric Assessment on Basketball, Handball and Volleyball Players

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Received: 10 Mar 2021 Revised: 13 Mar 2021 Accepted: 16 Mar 2021

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ABSTRACT

Volleyball is becoming the trending national sport in India that needs an efficient team of players. It is a performance-based sport depending on numerous factors such as positions of players and their regular training. The Anthropometric Parameters (AP) determines the appropriate playing positions of players as well as illustrates the body composition of the individuals. In addition, Motor Fitness Parameters (MFP) can enhance the quality of volleyball sport. In the existing literature, Relative Jump (RJ), Explosive Power (EP), Height in AP and Body Mass Index (BMI) The present work was successful in concluding that the BMI in MFP are exhibitive the positions of playing. While, RJ and EP are also important characteristics statistically. These can easily obtain through the latest techniques such as Support Vector Machine (SVM) as machine learning algorithm for the prediction of the appropriate playing positions. This paper presents a comprehensive review on the comparison study of numerous physiological and anthropometric between high-level volleyball players or with other sports (such as basketball, handball etc.).

Keywords: Volleyball, Anthropometric parameters, Support Vector Machine, Extreme gradient Boosting, Motor fitness parameter.

INTRODUCTION

Every single sport needs an appropriate profile for players in their training session for an efficient team formation. Recent years show several developments in providing research topics to enhance the performance of players. Team sport training requires to enhance the fitness levels and their relevance for achieving better results. It has a multifaceted approach towards the discerning of various performance factors significant for the competition. Due to

the requirement for each position and specific functions, each player has its certain physiological and anthropometric profile. However, different sports consists some common characteristics for performance comparison. Reference profiles of each discipline necessary for conducting the election processes on proficient talents. These are the essential component to properly coach the elite populations. The successful participation of basketball requires muscular power, strength and sprinting performance. Several studies show the results on notable average height of basketball players as anthropometric profile even from different nationalities. Literature studies depicts that anaerobic performance is critical with numerous crucial variables in basketball such as jumping ability, deceleration, acceleration and quick change of direction. However, all positions of the game are not necessarily connected homogenously with physical characteristics.

Previous Work with Performance Parameters

Forward and center positions in basketball depicts higher percentage of body fat due to heavier and taller nature than guards. High performance situations work on above-mentioned parameters for junior basketball players too with slightly different characteristics suggested by previous studies. Handball consists different terminologies and definitions for performance of sport proposed in literature. Gorostiaga et al. suggested that handball needed the high demands of physical capacity such as throwing abilities, jumping and stress running with high-intensity and intermittent nature. They proposed that this game needs great levels of strength for grabbing, changing speed, turning, pushing, blocking and hitting opponents during games. Hermassi et al. stated that handball is a strenuous contact sport that places emphasis on pushing, blocking, hitting, throwing, sprinting, jumping, and running. Marques explained the handball as a vigorous sport involving body contact with explosive ball throwing, change of directions, jumps and continuous prints. Numerous studies explain handball players consisting the anthropometric characteristics just like the basketball. It has the specific characteristics for the different positions during the existence of game. Goalkeepers have the higher percentage of body fat while backs and pivots incline to be the tallest players. Furthermore, hand-length of backs and body mass have the significant differences. Volleyball has to be similar physiological profiles as handball and basketball. Sheppard et al. explained volleyball as port characterized including the explosive activities (frequent and short) such as ball play, diving and jumping. Jumping activities are consisting the countermovement as blocking, jousts and jump setting. It includes the horizontal approaches for movement. Numerous research shows the relation between durable/remarkable jumping ability with an optimized fitness in volleyball. Fontani et al. recognized that high-level volleyball player performed an average of 96.5 jumps as per the previous findings during the course of a match. A proficient volleyball performance critically dependent on the tolerance of high stretch loads ability and stretch-shortening cycle performance. High-level volleyball players need the low fat percentage lean body, great height in their anthropometric profiles. Outside hitters and setters are comparatively lighter than middle blockers. Setters have the lowest standing reach, weight and average height players. However, most of the studies have the data analysis on weight behind the middle blockers, second group in height with point to these players and specific position to the right side hitters in game. Sheppard et al. performed an experiment with U-19 team of Brazil and the disciplines from U-21 teams of Brazil and Australia. Such research work consist the valuation of jump ability as the power expression that is highly correlated with squat exercise in form of strength output. Therefore, the ultimate aim of this exploration is to conduct a comparative analysis of physiological and anthropometric study between the players of high-level volleyball, handball and basketball. This study helps in the athletic orientation and in selection of future talent selection processes.

CONCLUSIONS

This research on previous works prove the significant differences in physiological and anthropometric characteristics of high-level male basketball, handball and volleyball players. It illustrated that the requirements of higher standing reach and more height for volleyball and basketball players. While lower fat mass and weight as compared to handball players. Furthermore, basketball and handball players depicted the higher power output of maximal upper

body. While, volleyball players supported the CMJ jump tests. Also, agility tests conducted on basketball players as compared with the volleyball and handball.

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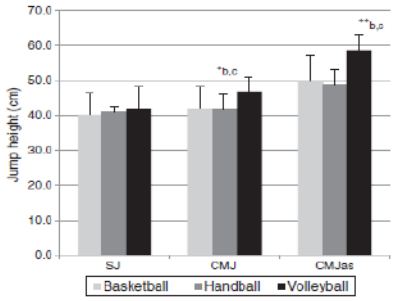
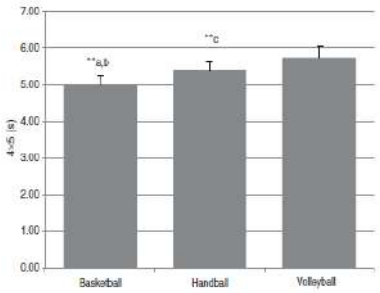


(a) Comparison of jump tests among the three sports



(b) Comparison of Agility tests among the three tests

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Green Synthesis of Nanoparticles from Moringa Seeds and Its Valuation to Adsorb Heavy Metals Nickel and Copper from Synthetic Effluent Is Imminent Promising Source to Control Water Pollution in Industrial Belt Area

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Received: 11 Feb 2021 Revised: 18 Feb 2021 Accepted: 26 Feb 2021

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ABSTRACT

Many industrial water bodies are polluted with organic and inorganic contaminants discharged into them as effluents. The quality and accessibility of drinking water are of paramount importance to human health and drinking polluted water may contain disease-causing agents and toxic chemicals and to control the risks to public health, systematic water quality monitoring and surveillance are required. Nickel (Ni) is released from smelting operations, battery industry, thermal power plants and others, acid batteries, E-waste, smelting operations, paints, coal- based thermal power plants, ceramics and bangle industry. It's very important to remove this element from contaminated water, even larger concentrations of copper in the effluent stream cause serious health problems in brain, kidney and anemia. Copper consumption at high dosages leads to serious toxicological concerns since it could be deposited in the brain, skin, liver and kidney. Bioremediation is a waste water management technique that facilitates removal or neutralization of pollutants from a contaminated site. Many plants and their extracts have been used for bioremediation of heavy metals in the process of phyto remediation. Among the various agents used for nanoparticle synthesis, plants have found important application and one such promising plant is *Moringa oleifera*, also known as drumstick is a fast growing, drought resistant plant that belongs to the family of Moringaceae. Nanobiotechnology gives emphasis to the synthesis of nanoparticles using living organisms such as microorganisms, plant extracts or plant biomass in an eco-friendly way. The biomolecules found in plants induce the reduction of Ag ions from silver nitrate to silver nanoparticles (AgNP's). The methanolic extract of *Moringa oleifera* seed was used as reducing and stabilizing agent for the synthesis of silver nanoparticle. This study aims at synthesizing zerovalent silver nanoparticles by environmentally ecofriendly method without using hazardous compounds. In this work, silver

nanoparticles were prepared using the *Moringa oleifera* seed extract (MOSE), this Synthesized nanoparticle is confirmed by the change of color from transparent yellow to dark brown which indicates the formation of silver nanoparticles. Nanoparticles were characterized by UV-Vis Spectroscopy and the capability of this nanoparticles to remove Nickel and copper from artificially synthesized synthetic effluents was then studied for Parameter like adsorbent dose, heavy metal concentration. The results suggest that AgNP's synthesized from *Moringa oleifera* seed extract have strong potential application in reducing the level of Ni (II) and copper from synthetic effluents. This studies shows *M. oleifera* as biosorbent, with the change in dosage and time exposure of this synthesized nanoparticle show the removal of copper and nickel in varying percentage, this indicate that *M.oleifera* is considered to be a promising type of nanoparticle for the removal of nickel and copper ions from aqueous solutions of pollutant water sources.

Keywords: Industrial water, Silver nanoparticles, *Moringa oleifera* seeds, Nickel, Copper, UV-Vis Spectroscopy, MOSE.

INTRODUCTION

Nanoparticles having the length within 1-100 nm scale [1] synthesized by chemical or biological methods, intends to increase the surface to volume ratio of particle [2]. Chemical synthesis methods have been showed critical effects due to the various toxic natures of chemicals absorbed or adsorbed on the surface material [3]. Synthesis of nanoparticles via biological methods promoted the cost effective and eco-friendly methods in the field of nanoscience and technology without any high pressure, energy, temperature and toxic chemicals [4]. Presently, metal nanoparticles especially gold and silver ones, are of particular interest to researchers due to their unique properties and wide application in medical field. Biosynthesis is a better alternative to physical and chemical methods [5, 6] due to eco-friendly, cost effective and less time required. Heavy metals such as Pb, Zn, Cu, Hg, etc. could pose a severe threat to human's health because they can be accumulated biologically in the food chain [7]. For example, heavy metals could cause damages to the kidneys, mental and central nervous functions, lungs, and other organs [8-10]. Moreover, heavy metals can also exert adverse effects on the environment and other ecological receptors, as they cannot be degraded by microorganisms once they are released into the environment, on the contrary, they will accumulate through the food chain. Heavy metals are highly toxic, most of which are even reported to be carcinogenic. Therefore, the removal of heavy metals from water is of great importance and has drawn tremendous attention. Up till now, numerous technologies have been developed to solve this problem, including chemical precipitation, ion exchange, adsorption, membrane filtration, electrochemical treatment, and so on. Besides, it is often the case that different techniques are combined for a better removal result. Among the techniques discussed above, adsorption is one of the most extensively used techniques due to its low cost and simple operation. This study focuses on utilizing silver nanoparticles as an adsorbent to remove heavy metals from synthetic effluents. Conventional physical and chemical methods presently have limited use in preparing metal nanoparticles due to toxic chemicals. Moreover, these methods are associated with high-energy input and costly downstream processing.

Moringa is one of the most useful trees in the world, with a huge amount of benefits. Moringa is used as a micronutrient to treat many diseases. Every parts of *Moringa oleifera* are used in a variety of traditional medicines. Accordingly, there is a strong need for effective determination of antioxidants from plant sources. The strong antioxidant properties of medicinal plants may improve the capability of plants to survive under polluted conditions. Such natural materials may provide exact advantages over synthetic ones, because they contain some essential compounds. Therefore, it is significant to verify the antioxidant compounds of *Moringa oleifera* seed extract. Antioxidative properties of phenolic acid in *Moringa oleifera* seeds arises from its great reactivity as electron or hydrogen donors from the ability to maintain, delocalize the unpaired electron (chain-breaking

function) and chelate metal ions (Fenton reaction terminator). While, flavonoids antioxidative power are resulted from distinct mechanisms includes scavenging of free radicals and chelation of metal ions. Drinking water may be contaminated by different contaminants which have an impact on the health and economic status of the consumers [3] & [4]. Contaminants such as bacteria, viruses, heavy metals, nitrates and salt have found their way into water supplies due to inadequate treatment and disposal of waste (human and livestock), industrial discharges, and over-use of limited water resources [5].Even if no sources of anthropogenic contamination exist, natural sources are also equally potential to contribute higher levels of metals and other chemicals that can harm human health, This is highlighted recently in Bangladesh where natural levels of arsenic in groundwater were found to be causing harmful effects on the population [6] & [7]. As per the periodic table the elements at the lower and middle section of the table, such as Iron, Copper, Zinc, Mercury, Lead and Manganese may be a nuisance in water or food but are dangerous. Lead and Mercury are considered to be more toxic elements as far as environmental agencies are concerned. In some tropical developing countries, the clarification of turbid river waters is an ancient practice, based on the use of natural materials that act as primary coagulants. One of these is the seed of the tropical tree *Moringa oleifera* Lam, which contains active agents with excellent coagulant activity. The extract of the seed has been mentioned by drastically reduce the sludge and amount of bacteria in waste water.

MATERIALS AND METHODS

Chemicals: Silver Nitrate, Distilled water, methanol, *M.oleifera* seeds.
Instruments Required: with hot plate, Magnetic bead, centrifuge (REMI), UV-visible spectrophotometer, Matured seeds of the *Moringa oleifera* plant were collected from the Village Bhandarkauthe, district Solapur, Maharashtra, India, in the month of December 2019. Magnetic stirrer *Moringa oleifera* seed and seed powder

Preparation of Aqueous Extract

The seeds collected from the farm were air dried at ambient temperatures (25°C) for a period of seven days before milling. They were kept away from high temperatures and direct sunlight to prevent degradation of some of the plant photochemical constituents. The seeds were separated from the membranes and broken to remove the kernel from the hard shell. The white kernel was crushed to a powder, using an electric grinder. The fine powder obtained was stored in a sterile air-tight container in a dark place to prevent oxidation.
Soxhlet Extraction of Plant Sample: The powdered kernel was then extracted with Soxhlet apparatus using sequential solvents that was 99.5%methanol for 4 to 5 hrs. The Soxhlet extraction process heats the solvent to the boiling point of that solvent.

Synthesis and Characterization of Nanoparticles

An aliquot (5 ml) of aqueous plant extract sample was added to 50 ml of 1mM aqueous $AgNO_3$. To drive nanoparticle formation the reaction mixtures were exposed to direct sunlight. Color change of the reaction mixtures were monitored to determine nanoparticle formation which is indicated by a dark brown color. Once color intensities of the solutions reached a maximum, the vessels were removed from sunlight and stored in darkness at room temperature to prevent agglomeration of the nanoparticles. A 50 ml aliquot of $AgNO_3$ containing 5ml distilled water was processed as described above and used as a negative control. Further confirmation of silver nanoparticle formation by the reduction of Ag^+ from $AgNO_3$ was achieved by UV-vis spectral analysis. Nanoparticle solutions were diluted 1:2 with distilled water and distilled water served as a blank. Nanoparticle solutions and the control were simultaneously scanned from 200 to700 nm using a UV-vis spectrometer. The green synthesized silver nanoparticles were characterized by the following methods: Color change and synthesis of silver nanoparticles from Moringa seeds. The formations of seeds extract mediated silver nanoparticles were confirmed by the spectral analysis. The UV spectra of the biosynthesized silver nanoparticles were recorded using Spectrophotometer by continuous scanning from 300nm to 700 nm and distilled water was used as the reference for the baseline correction.

Quantitative Phytochemical Analysis

Determination of Total phenolic Content

The phenolic content was determined by colorimetric assay

1. About 200 µL of Extracts, 800 µL deionized water, 100 µL of Folin- Ciocalteu reagent were mixed and incubated for 3 min at room temperature.
2. Add 300 µL of Sodium carbonate (Na_2CO_3) (20% w/v) and make up the volume up to 10ml with D/W and incubate the tubes for 2 hours at room temperature under dark condition.
3. The absorbance was determined using UV-Vis spectrophotometer at 765nm.
4. A blank was prepared with distilled water instead of aliquot extract.
5. A set of reference standard solutions of Gallic acid (10, 20, 40, 60, 80, 100 µg/ ml) were prepared. Absorbance for test and standard solutions were measured against the blank at 765 nm with an UV- Visible spectrophotometer.
6. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Standard Gallic acid solution.
7. The TPC was expressed as milligrams of Gallic acid equivalents (GAE)/g of dried sample.

Estimation of Total Tannin Content

The tannins were determined by Folin- Ciocalteu method: About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin- Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (10, 20, 40, 60, 80, 100 µg/ml) were prepared. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV-Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Estimation of Total Flavonoid Content

1. 100 mg quercet in was weighed and made up to 10ml with Methanol in a 100 ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100mcg/ml Quercet in standard solution.
2. From the stock solution, solutions of concentration 100, 200, 400, 600, and 800 mcg/ ml were prepared.
3. To 1 ml of each of these standard quercetin solution 4ml water was added followed by 0.3ml of 5% sodium nitrite.
4. After 5min 0.3ml of 10% Aluminum chloride solution and at the 6th minute 2ml of 1M Sodium hydroxide was added.
5. The total volume was made up to 10ml with distilled water. A blank was prepared without addition of aluminum chloride solution.
6. The solutions were allowed to stand for 15 min and the absorbance was measured against the blank at 510 nm using UV-Visible spectrophotometer.
7. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.
8. Preparation of sample Assay: Based on this method, each sample (1.0 ml) was mixed with 4 ml of distilled water and subsequently with 0.3 ml of NaNO2. After 5 min, 0.3 ml of 10% $AlCl_3$ solution was added followed by 2.0 ml of NaOH solution.
9. Immediately the mixture was thoroughly mixed and allowed to stand for 15 mins and absorbance was then determined at 510 nm versus blank. The results were expressed as quercet in equivalents (mg quercet in/ g dried extract).

Preparation of Synthetic Heavy Metals Solution

Synthetic heavy metals solution containing individual Cu, and Ni of known concentrations was prepared with deionized -distilled water. All chemicals used in the study, including copper sulphate, and Nickel chloride (hexahydrate) were of analytical grade and obtained from College Institute. The chemical stock solutions with a concentration of 1000ppm of each heavy metal were prepared and then different concentrations were prepared by adequate dilution of the stock solution with DDW as per need of the experiments. Dilute solutions of 0.1 M NaOH and 0.1 M HCl were used to adjust pH to give the required whole number values between 2 to 8. The pH adjustment of the solutions was made utilizing a pH meter, individual heavy metals solutions and mixture of heavy metals with known concentrations of nickel and copper were prepared, all the chemicals used in this experiment such as Nickel chloride, Copper sulphate, were of analytical grade. Different concentrations of working and standard solution of copper ions, nickel chloride from the stock solutions 1000mg/L were prepared using pure Copper sulphate, Nickel chloride and distilled water.

From the below solution a working concentration of 500 mg/L was prepared for experimental purpose.

Heavy Metals stock solutions

Standard assay for determination of Heavy Metals:

Different concentrations of heavy metal solutions were prepared from the stock solutions (100, 200, 300, 400, 500mg/l)

pH was adjusted to6

Absorbance of these solutions were taken at their respective wavelengths.

Observation

Estimation of Total Phenolic Content: Gallic acid was used as a standard and the total phenolic content were expressed in mg gallic acid equivalent (GAE) / g dry matter. Absorbance was measured using a spectrophotometer at 760 nm.

Estimation of Total Tannin Content: Tannic acid was used as a standard and the total phenolic content were expressed as TAE/ g dry matter. Absorbance was measured using a spectrophotometer at 700 nm.

Test Assay for Tannin Content

Estimation of Total Flavonoid Content: The total flavonoid content for aqueous and methanol extracts were measured with the aluminum chloride colorimetric assay using quercetin as standard. Aluminum chloride forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxide group of flavones and flavanols. Total flavonoid was expressed in mg Quercetin equivalent (QE) / g dry matter.

Effect of Contact Time and percent removal of heavy metals from AgNPs

Percent Removal of Nickel: The heavy metal removal by AgNPs and extracts were studied by varying the experimental conditions, namely effect of contact time (30 to 120 min) Furthermore, the removal efficiency was studied by varying the contact time (30 to 120 min.) with time interval of 30 minutes at 500 mg/L concentration of 50 ml heavy metal solution to optimize the time required for the removal of Nickel chloride, Copper sulphate, then 50 ul of AgNPs and Extracts were added to each metal solution and then the mixture was stirred according to the time interval on a shaker at 100rpm.

Standard Curve Determination: The heavy metal removal by AgNP‘s was studied by varying the experimental conditions, namely, effect of concentration of metal ion and effect of AgNP dosage.

Effect of concentration of metal ion: The biosorption experiments were carried out by mixing 0.05g AgNP‘ separately synthesized *Moringa oleifera* seed extract in 50 mL solution of Cu (II), Ni with concentration ranging from 30 to 150 ppm and they were kept on the shaker at 100 rpm stirring speed for 60 min.

Effect of AgNP's Dosage: The adsorbent amount, varying from (0.02g to 0.1g) was applied onto the Cu (II), Ni (II) solutions having Cu (II), Ni (II) concentration of 100ppm. The contact time was stated at 40 mins. The sample was then kept in centrifuge for about 2 min at 400-600 rpm for particles to settle down and then concentration of heavy metals in the supernatant after the treatment with AgNP‘s was analyzed using the Uv-Vis spectrophotometer at 445nm, 540nm for Ni, and cu respectively.

The percentage removal of heavy metal was determined by the formula:
% Removal= A_o -A / A_o × 100
Ao = Absorbance value before treatment, A= Absorbance value after treatment.

RESULT AND DISCUSSION

Characterization of Silver Nanoparticles by Visual and UV-visible spectroscopy Study: It is well known that silver nanoparticles exhibit yellowish brown color in aqueous and methanol solution due to excitation of surface Plasmon vibrations in silver nanoparticles. The seed extracts were mixed in the aqueous solution of the silver ion complex, it started to change the color from light green to dark brownish due to reduction of silver ion, which may be the indication of formation AgNPs. The UV- spectrum of *M. oleifera* Aqueous was recorded from the reaction medium. The results showed maximum absorption peak at 455 nm for Aqueous AgNPs.It is well known that silver nanoparticles exhibit yellowish brown color in aqueous and methanol solution due to excitation of surface Plasmon vibrations in silver nanoparticles.

1. Total Phenolics Content: Gallic acid was used as standard for total phenolic content. Total phenolic content of the seeds
2. Extracts and silver nanoparticles was comparable and it was greater in Aqueous AgNPs in comparison with other extracts and nanoparticles. The total phenolic content of Aqueous AgNPs solution was 77.03 mcg/ml ofGAE.
3. Total Flavonoid Content: Quercetin was used as standard for total flavonoid content. Total Flavonoid Content of aqueous AgNPs was more than methanol AgNPs and their seed extracts. Total Flavonoid Content of *Moringa oleifera* aqueous AgNPs was 80.33 mcg/ml of QCE.
4. Total Tannin Content: Tannic acid was used as standard for total tannin content. Aqueous AgNPs solution also showed more tannin content than other extracts and nanoparticle. Total Tannin Content of Aqueous AgNPs was 60.10 mcg/ml of TAE
5. Heavy Metals Removal: Effect of Contact Time for the removal of metal ions was studied at 500 mg/L concentration of heavy metals (Nickel, Copper heavy metals) at different times such as at 30 mins, 60 mins, 90 mins, and 120 mins. Contact time is an important parameter for determining the equilibrium time required for the adsorption of metal ions on a sorbent as it is directly proportional to amount of metal ions removed from aqueous solution. The results showed that as time increase the removal efficiency increases and *Moringa oleifera* aqueous AgNPs showed greater removal capacity than other extracts and nanoparticles. At time 120 mins the percent removal and concentration of nickel by Aqueous AgNPs was 72.78% (92.86 mg/L), for copper percent removal and concentration at 120 mins was 72.47% (123.45 mg/L) by Aqueous AgNPs .The effect of metal concentration on the nickel and copper removal by AgNP‟s: Effect of metal ion concentration on Ni (II) and cu(II) removal by AgNP‘s was studied at different concentrations of metal ion ranging from 30 to 150 mg/L keeping pH = 6 for Cu (II) and Ni (II) with, contact times for Cu(II) and Ni (II) (80 min). Increasing Cu (II), concentration showed a decrease in metal removal. Changing the initial concentration of Cu (II), Ni (II) in solution from 30 - 150 mg/L caused the percent removal to decrease from 78% to 51%, 70% to 43%

CONCLUSION

Safe water has become a competitive resource in many parts of the world due to increasing population, prolonged droughts, climate change, and so forth. Nanomaterials have unique characteristics, for example, large surface areas, size, shape, and dimensions, that make them particularly attractive for water/wastewater treatment. This study has shown that seeds of *Moringa oleifera* that has been used in traditional medicine contain high amount of antioxidant compounds. The number of bioactive components varied in plant seeds. The determination of active components from the plant seeds is significant and may lead to better utilization of the seeds by local folks. Silver Nanoparticles were prepared in the laboratory using *Moringa oleifera* seed extract and 1mM $AgNO_3$. *Moringa oleifera* seed extracts acts as a stabilizing agent for preparation of Ag+ nanoparticles which is inexpensive, and eco-friendly. These nanoparticles are free of toxic contaminations. The *Moringa oleifera* seed extracts can control the size and morphology of nanoparticles during synthesis process. The AgNPs were characterized with UV-Vis spectroscopy. The results show the effectiveness of the method used in the preparation of nanoparticles and the stability of the prepared particles and their effectiveness in water purification and environmental remediation. The preparation of AgNPs in this way has several good properties such as high reactivity, easy preparation and low cost compared to other methods of preparation of metal nanoparticles. Through the results, the % removal of nickel and copper ions depends on the pH and contact time. *Moringa oleifera* seed extracts were not that effective in removing heavy metals as compared with AgNPs synthesized from it. The efficiency for heavy metal removal is obtained maximum for Aqueous AgNPs. *Moringa oleifera* seed extract was proven to contain many effective functional groups that positively contribute to the metal ions adsorption process. Several experimental operating parameters have been found to influence the adsorption process including AgNP‘s dose and time exposure to metal ion concentration, changing the initial concentration of Cu(II), Ni (II) in solution from 30 - 150 mg/L caused the percent removal to decrease from 78% to 51%, 70% to 43% respectively for AgNP‘s. By increasing AgNP‘s dosage from 0.02g to 0.1g / 50 ml metal solution of conc. 100mg/L (100ppm) increased the Cu^{2+} removal percentage from about 18% to about 70% Similarly, there is increase in % removal of Pb^{2+} and Ni^{2+}. Pb^{2+} removal % increases from 20% to about 72% and Ni^{2+} removal % increases from 16% to about 59% -for green AgNP‘s. Thus AgNP‘s synthesized from *Moringa oleifera* seed extract can be used as an alternative adsorbent for metal ion removal from contaminated waters. The research team is currently working on the possibility of extending the study to other type metals removal Cd, Pb and Cr for comparison purpose with latest technology.

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Total Phenolic Content of *Moringa oleifera* seed extracts and Nanoparticles

Samples	Absorbance at 760 nm	Total phenolic content GAE (ug/ml)
Aqueous seed extract	0.524	50.047
Aqueous extract Silver Nanoparticles	0.694	77.031

Sr. No	Contact Time (mins)	% Removal of Nickel by Aqueous Extract	% Removal of Nickel by Aqueous Extract Silver Nanoparticles	Concentration of Nickel after treatment with Aqueous Extract (mg/L)	Concentration of Nickel after treatment with Aqueous Silver Nanoparticles (mg/L)
1.	30 mins	48.34%	53.73%	215.04	188.08
2.	60 mins	58.08%	60.17%	166.34	155.91
3.	90 mins	62.86%	65.73%	142.43	128.08
4.	120 mins	68.78%	72.78%	112.86	92.86

Total Tannin Content of *Moringa oleifera* seed extracts and Nanoparticles

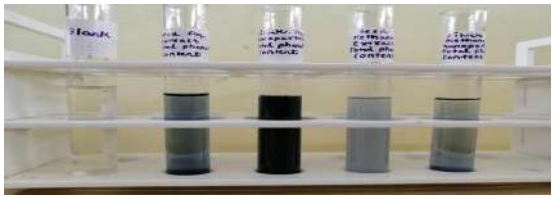
Samples	Absorbance at 700 nm	Total Tannin content TAE (ug/ml)
Seed extract in Distilled water	0.423	38.590
Silver extract Nanoparticles	0.565	60.106

Total Flavonoid Content of *Moringa oleifera* seed extracts and Nanoparticles:

Samples	Absorbance at 510 nm	Total Flavonoid content Quercetin Equivalent (ug/ml)
Aqueous seed extract	0.061	43.66
Aqueous extract Silver Nanoparticles	0.072	80.33

Percent of Copper Removal and determine the absorbance of Copper at different concentrations

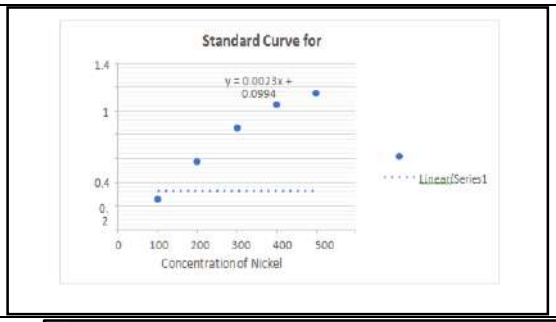
Sr.No	Contact Time (mins)	% Removal of Copper by Aqueous Extract	% Removal of Copper by Aqueous Extract Silver Nanoparticles	Concentration of Copper after treatment with Aqueous Extract (mg/L)	Concentration of Copper after treatment with Aqueous Silver Nanoparticles (mg/L)
1.	30 mins	49.33%	52.66%	244.95	227.45
2.	60 mins	55.14%	58.95%	214.45	194.45
3.	90 mins	61.71%	66.28%	179.95	155.95
4.	120 mins	68.19%	72.47%	145.95	123.45

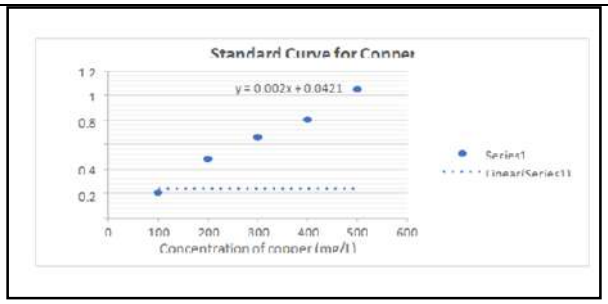


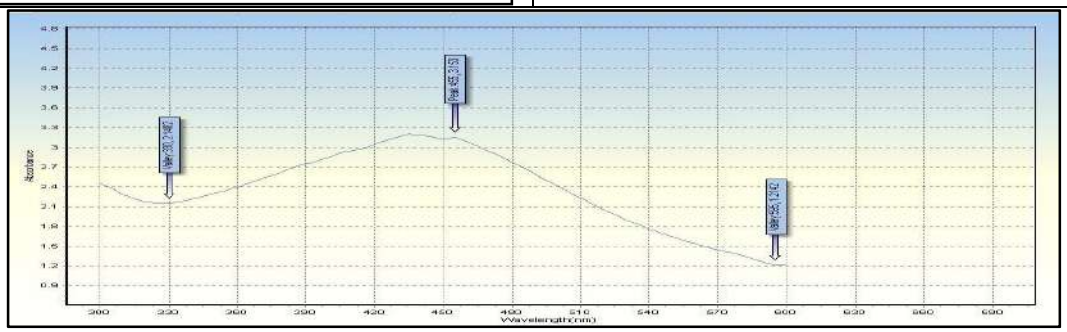
Standard Gallic acid assay Test assay for phenolic content

Quercetin Assay

Percent Removal and determination of nickel concentration at different time intervals







UV-Vis analysis of silver nanoparticles Heavy metal removal:

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Covid-19 and Health Care's Digital Technology Revolution

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Received: 12 Mar 2021 Revised: 15 Mar 2021 Accepted: 18 Mar 2021

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ABSTRACT

COVID-19's affliction has spared none regardless of country, economy, rich or poor, child or old age, salaried or entrepreneur, celebrity or common man, actor or politician, Policeman or thief, Teacher or student, Doctor or Patient. With the nature of high transmissibility and absence of effective vaccine, COVID-19 is now a global pandemic. In the twenty first century, the world has witnessed the most dreadful virus in the form of newly discovered virus called as Severe Acute Respiratory Syndrome Coronavirus 2, (SARS-CoV-2) which causes a respiratory illness disease called as Coronavirus disease 2019 (COVID-19). This COVID-19 has brought the healthcare industry in the complete spotlight probably for the first time in the history of mankind. Physical distancing, Environmental hygiene, isolation and quarantine, personal hygiene has become household terms due to the outbreak of the novel coronavirus COVID-19. The novel coronavirus has posed an unparalleled experience and challenging future by impacting more and more people around the world. Globally, from small clinics to large hospital chains are struggling to manage the COVID-19 patient population and non-COVID persons are restricted to stay in their homes. Undoubtedly, COVID-19 has impacted various sectors of the global economy including the healthcare industry. Undeniably, COVID-19 has created the necessity for the establishment of collaborative, robust digital healthcare systems with improved efficiency and this can be achieved with the help of latest technologies like Artificial Intelligence, Blockchain, Robots, Machine learning, Augmented Reality, Virtual Reality, Cloud Computing, telemedicine to secure medical data and to provide efficient, scalable healthcare solutions during uncertain times like COVID-19 Pandemic. This research paper discusses different digital technological applications adopted by healthcare tech companies with respect to COVID-19 pandemic management and response for pandemic surveillance, testing, contact tracing and effective healthcare delivery.

Keywords: COVID-19 Pandemic, SARS-CoV-2, Telemedicine, Digital Healthcare, Artificial Intelligence (AI), Machine learning (ML), Blockchain, Robots

INTRODUCTION

The COVID-19 Pandemic is devastating economies and livelihoods of people all over the world. Undeniably, it has created one of the unparalleled public health challenges in the last century by creating an acute strain on healthcare resources all over the world. Physical distancing and wearing mask will continue for a longer period of time to control the disease to save the lives of people. Healthcare tech companies in collaboration with healthcare providers are giving their best efforts to develop new means to control the pandemic. Globally, various Governments are taking coordinated efforts to contain and mitigate the COVID-19 and have achieved varied success rates. Moreover, successful strategies adopted by Government of various countries are shared among other countries related to effective surveillance, testing and contact tracing to contain and mitigate the COVID-19. Since humans are restricted within their homes due to COVI-19, digital tech companies in collaboration with healthcare providers are taking various necessary steps to effectively contain and mitigate COVID-19 by adopting latest technologies like Artificial Intelligence (AI), Machine Learning (ML), Blockchain, Telemedicine, Robots, Cloud Computing etc., In the ensuing paragraphs, various applications of digital technology in healthcare sector in response to COVID -19 Pandemic are discussed.

Redbird Company- Accra, Ghana

Each country all over the world is presently battling with the shortages of testing the COVID-19 virus that further adds to the difficulty of fighting this deadly COVID-19 disease. In Ghana, Redbird company has already been working to reduce the burden on hospitals with the help of technology to help pharmacies in order to diagnose and monitor chronic diseases by the way of saving user information on an app. The company has recently added a new feature which will help people to diagnose whether they have COVID-19 or not. The users of this app will be able to input symptoms into the app and they can keep track of their movements to identify whether they have been in contact with a confirmed COVID-19 case. Every person will be able to self-report presence of symptoms or absence of symptoms that is tagged to his/her phone number and location. This will certainly help the healthcare providers to easily map potential areas that need to be given attention and can follow-up with the high-risk patients. Meanwhile, coronavirus spreading in the workplace can also be very well prevented with the usage of this app by various companies in Ghana.

ZipLine – California, United States

It is a known fact that collection and delivery of COVID-19 test samples from the patients at rural health facilities and delivering them to the medical labs situated in the two large cities namely Accra and Kumasi located in Ghana by road transport practically will take long hours or sometimes even days. Adequate amount of samples from the hospitals need to be collected by the delivery truck before returning to the laboratory facilities and this may compromise the capability to respond rapidly. In addition to that, there is a possibility of the collected samples getting damaged during the transit especially when the cold chain is interrupted. Zipline, a California based drone-delivery start-up with the help of technology uses its drones to collect blood samples from rural health facilities in Ghana to deliver the same to the medical laboratories located in Accra and Kumasi. Healthcare providers will be able to order blood, vaccines and other medical supplies by SMS. A package of samples will be placed in a parachute in the belly of the drone located in one of the distribution centers of Zipline and once the drone reaches its particular destination, the healthcare provider will collect the required supplies from the dropped parachute and on an average it takes only 30-60 minutes.

AbCellera - Vancouver, Canada

The Canadian biotech company AbCellera is in the process of new antibody drug discovery advancements with the intersection of biology, technology, and Artificial Intelligence. Eli Lilly has partnered with Abcellara to co-develop antibodies for the treatment and prevention of COVID-19. Within 7 days from receiving a blood sample from a U.S. patient who was infected with COVID-19 and recovered from the disease, AbCellera was able to screen more than five million immune cells to identify the ones that produced antibodies to neutralize the virus and help that patient recover. AbCellara has developed its antibody identification technology in the form of a "lab on a chip" which is of the size of a credit card. It is used to consecutively test multiple antibody-producing cells at a time with the help of advanced sensors and machine learning. Potential targets are identified by the company tests with the help of hundreds of thousands of cells and then it can be narrowed down to more precise mechanisms that actually disrupt a virus' capability to infect a host. Partnering with Eli Lilly, AbCellera has examined the potential number of targets from 500 to 20. AbCellera Company's technology has already been validated by DARPA. Other partners now include pharmaceutical companies like Novartis, Gilead and Pfizer.

Scandit - Zurich, Switzerland

Scandit, is a Zurich based Enterprise barcode scanner company which offers a platform that combines technologies like machine learning and computer vision with augmented reality (AR), text recognition (OCR), barcode scanning, object recognition which is suitably designed for any camera operated smart device(s) like drones, smartphones, robots etc., There is an increase in the need for mobile computer vision on personal smart devices as the world all over is witnessing social distancing due to COVID-19 Pandemic. Thus, the company's technology will be more useful in the healthcare sector to scan medication, supplies, samples and even patient ids. For example, Augmented Reality (AR) and Mobile Scanner can scan a patient's wristband that will help the healthcare providers to access a patient's medical records and administration of medicines. Blood samples can be tracked real-time using Scandit powered mobile application and barcodes can be traced back to their origin thus helping to achieve an accurate chain-of-custody. In addition, blood bags with numerous barcodes can be scanned easily at a time rather than to scan each barcode discretely so that lot of time can be saved thus increasing efficiency.

Future Directions

Discovering highly effective vaccines is a long-term means of fighting the pandemic; it also demands large scale production and distribution apart from public acceptance. Further, there is no guarantee that a COVID-19 vaccine will protect the human beings from all types of virus that may emerge in future. May be, who knows, like the flu, yearly vaccinations will be administered in order to prevent the resurgence of COVID-19. Though, it is vital to continue developing various methods "Point-of-Care" diagnostics where patients need to visit health care centers or a centralized location to get treatment to fight COVID-19, adequate attention need to be given to continue to develop "Point-of-Need" diagnostics that can be used wherever the patient is located. When the restrictions on the lockdowns are relaxed in a phased manner or fully removed to revive the global economy and business, the employers may want their employees to undergo diagnostic tests either at their residence or in the workplace. Therefore, necessary steps need to be collectively taken by Health Organizations like World Health Organization (WHO), Centers for Disease Control and Prevention (CDC) and similar organizations/bodies, Government(s) of various countries, Health Care Policy makers, Pharmaceutical companies, Healthcare providers, Healthcare Experts, Hospitals, Healthcare Tech companies to discover affordable, portable, wide scale "Point-of-Need" COVID-19diagnostic so that human beings will be able to administer COVID-19 test themselves.

CONCLUSION

There is no second thought that the adoption of digital technology in various sectors due to COVID-19 pandemic has moved ahead of the curve during lockdown periods. Undeniably, COVID-19 pandemic is acting as catalyst for the rapid adoption of many sectors including healthcare sector. Adoption of technologies like Artificial Intelligence,

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Blockchain, Robots, Machine learning, Augmented Reality, Virtual Reality, Cloud Computing, telemedicine in the healthcare sector has certainly helped the healthcare providers and healthcare tech companies to expedite the testing of patients, surveillance, contact tracing etc., and will continue to do so in future too. Provision of Telemedicine, usage of robots for various medical facilities, remote monitoring have certainly helped both the healthcare providers and the patients to achieve a robust and an efficient health care delivery more effectively. Indisputably, COVID-19 Pandemic has accelerated not only the innovation but also the adoption of the latest digital technologies at a rapid pace to contain and mitigate COVID-19 Pandemic all over the world. Thus, healthcare providers and healthcare tech companies probably are placed in a relatively better position and far more confident to effectively manage and cure various other diseases including pandemic thus changing the landscape of healthcare delivery.

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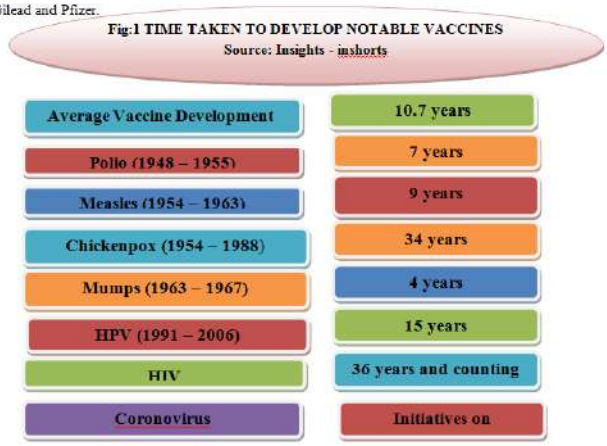
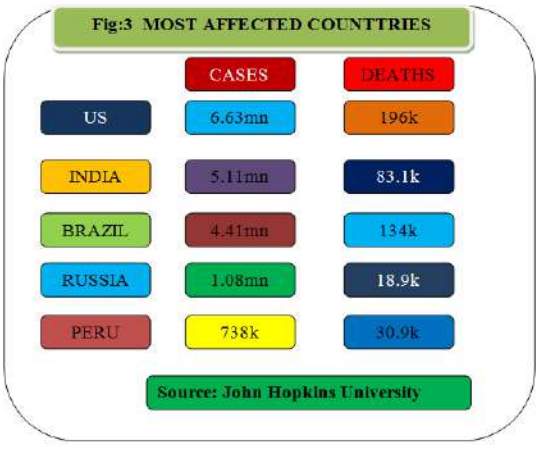
Table :1 COVID-19 Vaccine Status Tracker – Data as on September 13, 2020
Source: Insights- inshorts

Country	Vaccine		Status
United Kindgom	ChAdOx1nCoV-19	(Oxford – Astrazeneca)	Phase 3
United States	mRNA-1273	(Moderna)	Phase 3
United States	BNT162b2	(Pfizer/BioNTech)	Phase 3
China	CoronaVac	(Sinovac)	Phase 3
Russia	Sputnik V	(Gamaleya)	Final approval
India	ZyCov-D	(Zydus Cadila)	Phase 2
India	Covaxin	(Bharat Biotech)	Phase 2
170+ Vaccine Candidates in pre-clinical or clinical trials & 35 Vaccines in clinical trials			

Jilead and Pfizer.





Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluating the Acceptance Level of Cellular Service in Coimbatore District, Tamilnadu, India

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Received: 23 Jan 2021 Revised: 15 Feb 2021 Accepted: 02 Mar 2021

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ABSTRACT

Cellular service is changing the socio economic and political environment continuously and incorporates the removal of distance and communication barriers in Coimbatore district. Thus developing a model for customer's acceptance level of service is important and necessary in order to increase the sales volume in Coimbatore. The purpose of this study is to formulate a model for assessing the customer's acceptance level of service and validating a research model that best describes the customer's opinion about the service provided by the cellular service providers. This study proposes a hypothetical frame which is included the key determinants that influence the customer attitude towards the service. It has been tested through different parametric test and confirmatory factor analysis. Data analysis shows that service quality have a direct and positive influence on service guarantee and service fairness. Customer satisfactions have a positive influence on service loyalty and customer retention.

Keywords: Service quality, Service fairness, Service guarantee, Service loyalty, Customer relation, Customer satisfaction, customer retention

INTRODUCTION

The applications of quality services by telecom service providers are increasingly prevalent. Service Industry has a major role in cellular industry and the one of the significant area to do research in service loyalty. If service is properly delivered by the service providers to the customers then they can increase the customer loyalty as well as retain them. Research findings of other service industries cannot be generalized and applicable to cellular industry. The key determinants and moderators are service quality variables, service fairness, service guarantee variables and

customer relationship variables, etc. and moderators such as age, gender, income, occupation etc. The purchasers as postmodern subjects pursue the belief system of the market out of no-decision. It can't be expected that the market offers an opportunity for the decision to every one of them. Additionally, the market conveys different belief systems that go a long way past unimportant consumerism [26]. Service quality has a positive and strong effect on customer satisfaction which may attract the customer towards the cellular service provider. It has been revealed that the customer satisfaction is the most significant predictor of the brand loyalty [27]. Consumers are evaluating the quality of services at service deliveries. Service performance (SERVPERF) is measured through customer's perception of the performance of a service provider's adequate assessment for service quality (Cronin and Taylor, 1994; Bebko, 2000). Parasuraman, Zeithaml and Berry (1985) identified ten requirements useful for customers' evaluation of the quality of services: reliability, responsiveness, tangibles, communication, credibility, security, competence, courtesy, understanding the customers and service accessibility. The service quality factors namely tangibility, reliability, responsiveness, assurance, empathy and fees were noticed by Nimit and Monika (2007) to measure the service quality. A service guarantee is a set of two promises. The first is a promise to provide a certain level of service. The second is a promise to compensate the customer in a particular way if the first promise is not met (Hays and Hill, 2001; Kashyap, 2001). Ostrom and Iacobucci (1998) and Kennett et al. (1999) recognize that a service guarantee can reduce the perceived risk by setting the service standards that customers can expect. Customer satisfaction is increasingly growing for both consumer researchers and marketers from the theoretical and managerial point of view (Mishra, 2009). Several research studies uncovered the different types of satisfaction (e.g. Oliver, 1980; Vanhamme, 2002), approaches and concepts (e.g. Westbrook & Reilly, 1983; Oliver, 1993), antecedents (e.g. Fornell et al., 1996), and consequences (e.g. Fornell 1992; Fornell et al., 1996). The original scale concerned goods, so questions were modified to conform to a service exchange. Venkateswaran et al., (2015) stated that how much a satisfied customer reacts to brand, either loyalty or switching.

MATERIALS AND METHODS

Type of research

The primary objective of the study is to formulate a model for assessing the service level acceptance of customers and validating a research model that best describes the customers' opinion about the service provided by the cellular service providers. The research design for the study is descriptive. The data which were collected through a structured questionnaire from Coimbatore District.

Questionnaire design

The questions were arranged in five point Likert scale, i.e. assigning 5 point to the most positive response and 1 to the most negative response. The internal consistency of the variables was studied using Cronbach's alpha value. The Cronbach's alpha value is 0.938. To find out the factorial validity, the items were factor analysed using principle component analysis with varimax rotation. The sample was split into men (264) and women (279) and principle component analysis (PCA) was employed to identify the underling dimensionality of service in the study areas. The study was conducted in the period of September 2020 to December 2020 at Coimbatore District.

Validation of the measurement model

All internal consistency reliabilities based on Cronbach's alphas for measurement items (all interval scales) are better than 0.793, which shown a good consistency of the variables. Content validity for this research using Kaiser-Mayer-Olkin shows that the distribution of values is adequate for conducting Confirmatory Factor Analysis (CFA). The 't' statistics of the standardized factor loading of the variables in each variables are significant at five per cent and one percent level which reveals the convergent validity. It is also confirmed by the composite reliability and average variance extracted which is greater than its minimum threshold of 0.50 and 50.00 per cent respectively. The variables included in the model explain it to a reliable extent.

Results of regression analysis indicate that independent variables (service quality) account for 23.5% significant variance in service fairness (R2 = .962, F =8.869, p=.000). Standardized coefficient beta values as shown in Table-1 between service quality and service fairness are significant with significant T value when p<.001/p<.05. These results showed that service quality and service fairness are the predictors of customer satisfaction, hence, the hypotheses H1 to H9 are supported. The result similar to the studies of Wang et al. (2004) investigated the telecom industry in China, and Kim et al. (2004), Tung (2004), and Turel and Serenko (2006) investigated the mobile services in South Korea, Singapore, and Canada, respectively. These studies also supported that service quality positively influences customer satisfaction. Results of regression analysis indicate that independent variables (service quality) account for 28.2% significant variance in service guarantee (R2 = .55, F =5.362, p=.000). Standardized coefficient beta values as shown in Table-2 between service quality and service guarantee are significant with significant T value when p<.001/p<.05. These results showed that service quality and service guarantees are the predictors of customer satisfaction, hence, the hypotheses H10 to H18 are supported except H14 and H17 because their corresponding 't' value is 1.188(H14) and 1.176(H17) is less than < 2 ;p>0.001. This reflects that in cellular industry, customer expectation towards availability of network in all the time and data transfer confidentiality is high. Currently, customers are having poor opinion about the network coverage about data transfer confidentiality in certain areas.

Standardized coefficient beta values as shown in Table-3 between service fairness and demographic variables are significant with significant T value when p<.001/p<.05. These results showed that service fairness and demographic variables are the predictors of customer relationship variables, hence, the hypotheses H19 to H23 are supported with p>0.001. Adams (1965) revealed that people feel fairly treated when they perceive their economic outcomes, in proportion to their inputs, as in balance with the perceived ratio of the economic outcomes compared to the inputs of relevant others. So that fairly treated customer tries to create a relationship with the service providers. Service guarantee is not significant and not correlate with service fairness; p>0.001. Hence H24 is rejected.

Standardized coefficient beta values as shown in Table-4 between customer relationship and demographic variables are significant with significant T value when p<.001/p<.05. These results showed that customer relationships and demographic variables are the predictor of customer satisfaction hence, the hypotheses H25 to H29 are supported except H26 because their corresponding't' value is 1.58(H26) is less than < 2; p>0.001. Hence H26 is rejected. Sum et al. (2002) address the relationship between service guarantees and perceived service quality by analyzing empirical data obtained from employees and customers of a multinational hotel chain. They propose that service guarantee has a significant non-linear relationships exist between perceived service quality and its determinant variables. Standardized coefficient beta values as shown in Table-5 between service guarantee, Customer relationship and Service loyalty are significant with significant T value when p<.001/p<.05. These results showed that service guarantee is the predictor of customer relationships hence, the hypotheses H30 are supported well; p<0.001. Customer satisfaction are significant and shows a positive correlation with service loyalty and customer retention; p<0.001. Service loyalty is significantly associated with customer retention; p<0.001. Hence H31, H32 and H33 are supported. Customer relationship proneness (CRP) has an important affect on customer satisfaction (Odekerken-Schröder G., De Wulf ve Schumacher, 2003).

Confirmatory factor analysis

A confirmatory factor analysis (CFA) was required to determine the adequacy of the two models by evaluating the overall fit of the model. Estimates of the model fit indices from AMOS structural modeling shows that GFI of this study was 0.987 more than the recommended value of 0.90; the other measures fitted satisfactorily; AGFI=0.984, CFI=0.990, TLI=0.989, IFI=0.981 and NFI=0.987 with x2/DF < 5 at 1.7310 and RMSEA=0.0067 (Bagozzi and Yi, 1988) indicate a good absolute fit of the model. Goodness of fit indices supports the model and these emphasized indices indicate the acceptability of this structural model.

CONCLUSION

Besides theoretical implications, this study also provides managerial implications. This study offers managers a perspective for how consumers evaluate service from fairness point of view. How effective service guarantee can create an impact on customers relationship with the company and their satisfaction. This study also helps the managers to know about the influence of demographic variables. This study also points out that the reaction of a satisfied customers and the retention of them. i.e. once the customer is satisfied with the quality and loyal service then they may retain their customers. The study reveals that service quality, service fairness, service guarantee, customer satisfaction and service loyalty leads to customer retention for the cellular industry.

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Table 1- Regression analysis between service quality variables and service fairness

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Hypothesis
	B	Std. Error	Beta			
Reliability	.650	.056	.651	7.892	.000**	H1 - supported
Responsiveness	.252	.081	.238	3.876	.001**	H2 - supported
Assurance	-.247	.103	-.208	3.402	.001**	H3 - supported
Maintainability	-.567	.091	-.560	6.730	.000**	H4 - supported
Availability	.455	.081	.415	5.135	.000**	H5 - supported
Performance	.761	.054	.779	8.956	.000**	H6 - supported
Usability	-.204	.078	-.297	2.331	.021*	H7 - supported
Confidentiality	-.438	.098	-.413	5.403	.000**	H8 - supported
Data Integrity	.281	.078	.273	3.541	.001**	H9 - supported
R value- 0.832				F value-8.869		
R Square value-0.9622				Durbin-Watson-1.946		

**p<0.001 *p<.05

Table 2- Regression analysis between service quality variables and service guarantee

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Hypothesis
	B	Std. Error	Beta			
Reliability	.430	.062	.428	6.537	.000**	H10 - supported
Responsiveness	.211	.090	.209	2.288	.034*	H11- supported
Assurance	.312	.114	.310	3.309	.004*	H12 - supported
Maintainability	-.560	.101	-.550	5.592	.000**	H13 - supported
Availability	.144	.091	.134	1.188	.219	H14 - rejected
Performance	.402	.060	.422	5.565	.000**	H15 - supported
Usability	-.211	.087	-.209	4.356	.001**	H16 - supported
Confidentiality	.126	.107	.197	1.176	.420	H17 - rejected
Data Integrity	-.451	.086	-.427	4.568	.001**	H18 - supported
R value-0.742				F value-5.362		
R Square value-0.550				Durbin-Watson-2.219		

**p<0.001 *p<.05

Table 3- Regression analysis between service fairness, demographic variables and customer relationship variables

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Hypothesis
	B	Std. Error	Beta			
Service Fairness	.683	.050	.694	11.665	.000**	H19 - supported
Age	.519	.083	.504	6.447	.001**	H20 - supported
Sex	.253	.083	.247	2.634	.027*	H21 - supported
Income	.522	.081	.520	6.668	.001**	H22 - supported
Occupation	.285	.080	.253	2.295	.022*	H23 - supported
R value-0.813				F value-6.828		
R Square value-0.660				Durbin-Watson-1.835		

**p<0.001 *p<.05

Table 4- Regression analysis between Service guarantee, customer relationship, demographic variables and customer satisfaction

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Hypothesis
	B	Std. Error	Beta			
Service guarantee	.141	.037	.158	.110	.268	H24 - rejected
Customer relationship	-.622	.062	-.624	8.359	.000**	H25 - supported
Age	-.104	.063	-.114	-.158	.954	H26 - rejected
Sex	.329	.044	.337	2.658	.011*	H27 - supported
Income	-.446	.062	-.448	-4.352	.001**	H28 - supported
Occupation	.649	.060	.683	7.497	.000**	H29 - supported
R value-0.704				F value-4.120		
R Square value-0.495				Durbin-Watson-1.731		

**p<0.001 *p<.05

Table 5- Regression analysis between Service guarantee, customer satisfaction, Service loyalty and customer retention

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Hypothesis
	B	Std. Error	Beta			
Service guarantee	-.378	.084	-.392	4.633	.001**	H30 - supported
Customer satisfaction	.217	.094	.224	2.242	.000**	H31 - supported
Customer satisfaction	-.353	.080	-.352	3.662	.001**	H32 - supported
Service loyalty	.357	.056	.371	4.201	.000**	H33 - supported
R value-0.849				F value-9.48		
R Square value-0.720				Durbin-Watson-2.281		

**p<0.001 *p<.05

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Science of Ebonics – A Kaleidoscopic View

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Received: 26 Jan 2021 Revised: 15 Feb 2021 Accepted: 03 Mar 2021

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ABSTRACT

Ebonics refers to 'Black Sounds'. Traditionally, in Psychology, it's far mentioned as Black English. 'Ebonics' is mainly used for interacting by African Americans. In the beginning, black people lived in Africa and there were no black people in America until the slave trade began. Blacks in Africa spoke many languages, regularly wildly varying from village to village. During the age of imperialism, traders from Western Europe spoke with the natives of their colonies via simplified languages collectively known as pidgin, which mixed a European vocabulary with a native grammar. Many specific forms of pidgin have been devised and the forms of pidgin used in West Africa and the South Pacific are the most significant these days.

Keywords: Ebonics, Linguistics, Linguists, Black People, Slaves, Standard English, Creole, Dialects.

INTRODUCTION

After slaves were trapped, they were sent to forts on the West African Coast to be collected and shipped to the New World. They were surrounded by people speaking diverse languages and were taught the basics of pidgin by the slave traders that in turn laid the foundation for their communication upon arrival in America. 'Ebonics' is not similar to the slave languages of the Caribbean but is normally referred to as the progressed form of Pidgin English spoken in the United States. In the South, blacks originally spoke in pidgin and the whites spoke British English style. Since the white children were raised by a slave Nanny and had a slave playmate, the interactions during the formative stages of language acquisition meant that the white southerners' speech began to sound more like the Blacks and Ebonics more like Queen's English which the circles of aristocracy wanted to cover up. The African language structures and the black experience in America is clearly visible in the Ebonics speech structure. The end of civil war marked the beginning of freedom for the Blacks and the widespread of Ebonics throughout the nation which led to a lot of people communicating in Ebonics.

Coinage

'Ebonics is a term that was introduced by Black linguists in the mid-1970s. The word Ebonics was coined by Robert L. Williams from the words 'Ebony and Phonics' at the 1973 conference on Cognitive and Language Development of the Black English Vernacular (BEV) in the linguistic community. It refers not only to a particular grammar and syntax but also to paralinguistic (i.e., noises such as laughing and crying) and to the gesture (movement) of African American communication. This term is the offshoot of a broader outlook called Afrocentrism, an ideology that holds that identity based solely on skin color and that blacks have a unique culture, history, and even language.

Understanding Ebonics

'Ebonics' undeniably qualifies in the description of "language". There are two schools of thought regarding the origin of Ebonics- the Anglican based tradition and the African based tradition. The Anglican tradition believes that Ebonics is traceable to British dialects of Old and Middle English, which the slave picked up from white immigrants who settled in the south during the colonial era. As a result, they assert that Ebonics is just archaic white speech that has been sustained by linguistic isolation (Krapp 1925, Kurath 1949). According to the African-based tradition, Ebonics is really Africanized English, it could be traced to the formation of English pidgins and creoles during the slave trade. This perspective views Ebonics developing as a result of a language combining various European languages (Joycelyn Landrum-Brown Ph.D. 1995).

The following is an excerpt from a conversation between fourth grade Ebonics speakers (Clark, Eschholz, Rosa 1994):

Jimmy : Hey Ms. Smith, d'yaevvah watch Kung Fu on TV wifdat dude…wha's his name?
David : He have my name, Jimmy. He David, too.
Jimmy : Yeah, dat's right. Dat's duh dude's name.
Ms. Smith : Yes, I've watched it a few times. It's really an exciting show.
David : Did you evvah see how he throw all does dudes around', an' how he use his legs?
Jimmy : Yeah, You know what? He can really fight. He don't fight to be mean dough (though). He fight to be good, and he he'p people. An' he always duh good guy.
David : You know what? He one of does pries' or somefin'. Hey Ms. Smith, what is he? I can't remembah what dey call'.
Ms. Smith : Have you ever heard of the word "monk"?
Jimmy : No, what dat?
David : It's one does pries', I think, ain't it? Yea, it one does pries' dat live wifovvah pries', dose monks. He live in a convent like.
Jimmy : In wha'? Wha'sdat?
David : Ah man, ain't you know what dat is? It's where dey have people dat… people like pries' an' nuns live' dere.
Jimmy : No, but ain't he live in du desert? He always bewalkin' on a desert on TV.
David : No, but he ain't live in duh desert. He don't walk dere all duh time. He move aroun' a lot, you know. He travel all different places.
Jimmy : I'm wonderin' where he learn every fin'.
David : He learn' in duh convent when he young, I think. Dat's where dey say on TV one time.

Let's compare this excerpt to the properties of 1st language acquisition. The use of duh, dat, dose, dere, and dey is the same as the two-year-old usage of 'd' for 'th' during the 1st stages of acquisition. Another characteristic in the Ebonics' excerpt is the optional usage of the 's' ending for plurals. This is also a morphological characteristic during the 2nd stages of 1st language acquisition. There is confusion in the use of the 's' by 1st language learner at about the age of three where plurality is concerned and they add 's' to every word such as 'foots' or 'feetes' or ignoring the's'. Another shared characteristic between 1st language acquisition and Ebonics is the use of 'ed'. There is minor chaos

with this addition to where it is added to everything as in 'walkeded' and 'wented'.(G. Yule, 1996). As Blacks moved North during the Industrial Revolution displacing many immigrants in the slum areas of the big cities, their language became that of an isolated community. The limited social settings nurtured the continuation of structures of Black speech development. The absence of sustained social and cultural contact with mainstream America created a linguistic situation in which Back Speech was relatively free from White American English influences. Terms like "copping a plea" and "shucking" and "jiving" became terms used within the community of the Blacks when dealing with Whites. When providing themselves minimal comforts and opportunities in cities that allowed "restricted access" to hotels and restaurants, Blacks were often forced to recognize that it was to their advantage economically and the "part". Images of Blacks and their speech were perpetuated through the entertainment world. Radio also helped provide the stylized Black Speech and language to even a larger audience. The result was some degree of homogenization in at least the speech structures and styles used by those who had roles, which put them in association with whites. We must realize that Ebonics is a culturalised element of Black America and just as any other language; it carries the culture of its people. This is why so many Native American people are so concerned about losing their language because then they lose their history, their identity, and ultimately their culture is wiped out.

There are, for sure, a lot of terms that are not always consistently used. Many of the terms come into vogue quickly and go out of use just as they had come into use. To some extent, this problem is hard or even impossible to avoid. When so much about human language remains only partially understood, it should come as no surprise that new attempts to understand will succeed only partially- if at all. Even basic terms such as language and dialect are subject to innumerable controversies. A problem with the language/dialect distinction is that history shows, that today's dialect can become tomorrow's language. Many scholars in the social and behavioural sciences contend that the lexicons and grammars of different languages and sometimes of different dialects within the same language are associated with somewhat different cognitive patterns. Yet researchers report that groups who speak different languages often categorize their experiences in very different ways. This linguistic deviation apparently is not due to inherited difference among ethnic groups but instead due to changes in their cultural and environmental habituation. In 1976, Dr. Sheila Mayers, a black linguist, wrote that African-American modes of communication and expression, or what she called Ebonics, are grounded in African World View, a view which she claimed emphasizes rhythm, analogy, metaphor, and intuition. Black linguists have opined that Ebonics is based on an African perspective which, they say, differs radically from Euro-Western views of nature and reality.

If one actually researches the high concentration of specific tribes in geographically different places and then looks at how African language evolved in America, one notices that 'Ebonics' salvages cultural integrity from the European Wreck and honors the ancestors. It is an attempt by African-Americans to remember the African Holocaust and keep alive the tongues of their ancestors. Flagrant denigration of Ebonics is a demand by European-Americans that they deny their roots, their mother tongue, their ancestors, and their curious ability to survive. Many people are of the view that 'Ebonics' is actually a method for teaching standard English to children who speak Black English. They consider that the goal of Ebonics is to make teachers aware that children who speak Black English are using a different grammatical structure, which causes misunderstanding and allow teachers to use the children's knowledge of Black English as an aid in teaching Standard English. While some people consider Ebonics as slang, this is a stereotypical term, and therefore cannot be used as an absolute to describe a multitude of people. Standard African American English, or "Ebonics" as it is popularly known, is a legitimate, linguistically, and sociologically accepted dialect of standard American English. Its origin is a mixture of English and languages from the Niger-Congo linguistic group. The vocabulary is almost entirely English or modified English, but the grammar and pronunciation still reflect African language families in syntax and word order. It maintains other peculiarities like pronunciations that almost never end in a consonant or replacing "th" sounds with "S" or "F", which is something that new African immigrants still demonstrate in their efforts to speak English. From a social perspective, Black Vernacular English, or as it is often called, African American Vernacular English (AAVE), is fundamentally a spoken language, which encompasses the vernacular speech of blacks. Each of the black vernacular languages emerge within a particular

racial and cultural context. Most significantly, the roots of Black Vernacular English lie in the experience of slavery and in the cultural collision between a multitude of African languages and an English-speaking dominant culture.
The initial evaluation of Black Vernacular English was that it was merely an imperfectly learned approximation of "real" English, differing from it because the speakers were careless and lazy and didn't "follow the rules". It was seen as dialect, but was initially disapproved. The hypothesis was that Black children were "linguistically deprived," and that they were either not exposed to the English language as much as other children or that the language they were exposed to was in some way vastly inferior and didn't allow them to develop linguistically as they should have.
Grammatical characteristics of other English - based Creoles is more or less similar to Black English (or Black Vernacular English). On the other hand, it is noticed that as usual with the post creoles and their "parent" languages, there is a continuum found between Black Vernacular English and Standard English. Considering the individuals, there is an enormous difference between the race related dialect and Standard English. To the speakers of Standard English, Black Vernacular English is often in comprehensible and when a speaker of Standard English meets a person speaking Black Vernacular English a lot of misunderstanding, arising from wrong assumptions occurs. Black English vernacular has a regular systematic grammar of its own. There always is a difference in speaking a different variety of language which has a different grammar and this could be seen when the mistakes we make is slightly different from standard English. In African American Vernacular English, the copular verb 'be' can be omitted provided all of the following conditions are met:

- It must not be accented. 'is' should not be left out while saying, 'There already is one!'
- The sentence mustn't end with 'is', as in ' I don't know what it is'.
- The sentence mustn't begin without 'is' in a question like 'Is dat right?'
- It mustn't be an infinitive. 'be' is never left out as in something like 'You got to be strong' or an imperative like 'Be careful', or in one of those habitual aspect cases like 'He be laughin'.
- It must be in the past tense. 'was' or 'were' is not left out.
- It mustn't be negated. 'ain't' must not be left out from something like 'He ain't no fool'.
- It mustn't be first person singular. 'am' should never be left out in sentences like 'I'm yo' main man'.

Black Vernacular English was an "impoverished" version of Standard English. From these and other readily observable examples, it isn't difficult to conclude that the only reasons for the early results that implied that Black Vernacular English was an "impoverished" version of Standard English were simply artifacts of invalid methods of data gathering and interpretation. From these and other readily observable examples, it isn't difficult to conclude that the only reasons for the early results that implied that Black Vernacular English was an "impoverished" version of Standard English were simply artifacts of invalid methods of data gathering and interpretation. Actually, from a linguistics standpoint, it is still argued whether Ebonics is a form of slang or a dialect. It is a variant of English but bears enough differences in vocabulary and grammar to exceed slang. Furthermore, it is spoken by a small, ethnically distinct group, which reinforces the categorization. It is assumed that speakers of Ebonics are lazy or uneducated smacks of racism. Perhaps before we begin to decide whether "Ebonics" is a dialect or a slang, we should consider that African- American Vernacular English, also referred to as Ebonics, is a variety of English. It is a marker of ethnic identity and also a symbol of youth culture like rap. It is found among African-Americans but not all of them speak it and the features of Ebonics are more common in informal speech than in formal speech and it is more common among working-class than among middle-class speakers. The working-class speakers often see Ebonics as a marker of African-American identity and that the language can be used to gain a sense of community. They also feel that social networks may affect the influence of Ebonics.

Attitude towards Ebonics bears the stamp of the prejudice of what Ebonics is and what it says about the people who speak it. Many people think of Ebonics as broken English and the prejudices against Ebonics are closely related to racial and ethnic conflicts. There is a debate in the US among linguists about whether Ebonics is a dialect of English

or whether it is a Creole language. One important issue that all linguists seem to agree on is that Ebonics is "structured and rule-governed". According to the creole variety view, Ebonics was developed on the plantations in the southern states of America when Africans were brought to America as slaves. The African slaves are supposed to have learnt English from only a few native speakers and this would then have led to the development of a Pidgin language which expanded to a Creole language. Other linguists do not believe in this theory. They refer to a number of features of Ebonics which they claim come from older varieties of English that was once widely spoken. These linguists are the ones who argue that Ebonics is merely a dialect of Standard English. Some of the differences between Ebonics and Standard English could prove the reason why Ebonics is perceived as broken English or lazy English. However, it is important to point out that Ebonics is not broken or lazy English. It is spoken by most of the Black people in America.

Ebonics has the same vocabulary as other varieties of English. Of course, Ebonics speakers use some words that are present in other English dialects and have words that only exist in Ebonics. Sometimes it is hard to say where the words originally came from, some words can be traced to West African Languages, other words to English and some can be traced to more than one language (multiple etymologies). Pronunciation is one aspect that differentiates Ebonics from Standard English. One case where the pronunciation differs is when consonant clusters appear. The consonant cluster reduction in Ebonics is when two or more consonants, which appear at the end of a word, are reduced. This happens not only in Ebonics but also in other varieties of English. The difference is that in Ebonics the reduction is variable and systematic. In words where a nasal is followed by a vowel, Ebonics speaker nasalizes the vowel and deletes the nasal consonant. An example of this is the word man where the/n/ is deleted. Another feature of Ebonics is the merge of 'I' in 'pin' and in 'pen' before a nasal. Both the words are pronounced the same way. Only a distinctive feature in the northern US. A distinctive feature of Ebonics is the conjugated verb "be". This verb is often not used in Ebonics speech.

Ebonics (ebony + phonetics), after having been called coloured speech, Negro English, Black English, Black English Vernacular, and some abomination called Pan-African Communication Behaviours, is identified as slang, dialect, or language. But whatever it is, Ebonics is as controversial today as it was in 1855 when the first major linguistic study was done. While the term "Ebonics" goes back to 1973, the system described by that term goes back much farther. Some linguists believe that all facets of Ebonics can be found in Southern white speech. However, linguists who study the sounds, words, and grammars of languages and dialects, were more positive than the ordinary people. The distinct rules of grammar and pronunciation is obeyed by almost all human languages and dialects therefore, Ebonics is not slang. Slang refers just to a small set of new and usually short-lived words in the vocabulary of a dialect or language. Although Ebonics certainly has slang words such as chillin ("relaxing") or homey ("close friend"), to pick two that have found wide dissemination by the media- its linguistic identity is described by distinctive patterns of pronunciation and grammar. However, a look at Smitherman (1991) will reveal that Black English contains elements of Standard English, elements of West African languages, and elements unique to African-Americans, and researches on Ebonics suggests that it is not slang, but it has a distinct structure all its own.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Food Habits and Consumption Pattern of Bhil Tribe Children in Suwana Block, Bhilwara, Rajasthan

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Received: 27 Jan 2021 Revised: 15 Feb 2021 Accepted: 24 Feb 2021

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ABSTRACT

Good nutrition means maintaining a nutritional status that enables us to grow well and enjoy good fitness. The choice of food is deeply related to life style of an individual and above, in which he is living. However the food habits are highly influenced by thoughts, beliefs, notions, traditions, and taboos of the society apart from these socio-cultural barriers, the religion, education and economic factor do alter the food behaviors (habits). The study was conducted in Bhilwara district of Rajasthan state. One block was selected randomly i.e. Suwana having five gram panchayat of the block namely Bholi in Center, Kodukota in East, Gurla in West, Jodhdas in North and Hameergarh in South. Total 500 children, 50 children from each village from each gram panchayat namely Bholi, Kodukota, Gurla, Jodhdas and Hameergarh (Center, East, West, North, and South) were approached. Public tap water is the main source of water in East (58%), West (52%) North (66%) and South (60%). Maximum share of grocery expenditure is 83 percent in west zone. Majority of Bhil tribe children are non vegeterians (95%) in East in Suwana Block. Ninty four per cent children in North never carry lunch from home as there were alternate arrangement for mid-day meal. Consumption of cereals was quiet high among in Centre, East, West, North and South zone in Suwana Block. Data revealed that 100% of weekly once consumption of milk was provided by MDM only and no milk is consumed at home. Weekly one glass of milk is given by MDM. Consumption of desi ghee in Suwana block samities on daily, weekly and monthly basis were negligible. Consumption of other vegetables on daily basis was negligible except green chillies and tomato. Green chili and Tomato were consumed by 100 per cent of families daily in Suwana block generally as mix vegetables in dinner. The consumption of fruits was 68 percent in Centre, 45 per cent in East, 75 per cent in West, 54 percent in North and 84 per cent in South all because of Mid-Day Meal (MDM). Consumption of only egg was very low 5 percent in Centre, 2 percent in East, 4 percent in North and one per cent in South.

Keywords: Bhil Tribe, Children Food Habits, Consumption Pattern, Mid-Day-Meal (MDM)

INTRODUCTION

Food habits refers to why and how people eat, which foods they eat, and with whom they eat, as well as the ways people obtain, store, use and cancel food. Individual, social, culture, religious, economic, environmental and political factors all influence people's eating habits. Food habit and food beliefs are among the oldest and most entrenched aspects of any culture. Food is a requisite not only for attaining good health but also for maintaining adequate growth and body equilibrium. The choice of food is deeply related to life style of an individual and above, in which he is living. However the food habits are highly influenced by thoughts, beliefs, notions, traditions, and taboos of the society apart from these socio-cultural barriers, the religion, education and economic factor do alter the food behaviors (habits). These factors are the determinants of the food pattern of the individuals in a given society but bound to vary from a society to other, one area to other and so on. The Scheduled tribes inhabit the areas which may be included in the 'poverty square' of India, measured according to four indicators- infant mortality, female education, number below poverty line and per capita net domestic product and is centered mainly in the states of Uttar Pradesh, Bihar, Orissa, Rajasthan and Madhya Pradesh [1].

Good nutrition means maintaining a nutritional status that enables us to grow well and enjoy good fitness. Children are the wealth of any nation as they constitute one of the important segments of the populations. Good health and good nourishment are important factors in the child's growth and development. United Nations Educational Scientific and Cultural Organization (UNESCO) since 1972, for the purpose of statistics consider 6-11 years as primary school age and 12-17 years as secondary school age. Nutritional status of the population largely depends on the consumption of food in relation to their needs, which in turn is control by the availability of food and purchasing power. Consumption pattern and food habits of the tribes also need to be assessed because it differs from one tribe to another and from one region to another and it has a major issue on the nutritional status of the population. There is a rich habitat of natural foods in Indian tribal environments that need to be used to promote food surveillance, nutrition and health. Lead to development of poor nutrition and health challenges are geography, agricultural technology, cultural habits, lack of formal education, poor infrastructure, including health care facilities, and poverty. So there is an urgent need to assess as well as to document the data regarding the consumption pattern, food habits of the tribes, that will give an understanding of the existent health and nutrition related problems which will help to form various policies and programmes by the government for the welfare of tribals. This in turn will help to improve the health status of the tribes. The logic behind the acceptance of the Bhils for study rest on the fact that the Bhils constitute one of the largest tribal groups of India.

MATERIALS AND METHODS

The study was conducted in Bhilwara district of Rajasthan state. The Scheduled Caste and Scheduled Tribe population in Bhilwara district is 16.9 percent and 9.5 percent respectively whereas the State percentage of Scheduled Caste and Scheduled Tribe population is 17.8 and 13.5 respectively[2]. There are eleven block in Bhilwara district. List of blocks was obtained from the Block Development Officer (B.D.O.).From this list, one block was selected randomly i.e. Suwana having five gram panchayat of the block namely Bholi in Center, Kodukota in East, Gurla in West, Jodhdas in North and Hameergarh in South. Total 500 children, 50 children from each village from each gram panchayat namely Bholi, Kodukota, Gurla, Jodhdas and Hameergarh (Center, East, West, North, and South) were approached. This section dealt with information pertaining to the sanitary conditions, monthly expenditure on which items, the traditional food habits, daily meal pattern, carry lunch, alcoholic conditions, smoking practices, chewing materials and food consumption pattern. In food consumption pattern included information of daily, weekly, fortnightly, monthly and occasional consumption of major five food groups.

RESULTS AND DISCUSSION

The sample consists of 500 Bhil tribe children comprising of 237 boys and 263 girls aged between 4 to 12 year old. They are grouped in 4-6 year, 7-9 year and 10-12 year age group. Maximum percentage of girls (62%) is found in North Zone. For boys it is 55 percent in west zone in Suwana Block. (fig.1)

Source of drinking water

Public tap water is the main source of water in East (58%), West (52%) North (66%) and South (60%). Common Hand pump is main source in Center (58%) and West (57%). Tube well and well constitute a very small percentage of water uses. Some families are used both sources. (fig.2)

Electricity

Electricity availability was 88 per cent in Center, 86 per cent in East, 85 per cent in West, 82 per cent in North and 80 per cent in South. (fig 3.)

Lavatory facilities

Majority of families defecates in open with maximum 93% in South. Remaining Families used own W.C. built by Anganwadi.

Monthly expenditure on which items

Major part of monthly expenditure on grocery items like oil, pulses, wheat flour, rice and sugar. Maximum share of grocery expenditure is 83 percent in west zone.

Foods Habits

General food habits of Bhil Tribe Children in Suwana

Food habits are deeply related to life style of an individual and are influenced by various socio-cultural and economic factors. The general food habit of the Bhil tribe that 68 per cent of the children in West and follow two time daily meal pattern and 54 per cent children in Center follow three time daily meal pattern. (Fig 5.) In their study on Meena tribe of Madhya Pradesh reported that Meenas take their meal twice a day, one around 11-12 a.m. and another in the evening before it gets dark[3]. Majority of ninty five per cent children in East non-vegetarians. In non - vegetarian food items they prefer mutton, Meat, chicken. Consuming fleshes of rabbit, birds and animals. Chicken and mutton are used only in festival from own pet. It is a major part of their celebrations and various occasions. Seven per cent of children in Center and West were vegetarians. Bhil tribe children majority are non-vegetarians in Suwana block. Children prefer non veg only in home. (fig.6)

Eating Habits

Ninty four per cent children in North never carry lunch from home as there were alternate arrangement for mid-day meal. 100 per cent children skip morning breakfast in center, east west north and south. 19 percent children in Center were getting lunch from home. In Suwana block there was no difference in the food pattern of males, females and children in the family. All the members eat same kind of food. There is no practice of eating food in the same plate. Each member eats food in separate plate.

Alcoholic Practices

Alcohol consumption is a common practice among Bhils. It forms an indispensable part of their different celebrations. But in the case of children, mostly do not consume alcohol. No occasion is celebrated without alcohol. Women and girls consume alcohol occasionally during festivals and ceremonial days. In their study reported that

Kiad is popular local liquor of Pnar tribe of Jantia hill district. It plays an important role in their socio cultural life and is a major part of their occasions [4].

Smoking Practices

Maximum percentage of smoking was found in East zone (17%). Many children were habituated to smoking bidi due to undue influence from their parents. Still majority of the children remain non-smokers. Similarly, 22% of rural women in Kerala chew tobacco in pan (betel leaf) [5].

Chewing Practices

Maximum percentage of chewing practices were followed in West (37 %), Children are addicted due to bad influence from parents.

Food habits followed during different physiological conditions

The data depict that during various physiological conditions when the demand of body is high, majority of Bhil children follow the common meal pattern or there is no special consideration taken during various physiological conditions.

Food preferred during various physiological conditions

Illness: During illness foods that were easily digested were given to most of the patients. Foods that are liked by the patient were mostly preferred by Bhil children. Table. 1 reveals the information on foods that were most preferred during fever, Diahhroea, and cold and cough by the Bhil children. During fever *khichdi* was given to the patient as it provides energy and strength and easy to digest. In her study on traditional health care practice prevalent in tribal areas also reported that *neem* helps to develop resistance in the body to fight against fever. Curry of *neem* flowers was given to the patient with added sugar to make it edible. It helps to relieve fever and prevent further chances of infection [6]. *Moong dhal* water and *khichari* was also given as it is easy to digest. *Thuli* and *daliya* is preferred by most of the children, it is believed that it is easy to digest. Rice and curd helps to control diahhroea. *Shikanji* with added salt and sugar was preferred which helps to recover the water loss of the body. *O.R.S* taken after every loose motion helps to recover lost water. ORS Taken from school. In cold and cough black tea *(Kali chai)* with *tulsi* was preferred as it is good for health and gives relief to the body. Hot jaggery raabadi provides strength and helps in recovery.

Consumption pattern of Bhil Tribe Children in Suwana Block

Cereals

Maize and wheat were main staple cereals consumed by all the children. Consumption of cereals was quiet high among in Centre, East, West North and South zone in Suwana block. In cereals, wheat was consumed by 100 per cent children in Suwana block in the form of chapatti. Maize was used for making *Raabadi* and roti mostly in winter season. *Raabadi* is prepared by cooking maize porridge/ flour in buttermilk. 100 percent consumption of rice was twice or thrice per week in the form of Khichdi (Dal +Rice) in MDM. None of the children in Suwana block consumed rice on daily basis (fig.7). Regarding consumption of cereals, in their study on tribals of Jharkhand district reported that the consumption of cereals was significantly higher among tribals which support the above statement [7]. Similarly also found that cereals intake was higher in Baiga tribe [8].

Pulses

Among pulses, data revealed that green gram, black gram and bengal gram dal were consumed by Bhil children know as Mix dal. Bengal gram and black gram were consumed by most of the children. Majority weekly consumption of mix dal was found in children in Suwana block. Maximum of the children in East (64 %) and South (54%) were consuming mix dal (add black gram and Bengal gram) on weekly basis. (fig.7.) Pulses were sourced from market rather than cultivation therefore the consumption did not have enough variety. Similarly, lower intake of

pulses by tribal women of Ranchi as compared to RDI [7]. In her study also reported low intake of pulses in tribal of Rajasthan [9].

Milk and milk products

Data revealed that 100% of weekly once consumption of milk was provided by MDM only and no milk is consumed at home. Weekly one glass of milk is given by MDM. 76 per cent children of west and 84 per cent of North were consuming buttermilk on the weekly basis. Salt is added before eating. (fig 7.)

Fats and oils

In fig 7. Shows 100 percent children in Center, East, West, North and South has daily consumption of oil in the form of dal and vegetable. Consumption of Desi ghee in Suwana block samities on daily, weekly and monthly basis were negligible. In north 12 percent and 9 percent in south children consume *Desi ghee* occasionally. Children in Suwana block did not consume *Desi ghee* at all.

Green leafy vegetables

Bhaji is made by mixing all green leafy vegetable like meethi, radish leaves etc. Maximum green leafy vegetable were being consumed by maximum children in Suwana block. Consumption of green leafy vegetables was generally twice or thrice a week. Only Methi leaves consumption is 37% in both North and South on weekly basis. Green leafy vegetable is consumed only in winter season.(fig.7) The study of on Bhils of Gujarat was found supportive which states that the consumption of green leafy vegetables was done in large quantity when they are easily available[10].

Other vegetables

Bhils children were consuming low cost vegetables which were easily available in market and open field. The most commonly consumed other vegetables were green chillies. Consumption of other vegetables on daily basis was negligible except green chillies and tomato. Green chili and tomato were consumed by 100 per cent of families daily in Suwana block generally as mix veg in dinner. Karunda was consumed weekly by 53 percent children in North and 14 per cent in East as it is easily available from open fields. (fig 7). Brinjal was also consumed weekly by 26 per cent in Centre, 14 percent in East, 34 percent in West, 32 per cent in North and 57 per cent in South.

Roots

100 percent children in Suwana block consume potato twice or thrice per week. Only 17 per cent children in Centre, 56 per cent in East, 37 per cent in West, 49 per cent in North and 64 per cent in South children consume onion in daily basis. (fig 7.) 100 per cent families in Suwana block consume garlic daily in the form of dal and vegetable and chutney.

Fruits

The consumption of fruits was 68 percent in Centre, 45 per cent in East, 75 per cent in west, 54 percent in North and 84 per cent in South all because of MDM. Apart from MDM, it is very low due to poor socioeconomic status and seasonal availability. 45 percent in Centre, 12 percent in East, 57 percent in West, 16 percent in North and 26 percent in South consume zizphyus twice and thrice in a week.(fig 7.) It is wild fruit and is easily available. Low cost fruits like Guava and Banana are distributed as part of MDM in school. The study on diet of Oroan tribe was found supportive with our study [11].

Non vegetarian foods

Ninety five percent children in East are non-vegetarians. In non -vegetarian food items they prefer consuming rabbit, domestic birds and locally available animals twice or thrice in a week. Chicken and mutton is used only in festival. It is a major part of their celebrations and various occasions. 94 per cent children in south, 88 per cent in Centre, 93 percent in West, 92 per cent in North zone and 15 per cent in south use non veg only occasionally. Children prefer

non-veg only in home. Consumption of meat and chicken was very low and infrequent due to their low purchasing power. Consumption of egg was very low 5 percent in Centre, 2 percent in East, 4 percent in North and one per cent in South. Reported in their study on nutritional status of tribes of Rajasthan that all the tribes were non vegetarian but the intake of animal food was lacking because of its higher price and the low availability [12]. Also reported low consumption of animal products in tribal diet [13].

CONCLUSION

In this study Majority of ninety five per cent children in East non-vegetarians. Still majority of the children in Suwana were remain non-smokers. Ninety four per cent children in North never carry lunch from home as there were alternate arrangement for mid-day meal. 100 per cent children skip morning breakfast in Center, East West North and South. Wheat was consumed by 100 per cent children in Suwana block in the form of chapatti. Maize was used for making *Raabadi* and roti mostly in winter season. Majority weekly consumption of mix dal was found in children in Suwana block. 76 per cent children of west and 84 per cent of North were consuming buttermilk on the weekly basis. Cereals, pulses and milk and milk products consumption of Bhil tribe children in Suwana block is very much. Mainly reason of fruits, milk and pulses consumption is high because school going children had this in MDM. Potato, onion and green chili consumption are also highly in Bhil tribe children in Suwana Block.

ACKNOWLEDGMENTS

We would like to express our gratitude to Dr. Pramod Kumar Raghav, Department of Food Technology & Dairy Science, Jayoti Vidyapeeth Women's University, Jaipur for providing us the opportunity. We also extend our sincere thanks to the people and children of Suwana Block, Bhilwara, Rajasthan who participated in the study with full zeal & enthusiasm.

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Table 1: Food Preferred By Bhil Children during Various Physiological Conditions

S.No.	Condition	Type of Food	Reason
1.	Fever	1. *Khichari*	• Easy to digest
		2. *Thuli (dalia+moong dhal water)*	• Easy to digest
2.	Diahhroea	1.Shikanji	• Helps to recover lost water
		2.Rice+Curd	• Helps control diahhroea
		3.O.R.S taken after every loose motion	• Helps to recover lost water
3.	Cold and cough	1. Black tea with tulsi	• Good for health in cold
		2. Hot Raabadi	• Provides strength and helps in Recovery

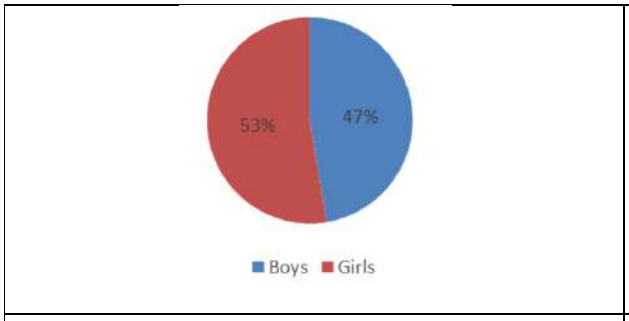

Fig 1: Boys And Girls ratio in percentage in Suwana Block

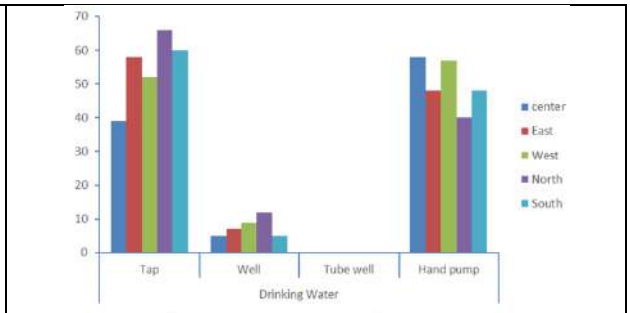

Fig 2: Sources of Drinking Water in Suwana Block

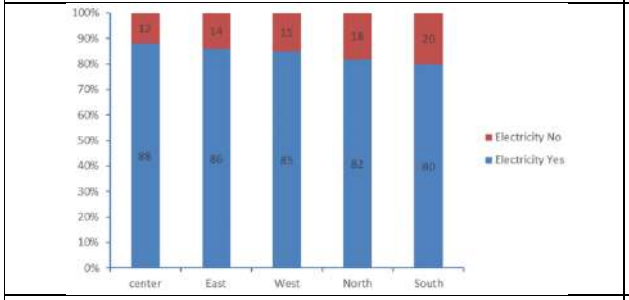

Fig 3: Electricity Availability and Status in Suwana Block

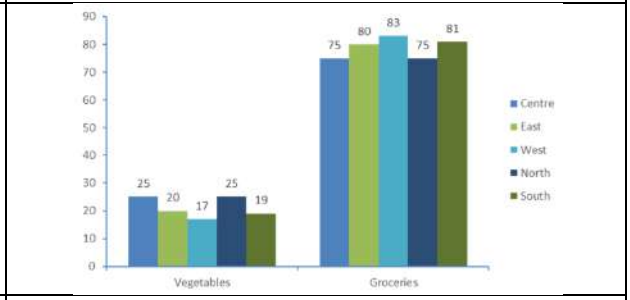

Fig 4: Monthly Expenditure Profile in Percentage on Vegetables and Groceries

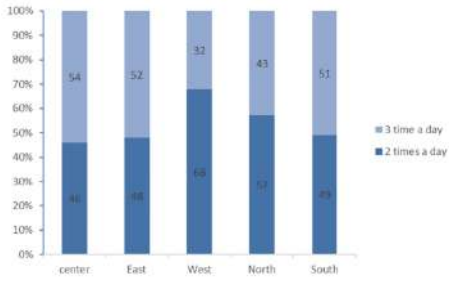

Fig. 5: Meal Pattern of Suwana Block Children

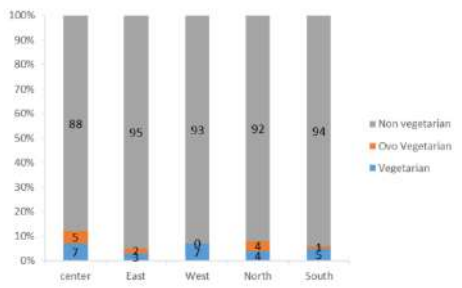

Fig.6: Food Habits of Children in Suwana Block

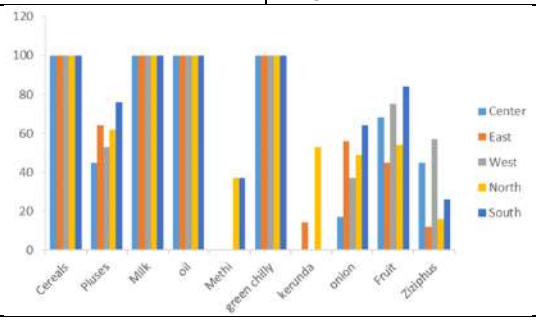

Fig 7: Bhil Tribe Children Food Consumption Pattern in Suwana Block

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Determination of Total Polyphenols and Flavonoids Contents and Iron Reducing Power of Aerial Parts of *Seseli praecox* Guss. from Algerian Flora

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Received: 21 Mar 2021 Revised: 24 Mar 2021 Accepted: 27 Mar 2021

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ABSTRACT

In this study, *Seseli praecox* of Algerian flora was investigated regarding their polyphenolic and flavonoids contents using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. TPC of crude aqueous ethanolic extract was 44mg± 0.09GAE/gDw, while, TFC have values of 1.53mg±0.01 QuercetinE/gDw and 6.93mg±0.01 RutinE/gDw for ethanolic extract, which mean that this plant is relatively poor source of polyphenols and flavonoids . In the other side, the polyphenolic extract of this plant was evaluated for its reducing power. The capacity of the extract to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex mention that *Seseli praecox* showed a very high iron reducing power activity with Ec_{50}=0.25mg/ml.

Keywords: *Seseli praecox,* polyphenols, flavonoids, reducing power activity.

INTRODUCTION

Polyphenols have recently aroused considerable interest because of their potential beneficial biochemical and antioxidant effects on human health. Commonly referred to as antioxidants, they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular diseases, inflammation and others. Most of the experimental results confirmed that polyphenols have several biological activities including radical scavenging, anti-inflammatory, anti-mutagenic, anti-cancer, anti-HIV, anti-allergic, anti-platelet and antioxidant activities[1]. Aromatic and medicinal plants have attracted attention as a significant source of natural products all over the world [2].The Apiaceae (or Umbelliferae) is a plant family comprising at the present time 466 genera and about 3800 species. It is distributed nearly worldwide, Africa has about 121 genera, where North Africa encompasses the largest occurrence of 82 genera, 13 of which are endemic [3]. This cosmopolitan family occupying an important area in algerian flora with 56 genra and 130 species (which 24 are endemic). *Bupleurum* and *Daucus* were more abundant with 14 and 11 species, respectively [4].The genus *Seseli* is an important genus of the Apiaceae family, with a large number of aromatic species ,comprised of 55 species distributed mainly in Europe. *Seseli* is a genus of herbaceous perennial plants. They are sometimes woody at base with a conic tap root. Leaf blades are 1–3-pinnate or pinnately decompound. Umbels are compound, with bracts few or absent. Petals are white or yellow, and the fruit ovoid or ellipsoid [5].Species from the genus *Seseli* L. have long been used extensively in tradition medicine in Mediterranean region, but there is quite limited information on their phytochemicals and biological activities [6].*Seseli* species are well-known source of the linear or angular pyranocoumarins possessing antiproliferative, antiviral and antibacterial activities [7].Traditional uses of *Seseli* species as anti-inflammatory agents were supported by the results of Kupeli [8].*Seseli praecox* is a rare species of algerian flora [9], herbaceous perennial plant with a thick and branched stems of 10 to 30cm, subline at the base, bears leaves divided into segments cut into lobes arranged in a fan pattern. The white flowers form umbels with poorly developed involucres. The high demand and curiosity for natural products, especially for the development of new drugs and in the course of our continuing search for bioactive natural plant products, this study is destined to the determination of total polyphenols and flavonoids contents of the plant *Seseli praecox* growing in Algeria, as well as to estimate their reducing power activity, were conducted for the very first time.

MATERIALS AND METHODS

Plant collection and identification

Seseli praecox was collected on April 2017 at Edough peninsula, at Annaba province in the north east of Algeria, and identified by Professor Tarek HAMEL, Laboratory of biodiversity and phytogenetic resources, University of Badji Mokhtar Annaba, Algeria. A voucher specimen is deposited at the herbarium of laboratory of chemistry of university Mohamed Boudiaf of M'sila, Algeria.

Extraction

The fresh, whole plant was collected and shade dried to obtain 100 g dry sample which was later coarsely powdered and used for solvent extraction. The sample was extracted at room temperature for 3 × 24 hours. The extraction was performed with 70% aqueous ethanol solution (70 ethanol /30 water, v/v). The extract were filtered through a Buchner funnel, The aqueous solution was extracted with hexane several times to eliminate lipids and the solvent was removed on rotary evaporator (BÜCHI, 461, Germany) to dryness under reduced pressure at 40° to give crude extract of polyphenols [10].

Determination of total phenolic content

The total phenolic content (TPC) was determined by using the Folin-Ciocalteu assay. This phenol reagent consists of a mixture of the heteropoly acids, phosphomolybdic and hosphotungstic acids in which the molybdenum and the tungsten are in the 6+ oxidation state. On reaction with a reductant, the molybdenum blue and the tungsten blue are

formed and the mean oxidation state of the metals is between 5 and 6[11]. In brief, standard curve using gallic acid (7.81 and 250 μg/ml) was made with following steps: 0.2 mL of the diluted sample extract was transferred in tubes containing 1.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. After waiting for 04 minutes, 0.8mL of a sodium carbonate ($NaCO_3$) solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min before absorbance at 765 nm was measured. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 mL of plant extract. The concentration of polyphenols in samples was derived from a standard curve of gallic acid using regression equation. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained [10] (Fig.1).

Determination of total flavonoid content

The total flavonoid content was determined according to the aluminium chloride colorimetric method. The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at $\lambda max = 430$ nm. The same procedure was repeated for the standard solution of rutin (2-64 μg/ml) and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of extract). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained [11] (Fig.2).

Reducing power

The reducing power of the crude mixture of polyphenols and BHT was determined according to the Topcu et al. method. The capacity of the extract to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by recording the absorbance at 700 nm after incubation. For this purposes, 250 μl of extract prepared at different concentrations were mixed with 250 μl of phosphate buffer (0.2 M, pH 6.6) and 250 μl of potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. 250 μl of Trichloro acetic acid (10%) was added. The supernatant was mixed with 1 ml of distilled water and 0.2 ml of ferric chloride was added, and then the solution's absorbance of the incident radiation was determined spectrophotometrically at 700 nm. The absorbance at 700 nm was used as the indicator of the reducing power. Increased absorbance of the reaction mixture indicated increased reduction capability.BHT was used as standard antioxidant [12] (Fig.3).

RESULTS AND DISCUSSIONS

Extraction yield and total polyphenols and flavonoids content

In the aerial parts of *Seseli praecox,* the extraction yield was obtained with 70% aqueous ethanol solvent which gives a brown mixture 10.75% o dry weight of plant. The total phenolic content (TPC) of the plant extract was measured using the Folin–Ciocalteu colorimetric method, based on the principle of reduction of phosphomolybdic acid by phenols in the presence of aqueous alkali [13]. *Seseli praecox* have 44mg±0.09 GAE/g Dw (expressed as gallic acid equivalents (GA) per gram of dry extract). This result is nearly the moiety when we compare it with previous studies concerning *Seseli* species. Data for the extraction yield of phenolic substances from some *Seseli* species have been published by Matejić *et al.* [14] where specific differences exist because of different methodologies of experimental work as well as different variety of *Seseli* samples. This can be explained probably because of the difference between species and other factors like culture conditions. Extraction time, temperature, solvent-to-feed ratio, the number of repeated extractions of the sample, as well as the choice of extraction solvents are the crucial parameters affecting the extraction yield. The yield and rate of polyphenolic extraction are related to the solvent characteristics. It has been observed that methanol is more efficient in the extraction of lower molecular weight polyphenols while aqueous acetone is a suitable solvent for the extraction of the higher molecular weight flavanols [15].The total flavonoidic content (TFC) of the plant extracts was determined by using the aluminum chloride colorimetric method, based on the reaction of aluminum ion with flavonoids at alkaline medium forming red chelates [13]. For *Seseli praecox*, TFC

have values of 1.53mg±0.01 Quercetin E/gDw and 6.93mg±0.01 Rutin E/gDw for ethanolic extract. For best extraction yield of phenolic compounds and flavonoids, we will discuss extraction conditions. In fact, recent studies of different *Seseli* species were focused on essential oil analyses [16, 17 and18]. This study mentions that the species *Seseli praecox* is a source of polyphenols and flavonoids.

Evaluation of reducing power

Antioxidants are important substances that possess the ability to protect the body from damage caused by free radical–induced oxidative stress. A variety of free-radical scavenging antioxidants exist within the body, many of which are derived from dietary sources such as fruits, vegetables, and teas. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity[19]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants[20]. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{+3}/ferricyanide complex used in this method to the ferrous form.

In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}) [20]. The results of this research showed that the plant *Seseli praecox* exhibited a significant iron reducing power with Ec50=0.25mg/ml. In fact, kæmpferol, rutin and quercetin (three flavonoids) have an important reducing power with Ec_{50} of 5.70**,** 6.15 and 1.48 μg/ml*,* respectively [21].It is widely accepted that some pathological events such as heart disease, chronic renal failure, diabetes mellitus, cancer, immune dysfunction, and aging are closely related to the peroxidation reactions in living organisms . Antioxidants are supposed to protect cell membranes against free-radical oxidative damages. There is growing interest to replace synthetic antioxidants by natural ones mostly found in plants. There is also a worldwide trend toward the use of natural additives in foods and cosmetics. For this reason, an extensive search for different types of antioxidants in various types of plants has been undertaken [19] (Fig.4).

CONCLUSION

A literature survey showed a few phytochemical and biological investigations concerning the plant *Seseli praecox* . Results of our study suggest that the species *Seseli praecox* which screened for the first time, is a source of polyphenols and flavonoids and exhibited a high iron reducing power with Ec50=0.25mg/ml which is near to the standard (BHT). We have, also, warranted the concentration dependent reducing ability of the extract. Further studies will help in identifying the individual compounds that aids in the reducing power and to identify the synergistic effect. Also a correlation between the reducing power and antioxidant activity can be derived. The extraction of the essential oil from this plant will carried out.

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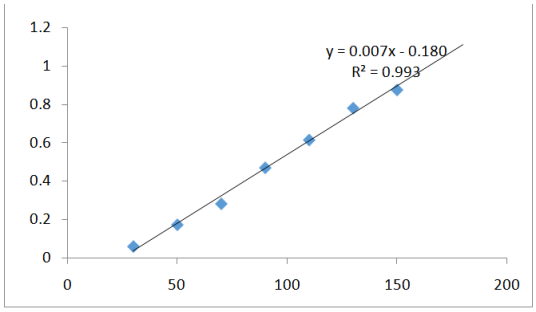

Figure 1: Calibration curve of standard gallic acid for determination of total phenolics content.

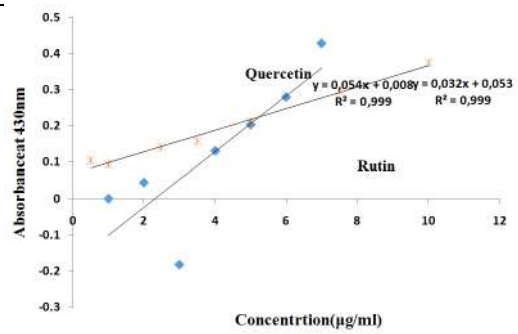

Figure 2: Calibration curves of standards quercetin and rutin for determination of total flavonoids content.

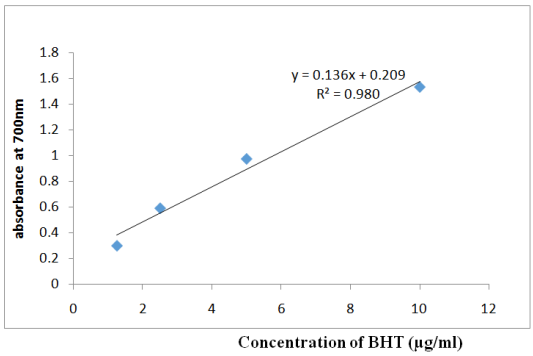

Figure 3: Reducing power of BHT

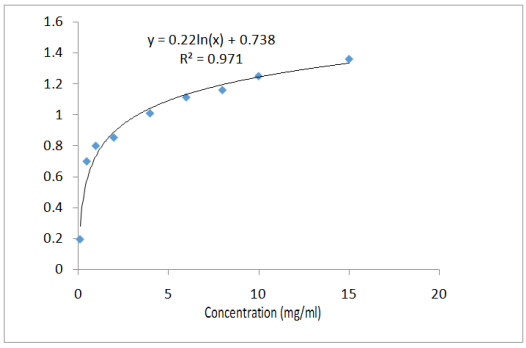

Figure 4: Reducing power of *Seseli praecox* extract

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Anti-Diabetic Effect and its Action on Relative PKB Gene Expression in Streptozotocin Induced Diabetic Rats Treated with *Begonia trichocarpa* Dalz Leaf

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Received: 23 Feb 2021 Revised: 28 Feb 2021 Accepted: 02 Mar 2021

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ABSTRACT

This work is designed to study the effect of *Begonia trichocarpa* Dalz leaf on diabetic and its influence on Relative PKB Gene expression in Streptozotocin induced diabetic rats by using selected methanol extract and ethyl acetate extract. Glibenclamide was used as a standard for comparing the anti-diabetic activity. A dose dependent activity observed. The present study proved that the animals treated with methanol extract and ethyl acetate extract at the dose levels of 200mg/kg, 400mg/kg showed reduction in blood glucose level, hypercholesterolemia and hypertriglyceridemia when compared with diabetic control. The effect of methanol extract and ethyl acetate extract of *Begonia trichocarpa* Dalz leaf on serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), glycosylated hemoglobin and serum alkaline phosphatase were found to be reduced. Hb level of treated animals were found to be recovered. The pancreas section showing hyperplasia of islet was recovered, mild congestion of pancreatic parenchyma was seen and number of islets increased. It was already reported that Streptozotocin induction will decrease the level of protein kinases such as PKB, which will lead to decreased pancreatic mass and subsequent insulin insufficiency. They showed a positive effect of Protein kinase B gene expression and has increased the levels of Protein kinase B in all treated groups. The results show the role of extracts in enhancing the expression of PKB, which in turn can support the theory of promoting beta-cell survival. *Begonia trichocarpa Dalz* leaf is a non toxic anti diabetic crude drug which improve insulin insufficiency through a positive effect of Protein kinase B gene expression.

Keywords: Protein kinase B, Glibenclamide, *Begonia trichocarpa Dalz,* Streptozotocin

INTRODUCTION

According to international diabetes federation, globally as of 2010, an estimated 285 million have type II diabetic and an increase of 90% of cases in 2013 1,2,3. The greatest prevalence are however expected to occur in Asia and Africa. The increase in incidence in developing countries is due to the change to western style in diet5,6. Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both7. It may due to autoimmune destruction of the β- cells of the pancreas with the consequent insulin deficiency that lead to abnormalities that result in resistance to insulin action8. Discovery of PCR brought enormous scientific development, such as genome sequencing, gene expression in a recombinant system. PCR has revolutionized the diagnosis of disease and investigation of their hidden factor at the genetic level9,10. The development of amplifying DNA sequencing has developed gene analysis as well as way to diagnosis many genetic diseases11,12. The gene has a property to switch on and off when they require. If the gene is not expressed means it should not show the character related to that gene. Gene expression can be identified by using markers 13,14,15. *Begonia trichocarpa Dalz* (Family: *Begoniaceae*) is a genus of *Begonia*, a perennial flowering plant, the genus contains 1,795 different plant species16,17. The hypoglycemic and anti-hyperglycemic activity of *Begonia malabarica Linn* was reported [1].Anti-oxidant [2] and anti-cancer [3] activity of *Begonia trichocarpa Dalz* was reported.

MATERIALS AND METHODS

Collection, Authentication and Preparation of *Begonia trichocarpa* Dalz Leaf Extract

The whole plant of *Begonia trichocarpa Dalz* was collected from the rural areas around the Kottayam district, Kerala, India. The whole plant material was identified, authenticated. About 1 kg of air dried, powdered leaf was extracted in soxhlet apparatus assembly successively with hexane, petroleum ether, chloroform, ethyl acetate and methanol and water (in the order of increasing polarity). The solvents were removed completely under reduced pressure and a semi solid mass was obtained 18,19.

Collection and Acclimatization of Rats

Healthy Wistar Albino rats of either sex, weighing about 150-200 gm were procured from animal house from K. M College of Pharmacy, Madurai. The entire study was approved by the Institutional Animal Ethical Committee (IAEC) which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

The animals were kept in clean and dry polycarbonate cages and maintained in a well-ventilated animal house with 12hours light – 12 hours dark cycle. The animals were fed with standard pellet diet and water was given *ad libitum*. The rats were maintained in this condition for a period of three weeks to acclimatize them prior to experimental uses. For experimental purposes the animals were kept fasting overnight, but allowed free access to water 20,21.

Experimental Design

Acute Toxicity Study of Methanol and Ethyl Acetate Extracts of *Begonia trichocarpa* Dalz Leaf

Acute toxicity study was performed according to OECD guidelines 423 acute toxicity class method. It is a stepwise procedure with three animals of single sex per step. Depending on the mortality and the morbidity status of the animal, on average, of 2-4 steps may be necessary to allow judgment on the test substance. The procedure is to fix a minimal number of animals, which allows acceptable database for scientific conclusion. The method uses different defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the "Globally Harmonized System" (GHS) for the classification of extracts which cause acute toxicity 22,23,24.

Evaluation of anti-diabetic activity of ethyl acetate and methanol extract of *Begonia trichocarpa* Dalz leaf

In the present investigation, a total of 42 rats (36 diabetic surviving rats and 6 normal rates) was taken and divided into seven groups of 6 rats each to determine the anti-diabetic activity of EABT and MEBT. The actions of the extracts were compared with that of the standard oral hypoglycemic agent, Glibenclamide. Group I: Rats are free access to water and food. Group II: Diabetic rats are free access to water and food. Group III: Diabetic rats treated with standard drug Glibenclamide at the dose of 0.5mg /kg b/w, orally for 28 days. Group IV: Diabetic rats treated with EABT 200 mg/kg b/w, orally for 28 days. Group V: Diabetic rats treated with EABT 400 mg/kg b/w, orally for 28 days. Group VI: Diabetic rats treated with MEBT 200 mg/kg b/w, orally for 28 days. Group VII: Diabetic rats treated with MEBT 400 mg/kg b/w, orally for 28 days. The rats were fasted for 12 hrs. Diabetes mellitus could be induced in experimental animals by single dose intra peritoneal administration of STZ at a dose of 60 mg/kg body weight. The animals were then kept for next 24 hrs on 5% glucose solution. The control rats were injected with saline only. After 72 hrs of STZ administration, the blood glucose content was measured by using glucometer, a blood sample from the tail vein. The animals with blood glucose levels ≥ 180 mg/dl were considered to be diabetic and used for the experiment 25,26,27.

Relative Expression of Protein Kinase Gene by the Effect of *Begonia trichocarpa* Dalz Leaf - PCR Technology.

Relative expression, protein kinase Gene expression in streptozotocin induced diabetic rats treated with ethyl acetate and methanol of the *Begonia trichocarpa Dalz* leaf was done reverse transcriptase polymerase chain reaction analysis. GAPDH was used as housekeeping gene 28,29.

Preparation of Tissues for Isolation of RNA

At the end of the fourth week of anti-diabetic study, all MEBT and EABT treated rats were sacrificed by cervical dislocation while fasting in the morning of the respective experimental day. Pancreas of the sacrificed animals was removed immediately and kept at 10% trizol reaction mixture.

Isolation of Total RNA (Trizol Method)

Tissue was homogenized with trizole reagent then treated with chloroform shaking was done vigorously for 15 seconds and incubated for 2-3minutes at room temperature, followed by centrifugation at 14000 rpm for 15 minutes at 4◦C.Aqueous layer treated with 100% isopropanol, incubated for 10 minutes ,centrifuged. The pellet was washed with ethanol by centrifugation. The RNA pellet was dried and suspended in TE buffer 30,31.

Synthesis of c DNA of Isolated RNA

iScript™cDNA Synthesis Kit (BIO-RAD Product code 170-889) reagent was used for the synthesis of cDNA .The cDNA synthesis was performed mixing 5x iScript Reaction Mix, iScript Reverse Transcriptase, RNA template, and nuclease free water. The thermal cycler (Eppendorf Master Cycler) was programmed to undergo cDNA synthesis. The following cycling conditions were employed for 5 minutes at 25ºC, 30minutes at 42ºC and 5 minutes at 85ºC 32.

Amplication of cDNA in Reverse Transcriptase Polymerase Chain Reaction

Primers

GAPDH

Forward 5'AATGCATCCTGCACCACCAACTGC

Reverse 5'GGAGGCCATGTAGGCCATGAGGTC

PKB forward 5'-GCC CAA CAC CTT CAT CAT-3'

PKB Reverse 5'-GGA CAC GGT TCT CAG TAA GC-3'

Reaction mixture was prepared by mixing PCR Master Mix, Forward primer, Reverse primer and Template DNA. Denaturation at 95°C and annealing at50-60 °C for 30 s and extension at 72°c for 1 minute, which was repeated for 35

cycles and the final extension at 72°C for1 5 minutes 33,34.

Separation and Visualization DNA Fragments by Agarose Gel Electrophoresis

After the amplification, the PCR product was separated by agarose gel electrophoresis**.** The gel was loaded with the samples and run at 50 V for 30 minutes. The stained gel was visualized using a gel documentation system (E gel imager, Invitrogen). Gel loading order of extracts 35.

Lane 1 G1C Lane 2 G5EA2 Lane 3 G6ME1 Lane 4 G2S
Lane 5G7ME2 Lane 6 G3PC Lane 7 G4EA1

RESULT AND DISCUSSION

Acute Toxicity Study of Selected Extracts of *Begonia trichocarpa Dalz* leaf

There was no mortality observed even at 2000 mg/Kg for the EABT and MEBT. All the animals were found to be normal and there were no behavioural changes till the end of the observation period. This observation revealed that the EABT and MEBT were found to be very safe up to 2000 mg/kg of body weight known as the maximum tolerated dose (MTD) by the acute toxicity model study as per OECD guidelines 423. Hence, from this 1/10th and 1/5th of MTD was selected and the effective doses were fixed at 200 and 400 mg/kg for further pharmacological studies. The toxicity studies of MEBT and EABT provide the indication that the *Begonia trichocarpa Dalz* is a less or no toxic to the animal 36,37.

Evaluation of Anti-Diabetic Activity of Ethyl Acetate and Methanol Extract of *Begonia trichocarpa Dalz* leaf

Anti-diabetic activity of the *Begonia trichocarpa Dalz* leaf was studied by determining the anti- diabetic effect of selected methanol extract and ethyl acetate extract on Streptozotocin induced diabetic rats 38. Glibenclamide was used as standard for comparing the anti-diabetic activity. A dose dependent activity observed. The observations are given in Table 1-3

Effect of Methanol and Ethyl Acetate Extracts of *Begonia trichocarpa* Dalz Leaf on Blood Glucose Level.

Administration of EABT and MEBT to the experimental groups showed highly significant ($p<0.001$) with MEBT (200mg/kg, 400mg/kg) and significant ($p<0.01$) with EABT (200mg/kg, 400mg/kg) reduction in blood glucose level when compare with diabetic control39.

Effect Of Methanol and Ethyl Acetate Extracts of *Begonia trichocarpa* Dalz Leaf on Lipid Profile

The Methanol extract showed significant effect on lipid profile of experimental animals. The levels of total cholesterol in the blood of STZ-induced diabetic rats were found to be significantly ($p<0.01$) elevated when compared with normal control rats .EABT and MEBT showed a significant ($p<0.001$) reduction in total cholesterol level when compared with diabetic control rats .Administration of two doses of EABT and MEBT restored the triglycerides highly significantly ($p<0.001$) in diabetic induced rats. In the restoration of HDL, high dose of MEBT ($p<0.001$) showed a highly significant effect. There was less significant effect ($p<0.05$) found with EABT (200mg/kg) on the reduction of LDL and significant ($p<0.01$) effect with 400mg/kg. Whereas low dose of MEBT showed significant ($p<0.01$) effect and higher dose of MEBT showed high significant ($p<0.001$) reduction of LDL in diabetic induced rats 40.

Effect of Methanol and Ethyl Acetate Extracts of *Begonia trichocarpa* Dalz Leaf on Hemoglobin and Liver Enzymes

The diabetic rats showed a significant increase in glycosylated hemoglobin ($p<0.001$) when compared with normal control rats. The higher and lower doses of MEBT showed a highly significant ($p<0.001$) decrease in the Hb and

glycosylated hemoglobin levels in diabetic treated rats. The level of liver marker enzymes such as SGOT, SGPT and ALP in the serum of diabetic rats were increased significantly ($p<0.001$) in diabetic rats when compared to a normal control group. Both two doses of EABT and MEBT restored the SGOT highly significantly ($p<0.001$) in diabetic induced rats. In the restoration of SGPT, two doses of MEBT ($p<0.001$) showed a highly significant effect, where as 200mg/kg, 400mg/kg of EABT showed only significant ($p<0.01$) effect. There was less significant effect ($p<0.05$) found with EABT (200mg/kg, 400mg/kg) on the reduction of ALP. The higher doses of MEBT significantly ($p<0.001$) reduced the ALP in diabetic induced rats. The anti-diabetic activity of the *Begonia trichocarpa* Dalz extracts supported by its promotion of beta-cell survival41.

Histopathological Investigation of Pancreas of Selected Extracts Treated On Streptozotocin Induced Diabetic Rats

Microscopic examination of pancreatic parenchyma cell and islets were examined. All the finding was presented in Fig 1. Histopathology of the pancreas in control animals showed normal pancreatic parenchyma cell and islet. In diabetic control, pancreas showed moderate hyperplasia of islet cells, more number of voculation, and severe congestion in pancreatic parenchyma, number of islet was reduced and mild infiltration of inflammatory cells. In diabetic animals treated with EABT, MEBT, pancreas section showing hyperplasia of islet was recovered, mild congestion of pancreatic parenchyma was seen and number of islet increased. In diabetic animal treated with Glibenclamide showed an increased number of pancreatic islets42.

Relative PKB Gene Expression in Streptozotocin Induced Diabetic Rats Treated With EABT and MEBT of *Begonia Trichocarpa* Dalz Leaf

The serine-threonine kinase PKB is required for various cellular processes, from the regulation of cell cycle, survival, and growth to glucose and protein metabolism, decreases in PKB lead to the malfunction of various cellular processes. PKB is highly expressed in insulin responsive tissues and pancreatic β cells. From our results it can be observe that the lane 4 shows less PKB expression which was in accordance with findings of workers such as43**.** It was already reported that Streptozotocin induction will decrease the level of protein kinase such as PKB, which will lead to decreased pancreatic mass and subsequent insulin insufficiency[44]. The present study showed a positive effect of Protein kinase B gene expression and has increased the levels of Protein kinase B in all treated groups. The result shows the role of extracts in enhancing the expression of PKB, which in turn can support the theory of promoting beta-cell survival

CONCLUSION

Begonia trichocarpa Dalz leaf is a non- toxic anti-diabetic crude drug, the extracts enhancing the expression of PKB, which in turn can support the theory of promoting beta-cell survival and observations of histopathology of the pancreas supporting the theory of promoting beta-cell survival by increasing the number of islet.

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Table 1 Effect of selected extract of *Begonia trichocarpa* Dalz leaf on blood glucose level of Streptozotocin induces diabetic experimental rats

Treatment groups	Blood glucose level mg/dl				
	Days of treatment				
	0	7	14	21	28
Normal control	82.17 ± 2.71	80.58 ±1.92	84.38 ± 2.14	81.53 ± 2.64	80.77 ± 1. 38
Diabetic control	201.65 ± 5.11	222. 23 ± 8. 31	236.18 ± 4.72	244.57 ± 7. 42	236.48 ± 5.26
Standard control Glibenclamide 0.5 mg/kg BW	203. 18 ± 2.35	158. 31 ± 2. 44	142. 54 ± 3.10	124.16 ± 1.85	103.37 ± 2.08
Treatment with EABT 200mg/kg	183.27 ± 3.87*	172.75 ± 4.17	148.36 ± 2.89	141.21 ± 4.75**	128.47±2.71**
Treatment with EABT 400mg/kg	181.21 ± 2.05*	169.74 ± 2.11	146.82 ± 1.56	140.45 ± 2.66**	125.58±3.90**
Treatment with MEBT 200mg/kg	200.61 ± 2.76*	148.71 ± 2.84*	138.34 ±3.18**	133.51 ± 2.51***	115.43±1.23***
Treatment with MEBT 400mg/kg	194.4 ±2.94**	145.24 ± 2.48*	130.74 ±2.37**	122.52 ± 2.63***	105.54±2.85***

All values are expressed as mean ± SEM for 6 animals in each group.*p<0.05, **p<0.01, ***p<0.001 significance between normal control vs diabetic control and drug treated groups

Table:2 Effect of selected extracts of *Begonia trichocarpa* Dalz leaf on lipid profile of Streptozotocin induced diabetic experimental rats

Treatment groups	Total cholesterol IU	Triglycerides IU	HDL IU	LDL IU
Normal control	1.95 ± 0.06	2.03 ± 0.11	1.15 ± 0.06	0.73 ± 0.06
Diabetic control	3.07 ± 0.08	3.08 ± 0.15	0.73 ± 0.06	1.08 ± 0.09
Standard control Glibenclamide 0.5 mg/kg BW	1.65 ± 0.65	2.05 ± 0.06	1.22 ± 0.03	0.78 ± 0.06
Treatment with EABT 200mg/kg	1.80 ± 0.04***	2.15 ± 0.65***	0.85 ± 0.05	0.88 ± 0.05*
Treatment with EABT 400mg/kg	1.95 ± 0.06***	2.53 ± 0.15***	0.83 ± 0.03	0.78 ± 0.40**
Treatment with MEBT 200mg/kg	2.20 ± 0.04***	2.28 ± 0.09***	0.83 ± 0.06*	0.8 ± 0.04**
Treatment with MEBT 400mg/kg	1.95 ± 0.06***	1.95 ± 0.10***	1.15±0.06***	0.5 ± 0.04***

All values are expressed as mean ± SEM for 6 animals in each group.*p<0.05, **p<0.01, ***p<0.001significance between normal control vs diabetic control and drug treated groups.

Table: 3 Effect of selected extracts of *Begonia trichocarpa Dalz* leaf on Hemoglobin and liver enzymes Streptozotocin diabetic experimental rats

Treatment groups	Hb	Glycosylatd hemoglobin	SGOT	SGPT	ALP
Normal control	14.53 ± 0.50	4.52 ± 0.23	25.50 ± 2.52	31.50 ± 1.44	144.75 ± 4.55
Diabetic control	10.55 ± 0.34	12.18 ± 2.36	57.00 ± 5.82	52.75 ± 3.59	167.75 ± 8.43
Standard control Glibenclamide 0.5 mg/kg BW	14.23 ± 0.38	5.78 ± 1.07	26.75 ± 2.43	33.75 ± 1.75	157.00 ± 4.22
Treatment with EABT 200mg/kg	12.90 ± 0.94*	7.43 ± 0.88*	27.50 ±1.04***	39.25 ± 6.54**	162.50±6.44*
Treatment with EABT 400mg/kg	13.38 ± 2.16*	5.85 ± 1.56**	24.25 ±1.11***	33.00 ± 0.70**	160.25 ± 5.02*
Treatment with MEBT 200mg/kg	12.98 ± 0.88*	5.13 ±0.65***	31.00±1.47***	36.75±0.85***	157.75 ± 3.83**
Treatment with MEBT 400mg/kg	14.63 ±1.48**	4.89 ±0.28***	28.25 ±2.69***	34.65 ±0.27***	147.75±15.80***

All values are expressed as mean ± SEM for 6 animals in each group.*p<0.05, **p<0.01, ***p<0.001significance between normal control vs diabetic control and drug treated groups.

