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EVALUATION OF PHYTOCHEMICAL CONSTITUENTS OF THE EXTRACTS OF *INDIGOFERA SUFFRUTICOSA* LEAVES

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ABSTRACT

Indigofera Suffruticosa is an Indian herb used for various ailments by traditional healers and it is a small tree plant in the family Fabaceae, traditionally used in treatment of several diseases (fever, headaches, hemorrhages, convulsions, acute cough and skin parasites). The present study was carried out to investigate the phytochemical profile of leaves of *Indigofera Suffruticosa*. The powder of the leaves of *Indigofera Suffruticosa* was successively extracted with acetone, benzene, chloroform, cyclohexane, ethylacetate and methanol. Phytochemical analysis shows the presence of carbohydrates, glycosides, proteins, amino acids, phenolic compounds, tannins, steroids, anthraquinone, anthocyanin, flavonoid, and alkaloid. The result of the study could be useful to description and foundation of monograph of the plant.

KEY WORDS

Tannins, Glycosides, Phytochemical analysis and *Indigofera Suffruticosa*.

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INTRODUCTION

Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis¹. Plants are known to be the source of many chemical compounds and also used medicinally in different countries and are a source of many potent and powerful drugs. *Indigofera suffruticosa* is a Wild indigo, also known as indigo, Guatamala indigo, anil, anil de pasto, and ti cafe, is a short-lived shrub that reaches 1 to 2 m in height and 1 to 2 cm in stem

diameter. The shrub may have multiple stems, especially if it has been disturbed by grazing or fire. The stems are gray-brown, pubescent, and more or less straight. The light green leaves are pinnately compound with 9 to 17 narrowly oblong, pubescent leaflets that are 1.5 to 2.5 cm long and about 9 mm wide. There are 6- to 8-mm lanciolate stipules at the base of the leaves. Crimson to rust-red flowers grow in short, many-flowered racemes. The curved legumes are short (1.1 to 2.5 cm) and contain three to seven seeds that are 1.5 mm wide and 1 mm thick^{2,3}. *Indigofera Suffruticosa* species became important commercial crops in various tropical and subtropical areas. The blue dye was produced by fermentation of the leaves, usually with caustic soda or sodium hydrosulfite, and the exudates processed into dry cake. The blue color developed as the cake was exposed to the air⁴. Poultices and extracts of wild *indigofera suffruticosa* leaves, alone or in combination with other ingredients, are used in herbal medicine to treat fever, headaches, hemorrhages, convulsions, acute cough, skin parasites, and boils⁵. Thus the present study was carried out to evaluate the phytochemical constituents present in the medicinal herb of *Indigofera Suffruticosa* leaves.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Indigofera Suffruticosa* were collected from Vaithiynathpuram Village in Perambalur District of Tamilnadu, India during January to December 2012 and authenticated by the Director of the Ranint Herbarium and Centre for Molecular Systematic, St. Joseph's college (campus), Trichirappalli, Tamilnadu, India. Fresh leaves were cleaned with running tap water and dried under the shade. Then the dried plant leaves were ground to fine powder mechanically and preserved in airtight containers for further analysis.

PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical evaluation of *Indigofera Suffruticosa* was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered leaves of

plant (100g) were extracted in a Soxhlet apparatus with acetone, chloroform, cyclohexane, ethanol, ethyl acetate and methanol successively. The resultant extracts were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids and steroids etc.

The percentage extractive yield was calculated by formula as mentioned below:

$$\% \text{ Extractive yield (w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant powder}} \times 100$$

Test for flavonoids

Shinoda test (Magnesium Hydrochloride reduction test)

To *Indigofera Suffruticosa* extract, 5ml. 95% ethanol was added. The mixture was treated with 0.5g magnesium turnings and few drops of conc. HCL. Pink color, if produced, may confirm the presence of flavonoids.

Alkaline reagent test

Small quantity of each extract sample was taken and added with lead acetate solution. After few minutes appearance of yellow color precipitates which indicated the presence of flavonoids.

