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Preliminary Phytochemical Screening of Methanol Extract of *Indigofera trita* Linn.

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Abstract

The aim of the study to phytochemical screening was carried out. The traditional medicine involves the use of different plant extracts or the bioactive constituents, qualitative phytochemical analysis of these plants contirm the presence of various phytochemicals like alkaloids, flavonoids, tannins, caponins, proteins, gums and mucilage, phytosterols. The result suggest that the phytochemical properties for curing various ailments and possess potential antioxidant and reads to the isolation of new and novel compounds.

Keywords: Secondary metabolites; Alkaloids; Flavonoids; Saponins; Phenolic compound

Introduction

Nearly 80% of the world's population relies on traditional medicines for primary health care, mast of which involve the use of plant extracts (i) In India almost 95% of the prescriptions were used in Unani, Ayurveda, Homeopathy and Siddha [1]. Phytochemicals are responsible for medicinal activity of plants [2] these are non-nutritive chemicals that have protected human from various diseases. The major constituent consists of alkaloids, flavonoids, saponins, phenolic compounds, phytosterols, proteins and amino acids, gums and mucilage and lignin [3]. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries the constituents are playing a significant role in the identification of crude drugs. The medicinal value of these plants lies in some chemical substances that produces a definite physiological action on the human body. The most important property of these bioactive constituents of plants is that they are more effective with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The anti-inflammatory, antispasmodic, anti-analgesic and can be attributed to their high steroids, tanning, terpenoids and saponins.

Indogofer trita is a dicot plant and phylum of tracheophyta. It is a class of magnoliopside and an order of fables, under a family of fabaceae. It is found throughout Africa, Asia and Australia widely distributed in South India of Tamil Nadu, Kerala, Karnataka. It has wide geographic and habitat range the population is inferred to be large and stable (Figure 1).

Figure 1: Photograph of *Indigofera trita.*

Taxonomic classification of *Indigofera trita*

Kingdom	:	Plantae
Sub Kingdom	:	Viridiplantae
Infra Kingdom	:	Streptophyta – land plants
Super division	:	Embryophyta
Division	:	Tracheophyta
Sub division	:	Spermatophytina
Class	:	Magnoliopsida
Super order	:	Rosanae
Order	:	Fabales

Family : Fabaceae

Genus : Indigofera L.

Species : *Indigofera trita* – Asian Indigo

Specific epithet : Trita

Other language of *Indigofera trita*

Common name : Asian Indigo

Tamil : Punal – Murunkai

Telugu : Jidi Vempali

Kannada : Goramti nili

The plant is known for being well perennial, erect or subs candent woody herb or shrub, 0.5-2 m tall. Taproot present, nodules present, stems and branches arching, spreading or decumbent. Branches becoming whitish, leaves are trifoliate, about 0.8-2.5 cm long. Leaflets are about 1.2-2.6 cm long, obovate of oblong, velvety on both sides inflorescencence is 6-12 flowered, 4.5 cm long or less. Flowers are zygomorphic. Calyx 5-lobed, flabrous, petal separate, clawed, pinkish to rose, corolla papilionaceous wing petals narrow, oblanceolate to oblong keel petals auriculate, spurred or gibbous, abruptly curved, or spirally coiled. Fruit a hairy legume, dehiscence oblong or ellipsoidal, coriaceous or becoming woody, 3-10 seeded. Seeds ovoid to rounded in outline, surface smooth, olive, brown or black in colour (USDA-NRCS, 2014) [4].

It occurs in secondary vegetation, is a weed of disturbed ground and often invasive. This is globally distributed tropical countries America, Australia, Asia and Africa, Bangladesh, Ethiopia. Within India, it has been recorded in Andhra Pradesh, Assam, Delhi, Goa, Jammu Kashmir, Karnataka, Tamil Nadu, West Bengal and Peninsular India.

Materials and Methods

Collection and authentication of plant

The plant *Indigofera trita* was collected from Ammapettai in Thanjavur district. The plant was authenticated by Dr. Jayaraman Director Plant anatomy and research centre, west Thambaram, Chennai. The plant identification number is (PAR/2015/3042).