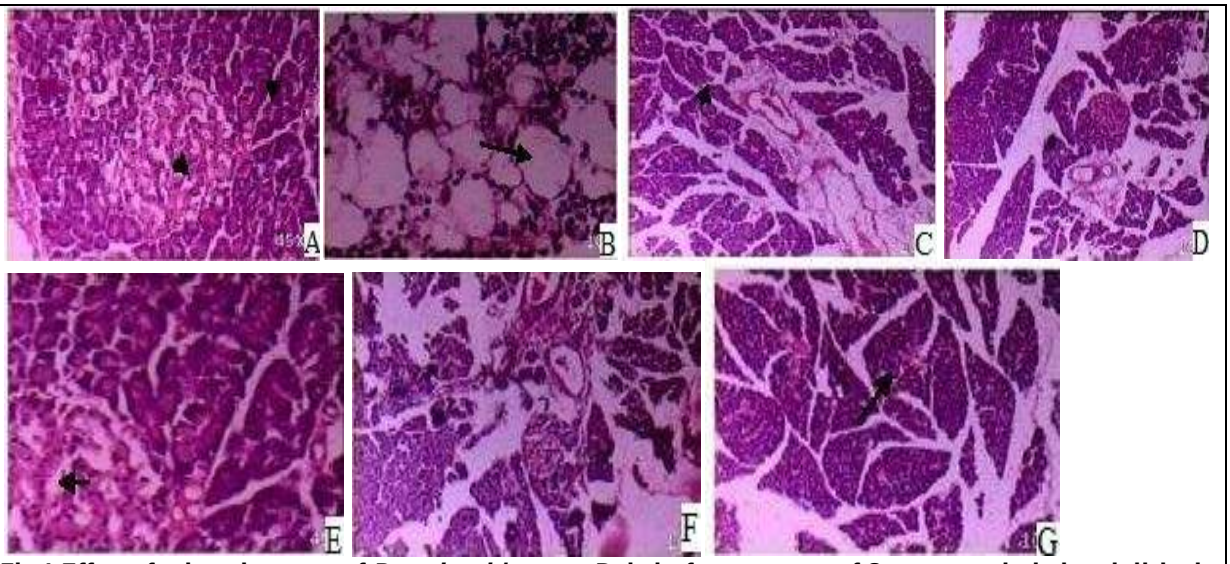
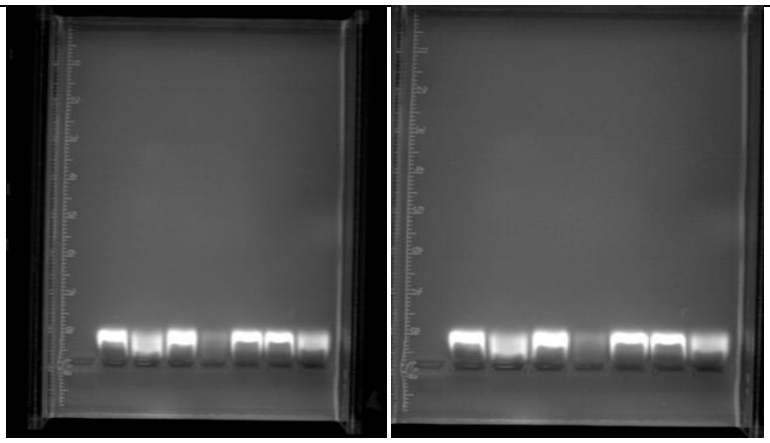


Fig 1 Effect of selected extracts of *Begonia trichocarpa* Dalz leaf on pancreas of Streptotozocin induced diabetic rats. Histological investigation: H&E Stained pancrease of (A) Normal group Rat (B) STZ treated group Rat (C) Glibenclamide treated group Rat (D) EABT low dose treated group Rat (E) EABT high dose treated group Rat (F) MEBT low dose treated group Rat (G) MEBT high dose treated group Rat .



(A) **(B)**

Fig 2.TIF of (A) GAPDH Gene (B) PKB Gene expression on MEBT &EABT treated STZ induced Diabetic rat

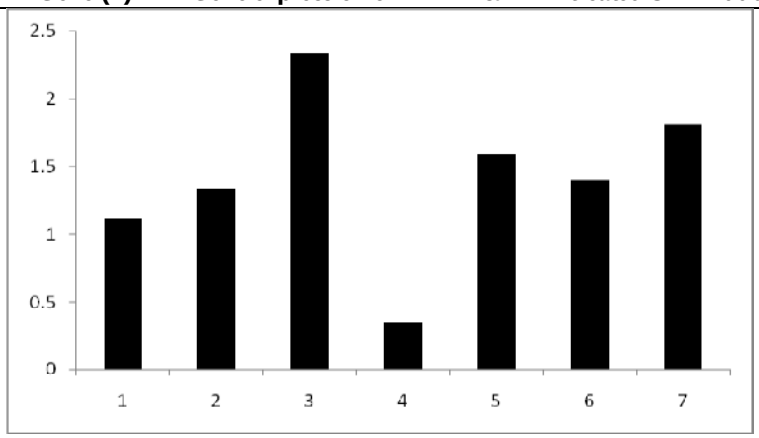


Fig 3 Relative expression of Protein Kinase B in Streptozotocin induced diabetic rats treated with selected extracts of the *Begonia trichocarpa* Dalz leaf. X axis: sample, Y axis: Relative difference in expression in arbitrary units (Image J analysis software).

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Prevalence of Oral Motor Problem among Cerebral Palsy Children

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Received: 20 Feb 2021 Revised: 24 Feb 2021 Accepted: 28 Feb 2021

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ABSTRACT

Aim: To learn the Prevalence of Oral motor problem among Cerebral Palsy children **Objective:** To examine the impenetrability in expression of communication and lip closure in the Cerebral Palsy children. To assess the oral sensitivity and arrangement of dentures among Cerebral Palsy children. **Methodology:** Fifteen Cerebral Palsy children with Age group of 7 – 10 years for both male and female children were selected based on the selection criteria. All the Cerebral Palsy children were assessed by using the Oral Motor Examination Tool (OMET) in order to examine oral motor the problems. Result: The result showed that, out of 15 Cerebral Palsy children, 73.3% are diagnosed to have Oral motor problem and 26.7% does not have Oral motor problem. So, it shows significant number of Cerebral Palsy children have suffered from Oral motor problem. From the statistical analysis, the mean value of oral motor problem among special children, problem in lip closure is 0.33, difficulty in articulation of speech is 0.53, problem in oral sensitivity is 0.4, and problem in arrangement of dentures is 0.26. From this mean value of oral motor problem, most of the children have difficulty in articulation of speech.. The statistical value shows that, the mean value of cerebral palsy is 77.7. Hence, the cerebral palsy children have significant prevalence in Oral motor problem. Conclusion: This study concluded that, the prevalence of oral motor problem is more in cerebral palsy children, and it is mainly due to the increased rate of difficulty in articulation of speech problem.

Keywords: Cerebral palsy, oral motor examination tool (OMET), difficulty in articulation of speech

INTRODUCTION

Cerebral Palsy

The cerebral palsy is a group of disorders of the development of movement and posture causing activity limitations that are attributed to non-progressive disturbance that occur in the developing fetal (or) infant brain .Normal development of sucking ,swallowing , and chewing require the correct Oral skills ,as well as coordination of voluntary and involuntary movement of Oral facial and tongue muscles .In children with cerebral palsy, normal functioning of group of muscles is impaired in a variety of ways. Abnormal tension and movement of the tongue, throat and palate, as well as sensory defects in these areas may lead to strangulation, as well as prevent swallow of saliva and thus cause drooling in these children. The two sided impairment of the upper motor neuron also usually causes swallowing disorder, which involves problem in the formation of food morsel and delays in the transfer the solid and liquid food from the Oral cavity to the digestive tract. Oral motor disorders, such as speech and swallowing disorders often occur in children. Generally parents complain that children refuse to eat hard food, drooling excess, and unable to speak clearly. Oral motor disturbance can occur due to the unavailability of maturation of Oral motor structure.

Oral motor is a co-ordination and movement of hard tissue, soft tissue, vascular system and the nervous control of face area and oral that forms the oral motor Function. The coordination of this structure is very important for the function of talking, chewing, and swallowing with large variety of food textures. Oral motor development started since the baby in the womb and continues until it reaches the age of 4 years baby already used muscles and movement that later needed to eat since the age 3 months pregnancy. Which continue with the initial reflex of children who seen from the process of breastfeeding, the process of breastfeeding is one of the various kinds of motor Function that will increasingly develop as you get order where the forming structure will be developed. The development and growth of structure -forming functions of the oral motor usually become mature at the age of 5 years, children who have been able to master the whole functions such as chewing, swallowing, and speaking, and shows maturity on oral motor function.

During the toddler, the children will experience the stages of making the function of oral motor has been done. The stages that must be done by the baby start from breast feeding then switch the foods with various textures in order to develop perfectly. The maturation of oral motor structures can be interrupted if there are obstacles in the introduction of food texture. It can be seen start from the transition of consumption intake of breast milk onto the solid food's texture. The children were introduced in various food textures like liquid, pulp, semi-solid and solid.

Mental Retardation

Mental retardation is one of the associated feature which can be seen in children with cerebral palsy. Which is characterized by below average intelligence (or) mental ability .Children with behavior problems such as explosive tantrums, poor Oral motor coordination, sensitive to touch and temperature the mouth, chokes or gags when trying to swallow and Oral facial defects, such as a cleft palate, aversive feeding behavior, prevent the achievement of development threaten clinical stability.

REVIEW OF LITERATURE

Studies Related To Oral Motor Problem in Cerebral Palsy Children

1. **Corrie Erasmus , Karen Vanhulst [2009]** ; Investigated whether drooling in children with cerebral palsy in general and in children cerebral palsy subtypes is due to hyper salivation. Saliva was collected from 61 healthy children and 100 children with cerebral palsy who drooled. The intensity of drooling was evaluated using the drooling quotient. Author concluded that no difference was found in the flow rates, Age [or] Sex between healthy children and children with cp who drooled. On additional such group analysis, the flow rates of children with dyskinetic cerebral palsy differed statistically from these of healthy children.

2. **Srishit Aggarwal, Ravinder Chadha [2015]** ; feeding difficulties among children with cerebral palsy ,the objective of this article was to review the studies conducted among children with cerebral palsy to assess their feeding problems and its impact on child's growth nutritional status . Emphasis should therefore be placed on an early identification, treatment correction of feeding problem. Significant improvement in child's health nutritional status can be achieved through intensive intervention programs.
3. **Mustapha Mouilly *et al* [2016];** Investigated the disorders of swallowing and feeding in children and teenagers suffering from cerebral palsy. This study with also highlighted the existence of correlation between Gross motor function classification system and which has occurred of false while eating and while drinking respectively .It is necessary to bring to this population of children and teenagers the adapted and affective care ,so that they will test a better quality of life .
4. **Farhad Sakhae [2019]**; The Author conducted a cross-sectional descriptive study, 60 children with cerebral palsy that referred to clinical centers were selected using random sampling feeding and swallowing skills of these children were investigated with using of pediatric assessment scale for server feeding problems findings indicated that children with spastic and flaccid cerebral palsy have feeding problems with similar severity. Also children athletic cerebral palsy showed lowest severity of feeding problems.

METHODOLOGY

Setting

This study was carried out at G.V Special School, C.C.W.E Trust and Department of Physical Medicine and Rehabilitation RMMC& H, Annamalai University, Annamali Nagar and Chidambaram.

Study Design

Observational study.

Selection Criteria

Children diagnosed with Cerebral Palsy for both male and female children with the age group of 7-14 were selected for this study.

Study Procedure

This study aimed to find out the prevalence of Oral motor problems among Cerebral Palsy children and it was conducted in G.V Special School, C.C.W.E. Trust and Raja Muthiah Medical College and Hospital at Annamalai Nagar and Chidambaram.

A total of 15 Cerebral Palsy, which include 9 male children and 4 female children were selected at certain age group of 7 – 15 years. All the 15 childrens were screened by using Oral Motor Examination Tool **(OMET)** to assessed, Lips, Soft Palate, Tongue, Jaw, Hard Palate, Diadochokinesis where the Cerebral Palsy children have problem in the oral motor skills and to provide statistical picture of this study.

DATA ANALYSIS AND RESULT

The above table exhibits that out of 9 children with cerebral palsy, 77.7% of children are found to have Oral Motor Problems and 22.2% of children do not have any Oral Motor Problems. So, it shows that significant number of children with cerebral palsy poses Oral Motor Problem. The above table shows that mean value of oral motor problem among special children ,problem in lip closure 0.33, difficulty in articulation of speech 0.53 ,problem in oral sensitivity 0.4 problem in arrangement of dentures0.26.From this mean value oral motor problem ,most of the children have difficulty in articulation of speech.

RESULT

The result showed that, out of 15 special children, 73.3% are diagnosed to have Oral motor problem and 26.7% does not have Oral motor problem. Thus, shows significant number of cerebral palsy children irrespective of their classification have Oral motor problem. From the statistical analysis, the mean value of oral motor problem among cerebral palsy children, problem in lip closure 0.33, difficulty in articulation of speech 0.53, problem in oral sensitivity 0.4, problem in arrangement of dentures 0.26. From this mean value of oral motor problem, most of the children have difficulty in articulation of speech. The statistical value shows that, the mean value of cerebral palsy is 77.7%,

CONCLUSION

This study concluded that, the prevalence of oral motor problem is more in cerebral palsy children, and it is mainly due to the increased rate of difficulty in articulation of speech problem.

ACKNOWLEDGEMENT

I Thankful to my bellowed Research Supervisor Dr. P. Swarna Kumari and my Family Members.

CONFLICT OF INTEREST

This work has done my own interest.

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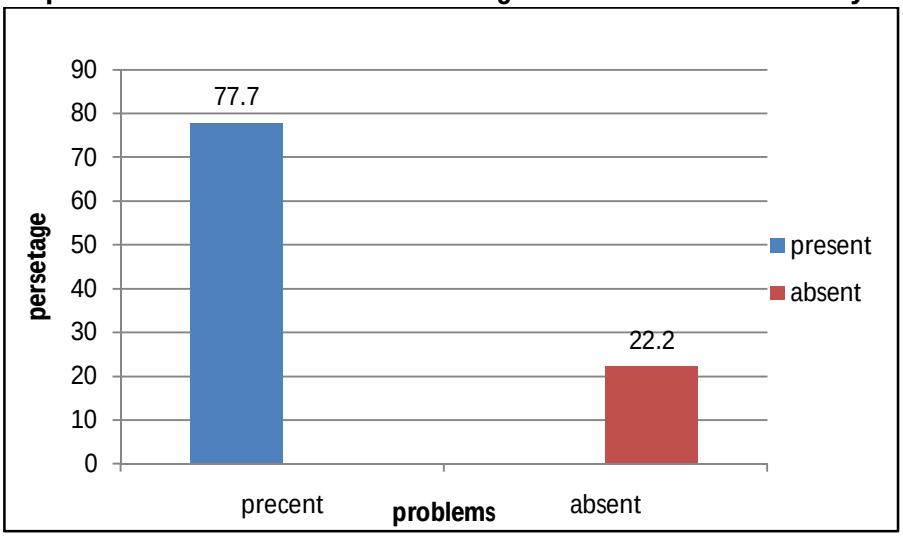
Table 1: Oral Motor Problems among Children with Cerebral Palsy: Primary data

Problems	N	Percentage
Present	7	77.7
Absent	2	22.2
Total	9	100.0

Table 2: Mean Value of Oral Motor Problems

	PROBLEM IN LIP CLOSURE	DIFFICULTY IN ARTICULATION OF SPEECH	PROBLEM IN ORAL SENSITIVITY	PROBLEM IN ARRANGEMENT OF DENTURES
Mean score	0.33	0.53	0.4	0.26

Graph 1 Show: Oral Motor Problem among Children with Cerebral Palsy



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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparison of Monomer Concentration Variation on Polymerization Rate in Absence and in Presence of Cyclodextrin - A Novel Study

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Received: 20 Feb 2021 Revised: 15 Feb 2021 Accepted: 25 Feb 2021

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ABSTRACT

The rate of polymerization was studied, by varying the concentration of monomer [methylmethacrylate]. Oxidant [Ce (IV)], reductant [LA] were used to study the rate of polymerization in the presence and in the absence of β-cyclodextrin and the results were compared. Monomer concentration variation was studied at a temperature 35ºC for 30 min. time duration. As the concentration of monomer increases rate of polymerization (R_p) increases. Due to the presence of cavity in β-cyclodextrin the rate of polymerization increases. The cavity in β-cyclodextrin brings the monomer molecules closer to one another in this redox system.

Keywords: polymerization, methylmethacrylate, β-cyclodextrin, cavity, redox system.

INTRODUCTION

Over half a century ago, Wolfgang Ostwald (1917) coined the term 'the land of neglected dimensions' to describe the range of sizes between molecular and macroscopic within which occur most colloidal particles. The term 'neglected dimensions' might have been applied equally well to the world of polymer molecules, the high molecular-weight compounds, so important to man and his modern technology.

Polymers

Polymers play a vital role in our day today life such as in food, clothing, shelter, transportation, communication and in modern technologies. Biological polymers such as muscles, sinews, genes and chromosomes constitute our body and it forms foundation of life. Compound with one or more monomeric structural units is known as polymer. The monomer molecules are bonded together to form a macro molecule, polymer and the process is known as polymerization. A polymer is a large molecule built up the repetition of small, simple chemical units called monomers. Thus the repeat unit of poly(vinyl chloride) is $–CH_2-CH-Cl$, its monomer is vinyl chloride, $CH_2=CHCl$.

Radical Initiators

Ever since Paneth demonstrated that free radicals can be formed by the thermal homolysis of lead tetra-alkyls, interest in the study of their formations, characterization and reactions has been growing by leaps and bounds. As a result of years of research, it has now been established that these reactive intermediates undergo a multitude of chemical reactions such as additions, substitution, rearrangement, dimerization and so on [1-6]. Their addition to vinyl double bonds often leads to polymerization reactions resulting in polymers having large molecular weights. The usefulness of such synthetic polymers has led to the development of newer and more efficient radical initiators. Thus besides organic peroxides and azonitriles, redox systems consisting of oxidant and reducing agents have been employed quite frequently for vinyl polymerization [7-9]. The work of Bacon,[10] Morgan[11] and Evans et.al.[12] represented that a redox reaction could be employed for initiating vinyl polymerization.

Special Features of Redox Initiators

Redox system generates free radicals through electron transfer reactions between oxidants and reducing agents. Such reaction requires relatively less activation energy to yield free radicals. This helps the polymerization can be carried out even at low temperatures. The earliest known redox initiator, used in the vinyl polymerization was Fenton's reagent, (H_2O_2-Fe^{2+}). The catalytic decomposition of hydrogen peroxide by the ferrous ion has been shown to proceed according to the following mechanism.[13]

$Fe(II) + H_2O_2 \longrightarrow Fe(III) + OH^- + OH^o$ ----------(1)

$Fe(II) + HO^o \longrightarrow Fe(III) + OH^-$ ----------(2)

$HO^o + H_2O_2 \longrightarrow HO_2^o + H_2O$ ----------(3)

$HO_2^o + Fe(II) \longrightarrow Fe(III) + HO_2^-$ ----------(4)

$HO_2^o + Fe(III) \longrightarrow Fe(II) + H^+ + O_2$ ----------(5)

The presence of monomers have the effect of suppressing step --- (2) since the hydroxyl radicals are trapped[14] in an initiating step such as

$^oOH + CH_2 = CHX \longrightarrow OH-CH_2-C^oHX$

Redox system based on transition metal ion oxidants such as Mn(III), V(V), Ce(IV) etc., and organic reducing agents have received much attention [15-18].

Cyclodextrin

Cyclodextrins are water soluble molecules they are composed of α – (1,4) linkages of a number of D(+) – glucosyl pyranose units. The number of glucose units is designated by a Greek letter, α for six, β for seven, γ for eight units and so on. Of these, the majority of research has focused on β-cyclodextrin. This is due to several reasons, namely: price, availability, approval status, and cavity dimensions, among others [19]. In fact they are nontoxic; biologically

degradable substances their utility, within well – defined limits, seems to be inexhaustible in a large number of chemical products. In CD, the Secondary hydroxyl groups (on the C-2 and C-3 atoms of glucose units) are located on one side of the torus, whereas the primary hydroxyl groups are located on the opposite side of torus. The interior of the torus consists of ring glycosidic oxygen and another ring of C-H groups. Therefore the interior of CD are relatively apolar compared to water. CD cavity is slightly 'V' shaped with the secondary hydroxyl side more open than the primary hydroxyl side. β-cyclodextrin contains six to twelve glucose units. Cyclodextrins are well-known host - guest molecules that find extensive use in complexation due to its well defined cavities and small size [20]. The structure of CD gives rise to the remarkable ability to form inclusion complexes with inorganic,[21-23] organic[24-27] and ionic compounds,[21,22] and also with polymers.[28-32] Cyclodextrins are mainly used to help solubilize poorly water soluble species by the formation of 'inclusion complexes' or 'host–guest' complexes [33-35]. Peroxodiphosphate ($P_2O_8^{4-}$) was first introduced by Meenakshi et.al [36]. $K_4P_2O_8$-ascorbic acid [37] and $K_4P_2O_8$-$Na_2S_2O_3$[38] are few redox systems used in the vinyl polymerization. A classic example is $KMnO_4$-oxalic acid redox pair which was employed in the polymerization of acrylonitrile.[39] Methylmethacrylate,[40] acrylic acid[41] and acrylamide[42] wherein the carboxyl radicals($C_2O_4^{2-}$ º or $COO^{-º}$) were the initiating species. Ce^{4+} is the first metal ion used for vinyl polymerization in the absence of any organic substrate because of its high oxidation potential ($E^º$ = 1.70). Ce(IV) in conjugation with alcohols,[43,44] aldehydes,[45] ketones,[46] etc., were used for initiation of vinyl polymerization.

In the polymerization of methylmethcrylate initiated by Mn(III)-alcohol redox systems, the order of reactivity of alcohols was found to be 1-propanol > propane-1,2,3 triol> ethane-1,2-diol > 2-butnol > 1-butanol > propane-1,2-doil >cycloheptanol>cyclohexanol>cyclopentanol. Mahadevan et. Al.[47] investigated the oxidation of pincol by Mn(III) sulphate and also used the redox pair for the polymerization of acrylamide. Rate of polymerization, was found to be independent of [Mn(III)] while being proportional to [monomer] and [pinacol] suggesting linear termination. The polymerization of methylmethacrylate in CCl_4 medium with ferric laurate, a metal soap in combination with n-hexylamine as the initiator system was studied by Saha and Chaudhuri[48]. The rate of polymerization was found to be linear with the monomer concentration and proportional to the square root of both ferric ion and amine concentration. In the absence of amines there was no polymerization even after 2 hours**.** The literature on vinyl polymerization initiated by metal ion-organic ligand redox system, shows that the transition metal ions with steady variable valency have been broadly used. And, the reactivity of reducing agents depended on resonance and hyperconjugation effects. In majority of the above said reactions, the rate of disappearance of metal ions followed the first order reactions during polymerization and terminated by mutual combination of growing chains. Moreover, because of the complexing ability of Ce(IV) with many organic ligands and also its high redox potential, this redox system has been chosen for the investigation. Ce(IV) – Lactic acid redox initiator can generate free radicals which requires less activation energy and polymerization occurs even at low temperature. So it was decided to study and compare the polymerization with Ce(IV) – Lactic acid redox pair in the presene and in the absence of β-cyclodextrin.

EXPERIMENTAL PROCEDURE

Monomer (methylmethacrylate) solution, lactic acid, sulphuric acid, sodium bisulphite and water were taken in the reaction vessel, thoroughly mixed and kept in the water bath. Nitrogen, freed from oxygen by passing through Fieser solution, was bubbled through the reaction mixture for 30 minutes and the system was thermostated at desired temperature. The reaction mixture takes about 10 minutes to attain equilibrium. After allowing ten minutes, ceric ammonium sulphate (already placed in the water bath for 10 minutes) was immediately added and the tube was sealed with rubber gaskets to ensure maintenance of an inert atmosphere and the time was noted. After 30 minutes, the reaction tube was opened and quenched with excess of ferrous ammonium sulphate (FAS) solution. The concentration of monomer was determined by the method of addition of bromine to the double bond. To 10 ml of 0.2M Winkler's solution in an Erlenmeyer flask, 3 ml of stock monomer solution and 20 ml of 2.5M H_2SO_4 were added. The contents of the flask were tightly stoppered and kept in dark for about 30 min. with intermittent shaking to allow the liberated bromine to add on to the double bonds in the monomer. KI was then added to the mixture and the iodine liberated by the excess bromine was titrated against std.$NaHSO_4$ using starch as an indicator. A blank titration was also made with 10 ml of

Winkler's solution as before and the difference in the time value was used to estimate the monomer concentration. Sodium bisulphite was used to maintain the ionic strength throughout the reaction.

The weight of the polymer was found out, from which the rate of polymerization was calculated. The overall rate of polymerization was calculated by using the formula

Rp = wt.of polymer/ mol.wt.of monomer × 1000/vol.taken × 1/time in sec.

RESULTS AND DISCUSSION

Effect of Monomer (Methylmethacrylate) Conc. on Polymerization Rate in Absence of β-CD

Rate of polymerization was measured by altering the monomer (methylmethacrylate) concentration from 0.01 to 0.05 mol.dm^{-3} at rigid concentration of oxidant [Ce(IV)], substrate [LA] and sulphuric acid [H$^+$] at 35ºC temperature for 30 min. time duration. The order of rate of polymerization was determined by logarithmic plot of R_P versus [methylmethacrylate] (Fig. 1). The rate of polymerization was increased from 12.7467 × 10^{-6} mol.dm^3.sec^{-1} to 83.6950 × 10^{-6} mol.dm^{-3}.sec^{-1}(Table 1) when concentration of monomer increased from 0.01 mol.dm^{-3} to 0.05 mol.dm^{-3}. The slope obtained was 1.15 (Fig.2). The order of reaction greater than unity with respect to monomer concentration denoted the dependence of the initiation rate on monomer concentration. The order nearly 1.5 dependence of the rate of polymerization on monomer concentration as authenticated by the double logarithmic plot has been reported in many redox initiated polymerization systems. The order 1.5 dependence of monomer concentration in the current study was dissimilar to the observations recorded in the case of polymerization initiated by the glycerol-V(V) redox system[49]. While V(V) and Ce(IV) were one electron oxidants, the oxidation potential of Ce(IV) was extremely greater than V(V) and ceric ion was able to initiate vinyl polymerization even in the absence of a reducing agent, while V(V) ion was not.

Effect of Monomer (Methylmethacrylate) Conc. on Polymerization Rate in Presence Of B-CD

The rate of polymerization of methylmethacrylate was studied by changing the concentration of monomer [methylmethacrylate] from 0.01 to 0.05 mol.dm^{-3} and keeping the concentration of [Ce(IV)], [LA], [H$^+$] and [β-CD] as constant which were 0.02 mol.dm^{-3}, 0.2 mol.dm^{-3}, 2.5 mol.dm^{-3}, 0.02 mol.dm^{-3} respectively at 35ºC temperature for 30 min. time duration. The rate of polymerization was increased from 12.7379 × 10^{-6} mol.dm^{-3}.sec^{-1} to 97.2299 × 10^{-6} mol.dm^{-3}.sec^{-1}(Table 2) in the presence of β-CD and it was shown by the plot of R_P versus [methylmethacrylate] (Fig.3). The increase in concentration of monomer increases the rate of polymerization (R_P). Linear slope with an increase in concentration of monomer showed the order dependence of the rate of polymerization on monomer concentration and it was decided by double logarithmic plot of R_P versus [MMA], the slope of which was 1.27(Fig.4). The order of reaction greater than one with respect to monomer concentration may be expressed to a dependence of the initiation rate on monomer concentration.[50] Nearly 1.5 order reliance of the rate of polymerization on monomer concentration was confirmed by the double logarithmic plot has widely been reported in redox initiated polymerization systems.[51] The order 1.27 dependence of monomer concentration in the present study was contrast to the observations given in the glycerol-V(V) redox system. Both V(V) and Ce(IV) were one electron oxidants, V(V) has low oxidation potential and hence it cannot initiate vinyl polymerization in the absence of reducing agent. But Ce(IV) has greater oxidation potential than V(V) and hence ceric ion can initiate vinyl polymerization even in the absence of reducing agent.

CONCLUSION

The rate of polymerization increases from 0.01 M to 0.05 M concentration both in absence and in presence of β – CD. In the absence of β – CD the monomer molecules are far away from one another but in the presence of β – CD, the monomer molecules are present inside the cavity structure of β – CD and thus are closer to each other hence the R_P is greater in presence of β – CD than in absence of β – CD.

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Table 1: Variation of methylmethacrylate conc. in absence of β-CD

[Ce(IV)] = 0.02 mol.dm^{-3} [LA] = 0.2 mol.dm^{-3} [H$^+$] = 2.5 mol.dm^{-3}

Temp. = 35ºC Time = 30 min.

[methylmeth acrylate] mol.dm^{-3}	3+log[methyl methacrylate]	Weight of polymer(g)	10^{-6} R_P mol.dm^{-3}. sec^{-1}	6+log R_p
0.01	1.0000	0.0459	12.7467	1.1054
0.015	1.1761	0.0744	20.6633	1.3152
0.02	1.3010	0.1059	29.4035	1.4684
0.025	1.3979	0.1228	34.0800	1.5325
0.03	1.4771	0.1480	41.0771	1.6136
0.035	1.5441	0.1840	51.0740	1.7082
0.04	1.6021	0.2298	63.7675	1.8046
0.045	1.6532	0.2649	73.5021	1.8663
0.05	1.6989	0.3016	83.6950	1.9227

Table 2: Variation of methylmethacrylate conc. in presence of β-CD

[Ce(IV)] = 0.02 mol.dm^{-3} [LA] = 0.2 mol.dm^{-3} [H$^+$] = 2.5 mol.dm^{-3}

[β-CD] = 0.02 mol.dm^{-3} Temp. = 35ºC Time = 30 min.

[methyl meth acrylate] mol.dm^{-3}	3+log[methyl meth acrylate]	Weight of polymer(g)	10^{-6} R_P mol.dm^{-3}. sec^{-1}	6+log R_P
0.01	1.0000	0.0459	12.7379	1.1051
0.015	1.1761	0.0669	18.5866	1.2692
0.02	1.3010	0.0953	26.4667	1.4227
0.025	1.3979	0.1263	35.0671	1.5449
0.03	1.4771	0.1608	44.6375	1.6497
0.035	1.5441	0.1969	54.6386	1.7375
0.04	1.6021	0.2414	66.9884	1.826
0.045	1.6532	0.2943	81.6770	1.9121
0.05	1.6989	0.3504	97.2299	1.9878

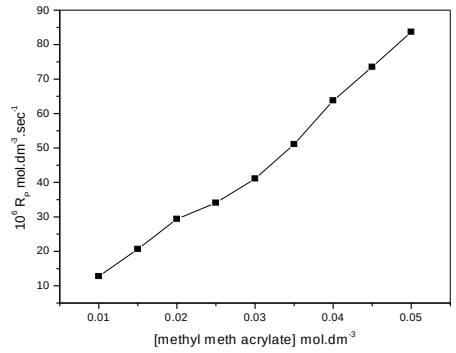

Fig. 1: Plot of rate of polymerization (R_P) versus [methylmethacrylate] in the absence of β-CD.

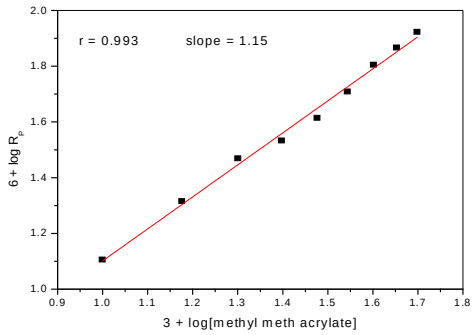

Fig. 2: Plot for variation of monomer (methylmethacrylate) concentration in the absence of β-CD.

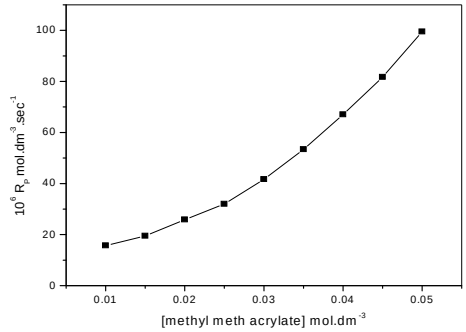

Fig.3: Plot of rate of polymerization (R_P) versus [methylmethacrylate] in presence of β-CD.

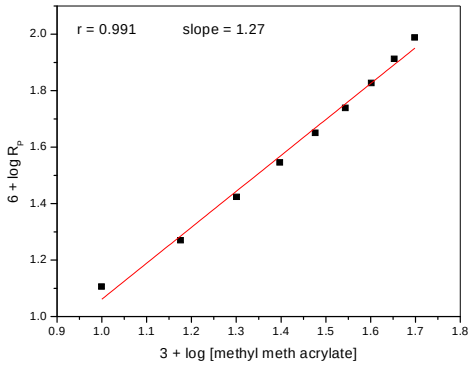

Fig. 4: Plot for variation of monomer (methylmethacrylate) concentration in presence of β-CD.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Boosted Weighted Optimized Neural Network Ensemble Classification Based Lung Cancer Prediction

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Received: 19 Feb 2021 Revised: 22 Feb 2021 Accepted: 28 Feb 2021

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ABSTRACT

Lung cancer is one of the world's leading cancer related diseases. The early prediction of lung cancer is critical as it can dramatically decrease mortality rates. Molecular biology presents an attractive method in microarray technology to use gene profiles for prediction of lung cancer. But the gene expression data has high dimensionality problem which is solved by using Dragonfly optimization algorithm (DA). It is inspired from the natural behavior of dragonflies in which they are flying randomly based on the model developed by using the Levy Flight Mechanism (LFM). LFM has some drawbacks such as overflowing of the search area and interruption of arbitrary flights due to its huge searching steps. So, an Improved Dragonfly optimization Algorithm (IDA) was proposed for dimensionality reduction of gene expression data. IDA used a Brownian motion to solve the issues of LFM. The particle best and global best concept of Particle Swarm Optimization (PSO) was used in IDA to avoid the premature convergence. The selected features by IDA were given as input to Random Subspace (RS), Artificial Neural Network (ANN) and Sequential Minimal Optimization (SMO) to predict the lung cancer. In this paper, a Boosted Weighted Optimized Neural Network Ensemble Classification (BWONNEC) algorithm is introduced to further refine the prediction accuracy and reduce the prediction error. At first in BWONNEC algorithm, weights are initialized to the selected features and then a weighted sum is calculated. Additionally, a conditional checking is made to check the weighted sum with the optimal weight. If the weighted sum is greater than optimal weight, the weighted sum value with the initialized various weight is attained. After that, a neural network classifier with minimum weighted error (weak classifier) is determined and combines the weak classifier with the optimal weight to predict the lung cancer. Thus, the accuracy for

lung cancer prediction is improved with minimum error rate. From the analysis of IDA-SMO and IDA-BWONNEC, it is proved that the accuracy, precision, recall and F-measure of IDA-BWONNEC is 1.94%, 0.85%, 1.6% and 1.72% better than IDA-SMO method respectively for lung cancer prediction.

Keywords: cancer prediction, feature selection, Improved Dragonfly optimization Algorithm, BWONNEC.

INTRODUCTION

DNA microarray technology [1] is able to concurrently determine large number of genes in an individual examination. In several areas of biological science, gene expression analysis is necessary to obtain the required knowledge. When time passes it has become more and more difficult and challenging to diagnosis, examine and treat the disease in general and lung cancer in particular. The research on lung cancer is one of the main fields of medical research. Prediction of lung cancer is a big concern and accurate prediction will have a great value to provide patients with improved care. Data mining algorithms are key elements and a more commonly used method for the achievement of important role for gene classification [2-4]. The profiles of gene expression reflect cell's molecular state that has incredible opportunities for clinical diagnosis. However, relative to the percentage of genes engaged, there is typically a very limited classification sample size required in the training sets. The restrictions on the training data pose an obstacle to some methodologies for classification. Feature selection methods remove the undesirable disruptive and repetitive features for extracting the marker genes those enhance the classification performance.

Nested Genetic Algorithm (NGA) [5] was used to select the important features from gene expression data. The NGA comprised of Outer GA (OGA) and Inner-GA (IGA) in which the gene expression datasets and DNA Methylation datasets were processed to select the features and those features were used in Random Subspace (RS), Artificial Neural Network (ANN) and Sequential Minimal Optimization (SMO) for diagnosis of lung cancer. However, NGA has high time complexity and random motion problem which affect the lung cancer diagnosis accuracy. So, a hybrid Dragonfly optimization Algorithm with Radial Basis Neural Network (DA-RBNN) [6] was proposed for lung cancer prediction. DA was used to reduce the dimensionality of gene expression data and RBNN was used for lung cancer prediction. In DA, the dragonflies were flying randomly based on LFM. But, it has some drawbacks like overflowing of search area and interruption of arbitrary flights. So, an Improved Dragonfly optimization Algorithm (IDA) [7] was introduced to solve the problems in DA. In IDA, a Brownian motion was used to model the search process to get an optimal solution of dragonflies. Additionally, particle best (pbest) and global best (gbest) concept of Particle Swarm Optimization (PSO) was used to further refine the search space and it avoids the pre-mature convergence. The selected features were fed into RS, ANN and SMO classifier for prediction of lung cancer. Though, the prediction performance of SMO classifier is better than the RS and ANN, the prediction error and accuracy are not much effective.

In this paper, BWONNEC algorithm is proposed to further reduce the prediction error and improve the prediction accuracy. Initially, the most relevant features are selected using IDA and the selected features are given as input to BWONNEC classifier. In BWONNEC, optimized weights corresponding to every ensemble classifier are progressively calculated with respect to the ensemble classifier outcome and connection between the outcomes of all ensemble classifiers. Primarily, a weak classifier with minimum weighted error is calculated and a new component is then achieved on the basis of error function. The new component is processed in final ensemble classifier for better lung cancer prediction. The continuation of this paper is given as follows: The researches related to gene expression data classification are analyzed in Section 2. Section 3 explains the BWONNEC classifier for gene expression data classification. Section 4 demonstrates the performance efficiency of BWONNEC classifier. Section 5 summarizes the paper with future scope.

LITERATURE SURVEY

Wang *et al.* [8] developed a strategy to choose the relevant features for categorization of cancer data. The basic theory of this strategy was to assign the features with various weights to choose the features for classification, which is measured by instances precisely or imprecisely categorized. However, it required a pre-defined feature size for feature selection.

Lu *et al.* [9] proposed an algorithm to select the most significant features those are used to accurately classify the gene expression data. This algorithm integrated Adaptive Genetic Algorithm (AGA) and Mutual Information Maximization (MIM) to reduce the computational burden for classification. When the volume of data is increased exponentially, the hybrid feature selection method takes a comparatively longer time for feature selection.

Iraji *et al.* [10] proposed a statistical model with soft computing to predict the autopsy recovery lifespan in lung cancer surgery. This model processed the collected in different classifiers for prediction of survival rate of lung cancer patient. Ramos-González *et al.* [11] proposed a Case-Based Reasoning (CBR) framework with gradient based feature selection method to predict and classify the lung cancer. In future, the pathway analysis in this framework will be used to provide better knowledge about the processes carried out in tumor cells.

Luo *et al.* [12] developed an approach for lung cancer prediction. The developed approach consisted of two steps. In the first step, appropriate biophysical predictors influencing local control were chosen using extended Markov blanket method which allowed probing of probabilistic relationship among different features. The chosen features were used to predict the tumor response and personalized radiation treatment.

Sun *et al.* [13] proposed a method depends on neighborhood rough sets for gene expression data classification. Initially, the noise and uncertainty parameters of neighborhood decision systems. After that, the neighborhood coverage and neighborhood credibility degrees were described and added with the mutual information and neighborhood entropy to reveal the efficiency of attributes for classification. At last, the Fisher score method was employed to preliminarily to remove the unnecessary genes to enhance the efficiency of cancer classification using gene expression data. However, it is not suitable for complex datasets.

Potharaju *et al.* [14] proposed a strategy for better classification of microarray gene expression data. This strategy used different measures among the various clusters to classify the gene expression data. However, this strategy is not much effective. García-Díaz *et al.* [15] proposed an unsupervised feature selection algorithm for multi-class cancer classification of gene expression data. The collected samples consisted of 20,531 genes. The most relevant genes were extracted by applying Grouping Genetic Algorithm (GGA). It was an alteration of GA for solving clustering and grouping issues of GA. The selected genes were processed in Extreme Learning Machine (ELM) for multi-class cancer classification. However, the downside of this unsupervised feature selection algorithm is possibly incomplete exploration of the solutions space.

Shukla *et al.* [16] proposed a hybrid binding approach based on Teaching Learning-Based-Optimization (TLBO) and Gravitational Search Algorithm (GSA) to select the genes for cancer type classification. A minimum Redundancy Maximum Relevance (mRMR) was used for choosing the most important genes and then binding approach was processed to get the informative genes from the genes obtained by mRMR. The gravitational search mechanism was combined with the teaching phase of TLBO for enhancing the searching capability at the evolution process. The selected genes were given as input to naïve Bayes for cancer type classification. However, it has pre-mature convergence problem.

METHODOLOGY

Here, the proposed BWONNEC is explained in detail for prediction of lung cancer. Initially, gene expression data is collected and then the IDA is applied to choose the most significant features in the gene expression data. The selected features are given as input to proposed BWONNEC classifier for prediction of lung cancer.

Improved Dragonfly Optimization Algorithm Based Feature Selection

IDA is an improved version of DA that selects the most optimal features from the gene expression data. In IDA, two important features are included in the conventional DA to improvise the performance of DA. One of the features is an internal memory which keep monitors the possible solutions i.e., most important features that has a potential to converge to global optima. Another feature is iteration level hybridization with Particle Swarm Optimization (PSO) which implements on this collection of saved solutions. IDA uses the social and cognitive behavior of PSO for faster convergence and to determine the global best solution. Additionally, IDA uses the exploration capability of DA to explore the search space effectively. The outcome of IDA is given as input to BWONNEC to accurately classify the gene expression data.

Boosted Weighted Optimized Neural Network Ensemble Classification Based Gene Expression Data Classification

Once the most important features are obtained, those features are processed in BWONNEC to improve the lung cancer prediction. In this paper, an ensemble of selected features is processed in BWONNEC classifier to improve the prediction accuracy and reduce the prediction error and computation time. Consider, m training data which consists of $\{(f_1, c_1), (f_2, c_2), \dots (f_m, c_m)\}$, where f_i is the selected features and c_i is the class label which represents the presence of lung cancer or absence of lung cancer. BWONNEC process is started with training a neural network using distribution D_i, D_i is the subset of selected features.

In a neural network, an artificial neuron has m synapses related to the selected features $(f_1, f_2, \dots f_m)$ and each feature has a corresponding weight w_n. In this case, the input at signal n is accumulated with the weight w_n, after that the addition of weighted inputs and its linear combination are achieved. In addition to this, a bias b is summated with the linear combination and a weighted sum w_s is calculated as,

$$w_s = b + w_1 f_1 + w_2 f_2 + \cdots w_m f_m \quad (1)$$

Following to this, a nonlinear activation function A is executed with w_s as shown in Eq. (2) that returns an result y as:

$$y = A(w_s) \quad (2)$$

After that, the neural network classifier with minimum weighted error is chosen which is called as weak classifier and is devised as,

$$\varepsilon_n = z = P_{D_i}[y + w_1 f_1 + w_2 f_2 + \cdots w_m f_m] \quad (3)$$

$$= P_{D_i}[A(w_s)] \quad (4)$$

From Eq. (3) and (4), the minimum weighted error ε_n is calculated according to the probability of distribution function P_{D_i} for a linear combination of weighted inputs (i.e., selected features) $A(w_s)$. At last, a new component k_n according to the error function is computed as follows:

$$k_n = \frac{1}{m}\sum_{n=1}^{m}(Actual\ error - observed\ error)^2 \quad (5)$$

In Eq. (5), m is the number of features which are selected by IDA.

Once the boosting iterations are completed, final ensemble classifier which has weighted error that is better than chance, is computed by integrating all weak classifiers with an optimal weight w_h which is

$$A(W_S) = SIGN\left(\sum_{h=1}^{H} w_h A(s)\right) \quad (6)$$

In Eq. (6), $SIGN(.)$ is the sign function, H denotes the number of weak classifiers. The optimal weight is calculated using optimal weight learning machine. The creation of neural network's optimal weight can be stated as the following optimization problem:

$$Min\left\{\frac{\gamma_1}{2}\|k_n\|^2 + \frac{d_1}{2}\|r\|^2\right\} \quad (7)$$

$$\text{Subject to } k = y_{ACE} - y_{OBE} = y_{ACE} - WF \quad (8)$$

In Eq. (7), γ_1 and d_1 are the positive real regularization parameters, $\|r\|^2$ represents the regularize that, by the suitable selection of the regularization parameters, the cost function is smoothen at the singular point of the correlation matrix of the feature vectors to improve the robustness of the neural network with respect to the noisy environment (i.e., missing data in the gene expression data). y_{ACE} is the actual error for lung cancer prediction, y_{OBE} is the observed error by the lung cancer prediction method, W is the $\{w_1, w_2, \dots w_m\}$ and F is the $\{f_1, f_2, \dots f_m\}$.

A Lagrange method is used to solve the Eq. (3.7). The Lagrange function is given as follows:

$$L_1 = \frac{\gamma_1}{2}\sum_{i=1}^{m}\sum_{j=1}^{\hat{N}} k_{nij}^2 + \frac{d_1}{2}\sum_{i=1}^{m}\sum_{j=1}^{\hat{N}} w_{ij}^2 - \sum_{q=1}^{\hat{N}}\sum_{p=1}^{m}\lambda_{ef}\left(y_{ef} - \sum_{i1=1}^{m} w_{i1e}f_{fi1} - k_{ef}\right) \quad (9)$$

In Eq. (9), $i = 1,2, \dots m$, $j = 1,2, \dots \hat{N}$, $\hat{N}$ denotes the count of hidden nodes in the neural network, k_{nij} is the ij-th element of the error matrix k, w_{ij} is the ij-th element of the weight matrix $\sum_{i1=1}^{m} w_{i1e}f_{pi1} = w_e f_f$ and λ_{ef} is the ef Lagrange multiplier.

Differentiating L_1 in relation with $w_{ij}$is given as follows:

$$\frac{\partial L_1}{\partial w_{ij}} = d_1 w_{ij} + \sum_{t=1}^{m}\lambda_{jt}f_{ti} \quad (10)$$

Assume $\frac{\partial L_1}{\partial w_{ij}} = 0$, will results to

$$d_1 w_{ij} = -\sum_{t=1}^{m}\lambda_{jt}f_{ti} = -\lambda_{j1}f_{1i} + \lambda_{j2}f_{2i} + \cdots + \lambda_{jm}f_{mi}$$

$$= -[\lambda_{j1} \quad \lambda_{j2} \quad \lambda_{jm}]\begin{bmatrix} f_{1i} \\ f_{2i} \\ \vdots \\ f_{mi} \end{bmatrix} \quad (11)$$

and

$$d_1[w_{11} \quad w_{21} \quad \dots \quad w_{m1}] = -[\lambda_{11} \quad \lambda_{12} \quad \dots \quad \lambda_{1m}] \times \begin{bmatrix} f_{11} & f_{12} & \cdots & f_{1\hat{N}} \\ f_{21} & f_{22} & \cdots & f_{2\hat{N}} \\ \vdots & \vdots & \cdots & \vdots \\ f_{m1} & f_{m1} & \cdots & f_{m\hat{N}} \end{bmatrix} \quad (12)$$

Hence,

$$d_1 w = -F\lambda^T \quad (13)$$

Additionally, differentiating L_1 with respect to k_{fij} is given as follows,

$$\frac{\partial L_1}{\partial k_{fij}} = \gamma_1 k_{fij} + \lambda_{ij} \quad (14)$$

Solving $\frac{\partial L_1}{\partial k_{fij}} = 0$, the following relationship is given as follows:

$$\lambda = \gamma_1 k_f \quad (15)$$

Considering the constraint in Eq. (3.8), the following Eq. (16) is expressed as,

$$\lambda = \gamma_1(y_{ACE} - w^T F) \quad (16)$$

and using (15) in (13) leads to

$$d_1 w = \gamma_1 F(y_{ACE} - w^T F)^T = \gamma_1 F y_{ACE}^T - \gamma_1 F F^T w \quad (17)$$

After that, the optimal weight matrix w is derived as follows:

$$w = \left(\frac{d_1}{\gamma_1}I + FF^T\right)^{-1} FY_{ACE}^T \quad (18)$$

Eq. (18) is applied in Eq. (6) and the final ensemble learning classifiers is calculated as weighted majority vote of the weak classifiers $A(s)$ where each classifier is allocated by weighting w_h. By ensembling weak classifier, the lung

cancer prediction accuracy is improved and error rate is reduced. The overall flow of IDA-BWONNEC based lung cancer prediction is shown in Figure 1.

IDA- BWONNEC Algorithm for Lung Cancer Prediction

Input: Gene expression data, b, $w = (w_1, w_2, \ldots w_m)$, iteration $i = 1,2, \ldots m$, optimal weight μ
Output: Lung cancer prediction

```
Begin
    Select the most important features F from the gene expression data using IDA
    Initialize w
        For each feature f_i in F and iteration i
            Calculate w_s using Eq. (1)
            if w_s ≤ μ then
                Calculate ε_n using Eq. (3) and Eq. (4)
            Get k_n using Eq. (3.5)
            Calculate the optimal weight of each weak classifier using Eq. (18)
            Obtain A(W_S) using Eq. (6)
            End if
            Else
                Go to step 5
End for
End
```

RESULTS AND DISCUSSION

The effectiveness of IDA-BWONNEC based lung cancer prediction method is implemented in MATLAB 2017b and compared with the DA-RBNN and IDA-SMO based lung cancer prediction method with respect to accuracy, precision, recall and F-measure. For the experimental purpose, a lung cancer dataset is used which is available in http://grafia.cs.ucsb.edu/autodecoder/dataset.html. This dataset consists of 12,625 genes and 56 samples. The instance in lung cancer dataset ranges from AD2 to AD384.

Accuracy

It is the fraction of correct lung cancer prediction over the total number of instances evaluated. It is calculated as,

$$Accuracy = \frac{True\ Positive\ (TP) + True\ Negative\ (TN)}{TP + False\ Positive\ (FP) + TN + False\ Negative\ (FN)}$$

where, TP is actual positive data (i.e., presence of lung cancer) which are exactly predicted as positive, TN is the actual negative data (i.e., absence of lung cancer) which are accurately predicted as negatives, FP is called as negative data which are incorrectly predicted, FN is the called as positive data which are incorrectly predicted.
Table 1 shows the comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of accuracy.

The lung cancer prediction accuracy of DA-RBNN, IDA-SMO and IDA-BWONNEC is shown in Figure 1. The accuracy of IDA-BWONNEC is 4.18% and 1.94% greater than DA-RBNN and IDA-SMO method respectively for lung cancer prediction. By ensembling weak classifier, the accuracy of IDA-BWONNEC is increased. From this analysis, it is concluded that the proposed IDA-BWONNEC has high accuracy than DA-RBNN and IDA-SMO method.

Precision

It measures the positive patterns (i.e., presence of lung cancer) which are accurately predicted from the total predicted patterns in the positive class. It is calculated as,

$$Precision = \frac{TP}{TP + FP}$$

Table 2 shows the comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of precision.

The comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC based lung cancer prediction in terms of precision is shown in Figure 2. The precision of IDA-BWONNEC is 4.77% and 0.85% greater than DA-RBNN and IDA-SMO method respectively for lung cancer prediction. It is found that the lung cancer prediction accuracy is improved using IDA- BWONNEC because of measurement of the weak classifier and new component based on error function through ensemble classification. From this analysis, it is proved that the proposed IDA-BWONNEC method has high precision than other methods for lung cancer prediction.

Recall

It measures the ratio of positive patterns which are correctly predicted. It is calculated as,

$$Recall = \frac{TP}{TP + TN}$$

Table 3 shows the comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of recall

Figure 3 shows the recall of DA-RBNN, IDA-SMO and IDA-BWONNEC methods. The recall of IDA-BWONNEC is 5.78% and 1.6% greater than DA-RBNN and IDA-SMO method respectively for lung cancer prediction. This result is achieved with the ensemble classification in BWONNEC where the weak classifier with minimum weighted error is used for lung cancer prediction. From this analysis, it is proved that the proposed IDA-BWONNEC method has high recall than other methods for lung cancer prediction.

F-measure

It is the dynamic average of precision and recall. In F-measure, precision and recall are combined to find an optimal blend of them. It is calculated as,

$$F - measure = 2 \cdot \left(\frac{Precision \cdot Recall}{Precision + Recall}\right)$$

Table 4 shows the comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of F-measure.

In Figure 4, the DA-RBNN, IDA-SMO and IDA-BWONNEC methods are compared in terms of F-measure. The F-measure of IDA-BWONNEC is 5.11% and 1.72% greater than DA-RBNN and IDA-SMO method respectively for lung cancer prediction. The ensembling of weak classifier minimizes the incorrect prediction of lung cancer which increases the F-measure of IDA-BWONNEC. From this analysis, it is concluded that the IDA-BWONNEC method has high F-measure than DA-RBNN and IDA-SMO methods for lung cancer prediction.

CONCLUSION

In this paper, a BWONNEC to further improve the lung cancer prediction accuracy and reduce the prediction error. Here, the lung cancer prediction undergoes two processes are feature selection and ensemble classification. In feature selection process, the most important features in the collected lung cancer data are selected using IDA. The selected features are given as input to the ensemble classification where BWONNEC is applied for lung cancer prediction. In BWONNEC, optimized weights with respect to the decision of every ensemble classifier are described aggressively, based on the outcome of all ensemble classifier and the connection between the outcomes of all ensemble classifiers. Therefore, the lung cancer prediction accuracy is enhanced with low error rate. The experimental analysis proves

that the proposed IDA-BWONNEC based lung cancer prediction method has high accuracy, precision, recall and F-measure compared to DA-RBNN and IDA-SMO method. In future, BWONNEC is extended as deep learning classifier to achieve sparse learning with less parameter for lung cancer prediction.

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Table1.Comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of Accuracy

	DA-RBNN	IDA-SMO	IDA-BWONNEC
Accuracy	0.91	0.93	0.948

Table 2.Comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of Precision

	DA-RBNN	IDA-SMO	IDA-BWONNEC
Precision	0.902	0.937	0.945

Table 3.Comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of Recall

	DA-RBNN	IDA-SMO	IDA-BWONNEC
Recall	0.90	0.937	0.952

Table 4 .Comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of F-measure

	DA-RBNN	IDA-SMO	IDA-BWONNEC
F-measure	0.90	0.93	0.946

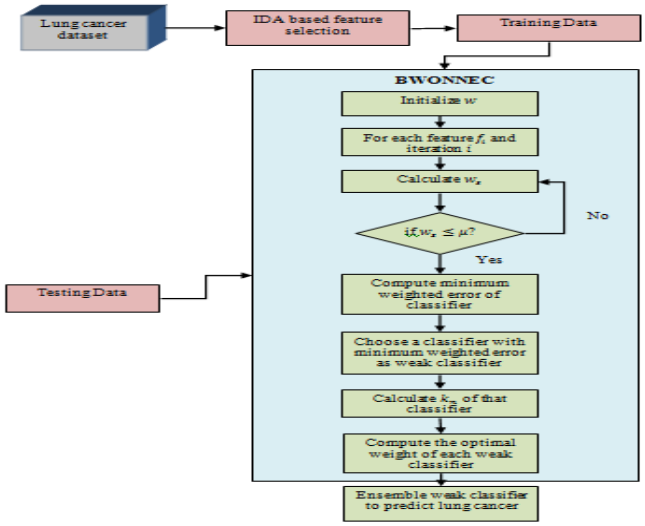

Figure.1 Overall flow of IDA-BWONNEC based lung cancer prediction

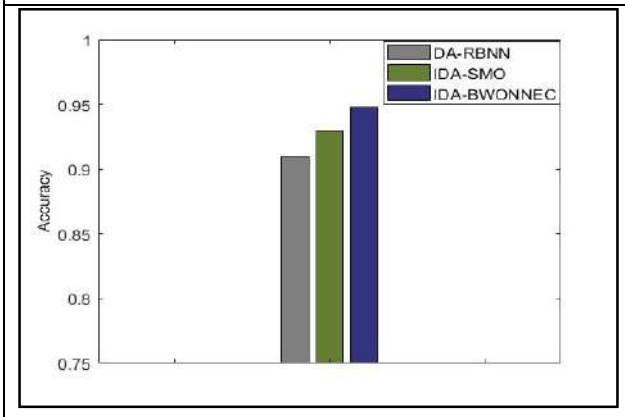

Figure 2. Comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of Accuracy

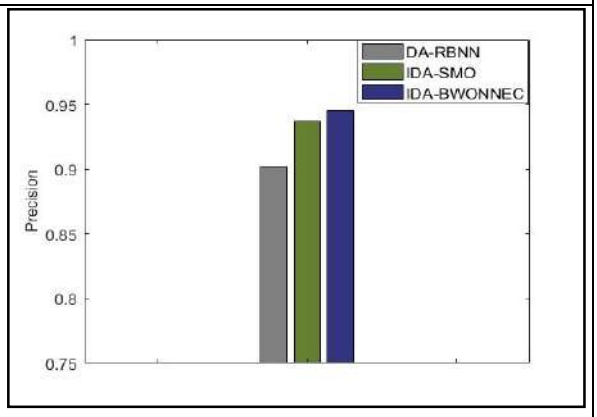

Figure 3.Comparison of IDA-SMO and IDA-BWONNEC in terms of Precision

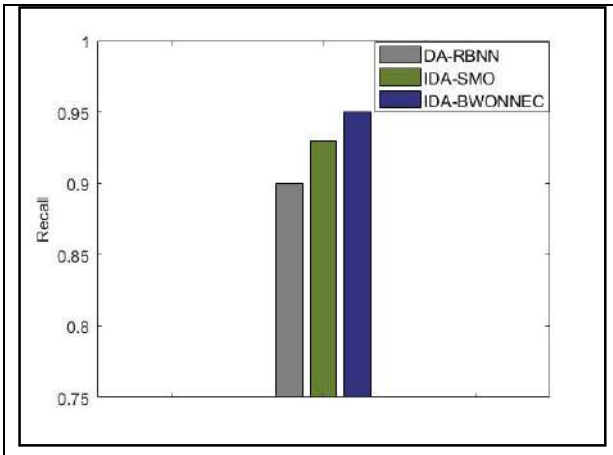

Figure 4.Comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of Recall

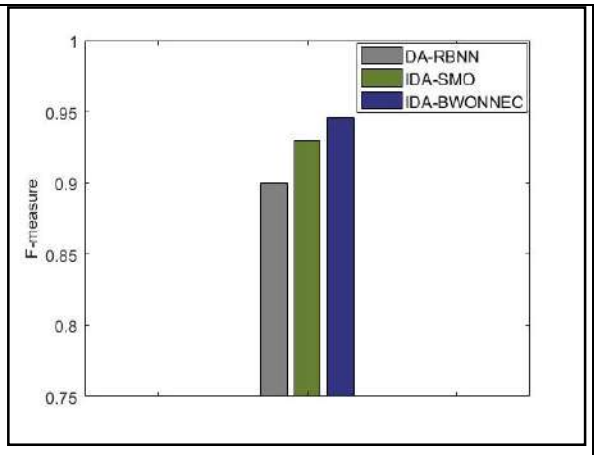

Figure 5.Comparison of DA-RBNN, IDA-SMO and IDA-BWONNEC in terms of Recall

Indian Journal of Natural Sciences *www.tnsroindia.org.in ©IJONS*

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Diversity, Dominance and Evenness of Butterflies in Southern Part of Western Ghats (Palani Hills)

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Received: 06 Mar 2021 Revised: 06 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

The present study has been done in southern part Western Ghats of Palani Hills in Tamilnadu. It was conferred as a global biodiversity hotspots that includes Kodaikanal Wildlife Sanctuary is to be proposed. We have studied the Diversity, Dominance and Evenness of butterflies in different habitats (forest area, crop area and river bank,) during the period of December 2018 to march 2019. A total number of 92 species, from 65 genera and 5 families were recorded. The diversity of Species and abundance have recorded maximum in March-May and dropped it to the minimum in December2018-January2019. Forest area habitats had a greater species diversity, while the river bank habitat had a greater number of butterflies; crop area had the least diversity of individuals and abundance present in the studied habitats. We have also been recorded the endemism and the flight period of some of the butterflies and their distribution range within the habitats with their nectar source plants. Analyses were also done to emphasize the importance of butterfly individuals and their need for the conservation.

Keywords: Butterflies, Dominance, Diversity.

INTRODUCTION

Butterflies *(Lepidoptera: Rhopalocera)* are the group of most plant dependent insects when compared to the other mega diverse group of insects. (Kristensen *et al.,* 1999) Butterflies are most beneficial as they are serving as a pollinators and the indicators of environmental quality and appreciated for their aesthetic value (Chakravarthy *et al.,* 1997). The holometabolous life history of the butterflies reveals that the family Lepidoptera are exposed to the wide range of environmental influences and are most sensitive to the climatic changes particularly in temperature, humidity and the light levels (Erhardt 1985; Warren *et al.,* 2001). Nearly 1500 butterflies (Smetacek 1992; Gay 1992) were identified from the Indian sub- continent, constituting 8.33% of 18,000 known species of the globe; most of the Indian butterflies were reported from the Himalayas and from the Western Ghats region (Larsen 1987a; 1988). The population status of butterflies in any study area would help us to understand the status of the ecosystem as they are good indicators of species (Karemen 1992). Nearly 300 species of the butterflies were recorded in a detailed survey of Nilgiri Biosphere Reserve (Larsen 1987a; 1988). At present Nilgiri Biosphere Reserve is one of the 18 hot-spots of the World, conferred as a UNESCO world heritage site.

Butterflies are the good indicators in terms of anthropogenic disturbance and a habitat quality (Kocher *et al.,* 2000). Especially in the forest ecosystem when these habitats are fragmented, butterflies that shift from one habitat to other have been increased chance of exposure to predators and they are vulnerable to disturbances related with human activity. The effects of the habitat lose can be seen clearly with the decreasing population of butterflies. Moreover, the butterflies are displaced after its habitat loss disappears subsequently. Climatic changes create a major impact in the diversity of species and they are expected to exacerbate the ecosystems (Scott *et al.,* 2005). The changes in parameters of rainfall patterns, temperature and extreme weather conditions such as heat waves, excessive rainfall, prolonged drought has to be taken it for consideration. Depletion of nectar and desiccation of the host plants causes direct mortality and induce migratory behaviors. Butterflies, being exothermal in nature, they are highly sensitive to climatic variation and a short generation time makes the individuals an appropriate model organisms to study.

The intensification of Agricultural is widely accepted as a cause of declining biodiversity. It is however a broad concept of encompassing many factors, such as loss of semi-natural habitat, ecosystem fragmentation , use of heavy machinery and increased inputs of insecticides, herbicides and pesticides (Tilman *et al.,* 2001). Of these, a chemical pesticide potentially affects the development of butterfly larva and nectar producing plants which adversely affects the adult butterfly diversity. Adult temperate butterfly species feeds primarily on nectar (Scoble 1992), supplemented to varying extents by dung or carrion and mud, (Boggs *et al.,* 2004). The agriculture development field in the forest ecosystem endangered many species throughout the world; at present the extinction rates are estimated to be 100 - 1,000 times the natural rate, depending upon the taxonomic group (Scriber *et al.,* 1995; Virtuosic *et al.,* 1997).

The diversity of butterfly species communities has been studied in different habitat types in different parts of the world including the Indian Great Himalayas Region. However, there are not more studies have done on the diversity of butterfly communities in tropical forests within the different habitat types from southern India especially in Western Ghats. Lien van Vu (2009) reported that the forest edges have greater diversity of butterflies which has more exposure to the open area. The gaps in the forest like the river path or stream have higher diversity of butterflies than the closed forest areas (Spitzer et al., 1997; Lien van Vu *et al.,* 2011).

The present study is to be aimed to examine the diversity, dominance and evenness of butterflies across three different habitats in Palani, namely forest area, river bank, and crop area, located in different altitudes from the southern part of Western Ghats and to correlate with the anthropological activity, availability of host plants and their nectar source.

METHODOLOGY

Transects and Butterfly Data

The sampling is based on standardized "Pollard walk" method (Pollard 1977; Pollard *et al.,* 1993). We have applied line transects of about 1000 Meters in length which has been divided into five segments of 200 meters. Each transect is being observed 3 times and the number of individuals of species as recorded from all the five segments. The butterflies are observed within 2.5 meters to the right and left side and five meters in front of the researcher or observer. The Butterflies are Photographed by using Canon DSRL (1500D) Camera and Identified through BNHS Field Guide. Details such endemism and mud puddling as habitat of occurrence, behavior were recorded. Also we have examined the vegetation in the transect line area.

Study Period

The butterfly species were collected using sweeps soft net and, photographed. The collection was done every month from December 2018 to March 2019 at 8.00 AM to 4 PM under perfect weather conditions (temp 18 ℃, (always >13℃) cloudless or just few clouds and speed of the wind\5 Beaufort (only leaves and thin branches are moved by wind).

Study Area

The study has been taken in different regions of the Southern Parts of Western Ghats, in Palani Hills. This Spot is most important biodiversity hot spots of our Western Ghats. The Palani Hills are the mountain range in the states of Tamil Nadu and Kerala in South part of India. The Palani Hills situated eastward extension of the Western Ghats ranges, which is runs parallel to the west coast of South India. Palani Hills (Figure – 1) adjoins the high Annamalai range in Kerala on the west, and extend towards east into the plains of South Tamil Nadu, covering in an area of 2,068 square kilometres (798 sq miles). The highest parts of this range are situated in the southwest, and reach into 1,800-2,500 meters (5,906-8,202 feet) elevation; the eastern extension of this range is made up of hills 1,000-1,500 m (3,281-4,921 ft) high. Different climatic gradients with various habitats like tropical moist deciduous forest, evergreen forest, moist deciduous forest, reservoirs, rivers, grass lands have been produced most favorable conditions for greater diversity of insects in Western Ghats.

The Western Ghats receives an annual rainfall of 7600 mm per year; it receives more of its rain from the southwest monsoon during June-September; this region usually gets fairly dry for the rest of the year. Annual mean minimum and maximum temperatures range from 9.6℃ to 20.7℃ to respectively. Annual relative humidity is 76.9% - 75.8%. A total number of 21 transects (one transect per study site) has been taken for this study representing three different types of habitats consisting the forest area, crop area (located within fragmented regions in the forest), and the river bank

Forest Area

Forest area is being represented by thick canopy with almost shady ground and high relative humidity. Fourteen sites were observed in this habitat seven sites were observed in this habitat. namely Kumbakarai (10°184883″ N 77.5157″ E Elevation 583m / 1925ft), Kombai (10°2945″ N 77°4370″ E Elevation 1020m / 3341ft), Pethuparai (10°2820″ N 77°5303″ E Elevation 1369m / 4490ft), Vellagavi (10°1970″ N 77°4992″ Elevation 1343m / 4408ft), Perumalmalai (10°2641″ N 77°5375″ E Elevation 1563m / 5127ft), Vattakanal (10°2153″ N 77°4853″ E Elevation 2080m / 6825ft), Bandichoolai (11°3720″ N 76°8194″ E Elevation 1979m / 6492ft), Shenbaganur (10°2317″ N 77°5030″ E Elevation 1798m/ 5899ft), Vegetation comprised of *Dahlia imperialis, Helichrysum bracteatum, Anaphalis leptophylla, Leucas martinicensis, Physalis peruviana, Rubus ellipticus, Citrus aurantifolia, O.chinensis, Crotalaria pallida, Triumfett aannua, Toddalia asiatica, Salvia coccinea, Eucalyptus globules, Bidenspilosa, Siegesbeckia orientalis, Erigeron karvinskianus, Calotropis gigantea, Musa paradisiaca, Ficus racemosa, Terminalia paniculata, Gloriosa superba, Malvastrum coromandelianum, Osbeckia octandra.*

River Bank

Rivers creates gaps in the forest; the river bank that lies on the open area within the forest support a variety of fauna including some kinds of insects. They have a microclimate that can be very different from the forest hence we have been considered in this area for this study. With a very thin vegetation of grass and shrubs, damp soil, the river bank was an ideal ground for many nectar containing plants. The present study considered one in this habitat; This was Elephant Valley Cascade (10°3116″ N 77°5205″ E Elevation 1148m / 3764 ft) located in 20 Km from the Palani Hills region. The Elephant Valley Cascade is one of the most important hotspots in the Western Ghats region. Dominant nectar source vegetations within the transect lines are *Hibiscus vitifolius, Chromolaena odorata, Helichrsysm bracteatum, Hibiscus rosasinensis, Capparis zeylanica, Tecoma stans, Osbekia octandra, Asystasia sp., Gnidiaglauca. Asclepias curassavica, Datura stramonium, Hyptis sp., Plum, Sarracenia alata, Sennatora.*

Crop Area

Various agricultural fields are chosen for this study to analyze the butterfly diversity among the fragmented area of the forest as well as to find the impact of pesticides and insecticides in their habitat. Line transect was fixed in the following agriculture fields and the study were conducted in the following sites are Shenbaganur (10°2317″ N 77°5030″ E Elevation 1798m / 5899ft), Pallangi (10°2732″ N 77°4523″ E Elevation 1665m / 5461ft), Poombarai (10°2471″ N 77°4040″ E Elevation 1926m / 6320ft). Dominant nectar plants were recorded *Citrus sinensis, Brassica oleracea, Beta vulgaris, Persea americana, Phaseoiuscoccineus, Piper nigrum, Camellia sinensis, Coffea arabica, Solanum tuberosum, Daucus carota, , pyrus* sp.

RESULTS

Diversity Calculation

The total number of butterfly species collected under each identified species in various habitats were recorded and the diversity indices namely dominance index, Shannon's diversity indices (H′), and evenness index (e^H/S) has been calculated by using PAST software (PAST; version=2.02).

Species Richness

There was a significant variance in number of species between the different types of habitats (Figure. 2). The Species Richness was greater in Forest Area (92) than in other two habitats, River Bank Habitat (78) and Crop Area Habitat (61). In all three habitats the maximum richness were recorded in the month of April and the minimum richness have been recorded in the month of December2018 and January2019.(Figure-3)

Species Abundance

In the totally recorded individuals from three different habitats in Forest Area (15927), River Bank Side (19230) and Crop Area (10371), the maximum abundance were noted in two seasons, March2018 – May2018 and September2018 – November2018 with the peak in April2018 and October2018 respectively. Maximum abundance within these habitats was observed in River Bank Side (4751) followed by Forest Area Side (3558) and Crop Area Side (2462). Minimum abundance is observed in the month of January (River Bank – 108, Crop Area– 79, Forest Area –19). Dominance, Simpson Diversity and Evenness has been studied using PAST version 2.0. Maximum dominance is recorded in River Bank area (0.11260) in the month of January2019 when compared to other two habitats, Forest Area side (0.09945) and Crop Area side (0.09123). In all three habitats the dominance showed similar kind of trend. Minimum dominance is recorded in the month of April followed by October. The River Bank habitat showed greater dominance among all three habitats.

Maximum Simpson diversity index was recorded in the River Bank (0.9743) in the month of April followed by Crop Area side (0.9819) and Forest Area side (0.9661). Simpson Diversity has showed the peaks in the months of April and October in all three habitats. Minimum Simpson diversity index was recorded in January for River Bank side (0.9088),

Crop Area side (0.9005), and Forest Area side (0.9088). The Trends in the Evenness index showed that there is a considerable dominance by few species in all three habitats. However in crop area the evenness was least modified throughout the whole season. Forest Area had the minimum evenness index with a higher degree of dominance particularly in the months of January (0.7792) and October (0.6128). Highest mean range Evenness has been found in River Bank side (0.6954) habitat followed by Crop Area side (0.6768) and Forest Area side (0.6054).

DISCUSSION

Western Ghats is to be considered one of the twelve mega biodiversity hotspots of the world (Larsen 1987a,b,c.); the climatic conditions and the tropical temperature with a very high rainfall throughout the year (Larsen 1988) has made it favorable for the richness of the species. Butterflies *(Lepidoptera: Rhophalocera)* are considered as a ecological indicators(Mc Geoch 1998; Rosenberg *et al.,* 1986; New *et al.,* 1995; Vu 2007). The butterflies helps in pollination (Johnson *et al.,* 1994; Johnson *et al.,* 1995). In this study, butterflies from all the families has been recorded; among them, family *Nymphalidae* is outnumbered with the maximum species throughout this period of study; this happens because of their ecological adaptation (Jiggins *et al.,* 1996), speciation and high dispersal ability (Adler *et al.,* 1994). Family *Nymphalidae* is the largest family representing nearly one-third of the known butterfly species of the world. Family *Nymphalidae* has been followed by *Lycaenidae, Papilionidae, Pieridae,* and *Hesperiidae* in the total number of species were observed. Similar findings has been reported by Mathew and Rahamathulla (1993) from the Western Ghats. Wynter- Blyth (1956) has identified two seasons, March-April and October is the peak periods in India for the species diversity and abundance. This study observed the maximum species diversity and abundance in the months of March-May, and October-November (Fig 2&3); and there are a gradual increase in the early summer from the month of March and it has reached the maximum in the month of May; a second peak has been recorded in the month of October, November. Butterfly species in all habitats have flight periods, and their abundance strongly been correlates with their different flight periods (Leather 1984; Norris 1935). Almost all butterflies are abundant in very short peak in particular seasons, and may or may not appear in the other seasons. Diversity and abundance of butterfly correlates with the flowering phenology of plants (Gutierrez *et al.,* 1995; Watt *et al.,* 1974; Kunte 2000). Some of the polyphagous species (Larsen 1988) like *Catopsilia pomona, Neptishylas and Euremaheceba*, were abundant throughout the year in all habitats. Species like *Papiliocrinohas* only short flight period and have been found only in the month of March in the river bank.

Eupolio core and *Papilio polymnester, Junonia almana*, has a long flight period. Species abundance and diversity is declined in two seasons, one in December-January due to the extreme cold and withering of flowers (nectar source) and again in late summer, June-July due to the non-availability of nectar source, over heat, and scarcity of water in the area. Among all the habitats studied, less abundance were recorded in the crop area habitat of the Western Ghats which can be due to non availability of the host plant. This needs to be studied further. Presence of the butterflies' in a particular habitat depends upon a wide range of factors; the availability of the food and microclimate are considered to be the most important (Janzen *et al.,* 1968). In these results, the river bank habitat had the greatest abundance of butterflies but lower in species number more than the forest and crop area habitats. The living environment of the river bank habitat is being diversified with the vegetation, animal dung, mud, rocks, sand, with water that attracts more butterflies (Janzen *et al.,* 1968), and thus the river bank habitat had the greatest abundance of the butterflies. The river bank habitat is less in diverse (78 species) than the forest habitat (91 species) probably due to the availability of host plant. Greater abundance (Fig 4) and less diversity (Fig 6) lead to the higher dominance (Fig 5) in the river bank habitat (0.1126) more than the other habitats. *Euremahecabe, Graphiun sarpedon, Catopsilia Pomona,* and *Ypthima ceylonica* were observed throughout the year in the river bank habitat.

Evenness index ranged between 0.4237 to 0.8341 in three different habitats (Fig 7). Optimum Evenness was found between June and July in this study, where the diversity and dominance of butterflies were even. Low rain fall, moderate availability of nectar source, and moderate temperature resulted in optimum evenness. High fluctuation in

Evenness in crop area is observed because of the different cultivable plants that have been varied in blooming period. In crop area *Lycaenidae* species *(Acytolepis puspa, Amblypodia anita,)* were dominantly found, because of their varied mechanism of feeding, ability to change their host plant, and symbiotic relationship with ant which is increased their caterpillar survival rate. The Western Ghats is unique in endemism (Holloway 1974, Sudheendrakumar *et al.,* 2000); each and every habitat has a specific set of microenvironment suitable for the endemic species (Holloway 1974). Seventeen endemic species of butterflies are observed and recorded in the present study. Among them, ten species namely *Papilio polymnestor, Vanessa indica, Vanessa cardui, Papilio helenus, Troides minos, Melanitis leda, Mycalesis axis, Cirrochroa thais, Cupha erymanthis, Colias nilagiriensis, Orsotrioena medus, letherohrianeelgiriensis, Tagiades litigiosa* and *Loxura atymnus* were observed in the forest habitat, and three other species were found such as *Hebomoia glaucippe, Phalanta phalantha, Papilio crino* from the river bank habitat. Maximum numbers of endemic species were found in the undisturbed evergreen forest when compared to other habitats; this may be observed due to the availability of host plant and least disturbance. Significantly, we couldn't record any endemic species in the crop area habitat side.

CONCLUSION

The statistical comparison of results in three different habitats shows that butterflies' diversity and abundance have significantly declined in the crop area habitats than the other two habitats, - river side and forest habitat. This is observed probably due to the destruction of host plant in the crop area habitat, usage of chemical pesticides, and human disturbance. Fragmentation of the forests for crop area could be certainly destroy the host plant and could greatly influenced the biodiversity of butterflies. Biodiversity laws alone cannot create an awareness and conserve the butterflies. It is most important to understand the relation between the host plants and the butterflies to protect them as they have co-evolved. Western Ghats being conferred as one of the biodiversity heritage sites needs more attention for effective conservation of butterflies.

ACKNOWLEDGEMENT

Authors were thankful to Dr. A.K. Khaja Nazeemudeen Sahib, Secretary and Correspondent, Dr. S. Ismail Mohideen, Principal and Dr. I. Joseph Antony Jerald, Head, P.G. and Research Department of Zoology, Jamal Mohamed College (Autonomous), Tiruchirappalli for Institutional support. We profoundly thank Principal Chief Conservator of Forests & Chief Wildlife Warden, Chennai for permitting to carry out the research work (Ref. No WL (A)/3378/2018-2019/Permit 26/2018-2019).we whole heartedly thank Dr. D. Paul Dhayabaran, Principal, Bishop Heber College (Autonomous),Tiruchirappalli for their support and help. We are also thankful to District Forest Officer & Wildlife Warden, Kodaikanal Wildlife Sanctuary and District Forest Officer, Dindigul Forest Division for their timely helps.

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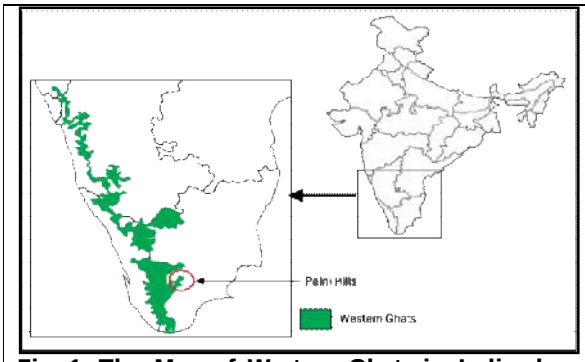

Fig. 1. The Map of Western Ghats in India shows biodiversity hotspot includes Palani Hills

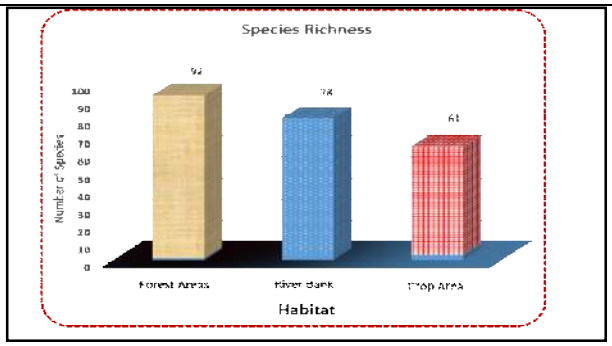

Figure. 2. Shows the species richness found in the forest area when compare to riverbank and crop area.

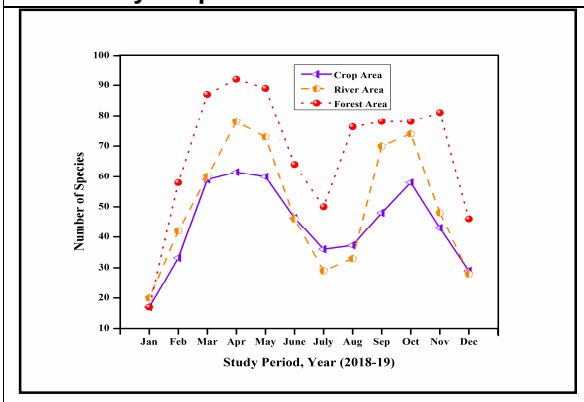

Figure. 3. Shows the Species richness found in the forest area in the month of April followed by riverbank and crop area

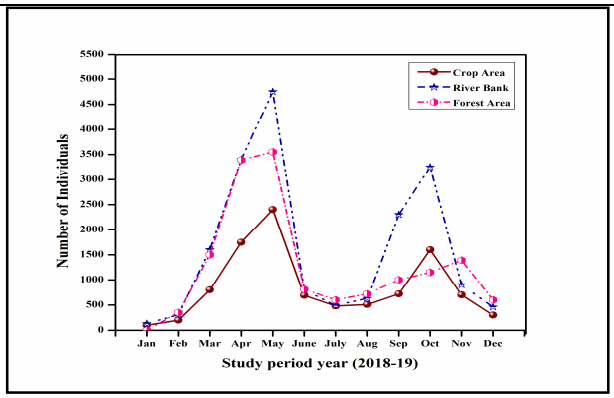

Figure 4. Graph showing the two peaks of abundance of individuals recorded in the month of March-May followed by September-November

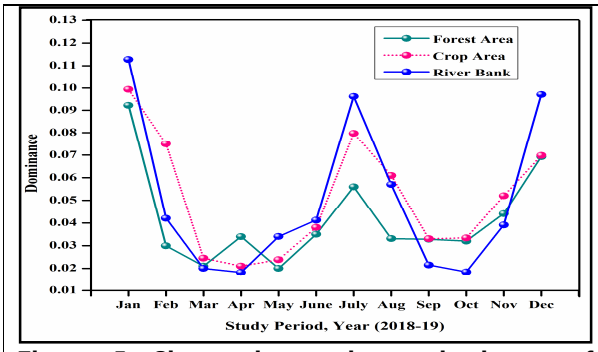

Figure. 5. Shows the maximum dominance of Butterflies in river bank habitat than the other study area

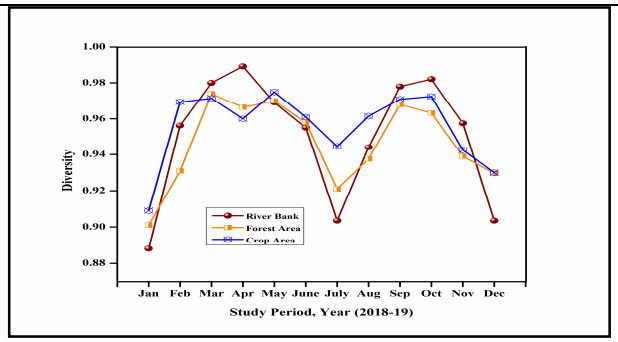

Figure. 6. Shows the greater diversity of butterflies in forest area followed by river bank and crop area in the month of April

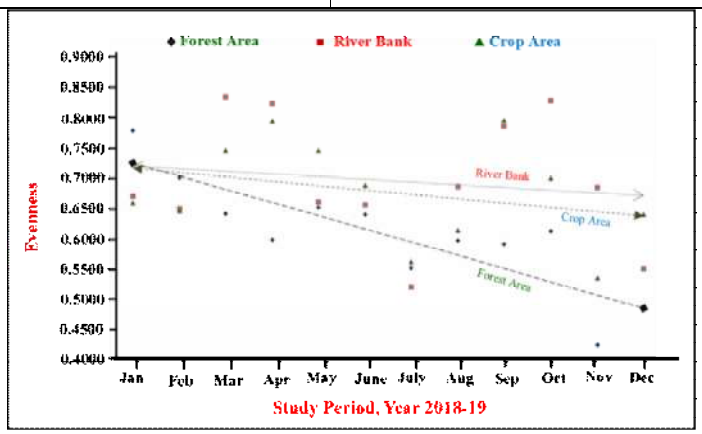

Figure. 7 shows the high degree of Evenness fluctuation throughout our study period

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Larvicidal Activity of Aqueous Extract of *Solanum trilobatum* L. Leaves and Stem against *Aedes aegypti*.

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Received: 05 Mar 2021 Revised: 05 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

To determine the larvicidal activity of aqueous extract of *S. trilobatum* L. leaves and stem against *Aedes aegypti.* Qualitative analysis of phytochemical constituents, protein and carbohydrate constituents, GCMS analysis and larvicidal activity against *Aedes aegypti* presents in aqueous extract of *S. trilobatum* L. Leaves and stem. The qualitative phytochemical analysis of aqueous extract of *S. trilobatum* L. Leaves and stem showed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Proteins, Carbohydrate, Phenol, Glycoside and Terpanoids, total protein content of *S. trilobatum* L. leaves and stem was 80µg and 82µg present respectively and total carbohydrate content of *S. trilobatum* L. leaves and stem was 620µg and 580µg present respectively.The GC-MS analysis of aqueous extract of *S. trilobatum L.* leaves revealed the presence of 29 compounds and stem revealed the presence of 30 compounds and the larval mortality of *A. aegypti* was most prominent and showed 50% mortality in 500 (µL/1L) concentration with the LC_{50} of 95.991(LCL=58.775;UCL=156.771) for 24 hours and 85% mortality in 500µL/1L concentration with the LC_{50} of 38.646 (LCL=26.947;UCL=55.423) for 48 hours for aqueous extract of *S. trilobatum* stem than leaves. In conclusion, the aqueous extract of *S. trilobatum* stem showed best response than leaves in larvicidal activity against *A. aegypti.*

Keywords: *Aedes aegypti,* GCMS, Larvicidial activity, Mosquitoes, Leaf extract, Stem extract, Mortality.

INTRODUCTION

Dengue fever is one of the most dangerous fever threatening humans in past years. Especially in developing countries, India is the main victim of this disease. 1.5 million Cases of dengue have reported [1]. Nowadays Aedes mosquitoes transmit dengue fever, yellow fever, chikungunya and Zika virus, along with many other diseases. Aedes mosquitoes are visually distinct compare to other mosquitoes because they have black and white markings on their bodies and legs. They are mostly active during the day in shaded areas and into the early evening. Female mosquitoes take blood as feed primarily on humans in order to lay eggs. This species is called as vector or carrier for the following diseases, yellow fever, dengue fever, chikungunya. These viruses are spread on to humans through the bites of an infected female Aedes mosquito through feeding on the blood of an infected person [2]. Naturally plants have a source of medicinal agents for thousands of years. Even now, approximately 80% of the world population is almost entirely dependent on traditional medicines for maintaining health and cure many diseases [3]. Herbal medicine is the foundation for about 75–80% of the world population, mainly targeting primary health care for in the developing countries because of better cultural acceptability, compatibility with human body and lesser side effects. However, there is a drastic increase in the usage of herbal medicine was found in last few years from the developed countries [4]. *Solanum trilobatum* Linn. was a Solanaceae family, a thorny creeper with bluish- white flower and grows as climbing under a shrub. It is one of the important medicinal plants, more commonly available in Southern India and has been used in herbal medicine to treat various diseases like respiratory problems, bronchial asthma, and tuberculosis [5]. This plant is well known in Ayurveda and Siddha systems. In Sanskrit it is known as 'Alarka', in Telugu 'Alarkapatramu', berries and flowers are used for cough [6]. So, the present study was investigated the larvicidal activity against *Aedes aegypti* using aqueous extracts of leaves and stem of *Solanum trilobatum* Linn.

MATERIALS AND METHODS

Extraction of Plant Material

Aqueous Extraction

The Fresh leaves were chopped into small pieces and weighed. The 100 g of the chopped leaves was completely soaked in 250 mL of distilled water at room temperature for 48h. After 48 h, the extract was filtered using muslin cloth as sieve and for filtration used No. 1 Whatman filter paper and the filtrate was collected in a beaker. The filtrate was concentrated to dryness using a water bath at 40°C until a dry and dark-green extract was obtained. The extract was properly stored in an air-tight container for further studies. This same extraction process is also followed for extraction of stem.

Yield of Extract: (Osonwan et al., 2012)

The percentage yield of the extracts was obtained after extraction using following formula

$$\text{Percentage Yield (\%)} = \frac{W_a}{W_b} \times 100$$

Where: W_a = Weight of obtained extract; W_b = Weight of extracted leaves.

Qualitative Analysis of Phytochemicals

Phytochemicals like alkaloids, Carbohydrate, Phenol, Glycosides, Terpenoids, Flavonoids, Saponins, Proteins, Steroids and Tannins of *Solanum trilobatum* extracts were identified [7].

Total Protein Content and Carbohydrate Content

The total protein content was estimated by the method of Lowry et al. [8]. The carbohydrate was estimated by Anthrone method [9].

Larvicidal Activity

Aqueous extract, *Aedes aegypti* mosquito larvae, beaker, water. The larva of *Aedes aegypti* was collected in ICMR-Vector Control Research Centre Field Unit. The larvicidal activity was conducted in the room temperature. The twenty 3rd and 4th instar larvae species were placed in a 200 ml water added beaker with the control set up and kept in the environment chamber 37°C with a 20 period of 16:8 hours light and dark cycle. Different concentration of aqueous extracts of *Solanum trilobatum L.* Leaf and stem (20,40,60,80 & 100µL) was added to the beaker. Observed the larvae species for 24-48 hours.

Gas Chromatography Mass Spectroscopy (GCMS)

The GCMS work was done in TUV SUD South Asia Pvt. Ltd. The aqueous extracts of *Solanum trilobatum* L. leaf and stem were subjected to GC-MS analysis to identify the presence of unknown compounds.

RESULTS AND DISCUSSION

Yield of Extracts

Weight of aqueous extract of *Solanum trilobatum* L. was obtained after extraction of stem (2.1253g) and Leaves (1.8635g) for 50g. The percentage of yield of extract in *Solanum trilobatum* L. leaves was obtained after extraction for 50g of leaves is 3.73%. The percentage of yield of extract in *Solanum trilobatum* L. stem was obtained after extraction for 50g of stem is 4.25%.

Phytochemical Screening

The phytochemical screening which carried out on aqueous extracts of stem and leaves results were shown in Table 1. The phytochemical analysis results of present study are comparable with similar reports of earlier workers. The presence of these metabolities in *Solanum trilobatum* had been reported that, preliminary phytochemical analysis of the *S. trilobatum* leaf extract of acetone solvent showed the presence of alkaloids, tannins, steroids, glycosides, and phenols [10 and 11]. *Solanum aethiopicum* L. contained higher levels of the beneficial agents than *S. macrocarpon* L. had been reported that, qualitative screening of phytochemical compounds in *S. trilobatum* aqueous extracts revealed the presence of saponins and tannins in leaves, stem, flowers and fruits [12]. Flavanoids, phenolic compounds and cardiac glycosides were present in all extracts except leaves. Similarly carbohydrates were present only in leaves and stem.

The GC-MS analysis of aqueous extract of *S. trilobatum* L. leaves revealed the presence of 29 compounds and stem revealed the presence of 30 compounds are shown in Figure 3 and 4 that contribute medicinal quality of the plant. The identified compounds were confirmed based on peak area and retention time was compared with data base of known compounds stored in the NIST library. In GCMS analysis of aqueous extract of *S. trilobatum* stem and leaves had bioactive compounds, so from that bioactive compounds may control *A. aegypti* in larvicidal activity. The GCMS analysis of present study are comparable with similar reports of eairler workers. Lalitha and Thangapandiyan [10] had been reported that, identify the phytochemicals present in acetone extracts of the leaves of *S. trilobatum* by qualitative photochemical testing and to identify the compounds present in the acetone extract of the leaves by GC–MS analysis . The GC–MS spectrum revealed that the acetone leaf extract of *S. trilobatum* contained the major phytochemical compounds such as cyclodecanol (12.42%), 2-tetradecycloxirane (6.07%), beta-sitosterol (10.25%), 1H-imidazole, 4,5-dimethyl-imidazole (4.65%), 1-octadecyne (4.30%), and phenol (4.05%). The other compounds also identified are diisoamylene (2.90%), chloro-5-iodo-benzoic acid pyridine-3-yl methylene-hydrazide (1.70%), 4-(2-oxo-bicyclo (3.1.0) hex-6-yl (1.57%), permethrin-1,3-phenoxybenzyl (1.36), 1H-pyrazole-5-carboxamide (2.38%), 2-cyclohexen-1-ol (1.38%), benzene,2-(tert-butyldimethylsilyl) oxy (2.63%), hydrochlorothiazide N-butylbronate (1.99%), silicic acid diethyl bis (trimethylsilyl)ester (2.83%), and isopropyl tris (3.48%).

In the present study, data pertaining to aqueous extract of *S. trilobatum* stem and leaves for 24 and 48 hours against third and fourth instar larvae of *A. aegypti* are shown in table 3.The larval mortality of *A. aegypti* was most prominent

as evidenced from the table 5, which showed best response of 85% mortality in 500µL/1L concentration with the LC_{50} of 38.646 (LCL=26.947; UCL=55.423) for 48 hours for aqueous extract of *S. trilobatum* stem compared to leaves extracts (Table 3). Similarly larvicidal activity for aqueous extract of *S. trilobatum* leaves showed best response of 75% mortality in 500µL/1L concentration with the LC_{50} of 57.923 (LCL=41.754;UCL=80.354) for 48 hours compared to leaves extracts (Table 3). The aqueous extraction of *S. trilobatum* L**.** 500 µL/1L stem exhibits significant larvicidal activity among various concentrations (100,200,300,400 and 500 µL/1L) against third and fourth instar larvae of *A. aegypti* in 48 hours**.**

In larvicidal activity no. of death larva were increased when concentration the range is increased like 100,200,300,400 & 500 (µL/1L) of aqueous extraction using *S.trilobatum L.* both in leaves and stem. Premalatha et al [13] had been reported that, methanol extracts of *S. trilobatum* was found to be more susceptible against the larvae of *A. aegypti, Cx. quinquefasciatus* and *An. stephensi* at 250 ppm with a LC50 value of 125.43, 127.77 and 116.64 ppm respectively. Crude petroleum ether, chloroform, and acetone aerial extracts of *Solanum trilobatum* were tested for larvicidal activity against the filarial vector, *Culex quinquefasciatus* [14]. Larvicidal activity .was evaluated at concentrations of 62.5, 125, 250, 500 and 1000 mg/L. Larval mortality was observed for 24 and 48 h. In the solvent extracts were tested, petroleum ether exhibited highest larvicidal activity and LC50 values was 203.87 and 165.04 followed by acetone and chloroform extract which were 295.87 and 186.38; 535.74 and 346.08 mg/L after 24 and 48 h respectively.

Araujo et al **[**15**]** had been reported that, the larvicidal activity of different crude extracts (methanolic, hydroethanolic and hexanic) from leaves of *Acmella oleracea* solubilized in fibroin solution, as an alternative to the organic solvents that are used against *Aedes aegypti*. Using the Probit analysis, the hexane extract showed LC 50 of 2.23µg/ mL af ter 24 h, whereas the hydroethanolic and the methanolic extracts showed LC50 of 28.42 µg/mL and 39.67 µg/mL. The *A.oleracea* extract dissolved in fibroin solution could be an alternative for controlling *Ae.aegypti* without causing damage to the environment.

CONCLUSION

In conclusion, the aqueous extract of *S. trilobatum* stem showed best response than leaves in larvicidal activity against *A. aegypti.* So the extract of *S. trilobatum* leaves or stem are used as spray for the welfare of human to control *A. aegypti*. Further studies of screening and isolation of specific compound will identify which acts effectively against mosquitoes.

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Table 1: Qualitative analysis Phytochemical constituents present in aqueous extract of *Solanum trilobatum L.* Leaves and stem.

SI.NO	Constituents	Aqueous extract of *Solanum trilobatum L.* Leaves	Aqueous extract of *Solanum trilobatum L.* stem.
1.	Alkaloids	presence	presence
2.	Flavonoids	Absence	Absence
3.	Tannins	presence	presence
4.	Saponins	presence	presence
5.	Proteins	presence	presence
6.	Carbohydrate	presence	presence
7.	Phenol	presence	presence
8.	Glycoside	presence	presence
9.	Terpanoids	presence	presence
10.	Steroids	presence	presence

Table 2: Quantitative analysis of protein and carbohydrate constituents present in aqueous extracts of *Solanum trilobatum L.* Leaves and stem.

SI.NO	Constituents	Aqueous extract of *Solanum trilobatum L.* Leaves (10 mg of extract)	Aqueous extract of *Solanum trilobatum L.* stem (10 mg of extract)
1.	Protein	80µg	82µg
2.	Carbohydrate	620µg	580µg

Table 3: Larvicidal Activity of *Solanum trilobatum* L. Leaves and stem.

S.No	Samples		Dosage (µL/1L)					LC50 (µL/1L)	95% of Fiducial limits		χ^2 (df=3)
			100	200	300	400	500		LCL	UCL	
1	***Solanum trilobatum* L.**(Aqueous Extract of stem)	24h	2 (10%)	6 (30%)	8 (40%)	8 (40%)	10 (50%)	95.991	58.775	156.771	0.964
2		48h	5 (25%)	10 (50%)	14 (70%)	15 (75%)	17 (85%)	38.646	26.947	55.423	1.000
3	***Solanum trilobatum* L.**(Aqueous Extract of leaf)	24h	2 (10%)	4 (20%)	5 (25%)	7 (35%)	8 (40%)	153.933	83.932	282.315	0.998
4		48h	6 (30%)	8 (40%)	10 (50%)	12 (60%)	15 (75%)	57.923	41.754	80.354	0.975

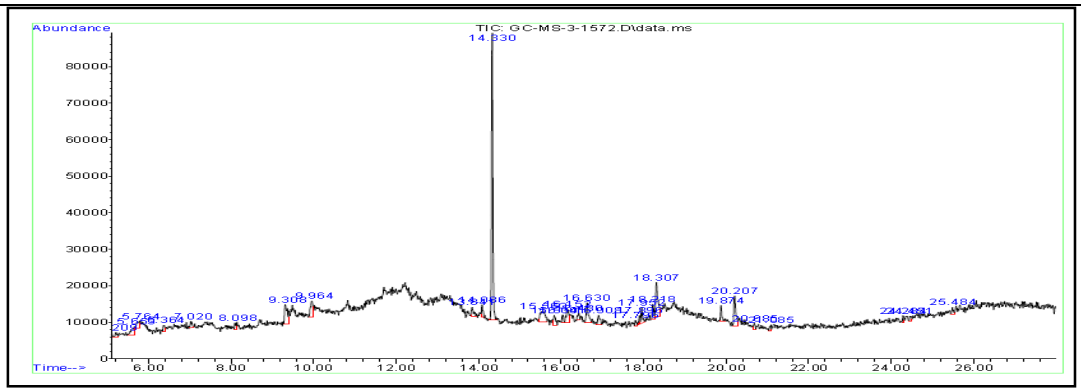

Figure 1.Chromatogram of GCMS results for Aqueous extract of leaves

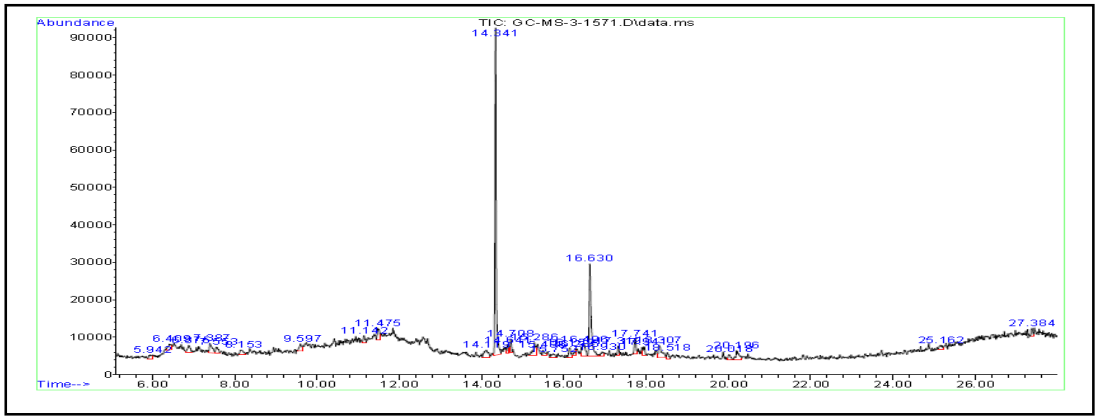

Figure 2. Chromatogram of GCMS results for Aqueous extract of stem

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Halophilic actinomycetes Inhabiting Marakanam Saltpan and their Potential Applications

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Received: 06 Mar 2021 Revised: 11 Mar 2021 Accepted: 17 Mar 2021

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ABSTRACT

Microbial diseases are caused by clinical pathogens to humans they are a big threat to public. Chemical drugs used as control agents of microbial diseases but it's induced some side effects. Hence the novel actinomycetes from natural sources produce antibiotics and controlling human pathogens. The potential of *Halophilic actinomycetes* evaluated by screening the Soil sample of Marakanam Saltpan. Five strains were isolated; two strains only produce amylase, gelatinase, protease, and cellulose enzymes. Preliminary and secondary screening performed against *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, proteus mirabilis* and *Klebsiella pneumoniae* test pathogens. Two potential actinomycetes out of five strains were identified as *Streptomyces sp* by pylogenetic analysis and submitted in NCBI with *Streptomyces* sp strain and accession number: MT211570.The potential hexane extract in GC-MS analysis were identified 3,7- Dimethyl-2,6-octadien-1-OL major compounds

Keywords: *Halophilic actinomycetes*, Enzymes, Antagonistic activity, 9-octadecenoic acid.

INTRODUCTION

Halophilic microbes are grown in salty environments salt pan, salt lake, salt mines, Brines, etc., They are categorized into three main groups slight, moderate and extreme conditions based upon the salinity [1]. Extreme environment are characterized by high salinity, low oxygen and high ionizing radiation concentrations [2]. Marakanam saltpan is one of the best sources of *Halophilic actinomycetes*. Few studies showing *Halophilic actinomycetes* produce Bioactive metabolites against the Clinical pathogens *staphylococcus aureus, Aspergillus niger*, and *Aspergillus fumigates.* They conclude *actinomycetes* act as an antibacterial and antifungal agent [3]. Pathogenic infection is a common health issue, and the least drugs only approved as antibiotics. Nowadays, less toxic antibiotics against Multidrug resistance pathogens need for human beings. Researchers developed the discovery of novel Bioactive metabolites from natural sources, [4] Microbial strains produce new emerging Bioactive substances from a soil source [5]. *Streptomyces* species produced although 1000 bioactive compounds for antibiotic production [6,7]. Secondary metabolites are largely produced by dominant group of *actinomycetes* [8]. *Actinomycetes* are the most widely distributed microbes inhabiting the soil environment [9]. The group of gram-positive bacteria is classified by actinobacteria have rich G+C content in their DNA molecule [10]. *Actinomycetes* are important products for the industrially economic enzymes like Cellulases, protease, amylase, chitinase from *Nocardia* sp, *Streptomyces* sp, *Thermomonospora* sp and *actinoplane* sp – [11]. *Halophilic actinomycetes* Produce Bioactive compounds against clinical Bacterial, fungal pathogens, Industrial Important Enzymes, pharmaceutical pigments, plant growth Hormones, and anticancer agents and Enzyme inhibitors [12,13]. Hence, in the present report focused on the *Halophilic actinomycetes* derived from Marakanam saltpan .the isolates were screened hydrolytic enzymes, antibacterial activities of the bioactive compounds.

MATERIALS AND METHODS

Isolation of *Halophilic actinomycetes*

Sediment samples were collected at the 10 cm depth and 20 meters distances at Marakanam salt pan, North latitudes 12.14'7.98" N and 790 56'9.81"^{o}E East longitudes. Samples were allowed to traditionally pre-treatment methods. Samples are carried out to serial dilutions up to 10^{5}, Aliquots of 0.1 ml of dilutions platting in starch casein agar by spread plate technique. To avoid the bacterial and fungal Contaminations using Nalidixic acid .After that incubate all plates incubated at 37℃ in the incubator for 7 days. The powdery Colonies were isolated and purified by streak plate technique Using Starch Caesin Agar [**14].**

Morphological & Biochemical Characterization

Halophilic actinomycetes isolates were performed and identified according to Bergey's Manual of Systematic Bacteriology. Gram staining, Biochemical tests of Indole, Methyl red, Voges Proskauer, Citrate, Urease, Catalase, and Oxidase tests were performed.

Screening of Industrially Important Enzymes

Amylase: All the isolates were inoculated in starch agar plates and incubated at 28±2℃ for 7 days. Plates were flooded with sterile iodine solution for 15 min. Amylase production is confirmed by zone around the media.

Protease: Skim milk agar plates prepared and introduced *actinomycetes* isolates and incubated for 48 h at 28℃, After incubation, the clear zones indicates Protease production

Cellulase: Nutrient agar plates containing (CMC) 1% Carboxy Methyl Cellulose and inoculated *actinomycetes* for 7 days at 280C. 0.1% Congo red of solution flooded on the plates for 20 min. The Clear Zone formed by the isolates was indicated Cellulase activity

Gelatinase: *Actinomycetes* isolates were streaked as a single line across the Center of the Nutrient plate Containing 1% gelatin, incubate for 2-4 days at 280C. The medium was flooded with 10 % Grams iodine solution. A clear zone around the growth of the isolates was indicated to gelatinase activity

Antagonistic Activity: To check the antimicrobial property of *actinomycetes* was screened by cross streak method followed by Lakshmipathy *et al* 2010. A single streak of *actinomycetes* on the surface of MHA and incubated at 28±2°C for 5 days. After the full growth of *actinomycetes* on the plates, the test pathogens (*streptococcus pyogenes*, *streptococcus pneumoniae*, *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, proteus mirabilis* and *Klebsiella pneumoniae)* were streaked at right angles to the original streak of *actinomycetes* and incubated at 37°C. The antagonistic *actinomycetes* were isolated after 24 and 48 hrs. Based on the presence and absence of inhibition zone, the antimicrobial producing *actinomycetes* were selected [15].

Antibacterial Screening of Bioactive Compounds: The primary positive isolates only inoculated in the Starch Casein broth and incubated at 28±2°C in a Rotary Shaker at 200 rpm for 7 days. Fermented potential culture allowed to centrifuge at 10,000 rpm for 20 min. The total culture filtrate of selected strain was used for solvent extractions using Chloroform, Hexane and ethyl acetate. The ratio 1:1 (v/v) of the solvent and filtrate was shaken ad mixed well. The upper layer was collected Using separating funnel and Solvents were evaporated using rotary evaporator followed by Gandhimathi et al [16]. Secondary metabolites of actinomycete isolate MACT 2 extracted with solvents were tested by agar well diffusion assay. Crude extract compounds dissolved in DMSO were loaded in the wells. All plates were incubated at 37°C for 24h for antimicrobial activity was measured based on Zone of inhibition.

Genome Sequencing: The MACT2 strain was further subjected to confirmation by 16S rRNA Sanger sequencing by following the standard protocol followed by (Smibert *et al*., 1994) (17). The alignment of the partial 16S rRNA sequence was performed by using the basic local alignment search tool (BLAST). The phylogenetic tree was constructed by using MEGA software version 7.0. Isolated strain was identified based upon the available data in online. The sequence obtained from the sample shows a 99% similarity with *streptomyces* sp.

Characterization of Bioactive Compounds by GCMS- analysis: The potential bioactive compound separated by using gas chromatography-mass spectrometer. The 30mL/ min of helium gas used as a carrier. To identify the compounds based on 90% similarity of MS spectrum of the unknown compounds are compared to reference compounds available in library of Ms spectra. (18)

RESULTS

Isolation of *Actinomycetes* from Saltpan Soil Samples

The *Actinomycetes* Were Isolated from the Saltpan soil sample were collected from areas of Marakanam Saltpan of Tamil Nadu. Pure Culture of *actinomycetes* isolates were maintained in Starch Casein Agar medium and named as according to the areas as MACT1 and MACT2.

Identification of Strains

Morphological Characterization: SCA plates show the two different Colonies of *actinomycetes* were obtained they are respectively Powdery white and Cream white Colonies obtained. Biochemical test results are shown in Table 1.

Screening of Enzyme Production: All the isolated *actinomycetes* were screened for amylase, Protease, Cellulase, Gelatinase enzyme activity using their respective substrates. Among them, isolate MACT1 showed production of cellulose, amylase and protease. MACT2 isolates showed Cellulase and protease enzyme. The results are tabulated in Table 2.

Antagonistic Activity: Antagonistic activity is summarized in Table 3. The results revealed that among two isolates only one isolate exhibited antagonistic activity against selected pathogens. The maximum antagonistic activity was given by isolate MACT2 Other isolates showed comparatively lesser antagonistic activity against tested pathogens.

Antibacterial activity of Bioactive Compounds: The MACT2 isolates were used for antibacterial activity. The DMSO was used for negative control. The different concentrations *viz*., 25,50,75,100 µg/ml of Hexane, choloroform, ethylacetate were tested against the eight selected clinical pathogens. The highest antibacterial activity of Hexane extracts was observed against *Pseudomonas aeroginisa* (21±0.4mm) followed by *Staphylococcus aureus* (20±0.2)**,** *Escherichia coli (19*±0.1), *Klebsiella pneumoniae* (17±0.4), *proteus mirabilis(16*±0.6) *and Klebsiella pneumonia* (17±0.3) *Bacillus cereus* (14±0.1)in 100 µg/ml concentration and the minimum Zone of inhibition was noted.

Molecular Identification: The 16S rRNA sequence obtained from PCR amplification of the gene that encodes the ribosomal RNA using universal primers. Isolate MACT2 closely associated with the members of the diverse *Streptomyces* spectrum.

GC-MS analysis: The potential Strain hexane extracts were identified by GC-MS. The results revealed 3,7-Dimethyl-2,6-octadien-1-OL : Molecular weight-154.25 g/mol ,Chemical formula -$C_{10}H_{18}O$ is the major compounds followed by Eicosanoic acid(CAS) Arachidic acid, Neophytadiene. These compounds are detected and showed against the clinical pathogens.

DISCUSSION

The present study was revealed on the isolation of halophilic actinomycetes produced industrially important enzymes Amylase, protease, cellulose, Gelatinase and also identify the antibacterial compound. Researchers explored to search novelty organisms to produce bioactive compounds. [19] In our study, totally five strains of actinomycetes were isolated from marine soil in Marakanam salt pan, Cuddalore district in Tamilnadu. A total of Five strains isolated from the location, Only two strains only showed good activity against clinical pathogens and produced industrially important enzymes. The Hexane as a best solvent for extracting the antibacterial compound from potential halophilic actinomycetes. 16s rRNA sequencing methods for determine the MACT2 strains showed *Streptomyces* sp. The compounds 9-octadecenoic acid were identified by GC-MS analysis.

CONCLUSION

In conclusion, MACT2 strain was identified *Streptomyces* sp against *streptococcus pyogenes*, *Streptococcus pneumoniae*, *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, proteus mirabilis* and *Klebsiella pneumoniae* pathogens. The effective methods of GC-MS used for detect presence of for **3,7- Dimethyl-2,6-octadien-1-OL** bioactive compounds. The present results suggest that the *Halophilic actinomycetes* could be used as antibiotics.

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Table 1: Biochemical Characterization of Actinomycetes –MACT1 & MACT2

S.NO	BIOCHEMICAL TEST	MACT1	MACT2
1.	Indole test	+	-
2.	Methyl red	-	-
3.	Voges Proskauer test	-	-
4.	Citrate utilization	-	+
5.	Urease test	-	-
6.	Catalase test	+	-
7.	Oxidase test	+	-
8.	Starch hydrolysis	+	+

Table 2: Qualitative Enzyme Screening

S.NO	ENZYMES	MACT1	MACT2
1	Amylase	+	-
2	Cellulase	+	+
3	Protease	+	+
4	Gelatinase	+	-

+ Presence of Zone of hydrolysis, - Absence of zone of hydrolysis

Table 3: Antagonistic activity against bacterial pathogens

S.NO	Pathogens	MACT2
1	*Streptococcus pyogenes*	++
2	*Streptococcus pneumoniae*	-
3	*Klebseilla pneumoniae*	+
4	*Staphylococcus aureus*	++
5	*Escherchia coli*	+
6	*Pseudomonas aeroginisa*	++
7	*Proteus mirabillis*	++
8	*Bacillus cereus*	+

-: No inhibition, +: moderate inhibition, ++: high inhibition

Table 4: Antibacterial activity of Bioactive compound.

Pathogens	Zone of inhibition (mm)												
	Chloroform				Hexane				Ethyl acetate				DMSO
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl	
Streptococci pyogenes	4	6	7	9	-	-	-	-	5	8	10	12	-
Streptococci pneumonia	6	7	8	10	10	12	14	16	2	3	5	6	-
Klebsiella pneumonia	5	7	9	11	12	14	16	17	3	4	5	7	-
Stapylococcus aureus	9	11	13	16	15	17	18	20	-	-	-	-	-
Esherchia coli	-	-	-	-	16	17	18	19	10	12	14	15	-
Pseudomonas aeruginosa	8	11	13	15	18	19	20	21	5	8	9	10	-
Proteus mirabilis	6	8	9	11	12	13	15	16	3	5	6	8	-
Bacillus cereus	2	4	6	8	5	7	10	14	-	-	-	-	-

Table 5: Chemical composition of *Streptomyces* strain using gas chromatography mass spectrometry (GC–MS) analysis

Peak Report TIC							
Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	5.572	147998	0.47	51033	0.96	2.90	trans-Geraniol
2	11.667	286833	0.92	46109	0.87	6.22	Hexadecanoic acid (CAS) Palmitic acid
3	11.808	284964	0.91	66975	1.26	4.25	Tetradecanoic acid (CAS) Myristic acid
4	11.919	122830	0.39	41556	0.78	2.96	3-HEXADECENE, (Z)-
5	12.512	1129291	3.62	224751	4.23	5.02	NEOPHYTADIENE
6	12.800	136964	0.44	40427	0.76	3.39	1-Dodecyne (CAS)
7	13.017	393639	1.26	82567	1.55	4.77	1-Hexadecyne (CAS)
8	13.567	114098	0.37	41662	0.78	2.74	Nonadecanoic acid, methyl ester (CAS) Methy
9	13.816	253637	0.81	57565	1.08	4.41	9,12,15-Octadecatrienoic acid, methyl ester, (Z
10	13.965	5414944	17.36	1177267	22.17	4.60	3,7-DIMETHYL-2,6-OCTADIEN-1-OL
11	14.214	436675	1.40	107629	2.03	4.06	Pentadecanoic acid, ethyl ester
12	15.397	296267	0.95	73856	1.39	4.01	9-Octadecenoic acid (Z)-, methyl ester (CAS)
13	15.541	405238	1.30	151791	2.86	2.67	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-
14	15.856	14926613	47.86	2144154	40.38	6.96	9-OCTADECENOIC ACID (Z)-
15	16.075	4781623	15.33	670134	12.62	7.14	Eicosanoic acid (CAS) Arachidic acid
16	16.364	800186	2.57	110537	2.08	7.24	Pentadecanoic acid, ethyl ester
17	16.875	295994	0.95	46312	0.87	6.39	3-Hexadecyne (CAS)
18	17.833	177279	0.57	47792	0.90	3.71	Docosanoic acid (CAS) Behenic acid
19	21.096	406797	1.30	62611	1.18	6.50	9,12-Octadecadienoyl chloride, (Z,Z)-
20	22.928	376895	1.21	65583	1.24	5.75	bis(2-Ethylhexyl) ether
		31188765	100.00	5310311	100.00		

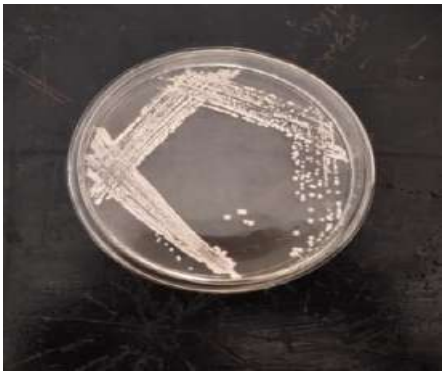

Fig 1: Pure culture of MACT2 (*Streptomyces* sp)

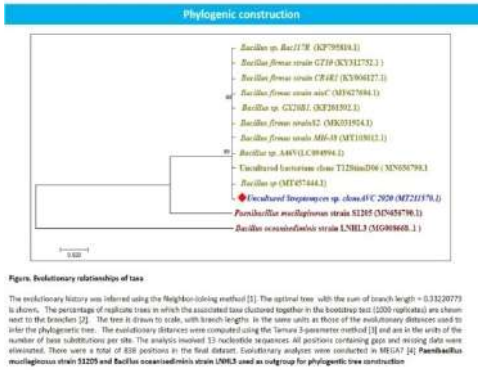

Fig- 2: *Actinomycetes* (MACT2) 16S rRNA partial sequence and accession number of MT211570

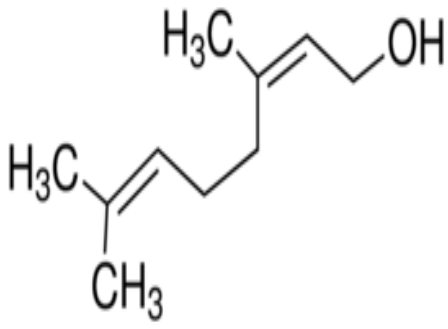

Fig- 3 3,7- Dimethyl-2,6-octadien-1-OL structure

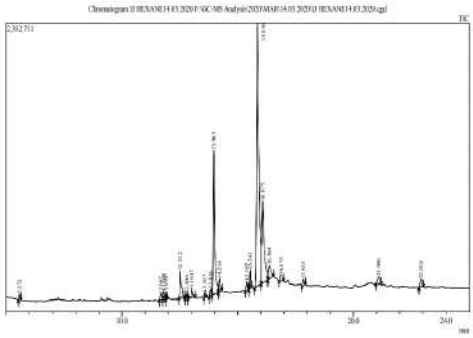

Fig – 4 GC- MS Spectrum of the bioactive compounds produced by *Streptomyces* Sp

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

The *8*-Aminoquinoline Assisted Copper-Catalyzed C(sp^2)-H Functionalization

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Received: 18 Feb 2021 Revised: 22 Feb 2021 Accepted: 28 Feb 2021

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ABSTRACT

During the past decades, directing group assisted C-H functionalization is an important tool for the expedient synthesis of C-C and C-hetero bonds. Among the various directing group strategies, the *8*-aminoquinoline as bidentate directing group are considered to be most efficient devices for C (sp^2)-H functionalization. In this review, we discuss copper catalyzed C-H activation for the construction of C-hetero bonds and tandem annulations with the help of *8*-aminoquinoline as bidentate directing group.

Keywords: C-H functionalization, Phosphorylation, Flourination, Selenylations

INTRODUCTION

The copper-mediated cross coupling is very well known chemistry in organic synthesis [1]. The Ullman and Goldberg developed pioneering work on copper-mediated cross coupling reactions [2].Generally, these methods require prefunctionalized starting materials such as halides, triflate, and organometallic compounds which have several drawbacks and decrease the overall efficiency of the transformations. To overcome these disadvantages, the direct metal mediated/catalyzed C-H functionalization is emerged as powerful transformation which obviates prefuntionalized starting materials. Generally, precious metals like Rh, Ru and Pd are being used for such type of transformations [3].From academic and industrial interests point of view, it would be highly desirable to explore cheap, abundant and inexpensive catalysts for C-H functionalization. Daugulis and co-worker first discovered palladium-catalyzed C-H bond functionalization using 8-aminoquinoline and picolinamide as bidentate directing groups[4] Shortly after, various research groups developed N, O- or N, N-based as well as N, S-based bidentate directing groups for C-H bond functionalizations [5].It has been hypothesized that transition metal-mediated/catalyzed C-H activations assisted by these bidentate directing groups go via metallacycle intermediate

(Scheme 1). Various research groups have disclosed bidentate directing groups assisted C-H bond functionalization using cheap metal catalysts such as Ni, Cu, Co and Fe [5] Recently, Copper catalyzed C-H Functionalization reactions using *8*-aminoquinoline as bidentateauxillary directing group has been paid significant attention due to inexpensive and low toxicity [6]. This review highlights major reactions on copper catalyzed C-H activation for the construction of C-hetero bonds and tandem annulations with the help of *8*-aminoquinoline as bidentate directing group.

C-Hetero Bond Formation

Phenoxylation/Alkoxylation

The Cu-catalyzed phenoxylation and alkoxylation of unactivated C(sp^2)-H bonds is also accomplished using *8*-aminoquinoline as bidentate directing group (Scheme 2)[7].This reaction utilizes $(CuOH)_2CO_3$ as catalyst, K_2CO_3, tetramethylguanidine, or K_3PO_4 as base, air as an oxidant, alcohol or phenol as coupling partner in presence of DMF, pyridine, or DMPU as solvent at 70-130 ^oC. A wide variety of functional groups on arenes such as amine, nitro, ester, nitrile, and halogen groups were tolerated under the optimized reaction conditions.

Selenylation

Jana *et al*. developed a copper-manganese dual catalytic system for a general chelation-assisted C (sp^2)-H selenation of benzamides and acrylamides (Scheme 3)[8] In addition, reaction with selenium powder in lieu of diaryldiselenide provided the cyclic ebselen derivatives via C-Se/N-Se bond formation cascade. A wide range of functionalized benzamides underwent selenation reaction with a variety of diaryl or dialkyldiselenides. Symmetrical benzamides provided diselenation products both at the *ortho* positions whereas *ortho* substituted benzamides afforded mono selenation products. Furans, indole, thiophene, provided the selenation products in high yields.

