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Preliminary Phytochemical analysis of *Indigofera trita* L. (Fabaceae)

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Abstract

The present study aims the chemical composition of the entire plant of *Indigofera trita* L. carried out by analyzing sample collected from Yercaud hills in the Eastern Ghats of Tamil Nadu, for chemical composition. The result of analysis shows that the presence of various chemical constituents like carbohydrates, glycosides, alkaloids, tannins, saponins, phenolic compounds, phytosterols and flavonoids were present. The significant amount of phyto-constituent of this plant may open the new window to pharmacological research in different dimensions.

Keywords: *Indigofera trita*, Chemical composition, Characterization, TLC

Introduction

Today lots of plant materials are used in the products of enormous array of medicines reported that 50 % of all modern chemical drugs are of natural product origin (Binu Thomas *et al*., 2011; Umapriya *et al*., 2011). It was reported that 60 - 80 % of the population in every developing countries of the world relics on medicinal plants in the treatment of some diseases. Although the actual numbers of the medicinal plant is not known but there is no doubt that most of the plants around us are medicinal (Ranganathan *et al*., 2012; Binu Thomas *et al*., 2012). The medicinal plants are the groups of plants in which one or more of its parts contain substances that can be for the synthesis of useful drugs Sharma and Kumar, 2011; Binu Thomas *et al*., 2012).

Indigofera trita L. is commonly known as '*Kattavuri*', growing widely in Southern India. It is an under shrub, branches hoary with fine appressed hairs. Leaves trifoliate, leaflets all obovate-oblong with fine appressed gray hairs, flowers small, 6-12 flowers in spicate racemes, salmon coloured. Pods rigid, straight, 4-gonous, spine pointed, not tortulose, silvery with appressed hairs. Seeds 6-10 oblong. It is used by the tribes and native medical practioners to treat much kind of diseases such as rheumatism, inflammation, tumor and liver diseases (Stuffness and Douror, 1982; Kirtikar and Basu, 1993; Safowora, 2000; Hailu *et al*., 2005).

This study is designed to determine the chemical composition and characterization of the isolated compounds of the entire plant of *Indigofera trita* L. for public and health awareness of its chemical status (Fig.1).

A. *Indigofera trita* L. Habit.

Materials and Methods

Plant material Preparation

The entire plant of *Indigofera trita* L. were collected from Yercaud Hills of Salem district of Tamil Nadu and identified with *Flora of the Presidency of the Madras* (Gamble, 1915- 1936) and *Flora of Tamilnadu Carnatic* (Matthew, 1983). The plant material was dried in shades, coarsely powdered and passed through No.40 sieve and was used for extraction. The shade dried coarsely powdered plants of *Indigofera trita* (500 gms) was extracted exhaustively in a soxhlet apparatus with alcohol 95 % v/v (75 - 78°C) for 72 hours until the extraction was completed. After completion of extraction, the solvent was removed by distillation. A dark brown colour residue was obtained and these residues were stored in desiccator.

Identification of phytochemical tests

The test were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, proteins, free aminoacids, phenolic compounds and tannins, fixed oils and fats.

Alkaloids

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added (Siddiqui and Ali, 1997). Most alkaloids are precipitated from natural or slightly acidic solution by Mayer's reagent (Evans, 2002). The alcoholic extract was evaporated to dryness and residue was heated on a boiling water bath 2 % hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were observed for the presence of turbidity or yellow precipitation

Glycosides

Glycosides are compound which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green colour in upper layer (Evans, 2002).

Flavonoids

Four milliliters of extract solution was treated with 1.5 ml. of 50 % methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones (Evans, 2002).

Tannins

To 0.5 ml. of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for cateholic tannins (Iyengar, 1995).

Carbohydrates

The filtrate was treated with 1 ml of Fehling's A and B and heated on a water bath. A reddish precipitate was obtained shows the presence of carbohydrates.

Fixed oils and fats

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantities of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats.

Proteins and free amino acids

A small quantity of various extracts was dissolved in few ml. of water and then they were subjected to treat with Millon's reagent. Red colour was formed shows the presence of proteins and frees amino acids.

Sapanins

The extracts were diluted with 20 ml. of distilled water and it was agitated in a measuring cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

Phytosterols

Small quantities of various extracts were dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to Libermann Burchard test, treated with a few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid and 3 ml of acetic anhydride. A bluish green colour was appeard indicates the presence or phytosterols.

Gums and Mucilage

A small quantity of various extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gum and mucilage.

Isolation of compounds

In view of the enormous medicinal value of the *Indigofera trita* L. is taken up for TLC studies. The TLC plates (20 × 20 cm) were coated with 1 mm thickness of silica gel G (Experiment-Merck). After airdrying the slurry on TLC plates in open for 30 minutes, the silica gel was activated by heating the plates in an oven at 110°c for 2 hr.