Plant processing and extraction

The entire plant was cut into small pieces dried under the shed for 4 weeks at room temperature. The entire plant was shaded and dried for grinding to get crude power. 100 g of crude powdered drug were taken and shifted into filter paper thimble. 250 ml of methanol were poured into round bottom flask (1000 ml capacity) followed by fitting in on soxhlet apparatus. The powdered drug was extracted with methanol for 24 hours. A semisolid extract was obtained after completed elimination of methanol under reduced pressure. The extract was stored in refrigerator until use.

Extraction

The entire plant was shade dried and pulverized. The coarse powder of 500 gm packed in a soxhlet apparatus to continuous hot percolation, using 1.5 litres of methanol as a solvent. The extract was concentrated under vacuum and dried in a desiccator yield and 23.25 g (6.7% w/w).

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures of identify the constituents as described by Sofowara [5].

Test for alkaloids

About 2 g of powdered sample was stirred with a few drops of dilute hydrochloric acid and then filtered. The filtrate was used for following test with various alkaloid reagents (Table 1).

Sr.No.	Alkaloid reagent	Observation
1.	Mayer's reagent	Cream precipitate
2.	Dragondroff's reagent	Orange brown precipitate
3.	Hager's reagent	Yellow precipitate
4.	Wagner's reagent	Reddish brown precipitate

Table 1: Alkaloid reagents.

Test for carbohydrates and glycosides

To 5 g of extracts were dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

Molisch's test: The filtrate was treated with 2-3 drops of 1% alcoholic naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test. Violet colour was formed which indicates the presence of carbohydrates.

Fehling's test: The filtrate was treated with 1 ml of Fehling's solution and heated in a boiling water bath. A reddish orange precipitate was obtained.

Test for glycosides

2 g of extract was hydrolysed with hydrochloric acid for a few hours on a water bath and the hydrolysate was subjected to Legal's, Borntrager's test to detect the presence of different glycosides [6].

Legal's test: To the hydrolysate 1 ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. None of the extract produced pink to red colour.

Borntrager's test: Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal oruantity of dilute ammonia solution was added. No colour change in ammonical layer was observed.

Test for phytosterol

1 gm of the extract was dissolved in few drops of dilute acetic acid, 3 ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid Appearance of bluish green colour shows the presence of phytosterol.

Test for fixed oils and fats

a) Small quantity of the extracts was separately pressed between two filter papers oil stain on the paper indicates the presence of fixed oil.

b) Few drops of 0.5 N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on water bath for 1.2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats [7].

The extract was diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The presence of saponins was indicated by formation of 1 cm layer of foam.

Test for tannins

i) 2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins [8].

ii) To 2 ml of the extract was added 5% dilute ferric chloride solution a violet colour was formed indicating the presence of tannins.

Test for proteins and amino acids

Dissolve small quantities of various extract in a few ml of water and treated with [9]:

Millon's reagent – Red colour

Ninhydrin reagent – Purple colour

Biuret test – Pink colour

Test for gums and mucilages

About 10 ml of the extracts was added to 25 ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates [10].

Results

The results obtained from the various *in vitro* and *in vivo* studies during the course of experiment are given in this chapter.

Preliminary phytochemical analysis of *Indigofera trita*

Qualitative determination of phytoconstiuents: The first part of some phytoconstituents in the methanolic extract of entire plant of *Indogfera trita* was analysed. The results were given in (Table 2).

S.No.	Phytoconstituents	Indication
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4.	Phytosterols	+
5.	Saponins	+
6.	Fixed oils and fats	-
7.	Tannins and phenols	+
8.	Proteins and free amino acids	+
9.	Gums and mucilage	-
10.	Lignin	+
11.	Flavonoids	+
12.	Volatile oils	+
13	Present	+
14	Absent	-

Table 2: Analysis of preliminary phytochemical analysis in methanolic extract of *Indigofera trita*.

In this investigation, the phytochemical analysis confirm the presence of alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, phenols, proteins, free amino acids, lignin, flavonoids volatile oils and absence of gums and mucilage and fats and fixed oils. Pharmacological activity of the plant is attributed to the presence of these compounds.

