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Phytochemical and HPTLC studies of methanolic extract of *Indigofera tinctoria* (Fabaceae)

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Abstract

The aerial parts (fresh leaves) of the plant Indigofera tinctoria were collected powdered and the powder was initially defatted with petroleum ether (60-80°C). The marc was further macerated with methanol for 72 hrs. The extract was preserved at room temperature for further studies.Preliminary Phytochemical screening was done which revealed the presence of sugars, tannins, polyphenols, alkaloids, aminoacids, triterpenoids, saponins, flavoniods.The HPTLC techniques were used for qualitative determination of possible number of components from the methanolic extract . A solvent system was optimized in order to get maximum separation on plate and presence of various phytochemicals present was confirmed by the use of different spraying reagents.

Key-Words: *Indigofera tinctoria, HPTLC of Indigofera tinctoria*

Introduction

Indigofera tinctoria is a widely distributed small erect medicinal shrub belonging to the family of fabaceae, found throughout India. The plant has proved to be more effective against chronic myelogenous and other leukemia's (Steriti, 2002). The roots, stems and leaves are bitter, thermogenic, laxative, trichogenous, expectorant, anthelminitic, tonic and diuretic, and are useful for promoting the growth of hair and in gastropathy, asthma, ulcers and skin diseases. Isolation of flavanoids apigenin, kaempferol, luteolin and quercetin from the plant has been reported (Kamal and Mangla, 1990). *Indigofera tinctoria* was found to contained carotenoids, coumarins and flavanoids (Mohmammed, *et al*., 1994).

Material and Methods

Plant material

The leaves of the plant material *Indigofera tinctoria* Linn. were collected from the Department of Agriculture, Annamalainagar Chidambaram, Cuddalore district, Tamil Nadu, India. The plant was authenticated by the Botanist, Department of Botany, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, and India.

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Preliminary Phytochemical Screening

The plants may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins, flavonoids etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of primary and secondary metabolites all the extracts were subjected to battery of chemical tests(Kandelkar).

Thin Layer Chromatography of Methanolic extract

Methanolic extract of *Indigofera tinctoria* was subjected to thin layer chromatographic studies, to find out the probable number of compounds present in them.

Extraction & Test solution preparation

The given dried Plant materials (5gm) were extracted with Methanol in Soxhlet apparatus for 3hrs. Cooled, filtered the content and concentrated using Vacuum flash evaporator. Dissolved the content with 1ml Methanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

Sample loading

1.5µl of the above test solution and 2µl of standard solutions were loaded as 6mm band length in the 4 x 10 Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Flavonoid) and

the plate was developed in the respective mobile phase up to 90mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm.

Derivatization: The developed plate was sprayed with respective spray reagent (Flavonoid) and dried at 100°C in Hot air oven. The plate was photo-documented at Day light and UV 366nm using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning

Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254nm. The Peak table, Peak display and Peak densitogram were noted.

HPTLC Study

HPTLC precoated, silica gel G 60 F25 (Merck, Germany) plates were used for application of sample. A small quantity of extract was dissolved in methanol and sample was applied in precoated plate with the help of Linomat IV applicator. Solvent system optimized for TLC study was chosen for HPTLC study. The details of HPTLC were as follows:-

Plate : Aluminium plate precoated with silica gel GF_{254}
Thickness : 0.2 mm
Plate size : 10 × 10 cms
Sample application : 10 µl
Solvent system : Toluene-acetone-formic acid (4.5: 4.5: 1)
Detection : U.V. (254,366 nm)

Instrument : CAMAG TLC Scanner 3 & LINOMAT- V

CAMAG TLC Scanner 3 & LINOMAT- V densitometric evaluation system with WINCAT software was used for scanning of thin layer chromatogram objects in reflectance or transmission mode by absorbance or by fluorescence at 254 and 366 nm respectively.