Test for Phenolics and Tannins

Ferric chloride test

Small amount of *Indigofera Suffruticosa* extract were shaken with water separately and warmed. Then about 2 ml of 5% ferric chloride solution was added and observed for the formation of green or blue color which may indicate the presence of phenols.

Gelatin test

1 % gelatin solution containing 10 % sodium chloride was added to each extract. Formation of precipitate indicated the presence of tannins and phenolic compounds.

Iodine test

Indigofera Suffruticosa extract were treated with diluted iodine solution separately. Appearance of transient red color indicated the presence of tannins and phenolic compounds.

Nitric acid test

Indigofera Suffruticosa extract were treated with dilute nitric acid separately. Formation of reddish to

yellowish color indicated the presence of tannins and phenolic compounds.

Test for Alkaloids

Dragendroff's test

Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added to each extract and observed for the formation of orange yellow precipitate which may indicate the presence of alkaloids.

Mayer's test

Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added to each extract and observed for the formation of white or cream color precipitate which may indicate the presence of alkaloids.

Hager's test

Few drops of Hager's reagent (saturated aqueous solution of picric acid) were added to each extract and observed for the formation of yellow precipitate which may indicate the presence of alkaloids.

Wagner's test

Few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to each extract and observed for the formation of reddish brown precipitate which may indicate the presence of alkaloids.

Test for Amino acid and Proteins

Biuret test

To each of the extract were treated with 1 ml 10 % sodium hydroxide solution separately and heated. A drop of 0.7 % copper sulphate solution to the above mixtures was added. The formation of purplish violet color may indicate the presence of proteins.

Million's test

3 ml test solutions were mixed with 5 ml Million's reagent separately. White precipitate was formed which on heating turned to brick red. It may indicate the presence of amino acids.

Test for Glycosides

Keller kelliani test

To each of the extract were treated with chloroform and evaporate it to dryness. Separately 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added and transferred to a small

test tube added with carefully 0.5 ml of conc.H₂SO₄ acid by the side of the test tube; blue color appears in the acetic acid layer indicating the presence of glycosides.

Borntrager's test

To each of the extract were boiled with 1 ml of dilute H₂SO₄ in a test tube separately for 5 min, filtered while hot, pipette out the supernatant or filtrate, cooled and shaken with an equal volume of dichloromethane. The lower levels of dichloromethane separated and shaken with half its volume with dilute ammonia. A rose pink to red color appeared in the ammonical layer, indicating the presence of glycosides.

Froth test

To each of the extracts were treated with water in a semi-micro tube separately shaken well. The froth appeared thus indicating the presence of glycosides.

Test for Carbohydrates

Molish's test

To each of the extract were treated with 2 drops of alcoholic α -naphthol solution in a test tube separately and 2 ml of conc.H₂SO₄ was added carefully along the sides of the test tubes. Formation of violet ring at the junction may indicate the presence of carbohydrates.

Fehling's test

To each of the extract were treated in equal volumes with 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute separately. The mixtures were boiled for 5-10 minutes on water bath. Reddish brown color was obtained due to formation of cuprous oxide which indicated the presence of reducing sugar.

Benedict's test

To each of the extract were treated with equal volumes of Benedict's reagent in test tubes separately. The mixtures were boiled for 5-10 minutes on water bath. Solution appeared green, yellow or red depending on amount of reducing sugar present in each filtrate.

Test for Steroids

Salkowsti's Test

To each of the extract was dissolved in 2ml of chloroform. Conc.H₂SO₄ was carefully added to

form a low layer. A reddish brown color in the interface is indicative of steroidal ring.

Test for Anthraquinone

Borntrager's test

To each of the extract was put in a test tube and 5ml of chloroform added and shaken for 5 minutes. This was filtered and shaken with equal volume of 10% Ammonium solution. A pink violet or red color in the ammonical layer (lower layer) is indicative of the presence of anthraquinone.