Fluorescence analysis

After detecting the phytochemicals, the fluorescence analysis of methanolic extract of *Indigofera trita* was done by various chemical tests by daylight and UV light was used to determine the compound was identified by various colours (Table 3).

S.No.	Chemical test	Methanolic Extract	
		Daylight	UV light
1.	Sample as such	Greenish brown	Light green
2.	Extract with aqueous NaOH	Yellow	Dark green
3.	Extract with alcoholic NaOH	Dark Yellow	Yellowish green
4.	Extract with HCL	Yellowish brown	Light green
5.	Extract with 50% HNO_3	Brownish yellow	Yellowish green
6.	Extract with 50% H_2SO_4	Brownish yellow	Greenish yellow
7.	Extract with methanol	Yellowish green	Green
8.	Extract with ammonia	Light yellow	Green
9.	Extract with I_2 solution	Dark brown	Fluorescent green
10.	Extract with $FeCl_3$	Yellowish brown	Fluorescent green

Table 3: Fluorescence analysis of methanolic extract of *Indigofera trita*.

The characteristic of fluorescence properties or colours emitted by the powdered *Indigofera trita* before and after treating with reagents were observed. The powdered *Indigofera trita* appeared greenish brown under daylight light green in ultraviolet radiation. After treating with various reagents such as aqueous NaOH and methanol dark the powdered *Indigofera trita* appeared yellow in day light and yellowish green in UV light.

Treating with HCL and HNO_3, H_2SO_4, ammonia and iodine under day light, it showed different shades of yellow and green. Light yellow colour showed in day light treated with ammonia and green colour in UV light. After treating with iodine solution and ferric chloride, dark brown, yellowish brown in daylight, fluorescent green in UV Light.

The characteristic fluorescent properties or colour observed through this study could be used as a standard in the identification and

authentication of the entire plant of *Indigofera trita* in its extracted form.

Proximate analysis

The proximate analysis in methanolic extract of *Indigofera trita* expressed the value in percentage (w/w) in total ash (4.2),water soluable ash (1.26), acid insoluble ash (1.8), sulphated ash (3.72), methanol soluble extractive (15.16), water soluble extractive (15.16), crude fibre content (1.6), foaming index (<100), and loss on drying (3.6) (Table 4).

S.No.	Parameters Determined	Values in (%) w/w
1	Total ash	4.2
2	Acid-insoluble ash	1.8
3	Water soluble ash	1.26
4	Sulphated ash	3.72
5	Methanol Solube extractive	18.26
6	Water soluble extractive	15.16
7	Loss on drying	3.6
8	Crude fibre content	1.6
9	Foaming index	< 100

Table 4: Proximate determination in methanolic extract of *Indigofera trita*.

These ash values are important pharmacognostic tool to standardize the crude drugs. The extracts obtained by exhausting plant materials with specific solvents are indicative of approximate measures of their chemical constituents extracted with those solvents from a specific amount of air-dried plant material.

Quantitative determination of phytonutreints

In this investigation, presence of quantitative phytochemicals in methanolic extract of *Indigofera trita* expressed the value in mg/g phenols was (5.36 mg/g), tannins (2.02 mg/g), flavonoids (3.42 mg/g) and alkaloids (4.20). Finding the natural substance of medicinal plant that decrease the inflammation and reduce oxidative stress and there by counteracting the macromolecular damage (Figure 2). Flavonoids and phenols in general are highly effective in scavenging free radical and providing antioxidant defence in living cells. Quantitative analysis of methanolic extract of *Indigofera trita* were given in Table 5.

Phytochemical(mg/g)	Values in (mg/g)
Phenols	5.36
Tannins	2.02
Flavonoids	3.42
Alkaloids	7.2

Table 5: Quantitative phytochemical analysis of methanolic extract of *Indigofera trita*.