The TLC plate was placed in the TLC chamber, which was saturated with mobile phase. The plate was run till the solvent attained ¾ th of the plate, then taken out and allowed to dry. An admixture of the extract (Ethyl acetate) with little amount of silica gel was prepared and it was loaded on the top of the stationary phase. Efluent is strongly passed through the column to advance the organic material. The individual components are retained by the stationary phase differently and separate from each other while they are running at different speeds through the column with the eluent. At the end of the column they elute at a time. During the entire chromatography process the eluent was collected in series of fractions.

Results and Conclusion

The results of the determination of the extractive values, the presence of various phyto constituents, fluorescence analysis, properties of common flavonols and flavones are given in Table-:1,2,3,4 and Plate 1). The presence of various phyto-constituents mainly is acetone, alcohol, aqueous extracts showed the acetone, alcohol and water extracts are same type of constituents. Hence, the alcohol extracts which has the polarity in between the acetone and aqueous, it has been taken for further studies.

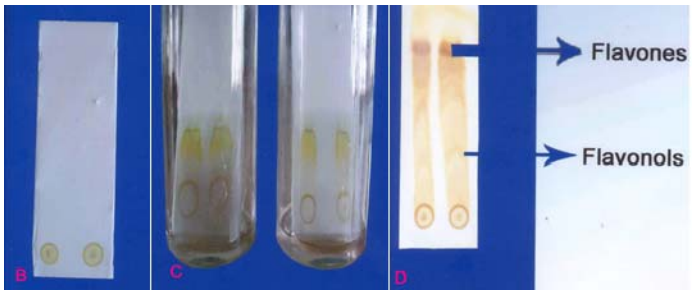

Fig. B, C and D: Ethyl acetate spot on TLC

Table- 1: Data showing the extractive values of *Indigofera trita* L.

Plant Name	Part Used	Method of extraction	Yield in Percentage					
			Petroleum ether	Chloroform	Acetone	Alcohol	Aqueous	Ethyl acetate
Indigofera trita Linn	Entire plant	Continuous hot percolation process	8.25	1.08	3.34	4.64	4.8	4.26

Table- 2: Data showing the various extracts of *Indigofera trita* L

Extract (s)	Carbohydrate	Glycosides	Fixed Oils & Fats	Proteins Oils & Amino acids	Saponins	Tannis	Phyto sterols	Alkaloids	Phenolic compounds	Flavonoids	Gums & Mucilages
Petroleum ether	+	-	+	-	-	-	+	-	-	-	-
Chloroform	+	-	-	+	-	-	-	+	-	-	-
Acetone	+	+	-	+	+	+	-		+	+	-
Alcohol	+	+	-	+	+	+	-	+	+	+	-
Aqueous	+	+	-	+	+	+	-	-	+	+	-

+ = Presence: - = Absence

Table 3: Fluorescence analysis of *Indigofera trita* powder

SI. No	Treatment	Day light	UV light
1	Powder as such	Light Brown	Grayish Brown
2	Powder + 1ml HCl	Dark Brown	Dark Brown
3	Powder + 1ml HNO_3	Yellowish Orange	Dark Yellowish red
4	Powder + 1ml HCl in Ethanol	Yellowish Green	Dark Brown
5	Powder + 50% H_2SO_4	Dark Brown	Black
6	Powder + Ethanol	Light Brown	Light Green
7	Powder + Acetone	Light Brown	Light Brown
8	Powder + Chloroform	Light Brown	Light Brown
9	Powder + H_2O	Light Brown	Light Green

Table - 4: Properties of common flavonols and flavones (Harborne-1973)

Flavonoid	Rf (*100) in Forestol	BAW	PhOH	Colour in UV and UV plus ammonia
Flavonols				
Kaempferol	55	83	58	
Quercetin	41	64	29	bright yellow
Myricetin	28	43	13	
Isorhamnetin	53	74	60	bright yellow fluorescent
Azaleatin	49	48	50	
Gossypertin	26	31	12	yellow dull black
Flavones				
Apigenin	83	89	88	
Luteolin	66	78	66	dull ochre → bright yellow or yellow green
Chrysoeriol	77	82	90	
Tricin	72	73	87	
Glycosylflavones	Water			
Vitexin	06	41	63	
Isovitexin	16	56	79	dull ochre → bright yellow or yellow green
Orientin	02	31	43	
Iso- orientin	09	41	51	
Orientin	02	31	43	yellow or yellow green
Iso-orientin	09	41	51	
Biflavonyl-Kayaflavone	00	98	99	dull brown

The composite chromatogram is obtained in ethyl acetate was formed with iodine. The colour of constituents was obtained yellow, bright yellow are quite characteristic for particular flavonoids. The result of the phytochemical screening of *Indigofera trita* L. indicates that carbohydrates, proteins, glycosides, saponins, tannins, phenolic compounds, flavonoides were the major phytochemicals identified in the entire plant extract. Such phytochemical screening sheds lights on the new way to pharmacological research. Nowa days this type research is an

urgent need for the validation of our natural resources for future generation.

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