Sulphoximination

In 2020, Baidya and his Co-workers reported copper-catalyzed *8*-aminoquinoline-directed oxidative C-H/N-H coupling for N-arylation of sulfoximines (Scheme 4)[9]. The reaction is scalable and has wide substrate scopes and tolerates different functional groups like methoxy, bromides, iodides etc. From mechanistic studies, the reaction involves organometallic pathway.

Phosphorylation

Chen and Yu developed a Cu-catalyzed direct sp^2 C-H phosphorylation of benzoicaicd derivatives with H-phosphonate reagents by using *8*-aminoquinoline bidentate directing group (Scheme 5)[10]. The reaction tolerates functional groups like ethers, halides, esters, nitriles and nitro. Moreover, heterocyclic amides are compatible with optimized reaction condition. This reaction is applicable to different diaryl H-phosphonates and dialkyl H-phosphonates which resulted easy route for the synthesis of *ortho*-phosphonated benzoic acid derivatives.

Fluorination

In 2013, Daugulis and co-workers discovered a Cu catalyzed, direct sp^2 C-H fluorination of benzoic acid derivatives using fluoride source AgF (Scheme 6)[11].Selectivemono- or difluorination products are achieved depending on reaction temperature, amount of CuI catalyst loading, fluoride source AgF, and oxidant. A wide variety of functional group and heterocyclic compounds tolerate under the optimized reaction conditions.

Tandem C-H functionalization/annulations

Huang and Yu also accomplished a copper catalyzed direct sp^2 C-H annulation reaction between arenes and terminal alkynes (Scheme 7)[12].From reaction screenings, they found that ligand has crucial role on the conversion and neocuproine used as ligand resulting almost quantitative conversion. The major improvement for the preparation of 3-methylene isoindoline derivatives is catalytic copper and molecular oxygen as a green oxidant.

Miura *et. al.* reported a Copper-catalyzed formal [4 + 1] cycloaddition of benzamides and isonitriles via directed C-H cleavage using 8-Aminoquinoline as a bidentate directing group (Scheme 8)[13].After screening of different copper catalysts, it was found that CuBr·SMe_2 as catalyst using both MnO_2 and O_2 as oxidant proved to be efficient for this transformation. The different functional groups like tert-butyl, methoxy and halogen groups were compatible under standard conditions. A plausible reaction pathway for copper-catalyzed formal [4+1] cycloaddition of benzamides with isonitriles is given in (Scheme 9). Initially, oxidation of Cu(I) to Cu(II) goes by MnO_2 and O_2. Then, the coordination of copper ion with isonitrile15a and sulfide results the active Cu(II) complex 16, which further reacts with 1a to form chelated metallacycle17. After *ortho*sp^2 C-H activation, the C-Cu complex 18 is formed and followed by insertion of isonitrile results the intermediate 19. Then, copper(III) complex 20 is obtained via Cu(II)-promoted oxidation of 19 and its reductive elimination will afford the desired iminoisoindolinoneproduct14. Finally, reoxidation of the liberated Cu(I) 21 by MnO_2 and O_2 results 16 to complete the catalytic cycle.

CONCLUSION

In this review, we have summarized major reactions in copper catalyzed 8-aminoquinoline assisted functionalization of unactivated C (sp^2-H) bonds. From a synthetic point of view, these reactions offer novel and efficient tools for C-hetero bond formation. However, the detailed mechanism of copper catalyzed auxiliary assisted functionalization of C-H bond needs to be elucidated, and the functionalization of C (sp^3)-H bonds remains underdeveloped. So, more efforts should be given to develop this unexplored area.

ACKNOWLEDGMENT

The authors are thankful to the Department of Chemistry, Aska Science College, Aska, Ganjam, for support and motivation during manuscript preparations.

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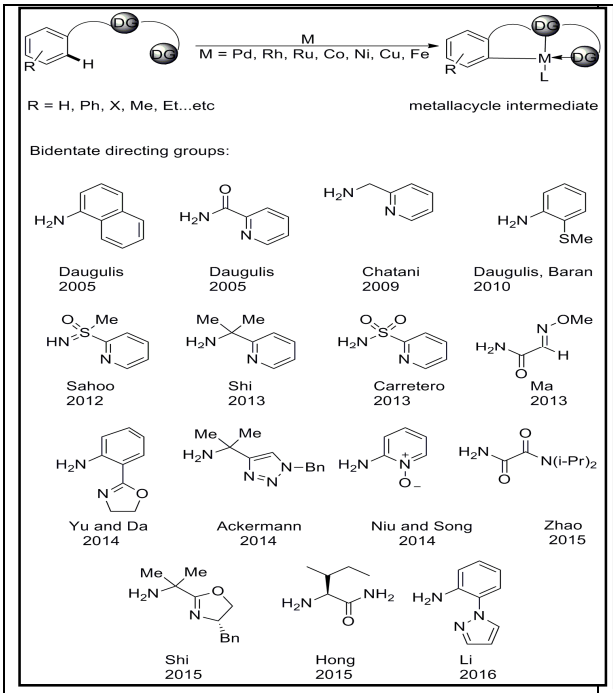
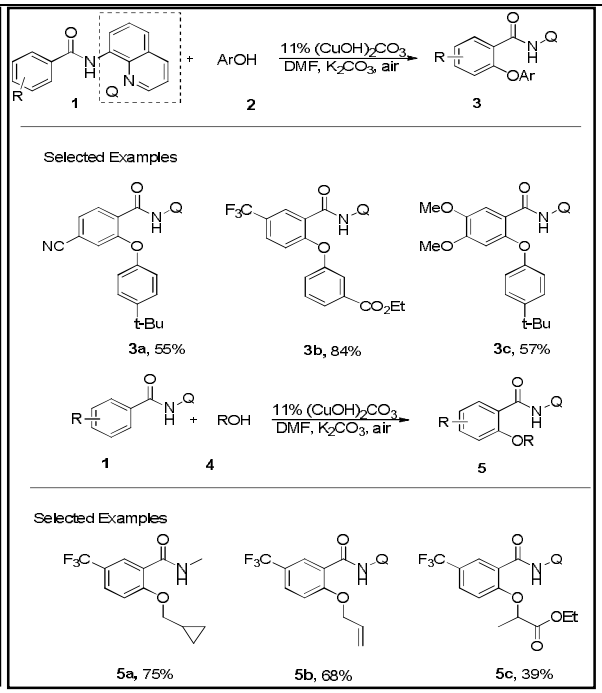
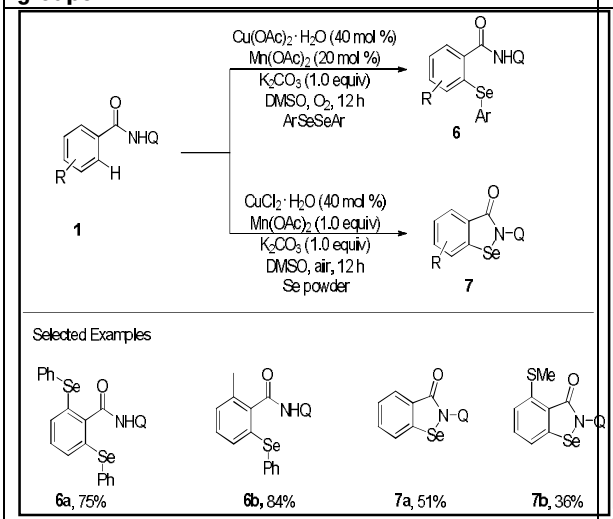


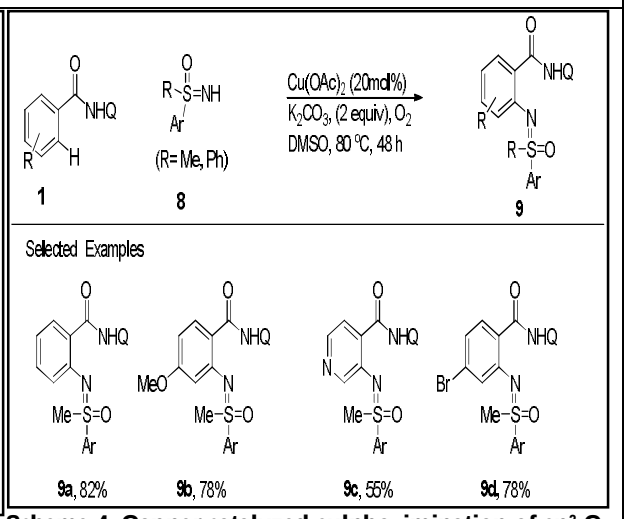
Scheme 1. Transition metal-mediated/catalyzed C-H bond activation assisted by bidentate directing groups.



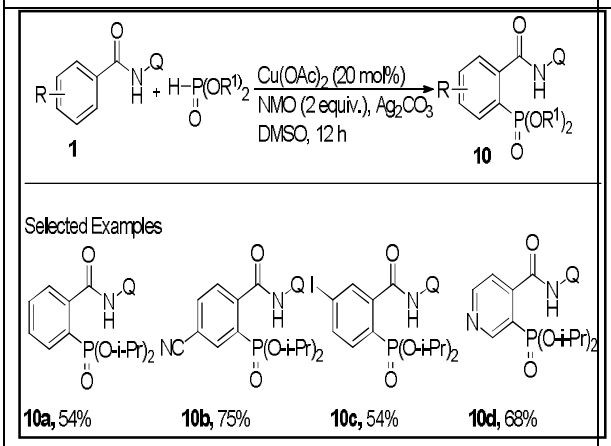
Scheme 2. Copper-catalyzed etherification of sp² C-H bonds of arenes.



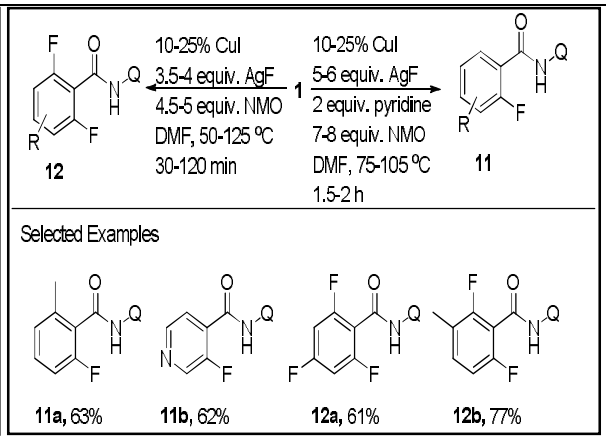
Scheme 3. Copper-catalyzed sp² C-H selenylation



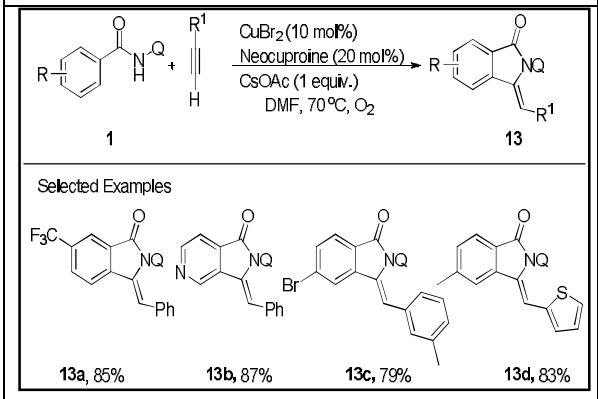
Scheme 4. Copper-catalyzed sulphoximination of sp² C-H bonds of arenes.



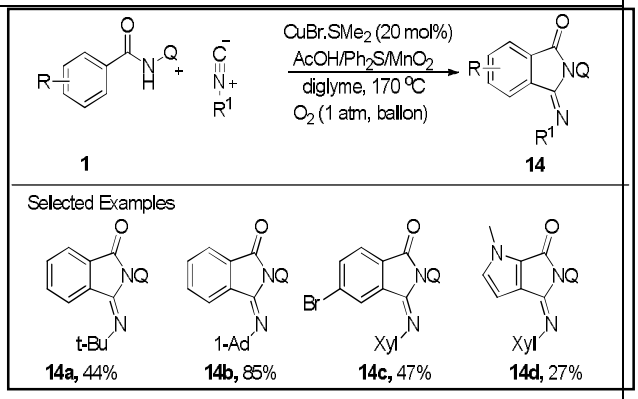
Scheme 5. Copper-catalyzed phosphorylation of sp² C-H bonds.



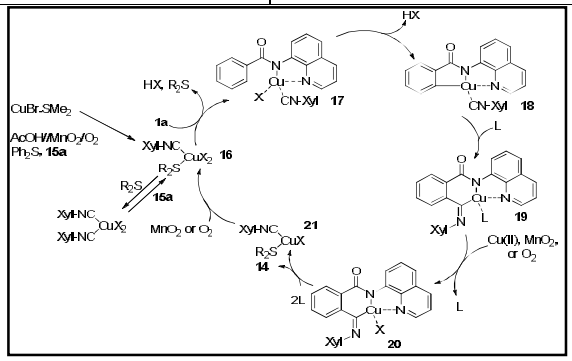
Scheme 6. Copper-catalyzed sp² C-H fluorination of arenes and heteroarenes



Scheme 7. Copper-catalyzed C-H activation based arene/ alkyne annulations..



Scheme 8. Copper-catalyzed formal [4+1] cycloaddtion of benzamides with isonitriles



Scheme 9. A plausible reaction mechanism for copper-catalyzed formal [4+1] cycloaddtion of benzamides with isonitriles.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Computing Off-Loading Techniques in Mobile Cloud Computing.

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Received: 25 Jan 2021 Revised: 01 Feb 2021 Accepted: 25 Feb 2021

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ABSTRACT

Computation offloading could be an assuring technique to enhance the depiction yet as decreasing the dissipating energy of a battery in mobile app by accomplishment the various elements of utilizing on distant server. In mobile cloud computing the advanced research primitively targets the code partitioning and offloading techniques, conceited that mobile data's square measure discharged to an equipped workstation. Moreover, the framework of a mobile implement, similar atmosphere, organization surroundings and acquirable cloud resources, evolves incessantly because it revolves throughout the day. The strategies also are entirely contrasting in assessment quality and coupling degree. This paper proposes a framework that deals with the mobile strategies with the context-aware predicting the discharging potentiality. First, a methodology blueprint is planned to convert the necessary application to be assessed the discharging technique as required. Subsequently, an evolution facsimile is bestowed associate in assisting the estimation model is bestowed inevitably preferable that the cloud resource for offloading. Additionally, scaffolding, both the consumer and host margins are enforced to provide security in the planning structure and therefore the assessment simulation.

Keywords: Mobile Computing, Cloud Computing, Smart watch, Data off-loading, Security

INTRODUCTION

The main aim of Cloud Computing is empowering IT departments to specialise in their businesses and comes rather than simply taking care of their information centres and keeping them operating. Cloud Computing could be a new

idea that enables to produce procedure amenities as concessions in an exceedingly rapid way, as acquired requisition, and efficacious the data services to the utilization of the consumers. The Cloud Computing criterion is bestowed in 3 cloud charging simulations: Infrastructure as a Service (IaaS), Platform as a Service (PaaS), and Software as a Service (SaaS). The CC can have in 2016 a worldwide combined Annual rate of growth (CAGR) of IaaS: forty first, PaaS: 26.6% and SaaS: seventeen.4%. The offloading will consult with each assessment of computing simulations. Consequently, assessed missions square measure dead remotely within the cloud to scale back process and power dissipation of the mobile implement. Offloading could be a viable blue print not just for power consumption, however may for increasing information storage and increasing simulating competency. Death penalty chores on a structural organization with enormous procedure efforts is very helpful for Central Processing Unit-persistent endeavours, like physical apprehending, a chore that lasts even up to thirty-eight minutes if dead domestically on good eye wear.

Offloading technique needs mobile tools to keep up information affiliation for human action with deserted systems that is dear regarding to the power dissipation. Understanding about the whole advantage of computation offloading, the wheeling and dealing between the rise in discharged power for data transferring and power absorption by circumventing domestic methodology ought to be contemplated. An in-depth evaluation of cloud power absorption has unconcealed that wireless access networks and not information centres area unit the foremost power thirsting elements of the cloud system. Computation offloading needs information to be broadcasted initially and encountered before acquiring back the corollaries, that executes offloading seconds overwhelming. On the contrary, we have tendency to scrutinize via perceptive speculating the benefits brought by computation offloading to the cloud to eye wear specializing in the power also enforcement the delay wheeling and dealing in commercial. Divergent jobs have already correlated power assimilation and battery constraints of smartphones and wearable devices. Moreover, to the most effective of our data, this one can be the primary work presuming coping evaluation of each procedure and clearing the issues of offloading. Additional exactly, the encountered evaluation postulates power and time required accomplishing jobs. Forecasting once, if it enables to commensurate dumping a chore is pivotal to increase power cell regulation and authorizes the throughput to the consumers. For instance, discharging elements of the delectability's deployment retains power, however have an effect on Quality of User expertise (QoUE). When we embody by investigating the thesis when wearable devices [1] destroy cellular proper victimization by utilizing the consumer smartphone as transceiver. Moreover, as smart-phones can be utilized for disburdening jobs in annexing to clouds, that brings vital to consider their power consumption. For the precedent explanations, the suggested model accounts for all three-elemental structural equipments: wearable devices, smartphones, and cloud data centres. From the smartphones' perspective view, two frameworks can be discovered. Initially, when they accomplish analysing domestically (s-l), medium mass of power is prerequisite with slightest assessment of communications elaborated, ascribed to contiguity to wearable accessories and least absorption of the Wi-Fi/Bluetooth interfaces. Subsequently, the elapsed seconds is vital for executing and radiating back to the wearable accessory's resolutions is increasing, as smartphones have restricted executing energy, that corollaries in enhancing seconds of executing. Preferably, the smartphone is edible for disburdening jobs to the cloud (s-c). In such state of affairs, no executing is necessary at the smart-watch, however broadcasting demand by perpetuating both LTE and Wi-Fi/Bluetooth interfaces, which counters increased level of power consumption.

Existing Work

The conception is splitting the term cloud computing into a lot of perceivable components that usually are outlines as follows: [2]

- Self-propelling of reserves and adaptability. -Cloud computing consumers enable to withstand, without customer facilitation, attain any cost of executing competences. For instance, communication cache, executing competences or software solutions, that brings acquirable (Hoang T.Dinh, 11, October 2016)at any moment and at any place.
- Pay peruse. - Expense of cloud executing solutions have relied upon deployment. Precisely, consistent rating of all times, despatching the packages or number of consumers increase.

- On demand availability - Cloud computing amenities are invariably approachable; platform freelance which includes unremarkably procured through internet or web service API.
- Versatility. - Cloud execution amenities are anticipated which have to be unrestricted simultaneously, combining any amenity stipulates which the consumer has. For instance, Data transmission rate, executional capabilities or accommodation for the data users. The cloud computing amenities should instantaneously capable of accustomed to implored deployment.
- Consolidation. - Compilations with reserves have splattered innumerous varieties of number of networks that practically are dispersed globally; the amenities are necessary and then pointed established on the executional requirement of the dispensation.

Cloud Computation offloading is the job of causation execution comprehensive modeling devices to an overseas server. Hitherto, countless executions offloading contexts already suggested with various strategies for strategies on wearable devices. These strategies are scattered at completely various roughness layers and therefore the parts are discharged to distant network for distant computation due to grow abundantly and upgraded the SMD's competences. Moreover, the execution offloading structures are pointing with various demands.

In the residual portion of this paper, the aim was presenting a synopsis throughout the MCC offloading analysis by deliberating below:

1. Deployment possible alternatives for discharging in Mobile Cloud Computing.
2. Methodologies functioning as accessed in transmitting.
3. An analysis about suggested transmitting contexts.

Cloud Execution transmitting moreover, its prospective approach that has been comprehensively examined simultaneously cordless transmission and Network rapidity altered comfortably upgraded that can be enabled to counterfeit. This paper predicts that the basics in wearable devices' offloading vitally relies upon communication web mechanisms equivalent to mobile communication as well as cordless communication. Both of the mobile communication and cordless technology predetermine this feasibility in cloud transmitting. At present, cordless network automation is adequate with increasing transferring rate of network acquaintances. Moreover, the data throughput utilising the mobile communication acquires a substantive measure of power from the cellular component in contrast with Wi-Fi network.

Proposed Technique

Mobile Cloud Computing (MCC) is the coalescence of Mobile Computing (MC), Cloud Computing (CC) and wireless communications to fetch high quality executional possessions to cellular consumers, network operators, including cloud computing benefactors. Hitherto extensively, MCC be contradicted as enhanced mobile computing technology that anchorages combined with the elastic resources of clouds and network mechanisms that sharply point out unlimited practicality, cache, and mobility to simulate a manifold mobile device wherever and whenever via the channel of local area network or web heedless of heterotypic surroundings and platforms. Practically, the offloading determination is the concluding pace previous distant simulation is initiated for off loadable devices. [5] (Fabrizio Granelli, December 2015). Whether an implanted distant device is utilised in the Surface Mount Device application and not relied upon generally on the simulation framework. If the decree is made at duration, plenty of data is acquirable that stands for scalability, for instance, the SMD not able to access network junction or the power absorption for transmitting the codes for the distant simulation would possibly be enriched.

The offloading can be transmitted, assuming the environment converts. When the resolution takes runout seconds, the deadline resolution provokes the aerial [4] constraints which naturally is absent. This comprehensive framework has analysed by Dinhet all fundamentally combined with wearable accessories interconnected with networks, the cellular connections that transmit the application from cellular devices to cloud for executing through network. The figure 4 explains the fundamental standardization for Mobile cloud. Entertainment like chess and Sudoku Solver which need quick computation and have numerous small datasets. Document Exploring applications require

abundant time to get specific document. Image modelling tasks and image fabricating chores are absolutely computationally persistent. Transpose programs enable save time and energy when offloaded to cloud. Antivirus software capable of transmitting cloud since it involves a complete scan of phone and comparison of several virus signatures. Depending on that decree be chosen about the transmitting execution within distant workstation, which discriminate a pair of elements which transmit contexts. Initially, the stationery transmitting contexts be encountered. Subsequently, overall encountered segments are executed at blueprint duration, before the software is initiated on the cellular network. Another types are variable transmitting contexts. From these contexts, moderately, concluding decree is made on regardless if transmitting single execution is taken at duration. One more a pair tiers enable to encounter at standardization else duration. Cordially, the structure which can be evolved from the consumer explications of off loadable elements which are pre equipped with devices upon distant workstation is termed as a unstable transmitting architecture, which concludes within duration lest the solution is fitted aptly for offloading execution or not. In spite of there are various offloading operations accessible for offloading calculation determined devices of cellular softwares to the cloud, which categorize the automations into a pair of wide classifications:

- Architectures derived from effective device replicating.
- Architectures derived from code offloading.

Architectures derived from data offloading offload determined application elements by imploring a distant methodology call using illusions, special synthesis or binary modulation. In contrast the effective device replicating and cellular phone's whole picture is conquered also reserved in cloud workstation. In course of transmitting, cell-phone's accomplishment be banned also transmitted to the Virtual Machine replicating in the cloud.

After inspected the architectures with their relevant attributes, we can distinguish and formulate them as follows:

- Composing: Whatever required developments before transmitting.
- Sectioning: Sectioning aided or not.
- Resolution: Static or Dynamic.
- Transmitting Automation: Automation used to offload methodology calculations.
- Coarseness altitude: Coarseness classification (i.e. division, approach, chore).
- Glossary: Technology with bifurcation methodology (Digital or hand operated).
- Benefaction: Elucidated conflicts?

As described earlier, executing mighty jobs can capitalize on cloud offloading. The assessment solutions depict that "normal" or day today jobs do most feasibly not deserve the discharging as the cellular device has enough simulation ability to set right jobs. The phenomenon of transmitting is visual elimination. Additionally, T visual footprints else converge movie to diverse dimensions have executed though the jobs are mighty compiling with high performance stationary computers. Henceforth it is not an apt job for cellular devices and cloud offloading become an optimal resolution. Divergently are small, simple simulation jobs not rewarding of transmitting to the cloud. Transmitting the chores to the cloud work station is delaying the seconds in examining simulating a single job in cellular device. Multiple consumers apportion the resources by tenants in CC. The mobile devices do not have tenants and usually not share the hardware resources in MC. The Constructional environment that is apt for the wearable cloud is innovation by cellular and vigorous clouds. This constructional architecture is explained as below.

The PTDs are rudiment collaborative components consist of cordless transmissions (e.g. WiFi, Bluetooth, ZigBee). [6] (AmandeepSingh, 2017) these components are not restricted with smart-phones, smart-watches, tablet computers, smart-glasses, health monitors, and other IoT devices. Here it is discriminated that the hardware specifications on the PTDs made be less, by acquiring rather 'dumb', cheap, and resource constraint devices, in regarding execution power, battery, memory, low-power short-range radio, and storage. The PTDs can collaborate through the wearable cloud for synthesised services. The CAP enhances positioned ensemble the least intersections allocated to process the wearable cloud. The Cloud Access Point can gratify processing the requests from the Personal Terminal Devices without future elastic resources. The Cloud Accessing Point can accomplish the keyhole for the Personal Terminal

Devices to interact with the wearable cloud for the consumer's service requests and transmitted jobs through wireless interfaces (e.g. WiFi, Bluetooth, ZigBee). The service requests and replies by acquiring structured manipulation and elastic time management and collaboration of least intersections. The CAP that combines with the following operational constraints to stimulate the wearable cloud context. The Manager acquires the requests from the Personal Terminal Devices; maintain the approach for the growth of the request which possesses the periods for manifold Personal Terminal Devices and penetrating requests. The response trainer contributes the solutions to the Manager, and substantially, manager contributes the executed results to the pointed Personal Terminal Device(s). The Task Allocator is evoked by the Manager calculating acquired reserves for provided commitment request. The task allocator is meant for pipe-lined operation in rapid way but split the desired back-up jobs into pockets for concurrent processing. The partitioned chores and input elements have already displaced on to the junction regulator for concurrent operation. Only when the job is executed, the OHL receives the response from the node controller. The OHL allocates the mobilised solution and controls the output for the given framework.

After the accomplishment, the result is then transmitted to the Manager. During the wearable cloud architecture, Node controller is competent and controls the elastic mini nodes. The Node controller controls the elastic resource disbursement and resourcing. The partitioned jobs and relevant input codes are received from the Task allocator. The Node controller then accommodates flexible least calculate reserve junctions based on resources allocated by the Task allocator. The assistant chores have accessed also placed least junctions and solutions are gathered after accomplishment. Mobile Cloud Computing is privilege for the consumers where they can earn more profits also experience without encumbrance about requirements on mobile devices. The offloading can be analysed completely and a comprehensive one, even for mathematical optimal solutions. In advance, the analysis for Mobile cloud computing is incommensurable for the optimal segmenting doctrine in all over the mobile controlling tribunes. In the mobile cloud computing, the datas are scattered when the transmission takes place. The widget's presence is conventionally restricted, so the basic obstacle for attaining optimality to scalability tackled with the mechanism is the occurrence of collisions. Hassles haven't yet entirely eradicated and they instigate over powering execution while numerous simulating segments grown. The offloading process is not centralized, controlling is difficult and also spells in lacking of security. The entire network is not reliable.

CONCLUSION AND FUTURE SCOPE

In cryptography analysis, the *encryption* is the procedure of encoding informations as demonstrated that only authenticated persons can comprehend it. Encryption never defends against hacking but it minimizes the execution of hacking process of encrypting the data. In an encryption synthesis, the data pocket is thoroughly encrypted using an encryption algorithm, converging it into an unreadable cipher text . Generally, this is performed with the use of an encryption key that examines how the message is to be encoded. Whether an irrelevant person, may take notice of that message he cannot discern the pioneer message or information that is already encrypted. However, an authenticated person, only reveals the encrypted information by utilizing a decryption algorithm and can decode the cipher text. Practically, the encryption synthesis acquires of the key-generation algorithm arbitrary generates the keys. The suggested framework is a convenient process of cloud which a consumer possesses with another cellular and wearable devices. The wearable cloud permits perceptive way of power consumption, comprehended and inexpensive wearable and mobile devices, and a plainer safety criterion. Practically it can be visualized, by providing of a hyper-wearable enriched execution cloud in cyclones and first output is the possibilities through multiple collaborating wearable clouds. The wearable cloud furnishes the comforts in accordance with resources that preserving the maximum power without compromising the consumers' involvement. The suggested theme is distinguished rather than utilising the electronic communication gadgets provide the static environment against the dynamic wearable devices.

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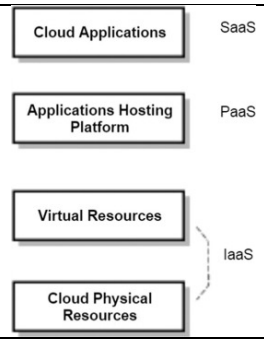

Fig 1. Mobile Cloud Computation Off-loading

Fig 2. Mobile cloud computing scenario for wearable devices

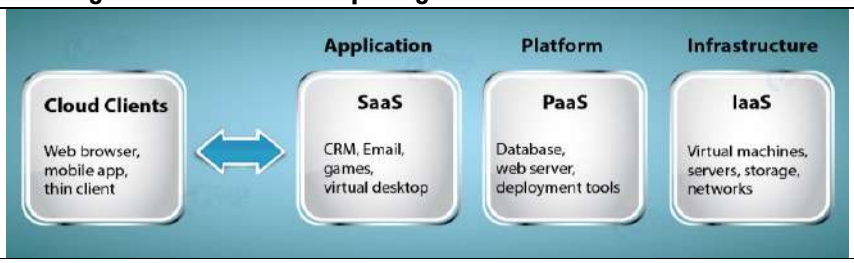

Fig 3. Off-loading techniques with Smart watch

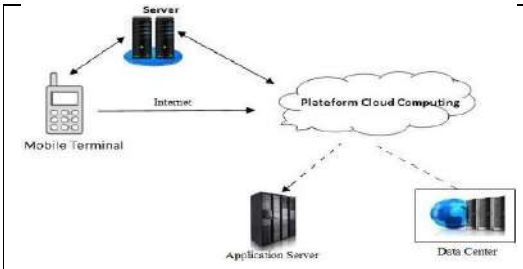

Fig 4. Basic Architecture of Mobile Cloud

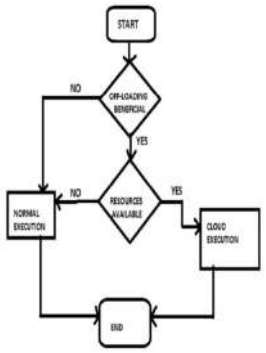

Fig 5. Flow-chart for offloading in Mobile Cloud Computing

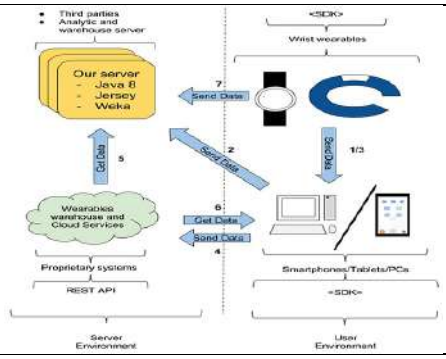

Fig. 6. Wearable smart watch for cloud operational architecture

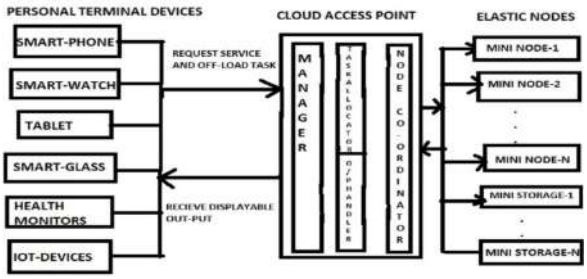

Fig.7. Operational Architecture of Cloud environment

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Consumer Buying Behaviour – A Contemporary Study in Hypermarkets, Tamil Nadu

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Received: 09 Mar 2021 Revised: 12 Mar 2021 Accepted: 15 Mar 2021

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ABSTRACT

The Indian retail market is one of the significant and promising market in the world and it is still growing. The retailing industry in India, specifically organized retail, seems poised for a noteworthy growth in the coming years owning to the presence of a vast market, growing consumer awareness concerning products and services, elevated disposable personal income of the consumers and the desire to try out new products. A hypermarket is a store combining a supermarket and a department store. The outcome is an expansive retail facility carrying a extensive range of products under one roof, including complete groceries lines and general merchandise. In theory, hypermarkets enable customers to fulfill all their routine shopping requirements in one visit. The study proposes to identify what factors that influence consumers and how is the current shopping behavior pattern in India. Study reveals that, physical factors (discounts, quality, local brands, display and visual appeal), social factors include (salesmen behavior and choice of children), and temporal factors (open space) should be considered by the hyper marketers while designing marketing strategy for Indian consumers as these factors are having influence upon the number of visits and amount spent in the hypermarkets.

Keywords: Hypermarkets, consumer behaviour, purchase patterns, customer satisfaction, Customer preference

INTRODUCTION

Retailing in India is one of the pillars of its economy and accounts for 14 to 15 percent of its GDP (Ramya,2015).The Indian retail market is estimated to be US$ 450 billion and it is one of the top five retail markets in the world by economic value (Dikshit,2011). India's retailing industry consists mainly of owner manned small shops. In 2010, large scale convenience stores and supermarkets reported for around 4 percent of the industry, and these are present only in major urban centers. India's retail and logistics segment employs about 40 million Indians nearly about 3.3% of Indian population (Sanjoy, 2011). Any retail passage chain that is professionally administered can be termed as organized retailing in India, if it has the following features (i) accounting transparency (with proper usage of MIS and accounting standards),(ii) organized supply chain management with centralized quality control and (iii) sourcing.(Zameer and Mukherjee,2011). Organizational learning is the organizations capability to maintain and improve performance and interests (A. Sabarirajan et al, 2015). Health care providers always try to make their customer's happy (T. Muthupandian et al, 2019). This accounts for 7 percent of India's roughly $435 billion retail market and is expected to reach 20 percent by 2020. In order to achieve long-term financial benefits, companies must design and deliver a service that satisfies customers so that they have a positive experience during the service encounter (Lovelock et al., 2004). Managers need to recognise the importance of creating value for their customers in the form of experiences. Contributing products or services alone is not enough; organization must provide their clients with adequate experience. It is said that organizations should be urged to produce market experiences by creating spaces (real or virtual) where they can try out contributions as they indulge themselves in the experience. Dramatic technology changes have contributed vastly to improve the shopping experience of customers. The customers as postmodern subjects pursue the belief system of the market out of no decision (P.S. Venkateswaran et al, 2019). The customer experience is defined as the "combination of everything you do, or fail to do for that matter, that underpins any interaction with a customer or potential customer" (Shaw, 2005).Obtaining and sustaining a competitive advantage in retailing, specifically in Indian retail environment today is a challenge and hence it is important to find the one aspect that is capable of differentiating one retailer from another. Retail establishments throughout the world are trying to identify that sustainable competitive advantage and it now seems achievable by strategically focusing on consumer experience which can perform as the key differentiator.

Hypermarkets

A supermarket, a form of grocery store, is a self-service store offering a wide variety of food and household merchandise, organized into departments. It is larger in size and has a wider selection than a traditional grocery store, also selling items typically found in a convenience store, but is smaller and more limited in the range of merchandise than a hypermarket. Most of the supermarkets are very large shops, which can store an extensive range of products. Supermarkets offer food and other household product to consumers. Consumers choose the items that they want to purchase from shelves as they go around a shop. On the contrary hypermarkets are very huge shopping areas where the customer can buy groceries, food, garments, home appliances, durables, toys, cosmetics, toiletries, books and music at a price that is always lower than the market price by 5-50 percent (Srivastava, 2008). E.g.: Big Bazaar, Star Bazaar, Saravana Stores etc. A hypermarket is a store combining a supermarket and a department store. The outcome is an expansive retail facility carrying a broad range of products under one roof, including complete groceries lines and general products. Indian hypermarket industry is more vibrant than ever, with major industry players vying for their share in the retail segment. The size and share of Indian hypermarket is expected to increase in the coming years, given the strong macro economic performance, favorable consumption pattern due to growing personal disposable personal income, availability of quality retail space, speedy expansion of Tier II and III cities, and recent entry of large industrial houses into retail market with focus on larger shop formats. Actually, the British government introduced the concept of Supermarkets to assist its officers with access of all household products under one roof. This led to the growth of Hypermarket or super supermarket or modem supermarket.

Hypermarkets in India address varied stores selling different types of necessary commodities along with luxury articles. These Hypermarkets are mainly focused in urban areas only. Hypermarkets operating in India typically have a heterogeneous mixture of large and small individual retailers (Pooja, 2014). Most of these hypermarkets sell private labels and products manufactured by domestic and international companies. Hypermarkets of India sell products with different price ranges to cater to the requirements of different segments of people. People prefer to buy from hypermarkets not only to buy goods but also for the experience, recreation and window shopping. Consumer behaviour is the scientific approach of the processes consumers apply to select, secure, employ and dispose of products and services that satisfy their requirement & Knowledge of customer behavior directly influences marketing strategy (Anderson, 2005). This is because of the marketing concept, i.e., the idea that firms exist to satisfy customer needs (Winter, 2000). Firms can satisfy those needs only to the extent that they understand their customer & for this reason, marketing strategies must incorporate knowledge of consumer behaviour into every facet of a strategic marketing plan (Solomon, 2002). In the past 25 years, consumers worldwide have become more knowledgeable, discriminating, and gained more purchasing ability. Simultaneously, retail forms have proliferated rather than diminished, and a complex set of consumption options are available (Berry, 1995 and Jones, 2003) concluded that consumers have changed and that the most notable change is in their patronage of food retail outlets other than supermarkets. Popkowski (2004) found that there are different segments of shoppers and different retail formats serve these different segments of shoppers. In present study, researcher tries to focus specifically on hypermarkets as emerging retail format in India

The literature refers to a great diversity of methodologies, to varying temporal and spatial contexts, and to different store types, which make both generalizations and typology construction difficult. Contradicting with Martineau's (1958) expressive and emotional perspective of shop patronage, experimental evidence illustrates a number of functional and objective characteristics as the most significant, such as: price (Doyle and Fenwick, 1974-1975; Bearden, 1977; Arnold et al., 1983; Hortman et al., 1990; Finn and Louviere, 1996); quality of product (Doyle and Fenwick, 1974-1975); variety (Stephenson, 1969; Doyle and Fenwick, 1974-1975; Schiffman et al., 1977; Bearden, 1977; Finn and Louviere, 1996) and location (Stephenson, 1969; Arnold et al., 1983; Hortman et al., 1990). Concerning intangible and emotional characteristics, the most mentioned ones are store environment (Martineau, 1958; Stephenson, 1969; Franca and Figueiredo, 1993); sales people (Martineau, 1958; Stephenson, 1969; Schiffman et al., 1977) and promotion activities (Martineau, 1958; Stephenson, 1969). Some empirical investigations provide important information about consumer behaviour and its reactions to the development of hypermarkets. Economical and social changes are major contributors for a growing fragmentation of consumers into multiple segments with different values and buying priorities. Consumers become more sensible, educated and challenging, learning how to manage funds and time more efficiently. The focus on low prices was gradually replaced by a value for money perspective. Concerning groceries, the most vital attribute mentioned was quality, followed by cost. Several investigations emphasize the possible coexistence of different store formats and others point out the relationship between the type of store and the type of products. These studies illustrate that, while specialized and conventional stores are preferred for fresh products, hypermarkets are preferred for the shopping in general, and also for frozen foodstuff, groceries and beverages. The purchase of fresh or perishables in hypermarkets is reduced.

Objectives of the Study

- To identify various physical, temporal and social factors affecting the consumer shopping experience in hypermarkets.
- The effect of above factors on number of trips to the hypermarket, and amount spent.

Physical factors include the variety of goods in the hypermarket, discounts, display of products, facilities like shopping carts, credit/debit cards, etc. Temporal factors are the time related factors like the location of the supermarket, ample parking space, open space in the supermarket, etc. Social factors include ambience, salesman behaviour and influence of children in buying goods. The identified factors were tested with the variables (i) Number of visits per month, (ii) Amount spent per visit.

RESEARCH METHODOLOGY

To analyze the various factors influencing purchase behaviour of consumers at hypermarkets in Coimbatore. The study was conducted through quantitative phase. The study used descriptive research design. A structured questioner was prepared and the data collected from 100 respondents. Respondents are regular customers of hypermarkets. The data was collected from the respondents in person. Their opinions were collected, analyzed and are presented in the form of tables. After the completion of the survey, a thorough check of the data has been made. The collected data has been analyzed with the help of SPSS statistical package.

Factors influencing Consumer Buying Behaviour

One way ANOVA was used to determine and to distinguish the factors which are critically affect the number of visits per month and amount spent per visit as measures of consumer buying behaviour.

Table: I - Physical Factors Influencing Consumer Buying Behaviour

The factors having value less than 0.05 shows a significant relationship with the two measure of consumer buying behaviour. Thus out of the physical factors, the critical one's affecting the buying behaviour are availability of local brands, and to some extend discounts,, quality display and visual appeal.

Table: II - Social Factors Influencing Consumer Buying Behaviour

The factors having value less than 0.05 shows a significant relationship with the two measure of consumer buying behaviour. Thus out of the social factors, the critical one's affecting the buying behaviour are ambience, salesman behaviour and influence of children.

Table: III - Temporal Factors influencing Consumer Buying Behaviour

The factors having value less than 0.05 shows a significant relationship with the two measure of consumer buying behaviour. Thus out of the temporal factors, the critical one's affecting the buying behaviour is Parking space, timings and spending time.

Findings and Suggestions

Study shows that various physical, social, temporal and demographical factors can be considered while analyzing a consumer supermarket buying behaviour, but only few of them are critical enough to affect the measures of consumer buying behaviour i.e. no. of trips per month and amount spent per trip. The principal contribution of this study for any retail organization is at three levels. First, physical, social and temporal factors which are found out in this study play an important role in the success of any retail organization.

CONCLUSION

Retailing in India has been increasing at a rapid speed over the last decade. More significantly it witnessed most important changes in terms of retail mix, quality and scale of retailing, varieties of retail formats and over above change in consumer preferences and shopping habits. Many Indian business houses have made their investment plans in this booming sector. Leading international hyper marketers are waiting in line to enter in the Indian retail market. So this research is an attempt to comprehend the shopping behaviour in this promising sector and thus we see that there are some particular factors that play the most crucial role in determining the consumer's shopping behaviour and these should be cautiously dealt with by the hyper markets considering the large potential that lies ahead of them in the retail industry in India.

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Table: I - Physical Factors influencing Consumer Buying Behaviour

S.No	Factors	Mean	No of visits per month (Sig values of F)*	Amount spent per visit (Sig values of F)*
1	Variety	3.01	0.093	0.602
2	Discounts	4.12	0.005	0.041
3	Quality	3.88	0.015	0.003
4	Local Brands	4.11	0.002	0.06
5	Facilities	2.57	0.826	0.075
6	Credit/debit card facility	3.88	0.721	0.039
7	Display	4.15	0.02	0.237
8	Visual Appeal	3.80	0.798	0.039

*Significance at 5% level

Table: II - Social Factors influencing Consumer Buying Behaviour

S.No	Factors	Mean	No of visits per month (Sig values of F)*	Amount spent per visit (Sig values of F)*
1	Ambience	4.37	0.281	0.31
2	Salesman Behaviour	4.22	0.008	0.44
3	Choice of Children	3.88	0.346	0.21

*Significance at 5% level

Table: III - Temporal Factors influencing Consumer Buying Behaviour

S.No	Factors	Mean	No of visits per month (Sig values of F)*	Amount spent per visit (Sig values of F)*
1	Parking Space	3.01	0.023	0.43
2	Open Space	3.80	0.801	0.39
3	Proximity	3.70	0.601	0.11
4	Timings	4.12	0.246	0.22
5	Spending Time	4.10	0.21	0.08

*Significance at 5% level

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Review on Green Human Resource Management Practices and Employee's Green Behavior

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Received: 01 Mar 2021 Revised: 04 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

Green HRM is a newly emerging concept in today's world. Developing worry for the worldwide climate and the advancement of global principles and conventions for ecological administration has made a requirement for business associations to embrace natural techniques and projects. The business is moving its focus from a conventional money related structure as far as possible based system which is set up to examine green monetary parts of the business as the corporate world is going around the world. The main objective of this review is to provide brief information about existing publications on GHRM practices and employee's green behavior of associations dependent on the existing studies.

Keywords: Green human resource management, GHRM practices, Employees green behavior

INTRODUCTION

In the course of the most recent couple of many years, there has been an overall acknowledgment of the significance of saving ecological wellbeing. Numerous accessible investigations have caused us to notice the ecological dangers related with developing commercialization and modern creation (Albino , Balice, & Dangelico, 2009; Olah, Popp, Haddad, Aburumman, Khan, & Kitukutha, 2020).The expanded mechanical yield has been considered mindful to a huge degree for some, negative ecological effects including loss of normal assets, air and water contamination, environmental change promoting an earth-wide temperature boost, dangerous sickness and eradication of species (Kolk & Pinkse, 2005; Behera & Reddy, 2002). Consequently, capable conduct by the people and associations is fundamental to accomplishing ecological supportability in the future.

These days it appears to be that an extensive number of associations practice green HRM practices in the worldwide context. Searching regarding the GHRM practices which are being drilled and can be polished by the business and different associations will contribute altogether to the human resource management field scholastically and basically. GHRM is an arising field of exploration in the associational investigations after the 1990s. Account to that, this study has its attention on investigating GHRM practices from the gleaming of existing hypothetical and exact examination works done by the researchers in the domain.

Focusing on the meaning or interpretation of GHRM starting with (Renwick, Redman, & Maguire, 2008)the incorporation of corporate ecological administration into HRM is named as "green human resource management". They additionally expressed that HR parts of ecological administration are GHRM. These researchers extensively determined that recognized approaches in the domain of selection and recruitment, performance appraisal, development and training, rewards are considered as amazing assets for adjusting workers to an association's environmental approach. GHRM can also be defined as "all the activities involved in the development, implementation and on-going maintenance of a system that aims at making employees of an organization green. It is the side of HRM that is concerned with transforming normal employees into green employees so as to achieve the environmental goals of the organization and finally to make a significant contribution to environmental sustainability. It refers to the policies, practices and systems that make employees of the organization green for the benefit of the individual, society, natural environment, and the business" (Opatha H. , 2013; Opatha & Arulrajaha, 2014).

There are various explanations behind considering GHRM as a vital test for the usage of a powerful maintainability approach of an association.

1) HRM assumes a significant part in greening authoritative strategies and practices at the actual heart of an association's maintainability through many practices like green - selection and recruitments, development and training, performance evaluation, just as in imparting esteems and corporate culture (Renwick, Redman, & Maguire, Green human resource management: A review and research agenda, 2012).

2) The progressions needed by the hierarchical move towards a supportability approach require the responsibility by both administration and all the representatives, not simply by those straightforwardly influenced by new green and human resource management practices, as these exercises can advance and support green practices among all the individuals from an association. (DuBois & Dubois, 2012).

3) A successful hierarchical way to deal with supportability requires consistency with formal standards as well as worker acknowledgment of and commitment with willful green activities in the working environment, for example, lessening power or paper utilization, and the utilization of steps rather than lifts, just to make reference to a couple. (Ren, Tang, & Jackson, 2018).

On account of creating overall biological concern and advancement of worldwide normal standards, there is a necessity for associations to get formal natural practices (Daily & Haung, 2001)"."HRM practices are the genuine human resource programs, cycles and procedures that get executed in the association or business unit (Gerhart, Wright, & MC, 2000)". Also, green HRM is a real GHRM project, procedure and approach which becomes actualized in the associations to decrease pessimistic ecological effects or upgrade constructive natural effects of the organizations.

LITERATURE REVIEW

HRM is the extensive arrangement of administrative exercises and errands worried about creating and keeping a certified labor force in manners that add to hierarchical viability. HRM is a notable and gotten idea; consequently the creators don't zero in additional on building up the significance or essential comprehension of this term. (Gupta, Aggarwal, & Arora, 2016) Adopting green HRM practices bring about diminished expense and improved incomes for the association by bringing down the overheads costs. Also receiving GHRM practices upgraded public picture and improved market position since it improves the connection among association and other stakeholders. (Nanda & Randhawa, 2020) For the organizations and particularly, the HR experts in a developing nation like India, current

computerized innovation is both a chance just as a test which ought to be embraced yet with some care. The connection among IT and human resource management can be named as harmonious. While the use of IT in HRM is accepted to change the part of the capacity, simultaneously HRM additionally permits innovation to create at its maximum capacity (Hempel, 2004). The little and medium organizations could profit in an incredible manner by embracing the different e-HRM works yet government and authoritative pioneers' help is a lot of needed for this gigantic assignment.

HRM have some elements which are basically contemplated as conventional and also there can be an assortment of green practices under every capacity. The accompanying segment brings up a wrap-up of the current and defined new GHRM practices under every capacity of GHRM.

1. Green selection and recruitment –

Green selection and recruitment is alluded to as a cycle of enlisting and choosing up and comers who are delicate to ecological issues and ready to focus on natural execution (G., Y., Y., P., & Jia, 2018). A few organizations decide to apply green rules while choosing candidates while some don't. Regardless, conveying an organization's ecological qualities and direction merits working on during green recruitment and selection. Past examinations have recognized some arbiters such as foreseen pride, seen esteem fit, an assumption for a great treatment, etc. that mediate between signs of the organizations' CES and a work searcher's view of associational allure. Although, the strength of this impact is affected by some mediators such as socio-ecological awareness, work searcher's aptitude, want to have a critical effect through one's work, etc. (Pham & Paillé, 2019)

2. Green development and training –

Green development and training is viewed as fundamental among Green HRM practices (Jabbour, Teixeira, & Jabbour, 2013), as it aids the improvement of natural administration practices and the making of association culture delicate to ecological administration (C.J.C., 2013). Well-prepared representatives with natural mindfulness are capable, for instance, to distinguish and lessen squander (Renwick, Redman, & Maguire, Green human resource management: A review and research agenda, 2012). Propose certain green development and training practices, preparing representatives to deliver green investigation of workspace, utilization of occupation pivot to prepare green chiefs of things to come, an arrangement of explicit preparing on ecological administration parts of wellbeing, energy proficiency, squander management, and reusing, the progress of green representatives' propensity, and formulation of staff leaving the position in significant polluter ventures (Renwick, Redman, & Maguire, 2008; Renwick, Redman, & Maguire, Green human resource management: A review and research agenda, 2012).

3. Green rewards and compensation –

This is also a vital capacity of GHRM. The manageability of an association's natural execution is profoundly subject to the green compensation administration practices of an association. To persuade directors and non-administrative representatives on administered climate management activities, green compensation administration has critical dedications. Companies can execute it in monetary and non-monetary ways. Green compensation can integrate the usage of working atmosphere and procedure of life ease, free bike, drawing in individuals in the green plan and proceeding to perceive their commitment. (Pillai & Sivathanu, 2014). Green rewards and pay essentially affect worker fulfillment for eco-activities (Renwick, Redman, & Maguire, Green human resource management: A review and research agenda, 2012)

4. Green performance appraisal –

Introducing corporate-wide ecological execution norms or setting up green execution markers into the performance management system, and evaluation isn't sufficient. Correspondence of green plans, execution markers and principles to all degrees of employees through execution assessment framework and setting up a business-wide discourse on green issues are likewise expected to emerge focused on ecological execution (Renwick, Redman, & Maguire, Green human resource management: A review and research agenda, 2012) (Renwick, Redman, & Maguire, 2008). Executives should regulate green targets, objectives and obligations regarding their segments or divisions or offices, they ought to survey the number of green occurrences, utilization of climate duty, and fruitful correspondence of ecological arrangement inside the extent of their activities (Renwick, Redman, & Maguire, Green human resource management: A review and research agenda, 2012; Renwick, Redman, & Maguire, 2008).

5. Green management of organizational culture – To completely react to ecological difficulties, associations should go through huge social change and alteration (Lillenluecke & Griffiths, 2010). There is an attention to an adjustment in the supported organization, as obtained from organization documentation, for example, statement of purpose, key plans, organizational reports, formal explanations of strategies and inside dispatches (Harris & Crane, 2002). Green authoritative culture is comprehensively laid out as the degree to which the suspicions, qualities, images and ancient rarities of the association mirrored a craving or need to work in an earth practical way. It has two measurements 1) the "greenness" of the formally supported organization line 2) the degree to which this uncovered position apparently was showed as organization antiques and practices. (Harris & Crane, 2002)

Green Management and Employees Green Behavior

During the previous decade, "environmental awareness" is introduced in the managers' structures as a strategy for regulating conditions through "coordinated activities" (Haden, Oyler, & Humphreys, Historical, practical, andtheoretical perspectives on green management: an exploratory analysis, 2009). In such a way, executives set up normal organizational systems and rules to show up and give their confirmation towards "being green" (Llic & Unnu, 2012). Researchers have fought on the meaning of green concern of the firm. Table 1 has examined the perspective of different specialists on green administration throughout the long term. Employees' green behavior is arriving at their exhibition objectives. Besides, when associations know the significance of EGB for in general occupation execution, they can assist in decreasing disparities between the craving for more EGB and the addition in execution assessment. Subsequently, they could help in a clear correspondence of assumptions to representatives. Also, by lessening these disparities, organizations can better prize EGB in execution assessments. Eventually, associations would appear as reasonable and straightforwardly which relates decidedly to work fulfillment, hierarchical responsibility, and OCB (Jason A., Donald E., Michael J., O.L.H., & Yee, 2001).

The benchmark of the underneath referenced structure is that presentation is the capacity of a person inside the general climate. As indicated by (Norton, Parker, Zacher, & Ashknay, 2015), relevant components are vital in getting down to business the individual exhibition including institutional, hierarchical, pioneer and group as these shapes up the presentation-based behavior of a person inside the surges of these variables. Besides, individual factors additionally assume an essential part in the presentation as to "Employee Green behavior (EGB)". "Between person" elements, for example, work, disposition and conduct and "within person" elements, for example, inspiration and goals can assume the critical part in choosing the EGB. Moreover, persuasive states can be either controlled or self-governing as to EGB. EGB is arriving at its exhibition objectives. Besides, when associations know the significance of EGB for in general occupation execution, they can help decrease disparities between the craving for more EGB and the additions in execution assessment. Subsequently, they could help an away from of assumptions to representatives. Also, by lessening these disparities, organizations can all the more likely prize EGB in execution assessments. Eventually, associations would appear as reasonable and straightforward, which relates decidedly to work fulfillment, hierarchical responsibility, and OCB (Colquitt, Conlon, Wesson, Porter, and Ng, 2001). It is also (Andersson, Shivarajan, & Blau, 2005) noticed that people with ecological qualities are bound to show climate amicable practices, while (Chou, 2014)noticed that people with green qualities display green conduct in the working environment. Essentially, (Cheema, Afsar, & Javed, Employees' corporate social responsibility perceptions and organizational citizenship behaviors for the environment: The mediating roles of organizational identification and environmental orientation fit, 2020) asserted that green qualities are probably going to affect a person's both extra–job and in-job practices, and if there is a fit among employees and hierarchical green qualities, compelling ecological administration arises. Nonetheless, these observational investigations have analyzed the immediate effect of green qualities on a person's practices.

Objective

The objective of this review paper is to provide brief information about existing publications on -

1) Green human resource management practices
2) Employees green behavior

Research Methodology

In order to accomplish the above expressed objectives, a review of literature was supervised with the help of an authentic strategy. This review paper utilizes a technique to review articles from various information bases, sites and other accessible sources with "green HRM practices" and "employees behavior". Consequently, the examination of this paper turns into a work area research as opposed to an overview investigation.

Bibliometric Analysis

Keywords: Green human resource management, Employees green behavior, Sustainability VOS viewer software was utilized " in light of the fact that it looks at the relation between the most cited authors, the collaboration between the different authors, coordination among countries, institutions, keywords and related knowledge to the topic" (Hoppen & Vanz, 2016). This software is also used for cluster analysis by picturing of geographical network maps through a matrix which tells a bunch of co-authorship and co-occurrence" (Waltman, Eck, & Noyons, 2010).

DISCUSSION

This review of literature confirms, to an immense degree, the natural limit of HRM practices in greening workers and organizational behavior. From work configuration capacity to representative relations, HRM has enormous capability in greening organizations and their activities. The above review of literature indicates, to a critical degree, the innate limit of HRM capacities in greening representatives and hierarchical tasks. The major issue in front of HR experts is to comprehend the extension and profundity of GHRM in changing their associations as a green element. It is also discovered that the green HR policies would assume an imperative function in any association to advance the natural related issues just as the social issues. It will likewise increase the confidence and execution of workers and furthermore would give the advantage to both the representatives just as to the organization. To make practices and keep up ecological related innovational practices of workers combined with the right demeanor of greening, GHRM practices are condemnatory. Without appropriate GHRM practices, it is hard to make and keep up economical natural execution. Accordingly, we attest that by understanding the extension and profundity of GHRM practices associations will have the ability to act in more harmless to the ecosystem way than any other time.

CONCLUSION

In light of this review, it is comprehensible to assume that by grasping and enlarging the extension & profundity of GHRM practices, associations can enhance their natural presentation in a better manageable way than before. The GHRM practices are all the more integral assets in making associations and their tasks green. Employees learn endless things from their working life and because of these learning singular conduct shifts towards the climate. This is just conceivable by the powerful usage of GHRM inside organizations. GHRM practices empower the gainful utilization of associations' space and framework. It improves the association and workers and workers-workers connections by sharing of assets and obligation to oversee and create green practices to teach civility of manageability. The green presentation, practices, disposition, and capabilities of HR can be molded and reshaped through the transformation of green human resource practices. Consequently, this is recommended that associations needed to provide greater need to construct each capacity of human resource management “green”. Future research study needs to give experimental proof while the green human resource management conveys the positive result in employee’s behavior.

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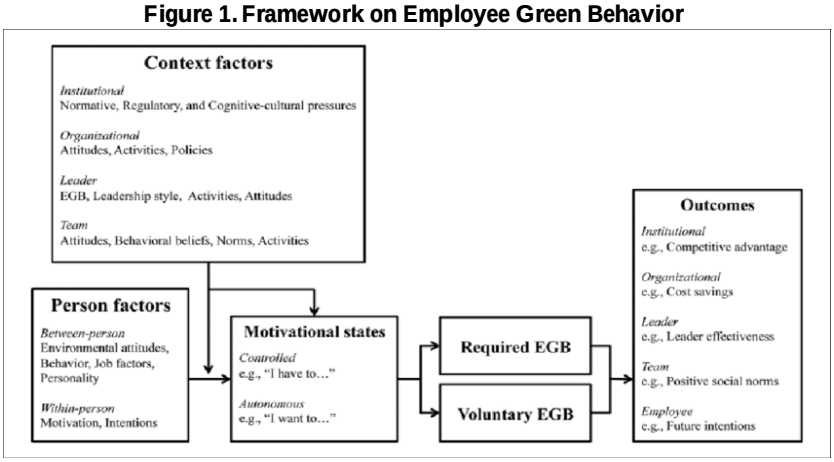
Table 1. Different perspective of different specialist on green administration

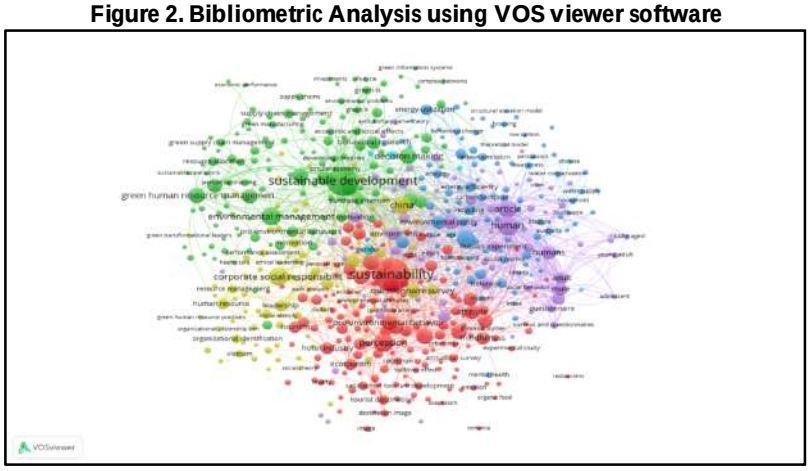
Authors	Findings/Conclusions
(Khan, Khan, Ahmed, & Ali, 2013)	Notice numerous business associations have re-marked their basic beliefs remembering social obligation regarding ecological greatness.
(Bruch & Walter, 2005; Porter & Kramer, 2006)	Suggest for an all the more close arrangement between an organization's center technique and its social obligation endeavors.
(Abbaspour, Karbassi, & Khadivi, 2006)	Talk about how the execution of green administration in game buildings of Iran can guarantee the constant plan and appraisal of exercises from the ecological viewpoint to control and forestall pollutions.
(Bergmiller & McCright, 2009)	Discover the qualities of the mix of creation cycle and green projects to enhance business outcomes.
(Haden, Oyler, & Humphreys, 2009)	Propose that green accomplishment can be made through constant learning and improvement through accepting natural objectives and techniques into the objectives and procedures of the association.

Figure 1. Framework on Employee Green Behavior



Source (Norton, Parker, Zacher, & Ashknay, 2015)

Figure 2. Bibliometric Analysis using VOS viewer software



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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Copper Mediated C (sp^2)-H Functionalization: 8-Aminoquinoline as Auxiliary Bidentate Directing Group

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Received: 17 Feb 2021 Revised: 24 Feb 2021 Accepted: 27 Feb 2021

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ABSTRACT

The development of copper mediated chemo- and regioselective sp^2 C-H bond functionalization with the help of bidentate directing group has made significant progress in last few years. These protocols involve powerful and atom economy transformations for introducing various functional groups via C-H bond activations. This review covers major achievements in bond formation like C-C, C-O, C-N, C-S, and tandem annulations via C-H bond activation.

Keywords: C-H functionalization, Arylation, Trifloromethylation, Hydroxylation, Amination

INTRODUCTION

Construction of aryl C-C and C-hetero bonds formation is key methods in organic synthesis due to the ubiquity of aryl and heteroaryl structural motifs in pharmaceuticals and natural products. But, the transition metal-catalyzed/mediated cross coupling between prefunctionalized starting materials such as aryl halides, triflates and aryl-metal intermediates is often chosen and reliable synthetic approach. However, the most recent focus on efficient synthetic routes and minimizing waste, multistep synthesis make new direct methods called as C-H bond activations for their construction particularly attractive (Scheme 1). Direct C-H activation continues to find a growing number of applications in the synthesis of bioactive molecules.

Copper-mediated sp^2 C-H functionalizations which obviates expensive metals like Pd, Rh, Ru [1]. The 8-aminoquinoline as auxiliary assisted, copper-mediated sp^2 C-H functionalization is attractive and reliable methods from the academic and industrial point of views. In 2005, Daugulis and co-worker first introduced 8-aminoquinoline and picolinamide as bidentate directing groups for palladium-catalyzed C-H bond functionalization [2].This

auxillary are commercially available and easily removable which enhance synthetic transformations. An extensive effort have been made to bidentate directing groups assisted C-H bond functionalization by using cheap metal catalysts such as Ni, Cu, Co and Fe by various research groups[3].Among plethora of transition metal catalysts, Copper catalysts gained much significant attention. These salts have been used as versatile catalysts for various reactions in organic synthesis due to inexpensive, abundant and possess low toxicity. Recently, Copper-mediated C-H Functionalization reactions has emerged as powerful synthetic tools for C-C and C-X bond formation[4].This review discusses on major achievements in this area and catalogued by types of bonds formed like C-C, C-O, C-N, C-S, bond formation and tandem C-H functionalization/annulation.

C-C Bond Formation

Heteroarylation

Miura group reported a copper-mediated dehydrogenative heteroarylation of benzamides using 8-aminoquinoline as bidentate directing group, which could be easily introduced and removed as a protecting group (Scheme 2)[5]. They screened various directing groups, among them, 8-aminoquinoline was found to be the best for the successful of this reaction. A wide variety of functional groups tolerate under standard conditions. But this methodology is limited to only azoles bearing acidic hydrogen. The removal of directing group was achieved via NH Boc protection followed by reflux with sodium ethoxide in ethanol solvent with 63% yield **3**a.The NH of benzamide derivatives is believed to play crucial role during transformation since N-Methyl benzamides did not furnish any products and starting materials intact. It has been observed that 2 equivalents of $Cu(OAc)_2 \cdot H_2O$ is essential for good conversion. Probable the excess copper salt acts as both catalyst and oxidant.

Arylation

Tan *et al.* reported copper mediated *ortho*-arylation of benzamides with arylboronic acid using 8-aminoquinoline as directing group (Scheme 3)[6]. Various biaryl has been synthesized with good yields and compatible with different functional groups. Notably, this protocol is selective monoarylation with excellent regioselectivity.

Trifluoromethylation

Organofluorine compounds are important in agrochemical and pharmaceutical research due to the changes of biological, physical and chemical properties. So, development of mild and efficient trifluoromethylation reaction is challenges to synthetic chemists. Zhu *et al* reported highly mono-selective *ortho*-trifluoromethylation of benzamides via *8*-aminoquinoline assisted Cu-promoted C-H activations (Scheme 4) [7]. This reaction tolerates a wide variety of functional groups and various *ortho*-trifluoromethylated benzamides were efficiently synthesized with high yields by simple using copper salts with Togni's reagent as the source of CF_3. Notably, this reaction depend two equivalent of water as additives. But, the exact role of water has not been explained.

C-O Bond Formation

Methoxylation

In 2013, Stahl and co-workers discovered oxidative methoxylation of *N*-(8-quinolinyl) benzamides using 2 equiv. of $Cu(OAc)_2$ by employing methanol as solvent (Scheme 5)[8].In order to understand the reaction mechanism, they did several experiments including deuterium-labelling experiments, Hammett studies and computational studies. From all the studies, it suggested that the reaction proceeds through Cu(II)-mediated C-H activation.

Hydroxylation

In 2016, Jana and singh developed *ortho* C-H hydroxylation of benzamides with inexpensive copper(II) acetate monohydrate and a pyridine ligand (Scheme 6)[9].An intra- and intermolecular ligand combination was explored to achieve regio- and chemo selective hydroxylation. Interestingly, typical regiochemical scrambling associated with the

C-H activation was further resolved by introducing a ligand-directed *ortho* hydroxylation of haloarenes and aryl methyl ethers.

C-N Bond Formation

Amination

Jana *et al* reported copper(II)-mediated C(sp^2)-H amination of benzamides with electronically neutral or electron-rich anilines (Scheme 7)[10] A dramatic influence of silver(I) and tetrabutylammonium bromide (TBAB), was observed on the reaction outcome. Using this methodology a number of non-steroidal anti-inflammatory drugs (NSAID's) were also synthesized. More interestingly, an acetyl group containing benzamide afforded the corresponding amination product in high yields which prefers facile imine formation with anilines under slightly acidic conditions.

Nitration

In 2014, the Gooβen group reported a copper-mediated *ortho*-nitration of arenes with the assistance of the removable 8-aminoquinoline as bidentate directing group (Scheme 8a)[11].The benzamides having both elctron-rich and electro-deficient groups tolerate under standard conditions. This reaction is highly regioselective and only mononitration product formed. Shortly after, the Tan group reported the copper-mediated AQ-directed nitration of aryl C-H bonds using sodium nitrite as the nitration agent. Interestingly, selectivity mono- and dinitration was observed by simply changing the reaction conditions (Scheme 8b)[12]. The standard reaction conditions for the mononitration were: 2 equiv. of $Cu(OAc)_2 \cdot H_2O$, 3 equiv. of $NaNO_2$, 2 equiv. of K_2HPO_4 as the base in anhydrous MeOH as solvent at 60 °C under air for 12 h. The standard reaction conditions for the dinitration were: 2 equiv. of $Cu(OAc)_2 \cdot H_2O$, 3 equiv. of $NaNO_2$, 2 equiv. of AgOAc as the base in anhydrous DMF as solvent at 80 °C under air for 12 h.

C-S Bond Formation

Sulfenylation

In 2012, Daugulis group discovered a copper-promoted auxiliary-assisted direct sulfenylation of sp^2 C-H bonds of arenes (Scheme 9)[13] The benzamides reacts with (trifluoromethyl) disulfide, alkyl or aryl disulfides as reactive partners in DMSO in the presence of $Cu(OAc)_2$(0.5 equiv.). The *ortho* substituted benzamides afford monosulfenylation while *meta* and *para* substituted benzamides form di-*ortho*-sulfenylation products under the optimized reaction condition. The reaction shows high generality, excellent selectivity toward ortho C-H bonds,as well as good functional group tolerance and offers a novel and easy synthetic procedure for aryl thioethers. Interestingly, this reaction provides a novel and straightforward approach for the preparation of aryl trifluoromethylthioethers. Tan group reported copper-mediated direct sulfonylation of C(sp^2)-H bonds with aryl sulfinate salts (Scheme 10)[14]. The reaction required stoichiometric amount of copper(II) acetate and aerial oxygen with assistant of 8-aminoquinoline as bidentate directing group. Notably, Mono sulfonylated product formed exclusively for all substrates.

Tandem C-H functionalization/annulations

In 2014, You and co-workers first disclosed copper-mediated tandem alkynyation/annulation of arenes with terminal alkynes (Scheme 11)[15]. After screening of copper salts, copper acetate was the ideal catalyst for the transformation and used as both reaction promoter and the terminal oxdant. A wide variety of substrate has been reported with high yields, tolerates different functional groups. This reaction shows exclusively chemo-, regio- as well as steroselectiveity. Moreover, they found that *ortho*-alkynylbenzamide could be transformed into the corresponding annulation product in the presence of either $Cu(OAc)_2$ or CuOAc, indicating that the domino reaction might proceed via an oxidative alkynylation/intramolecular annulation sequence.

In 2014, Liu and co-workers developed a copper-mediated annulation of benzamides with ethyl cyanoacetate using *8*-aminoquinoline as bidentate directing group (Scheme 12)[16]. A wide variety of isoquinolinone compounds were prepared via this tandem C(sp2)-C(sp3)/C(sp3)-N bonds formation sequence. Other cyano substrate derivates such as cyano substituted amides, phosphonates, and methylsulfonyls tolerate under normal reaction conditions.

In 2015, Copper-mediated oxidative coupling of benzamides bearing *8*-aminoquinoline with ma-leimides was reported by Miura and coworkers (Scheme 13)[17] From optimization table, it was found that organic bases could increase the yield of the desired product and proved Cy2NMe was essential for the success of this transformation. Based on control experiments, they observed Cy2NMe not only facilitated the C-H cleavages, but also accelerated other steps. The alkenes bearing *N*-methyl, *N*-benzyl- and *N*-phenylmaleimides and benzamides having different functional groups were compatible for the reaction. The major drawback of this reaction is its inaccessible to alkenes other than maleinides. For examples, other alkenes like maleic anhydride, acrylates, norbornenes, and styrenes were ineffective for the coupling.

CONCLUSION

Copper mediated reactions require stoichiometric amount of copper salts due to co-ordination effects. Despite remarkable progresses on this, several drawbacks need to be addressed. First, copper-catalyzed, directed C-H functionalization should be paid attention to minimize wastage of excess salts. Secondly, copper -catalysed sp^2 C-H based tandem reactions are still rare. So, more attention should be paid on these type reactions which could make complex molecular frameworks. Thirdly, most of the reactions are proposed to involve copper (III) species, however, no intermediated has been isolated so far. So, details reaction mechanism needs to be investigated for further understanding and designing efficient catalytic systems which may help to synthesis of complex molecular architectures and Pharmaceutical compounds.

ACKNOWLEDGMENT

The authors are thankful to the Department of Chemistry, Aska Science College, Aska, Ganjam, for support and motivation during manuscript preparations.

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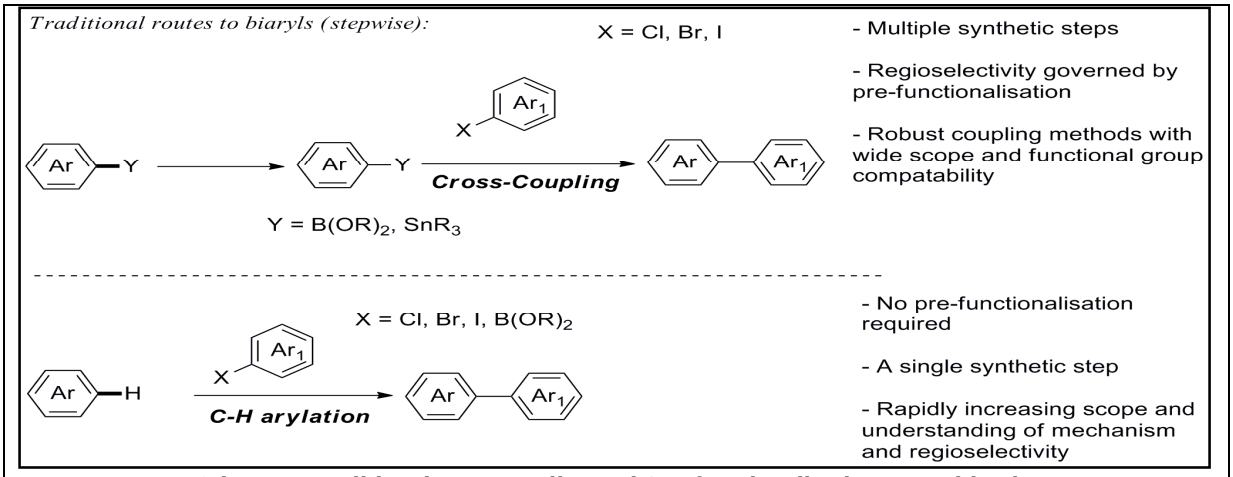
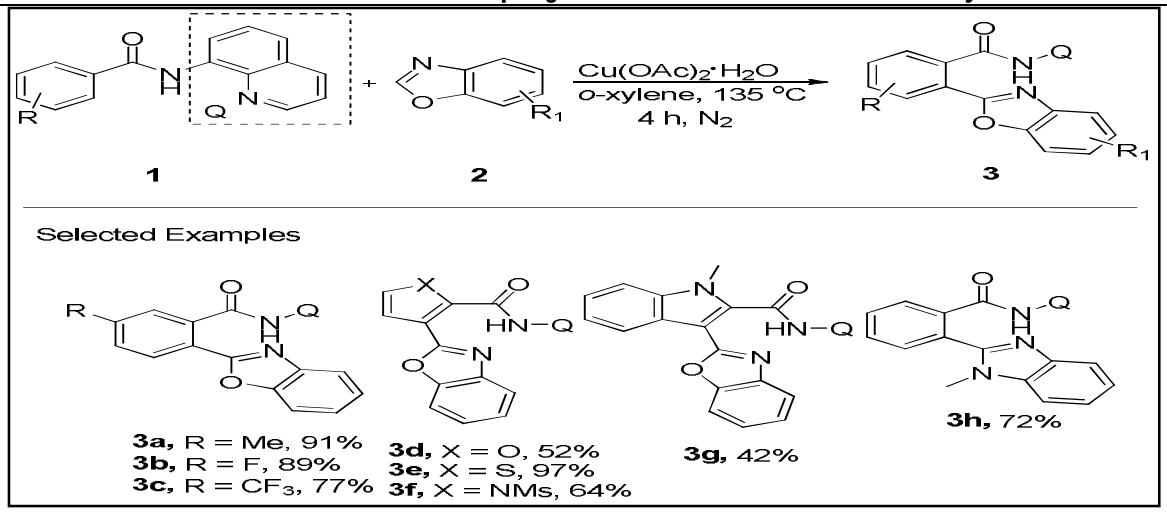
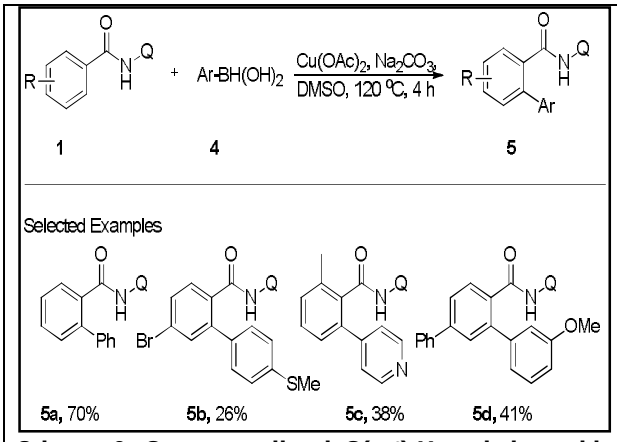


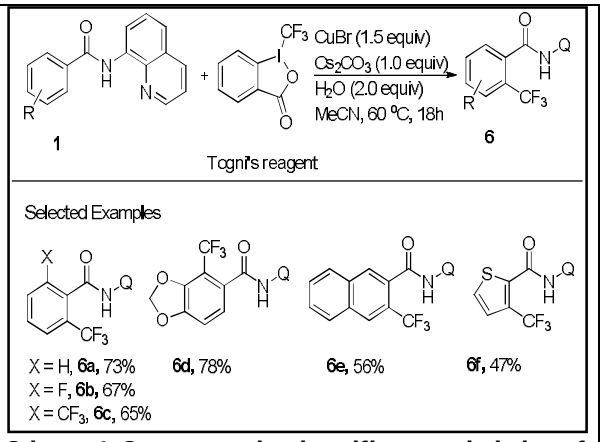
Scheme 1. Traditional cross-coupling and C-H functionalization routes biaryls.



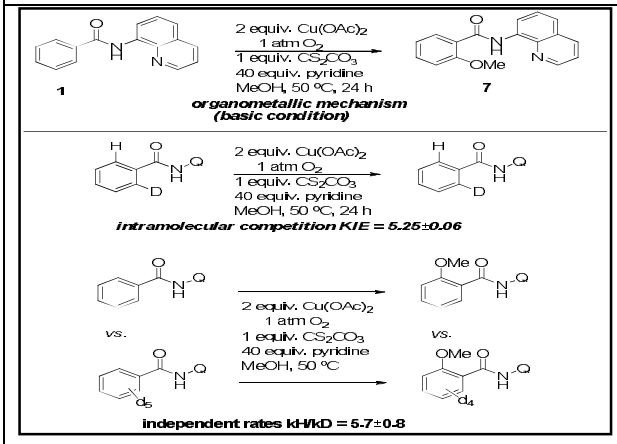
Scheme 2. Copper-mediated dehydrogenativeheteroarylation of C(sp^2)-H bonds with 1,3-azoles



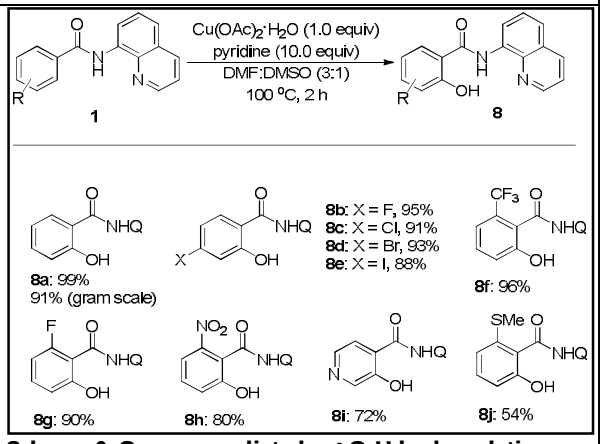
Scheme 3. Copper-mediated C(sp²)-H arylation with boronic acid



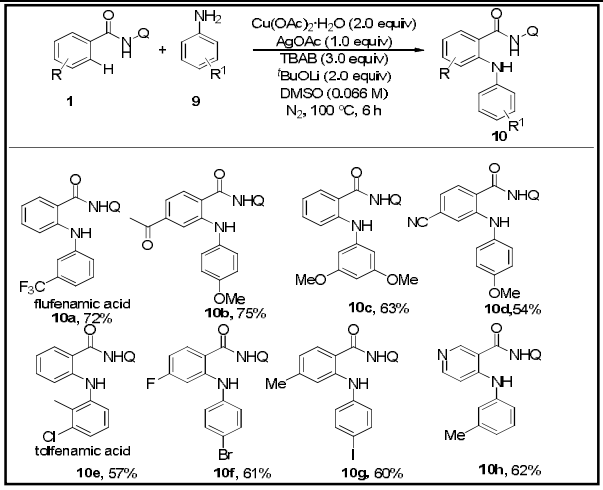
Scheme 4. Cu-promoted ortho-trifluoromethylation of substituted benzamides.



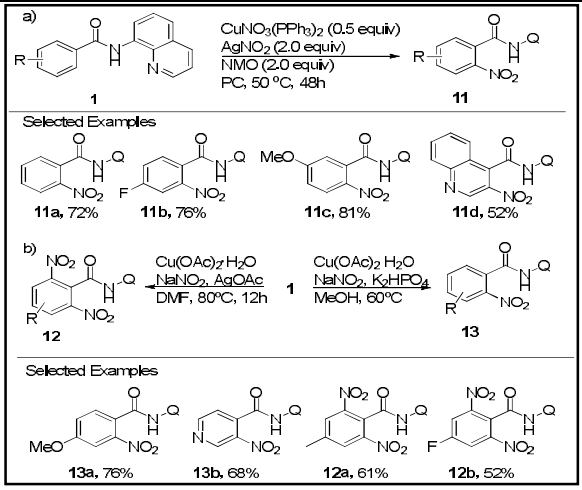
Scheme 5. Copper-mediated sp² C-H oxidative methoxylation of benzamides.



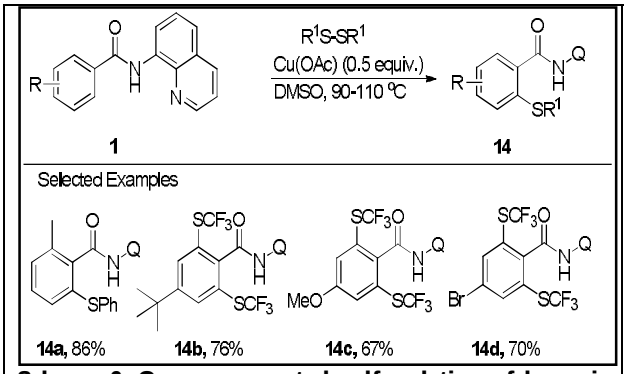
Scheme 6. Copper-mediated sp² C-H hydroxylation.



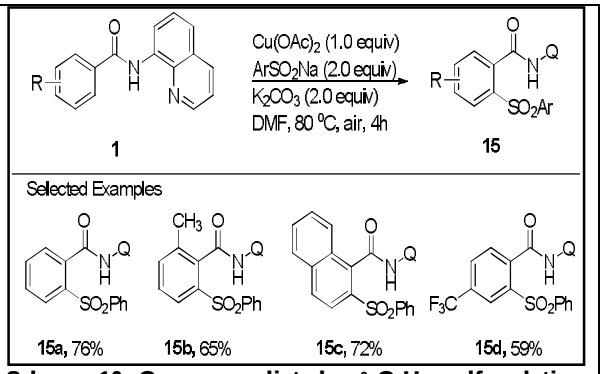
Scheme 7. Copper-mediated sp² C-H amination with electron rich anilines.



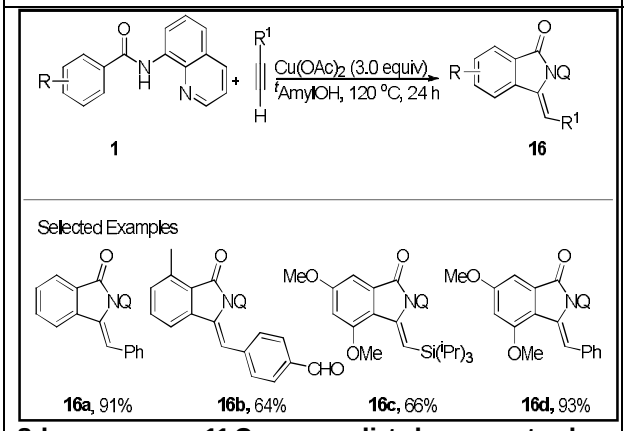
Scheme 8. Copper-mediated nitration of arenes and heteroarenes.



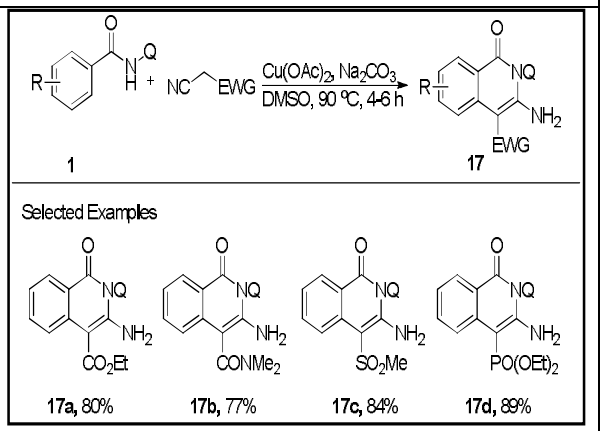
Scheme 9. Copper-promoted sulfenylation of benzoic acid derivatives.



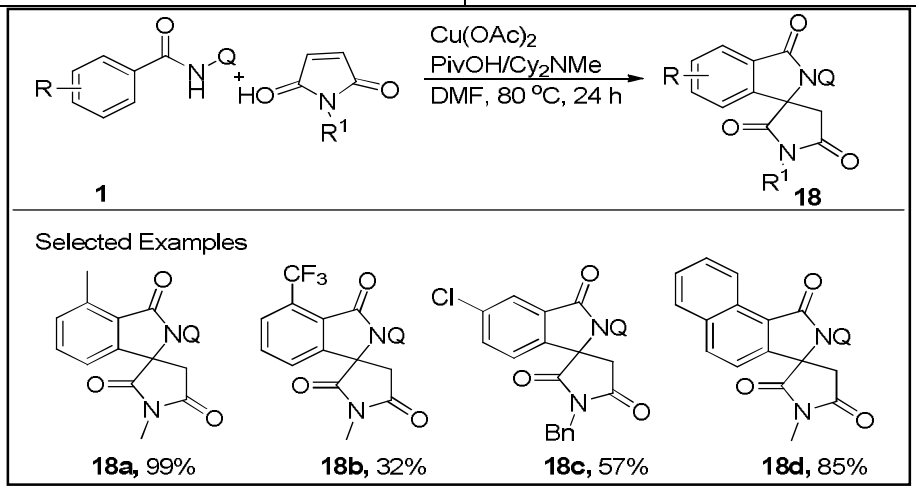
Scheme 10. Copper-mediated sp² C-H sulfenylation of arenes



Scheme 11.Copper-mediated tandem alkynyation/annulation of arenes with terminal alkynes.



Scheme 12. Copper-mediated annulation of benzamides with ethyl cyanoacetate



Scheme 13. Copper-mediated oxidative coupling of benzamides with maleimides.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Biology of Fresh Water Exotic Fish, *Oreochromis mossambicus* (Peters) in India: An Overview

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Received: 16 Feb 2021 Revised: 22 Feb 2021 Accepted: 27 Feb 2021

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ABSTRACT

The biology of freshwater cichlid exotic fish in the lentic and lotic water bodies of India as worked out by various researchers is reviewed based on published literature. The biological aspects of fishes includes, length-weight relationship, relative condition factor, food and feeding habits, reproductive biology, fecundity, age and growth, sex ratio and biochemical constituents. The present review study is aimed at enhancing the knowledge regarding the biology of fishes and to enable the formulation of suitable management measures towards a rational exploitation and management in water bodies in India.

Keywords: Biology, Cichlid fish, *Oreochromis mossambicus*, India.

INTRODUCTION

Biological invasions are increasingly recognized as a primary threat to global biodiversity after habitat degradation and that can bring out irreversible changes in an aquatic habitat that may lead to species extinctions (Nyman, 1991). Fishes are the most introduced (624 species) and threatened group of aquatic animal globally. In India more than 300 fish species were introduced for various purposes such as increasing fish production, sport fishing, aquarium trade and bio-control of mosquito (Singh and Lakra, 2011). The *Oreochromis mossambicus* is an cichlid fish (Figure 1) native to Southeastern Africa. Dull colored, the tilapia often lives up to a decade in its native habitats. It is a popular fish for aquaculture. Due to human introductions, it is now found in many tropical and subtropical habitats around the

globe, where it can become an exotic species because of its robustness. These same features make it a good species for aquaculture because it readily adapts to new situations and organoleptic quality. The study of biological aspects of cichlid fish, *O. mossambicus* will helps in mass culture and which in turn helps in conservation of the fish. Hence, in the present review study focused on the biology of the cichlid fish *O. mossambicus* undertaken from Indian lentic and lotic water bodies worked out by various researchers.

Biological Aspects (Table 1)

Length- Weight relationship and Relative Condition Factor

Ujjania and Sharma (1999) studied the length – weight relationship and relative condition factor of Tilapia (*Oreochromis mossambicus*) occurring in lake Jaisamand, Rajasthan and observed a linear relationship between length and weight. They found that the relative condition factor vary between 0.99 to 1.00 in Tilapia. Shendge (2005) studied the length- weight relationship and relative condition factor in *Oreochromis mossambicus* of Bhima river. The cubic law indicating the isometric growth of the fish. The correlation coefficient showed a good relationship. The mean relative condition factor was 1.00 indicated the well being of the fish. Dede and Deshmukh (2019) were undertaken to unveil information on the length weight relationship and Ponderal index of *Oreochromis mossambicus* from Bhima river tal. Pandharpur, Dist. Solapur. The total length recorded ranged from 10 to 25 cm. The weight of fish varied 23 to 286 gm. The Ponderal index value is greater than 1. Their results indicate the negative allometric growth and good condition of fish growth. Kiran et al (2006) worked on the length-weight relationship of *Oreochromis mossambica* from Jannapura pond, Bhadravathi taluk, Karnataka. In male and female fishes "b" values ranged 2.5225-2.5867 and 2.125-2.015 y. They reported that both sexes of fishes exhibited allometric growth pattern.

Age and Growth

Ujjania et al (2013) examined the scales of 90 tilapia *Oreochromis mossambicus* individuals from commercial catch from Jaisamand Lake, Udaipur (India). In their study population, age composition varied between 1+ to 4+ year class and 3+ age group was dominated. The growth constant and average growth constant during the initial year of life is high indicate that the fish had active growth period during first year.

Abundance, GSI and Feeding Habits

Mushtaq Ahmad Ganie et al.,(2013) investigated the population characteristics of non-indigenous tilapia, *O. mossambicus* in stretch of Yamuna river in India. The gonado-somatic index (GSI) and the presence of all 06 gonadal stages confirmed that *O. mossambicus* has established a breeding population. The GSI for females indicated increased spawning intensity in March to April and July to August.Males ranged from 142-280.0 mm total length and females from 130-265.0 mm TL. Small juvenile fishes were collected by them in the Yamuna River. Adult *O. mossambicus* consumed primarily detritus and plant matter, though the food of juveniles, was found to be carnivorous. Sakhare and Jetithor (2016) analysed gut content of 80 fishes of *Oreochromis mossambicus* from BornaReservoir,Maharashtra, India revealed that the food of juveniles mainly consisted of rotifers followed by copepods Chlorophyceae , Bacillariophyceae and aquatic insects . While the food of adults were Chlorophyceae followed by Bacillariophyceae , rotifers , copepods and aquatic insects . They found that the juveniles feed on zooplankton and adults on phytoplankton respectively. Severe feeding was noticed during summer season and juveniles were active feeders. Singh and Shukla (2014) noticed feeding intensity with ascend in water temperature and furthermore they saw that the medium fishes being more dynamic feeder than larger ones. Indira et al. (2013) additionally saw decline in feeding intensity with increment in size of *O. mossambicus.* Indira et al(2013) gathered the examples of *O. mossambicus* month to month during January 2010 to December 2010 utilizing gillnets of various cross section sizes from Pichavaram Mangrove territory. It was found to utilization of an assortment of substances of plant and animal inceptions. A sum of 10 diverse food segments was recorded in the gut of *O. mossambicus*. The diverse food materials were noticed for example, scavenger, fish, zooplankton, phytoplankton, polychaetes, nematodes, gastropods, bivalves, sand and various. The gonado somatic index (GSI) of the species changed from the male was

4.93±0.17 to 7.93±0.17 and the female was shifted from 5.78±0.36 to 8.73±0.36. The feeding intensity was varied consistently and the base was seen during June and August like the generating time frame. Aravindan (1980) and Panikker (2000) detailed the omnivorous feeding habit of *O. mossambicus*.

Bio Invasion

Ujjania et.al (2015) considered the populace design of fascinating fish tilapia and its effect on native ichthyofauna of Lake Jaisamand (India) is portrayed. Their outcomes show that fish production and local area construction of neighborhood fish fauna were unfavorably influenced by high thickness and bounty of this intriguing fish species tilapia. During 1990-91 fish production was 287 metric tons which was made by Indian significant carps , minor carps and catfishes however, because of attack of tilapia it was decreased 105 mt till 1996-97 and fish production organization was changed and it is contributed by Indian significant carps , minor carps , catfishes and tilapia . The decrease of native fauna was consistent and it is noticed that commitment of Indian significant carps were just 11%, minor carps (3%), catfishes (4%) and tilapia overwhelmed by 82% out of complete production 119 metric tons (2012-13). The sea-going climate of lake was truly appropriate for tilapia and its development regarding length/weight was least 25.0 cm/400 g and greatest 43.5 cm/1620 g during 1997. It shows the hindered development because of high thickness and noticed 17.5 cm/98 g least and 38.0 cm/932.5 g greatest during 2013. Study uncovered that tilapia is profoundly obtrusive because of high wealth and serious for food and space to Indigenous fish fauna. It is additionally suggested that there is need of point by point concentrates on tilapia bounty, enlistment and neighborhood natural conditions to comprehend the intrusion potential and ramifications for the endemic aqua biodiversity.

Fecundity

Sakhare and Chalak (2019) mentioned an objective fact on the fecundity of the *Oreochromis mossambicus* from reservoirs of Beed region, Maharashtra, India. The ovarian eggs were discovered to be of various sizes. The quantity of ova per gram develop ovary went from 235 to 390 and the quantity of ova per gram body weight, from 6.41 to 9.92, the normal being 9.01. The fertility expanded with the increment in size of fish. Fecundity in *O.mossambicus* is truly factor as detailed by various examiners. As per Hora and Pillay (1962), the female tilapia lays 75-250 eggs all at once. Vaas and Hoftstede (1952) found that the fertility of *T.mossambica* went from 80 to 300 ova for fish with length that goes from 8 to 11 cms in complete length. Dietmar (1966) checked eggs from ovaries of *T. mossmabica* and discovered them to go somewhere in the range of 390 and 910 with female body weight going from 92 to 365 gms. Sukumaran(1969) announced the scope of fertility in *T.mossambica* from 100 to 900 eggs in fishes going from 81 to 220 cm in complete length. Kulkarni(1984) revealed the absolute number of ova in fluctuated from 169 to 772 in species going from 103 to 179 cms long. Mironova (1969) revealed that the fecundity of tilapia went from 80 to 1000 eggs for each female. De Silva and Chandrasoma (1980) discovered fruitfulness of *O. mossambicus* going from 360 to 1775 eggs for every female for going from 20 to 31.9 cm in all out length.

Reproductive Biology

There are 06 maturity stages in *O. mossambicus*.Gonado-somatic index indicated that the breeding season extended from March to October. Ova-diameter frequency polygon showed 4 peaks suggesting 4 times of spawning of the fish. First sexual maturity observed - 5 to 10 cm length groups in female and 10 to 15 cm in male. 50 % of male mature at an average length of 12.5 cm and 50 % of female mature at an 7.5 cm length groups. Absolute fecundity was 100 to 850, Relative fecundity found to be 6 to 16. Positive correlation between GSR and condition factor (r = 0.88). High correlation was observed between fecundity and total length and fecundity and body weight (Hatikakoty and Biswas,1999).

Manivannan et al (2019) studied the gonads revealed the existence of 05 maturity stages of *O. mossambicus*. The breeding season extended from December to August the appearance of first sexual maturity was observed in female

than in male. They concluded that the 50% of male individual were mature than females which was assessed through histological analysis of the species.

Sex Ratio

Roshni and Renjithkumar (2020) studied the reproductive ecology of fish *Oreochromis mossambicus*from Vembanad Lake, Kerala. Females were dominant in the catches and the overall sex ratio was 1:1.40 ,which chi-square significantly deviated from theoretical 1:1 of which 41.74% were males and 58.26% were females. Males and females in reproductive activity occurred throughout the year, the peak spawning occurred between May to August and November to December. Information on the advantages and limitations of the techniques of hybridization and hormone treatment for the production of male tilapias was summarized by Pandian and Varadaraj (1987).. The production of triploid and tetraploid *O. mossambicus* through heat treatment of fertilized eggs was described by them.

Proximate Composition

PrakashShobha et al (2020) carried out the biochemical composition of *Oreochromis mossambicus*, *Lepidocephalichthys thermalis* and marine fishes; *Sardinellalemuru* and *Stolephorus indicus* fishes. Carbohydrate was high in *Oreochromis mossambicus*. Their results revealed that among the four tested fishes, the high amount of carbohydrate content present in the fish *Oreochromis mossambicus***.** The property of total proteins from fresh *Oreochromis mossambicus* have been assessed. The meat had elevated moisture with 81.34% and low lipid < 1% content.

Fishery

The fish fauna of Chalakudyriver is undermined by a scope of components like habitat change, over-abuse, contamination and presentation of exotic species. 05 intriguing fish species have been recorded from the stream framework up until this point. Despite the fact that few reports are accessible on the fish variety from this waterway, no endeavor has been made to measure the catch of non-local *Oreochromis mossambicus*. Roshni et al (2016) study on the monthwise exploitation of *O. mossambicus* from Poringalkuthu Reservoir situated in the midstream of Chalakudy River. A point by point month to month review was led at the supply to notice the bounty record and catch of the significant fish fauna alongside *O. mossambicus* for a time of June 2011 to May 2012. The information assortment depended on landing communities since test fishing was legitimately denied in the examination site and the fishing was permitted distinctly to the nearby clans. The month to month landing was assessed utilizing standard techniques and yearly catch was determined by summing up the arrivals, everything being equal. The total catch of the reservoir was discovered to be 8064 kg/year with a most elevated amount recorded for *O mossambicus* (2592 kg/year). The high abundance index was additionally recorded for *O. mossambicus* (31.71%) contrasted with other fish fauna. The ordinary experience of mature and ripe females of *O. mossambicus* demonstrates the fruitful foundation of the species in wild. The uncontrolled populace development of this potential fascinating fish might be checked since the species may harm the local fauna of the supply in a not so distant future.

CONCLUSION

The biological preference is an important for a making sure of the species suitability for aquaculture, since, it can help to determine the eligible species blend in culture systems with an inter species completion for natural food items. The natural environment providing the information on the biological profile of the species, it is mainly useful the species for small and large scale culture ones and maintain schedule of feeding their avoiding diseases and can benefit the environment.

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Table 1: Biology of cichlid exotic fish, *Oreochromis mossambicus* (Peters) in the lentic and lotic water bodies of India as worked out by different researchers

Sl. No.	Parameters	Water body	References
1	Age and growth	Jaisamand lake	Ujjania et al.,2013
2	Relative condition factor, GSI, Food and feeding habits	Yamuna river, Uttar Pradesh	Mushtaq Ahmad Ganie et al.,2013
3	Bio invasion	Jaisamand lake	Ujjania et al.,2015
4	Fecundity	Water bodies in Beed district (Maharashtra)	Sakhareb and Chalak,2019
5	Reproductive biology	Nazira pond, Assam	Hatikakoty and Biswas,1999
6	Fishery	Poringalkuthu reservoir, Kerala	Roshni et al.,2016
7	Food and feeding behaviour	Borna reservoir (Maharastra)	Sakhare and Jetithor,2016
8	Food and feeding habit	Pichavaram mangrove area	Indira et al.,2013
9	Length-weight relationship and Relative condition factor	Bhima river, Maharastra	Shendge,2005
10	Length-weight relationship and Ponderal index	Bhima river, Maharastra	Dede and Deshmukh,2019
11	Length-weight relationship	Jannapurapond,Karnataka	Kiran et al.,2006
12	Biochemical aspects	-	PrakashShobha et al.,2020
13	Biology	Marathwada water bodies	Kulkarni,1984
14	Length-weight relationship and Relative condition factor	Jaisamand lake	Ujjania and Sharma,1999
15	Reproductive ecology & sex ratio	Vembanad Lake, Kerala.	Roshni and Renjithkumar ,2020

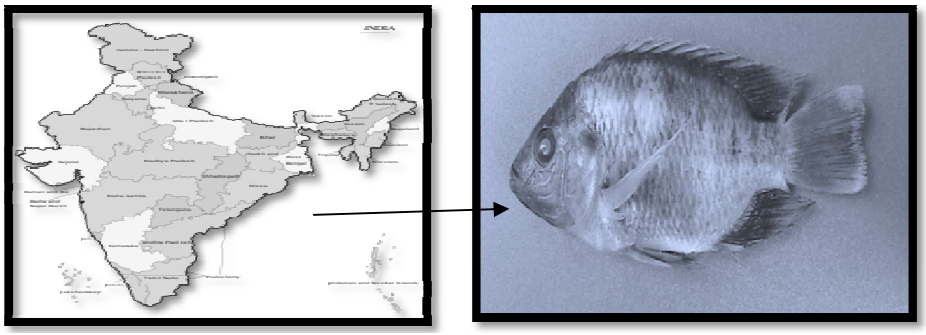

Figure 1: India map & *Oreochromis mossambicus* fish species

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Study on the Switch in the Attire Preferences of People Due to the COVID-19

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Received: 26 Feb 2021 Revised: 02 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

As a global pandemic has affected nearly every human being, thus affecting nearly every industry, the fashion industry remains no exception; the factories were shut down, retailers were out of business, millions of workers lost their source of income, thousands shifted industries, production came to absolute halt, a certain section of the market froze and thus the whole market seemed to have been shifted to the online platforms. Not only digital shopping is prioritized but also fashion shows and launches of new collections are being done in the online medium. Fashion giants chose to live stream his collections rather than invite people-effect of the rampant surge of cases in Italy during the first wave of the virus. A descriptive research design is chosen. All about 250 responses were received. And the method of sampling used in the study is simple random sampling. Qualitative research is done to know the shifts in the clothing preferences of people in the current period.

Keywords: Pandemic, lockdown, attire, preferences, switch

INTRODUCTION

During the lockdown, people all around the world, who dressed up and went to work or schools / colleges, were confined to their homes. In the pre COVID period, people dressed up, stepped out of their houses all prepped but the lockdown changed it all, they stopped stepping out of our house and were forced to stay inside. Classrooms were replaced by online classes and so was work. But as the time passed, started adapting to the new normal. Lifestyle got affected and so did the clothing preferences. The understanding and psychology of people towards fashion is

changing and will continue to change in the post COVID period. This change in the attitude/ behaviour of people i.e. consumers is affecting their preferences too. This study addresses the current flux in the population that chose is the Indian middleclass youth. This selected section of the population is mostly studying or working and thus contributing most to the overall of the industry giving an insight behind the same. The study is mainly conducted to know about how the COVID -19 pandemic affected peoples clothing preferences. And also to know the changed clothing preference of people and to analyse how peoples clothing preferences will be post pandemic. And to know how a global pandemic can change a person's views on self- representation and how the fashion industry gets affected by this. The COVID-19 pandemic has affected our daily lives. The understanding and psychology of people towards fashion is changing and will continue to change in the post COVID period. This change in the attitude/ behaviour of people i.e. consumers is affecting their preferences too. This study addresses the current flux in the industry, giving an insight behind the same.

REVIEW OF LITERATURE

Due to the unprecedented times as of today, industries around the globe has taken a hit and fashion industry is not an exception. Be it in Asia, Europe or America, the situation is the same, the busy catwalk shows to shopping for the latest fashion items, COVID-19 stands as a hindrance for the fashion industry. The big picture aside, the people on the workbench i.e. the fashion designers have to stand firm. Speaking about the designers Ravi Bajaj (Fashion Designer) (2020) states, "The picture is looking bleak! Currently, most of the Indian fashion designers rely on occasion wear. During the lockdown, nearly every person was in the comfort of his/her home. Due to this sudden shift in lifestyle, thus indirectly affecting the consumer behaviour, demand for a certain category in the industry skyrocketed. Nick Harper (Journalist) (2020) in his report for CNA states, "From crowded catwalk shows to shopping for the latest luxury items, COVID-19 is cutting into the fashion industry. High Street shopping is a thing of the past as the virus drives business online. Active wear brands like Lululemon and Nike are expected to perform well as people stuck at home choose casual or exercise ready clothes over office attire." Elaborating on the consumer behaviour Prof. Thomai Serdari (London Business Marketing, New York Stern Business School) (2020) says, "It's usually a major force that comes unexpectedly but actually gives the final push in something that perhaps was an innate desire for the population, to feel more comfortable, to behave in a different way. And with people needing less variety of clothes at home, I hope the virus will lead to a rethink on cheap mass produced fashion."

ANALYSIS OF RESPONSES

The clothing preferences of people due to the covid-19 pandemic have been determined by the use of the questionnaire by adopting mainly the scaling technique. The population that we chose is the Indian middleclass youth. This selected section of the population is mostly studying or working and thus contributing most to the overall of the industry. For the study, simple random sampling was used. Out of the total respondents, 46.1% of the respondents usual clothing preferences have changed due to the pandemic, 53.9% of the respondents clothing preferences have not changed due to the pandemic. Out of the total respondents, 13.9% choose online shopping, 18.4% choose offline shopping, 40.4% choose both but mostly offline, 27.3% choose both but mostly online. Out of the total respondents, 31.3% considered comfort as a factor while shopping clothes before the pandemic, 7.8% considered design as a factor, 8.2% consider price as a factor, 3.3% considered brand as a factor, 15.9% considered price, brand, design and comfort as factors. 33.5% considered multiple factors while shopping for clothes.

Findings

A good amount of 30.0% of people said that they don't shop very frequently, instead choosing to shop on special occasions. About 49.4% of people are neutral towards their approach of shopping of the latest trends. A whopping 71.4% of people shopped according to their personal choices prior to the pandemic. Comfort is a big factor that is

taken into consideration while buying clothes: 34.3% people shop according to the comfort of the clothes. About 56.3% of the people prefer casual clothing for their online class/meetings. A good amount of 44.1% of people are going completely casual in their current clothing attire. Majority of the people i.e. 53.9%, believe their attire has not changed due to the pandemic.40.4% of people prefer both, online and offline methods of clothes shopping however leaning more towards the offline mode.47.3% of the people say that they have adapted to the new normal while 37.1% are neutral about the adaption. From this it is clearly understood that the shift happens mainly due to the change in the economy. The lock down and the various changes that happened forced the people to shift the clothing preferences of the respondents.

CONCLUSION

Due to current unprecedented times, major elements of people's lives are facing some big changes and their clothing preferences could be one of it. The major factor of this shift in preference is the lockdown; thus a detail study of the situation is of utmost importance. The gist of this report provides whether really this shift is happening or not, when concerned with the selected population, the tested population mostly being young adult students. As the sample of the population mostly consist of students, their psychological thinking plays a major part in the selection of clothes. In the analysis of data done from the questionnaire, most people chose comfort and personal choices as the criteria for the selection of their clothes, and comfort being a key factor between for the preference between the formal and casual wear, and can see a rather temporary shift of the people from formal wear to casual wear. Even though there being slight change in the preference of clothes for work or school, this shift is not there to stay permanently. People surely are comfortable with the casual approach of clothing for their online sessions, they'll have to go back to the dress code once things go back to normal. Even though short lived, the shift towards the casual wear surely makes us think about how perceive comfort and in the crux of things, and how can integrate comfort with proper dress codes.

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Table No: 01

Does the clothing preferences changed due to the pandemic

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	132	53.9	53.9	53.9
	Yes	113	46.1	46.1	100.0
	Total	245	100.0	100.0	

[Source: primary data]

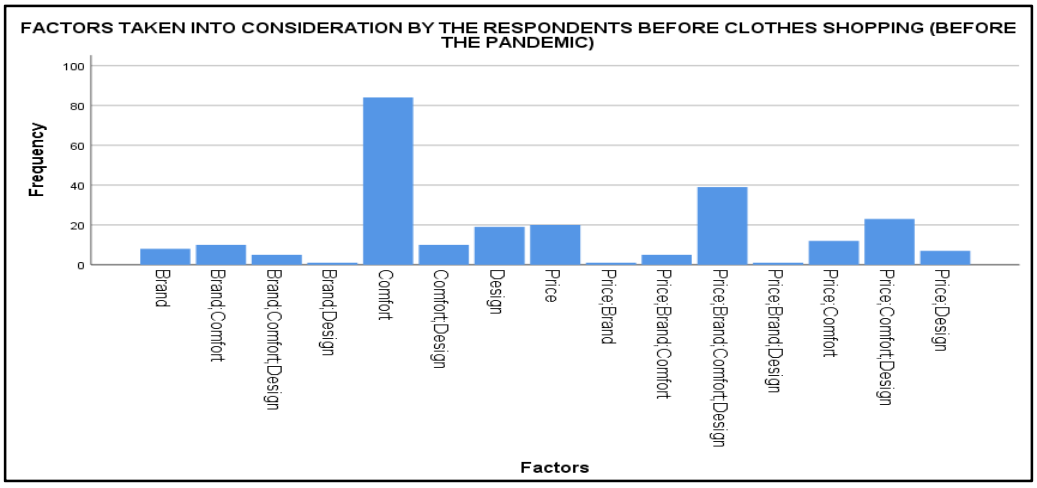
Table No: 02

Preferred mode of shopping for the respondents in the post COVID period

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Both but mostly Offline	99	40.4	40.4	40.4
	Both but mostly Online	67	27.3	27.3	67.8
	Offline Shopping	45	18.4	18.4	86.1
	Online Shopping	34	13.9	13.9	100.0
	Total	245	100.0	100.0	

[Source: primary data]

Chart No: 01



[Source: primary data]

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Waste Management System Evaluation in Dairy Farms of Kerala

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Received: 20 Jan 2021 Revised: 23 Jan 2021 Accepted: 25 Jan 2021

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ABSTRACT

The present study on "Waste Management System Evaluation in Dairy Farms of Kerala" was conducted to assess the usefulness of the different waste management methods adopted in dairy farms of Kerala. Forty five dairy farms were identified and visited. Data regarding general outlay of the farms, management practices in the farm, livestock details and existing waste management methods in the farms were collected and studied. The dairy farms under study were classified based on the animal holding capacity as those with less than six animals (class 1), 6-20 animals (class 2), 21-50 animals (class 3),and 51-100 animals (class 4), and above 100 animals (class 5). Among the 45 farms under study, four percent belonged to class 1, forty nine percent belonged to class 2, thirty five percent belonged to class 3, six percent came under the classes 4 and 5. Waste management was not commercialized in the farms. Majority farms had dung pit as waste disposal method and there was no regularity in dung removal from pit, as it was done upon demand. Compost units and biogas plants could become the most efficient waste management system in a dairy farm. The situation demands inputs from the local, state and central governments and the research bodies to popularize and incentivize them by way of technical and financial support. Value addition of dung has the potential to spawn a new genre of successful startups who can prove that waste is wealth.

Keywords: Dung pit, Compost, Biogas, Waste management

INTRODUCTION

Waste can be defined as unnecessary, unusable commodity at a given place, at a given time. The same substance became a usable commodity or a product, when properly managed and at a different place or different time (Singh *et al*., 2002)[1]. So the term waste is a misnomer, because ultimately it is a usable commodity or livestock product which must be utilized carefully and productively. Livestock waste management is important for the economic survival of an enterprise. The large quantity of manure generated when properly handled and utilized becomes an asset. Environmental issues relating to livestock farming are now a days increasing and create a bottleneck in establishment and running of animal farms. Since, livestock farming enterprises have great potential in employment generation, food security and sustainable development, it is highly essential to formulate strategies for designing environment friendly livestock production system. Presently commercial dairy farms are not following a pattern of waste management system. The pollution caused by different farms varies due to their difference in waste management system followed. So there is an emerging need for suggesting a cost efficient system for waste management in commercial dairy farms.

MATERIALS AND METHODS

Visits were made to the farms under study and details taken, regarding suitability of existing waste disposal method, different aspects of waste management like frequency of waste removal, separation of liquid and solid waste and quantity of waste generated in the farm. In the farms under study where manure pit was used as existing waste management method, the pits were classified as Earthen/ Concrete/ All nutt's manure pit. The distance of the pit from the farm, whether it was covered or not and frequency of waste removal from the manure pit were also recorded. In the farms under study where compost was used as existing waste management method the composting systems were classified as Trench/Raised. The measurement of the unit size and frequency of waste removal from the compost unit were also recorded. The farms where biogas unit was used as existing waste management method, the biogas units were classified based on type of the biogas unit that is Dome/ Drum. The measurement of the unit size (in m^3) and presence of slurry tank associated with unit as mode of utilization of slurry were studied. The farms using two or more waste disposal methods were classified as compost – manure pit method, manure pit-land fill, biogas-compost, and combination of manure pit, biogas and compost method. Based on the overall findings on waste management system followed the farms under study were randomly grouped into four groups that is group I (farms with manure pit as waste management method-conventional), group II (farms with compost units as waste management) group III (farms with biogas as waste management method) and group IV (farms with a combination of different waste management methods).

RESULTS AND DISCUSSION

The details of the classification of dairy farms are presented in Table 1 and the per cent of farms available in each class. Among the forty five farms under study, four per cent belonged to class 1, forty nine per cent belonged to class 2, thirty six per cent belonged to class 3, seven per cent in class 4 and four per cent in class 5 respectively. (Table 1) N=45

The dairy farms under study were classified based on the animal holding capacity. Among the 45 farms under study, four percent belonged to class 1, forty nine percent belonged to class 2, thirty five belonged to class 3, six percent farms came under the classes 4 and 5.(Table 1)The highest percent of farms came under the class 2. This finding is in accordance with the data furnished by *Farms/ Farmers According to Livestock's Data (2003)*[2] where it states that the average number of animal holding in dairy farms in India is around 17. The number of farms with more than hundred animals are three (seven percent) among the farms under study. These findings indicate that

there is a transitional change from the traditional small holder dairy units with less than five animals to medium or large scale units demanding new strategies for management and waste disposal. The different waste management systems adopted in various farms in each class are presented in Table 2 .The major existing waste management method adopted by commercial dairy farms is manure pit. Forty per cent of the farms had manure pit alone as the waste disposal method whereas eleven per cent of the total farms had biogas as the waste disposal method. The rest forty nine per cent of the farms had combined waste management methods. From the table 2 it is clear that the different types of waste management methods adopted in commercial dairy farms are manure pit, compost, biogas and land fill. Senthil kumar *et al*. (2008)[3] also reported the different waste management methods associated with commercial dairy farms as manure pit, compost, biogas and land fill. The highest percent of farms adopted conventional manure Pits. (Forty percent) This indicates that even though intensification occurred in cattle rearing, there is lack of scientific knowledge in the area of waste management. Linton, (1952)[4] observed the common practice of depositing the manure in a dump immediately outside the buildings.

From the Table 3 it is clear that eighty five percent of farms practiced three times removal of dung from the animal shed. In four percent of farms the frequency is only two per day where as in rest eleven percent, it was more than three per day. Linton (1952) [4] observed that the collection of solid manure in animal habitations under ordinary management is usually carried out once or twice daily. Sastry and Thomas (2008)[5] also stated under ideal management conditions solid manure is usually collected and removed from shed twice daily. The increase in the removal frequency is associated with the increase in the milking frequency. Usually dung removal is done in the farm just before milking. Most of the dairy farms were used wheelbarrow for collection and removal of dung from shed. Linton (1952) also observed that the practice of using wheelbarrow or similar vehicle for dung removal as and when the same accumulates. From the Table 3 that liquid separation facility in the waste management system existed only in eleven per cent of total farms. The rest eighty nine per cent farms had no facility to separate solid manure from liquid manure which consisted of urine voided and wash water from sheds. Sastry and Thomas (2008)[5] reported about the practice of direct application of liquid manure to fields of fodder grasses or can be fed as a slurry to bio-gas plants. They exemplified that in Arey Milk Colony, Bombay, fodder grasses are being cultivated economically by irrigating them with wash water from cattle sheds. In Kerala the intensification in the field of dairy sector is an emerging one adopting the conventional system of liquid waste treatment along with solid manure. The lack of separation of liquid manure consisting of urine and shed wash from solid waste leads to increased volume of waste. Hence a judicious separation of solids and liquid waste is essential for keeping high hygienic status.

Table 3 indicates the frequency of waste removal and facility for separating liquid and solid waste including fodder waste. Only eleven per cent of the farms under study had a separation facility. In most of the farms the removal of dung from the shed is mainly just before milking. Regarding the frequency of dung removal, eighty five per cent of farms practiced three times milking, and three times removal of dung. Four per cent of farms had a frequency of two per day where as in rest eleven per cent it was more than three times a day. The details of manure pits based on the type, distance of the pit from the farm, covered or not and frequency of the dung removal from the pit in different farms are presented in Table 4.The main type of manure pits in commercial dairy farms in Kerala is of concrete type (84.61 per cent). Rest was earthen type. In all the farms belonging to class 1 category the distance of the pit from the farm was less than five meters. In class four and five it was placed beyond five meters from the farm. Among class 2 farms, 25.64per cent of farms had their manure pits within a range of 5meter from the farm where as in class 3 the percentage of farms in the same group was only 12.82. In the rest of the farms in both groups the manure pit was located more than 5 meters from the farm.

Covered manure pit was observed in 74.35% of the farms under study had. More than half of the farms (69.23per cent) had no regular dung removal, and it was carried upon demand. But 30.76 per cent of the farms showed regularity in the removal of dung. Among this, 23.07 per cent of farms were removing the dung from the pit once in six months where as the remaining 7.69 per cent were practicing this twice in a year. From the Table 4it is clear that eighty four per cent of manure pits are concrete .Rest sixteen per cent was earthen type. In no farms there was an Allnutt's type of manure pit described by Linton (1952) [4] and Sastry and Thomas (2008)[5]. This finding reveals that

there was no scientific managemental practices like Allnutt's pit adopted by commercial dairy farms in Kerala. It is clear thatin all the farms belonging to class 1 category the distance of the pit from the farm is less than five meters. In class four and five it was placed beyond five meters from the farm. Among class 2 farms, 55.56 per cent of farms had their manure pits within a range of 5m. from the farm where as in class 3 the percentage of farms in the same group was only 33.33. In the rest of the farms in both groups the manure pit was located more than 5 meters from the farm. It is recommended in the Draft Proposal for Waste Disposal in Commercial Dairy farms of ministerial level conference (2006) the manure pit must be located at least 25 meter away from dwelling. It is also noted that out of forty five studied, 10 farms had no covering for manure pits. The lack of cover leads to accumulation of water during rainy season. From Table 4.4, it can be seen that more than half of the farms (71%) had no regularity in dung removal from the manure pit , and it was carried upon demand. Twenty nine per cent of the farms showed regularity in the removal of dung. Among this, twenty one per cent of farms were removing the dung from the pit once in six months whereas the remaining eight per cent were practicing this twice in a year. The above said ministerial level conference (2006) recommended the waste should not be allowed to accumulate in the pit, in order to avoid pollution issues. The waste disposals in dairy farms were carried out upon demand only. So this may sometimes lead to accumulation in the farm, if there is less demand for cow dung especially in rainy season. There should be a regular outlet for cow dung.

Compost units and biogas plants could become the most efficient waste management system in a dairy farm. The situation demands inputs from the local, state and central governments and the research bodies to popularize and incentivize them by way of technical and financial support. It will be a justifiable social investment on many counts. First of all, it can minimize the much demonized methane emissions from the dairy sector. Biogas plants can cater to the domestic energy needs, and can thus slow down the mounting pressure on the depleting fossil fuels. Slurry from the biogas plant is a good organic manure which does not burn the plants with methane, like the natural dung. Proper waste management can ensure that the dairy farm in the vicinity will no longer be anunpopular venture that cause pollution of the elements, as it as it used to be. Value addition of dung through composting, has the potential to spawn a new genre of successful startups who can prove that waste is wealth.

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Table 1 : Classification of farms based on the animal holding capacity.

Category	Animal holding capacity	Number of farms	Percent
Class 1	below 6	2	4.44
Class 2	6-20	22	48.88
Class 3	21-50	16	35.55
Class 4	51-100	3	6.66
Class 5	>100	2	4.44

Table 2. Classification of farms based on waste management systems.