Results and Discussion

The preliminary phytochemical screening of the methanolic extract revealed the presence of chemical constituents like Steroids, Tannins, Flavonoids, Carbohydrates, Saponins, Amino acids, alkaloids and flavanoids which was confirmed by performing TLC separation technique and different spraying reagents. The use of medicinal plants as Herbal Drugs, extractrs have significantly increased throughout the world in the recent decades. According to WHO 80% of world population uses herbal remedies to cure diseases. Taking into consideration the popularity of the medicinal plants, these traditional drugs should be evaluated in detail for their Pharmacognosy, Phytochemistry and Pharmacology. Such a standardization of traditional medicine is the step to tap the emerging export market for natural drugs. All these medicinally important traditional crude drugs and their extracts should be standardized for its quality, purity and safety by using techniques such as organoleptic, microscopical, physical, chemical, biological evaluation. Pharmacognostic study mainly involves correct identity of crude drug with the help of drug organoleptic (sensory characters), microscopical (histological characters), physical and preliminary chemical evaluation. *Indigofera tinctoria* has global market and so it has very great commercial importance. Present study has given very important information about standardization parameters of such a plant which will certainly useful in authentication and in avoiding adulteration.

The present studies on physicochemical parameters and preliminary phytochemical screening, phytochemical and elemental analysis provide useful information which may help in authenticating the plant along with nature of phytoconstituents present in it. HPTLC shows clear separation of components present in the methanol extract of the plant powder of *Indigofera tinctoria*. The method may be applied to identify the plant of *Indigofera tinctoria* from other species. HPTLC fingerprint enables a particular plant to be identified and distinguished from closely related species (Houghton 1998). The methanolic extract of *Indigofera tinctoria* having the potentiality to scavenge the free radical contains terpenoids which was analysed by using HPTLC. The peak table, peak display and peak densitogram were noted. Blue-violet colored zones were detected from the chromatogram after derivatization which confirmed the presence of Flavanoids (Figure 4). Thus, the presence of flavanoids in the methanolic extract of the aerial part of the plant of *Indigofera tinctoria* was confirmed by HPTLC analysis. The extract was run along with the standard flavanoid compound and it was observed that the extract showed the presence of presence of flavanoids and it was confirmed from the chromatogram after derivatization. The Rf value of the different compounds present in the extract was found to be 0.09, 0.02, 0.13, 0.17, 0.29, 0.51, 0.71, 0.79, 0.96 and 0.70 of peak 1, 2, 3, 4, 5, 6, 7 and 8 respectively. Among them peaks 1, 5, 6 and 1 were found to contain flavanoids. The peak height of the respective flavanoids was also given in the Figure 1 and Table 3.

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Table 1: Observation table of nature, colour, and yield of extracts

S/No.	Extract	Nature of Extract	Colour	Weight (g)	%Yield (w/w)
1.	Methanolic	Semisolid	Yellow-green	42.25	26.83
2.	N-hexane	Semisolid	Green-brown	69.20	28.18
3.	Petroleumether	Semisolid	Green- brown	25.7	12.8
4.	Chloroform	Semisolid	Yellow-green	13.32	7.6
5	Ethyl acetate	Semisolid	Yellow-green	23.42	12.9

Table 2: Results of qualitative chemical investigation

S/No.	Name of the test	N-hexane extract	Petroleum -ether extract	Chloroform extract	Ethylacetate extract	Methanol extract
1	**Test for sterols** a.Salkowaski test b.Liebermann Burchardt	–	+	–	–	+
2	**Test for Triterpenoids** a.Libermann Burchardt's b.Salkowaski test	–	+	–	–	+
3	**Test for glycosides** a.Keller-Killiani Test b.Baljet's Test	–	+	+	–	–
	Testforsaponins glycosides a. Foam Test	–	–	–	–	+
4	**Test for carbohydrates** a.Fehling's Test b.Benedict's test c.Molisch's Test d.Barfoed's Test	–	–	+	–	–

5	**Test for alkaloids** a.Dragendroff's Test b.Mayer's Test c.Hager's Test d.Wagner's Test	–	–	+	+	+
6	**Test for Flavanoids** a.Shinoda Test b.Ferric chloride Test c.Lead acetate Test	–	–	+	+	+
7	**Test for Tannins** a.Gelatin Test b.Ferric chloride Test c.Lead acetate Test d.Dil.HNO_3 Test	–	–	+	–	+
8	**Tests for Proteins** a.Xanthoprotein Test b.Million's Test c. Biuret Test	–	+	–	–	+
9	**Test for amino acids** Ninhydrin Test	–	–	+	–	+
10	**Test for fats& Volatile oils** Solubility Test	–	+	–	–	–