Test for Anthocyanin

Sodium Hydroxide Test

Weigh about 0.2gm of plant each extract in separate test tube, 1ml of 2N Sodium hydroxide was added, and heated for 5 minutes at $100^{\circ} \pm 2^{\circ}\text{C}$. A bluish green color is indicative of the presence of anthocyanin.

RESULT AND DISCUSSION

The study revealed that the plant extracts of *Indigofera suffruticosa* leaves were positive for carbohydrates, glycosides, proteins, amino acids, phenolic compounds, tannins, steroids, anthraquinone, anthocyanin, flavonoids and alkaloids. Most of the phytochemical constituents were present in the extracts of methanol, ethylacetate and cyclohexane. Similarly, the

acetone and chloroform extract of the plant revealed the presence of carbohydrates, glycosides, proteins, amino acids, phenolic compounds, alkaloids and tannins, but in the benzene extract of plant were found in alkaloids, glycosides, phenolic compounds and tannins. Table 2 showed the results of phytochemical screening of various extracts of *Indigofera suffruticosa* leaves. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities⁶. The result reveals that the plant extracts owing to their phytochemical constituents, are very important and beneficial in industrial and medicinal sciences. Tannins are anti- viral, anti-bacterial, and anti-tumor in effectiveness. Certain tannins are also able to inhibit HIV replication, selectivity, and diuretic⁷. Glycosides are a class of medications used to treat heart failure⁸. Anthraquinones are used as dyes and antibacterial agents. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity⁹. Flavonoids are effective antioxidant and show strong anticancer activities^{10, 11& 12}. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds^{9,10}.

Table No.1: Physical Characteristics and % Yield of Various Extracts of *Indigofera suffruticosa* Leaves

Solvent	Color of the extract	Odour	Consistency	Sense Of touch	Amount of extract(gm)	% of yield (w/w)
Methanol	Brown	Characteristic	Semisolid	Sticky	7.50	7.5
Cyclohexane	Yellowish black	Characteristic	Semisolid	Sticky	5.10	5.1
Ethylacetate	Light green	Characteristic	Semisolid	Sticky	6.10	6.1
Benzene	Greenish yellow	Characteristic	Semisolid	Sticky	3.20	3.2
Acetone	Yellowish green	Characteristic	Semisolid	Sticky	3.10	3.1
Chloroform	Brown	Characteristic	Semisolid	Sticky	2.50	2.5

The % yield was maximum (7.5%) obtained with methanol and least (3.1%) with acetone.

Table No.2: Phytochemical Screening of Various Extracts of *Indigofera suffruticosa* Leaves

Chemical test	Acetone extract	Benzene extract	Chloroform extract	Cyclohexane extract	Ethylacetate extract	Methanol extract
Flavonoids						
Shinoda test	+	-	-	+	+	+
Alkaline reagent	+	-	+	+	++	++
Phenolic compounds & Tannins						
Ferric chloride test	-	+	+	+	++	++
Gelatin test	-	-	++	-	-	+
Iodine test	-	+	-	+	+	+
Nitric acid test	+	-	-	+	-	-
Alkaloids						
Dragendroff's reagent	+	+	-	++	+	++
Mayer's reagent	-	+	+	-	++	-
Hager's reagent	+	+	+	+	+	++
Wagner's reagent	+	+	+	++	+	++
Amino acids & Proteins						
Biuret test	+	-	+	+	+	++
Million's reagent	+	-	+	+	++	+
Glycosides						
Molish's test	-	+	+	+	++	++
Fehiling's test	+	-	-	+	++	++
Benedict's test	+	-	+	-	+	+
Steroids						
Salkowsti's Test	-	-	-	+	+	-
Anthraquinone						
Borntrager's test	-	-	-	+	-	+
Anthocyanin						
Sodium Hydroxide Test	-	-	-	+	-	+

++ : Present in high concentration;

+ : Present in trace concentration;

- : Constituents not detectable using the specified assay method

CONCLUSION

The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

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