Farm category	Manure pit	Compost	Biogas	Combination
Class 1	2	_	_	-
Class 2	7	_	4	11
Class 3	9	_	1	6
Class 4	_	_	_	3
Class 5	_	_	_	2
Total	18 (40)		5 (11)	22 (49)

Table 3. Frequency of waste removal and separation of solid and liquid waste

Sl No	Frequency of waste removal from the farm in a day			Separation of liquid and solid waste including fodder waste	
	2 times	3 times	>3 times	Yes	No
Class 1	-	-	2	0	2
Class 2	-	20	2	2	20
Class 3	2	13	1	1	15
Class 4	-	3	-	1	2
Class 5	-	2	-	1	1
Total	2 (4.44)	38 (84.44)	5 (11.11)	5 (11.11)	40 (88.89)

Table 4. Details of manure pits in the farms

Class of farm	Type of manure pit			Distance of the pit from the farm		Covered		Frequency of the dung removal from the pit		
	Concrete	Earthen	Allnutt's manure pit	< 5 m	>5m	Yes	No	Once in 6 months or below	Within 6-12 months	Not regular
Class 1	1	1	-	2	-	1	1	-	-	2
Class 2	14	4	-	10(25.64)	8	12	6	2	2	14
Class 3	14	1	-	5(12.82)	10	12	3	3	1	11
Class 4	2	-	-	-	2	2	-	2	-	-
Class 5	2	-	-	-	2	2	-	2	-	-
Total	33 (84.61)	6 (15.38)		17	22	29 (74.35)	10 (25.64)	9 (23.07)	3 (7.69)	27 (69.23)

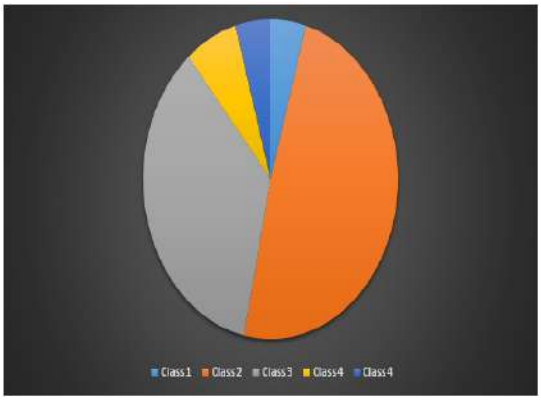

Figure 1 Classification of farms based on animal holding capacity

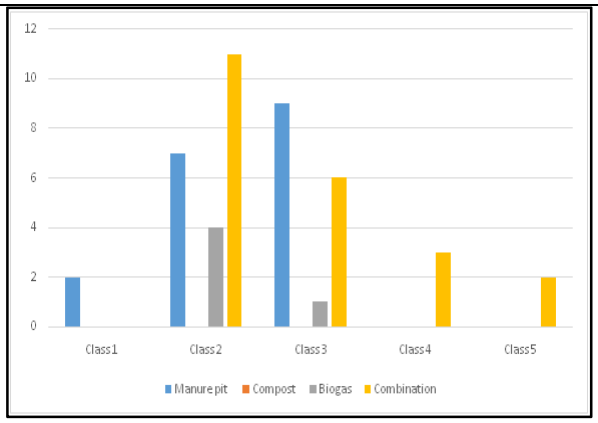

Figure 2 classification of farms based on waste management systems

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Embedding *Luffa acutangula* in the Biotic Cleanser

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Received: 04 Mar 2021 Revised: 05 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

Cleansers are essential toiletries that ensure healthy hygiene. The microbeads are used for exfoliation processes in the cosmetology and are small in size like 0.5mm, which leads to marine contamination. *Luffa acutangula* L., as an alternate source for the microbeads. The present investigation is to prepare the scrub by embedding the *L. acutangula*, with *Aloe vera* and Fuller's Earth and to determine the pH, moisture content, total fat molecules, alkali content, foam formation. The pH of the sample scrub that has been prepared is about 9 and percentage of TFM near grade 2. The foaming potential was valued to be more or less equal to other scrubs as per BIS. The results compared with the data from the BIS and it can be concluded that the values determined are within the limits set by BIS so, it could be used as an alternative for the products that contain microbeads.

Keywords: Cleanser, Exfoliation, BIS

INTRODUCTION

Cleansers are boiled version of sodium salts of fatty acids is certainly one of the most essential toiletries that ensure healthy hygiene. Not only does it remove dirt from the grimy body, and claims to keep bacteria at bay, its fragrant properties helps one feel fresher, cleaner and better. Although the first recorded evidence of the manufacturing of soap-like materials dates back to around 2800 BC in ancient Babylon, commercial soap production began in England around the end of the 12th century. There, because of the heavy taxes, soap remained an expensive item until 1853, when the tax was repealed. In the 19th century, soap gained popularity throughout Europe and across British-occupied regions.Today, India, more than 700 registered companies manufacture bar/cake and liquid soaps of various kinds with combined annual revenue of over Rs 1,700 corers. These soaps were embedded with plastic micro beads to enhance its potential of cleansing activity (Anonymous, 2016).

Plastics have a high rate of benefits and efficiency across the people living in the society. As a result of this the use of plastics has increased around 20 times compared in past 50 years. Some plastic micro beads are manufactured in purpose for or in the use of cosmetics. Micro beads reach and persist in the environment in large numbers because they are in products which are designed to be 'rinsed-off' and flushed down the drain. They are not captured by most wastewater treatment systems. In 2015 United Kingdom (Napper *et al*., 2015) estimated that between 4594 and 94,500 microbeads can reach the environment per use of just one facial scrub containing microbeads. In 2013 a study sampled for microbeads in the Laurentian Great Lakes, USA found that microbeads were present very high in number. It was estimated approximately 43,000 microbeads particles/ km^2, with one sampling location downstream from a major city containing 466,000 particles/km^2. In 2015, United Kingdom scientific study of facial scrubs found that the tested products could contain between 137,000 to 2,800,000 microbeads per bottle (Eriksen *et al.,* 2013).

Every year many whales are found dead in these oceanic areas because of the consumption of these microbeads and then these get cloated in their digestive systems. In 2013, a grey whale was found dead and examined due to which that it had consumed about 34 pounds of plastic in its stomach. These products consist of polyethylene, polypropylene, polysterene petrochemical products, polyethylene terephthalate, polymethlyl methacrylate (Fendell *et al*., 2009). They are found in products like Bioré, Deep Clean, Colgate max clean, Clearasil, Clean and clear etc., These microbeads are used for exfoliation processes in the cosmetology. Exfoliation is nothing but the removal dead skin from the skin's outermost surface. Plastic microbeads are very small in size like 0.5mm. Waste water treatment plants are designed to filter only human waste but they could not filter these plastic microbeads as because of their size (James *et al*., 2017). The marine species are not able to distinguish between their usual food and these microbeads. Marine species have been shown to uptake these particles, potentially introducing toxins to the base of the food chain. Microbeads have the potential to transfer up the food chain, which may lead to consumption by humans at the end. Humans may consume these microbeads either by consumption of fishes or by uptake of commercial salts (Ali karami *et al*., 2017). Micro plastics were taken up via the gills, and ingested into the stomach (Cole *et al.,* 2011). From there, they were taken up into cells, and translocated into the circulatory system. The impact of micro plastics on the marine environment has received significant attention from regulators in various parts of the world.

So, for this reason we could opt for the traditional methodology of using the *Luffa acutangula* Linn., commonly known as ridge gourd as an alternate for the microbeads. It belongs to the family Curcubitaceae (Brindha *et al*., 2016). The entire plant of *Luffaacutangula* is medicinally important and is used extensively in Indian traditional system of medicines. From Ayurvedic point of view, ridge gourd increases vata (the impulse principle necessary to mobilize the function of the nervous system) and kapha (the body fluid principle which relates to mucous, lubrication and the carrier of nutrients into the arterial system) and also it cools down and pacifies the dosha pitta (the energy principle which uses bile to direct digestion and hence metabolism into the venous system) in the body (Manikandaselvi *et al.,* 2016). Thoroughly ripened *L.acutangula* when its skin is removed, and divested of the seeds, is left with the skeleton of xylem fibers. It is this loofah with its network of fibers that becomes spongy when soaked in water. The loofah, in its spongy form, has been used for scrubbing and/or cleansing the skin during bathing or showering, making it an excellent natural exfoliating agent. Ridge gourd is allowed to dry and mature on the vine and it can be harvested as a sponge. This sponge has been used traditionally from ancestral period. They are considered to be useful in removing dead cells from the skin thus making the skin smooth and conditioned. The blood purifying properties of ridge gourd are helpful against pimples and acne problems. Loofah sponge is also effective in fighting off foot and body odour. Juice of ridge gourd mixed with other healthy vegetables taken daily helps in strengthening the immune system and helps the body in fighting against infections effectively. Ridge gourd loofah is a very good alternative that could be used as a replacement for the microplastics (Ananthan *et al.,* 2012).These ridge gourd loofah are grinded and then made to a corous powder which are used as an alternate for the microbeads that are present in the cosmetics and cleansers.

MATERIALS AND METHODS

Preparation of the Soap (Phansteil, 1998)

Here we use two types of oils and a base solution for the preparation of the soap. Taken 150 ml of olive oil and 150 ml of coconut oil and then measured 72 ml of NaOH solution and kept aside. This oils and the base solution are slowly heated to 32^{o}C and then both the oils and the NaOH solution were slowly mixed. The base solution is poured along the sides of the beaker to avoid bubble formation. The solutions were mixed using a blender. A thick aqueous solution is formed. At this stage the finely grinded *Luffa acutangula* fibers has been embedded and essential flavours like honey or cardoman flovours were added to it. Along with these Bentonite Clay (Multanimitti), *Aloe vera* gel was also added. This mixture was poured onto moulds and left for 24 hours for hardening purpose.

Analysis of pH (Viorica, 2012)

5 grams of soap sample has been taken and then completely dissolved in 100 ml of distilled water. This solution was tested with pH paper as well as pH meter. The colour change was observed in the pH paper. The reading was noted that was shown in pH meter.

Test for Moisture Content: (Viorica, 2012)

Firstly the china dish were washed and then dried in hot air oven and then cooled. Then 5 grams of test soap was taken and then placed in the dish and then weighed. Initial weight was noted. Then it has been dried for half an hour in hot air oven and then the weight was measured. The loss in weight and percentage of moisture was calculated by:
% moisture = mass of water ÷ dry mass of sample ×100

Determination of Total Fatty Molecules in soaps (Betsy, 2013)

5grams of soap sample was dissolved in 100ml hot water. About 40ml of 0.5N HNO_3 was added to make it acidic. The mixture is heated until fatty acids are floating as a layer above the solution. It was then cooled in ice water for the solidification of the fatty acids. The fatty acids were separated and the aqueous solution was treated with 50ml chloroform to remove the remaining fatty acids. The separated fatty matter was mixed together, solvent was evaporated. The total fatty matter present was calculated by the formula below:
Percentage of fatty matter = (y – x) × 100 ÷ weight of sample

Determination of Total Alkali Content (Betsy 2013)

5gm of soap sample was dissolved in 100ml hot distilled water. About 40ml of 0.5N HNO_3 is added to make the solution acidic. The mixture was heated until fatty acids found floating as a layer above the solution. It is cooled in ice water for the solidification of fatty acids. The fatty acids were separated and the aqueous solution was treated with 50ml chloroform to remove the remaining fatty acids. The aqueous solution was measured and 10ml of it was titrated against 0.5N NaOH using methyl orange as an indicator. The colour change from yellow to pink colour indicates the end point. The titration value was noted and then with that the alkali content present was calculated by the formula:
Total volume of the aqueous solution = V ml
10 ml of aqueous solution required t ml of NaOH
V ml of aqueous solution requires = V*t /10 = A ml.
Amount of NaOH required by acid in aqueous solution =A ml
Volume of HNO_3 required, B ml =A x Normality of NaOH / Normality of HNO_3
Volume of HNO_3 required for neutralizing NaOH = C=40 – B
Amount of NaOH in 1000 cc of soap solution (E) = (C x 40 x Normality of HNO_3 g) /1000
250 cc of soap solution contains (F) = (E x 250) / 1000 g
2 NaOH→ Na_2O + H_2O
80 gram of NaOH gives 62 g of Na_2O

F g of NaOH requires (Y) = (62 x F) / (80) g of Na_2O
Weight of soap taken = 5 g
% of alkalinity = (Y x 100) / w

Test for foam formation (Viorica, 2012)

The sample soap was taken and then washed in running water to check the formation of lather. This was done to verify the amount of time take by the soap for the formation of foam. These methodologies that were assayed for the prepared soap was given by the Bureau of Indian Standardsunder chemical section.The prepared sample soap was tested to satisfy the standards as prescribed by the BIS.

RESULTS

The usage of *Luffa acutangula* in the cleanser that has been prepared in the laboratory has satisfied the regulations of the Bureau of Indian Standards.The results were compared with those of other soaps that have been in markets at present.

There are different grades and percentage that the Bureau of Indian Standardscomprises. All these percentages have to be satisfied by the product that has been prepared.

Prepared Soap

Figure 1 shows **t**he soap has been prepared in the laboratory under sterile condition by using olive oil, coconut oil, *Aloe vera* gel, Mulltanimitti and embedding *Luffa acutangula*

pH Analysis

The pH of the sample soap that has been prepared is about 9. This pH of 9 indicates that the soap is basic in nature. According to the standards of BIS pH 8 to pH 10 of soaps are acceptable.

The table 5.1 shows the comparitive results between the commercial soaps and the sample soaps. The pH of the test soap has been around the grade 2 soaps, according to the BIS.

Moisture Content

The moisture content of the soap was estimated.

Initial weight of sample is 5 grams. Final weight of sample is 4.43 grams. The loss in mass of the 0.57 grams. By the formula,

% moisture = mass of water ÷ dry mass of sample × 100
% moisture = 0.57 ÷ 4.43 × 100 = 12.86 %

There is no specific requirement of moisture content as per the national standard. It should not be too high or too low. Here when compared to the Dove and Nivea product, the moisture content is higher, but when analyzed with Jhonson, Dermafex products the moisture content is low.

Determination of TFM

The most important factor to be considered in soap quality is its total fatty matter (TFM). Higher the TFM quantity in the soap, better is its quality. As per BIS, Grade 1 soaps should have 76% minimum TFM, while Grade 2 and Grade 3 must have 70% and 60% minimum TFM, respectively. The TFM level of the sample soap was analyzed:

% of TFM = (y – x) ×100 ÷ weight of sample
% of TFM = 31.67- 27.67 × 100 ÷ 5
% of TFM = 69.6

The sample soap that has been prepared shows a percentage of TFM near grade 2 soaps as per the norms of the BIS as prescribed. The TFM percentage is lower when compared to that of Dove, Pears and Jhonson's baby soap. The percentage of TFM recorded is slightly higher than that of Lux soap product.

Determination of Total Alkali content

Standard requirement of alkali content as prescribed is 3 per cent maximum. The free alkali in soap is usually made of hydroxide of sodium or potassium. Alkalinity may also be due to the presence of sodium silicate or other alkaline compounds that are sometimes added in the soap.

Total volume of the aqueous solution = V = 100 ml

10 ml of aqueous solution required 0.5 ml of NaOH

100 ml of aqueous solution requires = 100×0.5 /10 = 5 ml.

Amount of NaOH required by acid in aqueous solution =A= 5 ml

Volume of HNO_3 required, B ml =5 ×0.1 /0.5 = 1 ml

Volume of HNO_3 required for neutralizing NaOH = C=40 – 1= 39ml

Amount of NaOH in 1000 cc of soap solution (E) = (39 x 40 x 0.5 g) /1000= 0.78g

250 cc of soap solution contains (F) = (0.78 x 250) / 1000 g = 0.19 g

$2\ NaOH \rightarrow Na_2O + H_2O$

80 grams of NaOH gives 62 g of Na_2O

0.19 g of NaOH requires (Y) = (62 x 0.19) / (80) g of Na_2O = 0.14 g

Weight of soap taken = 5 g

% of alkalinity = (0.14 x 100) / 5= 2.8 %

The percentage of alkalinity when compared to other soaps the test sample percentage of alkalinity is higher (Table: 5.4). But this percentage is below the limits as prescribed by the BIS.

Foaming Potential

Lather is the foam or the froth created by soap when stirred in water or while bathing or washing hands. It is an important parameter for acceptability of soaps. To test the soap's ability to create lather, the soap is taken and washed in running water and the form is measured in measuring Jar. It took 7 seconds for the sample soap to make upto 103ml of foam in measuring jar. The foaming potential of the test soap was analyzed. This values were more or less equal to other soaps when compared (Table: 5.5). This was not too high or low when placed with comparison with other branded soaps. Hence, the test sample soap showed the results that has been satisfying to all the regulations that has been placed by the Bureau of Indian Standards,and it does not show any type of errors because of embedding the *Luffa acutangula.* By the preparation and usage of this soap which consist of *L. acutangula* instead of microbeads that has been present in other products, we could surely get rid of this microplastic pollution that has been found vastly in all parts of the nation. India is the country which still doesn't got affected by this microplastic pollution, but if we continue to adopt for the western culture, soon we would also get affected by this and all the aquatic regions surrounding the country will be affected. Ongoing by the words of the ancestors as prevention is better than cure, we should stop using the foreign products and has to go for Indian as well as natural products.

DISCUSSION

The pH of the Hotel soaps were higher than that of any compared soaps like, Palmolive, bubble magic, and the test sample soap. The pH of the hotel sample soaps were 10.3, whereas the pH of the prepared soap is 9, which is acceptable by the BIS.The high pH content indicated high percentage amount of unspecified and unsaponificable matter due to incomplete alkaline hydrolysis.Moisture content of the soap prepared was similar to the Dove, Nivea, Jhonson's baby soaps. There is no specific requirement of moisture content as per the national standard. It should not be too high or too low. The study indented to determine the total alkali content and total fatty matter of soaps, revealed that, the soaps which have high total fatty matter and low alkali content are having good quality. The low total fatty matter is associated with hardness and lower quality of soap and it is the most important characteristics describing the quality of soap. As per BIS, Grade 1 soaps should have 76% minimum TFM, while Grade 2 and Grade 3 must have 70% and 60% minimum (Betsy 2013). The TFM of the test cleanser is equal to the soaps of grade 2. The

higher TFM was found in Jhonson's baby soap. Among the bathing soaps that has been analyzed, it is observed that, all the soaps having alkali content in the range of 2-3% and the fatty matter content between 60-80%, shows an indication of good quality. Soap samples that have lower alkali content and higher TFM value, makes them good for health and environment. Lather is the foam or the froth created by soap when while bathing or washing hands. It is an important parameter for acceptability of soaps. All the brands had undergone the lather test. Higher scores of lather shows the good quality of the cleanser (Viorica, 2102). The sample cleanser that has been prepared has various positivity. It is suitable for all people as the pH is basic. The pH is low when compared to other hotel soaps. The moisturizing activity is also moderate, and the TFM is also in sufficient percentage that it could be also used for people who possess dry skin. The *L. acutangula* that has been embedded shows no toxicity (Anitha, 2014) neither to the people nor to the aquatic living beings. Altogether, soap prepared using *L. acutangula* hold numerous medicinal (Jyothi, 2010) value and hence it is safe for the use of public.

SUMMARY AND CONCLUSION

Microbeads are the plastic pollution found worldwide. It affects the marine ecosystem by causing hazards for the aquatic fishes living in the ecosystem. The fries and fishes couldn't identify their own food with these microplastics, and due to the consumption, it results in their fatal death. In order to replace these microbeads in the current study, *Luffa acutangula* has been used as an exfoliate in the cleanser that has been prepared. The soap prepared had undergone the tests as mentioned in the Bureau of Indian Standards, and it also elate the margins that has been prescribed in BIS. The work was carried for commercial soaps (Dove, Palmolive, lux, Fiama di wills, Nivea, Jhonson's baby soap) with the test sample soap that has been prepared. It was analzed for pH, TFM, Alkali content, Moisture content, and foaming property. A cursory look at the obtained results revealed similarities between the commercial soaps and the test cleanser. The results were compared with the data from the BIS and it can be concluded that the values determined are within the limits set by National Standards. As the test cleanser gratify the BIS, it could be used as an alternative for the products that contain microbeads.

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Table 1.Indicates the pH of test soap

Soaps	pH	BIS
Test Soap	9	The pH standard as per the Bureau of Indian Standards is from 8 to 10.
Palmolive aroma crme	9.5	
AMBR (Hotel brand)	10.3	
Clean and clear	8.2	
Bubble magic	7.2	

Table: 2. Moisture content present in the test samples

Product	Moisture %	BIS
Test soap	12.8	There is no certain percentage published by Bureau of Indian Standards for the moisture content of the soaps.
Dove	10	
Nivea	10.3	
Jhonson	16.2	
Dermafex	14	

Table: 3. Shows percentage of TFM present in samples

Soap	% of TFM	BIS
Test sample	69.6	The TFM level for grade 1 soaps are 76% to 80% as per the norms of Bureau of Indian Standards
LUX	68.2	
Jhonson's baby soap	77.6	
Pears	71.6	
Dove	73	

Table: 4. Shows percentage of alkalinity in samples

Soap	% of Alkalinity	BIS
Test sample	2.8	The percentage of alkalinity could be upto 3% as by the Bureau of Indian Standards
Lux	2.3	
Jhonson's baby soap	2.08	
Pears	1.61	
Fiama di wills	2.31	

Table: 5. Foaming potential of the soap

Soap	Foam height	BIS
Test sample	103	As per the regulations of Bureau of Indian Standards, the foaming level should not be too high or low
Dove	115	
Jhonson's baby soap	105	
Nivea men	107	
Park avenue	113	

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Antiulcer Activity of the Chloroform Extract from the Flowers of *Thespesia populnea*

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Received: 19 Jan 2021 Revised: 15 Feb 2021 Accepted: 24 Feb 2021

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ABSTRACT

Thespesia populnea (Fabaceae) is a native plant species of many Asian countries, including Malaysia and India. The root, stem, bark, and flowers of *Thespesia populnea* are used to treat various ailments, including ulcers and stomach cancer, we studied the antiulcer activity of lipid-soluble extract of *Thespesia populnea* obtained via extraction of air-dried flowers using chloroform. Anti ulcer activity of Chloroform extract was studied by pylorus ligated, aspirin induced gastric ulceration in albino rats the antiulcer activity chloroform of an extract of *Thespesia populnea* flowers was investigated by agar well diffusion method. Furthermore, our phytochemical studies indicated that the chloroform extract of *Thespesia populnea* flowers contains flavonoids, alkaloids, tannins and anthroquinone, glycosides. The anti ulcer activity was assessed by determining the ulcer index, pH and acidity. Chloroform extract (200mg/kg) produced a significant reduction in the ulcer index, PH and acidity when compared with ulcer control group and standard group. Chloroform extract reduces ulcer, there by the present study indicates that *Thespesia populnea* flowers showed Protective activity on gastric ulcer

Keywords: Anti ulcer, *Thespesia Populnea*, Chloroform Extract, GC-MS.

INTRODUCTION

Gastric ulcer, a serious gastrointestinal disorder that develops due to erosion on the inside lining of the stomach (1). Anti ulcer drugs in the treatment of gastric ulcer show usually over by various side effects. Medicinal plants have the main source of new drug candidates for the treatment of gastric ulcers. The medicines are considered safer because of the natural ingredients with now or less side effects. *Thespesia populnea* is an endemic plant that has been suggested as useful in the treatment of various diseases such as anti-diabetic (2) Herbal medicines, due to cheaper

accessibility, fewer or no side effects, and perceived effectiveness, are unfolding as substitute treatments to available synthetic drugs (3). *Thespesia populnea* (Malvaceae) is a large tree found in tropical regions and coastal forests of India and Tamilnadu. The different part of this plant was used in the treatment of cutaneous infections such as scabies, psoriasis, eczema, ringworm, guinea worm and inflammation (4). It is useful in dysentery, piles, diabetes, hemorrhoids. It cures ulcers, itching; scabies and other skin diseases and urinary disorders bark are astringent given internally as an alternative. In the form of hot poultice leaves are beneficial in painful joints. Fruits, leaves are applied externally to scabies, psoriasis, and other skin diseases. Parts is used has mainly been Root, leaves, flowers, fruits, bark [5]. Quantitative analysis of the complex mixtures of flavonoids encountered in Chloroform extract has been based on the amounts of pure compounds that have been isolated. Correlation of anti ulcer activity with the proportions of flavonoids present in crude preparations made an accurate. We report here a gas chromatography (GC) method that will quantify and identify almost all the compounds named, particularly the Antiulcer activity, If further confirmation is needed, mass spectrometry (MS) of the GC effluents Verifies identifications made from retention data.

MATERIALS AND METHODS

Plant material

The Flowers of *Thespesia populnea* were collected from the of the cuddalore region (Dist) Tamilnadu, India. The plant was identified the voucher specimen (SC 5/23) was deposited in herbarium the authenticated by Dr. N. Ramakrishnan assistant professor, department of botany, Govt arts college (Auto) Kumbakonam. The flowers of the plant were stored in the herbarium at the college for further reference.

Preparation of extract

The Flowers of *Thespesia Populnea* were collected, washed, cleaned and shade dried. The dried flowers were powdered with the help of mechanical mixer and passed through a 40 –mesh sieve to obtain coarse powder. The weighed quantity of coarse powdered material was extracted with methanol by using soxhlet apparatus. After completion of extraction, it was undergone a distillation under reduced pressure and the remaining solvent was removed by evaporation to dryness on a water bath. The Residue was obtained and it was kept in a desiccator and used for further experimentation (6). After filtration, the clear solution was consecutively partitioned with petroleum ether, chloroform and ethyl acetate. *Thespesia Populnea* flowers were reported on the identification chloroform crude extract was prepared by soaking a sample (50 g) of powdered flower material in 90% methanol (300 ml) for 72 h. The percentage yield from 50gm was found to be 1.98%.

Phytochemical screening

The presence of phytochemical constituents in the Chloroform extract was tested by using the standard methods (7).These standard methods revealed the presences of glycosides, flavanoids, steroids, alkaloids.

Spectroscopic methods

GC-MS analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and the components were separated using Helium as the carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1μL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 230 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library. The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Identification of Compounds

Interpretation of mass spectra of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials was as certained.

Animal

Albino rats (150-200g) of either sex were used for the experiment (8). Dr. Balasundram Department of Zoology , Annai college of arts & science, Bharathidasan University, Kumbakonam, Tamilnadu, India They were kept in the animal house in a controlled room temperature at 25+2 c, relative humidity 44-56%, light and dark cycles of 10 and 14 hr, respectively for 1 week before the experiment. The animals were grouped and housed in polyacrylic cages for the further experiment.

Extract

The Chloroform extract was dissolved in 1 % tween 80 as a vehicle and administered P.O in a dose of 200 mg/kg and 300mg/kg. The ranitidine was dissolved in 1% tween 80 and Administered i.p in a dose of 25mg/kg.

Acute Toxicity studies

The Acute Toxicity studies were performed in order to establish the therapeutic index of a test drug. The experiment was conducted according to the OECD, 423 guidelines. It was administered as 5,100, 1000 and 3000 mg/kg.

Assessment of Anti-Ulcer Activity

Rats were divided in to 4 groups. Each group contains 6 rats and treated with the following drug for five successive days and 6th day aspirin was administered and to evaluate the antiulcer activity.

Group 1: Control - Normal saline (1ml/kg)
Group 2: Ranitidine -25mg/kg (standard control)
Group 3: Chloroform extract -200mg/kg
Group 4: Chloroform extract -300mg/kg.

Aspirin induced ulcer method

Aspirin at a dose of 200mg/kg (20mg/ml suspension in 1%CMC (Carboxyl methyl cellulose)) was administered orally to 18hr fasted animals. After 1hr the test drug was administered on the 6th day. The ulcers were scored after 4hrs. The stomach was taken out and cut open along the greater curvature and ulcers were scored by a person in the glandular portion of the stomach. The ulcer index was calculated by adding the total number of ulcers per stomach and the total severity of ulcer per stomach (9).

Pylorus ligation induced ulcer method

This is the most acceptable method. Rats were divided into 4 groups. Each group contains 6 rats. Each weighing about 150-200gm and fasted for 4hr with free access to water. Pylorus ligation induced ulcer method was performed under diethyl ether anesthesia to each animal. Animals were given to chloroform extract of 200mg/kg and 300mg/kg B.w. ranitidine was prepared in 1% tween 80 suspension as a vehicle orally immediately after pylorus ligation. Animals were sacrificed 6hrs later the stomach was carefully removed and gastric contents were collected. The gastric juice was centrifuged at 3000 rpm for 30 mins and then the volume of gastric juice was measured. Acidity in the supernant was determined by titration with0.01N NaoH and expressed as m.eq/l. the stomach was cut open along the greature curvature and pinned on a soft board for evaluating gastric ulcers and ulcer index is calculated using formula:

The GC - MS chromatogram of the Chloroform Flower extract of *Thespesia populnea* showed 14 major peaks indicating the presence of eighteen compounds (Fig.1). The active principles with their peak, retention time (RT), and molecular

weight are presented in the Table 2. The 2-Isopropyl-5-Methylcyclohexyl 3-(1-(4-Chlorophenyl)-3-Oxobutyl)-C, 1-Pentacontanol, 2(3h)-Furanone 3-(15-Hexadecynylidene) Dihydro-4-Hydroxy-5-Methyl-, Cyclopentane (4-Octyldodecyl)-, Octadecanal 2-Bromo-, Octadecane 3-Ethyl-5-(2-Ethylbutyl), Heptacosane, 1-Chloro, Heptacosane 1-Hexyl-2-Nitrocyclohexane, 2,7-Octadiene-1,6-Diol, 2,6-Dimethyl- (Z)-, Cis-9,10-Epoxyoctadecan-1-Ol The GC-MS identified compound shows various pharmacological activities .

DISCUSSION

GCMS is one of the most precise methods to identify various secondary metabolites present in the plant extract The Chloroform Flower extract of *Thespesia populnea* was analyzed by GCMS to detect various compounds with the help of NIST library. The GC-MS analysis revealed The 14 chemical compounds. Cis-9,10-Epoxyoctadecan-1-Ol (29.75 RT) is the highest retention Time chemical compound and 2-Isopropyl-5-Methylcyclohexyl 3-(1-(4-Chlorophenyl)-3-Oxobutyl)-C, 1-Pentacontanol (18.3) as the lowest retention time chemical compound. Similarly, Octadecanal, 2bromo also showed the various biological activities as reported for *Mollugo pentaphylla* Linn [10]. However, isolation and characterization of individual phytochemical constituents may proceed to discover the novel drugs and their pharmacological activities. 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. The Causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the Stomach to the accumulating acid [11]. Pylorus ligation induced ulcer was used to study the effect of flower 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 An increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 36 hours, followed by ligation of the pyloric end of the stomach. The ulcer index is determined 5 hours after pylorus ligation. The lesions produced by This method is located in the lumen region of the stomach (12).From the above results, we observed that the Chloroform extract of flowers *Thespesia populnea* provides significant anti ulcer activity by pyloric ligation induced method & aspirin induced methods against gastric ulcers in rats.

RESULTS AND DISCUSSION

Thespesia populnea flower chloroform extract was studied for its antiulcer activity against Aspirin induced ulcer in rats and the results were shown in Table 1. A produce Gastric lesion in the glandular portion of the stomach. The rats treated with reference standard Ranitidine showed significant decrease in ulcer index and enhanced the percentage of ulcer protection. The rats treated with *Thespesia populnea* flower chloroform extract (200mg and 300mg/kg) also significantly ($P<0.001$ & $P<0.01$,respectively) decreased the intensity of gastric mucosal damage induced by Ranitidine the ulcer index and % protection of gastric lesion was 2.25+ 0.1041 and 47.63 %, respectively, in the groups of animals received *Thespesia populnea* flower chloroform extract (200mg/kg) as compare control. The ulcer index and % protection of gastric lesion was 1.95 + 0.1037 and 56.72% respectively, in the groups of animals received *Thespesia populnea* flower chloroform extract (300mg/kg) as compared control and the effect was equipotent with reference control Ranitidine. The ulcer index and % protection of gastric lesion was 1.06+ 0.1207 and 85.05%, respectively, in the groups of animals received Ranitidine.

CONCLUSION

From the above result, it was concluded that the *Thespesia populnea* flower chloroform extract exhibited antiulcer activity against Aspirin induced ulcer in rats. This property of chloroform extract of Flowers of *Thespesia populnea* act

would be highly beneficial for the treatment of various types of Gastric ulcers in human beings and reduce the cost burden of the society. However further the active principles of *Thespesia populnea* flowers responsible for this property is too isolated phytochemical and studies with these purified constituents on different ulcer models are waiting to understand the complete mechanism of antiulcer activity.

ACKNOWLEDGEMENT

This work supported in part by the, Department of chemistry under grant Annai College of arts & science.

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Ulcer index = 10/x

Where x = Total mucosal area / Total ulcerated area.

Table 1: Values are expressed in Mean ± SEM, (n=6), when compared with control, P<0.05, P<0.01, P<0.001 *, **, * respectively, One way ANOVA followed by Dunnet's t – Pylorus Ligated induced ulcer Method**

Sl. No.	Groups	ASPIRIN (mg/kg)	Ulcer Index	Ulcer Inhibition (%)
01	Ulcer Control 0.9 % saline	0.2	3.08320.1396	---
02	Ranitidine (27 mg/kg)	0.2	1.73±0.1657 **	32.52 %
03	Chloroform extract (200mg/kg)	0.2	2.3+ 0.2382 **	21.03 %
04	Chloroform extract (300mg/kg)	0.2	3.32+ 1.537 **	31.14%

Chloroform Extract of *Thespesia populnea* flower

Table 2: Values are expressed in Mean ± SEM, (n=6), when compared with control, P<0.05, P<0.01, P<0.001, *, **, * respectively, One way ANOVA followed by Dunnet's t – Test**

Sl. No.	Groups	Dose (Mg/Kg)	Gastric Volume (Ml)	PH	Free Acidity
01	Ulcer Control	0.9 % saline	2.36± 0.5578	2.35±0.09916	53.83±0.8133
02	Ranitidine	27	3.21± 0.1078 **	4.13±0.09054 **	31.16±1.004 **
03	Chloroform extract	200	2.63±0.08545 **	3.48± 0.03061 **	39.5±0.5638 **
04	Chloroform extract	300	3.18±0.07098 **	3.25±0.01065 **	32.16±0.1073 **

Chloroform extract of *Thespesia populnea* flower

Table 3: Values are expressed in Mean ± SEM, (n=6), when compared with control, P<0.05, P<0.01, P<0.001 *, **, * respectively, One way ANOVA followed by Dunnet's t – Test**

Sl. No.	Groups	Dose (mg/kg)	Ulcer Index	Ulcer Inhibition (%)
01	Ulcer Control	0.9 % saline	5.58± 0.2386	---
02	Ranitidine	27	1.06± 0.1207 **	85.05 %
03	Chloroform extract	200	2.25± 0.1041 **	47.63 %
04	Chloroform extract	300	1.95±0.1037 **	56.72 %

N= 6 animals in each group.

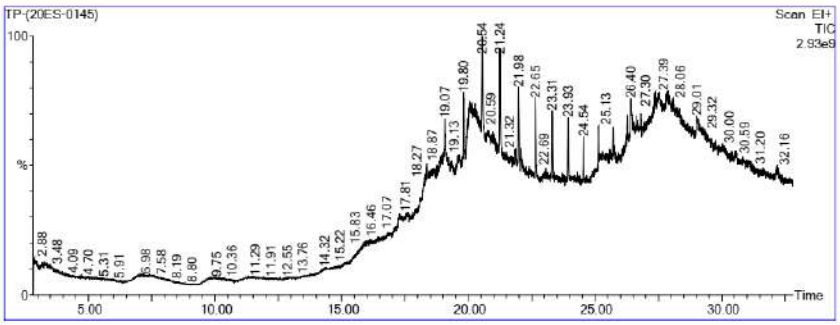
Chloroform extract of *Thespesia populnea* flower

Figure: *Thespesia populnea* Flower

Qualitative Report

File: C:\TURBOMASS\2019.PRO\Data\TP-(20ES-0145).raw
Acquired: 21-Feb-20 10:09:30 PM
Description:
GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP
Sample ID: TP-(20ES-0145)

Printed: 26-Feb-20 03:17 PM
Page 1 of 1
Vial Number: 61



#	RT	Scan	Height	Area	Area %	Norm %
1	18.349	3109	550,804,352	248,324,528.0	9.178	52.14
2	19.070	3253	946,782,080	334,439,744.0	12.361	70.22
3	19.635	3366	452,284,320	88,496,160.0	3.271	18.58
4	19.800	3399	1,135,563,520	94,814,640.0	3.504	19.91
5	20.055	3450	990,342,080	476,259,072.0	17.603	100.00
6	20.535	3546	1,660,311,680	363,764,096.0	13.445	76.38
7	21.241	3687	1,416,052,736	119,680,128.0	4.423	25.13
8	21.976	3834	920,055,808	33,794,724.0	1.249	7.10
9	23.932	4225	756,948,480	36,699,728.0	1.356	7.71
10	26.403	4719	455,950,816	35,200,168.0	1.301	7.39
11	27.388	4916	550,600,128	229,756,736.0	8.492	48.24
12	27.873	5013	593,330,496	444,476,832.0	16.428	93.33
13	29.009	5240	391,010,624	154,160,800.0	5.698	32.37
14	29.759	5390	172,695,392	45,705,812.0	1.689	9.60

Inst() ACQUISITION PARAMETERS
Oven: Initial temp 60°C for 2.80 min, ramp 10°C/min to 300°C, hold 6 min, InjAauto=260°C, Volume=0 µL, Split=10:1, Carrier Gas=He, Solvent Delay=2.80 min, Transfer Temp=230°C, Source Temp=230°C, Scan: 40 to 600Da, Column 30.0m x 250µm

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Matrix Rhythm Therapy on Aerobic Endurance in Young Male Athletes

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Received: 28 Feb 2021 Revised: 03 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

This study analyses the influence of Matrix Rhythm Therapy in improving the aerobic endurance of young male athletes. Thirty young, psychologically and medically fit male athletes between the age group of 18 and 22 at Vinayaka Missions Research Foundation- Deemed to be University, Salem with Form – 3 Certification were randomly selected. Pretest assessment of Maximal oxygen uptake was estimated using Queen's College Step test. All the subjects received Matrix rhythm therapy for 4 days in a week for 2 weeks following pre test assessment and on the 14th day, Queen's College Step test was administered similar to that of pre test assessment to record the post test assessment of Maximal oxygen uptake. The results of the study indicate that Maximal oxygen uptake and in turn aerobic endurance improved in Young male athletes following Matrix rhythm therapy for 2 weeks.

Keywords: Matrix Rhythm Therapy, Aerobic endurance, Maximal oxygen uptake, Queen's college step test, Cardio-respiratory fitness.

INTRODUCTION

Every human being has got a particular aim or goal in life and they work hard to achieve the same. Likewise a sportsperson also aims in achieving the peak performance and wants to maintain it. A good preparation before competition needs fitness training, sports specific training and maintenance of optimal size, weight and proportion of human body. Athletes and coaches incorporate one or more of the pre competition preparation strategies like static stretching, dynamic stretching, warm up drills, game simulation and mental imagery to enhance performance. They constantly fine tune their training strategies in order to develop a competitive edge. Athletic skills depend on

various fitness components like strength, flexibility, agility, cardiovascular endurance, speed etc. The use of therapeutic modalities such as thermal agents, electrical stimulation and massage are often performed for this purpose. Effects of all these physiotherapeutic modalities are inconsistent and vary for different fitness components and last for variable duration. Athletic performance is the final goal of every athlete and in most of the games a small difference in performance can change the whole scenario of the game. Hence there is a great need to find the effects of physiotherapeutic modalities in enhancing various fitness components and thereby athletic performance.

Any activity which is rhythmic, maintained continuously and uses large muscle groups is referred as aerobic exercise by The American College of Sports Medicine (ACSM) [1]. As the name implies, muscle groups activated by this type of exercise rely on aerobic metabolism to extract energy in the form of adenosine triphosphate (ATP) from amino acids, carbohydrates and fatty acids. Examples of aerobic exercise include cycling, dancing, hiking, jogging/long distance running, swimming and walking. These activities can best be accessed *via* the aerobic capacity, which is defined by the ACSM as the product of the capacity of the cardio respiratory system to supply oxygen and the capacity of the skeletal muscles to utilize oxygen [2]. Aerobic endurance refers to the ability of cardiovascular and respiratory systems of the body to sustain moderate intensity exercise over extended periods. A person's aerobic fitness level is dependent upon the amount of oxygen which can be transported by the body to the working muscles via the lungs and blood system and the efficiency of the muscles to use that oxygen. Aerobic endurance can be measured by the amount of oxygen utilized while exercising at your maximal capacity. Aerobic endurance can be assessed by using Cooper's 12 minute run test, Bruce and Balke wave treadmill protocol, Queen' College step test etc.

S Chatterjee et al suggested that Queen's College step test can be applied in the studied population to produce a good estimation of maximum oxygen uptake, especially in the field where large numbers of participants are tobe evaluated without a well equipped laboratory [3]. Dr. Ulrich G. Randoll postulated Matrix Rhythm Therapy and the principle behind is that, all tissues in the body vibrate/oscillate at 8 to 12 Hz frequency and the normal physiologic function of the body is maintained by the rhythm and it gets interrupted by any disturbance such as injury, inflammation, trauma which leads to pain in the body and further loss of function [4, 5]. Matrix rhythm therapy propagates phase-synchronized magneto-mechanical vibrations deep into the body tissues modulated between 8 and 12 Hz. This study aims to analyze the usefulness of Matrix Rhythm Therapy in improving the aerobic endurance of young male athletes. The results of the study will help athletes, physical trainers and physical therapists choose right methods to enhance athletic performance.

MATERIALS AND METHODS

This study is a pre post experimental trial. Thirty young, psychologically and medically fit male athletes between the age group of 18 and 22 at Vinayaka Missions Research Foundation- Deemed to be University, Salem with Form – 3 Certification were randomly selected. Subjects with Mini mental Examination score > 24 and Medical fitness for participation in sports from a recognized Government doctor were only included. Subjects were detailed about the purpose of the study and a written consent was obtained before inclusion into the study. Pretest assessment of Maximal oxygen uptake was estimated using Queen's College Step test. A measure of aerobic endurance is provided by this sub maximal test through the recovery heart rate measured after the test. This test requires a step with a height of 16.25 inches/41.3 cm, metronome and a stop watch. The subjects steps at a rate of 24 steps per minute guided by the metronome up and down on the step in the following fashion i.e four-step cadence, 'One leg up-Next leg up-One leg down-Next leg down' for a period of 3 minutes. The subject stops stepping immediately at the end of 3 minutes, and 15 seconds heartbeat from the first 5-20 seconds in recovery period was recorded. This heartbeat value for15 second is multiplied by 4 to get the beats per minute (bpm) value. Beats per minute can be used to estimate VO2max with the help of the following formula,

VO2max (ml/kg/min) = 111.33 - (0.42 x heart beat per minute (bpm)) [6]

Subjects were allowed to take some trials to familiarize the test few days before the pre-test assessment. Research assistants were used to calculate the time duration of the test using stop watch and proper execution of the test in accordance with the metronome. The heart rate of the subjects was counted for 15 seconds from 5 – 20 seconds of recovery manually with the help of assistants and recorded.

After the pre-test assessment was over, Matrix rhythm therapy was given for 4 days in a week for 2 weeks. It was given for duration of 60 minutes. The areas covered were

- Para spinal regions to balance the sympathetic and parasympathetic system
- Thoracic regions & Pelvic regions to improve lymphatic drainage
- Upper limb to improve circulation and oxygenation
- Lower limb to improve circulation and oxygenation

Post-test assessment for Maximal oxygen uptake was estimated after a period of 2 weeks of Matrix rhythm therapy using Queen's College Step test done similarly to pretest measurement and was recorded individually. Feasibility of the study was analyzed by a pilot study with six subjects. Statistical analysis was performed on the collected data. Normality of the data was estimated using Shapiro-Wilk test and was found to be normally distributed. Paired t test was used as a statistical test to analyse the influence of Matrix rhythm therapy on aerobic endurance.

RESULTS

Statistical analysis using a paired t-test revealed that there is a significant improvement of Maximal oxygen uptake in the subjects who received Matrix rhythm therapy. Table I listed in the list of tables represents the results of the statistical analysis. The result of the study state that there is a statistically significant improvement of Maximal oxygen uptake and in turn the aerobic endurance in the young, psychologically and medically fit male athletes who received Matrix rhythm therapy for a period of 2 weeks.

DISCUSSION

Matrix rhythm therapy is favored by the results of the study as a contributor to improve Maximal oxygen uptake of young, psychologically and medically fit male athletes between the age group of 18 and 22. Skeletal muscle rhythm is an important factor in strengthening the drainage of fluids which makes room for fresh metabolites and oxygen. Hence maintaining proper rhythm of skeletal muscle is essential and Matrix rhythm therapy aims to normalize the rhythm and thereby the cellular environment so that the cell can function optimally. Depth-effective rhythmical micro-extensions of Matrix rhythm therapy makes the cell metabolism to be reactivated in the tissue and inductive relaxation of the muscles that are contracted in the following manner i.e Circulation> Oxygen > ATP> Dissolution of the tension [7]. Matrix rhythm therapy has been proved to be effective in all cases related to microcirculation disturbances. Matrix rhythm therapy improves the supply of oxygen and heat, trace elements, vitamins, electrolytes and nutrients and thereby helps to optimize the external environment of the cells [8]. Taspinar F, Aslan UB, Sabir N, Cavlak U (2013) concluded that peripheral blood flow in young women increased following Matrix rhythm therapy and massage but resulted in more prominent increases with Matrix rhythm therapy method [9]. Matrix rhythm therapy since its development at the Erlangen University clinic was tested in over five years particularly in the rehabilitation range, successfully in the high-speed sport and in the veterinary medicine showed how meaningful rhythms are in humans if they are used therapeutically and purposefully [10].

CONCLUSION

The study concludes that Matrix rhythm therapy effectively improves the maximal oxygen uptake and in turn the aerobic endurance of young, psychologically and medically fit male athletes.

ACKNOWLEDGEMENT

Our sincere gratitude to Vinayaka Mission's College of Physiotherapy and Vinayaka Mission's Research Foundation (Deemed to be University), Salem for their encouragement and support with facilities required for the study.

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Table No. 1. Descriptive statistics of Experimental Group

VO2 MAX IN ML/ KG/ MINUTE	N	Range	Mean ± SD	t value	P value
Pre-Test	30	37.41 – 54.21	45.59 ± 4.49	-18.107	0.0001 Significant
Post-Test	30	44.13 – 59.25	53.37 ± 4.45		

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Biodiversity of Phytoplankton in Theroor Wetland Ecosystem, Kanyakumari, India

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Received: 12 Feb 2021 Revised: 22 Feb 2021 Accepted: 27 Feb 2021

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ABSTRACT

The present study was aimed to understand the diversity, distribution pattern of phytoplankton in Theroor wetland ecosystems, Kanyakumari, Southeast India. Standard methodologies were followed from collection till analysis. In the present investigations period (March 2015- February 2016) a total of 65 phytoplankton genera, representative of six families were identified such Chlorophyceae (22), Cyanophyceae (17), Bacillariophyceae (14), Euglenophyceae (6), Dinophyceae (4) and Coscinodiphyceae (2) and recorded seasonally. Among the six groups, Chlorophyceae was being dominant. The percentage composition of phytoplankton recorded at all three selected stations showed dominant group as chlorophyceae which comprised (44.14%) followed by Cyanophyceae (21.93%), Bacillariophyceae (19.45%), Euglenophyceae (6.97%), Dinophyceae (6.24%) and Coscinodiphyceae (1.27%.). Thus, the aim of the present analysis in the selected Theroor wetland ecosystems show phytoplankton community species richness which provides a primary documentation of existing diversity of phytoplankton which could be considered a baseline data base for understanding of current status of the lake ecosystem for researchers.

Keywords: Phytoplankton; Diversity; Wetland; Microalgae; Chlorophyll; Water quality.

INTRODUCTION

A wetland ecosystem as an ecological barometer play an important role in the social ecology of the region in which they are located and also helps in determining the health of a city. They are the primary shelter for aquatic biodiversity including aquatic flora, fauna and other microorganisms. In general the wetlands are more complex and fragile ecosystem as it does not have a self-cleaning ability and therefore readily accumulate pollutants. In addition, currently due to the rise in anthropogenic activities in and around these fresh water aquatic systems and their catchment areas have added to a large extent in deterioration of their water quality and dwindling of water bodies which in turn leading to their accelerated eutrophication. Moreover owing to inadequate managements, these prime wetlands in and around human dominating areas is recently facing great threat. Despite appropriate measures the contamination of the aquatic ecosystem is on rise.

Large wetland called Theroor a prime land mark surrounded by majestic hills, fringed with coconut trees and paddy fields located in Agastheeswaram, a pre-urban town in Kanayakumari district, Tamilnadu is good example of such situation. It is located in the vicinity of Pachipari reservoirs. The water drain through streams and small rivulets amidst of urbanizing and agricultural towns like Thearikalpudur, Thathyarkulam etc., via Pudugramam reaches Theroor. Due to unplanned industrialization, improper effluent management, excessive exploitation and mounting anthropogenic influences in and around Theroor wetland have generated great amount of wastewater. These waste waters getting discharged constantly in to Theroor wetland not only deteriorating water for human consumption but also spoils the roosting site for many migratory birds and fish population by accumulating in inhabitant. Even though nature is somewhat effectively maintaining a balance in the population of flora and fauna through biological control, the phytoplankton and zooplanktons population nowadays is hampered due to multitude of anthropogenic disturbances, advancement of the technology and urban industrialization that has already become a serious problem in many areas of the country like India. Phytoplankton, half of the earth's primary producer is the integral component of wetland ecosystem which determines the primary productivity of the water body was also used as indicators of water quality of aquatic systems [1-2]. It form the basis of the whole food chain in open waters and serve as direct food source for aquatic animals [3] . Thus, it plays a crucial role in maintaining the biological balance and quality of water [4] Abdar [5].

Many ecologist like Chinnaiah and Madu, [6]; Feresin*et al*. [7]; Vasanthakumar and Kumar [8]: Uveges *et al*. [9]; Sharma *et al*. [10] through their research works have laid emphasis that these primary producers like blue green algae, green algae, diatoms, desmids, euglenoids etc. are very sensitive to environment especially for physical, chemical and nutrient changes. Thus, plankton in general serves as bio-indicators mainly its abundance and species composition, can be an excellent indicator [9] with reference to water quality. Moreover, it is considered as a best tool for assessing the health of the aquatic ecosystems [11] because of its sensitivity to environmental changes and is useful as water bioremediations agent [12]. Their appearance, disappearance, density and pattern of distribution depend on biotic and a biotic factor and in various aquatic ecosystems [11-12]. Thus, maintenance of fertile and healthy aquatic ecosystem depends on the biotic properties of water and the biological diversity of ecosystem [13] especially reflected through productivity of the phytoplankton, the primary producers. So, it is prime to study on species communities in any aquatic systems as it assists to understand the life of living organisms and helps in monitoring the conditions congenial for their life in the environment they live in. In concordance to the statement of Mathivanan *et al*. [14] and Rajagopal *et al*. [15], that plankton population observation may be used as a reliable tool for bio monitoring studies to assess the pollution status of fresh water bodies, In the present study phytoplankton biodiversity assessed following standard indexing methodologies to understand the current status of Theroor wetland water.

MATERIALS AND METHODS

Collection and Identification of Plankton Samples

The present study was carried out on seasonal basis from March 2015- February 2016. The selected wetland, Theroor in Agastheeswaram taluk, Kanyakumari district has a wide spread area of 80Km^2 encompassing 12 villages, fall between the coordinate 33°10'N; 77°10"E. Three sampling stations (Stn.1, Stn.2 and Stn.3) were selected for plankton analysis. The plankton samples were collected during the morning hours (6.00 – 10.00 AM) from the selected sites every month first week of the study period. A standard vertical and horizontal sampling method was followed during the present study for the collection of plankton sample from selected stations surface waters using standard plankton net (mouth diameter 0.35nm) made up of bolting silk mesh size (48µm). 100 L of water was filtered through plankton net (48µm) for quantitative analysis of plankton. Samples with three replicates were collected during the fixed timing from each sampling stations and the plankton biomass was preserved in formalin of ratio 5-80+0.90 parts (V/V) and stored in labeled capped bottles, transported in cool box to the laboratory for microscopic analysis and identification. The collected samples were poured in graduated tubes of 10-20 mL capacity and centrifuged for 20 minutes at 2000rpm. Later the supernatant was discarded. From each concentrated samples 1ml of quantitative sub sample of phytoplankton was examined in Sedgwick rafter chamber under 400X magnification following Hosmani, [16]; Manickam *et al*. [17] modified Sedgwick rafter method using fine needle and brush Santhanam *et al*. [19]. Detailed taxonomic identification, classification and description was done with the help of Olympus microscope having different magnifications following the methods described by Anand [18], Hosmani [16]. Manickam *et al*. [17].

Calculation

$$N = n \times v/V$$

Where,

N = Total number of plankton cells / L,
n = Average number of plankton cells in 1 ml of sample
v = Volume of plankton concentrate,
V = Total volume of water filtered (L).

Diversity Analysis of Plankton Samples

The diversity analysis of phytoplankton of Theroor wetland in Kannyakumari district was analyzed by calculating different diversity indices (DI). Shannon Weiner's Index (H'), Simpson richness index (D') and Evenness index (E') was calculated by using diversity software package (PAST–Palaeontological Statistics Ver. 2.00) according to Shannon and Wiener [20], Simpson [21], Pielou [22].

Estimation of Chlorophyll - 'a'

For the Chlorophyll-'a' estimation, five liters of water samples were collected from study area from which, 250ml of water was filtered through the millipore filtering unit filtered with 0.45 diameter GF/C filter paper and the filtered sample a was used for chlorophyll 'a' estimation following Spectrophotometric method [11].

RESULTS

Results of quantitative and qualitative analysis of phytoplankton from all three stations comprised of Chlorophyceea, Cyanophyceae, Bacillariophyceae, Euglenophyceae, Dinophyceae and Coscinodiphyceae (Fig. 1). The quantitative and qualitative analyses of phytoplankton showed that the population density of Chlorophyceae was found to be maximum in all the seasons at all stations. Over all the total population density of phytoplankton (including all classes) was maximum during summer season, followed by pre-monsoon, post-monsoon and minimum during monsoon.

Species Composition

During the one-year study period (March 2015-February 2016) a total of 65 phytoplankton species were recorded from Theroor wetland. Of the 65 species of composition of phytoplankton 22 species of Chlorophyceae, 17 species Cyanophyceae, 14 species of Bacillariophyceae, 6 species of Euglenophyceae, 4 species of Dinophyceae and 2 species of Coscinodiphyceae were recorded. Phytoplankton was observed to be maximum during summer followed by pre monsoon season, post monsoon and minimum during monsoon season while station wise Stn.3 recorded maximum phytoplankton followed by Stn.2 and Stn.1.

Percentage Composition

The phytoplankton of Theroor wetland at three stations (Station-1, 2, and 3) are found in six classes Chlorophyceae (Green algae), Cyanophyceae (Blue green algae), Bacillarophyceae (Diatoms), Euglenophyceae (Flagellates), Dinophyceae (dinoflagellates) and Coscinodiphyceae. Percentage composition of phytoplankton recorded during the study period from Theroor wetland at all three selected stations showed dominant group as Chlorophyceae (green algae) which comprised (44.14%) followed by Cyanophyceae (Blue green algae) (21.93%), Bacillariophyceae (Diatoms) (19.45%), Euglenophyceae (6.97%), Dinophyceae 6.24% and Coscinodiphyceae 1.27%. In class Chlorophyceae phytoplankton's are found to show (order 13), class Cyanophyceae shows (9) orders, class Bacillarophyceae shows (7) order, class Euglenophyceae shows (3) order, i.e. Dinophyceae shows (3) order and Coscinodiphyceae shows order. During the one year of study period, a total of 65 species of phytoplankton species were recorded. 22 species of the 65 species Chlorophyceaea, 17 species of Cyanophyceae, 14 species Bacilarophyceae, 6 species of Euglenophyceae, 4 species of Dinophyceae and 2 species of Coscinodiphyceae were recorded. The maximum recorded phytoplankton number at Stn.1 composed (48.97%), of Chlorophyceae (25.31%), of Cyanophyceae (20.34%), of Bacilarophyceae (10.88%), of Euglenophyceae (8.39%), of Dinophyceae and (2.09%), of Coscinodiphyceae while the minimum value recorded at Stn.1 were (34.96%), of Chlorophyceae, (13.08%), of Cyanophyceae, (14.00%), of Bacilarophyceae (2.17%), of Euglenophyceae (1.98%), of Dinophyceae and (0.84%), of Coscinodiphyceae respectively . At Stn.2 the percentage value of Chlorophyceae was observed to fall between range (55.7%-35.6%); Cyanophyceae range was between (26.63%-13.85%); the range of Bacilarophyceae was between (20.49%- 14.28%); maximum and minimum Euglenophyceae at Stn.2 was noted range between (11.31%-4.36%); Dinophyceae recorded at Stn.2 was noted to be (8.62-3.55%); while class Coscinodiphyceae was observed to fall within the range (2.57%-1.77%) respectively. The annual average percentage composition of different groups of phytoplankton at Stn.3 revealed to compute maximum Chlorophycae (53.63%), Cyanophycea (27.91%), Bacilarophycea (27.91%), Euglenophycea (13%), Dinophycea (7.63%) and Coscinodiphycea (3.38%). while, minimum of (36.53%), of Chlorophycae (16.78%), of Cyanophycea (14.66%), of Bacilarophycea (5.07%), of Euglenophyceae (4.1%), of Dinophycea and (1.81%), of Coscinodiphycea was observed during the study period at Stn.3 respectively. Thus the result of present study revealed that maximum percentage of phytoplankton was recorded in summer season followed by pre monsoon, post monsoon and monsoon and among the stations maximum percentage composition was recorded in Stn.3 and Stn.2, while minimum at Stn.1 respectively.

Population Density

In the present investigation population density of phytoplankton recorded at selected three stations ranged between (29833±150.25-4346±55.14cells/L) at Stn.1; (31553± 126.85-1183±18.65cells/L) at Stn.2 and (35733±156.24-10766±49.24cells/L) at Stn.3. The minimum density of phytoplankton was recorded during monsoon (4346±55.14 cells/L) at Stn.1, while the maximum value (35733±156.24 cells/L) at Stn.3.Thus the peak value of phytoplankton was recorded during summer and lower value during in monsoon season in the present investigation respectively (Fig. 2).

Species Diversity

On analysis of species diversity of phytoplankton it was noticed to fall between ranges of (5.714±0.63-5.053±0.75) in Stn.1; (5.801±0.80–5.11±0.85) in Stn.2 and (5.583±0.45- 5.206±0.65) in Stn.3. The minimum (5.053±0.75) diversity was recorded during monsoon in Stn.1, whereas the maximum value (5.801±0.80) was noticed during summer in Stn.3

(Fig 3). Overall the maximum value of species diversity was noted in summer followed by pre monsoon season, post monsoon at Stn.3 and minimum value recorded by monsoon season at Stn.1 during the study period.

Species Richness

Species richness of phytoplankton recorded at three different stations ranged between (0.979±0.18 - 0.914±0.14) at Stn.1, (0.980±0.14-0.930±0.12) at Stn.2, (0.981±0.15-0.967±0.14) at Stn.3 During the study period among the selected stations high species richness value (0.981±0.15 and 0.979±0.18) for both phytoplankton was recorded during summer season at Stn.3 and lowest species richness value (0.914±0.14 and 0.342±0.08) was recorded in monsoon season at Stn.1 (Fig. 4).

Species Evenness

Species evenness of phytoplankton recorded at Theroor wetland ranged between (0.994 ± 0.13- 0.977±0.09) at Stn.1: (0.997±0.18-0.981±0.14) at Stn.2 and (0.998±0.11-0.955±0.13) at Stn.3. Among the stations maximum (0.998±0.11 and 0.997 ±0.18) evenness value of phytoplankton plankton was noted at Stn.3 during summer season while minimum (0.977±0.09 and 0.9730.832±0.16) evenness value of phytoplankton was observed at Stn.1 during monsoon season (Fig. 5).

Chlorophyll 'a'

During the present investigation the Chlorophyll 'a' was observed to fall between the range (2.903/$m^{3)}$ in Stn.3, (2.511/m^3) in Stn.2 and (2.122mg/m^3) in Stn.1. The minimum (0.181 mg/m3) chlorophyll 'a' was recorded during monsoon while maximum value (2.903 mg/m^3) was observed during summer (Fig. 6). Among the stations maximum Chlorophyll 'a' (2.903 mg/m3) was recorded in Stn.3 while minimum Chlorophyll 'a' (0.181 mg/m^3) was recorded in Stn.1

DISCUSSION

In most inland waters the bulk of living matter found is phytoplankton. Their biological importance is immense as these are the main sources of food directly or indirectly to freshwater fish which feed on the plankton at one or other stage of their life cycle. These primary producers which are the most essential component of aquatic food chain are largely governed by water quality parameters (Table-1). They fix the energy and it subsequently transfer to higher tropic levels [23] and they react fast to the pollution in the aquatic environment. Therefore, plankton population observation may be used as a reliable tool for bio-monitoring studies to assess the pollution status of aquatic bodies [24-25]. According to Olele and Ekelemu [26], the phytoplankton not only influences the food chain of freshwater bodies but also have economic value and biological significance to man. So the diversity, species composition and abundance of phytoplankton along with environmental factors like physicochemical properties of water, meteorological properties of the particular region, morphometric and hydrographic characters of the wetland ecosystem are to be monitored regularly [27] to preserve it for future. But nowadays Theroor wetland is getting much affected due to discharge of effluents from industries, influx of domestic sewage, and municipal waste, discharge from agricultural land etc. patricidal and chemical fertilizers.

The indicator properties of phytoplankton are determined by its species composition and quantitative parameters of developments, so the species composition, abundance, biomass and distribution of algae over Theroor wetland area are recorded completely during the year (March 2015- February 2016). Since plankton study being the reliable tool to estimate the wetland status in the present study period qualitative observation of phytoplankton was carried out in Theroor wetland. Totally 65 number of phytoplankton species were observed and identified in water at three stations. Phytoplankton from all three stations represented by six groups namely Chlorophyceae (22), Cyanophyceae (17), Bacillariophyceae (14), Euglenophyceae (6), Dinophyceae (4) and Coscinodiphyceae (2). Different phytoplankton in different water bodies has been observed by several reporters like Zuber [28], Verma [29]. Total of

44 genera of phytoplankton population were quantified belonging to Chlorophyceae (18), Cyanophyceae (10) Bacillariophyceae (9) Euglenophyceae (4) and Dinophyceae (1) was recorded by Ratna *et al*. [30] in Shetterlake. Gupta and Devi [31] recorded 30 phytoplankton taxa belonging to Chlorophycea, Cyanophyceae and Bacillariophyceae. Borics *et al*. [32] recorded 21 species of chlorophyceae in oxbow lake and 47 species of Chlorophyceae in Rewalsarlake. Yao *et al*. [33] recorded 46 species in lake Yueya, which was reduced to 33 species and then to 21 species in lake Yueya. This result agrees with the work by Abdullahi and Indabawa [33], on the phytoplankton content of Ngurulake. The dominating presence of Chlorophyta in study areas shows gradual deterioration of the water quality. This could be as a result of anthropogenic activities, such as chemicals and wastes washed into it, washing of clothes and bathing done sometimes around the lake. [34-35]. Anago *et al*. [36] reported that in lakes where domestic, agricultural and industrial pollution is accelerated, growth of Chlorophyta and Cyanophyta result.

The phytoplankton community in Wawan-rafi lake was dominated by Chlorophyta followed by Cyanophyta with Bacilariophyta been the least represented by Usman [37]. Forty one genera of phytoplankton, belonging to 5 groups (Chlorophyceae, Cyanobacteria, Bacillariophyceae, Euglenophyceae and Dinophyceae) were encountered in the Baskandianualake by Devi *et al*. [38-39]. In the present investigation Chlorophyceae (44.14%), the most prominent phytoplankton recorded during the study period was represented with (*Volvox*, *Ulothrix* sp, *Pediastrum*, *Microcysti* and *Ankistrodesmus*) followed by second largest group Cyanophyceae (21.93%), comprised of (*Nostoc*, *Oscillatoria* and *Sipurilina*) followed by Bacillariophyceae (19.45%), dominate with *Synedraulna*, *Nitzschia* and *Fragilaria* then by Euglenophyceae (6.97%), Dinophycea (6.24%) and Coscinodiphyceae (1.27%). All the members of Chlorophyceae, Cyanophyceae were found to present in all study stations of the wetland. The present status of phytoplankton composition and diversity that in Theroor water ecosystems the Chlorophyceae is dominated over Bacillariophyceae, the second largest groups in all the stations. Similar observations were recorded by Tiwari *et al*. [40], Verma and Singh [41]. The presence of pollution indicator species viz *Ankistro desmus* in Chlorophyceae, among Cyanophycea (*Oscillatoria*, *Spirulina*) and Bacillariophyceae (*Nitzschia*) these are pollution indicator species which implies that the nature of anthropogenic stress and pollution threat arising as this species have already been identified as pollution indicator species by Rai [42], Chandermohan [43], Zuber [44], Thirupathaiah *et al*.[45].

In the study area during the study period maximum phytoplankton density was recorded in summer season at Stn-3 (35733 cells/L) followed by post monsoon season and minimum values of phytoplankton recorded in monsoon season at Stn.1 (4346 ±55.14 cells/L). During the one year diversity assessment of phytoplankton it was observed that at increase in diversity was observed in Stn.1 (5.583±0.45cells/L), Stn.2 (5.714 ±0.63cells/L) and Stn.3 (5.801 ±0.80cells/L). Similarly the density of phytoplankton also increased during the study period at Stn.1 (29833±150.25 cells/L); Stn.2 (31553 ± 126.85 cells/L) and Stn.3 (35733±156.24 cells/L) respectively and the present result was in agreement with the report of Bhat *et al*. [46]. During the present study maximum density of phytoplankton was noted in summer which may be attributed by maximum sunlight and higher temperature which is reported to stimulate growth of the aquatic autotrophy which was supported by early reports [43-48] who all reported that during their study among the period phytoplankton, Chlorophyceae remains dominant throughout the study followed by Bacillariophycea and Cyanophyceae. An opposite position occurs during monsoon when the water cover is highest, plankton get more distributed resulting in decline in their density when the water column was remarkably stratified to a large extent because of heavy rainfall, high turbidity caused by run-off, decreased temperature and pH, overcast sky and cool conditions. However, Hassan *et al*. [49-54] study result too showed that minimum density of phytoplankton during monsoon and maximum during summer in Euphrates River, Iraq and similarly Laskar and Gupta [55] reported minimum density of phytoplankton during monsoon and maximum during summer in Chatlalake, Assam. High density of phytoplankton in summer was observed by Wojciechowska *et al*. [56].

During the present investigation maximum species diversity value (5.801±0.80) was recorded in summer seasons followed by pre monsoon season at Stn.3 while minimum species diversity as value (5.053±0.75) was recorded in monsoon at Stn.1, due to rainfall, resulting in enhanced concentrations of suspended solids, inorganic particles and dissolved organic matter, thereby adversely affecting seasonal abundance and phytoplankton's diversity reported by

Chattopadhyay and Banerjee [57]. Similar seasonal report on phytoplankton diversity was given by Ahmad [58], Vareethiah and Haniffa [59], Sivakumar and Karuppasamy [60]. One of the major components the species richness is expressed by simple ratio between total number of species (s) and total number of individual (n). The species richness index (*D')* of phytoplankton was noted to be minimum during monsoon (June, July) at Stn.1 (0.97±0.13) and maximum richness were recorded in summer (April, May) at Stn.3 (0.981±0.15). Next major component of diversity indices is Specie evenness (*E'*) which refers to how the species abundance is distributed among the species. In the study area maximum Species evenness value (0.998±0.11) was observed in March i.e. in summer season in Stn.3 while minimum value (0.977±0.18) was recorded in August i.e. in monsoon season at Stn.1. With this regards a few studies were conducted on phytoplankton communities of different fresh water systems reported by Das *et al*. [61]; Dalal and Gupta [62]; Devi and Antal [63]; Gupta and Devi [64].

Species evenness of phytoplankton recorded was in the range between (0.998 ±0.11- 0.984 ±0.14) at Stn.3 and (0.994±0.13 - 0.977 ± 0.09) at Stn.1. Maximum value of Species evenness was recorded in summer season and minimum value was noted in monsoon season. According to Welch [65-67], the diversity and density or distribution of plankton is mainly affected by wind flow, inflowing streams, dilution, qualitative variation of water, physicochemical alteration of water, depth of water, shoreline, current plankton swarms and action of predators and diurnal migration of plankton.

CONCLUSION

The overall view of this study reveals that the fluctuation of phytoplankton occurs distinctly in the study area and normally in rainy season. The less population during the study period may be due to the dilution factors and which in turn leads to less photosynthetic activity by primary producers. But in summer stability of water body, availability of nutrients, favorable temperature, light penetration, clarity of water and availability of more food due to decomposition of organic matter may favor the abundance, rich density, evenness of phytoplankton and high number of zooplankton might be due to less predators.

ACKNOWLEDGEMENT

Authors are thankful to the Head, PG Research Department of Zoology and authorities of Nirmala College for Women, Coimbatore for the facilities provided. One of the authors (SDK) thanks the UGC Govt. of India for providing post-doctoral Fellowship (Ref No. F./31-1/2017/PDFSS-2017-18-TAM-13681 dated 19.06.2017).

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Table-1. Correlation coefficient of biological characteristics in Theroor wetland waters at Station-1, 2 and 3.

	Population Density	Species Diversity	Species Richness	Species Evenness	Chlorophyll	CR	Net Primary Productivity	Gross Primary Productivity
Station-I								
Population Density	1							
Species Diversity	.886**	1						
Species Richness	.375	.480	1					
Species Evenness	.630*	.730**	.664*	1				
Chlorophyll	.691*	.688*	.708*	.909**	1			
CR	.626*	.801**	.645*	.904**	.821**	1		
Net Primary Productivity	.540	.648*	.865**	.891**	.928**	.845**	1	
Gross Primary Productivity	.700*	.776**	.673*	.951**	.944**	.943**	.926**	1
Station-II								
Population Density	1							
Species Diversity	.910**	1						
Species Richness	.382	.474	1					
Species Evenness	.858**	.856**	.348	1				
Chlorophyll	.516	.662*	.693*	.624*	1			
CR	.849**	.872**	.610*	.810**	.686*	1		

Net Productivity	Primary	.875**	.868**	.636*	.833**	.827**	.877**	1	**
Gross Productivity	Primary	.880**	.891**	.659*	.850**	.796**	.951**	.979**	1
Station-III									
Population Density		1							
Species Diversity		.876**	1						
Species Richness		.870**	.927**	1					
Species Evenness		.428	.202	.258	1				
Chlorophyll		.627*	.802**	.722**	-.063	1			
CR		.958**	.883**	.867**	.456	.583*	1		
Net Productivity	Primary	.958**	.888**	.871**	.331	.756**	.889**	1	
Gross Productivity	Primary	.773**	.725**	.661*	.362	.668*	.819**	.709**	1

**. P< 0.01 *. P<0.05

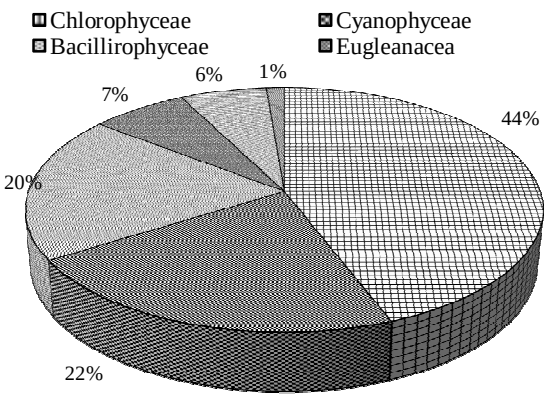

Fig. 1. Percentage composition of phytoplankton at three stations in Theroor wetland waters during March 2015-February 2016.

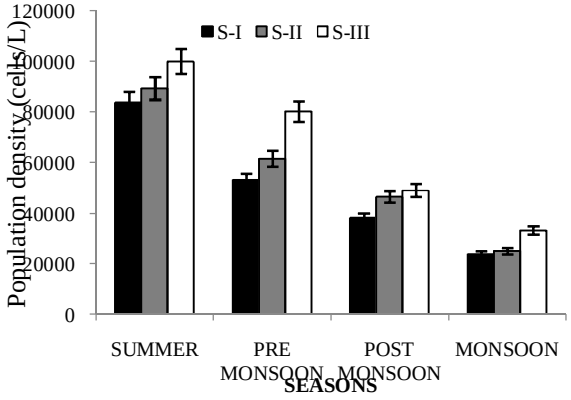

Fig. 2. Seasonal variation in of population density of phytoplankton at three stations in Theroor wetland waters during March 2015-February 2016.

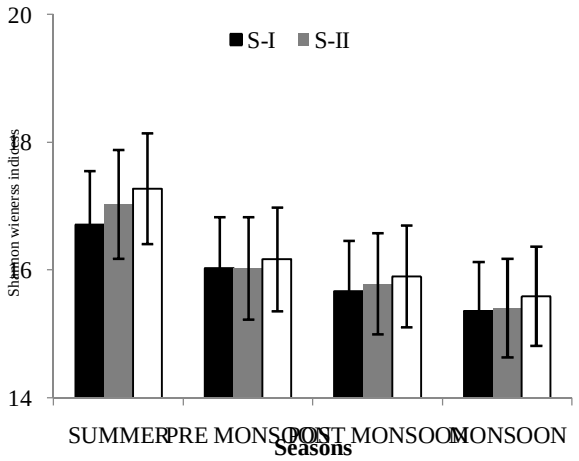

Fig. 3. Seasonal variation in of species diversity of phytoplankton at three stations in Theroor wetland waters during March 2015-February 2016

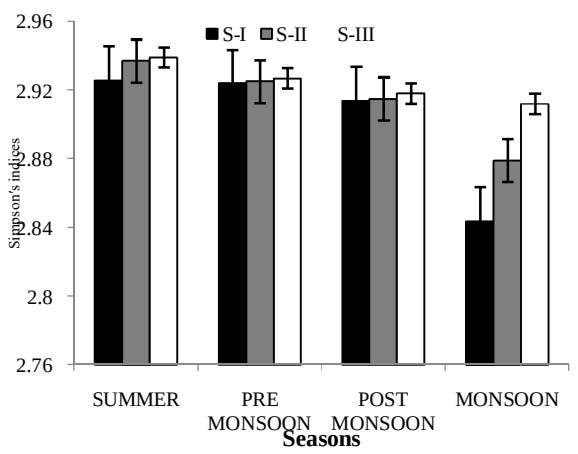

Fig. 4. Seasonal variation in of species richness of phytoplankton at three stations in Theroor wetland waters during March 2015-February 2016.

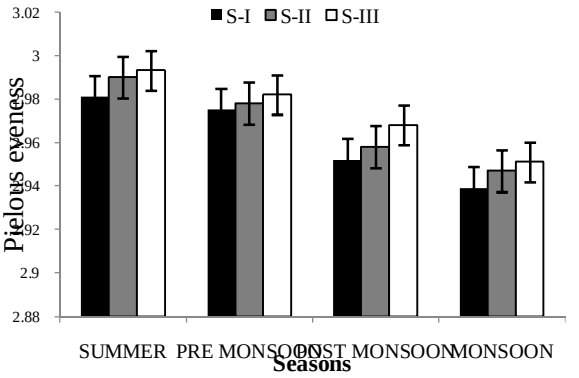

Fig. 5. Seasonal variation in of species evenness of phytoplankton at three stations in Theroor wetland waters during March 2015-February 2016.

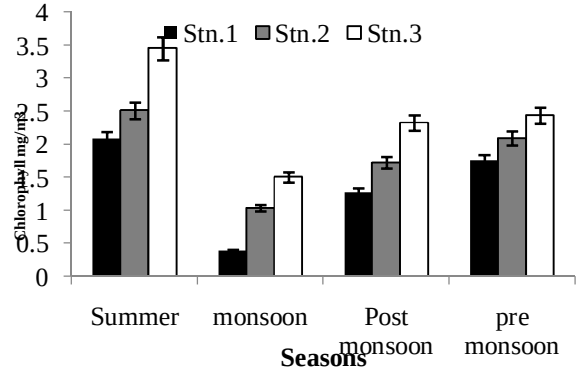

Fig. 6. Seasonal variation in of chlorophyll 'a' at three stations in Theroor wetland waters during March 2015-February 2016.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Analysis of Nutritional Composition of Three Aquaculture Important Live Feeds

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Received: 04 Mar 2021 Revised: 05 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

The present study aimed to understand the proportion of nutritional value of marine copepod *Nannocalanus minor* harvested from outdoor mass culture system and its composition were compared with traditional live feeds such as Artemia and rotifer. The study includes the analyses of proximate composition like moisture, protein, carbohydrate, lipid, ash, amino acids, and fatty acids by adopting standard methods. Among the three live feeds analyzed, protein and carbohydrate was high in copepod whereas lipid and ash was found to be high in Artemia and moisture was found to be high in rotifer. The total amino acids were higher in *N. minor* (100%) followed by rotifer (92.82%) and Artemia (85.71). The saturated fatty acids (SFA) were found to be higher *N. minor* (67.22%) than *B. plicatilis* (44.63%) and *Artemia* nauplii (30.69%).This proximate composition data may cause an essential base for enhancing the combination in live feeds or formulated feeds while using the larval stage of marine finfish and shellfish culture.

Keywords: Copepod; Artemia; Rotifer; Live feeds; Nutritional value; Aquaculture

INTRODUCTION

Copepods may be necessary to optimize production of marine fish species. Furthermore, copepods contain the appropriate HUFA composition required for the growth and development of fish larvae. Among other factors, normal egg development and subsequent hatching of fish depends on their chemical constituents of food. Studies on biochemical composition of cultured copepod particularly in Indian sub-continent are seemed to be very limited and restricted [1-5]. The marine copepods are considered to be "nutritionally superior" for marine fish and prawn larvae and they are valuable source of proteins, lipids, carbohydrates, amino acids, fatty acids and enzymes (amylase, protease, exonuclease and esterase), which are essential for larval digestion and metamorphosis [6]. Further the nutritional quality of copepods is important in determining the survival and growth of fish larvae [7]. In many marine fish and shrimp hatcheries high mortality has been encountered due to the lack of nutritionally adequate live feed organisms at the first feeding stage and the copepods are helpful in solving these problems. Thus copepods could be an inexpensive ingredient and they serve as an alternative to more expensive brine shrimp. The fish larvae require food organisms with high concentrations of highly unsaturated fatty acids (HUFA). *Artemia* and rotifer synthesize significantly minimum level of the essential fatty acids, DHA and EPA compared to copepods.

Copepods have been shown to have a high natural omega-3 profile, which exceeds that provided by enriched rotifers or *Artemia* [8]. Fatty acid content of different diets used to rear copepod determined by Payne and Rippingale [9]. Especially the three long chain polyunsaturated fatty acids such as arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are required in order to obtain normal growth and development, through maintaining the structural and functional integrity of cell membranes, and as precursors of eicosanoids [8]. Marine copepods, especially calanoids, have been proven as ideal food for many cultured marine larvae [10]. Improvements in growth, survival, and stress resistance are attained when suitable absolute amounts or ratios of these fatty acids are present in larval diets [8]. However, there is no much work on nutritional estimation in cultured copepods from Indian waters except Santhanam [11], Rajkumar and Vasagam [12], Ashok Prabu and Rajkumar [13], Vengadeshperumal *et al*. [14], Ananth and Santhanam [2], Jeyaraj and Santhanam [15] and Ananthi *et al.*[16], Santhanam and Perumal[3]. In this line, the present study was made on the evaluation of nutritional composition *viz* moisture, protein, carbohydrate, lipid, ash, aminoacids and fatty acids of cultured copepod *N. minor* and the data compared with Artemia and rotifer.

MATERIALS AND METHODS

Analyses of biochemical compositions in live feeds The moisture content was estimated according to methods explained by Rajendran [17] using following formula:

Moisture %= Wet weight of sample - Dry weight of sample / Wet weight of the sample x 100.
The protein content was estimated according to the Biuret method described by Raymont *et al*. [18] and the protein content was calculated as follows:

Protein (%) = OD of sample x standard value x total volume/Weight of the sample x Volume of extract x 100. The concentration of carbohydrate was estimated according to the procedure of Dubois *et al.*[19] with the help of 5% phenol solution and concentrated sulphuric acid. The percentage of carbohydrate content was calculated as follows:Carbohydrate (%) = Standard value x OD of the sample/Weight of the sample taken x 100. To estimate the lipid concentration, the chloroform: methanol method was followed [20]. The percentage of lipid content was calculated as follows:Lipid (%) = Amount of lipid in the sample/Weight of the sample taken x100.The ash content was determined by burning oven dried sample in a muffle furnace at 550^{o}C according to the produce by AOAC [21]. The percentage of ash content was determined by using the following formula:Ash (%) = Ash content/Dry weightx100.
Analyses of amino acids and fatty acids concentration in live feeds

The analyses of amino acids concentration in live feeds was done by following the procedures of Yamamoto *et al*. [22]. In short, the sample was digested with 6-N HCl at 110º C for 22 hours. Then the copepod was dissolved in 2ml of sample diluents. The acid hydrolysate was dried using speed vacuum concentrator and the sample filtered. The amino acids were determined by an automatic amino acid analyzer (Shimatzu-High Performance Liquid Chromatography (LC 4A). To estimate the fatty acids concentration in the live feeds, the sample was homogenized with chloroform: methanol (2:1 v/v) mixture and they were extracted using the method of Bligh and Dyer [23]. After the fat extraction, they were esterified with 1% H_2SO_4 and fatty acid methyl esters were prepared by following the procedure of AOAC [21]. Identification and quantification of fatty acids were done by using a Gas Chromatograph (Hewlett Packard, 5890 Model).

RESULTS AND DISCUSSION

The percentage of moisture, protein, carbohydrate, lipid and ash contents of cultured copepod, *N.minor* were: 71.41, 63.16, 20.18, 12.53 and 4.19 respectively. The mean values of biochemical composition of *N. minor* was given in Table-1. The biochemical composition of rotifer, *Brachionus plicatilis* showed 85.22% of moisture, 62.84 % of protein, 13.4 % of lipid, 18.87 % of carbohydrate and 4.89% of ash. The laboratory hatched *Artemia* nauplii showed the moisture, protein, lipid, carbohydrate and ash content of 83.45%, 62.89%, 15.11%, 15.12% and 6.88%, respectively.

Table-1. Biochemical composition of live feeds

The nutritional profile of copepod *N.minor* showed a remarkable values of protein (63.16%) and carbohydrate (20.18%) and a lower percentage of lipids (12.53%) compared to other live feeds *viz.,B. plicatilis* and *Artemia* nauplii. The nutritional superiority of copepod for marine fish larvae over traditional live food organisms such as rotifer and *Artemia* is well confirmed earlier by Witt *et al*. [24]; Sargent *et al*. [8]; Toledo *et al*. [25] and Woods [26].The nutritional value of any food organisms is usually related to their conversion efficiency of biochemical composition to their host organisms [27-29]. However, *Artemia* and rotifers have limited abilities to convert the short-chain n-3 PUFA into long-chain DHA and EPA as stated by Lubzens *et al*. [30]. Vital for the persistence of copepod populations is the production of viable eggs. Among other factors, normal egg development and subsequent hatching depends on their chemical constituents. For example, carbohydrates are mainly used as energy source during hatching [31], proteins modulate cellular events such as gene expression and growth while fatty acids (specifically polyunsaturated fatty acids) are involved in the metabolism of chemicals responsible for regulating cell differentiation, and hatching [32-33]. Often these roles are specific to individual biochemical constituents, and are typically not interchangeable. Several studies have demonstrated the effect of prey biochemical contents on egg viability [34-35]. It is however not clear whether egg viability is also affected by food availability [36-37].

Several studies have demonstrated that the effect of food availability on the biochemical composition of the cultivable species [38-40]. The nutritional superiority of copepods is well documented due to the high levels of long chained fatty acids, phospholipids and natural antioxidants [8]. The protein content of copepods showed greater variations. These values are higher comparable with those reported earlier for mixed zooplankton from higher latitudes [41]. The protein was found to be the major biochemical component in *N. minor*. It is comparable with the values reported earlier for wild copepods [42]. Ferguson and Raymont [43] have observed high protein content in copepod, *Calanus finmarchicus* and Krill, *Euphasia superba,* respectively. The protein formed the major fraction compared with lipid and carbohydrate, indicating the usefulness as energy reserve [44]. The observed marked variations in the protein content might be due to the fact that it is utilized as a metabolic substrate. The similar statement was also given by Drillet *et al.,* [45].

The lipid content was lower than that of carbohydrate and protein. The continuous supply of phytoplankton would render lipid reserve unnecessary, which might account for the low content [11, 46]. The lipid content of tropical zooplankton when compared to temperate zooplankton is significantly low as proved by the findings of Sreepada *et*

al. [47]. The variations in the lipid content can also be attributed to its storage and utilization during the period of starvation, when it serves as an effective energy reserve [48]. The function of protein as an important energy reserve may be true for zooplankton having low lipid content [49]. The similar findings was also obtained by Stottrup [50]; Evjemo and Olsen [51] and Giesbert *et al*. [52].

The recorded total amino acid levels of *B. plicatilis* were 92.82%. Among these, amino acids such as Glutamic acid (11.40%), Isoleucine (5.21%), Leucine (6.51%), Tyrosine (4.92%), Phenylalanine (5.13%), Histidine (4.21%) and Lysine (9.75%) were found to be higher. The laboratory hatched *Artemia* nauplii showed the total amino acid level of 85.71%. Off these, amino acids such as Glutamic acid (13.24%), Glycine (8.20%), Isoleucine (4.44%), Leucine (8.65%), Tyrosine (6.96%), Phenylalanine (3.55%) and Lysine (10.05%) were noticed in higher percentage. The copepod *N. minor* was containing comparatively higher amino acid content than rotifer and *Artemia* nauplii. The recorded total amino acid level of *N.minor* was 100%. Among these, the amino acids such as Aspartic acid (9.57%), Threonine (10.50%), Alanine (10.12%), Valine (8.57%), Serine (6.37%) and Methionine (8.89%) were found to higher in copepod, *N.minor.* The essential amino acids (EAA) were recorded too high in *N. minor* (57.28%) than rotifer (48.17%) and *Artemia* nauplii (40.62%) (Table-2).

The total amino acid content of *N. minor* were higher than *B. plicatilis* and *Artemia*nauplii might be due to the physiological differences as reported earlier by Safiullah [53]. Ogino [54] stated that the various kinds of natural zooplankton are valuable protein sources judging from their amino acid composition. The present results showed that proportion of essential amino acids are higher in *N. minor* than *B.plicatilis* and *Artemia* nauplii. The results of amino acid content of copepods showed the adequate levels recommended for the finfish and shellfish larvae as agreed by Watanabe *et al*. [28]. Similar result was also reported by Santhanam [11] (2002); McAllen [55]; Aragao *et al*. [56]; Rajkumar and Vaasagam [12]; and Perumal *et al*., [57]. Similar amino acid dominance was reported earlier by Santhanam and Perumal[1] who found ten aminoacids in the cultured copepod, *O. rigida* with the high proportion of norvaline. Ashok Prabu and Rajkumar [13] who reported ten amino acids from the cultured copepod; *A. spinicauda* with the major component was asparagines.

The fatty acid content was also comparatively higher in copepod than rotifer and *Artemia* nauplii (Table-3). The recorded total fatty acid content of *N.minor* was 100%. The fatty acids such as 12:00; 14:00; 15:00; 16:00; 16:1n-7; 17:00; 18:00; 20:4n-6; 20:5n-3; 22:6n-3 was noticed predominantly in the percentages of 2.29, 26.58, 1.97, 21.64, 7.36, 3.78, 10.96, 2.84, 6.78, and 8.43 respectively. Particularly highly unsaturated fatty acids such as eicosapentaenoic acid(EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3) were reported to be high with 6.78%, and 8.43% respectively in copepod (Table-3) whereas the total fatty acid contents of rotifer and *Artemia* nauplii were 100% each. The fatty acids such as 16:00, 16:1n-7, 18:01, 14:00 and 18:2n-6 was noticed in the percentages of 26.01, 22.68, 17.3, 10.09 and 6.51 in rotifer *B. plicatilis.* The fatty acids *viz,* 18:3n-3; 16:00; 18:01 and 18:1n-9 was recorded to be high in the level of 25.51%; 22.45%; 19.2%; and 8.6% respectively in *Artemia* nauplii.

The highly unsaturated fatty acids such as EPA and DHA were recorded in the percentages of 3.31 and 0.2 in *B. plicatilis* while in *Artemia* nauplii it was reported as 4% and 2.9% respectively. Among the total fatty acids recorded in the live feeds, the copepod has been found that the maximum (67.22%) of saturated fatty acids followed by rotifer (44.63%) and *Artemia* nauplii (30.69%). However, the mono saturated fatty acids were noticed to be more (19.2%) in *Artemia* nauplii followed by rotifer (17.3%) and copepod (2.52%).The fatty acid content was higher in *N.minor* comparatively than other live feeds such as *B. plicatilis* and *Artemia*nauplii. Similar results were also observed in other copepod species by Watanabe *et al*. [58] in harpacticoid copepod *Tigriopus*; Norsker and Stottrup[59] and Nanton and Castell [60] in *Tisbe* sp. The copepods are able to produce large amounts of EPA and DHA and it may be due to the desaturating ability of copepods. This could be an adaptation to convertion of long chain essential fatty acids. Similar trend has been demonstrated for calanoid copepod species, *Acartia* sp. and *Pseudodiaptomu s*sp. which have higher fatty acid about 2 to 3 times higher than rotifers as agreed by Toledo *et al*. [25]; Santhanam *et al*. [11]; Rajkumar and Vasagam [12], Raju et al. [61], and Santhanam and Perumal [3].

The fatty acid content of copepod may be influenced by the type of algal species given as feed [45, 60]. The dietary influence on the lipid production in copepods has also been documented by Sargent and Falk Person [62] and Stottrup and Jensen [63]. In the present investigation, the docosahexaneoic acid (DHA) content of *N.minor* was higher than those found in *Artemia* and it is similar to the findings of Santhanam [11]. In the present study, palmitic acid was occupying the highest value in copepod. This has been evidenced by Kanazawa *et al*. [64-65] and Kattner *et al*. [66]. Kim and Lee [67] demonstrated that combination of DHA and EPA in the diet was more helpful to increase the growth of juvenile flounder than the diet rich in EPA. The superior nutritional value of copepods compared with *Artemia* and rotifers has traditionally been attributed to their high PUFA and particularly HUFA contents. Similar results also made earlier by several workers including Bell *et al*. [68], Rajkumar and Vasagam [12]. HUFAs are extremely high in copepod which is an important in improving the stress resistance of marine fish larvae as reported by Tocher *et al* [69].

CONCLUSION

Marine copepods are minute crustaceans and it forms a major component (more than 80%) of the ocean. Among the copepods, the sub-order calanoida are dominant in the plankton in many parts of the world's oceans. The nutritional profile of copepod *N.minor* showed a higher percentage compared to other feeds such as rotifer and Artemia. The present study concluded that the calanoid copepod *Nannocalanus minor* contains good quality of nutritional profile and can be considered as the most suitable candidate species for larval rearing of fish and shrimp. Further investigations are needed on large scale production of this species and evaluation of their suitability as live feed in commercial production of healthy seeds.

ACKNOWLEDGEMENTS

The Secretary, Principal and the authorities of Kandaswami Kandar's College (Affiliated to Periyar University, Salem), gratefully acknowledged for the facilities provided. One of the author (PS), highly acknowledged the Department of Biotechnology (DBT), Govt. of India, for funding to develop the indoor copepod culture facility through a Major Research project (BT/PR10161/AAQ/3/371/2007; dated20.06.2008).

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Table-1. Biochemical Composition of Live Feeds

Composition (%)	*B. plicatilis*	*Artemia* nauplii	*N.minor*
Moisture	85.22	83.45	71.41
Protein	62.84	62.89	63.16
Carbohydrate	18.87	15.12	20.18
Lipid	13.4	15.11	12.53
Ash	4.89	6.88	4.196

Table-2. Amino Acids Composition of Live Feeds

Amino acids	*B. plicatilis*	*Artemia* nauplii	*N. minor*
Aspartic acid	7.98	7.06	9.57
Threonine	3.6	3.51	10.5
Serine	4.7	5.31	6.37
Glutamic acid	11.4	13.24	10.1
Glycine	7.33	8.2	4.25
Alanine	5.11	4.32	10.12
Valine	6.73	3.09	8.57
Methionine	1.93	1.6	8.89
Isoleucine	5.21	4.44	2.17
Leucine	6.51	8.65	4.92
Tyrosine	4.92	6.96	2.31
Phenylalanine	5.13	3.55	2.05
Histidine	4.21	1.71	3.5
Lysine	9.75	10.05	9.04
Arginine	5.1	4.02	7.57
Total EAA	**48.17**	**40.62**	**57.28**
Total NEAA	**44.65**	**45.09**	**42.72**
Total	**92.82**	**85.71**	**100**

Table-3. Fatty Acids Composition of Various Live Feeds[a]

Fatty acids	*B. plicatilis*	*Artemia* nauplii	*N. minor*
12:00	-	-	2.29
14:00	10.09	1.53	26.58
15:00	-	-	1.97
16:00	26.01	22.45	21.64
16:1n-7	22.68	5.8	7.36
17:00	-	0.61	3.78
18:00	5.2	4.76	10.96
18:01	17.3	19.2	2.52
18:1n-9	-	8.6	3.83
18:2n-6	6.51	-	0.64
18:3n-6	0.57	-	0.38
18:3n-3	0.16	25.51	-
18:4n-3	0.16	2.2	-
20:00	3.07	0.63	-
20:2n-6	0.21	-	-
20:4n-6	3.72	1.1	2.84

20:4n-3	0.55	-	-
20:5n-3	3.31	4	6.78
22:6n-3	0.2	2.9	8.43
24:00:00	0.26	0.71	-
SFA[b]	44.63	30.69	67.22
MUFA[c]	17.3	19.2	2.52
Total n-3[d]	4.38	34.61	15.21
Total n-6[d]	11.05	1.1	3.86
Others	22.64	14.4	11.19
Total	**100**	**100**	**100**

Note: - Denotes not detected.

[a]Fatty acid values (percentage of total fatty acid methyl esters) were adjusted to express a percent of the total area identified in the chromatograms, unidentified peaks were not considered in the computations.

[b]Saturated fatty acids (SFA):12:00,14:00, 15:00, 16:00, 17:00, 18:00, 24:00.

[c]Monounsaturates fatty acids (MUFA): 18:01.

[d]Total n-3: 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:6n-3.

[e]Total n-6: 18:2n-6, 18:3n-6, 20:2n-6, 20:4n-6.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

In Silico Molecular Docking Analysis of Anti HIV-1 Rt from Indian Medicinal Plant *Hybanthus enneaspermus*

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Received: 10 Feb 2021 Revised: 18 Feb 2021 Accepted: 26 Feb 2021

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ABSTRACT

Introduction: The virtual screening approach for molecular docking small compounds into a specific protein structure could likewise be a solid apparatus for the planning of new drugs. During this work, a blended docking and neural framework approach, using a self sifting through guide, has been made and applied to screen against HIV-1 inhibitors of, HIV-1 RT, from active compounds from *Hybanthus enneaspermus* Indian medicinal plant.
Objective: This research work aimed to apply the virtual screening of the isolated bioactive compounds from *Hybanthus enneaspermus* against HIV-1 reverse transcriptase enzyme by molecular docking studies.
Methods: A total of five bioactive compounds viz. Apigenin, Quercetin, Luteolin, Quercetin 3-o-α-d-rhamnoside, Kaempferol-3-o-a-rhamnoside, were used for molecular docking studies in the direction of drug improvement towards Human Immunodeficiency Virus-1 Reverse Transcriptase (HIV-1 RT- PDB - 1FK9) by Autodock software.
Results: Among the five biomolecules, Apigenin, Quercetin, Luteolin, Quercetin 3-o-α-d-rhamnoside and Kaempferol-3-o-a-rhamnoside, the computational drug ligand binder assay reveals the bioactive (antiviral) compound as Apigenin which has highest dock score of -7.21, which has a great scope of being formulated in designing novel anti-HIV drug.

Conclusion: Molecular docking study demonstrated the bioactive of *Hybanthus enneaspermus* exhibit potential anti-HIV-1 Reverse Transcriptase enzyme endeavour and for this reason can be optimized to enhance new drug with computer-aided drug designing bioinformatics tools in the future.

Keywords: Molecular docking, HIV-1 RT enzyme, *Hybanthus enneaspermus*, Bioactive compound.

INTRODUCTION

Computer-aided molecular docking and docking research would possibly speed up drug discovery with the aid of predicting which drug-like structure must be synthesized. Molecular docking performs a necessary function in structure- based drug design [1-2].The docking helps in medicate structure and gives a decent comprehension of the system of the collaboration of the medication and target protein [3]. In molecular docking studies, the energy scores can be evaluated by the different bound conformations, with a scoring function to predict the binding mode of the receptors and ligands [4].The receptor structure is available in a precondition for the process of molecular docking [5-6]. Globally the peoples affected by Human Immunodeficiency Virus type 1 (HIV-1) infections tend to be in increasing order [7-8]. The major problem in the medical management of HIV-infected patients is the increasing potential of the resistant nature of antiviral agents in the antiretroviral therapy. To inhibit HIV-1needed the golden tool - HIV-1 Reverse Transcriptase (RT). Therefore, the scientists paying more attention to the structural changes in RT to combat the HIV resistance [9].

Without the help of Reverse transcriptase (RT), the retroviruses could not complete their life cycle. HIV-1 RT is an asymmetric heterodimer with 1000 amino acids. The enzymatic activities of the Reverse transcriptase has occurred in the p66 (largest subunit) of RT with 560 amino acid [10]. On the subunit (p66) (560aa) and p51 (440 aa) two active sites namely polymerase and ribonuclease H have been found [4, 11].. Due to their structural diversity and anti-viral mechanisms, the natural products provides the best screening for anti-HIV agents. It starts from viral entry, integration, protease inhibition and even in the intervention of the reverse transcription process, so many natural products show inhibition of the unique enzymes and proteins crucial to the life cycle of HIV. For various diseases and conditions, medicinal plants emerging as a promising alternative [12-14].

Many compounds from natural sources possessing anti-HIV-1 effects like alkaloids, sulfated polysaccharides, polyphenolics, flavonoids, coumarins, phenolics, tannins, triterpenes have been isolated and screened to inhibit HIV in every stage of the viral life cycle [14-15]. *Hybanthus enneaspermus* Linn F. Muell. (Violaceae) is a perennial herb or a shrub distributed in tropical Asia, America, Africa and Australia and occurs mostly in the warmer parts throughout the Deccan plateau of India. Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhoea, cholera, leucorrhoea, gonorrhea, dysuria, inflammation, and sterility. The plant has also been reported to have anti-inflammatory, antitussive, antiplasmodial, antimicrobial, antiviral activities [16-19]. Approximately 119 pure chemicals have been extracted from *H. enneaspermus* plants that are used in medicine throughout the world. They are very effective in the treatment of infectious disease [20]. This work is focussed on the molecular docking approaches of the HIV-1 resistance, associated with RT structure changes to find the novel molecules active HIV-1 RT with bioactive compounds.

METHODOLOGY

Bioactive Phyto Compounds Used for Study against HIV-1 RT Receptor

The selected bioactive compounds isolated from the Indian medicinal plant of *Hybanthus enneaspermus. H. enneaspermus* consist a rich in flavonoids is found to be a potential agent against HIV. The following flavonoids compounds such as, Apigenin, Quercetin, Luteolin, Quercetin 3-o-α-d-rhamnoside and Kaempferol-3-o-a-rhamnoside were isolated and used for molecular docking learn about towards HIV-1 RT. Ligand preparation was summarized in Table 1.

Crystal Structure of Reverse Transcriptase Enzyme

The 3D-structure of HIV-1 Reverse Transcriptase was retrieved from the PDB database and its PDB id is 1FK9 from organism Human Immunodeficiency Virus I (Fig. 1).

Molecular Docking Screening of Bioactive Compounds with HIV-1 RT

The 3D structure of the target HIV-1 RT enzyme (PDB ID: 1FK9) was retrieved from protein data bank 2.6 Å and the ligand molecules had been retrieved from the PubChem database. Docking research had been carried out with the aid of AutoDock. Computational procedures are progressively utilized in sedate disclosure and advancement. Procedures applied to hostile to HIV medicate investigate are delegated, ligand techniques dependent on known dynamic exacerbates that can surmise natural action, for example, old-style Quantitative Structure–Movement Relationship (QSAR), structure-put together strategies that depend concerning the 3D structure of protein receptors, for example, sub-atomic docking and sub-atomic elements, and general techniques, structure-or ligand-based, for example, 3D QSAR or 3D pharmacophore clarification. Homology displaying is normally valuable when a test 3D structure of the protein receptor isn't accessible. Computational strategies utilized in hostile to viral operator advancement and various techniques are applied to against HIV sedate improvement, receptor structure-based sub-atomic docking and ligand-based QSAR are the most much of the time utilized strategies [21-23]. For docking explores different avenues regarding AutoDock, the ligands were pre-prepared by expansion of Gasteiger fractional charges and by converging of non-polar hydrogen iotas with the assistance of AutoDock devices. Auto grid program was utilized to make the Affinity maps with 0.375 dividing. Each docking test was gotten from ten unique runs and reproductions were performed utilizing the Lamarckian hereditary calculation (LGA). The docking tests via AutoDock were both centered around the docking site on the predefined restricting site by ligand [24-25].Accelrys is a product organization headquartered in the US, with the portrayal in Europe and Japan. It gives programming to concoction look into, particularly in the territories of drug discovery and materials science [26-28].

RESULTS

Molecular docking was performed to investigate the inhibitory activity of each bioactive compound with the HIV-1 RT enzyme. To locate the best restricting posture of ligand at the dynamic site of the protein, the coupling vitality was determined by including the last intermolecular vitality and torsional free vitality. In Our exploration work uncovered that, Apigenin, Quercetin, Luteolin, Quercetin 3-o-α-d-rhamnoside and Kaempferol-3-o-a-rhamnoside docked against HIV-1 reverse transcriptase. The top scorer was found to be Apigenin with maximum binding energy -7.21 kcal/mol, in spite of despite that it was unable to make H-bond with the respective target; this showed that other intermolecular interactions were predominantly working in this case. Similarly, Luteolin was second top scorer with binding energy -7.12kcal/mol without making any H-bond. Querctin –O–α–D- Rhamnoside and Kaempferol-3-o-a-rhamnoside was were found to be top scorer with the binding energy of -6.42 kcal/mol, and -6.21 kcal/mol respectively. Finally, Quercetin was found binding energy of -5.49 kcal/ mol. The docking results of HIV-1 Reverse Transcriptase and all 5 compounds are summarized in Table: 2. In Autodock with Acceryls Discovery Studio Visualizer, Apigenin interacted well with 1FK9 of HIV 1 Reverse Transcriptase and it gave a docking score of –7.21. (Fig. 2). It made interactions with six residues, interacting with Lys102 (H-bond length 2.16Å; 3.14 Å; 3.19 Å), Lys347 (H-bond length 1.68 Å), Glu344 (H-bond lengths 2.98 Å), and Gln343 (H-bond length: 3.06Å)

In Autodock with Acceryls Discovery Studio Visualizer, Quercetin interacted well with 1FK9 of HIV 1 Reverse Transcriptase and it gave a docking score of –5.49. (Fig. 3). It made interactions with four residues, interacting with Lys190 (H-bond length 3.08Å), Tyr181 (H-bond length 3.10 Å), Pro236 (H-bond lengths 2.05 Å), and Lys101 (H-bond length: 3.12Å). In Autodock with Acceryls Discovery Studio Visualizer, Luteolin interacted well with 1FK9 of HIV 1 Reverse Transcriptase and it gave a docking score of –7.15. (Fig. 4). It made interactions with three residues, interacting with Pro95 (H-bond lengths 1.92Å), Lys103 (H-bond length 3.17Å) and Pro236 (H-bond lengths 1.91 Å).

In Autodock with Acceryls Discovery Studio Visualizer, Quercetin 3-o-α-d-rhamnoside interacted well with 1FK9 of HIV 1 Reverse Transcriptase and it gave a docking score of –6.42. (Fig. 5). It made interactions with six residues His96 (H-bond length 2.02Å; 2.59 Å; 2.72 Å), Gly93 (H-bond length 3.17Å), Gly39 (H-bond lengths 2.08Å) and Ile94 (H-bond lengths 2.18Å). In Autodock with Acceryls Discovery Studio Visualizer, Kaempferol-3-o-a-rhamnoside interacted well with 1FK9 of HIV 1 Reverse Transcriptase and it gave a docking score of –6.21 (Fig. 6). It made interactions with five residues His96 (H-bond length 2.02Å; 2.59 Å; 2.72 Å), Gly93 (H-bond length 3.17Å), Gly39 (H-bond lengths 2.08Å).and Ile94 (H-bond lengths 2.18Å).

DISCUSSION

Computational strategies give bits of knowledge into the itemized collaboration among bioactive compounds and targets, giving an exhaustive comprehension of the pharmacological exercises of mixes and data after adjustment of the drug. Different examinations have concentrated on the disclosure of potential RT inhibitors using molecular docking. The unliganded HIV-1 RT (1DLO) was utilized for the virtual screening of 4-thiazolidinone and its subordinates (Chem Bank database) by utilizing Auto Dock. One subsidiary, (5E)- 3-(2-aminoethyl)- 5-(2-thienylmethylene)- 1,3-thiazolidine-2,4-dione (CID 3087795), was found to be a promising inhibitor for HIV-1 RT with a base vitality score and the most elevated number of collaborations with dynamic site buildups. Atomic docking is additionally generally utilized in SAR contemplates, as an approach to assess the counter popular movement of newfound or synthesized compounds [24, 29-30]. The Reverse transcriptase was docked to compounds such as, 3,6-Pyridazinedione, 1,2-dihydro-4-methyl, 4-H-pyran-4 one , 2,3 dihydro-3,5-dihydroxy-6-methyl-, 1H-inden-1- one,2,3,dihydro, d-Mannose, 1,6-anhydro-a-D-gluco pyranose (Levoglucosan), m-toluicacid, allylhydrazone 2H-Pyra-2-one,5,6-dihydro-4-(2-methyl-2- propen-3yl)-, from *Enicostema littorale* [31-33]. Srivastava et al. [34] revealed that molecular docking concentrates with HIV-1RT (PDB ID 1FK9) were completed utilizing certain succinic corrosive subsidiaries. One of the subordinates 2-(diphenyl methylene) succinic corrosive was found to have restricting vitality and docking design practically identical to the initially docked efavirenz particle. On docking against HIV-1 IN, the compound of *Andrographis panniculata* (1,2-Benzenedicarboxylic acid, Diundecyl ester) showed greater binding affinity towards the enzyme and got the best ligand pose energy of -13.7605 with low toxicity. Further research on the plant *Andrographis panniculata* will be valuable in structuring drugs for hindering HIV-1 RT[3, 35].

Plant alkaloids from *Tinospora cordifolia* displays hostile to HIV action utilizing molecular docking considers. The HIV-1 protease was docked by three alkaloids in particular; jatrorrhizine, magnoflorine, and tinosporide utilizing Igemdock v2.1 The outcome shows that all the chose alkaloids had bound to the protease restraining its movement [36]. According to Ganguly and Yadav [37], Molecular docking studies of 484 pyrazole analogs were performed on NNIBP of HIV-1RT2 by using Glide-5 and eight compounds with substituents exhibited highest docking score is -13.06. Compound one also showed two hydrogen bond interactions in the NNIBP of Reverse Transcriptase (PDB ID 1RT2). HIV-1 Reverse Transcriptase (RT) is a significant catalyst supporting replication pattern of HIV. Endeavor has been made to screen zinc compound Library, Using e-HiTS software in docking process with nevirapine bound HIV-RT, 884 ligands with like properties were tested for their binding affinity towards targeted enzyme and at last 39 hits were reported as effective inhibitors of HIV-RT [38].

According to Elgindiet al. [39], molecular docking predicts the coupling proclivity of the Ligand to the protein-dependent on complex geometry. The mixes of *Mappia foetida* were docked against HIV - 1 Protease utilizing Argus Lab 4.0.1. Physiochemical properties of mixes from *Mappia foetida* were checked. 3-benzyl-6,7-diphenyl-2-methyl-4h-pyrazolo(5,1 c)(1,4)oxazine, the compound of *Mappia foeida* got best ligand present vitality of - 10.8761 kcal/mol. Comparable research was completed by Ravindra et al. [40] wherein flexible docking reproductions were performed on two arrangements of 4-thiazolidinones as HIV-1 RT inhibitors. The coupling scores for these compounds were additionally harmonious with the counter HIV action of *Hybanthus enneaspermus.* The consequences of docking examine give a knowledge into the pharmacopeia basic prerequisites for the HIV-1 RT inhibitory action of this class of molecules. From this research work, the top scorer was found to be a pigenin with maximum binding energy -7.21

kcal/mol, despite that it was unable to make H-bond with the respective target; this showed that other intermolecular interactions were predominantly working in this case. Similarly, Luteolin was second top scorer with binding energy -7.12kcal/mol without making any H-bond.

CONCLUSION

The molecular docking analysis helps in predict the new drug and gives a decent comprehension of the system of cooperation of the drug and the target protein. The current research work, molecular docking was performed by phytocompounds from *Hybanthus enneaspermus* docked with HIV-1 RT enzyme. In light of the 3D structure acquired from PDB, the molecular docking was utilized to deliver the coupling posture of best-docked structure. All out five mixes such, Apigenin, Quercetin, Luteolin, Quercetin 3-o-α-d-rhamnoside, Kaempferol-3-o-a-rhamnoside, were utilized for molecular docking concentrates against HIV-1 Reverse Transcriptase (PDB -1FK9). The top scorer was found to be apigenin with maximum binding energy -7.21 kcal/ mol, despite that it was unable to make H-bond with the respective target; this showed that other intermolecular interactions were predominantly working in this case. Similarly, Luteolin was second top scorer with binding energy -7.12kcal/mol without making any H-bond. Subsequently, this outcome it very well may be misused to build up another powerful and potential anti- HIV drug.

ACKNOWLEDGMENT

The authors would like to express their heartfelt obligation, indebtedness, gramercy and profound appreciation are made to Department of Microbiology, DST and FIST, Muthayammal College of Arts & Science (MCAS), Rasipuram, Namakkal, Tamil Nadu, India, and the authors extend their appreciation to the Researchers Supporting Project number (RSP-2020/70), King Saud University, Riyadh, Saudi Arabia, for their general facilities continuous support, for the conduction of this study.

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Table-1. Ligand preparation of docking study

Name of the bioactive compound	Molecular formula	Molecular weight	Hydrogen bond donor	Hydrogen bond acceptor	X log p3
Apigenin	$C_{15}H_{10}O_5$	270.24	3	5	1.7
Quercetin	$C_{15}H_{10}O_7$	302.23	5	7	1.5
Luteolin	$C_{15}H_{10}O_6$	286.23	4	6	1.4
Quercetin 3-o-α-d-rhamnoside	$C_{21}H_{20}O_{11}$	448.38	7	11	0.9
Kaempferol-3-o-a-rhamnoside	$C_{21}H_{20}O_{10}$	432.38	6	10	1.2

Table: 2 Docking energy of HIV-1 Reverse Transcriptase

Compound name	**Docking energy (kcal/mol)**
Apigenin	-7.21
Quercetin	-5.49
Luteolin	-7.12
Quercetin 3-o-α-d-rhamnoside	-6.42
Kaempferol-3-o-a-rhamnoside	-6.21

Fig. 1. 3D Structure of HIV-1 RT enzyme Visualized Using Rasmol (1FK9)

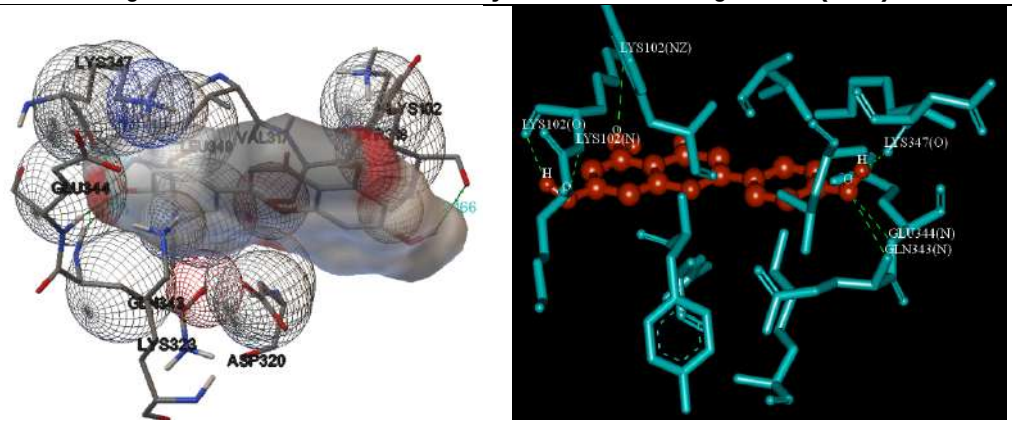

Fig. 2. (a) Interaction of Apigeninwith 1FK9interacting with docking score –7.21interacting with Lys102 (H-bond length 2.16Å;3.14 Å;3.19 Å), Lys347 (H-bond length 1.68 Å), Glu344 (H-bond lengths 2.98 Å), and Gln343 (H-bond length: 3.06Å). (b) Apigenin Binding energy, active pocket residues and hydrogen bonding revealed through docking with 1FK9.

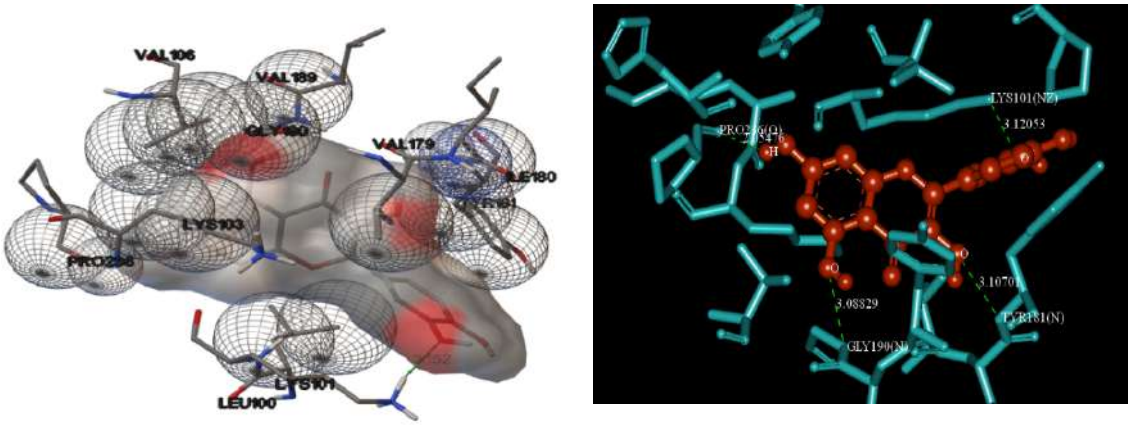

Fig. 3. (a) Interaction of Quercetin with 1FK9 interacting with docking score –5.49 interacting with Lys190 (H-bond length 3.08Å), Tyr181 (H-bond length 3.10 Å), Pro236 (H-bond lengths 2.05 Å), and Lys101 (H-bond length: 3.12Å). (b) Quercetin

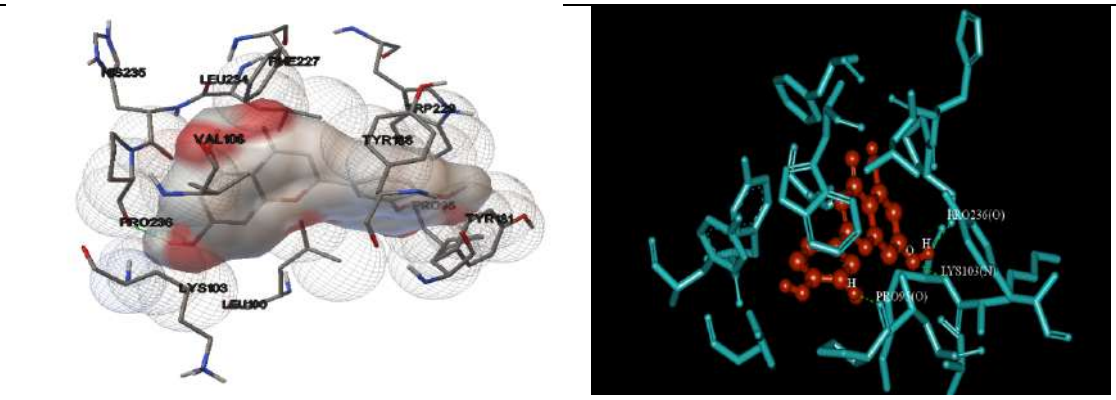

Fig. 4. (a) Interaction of Luteolin with 1FK9 interacting with docking score –7.15 interacting with Pro95 (H-bond length 1.92Å), Lys103 (H-bond length 3.17Å) and Pro236 (H-bond lengths 1.91 Å). (b) Luteolin Binding energy, active pocket residues and hydrogen bonding revealed through docking with 1FK9

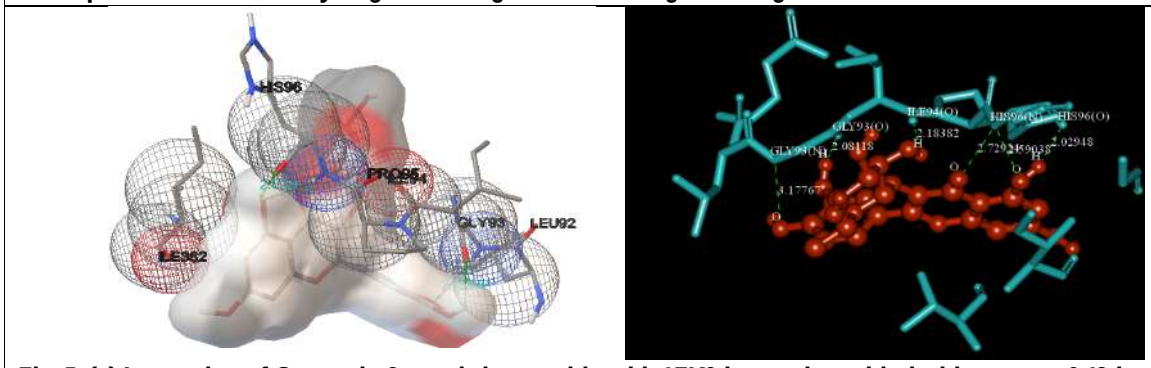

Fig. 5. (a) Interaction of Quercetin 3-o-α-d-rhamnoside with 1FK9 interacting with docking score –6.42 interacting with His96 (H-bond length 2.02Å; 2.59 Å; 2.72 Å), Gly93 (H-bond length 3.17Å), Gly39 (H-bond lengths 2.08Å) and Ile94 (H-bond lengths 2.18Å). (b) Quercetin 3-o-α-d-rhamnosideBinding energy, active pocket residues and hydrogen bonding revealed through docking with 1FK9

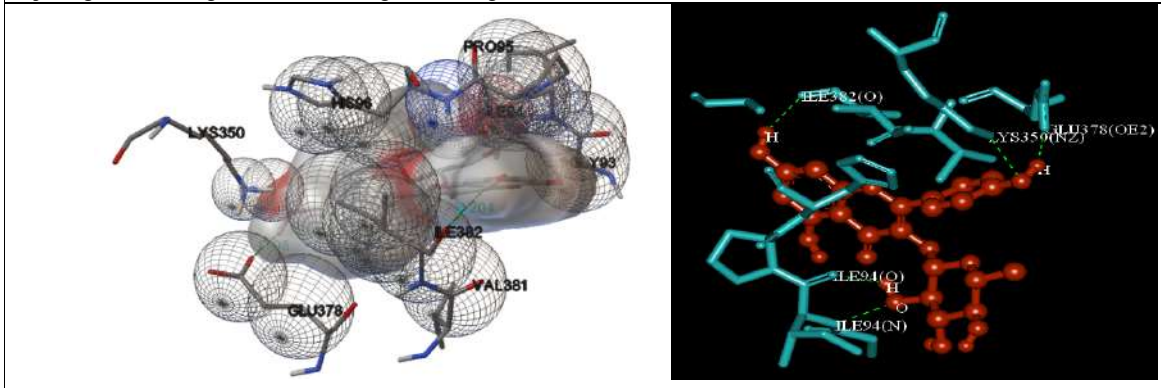

Fig. 6. (a) Interaction of Kaempferol-3-o-a-rhamnoside with 1FK9 interacting with docking score –6.21 interacting with Ile94 (H-bond length 2.50Å and 2.31Å), Ile382 (H-bond length 2.20Å), Glu378 (H-bond lengths 1.79Å) and Lys350 (H-bond lengths 2.97Å). (b) Kaempferol-3-o-a-rhamnoside, Binding energy, active pocket residues and hydrogen bonding revealed through docking with 1FK9

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Mass Culture of Marine Copepod *Nannocalanus minor*

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Received: 04 Mar 2021 Revised: 05 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

The present study aimed to develop the mass culture technique for marine copepod *Nannocalanus minor* (Claus, 1863) which is suitable live feed for larval fish. The culture has been carried out in 100 L FRP tanks for 14 days under the room temperature (28 to 32°C) and a salinity value of 35 PSU. During the culture, the copepod was fed using mixed microalgae (*Chlorella marina*, *Dunaliella salina Isochrysis galbana, Nannochloropsis salina*, *Coscinodiscus centralis, Chaetocerous affinis* and *Skeletonema costatum*) four times per day. The water exchange and uneaten food were removed weekly once. The production of copepod was estimated by sub sampling and daily mean production also estimated. After 14 days of culture period, the total population density was1, 56, 262 nos/L consisting 76265 nos/L, nauplii 58146nos/L copepodites and 21, 850 nos/L of adults. This study clearly indicate that the, current culture technique ensuring a good result in terms of copepod population and enhancement of this population might be due to the favorable water quality and food conditions provided.