Table 3: Peak table

Track	Peak	Rf	Height	Area	Assigned substance
RUT	1	0.09	248.2	6618.5	Rutin
Sample A	1	0.02	208.1	2590.3	Unknown
Sample A	2	0.13	103.9	4351.3	Unknown
Sample A	3	0.17	80.2	1389.9	Unknown
Sample A	4	0.29	382.6	14779.1	Unknown
Sample A	5	0.51	117.6	3398.5	β-sitosterol
Sample A	6	0.71	115.8	2539.4	Eugenol
Sample A	7	0.79	69.8	1619.7	Unknown
Sample A	8	0.96	118.3	3280.0	Unknown
QUE	1	0.70	134.7	3737.6	Quercetin

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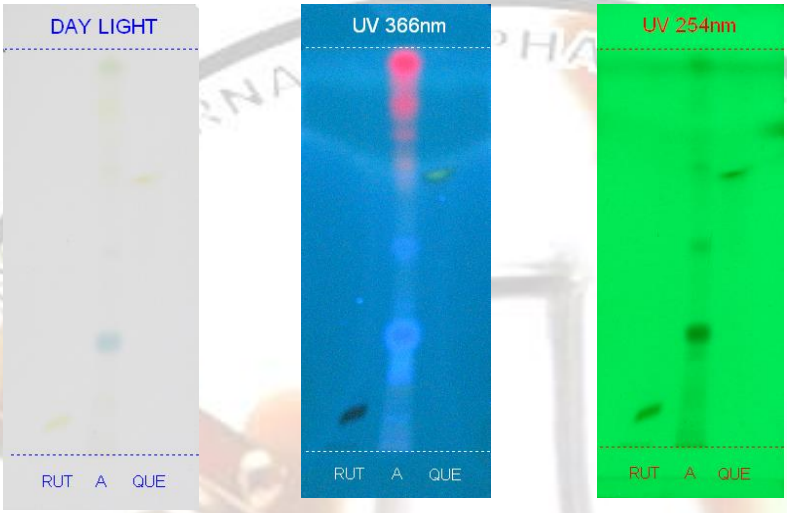
Chromatogram before derivatization



After Derivatization

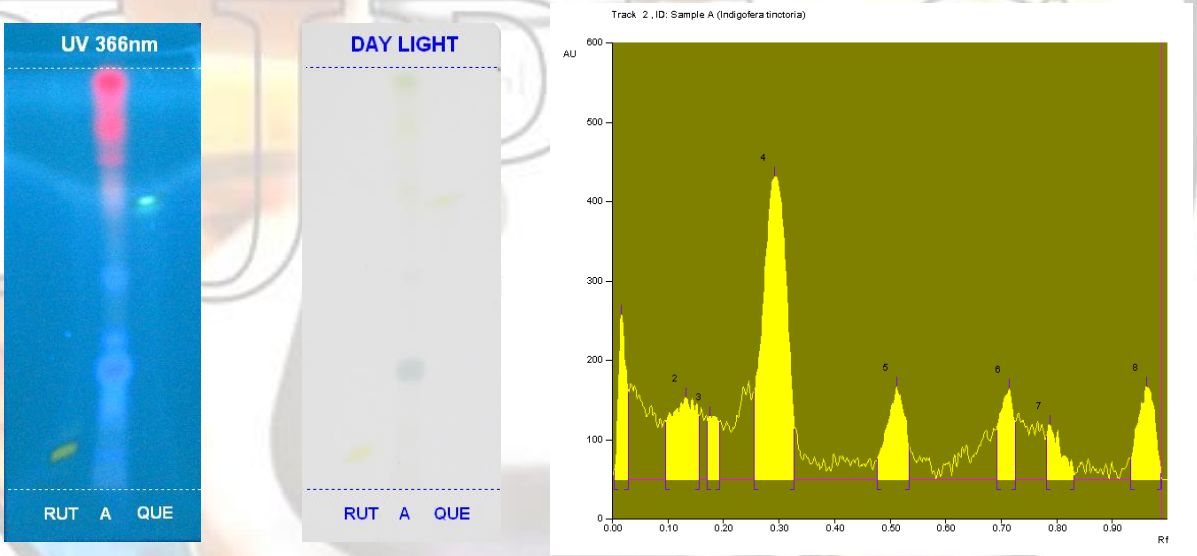


Fig. 1: Track A – Sample A *Indigofera tinctoria* Methanolic extract Plant sample Peak densitogram display (Scanned at 254nm)