In medicine, it is used in antioxidant, anti-cancer, anti-inflammatory [11] central nervous system activities and weight loss etc. The plant having saponins are *Indigofera trita* Linn. Glycosides, Volatile oils and fats were absent in *Indigofera trita* Linn. Tannins were reported that certain tanning were reported that certain tannings were able to inhibit HIV replication selectively besides uses as diuretics and it is recognized for their pharmacological properties and are known to make trees and shrubs a different meal for caterpillars [12]. Apart from tannin and phenolic compound alkaloids and flavonoids are potent water soluble anti-oxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity [13] and also anti-arthritic activity [14].

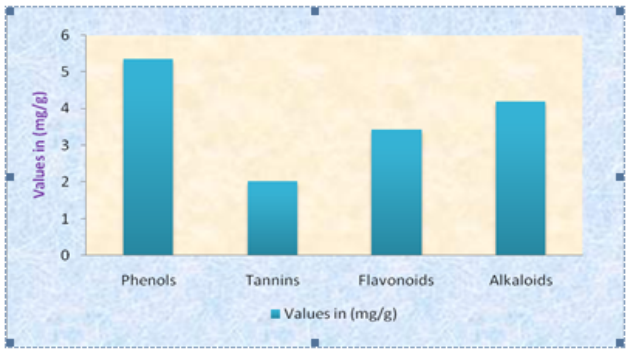

Figure 2: Quantitative phytochemical analysis on methanolic extract of *Indigofera trita*.

Discussion and Conclusion

Rheumatoid arthritis (RA) is a common severe joint disease affecting in all age groups. It is great importance to develop new strategies for its treatment. As a number of disease –modifying anti-rheumatic drugs (DMARDS) often have side effects at high doses and during long term administration, the search for new pharmacologically active agents obtained by screening natural sources is warranted.

Preliminary determination of phytochemicals

The Phytochemical screening in the present study has revealed the presence of alkaloids, carbohydrates, glycoside, phytosterols, saponins, tannins, phenols, proteins, amino acids, lignin, terpenoids, volatile oils. Flavonoids are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found in the methanolic extracts of *Indigofera trita*, it might be responsible for the potent antioxidant capacity.

In Quantitative analysis of *Indigofera trita* presence of phenols and flavonoids alkaloids, terpenoids, tannins, are one of the most diverse and widespread groups of natural compounds in plant species. In the present study the phenols and flavonoids content seems to be more in methanolic extract of *Indigofera trita*. Reported by Yanishlieva [8], flavonoids are found to be better antioxidants and have multiple biological activities including vasodilatory, anti-carcinogenic, anti-inflammatory, antibacterial, immune-stimulating, anti-allergic, antiviral and radioprotective effects.

The results show that the extractable high molecular weight phenolic compounds in methanol extract of *Indigofera trita*. Tannins

are phenols known for scavenging the hydroxyl radical by in direct interaction with radical. Tannin-protein complex was also found to be potential free radical scavenger, radical sinks and prevent the radical mediated diseases occurring in the gastrointestinal tract including peptic ulcer.

Polyphenolic compounds is a highly inclusive term that covers a wide group of phytochemicals, including well known subgroups of phenolic acids, flavonoids, natural dye, lignins etc., it is produced by plant as a secondary metabolites is represent a potential source with significant amount of antioxidants to prevent oxidative stress caused by free radicals. In the present study, methanol extract of *Indigofera trita* was reported to possess polyphenoilc compounds exhibits its antioxidant activity by chelating redox- active metal ions, in activating lipid free radical chains and preventing hydroperoxide conversion in to reactive oxyradicals and other biological properties includes diffusion of toxic free radicals, altering signal transduction, activation of transcription factors and genes expression by Raju Senthilkumar et al. [9].

Determination of fluorecence analysis

Fluorescence is the phenomenon the colours emitted by treating with various chemical regents by various chemical. Some phytochemical constituents show fluorescence of the visible range in daylight and UV light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. The substances themselves are not fluorescent; they may often be converted into fluorescent derivatives or decomposition of products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [9].

After detecting the phytochemicals, the flurescencce analysis of *Indigofera trita* in chemical test by daylight and UV light the extract appeared greenish brown and green colour. The plant is reliable possess 2 large number of medicinal value and the colour was undertaken as a phamacognostic standardization.

Determination of proximate analysis

The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash. These ash values are important pharmcognostic to standardize the crude drugs [10].

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