Keywords: *Nannocalanus minor*; copepod; mass culture; microalgae; zooplankton; aquaculture

INTRODUCTION

Copepod cultivation is a useful basic procedure for aqua culturists, as they provide an important source of nutrition for fish fry. Copepods are small crustaceans, one of the most diverse and numerous aquatic life forms, and can be

found in both fresh and salt water environments at all stratifications. Copepods occupy a key trophic position in marine food webs, and they are responsible for the transfer of a large proportion of the energy that moves between primary producers and tertiary (and higher) trophic levels; they are a common food source for adult fishes, and their eggs (and nauplii) are preyed upon by first-feeding larval fish[1]. Continuous culture of copepod at large scale is necessary [2-3]. for supply to larval fish. Establishing larvi culture protocols that include copepods may be necessary to optimize the production of marine fish species. Copepods are the predominant prey item for a vast majority of wild marine fish larvae [4-7]. Most of available works on marine copepods culture so far reported in temperate countries. Favorable traits for mass culture of harpacticoid copepod under laboratory condition were studied by Fleeger [8]. To optimize the mass culture methods of *Nitokrala custris*in batch cultures. First, live algae, frozen algae paste and a formulated feed were analysed for their effect on the overall yield and population growth rates *of N. lacustris*in small containers of two types, trays and carboys standardized by Rhodes [9]. Payne and Rippingale [10] have studied the intensive culture of calanoid copepod *Gladioferen simparipes*. Camus [11] studied the paracalanid copepod *Bestiolina similis* in laboratory condition. Payne and Rippingale [10] have standardized the culture technique for the calanoid copepod *Gladioferen simparipes*. The first attempt for a large scale culture of *Schmackeria poplesia* and the technique was studied by Liu and Xu [12]. Ohset al. [13] has cultured the calanoid copepod *Pseudodiaptomus pelagicus.* Olivotto *et al*. [14] have cultured the harpacticoid copepod *Tisbe* spp. and maintained in the laboratory. Abolghasem *et al.* [15] have cultured the copepod *Acartiaclausi* at different salinity of the seawater. As India is concern very limited works have been done that including Merrylal James and Martin Thompson [16] who cultured the brackish water cyclopoids and used them in mariculture hatchery system. Santhanamand Perumal [17] who cultured the cyclopoid copepod *Oithonarigida* and used them as feed for *Latescalcarifer* larval rearing. Similarly, Rajkumar and Vasagam [18]; Ananth and Santhanam [19] and Ananthi *et al.*[20] have cultured calanoid, harpacticoid and cyclopod copepods respectively in laboratory scale. In this line, the present study has been made on the culture of calanoid copepod *Nannocalanus minor* in the laboratory condition.

MATERIALS AND METHODS

Algal Culture

The stock culture of marine microalgaestrains such as *Chlorella marina*, *Dunaliella salina, Isochrysis galbana, Nannochloropsis salina*, *Coscinodiscus centralis, Chaetocerou saffinis* and *Skeletonema costatum* were maintained separately in 250ml, 500ml, 1 and 2 liters of round bottom conical flasks (Fig. 1a), 5litres plastic jars and 15 liters transparent plastic buckets (Fig. 1b). The culture container contains filtered seawater at 20-23^{o}C temperature, 30‰ salinity and 7000-9000 light intensity and fertilized with Conway's medium [21] and the detailed composition were provided in Table-1. During the culture experiment, the photoperiod was provided in 12:12 hours L:D. Outdoor algal culture was maintained at 100L FRP tanks (Fig. 1c) by using commercial fertilizers *viz*., ammonium sulphate, urea and super phosphate in the ratio of 10:1:1 and the culturing techniques of algae were followed as per the Perumal et al [22].

Collection and Identification of Copepods

Copepod samples were collected (Fig. 1d) from the coastal waters of Nagapattinam, Muthupet, Parangipettai and Pichavaram during early in the morning using plankton net with 158µm mesh (0.35m mouth diameter). The collected samples were immediately transported (Fig. 1e) to the laboratory provided with vigorous aeration by using battery aerators and in the laboratory samples thoroughly rinsed to reduce the contamination from other zooplankters. From the samples, *N. minor* was identified under microscope using the key of Kasturirangan [23]. After the confirmation, 200 numbers including male and females of *N. minor* were isolated by using fine capillary tube and stocked initially in 2 and 3L plastic containers (Fig. 1f). Later copepods were transferred to an oval shaped, flat-bottomed fiberglass tank (0.54m dia, 0.81m length) filled with 100 litres of filtered seawater and vigorous aeration was given to culture.

Culture of Copepods

Seawater filtered through a membrane filter (1µm) was used for the culture of copepod. The water quality parameters such as temperature, salinity, pH and dissolved oxygen were maintained in the ranges of: 26-30^{o}C; 28-32 PSU, 7.5-8.5; 5.0-7.5 ml/l respectively (during entire rearing period) fed with a daily ration of mixed microalgae *viz*., *C. marina*,*D. salinaI. galbana, N. salina, C. centralis,C. affinis* and *S. costatum* in the concentration of 30,000cells/ml. The copepod cultures were harvested at every 14 days by gentle siphoning. The generation time of *N. minor* is about 12-14 days under optimal conditions at 26-30^{o}C and having 6 naupliar and 6 copepodite stages including the adult. Finally the adult male and female copepods were used to restart stock culture. The population density, growth and survival of nauplii, copepodite and adults were observed daily. For biological observations triplicate samples of 1ml was taken and counted for the different stages of copepods for average results.

RESULTS AND DISCUSSION

Over 14 days operation, the culture system produced an average of 2678 naulpii, 993.3 copepodites and 5,417 adults per litre during 14th day. The maximum densities of *N. minor* were 11, 265 nauplii, 10,167 copepodites and 5,417 adults per litre. For the entire culture period (2 months) the total average production 1, 56, 262 nos/l. Of these, 76, 265 nauplii, 58, 146 copepodites and 21, 850 adults per liter were produced. Copepod *N. minor* had a generation period of 12-14 days depending upon the temperature and the availability of food. The daily production rate of *N. minor* is given in Table-1. Experiment on laboratory culture indicated that a 14 days culture cycle was sufficient for maximum copepod density. In the present study, the maximum production of nauplii (11, 265 nos/l) and copepodite (10, 167 nos/ml) was attained on 11th day of culture and it could be noticed that the nauplii and copepodite production decreased 12th day onwards. However the maximum adult density (5, 417 nos/l) was noticed on final day (12th day) of the experiment. The total mean production for the 2 months period of copepod is shown in Fig. 2. The newly hatched nauplii, copepodites and the adult copepods (Fig.3a-d) were harvested from the cultures and stocked directly into larval rearing tanks.

In our system, only adult copepods were used to restart the cultures. Eggs and nauplii were not used due to the problem in separation. In the present experiment, individuals of adult male and females of copepods of the species *N. minor* were stocked in culture tanks and the mating occurred between the pairs and after mating the eggs were released and nauplii were hatched out within 24 hours. Our method is similar to previous workers who made attempts for other species *viz*. *Paracalanus* sp., *Eurytemora* sp., and *Tigriopus* sp. [24-25].The present study is similar to the work of Schipp *et al*. [26]. However, controvert from the earlier work of Stottrup*et al*. [17] who started the culture from the separated eggs of culturing animals. Stottrup *et al*. [17] reported that adults of *Acartiatonsa* were stocked in culture tank and the eggs were released in tank and they were siphoned from the bottom of the culture tanks and then transported to hatching tanks within 24 hours to avoid egg cannibalism. There are numerous laboratory studies on the effect of the quality and food concentration on the egg- production rates, growth and development on copepod *N.minor* also made in the present experiment which provided ideas for maintenance of the copepod as supported by Calliari and Tiselius [28]; Hassett [29] and Stottrup and Jensen [30].

A few studies have examined the production of copepods at a large commercial scale [31]. Culture of *Oithona aculata* as a food for marine fish larvae was made by Hernandez Molejon and Alvarez- Lajonchere [3]. Copepod stock cultures of *Tisbe* spp. were maintained in a temperature controlled room (26 ± 0.5 °C) in four different 100 L tanks with gentle aeration under the following conditions: 30‰ salinity, pH 8.2, NO_2 and NH_3<0.03 mg/L. A 14:10 light-dark cycle was maintained in the culture room and water quality was continuously monitored [33]. In the present study, the mixed microalgae have been used to feed copepod which shown maximum density as agreed by Camus *et al.* [11] who used mixed microalgae for the culture of *Bestiolina similis*.

Liu and Xu[12]suggested that the optimal culture condition for *Schmackeria poplesia* is to maintain the copepods at a salinity of 20g L^{-1}, temperature of 25°C and feed with mixed algal prey of *Isochrysis galbana and Phaeodactylumtricor nutum* with the cell ratio of 1:1 at a concentration of 10^5 cells/ml, this optimal condition has been used for the large scale of this copepod. Calanoid copepod *Gladioferen simparipes* nauplii were collected and culture maintained at five algal diets by Payne and Rippingale [34]. The effects of feeding different densities of T-iso diet on the development of the calanoid copepod *Pseudodiaptomus pelagicus* was studied by *Ohs et al* [13]. The attempt has been made to cultured copepod in large outdoor tanks by Ohno *et al* [35]. The laboratory culture of copepod *A. clausi* was achieved with maximum density at 35‰ salinity [15]. Matias-Peralta *et al* [36] cultured the marine copepod *Nitocraaffinis* and established a reliable, highly productive system. In this system, within six weeks (42 days) author has found a minimum of 43.6×10^3 copepod.L^{-1} and maximum of 44.5×10^3 copepod.L^{-1}.

Drillet *et al.*[37]stated that many important and novel parameters that could help the improvement of copepod cultivation techniques. The author recommended that scientific research to understand catabolic liquid pathways in various copepod species would help in developing inexpensive food items for copepods able to bio-convert fatty acids. Also the need for understanding mating process, sex changes, sex ratio and chemical communication between copepods seems obvious and the amount of effort should needed in these areas. In the present study the male and females were stocked and copepods were allowed to mate and the spawned eggs were left in the same tank until they hatched out, because our species of *N. minor* are herbivores. The present culture method is more similar to Santhanam [38] who cultured copepod *O.rigida*, Rajkumar *et al.*[39]in *Acartiaclausi*, Ananth and Santhanam [19]in *Macrosetella gracilis* and Ananthi *et al.*[20] in *Pseudodiaptomous* sp. with mixed microalgae such as *C.marina*, *I.galbana*, *Nannochloropsis* sp.,*Dunaliella* sp.,and *Tetraselmis* sp. The presently obtained copepod density is enough to produce require numbers of fish larvae as agreed by Schipp*et al.*[26]; Payne and Rippinglae [31]and Santhanam [38].

CONCLUSION

Marine copepods are minute crustaceans and it forms a major component (more than 80%) of the ocean. Among the copepods, the sub-order calanoida are dominant in the plankton in many parts of the world's oceans, making up 55-95% of plankton samples. The present study result the successful culture of calanoid copepod *Nannocalanus minor* in mass scale and they have good survival, growth, high reproductive capacity and it can be yielded more density under culture condition. Therefore, copepod *Nannocalanus minor* can be considered as the most suitable candidate species for pilot scale production for sustainable aquaculture practice.

ACKNOWLEDGEMENTS

The Secretary, Principal and the authorities of Kandaswami Kandar's College (Affiliated to Periyar University, Salem), gratefully acknowledged for the facilities provided. One of the author (PS), highly acknowledged the Department of Biotechnology (DBT), Govt. of India, for funding to develop the indoor copepod culture facility through a Major Research project (BT/PR10161/AAQ/3/371/2007; dated 20.06.2008).

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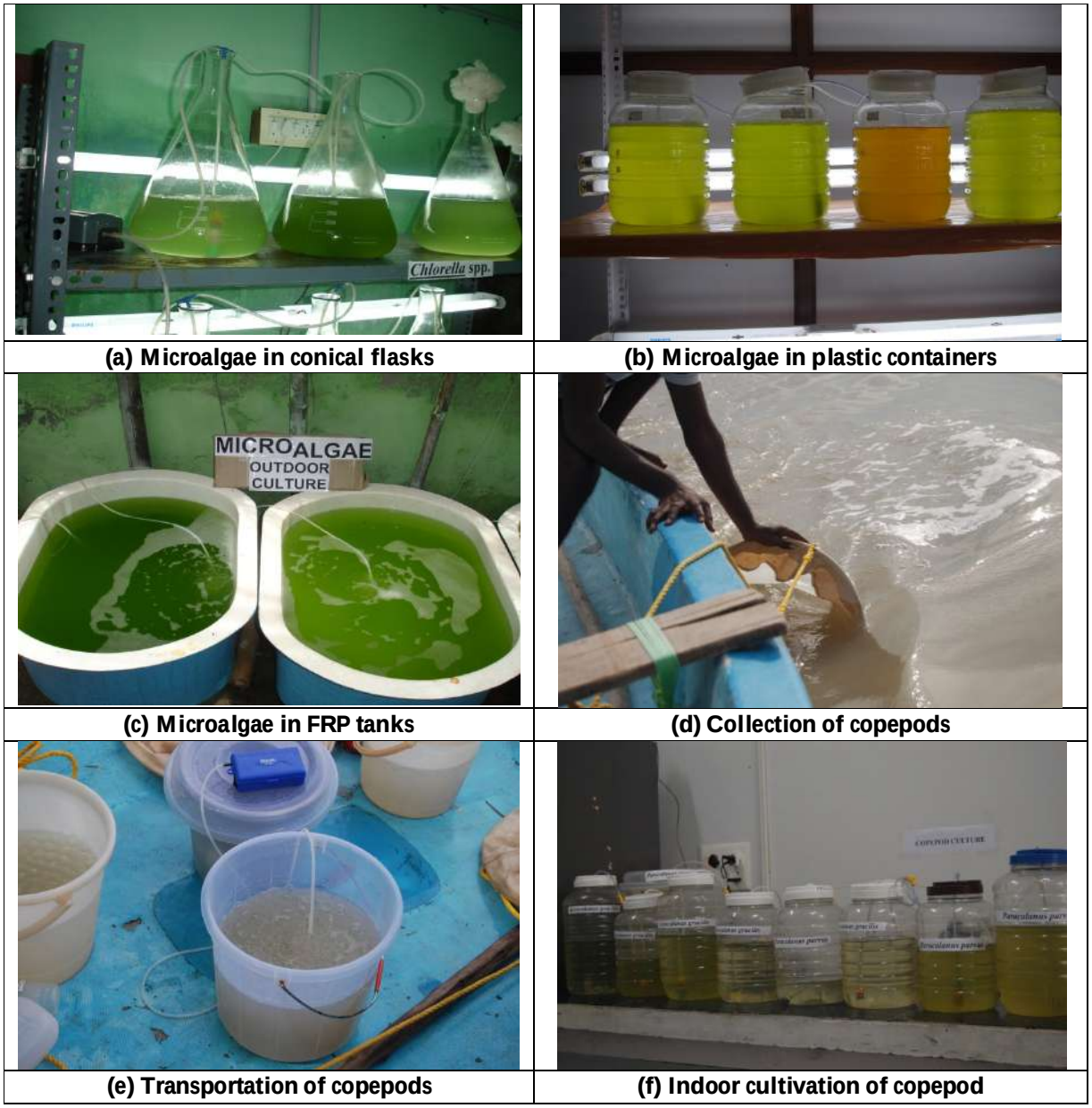
Table- 1. Culture media compositions

Components	Quantity
Solution - A	
Potassium nitrate	100gm
Sodium orthophosphate	20gm
EDTA (Na)	45gm
Boric acid	33.4gm
Ferric chloride	1.3gm
Manganese chloride	0.36gm
Dissolved in 1000 mL of double distilled water	
Solution - B	
Zinc chloride	4.2gm
Cobalt chloride	4.0gm
Copper sulphate	4.0gm
Ammonium molybdate	1.8gm
Dissolved in 1000 mL of double distilled water	
Solution - C	
Vitamin B1 (Thiamin)	0.01g
Vitamin B12 (Cyanocobalamine)	0.01g
Biotin	0.0002g
Dissolved in 100 mL of double distilled water	

Table-1. Daily mean production of copepod, *N. minor*

Rearing days	Nauplii(nos./L)	Copepodite(nos./L)	Adults (nos./L)
0	0	0	200±0
1	0	0	200±0
2	1153±2.309	0	200±0
3	2236±1.528	1039±13.32	193±4.726
4	3658±1.528	2289±5981	153±6.11
5	4586±3.512	3300±53.05	0
6	5236±2.52	4646±117	0
7	6908±4.041	5347±129.87	0
8	7596±2	7089±92.418	975±49.003
9	8364±3	7214±50.123	1440±87.74
10	10627±5.131	9152±330.94	1801±67.35
11	11265±3.214	10167±494.79	2395±433.68
12	6595±4.041	4659±369.5	3575±103
13	5363±3.79	2251±206	5302±181
14	2678±3.512	993±54.64	5417±261



(a) Microalgae in conical flasks **(b) Microalgae in plastic containers**

(c) Microalgae in FRP tanks **(d) Collection of copepods**

(e) Transportation of copepods **(f) Indoor cultivation of copepod**

Fig 1. Maintenance of indoor cultivation of microalgae and copepod

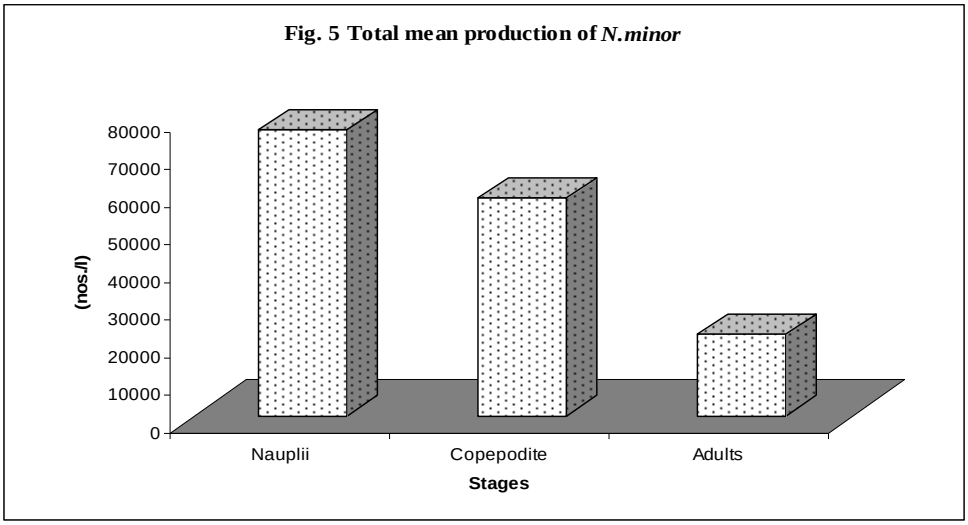


Fig 2. Total mean production of *N. minor*

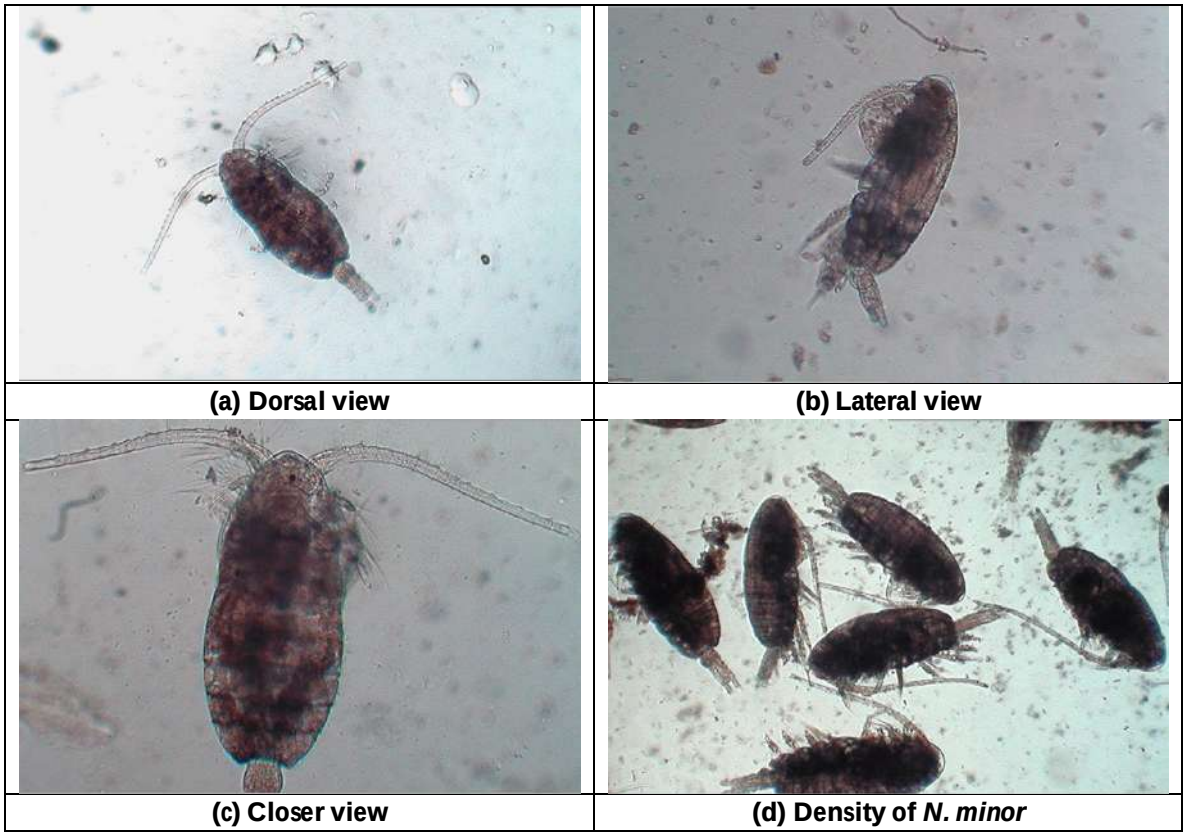


Fig 3. Microscopic views of *N. minor*: Dorsal view (a); Lateral view (b); Closer view (c); Density of *N. minor* (d)

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Design, Development and Validation of a Green Ultraviolet Spectrophotometric Analytical Method for the Evaluation of Metformin HCl - Okra Gum Composite Mucoadhesive Microspheres

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Received: 16 Jan 2021 Revised: 01 Feb 2021 Accepted: 24 Feb 2021

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ABSTRACT

Introduction: Diabetes mellitus, a metabolic disorder characterized by increased blood sugar level. Metformin hydrochloride is used to treat type I Diabetes mellitus. Metformin hydrochloride chemically 1, 1-dimethylbiguanide hydrochloride, is white crystalline powder, hygroscopic and freely soluble in water, Officially UV spectrophotometric method used for estimation of Metformin Hydrochloride from the bulk and tablets formulations.

Objective: Develop and validate a simple, rapid, accurate, economic and precise UV/VIS method for Metformin Hydrochloride in bulk and microsphere formulation.

Methodology: A cheaper ecofriendly solvent is essential so various aqueous solvent ranges including water, 0.3% NaCl, 0.1N HCl and 0.3% NaCl in 0.1N HCl (Simulated gastric fluid) were analyzed. Mucoadhesive microspheres were formulated using Okra gum and Metformin HCl by ionotropic gelation technique.

Conclusion: Among different solvents simulated gastric fluid (SGF) showed better results, hence water was selected as a solvent for the proposed method. Metformin Hydrochloride showed maximum absorbance at 221 nm. The recovery studies found in the range of 99- 101 %. The validation study performed conformed linearity, accuracy, precision, ruggedness, robustness, and solution stability. The method will serve as a alternative green estimation method were no hazardous solvents were used

sample preparation, cost effective and suitable for the evaluation of Metformin Hydrochloride from bulk and polymer based dosage forms.

Keywords: Metformin HCl; UV-Spectrophotometry; Okra gum; Mucoadhesive microsphere.

INTRODUCTION

Metformin HCl is a biguanidine class of antidiabetic drug (Fig.1) prescribed orally for the treatment of non-insulin-dependent diabetes mellitus [1-4]. UV spectrophotometric assay of metformin HCl in immediate release tablets is is done as per British Pharmacopoeia (BP) and United States Pharmacopoeia (USP) [5]. RP-HPLC method was utilized for the assay as per USP monograph for Metformin tablets [6]. Few analytical methods have been published in the literature for the estimation of Metformin HCl in combined dosage forms [7-19]. These methods work well for Metformin tablets which do not contain controlled release polymers used for microspheres and found unsatisfactory for the assay of Metformin HCl in Metformin polymer based formulations due to poor recovery of Metformin during the preparation of sample solution. Many methods were reported for the evaluation of commercial tablets of Metformin HCl but there were no method for the quantification of Metformin HCl microspheres using Okra gum natural polymers. The reported method uses water or 0.1N Hcl as solvent were electron withdrawing chloride groups will interfere with the absorbance characteristics of Metformin HCl. Moreover simulated gastric fluid contains sodium chloride which will interfere with the absorbance characteristics. It was found by extensive review that the solvent problem was not studied in detail. Hence development of UV spectrophotometric method using the exact simulated gastric fluid prepared will give accurate results which can be correlated with the human gastric fluid for the quantification Metformin HCl. Therefore, the objective of the present study was to replace the solvents of the BP and USP methods with exact simulated gastric fluid to obtain accurate quantification of Metformin HCl in bulk and natural polymer based microsphere formulations.

MATERIALS AND METHODS

Reagents and materials

Metformin HCl pure drug was obtained from Micro Labs Ltd, Hosur, India. The Calcium chloride and sodium alginate used was of analytical reagent (AR) grade (Spectrochem, India). Sodium chloride is of general purpose (GR) grade (Merck, India), ethanol (99 % v/v, GR grade, Qualigens, Mumbai, India), acetone (Merck, USA) were also used. Purified water was collected from Millipore water system (Elix 10 C model) was used for the solvent system. UV Spectrophotometer a Systronics UV-visible spectrophotometer (model 2202) was used for UV spectrophotometric experiments.

Isolation of Okra gum

Procedure: About 1kg of fresh immature fruit of Okra (*Abelmoschus esculentus*) was purchased from a local market of Salem, Tamilnadu, India. After removal of the seeds, the fresh immature fruits were sliced, and chopped in to fine pieces nd soaked in deionised water. It was boiled at 60^{o}C for 6 hours and cooled at room temperature. The crude mucilage was collected without cell debris. Equal volume of acetone was added to equal volume (1:1) Okra mucilage and the precipitated mucilage was collected. The precipitated gum was washed several times with acetone; the obtained cream colored mucilage was dried in oven and stored in a desiccators. A light brown colored powder was obtained after complete removal of moisture. The dried gum was pulverized using Grinder and screened through 80# stainless steel sieve. This was stored in a well closed amber colored specimen bottle till ready for use. The yield of crude *Abelmoschus esculentus* gum was 8-10% from fruits [20].

Formulation of Metformin Loaded Okra gum based Mucoadhesive microspheres

Procedure: Mucoadhesive microspheres of Metformin Hydrochloride was prepared as per the formulation design depicted in the table

Inotropic gelation method: Aqueous phase was formed by slowly adding 2% sodium alginate in 20ml of distilled water and stirred with magnetic bead at constant temperature of 50-60°C. To the above mixture Okra gum powder at different concentration was added as per the formulation design given in the Table.1 with continuous stirring. Accurately weighed 500mg of Metformin Hydrochloride (drug) was added in each formulation with continuous stirring at 35-40°C. Stirring is continued to dissolve and make a uniform viscous solution. The mixture was sonicated for 15 m minutes to remove entrapped oxygen. Prepare a 5% w/v solution of calcium chloride in deionizer water and sonicated for ten minutes for complete dissolution. Aqueous phase was slowly added through 22# needle in the 3 and 5% calcium chloride solution within 15min with continuous stirring through magnetic bead at room temperature. The prepared beads were given a curing time of 30min in the cross linking solution for the completion of formulation process. The procedure was repeated for all the mucoadhesive microsphere formulations. After the stipulated curing time the microspheres were filtered and washed two times with 50 ml distilled water and collected. Collected microspheres were dried in a Hot air oven at 37-40°C till completely dried [21].

Preparation of standard solutions for the proposed method

Preparation of simulated gastric fluid: Accurately weigh 3 gm of sodium chloride in 100ml of 0.1N HCl and sonicated for 15 minutes to aid complete dissolution of sodium chloride. The entire content is diluted in to a 1450ml with deionised water. Transfer adequate amount of 0.1N HCl slowly in to the container containing sodium chloride until to adjust the pH of 1.2 ± 0.1. This composition is used as simulated gastric fluid for further course of UV Spectrophotometric analytical method development [22].The effect of shift in absorption maxima was determined for future reference. MFN in 0.3% Sodium chloride in 0.1N HCl The absorption maxima of the simulated gastric fluid (0.3% Sodium chloride in 0.1N HCl) with pH 1.2 was investigated and utilized to derive linear calibration curve of Metformin Hydrochloride.

Determination of λmax in SGF

Measurement of spectral characteristics: A 10µg/mL concentration of Metformin Hydrochloride was prepared from the mother stock of 1000µg/mL concentration in the selected solvents to study the effect of different solvents and select the solvent for the research work. The sample was scanned in the UV region from 200-400nm and their characteristic wavelength with maximum absorbance (λmax) was determined.

Preparation of standard Metformin HCl solution using SGF (Solution 1): Accurately weighed 100 mg of Metformin HCl API was transferred into a clean dry 100 mL volumetric flask with 70 mL of SGF and made up to 100 mL. The mixture was sonicated for 15 minutes. It was followed by dilution with SGF (99 %v/v) and the filtrate was diluted to 100 mL with SGF (standard solution containing 100 µg/mL of Metformin HCl). !0mL of the standard stock was diluted to 100 mL using SGF to obtain the standard solution with the working stock concentration of 10 µg/mL of Metformin HCl.

Preparation of microsphere sample solution using SGF for UV spectrophotometric determinations

Accurately weighrfd 100mg of MFN was powdered and the microsphere powder equivalent to about 100 mg of Metformin HCl was accurately weighed into a dry 100 mL volumetric flask. About 70 mL of SGF was added, sonicated for 15 min with intermittent shaking and the volume was made up to 100 mL with ethanol (99 %v/v). This solution was filtered and the filtrate (10 mL) was diluted to 100 mL with water to get a solution of 100 µg/mL of

Metformin HCl. Further, 10 mL of Solution 5 was diluted to 100 mL with water to obtain a solution containing 10 µg/mL of Metformin HCl for UV spectrophotometric method.

Assay of Metformin Hydrochloride Mucoadhesive Microspheres

Accurately weighed 100mg equivalent of MFN in Mucoadhesive microspheres was transferred to a clean dry glass mortar and thoroughly homogenized. Appropriate amount was mixed with SGF to get a concentration 1.0mg/ml. It was sonicated for 15 minutes and filtered through a whatmann filter paper No.1. Specified amount of standard stock was transferred to get a concentration of 10, 20 and 30 µg/ml. The absorbance of the sample is measured at 221nm. The amount of MFN in mucoadhesive microspheres was determined by interpolating the sample absorbance in the standard calibration curve. Then amount and percentage purity of tablet formulation was determined.

Method validation [23]

The UV spectrophotometric was validated for specificity, linearity and range, precision, accuracy, robustness and solution stability according to USP and ICH guidelines.

Specificity: The specificity of the method was evaluated by spiking Metformin hydrochloride into the placebo containing starch as an additive and checking the interference of the placebo in the assay of Metformin HCl.

Linearity and range: The linearity and range of the method was established by recording the response of standard preparation at 5 different concentrations prepared in the range of 1 to 50 µg/mL of median concentration. The linear regression analysis of the data was done by the method of least squares. The correlation coefficient values have been reported.

Precision: The reproducibility of the method was determined by measuring the repeatability (intra-day precision) and the intermediate precision (inter-day precision) studies. Precision was calculated from nine determinations of the test Metformin HCl microspheres on same day (intra-day precision) and on two different days (interday precision). The SD and inturn % RSD of the estimated content of Metformin HCl was determined.

Accuracy: A known amount of pure Metformin HCl was added to the formulations and the accuracy of the method was determined by estimating the percentage recovery of Metformin HCl the spiking of Metformin HCl was done at three different concentration levels (i.e., 80 to 120 % of median concentration). The percentage recovery range and the corresponding % RSD at each level was determined.

Robustness: Robustness was established by varying the chromatographic conditions with respect to flow rate, pH of buffer, % of organic modifier and wavelength. Standard and sample solutions were injected and % Metformin content was calculated under these modified conditions.

Solution stability: The solution stability of the standard and sample solutions was studied for 24 hours at ambient temperature in the lab. The percentage difference absorbance of standard solution and sample solution were analysed.

Statistical analysis: The % RSD value and linear regression analysis by the method of least squares were calculated using Microsoft Excel 2003 application.

RESULTS

Linearity range for Metformin HCl is in the range of 1-50 µg/ml. The coefficient of correlation was at 221 nm is 0.997 (Fig. 1 & 2). The assay estimated an amount of 497.00mg with the percentage drug content of 99.40±0.60 mg (Table. 2)

.The results of % recovery of Metformin HCl from a microsphere are presented in Table 3. The results of precision studies were derived as repeatability and intermediate precision is shown in Table 4 & 5. The relative standard deviation of robustness, solution stability and system suitability (Table 6, 7 & 8) was within the were all within the acceptance criteria. The collective data of validated validation are presented in Table 9.

DISCUSSION

The recovery of Metformin HCl from a mixture of Metformin HCl microsphere at simulated gastric fluid 99.58 to 101.40 % w/w. Okra gum and alginate used for microsphere preparation was totally removed by filtration in the first step of sample preparation in the proposed method, thereby preventing interaction. The simulated gastric fluid was used as solvent in the research work will match the exact human gastric contents and delivers an accurate result for the quantification of Metformin HCl in mucoadhesive microsphere formulations. Thus, the proposed method which utilized SGF as solvent and validated in terms of accuracy, linearity, specificity, range, robustness and stability of solution.

CONCLUSION

The developed method which incorporates a change in the solvent used in the BP/USP methods for the preparation of microsphere samples would lead to accurate quantification of Metformin HCl in the presence of polymers like Okra gum and sodium alginate. The developed method was successfully validated and should be used for the assay of Metformin hydrochloride in Metformin HCl loaded Okra gum based mucoadhesive microspheres.

ACKNOWLEDGEMENT

This work was supported by Vinayaka Mission's College of Pharmacy, VMRF (DU) Salem, and Tamilnadu, India.

CONFLICT OF INTEREST

The authors don't have any conflict of interest.

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Table 1: Linearity Data

Concentration (µg/mL)	Absorbance at 221nm
1	0.014
3	0.046
5	0.082
7	0.108
9	0.132
10	0.144
20	0.307
30	0.459
40	0.599
50	0.709

Table 2: Analysis of Metformin loaded Microspheres and tablet dosage forms

S.No	Formulations	Labeled claim (mg)	Estimated amount (mg)	Percentage Content (%w/w)
1.	MFNMA5	500	497.00	99.40±0.60
2.	MFNT1(GLYCOMET)	500	502.95	100.50±0.50
3.	MFNT2(GLCYIPHAGE)	500	505.10	100.59±0.41

Table 3: % Recovery Data

Levels	MFNMA5 (mg)	MFN added (mg)	Estd. Amt. (mg)	Amnt. Recovered (mg)	Recovery		SD	% RSD
					% w/w	Mean % w/w		
80%	100	80	179.68	79.68	99.60	**99.58**	0.3151	0.3955
	100	80	179.98	79.98	99.97			
	100	80	179.35	79.35	99.18			
100%	100	100	198.65	98.65	98.65	**99.35**	0.6310	0.6351
	100	100	199.54	99.54	99.54			
	100	100	199.87	99.87	99.87			
120%	100	120	220.24	120.24	100.20	**101.04**	1.0359	0.8543
	100	120	221.20	121.20	101.00			
	100	120	222.31	122.31	101.92			

Table 4: Repeatability Data

Concentration (µg/mL)	Absorbance At 221nm			SD	% RSD
10	0.144	0.143	0.144	0.000577	0.401623
20	0.307	0.310	0.309	0.001528	0.495031
30	0.459	0.459	0.458	0.000577	0.458667

Table 5: Intermediate precision Data

SET	1.	2.	3.	4.	5.	6.	7.	SD	% RSD
Interday	0.144	0.144	0.145	0.144	0.145	0.144	0.144	**0.0009**	**0.6826**
Intraday	0.144	0.142	0.143	0.144	0.145	0.144	0.143	**0.0012**	**0.8461**

Table 6: Robustness Data

Sl.no	220nm	221nm	222nm
1	0.141	0.144	0.141
2	0.140	0.144	0.141
3	0.141	0.144	0.142
4	0.141	0.143	0.143
5	0.142	0.141	0.144
6	0.143	0.142	0.142
Mean	**0.141**	**0.143**	**0.142**
SD	**0.001033**	**0.00126**	**0.00116**
%RSD	**0.7326**	**0.8846**	**0.8169**

Table 7: System Suitability Data

SET	221nm
1	0.144
2	0.144
3	0.144
4	0.143
5	0.144
6	0.144
Mean	**0.144**
SD	**0.0004082**
%RSD	**0.2833**

Table 8: Solution stability stud

Time	Standard	Sample
0	0.144	0.144
1	0.144	0.142
2	0.143	0.143
3	0.145	0.142
4	0.144	0.142
5	0.144	0.142
6	0.142	0.141
Mean	**0.142**	**0.142**
SD	**0.000951**	**0.000951**
% RSD	**0.6697**	**0.6697**

Table 9: Comparative Validation Data of UV Spectroscopic method

S.No	Parameters	Units	Data
1.	Accuracy	%w/w	99.35-101.04
2.	Precision		
	Repeatability	% RSD	<0.5%
	Interday	% RSD	0.6826
	Intraday	% RSD	0.8461
3.	Linearity	(µg/mL)	1-50
4.	Range	(µg/mL)	1-50
5.	LOD	(µg/mL)	0.05822
6.	LOQ	(µg/mL)	0.1764
7.	Specificity	-Specific	Specific
8.	Robustness	% RSD	< 2%
9.	System suitability	% RSD	0.2833
10.	Solution Stability	% RSD	0.6697

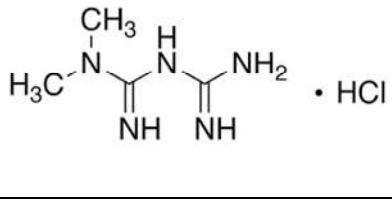

Figure 1: Structure of Metformin HCl

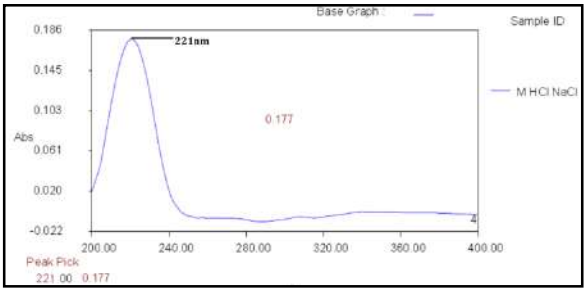

Figure 1: UV Spectrum of Metformin Hydrochloride in 0.3% NaCl in 0.1N HCl

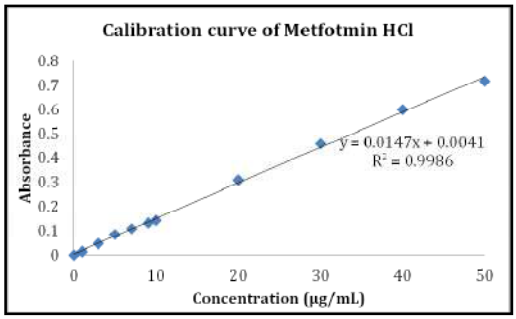

Figure 2: Linearity curve of Metformin hydrochloride pure drug

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Formulation and Assessment of Ofloxacin Hydrogel for Ophthalmic Use

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Received: 12Nov 2020 Revised: 24 Nov 2020 Accepted: 31 Dec 2020

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ABSTRACT

The present research work deals with the formulation and evaluation of hydrogel system based on sol-to-gel transition for ophthalmic delivery of an antibiotic ofloxacin, to overcome the problems of poor bioavailability and therapeutic response exhibited by conventional formulations. ofloxacin an antibiotic drug preferentially used in the treatment of infections caused by herpes simplex virus, which is the main cause of viral conjunctivitis .so in the present study ophthalmic hydogels of ofloxacin were prepared to treat viral conjunctivitis. Carboxymethyl cellulose was used as the thickening agent. The prepared formulations were evaluated for pH, viscosity, drug content, gelling strength, *In vitro* drug release, antimicrobial studies, of the developed formulations respectively. The developed formulation can be used as an hydrogel vehicle to enhance ophthalmic bioavailability and helped in the reduction in the frequency of instillation thereby resulting in better bioavailability

Keywords: Hydrogel, Carboxymethyl Cellulose, Ofloxacin, Ophthalmic Drug Delivery

INTRODUCTION

Delivery of medication to the human eye is an integral a part of medical treatment. Ophthalmic drug delivery is one of the foremost interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology, and biochemistry of the attention render this organ highly impervious to foreign substances. a big challenge to the formulator is to bypass the protective barriers of the eye without causing permanent tissue damage. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents still provide ocular delivery systems with high therapeutic efficacy. In general, hydrogels can be prepared from either synthetic polymers or natural polymers. The synthetic polymers are hydrophobic in nature and chemically stronger compared to natural polymers. Their

mechanical strength results in slow degradation rate, but on the other hand, mechanical strength provides the durability as well. These two opposite properties should be balanced through optimal design Water-soluble linear polymers of both natural and synthetic origin are cross-linked to form hydrogels in a number of ways. Ofloxacin is a quinolone/fluoroquinolone antibiotic. Ofloxacin is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. Ofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria [1].

MATERIALS AND METHODS

Materials

Ofloxacin pure drug was were given gifted sample from Micro Lab, Hosur. CMC (carboxymethyl cellulose) Rasayan Trading Co., Ahmedabad, Propylene Glycol Acuro Organics Limited., New Delhi, Glycerin, Shreya Chemicals., Bangalore, Tween 80 Mohini Organics Pvt. Ltd., Mumbai, Methyl Paraben Heera Lakshmi Pharma Agencies., Mumbai, pH Phosphate buffer pH 6.8 Sisco research Laboratories., Mumbai, Distilled Water (q.s) VMCP., Salem.

Scanning of Model Drug (Ofloxacin)

10 mg of pure model drug (Ofloxacin) was dissolved in water and was diluted to give concentration of 10µg/ml and was scanned 290 nm to 300 nm fort the determination of λ_{max}. The wavelength of 292 nm was selected as for λ_{max}. The same was used for further analysis of drug solution and absorbance of final standard solution was also measured at 292 nm[2].

Preparation of pH 6.8 Phosphate Buffer

Dissolve 28.8g of disodium hydrogen phosphate and 11.45g of potassium Di hydrogen phosphate in distilled water and then make up the volume to 1000ml with distilled water.

Preparation of Calibration Curve

10mg of pure Ofloxacin drug was taken in a 10ml standard flask and dissolved in distilled water. The volume of stock solution was made up to 10 ml with pH 6.8 phosphate buffer. From the above stock solution, 1 ml was transferred into a 10ml volumetric flask and volume was adjusted to 10 ml that corresponded to 100µg/ml Losartan in solution. From that solution different aliquots of 0.2, 0.4, 0.6, 0.8, 1.0 and1.2 ml were transferred to 10ml volumetric flask and volume was adjusted to 10ml with ph 6.8 phosphate buffer, which gave a concentration of 2, 4, 6, 8,10 and 12 µg/ml respectively of final standard[3].

Fourier Transform Infrared (FTIR) spectroscopy

Drug excipient interaction studies are significant for the successful formulation of every dosage form. Fourier Transform Infrared (FTIR) Spectroscopy studies were used for the assessment of physicochemical compatibility and interactions, which helps in the prediction of interaction between drug and other excipients. In the current study 1:1 ratio was used for preparation of physical mixtures used for analyzing of compatibility studies. FT-IR studies were carried out with a Bruker, ATR FTIR facility using direct sample technique [4]

Composition of Ofloxacin Hydrogel Formulation

Different formulations were prepared with a various concentration of CMC as described in the drug solution was added to the hydrogel base while stirred. So that no foam was observed. The buffer solution was added to the formulation, and the following by addition of distilled water up to 100 ml. Formulations that have been made were stored in 10 ml closed vials. This formulation was terminally sterilized by autoclaving at 121°C for 15 mins [5].

Evaluation of Hydrogel

Gel Strength Determination

Gel strength is indicative of the tensile strength of the gelled mass. It signifies the ability of the gelled mass to with stand the peristaltic movement. The gel strength of the formulation is an important variable dependent on the type and concentration of the polymer, combination of polymers, gas generating agent and cation source (CaCO3).The method to measure gel strength of gelled mass was modified; by using fabricated gel strength apparatus and it was done triplicate as shown in Solution of 5 ml was taken in the cylinder followed by addition of 25 ml of GF 0.1 N HCl (pH 1.2) for gelation. After gelation the HCl was drained off leaving the formed gel mass, and then the device was rested on to surface of the gel. At the free end of the device a light weight pan (4 g) was attached to which the weights were added. The gel strength was reported in terms of weight required to pass the apparatus through the formed gel mass

Viscosity Measurements

The viscosity of the prepared solutions were measured out using sample of 100ml. Measurements were performed using suitable spindle number 64 and sheared at a rate of 3, 4, 5, 6, 10, 12, 20, 30, 50, 60, 100rpm, and the temperature was maintained at 37°C. The viscosity was read directly after 30 seconds. All measurements were made in triplicate. The rheological velocity was explained by plotting viscosity against angular velocity. This method is applied for the prepared formulations and for the marketed conventional Ofloxacin solution (20mg/5ml) [6].

pH Measurement

The pH of the prepared solution for all formulations was measured by digital pH meter at 25 + 0.5^{o} C after it is calibration using standard buffer solutions of pH 4, 7, 9 then the measurements of pH were recorded [7].

Determination of Drug Content

Accurately, 5 ml of liquid solution (containing 0.20 mg of the drug) from all formulations was taken and to which 70 ml of 0.1N HCl was added, then the sample was sonicated for 30 min until clear solution is made. The volume completed to 100 ml and filtered using whatman filter paper No. 41. From this solution, 1ml sample was withdrawn and diluted to 10 ml with 0.1N HCl. Contents of Ofloxacin was determined spectrophotometrically at 302 nm using double beam UV-Visible spectrophotometer [8].

In-Vitro Drug Diffusion Study

In-vitro drug release studies of the formulations were conducted using Franz diffusion cell apparatus. Egg membrane isolated from egg by placing in dil. HCl was used to study the invitro diffusion of the formulations. The receptor chamber was filled with freshly prepared buffer of pH 6.4. The donor compartment was loaded with 1gm of formulation. Aliquots of receptor medium were withdrawn and replenished with fresh medium at specific intervals of time and analyzed spectrophotometrically for the drug content [9].

Release Rate Kinetics to Dissolution Data

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, and Korsmeyer-Peppas release model.

Zero Order Release Rate Kinetics

To study the zero–order release kinetics the release rate data are fitted to the following equation.

$F = K_o t$

Where, 'F' is the drug release at time't', and 'K_o' is the zero order release rate constant. The plot of % drug release versus time is linear.

First Order Release Rate Kinetics

The release rate data are fitted to the following equation

Log (100-F) = kt

A plot of log cumulative percent of drug remaining to be released vs. time is plotted then it gives first order release.

Higuchi Release Model

To study the Higuchi release kinetics, the release rate data were fitted to the following equation.

F = k t1/2

Where, 'k' is the Higuchi constant.

In higuchi model, a plot of % drug release versus square root of time is linear.

Korsmeyer and Peppas Release Model

The mechanism of drug release was evaluated by plotting the log percentage of drug released versus log time according to Korsmeyer- Peppas equation. The exponent 'n' indicates the mechanism of drug release calculated through the slope of the straight Line.

$M_t / M_\infty = K t^n$

Where, M_t / M_∞ is fraction of drug released at time 't', k represents a constant, and 'n' is the diffusional exponent, which characterizes the type of release mechanism during the dissolution process. For non-Fickian release, the value of n falls between 0.5 and 1.0; while in case of Fickian diffusion, n = 0.5; for zero-order release (case I I transport),n=1; and for supercase II transport, n > 1.In this model, a plot of log (M_t / M_∞) versus log (time) is linear[10].

Antimicrobial Activity

Antimicrobial efficiency studies were carried out to ascertain the biological activity of hydrogel systems. This was determined in the agar diffusion medium employing Cup plate technique. Sterile solution of marketed formulation was used as a standard. The standard solution and the developed formulations (test solution) were taken into separate cups bored into sterile nutrient agar previously seeded with *Staphylococcus aureus* organisms. The gels were allowed to diffuse for two hours and then the plates were incubated for 24 hrs at 37°C. The zone of inhibition (ZOI) was compared with that of the standard [11]

Stability Studies

Stability of a drug can be define as the time from the date of manufacture and the packaging of the formulation, until its chemical or biological activity is not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously.

In any design and evaluation of dosage forms for drugs, the stability of the active component must be a major criterion in determining their acceptance or rejection [12]

RESULTS AND DISCUSSION

UV- Spectra Photometric Study

Standard Curve (Λ –Max) Ofloxacin Hydrogel

Antimicrobial Activity

The optimized hydrogel formulations showed antimicrobial activity were studied in microbiologically by the Cup-Plate technique. The results are shown in Fig. 19 indicate that marketed formulation retained its antimicrobial efficacy when formulated as an Optimized hydrogel formulation.

Stability Studies

Stability study test are used to find out whether the formulations are maintaining their quality during the storage period or not. Stability study tests are used to find out the best formulation. It can be studied by applying a stress to the formulation such as temperature, humidity and light. Here stability study was conducted for F4 at 40ºC/75%RH.The control tests were carried out at the end of the one month.

CONCLUSION

The ofloxacin hydrogel ophthalmic preparations formulated with CMC have shown a good characteristics, and acceptable sustained released profile that may extend absorption of the drug for ensuring an optimum bioavailability at the site of action. The finding of this study indicated that ofloxacin hydrogel ophthalmic preparations has to give the better results.

ACKNOWLEDGMENT

The authors are thankful to Vinayaka Mission's Research Foundation (Deemed to be University) & Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu for extending their support and facilities for this research.

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S. No	Ingredients (%)	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)
	Oofloxacin	0.5	0.5	0.5	0.5
2	CMC	0.2	0.3	0.4	0.5
3	Propylene Glycol	5.0	5.0	5.0	5.0
4	Glycerin	2.0	2.0	2.0	2.0
5	Tween 80	0.5	0.5	0.5	0.5
6	Methyl Paraben	0.02	0.02	0.02	0.02
7	pH Phosphate buffer ph 6.8	5	5	5	5
8	Distilled Water (q . s)	100	100	100	100

Table No: 1 Result of identification of drug.

S.No	Properties	Results
1	Description	Powder
2	Odour	Odourless
3	Colour	Off White
4	Reading in thermometer(°C)	178-180°C

Table No: 2 Results of Melting point determination test of drug

Reported melting point	Observed melting point
184-189°C	184-186°C

Table No: 3 Evaluations of Ofloxacin Hydrogel

Formula No.	Gel strength (N/m^2)	Content uniformity (%)	pH	Viscosity (cps)
F1	12.14	98.66	6.58	3498
F2	10.24	97.25	7.12	5621
F3	11.02	95.79	7.34	6874
F4	13.69	99.12	7.25	9756

Table No: 4 In-vitro release data for Ofloxacin Hydrogel

Time(hrs)	F1	F2	F3	F4
0	0	0	0	**0**
0.5	20.32	30.04	13.15	**26.63**
1	29.53	46.56	17.41	**32.63**
2	48.90	52.35	22.98	**46.52**
3	45.96	56.52	25.09	**51.31**
4	54.14	63.75	28.54	**57.25**
5	63.85	75.54	35.36	**65.77**
6	79.92	84.26	42.67	**68.15**
7	83.51	86.64	48.95	**73.56**
8	85.36	87.54	53.77	**78.28**
9	87.28	88.54	56.42	**86.65**
10	89.14	90.14	62.02	**89.85**
11	91.60	93.15	65.46	**93.28**
12	92.14	95.42	78.39	**98.86**

Table No: 5 Release kinetics data for optimized formulation (F4)

Time(hr)	% of Drug Release	% of Drug Remaining	Log% Drug Release	Log% Drug Remaining	Log Time	Square root of time
0	0	100	0	2.000	0	0
0.5	3.11	75.37	0.493	1.986	-0.301	0.707
1	7.15	69.37	0.854	1.968	0.000	1.000
2	14.21	57.48	1.153	1.933	0.301	1.414
3	27.54	49.69	1.440	1.860	0.477	1.732
4	35.45	41.75	1.550	1.810	0.602	2.000
5	45.21	34.22	1.655	1.739	0.699	2.236
6	53.77	31.83	1.731	1.665	0.778	2.449
7	59.34	26.43	1.773	1.609	0.845	2.646
8	66.73	21.73	1.824	1.522	0.903	2.828
9	77.69	13.36	1.890	1.348	0.954	3.000
10	85.54	10.13	1.932	1.160	1.000	3.162
11	91.15	6.71	1.960	0.947	1.041	3.317
12	95.49	1.15	1.980	0.654	1.079	3.464

Table No: 6 Stability studies

Parameters	Controlled	After 15 days	After 1 month
Appearance	White	white	White
Ph	7.0	7.0	7.0
Viscosity	33254±516 cps	33255±515 cps	33255±515 cps
MOISTURE CONTENT %	35.69	35.70	35.69
SPREADIBILITY (cm)	7.8	7.8	7.7

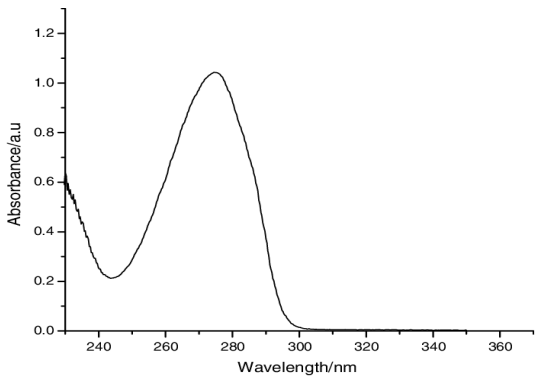

Fig. No: 1 Standard curve of ofloxacin

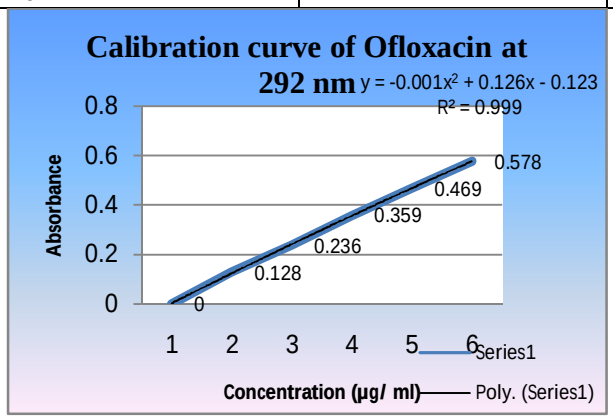

Fig.No:2 Calibration curve of Ofloxacin at 292 nm

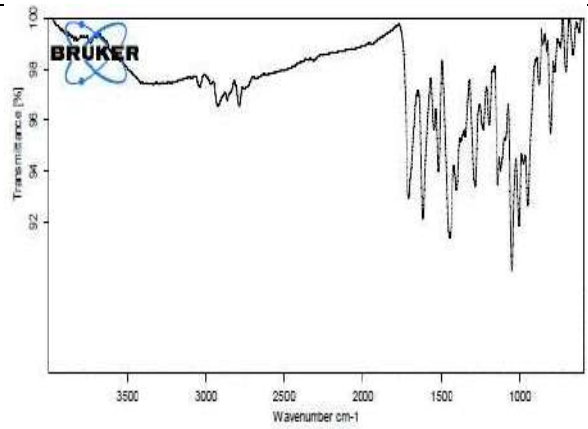

Fig.No:3 FTIR Graph of Pure Drug

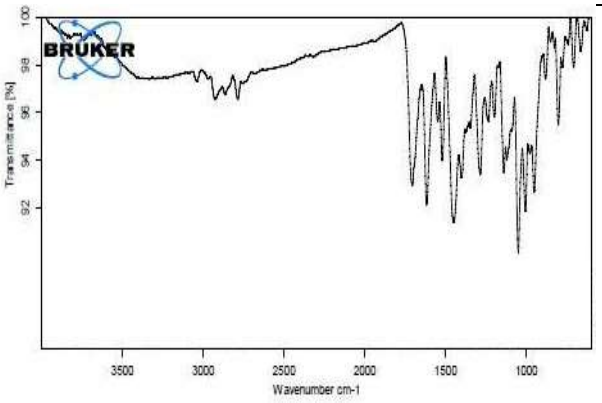

Fig.No.4: FTIR Graph of Drug with Excipients Formulation

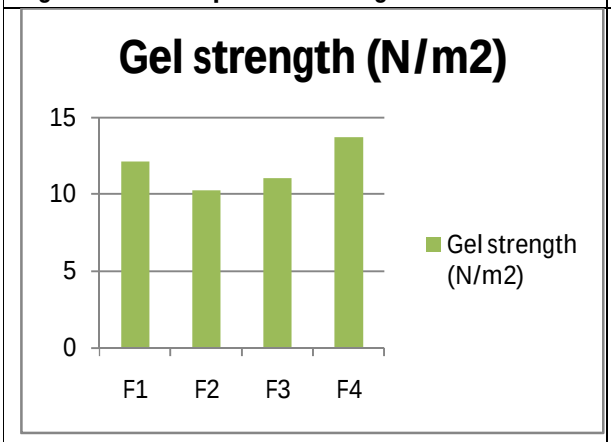

Fig.No:3 Gel strength

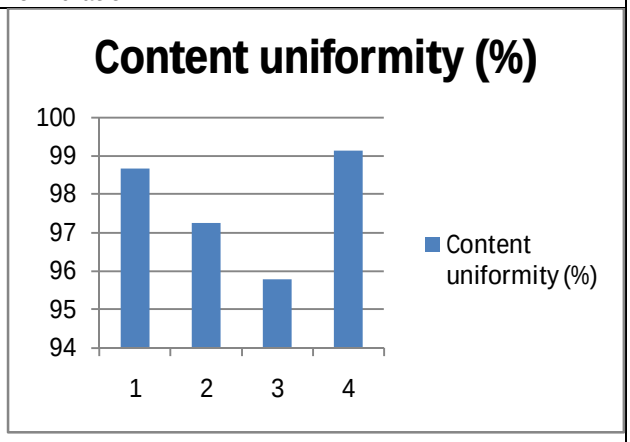

Fig.No:4 Content uniformity

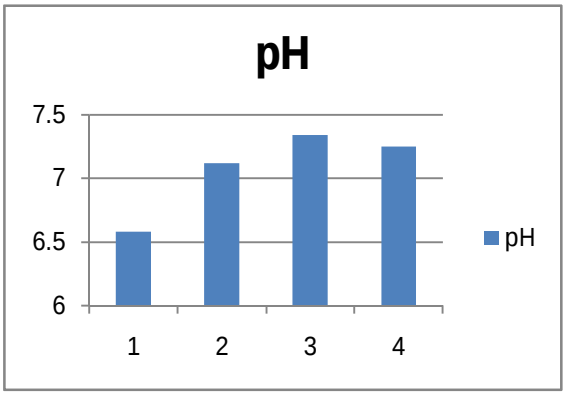

Fig.No:5 pH

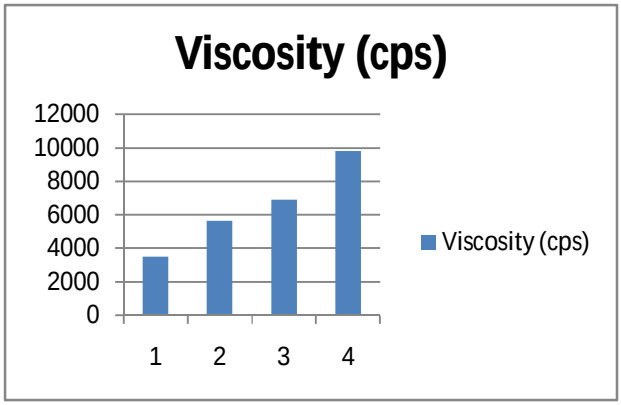

Fig.No:6 Viscosity

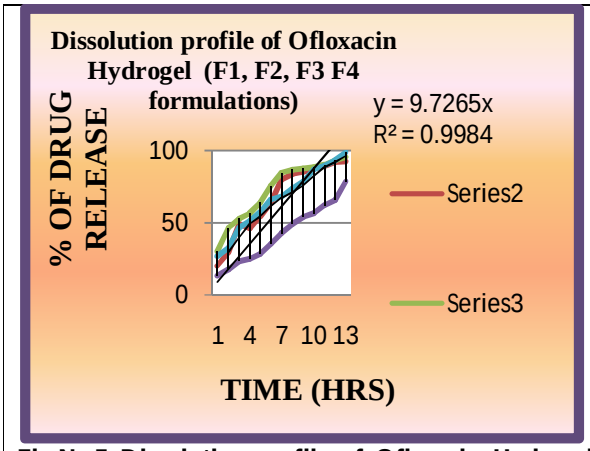

Fig.No:7 Dissolution profile of Ofloxacin Hydrogel (F1, F2, F3 F4 formulations)

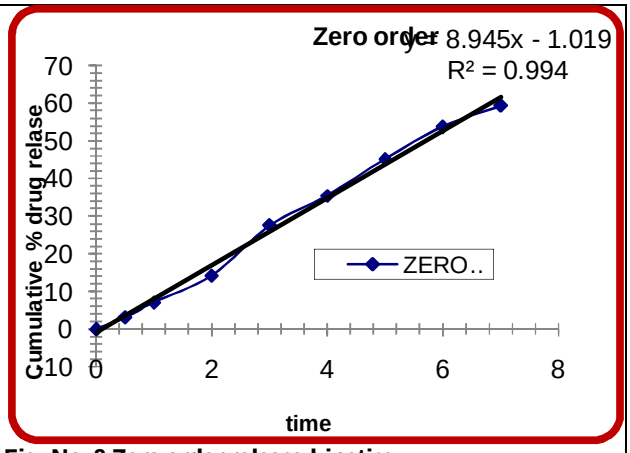

Fig. No: 8 Zero order release kinetics

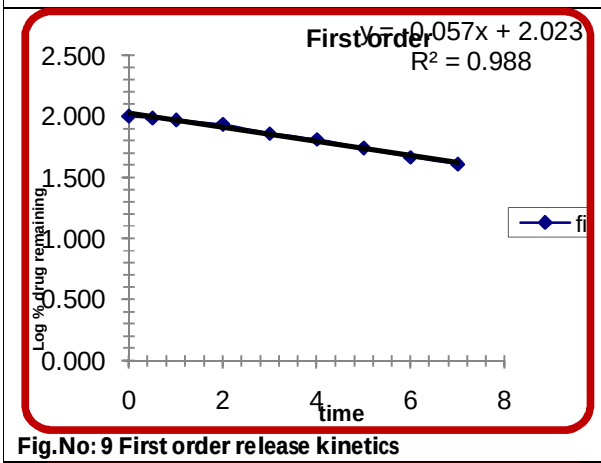

Fig.No: 9 First order release kinetics

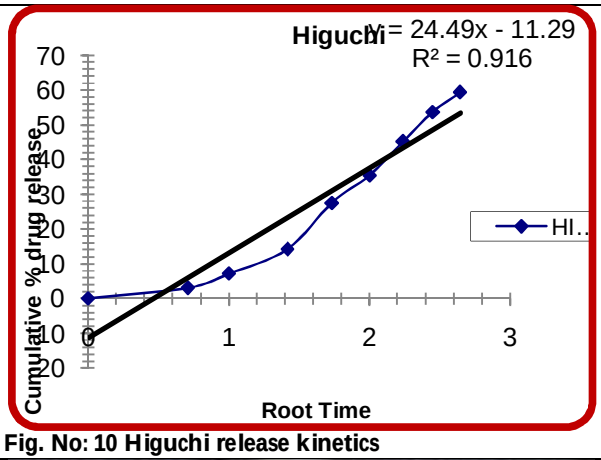

Fig. No: 10 Higuchi release kinetics

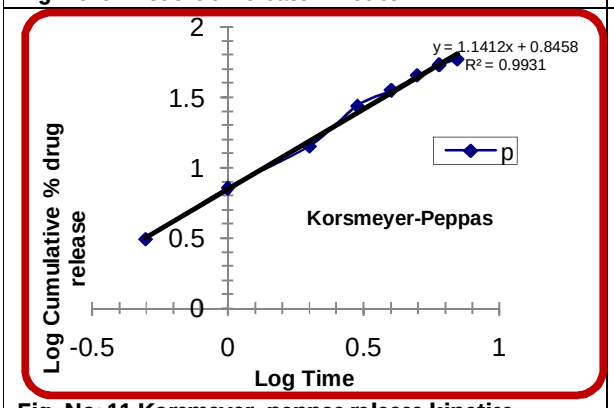

Fig. No: 11 Korsmeyer -peppas release kinetics.

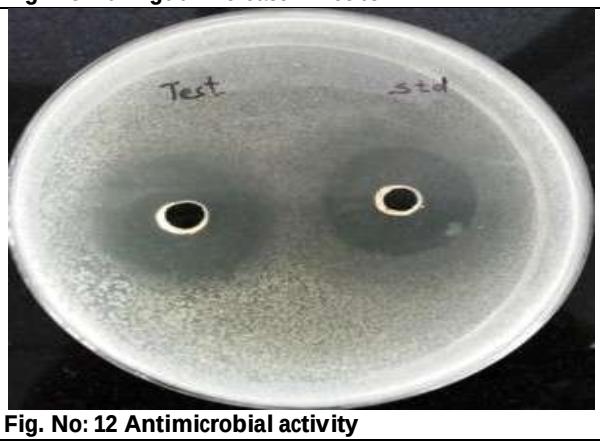

Fig. No: 12 Antimicrobial activity

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Weed seeds Tolerance to Water Stress at the Germination Stage using *Polyethylene Glycol 6000*.

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Received: 01 Mar 2021 Revised: 04 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

The present study was conducted to evaluate the behavior of different weed species under water stress effects at the germination stage. Nine species (*Torilis arvensis* (Huds.) LinK., *Lactuca serriola* L., *Bromus madritensis* L., *Centaurea diluta* Ait. *Algeriensis* Cross. & Dur., *Convolvulus arvensis* L., *Vicia monentha* Retz., *Hordeum murinum* L., *Sinapis arvensis* L.,*Datura stramonium* L.) were used for germination under controlled conditions. Water stress was simulated using polyethylene glycol 6000 (PEG 6000), according to different levels of water potential (-0.03, -0.1, -0.7, -1, -1.6 MPa).Water stress has a negative effect on the germination potential of the nine species; the germinative behavior of these species under water stress, differs according to the species and the concentration of the PEG 6000 applied for most studied variables. High water potential (-0.03 MPa) increase the germination of *Bromus madritensis* L and *Hordeum murinum* L. Indeed, a maximum of 86% was achieved for the same species to High water potential (-0.03 MPa). By contrast, at the lowest water potential (-1.6 MPa), the percentage of germination was reduced and was less than 4% (*Lactuca serriola* L. et *Vicia monentha* Retz. and *Sinapis arvensis* L.). With these results, we have found a superiority of tolerance between species; there are tolerant, moderately tolerant and sensitive species. However, germination time is slightly higher for all species in high water stress levels.

Keywords: water stress, germination, weeds, tolerance, polyethylene glycol 6000.

INTRODUCTION

One of the biggest challenges in agriculture is the management of water, widely considered the greatest limiting resource for crops [1]. This limitation is especially important in the arid environments. In the field, the cultivated

plants and weeds take share in influencing the water balance [2]. The presence of weeds in crops may be harmful in many ways. The competition for water, mineral elements and light, directly affects the growth of the crop and its yield [3]. They are adapted to the same soil and climatic conditions similar crops. Practices that promote cultures also favor weeds [4] Persistence of a weed is largely influenced by climatic, edaphic (soil) and biotic factors which affect its occurrence, abundance, range and distribution [5]. Plants improve their adaptability to thrive in arid environments mainly by evolving several mechanisms of drought escape, tolerance, and avoidance [6]. Successful establishment of weeds depends heavily on their ability to evolve stress tolerance, and weeds tend to exhibit an increasing tolerance to stress than crops [7]. Weeds compete for water, cut water availability, and contribute to crop water stress [8]. In most cases, weeds are more tolerant to drought than crops, leading to reduced crop and increased weeds in drought years [9]. Drought stress is one factor that can affect the germination of resistant and susceptible weeds [10], because water is not only an obligatory condition but also a trigger of germination [11].The answer of seeds to drought could also be an indicator of the tolerance of plants for the later stages of development. Therefore, there have been attempts at germinating under variable stress conditions to identify the populations which adapt to dryness [12].The present work consists of evaluating the behavior and tolerance of 09 species weeds at the germination stage under different levels of simulated water potential using a solution of polyethylene glycol 6000 (PEG 6000)

MATERIAL AND METHODS

Plant Material

Mature, healthy and equal sized seeds of weed were previously disinfected by immersion in a calcium hypochlorite solution, containing 5% active chlorine, for one minute. The seeds were then washed three times with sterilized distilled water. The experiments were carried out on seeds of Nine weed species (*Torilis arvensis* (TOAR), *Lactuca serriola* (LASE), *Bromus madritensis* (BRMA), *Centaurea diluta* Ait. Algeriensis Cross. (CEDI), *Convolvulus arvensis* (COAR), *Vicia monentha* (VIMO), *Hordeum murinum* (HOMU),*Sinapis arvensis* (SIAR),*Daturastramonium* (DAST). The seeds used in the germination tests were collected from cultivated fields of the Dehamcha region (located 45 km northeast of the city of Sétif, 36 ° 22 ′ 56 ″ North, 5 ° 35 ′ 43 ″ East, Latitude: 36.3821, Longitude: 5.5953, Altitude: 933 m, Mediterranean climate with hot summer). These tests were carried in the Plant Resource Valorization laboratory at the level of the research unit of Ferhat Abbas University (Sétif 1).

Water Stress with Peg 6000

Osmotic solutions are used to impose water stress reproducibly under in vitro conditions [13]. Water stress was simulated using polyethylene glycol 6000 (PEG 6000), according to different levels of water potential (-0.03, -0.1, -0.7, -1, -1.6 MPa), which were prepared adding polyethylene glycol (PEG 6000) to distilled water according to Michel and Kaufman (1973) [14] to have the osmotic potential in PEG. PEG 6000 is a relatively stable, inert, nonionic, but well soluble in water agent. It is non-toxic even in high concentrations, in the dark and at the optimum temperature for germination (25 ° C). The PEG maintains a stable and uniform water potential throughout the experimental period. Indeed, the molecules of PEG 6000 constitute a more efficient means to simulate a water stress. The choice of this osmotic agent is justified by its advantages, namely an inert, neutral product, not affecting the pH and having a high molecular weight. It does not penetrate the seeds and does not seem to have any interference or side effects[15].

Germination Tests

Seed germination tests were carried out in sterilized petri dishes with Whatman No.1 filter paper.The dishes were placed in germination chamber for ten days, in dark and at 25°C. Seed germination results from the elongation of embryonic axis cells [11].Seeds were considered germinated when the radicle emerged with at least 2 mm long. Number of seeds germinated was manually counted on each day up to 10 days and the final germination percentage and rate were estimated.

Measured Variables

The following parameters were calculated for all nine species:

Percentage of germination or germination capacity (Pg): This is the percentage of seeds capable of germinating under well-defined conditions:

Average germination time (AGT) (days): Estimated by the formula of Kotowoski (1926) in [16]:

$AGT = (\Sigma niti)/ \Sigma ni$,

where: n = number of germinated seeds per day and ti = incubation time (days).

Statistical Analysis

The data were processed by analysis of variance, and the means were compared using SPSS V.23 test. Software package was used for the calculation and graphing.

RESULTS AND DISCUSSION

Water stress has a negative effect on the germination potential of the nine species; the germinative behavior of these species under water stress, differs according to the species and the concentration of the PEG 6000 applied for most studied variables. However, germination time is slightly higher for all species in high water stress levels.Significant differences have been found among both PEG treatments and species weeds in terms of germination percentage, with species × treatment interactions being also significant ($p < 0.00$). Although the Average germination time (AGT) considerably varied among the species. Results showed the final germination rate and percent germination of weed seed significantly affected by PEG 6000 (Figure 1). By increasing osmotic potential of PEG 6000, percent, rate and final germination were decreased. In distilled water, percentage of seed germination was highest. High water potential (-0.03 MPa) increase the germination. Indeed, a maximum of 86% was achieved for the same species to High water potential (-0.03 MPa). By contrast, at the lowest water potential (-1.6 MPa), the percentage of germination was reduced and was less than 40%. Drought is an important factor that negatively affects plant growth. PEG-6000 was used to induce drought stress as it can modify the osmotic potential of nutrient solution cultures [17].

The results obtained show, in the species studied, very clear differences in germination capacity in each treatment (Figure 2). However, according to the results of analysis of variance, these are the significance for the interactions. The steady decrease in germination rates, depending on the treatments and also the species in the rows, probably made the interaction of the species * treatment statistically significant. The polyethylene glycol (PEG)-induced inhibition of germination has been attributed to osmotic stress [18]. According to Ayaz *et al.*[19], decrease in seed germination under stress conditions is due to some metabolic disorders. Increasing drought stress levels caused delay in seedling emergence as a result of reduced cell division and plant growth metabolism. The results that we have shown in Figure 3 correspond to the average germination time for the 9 weed species. The average germination time (AGT) considerably varied among the species. It is slightly higher for all species in high water stress levels and more particularly for species with the lowest rate

Concerning the average germination time, there are no significant differences between the species studied, it lengthens slightly depending on the intensity of the water stress (Figure 4).The first physiological disorder, which takes place during germination, is the reduction in imbibition of water by seeds which leads to a series of metabolic changes, including general reduction in hydrolysis and utilization of the seed reserve [20]. The germination delay caused by the increasing concentrations of PEG 6000 would result from a difficulty in hydration of the seeds due to a high osmotic potential and can be explained by time required for the seed to set up mechanisms allowing it to adjust its inert osmotic [21]. It could also be a lack of hydration of the seeds following a high osmotic potential leading to an

inhibition of the mechanisms leading to the exit of the radicle out of the seed coats and consequently a delayed germination of the seeds [22,23].

Effect of Water Stress on the Germination Behavior of Seeds of the 09 Species Studied

Significant differences in germination percentage between the weed species under the different water stress levels were observed (Figure 5). High water potential (-0.03 MPa) increase the germination of (TOAR) and (DAST). Indeed, a maximum of 86% was achieved for (BRMA) and (HOMU) to High water potential (-0.03 MPa). By contrast, at the lowest water potential (-1.6 MPa), the percentage of germination was reduced and was less than 4% (LASE, VIMO and SIAR).

By increasing osmotic potential of PEG 6000, percent final germination was decreased. In distilled water, percentage of seed germination was highest. The higher amount of PEG 6000 concentration in this research (-1.6 MPa) completely inhibit seed germination (SIAR). According to Mirbahar *et al.* [24] it has been suggested that several factors involved in seed quality such as the age of seed, environmental conditions during development, harvest and storage conditions may also affect the germination.

Lowering the water potential decreases the germination capacity and increases the average germination time for different species. These results allow us to classify the species into 3 groups: Referring to Mnif *et al.* [25]:

- Group 1: species tolerant to water stress this group contains the species *Bromus madritensis* L. and *Hordeum murinum* L. which show good germination (about 97%) up to P4 (-1.6 MPa).

- Group 2: moderately tolerant species. The species of this group *Torilis arvensis*, *Centaurea diluta* Ait.*Algeriensis* Cross. &Dur., *Convolvulu sarvensis* are characterized by an average germination rate compared to the species of group 1. They are qualified as species relatively sensitive to water stress since a decrease in germination rates is noticed from the level of -1.0 MPa. This decrease is accentuated towards -1.6 MPa.

- Group 3: species sensitive to water stress. This group is represented by the species *Lactuca serriola*,*Sinapis arvensis* L., *Daturastramonium* L**.** and *Vicia monentha* which shows low germination rates (3.3%) especially at the level of -1.6 MPa.

CONCLUSIONS

The present study was conducted to evaluate the behavior of different weed species under water stress effects at the germination stage. Nine species (*Torilis arvensis*(Huds.) LinK.,*Lactuca serriola* L.*, Bromus madritensis* L., *Centaurea diluta*Ait. *Algeriensis* Cross. & Dur.,*Convolvulus arvensis* L., *Vicia monentha* Retz.*, Hordeum murinum* L.,*Sinapis arvensis* L., *Daturastramonium* L**.**) were used for germination under controlled conditions. Water stress was simulated using polyethylene glycol 6000 (PEG 6000), according to different levels of water potential (-0.03, -0.1, -0.7, -1, -1.6 MPa).Water stress has a negative effect on the germination potential of the nine species; the germinative behavior of these species under water stress, differs according to the species and the concentration of the PEG 6000 applied for most studied variables. With these results, we have found a superiority of tolerance between species; there are tolerant ($Pg \geq 50\%$), moderately tolerant ($10\% \geq Pg \geq 50\%$) and sensitive species ($Pg \leq 10\%$). On the other hand, the widespread prevalence and great competition imposed by weeds is also due to the ability of their seeds to exploit the water in the soil, which explains its high competitiveness. Our study suggests that, based on the analysis of osmotic stress tolerance, seeds of weedshould not have great difficulty in germinating in arid or semi-arid regions. In conclusion, we can say from our study that once the seeds of weedare released from their dormancy, they are able to germinate in a wide range of water stress. Consequently, any near or long-term strategy for weed management should take into account species that are resistant to water stress, or in other words the most exploited water sources in the soil.

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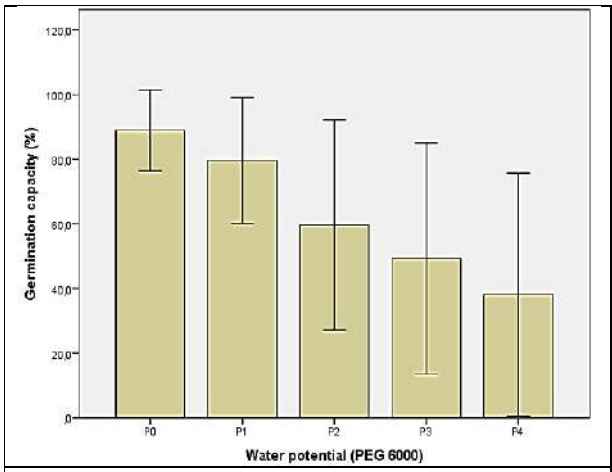

Figure 1: Effect of water potential (P_1:-0.03, P_2:-0.7, P_3:-1, P_4:-1.6 MPa). on the percentage of germination (Pg).

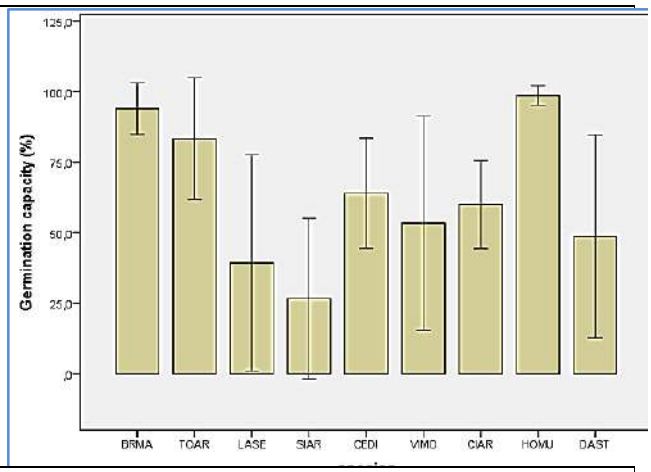

Figure 2: Effect of the species on the percentage of germination (Pg).

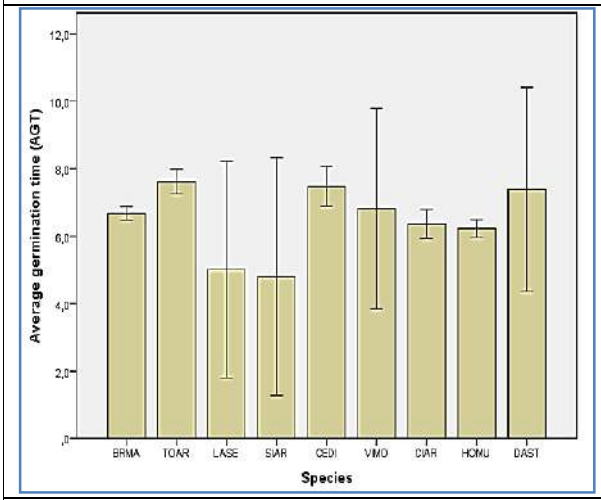

Figure 3: Effect of the species on the Average germination time (AGT)

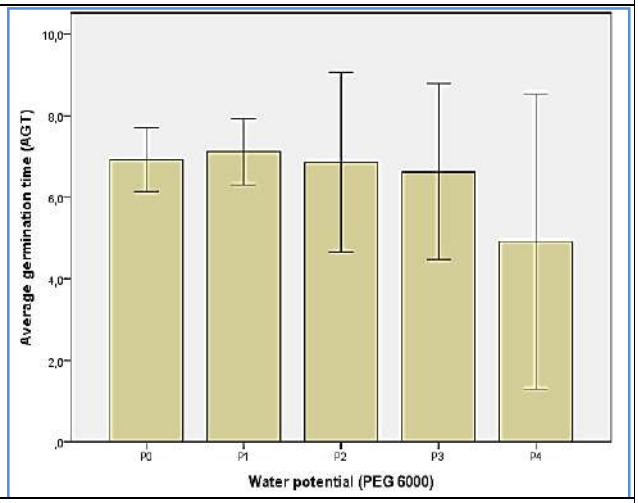

Figure 4: Effect of water potential (P_1: -0.03, P_2:-0.7, P_3:-1, P_4:-1.6 MPa) on the Average germination time (AGT)

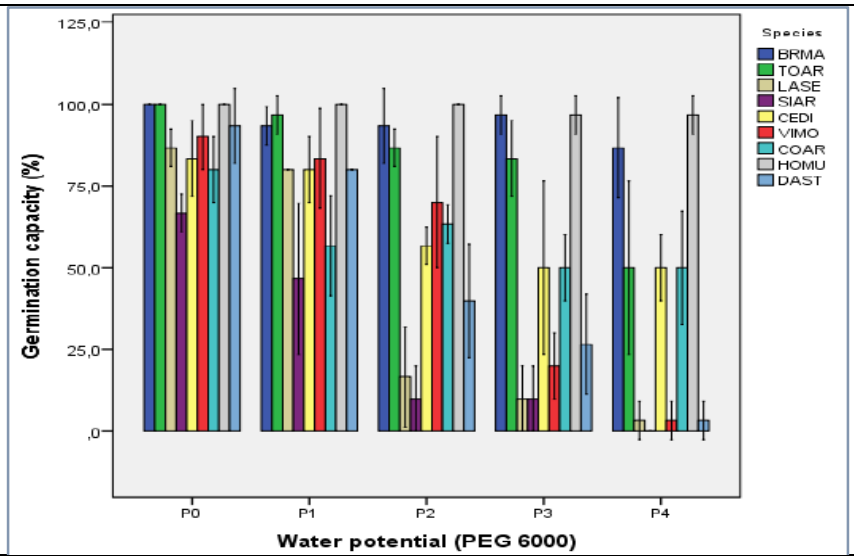

Figure 5: Effect of water potential (P_1: -0.03, P_2:-0.7, P_3:-1, P_4:-1.6 MPa) on the germination behavior of seeds of the 09 species studied.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

An Efficient Method for Image Mosaicing using Invariant Features

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Received: 22 Feb 2021 Revised: 26 Feb 2021 Accepted: 01 Mar 2021

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ABSTRACT

Many times, it is not possible to capture an entire image in a single exposure as the image-capturing media works with a definite size. In such cases, the images have to be captured separately. It is difficult to analyze such images. Hence, mosaicing helps to analyze those kinds of images. Image mosaicing is the process of combining split images into a single large image with the help of some overlapping regions. This article presents a process for mosaicing images using feature extraction, matching correspondence between those images, computing homography, warping of images, and finally stitching them. The mosaiced image is evaluated using image quality assessment metric BRISQUE and achieved an accuracy of 90%.

Keywords: Image mosaicing, feature extraction, matching correspondence, computing homography, warping, stitching.

INTRODUCTION

Nowadays, Image Mosaicing has become the most popular technology in fields like multimedia, image processing, computer vision, and computer graphics. Capturing a long image that covers a long space is difficult with the cameras, Image Mosaicing is being used in such cases but the numbers of images taken as input are restricted to only two which is commonly known as panorama images in mobiles. The construction of the large satellite and aerial photographs from the collection of images are the most traditional applications and change detection, video indexing, video compression, and scene stabilization are the most recent applications of image mosaicing. Image Mosaicing clutches a group of images that have some overlapping regions and forms a wide-angle image that holds information from the considered input images. The resolution images can be expanded using image mosaicing. Hence, it is extensively used in criminal image analysis, video monitoring, remote sensing image processing, and medical images, etc. The complexity of the mosaicing process increases, when the number of images to be mosaiced is increased which results in the quality of mosaiced images. Therefore, a new methodology has to be developed to

improve the quality of mosaiced images. This article explores the invariant feature-based approach using features of the images for enhancing the process of mosaicing images. The article is further ordered as follows, section2 discussed literature work, section 3 presents the proposed methodology, section 4 describes the environmental setup, section 5 deals with evaluation metrics, and section 6 discussed the conclusion and future work.

Related Work

In the research literature, many methods for automatic image alignment and stitching are proposed which fall broadly into two categories – direct [9, 10, 11, 12] and feature-based [13, 14, 15]. Direct methods have the advantage that they use all of the available image data and hence can provide very accurate registration, but they require a close initialization. Feature-based registration does not require initialization, but traditional feature matching methods (e.g., correlation of image patches around Harris corners [16, 17]) lack the invariance properties needed to enable reliable matching of arbitrary panoramic image sequences. R. Szeliski [1] reviewed different geometric transformation models, direct intensity-based models, feature-based registration algorithms which undergo panoramic image stitching and discussed various methods for high-quality panoramas for static or interactive viewing. M. Brown et al [2] described a framework for multi-view matching using invariant features of a new type in discrete scale-space at Harris corners and obtained better results than the state-of-art methods. J. Davis [3] illustrated a method for mosaicing framed in moving objects using projective transformation, solving linear system equations for matrices of pair wise registration and yielded good results. Helge Seetzen et al [4] experimented with two different methods using LCD panel, DLP projector and LED panel and designed systems for lab and office purposes. Guangquan Cheng et al [5] described a seamless image stitching algorithm using the wavelet-based methodology and obtained better results in conditions of trivial misalignment exists. Tappen M et al [7] experimented with an algorithm to form a single image via recovering reflectance and shadings from intrinsic images. As per the literature, many methods were proposed by the researchers for image mosaicing. This article was concentrated on panoramic image mosaicing using invariant features for improvement of the performance of the existing system.

METHODOLOGY

One of the most prominent techniques of image processing used for tiling digital images is called image mosaicing, which blends together many arbitrarily shaped images to form a single large image. Hence, this article proposes an efficient method for mosaicing of images using invariant feature analysis.
The following steps that are to be followed for developing a system which produces mosaiced image Fig.1.

Initially, the resolutions of the images are changed to a particular size and then the features are extracted from those images. The correspondence between those images is found with the help of extracted features to recognize panoramas in those images. Then homography matrix is computed with the overlapped points for bundle alignment to achieve planar warping of the images and then after doing some corrections like color corrections etc., the mosaiced panorama image will be the output.

Feature Extraction

Multiple images should be given as an input for the process and in the first step the features are extracted from the image using the SURF(Speed Up Robust Features) Algorithm which is shown in Figure 2.
This Algorithm contains three modules namely:

Scale Space Extrema Detection: This process finds the key points and descriptors present on the boundaries of an image.
Key Point Localization: This process finds the key points and descriptors present on the influential parts like in the centre of the image.

Key Point Matching: Matching on the key points takes place which are greater than the threshold**.**

Matching Correspondence

In order to join any two images, their overlapped points must be extracted. These overlapped points must give us an idea of the orientation of the second image w.r.t first image. The correspondence between those images can be found by using the FLANN (Fast Library for approximate nearest neighbour) algorithm with the extracted features. There were a large number of key points that were obtained from feature extraction; hence the inliers should be placed. The inliers that are placed in this system are 6 inliers. Another approach exists to find correspondence between images and it is BFMatcher, and this algorithm performance is very low compared to FLANN Algorithm.

Computing Homography

The Homography matrix will use the matching points to estimate a relative orientation within two images. Computation of the homography matrix is achieved by using RANSAC (Random Sample Consensus) algorithm. RANSAC algorithm is a random estimation procedure that uses randomly sampled relations or correspondence between images which are used to estimate the parameters of the image transformation as shown in equation (1). To get panoramas we select n=4 feature correspondence and n=500 trails to compute homography along with Direct Linear Transform (DTL).

$$P(H \text{ is correct}) = 1 - (1 - (pi)\, r)\, n \qquad (1)$$

This probability defines the computed homography by using the values of r and n.

Warping

Warping is the process of changing the shape of the image. To merge one image along with another image. One image has to change the shape and the change in shape is done through warping. Different types of warping that are existing are planar, cylindrical, 3600, and geometrical warping. When dealing with plane images, it is recommended to use planar warping for getting results for the warped image.

Stitching

The final phase of the process is stitching the warped image. The warped image is the input for this process where it is able to perform leftward stitching and right ward stitching while taking the center image as a reference. Since a warped image is input for this process, it checks for the coordinate values along with the pixel values. When both images pixel and coordinate values got matched and at that point, both the images got merged and able to produce the final image as the outcome of the panoramic mosaicked image.

Environmental Setup

The overall experimentation was carried out in a system with the configuration of Windows 10 Operating System (64-bit) with Intel® Core™ i5-8250 CPU @ 1.80 GHz Processor, 8.00 GB Ram and 1 TB HDD installed with Visual Studio platform with supporting image processing packages. The experiment was carried out using the dataset which contains 400 images. The images are arranged as frames in an order where there is a common region between the subsequent frames.

EVALUATION METRICS AND RESULTS

The proposed model is evaluated based on Brisque image quality assessment metric and compared the obtained results with existing ORB model and found that the proposed model has achieved an accuracy of 90% where existing model obtained 68% which are shown in Table-1.

CONCLUSION AND FUTURE WORK

In this paper, an effective model for image mosaicing using image processing techniques has been proposed to improve the quality of the mosaiced image. The results showcased that the proposed model has obtained an accuracy of 90% when compared to the existing model which obtained an accuracy of 68%. Hence, from the results, it can be inferred that the quality of the mosaiced image has been improved. The application of the proposed model in video processing for better outcomes is considered for future work.

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Table1. Accuracy comparison of different models

METRIC\METHOD	ORB MODEL	PROPOSED MODEL
ACCURACY	68%	90%

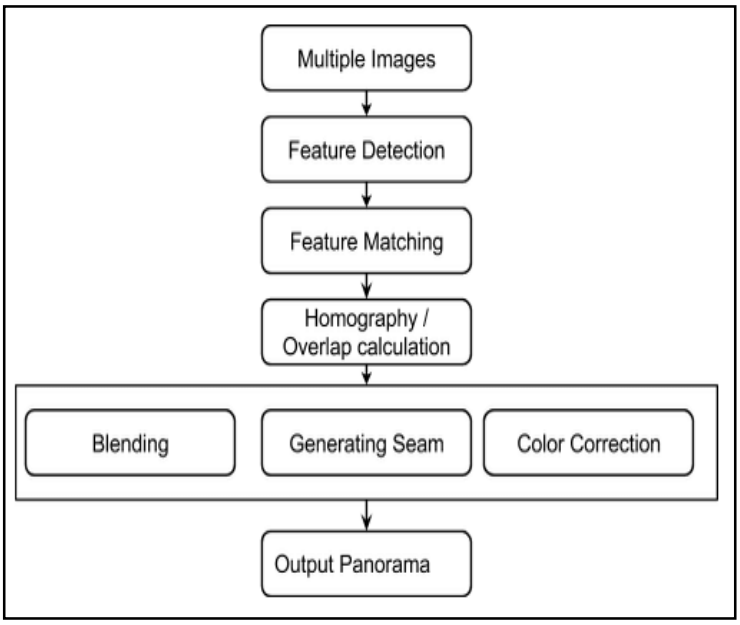


Figure 1. Flow diagram of the system

PSEUDO CODE

```
do
   for each pixel use decision tree classification
   Initialize: Set value for maximum no. of iteration. Set
   maxItem(i)=-1                          for each pixel i.
   while item<maxItem
      Start: for each pixel Ni
         Initialize: Set the initial maximum similarity
   maxSimilarity=0
         For each tile center Nj from a 2 region Size*2
   regionSizesquare                       neighborhood around Ni
         1.Determine the 3×3 image patches INi and INj which
   include                                the central pixel Nj, respectively
         2.Calculate the pixel Similarity S(I,j) for Ni and Nj
            If maxSimilarity < S(I,j)
            maxSimilarity=S(I,j); I(i)=j
      Item=item+1;
end
```

Figure 2. SURF Algorithm Pseudo code

Figure 3. Feature extraction on image

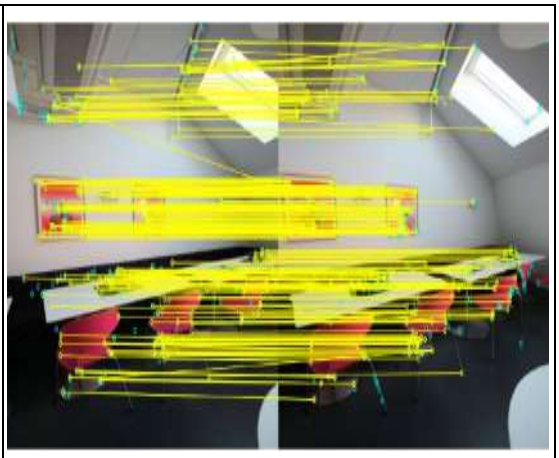


Figure 4.Matchingcorrespondences on images

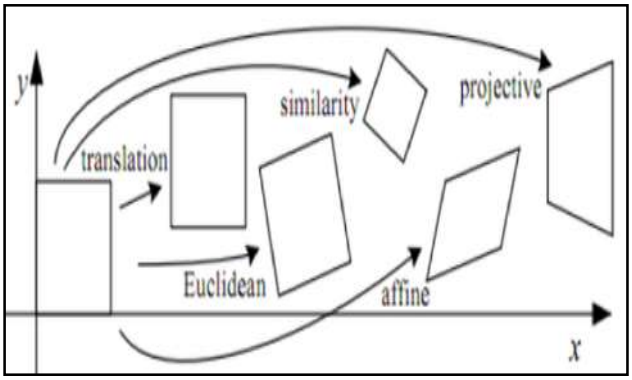


Figure 5. Transformation of images using warping

Figure 6. Warped plane image

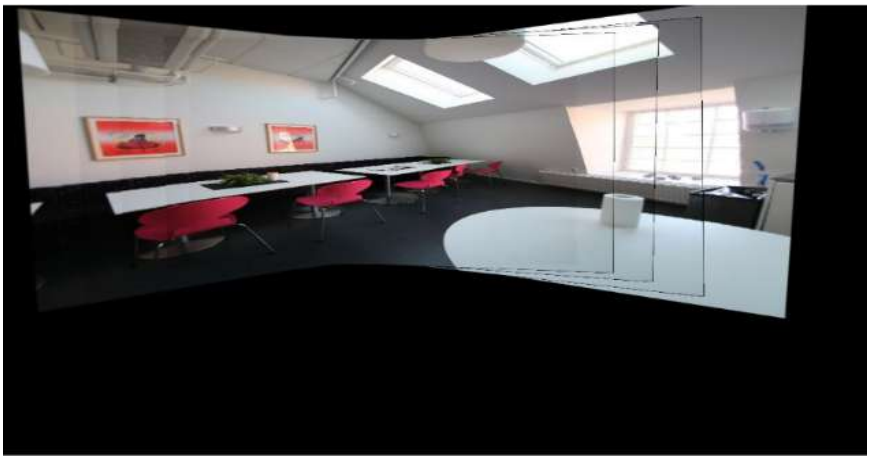


Figure 7. Final stitched image

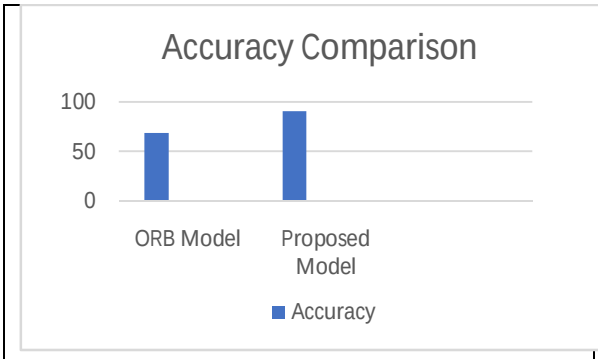
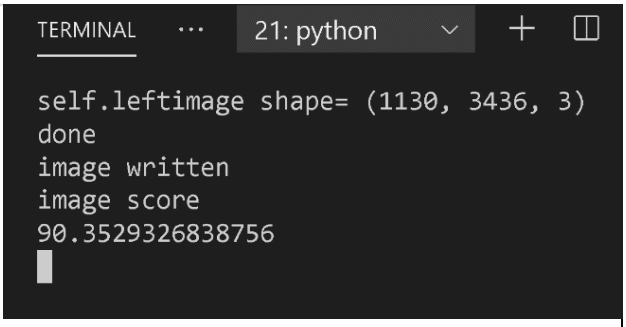


Figure 8. Accuracy comparison of different models



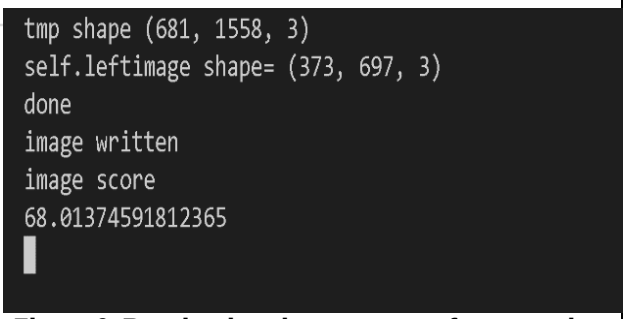


Figure 9. Results showing accuracy of proposed and existing models.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Formulation and Evaluation of Topical Gel of Tacrolimus

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Received: 22 Dec 2020 Revised: 26 Dec 2020 Accepted: 31 Dec 2020

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ABSTRACT

In the present work an attempt was being made to formulate and evaluate topical gel containing, Tacrolimus anti-psoriasis drug and to improve its bioavailability with reduction in dosing frequency and dose related side effects. Nine formulations were developed with varying concentrations of polymers like Carbopol 971, Sodium CMC and carbopol 934 were selected as polymers. The drug and excipient compatibility was studied by using FTIR. Nine formulations of gels were prepared by taking different quantities of polymers. The prepared gel was subjected to various evaluation tests like pH, Drug content, Spreadability, viscosity, and *in-vitro* diffusion studies conducted upto 12hrs. FTIR studies showed no evidence on interactions between drug, polymers and excipients. The best in-vitro drug release profile was achieved with the formulation F8 containing 10gm of exhibited 12 hr drug release i.e. 95.49% with desired therapeutic concentration which contains the drug and Carbopol 934. In diffusion studies it was observed that formulation F8 shown maximum drug release of 95.49% which was considered as optimized formulation. The surface pH, drug content and viscosity of the formulation F8 was found to be 7.1, 97%and 97,489cps respectively. The *in-vitro* release kinetics studies reveal that all formulations fit well with zero order kinetics followed by non-Fickian diffusion mechanism.

Keywords: Tacrolimus, Topical gel, Carbopol 971, Sodium CMC and *in-vitro* release kinetics.

INTRODUCTION

Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. The main advantage of topical delivery system is to bypass first

pass metabolism. Avoidance of the risk and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time are other advantage of topical preparations. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solution, and even medicated adhesive systems are in use [1,2]. The topical drug delivery system is generally used where the others system of drug administration fails or it is mainly used in pain management, contraception, and urinary incontinence3. Over the last decades the treatment of illness has been accomplished by administrating drugs to human body via various routes namely oral, sublingual, rectal, parental, topical, inhalation etc. Topical drug delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of confining the pharmacological or other effect of the drug to the surface of the skin or within the skin. Topical activities may or may not require intra-cutaneous penetration or deposition.4,5 Topical drug delivery systems include a large variety of pharmaceutical dosage form like semisolids, liquid preparation, sprays and solid powders. Topical application of gel at pathological sites offer great advantage in a faster release of drug directly to site of action, independent of water solubility of the drug as compared to cream and ointments[6]. Some of these systems are as clear as water in appearance, visually aesthetically pleasing as in gelatin deserts and other are turbid. Tacrolimus (FK 506) is an effective and well-tolerated primary immunosuppressant drug used in solid organ transplantation. Generally the formulations of Tacrolimus commercially available are in oral and ointment form. More recently, a topical gel formulation will be introduced specifically for the treatment of localized painful and inflammatory condition [7,8].

MATERIALS AND METHOD

Materials

Tacrolimus was a gift sample from Natco Pharma, Hyderabad. Carbopol 934, Sodium CMC and Carbopol 971 were purchased from S.D. Fine chem. Ltd, Mumbai. All other reagents used were of analytical grade.

Preformulation Studies

Characterization of Tacrolimus

Description

The sample of tacrolimus was analyzed for its nature, colour.

Solubility

The solubility of tacrolimus in various vehicles, including oils, surfactants was determined by the shake flask method**.**

Melting Point

The melting point of Tacrolimus was determined by capillary tube method.

Drug Excipient Compatibility Study

The compatibility studies were carried out to investigate and predict Physio-chemical interaction between drug and excipients and therefore to select suitability of chemically compatible excipients. The compatibility between the pure drug and excipients was detected by FTIR spectra obtained on Bruker FTIR Germany (Alpha T).The potassium bromide pellets were prepared on KBr press by grounding the solid powder sample with 100 times the quantity of KBr in a mortar. The finely grounded powder was then introduced into a stainless steel die and was compressed between polished steel anvils at a pressure of about 8t/in2. The spectra were recorded over the wave number of 8000 to 400cm-1.

Preparation of Tacrolimus Gel

Above mentioned quantity of carbopol 934, Carbopol 971was soaked in water for a period of 2 hours. Carbopol was then neutralized with triethanolamine (TEA) with stirring. Then specified amount of drug was dissolved in appropriate and pre weighted amounts of propylene glycol and methanol. Solvent blend was transferred to carbopol container and agitated for additional 20 min. The dispersion was then allowed to hydrate and swell for 60 min, finally adjusted the pH with 98% TEA until the desired pH value was approximately reached (6.8-7).During pH adjustment, the mixture was stirred gently with a spatula until homogeneous gel was formed. All the samples were allowed to equilibrate for at least 24 hours at room temperature prior to performing rheological measurements.

Evaluation of Gel

About Nine formulations i.e. F1 to F9 were conducted. Gels were evaluated for their percentage yield, pH, viscosity, Spread ability, percentage drug content, in-vitro diffusion studies, in-vitro drug release kinetic study, ex-vivo and stability studies by using standard procedure. All studies were carried out in triplicate and average values were reported.

Percentage Yield [9]

The empty container was Weighed in which the gel formulation was stored then again the container was weighed with gel formulation. Then substracted the empty container weighed with the container with gel formulation then it gives the practical yield. Then the percentage yield was calculated by the formula. The results are shown in Table. No: 2 Percentage yield = practical yield / Theoretical yield x 100

Determination of pH [9]

2.5 gm of gel was accurately weighed and dispersed in 25ml of distilled water. The pH of the dispersion was determined by using digital pH meter. The results are shown in Table. No: 2

Drug Content Studies [10,11]

To ensure uniform formulation of the gel, it was sampled from the different locations in the mixer and assayed for the drug content. Drug content of the gels was determined by dissolving an accurately weighed quantity of gel (about 1 gm) in about 100 ml of pH 6.8- phosphate buffer. These solutions were quantitatively transferred to volumetric flasks and appropriate dilutions were made with the same buffer solution. The resulting solutions were then filtered 0.45 mm membrane filters before subjecting the solution to spectrophotometric analysis for Tacrolimus at 291 nm. Drug content was determined from the standard curve of Tacrolimus. The results are shown in Table. No: 2

Viscosity Measurement [12]

A viscometer (Brookfield digital viscometer DV II RVTDV-II USA) was used to measure the viscosities (in cPs) of the gels. The spindle (TF 96) was rotated at 0.5 rpm. Samples of the gels were to settle over 30 min at the assay temperature (25 ±/1 oC) before the measurements were taken. The results are shown in Table. No: 2

Spreadability [13,14]

It was determined by wooden block and glass slide apparatus. For the determination of spreadability, excess of sample was applied in between two glass slides and then was compressed to uniform thickness. The weight (50gm) was added to pan. The time required to separate the two slides i.e., the time in which upper glass slide moves over the lower plates was taken as a measure of spreadability (S).

$S = M . L / T$

Where M = wt. tied to upper slide

L = length of glass slides

T = time taken to separate the slides

The results are shown in Table. No: 2

***In-Vitro* Diffusion Study [**15,16].

The skin permeation of Tacrolimus from gel formulation was studied by using an open ended diffusion cell specially designed laboratory according to the literates. The effective permeation area of the diffusion cell and receptor cell volume was 2.4 cm and 200 ml respectively. The temperature was maintained at 37 ± 0.5°C.The receptor compartment contained 200 ml of pH 6.8 phosphate buffer and was constantly stirred by magnetic stirrer at 100 rpm. The dialysis was prepared by using semi permeable membrane from egg. The membrane was tied to an open end tube. This served as the donor compartment where as the beaker containing phosphate buffer served as the receptor compartment. Gel formulation [F1-F9 (20ml suspension) and for optimized gel (10gm)] was applied to the dialysis membrane and the content of diffusion cell was kept under constant stirring. Then 5 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals and analyzed by spectrometric method at 291 nm after suitable dilution. The receptor phase was immediately replenished with equal volume of fresh pH 6.8 buffer. Triplicate experiments were conducted for drug release studies .The results are shown in Table No: 3

Release Kinetic Study [17,18,19]

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, and Korsmeyer-Peppas release model.

Zero Order Kinetics

It refers to the process of constant drug release from a drug delivery device independent of the concentration. Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions are obtained) can be represented by the following equation, $Q_t = Q_o + k_o t$

Where, Q_t= amount of drug released in time 't, Q_o= initial amount of drug in the solution and k_t= zero order release constant. The data is given in Table. No: 4

First Order Kinetics

The first order equation describes the release from system where release rate is concentration dependent, the following relation can express this model:

$Log\ Q_t = Log\ Q_o + k_t t/2.303$

Where, Q_t= amount of drug released in time 't', Q_o= initial amount of drug in the solution, k_t= first order release constant. The data is given in Table. No: 4

Higuchi Model

Higuchi developed several theoretical models to study the release of water soluble drugs incorporated in semisolid and/or solid matrixes. Simplified Higuchi model can be expressed by following equation:

$Q = k_H t^{1/2}$

Where, k_H= Higuchi diffusion constant, Q= Amount of drug release in time't'. Higuchi describes drug release as a diffusion process based on the Fick's law, square root time dependent. It is used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms. The data is given in Table. No: 4

Korsemeyer-Peppas Model

The mechanism of drug release was evaluated by plotting the log percentage of drug released versus log time according to Korsmeyer- Peppas equation. The exponent 'n' indicates the mechanism of drug release calculated through the slope of the straight Line.

$M_t/M_\infty = ktn$

Where, $Mt / M\infty$ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. Is the diffusional exponent, which characterizes the type of release mechanism during the dissolution process. For non-

Fickian release, the value of n falls between 0.5 and 1.0; while in case of Fickian diffusion n = 0.5; for zero-order release (case I transport),n=1; and for supercase II transport, n > 1.In this model, a plot of log (M_t/ M_∞) versus log (time) is linear. To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release versus log time. The data is given in Table .No: 4

Stability Studies [20, 21, 22]

The optimized formulation F8 was subjected to a stability testing for the period of three months as per ICH norms at a temperature of 25°±2°C with relative humidity RH= 60±5% and 40º ± 2°C with relative humidity RH= 75±5%. The optimized formulation F8 was analyzed for the changes in appearance, pH, viscosity, spreadability by procedure stated earlier. The data is given in Table .No: 5

RESULT AND DISCUSSION

The objective of the present study was to formulate Topical gels of Tacrolimus. Total Nine different Tacrolimus topical gels with different polymer ratios were prepared. In order to select the optimized formulation, various evaluation parameters were checked and subjected to in-vitro diffusion study and release kinetic study were observed.

Pre formulation Studies

The following tests were performed according to British Pharmacopoeia.
Description: A white or almost white powder
Solubility: Ethanol and Methanol
Melting Point: 113-115°C
From these tests it was confirmed that the sample complies with the monograph.

Compatibility Study

Compatibility of the drug with recipients was determined by FT-IR spectral analysis, this study was carried out to detect any changes on chemical constitution of drug after combined it with the recipients . The samples were taken for FT-IR study. The results are shown in Figure. No: 1 to 2

Evaluation of Topical Gel

All the formulations (F1-F9) prepared were evaluated for different parameters like percentage yield, pH, drug content, viscosity, spreadability. Results are shown in Table .No:9

In- Vitro Drug Release Study

The prepared formulations were subjected for *in vitro* dissolution study to study the effect of different variables on percentage of drug release**.** From the results of above parameters, formulation F8 was chosen for final Optimized formulation of evaluation. The development of *in-vitro* diffusion tests is to show the release rate and extent of drug from the dosage form. So, F8 selected for optimized formulation. It was giving drug release of 95.49 in 12h respectively, Viscosity is also considered as crucial factor for batch selection where F8 showed very Optimum viscosity 97.489 cps. The release data was obtained for all the gel formulations. Spectrometric results were obtained and given consideration to sampling loss, to calculate actual cumulative drug diffused was calculated since the volume of receptor cell was only 20 ml. The obtained diffused amount of drug was extrapolated to diffusion by unit surface area of semi permeable membrane. Results are shown in Table. No: 3

Drug Release Kinetics

Data of *in vitro* release studies of formulations which were showing better drug release were fit into different equations to explain the release kinetics of Tacrolimus release from gel. The data was fitted into various kinetic

models such as zero, first order kinetics, higuchi and korsmeyer –pepp as mechanisms. The batch F8 had appreciable correlation with zero order plots (R^2 =0.994) and simultaneously apparent to Higuchi drug release profile (R^2 = 0.916). As per data fitting with Korsmeyer Peppas model, value of n for each batch was calculated and for batch F8, then value was 0.845 describing Non-Fickian drug release mechanism. All the formulations exhibited anomalous (Non-Fickian transport) diffusion mechanism and followed zero order kinetics. Based on the found data of *in vitro* drug release and kinetic data modeling, formulation F8 was selected for the stability studies. The results were shown in below Table. No: 4 and they graphically represented in Figure.No:3to5.

Stability Studies

Stability study test are used to find out whether the formulations are maintaining their quality during the storage period. Stability study tests are used to find out the best formulation. It can be studied by applying a stress to the formulation such as temperature, humidity and light. Here stability study was conducted for F8 at 40ºC/75%RH. The control tests were carried out at the end of the one month. Results are shown in Table .No:5.

CONCLUSION

It was observed that carbopol 934 gel containing tacrolimus F8 produced better spreadability and consistency as compared to other formulations .The F4 gel showed suitable Ph, good viscosity, good stability. The maximum percentage of drug release was found to be 95.49% in 12hrs in formulation F8. The optimized formulation followed zero order kinetics. Thus, the Topical gel formulation of Tacrolimus using Carbopol 934 as a polymer developed and optimized, showed better diffusivity of drug across skin layers and has wider prospects to be used as a topical drug delivery system.

ACKNOWLEDGEMENT

Authors are thankful to guide prof & principal Dr.B.S.Venkateswarlu, prof. Dr.R. Margret Chandira Vinayaka missions college of pharmacy, Salem, Tamilnadu.

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Table.no.1 Formula for the preparation of Tacrolimus Topical gel

S.No	INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Tacrolimus (mg)	02	02	02	02	02	02	02	02	02
2	Carbopol 971 (mg)	1	1.5	02	-	-	-	-	-	-
3	Sodium CMC (mg)	-	-	-	1	1.5	02	-	-	-
4	Carbopol 934 (mg)	-	-	-	-	-	-	1	1.5	02
5	Methanol (ml)	10	10	10	10	10	10	10	10	10
6	Triethanolamine (ml)	5	5	5	5	5	5	5	5	5
7	Polyethylene glycol (mg)	10	10	10	10	10	10	10	10	10
8	Methyl paraben (mg)	5	5	5	5	5	5	5	5	5
9	Water	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s

Table no. 2 Percentage yield, pH, Drug content, Viscosity, Spreadability

Formulation	Percentage yield %	pH	Drug content	Viscosity (cps)	Spreadability gm.cm²
F1	90.54	6.8	92	63,540.06	11.06
F2	95.67	7	96	91,669.03	11.65
F3	93.48	6.4	91	92362.37	10.72
F4	92.33	6.2	95	1,19,000.01	11.88
F5	93.75	6.8	94	1,35,677.30	10.57
F6	94.28	6.4	93	61,552.01	11.10
F7	91.22	6.8	92	99,882.04	10.84
F8	**95.89**	**7.1**	**97**	**97,489.04**	**11.95**
F9	91.33	6.9	94	1,27.023.04	11.09

Table .no:3 In-vitro cumulative % drug release profile for Tacrolimus Time	**Cumulative % drug release**								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	**F_8**	F_9
0	0	0	0	0	0	0	0	**0**	0
30min	13.56	18.5	11.09	18.09	12.39	12.01	10.21	**3.11**	5.54
1hr	24.55	33.52	19.26	32.51	22.21	17.09	20.62	**7.15**	12.17
2hr	31.86	35.3	25.21	37.42	26.22	25.31	30.72	**14.21**	24.58
3hr	34.22	40.52	31.71	46.42	32.09	29.69	33.32	**27.54**	33.19
4hr	39.26	45.81	35.21	50.31	35.21	31.03	37.29	**35.45**	39.79
5hr	41.62	55.32	39.05	56.51	38.02	33.61	40.25	**45.21**	48.69
6hr	44.72	59.5	45.02	59.41	43.3	35.3	44.91	**53.77**	52.75
7hr	49.25	62.32	49.05	61.21	47.31	41.65	52.41	**59.34**	61.38
8hr	53.45	66.92	55.51	65.72	49.85	43.32	57.86	**66.73**	67.54
9h	60.53	70.07	59.37	72.46	55.31	47.32	59.92	**77.69**	75.28
10hr	67.02	75.41	68.42	78.32	65.21	51.09	62.59	**85.54**	79.19
11hr	73.52	79.2	71.31	85.31	69.71	56.31	65.43	**91.15**	81.14
12hr	76.89	82.41	74.62	87.42	73.09	65.21	67.19	**95.49**	86.68

Table .no: 4 Release Kinetic Data for optimized formulation (F8)

Time (hr)	% of Drug Release	% of Drug Remaining	Log% Drug Release	Log% Drug Remaining	Log Time	Square root of time
0	0	100	0	2.000	0	0
0.5	3.11	75.37	0.493	1.986	-0.301	0.707
1	7.15	69.37	0.854	1.968	0.000	1.000
2	14.21	57.48	1.153	1.933	0.301	1.414
3	27.54	49.69	1.440	1.860	0.477	1.732
4	35.45	41.75	1.550	1.810	0.602	2.000
5	45.21	34.22	1.655	1.739	0.699	2.236
6	53.77	31.83	1.731	1.665	0.778	2.449
7	59.34	26.43	1.773	1.609	0.845	2.646
8	66.73	21.73	1.824	1.522	0.903	2.828

9	77.69	13.36	1.890	1.348	0.954	3.000
10	85.54	10.13	1.932	1.160	1.000	3.162
11	91.15	6.71	1.960	0.947	1.041	3.317
12	95.49	1.15	1.980	0.654	1.079	3.464

Table .no.5: Stability studies of optimized formulation F8

Parameters	Controlled	After 15 days	After 1 month
Appearance	White	white	White
pH	7.1	7.1	7.1
Viscosity	97489±515cps	97489±515 cps	97489±515 cps
Spreadability (cm)	11.95	11.95	11.95

*Mean±SD (n=6)

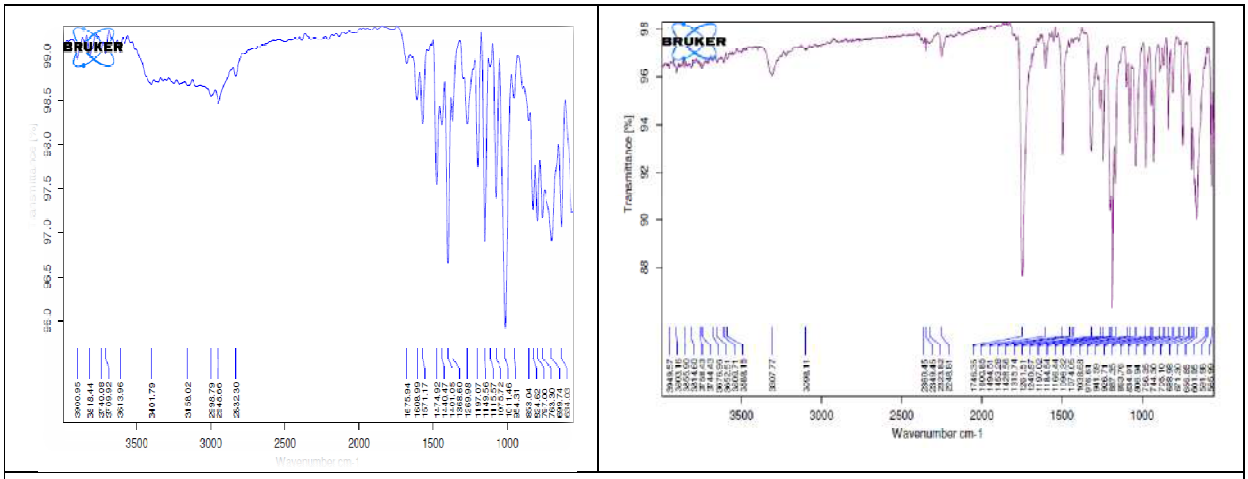
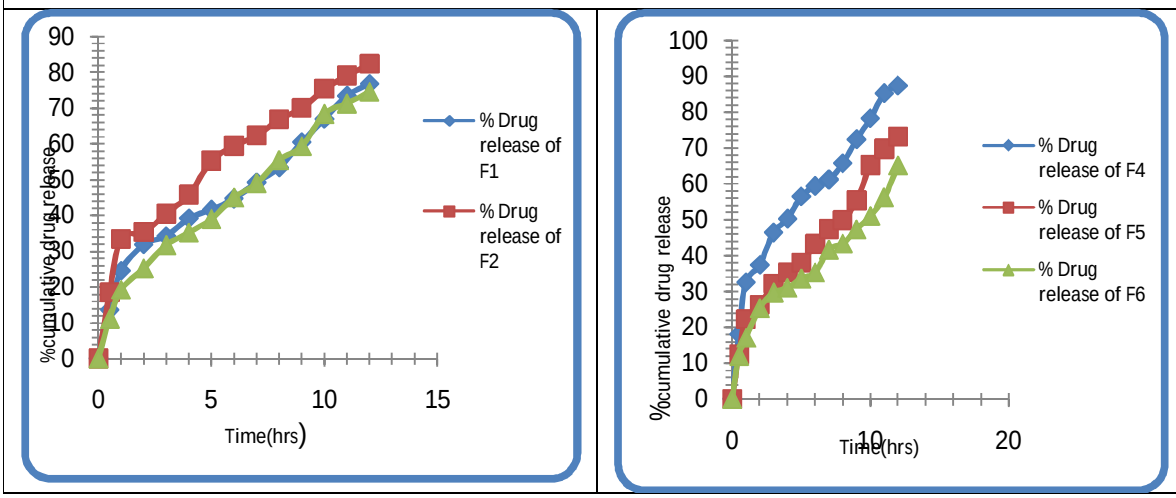


Figure .no: 1&2 FTIR spectra



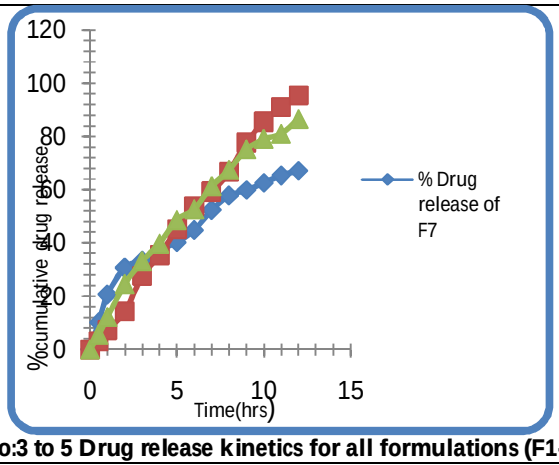


Figure.no:3 to 5 Drug release kinetics for all formulations (F1-F9)

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antimicrobial Activities of Synthesized Gold Nanoparticles against *Escherichia coli* and their *In-vitro* Toxicity Assessment

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Received: 28 Sep 2020 Revised: 28 Feb 2021 Accepted: 06 Mar 2021

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ABSTRACT

Safe drinking water in an urban city has been a great challenge for human population. Consumption of contaminated water may lead to occurrence of several diseases. Enterotoxigenic *Escherichia coli* (ETEC) is an enteric pathogen which can survive even in treated drinking water and cause watery diarrhea. The pathogen harbours virulent and drug-resistant genes responsible for its virulence and antibiotic resistance. Persistence of such strains of ETEC are often associated with disease outbreaks. The present work involves antimicrobial activity of synthesised Gold nanoparticles against ETEC, isolated from drinking water. Gold nanoparticles (AuNPs) were synthesized using biological approach and characterised by UV-visible spectroscopy and Electron Microscopy. The morphology of nanoparticles was found to be quite spherical within the range of 20-30 nm. AuNPs were effective as antimicrobial agent against ETEC.Cytotoxicity assay was performed to evaluate the toxicity of synthesized nanoparticles. It was evaluated by using sulforhodamine B (SRB) assay on Vero cell line.Study revealed that the synthesized AuNPs and do not exhibit any toxicity within the experimental range of antimicrobial concentration of AUNPs and therefore these are safe.

Keywords: Enterotoxigenic *Escherichia coli*, Drug-resistance, Gold nanoparticles and Cytotoxicity.

INTRODUCTION

Microbial contamination of safe water is one of the most serious public-health problems. Presence of bacterial pathogens in water is often related to disease outbreaks in a population [1,2,3]. These include diarrhoea, typhoid, cholera etc. [4,5,6,7]. Even in urban areas, the supply of safe drinking water is a challenge. *Escherichia coli* and its

pathotypes, particularly, enterotoxigenic *Escherichia coli* (ETEC) is mainly responsible for diarrhoea and other enteric diseases. Countries like India contribute more than half of total deaths due to infections caused by ETEC [8,9,10,11]. Almost 17 million cases of watery diarrhea and 600,000 associated deaths occur annually. Consequently, identification of critical contamination factors for the distribution of drinking water is a necessary [12,13]. ETEC bears*LT-1* gene, which encodes the heat labile enterotoxin [14,15]. Previous studies have reported the identification and quantification of ETEC in aquatic as well as in other samples [16,17,18,19]. Prevalence of ETEC in potable water has become a major threat for public health. High dose of ETEC (~10^8 CFU) is required for causing infection. Children below 5 years are often susceptible to get infection with ETEC. In a developing country like India, antimicrobial drug resistance has become serious problem. Frequent and over use of antibiotics to kill infectious agents over the years has led the emergence of resistant strains. This has caused the prevalence of drug resistant-Enterotoxigenic *Escherichia coli* and other pathogens in environment [20]. Genes responsible for antibiotic resistance are often disseminated to other pathogens through horizontal gene transfer. Nanomaterials exhibit outstanding physical and chemical properties. They have shown promising applications in biomedical and therapeutic applications [21,22,23]. Tiny size and high surface volume ratio are the unique properties of metallic nanoparticles. These attributes make them a suitable candidate to actively interact with biological system [24,25,26].

Novel antimicrobial agents should be safe for end user. Although, well established experimental models for genotoxicity and cytotoxicity exist, still their correlation with respect to antimicrobial nanoparticles is meager. Generally, toxicity is the degree to which a chemical can harm human or animal. In general, toxicity studies are performed *In-vivo*, but before evaluating the acute or sub-acute toxicity, they should be checked for *In-vitro* cytotoxicity in order to save the laboratory animals. Cell culture experiments are used to evaluate the toxicity of several compounds at cellular level. *In-vivo* cytotoxicity assays took much time for completion, are expensive and also involve ethical problems while *In-vitro* experiments are devoid of such problems and are much faster, cheaper and easy to perform. This makes *In-vitro* experiments the first choice for cytotoxicity evaluation [27]. They may include the integrity of the cell membrane such as the MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) and SRB (sulforhodamine B) assays [28]. The SRB assay has been used since 1990. It is based on the property of SRB, which binds to proteins in acidic condition is extracted in basic condition [29]. In the present work, we report the antimicrobial activity of biologically synthesised gold nanoparticles against Enterotoxigenic *Escherichia coli* and their cytotoxicity assessment on Vero cell line.

MATERIALS AND METHODS

Bio-assisted Synthesis of Gold Nanoparticles (Au NPs)

Gold nanoparticles were synthesised by Auric Chloride ($HAuCl_4$) and extract of Lemongrass (*Cymbopogan citratus)* leaf. Fresh leaves from the plant were collected from local farm of Gwalior, M.P, India. Leaves were thoroughly washed with double distilled water and were cut (50 g) into fine pieces. This was followed by dipping into 200 mL double distilled water. Flask was heated up at 60^{o}C for 15 minutes. Leaf extract was filtered and filtrate was stored at 4^{o}C. Isolated leaf extract of *C.citratus* was allowed to mix with aqueous solution of 1 mM Auric Chloride (1:4 ratio). The conical flask was further incubated at 37^{o}C for 24 h. Change in the solution colour from yellow to light pink and then to darker pink was observed. The gold nanoparticle was purified by centrifugation at 15000 x g for 15 min followed by re-dispersion of pellet in deionized water for repetitive wash and were dried at 55^{o}C. Synthesized AuNP were subjected to mild sonication for 10-15 minutes.

Characterisation of Nanoparticles

Synthesised nanoparticles were characterised using various biophysical techniques. UV-Visible spectroscopy (between 300-700 nm) was used to observe the spectra of synthesised gold nanoparticles. Size and morphology of AuNPs were determined by Electron Microscope at the 120 KV (JEOL 2000). Samples after preparation and preliminary characterization were sent to sophisticated test and instrumentation centre (STIC), Cochin, Kerala. The

size and particle distribution of nanoparticles was confirmed using transmission electron microscopy (TEM). Briefly, sample was prepared by placing a drop of synthesized nanoparticles on a carbon coated copper grid followed by drying the sample in an oven at 60°C before transferring it into microscope. Size and morphology was characterized by TEM (JEOL 2000).

In-vitro Experiment For Antimicrobial Activity of Nanoparticles against ETEC

Enterotoxigenic *Escherichia coli* MTCC 723 was used as reference strain and procured from Microbial Type Culture Collection (CSIR-IMTech, Chandigarh). ETEC was grown at 37°C in Luria Bertani broth. Various concentrations of gold nanoparticles (0, 10, 20, 30, 40, 50 and 100 µg/mL) were added to the grown bacterial culture (~1 x 10^8 CFU), followed by incubation at 37°C for 16-20 h in shaking incubator at 120 rpm. Bacterial cultures showing poor growth were spread (100 µL aliquots) onto the control MH agar plates (without any antibiotics) to further examine the culturability of culture and bactericidal effect of nanoparticles.

Estimation of Minimum Inhibitory Concentration for ETEC Using Agar Dilution Method

MIC and MBC were evaluated by agar dilution method. Agar dilution was followed using Wiegand et al 2008[30]. Briefly, the Muller Hinton Agar (MHA) was prepared and sterilized. After autoclaving, the AuNPs in different concentrations were added before the agar solidified. This was followed by mixing the bacterial suspension (~1 x 10^8 CFU/ mL). The surface of the agar along with bacterial culture was dried and incubated at 37°C for 20 h. Grown colonies after incubation were counted next day.

Cytotoxicity of Nanoparticles

Cytotoxicity was done to check the toxicity of nanoparticles on mammalian cell lines under lab conditions. All the toxicity studies were performed in CSIR-Central Drug Research Institute, Lucknow. Cells were cultured *in-vitro* and growth rate of cell were measured by the change in colour and its intensity.

SRB Assay on Vero Cell line

Twenty thousand cells were seeded into every well of a 96-well plate. Cells were treated with varying concentrations of biologically synthesized gold nanoparticles (0, 6.25, 12.5, 25, 50, and 100 µg/ml) for 24 h. Specific concentration of nanoparticles was mixed in normal saline (0.85% NaCl). Toxicity assays were also performed for vehicle. All the stock solutions were steam sterilized followed by filtration (0.22 µm, Millipore) and storage at 20°C. Cell line was procured from National Centre for Cell Science, Pune. All the cells were allowed to culture in prescribed media and were supplemented with 10% foetal bovine serum, 1% penicillin (10^4 U/ml)/streptomycin (10 mg/ml) and 1% L-glutamine (200 mM) (Sigma–Aldrich). Cells were incubated in an appropriate culture medium at 5% CO_2. Cells were rinsed with 5 ml phosphate-buffered saline (Ca^{2+} and Mg^{2+} free) and incubated with 2 ml 0.05% trypsin/0.5 mM EDTA (Sigma–Aldrich) for 3 min at 37°C. Cell culture flask was tapped for the detachment of cells, followed by mixing of detached cells with one ml pipette several times to avoid clumping of cells and also to produce single cell suspension. About 5 ml medium containing 10% foetal bovine serum was added to inactivate the activity of trypsin. Cell suspension was transferred to conical tube and centrifuge at 100 x g for 5 min. After centrifugation the supernatant was discarded and cells were re-suspended in the culture medium. Cytotoxicity was determined by 'measuring the absorbance at 570 nm on ELISA reader'. The percentage of cell proliferation was calculated.

RESULTS AND DISCUSSION

Synthesis and Characterisation of Au Nanoparticles

Highly stable Au nanoparticles were synthesized using Sodium citrate as capping agent. Sodium borohydrate was used as reducing agent. Finally, synthesized product was characterized using standard characterization techniques, to check the shape size and morphology of the particle.

Characterization of Nanoparticles was done Using Different Methods

UV-Visible Spectroscopy

UV-Visible spectrum was taken in an optical quality quartz cuvette with a 1 cm path length. A visible colour changes from yellow to pink due to Surface Plasmon Resonance (SPR) vibration was observed indicating the formation of nanoparticles. Spectra were seen at room temperature, while double distilled water was used as a blank. The absorption spectrum was recorded from of 300 to 700 nm. The Gold nanoparticles synthesized by chemical method exhibit Surface plasmon resonance spectra at 515 nm.

Transmission Electron Microscopy

Gold NPs size was found in the range of 20 to 30 nm as evident from Transmission Electron Microscopy. Nanoparticles were prominently spherical in shape (Figure 1). The selected area diffraction pattern of Au nanoparticles evidenced the crystalline planes of the face-centred-cubic structured gold (Figure 2), which suggested the crystalline nature of synthesised gold nanoparticles.

In-Vitro Experiment for Antimicrobial Activity of Gold Nanoparticles against ETEC and Evaluation of MIC

Growth inhibition of ETEC was examined in broth containing varying concentrations (0, 10, 20, 30, 40, 50 and 100 µg/mL) of AuNPs. Gold nanoparticles were highly effective at concentration ≥ 50 µg/ml. At concentration of 40 µg/ml, the gold nanoparticles showed modest antimicrobial property and fewer viable cells were observed as compared to control samples. Further, it was observed that at concentrations ≥40 µg/ml of the nanoparticles ETEC lost its culturability. The MIC of gold nanoparticles was evaluated to be 50 µg/mL. Previous reports have demonstrated that NPs of size less than 5 nm can enter human tissues easily and may disrupt the cell normal biochemical environment [31,32].

Cytotoxicity Testing of Nanoparticles

SRB Assay on Vero cell lines

To assess the cell activity, the intracellular dose of Sulforhodamine was quantified. Gold nanoparticles were tested over a 24h exposure. The particles showing MIC less than 64µg/ml were considered further to check the cytotoxicity on Vero cell line using SRB assay. SRB result demonstrated that higher concentration has generated more toxicity (Kong et al., 2011). For the biologically synthesized Gold nanoparticles, there was no any significant reduction in the percentage of SRB of Vero cells. At higher concentration 100 µg/ml cells showed very less reduction i.e., was only 4.9%, whereas at 50µg/ml, it was 2.3 %, while at lower concentration cells were proliferating as shown (Table 1 and Figure 3). The relevant IC_{50} value on Vero cells was 100 µg/ml. This was an edge over other previous reports, where phyto-synthesized gold nanoparticles from *C. roxburghii* DC. leaf had IC50 values of 30 µg/ml on HepG2 cells lines, 50 µg/ml on HeLa and MCF7 cell line and more than 75 µg/ml for vero cell lines [33].

CONCLUSION

In conclusion, the synthesized AuNPs were effective and showed antimicrobial activity against ETEC. The nanoparticles did not exhibit any toxicity within their antimicrobial concentration range.

ACKNOWLEDGEMENT

We wish to express our sincere acknowledgment to Dr. Ashok Kumar Chauhan, President, RBEF parent organization of Amity University Madhya Pradesh (AUMP), Dr. Aseem Chauhan, Additional President, RBEF and Chancellor, AUMP, Gwalior, Lt. Gen. V.K. Sharma, AVSM (Retd.), Vice Chancellor of AUMP, Gwalior for providing necessary facilities, their Valuable support and encouragement throughout the work.

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Table 1: Percent cell inhibition and cell viability of biologically synthesized Gold nanoparticles

S. No.	Concentration	Vero Cell Line	
	(µg/ml)	% Cell Viability	% Cell Inhibition
1	6.25	103.3	-3.3
2	12.5	102.4	-2.4
3	25	101.7	-1.7
4	50	97.7	2.3
5	100	95.1	4.9

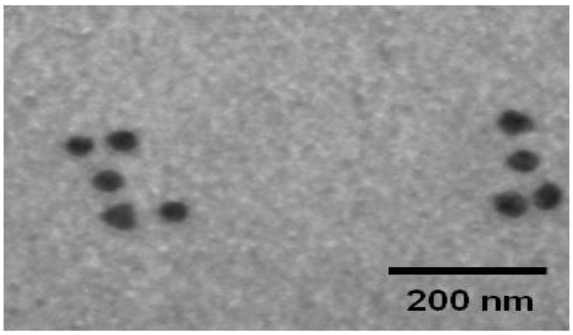

Figure 1: TEM micrograph of synthesized AuNPs with the leaf extract of *Cymbopogan citratus*.

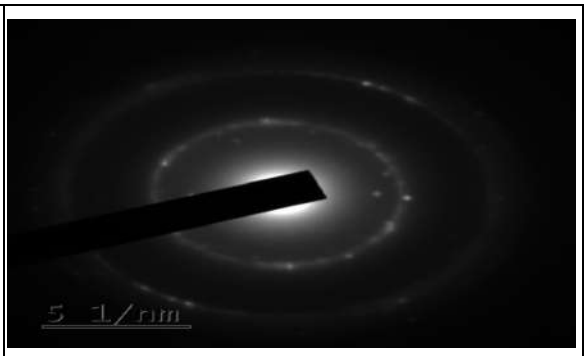

Figure 2: SAED Pattern of synthesized AuNPs with the leaf extract of *Cymbopogan citratus*.]

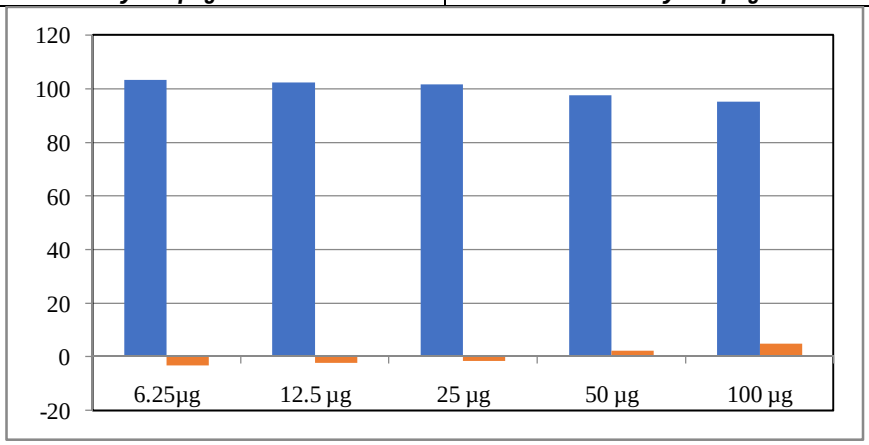

Figure 3. Graphical representation of Percentage cell inhibition and cell viability of biologically synthesized Gold nanoparticles (blue bar denotes % cell viability, orange bar denotes % cell inhibition)

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

IoT Enabled Face Mask Detection System for Ensuring Covid-19 Social Norms

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Received: 28 Feb 2021 Revised: 03 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

COVID-19 coronavirus pandemic is a global health issue and has caused a major change in the lifestyle of people throughout the world. Public health experts are emphasizing the necessity to practice social distancing and wearing face masks. The face mask has become mandatory in every place and any crowded environment like educational institutions, shopping malls, function halls, auditoriums will have to examine the crowd to trace persons without face masks. This paper proposes a face mask detection system that will help identify persons without face masks and thereby reduce the possibility of the spread of virus and infection. In this research, a deep learning convolution neural network (CNN) and Open CV algorithms are proposed to detect the unavailability of facemasks. The CNN algorithm is employed to train the model and identify the face. The convolution neural network model is then integrated with an Internet of Things (IoT) based sensor device which helps to identify a person not wearing a facemask. The CNN model is trained using benchmarked facemask dataset. The experimental results are highly promising with an acceptable accuracy of 86%.

Keywords: Deep Learning, Convolution Neural Network, Covid-19, Face Mask Detection, Internet of Things (IoT)

INTRODUCTION

A global health crisis is being produced by the coronavirus (COVID-19) pandemic. The rapid spread of COVID-19 in 2020 prompted the World Health Organization to declare COVID-19 a pandemic worldwide. According to WHO, more than five million cases across 188 countries have been infected with COVID-19 in less than six months [1]. The virus spreads in crowded and overcrowded areas and by close contact. The WHO has released numerous

recommendations for protecting against the spread of coronavirus [2]. One of the important methods of defence, according to the WHO, is to wear a face mask in public places. People used to wear masks prior to Covid-19 to safeguard their health from air pollution. While most individuals are self-conscious about their looks, by covering their faces, they shield their feelings from the public. Health experts have proved that wearing face masks works by preventing the transmission of COVID-19 [3]. The coronavirus outbreak has given rise to an exceptional degree of scientific collaboration worldwide. It is a known fact that manual monitoring to ensure covid-19 social norms is an extremely challenging and unrealistic task. Automated systems with machine learning and deep learning approaches can help combat Covid-19 in several ways [4]. In this paper, the Internet of Things (IoT) based face mask detection system is proposed. The proposed system consists of two parts: 1) the development of a deep learning model for face mask detection; 2) the development of an IoT-based face mask detection alarm system. In this study, datasets of face mask detection that are publicly accessible are used to train the model. The input images are cropped in preprocessing and transformed into greyscale images. To train the face mask detection model, the convolution neural network (CNN) algorithm is employed. The trained model is then implemented in the IoT system. The test image is fed to the IoT system from the surveillance camera as an input. If the mask in the captured image is not detected, then some warning sound is provided. The experimental results indicate that the CNN model detects the face mask effectively. The pros of the proposed system that helps to confirm that, individuals can enter any institutions, shopping malls, industry, and theatre in safe mode. The proposed architecture is shown in Figure 1.The paper is organized as follows: Section 2 describes the image acquisition and preprocessing. The CNN architecture is described in Section 3. The proposed framework is described in Section 4. The CNN model integration is explained in Section 5. The conclusion and future work is presented in Section 6.

Image Acquisition and Pre-Processing

This face mask dataset [6,7] contains 1,376 images from two groups: the first one with 690 images consists of images of faces with a mask, the other one has 686 images of faces without a mask. The idea is to train a custom model of deep learning to detect a face of a person from a live webcam feed and then classify them as either wearing a mask or not wearing a mask. For the model to be efficient, a series of preprocessing steps are required. Two stages of pre-processing are done to improve efficiency. The first step is to convert to a grayscale picture from RGB (Red, Green, Blue). There is one colour channel for grayscale images and three channels for colour images. In the next step, the images are resized to reduce the complexity of the CNN model and thus reduce the computing power needed to train the model. The images are stored in a directory with two subdirectories, one titled 'with mask' and one titled 'without a mask'. These directory names are also used as class labels during classification. For resizing the images, the image size component has been set to 100, so that each image becomes a square image of 100 x 100. The images are stores in an array to decrease model complexity and maximize training time, and then divide the array by 255. This normalizes the range of pixels to fall between 0 and 1.

CNN Architecture

The architecture of CNN [8] includes 2 convolution layers, each with a 3 x 3 convolution window and an activation feature for the Rectified Linear Unit. About 200 feature maps are generated by the first convolution layer, while the second produces 100 feature maps. The procedure for padding and other processing is the same as the convolutional model. The Max Pooling layers have 2 x 2 window sizes and the fully-Connected layers have one hidden layer and the last layer is the output layer. The hidden layer does have 50 neurons, and for each class, the output layer has only two nodes. For the estimation of class probabilities, the Softmax activation function was used. The architecture uses a dropout to help prevent over fitting of the model to the training data. In this implementation, 90% of the data is used to train the model, and 10% is used to test the model. The CNN architecture is shown in Figure 2.

IoT Based Mask Detection – The Proposed Architecture

This IoT -2 Architecture [9] does have a single node for sensing and analysis as shown in Figure3. It is a cloud-based architecture and data gets stored and processed in a cloud-based application. Level-2 IoT systems are suitable for

solutions where the data involved is significantly large, but the primary requirement for the analysis is not computer-intensive and can be performed locally. To store and retrieve face data which is stored in the cloud database, a cloud-based REST web service is used.

CNN Model Integration

The model was trained with training datasets of about 1000 images. and the output of the unseen images from the test set was evaluated. The live images from the camera can be fed as input to the prediction model. All images from the feed need to be pre-processed using the same technique as with the training data and then proceed to the identification stage. The feed from the original picture is processed to crop only the face which is then classified. As far as the visual input from the classification task is concerned, it shows the results as two rectangular boxes. If a mask is present, we will have a Green contour, and if no mask is detected then a red contour is displayed along with the class label 'Mask' or 'No Mask'. The result is shown in Figures 4 and 5.

CONCLUSION

In this research work, an IoT based face mask detection using deep learning techniques was developed. In this method, an IoT based sensing module is integrated with Convolution Neural Network (CNN) to detect the presence of a face mask on any individual from the life we streaming video and the predicted results are stored in the cloud for further analysis. The proposed face mask detection system alerts people who do not wear a face mask. The proposed CNN model efficiently detects a face mask and ensures the practice of wearing face masks by alerting individuals on their rule-breaking behaviour. In the future, this research can be further extended to integrate smart intelligent devices to detect social distancing and perform analytics on the huge repository in the cloud. The repository can also be utilized to analyse the social and behavioural patterns in people belonging to various demographic and geographical locations.

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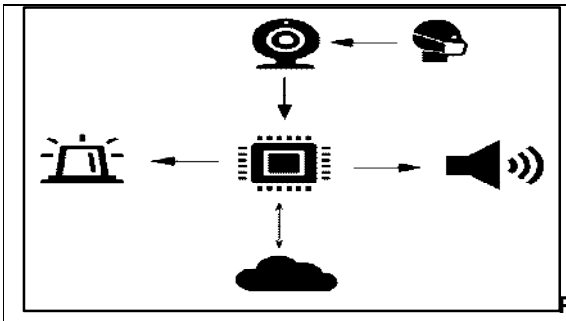

Fig. 1 The Proposed Architecture

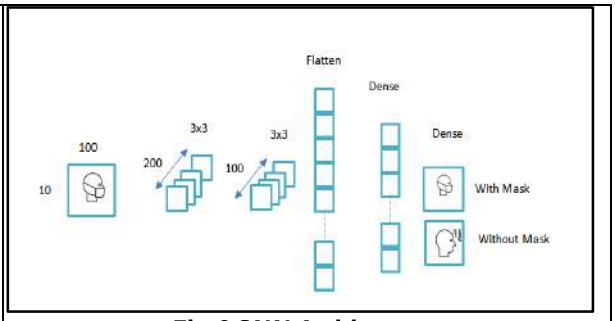

Fig. 2 CNN Architecture

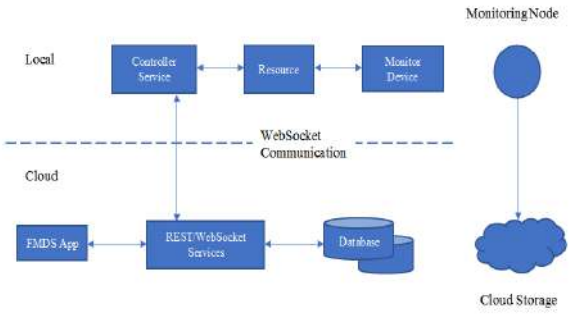

Fig. 3 IoT Architecture

Fig. 4 Predicted result (With Mask)

Fig 5 Predicted result (Without Mask)

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Correlating the Impact of Various Polymers with Few Pharmacokinetic Parameters in Oral Controlled Release Matrix Tablets of Nisoldipine

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Received: 22 Dec 2020 Revised: 26 Dec 2020 Accepted: 02 Jan 2021

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ABSTRACT

The objective of this study was to Correlating the Impact of various polymers with few pharmacokinetic parameters in oral controlled release matrix tablets of Nisoldipine, which is expected to deliver the drug in controlled release manner with reduce frequency of drug administration, reduce GI tract side effects and improve patient compliance. The half-life of nisoldipine occurs about 7 – 12 hrs. So a controlled release formulation of nisoldipine would increase the length of time release in which nisoldipine achieves an effective concentration in the body. The present work aimed at pharmacokinetic modification of controlled release matrix tablets of nisoldipine using different polymers. Nine batches of matrix tablets of nisoldipine were successfully prepared using Eudragit RS-100, Eudragit RL-100, and other excipients by direct compression method. Based on results formulation containing Eudragit RS-100 (7.5%) and Eudragit RL-100 (7.5%) F(8), was identified as ideal and better formulation among all formulations developed for Nisoldipine tablets. All the formulations exhibited anomalous (Non-Fickian transport) erosion mechanism and followed zero order kinetics. Based on the found data of *in vitro* drug release and kinetic data modeling, formulation FN8 was selected for the stability studies From study it is concluded that the formulated matrix tablets of Nisoldipine of batch F8 (optimized formulation), were superior and effective in achieving better patient compliance.

Keywords: Nisoldipine, Matrix tablets, Direct compression method, controlled release.

INTRODUCTION

Controlled drug delivery occurs when a polymer is combined with a drug or active agent such that the release from the bulk material is pre-designed. Controlled and Sustained Release, both has been used in consistent and confusing manner. Both represent separate delivery process. Sustained release constitutes any dosage form that provides medication over an extended time or denotes that the system is able to provide some actual therapeutic control whether this is of a temporal nature, spatial nature or both. Sustained release system generally don't attain zero order type release and usually try to mimic zero order release by providing drug in a slow first order. The basic rationale for controlled drug delivery is to alter the pharmacokinetics and pharmacodynamics of pharmacologically active moieties by using novel drug delivery system or by modifying the molecular structure and /or physiological parameters [1]. Controlled release dosage forms are mainly designed to maintain therapeutic blood or tissue levels of the drugs that have a short elimination half-life. The controlled release dosage forms offer a number of advantages over immediate release products, such as better patient compliance due to decrease in dosing frequency, portability, convenience and fewer side-effects. Such dosage forms exhibit better pharmacological effect and prolonged therapeutic activity. Matrix tablets are one of the foremost commonly used controlled release dosage forms as they release the drug in a controlled manner.

In global market of the pharmaceutical industry, tablets are the most popular method of oral drug administration. Furthermore, the speed of prolonged/controlled release matrix tablet has grown gradually in recent years. Controlled release oral delivery systems are designed to: achieve therapeutically effective concentrations of drug in systemic circulation over an extended period of time; decrease the frequency of taking medicine; increase convenience to patients [2]. Hypertension or high blood pressure is a cardiac chronic medical condition inwhich the systemic arterial blood pressure is elevated. What that means is that the heart ishaving to work harder than it should to pump the blood around the body. Blood pressure involves two measurements, systolic and diastolic. Normal blood pressure is at or below120/80 mmHg. Hypertension is the opposite of hypotension. Hypertension is classified as either primary (essential) hypertension or secondary hypertension; about 90–95% of cases are categorized as "primary hypertension," which means high blood pressure with no obvious medical cause. The remaining 5–10% of cases (Secondary hypertension) are caused by other conditions that affect the kidneys, arteries, heart or endocrine system [3,4].

Persistent hypertension is one of the risk factors for stroke, myocardial infarction, heart failure and arterial aneurysm, and is a leading cause of chronic kidney failure. Moderate elevation of arterial blood pressure leads to shortened life expectancy. Dietary and lifestyle changes can improve blood pressure control and decrease the risk of associated health complications, although drug treatment may prove necessary in patients for whom lifestyle changes prove ineffective or insufficient [5]. Nisoldipine is a member of the dihydropyridine class of calcium channel antagonists (calcium ion antagonists or slow channel blockers) that inhibit the transmembrane influx of calcium into vascular smooth muscle and cardiac muscle. It reversibly competes with other dihydropyridines for binding to the calcium channel. Because the contractile process of vascular smooth muscle is dependent upon the movement of extracellular calcium into the muscle through specific ion channels, inhibition of the calcium channel results in dilation of the arterioles. *In vitro* studies show that the effects of nisoldipine on contractile processes are selective, with greater potency on vascular smooth muscle than on cardiac muscle. Although, like other dihydropyridine calcium channel blockers, nisoldipine has negative inotropic effects *in vitro*, studies conducted in intact anesthetized animals have shown that the vasodilating effect occurs at doses lower than those that affect cardiac contractility. The effect of nisoldipine on blood pressure is principally a consequence of a dose-related decrease of peripheral vascular resistance. While nisoldipine, like other dihydropyridines, exhibits a mild diuretic effect, most of the antihypertensive activity is attributed to its effect on peripheral vascular resistance [6].

MATERIALS AND METHODS

Nisoldipine were kindly donated by Cadila Pharmaceuticals, Eudragit RS-100, Eudragit RL-100, Lactose Monohydrate DCL11, Microcrystalline Cellulose pH102, were donated by FMC Bio-Polymer FMC Bio-Polymer. Purified Talc were donated by Nitica Chemicals. Sodium Lauryl Sulphate were donated by Chetan & Chetan. Colloidal Anhydrous Silica (Aerosil) were donated by CabotSanmer. Magnesium Stearate, Sodium Starch Glycolate were donated by Amishi Drugs and Chemicals. Sodium Starch Glycolate. All other materials and reagents used were of analytical grade of purity.

UV Spectrophotometric Analysis [20]
UV Spectrophotometric Analysis

Preparation of 0.1 N Hydrochloric acid
0.1 N Hydrochloric acid prepared by diluting 8.5ml of concentrated hydrochloric acid to 1000ml with distilled water [14].

Preparation of Phosphate Buffer pH 6.8
Dissolve 28.80 g of disodium hydrogen phosphate and 11.45 g of potassium dihydrogen phosphate in sufficient water to produce 1000 ml [15].

Preparation of Standard Stock Solution
100mg of Drug (Nisoldipine) transferred into 100ml volumetric flask. It was dissolved in 0.1N HCL and Phosphate buffer pH 6.8 and volume was made up to the mark with same buffer medium. This gives stock solution of concentration (1000mg/ml), from this 10ml was withdrawn and diluted to 100ml to get a concentration of (100mg/ml) [16].

Standard Curve Preparation of Nisoldipone
From this standard solution stock solution, aliquots 1 to 10ml were withdrawn and made up to 10ml 0.1N HCL and Phosphate buffer pH 6.8 to give a concentration of i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10μg/ml for pH 6.8 buffer and 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μg/ml. Absorbance of these solution was measured 237 nm. The absorbance was taken on double beam U.V. spectrophotometer (1601 UV spectrophotometer SHIMADZU, Japan) using λ_{max} at 237 nm. The absorbance values were plotted against concentration (μg/ml) to obtain the standard calibration curve.

FT IR Absorption Spectra
FT IR study was done as a part of preformulation study for the selection of excipients and to check compatibility of the drug with other excipients. 2 mg of the substance being examined was triturated with 300 mg of finely powdered and dried IR grade potassium bromide. These quantities were usually sufficient to give disc of 13 mm diameter and a spectrum of suitable intensity. The mixture was grinded carefully spreaded uniformly in a die, and applied to a pressure of about 800 MPa (8 t. cm^{-2}). Several factors may cause the formation of faulty discs, such as insufficient or excessive grinding, humidity or other impurities in the dispersion medium and an insufficient reduction of particle size. A disc was rejected if visual examination shows lack of uniform transparency or when transmittance at about 2000 cm^{-1} (5μm) in the absences of specific absorption band was less than 75 % without compensation. The discs were scanned over a wave number range of 400 to 4000 cm^{-1}in FTIR instrument.

Preparation of the Controlled Release Matrix Tablets of Nisoldipine
The matrix tablets containing Nisoldipine was formulated by direct compression method using different viscosity grades of Eudragit RL – 100 & Eudragit RS – 100. All the ingredients were weighed according to the formula and blended in mortar and sifted through sieve #30. The powder blend was mixed uniformly in polybag for 10 minutes.

The blend was compressed into tablet using instrumented Cadmach single punch tablet machine using 12 mm punches for Nisoldipine to get tablets of average weight 100 mg. The punched tablets were subjected to various evaluations [10].

Evaluation of Nisoldipine Matrix Tablets

Evaluation of pre-compression parameters of powder Prior to compression, powder were evaluated for their flow and compressibility parameters. Flow properties of powder were determined by angle of repose method. Compressibility index of powder were determined by Carr's index and Hauser ratio [7-8].

Angle of Repose [9]

It was measured by fixed funnel. The fixed funnel method employs a funnel that was secured with its tips at a given height H, above graph paper that was placed on a flat horizontal surface. Granules were carefully poured through the funnel until the apex of the conical pile just touches the tips of the funnel. Thus, with R being the radius of the base of the conical pile.

$\tan \theta = H/R$

Where, θ = angle of repose

H =Height of pile

R = Radius of pile

Determination of Tap Density and Bulk Density

An accurately weighed quantity of the powder (W), was carefully poured into the graduated cylinder and the volume (V_0) was measured. Then the graduated cylinder was closed with lid, set in to the density determination apparatus, (Erweka Pvt. Ltd). The density apparatus was set for 100 taps and after that the volume (V_f) was measured and continued operation till the two consecutive readings were equal. The bulk density, and tapped density were calculated using the following formulas,

Bulk density = W/V_o

Tapped density = W/V_f

Where,

W = weight of powder

V_o = Initial volume

V_f = final volume

Compressibility Index (Carr Index)

Compressibility index is an important measure that can be obtained from the bulk and tapped densities. carrs index a material having values of less than 15 to 20 % is defined as the free flowing material.

$$Ci = \frac{(V_0 - V_f)}{V_o} \times 100$$

Hausner Ratio

It indicates the flow properties of the powder and is measured by the ratio of tapped density to bulk density.

Hausner ratio =tapped density/ bulk density

Sieve Analysis

A series of sieves were arranged in the order of decreasing pore diameter. (Increasing in sieve number) i.e. sieve numbers #20, #40, #60, #100. 100gm of drugs were weighed accurately and transferred to sieve #20 which kept on top. The sieves were shaken for about 5 minutes. Then the drug retained on each sieve were taken, weighed separately and expressed in terms of percentage.

Evaluation of Post-Compression Parameters of Tablets

Appearance

Tablet from each formulation were randomly selected and organoleptic properties such as color, odour, taste, and shape were evaluated.

Weight Variation Test

20 tablets were selected randomly from the lot and weighted individually to check for weight variation.

Hardness Test

Hardness or tablet crushing strength (fc), the force required to break a tablet in a diametric compression was measured using Monsanto tablet hardness tester. It is expressed in kg/cm2.

Thickness

The thickness of tablets was determined suing a Digimatic veriner caliper (Mitutoya, Japan). Three tablets from each batch were used, and average values were calculated.

Friability (F)

Friability of the tablet determined using Roche friabilator. This device subjects the tablet to the combined effect of abrasion and shock in a plastic chamber revolving at 25 rpm and dropping a tablet at height of 6 inches in each revolution. Pre-weighted sample of tablets was placed in the friabilator and were subjected to the 100 revolutions. Tablets were dusted using a soft muslin cloth and reweighed. The friability (F) is given by the formula.

$$F=W_{initial}-W_{final}X100/W_{initial}$$

Acceptance criteria for % friability, %weight loss should be less than 1%

Assay (Nisoldipone)

Twenty tablets from each batch were weighed accurately and powdered powder equivalent to 100mg Nisoldipone was shaken with 100ml of phosphate buffer pH 6.8 in 100ml amber colored volumetric flask and from this 10ml pipette out and dilute upto 100ml.from standard solution again 10ml pipette out and diluted upto 100ml in 100ml amber colored volumetric flask resulting solution was filtered and assayed at 330 nm and content of Nisoldipone was calculated.

***In Vitro* Drug Release Study (Nisoldipone)**

The release rate of drug from CR was determined using USP Dissolution testing apparatus II (paddle method). The dissolution medium was Hydrochloric acid (0.1N) followed by phosphate buffer pH 6.8 were used as release media, the volume being 900ml. The temperature was maintained at 37±0.5ºc. The rotation speed was 50rpm. A sample (5ml) of the solution was withdrawn from the dissolution apparatus at 0.5, 1 1.5, 2, 3, 4, 6, 8, 12, 16, 20 & 24 Hours. And the samples were replaced with fresh dissolution medium. The samples were filtered through a membrane filter and absorbance of these solutions was measured at 237 nm using a UV/V is double-beam spectrophotometer of Cumulative percentage drug release was calculated using linear equation obtained from a standard curve.

Kinetic Modeling [11-13]

Zero Order Kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions are obtained) can be represented by the following equation;

$Q_t = Q_o + k_o t$

Where, Q_t = amount of drug released in time't', Q_o = initial amount of drug in the solution, k_t = zero order release constant.

The pharmaceutical dosage forms following this profile, release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. This relation can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage form, as in the case of some transdermal system, as well as matrix tablets with low soluble drugs coated form, osmotic systems, etc.

First Order Kinetics

The application of this model to drug dissolution studies was first proposed by Gibaldi and Feldman. The following relation can express this model:

$\text{Log } Q_t = \text{Log } Q_o + k_t t/2.303$

Where, Q_t = amount of drug released in time't', Q_o = initial amount of drug in the solution, k_t = first order release constant.

The pharmaceutical dosage forms following this dissolution profile, such as those containing water soluble drugs in porous matrices, release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amounts of drug released by unit of time diminish.

Higuchi Model

Higuchi developed several theoretical models to study the release of water soluble drugs incorporated in semisolid and/or solid matrixes. Simplified Higuchi model can be expressed by following equation:

$f_t = k_H t^{1/2}$

Where, k_H = Higuchi diffusion constant, f_t = fraction of drug dissolved in time't'.

Higuchi describes drug release as a diffusion process based on the Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs.

Hixson-Crowell Cube Root Law

The Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of particles or tablets

$Q_0 1/3 - Q_t 1/3 = KHC_t$

Where, Q_t is the remaining amount of drug in the dosage form at time t, Q_0 is the initial amount of the drug in tablet and KHC_t is the rate constant for Hixson-Crowell rate equation. A graphical representation of the cube root of the amount remaining versus time will be linear if the equilibrium conditions are not reached and if the geometrical shape of the dosage form diminishes proportionally overtime (Cube root of initial drug load minus cube root of % drug remaining) are plotted against time (hour) to demonstrate the Hixson Crowell plot. This model is used by assuming that release rate is limited by the drug particles dissolution rate and not by the diffusion.

Korsmeyer-Peppas Model

Korsmeyer developed a simple, semi empirical model, relating exponentially the drug release to the elapsed time (t);$f_t = at^n$

Where, a = constant incorporating structural and geometric characteristics of the drug dosage form, n = release exponent, $f_t = M_t/M_\infty$ = fraction release of drug.

Stability Studies [18-20]

Stability of a drug can be define as the time from the date of manufacture and the packaging of the formulation, until its chemical or biological activity is not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously. In any design and evaluation of dosage forms for drugs, the stability of the active component must be a major criterion in determining their acceptance or rejection.

RESULTS AND DISCUSSION

UV Spectrophotometric Analysis
Drug and Excipient compatibility studies

FT – IR Studies

The pre-compression parameters were also studied for the physical mixture blend batches FN1 to FN9,as reported in Table No. 2. The study results showed that the Angle of repose for all the study formulations were in range of 26º.96' to 29º.93', Compressibility index in range of 15.82 to 19.42%, Bulk Density in the range of 0.469 to 0.538 g/ml, Tapped Density in the range of0.552 to 0.698 (g/ml) and Hausner's Ratio in the range of 1.10 to 1.19. The physical mixture characterization of the drug as well as excipients, shows considerably good compatibility, thus helpful to formulate the Controlled Release Matrix Tablets using dry granulation technique. Matrix tablets of Nisoldipine were prepared by the direct compression method and subjected to different evaluation tests as reported in Table No. 3. Each compressed tabletaveragely weighed 100mg. The maximum weight variation of the tablets evaluated was between 99.8% to 103.4%. The percentage of average weight variation for the 20 tablets studied from each formulation batch was less than ±10%. The Hardness for tested tablets from all formulated batches were in the range of 3.37 to 3.67 kg/cm². The thickness of the tablets of all the batches was found in the range of 3.18to 3.38mm. The friability of the tablets of all batches was found in the range of 0.31 to 0.27 %. The results indicated that the, physical parameters of formulated tablets were within the Pharmacopeial specifications.

ASSAY

Matrix tablet of Nisoldipine were prepared by the direct compression method and subjected to different evaluation tests reported in Table no. 4. As per pharmacopeial, drug content of each tablet should be in the range of 90-110% of the theoretical label claim. Formulations FN1, FN2 & FN3 developed using single polymer Eudragit RS-100 at 10%, 15% and 20% respectively showed release but not in controlled manner. Similarly, Formulation FN4, FN5 and FN6 developed using Eudragit RL-100 at 10%, 15% and 20% respectively also failed to exhibit controlled release. Formulation FN7, FN8 and FN9 developed using combination of polymers at 10% (Eudragit RS-100 5% & Eudragit RL-100 5%), 15% (Eudragit RS-100 7.5% & Eudragit RL-100 7.5%) and 20% (Eudragit RS-100 10% & Eudragit RL-100 10%) respectively, were also studies for release kinetics in which the FN8 showed controlled release kinetics but not the FN7 and FN9. Thus, Formulation FN8 was optimized and selected for further studies.

Kinetic Data

In vitro release kinetic study results, as shown in Table. No: 10, for the optimized formulationFN8 had appreciable correlation with Zero order plot (R^2=0.983) and simultaneously apparent to Higuchi drug release profile (R^2=0.991) thus, presenting a controlled drug release pattern as a CR tablets. The data fitting with Korsmeyer Peppas model and value of 0.848 for the variable n calculated for each batch including FN8, confirms that the release kinetics followed non-Fickian drug release mechanism. All the formulations exhibited anomalous (Non - Fickian transport) erosion mechanism and followed zero order kinetics. Based on the found data of *In vitro* drug release and kinetic data modeling, formulation FN8 was selected for the stability studies

CONCLUSION

Formulations were prepared using varying concentrations of Eudragit RS100 & Eudragit RL100 polymers at 10, 15, 20% and different ratios of other excipients. The prepared tablets, by direct compression, were subjected to various test of characterizations like hardness, friability, weight variation, thickness, and i*n vitro* drug release kinetics in simulated gastric fluid (Phosphate buffer pH 6.8). The hardness of all the formulations was in the range of 3.37 to 3.67 kg/cm². By comparing the values of *in vitro* dissolution studies, the maximum drug release was exhibited by FN8

of 98.87%. The rate of drug release from all the formulations is in the following order at the end of 24 hrs of *in vitro* dissolution studies: Two polymers (7.5% + 7.5% Optimized Formulation). The i*n vitro* dissolution study data for the optimized formulation FN8 showed promising results. As per data fitting with Korsmeyer Peppas model, value of n for each batch was calculated and for batch FN8, the n value was 0.848 describing Non-Fickian drug release mechanism. From, these it was concluded that the controlled release tablets of Nisoldipine (FN8) showed improved dissolution, minimizes the dose and improves the patient compliance and effective controlled release matrix tablets in oral route of administration.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. B.Jaykar, Professor & Registrar, Vinayaka Mission's Research Foundation (Deemed to be University) & Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu for extending their support and facilities for this research.

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Table. No: 1. Composition of Nisoldipine matrix Tablets

INGREDIENTS	FN1	FN2	FN3	FN4	FN5	FN6	FN7	FN8	FN9
Nisoldipone (CCB)	10	10	10	10	10	10	10	10	10
Eudragit RS-100	10	15	20	-	-	-	5	7.5	10
Eudragit RL-100	-	-	-	10	15	20	5	7.5	10
Lactose Monohydrate	58	53	48	58	53	48	58	53	48
Sodium Lauryl Sulphate	1	1	1	1	1	1	1	1	1
Aerosil	1	1	1	1	1	1	1	1	1
Magnesium Stearate	3	3	3	3	3	3	3	3	3
Micro-Crystalline Cellulose (MCC pH 105)	15	15	15	15	15	15	15	15	15
TALC	2	2	2	2	2	2	2	2	2
Total Weight	**100 mg/tablet**								

Table No. 2: Pre-Compression Parameters of Nisoldipine controlled release matrix Tablets

Batch Code	FN1	FN2	FN3	FN4	FN5	FN6	FN7	FN8	FN9
Bulk Density (g/ml)*	0.499	0.487	0.485	0.488	0.520	0.538	0.489	**0.469**	0.478
Tapped Density (g/ml) *	0.612	0.588	0.578	0.638	0.641	0.688	0.698	**0.552**	0.589
Carr's Index %	18.42	19.32	16.85	19.42	18.29	17.29	18.72	**15.82**	16.98
Hausner Ratio	1.14	1.12	1.15	1.19	1.18	1.128	1.11	**1.10**	1.12
Angle of Repose (θ)	28º.41'	29º.93'	28º.13'	29º.41'	29º.47'	27º.47'	28º.71'	**26º.96'**	29º.13'

Table No. 3: Post-Compression Parameters of Nisoldipine controlled release matrix Tablets

S. No	SPECIFICATION	FN1	FN2	FN3	FN4	FN5	FN6	FN7	FN8	FN9
1.	Weight Variation (mg) **	102.7±0.08	101.6±0.06	101.1±0.06	102.9±1.25	102.3±1.25	103.4±1.18	102.6±1.17	**101.8±1.17**	99.8±1.25
2.	Hardness (kg/cm2) *	3.45±0.21	3.43±0.22	3.41±0.21	3.51±0.20	3.47±0.18	3.45±0.14	3.35±0.12	**3.37±0.13**	3.67±0.12
3.	Thickness(mm) *	3.23±0.04	3.21±0.03	3.18±0.01	3.34±0.04	3.28±0.03	3.25±0.05	3.38±0.05	**3.28±0.05**	3.36±0.04
4.	Friability (%) *	0.29%	0.28%	0.28%	0.28%	0.27%	0.31%	0.31%	**0.27%**	0.31%

Table No. 4: Assay of different formulations

Formulations	% Assay of Nisoldipine**
FN1	102.22
FN2	102.02
FN3	101.89
FN4	104.55
FN5	99.59
FN6	98.88
FN7	102.25
FN8	101.02
FN9	102.32

Table No. 5: *In vitro* percentage dissolution studies of controlled release matrix tablets of Nisoldipine

Time (h)	FN1	FN2	FN3	FN4	FN5	FN6	FN7	FN8	FN9	Marketed Formulation
0	0	0	0	0	0	0	0	**0**	0	0
0.5	8.38	9.88	3.69	4.55	7.88	8.99	6.58	**3.58**	2.58	5.55
1	16.55	18.55	6.98	8.98	13.55	14.55	9.85	**7.58**	6.87	8.99
1.5	28.55	27.55	9.88	12.55	18.22	19.68	16.55	**9.52**	10.25	12.22
2	36.55	35.55	13.55	16.88	26.55	21.55	21.55	**13.65**	14.58	15.68
3	56.55	51.66	21.55	21.58	31.55	32.59	23.58	**19.85**	15.85	21.22
4	62.22	54.98	24.99	26.88	36.85	37.89	33.54	**24.98**	19.58	26.58
6	70.28	64.58	36.55	31.55	45.98	46.85	38.98	**36.98**	26.58	38.98
8	74.58	81.22	38.95	41.87	56.88	59.55	48.95	**46.89**	31.25	49.55
12	76.55	82.55	63.55	61.85	73.55	71.58	61.25	**56.89**	48.58	58.98
16	81.22	84.55	75.68	69.59	74.98	82.55	73.55	**73.58**	61.25	63.55
20	83.55	86.55	81.54	79.55	76.87	86.55	83.55	**88.98**	66.53	68.98
24	84.68	88.55	86.55	94.55	79.88	89.88	85.55	**98.87**	74.58	75.85

Table no.10: Kinetic study

Code	Zero order		First order		Higuchi		Peppas		Hixson Crowell	
	R^2	Slope	R^2	Slope	R^2	Slope	R^2	N	R^2	Slope
FN1	0.655	3.080	0.828	-0.031	0.814	17.380	0.848	0.548	0.771	-0.079
FN2	0.795	3.652	0.871	-0.038	0.872	18.850	0.903	0.539	0.826	-0.093
FN3	0.958	3.811	0.993	-0.037	0.987	21.47	0.99	0.829	0.99	-0.098
FN4	0.978	3.806	0.916	-0.043	0.989	21.160	0.993	0.752	0.973	-0.106
FN5	0.852	3.269	0.936	-0.029	0.956	18.38	0.969	0.599	0.916	-0.079
FN6	0.982	3.81	0.918	-0.042	0.986	21.2	0.993	0.785	0.974	-0.104
FN7	0.942	3.575	0.993	-0.035	0.994	19.94	0.986	0.669	0.99	-0.092
FN8	**0.983**	**4.145**	**0.841**	**-0.064**	**0.991**	**23.19**	**0.994**	**0.848**	**0.956**	**-0.133**
FN9	0.977	3.141	0.994	-0.024	0.986	17.51	0.978	0.805	0.994	-0.07

Table No. 11: Stability parameters of Optimized Formulation FN8

Parameters	1st Month		2nd Month		3rd Month	
	RT	40°C/75%RH	RT	40°C/75%RH	RT	40°C/75%RH
Weight variation test**	101.8±1.17	101.7±1.15	101.6±1.15	101.5±1.14	101.1±1.11	101.0±1.08
Thickness*	3.28±0.05	3.27±0.04	3.26±0.07	3.25±0.05	3.24±0.05	3.23±0.03
Hardness*	3.37±0.13	3.37±0.11	3.35±0.11	3.34±0.10	3.33±0.10	3.33±0.07
Friability*	0.27%	0.27%	0.26%	0.25%	0.25%	0.23%
Percentage Drug Content**	101.02	101.02	101.01	101.00	100.89	100.77
Percentage Drug Release*	98.87	98.85	98.76	98.73	98.76	98.57

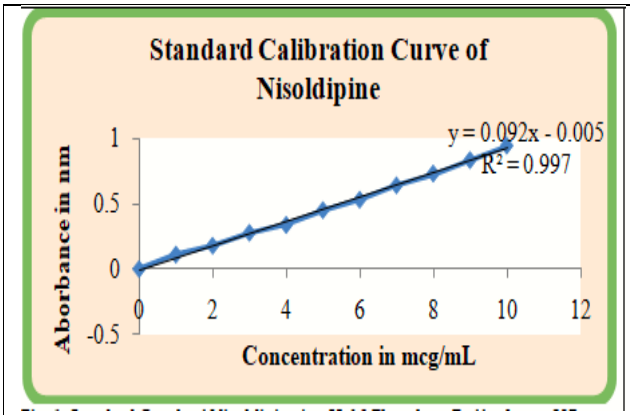

Fig. 1: Standard Graph of Nisoldipine in pH 6.8 Phosphate Buffer λ_{max} at 237 nm

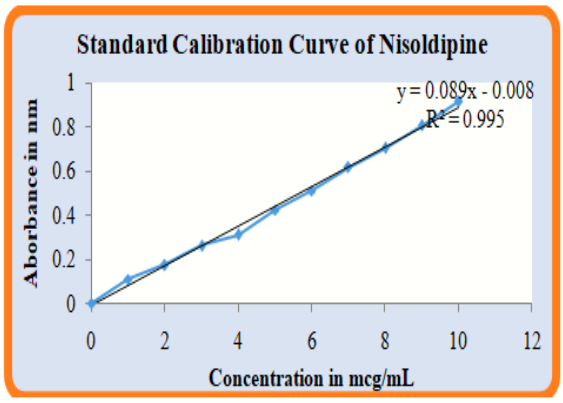

Fig. 2: Standard Graph of Nisoldipine in 0.1N HClλ_{max} at 237 nm

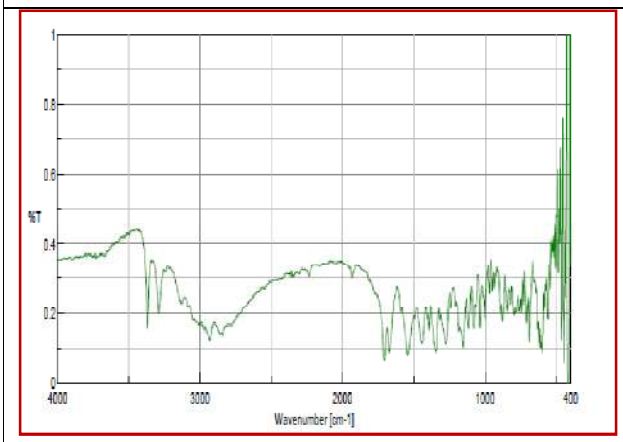

Fig. 3: FTIR Spectarum of Pure Nisoldipine

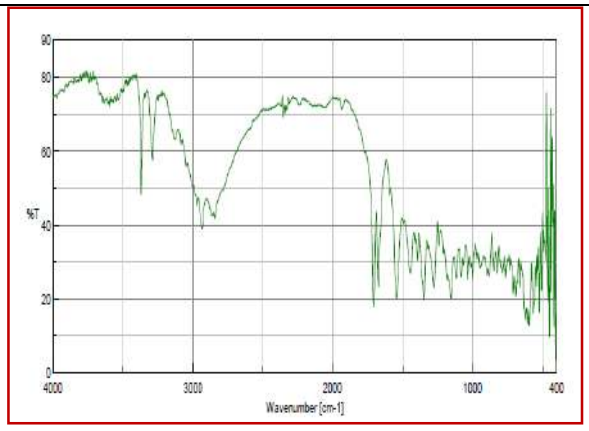

Fig. 4: FT IR Spectarum of Pure Nisoldipine and Eudragit RL – 100

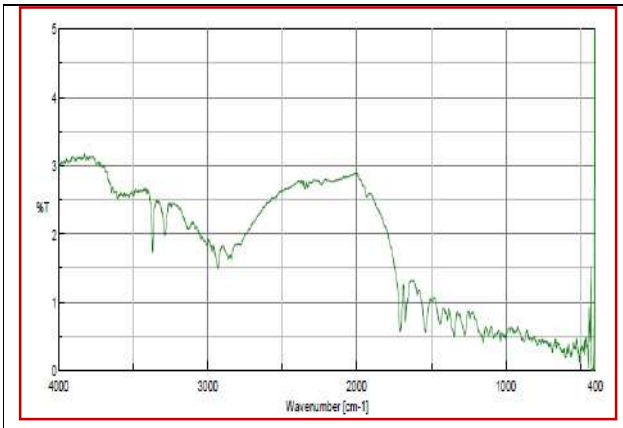

Fig. 5: FT IR Spectarum of Pure Nisoldipine and Eudragit RS – 100

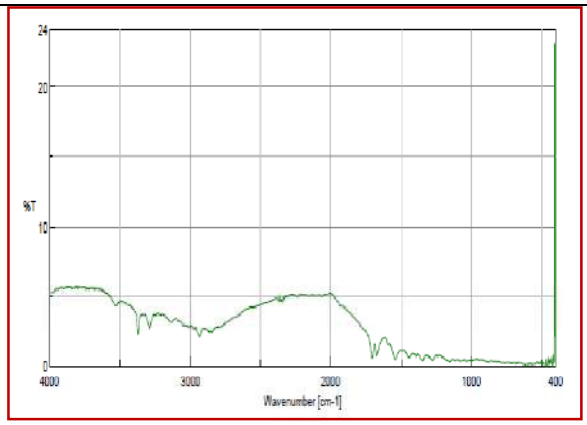

Fig. 6 : FT IR Spectarum of Pure Nisoldipine and Lactose monohydrate with Microcrystalline cellulose pH 101

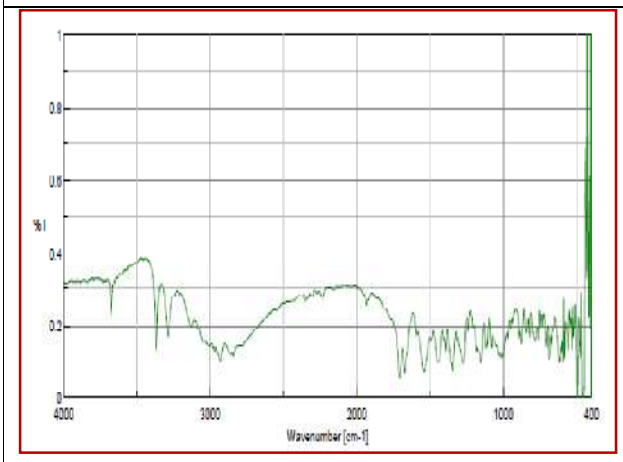

Fig. 7 : FT IR Spectarum of Pure Nisoldipine and Glidant

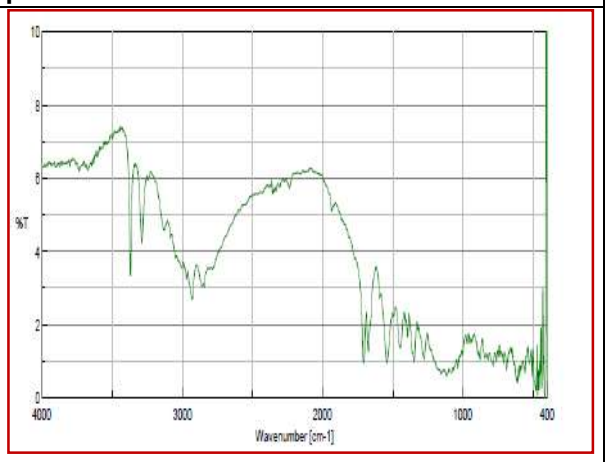

Fig. 5 : FT IR Spectarum of Pure Nisoldipine and Sodium Lauryl Sulphate

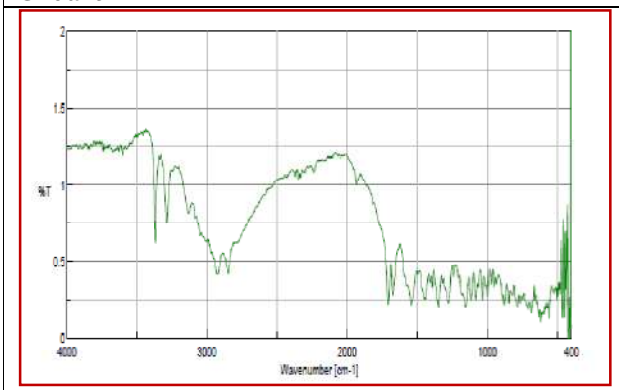

Fig. 8 : FT IR Spectarum of Pure Nisoldipine and Magnesium Stearate

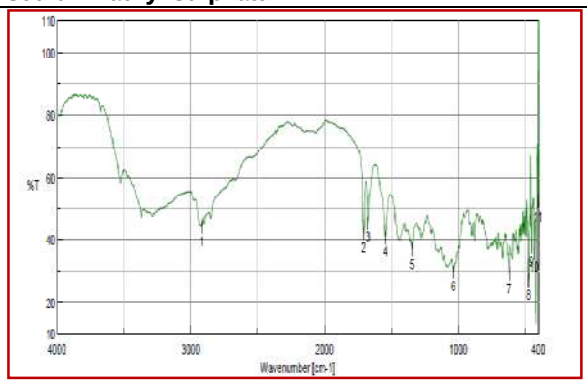

Fig. 9: FT IR Spectarum of Pure Nisoldipine and Physical Mixture of Nisoldipine and Optimized Formulations

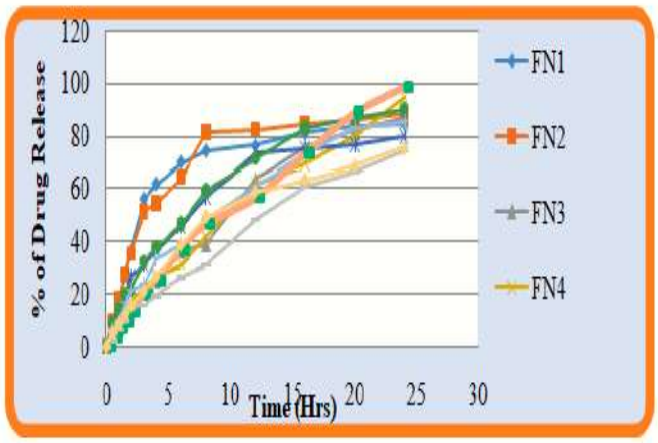

Fig. 10: *In vitro* percentage dissolution studies of controlled release matrix tablets of Nisoldipine FN1 – FN9 with Marketed Formulation

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of *Pisonia alba* (Cabbage Tree) Extract on Corrosion Inhibition of Carbon Steel in Well-Water

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Received: 18 Jan 2021 Revised: 15 Feb 2021 Accepted: 24 Feb 2021

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ABSTRACT

The corrosion protection effects of *Pisonia alba*(PA) with Zn^{2+} on carbon steel in well-water has been investigated by weight-loss studies using various immersion period, electrochemical studies, and surface morphology was studied using metallurgical microscope techniques. The highest inhibition efficiency of 91% was obtained at the optimum concentration of 500ppm of PA extract using weight-loss studies. Polarization curves indicate that the inhibition action of PA extract has mixed type on carbon steel surface in well-water. The electrochemical impedance spectroscopy indicates that the formation of safe layer by the charge transfer resistance is stimulated in controlling the corrosion of carbon steel. F- Test indicates that the effect of Zn^{2+} on the inhibition efficiency of various concentrations of *Pisonia alba* is statistically significant. The influence of pH reveals that the highest inhibition efficiency of 91% was achieved in pH 8. The kinetic and thermodynamic parameters were calculated for various temperatures using weight-loss studies such as activation energy (Ea), enthalpy of adsorption (ΔH), free energy of adsorption (ΔG_{ads}), heat of adsorption (Q_{ads}), entropy of adsorption (ΔS_{ads}), and K_{ads}.. From these values, the adsorption process of PA leaves extract on carbon steel surface is spontaneous and endothermic. the protective layer forming on the metal surface has been studied by SEM and AFM Surface analysis, it is producing the safe layer consist of Fe^{2+} -PA extract complex and $Zn(OH)_2$. The EDS Spectra studies indicating the existing of elements in inhibitor molecules and Zn^{2+} ions over carbon steel surface in well-water.

Keywords: carbon steel, weight-loss, PDP, EIS Studies, F-Test, SEM, AFM, EDS spectra.

INTRODUCTION

Plant extracts are important as an environmentally acceptable, readily available, and renewable source for a wide range of inhibitors [1]. In general, plant extracts are inhibitors with high inhibition efficiency and non-toxicants. Extract several plants [2-6]. Steel finds tonnes of applications in industries such as metal finishing, boiler removal, pickling baths, etc., steel rust when in contact with an aqueous medium. The use of inhibitors is one of the simplest methods for shielding metals from corrosion. Corrosion may be a chemical or electrochemical process in nature with four components, including an anode, cathode, electrolyte, and a few direct electrical connections between anode and cathode. The adsorbed inhibitor then acts to slow the corrosion process by either: increasing the behaviour of anodized or cathodic polarisation or reducing the movement or diffusion of ions to the metallic surface. Corrosion inhibitors are not recommended to prevent corrosion in such cases. The majority of known inhibitors are organic compounds containing heteroatoms, such as O, N, S, and multiple bonds [7]. Corrosion is the deterioration of metals and alloys by electrochemical reactions with their environment. This is a phenomenon that cannot be avoided, but is often controlled and prevented by appropriate preventive measures such as metallic coating, anodic protection, cathode side protection and the use of inhibitors, etc. Inhibitors have a very good role to play in the corrosion control process. Previously, tonnes of natural products have been used as corrosion inhibitors for various metals in different environments [8-11]. Research on whether or not the synergism effect of this inhibitor system is statistically significant by performing an F-test was used by the Variance analysis (ANOVA) to develop an appropriate mechanism for inhibiting corrosion based on the results of the weight-loss, polarisation, and AC impedance spectra studies. The protective film formed on the metal surface is characterized by surface analytical techniques such as Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM).

EXPERIMENTAL METHODS

Composition of the Carbon Steel Specimen

Preparation of the *Pisonia alba Extract*

By refluxing 10g of dried *Pisonia alba* tends to leave in presence of 200ml of ethanol for 3h, the plant extract was prepared and then filtered off using Whatmann No.1 filter paper. By using distilled water, these crude extracts were dried and then a dense solid mass was obtained. 1g of *Pisonia alba* crushed up leaves has been decided to make with a 100ml Standard Measuring Flask with double distilled water up to the mark. Various amounts of leaf extracts are decided to make from this solution.

Weight-Loss Method

Different composition of *Pisonia alba* was dipped in a solution of well water which contained various concentrations of Zn^{2+} ions. This was performed for a day, with and without Zn^{2+}. The specimens were weighed with a digital balance (Shimadzu AY62) before and after they were immersed in well-water, before calculation the inhibition efficiency of corrosion.

$$IE\ \% = \frac{W_1 - W_2}{W_1} X\ 100$$

Where,

W_1 and W_2 are weight loss values with and without the inhibitor

Preparation of stock solutions

Preparation of Zn^{2+} Solution

4.4g of zinc sulphate was correctly dissolved in double-distilled water and up to 1 litre was produced. A hundred-fold dilution yields a concentration of Zn^{2+}exactly 10ppm.

Preparation of Sodium Hydroxide Solution

10g sodium hydroxide was correctly dissolved inside of 250ml standard measuring flask to make a 1N solution from transferring the essential double distilled water up to the mark. this solution was utilized to exact strength was calculated by titrating against standard oxalic acid solution using phenolphthalein as an indicator, to accomplish an ordinary solution marginally greater than 1N

RESULT AND DISCUSSIONS

Weight-loss Measurement Study

Weight-loss method was used to calculation of inhibition efficiency and corrosion rate for an ethanolic extract of *Pisonia alba* on carbon steel immersed in well-water to one day period. The details of result are provided in Table 3 Inhibition efficiency of various concentration of PA in absence and presence of Zn^{2+} were plotted in Figure 2

Effect of PH on PA-Zn^{2+}system

The inhibition efficiency in pH 3 is 62%; the highest inhibition efficiency of 91% was achieved in pH 8 when compared with pH 3,12.

Effect of Immersion Period on PA-Zn^{2+}System

The effects of immersion period from 1 to 7 days on the corrosion inhibition behaviour of carbon steel in well- water containing various concentrations of PA with Zn^{2+}. The inhibition efficiency and corrosion rate was calculated using weight-loss study. The results are given in Table 5. The inhibition efficiency and immersion period are plotted in Figure 3. When the immersion period is increased, the inhibition efficiency is decreased and also corrosion rate was increased. The results are indicating the safe layer deteriorates and desorption in the solution on the metal surface due to increasing the corrosion process in the inhibitor systems.

The Effect of Temperature on Inhibition Efficiency and Corrosion Rate

The influence of temperature on the corrosion behaviour of carbon steel in well-water containing various concentrations of *Pisonia alba* leaves extract was investigated using weight-loss study [12-14]. The results are given in Table 6. The effect of various temperatures in well-water with and without *Pisonia alba* leaves extract is plotted in Figure 4.

The Effect of Kinetic and Thermodynamic Parameters

The effects of kinetic and thermodynamic parameters are used to determine the type of adsorption and reaction (endothermic (or) exothermic) in well-water containing various concentrations of *Pisonia alba*. The results are given in Table 7. The activation energy value is increased from 17.14kJmol^{-1} to 117.80kJmol^{-1}. When concentration of *Pisonia alba* is increased from 100ppm to 500ppm. The results are indicating the physical adsorption on the carbon steel surface [16]. The values of enthalpy of adsorption (ΔH) are found to be 14.57kJmol^{-1} to 115.24kJmol^{-1}. The Positive values of ΔH indicate that the reaction is endothermic on carbon steel surface [17]. The free energy of adsorption values are around from -16.23KJmol^{-1} to -17.69KJmol^{-1}. The ΔG_{ads} values ranging is suggests that the adsorption of *Pisonia alba* on carbon steel belongs to the physisorption as well as the adsorption layer has an electrostatic character [18-23]. The negative values of ΔG_{ads} and Q_{ads} indicate that the adsorption of the inhibitors on carbon steel is a spontaneous process and there is a strong interaction between the inhibitor molecules and carbon steel surface [24-25].The negative values of ΔS_{ads} are confirmed to forming an ordered stable film of the inhibitor molecule on carbon steel surface as well as a stable equilibrium between the adsorption and desorption processes [26]. The K_{ads} values are decreased from 20.00mol^{-1} to 4.45mol^{-1}, when temperatures are increased from 303K to 333K. These values are indicating that adsorption of PA leaves extract on the carbon steel surface. It is also confirms to physisorption onto carbon steel surface [27].

F-STUDY

F-test was utilized to study the effect of Zn^{2+} on the inhibition efficiency of *Pisonia alba* is statistically significant or not. The F-test results are shown in table 9. The F-value obtained 4.42 for 10ppm of Zn^{2+} is not statistically significant [27]. Since, this value is less than critical F-value 5.32 for 1.8 degree of freedom at 0.05level of significance. The F-values obtained 10.09 and 14.55 for 20ppm and 30ppm of Zn^{2+}. Hence, these values are greater than critical F-value 5.32 for 1.8 degree of freedom at 0.05level of significance. Therefore, it is concluded that the effect of 10ppm of Zn^{2+} over the inhibition efficiency with different concentrations of *Pisonia alba* has statistically insignificant but the F-values for 20ppm and 30ppm of Zn^{2+} with various concentrations of *Pisonia alba* has been statistically significant [28-30].

Analysis for Polarization Curves

Polarization study was utilized to determine the type of inhibitor action on metal surface through tafel slopes values and calculate the corrosion potential, anodic, cathodic slope values, linear polarization resistance, and corrosion current. The corrosion potential value was shifted from -580Mv Vs SCE to -590Mv. Vs SCE. The anodic and cathodic slope value is increased from 116.23mV/dec to 185.89mV/dec and 110.88mV/dec to 180.32mV/dec. The linear polarization resistance value is increased from $963\Omega cm^2$ to $3671\Omega cm^2$ in with and without of PA-Zn^{2+} system (500-30 ppm).The current density value is decreased from $5.5431x10^{-5}A/cm^2$ to $0.06897x10^{-5}A/cm^2$ in absence and presence of PA-Zn^{2+} system (500-30 ppm). From the above results shows that inhibitor acts as a mixed type of inhibitor on carbon steel surface [31].

Ac Impedance Spectra

Impedance spectra are utilized to identify the protective layer formed on the metal surface in absence and presence of inhibitor system and zinc ions.

Scanning Electron Microscopy Technique

The SEM image 7(a) for polished carbon steel surface is indicating the smooth surface of the metal without any corrosion products. The SEM image 7(b) for carbon steel immersed in well water is confirmed that the existing highly corroded area on carbon steel surface. The SEM image 7(c) for carbon steel immersed in presence of 500ppm of PA and 30 ppm of Zn^{2+} within well water gives insoluble complex on the carbon steel surface because of reduced the rate of corrosion on the carbon steel surface. The SEM analysis is mostly used to investigating for metal surface in various inhibitor systems [33-34].

Energy Dispersive X-Ray Study

EDAX spectra are used to determine the compositions of elements present in the inhibited and uninhibited system on carbon steel surface in before and after corrosion process. The EDAX spectrum of polished carbon steel is shown in figure 8(a), in this spectrum contains mainly Fe with small percentages of carbon and oxygen. The EDAX spectrum of carbon steel in well water for one day period is provide in figure 8(b), this spectrum contains elements of iron oxides, iron hydroxides like Fe, C, O and Cl etc., The EDAX spectrum of carbon steel in presence of 500ppm of PA and 30 ppm of Zn^{2+} for one day period in well water is shown Figure 8(c), this spectrum study indicated the presence of elements in inhibitor molecules and Zn^{2+} on the metal surface [34-39].

Atomic Force Microscopy Techniques

AFM study was used to analyse the roughness parameters and image morphology on carbon steel surface in well-water containing 500ppm of PA and 30ppm of Zn^{2+}. From above AFM images, Figure 9(a) for polished carbon steel surface is uncorroded carbon steel surface due to small roughness was obtained on polished carbon steel surface by atmospheric corrosion. Figure 9(b) for carbon steel is immersed in well-water, this image displays highly corroded on metal surface with maximum roughness area. Figure 9(c) for carbon steel is immersed in well-water containing 500ppm of PA extract and 30ppm of Zn^{2+}. Therefore, these images are confirmed that the protective layer formed on the metal surface for good corrosion resistance.

CONCLUSION

It is observed that the immersion time increases, while the corrosion rate increases. *Pisonia Alba* report that inhibits efficiencies increases as well as Zn^{2+} concentration increases. F-test confirms a synergistic effect between the PA and Zn^{2+}Systems. The study of Potentiodynamic Polarization curves indicates the complex of *Pisonia alba* and Zn^{2+} behaves as a mixed type of inhibitor. EIS measurements indicating the steel corrosion was widely regulated by charge-transfer resistance. The kinetic and thermodynamic parameters are confirmed that the inhibitor molecules adsorbed on carbon steel surface through physical adsorption, the adsorption process is spontaneous and endothermic. SEM studies indicate the safe layer was established onto metal surface. EDAX spectra support an inhibitive action as well as unrestricted process composition of its element present on the surface of carbon steel. AFM tests confirm that the smooth barrier film. Because of the formation of Fe^{2+}-PA and $Zn(OH)_2$ shielding layer around the metal surface.

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Composition of the Carbon Steel Specimen

Table 1 Percentage of Composition for Carbon steel specimen

Types of Elements	Phosphorous	Manganese	Sulphur	Carbon	Iron
Percentage of composition (%)	0.06	0.4	0.0267	0.1	99.287

Table 2 Taxonomy of *Pisonia alba*

S.No.	Kingdom	Plantae
1	Phylum	Tracheophyta
2	Class	Magnolipsida
3	Order	Caryophyllales
4	Family	Nyctaginaceae
5	Common Name	Cabbage tree
6	Tamil Name	Nanju Murichchan [poison cutter]

Table 3 Determination of IE and CR for PA-Zn^{2+}system (Period of immersion: 1 day)

PA (ppm)	IE%				CR (mmpy)			
	Zn^{2+} (ppm)				Zn^{2+} (ppm)			
	0	10	20	30	0	10	20	30
0	----	11	23	27	0.1809	0.1610	0.1393	0.1321
100	15	29	38	40	0.1538	0.1284	0.1122	0.1085
200	17	31	41	56	0.1501	0.1248	0.1067	0.0796
300	25	42	54	67	0.1357	0.1049	0.0832	0.0597
400	35	58	69	87	0.1176	0.0759	0.0561	0.0235
500	41	64	78	91	0.1067	0.0651	0.0398	0.0163

From the above results the maximum inhibition efficiency of 91% was achieved for 500ppm of PA and 30ppm of Zn^{2+} system. The inhibition efficiency is increases and also corrosion rate was decreases for various concentration of PA in absence and presence of Zn^{2+}. The table shows that the combination of these two inhibitors system has achieved better inhibition efficiency when compared to individual inhibitor

Table 4 Influence of pH on IE and CR for PA-Zn^{2+}inhibitor system Period of Immersion: one day

System	pH		
	3	8	12
Well-water CR (mmpy)	0.2067	0.1809	0.1912
PA-Zn^{2+} system (500:30 ppm)CR (mmpy)	0.0785	0.0163	0.0325
IE (%)	62	91	83

Table 5 Effect of Immersion Period on PA-Zn^{2+}System

Immersion Period (days)	Corrosion Rate		IE%
	Well-water (mmpy)	PA+ Zn^{2+} (500:30 ppm) (mmpy)	
1	0.1809	0.0163	91
3	0.2013	0.0362	82
5	0.2124	0.0467	78
7	0.2158	0.0626	71

Table 6 Corrosion rate and Percentage of Inhibition efficiency for different temperatures (30ppm of Zn^{2+})

Concentration of PA (ppm)	303K		313K		323K		333K		343K	
	IE %	CR	IE%	CR	IE%	CR	IE%	CR	IE %	CR
Blank	-	0.1809	-	0.2248	-	0.2437	-	0.2728	-	0.3102
100	40	0.1085	36	0.1802	31	0.2118	27	0.2411	21	0.2701
200	56	0.0796	52	0.1612	48	0.1898	42	0.2118	35	0.2321
300	67	0.0597	63	0.1324	59	0.1462	53	0.1742	46	0.1826
400	87	0.0235	83	0.0923	77	0.0966	71	0.1249	65	0.1325
500	91	0.0163	87	0.0726	83	0.0299	76	0.0429	69	0.1192

From the above results, the temperature increased from 303K to 343K, The inhibition efficiency has obtained to slightly reduces from 91% to 69% as well as the corrosion rate is increased from 0.0163 to 0.1192. This results are indicating that adsorption of the extract of PA onto carbon steel may be due to physical adsorption [15].

Table 7: Kinetic parameters for PA-Zn^{2+} System (30ppm of Zn^{2+})

Concentration of PA	Ea $KJmol^{-1}$	ΔH $KJmol^{-1}$
Blank	17.14	14.57
100	40.00	37.44
200	55.65	53.09
300	62.81	60.25
400	107.88	105.32
500	117.80	115.24

Table 8: Thermodynamic Parameters for *Pisonia alba* extract (500ppm) in well-water

Temperatures (K)	Q_{ads} ($KJmol^{-1}$)	K_{ads} (mol^{-1})	ΔG_{ads} ($KJmol^{-1}$)	ΔS_{ads} ($KJmol^{-1}$)
303	-32.50	20.22	-17.69	-0.1656
313	-26.52	13038	-17.20	-0.1397
323	-38.72	9.76	-16.90	-0.1722
333	-33.48	6.33	-16.23	-0.1493

Table 9 F-values distributed between the IE of PA-Zn^{2+} System

Zn^{2+} (ppm)	level of significance of F	source of variance	sum of squares	degree of freedom	mean square	F
10	P > 0.05	Between	828.1	1	828.1	4.42
		Within	1498	8	187.3	
20	P > 0.05	Between	2160.9	1	2160.9	10.09
		Within	1713.2	8	214.15	
30	P> 0.05	Between	4231.5	1	4231.5	14.55
		Within	2326	8	290.8	

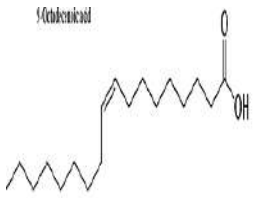
Table 10 Polarization Parameters for PA-Zn^{2+} System

System	E_{corr} [mV] Vs SCE	ba [mV/dec]	bc (mV/dec]	LPR (Ω cm^2)	I_{corr} (A/cm^2)
Blank (WW)	-580	116.23	110.88	963	5.5431×10^{-5}
PA-Zn^{2+} (500:30 ppm)	-590	185.89	180.32	3671	0.6897×10^{-5}

Table 11 AC Impedance Parameters of well-water and PA-Zn^{2+} System

System	R_{ct} (Ω cm^2)	C_{dl} ($\mu F/cm^2$)
Blank (WW)	391	1.3137×10^{-6}
PA-Zn^{2+} (500:30 ppm)	3018	0.0253×10^{-6}

From the above table shows that R_{ct} value is increased and also C_{dl} value is decreased from 1.3137 to 0.0253x 10-6. The From 391ohm cm^2 to 3018ohm cm^2 above results are indicating strong layer on the metal surface [32]. The R_{ct} value gives 87.04 inhibition efficiency for PA-Zn^{2+} system (500ppm-30ppm) with well water.



Major active components observed in ethanolic extract of *Pisonia alba*

Figure 1: Apperance of Pisonia alba

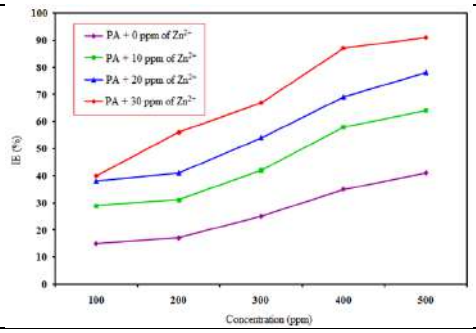


Figure 2 Graphical representation to Concentration (ppm) Vs IE (%) for PA-Zn^{2+} System

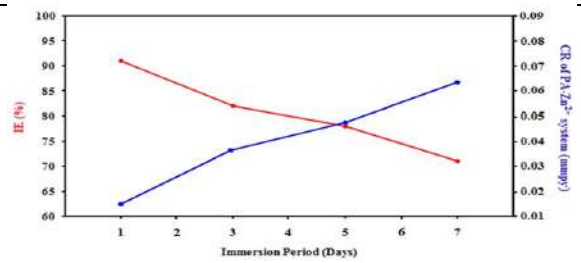


Figure 3 Graphical illustration of Effect of Immersion Period for PA-Zn^{2+}System

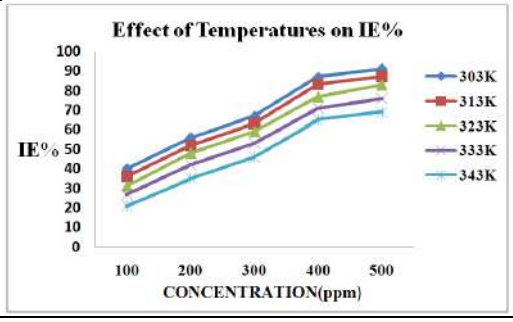


Figure 4: Effect of temperatures on inhibition efficiency in well-water containing various concentration of PA Leaves extract and 30ppm of Zn^{2+}

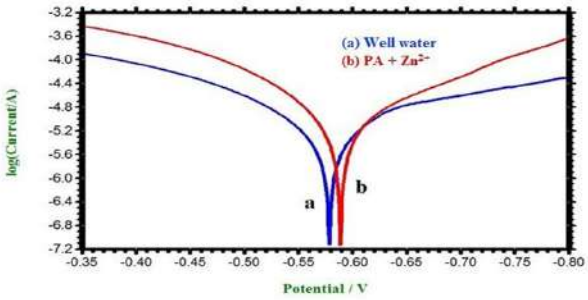


Figure 5 Tafel Curves Correlation for PA-Zn^{2+} System onto carbon steel with well-water

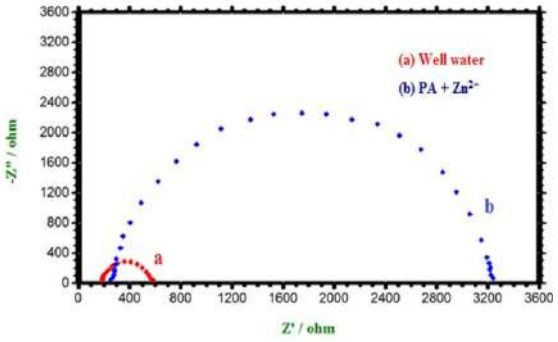


Figure 6AC Impedance Spectra (a) Blank-well-water (b) PA-Zn^{2+} System

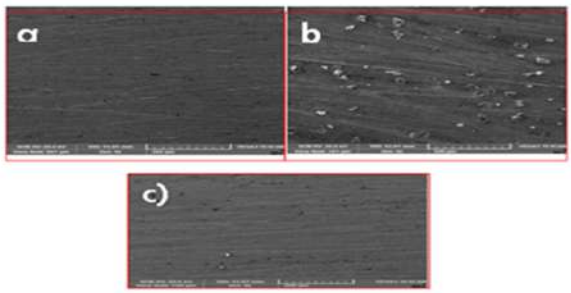


Figure 7 SEM images for Polished-Carbon steel (a), blank (well-water) (b), and PA-Zn^{2+} system (c)

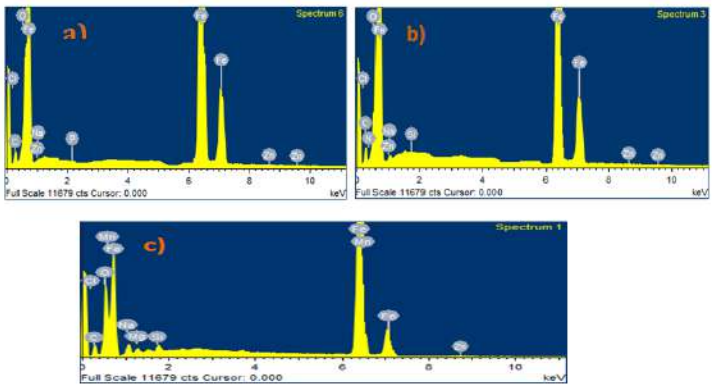


Figure 8 EDX Spectra for (a) pure carbon steel, (b) Blank (well-water) and (c) PA-Zn^{2+} (500:30ppm)

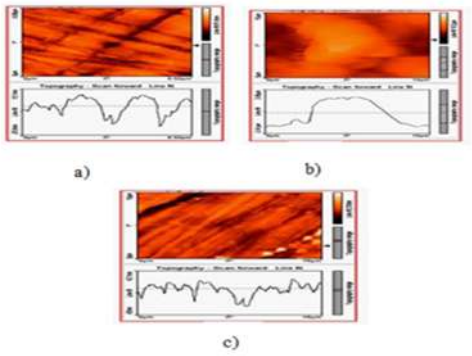


Figure 9 AFM cross sectional AFM images of polished carbon steel (a), blank (well-water) (b), and PA-Zn^{2+} (500-30ppm) (c)

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Landslide Hazard Zonation along Sesawng Village and Seling Village Thingsulthliah Rural Development Block, Aizawl District.

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ABSTRACT

Landslides are the most well-known hazard in hilly state of Mizoram. Development, expansion of human settlement and the uncontrolled interaction with the nature are the reason for landslide in Mizoram. Vulnerability of landslide Mizoram is already high due to lithology, land use and land cover, geomorphology and slope factor. The extensive mining activities along the highways for road construction, building materials and unplanned settlement also cause one of major landslide in the state of Mizoram. The study investigates the Landslide Hazard Zone in the highway number 150 and 54 of Thingsulthliah rural development block. The National Using Remote Sensing and Geographic Information System (GIS), thematic layers like slope morphometry, geomorphology, lithology and land use / land cover were generated. The weightage rating framework subject to relative criticalness of various causative factor is used on the different classes of thematic layers. The classes were assigned to the relating rating value as attribute information in the GIS. The thematic layer of each classe was assigned to an ordinal rating from 0 to 10.

Keywords: Landslide hazard zonation, GIS, Remote sensing, mitigation and Thingsulthliah Rural Development Block

INTRODUCTION

Landslide is one of the major geo-environmental dangers in the Indian Himalayan Region including the province of Mizoram. Landslides are straightforwardly connected with the structurally dynamic Himalayan districts, and can be considered as the most widely recognized characteristic dangers which lead to harm in the street area and neighborhoods in the sloping landscapes (Gurugnanam *et al.,* 2012). Geologically, Mizoram is young formation with North-South moving anticlinal edges with steep slope and mediating synclinal valleys. Faulting and folding in

numerous regions has delivered steep flaw scarps (GSI, 2011). Accordingly, the whole zone is commonly inclined to landslide. Due to increase of population and development in activities in the state i,e urban and rural areas. The vulnerability of human settlements to landslide is ceaselessly expanding. Thus, when it happens in residencential area, Landslide become calamities (Chandel *et al.,* 2011). Also, the human settlement can be exceptionally helpless against cataclysmic events because of high thickness and areas on steep slope (Rawat *et al.,* 2010). A few landslide disasters have been recorded from Aizawl area throughout the previous twenty years. Enormous landslide in the stone-quarry at South Hlimen town in 1992 killed 66 occupants and 17 houses were obliterated. Land subsidence happened in 1994, in Aizawl Venglai , Ramthar and Armed veng regions which caused severe damaged to 65 houses. In 1995, at Hunthar area close by Aizawl to Sairang (National Highway 54),due to slip surface along the road, around 17 houses were destroyed.

The subsidence at Hunthar locality wich took place in 1995 occurred again in 1999 endangering the structures of about 12 houses and 11 families within this area were evacuated. During the monsoon of 2011, Lengpui Airport road was blocked by landslide causing havoc to commuters and, within Aizawl city around 10 houses were dismantled and about 15 families were evacuated. In 2012, a huge rockslide at the stone-quarry near Keifang locality (Saitual town) claimed the lives of 18 people which is among the worst tragedies in terms of geo-environmental catastrophes in Mizoram. The same year experienced a logline crack at Ramhlun Sport Complex in which 10 houses were dismantled and almost 60 families vacated their houses. During the month of May 2013, there was a massive landslide at Laipuitlang locality within Aizawl city claiming the lives of 17 persons. More than 10 persons were injured, about 12 houses and 16 vehicles were damaged. Due to the manifold miseries and problems it causes, attempts to study landslide within the state of Mizoram is one of the most important and challenging aspect in the field of geology.

Study Area

The study area lies under Thingsulthliah Rural development Block in the state of Mizoram between 92° 85.33'E to 92° 85.90"E and 23° 75.0'N to 23° 68.79'N in Aizawl district and falls under Survey of India topographical map No. 84A/14 and No 84A/13

MATERIALS AND METHODS

Data Used

Indian Remote Sensing Satellite (IRS-P5) stereo- matched Cartosat-I information having spatial goal of 2.5 m and Quick bird data having spatial resolution of 0.8 m were utilized as the main data. SOI topographical maps and different subordinate information were additionally alluded to.

Thematic Layers

There are a few causative elements that actuated landslide (Bijukchhen *et al.,* 2009). Determination of these components and planning of thematic data layers are profoundly significant for landslide susceptibility mapping (Sarkar and Kanungo, 2004). Joining of multi-sources of data means a major and Kausik, 2013). In the current investigation, five significant thematic layers were set up from satellite information and field work. These layers were then used for Landslide Hazard Zonation. The various layers are as per the following

Slope

Landslide are more boundless in the precarious slope zone than in moderate and low incline zones (Sharma et al.,2011). This is because of the way that the shear stress in soil or other unconsolidated material increases as the incline point increases. Therefore, slope is one of the most important parameter for stability consideration (Lee *et al.,* 2004; Nithya and Prasanna, 2010). Slope map was generated using the IRS-P5 stereo-paired Cartosat-I data and Digital Elevation Model (DEM) in a GIS environment. The inclines zone represent in degrees, and are

advantageously isolated into eight slope aspects, viz 30-35, 35-40,40-45,45-60 and above 65 degree. Weightage values are assigned in accordance with the steepness of the slope. The slope map of the study area is shown in Fig 2.

Land Use / Land Cover

The landslides are found commonly in and out around the area where there are excessive land use along the highways and human settlements. Land use and landslides may be helpful in understanding the role of land use that induces landslides. Abandoned land in the form of old jhum with sparse vegetation cover are basically potential site of landslide .As land use/land cover controls the pace of enduring and disintegration; it is one of the most significant factor in Landslide Hazard Zonation. The study area was divided into five classes, viz., Heavy, Light Vegetation, Scrubland, barren land and Built up. Areas with thick vegetation spread were seen as less prone to landslide (Mohammad Onargh *et al.,* 2012), thus, Heavy Vegetation class was allocated low weightage value. Built up areas were more prone to landslide as compare other the wide range of various classes (Pandey *et al.,* 2008) and were given high weightage. The study area was divided into five classes, viz., Heavy Vegetation, Light Vegetation, and Scrubland, Built-up and barren land. Areas with dense vegetation cover were considered less prone to the occurrence of landslides (Mohammad Onargh *et al.,* 2012), hence, Heavy Vegetation class was assigned low weightage value. Built-up areas were more prone to landslide than all the other classes (Pandey *et al.,* 2008) and were given high weightage. Land use / land cover is shown in Fig 3.

Lithology

Lithology is one of the factor for landslide hazard zonation (Sharma et al.,2011). The geology of Mizoram comprised of incredible flysch facies of rocks involving dreary groupings of shale and sandstone (La Touche, 1891). The area situated in Surma Group of Bhuban formation (GSI, 2011) and this Bhuban formation was sub-isolated into Lower, Middle and Upper. Middle Bhuban which consist of mainly argillaceous rock is exposed and Upper Bhuban of Arenaceous rock is also exposed within the study area. The area is made up of three different types of sedimentary rocks viz; sandstone, shale and silty shale. Sandstone occupied the major portion of the formation which are highly compact with fine to medium grained having two distint colors. The weathered sandstone horizons are brownish in colours while the less weathered sandstones are grayish in color. Three litho-units have been established for the study area purely based on the exposed rock types. These are named as Shale-sandstone, Silty shale unit and Sandstone-shale unit. Lithological units including shale and siltstone are more susceptible to landslide than the hard and compacted sandstone - shale units. Silty shale is the most susceptible unit to landslide. As per this, weightage values are alloted for analysis.

Geomorphology

Geomorphic units assume significant part in the vulnerability of settlements and transport communication. Consequently, it is a significant factor in landslide hazard zonation (Chandel *et al.,* 2011). The area has high allveation or nearby allevation and was arranged to highly dissected and Less dissected structural slopes. High allevation areas are more vulnerable to landslide than areas with lower allevation (Lee *et al.,* 2004) and following this example, weightage values were given to each of the geomorphic classes. The geomorphological map of the study area is appeared in Fig 4.

DATA ANALYSIS

The road along Sesawng village and Seling village was buffered 100m on both side to delineate the study area keeping in mind that any landside incident within that vicinity may damage the road and disrupt any kind of transportation activities. Landside inventory was done along the highway in which later and dormant landslide were recognized, anaylse and plotted in a GIS. The geo-environment factor like incline morphometry, land use/land spread, lithology and geomorphology are assuming significant roles in causing landslide in the study area. These four play the significant parameters for hazard zoantion and are independently isolated into suitable classes.

Singular classes in every parameters are painstakingly analysed to set up their connection to landslide susceptibility. Weightage value is attributed for each classes on the basis of susceptibilty to landslide in such a way that less weightage exhibits to minimal impact towards landslide occurance, and more weightage, the most higest. The task of weightage value for the various classes inside a parameter is done with the accepted or expected significance in prompting landslide dependent on the apriority information on the experts. All the thematic layers were incorporated and dissected in a GIS utilizing ARCINFO (10.4 rendition) to determine a Landslide Hazard Zonation map. The plan of giving weightages by National Remote Sensing Agency (NRSA, 2001) and dependability rating as formulated by Joyce and Evans (Joyce and Evans1976) were embraced in the study as appeared in the table 1.

RESULTS AND DISCUSSION

Very High Hazard Zone

This zone is highly unstable due to wedge failure of slope. The area is steep slopes with rock fall prone area due to illegal quarrying. Tlungvel old quarry lies in the area. Unscientific extraction of boulder for construction materials and due to high angle cutting which resulted the upper rock to fall.

High Hazard Zone

It principally incorporates areas where the debris sliding probability is at a high risk. It is areas of steep inclines which when upset is inclined to landslide. Besides, this zone comprises areas where the dip of the rocks and slope of the area, which are usually very steep, are in the same direction. The human settlement also come under this zone .Subsidence of road occur in the area due to landslip. Landslide also occur due to rainfall and its saturate soil, lithology in soft nature and unconsolidated debris.

Moderate Hazard Zone

This zone is viewed as stable and albeit steep slope may include for this area, less overlying debris and non appearance of anthropogenic action make the zone less unsafe. . It is best not to upset the natural drain, and at the same time, slope modification ought to be dodged quite far.

Low Hazard Zone

In this zone, the area of different controlling parameter is commonly unlikely to antagonistically impact the slope stability. Vegetation is moderately thick, the slope angles are commonly low, around 30 degrees and below. Large part of this zone lies over consolidated debris, hard and compact rock type. This zone is mainly confined to areas where human activities are less or absent.

The present study area proved that physical factors like slope, geomorphology, land use/land cover and lithology are directly linked with landslide hazard. The study area has been categorized into four different hazard zones i.e. very high hazard, high hazard zone, moderate hazard zone and Low hazard zone. The study area is mostly moderate hazard and low hazard zones.

Mitigation Measure

Complete prevention of landslide is a very difficult task. However, the effects of landslides especially the smaller ones and those provoked by human activities, can be minimized. So the study revealed the following

i) The study areas fall under Tertiary sediments composed mostly of argillaceous shale and sandstone interbedded.

ii) The identified slip surfaces are to be treated for conservation measures in order to avoid triggering of new slides in the area.

iii) Area required preventing soil erosion and extensive gully erosion.

iv) Rock excavation and extraction have been done primarily from bottom of the rock formation i.e. toe cutting. This type of excavation and extraction triggered and exacerbated the rock fall. So, this kind of excavation and extraction of rock must be banned.

v) Develop good and effective drainage system so that slope materials do not become water logged and trigger the slide.

CONCLUSION

The present study proved that the physical factors like land use/ land cover, lithology, slope, and geomorphology are directly linked with landslide hazard. Fortunately, due to low slope angles and limited human activities, large part of the study area falls under Low to Very low landslide hazard zones. Due to increase in anthropogenic activities which may cause hazard in settlement and uncontrolled widening of roads can induce the occurence of landslides. Therefore, the landslide hazard map prepared through the present study can be utilized for identifying the critical areas for implementing suitable mitigation measures as well as for selecting sites further expansion of settlement. It is also significant to bear in mind that though very high and high hazard zones are confined in small areas, the major part of moderate hazard zone may also become highly unstable if unplanned anthopogenic activities are continued without considering the geo-environmental condition.

ACKNOWLEDGEMENTS

The authors are thankful to their colleagues of Mizoram University for their cooperation and support during the course of study.

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Table: Ratings for Parameters on a scale of 1-10

Parameter	Rank in percent	Category/Unit	Weight
Lithology	30	Shale-sandstone	8
		Sandstone-shale	4
		Silty-shale	6
Slope in degree	40	30-35	4
		35-45	4
		45-60	6
		>61	8
Geomorphology	20	Highly dissected structural hill	7
		Less dissected structural hill	4
Land Use / Land Cover	10	Heavy vegetation	2
		Light vegetation	7
		Scrub land	7
		Built up	6
Total	**100%**		

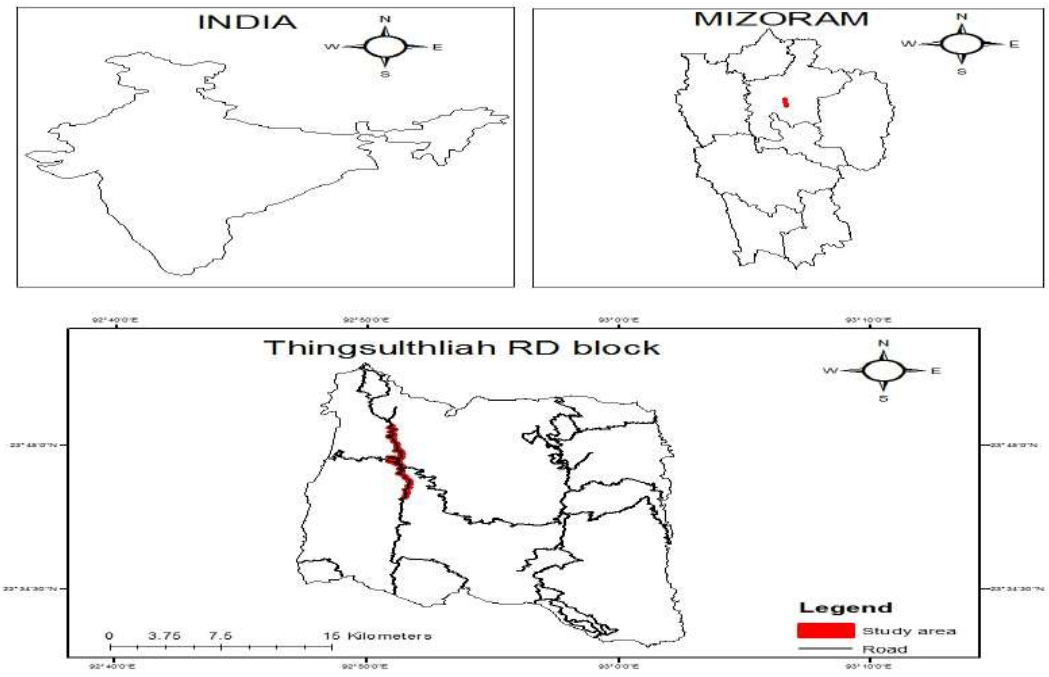

Figure 1. Study Area

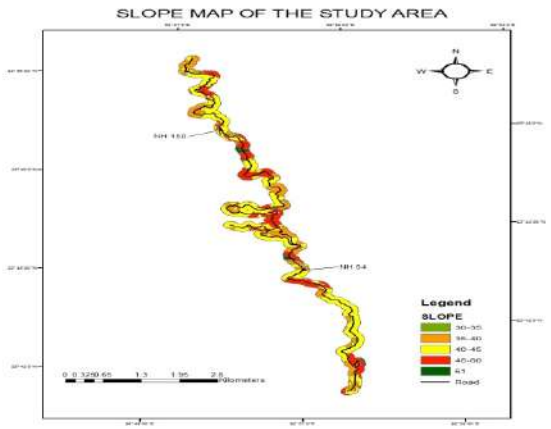

Figure 2. Slope Map of the Study Area

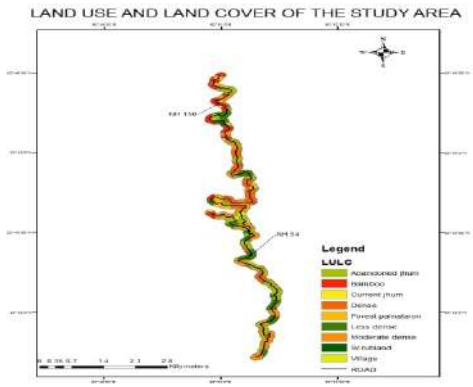

Figure 3.Land Use And Land Cover Map of the Study Area

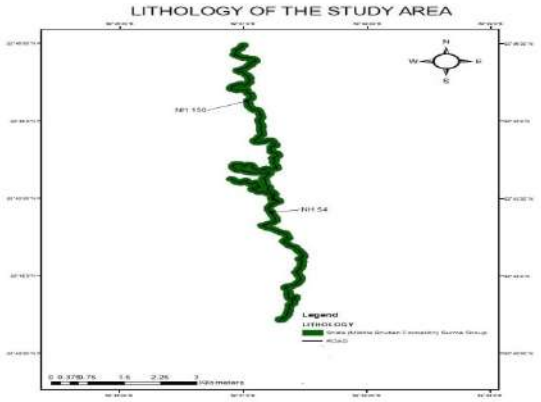

Figure 4. Lithology of the Study Area

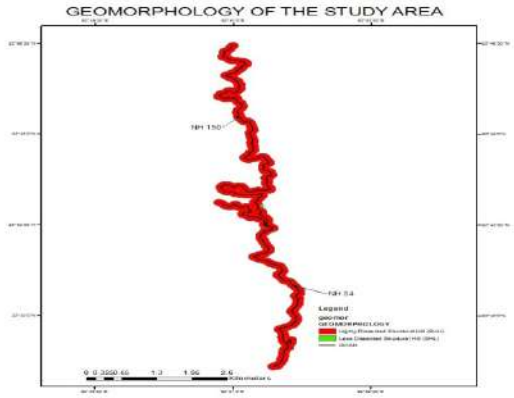

Figure 5.Geomorphological Map of the Study Area

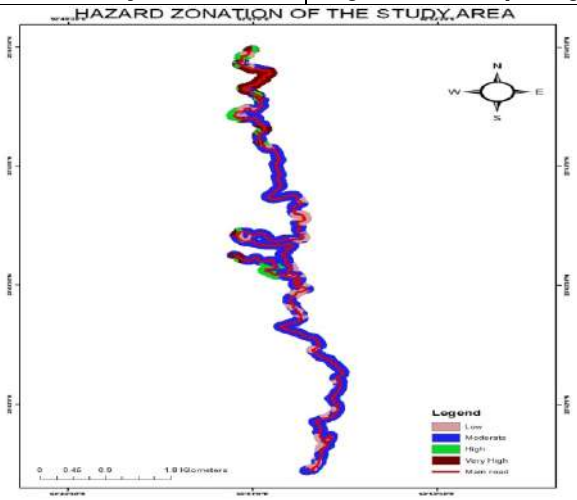

Figure 6: Hazard Zonation Map of the Study Area

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Antibiotic Resistant activity of *Escherichia coli* Isolates from Poultry Farm against Selected Antibiotics

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Received: 02 Mar 2021 Revised: 04 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

In the present study antibacterial resistance of *Escherichia coli* isolated from poultry form was conducted. In this study there were 400 microbes were isolated from poultry farm. Among this 230 *E. coli* isolates were identified through the biochemical and serological study. Antibiotic used in this study no one was found cent percent of antibacterial activity. *E. coli* isolates were developed resistance to the antibiotics. High level of resistance was showed against penicillin G (P) 10µg (95%) and low level of resistance was found in tetracycline (TE) 30µg. From the study it is concluded that the *E. coli* isolates were developed resistances to the antibiotics used in poultry form for the control of poultry diseases. It is due to the indiscriminate uses of antibiotics in poultry form to prevent the diseases.

Keywords: Poultry form, *E. coli,* serology, antibiotics and antibiotic resistance

INTRODUCTION

Escherichia coli are common microbial flora present in the gastrointestinal tract of poultry, human being and other animals and, may become pathogenic to both [1, 2].However, most of the isolates *E. coli* are nonpathogenic but considered as indicator of contamination in food. The *E. coli* infection in poultry form is usually considered as a secondary infection, which is triggered by various factors particularly environmental factors including overcrowding, poor ventilation and other biological predisposing factors such as parasitic or viral infections [3].About 10 to 15% of intestinal coliforms are pathogenic [4] and cause a variety of lesions in immune compromised hosts as well as in poultry. Among the diseases some are often severe and sometimes lethal infections such as urinary tract infection, epidemic diarrhea of adults and children [5] and yolk sac infection, omphalitis, cellulitis, coli

granuloma, and coli bacillosis [6].Thus, antibiotics have been introduced into animal populations mainly for disease treatment for many years, just after the first antibiotic, tetracycline, were introduced to human health. Antibiotics are extensively used to poultry production for growth promoters or to control infectious disease. Antibiotic is most important issue that promotes the emergence, selection and spreading of antibiotic-resistant microorganisms in both veterinary and human medicine [7, 8]. It was proved by well established evidence that antibiotics can lead to emergence and dissemination of resistant *E. coli* which can passed into people *via* food or direct contact. Resistant microbes may act as a potential source in the transportation of antimicrobial resistance to human pathogens [9, 10].In recent years, due to enormous uses of antibiotics in veterinary field, large number of resistant bacterial strains would develop resistant to antibiotics. The transmission of plasmid mediated resistance between different bacterial species and genera are now widely occurred [11]. Thus the present study was focused to evaluate the antibiotic resistances of *E. coli* strains isolated from poultry form.

MATERIALS AND METHODS

Collection of Samples

The tissues were collected based on the methodology provided by Department of Pathology, Veterinary College, Bangalore, Poultry Disease Diagnostic Laboratory, Bangalore and Central Disease Investigation unit, Bangalore. Totally 400 samples from 150 birds of 2-7 weeks of age were collected in sterile containers following aseptic precautions and transported to laboratory. Samples were collected from different organs such as liver, heart, intestine and faecal material.

Isolation and Identification

The tissue samples were placed on Macconkey agar (HIMEDIA) and incubated 24 hours at 37^{o}C. The lactose fermenting colonies were reinoculated to another media containing Eosin Methylene Blue (HIMEDIA) agar. Colonies produced metallic sheen was transferred to Nutrient agar slants and it was incubated for 24 hours at 37^{0}C and stored at 4^{o}C for further identification.

Bacteriological Analysis

Identification of isolated *E. coli* strain was done according to Buchanan and Gibbons [12]following a series of biochemical test and straining technique[13]included gram staining, tests for oxidase, methyl red, Voges-Proskauer reactions, indole, citrate, catalase, urea hydrolysis, gelatin hydrolysis, lactose fermentation, nitrate reduction, casein hydrolysis and sugar fermentation.

Antimicrobial Sensitivity

Antibiotic resistance activity was done by the method of Bauer *et al* (1966) utilizingMueller Hinton Agar plates (HIMEDIA) by placing 20 mmantibiotic discs purchased from local medical shop. The antibiotic discs such as Amikacin (AK) 10µg, Ciprofloxacin(CIP) 5µg, Erythromycin (E)15µg, Pencillin G (P) 10µg, Streptomycin (S) 10µg, Tetracycline (TE) 30µg and Levofloxacin (LE) 5µgwere used for the antibiotic resistance evaluation.

RESULTS AND DISCUSSION

In the present investigation, identification of antimicrobial resistance activity of *E. coli*was conducted. *E. coli* samples were collected from liver, heart, faecal material and intestine of poultry form. There were 400 samples were collected from chicken and 150 chicken were used for the collection of much samples. Among them totally 230 *E. coli* samples were isolated through the biochemical and staining analysis. Highest percent of *E. coli* was isolated in faecal material (74%) followed by intestine (68%) liver (50%) and heart (30%)(Table 1). Biochemical analysis of the collected samples revealed that more or less 85% isolates of *E. coli* were present in the collected samples (Table 2).

The sensitivity and resistance pattern of these isolates for various antibiotics are presented in Table 3 and Fig. 1, 2. It was observed that no one of the antibiotics used in the present study were found to cent percent effective. Multiple antibiotic sensitivity and resistance pattern was observed in all of the *E. coli* strains and it was coincided with previous studies [14, 15].*E. coli* isolates were showed that the different percentages of sensitivity and resistance to the antibiotics. High level of bacterial resistance was recorded against Penicillin G (95.19%) followed by Ciprofloxacin (85%), Levofloxacin (80%), Amikacin and Erythromycin (70%) and Streptomycin (65%). Low level of antibiotic resistance was observed in Tetracycline (55%). Rahman *et al*. [16] reported that the *E. coli*isolates from broiler and layer poultry form were found resistant to chloramphenicol, ampicillin, ciprofloxacin, tetracycline and streptomycin in the range of 37-87.5%; and 50-66.6% of strains were highly sensitive to chloramphenicol and gentamicin. Islam *et al.* [17] studied antibacterial resistance of *E.coli* isolated from poultry in Bangladesh. They reported that 66-100% *E. coli* strains showed resistance to tetracycline, penicillin, erythromycin and chloramphenicol. Tricia *et al*. [18]reported 43% isolates of *E. coli* showed resistant to ampicillin but not to gentamicin. The antibacterial resistant of these drugs was due to the regular usage in poultry industry for the control of pathogenic avian colibacillosis. The present results of the study were varied with other workers, indicating that antibiotic pattern varied with different isolates, time and development of multiple resistances of the drug among *E. coli* isolates related to transmission plasmid [19]. The resistance plasmids of *E. coli* from poultry to human transmission have also been reported [20]. The result of the present study was coincided with above mentioned results. From the study it is concluded that *E. coli* isolates from poultry form possessed antibacterial resistance activity against the antibiotics used in poultry form. Moreover, high level of resistance was recorded in Penicilin G. It is due to the continuous usage of antibiotics in the poultry form. Further study will be carried out to identify the effective and less or side effect free antibiotics for the control of poultry diseases.

ACKNOWLEDGEMENT

The authors thanks to the Management, the Principal and the Head, Department of Microbiology, J.J College of Arts & Science (Autonomous), Pudukkottai, for providing necessary facilities to do this research work

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.Successfully.

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Table 1: Distribution of *Escherichia coli* in various samples of poultry form

Sample Source	No. of Samples Tested	No. of Samples Positive for *E. coli* Detection	Percentage positive samples
Liver	100	50	50
Heart	100	38	38
Intestinal fluid	100	68	68
Faecal	100	74	74
Total	400	230	58

Table 2: Biochemical tests used for identification of *Escherichia coli*

Biochemical Test	*Escherichia coli* Reaction
Oxidase	Negative
Catalase	Positive
Indole	Positive
Methyl red	Positive
Vogasproskauer's	Negative
Citrate utilization	Negative
Glucose	Positive
Adonitol	Negative
Arabinose	Positive

Lactose	Positive
Sorbitol	Positive
Mannitol	Positive
Rhamnose	Positive
Sucrose	Positive

Table 3: Antibiotic resistance activity of selected strains of *Escherichia coli*

Antibiotic Disc	Sensitivity Groups of *Escherichia coli* Isolates					
	Resistant		Intermediate		Sensitivity	
	% of strains positive	Inhibition zone (mm)	% of strains positive	Inhibition zone (mm)	% of strains Positive	Inhibition zone (mm)
Amikacin (AK) 10 µg	70.00	<18	20.00	15-17	10.00	>18
Ciprofloxacin(CIP)5 µg	85.00	<21	00.00	16-20	15.00	>21
Erythromycin(E)15 µg	70.00	<23	00.00	14-22	05.00	>23
PencillinG (P) 10 µg	95.00	<15	15.00	NIL	05.00	>15
Streptomycin(S) 10 µg	65.00	<15	25.00	12-14	20.00	>15
Tetracycline (TE)30 µg	55.00	<15	00.00	12-14	30.00	>15
Levofloxacin(LE) 5 µg	80.00	<17	15.00	14-16	20.00	>17

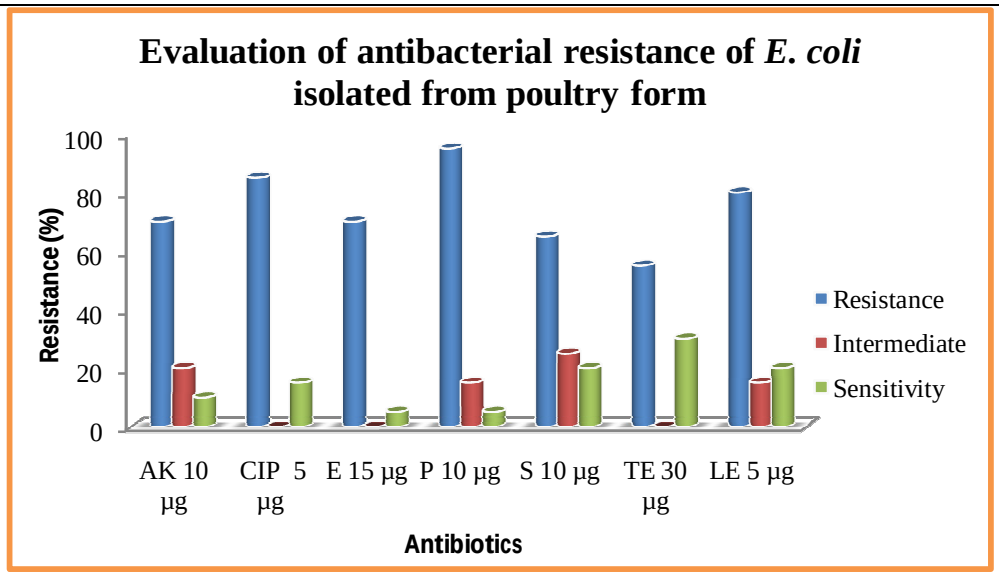

Fig. 1.Evaluation of antibacterial resistance of *E. coli* isolated from poultry form

AK- Amikacin10µg, CIP – Ciprofloxacin 5µg, E – Erythromycin 15µg, P – PencillinG 10µg, S – Streptomycin 10µg, TE – Tetracycline 30µg LE – Levofloxacin 5µg

Fig.2.Antibiotic resistant activity of *E. coli* strain isolated from poultry form

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Food and Feeding Habits of Tilapia, *Oreochromis mossambicus* (Peters, 1852) From Jannapura Tank of Bhadravathi, Karnataka

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Received: 09 Mar 2021 Revised: 12 Mar 2021 Accepted: 15 Mar 2021

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ABSTRACT

The food study of *Oreochromis mossambicus* collected from Jannapura tank of Karnataka, India revealed that the food of juveniles mainly consisted of rotifers (30%), followed by copepods (25%), Chlorophycean algae (20%), Diatoms (15%), aquatic insects (5%) and miscellaneous items (5%). While, the food items recorded in the gut of adults were Chlorophycean algae (30%), followed by Diatoms (20%), rotifers (25%), copepods (15%), aquatic insects (5%) and miscellaneous items (5%). During this study, it was found that the juveniles of *O. mossamobicus* mainly feed mainly on zooplankton, and the adults feed on phytoplankton. Intense feeding was noticed during summer and juveniles were the active feeders. The stomachs of fishes collected for the study of food and feeding habits were classified depending on their relative fullness into gorged, full, ¾ full, ½ full, ¼ full, little and empty. Fishes with stomachs classified as gorged, full, ¾ full, ½ full were considered to have actively fed, whereas those with ¼ full and little as poorly fed. Empty stomachs were found in all the months with peaks in June-July and January in both juveniles and adults.

Keywords: Food and feeding, *Oreochromis mossambicus*, exotic fish, Jannapura tank, Karnataka.

INTRODUCTION

Food assumes a significant part in the development, movement and generating conduct of the fish. As the idea of food relies on the idea of a few biotic and abiotic factors, the issue is interesting from explicit, just as environmental perspective (Bhuiyan et al., 2006; Sakhare and Jetithor, 2016). Studies on the food and taking care of propensities

show the species specialty in the environment, their food inclinations and food range covers. The investigation of food and taking care of propensities for freshwater fish species is a subject of consistent exploration since it comprises the reason for the improvement of an effective fisheries the board. Various fishes devour various sorts of food things. In this way, the food and taking care of propensities for fish have tremendous biological worth. By examining the food and taking care of propensities, the example of between explicit competition of fishes can be evaluated (Sakhare and Jetithor, 2016)**.** Cichlid fish *Oreochromis mossambicus* (Figure 1) is confined to Africa and the Middle East. They have spread primarily through presentations for fish cultivating and are currently found in all tropical and semitropical continents. *Oreochromis* is presently being cultivated in excess of 90 nations. It is found in India, Thailand, Sri Lanka, Vietnam, South Africa, Java, Malaysia, Uganda and so on and the backwater lakes of Indonesia (Sakhare and Jetithor,2016**)**. The common name in this locale is '*Jilabi meenu'*, because of its accessibility to develop rapidly with low quality information sources. There is no scientific literature on food and feeding habits of *O. mossambicus* from Malnad region of Karnataka. Hence, the present study is carried out.

MATERIALS AND METHODS

Study Area

Jannapura tank is located at about 3 Kms away from Bhadravathi town (Figure 2) in Shivamogga district, Karnataka, This tank is situated at 13°48'37''-13°52'30''N latitude & 75°40'42''-75°43'33''E longitude and it is a perennial tank and receives the water from Bhadra left channel water also as rain fall water .The area of the tank is about 20 ha and depth is around 5-10mt respectively. This water body is used for irrigation, human activities and fisheries.

Food and Feeding Habits

Both qualitative and quantitative analysis were carried out during July 2016 to June 2017 to study the food contents of *Oreochromis mossambicus.* Each fish gut was observed under a binocular microscope in the laboratory. For qualitative analysis, the identification of food items was made up to the generic level.

Quantitative Analysis

The stomach contents were washed and points were given depending on the relative volume of each food item, taking into consideration the extent of fullness of stomach and the amount of food consumed. In the present study, the fullness of the stomach was classified as (i) gorged ; (ii) full; (iii) ¾ full; (iv) ½ full; (v) ¼ full; (vi) little; and (vii) empty based on the amount of feeding and the grade of distention of the stomach.

Statistical Analysis

One way ANOVA is calculated to know the significance difference in the food items of juveniles and adults by using good calculator software.

RESULTS AND DISCUSSION

Diet composition of various food items found in the gut of *O. mossambicus* is depicted in Table 1. A total of 60 fishes were examined. The length of juveniles ranged from 1.6 cm to 8.3 cm and While, the length of adults varied from 17 cm to 26 cm respectively.

Juvenile Fishes

Rotifers (35%) formed the main diet in juveniles of *O. mossambicus* (Figure 3). Copepoda (25%) formed the next important item of diet of juveniles followed by Chlorophycean algae (20%). Diatoms formed 15% of the diet. Aquatic insects (5%) found in the gut were insect larvae and mosquito larvae. Miscellaneous items contributed to 5% of the gut content of *O. mossambicus***.**

Adult Fishes

Chlorophycean algae formed the main item of gut contents forming 30% (Figure 4). Diatoms was next in the order of dominance forming 25% . Rotifers formed 20% of the food items. Copepods formed 15% of the gut contents . Aquatic insects formed 5% and miscellaneous items contributed to 5% and consists of debris, plant material, mud, sand, fish parts etc.

Feeding Intensity

Feeding intensity fluctuated throughout the year showing two peaks . The degree of fullness of stomach in *O. mossambicus* during the study period is depicted in Figures 5 and 6. Percentage of empty stomachs was higher during June and January in juveniles and during July and January in adults. The feeding rate was reduced drastically during spawning months.

DISCUSSION

Green et al.(1978) found that juvenile with 8-18mm of *O. mossambicus* in Java were feed on zooplankton. A similar finding was reported by Le Roux (1956) where, juvenile with 5-7.5cm in freshwater ponds were feed mainly on Entomostraca. Both juveniles and adults were reported to be opportunistic feeders (Schuster,1951; Neil,1966; Man and Hodgkiss,1977). Aravindan (1980) reported that *O. mossambicus* is selective in feeding and prefers aquatic plants even though filamentous and unicellular algae, copepods and detritus are abundant in the tank. Singh and Shukla (2014) observed an increase in feeding intensity with increase in water temperature and also observed that the medium sized fishes were more active feeder than larger sized fishes. Sakhare and Jetithor (2016) observed intense feeding during summer season .While, Indira et al.(2013) noticed low feeding intensity with an increase in size of *O. mossambicus*. Similar observation noticed in the current study also. Ramesh and Kiran (2016) studied the pattern of food and feeding habits of cat fish, *Clarias batrachus*. They reported that the food of *Clarias batrachus* consisted of zooplankton, insect larvae, fish larvae, small shrimps and organic debris. Fish larvae and insect larvae were preferred as the major food. On average for all months, insect larvae dominated. The feeding intensity is poorer from pre-spawning and spawning period but remarkably higher from post spawning period.

CONCLUSION

Along these lines, there is an urgent need to observing of the populace status of this fish and reach extension of *O. mossambicus* in the investigated tank and related parts of the region. The assessment on the historical backdrop of intrusion, natural surroundings explicitness and eco-biology of the fish is recommended to assess its possible effects on local fishes and for creating viable administration approach. Additionally, attention to the ramifications concerning this intrusive species ought to be produced among researchers, fish farmers, lawmakers and the public peoples to accommodate the thorough use of such administrative measures.

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Table 1: Food items recorded in the gut of *Oreochromis mossambicus*

Groups	**Organisms**
Rotifera	*Brachionus, Lecane, Keratella, Filinia* sp.
Copepoda	*Cyclops, Mesocyclops* , *Diaptomus* sp.
Diatoms	*Fragillaria*, *Synedra, Nitzschia, Navicula, Melosira, Pinnularia Gomphonema* sp
Chlorophycean algae	*Spirogyra, Ulothrix, Chaetophora, Scenedesmus, Pediastrum* sp.
Insects	Insect larvae, mosquito larvae
Miscellaneous	Debris, plant material, mud, sand, fish parts

Table 2: One way ANOVA data for degrees of fullness of food items of Juveniles and Adults

Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**	
Juveniles	72	16.6665	22.6745	2.6722	
Adults	72	16.6342	24.4211	2.8781	
Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F-Stat**	**P-Value**
Between fishes	1	0.0376	0.0376	**0.0001**	**0.9934**
Within fishes	142	78847.1384	555.2615		
Total:	143	78847.1759			

F-statistic value = 0.00007 ; P-value = 0.99345

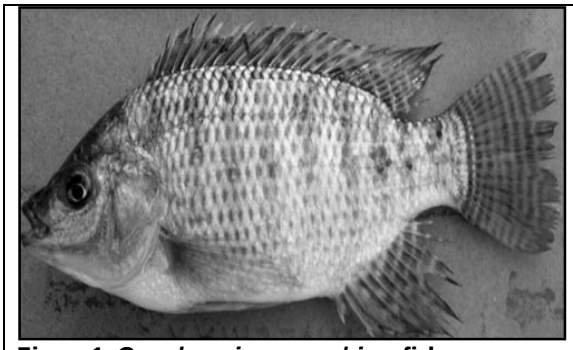

Figure 1: *Oreochromis mossambicus* fish

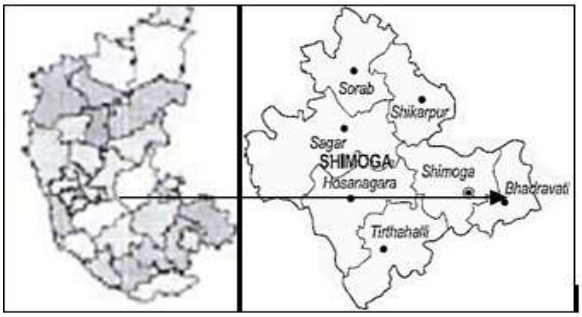

Figure 2: Location of Jannapura tank in Bhadravathi area

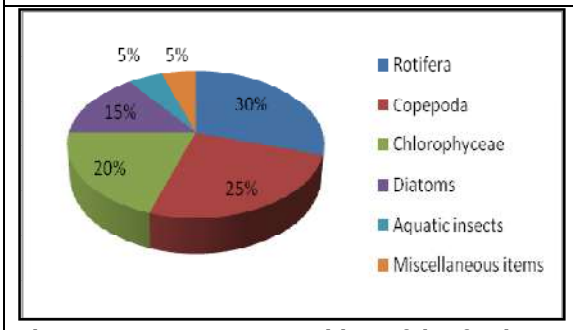

Figure 3: Percentage composition of the food items in the juveniles of *O. mossambicus*

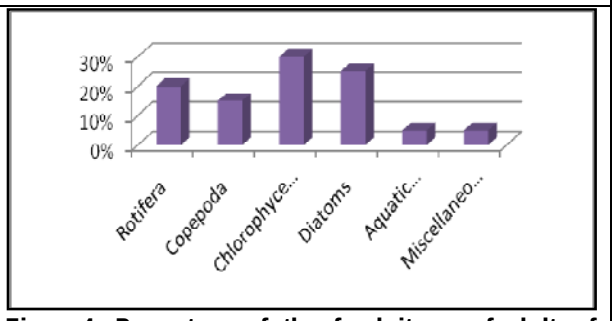

Figure 4: Percentage of the food items of adults of *Oreochromis mossambicus*

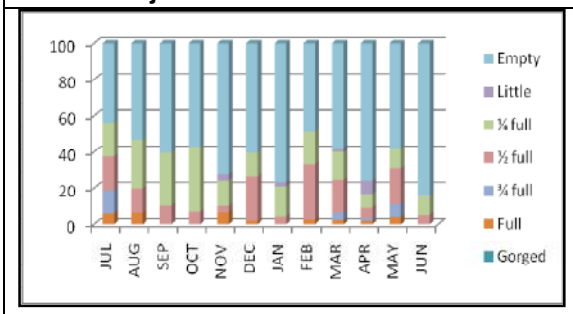

Figure 5: Degree of fullness of stomach in Juvenile of *O. mossambicus*.

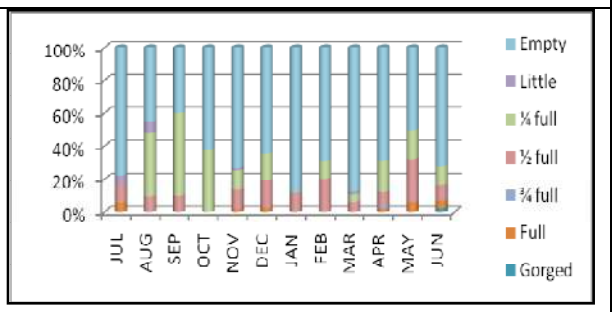

Figure 6. Degree of fullness of stomach in the adult of *O. mossambicus*.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Review on Fish Composition in the Rivers of Udupi and Shivamogga Districts, Karnataka

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Received: 11 Feb 2021 Revised: 18 Feb 2021 Accepted: 26 Feb 2021

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ABSTRACT

This review paper is deals with the fish diversity in the rivers of Udupi and Shivamogga district as worked out by several researchers. In Udupi district 8 rivers were selected. Among these rivers Sita, Swarna and Varahi consists of 82 species belongs to 20 families and 42 genera. While, Nethravathi, Kumaradhara, Phalguni, Shambhavi, Swarna & Sowparnika rivers harbor 78 species with 36 families having 58 genera. In Shivamogga district Six rivers were selected for this review i.e.,Tunga, Bhadra, Tungabhadra, Varada, Varahi and Sharavathi. A total of 31 families and 8 orders are recorded. Among the fish families Cyprinidae was dominant with 47 species at Tungabhadra river followed by Tunga, Bhadra , Tungabhadra (40 species) and Varada river with 35 species. While, Bagridae was dominant at Varada river with 10 species .As per as fish order is concerned Cypriniformes was dominant in Varada, Tunga, Bhadra and Tungabhadra rivers with 40 species proceeded by Varahi with 33 species. Siluriformes is dominant with 24 species at Varada river followed by Tunga, Bhadra, Tungabhadra with 14 species. However, Perciformes was dominant at Varahi river with 11 species.

Keywords: Fish fauna, Rivers, Udupi and Shivamogga district, Fish families, Orders.

INTRODUCTION

Water bodies are the fundamental assets abused for inland fisheries and comprehension of fish faunal assorted variety which is a significant viewpoint for its improvement and the sustainable management. Wetlands in India support rich assortment of fish species, which thusly encourage the business capability of the fisheries (Krishna and Piska, 2006). Other than to these credits, fishes are considered as one of the significant protein rich nourishment source among the water fauna (Sukla and Upadhay,2000;Shanawaz et al.,2011; Thirumala and Kiran,2017). Fishes are not just significant pointers of environmental wellbeing and the wealth, yet additionally keep up a parity in the natural way of life by expending microscopic fish and little creatures and structure nourishment for some creatures. This balance in natural way of life might be influenced because of contamination in aquatic system. Furthermore, there are numerous dangers to fish variety, for example, development of dam, which hinder the producing relocations and presentation of fascinating species and over fishing. In this way, knowing the status of fish fauna is vital to prevent the loss of specific species (Ramanjaneya and Ganesh,2016)**.** Fish is exploited from both inland and marine habitats. There is no proper documentation of sources of freshwater fishes of India due to irrational fishing practices, water abstraction, increased sedimentation and pollution over the years (Narasimhaiah et al., 2016). However, the study of fish fauna with respect to rivers of Shivamogga and Udupi districts of Karnataka in particular is lacking. Hence, the present review investigation is a scientific documentation of fish fauna of rivers of above districts, Karnataka.

Fish Fauna in Udupi District

Seeta River

Narasimhaiah et al (2016) made an attempt to understand the diversity of fishes in the Seeta river of Udupi district. A total of 20 species belonging to 11 different families were identified. In their study, the largest fish *Clarias dossumieri* (37 cm) and the smallest fish *Etroplus suratensis* (8.6 cm).

Nethravathi, Kumaradhara, Phalguni, Shambhavi, Swarna & Sowparnika Rivers

Sushmitha Rao et al (2013) studied a systematic survey to record the diversity of fishes in Dakshina Kannada and Udupi districts. 78 fish species,58 genera of 36 families have been recorded from them. About 40 species are found in rivers and 26 species are common in tanks and in rivers. Nearly 21 species are to be evaluated for their conservation status. The species distribution along with the current status of the water bodies are presented.

Sita, Swarna and Varahi Rivers

Venkateshwarlu et al (2014) made an attempt to study the conservation status of fish diversity in rivers-Sita, Swarna and Varahi of Udupi district. The richness of fishes consists of 82 species with 8 orders, 20 families and 42 genera. Among them the order Cypriniformes was the most dominant group in the assemblage composition with 52.43% followed by order Siluriformes with 17.07%. 3 species are Critically Endangered; 10 species are Endangered; 9 species are Vulnerable; 70.37% are under Low Risk near threatened and only 2 species are in Low Risk least concern. The fish diversity in relation to water quality of 03 rivers Sita, Swarna and Varahi of Udupi district, Karnataka, India was studied by Arun Kumar Shetty et al (2015).They recorded 71 species,7 orders, 20 families and 41 genera from 21 sites. The species richness and abundance of fishes were correlated with land-use cover, canopy, pH and turbidity. Discharge of sewage, surface runoff from agricultural lands and water diversion that alter the quality of habitat. Range extension of *Horabagrus brachysoma* in Udupi district of Karnataka was studied by Venkateshwarlu and Arun Kumar Shetty (2013) in Sita, Swarna and Varahi rivers. *Horabagrus brachysoma* belonging to the family Bagridae. Their paper extends the geographical range of this fish in North of Kerala to the Western parts of Karnataka. This catfish was found in upstream, downstream as well as midstream reaches of all the three rivers.

Occurrence of Fishes in Rivers of Shivamogga District

Gangadhara Gowda et al (2015) recorded a total of 34, 42 and 48 fish species in Tunga, Bhadra and Tungabhadra rivers, separately. In their examination, the order Cypriniforms was seen as most predominant followed by the Siluriformes, Perciformes, Osteoglossiformes, Synbranchiformes, Beloniformes and Cyprinodontiformes. The non-native species are commonly progressively effective where local species are drained because of anthropogenically changed water systems. Their discoveries will profit the arranging and the board of sustainable fisheries and protection of natural assets at national level.

River Tunga

The fish species with cypriniformes were predominant and have indicated constant conveyance speaking to the species.The other species represented by *Oreochromis mossambicus, Notopterus, Parambassis ranga* and *Aplocheilus lineatus* were likewise recorded by Gangadhara Gowda et al (2015).

Kumar Naik et al.(2013) documented 37 fish species belonging 11 families and 4 orders in Tunga river stretch from Gajanoor fishing village to Kudali of Shivamogga district. Gangadhara Gowda et al (2015) infer that the upper stretches of river Tunga supported great fishery yield, while the lower stretches of waterway at Shivamogga didn't support great fishery because of weakened water quality as demonstrated by the low DO content, higher BOD, alkalinity, nitrate and phosphate levels. The lower starches of Tunga river upheld progressively exotic, tolerant, omnivorous and carnivorous fishes with a uneven conveyance in existence. Arunachalam and Muralidharan (2007) have reported a new fish species like Batasio sharavatiensis from Tunga river, Karnataka.

River Bhadra

A several studies have been done on fish assorted variety in connection to water nature of Bhadra river of Karnataka. The total number of fish species announced by Thirumala et al.(2011) was similarly low (33 species), while number of fish species revealed by Shahnawaz et al.(2010) and Shivashankar and Venkataramana (2012) were relatively high (56 and 48 species, separately).Their investigation revealed the predominance of cyprinids in Bhadra river. This demonstrated cyprinidae fishes have wide scope of distribution and arrangement. The order cypriniforms was dominant with continuous distribution. Cat fishes were also recorded by above researchers.

Gangadhara Gowda et al (2015) opined that the upper and lower stretches of river Bhadra upheld great fishery production, while the center stretches of river Bhadra at Bhadravathi didn't support great fishery because of debilitated water quality as demonstrated by the low DO content, raised convergences of BOD, ammonia, nitrate and phosphate. The middle stretch of Bhadra waterway have upheld progressively exotic, tolerant, omnivorous and voracious fishes with an uneven distribution in the river.

Shahnawaz and Venkateshwarlu (2009) were surveying the fish variety of two significant rivers of Karnataka to be specific Tunga and Bhadra. Their examination has indicated that both the rivers upheld rich fish variety and 77 fish species were recorded having a place with 5 orders, 18 families and 44 genera. To the extent preservation status is concerned (IUCN,1994), 20 fish species are classified into LR-nt, 33 NA, 10 VU, 7 EN, 2 DD, 4 and 1 as CR and LR-lc individually. They opined that for the best possible management and use of this fish wealth, it is important to find a way to sustainable steps and monitor this fish wellbeing. The fish species like *Garra mullya, Cirrhinus fulungee, Cirrhinus reba, Salmostoma*, *Rasbora* and *Puntius* groups were more dominant. Therefore, their study indicates that cyprinid fishes are found to be the more dominant group than others which is supported by Shanawaz and Venkateshwarlu (2009).

River Tungabhadra

The fish species with cypriniforms were predominant and have demonstrated constant distribution, representing to the species, for example, *Barilius barna, B. bendelisis, Cirrhinus mrigala, Danio aequipinnatus, D. malabaricus, D. devario, Gerra gotyla, Labeo angra, L. fimbriatus, Puntius jerdoni, P. sarana, P. sophore, Rasbora* and

Salmostoma boopis. Among catfishes, *Sperata aor, A. seenghala, Mystus cavasius, Rita pavimentata, Clarius batrachus* and *Wallago attu* were prevailing. The various species like *Pangasius, Mastacembelus armatus, Channa marulis, C. striatus, Etroplus maculates, Oreochromis mossambicus, Glossogobius giuris* and *Notopterus* were dominant at the lower stretches of the river (Gangadhara Gowda et al.,2015). Recently, Chaudhary (2014) studied on the impact of effluent and sewage release on the aquatic life and the spatial conveyance of fish species in 200 km stretch of Tungabhadra river based on oxygen inconstancy all through the river utilizing QUAL2K model. This reproduced model was utilized to find the indicator species on the river based on disintegrated oxygen level. Likewise this model revealed that the spatial distribution and species variety of some fishes is fluctuating with the variation in effluent load and movement of water in the river.

River Varada

Kumar Naik et al.(2013) has put forth an effort to explore the fish assets quantitatively by examining the ichthyofaunal biodiversity of Varada river stretch from Karehonda fishing town to Bankasana of Soraba. During their investigation, a sum of 78 species with 18 families and 6orders were recorded. The order Cypriniformes was seen as predominant with 40 fish species (51.28%) followed by Siluriformes 24 species (30.76%) and Perciformes 10 species (12.82%). albeit, 78 species were recorded, the Cyprinidae recorded with 35 fish species (44.87%) preceded by Bagridae 10 (12.82%),Schilbeidae and Ambassidae with 5 fish species (6.41%) each to total fish landing.

River Varahi

ArunKumar Shetty (2013) recorded a complete of 61 fish species representing 6 orders, 15 families and 35 genera in Varahi river of Karnataka. The dominated cyprinid fishes contributed 43.55% in the assemblage group and among the cyprinids only 15.55% species like *Puntius chola ,Puntius parrah ,Rasbora daniconius, Rasbora rasbora, Garra gotyla gotyla*, were reported by him. Species uniqueness is extremely less. The three species like *Gerres erythrourus, Gerres limbatus* and *Gerres filamentosus* are the fish species which were recorded only from the river Varahi and showed the exceptionality in distribution. Bhat (2003) studied the fish diversity within the four rivers viz., Sharavati, Aghanashini, Bedti and Kali and reported 63 species in Bedti, 53 species in Kali, Aghanashini (52 species) and Sharavathi river with 51 species respectively.

STATISTICAL ANALYSIS

Table 3 and 4 shows one way ANOVA and Tukey HSD data**.** The F-value is 0.94713 and the p-value is .44847. The Tukey's HSD (honestly significant difference) procedure facilitates pair wise comparisons within your ANOVA data. The F statistic tells whether there is an overall difference between sample means. Tukey's HSD test allows to determine between which of the various pairs of means - if any of them - there is a significant difference. Tukey's HSD is appropriate if the F-ratio score has not reached significance.

CONCLUSION

Many factors like water quality, human activities, habitat , flow rate and nutrients supplies from riparian habitats control the abundance and distribution of river fishes. The diversity and distribution of fishes of aquatic ecosystem depends on the many features like, water level variations, morphometric features and bottom that have immense implication. The distinctive distribution and variation in fish species among the rivers is likely because of variation in geographic and geological conditions (Mathew and Robinson,1998). Effective measures in controlling these activities rely on the local bodies that govern the stretches of water. Conservation priorities that may yield promising results include strengthening and directing resources to organization and institutions meant for resource management.

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Table 1: Species, Genera, Families and Order wise occurrence of fish species in rivers of Udupi district by various researchers

Rivers	Species	Genera	Families	Order	References
Seeta river	20	-	11	-	Narasimhaiah et al.,2016
Nethravathi, Kumaradhara, Phalguni, Shambhavi, Swarna & Sowparnika rivers	78	58	36	-	Sushmitha Rao et al.,2013
Sita,Swarna & Varahi	82	42	20	8	Venkateshwarlu et al.,2014
Sita,Swarna & Varahi	71	41	20	7	Arun Kumar Shetty et al.,2015
Sita,Swarna & Varahi	01	01	01	01	Venkateshwarlu & ArunKumar Shetty,2013

Table 2: Order wise occurrence of fish species in rivers of Shivamogga district by various researchers

Orders	Tunga	Varada	Sharavathi	Tunga, Bhadra, Tungabhadra	Varahi
Cypriniformes	23	40	2	40	33
Siluriformes	11	24	0	14	10
Perciformes	2	10	0	7	11
Synbranchiformes	1	1	0	1	5
Osteoglassiformes	0	2	0	1	0
Clupiformes	0	1	0	0	0
Beloniformes	0	0	0	2	1
Cyprinodontiformes	0	0	0	1	1
References	KumarNaik etal,2013	Kumar Naik etal,2013	SreeKantha et al,2006	Gangadhara Gowda etal,2015	ArunKumar Shetty,2013

(Source: Thirumala and Kiran,2020)

Table 3: One Way ANOVA and Tukey HSD data for fish orders in rivers of Shivamogga district by various researchers

	Tunga	Varada	Sharavathi	Tunga,Bhadra, Tunga Bhadra	Varahi	Total
N	8	8	8	8	8	40
∑X	37	78	2	66	61	244
Mean	4.625	9.75	0.25	8.25	7.625	6.1
$\sum X^2$	655	2282	4	1852	1337	6130
Std.Dev.	8.3141	14.743	0.7071	13.667	11.1604	10.9094

Source	*SS*	*df*	*MS*	

Between-orders	453.35	4	113.3375	*F* = 0.94713
Within-orders	4188.25	35	119.6643	
Total	4641.6	39	The result is not significant at p < .05.	

Table 4: Post Hoc Tukey HSD (beta) values

Pair wise Comparisons		HSD.05 =15.7251 HSD.01 = 19.2620	Q.05 = 4.0659 Q.01 = 4.9804
T_1:T_2	M_1 = 4.63 M_2 = 9.75	5.13	Q = 1.33 (*p* = .88048)
T_1:T_3	M_1 = 4.63 M_3 = 0.25	4.38	Q = 1.13 (*p* = .92888)
T_1:T_4	M_1 = 4.63 M_4 = 8.25	3.63	Q = 0.94 (*p* = .96302)
T_1:T_5	M_1 = 4.63 M_5 = 7.63	3.00	Q = 0.78 (*p* = .98141)
T_2:T_3	M_2 = 9.75 M_3 = 0.25	9.50	Q = 2.46 (*p* = .42551)
T_2:T_4	M_2 = 9.75 M_4 = 8.25	1.50	Q = 0.39 (*p* = .99870)
T_2:T_5	M_2 = 9.75 M_5 = 7.63	2.13	Q = 0.55 (*p* = .99495)
T_3:T_4	M_3 = 0.25 M_4 = 8.25	8.00	Q = 2.07 (*p* = .59268)
T_3:T_5	M_3 = 0.25 M_5 = 7.63	7.38	Q = 1.91 (*p* = .66358)
T_4:T_5	M_4 = 8.25 M_5 = 7.63	0.63	Q = 0.16 (*p* = .99996)

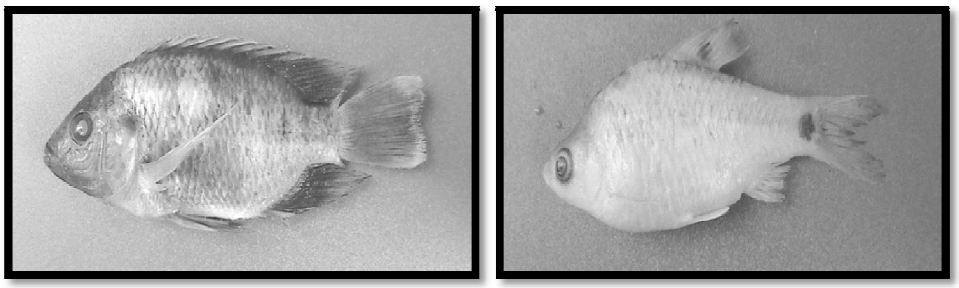

Figure1: ***Oreochromis mossambicus*** **and** ***Puntius sophore*** **fishes**

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antimicrobial Activities of *Pergularia daemia* by Microdilution Bio-assay Method

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Received: 19 Feb 2021 Revised: 23 Feb 2021 Accepted: 28 Feb 2021

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ABSTRACT

The hexane, acetone, ethyl acetate and methanol extracts of *Pergularia daemia* were examined for antibacterial and antifungal activities. Antimicrobial activities of the extracts were determined by micro-dilution bioassay method. Methanol extract was found be more effective in controlling the growth of both bacterial and fungal pathogens. Among the bacterial pathogens tested the gram negative bacteria such as *Klebsiella pneumoniae* and *Vibrio cholerae* were inhibited at MIC value of 0.78 µg/ml and *Pseudomonas aeruginosa* at MIC value of 1.56 µg/ml by the methanol extract. The fungal pathogen *Aspergillus fumigatus* was more susceptible to the extracts and it was inhibited at MIC value of 0.39 µg/ml by the methanol extract, followed by *Aspergillus flavus* and *A.niger* at MIC value of 0.78 µg/ml by the same extract. The results show that the test plant *P.daemia* is a suitable candidate for the investigation and isolation of potent antimicrobial compounds for human well being.

Keywords: Antimicrobial activity, Microdilution method, MIC value, *Pergularia daemia*.

INTRODUCTION

The continued evolution of infectious diseases and the development of resistance by pathogens to existing pharmaceuticals have led to the intensification of the search for new novel leads, against fungal, parasitic, bacterial, and viral infections [1]. Despite the recent advances in drug development through molecular modeling, combinatorial and synthetic chemistry, natural plant-derived compounds are still proving to be an invaluable source of medicines for humans [2]. Plant-derived antimicrobials have a long history of providing the much needed novel therapeutics [3].

Plants are still the reservoirs of raw materials that are needed for the manufacture of drugs for treating various diseases of humans and animals. Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day [4]. Several synthetic antibiotics and drugs are employed in the treatment of the microbial infections and communicable diseases; but, the microbial pathogens develop resistance to the synthetic antibiotics. The increasing incidence of resistance to antibiotics and their side effects on the functioning of different parts of the body organ systems necessitate to finding out substitutes for the existing antibiotics [5].

Pergularia daemia (Forssk.) Chiov., *Res. Sci. Somalia Ital. 1: 115 (1916)* (Asclepiadaceae) is a well known Indian herbal drug used in ancient medicines. It is distributed all over India and has been used in many ways in medicine. It was mainly found in tropical and sub-tropical areas, secreting milky latex. Leaves are thin, broadly ovate and heart-shaped, covered with soft hairs. The leaves were used in folk medicine to treat various diseases including liver disorders [6], diabetes [7] and fungal infection[8]. Bioactive compounds of *P.daemia* such as quercetin, α-sitosterol, β-amyrin, betaine, isorhamnetin, chrysoeriol, taxifolin, naringenin etc. are responsible for its wonderful therapeutic potential and free radical scavenging activity [9],[10].

MATERIALS AND METHODS

Plant Material

Pergularia daemia plants were collected from Arivelur village Mayiladuthurai, Nagapattinam district during the month of February 2018. The aerial parts of the plant samples were separated, and first washed under running water to remove the soil particles, then washed with distilled water, cut into small pieces, and shade dried. The dried parts were ground to fine powder using a mixture grinder.

Preparation of Extracts

One hundred grams of powdered leaf material was successively extracted with hexane, acetone, ethyl acetate and methanol by using Soxhlet apparatus for 8 hours [11]. The extracts were filtered, pooled and the solvents were evaporated with the help of rotary evaporation (Heidolph, Germany) under reduced pressure at 40^{o}C and the crude extracts were kept at 4^{o}C in refrigerator for antimicrobial assay.

Antibacterial Activity

Minimum inhibitory concentrations (MIC) of the extracts for antibacterial activity were determined using the microdilution bioassay as described by Eloff (1998)[12]. Overnight cultures (incubated at 37◦C in a water bath with an orbital shaker) of Gram-positive (*Staphylococcus aureus, Bacillus subtilis,*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Vibrio cholerae*) bacterial strains were diluted with sterile Mueller–Hinton (MH) broth to give final inocula of approximately 106 CFU/ml (colony forming units) were subjected to inhibition study. The different extracts were resuspended in respective solvents to known concentrations (50mg/ml). One hundred microliters of each extract were serially diluted two-fold with sterile distilled water in a 96-well microtiter plate for each of the six bacterial strains. A similar two-fold serial dilution of Streptomycin (Sigma Aldrich) (0.1mg/ml) was used as a positive control against each bacterium. One hundred microliter of each bacterial culture were added to each well. Water and different extracts used in the present study were included as negative and solvent controls respectively. The plates were covered with parafilm and incubated at 37^{o}C for 24 hours. Bacterial growth was indicated by adding 50µl of 0.2 mg/ml p-iodonitrotetrazolium chloride (INT) (Sigma–Aldrich) with further incubation at 37°C for 2 hours. Since the colourless tetrazolium salt is biologically reduced to a red product due to the presence of active organisms, the MIC values were recorded as the concentrations in the last well in which no colour change was observed after adding the INT indicator. Bacterial growth in the wells was indicated by a reddish-pink colour. The assay was repeated twice with two replicates per assay.

Antifungal Activity

The microdiluton method described by Eloff (1998)[12] and modified for fungi[13] was used to determine the anifungal activity. Fungal strains viz., *Mucor racemosus*, *Aspergillus flavus, A.fumigatus, A.niger, Penicillium notatum* and *Candida albicans* obtained from Rajah Muthiah Medical College, Annamalai University were used in the present study. An overnight fungal culture was prepared in Yeast Malt (YM) broth. Four hundred microliters of the overnight culture were added to 4ml of sterile distilled water and absorbance was read at 350nm. The absorbance adjusted with sterile saline to match that of 0.5 M McFarland standard solution. From this standardised fungal stock, a 1:1000 dilution with sterile YM broth was prepared giving final inoculums of approximately 106 CFU/ ml. Dried crude organic plant extracts (hexane, acetone, ethyl acetate and methanol) were resuspended in 70% ethanol to known concentration while saponin and phenolic extracts were redisolved in 50% methanol and water extracts in distilled water to the same concentrations. One hundred millilitres of each extracts were serially diluted two-fold with sterile water in a 96-well microlitre plate. A similar two-fold dilution of Amphotericin B(Sigma- Aldrich, Germany) (2.5mg/ml) was used as the positive control while water and 70% ethanol were used as negative and solvent controls respectively. One hundred microlitre of the dilute fungal stains were added to each well. The plates were covered with parafilm and incubated at 37ºC for 24 hours, after which 50µl (0.2mg/ml) INT were added and incubated for a further 24 hours at 37oC. The wells remained clear where there was inhibition of fungal growth. MIC values were recorded as the lowest concentration that inhibited fungal growth after 48 hours. To determine the fungicidal activity, 50µl of sterile YM broth were added to all the clear wells and further incubated at 37oC for 24 hours after which the minimum fungicidal concentration (MFC) were recorded as the last clear wells. The assay was repeated twice with two replicates per assay.

RESULTS

In the present investigation the antibacterial and antifungal activities of hexane, acetone, ethyl acetate and methanol extracts of leaves of *Pergularia daemia* were studied against gram positive bacteria (*Staphylococcus aureus and Bacillus subtilis),* gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Vibrio cholerae)* and fungal strains *Mucor racemosus*, *Aspergillus flavus*, *A.fumigatus*, *A.niger*, *Penicillium notatum* and *Candida albicans*. In general all the extracts process antimicrobial activities. Minimum Inhibitory Concentration (MIC) was estimated for all the compounds over the test pathogens. The range of MIC to inhibit bacterial strains are *Staphylococcus aureus* (6.25-3.125 µg/ml), *Escherichia coli* (6.25-3.125 µg/ml), *Pseudomonas aeruginosa* (3.125 -1.56 µg/ml), *Bacillus subtilis* (3.125-1.56 µg/ml), *Klebsiella pneumoniae* (3.125 -0.78 µg/ml) and *Vibrio cholerae* (3.125-0.78 µg/ml) (Table 1.). Among the extracts methanol was found to be more active in the inhibition of growth of the bacterial pathogens. Interestingly methanol extract inhibit the growth of gram negative bacteria such as *Klebsiella pneumoniae* and *Vibrio cholerae* at MIC of 0.78 µg/ml and *Pseudomonas aeruginosa* at MIC of 1.56 µg/ml along with ethyl acetate extract. Moreover methanol extract is also active against gram positive bacteria *Bacillus subtilis* at MIC value of 1.56 µg/ml. Among the tested pathogens *Klebsiella pneumoniae* and *Vibrio cholerae* were more susceptible to the extracts compare to other tested pathogens.

In general all the extracts were active in inhibiting the growth of fungal pathogens used in the present study. The range of MIC to inhibit fungal strains are *Mucor racemosus* (6.25 - 1.56 µg/ml), *Aspergillus flavus* (3.125 - 0.78 µg/ml), *A.fumigatus* (3.125 - 0.39 µg/ml), *A.niger* (3.125 - 0.78 µg/ml), *Penicillium notatum* (6.25 - 1.56 µg/ml), and *Candida albicans* (6.25 - 1.56 µg/ml) (Table-2). In the present study like antibacterial activity, methanol extract was more active in inhibition of fungal pathogens compare to other tested extracts. Among the test pathogens *A.fumigatus* was more susceptible the extracts and it was inhibited at MIC value of 0.39 µg/ml by the methanol extract, followed by *Aspergillus flavus and A.niger* at MIC value of 0.78 µg/ml by the same extract. The fungal pathogens were also subjected to maximum inhibitory concentration and the results are presented in table 3. The range of maximum inhibitory concentration to inhibit fungal strains are *Mucor racemosus* (12.50 - 3.125 µg/ml), *Aspergillus flavus* (6.25 - 1.56, µg/ml), *A.fumigatus* (6.25 - 0.78µg/ml), *A.niger* (6.25 - 1.56, µg/ml), *Penicillium notatum* (12.50 - 3.125 µg/ml) and

Candida albicans (12.50 - 3.125, µg/ml). Among the twelve pathogens tested the fungi *Aspergillus fumigatus* (0.39µg/ml) was most susceptible to the extracts and the rest of the pathogens showed good to moderate MIC values. Among the different solvents tested, methanol was most active followed by hexane, acetone and ethyl acetate extract in the inhibitory effect of test pathogens.

DISCUSSION

Medicinal plants are used by billions of people in most developing countries because of the frequently inadequate provision of modern medicine, their low cost, effectiveness, as well as cultural believes and preference[14]. Iwu, et al. (1999)[15] stated, plant based antimicrobial shows more effect than synthetic antimicrobials Packirisamy and Moorthy (2014)[16] reported the antimicrobial activities of 3 different concentrations of hydro alcohol (1:1) extracts of *P.daemia.* It showed wide spectrum of activity against test organisms namely *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus pyogenes* (gram positive), *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Aeromonas hydrophila* and *Vibrio harveyi* (gram negative). Similarly in the present study, it is observed that the methanol extract showed more activities against *Vibrio cholerae* and *Klebsiella pneumoniae* (Gram Negative). However, Senthilkumar et al. (2005)[17] reported significant antibacterial activity in ethyl acetate and ethanol extracts of *P.daemia* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Escherichia coli* and *Salmonella typhi*. Ignacimuthu et al. (2009)[18] found that the hexane and chloroform extracts of *P.daemia* did not inhibit bacterial growth at 500µg whereas ethyl acetate extract showed growth inhibitory activity against *Bacillus subtilis* (15 mm), *Staphylococcus aureus* (17 mm) and *Proteus vulgaris* (20mm) at 400µg/disc. In the present study the methanol extract was found to be more active (MIC ranged between 3.125 and 0.78 µg/ml) in inhibiting the growth of test pathogens.

Ramanathan et al. (2013)[19] reported that *Penicillium* sp. and *Aspergillus niger* were highly susceptible to aqueous extract of *P.daemia* (21 and 18 mm) followed by chloroform extract (20 and 18mm) and methanolic extract (19 and 17mm) at 125µL concentration respectively, while in this study it was observed that *Aspergillus fumigatus* is highly susceptible to methanol extract followed by *Aspergillus niger* and *Aspergillus flavus*. Dosumu et al. 2019[20] stated that all tested fungal species were resistant to the action of extracts at low concentrations, but ethanol extract of the leaf, showed inhibition at all levels, but this study suggests that methanol extract of the plant shows better activity against Fungi. Savitha et al. 2014[21] observed that the maximum antifungal activities were observed in methanol extract (13mm) other than chloroform extract (8mm) and hexane extract (10mm), which is in accordance with this experiment. In the present study, it is observed that no other extracts of *P.daemia* are as effective as methanol extract. According to Deepika Thenmozhi et al. (2011)[22], antimicrobial activity is probably due to the membrane disruption by terpenes and their activity might be due to their ability to form complex with extra cellular, soluble proteins and bacterial cell wall and disrupt microbial membrane.

CONCLUSION

Pergularia daemia is a highly valuable medicinal plant. The antimicrobial study showed that the extracts were biologically active against both bacteria and fungi. The methanol extract was found to be more active than the other extracts. This investigation can be used in clinical application to produce antifungal and antibacterial substances for possible treatment of many diseases including bacterial and fungal infections.

ACKNOWLEDGEMENTS

We sincerely thank Rajah Muthiah Medical College, Annamalai University for providing the pathogens for my research. I also thank Department of Botany, Annamalai University for the support given to my research. No funding has been received for this research. There are no conflicts of interest for any of the authors.

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Table 1: Minimum inhibitory concentrations (MIC-µg/ml) of the different extracts of leaves of *Pergularia daemia* against the bacterial strains.

S.No	Pathogens/solvents	Hexane	Acetone	Ethyl acetate	Methanol
1	*Staphylococcus aureus*	6.25	3.125	3.125	3.125
2	*Bacillus subtilis*	3.125	3.125	3.125	1.56
3	*Escherichia coli*	3.125	6.25	3.125	3.125
4	*Pseudomonas aeruginosa*	3.125	3.125	1.56	1.56
5	*Klebsiella pneumoniae*	3.125	3.125	3.125	0.78
6	*Vibrio cholerae*	3.125	3.125	3.125	0.78

Table 2: Minimum inhibitory concentrations (MIC-µg/ml) of the different extracts of leaves of *Pergularia daemia* against the fungal strains.

S.No	Pathogens/solvents	Hexane	Acetone	Ethyl acetate	Methanol
1	*Mucor racemosus*	6.25	1.56	3.125	1.56
2	*Aspergillus flavus*	3.125	3.125	1.56	0.78
3	*A.fumigatus*	3.125	3.125	1.56	0.39
4	*A.niger*	3.125	1.56	3.125	0.78
5	*Penicillium notatum*	6.25	3.125	3.125	1.56
6	*Candida albicans*	6.25	3.125	3.125	1.56

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Isolation of Calcium Ions from Oyster Shell to Form as Paste for Remineralization of Demineralized Teeth

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Received: 12Nov 2020 Revised: 24 Nov 2020 Accepted: 02 Jan 2021

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ABSTRACT

Calcium carbonate is one of the most used raw materials in various industries, such as construction materials, food supplement, pharmaceutics, animal feed, plastic production, and others. Calcium carbonate can derive from marine wastes, like crustaceans and bivalve's shells. There is a high content of calcium carbonate in oyster shells, which can be used in the formulation of medicine. This work has as its main objective to obtain calcium carbonate from oyster shells and formulate as paste for remineralization of demineralized teeth. The research demonstrate that oyster-shells can be resources of pure calcium carbonate materials and effective in replacement of remineralization of demineralized teeth.

Keywords: oyster shell paste, calcium carbonate, remineralization of demineralized teeth

INTRODUCTION

This invention relates to dentifrice products and methods for remineralizing and/or mineralizing teeth. More particularly, this invention relates to calcium containing dentifrice products and methods of using same to achieve improved remineralization of subsurface carious lesions and/or mineralization of exposed dentinal tubules. Dental caries tooth decay, is a leading cause of tooth damage in humans1. Dental caries begins with lesions of so-called white spots. which are demineralized areas below the surface of intact dental enamel. Such subsurface lesions are formed before a cavity is detectable. If unchecked, surface enamel above a subsurface lesion eventually collapses, resulting in cavitation and subsequent loss of tooth structure. Tooth decay is that the softening of your enamel and refers to the damage of the structure of the tooth caused by acids that are created when plaque bacteria break down sugar in your mouth. If this loss of mineral from the enamel is left untreated, a cavity, or hole within the tooth, can

eventually occur. Without treatment, these holes can grow larger over time and should even destroy the entire tooth. Sugar combines with plaque to weaken the enamel leaving you vulnerable to tooth decay. There are many phosphate delivery systems which may promote re-mineralization. Toothpaste or creams or gels that contain sodium fluoride or tri-calcium phosphate are excellent media to provide inorganic ions and can be considered to be the most effective source of required ions for teeth re-mineralization2. At neutral pH without acid challenge, the main driving force for re-mineralization is the passive transport of salivary or plaque calcium and phosphate ions down their concentration gradient. Soluble calcium phosphate phases are transformed to a solid and less acid soluble phase through the precipitation of fluorapatite (FAP) or fluoridated hydroxyapatite (F-OHAp) on existing demineralized crystallites or through the nucleation of new crystallites3. This re-mineralization process is a natural chemically inorganic process that does not require soft-tissue or cellular biological processes, as in bone and dentin re-mineralizing mechanisms. In general, most of the oyster shells are discarded with no further use once the flesh is stripped off; except that a little amount is employed for art creation. As a result, shell piles are common in areas of oyster production with no further utilization. Chemical and microstructure analyses reveal that oyster shells are predominantly composed of CaO3, almost like that of lime, which has been used for soil stabilization. In addition, oyster shells are often utilized for producing medium- and high-quality cement. The expandable nature of clay is reduced by the natural process from the interaction between calcium ions and clay. In this study revealed that the oyster shell powder calcium carbonate containing paste has given the remineralized compounds to the demineralized teeth 4.

MATERIALS AND METHODS

Method of Processing of Oyster Shell Powder

We used oyster shell waste collected from sea shore in Chennai, Tamil Nadu, and cleaned with alcohol and water to remove impurities attached to the surfaces. The cleaned and dried oyster shells were milled using comminution equipment until a fine powder was obtained with a particle size of less than 100 μm. This fine oyster shell powder was calcined in an electric furnace at a constant temperature of 1,000^{0}C for 2 h, which was increased to this point at a rate of 10^{0}C/min. The calcined raw materials were mechanically ground for 1 h until the particle size was less than 70μm. The fine powder was processed to hydration (40 g/lit) with distilled water at 80^{0}C for 1h and filtered with a 200 mesh. The filtrate was collected and washed three times with distilled water and filtered again with a 325 mesh and dried at 80^o C for 12 h.

Infra-Red Spectrophotometric Analysis

The pellets were made with mixing 1gm of oyster shell and 100gm of dried potassium bromide powder. Mixer was then compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The thin pallet was put on pellet disc to get IR Spectra.

SEM Analysis

The scanning electron microscopy (SEM) is one of the most important techniques used for analysis of surface morphology such as spherical shape; smoothness and formation of aggregates and size distribution of (oyster shell) were studied by (Hitachi, Japan). Oyster shell were sprinkled onto the double- sided tape and coated with gold film of thickness of 200 nm under reduced pressure of 0.001 mmHg that was affixed on aluminum stubs. The aluminum stub was placed in the vacuum chamber of a scanning electron microscope. Photographs were taken at suitable magnification.

Preparation of Paste (Trituration and Fusion Method)

Oyster shell powder, calcitriol and xanthan gum are mixed as a dry powder on mortar and with the help of pestle it was mixed. Then a hot solution of Glycerol, gum tragacanth and distilled water is added slowly with mixing of the dry powder. After that solution of sodium lauryl sulfate was added to form homogeneous paste**.**

RESULT AND DISCUSSION

Scanning Electron Microscopic (Sem) Analysis

Morphological structures of oyster shell with all excipients and purified oyster shell are outlined. As observed, all particles are irregular in shape with a wide range of particle sizes due to the crushing processes. The organic membranes on the surface of the oyster shell particles can be observed. The tensile fractured surface morphologies of pure bio-epoxy, bio-epoxy filled with oyster shell and purified oyster shell under different filler loadings were taken at a magnification of X1000.

Ex Vivo - Diffusion Studies

Re-Mineralization of De-Mineralized Teeth

2 groups were made which was as follows: (Fig 8 c& d)

Group C (n=10)- untreated sample.
Group D (n=10)-(demineralized) teeth with oyster shell paste
Were carried out and placed in artificial saliva (pH 6.7) for re-mineralizing which contains teeth with oyster shell paste for 21 hrs.

Re-Mineralization of Demineralized Teeth

Group D (n=10)- the demineralized teeth with oyster shell paste has give the possible results compared to the untreated (Group C) sample. (Fig 8)

Anti-Microbial Activity Observation

Oral Bacteria

The test organisms were isolated and identified and the results were recorded based on the morphological and gram stain microscopic.

Anti-Microbial Activity Observation

The formulated paste exhibited fairly good anti- *S.aureus* activity as compared to the oyster shell paste. The formulation exhibited an impressive ZOI of 7 mm at MIC of 25µg/mL, whereas oyster shell paste exhibited 10 mm ZOI at MIC of 6.25µg/ml. Therefore, it may be concluded that formulated paste have potential to exhibit anti-microbial activity.

DISCUSSION

In the present investigation, an attempt has been made to oyster shell paste by using different binder concentration. The optimized formulation (F6) no significant change was observed in the paste content, physical properties and *ex-vivo* absorption rate of these paste after the storage period of 1 month at 40ºC and 75%RH.The modern dental practice is now continuously shifting towards the concept of minimal invasion dentistry (MID) which is a conservative concept that mainly emphasizes upon early detection of carious lesions.

CONCLUSIONS

The production of molluscs, particularly the, Ostreida (Ostreinae) generates thousands of tons of waste each year. The careless disposal of this massive amount of waste impacts the soil, water, and air quality and represents an environmental and public health problem. But the oyster shell has a bio medical application (calcium). In this study we are used oyster shell powder to formulate as paste for remineralization of demineralized teeth. Oyster shell paste

have remarkably minimized risk factors associated with demineralized teeth. They have significantly increased total calcium level. Almost absolute recovery for demineralized teeth was noticeable in group D. This study clearly indicates that consuming oyster shell paste in remineralization of demineralized teeth does have a potential effect in restoring calcium level in the teeth. This could be used for prevention and treatment of demineralized teeth.

ACKNOWLEDGEMENTS

The authors are thankful to Vinayaka Mission's Research Foundation (Deemed to be University) & Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu for extending their support and facilities for this research.

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Table No 1: Formulation of Oyster shell Paste (50gm)

S.No	Ingredients in (gm)	F1 (gm)	F2 (gm)	F3 (gm)	F4 (gm)	F5 (gm)	F6 (gm)	F7 (gm)	F8 (gm)	F9 (gm)
1	**Oyster shell powder**	15	20	25	15	20	25	15	20	25
2	**Glycerol**	10	10	10	10	10	10	10	10	10
3	**Sodium lauryl sulphate**	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
4	**Gum tragacanth**	0.75	1.0	1.25	-	-	-	0.75	1.0	1.25
5	**Xanthan gum**	-	-	-	0.75	1.0	1.25	0.75	1.0	1.25
6	**Calcitriol**	1.00	1.25	1.50	1.00	1.25	1.50	1.00	1.25	1.50
7	**Purified Water**	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

*Mean±SD(n=6

Table No: 2 % of $CaCO_3$ present in the oyster shell

Source	$CaCO_3$ present
Oyster shell	95.994%

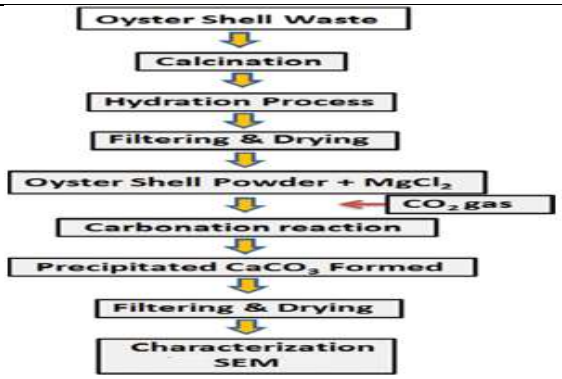
Table No.3 Preformulation Study of the blend

Batch Code (Powder blend)	Bulk Density (gm/cm³)*	Tapped Density (gm/cm³)*	Angle of repose (θ)*	% Compressibility*	Hausner's Ratio*
F1	0.262	0.337	30^0 78'	25.87	1.33
F2	0.233	0.348	31^0 14'	26.70	1.35
F3	0.236	0.336	30^0 13'	26.89	1.36
F4	0.237	0.337	29^0 54'	26.80	1.31
F5	0.244	0.339	32^0 33'	27.20	1.30
F6	0.245	0.338	31^0 69'	26.10	1.38
F7	0.266	0.335	31^0 02'	26.30	1.35
F8	0.269	0.324	30^0 19'	27.20	1.36
F9	0.279	0.348	31^0 10'	26.18	1.29

Table No 4 Evaluation of Oyster Shell Paste

S. No	Evaluation test	F1	F2	F3	F4	F5	**F6**	F7	F8	F9
1	Appearance	Brown	Brown	Brown	Brown	Brown	**Brown**	Brown	Brown	Brown
2	Moisture Content %	31.33	32.46	31.96	33.63	33.96	**36.23**	33.55	33.88	32.98
3	Homogeneity	Pass	Pass	Pass	Pass	Pass	**Pass**	Pass	Pass	Pass
4	SPREADIBILITY (Cm)	6.4	6.6	6.9	7.3	7.7	**8.1**	7.4	7.7	7.8
5	Tube Extrudability(%)	89.6	91.1	89.7	90.7	89.9	**90.8**	90.7	90.8	91.3
6	Viscosity	35447± 332cps	34247± 354cps	33337± 467cps	31747± 297cps	32347± 490cps	**36887± 776 cps**	33457± 524cps	35125± 756cps	33657±4 89cps
7	Abrasiveness	1.1	1.3	1.2	1.1	1.1	**1.0**	1.3	1.2	1.2
8	Gritty Matter	Absent	Absent	Absent	Absent	Absent	**Absent**	Absent	Absent	Absent

*Mean±SD(n=6)**Mean±SD(n=20)



Determination of $CaCO_3$ in an oyster shell (Fig. No: 1)

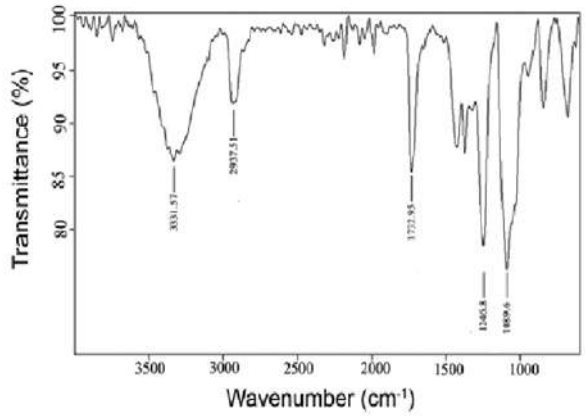


Fig. No: 2 FTIR of Pure Oyster Shell

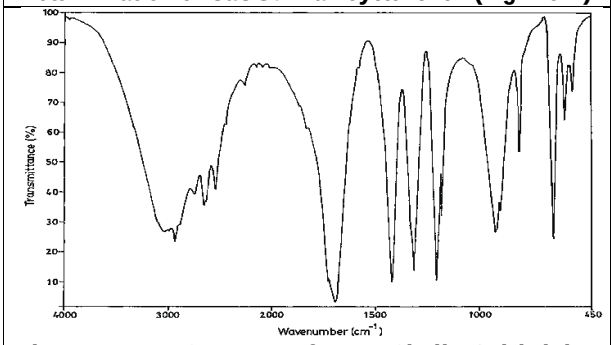


Fig. No: 3 FTIR Spectrum of oyster Shell + Calcitriol

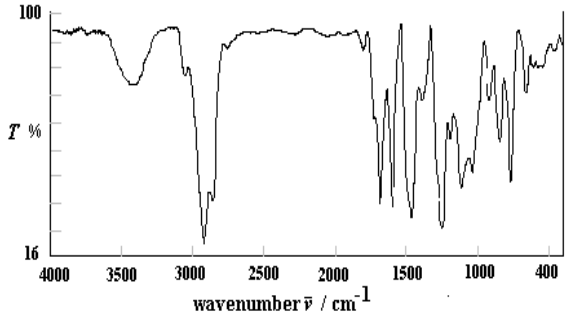


Fig. No: 4 FTIR Spectrum of Oyster Shell + SLS

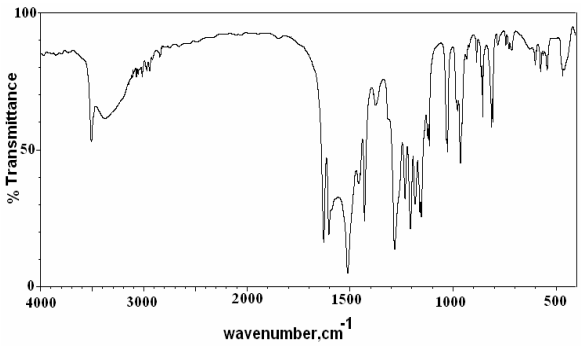


Fig. No: 5 FTIR Spectrum of oyster Shell+ All excipients

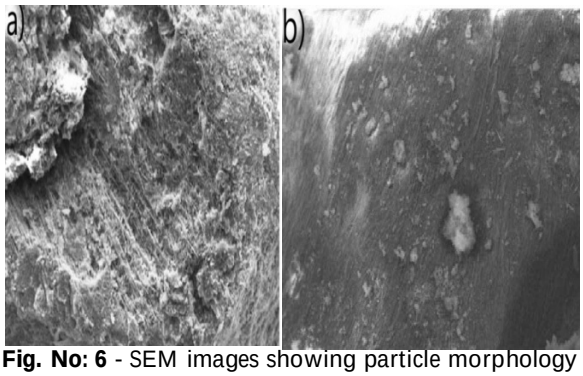


Fig. No: 6 - SEM images showing particle morphology of (a) oyster shell + all excipients (b) purified oyster shell

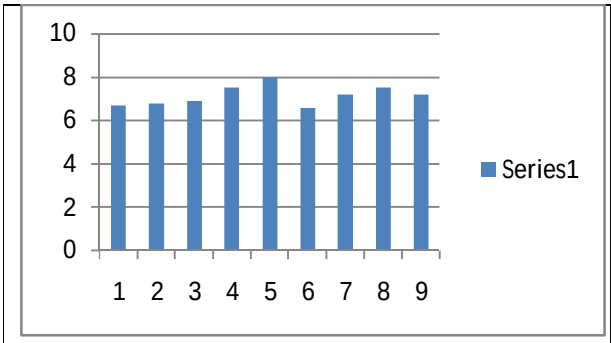


Fig.No:7 Determination of pH

Figure No:8Re-mineralization of De-Mineralized teeth

Figure No: 9- 1 & 2 Oral Bacteria plate and gram stain result

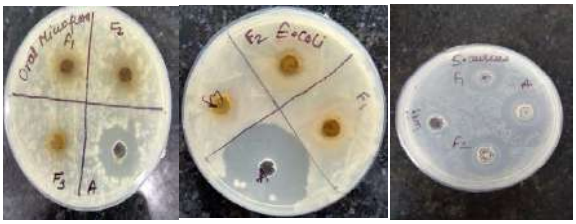


Figure No: 10- Fig Zone of inhibition of F1, F2 and F3 against 1. OralBacteria, 2. *E.coli* 3. *S. aureus*

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Analysis of Geomagnetic Storm Effects on the Ionospheric F Layer Over Northern and Southern Hemisphere

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Received: 21 Feb 2021 Revised: 28 Feb 2021 Accepted: 06 Mar 2021

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ABSTRACT

In the present work, we have studied the response of ionospheric F layer critical frequency (foF2) to geomagnetic storms at the northern and southern hemispheres. To accomplish this study, we have selected two stations, one in the northern hemisphere, I-Cheon (37.40^0 N; 127.54^0 E) in South Korea, and another in the southern hemisphere, Hermanus (-34.42^0 N; 19.22^0 E) of South Africa. For this work, we have taken two geomagnetic storms i.e. on 17 March 2015 and 08 September 2017. From our study, we observed that during the main phase of the geomagnetic storm, the foF2 values obtained its maxima or minima depending on the storm's arrival time.Such studies are important for accurate forecasting and modeling of ionospheric F2 layer critical frequency, which is very useful for high-frequency communication.

Keywords: Ionosphere, Geomagnetic storm, Critical frequency (foF2), X-ray flux.

INTRODUCTION

The primary source of disturbances in the earth's upper atmosphere is solar emissions and extremely energetic solar particles. Solar corona and solar wind produce energetic particles of 10Mev -1Gev that accelerated for a short time. When coronal mass ejections (CMEs) enter the earth's atmosphere, they produce ram pressure. It induces a sudden increase in solar wind temperature, velocity, density, and it also causes a change in the north-south inclination of the interplanetary magnetic field (Bz) [Shweta *et al.,* 2012]. This interplanetary magnetic Bz is the main cause of geomagnetic storms [Adebesin and Chukwuma; 2008 Tsurutani *et al.,* 1990]. The main phases of any geomagnetic

storm event is observed in three phases, Initial phase (increasing from the average value of the index), Main phase (maximum value of the index), and the recovery phase (decreasing from maximum) [Adeniyi, 1986 and Joshua *et al.,* 2013a,b]. The geomagnetic storm is characterized by the change in ring current and then recovery after the event. H component of geomagnetism is depressed during storm period, and this depresses measured at Dst (disturbance storm time) scale [Kamide *et al.,* 1998 a, b]. It is the magnetic index used for categorized geomagnetic storms [Gonzalez *et al.,* 1994]. The storm can be positive or negative [Prölss, 1995; Förster and Jakowski, 2000] according to the increase or decrease in total electron content (TEC), electron plasma densities in the F2 layer. The negative storm can be characterized by a decline in the neutral density ratio of O/N [Prölss, 1995]. And the positive storm is recognized by a raise in neutral density ratio of O/N, plasmaspheric downward flux, thermospheric winds, and an increase in storm time ionospheric plasma density [Fuller Rowell et al.,1996; Huaung *et al.,* 2005; Danilov, 2013]. In this study, we have taken the responses of ionospheric F-2 layer peak parameter or maximum plasma frequency foF2 for two extreme geomagnetic storms of solar cycle 24. For this, we have used geomagnetic storm parameters such as B_Z component of interplanetary magnetic field (IMF) and Disturbance storm time (Dst).

DATA AND METHODS USED

We have picked two events of extreme geomagnetic storms of 24 solar cycles, first one occurred on 17 March 2015 and second on 08 September 2017, and data set for these events for the two different hemispheres. Hourly data of Ionospheric F-2 layer maximum peak frequency (foF2) in MHz was taken from http://giro.uml.edu/didbase/scaled.php, which is the Global Ionospheric Radio Observatory (GIRO) site that provides Digisonde data of ionosphere, the site is open for public access. For our study, we have been taken data of two different hemispheric stations, one from the northern hemisphere I-Cheon (37.40^0 N; 127.54^0 E), North Korea, and the other from the southern hemisphere Hermanus (-34.42^0 N; 19.22^0 E), South Africa. We took the Bz componat of IMF in GSM co-ordinates and Geomagnetic index (Dst) from the OMNI database at the Goddard Space Flight Centre Space Physics Data Facility (GSFC/SPDF) OMNIWeb interface http://omniweb.gsfc.nasa.gov/form/dx1.html.

EUV Solar Flux Data

The EUV data were taken from https://dornsifecms.usc.edu/space-sciences-center/ download-sem-data/. It gives the satellite data of Solar and Heliospheric Observatory (SOHO), the satellite has been measuring solar EUV flux data since 1996 [Judge *et al.,* 1998]. Data include two channels: narrowband (26–34 nm) and broadband (0.1–50 nm) for our study, and we use broadband data of 15-second average.

X-ray Solar Flux Data

X-ray solar flux data have been taken from www.ngdc.noaa.gov website gives the X-ray flux measurement by onboard Geosynchronous Operational Environmental Satellite GOES-15, it has two X-ray Sensors (XRS) which provide wavelength of 0.05 nm to 0.4 nm (short channel) and 0.1 to 0.8 nm (long channel) of high-quality X-ray flux data. GOES X-ray flux data available in 5 minute and 1-minute averages data sets; for our study, we use long channel data of 1 minutes average. The plots for each event composed of 5 panels from top to bottom X-ray flux, EUV flux, DST index, IMF Bz, and foF2, all the parameters were plotted against universal time (UT) in an hour, each plot consists of 5 days (two days prior and two days post the storm event). For the 17 March 2015 storm, we have taken data for 11 – 19 March 2015 and 08 September 2017, 06-10 September 2017.

RESULTS AND DISCUSSIONS

Fig.(1) shows the time variation of ionospheric peak frequency and geomagnetic storm parameters for 15-19 March 2015. foF2 enhancement was on 17 March 2015 over Hermanus. These changes were observed when the DST value decreases to its lowest value of -223 nT at 22:00 UT and IMF Bz turns northward from 12:00 UT to the next day till 07:00 UT in the morning same day and minimum value -18.10 nT was observed at 14:00 UT. These changes were

noticed followed by two M class X-ray flux, first on 15 March 2015 at 23:44 UT and second on 16 March 2015 at 10:52 UT and, EUV flux recorded 3.07 photons cm $^{-2}$ s^{-1} at 20:53 UT on 16 March 2015. During the recovery phase (23:00 UT on 17 March 2015) foF2 curve for the next day decreased in peak frequency as compared to the average of the quiet day values.

Fig.(2) shows the variation of the Ionospheric peak parameters and the geomagnetic storm parameters for 15-19 March 2015 of northern hemispheric station I-Cheon. The initial phase of the storm started on 17 March around 0500 UT with a sudden storm commencement. The pre-storm enhancement was shown in Fof2 value at 03:00 on 17 March 2015, and the Dst value goes a positive maximum of 55 nT at 05:00 on 17 March and the same day it goes negative maximum -223 nT at 22:00 UT. The Bz component of IMF moved southwards and reduced slowly from 20.1 nT to the lowest minima of -16 nT around 0800 UT on 17 March. It continued the negative value for about 2 hrs, then turned northward. It obtains the value of 9.3 nT at 1100 UT, and again it obtains the peak of -18.1 nT at 1400 UT and remains negative about 12 hours. Later it turned northward, indicative of the commencement of the recovery phase. During the recovery phase (form 20:00 UT of 17 March 2015) of storm minimum value of foF2 peak frequency as compared to the average of the quite day values but there is the exceptional case on 02:00 UT of 18 March 2015 when foF2 value goes sharp peak of 11.77 MHz it was due to another M class X-ray flux and EUV flux on 23:30UT of 17 March 2015.

Fig.(3) represents the variations in the parameters of the geomagnetic storm and the Ionospheric peak parameters for September 06 – 10, 2017, for the northern hemispheric station Hermanus. During this period, there was two X class X-ray flux was first on 06 September at 12:00 UT and other on 07 September at 14:36 UT, EUV flux was 5.62 x10^{10} photons cm $^{-2}$ s^{-1} on 12:04 UT at 06 September and 5.50 x10^{10} photons cm $^{-2}$ s^{-1} on 05:50 UT at 08 September respectively. The Bz component of IMF turned southward and falled gradually from 7.5 nT around 1700 UT on 07 September to -24.2 nT around 00:00 UT on 08 September. It remained negative for about two h. DST on 06 September was minimum of -15 nT at 15:00 UT, and then on 07 September it reaches the maximum of 32nT at 01:00, due to second flares on 07 September DST decreases from -7.0 nT at 19:00 UT (07 September) to -142 nT at 01:00 UT (08 September). The effect of the storm impacted on Fof2 values on 06 September, when the value of foF2 sharply decreases from 8.40 nT at 03:00 UT to 3.02 nT at 18:00 UT, and during the recovery phase, foF2 value remains almost the same till 10 hours, due to the second storm foF2 value reaches 9.913 MHz at 11:00 UT on 08 September and next day during the recovery phase, foF2 value shows the almost same value from 06:00 to 16:00 UT.

Fig(4) illustrate the storm parameter against time (UT) for September 06-10, 2017, for Southern hemispheric station I-Cheon, on 06 September when the first X class flares produce, the foF2 value decreases from 6.55 MHz at 12:00 UT to 3.75 MHz at 20:00 UT, when second X class flares produce on 08 September DST goes a minimum of -142 nT, and foF2 value first reaches to a maximum of 9.68 MHz at 04:00 UT on 08 September, during the recovery phase, foF2 decrease to 2.52 MHz at 19:00 UT and remain between 4-6 MHz form 21:00 UT of 08 September to 12:00 UT of 09 September 2017.

CONCLUSION

During the main phase of storm, when the Dst value was minimum, foF2 obtained its maxima or minima values depending on the storm's arrival time. For the storm on 17th March, foF2 showed strong effects with a minimum of 1.9 MHz at 04:00 UT (06:00 LT) over Hermanus on 18 March 2015 and 2.05 MHz at 20:00 UT (05:00 LT) over I-Cheon on 17 March 2015. On 08 September 2017, storm values of foF2 were maximum with 9.91 MHz at 11:00 UT (13:00 LT) on 08 September 2017 over Hermanus and 9.68 MHz at 04:00 UT (13:00 LT) over I-Cheon. During the recovery phase of both the storms for both the station, the foF2 curve values were lower than the quiet day curve and clearly showed in the graph of foF2 on 18 March 2015 and 09 September 2017.

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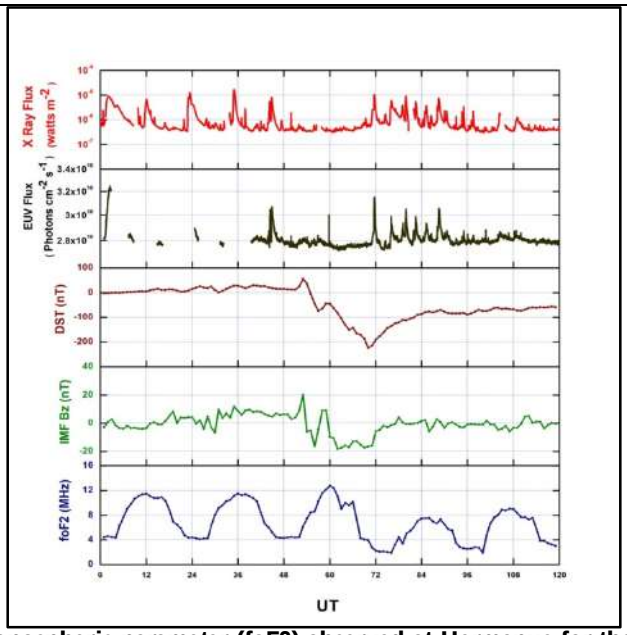

Fig. (1). Variations of the ionospheric parameter (foF2) observed at Hermanus for the period from 15-19, March 2015, from top: X-ray flux, EUV flux, Dst index, IMF Bz, and foF2 with universal time (UT) in hours.

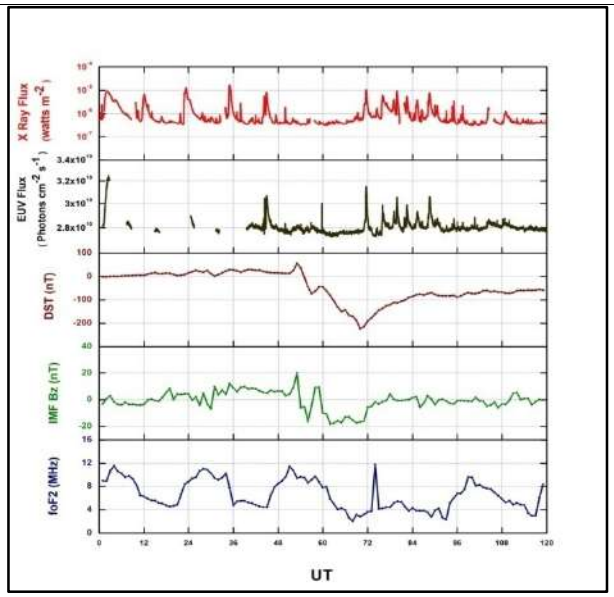

Fig. (2). Variations of the ionospheric parameter (foF2) observed at I-Cheon for the period from 15-19, March 2015, from top: X-ray flux, EUV flux, Dst index, IMF Bz, and foF2 with universal time (UT).

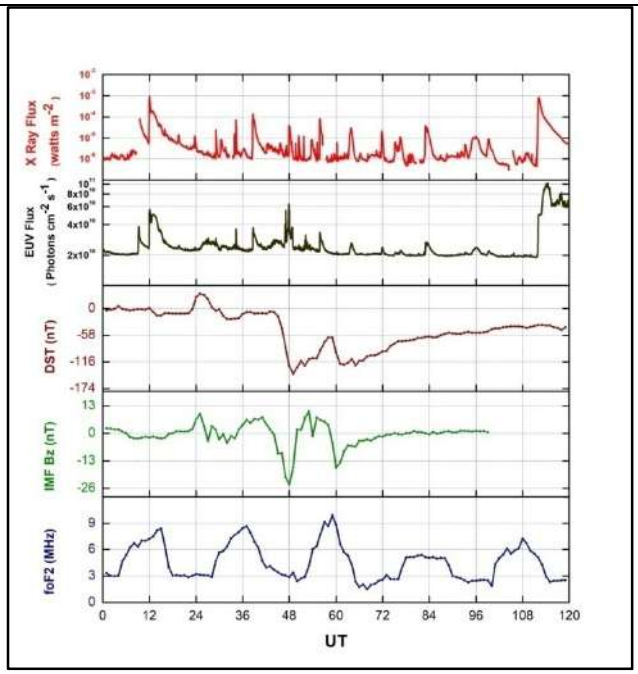

Fig. (3). Variations of the ionospheric parameter (foF2) observed at Hermanus for the period from 06-10, September 2017, from top: X-ray flux, EUV flux, Dst index, IMF Bz, and foF2 with universal time (UT).

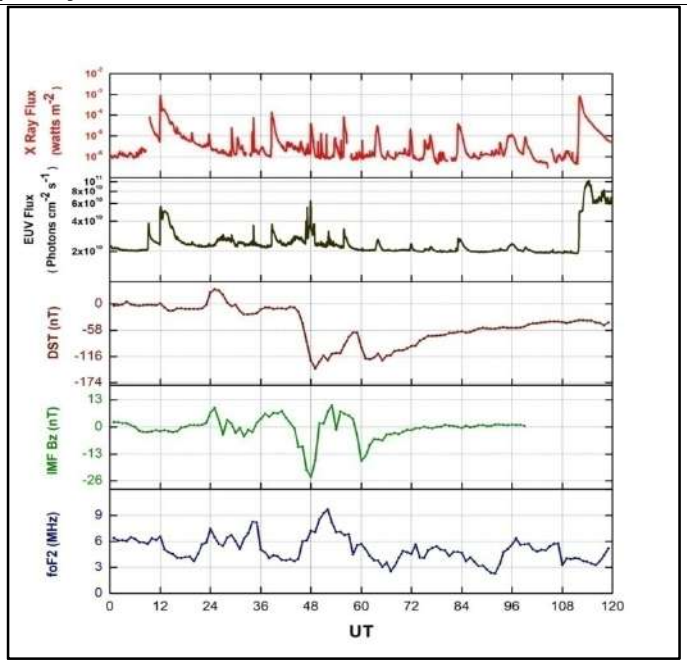

Fig. (4). Variations of the ionospheric parameter (foF2) observed at I-Cheon for the period from 06-10, September 2017, from top: X-ray flux, EUV flux, Dst index, IMF Bz, and foF2 with universal time (UT).

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Amyloid β- Peptide: Structure and Therapeutic Approaches

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Received: 09 Mar 2021 Revised: 12 Mar 2021 Accepted: 18 Mar 2021

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ABSTRACT

β- and γ-secretase mediated proteolytic processing of amyloid precursor protein, leads to the synthesis of Amyloid beta peptide (Aβ). In pathogenesis of Alzheimer's disease, Aβ accumulation in brain is thought to be an early toxic phenomenon. AD is most common form of dementia associated with deposition of plaque and results in brain confusion. Physiological and pathological forms of Aß as well as mechanism of AP are still not clear. In addition, no such reports are available for the effective drug that hinders Alzheimer's disease progression. In this article, Aβ structure, metabolism of Aβ as well as various nano based treatment aspects has been summarized.

Keywords: Amyloid beta peptide (Aβ), Alzhiemer's Disease, β- and γ-secretase, Aβ monomers, Neuro Imaging

INTRODUCTION

Epidemiology

Alzheimer's is a deadly disease which affects a large part of world's population that is 20 million people. And it is expected that this number will grow by the year 2050 and will probably reach 135 million. Alzheimer's sickness is common dementia which influences individuals more established than 60 years old [1]. In AD, the dysregulation of the amyloid-beta (Aβ) level prompts the presence of decrepit plaques which contains depositions of Aβ [2]. Aβ is a complex natural particle which communicates with numerous kinds of receptors and additionally frames insoluble congregations and, in the long run, its non-physiological statements substitute with the ordinary neuronal conditions [3]. Right now, signs show up and the patients experience checked cognitional incapacities. When all is said in done, mind, social abilities, character, and memory are affected by this malady and, over the long haul, it prompts a decrease in personal satisfaction and future [4]. Because of the crucial job of Aβ in the pathobiology of AD, a lot of exertion has been made to uncover its precise job in neuronal dysfunctions and to finding effectual restorative techniques against its antagonistic neuronal results [5]. Thus, the assurance of its distinctive sub-atomic gatherings and the components hidden its neurotic impacts are of intrigue. In the present paper, a portion of the entrenched

auxiliary types of Aβ, its communications with different receptors and conceivable sub-atomic and cell components hidden its neurotoxicity are examined. What's more, a few Aβ-based rat models of AD are audited [6]. Alzheimer's illness (AD) was first portrayed by the German specialist, Alois Alzheimer, in the mid1900s and is currently viewed as the most pervasive dynamic neurodegenerative issue, liable for 75% of all dementia cases [7]. It is found around 35.6 million individuals all over the world. This will increment with populace maturing and will presumably influence almost 106.8 million individuals by 2050 [8]. It causes mental and intellectual shortages, for example, impeded memory, astuteness in individuals more established than 65 years old. In the propelled phases of the malady focal tactile techniques, including the visual framework, get influenced, as well [9]. All things considered, AD-related issues decline future, lessen personal satisfaction, cause physical incapacity, and in the end lead to significant issues in everyday life exercises [10]. To diminish the social and monetary expenses and the weight of the illness on patients and their families, some momentous endeavors have recently been made to discover analytic markers which anticipate the sickness prior [11]. Neuroimaging strategies, for example, magnetic resonance imaging and positron emission tomography have been created to empower analysts to analyze AD in its beginning periods. Likewise, a few biomarkers, which are critical in recognizing obsessive highlights of AD, have been found in cerebrospinal liquid (CSF) and can be surveyed [12]. From a histological perspective, the movement of AD is related with 3 cardinal neuropathological highlights: the collection of extracellular decrepit plaques which is interceded by Aβ [13]. These occasions predominantly happen in the neocortex, hippocampus, and other subcortical districts which are important for psychological capacity. The presence of these markers evidently happens numerous years preceding the clinical signs and manifestations of the ailment, henceforth they could be acceptable markers for AD forecast [14]. In the meantime, Aβ peptide is a significant hazard factor and has a focal job in the beginning and movement of AD. Aβ is created in typical people in any case, in specific situations, this particle may total and begin infection movement [15]. There is a huge assemblage of proof stressing that Aβ oligomers assume the fundamental job in neuronal brokenness and AD.

Amyloid β-peptide Structure and AD Pathogenesis

Amyloid fibrils are the filamentous structures, with the normal diameter of 10nm and up to few micrometers. It is formed by large number of proteins and peptides with different molecular weights and sequences [16]. Since Amyloid fibrils are nanocrystalline solid materials and are incompatible with liquid state NMR and X-Ray crystallography, it is almost impossible to get a high-resolution structure of amyloid fibrils at its molecular level [17]. In-vivo length of the β -amyloid peptide concerned with AD, ranges from 39 to 43 residues [18]. Structural domains of amyloid fibrils include crystallographic and NMR-structures of the growth-factor like and copper-binding domains of APP, its E1-domain, of its KPI (Kunitz type protease inhibitor) domain, its E2 or central APP domain, its intracellular domain (Fig. 1) [19].

GFLD is Growth factor-like domain and CuBD is Copper binding domain are the two sub-domains of the E1 domain. If these subdomains are observed at slight acidic pH, the entire 3-D structure of the E1 domain is visible that include the interaction core, the contacts mediated by H2O molecules, one predominant salt bridge and the several hydrogen bonds present [20]. The Kunitz type protease inhibitor domain is only known to be found in the longer Amyloid β precursor protein splicing isoforms APP770 and APP751. The structure of KPI and Bovine Pancreatic Trypsin Inhibitor are more or less similar [21]. The structure of KPI consists of three conserved disulphide bridges that dominate the fold that consists of a two stranded, twisted β-sheet and an α-helix that face against the pack of β-sheet [22]. The second region of the 3-D structure of the neuronal isoform APP695 is represented by the E2-domain [23]. It exclusively consists of the secondary structure of α-helix, which can be sub-divided into an N-terminal coil that contains helices of A β [24]. The helix that connects the two motifs is ~90 Å long with 15 turns enclosed by α C helix that belongs to both the regions [25].

The APP is Amyloid precursor protein during apoptosis is productively and straightforwardly cleaved, bringing about increased A β arrangement [26]. The dominating caspase site interceded lysis is inside APP cytoplasmic tail, and cleavage at this site happens in hippocampas neurons *in vivo* following intense excitotoxicity or ischemic mind

injury [27]. Caspase-3 is actively involved in APP cleavage, reliable with its stamped rise in kicking the bucket neurons of Alzheimer's illness minds and colocalization of its APP cleavage item with Aβ plaques [28]. Caspases in this way seem to assume a double job in proteolytic handling of APP and the subsequent penchant for Aβ peptide arrangement, just as in a definitive apoptotic passing of neurons in Alzheimer's infection [29]. The name Beta Amyloid protein, along with tau protein, is associated with the pathogenesis of Dementia and AD. It is due to the deposition of these two proteins, which lead to the neural deterioration of the brain tissue.

On the surface of peripheral tissues like blood cells and epithelium, a transmembrane glycoprotein known as Amyloid Precursor (APP) is present, which on the activity of two proteolytic enzymes, namely, beta and gamma secretase, gets metabolized involving two pathway, namely- amyloidogenic and non amyloidogenic to give Amyloid beta (Aβ) [30]. In the former, Amyloid Precursor Protein is lysed with the help of β-secretase giving a N- terminal fragment (APPβ) and a C-terminal fragment (C99) that, on subsequent cleavage by γ-secretase gives the complete β-amyloid peptides (Aβ) [31]. Alternatively, the non amyloidogenic involves cleaving of APP by γ-secretase, that gives a C-terminal fragment (C83), and soluble N-terminal fragment (sAPPα) [32]. Ultimately α-secretase acts on the fragment releasing a C-terminal fragment of 3KDa (C3) [33]. Normally Aβ monomer is cleared within the body. However in case of Alzheimer's disease an imbalance results into accumulation of Aβ monomers into oligomers and longer polypeptide chains, that gives rise the senile plaque. The monomers of beta amyloid are known to be non-toxic and are present in the cerebrospinal fluid (CSF) of any healthy individual [34]. However, it is still unknown that at what stage their aggregation turns to be harmful. Some school of thoughts suggest that beta amyloid can in fact, be helpful for the healthy functioning of the neurons. Problem arises when they do not get cleared as swiftly as they ae getting produced from the APP. This further suggests that the excessive concentration of beta and gamma secretase may be responsible of the conversion of monomers into amyloid oligomers, and ultimately for the senile plaque formation [35].

Though researches are under process to know the exact role of the beta amyloid monomers, the exact answer is still to be known. Answer to this question may also give new directions to drug developments targeting definite cure for Alzheimer's and dementia [36]. The receptor is a glutamate receptor and ion channel protein found in nerve cell. The major functions of these receptors are they help in memorizing and learning and are critical for spatial memory. These type receptors are preeminent neurotransmitter-gates channels in the central nervous system [37]. The receptor plays a major role in excitatory neurotransmission and synaptic plasticity. If they are hypo or hyper activated diseases there is a risk of brain disease, schizophrenia, depression or chances of Alzheimer's disease [38]. They belong to L-glutamine family. NMDARs have numerous regulatory sites which are sensitive to polyamines, protons and glutathione [39]. The receptors contain GluN2A and GluN2B subunits. The subunits express highly in cerebral cortex and hippocampus. There is a differential spatiotemporal articulation and conveyance of the different NMDA receptor subunits inside the cerebrum recommending the nearness of various NMDA receptor populaces [40]. The GluN2B subunit is profoundly communicated all through the cerebrum during beginning periods of advancement and decays at the beginning of sexual development: GluN2A subunit-containing NMDA receptors increment over a similar life expectancy40. Right now, it isn't notable how the various subunits of NMDA receptors change may impact the subjective capacity. Notwithstanding, proof is mounting to demonstrate that exceptional age is related with a decrease in NMDA receptor capacity and subunit articulation inside cerebrum locales engaged with higher mind work including synaptic versatility, learning and memory [41].

Alzheimer disease is caused by the accumulation of Amyloid-β in the brain. This accumulation happens when there is an imbalance between the generation of Aβ from the precursor molecule which is APP and its further removal. The Aβ overproduction is not the major cause of Alzheimer Disease as accumulation of Aβ. From the brain interstitial fluid and cerebrospinal fluid, this accumulated Aβ can be removed by various mechanism discovered in the brain in order to eradicate extracellular Aβ [42]. In astrocytes and neurons, LDL receptor associated protein-1 clear up Aβ by up taking Aβ. The whole mechanism leads to Aβ transcytosis, across the BBB, together with P-gp [43]. LRP-1 is the APP modulator driving generation of Aβ through intracellular and extracellular domains interaction of

the LRP-1 and APP transmembrane proteins [44]. Depending on the relative expression levels of LRP-1, it is possible to modulate the APP integration and Aβ accumulation [45-46]. Reduction of LRP-1 endocytosis affects Aß generation by cleaving alpha secretase and preventing Aß production after endocytosis. Studies also show that LRP-1 plays a significant function in APP metabolism and Aβ clearance.47 Functional overproduction of LRP-1 receptors in the transgenic APP mouse model has been observed to cause an age-dependent rise in soluble Aβ in the brain.48 Inhibition of endogenous LRP-1 is expected after Aβ generation and deletion. LRP-1 ablation causes net accumulation of Aβ in the brain. LRP-1 is involved in other functions such as cell signaling, all migrations, proliferation, angiogenesis and wnt signaling. LRP-1 plays an important role in the regulation of APP treatment in vivo [49-50].

Metabotropic glutamate receptors (mGluR) are a family of G-protein coupled receptors that allow the binging of Glutamate, which is an amino acid that is responsible for excitatory neurotransmission. By exerting control on the release of Glutamate, mGluRs are capable of regulating neuron functions. Pr PC is a cell surface glycoprotein that can convert into an isoform that causes prion diseases [51]. Both PrPC and a member of the mGluR family, mGluR-5, have been identified as Amyloid beta receptors for AD. A plethora of evidence exists that point to PrPC as being a receptor for Amyloid beta oligomers. PrPC bound- Amyloid beta complexes are shown to reduce synaptic density, LTP inhibition and synaptotoxicity [52]. The mGluR-5 receptor is responsible for the activation of Fyn kinase and subsequent phosphorylation of NMDA receptor, due to the influence of the Amyloid-beta - PrPC complex [53]. The activation of Fyn kinase is responsible for the linking of the Amyloid Beta and Tau pathologies associated with Alzheimer's disease [54].

A simple therapy to reduce the symptomatic spread of AD would be to inhibit the function of the mGluR-5 receptor. What makes this solution not simple is the fact that this receptor is also involved in normal neuronal functioning like learning and memory, making the inhibition of the receptor problematic [55]. A strategy to bypass the problem would be to make use of antagonists /agonists /regulators that selectively inhibits the interaction with PrPC-Amyloid beta complex. The silent allosteric modulator (SAM) of mGluR-5 receptor can be used to selectively inhibit the effects of Amyloid Beta peptide while leaving normal mGluR-5 functions intact. [56].

FcγRIIb was immunoglobulin receptor (IgG), which binds to IgG complex containing antigen (foreign to body) expressed on the cell surface of neutrophils, macrophages, and B cells [57]. In B cells these receptors inhibit the B cell mediated immune response and prevent autoimmunity. It is also reported that FcγRIIb in purkinje cells regulates the cerebellar functions of brain [58]. Some studies states that Aβ effectively up-regulated the cγRIIb in cortical neurons and and its deficiency leads to inhibition of long term potentiation (LTP) and synaptic dysfunction[59]. The study of Alzheimer's disease by this approach is quiet relevant in Alzheimer's pathogenesis.The murine PirB (paired immunoglobulin-like receptor B) and in human brain it is LilrB2 (leukocyte immunoglobulin-like receptor B2) they are receptors for amyloid beta in Nano molar concentration.[60] These two immunoglobulin receptor are extra cellular and mediate cofilinsignaling (in this signaling the cofilin molecule play a crucial role in regulation of actin dynamics, which affects locomotion, migration and cell viability) which is seen in Alzheimer's patient brain[61]. In mice, the dysfunction of amyloid beta oligomers on hippocampal long term potentiation required PirB, which further contributed to memory loss and loss of synaptic plasticity in visual cortex of juvenile [62].

β Amyloid (Aβ) protein is a breakdown product of the Amyloid Precursor Protein (APP), consisting of around 36-43 amino acids and is the core component involved in the Alzheimer's disease. APP is an integral membrane protein found in a high concentration within the neurons [63]. B-Amyloid is one of the breakdown products of this protein by the action of enzymes β & γ secretases, in the abnormal or diseased condition. In the normal condition the acting enzymes include α-secretase instead of β-secretase [64].

The β-amyloid after the degradation is released into the plasma and CSF (cerebrospinal fluid) at a very fast rate. These are in the Nano molar concentrations and get internalized on the receptors on the surface of the Neuronal and

Glial cells [65]. Now these β-amyloids get internalized within the p75 receptors (a member of the death domain receptor family) leading to the functionally required structural changes, i.e., folding in the form of β-pleated sheets [66]. The most toxic forms of Aβ are the oligomers (insoluble forms) involved in the serious deleterious cellular events like neurotic death, neuronal degeneration, etc. in the serious neurodegenerative diseases like the Alzheimer's disease. Although the exact structure involved in binding is yet to be known. Followed by the degradative pathway of APP, Aβ within the CSF and plasma binds to the p75 receptor as the death binding ligand [67].

Followed by the structural changes in Aβ and its binding to it, p75NTR induces the neuronal degeneration and death by the mediation of c-Jun Kinase (JNK) and c-Jun (transcription factor)[68] Another supposedly deleterious consequence of Aβ binding to p75NTR is the abnormal neurotic outgrowth at the low concentrations of Aβ[69]. P75-NTR is particularly expressed in some specific regional neurons like Basal forebrain cholinergic, hippocampal, cortical and entorhinal neurons; in the people highly prone to the Alzheimer's disease [70]. For cellular signaling, the p75 receptor requires to be cross-linked and in turn for the processing of Aβ protein. Now the binding of the Aβ protein to the p75 induces the neurodegeneration within that neuronal tissue; also called as Aβ induced cellular apoptosis.[70] This leads to the structural changes in the β-amyloid: Foldings in the form of β-pleated sheets leading to the stacked formation of long fibrillary aggregates known as senile or neurotic plaques [71].

Normally the host cells involved in this binding is the ones that usually lack the expression of the neurotrophic receptors, but are genetically engineered to express them. Examples of the p75 expressing cells are Primary rat cortical neurons and Human epidermal melanocytes. These cells also involve the expression of other neurotrophic receptors [72]. For cellular signaling, the p75 receptor requires to be cross-linked and in turn for the processing of Aβ protein. Now the binding of the Aβ protein to the p75 induces the neurodegeneration within that neuronal tissue; also called as Aβ induced cellular apoptosis. This leads to the structural changes in the β-amyloid: Foldings in the form of β-pleated sheets leading to the stacked formation of long fibrillary aggregates known as senile or neurotic plaques [73].

Normally the host cells involved in this binding is the ones that usually lack the expression of the neurotrophic receptors, but are genetically engineered to express them. Examples of the p75 expressing cells are Primary rat cortical neurons and Human epidermal melanocytes. These cells also involve the expression of other neurotrophic receptors [74]. Amyloid beta form has antagonistic property on one side of the protein and it also has antioxidant properties on another side. Toxic properties amyloid beta form is mediated by different mechanism like oxidative stress, mitochondrial diffusion, change in membrane permeability, etc. Amyloid beta has some metal binding locations in its short amino acid sequences like histamine and tyrosine in the 10th position, all have copper metal binding sites[75]. Amyloid has the ability to convert Cu^{2+} and Fe^{3+} to Cu^{+} and Fe^{3+}. These reduced metals now available to oxygen to react and forms superoxide anion which react with two hydrogen atom and result in the formation of hydrogen peroxide which react with metallic ion which are in reduced form , result in the formation of hydroxyl radical [76]. Amyloid beta in its radical form able to extract H^{+} from lipids or protein and result in generation of peroxide. Reduced metal ions plays role in amyloid beta toxicity. Metal reduction is mediated by methionine because it has sulfide group which has the ability of oxidation that is it can donate electrons. An oxidative property of amyloid beta is depending upon methionine amino acid [77].

Therapeutic Approaches for Treatment of AD

The cholinergic hypothesis states that the disease is caused by the reduced synthesis of the neurotransmitter acetylcholine [78] Currently in order to alleviate symptoms, some drugs like acetyl cholinesterase inhibitors or N-methyl-D aspartate receptor antagonists, are available [79]. An experimental vaccine was found to clear the amyloid plaques using the small molecules but it did not have any significant effect on the dementia. Small molecules that are inhibitory in nature which will restrict the formation of the beta amyloid deposition in the brain or which can increase the synthesis of the neurotransmitter acetylcholine can help developing in the synthesis of the Alzheimer's drug [80].

AD is a neurodegenerative disorder which is caused due to accumulation of β amyloid as extracellular amyloid β (Aβ) plaques and aggregates of tau protein in the form of intracellular neurofibrillary tangles (NFT) [81]. The deposition of Aβ species leads to cell death. Both Aβ and tau oligomers have similar structures but are not identical. They have large β-sheet content which is not affected by proteolytic degradation. Immunotherapy method is one such approach that can be utilized to reduce the collection of Aβ and hopefully cure the cause of this disease. This approach can be performed in 2 forms. The first one being Active Immunization and second one is being Passive Immunization [82].

Active Immunization basically means to introduce some outside substance into the body of the person which can be recognized as an antigen so that the person's body start acting against it and give a suitable response. This method is widely used for curing many diseases such as tetanus, influenza, chickenpox, typhoid, hepatitis etc. But in this case there are various hurdles such as immune system insures that it does not produce antibodies against its own molecules. For this, a process called, B- cell tolerance is performed by the body. And if any antibody could possibly act against its own body then it is removed from the system. For this immunotherapy method, we would need to have response against the patient's own Aβ protein and thus require breaking the self- tolerance [83]. Also, the response against A β protein is also unsure as it is a neuro-protein because the movement of the antibodies to the brain is very much limited and the brain is immune- protected organ. The blood brain barrier blocks the passage of small molecules and majority of the proteins. But, to a surprise, an experiment on a mouse was performed which showed that immunization of the PDGF driven APP with Aβ42 could stop the plaque creation in mice if performed in early stages of their life cycle [84]. And in older mice, it was seen that, the creation of plaque has slowed down. This method was reproduced in many other animals. After that, Clinical trial AN1792 was performed on 372 patients and an active vaccine with an adjuvant was added to the immune systems of the patients. The trial was however stopped in between and the results were observed. It was seen that a lot of progression has been made in the case of patients where an antibody response to the Aβ antigen is shown. Their Aβ plaque density becomes less than the untreated patients [85].

Passive immunization however has shown more results in treating this deadly disease. In this approach, epitopes are recognized in the laboratory and antibodies are produced outside the body of the patients that could be injected directly into them. However one major setback of this immunotherapy is that it requires multiple dosages of the antibodies in a particular time interval. This method proved to be successful in treating autoimmune diseases, transplant rejection and cancer. Antibodies were prepared for direct injection against N-terminus of the Aβ protein. Treatment of APPtg mouse models of AD showed reduction in the amount of Aβ. Hence, we can say that, Aβ immunotherapy promises to show improvement in the treatment of AD (Table 1) [86].

Nanotechnology Based Sensing and Therapeutic Approaches

Classical studies have shown the therapeutic effect of acetyl cholinesterase (AChE). Recently, scientists are more focused towards the treatment of AD by employing interaction of acetyl cholinesterase with Aβ peptide for disaggregating A β plaques [128]. Previous reports have shown the selective labeling of Aβ1-40 fibrils with non-fluorescent or fluorescent rhodamine tags or through Congo red-encapsulated magnetic γFe2O3 nanoparticles [129]. Various *in vivo* studies have been done, which demonstrates the effect of modified carboxylated poly (glycidyl methacrylate)/ PE154- loaded core-shell polystyrene-block-poly (n-butyl cyanoacrylate) NPs on Aβ deposits.PE154 acts as an inhibitor, showing structural resemblance with acetyl cholinesterase. PE154 acts as a fluorescent probe for Aβ plaques present in brain samples collected from both transgenic animals as well as human subjects. Still they are inefficient in crossing BBB is Blood brain barrier [130]. For biological labeling as well as several diagnostic purposes, fluorescent semiconductor nanocrystals i.e. quantum dots have recently been used. Fluorescent semiconductor nanocrystals have shown enhanced photos as well as physicochemical stability. Aβ peptide aggregation kinetics can also be assessed using gold nanoparticles. The important parameter that needs to be assessed is the detection of *in vitro-in vivo* cytotoxicity level of the administered drug [131].

Cholinergic transmission deficiency is known to be an important factor, affecting the learning and memory of an individual. Thus, any therapeutic means that enhances the cholinogernic transmission kinetics could be a newer therapeutic approach for treating AD. The drug may at least provide homeostasis, if not improvise that much cognitive function may be due to poor brain translocation etc. Polymeric PnBCA NPs coated with polysorbate 80 loaded with Rivastigmine, is actively involved in modifying the brain delivery systems with reduced side effects and increased bioavailability. Polysorbate 80 has greater affinity for apolipoproteins E and I, which makes nano complex more prone to get internalized through low-density lipoprotein receptors available on BBB [132].

Tacrine, which is another AChE inhibitor, gets bio-absorbed through the same mechanism, with minor difference where PnBCAnanocarriers have been used as a delivery system. Studies have shown that as compared to free drug, the nano-based therapeutic approach may enhance the drug uptake by a factor of 4.Single-wall carbon nanotubes can also be used as an altered therapeutic system, as they are nonbiodegradable and until now, no such reports have been identified having their therapeutic effects with acute and chronic toxicity [133].

Estrogen and androgen (gonadal steroids) therapies may also participate actively in CNS development and regulation, by promoting the growth of cholinergic neurons along with reducing cerebral amyloid deposition and ultimately decreasing the risk of AD. They usually act by slowing the progression of cognitive decline in AD patients, by affecting P-glycoprotein efflux system. Both hormone and anti-hormone molecules can be bioabsorbed at a higher rate when administered in combination with nanosystems [134]. *Curcuma longa* has active phyto-constituents called curcuminoids, used widely in the food industry. Reports have shown that they have potential therapeutic efficacy as an anti-inflammatory, antioxidant, antimicrobial agent. Thus to overcome the drawback, non-functionalized NPs can be a better alternative approach. As far as toxicity parameters are concerned, the results showed that this nanosystem showed a significant reduction in the ROS level as well as Aβ1-42-related toxicity [135].

For AD treatment, several immunotherapies have been carried out against Aβ1-42 peptide, but during clinical trials, severe complications related to therapies have been obtained. Polyethyleneimine complex with encapsulated Cu (II) ions, are known to have great bioabsorption thus ultimately resulting in its rise in cytosolic concentrations. Due to the high toxicity issues of polyethyleneimine when tested in *in vivo* experiments, no further studies have been carried out on this. Various postmortem reports have shown that co-incubation of AD brains with ion chelators like ethylenediaminetetraacetic acid salts, desferrioxamine, and clioquinol could resolubilize the same. Thus it can be said that this therapeutic system may improvise the condition of AD patients, but still, toxicity associated parameters have reduced their therapeutic efficacy [136].

CONCLUSION

Alzheimer's is a progressive neurodegenerative disease characterized by memory loss, dementia and cognitive impairment. Unfortunately, there is no effective drug discovered till now which can cure this disease. The cause of the disease is poorly understood, many scientists concluded that it is inherited from a person's parent with many genes usually involved. Amyloid hypothesis postulated that extracellular beta amyloid deposits are the fundamental cause of the disease. The nanotechnology based treatment emerges as an alternative therapeutic approach for providing improved therapy with less side effects and toxicity. Because of high surface area to volume ratio, more sensitivity and less toxicity-related issues, magnetic iron oxide nanoparticles are the current focus of researchers these days. The concept is quite very much encouraging but manipulation at the level of blood-brain barrier is still a challenging area.

Author Contribution

All authors are equally contributed in data collection, review, and preparation of this manuscript and approved the final version for the publication.

Statement of Funding Source

We have not received any funding from government or private or any other organizations.

Declaration of Competing Interest

No conflicts of interest.

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Table 1: Showing Orthodox Medical Regimes for Treatment of AD, Their Therapeutic Effect and Side Effects

Types	Targets	Effects	Drugs	Side effects	Reference
Approved drugs	Cholinesterase inhibitor	Blocks acetylcholine neurotransmitter	Donepezil, Rivastigmine, Galantamine	Gastrointestinal problems, Nausea, headache, vomiting, anorexia etc.	87
	Cholinesterase inhibitor and NMDA receptor antagonist	Blocks acetylcholine and glutamate neurotransmitters	Donepezil	Insomnia, muscle cramps, fatigue	88
	NMDA receptor antagonist	Blocks glutamate neurotransmitter	Memantine (Namenda)	Dizziness and constipation	89
Small molecule inhibitors of amyloid	Blocks Aβ oligomerization and fibrillization	Blocks neurotoxicity	2-Amino-4-chlorophenol, 3,4-Dihydroxybenzoic acid, 4-Aminophenol	Carcinogenicity, basal oxidative, DNA damage, gastrointestinal hemorrhage, deteriorated liver function etc.	90

	Remodels soluble oligomers and amyloid fibrils into nontoxic species	Attenuates cognitive deterioration, Reduces Aβ aggregation, Prevents cognitive decline, Increases the life span, Prevents toxicity and ROS accumulation	Resveratrol,NQTrp, ClNQTrp, Coumarin, Furosemide, D737	Interact with blood thinners such as warfarin resulted in continuous bleeding, Non-specific, Less inhibition potential, slight nausea, diarrheahypochloremia, hypokalemia, hyponatremia, gastrointestinal uneasiness	91-94
Secretase inhibitors	γ-Secretase inhibitor	Reduces Aβ formation	Semagacestat (LY450139)	Adverse influence on cognition	95
	β-Secretase inhibitor	Reduces Aβ levels	MK-8931, Tarenflurbil	Hepatotoxicity	95
	α-Secretase agonist	Biases APP processing towards the non-amyloidogenic pathway	EHT-0202	Adverse effect on central nervous system	96
Immunotherapeutic approaches	Aβ antibody	Inhibits Aβ oligomerization and cytotoxicity	CAD106, SDPM1	Fever, chills, and fatigue.	97,98
	T-cell-mediated autoimmune meningoencephalitis	Clears Aβ plaques and improves cognitive performance	PD-1 inhibitors	Swelling, weight increase from extra fluids	99
	Humanized monoclonal antibody to Aβ	Binds to aggregated Aβ and reduces Aβ plaques in the brain	Gantenerumab,Solanezumab, Crenezumab	Heart palpitations, a stuffy head, and diarrhea	100-102
	Human immunoglobulin preparation containing endogenous polyclonal antibodies to Aβ	Binds to Aβ and reduces neurotoxicity	IVIG	Dizziness	103
Anti-aggregation agents	Prevents Aβ aggregation	Reduces cellular toxicity	Apomorphine	Severe headache; daytime sleepiness or drowsiness	104
	Inhibits Aβ aggregation	Reverts amyloid deposition	Hormone melatonin	Stomach cramps, and irritability	105
	Blocks microglial activation	Prevents Aβ-promoted inflammation	Cannabinoid, HU-210	Intense anxiety, fear, distrust, or panic	106
	Binds Aβ fibrils	Inhibits Aβ aggregation and cytotoxicity	Tannic acid	Augmented chance of developing nose or throat cancer	107
	Polysaccharide that blocks Aβ fibril formation	Reduces neurotoxicity	LJW0F2	Sleepiness or drowsiness	108
	Inhibits Aβ aggregation	Reduces neurotoxicity	EGb761	Bleeding disorders and constipation	109
			Isoliquiritigenin	Lower solubility, short elimination half-life, and low bioavailability	110
			Protocatechuic acid	Stuffy head, and	111

				diarrhea	
			Atractylenolide III	Mild nausea and diarrhea	112
			Chlorogenic acid	Insomnia and nervousness	113
			Euphorbiafactor L3, L2	Hay fever	114
			Ganoderic acid D, DM	Dizziness and constipation	115
			Substituted bisphenol A derivatives	Heart attack, hypertension	116
β-degrading proteases	Endogenous regulator of Aβ	Degrades Aβ oligomers	NEP 2, hMMEL	Nausea and diarrhea	117
	Endothelin converting enzyme	Endogenous regulator of Aβ	ECE1, ECE2, ACE	Fatigue and rash	118
	Serine protease	Degrades both monomeric and fibrillar forms of Aβ	Plasmin, Acylpeptide hydrolase	Diarrhea	119
	Cysteine protease	Degradation of Aβ	Cathepsin D, BACE1, BACE2, Cathepsin B	Insomnia and fatigue	120
Novel therapeutic approaches directed against the tau protein Approaches against the tau protein	Inhibitor of tau hyperphosphorylation	Facilitates clearance of tau from the brain and anti-Aβ aggregation	LMTX	Nausea, headache, vomiting	121
	Microtubule stabilizer	Increases BBB permeability and microtubule stability	Epothilone D	Nausea	122
			TPI-287	Dizziness	123
	Tau active vaccination	Improves neurobehavioral deficits, and reduces neurofibrillary degeneration and mortality	AADvac1	Drowsiness	124
	Anti-tau vaccine	Stimulates the immune system to produce antibodies which	ACI-35	Nausea and diarrhea	125
	GSK-3β inhibitor	Blocks phosphorylation of tau protein	Tideglusib	Cough, fatigue, and headache	126
			Humulin R	Palpitation and tremor	127

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Social Media Addiction for Children with Sensory Processing Disorder (SPD)

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Received: 05 Mar 2021 Revised: 06 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

Aim: To observe the social media addiction and its impact on attention for children with Sensory Processing Disorder (SPD) **Objectives:** To identify the level of addiction for children with sensory processing disorder (SPD) and To access the level of attention with mindful attention awareness scale (MAAS) for children with sensory processing disorder (SPD) **Methodology:** The main purpose of this study is to examine the relationship between the level of addition towards the social media and its impact on attention for children with Sensory Processing Disorder (SPD). A total of 30 participants with special school students between the ages of (12-17) (Mean Age 14.50) both male and female were selected for this study. All the participants were screened by using social media addition scale (SMAS) and Mindful attention awareness scale (MAAS). **Results:** The result showed that out of 30 students, 18 students were getting highly addicted in social media, 12 students moderately addicted in social media. This statistical picture shows that, due to social media addiction most of the children with SPD have severe level in their attention. **Conclusion**: This study concluded that, due to social media addiction; most of the children with SPD have severe level in attention.

Keywords: Social Media Addition Scale (SMAS), Mindful Attention Awareness Scale (MAAS),

INTRODUCTION

In the present day fast moving and globalised world it is almost impossible to imagine our day to day life without mobile phones. It is one of the most successful inventions of the 20th century. This has become a convenient means of communication. Modern mobile phones perform many other functions as well. They can substitute for such devices as music player, camera and organizers. Most of them also provide internet access and texting as modern people need. Phones in all spheres of their lives professional and personal, but we do not notice how much we depend on cell phones and what effects their excessive use might bring.

Addiction

People with addictions are not in control over things that are doing, using or taking. The addiction thus reaches a point that is harmful to a person physically, emotionally and socially and inturn affecting the well being of a person in society. An addiction not only includes physical attributes such as drugs or alcohol. It also includes mobile addiction and social media addiction is considered as a kind of internal addiction those individuals who spend too much time on social media.

Difference between Habit and Addiction

Addiction involves psychological, physical, social and environmental component where the person is spending too much of time in social media called as addiction.

The person with the habit can choose to stop and will subsequently stop if they want. In term of the habit with person is in control of their choices.

Impacts:

- **Positive:** Mobile and social media are useful for studies such as Academic performance, searching information and communication with friends and family members among them mobile is playing an important role.
- **Negative:** Now a day's people communicate with families and friends using social media. It decrease the quality of inter personal communication (verbal and non-verbal) and also affect the education, health and social life. Most of the students using mobile phone for chatting typically playing online games and watching videos .This is one of the sources of distraction from studies. Social media addiction also causes the psychological problems like anxiety, depression, loneliness, lack of attention this will also affect the education.

Social Medial

Social media is computer based technology that facilitates the sharing of ideas, thought and information through the building of virtual network and communities. Social media originated as a way to interact with friends and family but was later adopted by business which wanted to take advantage of a popular new communication method to reach out to customers .The power of social media is the ability to connect and share information with anyone on earth or with many people simultaneously.

Social media is an ever-changing and ever evolving web based platform.

Social Media Addiction

Social media use one of the most popular leisure activities among adulterants. Use of the social media has become a part of life of every student. Social media addiction is a behavioral addiction that is characterized as being overly concerned about social media and devoting so much time and effect to social media that it impairs other important life areas. Addictive social medial use will look much like that of any other substance use disorder, including mood modification. (Example): changes in emotional states, behavioral, cognitive and emotional preoccupation with social

media. The main objectives of this study are to find out the social media addiction among people and evaluating social media addiction through measuring social media addiction scale. Causes of attention problem among social media addictors through "Mindful attention awareness skill" program.

REVIEW OF LITERATURE

Aylin Tutgun-Unal *et al* (2015)

The aim of the research was to develop a scale to detect the social media addiction of university students. The data collection from 775 university students revealed that the scale was composed of four factors. Of these four factor, the first one called as "occupation" explained itself 17% of the variance, the second one called as "mood modification" explained itself 9.8%of the variance, the third factor called as "relapse" 8.8% of the variance itself, the fourth factor called as "conflict" explained itself 23.5% of the variance .these four factor composed of 41 items totally explained 59% of the variance altogether. As a result of the studies conducted, the scale was found to be valid and reliable and named "social media addiction scale" (SMAS).

Kanokporn Sri Wilai *et al* (2016)

The aim of this study is to investigate the impacts that social media addiction has on mindfulness and choice of coping strategy ,as well as to explore the consequence on emotional exhaustion .the survived data were collected from 211 employees in 13 enterprises in Thailand. The result from partial least square structural equation modeling revealed that people who are highly addicted to social media tended to have lower mindfulness and tended to use emotion coping strategy are also subsequently associated with higher emotional exhaustion.

Cengiz Sahin (2018)

This study is to develop a valid and reliable measurement tool to determine the social media addiction of school and university students -998 students participated in the study. Test related method was used to determine .the consistency of the scale with the participation of 224 students; exploratory factor analysis , confirmatory factor analysis ,total item correlation , means difference between upper and lower groups , internal consistency coefficient and test-retest correlation coefficient were calculated within the scope of assessing the validity and reliability of the research.tn this conclusion SMA-SF is a 5 point likert –type scale consisting of 2g items grouped under 4 factors. The statistical analysis indicated that the scale is valid and reliable enough to be used in determining the some add of school and university students.

Mindfulness Attention Awareness Scale
James Mackillop .Emily J. Andreson N (2007)

Esther I .De Bruin *et al* (2011)

In total number of 781 high school students from to high school in nether land one in the most urban area other in the rural area. This questioner was administered in the class room and two of the project members were present to supervise the administration and to assist the adolescent where necessary males and females did not differ in MAAS - A scores. Mindfulness as measure by their MASS – A correlated positively with quality of life ,but an expected positive relationship and reliable Dutch measure of mindfulness for adolescents. The factor structural, internal consistency, convergent and divergent validity as well as their relationship to quality of life are comparable to the original MASS – A.

Kirk Warren Brown, *et al* (2011).

The present studies were designed to valid among 102 adolescents a measure of mindfulness previously validated for adults 14 -18 years. participants randomized to a mindfulness based stress reduction intervention showed significant increases in MAAS score. From base line to three month follow up, relative to non significant score

changes among treatment as usual participant. increase in MAAS -A score among mindfulness based stress reduction participant were significantly related to beneficial changes in numerous mental health indicators.

MATERIALS AND METHODS

The study was conducted in the special school at Cuddalore, between the ages of 16 to 20 years old (Both male and female). The purpose of the study was explained to the student and an informed consent was given in there known language. Social media addiction scale and mindful attention awareness scale was to assess the social media addiction and its impact on attention.

Social Media Addiction Scale

In this experiment, social media addiction is identified using "social media addiction scale". Participants each completed a set of questionnaires' in class.

Scoring of the Scale

Social media addiction scale-student form is a 5 point scale which consists of 29 items and 4 sub dimensions. The highest point is 145 and the least one is 29.
The highest score indicated that agent perceives himself as a "social media addict".

Mindful Attention Awareness Scale

Mindful attention awareness scale is used to measure individual's mindfulness level. Depend upon the social media addiction scale –student form Identified the addicted people who are all addicted to social media they completed, another set of "mindful attention awareness scale" questionnaires.

Scoring

Mindful attention awareness scale consists of 14 items on 6 point scale. Ranging from 1"almost always" to "almost never" Higher scores are indicated of higher trait mindfulness.

DATA ANALYSIS AND RESULT

For this study 30 students were selected with the age group of 11-17 years .They were assessed by using SMAS and MAAS-A. From that, SMAS-SF was found, that high level of addiction (13) moderate level of addiction(11) ,less level of addiction (5) ,and no addiction (1).From this SMAS, high and moderate addicted children were selected, MAAS-A was given and assess the mindfulness of addicted students, where almost always (0.13) ,very frequently (1.70), somewhat frequently (6.95), somewhat infrequently (2.32), very infrequently (0.05), almost never (0) Most of the students was high in somewhat frequent than other, Hence, through this result concluded that, most of the students have high and moderate level in attention.

The result showed that out of 30 students, 13 children highly addicted in social media, 11 children moderately addicted in social media .The statistical value shows that, due to social media addiction most of the students have high level in attention.

CONCLUSION

Through this study concluded that, due to social media addiction; most of the students have high and moderate level in attention.

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ACKNOWLEDGEMENT

I Thankful to my bellowed Research Supervisor Dr. P. Swarna Kumari and my Family Members.

CONFLICT OF INTEREST

This work has done my own interest.

SOURCE OF FUNDING

Nil

Table 1, show mean score of high addiction

Mean Score	Age	Never	Rarely	Sometime	Often	Always
	14.50	1.212	5.090	13.727	2.454	7.452

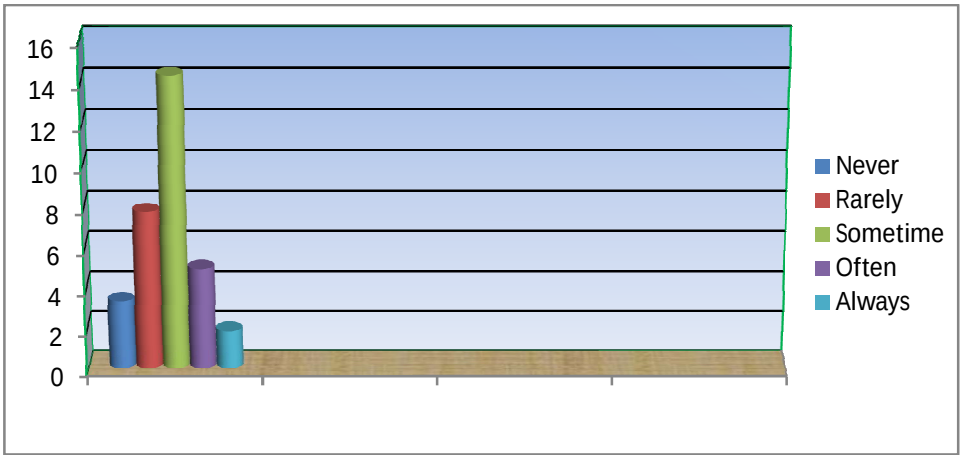
Table 2, show mean score of moderate addiction

Mean Score	Age	Never	Rarely	Sometime	Often	Always
	12.546	3.323	7.733	14.331	4.933	1.833

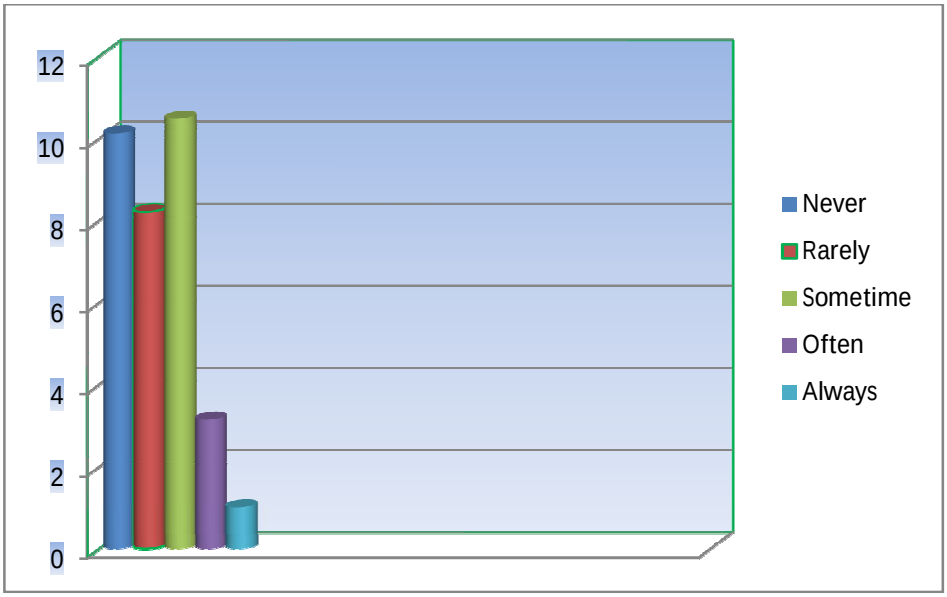
Table 3, show mean score of less addiction

Mean Score	Age	Never	Rarely	Sometime	Often	Always
	11.897	10.120	8.213	10.487	3.1538	1.012

Graph 1, showing ratings on social media addition scale **(SMAS)** among the participants of **Moderate Addiction**



Graph 2, showing ratings on social media addition scale **(SMAS)** among the participants of **Less Addiction**



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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Comparative Study on the Effectiveness of Phonophoresis Versus Trigger Point Release Technique in Reducing Myofascial Pain of Levator Scapula.

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Received: 28 Feb 2021 Revised: 03 Mar 2021 Accepted: 06 Mar 2021

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ABSTRACT

The study was aimed to compare the effects of Trigger point release and phonophoresis of hydrocortisone 1%, on patients with myofascial pain of levator scapulae. The randomized controlled trial design with two experimental groups was used. 20 Subjects with Myofascial pain of Levator scapulae were selected using simple random sampling and were further divided into two equal groups. The myofascial pain was treated by Phoonophoresis and Trigger Point Release. The results showed both the treatment groups were decreased in pain. Our results indicate that both the treatments used in this study were effective for treating Myofascial pain of Levator scapula but Trigger point Release was significantly more effective than Phonophoresis in reducing pain.

Keywords: Myofascial pain; Phonophoresis; Trigger point Release;

INTRODUCTION

One of the most commonly involved shoulder-neck muscles that are injured by acute or chronic stress is the levator scapula muscle. This muscle starts at the upper inside corner of your shoulder blade and runs up the side of your neck to connect to the upper four bones (vertebra) of your spine. It was found that 1/5 of healthy individuals had trigger points in levator scapula without even being conscious of it. It is also one of the most common trigger points found in the research studies. Levator scapulae myofascial pain is frequently caused by utilizing a console or keyboards in a strange situation with the neck pivoted however can happen in sports, for example, swimming, where continuous neck revolution is required [1]. The conventional definition of myofascial pain syndrome (MPS) is characterized by regional pain originating from hyperirritable spots located within taut bands of skeletal muscle, known as myofascial trigger points (MTrPs) [2]. MTrPs can lead to muscle weakness of the involved painful muscle and reorganization of motor activation patterns. Restricted range of motion may be observed secondary to a contracted taut band [3]. Myofascial pain syndrome is one of the common disorders in the world population. In a study on patients with pain complaints, 31% of them had acute trigger points. Trigger points have been described in all age groups and both sexes. It seems to occur more with increasing age until mid-life. An impaired blood flow in the immediate vicinity of TrPs may cause a decreased energy replenishment [4]. Janet Travel, a pioneer researcher has described this syndrome as a regional muscle pain disorder that is characterized by a tender spot in the taut band of muscles that refers to pain to areas overlying or distant to the tenderness. Ultrasound is a technique that has been proposed to treat myofascial pain by converting electrical energy to sound waves in order to provide heat energy to muscles [5]. Ultrasound is an effective modality widely used for soft tissue injuries. Phonophoresis is the introduction of drugs into the tissues through ultrasound for treatment purposes. Phonophoresis can be effective in the relief of myofascial pain and restore the normal range of motion. Trigger point therapy is a soft tissue manipulation done to desensitize trigger points, improve circulation and thereby aid in pain reduction.

DESIGN AND METHODOLOGY

Research Design

The study is a randomized controlled trial with two experimental groups.

A Pre-Test Assessment Of Pain Was Taken Using A Visual Analog Scale.

After the pre-test, the experimental group A received phonophoresis whereas the Experimental group B received the Trigger point release technique for a period of one week. The treatment was given once a day for both groups.
On the 7th-day post-test measurement were taken for both groups.

Criteria for Selection

Inclusion Criteria

- Patients with myofascial pain of levator scapula
- Patients with the age group of 20-40 years
- Patients with the palpable taut band and active trigger point
- Patients with exquisite spot tenderness of a nodule on a taut band
- Subjects who were psychologically fit

Exclusion Criteria

- Patient with dermatitis
- Any open wounds and scars over the levator scapula
- Patients with any associated problems
- Sensory deficits
- Degenerative changes of the cervical spine.

Population

All the patients who fulfilled the selection criteria were taken as the population of the study.

Sample Size and Method of Selection

Twenty samples were selected from the population using a simple random sampling method.

METHODOLOGY

Twenty samples were selected using a simple random sampling method and were further divided into two equal groups. Both the groups underwent pretest assessment of pain using a visual analog scale. Pretest assessment of pain was taken for both the groups using a visual analog scale, which consisted of a 10cm unmarked line marked as no pain on one end and another end as maximum pain. The patients were requested to turn the neck to the side of pain and mark on the line which corresponds to the level of pain intensity, he presently felt.

PROCEDURE

Experimental Group-A

Phonophoresis Therapy

After the pre-test analysis of pain, the experimental group A received phonophoresis. Patients were seated in a chair with shoulders relaxed. The area was cleaned and phonophoresis with 1% hydrocortisone and a frequency of 1 MHZ was given by direct contact method in a pulsed mode 1:1 with an intensity of 0.25 – 0.75 w/cm2 for 8 minutes duration once a day for a period of seven days. The post-test measurements of pain were collected at the end of the seventh day in a similar manner as that of pretest measurement.

Experimental Group-B

Trigger Point Release Technique

After locating the trigger point, a Firm digital compression was applied with a single finger pad. The pressure was gentle at the beginning and was gradually progressed deeper into tissues and clocked approximately up to 4 kilograms of force it was performed very slowly to accommodate the patient's pain threshold level. The compression was maintained for 5 seconds and released it for 2-3 seconds. This same cycle was repeated till the patient has reported a reduction in local or referred pain or an increase in pain or until 2 minutes has passed without any change in pain level. A small amount of talcum powder was applied over the trigger point, before the procedure in order to reduce the noxious Skin friction, after this method subjects were brought to the position of comfort.

Unilateral Stretching

The patient was made to sit in a chair and place the contra lateral hand on the head and gently pull forward and to the side (the side opposite to the side of pain) at the same time until a stretch is felt and was held for 30 seconds. The cycle was repeated three times at least three times a day.

The post-test measurements of pain were collected at the end of the seventh day in a similar manner as that of pretest measurement.

Observation and Analysis

The collected data were analyzed using paired "t" test and independent "t" test.

RESULTS AND DISCUSSION

20 samples were selected using simple random sampling and they were allotted into two equal groups randomly. Group A received phonophoresis and group B received Trigger point release. The analysis of the collected data which was done using paired "t" test has revealed that both phonophoresis and Trigger point release was significantly effective in reducing myofascial pain of levator scapula. The analysis of the collected data using independent "t" test has revealed that the Trigger point release technique is significantly more effective in reducing myofascial pain of levator scapula than phonophoresis

DISCUSSION

Phonophoresis is significantly effective in reducing myofascial pain in the levator scapula. The reduction of pain following phonophoresis therapy with 10% hydrocortisone gel may be due to heat produced by phonophoresis in large myelinated nerve fibers which may reduce pain through the gating mechanism. It is also stated that during the early inflammatory phase of repair macrophages and mast cells occupy the inflamed site and it has been shown that these cells are responsive to therapeutic phonophoresis. Therapeutic phonophoresis accelerates the inflammatory phase, resulting in a more rapid entry into the proliferative phase. the introduction of the drug also reduces the pain. Ultrasound accelerates metabolism, blood supply, recovery, and extensibility of connective tissue with its warmth and mechanical impacts [6]. While utilizing ultrasound, the pain gate theory could be referenced again because of the excitation of A mechanoreceptors. In this condition, messages delivered from mechanoreceptors enter the spinal line and brisk torment driving forces at the spinal cord become repressed and pain would be suppressed [7]. The trigger point release is significantly effective in reducing myofascial pain of the levator scapula. The trigger point release technique alters the dynamics of circulatory imbalance affecting trigger points and deactivates them. It also increases the pain threshold level and extensibility of soft tissues. Trigger point release massage (or ischemic compression) provides an external force that could separate sarcomeres and reduce compression on blood vessels. MTrPs are believed to be important elements in MPS [8]. Reduced Pain pressure threshold at the MTrP is an indicator of increased sensitivity and treatments directed at MTrPs have shown promise at improving clinical outcomes, including improved muscle strength and range of motion, and reduced pain [9]. Trigger point release massage (or ischemic compression) provides an external force that could separate sarcomeres and reduce compression on blood vessels. For these reasons, the trigger point release technique was significantly more effective in reducing the myofascial pain of the levator scapula.

CONCLUSION

The results of this study make us conclude that Trigger Point Release Technique is significantly more effective in reducing myofascial pain of levator scapula than phonophoresis.

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Table. 1. Phonophoresis Versus Trigger Point Release Technique with paired 't' test and independent't' test

Group	Variable	"t" cal value	"t" table value
A (Phonophoresis)	Myofascial pain	8.24	2.262
B (Trigger point release technique)	Myofascial pain	12.78	2.262
Independent "t" test	Myofascial Pain	5.24	2.101

- "t" calculated value > "t" table value
- Significant at 5% level.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Formulation and Evaluation of Fexofenadine Hydrochloride Fast Dissolving Tablet

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Received: 22 Dec 2020 Revised: 26 Dec 2020 Accepted: 02 Jan 2021

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ABSTRACT

The aim of present study was to formulate fast dissolving tablets of Fexofenadine hydrochloride by using different super disintegrates like Crospovidone, Crosscarmellose sodium etc. Fexofenadine HCl, is a non-sedating anti histamine used in the symptomatic relief of allergic conditions including seasonal allergic rhinitis and urticaria with poor aqueous solubility. The formulation were done by the fast dissolving with the direct compression method with different super disintegrates and evaluation Bulk density ,Tapped density ,Carr's index , Hausner's ratio, Angle of repose, and evalution study were Tablet evaluation parameters ,Weight variation, Hardness ,Thickness ,Friability, Disintegration time, Wetting time, Water absorption ratio ,*In vitro* dispersion time, Content uniformity test. The Tablets prepared were found to be good, without chipping and sticking with optimum hardness and thickness. The tablets prepared by using Cross carmellose sodium and Crosspovidine ratio were found to be disintegrate faster and there is a rapid drug release within short time and found to be stable . The rate of drug release is increased and disintegration time is decreased to a considerable effect by increasing the concentration of superdisintegrants. Thus it can be concluded that as the concentration of the superdisintegrant is increased the rate of drug release and disintegration time is enhanced. This study demonstrated that disintegration of Fexofenadine HCL can be enhanced to a great extent by direct compression technique with the addition superdisintegrants.

Keywords: Fexofenadine HCL, super disinter grants, direct compression technique

INTRODUCTION

Oral routes of drug administration have wide acceptance up to 50-60% of total dosage forms. Solid dosage forms are popular due to simple administration, accurate dosage, self-medication, pain avoidance and most significantly the patient compliance. The most popular solid dosage forms are being tablets and capsules; one important drawback of this dosage forms for a few patients, is that the difficulty to swallow. Drinking water plays a crucial role within the swallowing of oral dosage forms. Often times people experience inconvenience in swallowing conventional dosage forms like tablet when water isn't available, within the case of the kinetosis (kinetosis) and sudden episodes of coughing during the common cold, allergic condition and bronchitis [1]. For these reason, tablets which will rapidly dissolve or disintegrate within the mouth have attracted an excellent deal of attention. Ordispersible tablets are not only indicated for people who have swallowing difficulties, but also are ideal for active people. Fast dissolving tablets also are called as mouth-dissolving tablets, melt-in mouth tablets, Orodispersible tablets, rapimelts, porous tablets, quick dissolving etc. Fast dissolving tablets are those when put on tongue disintegrate instantaneously releasing the drug which dissolve or disperses in the saliva [2].

Fast Dissolving Tablet

Despite disadvantage, oral drug delivery remains the preferred route of drug delivery. Oral fast dissolving tablets have received important acceptance as novel drug delivery system for treatment of various diseases upon introduction in the mouth disintegrate in the mouth in the absence of additional water for easy administration of active pharmaceutical ingredients [3].

Drug delivery systems are strategic tool for expanding markets, extending product life cycle. Oral routes of drug administration have a wide acceptance up to 50-60% of total dosage forms. It is most popular route for systemic effects due to ease of ingestion, pain, versatility, less patient compliance. The demand for solid do-sage forms can be dissolved & suspended in water, chewed or rapidly dissolved in mouth. The dosage forms are placed in mouth, allowed to dissolve in saliva and swallowed in normal way. Most fast dissolving tablets include substances to mask bitter taste of active ingredient. Faster the dissolution, quick absorption [only in ionized form of drug] and quick on set of action. Fast dissolving tablets are also known as mouth dissolving tablets, melt-in mouth dissolving tablets, oral dispersible tablets, rap melts, porous tablets, quick dissolving tablet. Fast dissolving tablets dissolve or disintegrate in oral cavity without need of water. Some tablets are designed in saliva within a few seconds, and so called ‘true fast dissolving’ tablets [4]

Pharmacokinetics of FDT

It deals with absorption, distribution, metabolism, ex-cretion. After absorption drug attains therapeutic level and elicit pharmacological effect. So both rate and ex-tent of absorption is important. In conventional dosage form there is delay in disintegration and dissolution. But in case of fast dissolving tablets rapidly disintegra-tion in oral cavity and dissolution is fast. The faster dissolution of tablet takes place in mouth absorption from mouth, pharynx, and esophagus. Some factors like age, sex, pH, blood flow through gastrointestinal taken into consideration because elders may be considered as separate unique medical care preparation.11 Drug distribution depends on many factors like tissue permeability, perfusion rate, binding of drug to tissue, disease state drug interaction. In geriatric patients, decrease in body mass and total body water result in decreased volume of distribution of lipid soluble drugs. Duration and intensity of action depends upon rate of drug removal from body i. e. biotransformation. De-crease in renal volume, regional blood flow to liver reduces bio transformation of drug through oxidation, reduction, and hydrolysis. Excretion by renal clearance is slowed, thus half life of renal excreted drugs in-crease. The metabolism of fast dissolving tablets is very easy and can be obtained very faster. Drinking water plays an important role in swallowing of oral dosage forms [5].

Pharmacodynamic of FDT

Drug reception interaction impaired in elderly also as in young adult thanks to undue development of organ. Decreased ability of body to respond baro reflexive stimuli, cardiac output, and orthostatic hypotension may see in taking anti hypertensive like prazosin. Decreased sensitivity of CVS to beta adrenergic agonist and antagonist. Immunity is less and taken into consideration while administered antibiotics [6].

Advantages of Fast Dissolving Tablets [7]

Ability to supply advantages of liquid medication within the sort of solid preparation

- Improved compliance/added convenience
- Better taste
- No chewing needed
- Allows high drug loading
- Improved stability
- Suitable for controlled as well as fast release actives
- Cost-effective

Idea Characteristics of Fast Dissolving Delivery System [8]

- It allows high drug loading.
- Improved patient compliance.
- Leave little or no residue in mouth after oral administration.
- Pleasant mouth feels properties, adequate hardness
- Should have adequate taste masking properties.
- No need of water for oral administration

Mechanism of Fast Dissolving Tablets [9]

To Achieve the Tablets Fast Dissolving Properties

Water must quickly enter into the tablet matrix to cause rapid disintegration and instantaneous dissolution of the tablet. Incorporation of an appropriate disintegration agent or highly water soluble excipients in the tablet formulation

These are some under mentioned mechanisms by which the tablet is broken suspension of drug.

The mechanisms are

- High swell ability of disintegrates
- Chemical reaction.
- Capillary action.

MATERIALS AND METHOD

Preformulation Studies

Preformulation study relates to pharmaceutical and analytical investigation carried out proceeding and supporting formulation development efforts of the dosage form of the drug substance. Preformulation yields basic knowledge necessary to develop suitable formulation for the toxicological use. It gives information needed to define the nature of the drug substance and provide frame work for the drug combination with pharmaceutical excipients in the dosage form. Hence, the following preformulation studies were performed on the obtained sample of drug.

The goals of the program therefore are:

To establish the necessary physio-chemical characteristics of a new drug substance.

To establish its compatibility with different excipients.

Hence, preformulation studies on the obtained sample of drug include color, tastes, solubility analysis, melting point determination and compatibility studies.

Bulk Density (Db)

It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weight powder (passed through standard sieve # 20) into a measuring cylinder and initial weight was noted. This initial volume is called the bulk volume. From this the bulk density is calculated according to the formula mentioned below. It is expressed in g/ml and is given by [11]

Db = M/ Vb

Where, M is the mass of powder

Vb is the bulk volume of the powder.

Tapped Density (Dt)

It is the ratio of total mass of the powder to the tapped volume of the powder. Volume was measured by tapping the powder for 750 times and the tapped volume was noted if the difference between these two volumes is less than 2%. If it is more than 2%, tapping is continued for 1250 times and tapped volume was noted. Tapping was continued until the difference between successive volumes is less than 2 % (in a bulk density apparatus). It is expressed in g/ml and is given by

Dt = M / Vt

Where, M is the mass of powder

Vt is the tapped volume of the powder.

Angle of Repose (θ)

he friction forces in a loose powder can be measured by the angle of repose (θ). It is an indicative of the flow properties of the powder. It is defined as maximum angle possible between the surface of the pile of powder and the horizontal plane

tan θ= h / r

θ = tan-1 (h / r)

Where, θ is the angle of repose.

h is the height in cms

r is the radius in cms.

Carr's index (or) % Compressibility

It indicates powder flow properties. It is expressed in percentage and is give

$$I = \frac{Dt - Db}{Dt} \times 100$$

Where, Dt is the tapped density of the powder and

Db is the bulk density of the powder.

Hausner Ratio

Hausner ratio is an indirect index of ease of powder flow. It is calculated by the following formula.

Hausner ratio =Dt/Db

Where, Dt is the tapped density.

Db is the bulk

density.

Direct Compression Technique

- Weigh the ingredients accurately.
- Shift the drug, crospovidone, Mannitol, sucrose, through sieve no 60 and mix them for 5 min in a poly bag.
- Pass talc and magnesium stearate through sieve no 60.

❖ Powder blend is lubricated with talc and magnesium stearate and blend it for 20 min. it is ready for compression.

The blend is compressed using multiple tolling twenty station single rotary with single puch (8mm), on labpress machine to produce round shaped tablets. Weighing 180 mg each. The compression force is adjusted to obtain tablets with hardness 4-5kg/cm^2

Evaluation of Fast Dissolving Tablets

In Process Parameters Evaluation

Appearance

Tablet from each formulation were randomly selected and organoleptic properties such as color, odour, taste, and shape were evaluated. The results were mentioned in the

Weight Variation Test

20 tablets were selected randomly from the lot and weighted individually to check for weight variation. Weight variation specification as per I.P. is shown in

Hardness Test

Hardness or tablet crushing strength (fc), the force required to break a tablet in a diametric compression was measured using Monsanto tablet hardness tester. It is expressed in kg/cm2.

Thickness

The thickness of tablets was determined suing a Digimatic veriner caliper (Mitutoya, Japan). Three tablets from each batch were used, and average values were calculated.

Friability (F)

Friability of the tablet determined using Roche friabilator. This device subjects the tablet to the combined effect of abrasion and shock in a plastic chamber revolving at 25 rpm and dropping a tablet at height of 6 inches in each revolution. Pre-weighted sample of tablets was placed in the friabilator and were subjected to the 100 revolutions. Tablets were dusted using a soft muslin cloth and reweighed. The friability (F) is given by the formula.

$$F=W_{initial}-W_{final}X100/W_{initial}$$

Acceptance criteria for % friability, %weight loss should be less than 1%

Disintegration Time Testing

It was determine using USP tablet disintegration test apparatus, using 900ml of distilled water without disk at room temperature. Test was performed on 6 tablets. Limit set for the disintegration time: not more than 30 seconds.

***In vitro* Dispersion Time**

For determination of *In vitro* dispersion time, one tablet was placed in a beaker containing 10ml of PH 6.8 phosphate buffer at 37±0.5ºc and the time required for complete dispersion was determined. The test was repeated on three other tablets of same batch, the average gives *In vitro* dispersion time.

Wetting Time of Tablet

Wetting time test gives the idea on porosity, compressibility as well as absorption capacity of the tablets. Since the dissolution process of a tablet depends upon the wetting followed by disintegration of the tablet, the measurement of wetting times may be used as another confirmative test for the evaluation of tablets.

Water Absorption Ratio

A piece of tissue paper folded twice was placed in a small Petri dish containing 6 ml of water. A tablet was put on the paper & the time required for complete wetting was measured . The wetted tablet was then weighed. Water absorption ratio, R, was determined using following equation,

R=100(Wb-Wa) /Wa

where, Wa is weight of tablet before water absorption

Wb is weight of tablet after water absorption.

***In Vitro* Drug Release Study (Fexofenadine HCL):**

The release rate of drug from FDT was determined using USP Dissolution testing apparatus II (paddle method). The dissolution medium was pH 6.8 phosphate buffer, the volume being 900ml. the temperature was maintained at 37±0.5ºc. The rotation speed was 50rpm. A sample (5ml) of the solution was withdrawn from the dissolution apparatus at 5,10,15,20 and 25 minutes. And the samples were replaced with fresh dissolution medium. The samples were filtered through a membrane filter and absorbance of these solutions was measured at 225nm using a UV/V is double-beam spectrophotometer of Cumulative percentage drug release was calculated using linear equation obtained from a standard curve.

Stability Studies

Stability of a drug can be define as the time from the date of manufacture and the packaging of the formulation, until its chemical or biological activity is not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously. In any design and evaluation of dosage forms for drugs, the stability of the active component must be a major criterion in determining their acceptance or rejection.

RESULTS AND DISCUSSION

Determination of UV absorption maxima of Fexofenadine HCL

UV scanning was done for 10 mg/ml drug solution from 200-400 nm in Distilled Water as a blank using double beams UV/VIS spectrophotometer. The wavelength maximum was found to be at 225 nm

Calibration Curve

The different concentration (1 to 10µg/ml) of Fexofenadine HCL were prepared with Distiled Water and analyzed through UV at 225 nm using corresponding media as a blank. The absorbance obeys the Beers-Lamberts law at the range 1 to 10 µg/ml.

Drug Exipient Compatibility Study

Compatibility of the drug with recipients was determined by FT-IR spectral analysis, this study was carried out to detect any changes on chemical constitution of the drug after combined it with the recipients. The samples were taken for FT-IR study.

CONCLUSION

As per flow ability scale, the drug has good characteristics to flow . The excipients did not make any effect on the flow of blend. Thus it was decided to use direct compression method.

***In vitro* dissolution profile of Fast Dissolving tablets of Fexofenadine HCL:**

Release of drug *In vitro*, is determined by estimating the dissolution profile. *In vitro* drug release studies were performed as per the procedure described in the experimental section. The results of *In-vitro* drug release studies of

all the developed formulation in respective tables. The percentage cumulative drug release was plotted against time to obtained drug release profiles. It is the responsibility of the manufacturing company that products come to customer must be in the active form. So the stability of pharmaceuticals is an important criteria. Stability of medicinal products may be defined as the capability of particular formulation in a specific container to remain in its physical, chemical, microbial, therapeutic and toxicological specification i.e. stability of drug is its ability to resists Detoriation. 90% is the minimum potency required which is acceptable potency label. Detoriation may take place which results in change of physical, chemical, microbial, therapeutic and toxicological properties. That's why stability testing is necessary for medicinal products. The Tablets prepared were found to be good , without chipping and sticking with optimum hardness and thickness. The tablets prepared by using Cross carmellose sodium and Crosspovidine ratio were found to be disintegrate faster and there is a rapid drug release within short time and found to be stable . The rate of drug release is increased and disintegration time is decreased to a considerable effect by increasing the concentration of superdisintegrants. Thus it can be concluded that as the concentration of the superdisintegrant is increased the rate of drug release and disintegration time is enhanced. This study demonstrated that disintegration of Fexofenadine HCL can be enhanced to a great extent by direct compression technique with the addition of combination of superdisintegrants. Thus it was concluded that Fexofenadine HCL fast dissolving tablets can be formulated by using direct compression method. Which is simple and economic.

ACKNOWLEDGMENT

The authors are thankful to Vinayaka Mission's Research Foundation (Deemed to be University) & Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu for extending their support and facilities for this research.

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Table 1: Preparation of Fexofenadine HCL Fast Dissolving Tablet

S.No	Ingredients in (mg)	F1 (mg/tab)	F2 (mg/tab)	F3 (mg/tab)	F4 (mg/tab)	F5 (mg/tab)	F6 (mg/tab)
1	Fexofenadine HCL	180	180	180	180	180	180
2	Cross Carmellous Sodium	2.5	5	7.5	-	-	-
3	Crospovidone	-	-	-	2.5	5	7.5
4	Microcrystalline Cellulose	45	45	45	45	45	45
5	Mannitol	35	35	35	35	35	35
6	Magnesium Stearate	6	6	6	6	6	6
7	Ethyl Vinyl Acetate	2.5	2.5	2.5	2.5	2.5	2.5
8	Sucrose	9	9	9	9	9	9
Total weight in (mg)		280	280	280	280	280	280

*Mean±SD (n=6)

Table No. 2 : Standard calibration curve of Fexofenadine HCL

S.No.	Concentration (mcg/ml)	Absorbance
1	1	0.0089
2	2	0.031
3	3	0.0544
4	4	0.0841
5	5	0.1051
6	6	0.1233
7	7	0.1496
8	8	0.1655
9	9	0.1833
10	10	0.1996

Table no: 3 Preformulation Study of Pure Drug (Fexofenadine HCL).

S. No.	Parameters	Result
1	Bulk Density (gm/cm^3)	0.255
2	Tapped Density (gm/cm^3)	0.334
3	Angle of Repose (θ)	30^0 40’
4	Carr’s Index (%)	23.6
5	Hausner Ratio	1.11

Table no: 4: Preformulation Study of the blend

Batch Code	Bulk Density (gm/cm^3)*	Tapped Density (gm/cm^3)*	Angle of repose (θ)*	% Compressibility*	Hausner’s Ratio *
F1	0.261	0.335	30^0 04’	25.58	1.32
F2	0.265	0.324	30^0 09’	26.76	1.33
F3	0.274	0.345	30^0 13’	27.74	1.25
F4	0.236	0.335	29^0 28’	26.37	1.29
F5	0.240	0.350	32^0 10’	26.85	1.27
F6	0.239	0.345	31^0 15’	25.25	1.26

Table No. : 5 Evaluation of Post compression parameters of Fast Dissolving tablets of Fexofenadine HCL

Test	Weight Variation (mg)**	Hardness (kg/cm²)*	Thickness (mm)*	Friability (%)*	Disintegration Time (sec)*	*In vitro* dispersion time (Sec)*	Wetting Time (sec)*	Water Absorption Ratio*	Drug-Content (%)*
F1	285±1.39	3.35 ± 0.23	3.46 ± 0.052	0.41	13	97.10	18.33±1.527	65.2 ±1.637	99.23
F2	**282±1.45**	**3.39 ±0.12**	**3.49±0.0133**	**0.42**	**10**	**100.05**	**12.3 ±1.527**	**59.2 ±1.527**	**99.89**
F3	284±1.31	3.42±0.113	3.39±0.0132	0.56	11	97.10	14.12 ±0.89	63.6 ±0.577	99.76
F4	279±1.35	3.69±0.226	3.32±0.0132	0.49	15	92.10	48.1±1.527	73.66±1.527	97.30
F5	281±1.48	3.78±0.188	3.42±0.0132	0.44	14	95.06	43.6 ±1.577	86.01 ± 1.01	98.76
F6	283±1.38	3.95±0.100	3.51 ±0.0132	0.59	16	95.89	26.3 ±0.577	60.6 ±1.527	97.87

Table. No 6: *In-vitro* percentage drug release profile of the Fexofenadine HCL Tablets F1 to F6

Time in (min)	F1	F2	F3	F4	F5	F6
0	0	**0**	0	0	0	0
1	32.67	**50.31**	45.85	29.67	36.65	47.89
3	49.36	**55.01**	58.89	39.36	49.90	49.09
5	55.79	**77.92**	67.89	47.79	55.50	55.08
7	70.08	**86.43**	70.08	69.08	77.88	80.90
10	73.28	**100.05**	80.89	72.28	82.90	87.08
15	89.10	**100.04**	89.10	83.70	92.03	94.90
20	97.10	**100.04**	97.10	92.10	95.06	95.89

Table No. 7 : Stability studies of optimized formulation F2 at room temperature 25⁰C.

Parameters	Controlled	After 15 days	After 1 month
Drug content (%)*	98.89	98.09	97.79
Disint. Time (sec.)*	9	10	10
Wetting Time (sec.)*	14.3±1.527	14.2±1.517	14.01±1.501
Hardness (kg/cm²)*	3.39±0.12	3.28±0.11	3.21±0.01
Friability%*	0.40	0.39	0.36
	Cumulative %Drug Release		
Time	**Controlled**	**After 15 days**	**After 1 month**
0	0	0	0
1	49.31	49.01	48.81
3	54.01	53.81	53.21
5	76.92	76.02	75.82
7	85.43	84.03	83.83
10	99.05	99.00	98.70
15	99.04	99.00	98.75
20	99.04	99.00	98.75

*Mean±SD (n=6)

Table No.8 : Stability studies of formulation F2 at accelerated temperature 40°C.

Parameters	Controlled	After 15 days	After 1 month
Drug content (%)*	98.89	98.01	98.01
Disint. Time (sec.)*	9	10	11
Wetting Time (sec.)*	14.3±1.527	14.1±1.501	14.0±1.401
Hardness (kg/cm²)*	3.39±0.12	3.29±0.02	3.19±0.02
Friability%*	0.40	0.39	0.39
Time	Cumulative %Drug Release		
	Controlled	After 15 days	After 1 month
0	0	0	0
1	49.31	48.08	48.01
3	54.01	53.48	53.37
5	76.92	75.93	75.73
7	85.43	84.03	84.01
10	99.05	98.02	98.01
15	99.04	98.01	98.01
20	99.04	98.01	98.01

*Mean±SD (n=6)

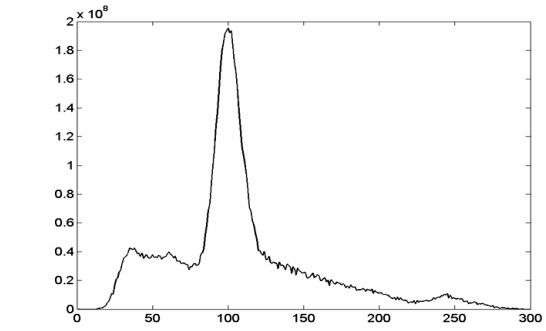

Fig No 1: Wavelength maxima for Fexofenadine HCL

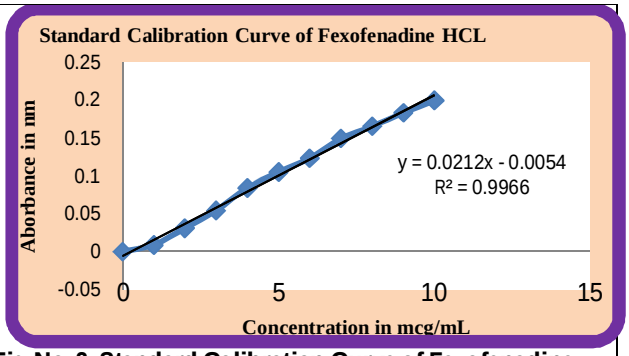

Fig No. 2: Standard Calibration Curve of Fexofenadine HCL

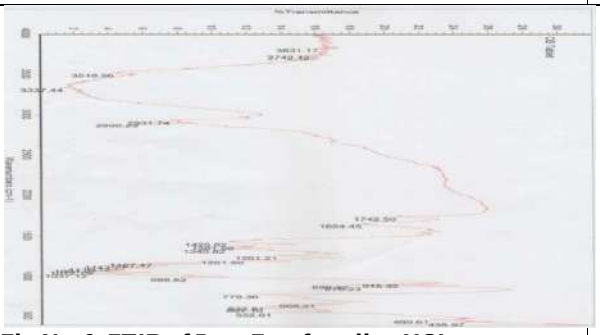

Fig No. 3: FTIR of Pure Fexofenadine HCL

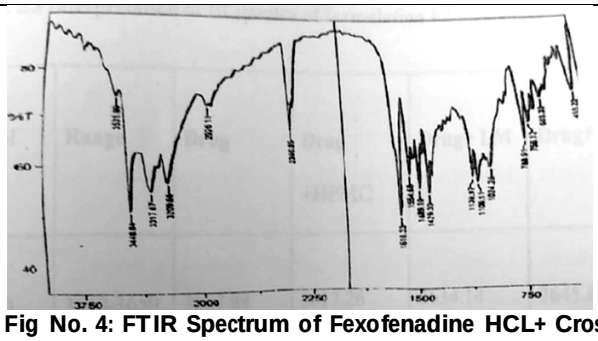

Fig No. 4: FTIR Spectrum of Fexofenadine HCL+ Cross Povidone

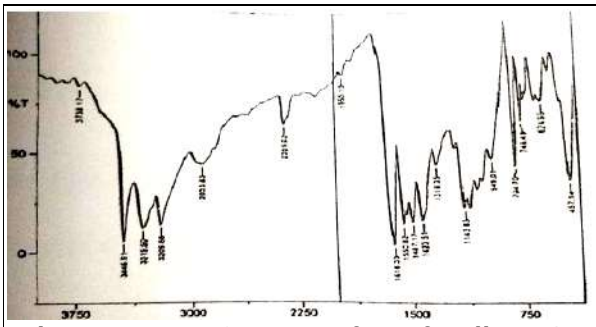

Fig No. 5: FTIR Spectrum of Fexofenadine HCL+ Croscarmellose Sodium

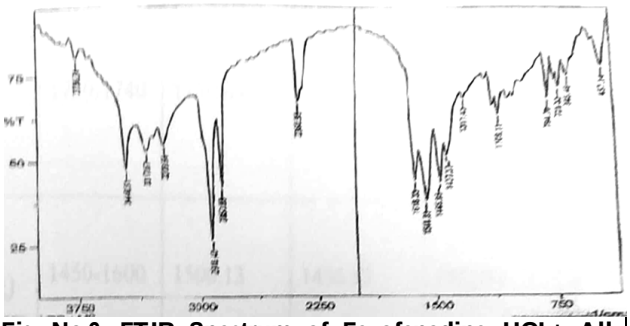

Fig No.6: FTIR Spectrum of Fexofenadine HCL+ All excipients (Optimized tablet)

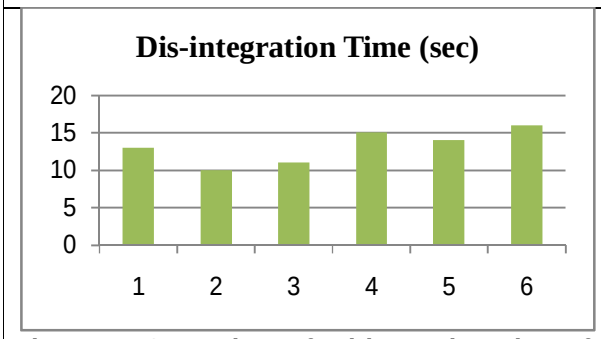

Fig No 7: Comparison of Disintegration Time of various formulations

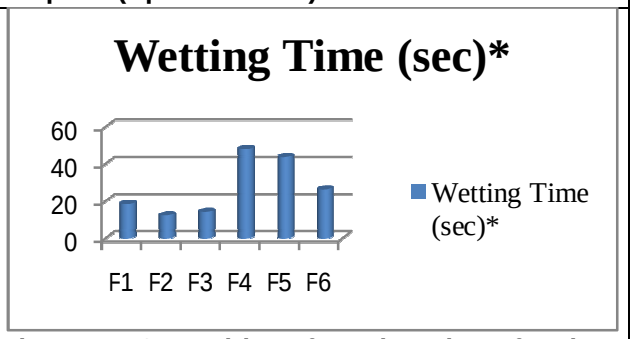

Fig No. 8: Comparision of Wetting Time of various formulations.

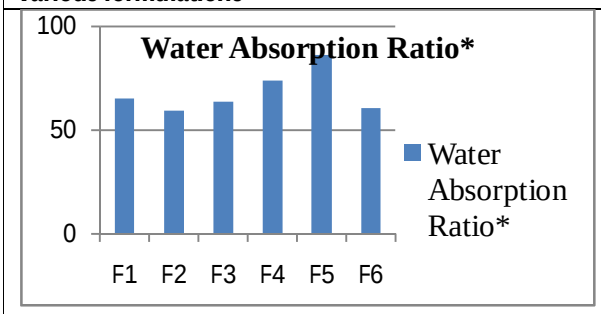

Fig No. 9: Comparision of Water absorption Ratio of various formulations.

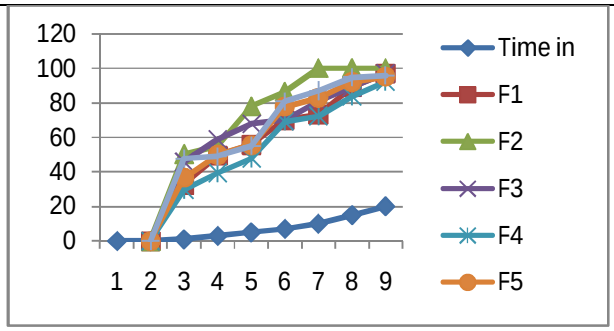

Fig No.10: *In-vitro* percentage drug release profile of the Fexofenadine HCL Tablets F1 to F6

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Study of Ground Water Analysis at Selected Areas on Tiruchirappalli Town

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Received: 04 Feb 2021 Revised: 24 Feb 2021 Accepted: 16 Mar 2021

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ABSTRACT

There exists a need to improve the water facilities due to rise of a rise in pollution on a worldwide scale caused by increasing population, urbanization and industrialization. The ground water quality in Tiruchirappalli town were studied in samples collected in three different Residential areas of Trichy namely Residential area (Morais city), Residential area (Allur), Residential area (Veereshwaram) by determining the physiochemical parameters, Temperature, Chloride, Nitrate, phosphate, Sulphate, Sodium, Potassium, Calcium and Magnesium .Studies revealed all the parameters were in the normal limits and the water is safe for drinking purpose.

Keywords: Calcium; Ground water; Physico-chemical; Potable water; Tiruchirappalli; Water quality

INTRODUCTION

Ground water could be a good source of freshwater resource. It is a biggest issue before the policy makers for its sustainable utilization [1].Groundwater is a very important source of water for human kind. It contains over 90% of the H2O resources and is vital reserve of fine quality water [2]. It is used for agricultural, industrial, household, recreational and environmental activities everywhere on the planet [3]. Within the previous few decades the ground water potential and its quality is reduced because of the population, explosion, urbanization, industrialization and Also the failure of monsoon and improper management of rain water [4].The chemical composition of groundwater could be a measure of its suitability as a source of water for human and animal consumption, irrigation

and for industrial and other purposes. The bottom water quality is often characterized by physical characteristics, chemical composition and biological parameters [5]. Underground water is the Principle source of water in rural areas of India and its indispensable source of life. The issues of underground water quality are more acute within the areas which are densely populated and thickly industrialized. Once the bottom water is polluted its purification is simply too difficult. So as to keep up equilibrium in bio chemical process going down in living organism .Certain chemical methods are needed for maintaining physiology of masses or living organisms. The presence of too much of chemical elements is additionally harmful [6]. A clean facility is one amongst the key indicators for development in any country: However, the situation of most Trichy cities isn't encouraging. Trichy city need to improve the standard of public facility has been identified as a result of a rise in pollution on a worldwide scale caused by increasing population, urbanization and industrialization [7]. Therefore an attempt has been made to assess the standard of water in selected areas of Trichy District.

Significance of the Study

Water is an absolute necessity always. Water bodies may host harmful biological and chemical agents that impact the health of the humans. There exist a powerful correlation between water borne biological agents and human disease. In the present study various Physico-chemical analysis studies are done in underground water collected from different area. Water quality is critical to grasp the ecological quality of Underground water and successive revitalization of ecosystem. Protecting of water resources is of paramount importance thus assessing the water quality at regular intervals is critical for the choice makers to require remedial actions and solve the matter.

MATERIALS AND METHODS

Study Area

Tiruchirappalli city is found within the part of Tamilnadu state, between 1038′and 1138′N latitude and 7828′ and 7938′E longitude with a region of 51.95 km with population of 866354 (as per 2001 survey).The city is found on the southern bank of river Cauvery. In the top edge of the city, the river splits into two branches towards north and south direction. kollidam is that the name of the northern branch and south gets the name Cauvery. Tiruchirappalli is one amongst the foremost important industrial cities in Tamilnadu. Tiruchirappalli city is found at the pinnacle of the Cauvery delta and its altitude is low with 78.8mts above the mean water level and also the city is 120kms away from Bay of Bengal. It forms an element of a vast plain of fertile alluvia soil with a delicate but gradual slope form the west.

Water Sampling

Areas choosen for study were,

1 - Residential area, Morais city (Water Sample 1)
2 - Residential area, Allur (Water Sample 2)
3 – Residential area, Veereshwaram (Water Sample 3)

Sample Collection

Water samples were collected in pre-cleaned and rinsed polythene bottles of the half litre capacity with necessary precautions [8].The ground water samples were collected, during December 19th2019

Temperature

Temperature measurement is made by taking a portion of the water sample (about 1litre) and immersing the thermometer into it for a sufficient period of time (till the reading stabilizes) and the reading is taken, expressed as °C

pH (Electrometric Method)

The electrode of the pH meter is dipped into the sample, and the readings are noted. The electrode is allowed to stand for 2 minutes to stabilize before taking reading for reproducible results (at least ±0.1 pH units).

Turbidity

The sample was placed on the turbidity meter and the value was recorded.

Electrical Conductivity

The electrode of the conductivity meter was dipped into the sample, and the readings were noted for stable value shown as mho or Siemens /cm.

Total Dissolved Solids

The electrode of the TDS meter is dipped into the sample, and the readings were noted for stable value gives Total Dissolved Solids (TDS).

Dissolved Oxygen (Iodometric Method)

Winkler bottles of 10ml capacity were used for oxygen estimation. The bottle was filled with the water sample by dipping it well below the water level. To this 0.1ml of manganous sulphate and 0.ml of alkaline potassium iodide were added. The bottle was carefully closed without trapping air bubbles. After settlement of precipitte 0.2ml of sulphuric acid was added through the sided of the bottle .The resulting solution was titrated against the sodium thiosulphate solution. The end point is the disappearance of the blue colour [9].

Calulation;

$$\text{Dissolved Oxygen(as mg/l)} = \frac{(0.2)\ (1000 \text{ ml of Sodium thiosulphate})}{200}$$

Nitrate (Phenoldisulphonic Method)

1ml of each of the samples, standard and blank was pipetted out into test tubes. To each of the test tube,.0.5ml of brucine sulphanilic acid solution was added . To a second series of test tubes, 5ml sulphuric acid solution was added. The contents of the first series of test tubes were added to the second series. This content was mixed well. The tubes were kept in dark for 10minutes. 10ml of distilled water was added to each tube. They were cooled in dark for 20 minutes and the OD was measured at a wavelength of 510nm. [10].

Calculation

$$\text{Nitrate N} = \frac{\text{OD sample} \times \text{con.std} \times 1000}{\text{OD std} \times \text{volume of sample}} \text{ (mg/l)}$$

Calcium (EDTA TITRIMETRIC METHOD)

The tube is embedded in an exceedingly clean cone like flask of known size (50 mL), containing one milliliter hydroxide and 1milliliter iso-propyl alcohol. A pinch murexide indicator was added to the mixture and titrated against EDTA till the pink color turns purple colour [11].

Calculation

$$\text{Calcium as Ca (mg/l)} = \frac{T \times 400.5 \times 1.05}{\text{Sample taken, ml}}$$

Total Hardness (EDTA TITRIMETRIC METHOD)

Exactly 50ml of the well-mixed sample was pipetted into a conical flask, to which 1ml of ammonium buffer and 2-3 drops of Eriochrome black -T indicator was added. The mixture was titrated against standard 0.01M EDTA until the wine red colour of the solution turns pale blue at the end point [11].

Calculation

$$\text{Total hardness (mg/l)} = \frac{(T)\ (1000)}{V}$$

Magnesium (EDTA TITRIMETRIC METHOD)

The tube was embedded in an exceedingly clean cone like flask of known size (50 ml), containing one milliliter hydroxide and 1 ml iso-propyl alcohol. A pinch murexide indicator was added to the mixture and titrated against EDTA till the pink color turns purple colour [11].

Calculation:

$$\text{Magnesium} = (T - C) \times 0.243 \text{ (as mg/l)}$$

Sulphates (TURBIDOMETRIC METHOD)

A 100 ml sample was filtered in an exceedingly Nessler tube with 5 ml of learning chemical agent. About 0.2 g of metallic element chloride crystals were added with constant stirring. to administer a 100 NTU, a operating customary is ready by taking 1 ml of quality, 5ml of learning chemical agent adding up to a100ml. The turbulence generated by the model and standards were measured employing a nephrolo meter and therefore the results were tabulated [11].

Calculation: Sulphate = (Nephlometric reading) (0.4) (Dilution factor)(mg / l)

Chlorides (ARGENTOMETRIC METHOD)

A known volume of filtered sample (50ml) was taken in a conical flask, to which about 0.5ml of potassium chromate indicator is added and titrated against standard silver nitrate till silver dichromate ($AgCrO_4$) starts precipitating [9] .

Calculation:

$$\text{Chlorides } (Cl^-) = \frac{(A-B)\ (N)\ (35.45)}{\text{Sample taken in ml}}$$

Potassium (FLAME PHOTOMETRIC METHOD)

The filter of the flame photometer was set at 766.5nm (marked for Potassium, K) the flame is adjusted for blue colour. The scale is set to zero and maximum using the highest standard value. A standard curve of different concentration was prepared by feeding the standard solutions. The sample was filtered through the filter paper and fed into the flame photometer. The concentration is found from the standard curve or as direct reading [12].

Sodium (FLAME PHOTOMETRIC METHOD)

The filter of the flame photometer was set to 589nm (marked for Sodium, Na). By feeding distilled water the scale was set to zero and maximum using the standard of highest value. A standard curve between concentration and emission was prepared by feeding the standard solutions. The sample was filtered through filter paper and fed into the flame photometer and the concentration was found by direct readings [12].

RESULTS AND DISCUSSSION

The physico-chemical characteristics were determined. Highest Temperature values were recorded in Veereshwaram (Residential area) (29.9°c) (Table 1) .Water samples in Residential area (Morais city) , Residential area (Allur), had a values of 27.**7°**c ,28°c Respectively (Table 1 , Figure 3). The pH value is an important consideration in maintaining carbonate and bicarbonate levels in water. Sample studied were found to have pH values in within the permitted range of WHO (6.5-8.5). pH was found to be 7.6,7.4,7.3. in Sample1 Residential area (Morai city) , Sample 2 Residential area (Allur) , and Sample 3 Residential area (Veereshwaram) respectively. In this present study Turbidity value where found to be zero for all the samples studied. In Sample1 Residential area (Morais city), Sample 2 Residential area (Allur) , and Sample 3 Residential area (Veereshwaram) respectively . (Table 1) It is ideal if turbididity value is below 1 . According to WHO turbidity of drinking water should not be more than 5 NTU (EPA 2009). Electrical conductivity values in the Residential area (Morais city) ,Residential area (Allur) , Residential area (Veereshwaram)were 1362 micro mho cm, 664 micro mho cm , 613 micro ohm cm respectively. Normal range is 600 micro mho cm . (Table 1 , Figure 5) . Electrical conductivity is high in Morais city residential area. This indicates the presence of large quantities of dissolved mineral substances, ionic elements and dissolved minerals within the water samples.High electrical Conductivity affected the germination of crops, and this could lead to reduced yields. Highest TDS value of 640mg/l was found in Morais city residential area. (Table 1) (Figure 6). The Residential area (Allur), Residential area (Veereshwaram)water samples had values of TDS values326 mg/l and 289 mg/l respectively . (Table 1 , Figure 6) . In all the water samples TDS was found in the permissible limits only. Dissolved oxygen one of every important parameters for water quality. The Residential area (Morais city) water sample had value of DO value 2.21 mg/l(slightly above the normal table 2). The Residential area (Allur), Residential area (Veereshwaram)water samples are contain Normal DO values respectively 1.23 mg/l , 1.4mg/l (Table 2 , Figure 7).

Nitrate in The Residential area (Morais city) ,Residential area (Allur) , Residential area (Veereshwaram) water samples were No.2 values are respectively43.7 mg/l,12.5mg/l, 18.7 mg/l (Table 2 , Figure 8) . The Nitrate permissible limit 45 mg/l .

Among the sample studied highest magnesium values found in sample 1 (Morais city) 7.46 mg/l(Table 2 , Figure 9) . Sample 2 (Allur) , Sample 3(Veereshwaram) water samples were Mg values respectively 3.42mg/l, 1.96mg/l (Table 2 , Figure 9) . The Magnesium Permissible limit 150 mg/l. Groundwater samples Indicates that the water is suitable for domestic use. The value of calcium for all groundwater samples ranges from 15-32 mg/l (Table 2). The Residential area (Morais city) water samples contain a Normal Ca value 32.80 mg/l (Table 2). The residential area (Allur) water samples contain a Normal Ca value 20.18 mg/l (Table 2). The Residential area (Veereshwaram) water samples contain a normal ca value 15.97 mg/l(Table2 , Figure 10) . In the present study, calcium values were found to be within the most permissible range (200 mg/l). Highest Sulphate value of 14.6 mg/l was found in Allur Residential area (Table 2 ,Figure 11) . The Residential area (Morais city), Residential area (Veereshwaram) values are respectively 8.2 mg/l , 7.8 mg/l (Table 2 , Figure 11) . All Ground water samples were found in the WHO permitted range (200 – 400 mg/l). The value of chloride for all Ground water samples ranges from 0.00099-0.01 mg/l .The Residential area (Morais city), The Residential area (Allur) , The Residential area (Veereshwaram) water samples contain Cl values are 0.01 mg/l , 0.00099 mg/l , 0.009 mg/l respectively (Table 2 , Figure 12) Normal range is 250 - 1000 mg/l (WHO) .

Chloride over 250 mg/l gives the water a salty taste. Increase in chloride Water levels can be harmful for people with heart and kidney disease. High concentrations of chloride are taken into account as an indicator of pollution by animal organic matter and industrial appearance. Highest Potassium values of 25 mg/l was found in Residential area (Allur) (Table 2) .The Next higher Value 15 mg/l were found in the Residential area (Veereshwaram) .The Residential area (Morais city) water samples contain a potassium values are 13.75 mg/l (Table 2 , Figure 13) . High concentration of potassium is also responsible for the contamination of wastewater. The WHO acceptable limit 20 mg/l .The low Sodium values of 4.62 mg/l was found in Residential area (Veereshwaram) (Table 2, Figure 14) The lower values were found in the Residential area (Allur) (Table 2 , Figure 14) . The highest value 22.12 mg/l was found in Residential area (Morais city) (Table 2 , Figure 14). The acceptable limit 200-400 mg/l .In the present study water samples studied were found in good quality and can be used for drinking and domestic purpose. In future studies

more number of samples at different seasons should be taken and studies has to be carried out The rain water harvesting structures should be installed to restore the ground water aquifers for improvement of ground water resources in order to maintain the quality and quantity of ground water . Public awareness program should be begun to enhance the knowledge and awareness to save water pollution on human being around their dweller.

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Table 1.Results of Physical Parameters of the Water Samples

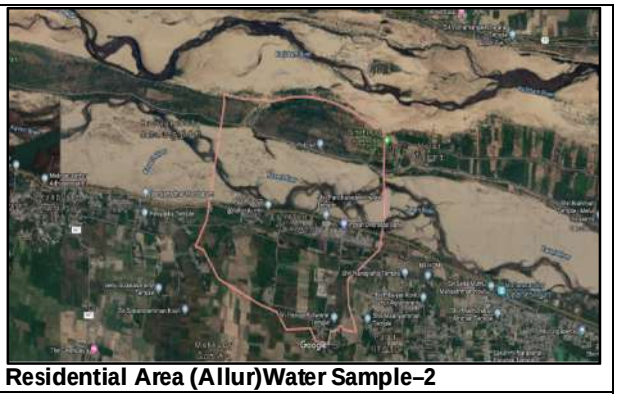
S. No	SAMPLES	pH	TDS (mg/l)	EC (µs/cm)	TEMPERATURE (°C)	TURBIDITY (OD)
1.	WS I	7.6	0640	1362	27.7	0.0
2.	WS II	7.3	0326	0664	28	0.0
3.	WS III	7.4	0289	0613	27.9	0.0
4.	PERMISSIBLE LIMIT	6.5-8.5	500 – 1000	300	12-25	5 -10

Table 2.Results of Chemical Parameters of the Water Samples

Chemicals	Sample 1 mgl 1	Sample 2 mgl 1	Sample 3 mgl 1	Permissible Limit mgl 1
Dissolved oxygen	2.21	1.23	1.47	6.5 – 8.5
Nitrate	43.7	18.7	12.5	45
Magnesium	7.46	3.42	1.96	30
Calcium	32.80	20.18	15.97	75
Sulphate	8.2	14.6	7.8	200 - 400
Chloride	0.01	0.00099	0.009	250 - 1000
Potassium	13.75	25.00	15.00	20
Sodium	22.12	7.12	4.62	200

1 - Residential area, Morais city (Water Sample 1) 2 - Residential area, Allur (Water Sample 2) 3 - Residential area, Veereshwaram (Water Sample 3)

Residential Area (Morais City) Water Sample - 1



Residential Area (Allur)Water Sample–2

Residential Area(Veereshwaram)Water Sample - 3

Figure 1 . (pH)

Figure 2 .(Total Dissolved Solids And Electrical Condictivity)

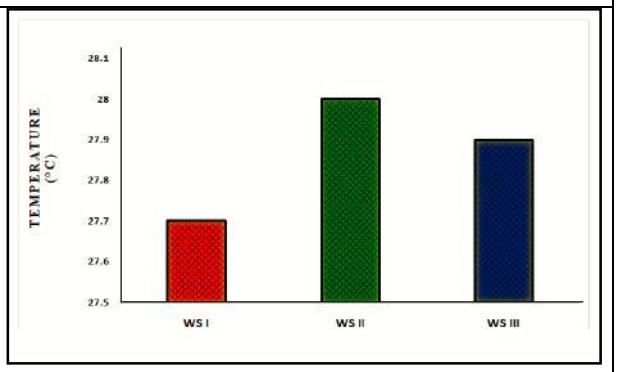


Figure 3. Levels of Temperature

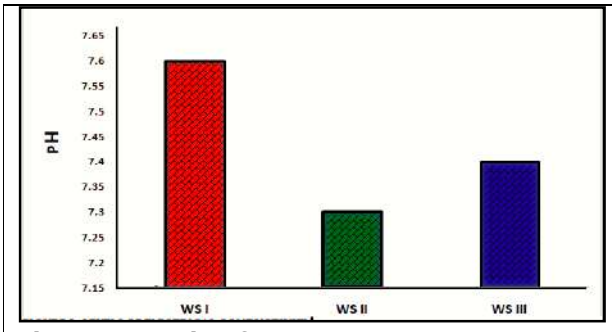


Figure 4 . Levels Of pH

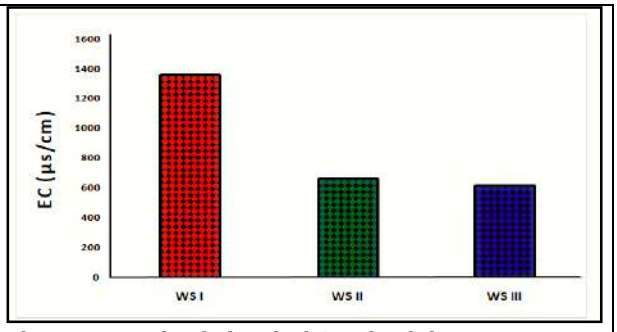


Figure 5 . Levels of Electrical Conductivity

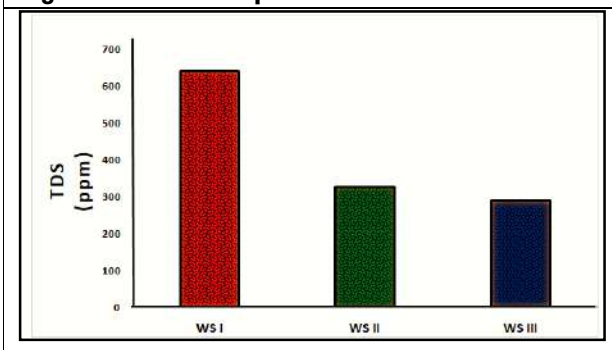


Figure 6 . Levels of Total Dissolved Solids

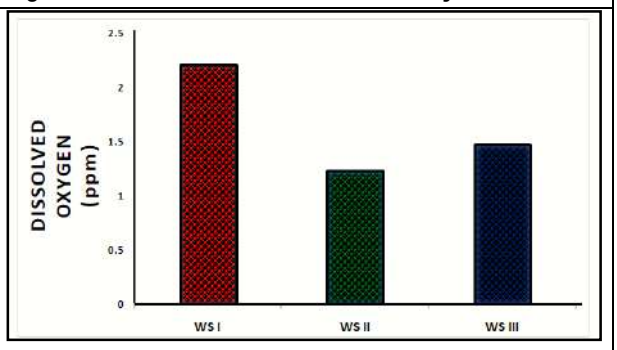


Figure 7 . Levels of Dissolved Oxygen

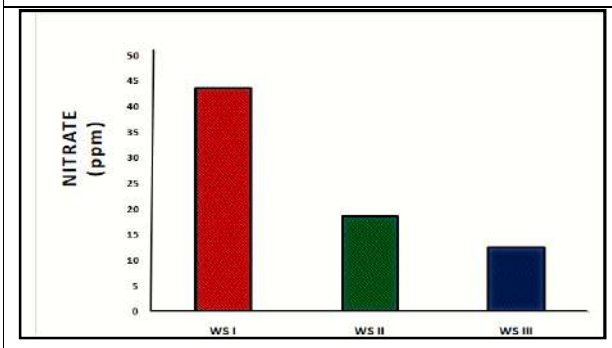


Figure 8 . Levels of Nitrate

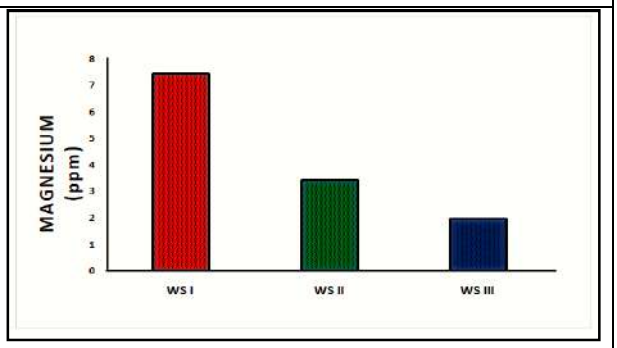


Figure 9 . Levels of Magnesium

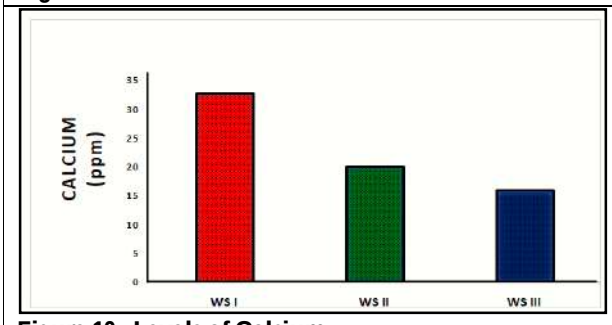


Figure 10 . Levels of Calcium

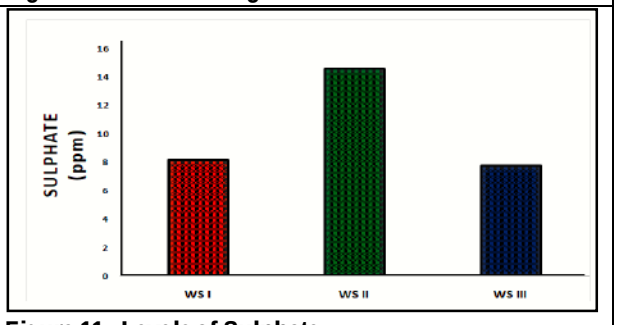


Figure 11 . Levels of Sulphate

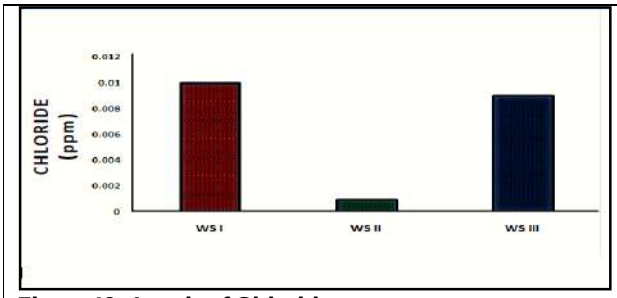


Figure 12 . Levels of Chloride

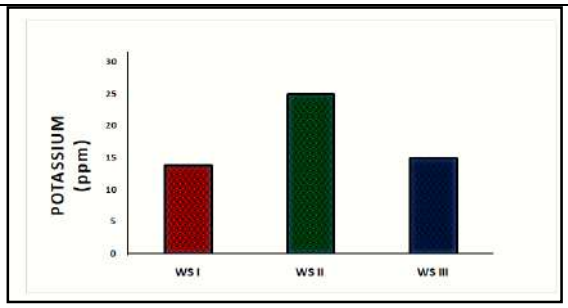


Figure 13 . Levels of Potassium

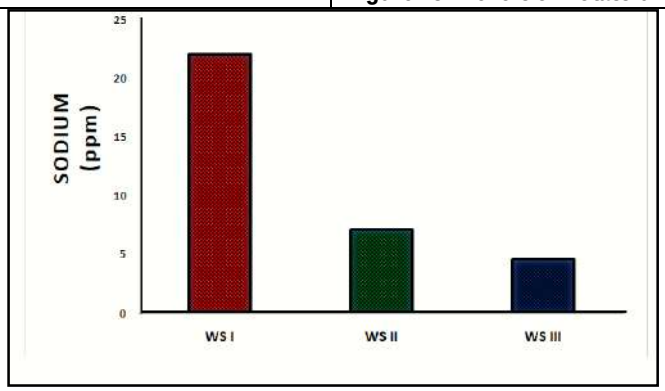


Figure 14 . Levels of Sodium

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Prevalence of Vitamin D Deficiency among Athletes in Kozhikode and Malappuram Districts of Kerala State - A Cross Sectional Study

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Received: 09 Mar 2021 Revised: 12 Mar 2021 Accepted: 15 Mar 2021

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ABSTRACT

The objective of the study is to find the prevalence of vitamin D deficiency in athletes living in Kozhikode and Malappuram districts of Kerala State in India. 220 subjects belonging to age group 15-30 years old were screened for inclusion and exclusion criteria and 97 subjects were taken into the Cross- sectional study. Anthropometric assessment and Blood analysis for Vitamin D and Calcium was done on them and among them 73 subjects had insufficient/deficient Vitamin D level were the actual samples for cross sectional study. The data collection spanned for 6 months (March – August 2018). Among 97 subjects screened, a genera prevalence of 77%of Vitamin D insufficiency (<30 ng/ml) was observed in male and 71% among female athletes in selected districts of Kerala, India. Among this 73 subjects 27% had Vitamin D deficiency (< 20 ng/ml). Significant correlation between Vitamin D deficiency and serum calcium levels of the athletes was established with P value <.001. No statistically significant correlation between 25(OH)D levels and sun exposure, physical activity, practice area or anthropometric levels could be established. The study concluded that healthy male and female athletes in Kerala have a high prevalence of vitamin D deficiency. The results of this study suggest that there is a need for regular supplementation and vitamin D awareness campaigns for athletes in Kerala, India.

Keywords: Vitamin D, 25-hydroxyvitamin D (25(OH) D), Calcium, athletes, vitamin deficiency, vitamin Insufficiency, BMI, Hip waist ratio

INTRODUCTION

Vitamin D has a key function in the maintenance of the human musculoskeletal and other systemic functions. Sun exposure has been regarded the main source of vitamin D to the human body, as the food sources are very limited. Naeem Z stated that Vitamin D deficiency is now a major worldwide concern and epidemic and it has been estimated that about one billion people worldwide are running short of serum 25(OH) D concentrations. In the past there are many researches which were done analyzing the vitamin D deficiency in a geographical area [1]. Ogan Det.al concluded that Vitamin D deficiency is very prevalent among both non-athletes and athletes, and this nutrient is suggested to play a very vital role in their health and performance [2]. Halliday T.M et.al reported that vitamin D was inadequate among 56% of the athletes worldwide. Results from studies carried out in Middle Eastern part of world also share similar weather with athletes in Kuwait showing high prevalence of vitamin D levels compared to all other Middle East countries [3]. About 84% of the athletes from Qatar and national players of Israel had inadequate concentrations of vitamin D. Results from Tunisia reported a very high rate of prevalence (over 90%) of vitamin D deficiency (serum 25(OH)D concentration <75 nmol/L) among their countries young athletes particularly during winter months, with concentrations significantly less in athletes involved in indoor games than the outdoor gamers. Badawi, A et.al suggested that it is expected that athletes who does exercises outdoor will have better serum 25(OH)D levels compared tonon-athletes [4].

There is a very limited data available assessing the effect of vitamin D levels on an athlete's physical performance. Zittermann A study proved that exercise is associated with increased vitamin D concentrations and that young athletes have higher calcium absorption rates compared to others and 1,25 (OH)2D plasma levels compared to controls [5]. Daly RMet.al stated that Vitamin D deficiency is common in Australia affecting nearly one-third of adults aged ≥25 years. This indicates that strategies are needed at the population level to improve vitamin D status of Australians. [6]. A recent meta analysis by Forough Fet.al brings out the fact that Despite the limitations of the current evidence, the prevalence of vitamin D inadequacy in athletes is prominent. The risk significantly increases in higher latitudes, in winter and early spring seasons, and for indoor sport activities. Regular investigation of vitamin D status using reliable assays and supplementation is essential to ensure healthy athletes. The prevalence of injuries in athletes is notable but its association with vitamin D status is unclear. A well-designed longitudinal study is needed to answer this possible association [7]. This study tried to bring out the real scenario among athletes in Kerala's selected district. Kerala is located in the south most part of India. We have selected Kerala not because the primary researcher is associated with Kerala but the region itself is unique in many was compared to other parts of India. Kerala known a "Gods own country" is a naturally blessed place with great natural resources. The practices here differ vastly from the adjacent states which have more similar practices. Kerala also differs in the cultural background particularly their dressing. The dietary habits also differ predominantly from other parts of the India with coconut being the major ingredient and predominant ingredient. The main objectives of the study was to find the prevalence of vitamin D deficiency among athletes from selected districts of Kerala with age 15 to 30 years and to find the association of selected factors (Sex, age, practice area, years of experience, practice costume, waste hip ratio, basal metabolic index, type of exercise training, time of practice, diet and serum calcium levels) with Vitamin D levels.

METHODOLOGY

In this phase a total number of 220 subjects between age of 15 to 30 years of both sex were screened for inclusion and exclusion criteria from 8 registered sports academy. Out of this 97 athletes were selected and were screened for vitamin D levels for the prevalence. All the subjects were provided an information sheet containing the study

intension and objectives. The athletes were also elaborate about the importance of vitamin D and its significance in enhancing the athletic performance. If the subjects are willing to participate, they will the informed consent form. The subjects were included in the study if the full fill the following criteria. The subjects were selected if they fulfilled the following selection criteria. Both indoor and outdoor player and athletes who had atleast 3 years of academic registration were selected. Both male and female of age 15 to 30 years belonging to the state of Kerala (natives) who were not tested already for Vitamin D deficiency were selected. Subjects with any other health ailment like endocrine disorder, cardiac disease, neurological diseases, liver diseases and chronic skin diseases. (as reported by the athletes), who had quit practice for the past 3 months or who have irregular career, who have migrated to Kerala less than 10 years ago were excluded from the study. Already diagnosed subjects who are on regular vitamin D supplementation, Female athletes who had pregnancy or miscarriage within last 2 years or who are planning for child birth or already pregnant, Athletes who are on performance enhancing medication that may influence the levels of 1, 25(OH) 2D in plasma as perceived by the researcher, Subjects who were on parathyroid or thyroid medication, Subjects who are on injury list or on immobilization in the past 2 years which might have affected their activity levels for at least 3 months were also excluded.

Both healthy male and female athletes playing different indoor and outdoor sports, who were registered as professional or amateur players in official sports clubs in Kerala were identified by the primary researcher and two other researchers utilized only for this phase of the study. (one physiotherapist with 5 years of clinical experience and one social worker with 10 years of experience in region of Kerala). Athletes were considered healthy if only they were free of systemic disease like diabetes, cardiovascular diseases, gastrointestinal or nutritional disorder other than vitamin D deficiency and kidney diseases or already known endocrine disorders. Athletes who received prescribed high vit-D supplementation within 3 months prior to testing were also excluded. 8 out of 24 sport clubs were randomly selected with 4 each from two districts of Kerala. A lottery method was adopted to select the academy which had existed for at least past 3 years. The districts were selected conveniently. The selected districts were Malappuram and Kozhikode Districts. A total of 220 athletes were invited to participate in the study. Informed consent was obtained after a complete explanation about the following in the form of a PPT. the athletes who fulfilled the inclusion criteria and exclusion criteria were grouped into 5 members each batch and were elaborated about the following using statistics, pictures and videos.

1. The role of micro and macro nutrients
2. Vitamin D sources, function and deficiency effects,
3. Vitamin D deficiency among the athletes and the epidemiology of the same,
4. Role of vitamin D in athletic performance,
5. What is the significance of screening for vitamin D globally and locally and
6. The study aim and objectives.

To ensure confidentiality, every subject was assigned a unique individualized identification code, which was specific to their sports, indoor or outdoor training predominance, male or female which was used in all documents. E.g. M/FT/DIS/OD/11. M- male. FT- foot ball, DIS don'ts first three letters of the district name, OD- out door and 11 denotes the number of the sample. Out of 220 subjects 97 agreed to participate in the study. The acceptance rate was very less even after so much of elaboration because the athletes at professional level had many constraints to get into any studies as they have signed contracts with various sponsors and organizations which had its own norms. The subjects who came from remote areas of the district asked for commutation charges which the primary researcher was not able bare. The selected 97 subjects were predominantly from Malappuram66% and and Kozhikode Districts 34%.

Procedure

The athletes were recruited into the study ounce the sports club provided its approval. All the athletes after fulfilling the criteria for inclusion were subjected to a blood analysis for finding the levels of vitamin D. the bio marker used for this analysis was Serum 25(OH)D level .

A secondary analyzing was done for finding the following criteria that were perceived to be determinants of vitamin D levels.

1. Age categories – 15-19 years, 20-25 years, 26 – 30years
2. Sex – Male and female
3. Practice area – Predominantly out door, Predominantly indoor, not specific
4. Years of sporting involvement
5. Practice costume – fully covered, moderately covered, minimal covered.
6. Type of sporting event – Football, Volleyball, basketball, track events, field events
7. Type of exercises training – Predominantly aerobic, predominantly anaerobic, equal combination.
8. Diet – strict vegetarian and non vegetarian
9. BMI
10. Hip waist ratio
11. Blood Calcium levels

The time of data collection in a day was different for individual subject, which was according to their availability and convenience. Blood sample was collected as "random non-fasting venous blood sample" in a sterile tube from each subject. The serum was isolated from blood cells and centrifuged at a rate of 3000rmp for five minutes. Following this, the quantitative determination of serum levels of 25(OH)D was measured with "Roche/Hitachi Elecsys 2010 immunoassay" analyzer. Plasma quantitative determination of calcium in serum was measured. A private lab was contracted, who volunteered to do all the blood analysis at a subsidized coast as an initiative to help for a novel cause. The institute of Medicine in the year 2011 reported that 50 nmol/L is the serum 25(OH)D level which is sufficient for all the needs of human body for 97.5% of the population, but based on recommendations provided by the Endocrine society, the definition for 'vitamin D inadequacy' (also called insufficiency) was serum 25(OH)D levels < 75 nmol/L) and additionally, inadequacy is further subdivided into 'vitamin D deficiency' as < 50 nmol/L and 'severe vitamin D deficiency' as < 25 nmol/L) as mentioned by Holick MF[8,9].

Statistics

All statistical procedures were performed with the help of SPSS software version 23.0 (SPSS, Inc., Chicago. IL). The P-values < 0.05 were considered statistically significant. When the variables are non-parametric in nature and are not normally distributed, non-parametric analyses were used (Spearman's correlation) when parametric values were used then Pearsons correlation was used. Correlation was used to examine the relationship between vitamin D and Age categories, Sex, Practice area, Years of sporting involvement, Skin color practice costume, hip waist ratio, type of exercises training, time of practice, occupation, diet, BMI, type of sporting event. Multivariate regression analysis with serum 25(OH)D as independent variable (X) all other variables are dependent variable and considered Y. As there is one X and many Y we preferred a multivariate analysis for the current study.

RESULTS

The study was conducted with 97 subjects and two were dropped due to participants being diagnosed as having diabetes mellitus which was unidentified before, and one failed to give complete information. Finally, 73 participants who were insufficient and deficient in Vitamin D levels were included and accounted for the statistical inferences in the study. Figure 1 shows the flow of participants through the cross sectional study. Data collection occurred from March 2018 (average temperature was 32^{o}C, temperature ranging from 27^{o}C to 37^{o}C) to August 2018 (average temperature 41^{o}C, and temperature ranging from 37^{o}C to 44^{o}C). The data collection spanned for 6 months in 2018 with 11% (n = 10) data collected in March, 13.5% (n = 14) data in April, 7% (n = 7) data in May, 24.5% (n = 24) data in June, 15% (n = 14) data in July and 29% (n =28) data in August. Among 97 subjects screened, a genera prevalence of 77%of Vitamin D insufficiency (<30 ng/ml) was observed in male and 71% among female athletes in selected districts of Kerala, India. Among this 73 subjects 27% had Vitamin D deficiency (< 20 ng/ml). Significant correlation between

Vitamin D deficiency and serum calcium levels of the athletes was established with P value <.001, ref. Table 1. No statistically significant correlation between 25(OH) D levels and sun exposure, physical activity, practice area or anthropometric levels could be established as shown in Table 1 & 2.

DISCUSSION

The study was designed to analyse the prevalence of vitamin D deficiency among athletes of two selected districts of Kerala. The researcher approached all the registered sports academies in these two district and based upon the standards and the year of registration of the academies. The researcher selected 14 Academy for the study. There were four academy from each district exclusively selected through random sampling from 14 Academy. The researcher and team approached all the athletes personally explaining to them the importance of the study and taking informed consent for the study. The objective of the study was not only to find the prevalence of vitamin D deficiency among athletes but also to find the correlation of certain factors which were perceived by the researcher as determining factors. The determinant factors were also selected from previous studies were significant relationship was established between vitamin D deficiency and these factors. Study results can be summarised as the vitamin D deficiency being more commonly present among athletes of both sex which was slightly more in female than in male. A multiple regression model described by Kimlin MG et al showed that 40% of the variance in 25(OH)D concentration; modifiable behavioral factors contributed 52% of the explained variance, and environmental and demographic or constitutional variables contributed 38% and 10%, respectively. The amount of skin exposed was the single strongest contributor to the explained variance (27%), followed by location (20%), season (17%), personal ultraviolet radiation exposure (8%), vitamin D supplementation (7%), body mass index (weight (kg)/height (m)(2)) (4%), and physical activity (4%). Modifiable behavioral factors strongly influence serum 25(OH)D concentrations in Australian adults. In addition, latitude was a strong determinant of the relative contribution of different behavioral factors [10].

The study also showed that there was an overall prevalence of vitamin D deficiency among sport professionals of Kerala which did not had a significant correlation with the diet pattern sex age the type of tractors. Only a significant correlation between vitamin D deficiency and serum calcium levels of the athletes. This is very much understood because vitamin D is a important ingredient for absorption of calcium in human. The limitations of the study are that it is performed in a very limited population and in a very limited geographical area of this Kerala state which is a wide geographical territory in South India. The informations drawn from a small population cannot be generalized to sporting population of entire Kerala. We feel there is a need for future studies about the prevalence of vitamin D deficiency in other states of India as well as other districts of Kerala to come aap with an inclusive information regarding the prevalence of vitamin D in India and interested difference among vitamin D deficiency. Like our study many other studies have also come up with the idea that takes do not influence the vitamin D status among athletes. There are studies which have stated that vitamin D deficiency is significantly associated with age group but in the current study the age group included is less compared to the other studies which have claimed that age can influence vitamin D status. Only few Sporting activities for available in the registered Academy and football being the predominant Sporting activity of Kerala there were no variety of Sporting professional available to be screened in the study. In the current study football was the predominant game compared to other games which can also influence the results of the study.

Practice area was in analysis because many studies have come out with an idea that people who practice in the open area are less vulnerable for vitamin D deficiency compared to people who work out inside gymnasiums and indoor training activities. But the current study did not show any significant correlation with practice area and vitamin D deficiency. It was also seen that the predominant subjects were outdoor practitioners as most of them were football players whose workout routine was mainly on-field activities that is in open area. Experience or years of sporting was taken into consideration because many studies stated that more sporting years reduce the risk of vitamin D

deficiency because of the physical activity and the sun exposure which was involved but in the current study that there was no significant correlation between years of sporting activity and vitamin D deficiency. Practice costume was also discussed in the previous studies, which claimed that usage of less clothing resulted in more exposure of the body part to the sunlight which resulted in better absorption of Vitamin D. In the current study hip waiste ration and BMI were considered as components defining the body composition which was found to be positively correlated with many bio markers of the body. Yet in the current study the body composition did not influence the vitamin D levels. When it comes to nutrients the type of exercise training does always matters. In the current study there was an analysis performed to know whether the type of exercise program and training performed influence the metabolic status of the athletic population. The result stated that there was no correlation between the nutritional status and type of exercise program performed by the Sporting population. As we are dealing with the nutrient we also wanted to know about the influence of the diet in the occurrence of Vitamin D deficiency. Any vitamin is a micronutrient it is accumulated in the body by means of the food sources but Vitamin D is an exception from the other vitamins because vitamin D is a sunshine vitamin which is abundantly available only in the sun. This was substantiated by the study results that there was no correlation between the vitamin D and the dietary intake. Both vegetarian diet and non vegetarian diets did not show any correlation with the vitamin D occurrence.

Only determinant that established a significant correlation with the Vitamin D3 deficiency was the serum calcium level which was found to be highly significantly related to the Vitamin D levels. There are many previous studies which have stated that Vitamin D level is critical for the calcium level in human, but contradicting studies are also available which states that both these values are independent of each other and it is always the coincidence that find the deficiency of both in individual. In the current study the later statement has been declined. Though the results of the study cannot be generalized to a larger population of Kerala, yet the results of the study very clearly states that Vitamin D levels in the body is an independent variable and it is not dependent on almost many factors that are perceived as determining the Vitamin D3 level. The study also clearly explains that the common scenario that persist among the general population is also prevailing among the sporting population. There are claims and proofs that vitamin D level can influence the performance of the sporting population but levels of vitamin D in the body cannot be determined by almost many of the factors dealt with in the study. There are a lot of future recommendations from this study. The first and foremost recommendation being exploring the influence of exercise in the absorption of vitamin D which has not been touched so far by any of the researchers to our knowledge. This is carried out as a second phase in the current study. Apart from this there is a great scope of research in vitamin D deficiency in India. There are very few studies till date which could explain why Vitamin D3 deficiency is problem in the community particularly in a tropical country like India. Future study can concentrate on finding out what are all the dependant factors which may influence the vitamin D deficiency of individuals for example usage of cosmetics, sophistication in transportation, lifestyle, work style and so on. If such variables are identified they may be considered as a confounding variables in most of the previous studies don on vitamin D deficiency in India and abroad.

CONCLUSION

The study concluded from the sporting population in two selected districts of Kerala that vitamin D deficiency is highly prevalent among the sample population. Apart from the calcium levels which is directly dependent on the Vitamin D levels, no other factors were found to be associated with Vitamin D3 levels from the current study. Future studies are recommended in the same topic with more number of population including a wider geographic area and a long-term follow-up among variety of sporting population.

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Table 1: Pearson's Correlation of vitamin D levels with Age, Years of Sports, Hip Waist Ratio, BMI and Serum Calcium Levels

Pearsons Correlation of vitamin D levels with Age, Years Of Sports, Hip Waist Ratio, BMI and Serum Calcium Levels						
	25(OH)D	Age	Years of sports	Hip Waist ratio	BMI	Serum Calcium mg/dl
Pearson Correlation	1	0.004	-0.038	-0.117	-0.127	.563**
Sig. (2-tailed)	.	0.969	0.71	0.254	0.215	0**
N	97	97	97	97	97	97

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2: Spearman's Rho Correlation of vitamin D levels with Sex, Practice Area, Practice Costume , Type of Training and Diet

Spearman's Rho Correlation of vitamin D levels with Sex, Practice Area, Practice Costume ,Type of Training and Diet						
	25(OH)D	Sex	Practice area	Practice costume	Type of training	Diet
Correlation Coefficient	1	0.165	-0.015	-0.004	0.09	-0.047
Sig. (2-tailed)	.	0.107	0.885	0.969	0.381	0.648
N	97	97	97	97	97	97

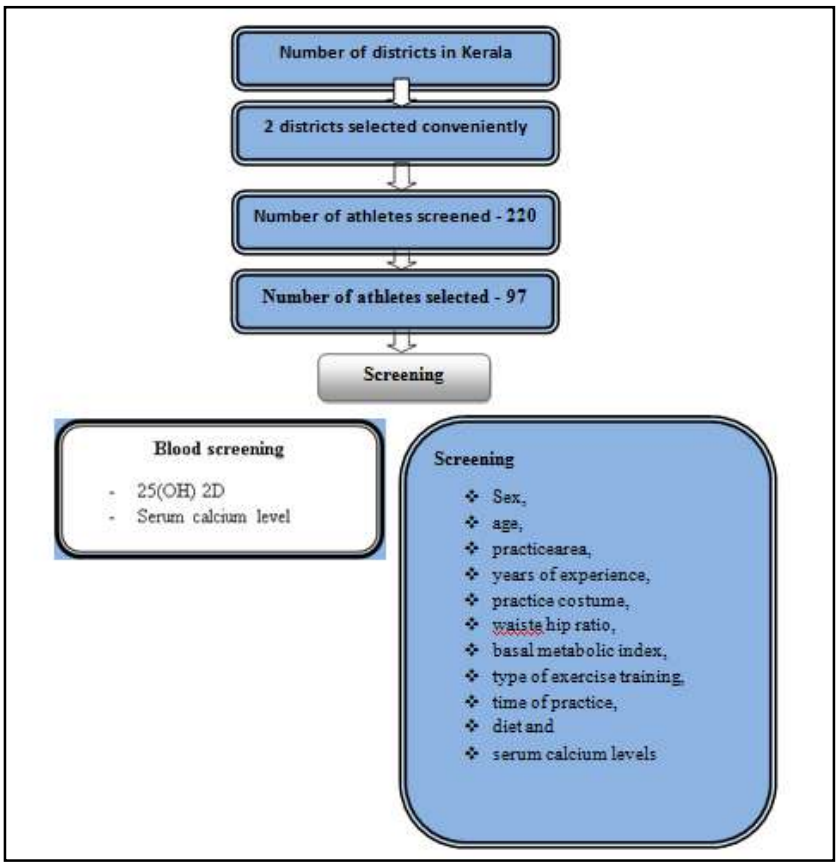

Figure 1: An illustrated flow chart of the study procedure.

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Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A New Approach to Electrolytic Refining of Silver

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Received: 22 Feb 2021 Revised: 28 Feb 2021 Accepted: 01 Mar 2021

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ABSTRACT

This research paper reveals a new approach to refining of Silver electrolytically. The basic principle involved to refining of Silver is "Electrolysis" method. In this process, the standard Silver alloy of fineness (purity in parts per thousand) 890-920 parts per thousand is anodically dissolved and spongy refined Silver / fine Silver of fineness 999.0 – 999.9 parts per thousand is cathodically deposited using Silver nitrate-nitric acid electrolytic medium. The unique part of our research is the use of Ferritic stainless steel (F.S.S.) plate as cathode instead of fine Silver cathode which is traditionally used. Use of F.S.S. plate as inert cathode is to minimise the process loss of Silver during electrolysis from cathodic half cell which ultimately leading to corrosion. The supplied current is AC to DC 10-12volt with 70-100amperes per voltameter consisting of two cells. When the supplied current exceeds 10-12 amperes of potential difference, the colloidal form of silver predominates and the temperature of the electrolytic cell increases the production of spongy refined Silver. The crude anodic Silver contains a significant percentage of Copper and other metals as impurity. This major impurity Copper present in standard Silver is electrolytically separated from the crude standard silver alloy by ion exchange mechanism between electrolyte used and cathode-anode reaction. Due to significant difference in standard reduction potential (electro motive force) between Silver (Ag^{+}/Ag) & Ferric ion (Fe^{3+}/Fe) the electrolytic cell reaction is smoothly occurs. After separation of copper from crude standard Silver, spongy refined silver is periodically collected from F.S.S. cathode plate and it is chemically processed using nitric acid to enhance the fineness where Silver ion is converted to Silver metal and dissolved copper particle is converted to copper ion (Cu^{2+}) which goes into the solution. After chemical processing it is filtered and melted. The melted Silver sample is assayed and fineness report is generated by using BIS method (IS 2113 : 2002) and using X-Ray fluorescence (XRF) instrument. Now this fine Silver of high fineness may be used for manufacturing of Jewellery, Medals, Medallions or commemorative coins, mementoes etc. according to the requirements.

Keywords: Fineness, Ferritic stainless steel, Standard reduction potential, Assay, BIS method (IS 2113: 2002) and X-Ray fluorescence

INTRODUCTION

Out of the seven metals of antiquity of prehistoric humans, Silver is one of the precious metal recorded. Silver is belongs to group-11 or second transition metal series of the modern periodic table. Its Latin name is "**Argentum**" with the symbol "**Ag**". Its atomic number is '**47**' with atomic weight of 107.8682 atomic mass unit. Its electronic configuration is $[Kr]4d^{10}5s^{1}$ It is a white, lustrous, ductile, malleable and soft metal. With Copper and gold, Silver belongs to the noble metal group. At normal and high temperature it does not react with air and thus it is considered to be a noble metal by alchemists. Silver crystallizes in a face-centred cubic (FCC) lattice with bulk coordination number of 12. It has the highest thermal conductivity, electrical conductivity and reflectivity of any metal. Its purity is typically measured on a per-mille basis or parts per thousand basis (Called as fineness).

The metal is found in the Earth's crust in the pure and free elemental form. It is found as alloy with gold and other metals. The abundance of silver in the Earth's crust is 0.08 parts per million, almost exactly the same as that of mercury. Naturally occurring silver is composed of two stable isotopes, ^{107}Ag and ^{109}Ag, with ^{107}Ag being slightly more abundant (51.839% natural abundance). It does not decompose and oxidizes easily. It is generally used for the manufacturing of jewellery, medals, medallions, commemorative coins etc. Most silver is produced as a byproduct of copper, gold, lead and zinc refining. The electrolytic silver deposition by refining process is based on Faraday's laws of electrolysis in which impure or crude silver is anodically dissolved and refined silver is cathodically deposited in the proportion of current and at the same time accompanying metals are selectively extracted from the spent electrolyte and separately cathodically deposited after having been transferred into an aqueous phase and the regenerated electrolyte stripped of accompanying metals is recycled to the refining process and in which further the spent electrolyte is anodically enriched in silver and accompanying metals are cathodically deposited from the aqueous phase in a joint electrolysis step.

The advent of this innovative development occurred during the exercise of electro-refining of impure silver in the Assay department of India Government Mint, Kolkata.

Experimental Method

This research project is carried out in Assay department of India Government Mint, Kolkata, West Bengal, India. The overall experimental steps operated in this research project are presented here through a flow diagram.

Flow chart of Silver Refining Project in IGM, Kolkata, India

Stock Standard Silver of fineness ≈ 890.0 to 920.0 with different dimensions

↓

Standard Silver bars are melted to Silver bars

↓

Hanging of Standard Silver bars in Vat as anode & Ferritic Stainless Steel (F.S.S.) plates as Cathode with supply D.C. of 10-12 Volts in nitric acid medium

↓

Spongy Silver is deposited at F.S.S. cathode during electrolysis

↓

Spongy Silver periodically collected from F.S.S. cathode into a bucket

↓

Acid processing to produce high fineness Silver

↓

Filtration of spongy Silver by PVC funnel, Cotton & graduated jar

↓

Melting of spongy Silver using carbon to form Silver pig bars with high fineness

↓

Assay of melted Silver samples using IS 2113 : 2002 and X-Ray fluorescence (XRF) instrument

↓

Final assay report i.e. chemical analysis report is generated for fineness of silver in parts per thousand unit (PPT)

The different steps of electrolytic silver refining is clearly depicted from the above flow chart. The crude silver of low fineness of ≈ 890.0 to 920.0 parts per thousand is converted to refined silver of high fineness using Ferritic Stainless Steel (F.S.S.) as cathode is an innovative process in our project. Previous research work on electrolytic Silver refining was done using Silver or steel as cathode **(1,2).** The composition of F.S.S. is Cr (16-17) % + C (≈0.12%) + S (0.03%) + Si (1.0%) + Fe (Rest). The crude silver bars are melted to silver bars of dimension (size- 4inch x 10inch x 10mm). The melted crude silver bars are fitted with F.S.S. plates by nut and bolt and hanged by a "S" shaped angular rod to a solid rod placed over an Iron stand. The average weight of crude silver bar per Vat is 18 to 20 kg and this is acts as anodic compartment of the electrolytic system. The cathodic compartment is F.S.S. plate of dimension (Size- 6inch x 8inch x 1mm approx.). The electrolytic process is carried out in 1% silver nitrate electrolytic medium with electricity DC supply of 10-12Volts / 70-100amperes per voltameter consisting of two cells using stainless steel clips. Use of over voltage leads to coagulation of Silver. The refined silver floating at F.S.S. cathode is collected in spongy silver form and it is collected periodically using a wooden spoon in a plastic bucket. As electrolysis process goes on, gradually colour of the electrolytic solution in the vat becomes blue due to formation of Copper nitrate in the medium [$Cu(NO_3)_2$]. As Copper ion (Cu^{2+}) concentration gradually increases as the electrolysis process goes on ,200-250ml Nitric acid (3) is added after 24hrs or 30hrs depending upon the spongy silver deposition. The spongy silver collected in the bucket is undergoes chemical processing with constant stirring using nitric acid to enhance the fineness of silver where Silver ion is converted to Silver metal and dissolved copper particle is converted in situ to copper ion (Cu^{2+}) and goes into the solution. After final processing the spongy silver is filtered with the help of PVC funnel, cotton and graduated glass jar using suction pump .During filtration the spongy silver is washed multiple times till the filtrate becomes transparent clean. The filtered processed spongy silver is melted using induction furnace using charcoal forming fine silver pig bars as the final product. The samples of melted silver pig bars are assayed or tested by gravimetric chemical analysis method using IS: 2113: 2002 norms and spectroscopic analysis is done by X-Ray Fluorescence spectrometer (XRF) .The final report is generated and the reported fineness is embossed on the respective refined silver pig bars.

Chemical Analysis

The analysis of fineness or purity of refined silver bars are carried out mainly by two methods available in India government mint, Kolkata. Such as

ASTM Method

This method is abbreviated as "American Society for Testing Metals"(5,6). This method of analysis is also known as IS 2113:2005. This is a gravimetric chemical analysis in which silver metal is chemically converted to silver chloride by using nitric acid and hydrochloric acid. The fineness or purity is calculated from the weight of silver chloride formed. i.e. 1gram of silver chloride contains 0.7526gram of metallic silver.

XRF Analysis Method

The fineness or purity of refined Silver sample is tested by X-Ray Fluorescence spectrometer (XRF) make Bowmann, U.S.A. Maximum refined silver samples found 999.9 parts per thousand of purity confirmed by XRF spectrometer.

RESULT AND DISCUSSION

The electrolytic refining of silver from crude or impure silver alloy using F.S.S. plate as cathode is an innovative step of our research project work. The use of F.S.S. not only decrease the overall cost of the project but also has almost the equal productivity like silver electrode as cathode. The standard reduction potential difference between silver ion (Ag^{+}/Ag) and Ferric ion (Fe^{3+}/Fe) is responsible for the spontaneous flow of electron from anode to cathode. The final fineness or purity of the refined silver is above 999.0 (in maximum case the fineness is 999.9) reported from the method of ASTM (American Method for Testing Metals) or IS 2113:2002 & XRF data. The different parts use in our research is rejection materials from different departments of India Government Mint, Kolkata. The overall performance of the project related to electrolytic refining of silver is very much satisfactory.

CONCLUSION

This innovation reduces cost of cathode by replacing pure silver with F.S.S. cathode. Electrolyte contains nitric acid in which F.S.S. alloy acts as an inert alloy whereas pure silver readily dissolves in this medium at the interface of collection of spongy silver at zero DC supply and the fact leads to the recovery of the same. Thus the introduction of the F.S.S. cathode inhibits corrosion of pure silver as cathode.

ACKNOWLEDGEMENT

This research project work is funded & supported by India government Mint, Kolkata- A unit of Security Printing and Minting Corporation of India Limited. We acknowledge all the staffs of Assay department of India Government Mint, Kolkata for their whole hearted support and cooperation towards the project.

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Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of *In vitro* and *In vivo* Antidiabetic Activity of *Lepisanthes tetraphylla* (Vahl) Radlk against Streptozotocin Induced diabetic Wistar Albino Rats

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Received: 23 Jan 2021 Revised: 01 Feb 2021 Accepted: 24 Feb 2021

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ABSTRACT

Across the planet, diabetes mellitus has become a significant public health and economic issue. Lack of adequacy and extreme adverse reactions associated with traditional pharmaceutical products have contributed to the determined quest for alternative natural therapeutic agents. The current study evaluated *in vitro* α-amylase & α-glucosidase inhibitory activity and *in vivo* antidiabetic activity of ethanolic extract of *Lepisanthes tetraphylla* (EELT) in streptozotocin induced diabetic rat model. By adhering OECD-423 guidelines safe doses of EELT were assessed in rats for the main study. Oral glucose tolerance test, blood glucose and body weight estimation were conducted to assess antidiabetic efficacy of EELT. IC_{50} of EELT was found to be 45.32µg/ml and 53.74µg/ml against α-amylase & α-glucosidase respectively. EELT was found to be safe up to the dose of 2000 mg/kg. Significant reduction in glucose levels was observed in EELT treatment groups. The loss of body weight due to diabetes was prevented significantly in diabetic rats treated with EELT. Further studies to standardize the extract and evaluation of safety profile in long-term toxicity studies are recommended for safe and effective antidiabetic nutraceutical development.

Keywords: *Lepisanthes tetraphylla,* Diabetes mellitus, Streptozotocin, Antidiabetic, Medicinal plant

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic condition of chronic hyperglycemia triggered by insulin secretion deficiencies, insulin action defects or both (Alberti *et al.,* 1998). The International Diabetes Federation estimated that 415 million people worldwide have diabetes in 2015; by 2040 this will rise to 642 million (International Diabetes Federation, 2015). It is linked to acute and chronic complications that are responsible for the majority of morbidity and mortality associated with DM, financial stress and poor quality of life. In addition, diabetic complications caused by persistently high sugar levels result in damage to multiple organs, especially the skin, kidneys, nerves and blood vessels (Soumya, 2011). Despite the fact that many antidiabetic agents from natural and industrial source have been added to the market to diabetes, as well as its micro and macro risks, appears to be a significant medical concern worldwide (Piero *et al.,* 2015). Herbal plants are believed to be a source of herbal medicinal goods. A literature survey found that a significant number of plants with either little to no side effects are used for their hypoglycemic behaviours (Wachtel-Galor and Benzie., 2011). Hence, primary emphasis is therefore on the quest for phytoconstituents in novel antidiabetic plants without side effects.

Lepisanthes tetraphylla (Vahl) Radlk, commonly called as Kurpa, Nekota (Tamil name), is a shrub growing in India which belongs to the family Sapindaceae was used traditionally for the treatment of elephantiasis, skin disease, anti-emetic, contraceptive, fever, anti-microbial and anti-convulsing property. In Ayurveda, this plant is used in the treatment of eczema, psoriasis and for fretchlet removal. Isolated constituents and many medicinal plants have demonstrated beneficial therapeutic potentials (Katiyar *et al*., 2012; Meena *et al*., 2012; Ranjini *et al*., 2013). Our previous study confirmed the presence of carbohydrates, proteins and amino acids, saponins, tannins, flavonoids, gums in ethanolic extract of *Lepisanthes tetraphylla* (Sudha Kesavarthini *et al*., 2018). From our previous *in silico* study on the phytochemicals of *Lepisanthes tetraphylla* showed better binding interaction with PPAR-gamma inhibitor (Sudha Kesavarthini *et al*., 2019). Therefore, the present study aimed to investigate the *in vitro* and *in vivo* antidiabetic potency of the crude extract of *Lepisanthes tetraphylla*.

MATERIALS AND METHODS

Chemicals and standard drugs

Streptozotocin (STZ) and Glibenclamide were obtained from Sigma–Aldrich, USA. All other chemical and solvents were procured from Hi-media Laboratories Pvt., Ltd., India.

Collection and Extraction

The leaves of plant was collected from the natural habitats of Tirunelveli District of Tamilnadu, the leaves of plant were shadow dried and made as coarse powder using electrical blender. This powder was pre-extracted with hexane and the lipids had strictly removed at an early stage of the extraction and then extraction was continued with 99%v/v alcohol in a Soxhlet extractor for 48 h.

In vitro anti-diabetic activity

***In vitro* α -amylase inhibition activity (Bernfeld P.,1955):** The α-amylase (0.5 mg/mL) was premixed with extract at various concentrations (20-100 μg/ml) and to initiate the reaction, a 0.5% starch solution was added as a substrate. The reaction was conducted at 37°C for 5 min and stopped with the addition of 2 mL of DNS (3,5-dinitrosalicylic acid) reagent. The reaction blend was heated at 100°C for 15 minutes and dissolved with 10 ml of distilled water in an ice bath. The alpha-amylase activity was determined by a 540 nm spectrum measurement. The % α-amylase inhibitory activity was calculated by the following formula

% Inhibition = (Control OD-Sample OD / Control OD) × 100

***In vitro* α-glucosidase inhibition activity (Collins *et al.*, 1997):** Premixing alpha-glucosidase (0.07 Units) with 20-100 μg/mL of extract was used to assess the enzyme alpha-glucosidase inhibitory function. Then, as a substrate, 3mM p-nitrophenyl glucopyranoside was added. For 30 minutes at 37°C this reaction mixture was incubated and 2 mL of sodium carbonate had been added to terminate the process. The p-nitrophenyl release from p-nitrophenyl glucopyranoside was calculated at 400 nm to determine the of α-glucosidase activity. The % α-glucosidase inhibitory activity was calculated by the following formula

% Inhibition = (Control OD-Sample OD / Control OD) × 100

In vivo anti-diabetic activity

Animals and treatments: Swiss albino Wistar rats (male and female), 175 to 200 g body weight, were kept in individual polyethylene cages and housed in an air conditioned room at 20 ± 2 °C; 40–60% humidity with 12 h light and 12 h dark circle; at the animal house facility of Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, Karnataka, India, in accordance with Animal Institutional Ethical Committee of Aditya Bangalore Institute of Pharmacy Education and Research (1611/PO/a/12/CPCSEA) recognized by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, for care and use of laboratory animals.

Acute toxicity studies: According to the Organization for Economic Co-operation and Development (OECD) guideline number 423, the acute toxicity test of the extract was determined. The male and female Wistar rats (175-200g) have been used in this study. After the sighting study, the starting sample dose is 2000 mg/kg per (p.o.) assay from different groups comprising six animals from each group. Mortality and various responses such as conduct, nervous and autonomic responses were followed up on treated animals for 14 days. Till the end of the study, no death has been observed. The test samples were found to be healthy up to a dosage of 2000 mg/kg and from the results 200 mg/kg/b.w and 400 mg/kg/b.w doses were chosen for further experimentation as the minimum and maximum doses.

Experimental design

The animals were divided into five groups and each group consisted of six animals.

Groups		**Treatment**
Group I	:	Untreated normal rats received saline
Group II	:	Untreated diabetic rats received saline
Group III	:	Diabetic rats treated with Glibenclamide (10 mg/kgb.w, i.p.)
Group IV	:	Diabetic rats treated with EELT (200mg/kg b.w, p.o.)
Group V	:	Diabetic rats treated with EELT (400mg/kg b.w, p.o.)

Oral Glucose Tolerance Test (OGTT): OGTT was performed according to the method of Du Vigneaud and Karr, 1925. Following the overnight fasting protocol, '0' min of the blood measure (0.2 ml) was obtained by tail vein. A glucose solution (2g/kg weight) was administered without delay by gavage. After administration of glucose, four additional samples were taken at 30, 60, 90 and 120 min intervels with potassium oxalate and sodium fluoride. Blood glucose concentration was estimated using a commercial glucometer and test-strips (Accu-chek Active™ test meter).

Induction of diabetes: For experimental study, Swiss albino (Wistar strain) rats were used. All the rats were starved overnight (12 h) before the experimentation. Single STZ (Streptozotocin, 60 mg/kg, body weight) intraperitoneal injection primed by STZ dissolving in a 0.1 M citrate buffer (pH = 4.5) and administered through intra-peritoneal route.

Estimation of body weight and blood glucose: The body weight was calculated for the 1st and 28th days. The changes in weight were recorded. Blood glucose was estimated with the o-toluidine reagent by the method of Sasaki *et al.,* 1972. The glucose aldehyde group condenses with o-toluidine in glacial acetic acid when heated, creating a bluish green tint, calculated calorimetrically at 630 nm. With 1.9 mL of 10 % TCA, 0.1 mL of blood was precipitated, permitted to stand for 5 min, and centrifuged. In a dry test tube, 1 mL of the supernatant was taken and 4 mL of the o-toluidine reagent was added. The test tubes were placed for 10 minutes in a boiling water bath and cooled. Standards in the range of 20-100 mg were rendered up to 1mL, along with a blank of 1 mL of distilled water, and were processed as test. The values were expressed as mg/dL of blood.

Statistical analysis

The values are expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's 't' test. $P < 0.001$ was considered significant.

RESULTS

In vitro anti-diabetic activity

Alpha amylase inhibitory activity for the EELT is presented in table 1. The inhibition of α-amylase potential shown maximum activity of 93.51% at 100µg/ml. The IC_{50} EELT was found to be 45.32µg/ml. Alpha glucosidase inhibitory activity for the EELT is presented in table 1. The inhibition of α-glucosidase potential shown maximum activity of 91.19% at 100µg/ml. The IC_{50} EELT was found to be 53.74µg/ml.

In vivo anti-diabetic activity

Estimation of Oral Glucose Tolerance Test (OGTT)

The impact of EELT in regular and experimental rats on oral glucose tolerance is given in table 2. Diabetic rats treated with glucose reported substantial changes in blood glucose levels relative to regular rats. The blood glucose levels increased from 253.21 to 290.47 at 120m. Blood glucose was substantially decreased in contrast to diabetic control rats after oral dosing of EELT in 200mg/kg and 400mg/kg. The blood glucose level of EELT at the dose of 200 &400mg/kg decreased from 271.32 to 118.26 and 267.09 to 94.78 respectively. The standard drug showed the significant decrease in the blood glucose level after 120m (273.89 to 83.33). Finally, a substantial drop in the blood glucose level in diabetic rats after 120 m of therapy was seen in dose-based manners in rats after 400mg/kg of extract and the standard drug.

Estimation of fasting blood glucose levels

The impact of repeated oral EELT administration on blood glucose levels was assessed on 1, 7, 14, 21 and 28 post-induction days relative to regular and diabetic control groups. The results were presented in the table 3. Diabetic rats treated with STZ reported significant changes in blood glucose levels compared with regular rats. So, the blood glucose levels increased from 250.13 to 265.89 on 28th day. After treatment with oral EELT doses of 200mg/kg and 400mg/kg, blood glucose was greatly decreased relative to rats with diabetic regulation. The blood glucose level of EELT at the dose of 200 & 400mg/kg decreased from 263.57 to 141.46 and 261.32 to 123.43 respectively. The standard drug (Glibenclamide) also demonstrated a substantial reduction in blood glucose after 28 days of therapy (261.91 to 114.76). After 28 days of dose-dependent therapy in rats, 400mg/kg of extract and the standard drug demonstrated a substantial decrease in the blood glucose level of diabetic rats.

Estimation of Body Weight and Blood Glucose level

Table 4 shows the effect of EELT in normal and experimental rats on body weight and blood glucose levels. In normal control rats, the body weight increased slightly from 186.84 to 202.93, while in diabetic rats, there was a significant reduction in body weight from 184.02 to 152.68. After treatment with extracts EELT, they showed an

improvement in (193.23 & 207.72 for EELT) body weight when compared to the diabetic control and standard glibenclamide treated group. In diabetic rats, the blood glucose increased significantly and body weight decreased compared with normal control rats. In comparison to normal rats, STZ-treated diabetic rats showed a significant increase in blood glucose levels. So, the blood glucose levels increased from 247.86 to 294.08 on 28th day. The blood glucose was significantly reduced compared to diabetic rats following oral administration of EELT at 200mg/kg and 400mg/kg. The blood glucose level of EELT at the dose of 200 & 400mg/kg decreased from 239.09 to 165.66 and 241.47 to 119.31 respectively. The standard drug also showed that blood glucose levels were significantly lower after 28 days of treatment (249.65 to 113.30). Finally, after 28 days of treatment, 400mg/kg of extract and the standard drug showed a significant decrease in blood glucose levels in diabetic rats in dose-dependent mode in rats.

DISCUSSION

Administration of glucose (2gm/kg) showed sharp rise in blood glucose level in normal rats after 30min. The rats treated with extracts and standard, the blood glucose level reached peak level at 30min and return to normal at the end of 120min. Administration of EELT showed significantly lower the glucose values when compared to normal control rats. It can be inferred from the oral glucose tolerance examination that the 400mg/kg EELT dosage demonstrated the highest increase in glucose tolerance. The impact of repeated oral EELT administration on fasting blood glucose levels were assessed on post-induction days 1, 7, 14, 21 and 28, and compared with the normal and diabetic control groups. STZ-treated diabetic rats reported substantial changes in blood glucose levels when compared to control rats. Following oral administration of EELT at doses of 200 mg/kg and 400 mg/kg, blood glucose was substantially decreased when compared to diabetic control rats. After 28 days of therapy, the standard drug (Glibenclamide) showed a substantial reduction in blood glucose levels. After 28 days of dose-dependent therapy, 400 mg/kg and standard drug showed decrease in the amount of blood glucose in diabetic rats. During insulin deficiency, the excessive catabolism of protein to provide amino acids for gluconeogenesis results in muscle wasting and weight loss in diabetic control rats. In normal control rats, the body weight was slightly increased, while in diabetic rats, the body weight decreased considerably. A significant loss of body weight is characterized by STZ caused diabetes. Since treatment with extracts, there was an increase in body weight relative to the diabetic management and normal category controlled with glibenclamide. A rise in body weight attributed to glycemic regulation changes and improved structural protein synthesis. As a consequence of its potential to suppress hyperglycemia, extracts tend to be capable of protecting body weight loss. Compared to control rats, the amount of blood glucose in diabetic rats increased dramatically. In diabetic rats, EELT therapy greatly decreased plasma glucose in comparison to diabetic control rats. Glibenclamide and 400 mg/kg dose of EELT have greatly restored plasma glucose levels to near normal levels. Diabetic induced groups treated with EELT produced potent anti-diabetic activity in a dose dependent manner, dose level of 400mg/kg produced potent activity when compared with the groups treated with 200mg/kg of EELT.

CONCLUSION

The present research suggests that EELT have substantial antidiabetic function on STZ induced diabetic rats. The antidiabetic activity may be due to improvement in glucose tolerance at the dose level of 200 & 400 mg/kg. Administration of EELT (200 & 400 mg/kg) to STZ-diabetic rats lowered near normal blood glucose levels and after 28 days of therapy, recovered the body weight. EELT at a dose level of 400 mg/kg showed an equivalent antidiabetic activity in comparison with the standard glibenclamide treated group. Based on the results, we concluded that the ethanolic extracts of selected the plant *Lepisanthes tetraphylla* may have a promising role in the management of diabetes mellitus especially in a country like India where conventional treatment is not easily accessible to the general population due to cost factors. In future, the active constituents responsible for antidiabetic activity can be isolated from the plant extracts and their structures may be elucidated. Further, a detailed mechanism of action of these extracts and clinical trials may be carried out which may be useful for the society for the management of diabetes and other oxidative stress related diseases.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENT

The author P. Sudha Kesavarthini was thankful to the management of Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore for carrying out the research work.

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Table 1: *In vitro* anti-diabetic activity

S. No	Concentration (µg/mL)	% of EELT inhibition	
		α -amylase	α-glucosidase
1	20	28.23	33.5
2	40	49.45	48.26
3	60	67.75	57.61
4	80	80.06	72.45
5	100	93.51	91.19
IC_{50}		**45.32**	**53.74**

All values are expressed as mean ± SEM for three determinations

Table 2: Estimation of Oral Glucose Tolerance Test (OGTT) in normal and diabetic rats

Group	Blood glucose concentration (mg/dL)			
	0m	30m	60m	120m
Group I	82.67±1.77	84.65±1.52	82.83±1.80	80.48±1.55
Group II	253.21±2.77[a]	267.22±3.17[a]	278.49±3.64[a]	290.47±3.28[a]
Group III	273.89±2.91[a]	153.74±3.55[a,b]	127.26±2.72[a,b]	83.33±2.19[b]
Group IV	271.32±3.54[a]	189.56±2.84[a,b]	163.40±3.06[a,b]	118.26±3.69[a,b]
Group V	267.09±3.72[a]	173.68±2.60[a,b]	140.91±3.34[a,b]	94.78±1.90[b]

Values are mean ± SEM; n = 6 in each group;

[a]P< 0.001 when compared to normal control;

[b]P< 0.001, when compared to diabetic control (one-way ANOVA followed by Dunnett's 't' test)

Table 3: Effect of EELT on fasting blood glucose level in control and diabetic rats

Group	Fasting blood glucose level (mg/dl)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Group I	80.48±2.06	81.18±2.26	82.14±2.05	82.83±2.21	84.78±2.08
Group II	250.13 ±5.17[a]	259.59±3.44[a]	266.76±3.83[a]	288.14±3.34[a]	265.89±3.02[a]
Group III	261.91±5.35[a]	200.38±3.94[a]	158.74±4.21[a,b]	123.09±2.83[a,b]	114.76±2.10[a,b]
Group IV	263.57±6.72[a]	221.49±4.77[a]	198.71±4.59[a,b]	171.24±3.73[a,b]	141.46±3.06[a,b]
Group V	261.32±4.71[a]	205.27±3.73[a]	176.68±5.03[a,b]	146.8±3.01[a,b]	123.43±3.73[a,b]

Values are mean ± SEM; n = 6 in each group;

[a]P< 0.001 when compared to normal control;

[b]P< 0.001, when compared to diabetic control (one-way ANOVA followed by Dunnett's 't' test)

Table 4: Effect of EELT on body weight and blood glucose level in control and diabetic rats

Group	Body weight (g)		Blood glucose (mg/dL)	
	1st Day	28th Day	1st Day	28th Day
Group I	186.84±4.95	202.93±4.62	86.98±2.63	88.19±2.36
Group II	184.02±3.59	152.68±3.6[a]	247.86±4.93[a]	294.08±4.72[a]
Group III	183.66±4.26	208.95±4.3[b]	249.65±4.71[a]	113.30±4.46[b]
Group IV	185.74±4.84	193.23±3.8[b]	239.09±5.07[a]	165.66±5.38[a]
Group V	183.59±4.03	207.72±4.3[b]	241.47±4.65[a]	119.31±5.27[b]

Values are mean ± SEM; n = 6 in each group;

[a]P< 0.001 when compared to normal control;

[b]P< 0.001, when compared to diabetic control (one-way ANOVA followed by Dunnett's 't' test)

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.12 / Issue 65 / April / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Enriching Primary Learning with Eco Centric Graphic Narratives: A Semio-Pragmatic Study

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Received: 22 Feb 2021 Revised: 27 Feb 2021 Accepted: 01 Mar 2021

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ABSTRACT

The first eight years of a child's education are considered to be formative in laying the foundation of their approach and attitude towards their surroundings. The activities performed inside and outside the classroom play a functional role in shaping their behavior meaningfully. Thus, it is a matter of great significance and responsibility to feed such fluid minds with conscientiousness and appreciation towards nature. Further, the choice of the content or study material these children are exposed to in school also essentially mirrors our own culture as well as the future we wish to create with and for these children. Graphic narratives pave the way for eclectic learning, developing critical thinking and forming associations in young minds. The colorful world of contextual storytelling not only productively engages its audience but also leaves an everlasting impression on their senses. Therefore, issues those are of great concern e.g. ecological crisis, endangered species, when rolled into a visual narration have a deeper impact.

This study qualitatively analyses the NCERT EVS texts of grades 1 to 5 (prescribed under CBSE board) for their content centered on nature and its concerns. It also delves into the graphic narrative content in the syllabi emphasizing proximity to environment. Additionally, the paper applies the communicative theory of relevance and the principles of Peircian semiotics to explain the significance of incorporating ecocentric graphic narratives as a formal part of kids' education. In order to cohesively bind the study into a context, *Plants and Trees, Heal the World Little Bunny, What a Waste?* and *The Magic and Mystery of Trees* are considered as primary texts for this paper. These texts contain graphic narratives a few of which will subsequently be used to examine the semio-pragmatic side of eco-graphic narratives. Thus, this paper proposes prescribing ecocentric graphic narratives in primary division of school education in order

to sensitize kids towards ecological themes. The aim is to 'assimilate' empathy towards nature in the mental schema of kids at an early age (4-8 years) so that they internalize it as a way of living. It is about inducing a caring attitude towards earth's ecology as a conscious cultural decision.

Keywords: theory of relevance, Peircian semiotics, graphic narratives, environment, semio-pagmatic

INTRODUCTION

'Nature for nature's sake' as an approach seeks to explore nature as an entity per se keeping ecology and environment at the centre of the study. Interaction between living and non-living organisms, the constituents of ecosystem, and encouraging biodiversity, perceiving nature as a creator, guide, healer, provider, nurturer, inspiration are its major focus areas. It demands a dynamic shift from an anthropocentric and biocentric attitude to ecocentrism. It is not only about what sort of future we are creating for our next generations but also about what kind of future generations are we creating to exist on this planet earth! Failing to do so will not only be a failure of imagination but a cultural failure [1]. The new learners look up to us, observe us and follow our footsteps. It therefore becomes our moral responsibility to show them the virtuous path of symbiosis and not instrumentality. Just as the first words on a blank slate leave an indelible impression behind, so do the childhood memories have an ever-lasting impact on the human mind. The formative years (0-8years) are the foundational period in a child's cognitive, emotional and physical development. Mental schemas and habits formed during this span play an integral role in moulding the personality of a child as an adult.

"The Convention on the Rights of the Child clearly highlights the importance of early childhood development (ECD), stating that a child has a right to develop to "the maximum extent possible" (Article 6) and that "States Parties recognize the right of every child to a standard of living adequate for the child's physical, mental, spiritual, moral and social development" (Article 27)"[2] (UNICEF unite for children). One such right is "to be amazed" by the world about and around. Nature is a generous educator, mentor, nurturer and healer. It is both mysterious and simple. It is at once a historian, scientist, artist, financer. It is a patient storyteller and offers a universe of learning- of virtues, kindness, empathy. It generates empathy, amazement, humility- all at once. It can stimulate eighth intelligence. There is a direct connection between mystery of nature and imagination.

Ecocentrism [3] is a concept based on the ecological philosophy where all organisms are not only internally related with their environment but also shaped by those very environmental interrelationships. It assigns value to systems and actions like waste management, protecting biodiversity, endangered species, restoring forests that make our world beautiful and thriving. Ecocentrism offers a holistic approach towards dealing with the individual components of the environment as a synthesized whole and having their due intrinsic value, all at once.

Objectives

a. To suggest a necessary shift towards ecocentrism in CBSE prescribed environment education content for primary education
b. To propose introducing a dedicated space for ecology and environment based graphic narratives in the prescribed texts
c. To explain the semio-pragmatic significance of graphic narratives at primary level

MATERIALS AND METHODS

This study qualitatively and quantitatively scrutinizes CBSE prescribed NCERT Environmental Studies texts [4][5][6][7][8] for grades first through fifth for the presence of ecocentric content and graphic narratives based on

nature and environment. Further, the paper discusses the theory of relevance and the Peircean trichotomy of sign-object relationship. Subsequently, excerpts drawn from four primary texts -- *Plants and trees*[9], *Heal the world little bunny*[10], *What a waste? rubbish, recycling and protecting our planet* [11], and *The magic and mystery of trees*[12]-- are semio-pragmatically analyzed for some of the overlapping themes as found in the aforesaid school textbooks.

RESULTS AND DISCUSSIONS

Analysis of NCERT environmental studies texts [grade 1-5]

The currently prescribed NCERT Environmental Studies books for grade 1 through Grade 5 in CBSE board were analyzed for their approach towards ecocentrism and the usage of graphic narrative content. The study shows that most of the chapters of the reading material are about awareness or observation of self or others/ surroundings (Fig.2). Only a small chunk is further divided between biocentric and ecocentric (Fig.4). Further, the comparative analysis of the quantum graphic narratives showed the percentage proportion of Visual narratives as 54.5%, Visual and Verbal narratives as 28.7%, and Ecocentric visual and verbal narratives as 16.8% (Fig.3).

Graphic narratives and early childhood

The multimodality of human expression involves making utterances, moving bodies, creating graphic representations. These modalities are a blue print of human communication inherited by children since their birth. Neil Cohn in his *The Visual Language of Comics* proposes that when any of these modalities is structurally sequenced 'governed by rules that constrain the output—i.e. a grammar', a type of language is yielded. This is how structured sequential images literally become visual languages. Visual languages are timeless by nature. They are dynamic as they can range from being concrete to abstract; they can also range between being purely aesthetic and specifically educational.

Cognitive-Gestalt perspective

In modern elementary education, graphic narratives like cartoons, comics, info graphics carry enormous potential of visual language to capture attention instantly, to engage and to sustain longer in human memory. They can impart useful knowledge in a vibrant, simplified fashion. In early childhood, children gradually master class inclusion, seriation problems and transitive inference during their concrete operational stage. They can solve verbal appearance- reality problems, signifying a more secure understanding [13].Such cognitive capacities have a direct relation with the growing ability to make meaning of sequential verbal and visual structures or narratives within a context. Robbie Case, a Neo-Piagetian, attributes this ability to make meaning to the formation and automization of mental strategies and central conceptual structures permitting children "to think about a wide range of situations in a more advanced way" (Laura Berk, 276). Case also ascribes such a cognitive development to the gains in working memory capacity.

The Gestalten view of learning explains the formation of connections from the sensation and perception perspective where the whole is different from the sum of its parts. The common principle between cognitive development and Gestalt therapy theory [14] is 'assimilation' meaning thereby, children assimilate new learning with the existing schemas. Talking about assimilation, the already existing schemas or mental representations of human body parts can be overlapped with the reference made to tree parts (Fig.5 & 6). As an emphasis on the principle of conservation, trees have their the same parts as roots, stem, branches, leaves, flowers, fruits wherever they grow.

Thus, formation of new schemas (Piaget), or central conceptual structures (Case), or re-equilibration (Perls et al) calls for assimilation in order to form new approximations of reality. Once formed, these connections are difficult to unlearn. A false understanding or misinterpretation of reality during the formative years of development can lead to difficulty in reprogramming the mind to work otherwise and that can lead to long term learning problems, for our learning and thoughts turn into habits. Having said this, it becomes our responsibility to prepare/ condition our children to look at the natural environment to be observed and cared for.

Semio-Pragmatic meaning of graphic narratives

Cognitive theory of communication: The theory of relevance

Proposed by Dan Sperber and Dierdre Wilson (1986) Relevance Theory aims to explain that the communicators usually convey much more information than the literal sense of their utterances. The theory argues that the acts of human verbal communication are ostensive in drawing attention of the addressee to the relevance of the information conveyed. The relevance of an utterance in this technical sense is one from which many conclusions can be drawn at a low processing cost or, in other words, the relevance of the address can be maximized with relatively less mental processing effort [15]. To infer conclusions from a communication, the addressee uses the information contained in the utterance together with his expectations about its relevance, his real-world knowledge, as well as sensory input. Here, differences in cultural, educational background as well as intelligence can affect the inference drawing process of communication. Therefore, the addressee can draw more conclusions if the utterance contains information that they already know or believe in. Such literal meaning is a mere part of the entire inference process. Sperber and Wilson characterize these features of verbal communication as ostensive-inferential communication comprising of a double layered intention, such as:

a. The informative intention
b. The communicative intention

Here, the informative intention is the intent to inform an addressee of the communicative content. On the other hand, the intent to inform the addressee of one's intention to communicate some information is termed as the communicative intention. Research shows that kids between 3 and 5 years begin to grasp illocutionary intent and continue to increase their understanding of the same further (Laura Berk, pg 273). Further, the relevance theory operates on the principle of maximum relevance [16] of an observed phenomenon with less mental processing effort. In other words, the extent of relevance of a communicative activity to an individual is defined by its larger positive cognitive effects (value addition to one's representational world) and low processing effort. It is like the cost benefit analysis our cognitive capacities do for an utterance.

This paper touches mostly upon inference drawn in the form of explicature (its counterpart being implicature). The example is taken from Henrich's *Heal the World Little Bunny* where the Daddy bunny consoles his little bunny by asking him to imagine growing bigger and bigger till the moment the kid bunny can hug the planet earth and send his love to all abiotic and biotic entities (Fig.7). The little bunny can understand the topographical categories like 'rivers', 'oceans', etc. and even the binaries like 'mountains', 'plains', 'deserts', 'jungles'. However, when the little bunny is asked to send his love to all the creatures on earth he very well understands his father's intent but wonders if he should really be praying for the humans. It was only due to the unthoughtful humans that the little bunny could not go out and play. The image shows the upfixes [18] of curiosity- question marks. That the little bunny understands this explicature is conveyed by the consecutive narrative where the Daddy bunny insists on him sending love to 'the human varmints.'

Peircean semiotic relationship between sign and object

Peirce's triadic approach of sign reference states three forms:[17]

a. Icon: some signs derive their meaning by resembling what they mean [Iconic]
b. Index: some signs derive their meaning by causing or indicating meaning in something else [Indexical]
c. Symbol: some signs can be considered meaningful through a conventional relationship between a stimulus and its meaning [Symbolic]

Thus, these three define the relation of a sign to its object in the form of resemblance, indication and convention respectively.

(Fig.8) displays a red four wheeler that resembles a car (iconic). The back of the car exudes a tail of smoke (indexical) which finally turns into a huge black figure with diabolical features symbolizing itself as a demon (symbolic). In the

backdrop we again see a cloud of smoke indicating chimneys as its source. In the left forefront, a human figure (iconic) is overshadowed by the fear inducing demonic smoke. The figure thus has a predominance of indices. (Fig.9) iconically contains a little bunny, blue barrels with upfixes indexing radiation, a cock with a blue crown and four legs (an anomaly indicative of the hazardous effects of radiation) walking over the spilled green contents of a fallen barrel. Each barrel has a sign icon which conventionally symbolizes radiation. The little bunny cautiously observes the scene and gets the informative intent of his mother implying to not go any near to the dangerous site of radioactive wastes.

CONCLUSION

Ecocentrism as a philosophy if made the core context around which a major proportion of our education is centered, we will certainly be able to form pro-ecology attitudes during the foundational years of learning. These energize the central conceptual structures of a child's mind generating awareness, empathy and drawing connections with the surroundings and beyond. The importance of a learning in which human life becomes a subsystem in the larger scheme of nature than the other way round can be a major breakthrough in changing the mindsets. When children learn to correlate the impact of human actions, they become conscious of their choices. This is a way forward to turning our mindsets green. The children learn that all biotic and abiotic constituents of nature have their own intrinsic value rather than just being instrumental in fulfilling human needs.

The above observations explain how we can club ecocentric approach with the primary education themes. Themes like Parts of a plant / tree, Caring for all the creatures, towards a safer planet can be pulled into the contextual frames of parts of human body, caring for others, safety of self respectively. In fact, this association can be enhanced to Means of communication with Wood wide web, Save the earth with saving the oceans and forests, good habits with minimising plastic use. The common themes could be Senses, Shelter/Home, Family Together, Food and Nutrition, Defences, and others which would be dealt with in another paper. The assimilation and accommodation of the associated themes form connections with the existing mental schemas while synthesizing new ones. Thus, this paper proposes to bring more eco-centered learning to kids especially in their formative years. As Willaim Wordsworth rightly averred "*The Child is father of the Man"* the hope not only for a better nation but also better nature lies in the hands of our future generations.

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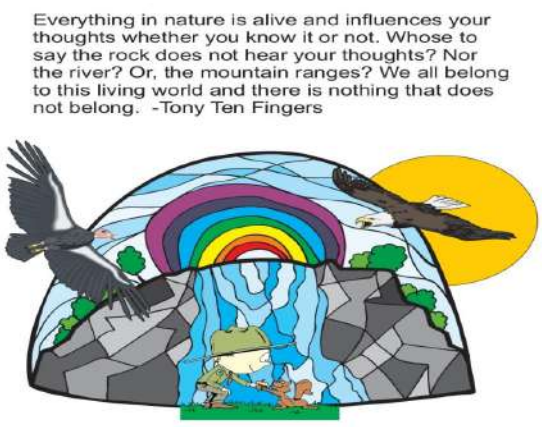

Fig.1:courtesy Henrich's *Heal the World Little Bunny*

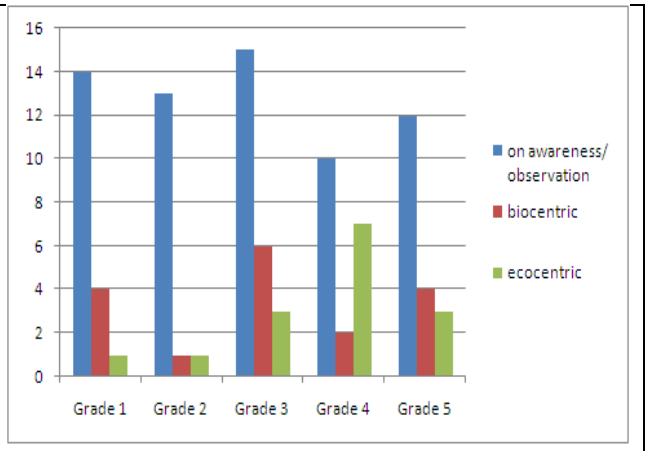

Fig.2: Grade content focus in primary grades

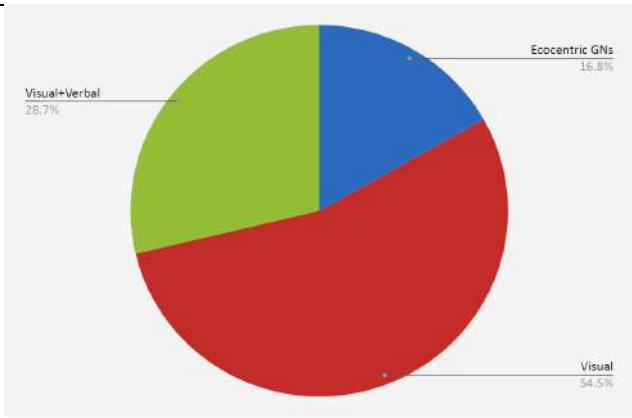

Fig.3: Distribution of Ecocentric Graphic Narratives (GNs), Visual & Verbal,Visual Narratives in all four grades combined.

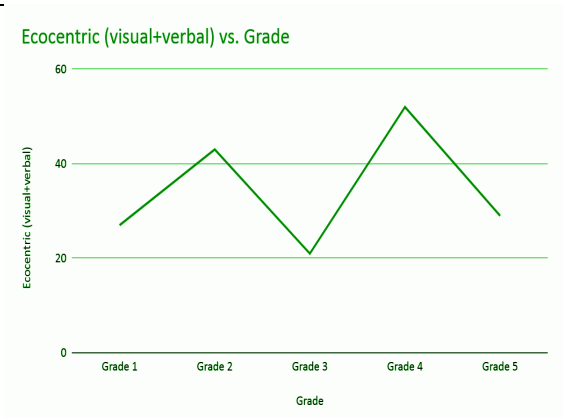

Fig.4: Distribution of only Ecocentric Visual and/or verbal narratives across the four grades.

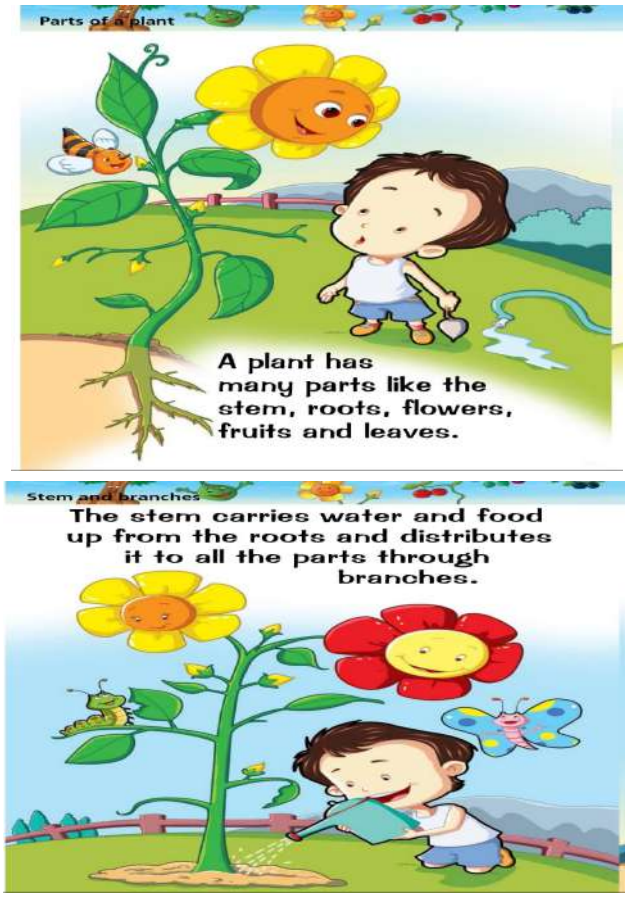

Fig.5: courtesy My Knowledge Book- *Plants & Trees*

Fig.6: courtesy Green's *The Magic & Mystery of Trees*

Theme 1.Parts of Body -- Parts of a Plant / Tree (Fig.5&6)

Theme 2 Caring for Others-- Caring for all the creatures

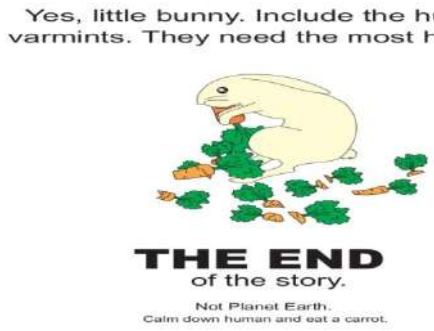

Fig 7: courtesy Henrich's *Heal the World Little Bunny*

Fig. 8: French's What a Waste? Rubbish, recycling and protecting our planet

Fig.9: courtesy Henrich's Heal the World Little Bunny.