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Research Article

IDENTIFICATION AND PHYTOCHEMICAL EVALUATION OF ETHANOLIC EXTRACT OF *INDIGOFERA ASPALATHOIDES* (SHIVANAR VEMBU)

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ABSTRACT

Objective: *Indigofera aspalathoides* is commonly called as 'Shivanar Vembu' in Tamil. The aim of the present work was to identify and phytochemical evaluation of ethanolic extracts of *Indigofera aspalathoides* leaves and its aerial parts by using HPTLC and FT-IR spectroscopic technique.

Methods: The leaves and aerial parts of *Indigofera aspalathoides* were dried, powdered and extracted using n-hexane, chloroform, ethyl acetate and ethanol, respectively. Extracts were then subjected to phytochemical screening for flavonoids and further confirmed using FT-IR and HPTLC techniques.

Results: Phytochemical studies revealed the presence of alkaloids, flavonoids, glycosides, tannins, phenol, terpenoids and steroids. Among bioactive compounds naturally occurring flavonoids have gained a particular interest because of the broad pharmacological activity. The FT-IR results shown that the sharp absorption peaks at 1616.73 cm^{-1} and 1618.66 cm^{-1} are assigned to C=O stretching vibration in carbonyl compounds which may be characterized by the presence of high content of flavonoids and terpenoids. The HPTLC procedure was identified the flavonoid group present in both sample extracts (T2&T3), when compare to Quercetin standard (T1). The mobile phase consisting of Toluene: Ethyl acetate: Acetic acid: Methanol (2.5:7.0:0.25:0.25) gave a visible band (Rf 0.74) for sample solution corresponding to Quercetin standard solution.

Conclusion: This study revealed the identification of flavonoids in ethanolic extract of *Indigofera asphalathoides* leaves and its aerial parts.

Keywords: Flavonoids, FT-IR analysis, HPTLC analysis, Shivanar Vembu (in Tamil)

INTRODUCTION

Indigofera is large genus of about 700 species of flowering plants belonging to the family Leguminosae (Fabaceae). It is widely distributed in tropical and subtropical regions. In the most recent treatment 81 species are distributed in China, of which three have simple or unifoliate leaves [1]. *Indigofera aspalathoides* is commonly called as 'Shivanar Vembu' in Tamil. In the traditional medicinal system the leaves, flowers and tender shoots are said to be cooling and demulcent, they are used in the form of decoction for leprosy and cancerous affection. The entire plant is traditionally used for various ailments including liver disorder and tumours [2]. The plant is very specific for psoriasis, secondary syphilis and viral hepatitis [3]. Roots are chewed as a remedy for toothache and aphthae [4]. Leaves are used to obtain blue black dye and the plant shows positive effect on colonization with arbuscular microflora fungi [5]. Phytochemical analysis shows the presence of alkaloids, flavonoids, glycosides, tannins, phenol, terpenoids and steroidal compounds. Among bioactive compounds naturally occurring flavonoids have gained a particular interest because of the broad pharmacological activity. Flavonoids are ubiquitous in plant foods and drinks and therefore a significant quantity is consumed in our daily diet. The *in-vitro* anti-oxidant activities have been recognised for decades, but still not clear whether they are *in-vivo* beneficial effects [6]. Flavonoids have been shown to be potent inhibitors of the oxidative modification of low density lipoproteins by macrophages [7]. The analysis of these phytoconstituents would help in determining various biological activities of plants. A variety of techniques can be used to determine and estimate the presence of such phytoconstituents in medicinal plants. Chromatography and spectroscopic techniques are the most useful and popular tools used for this purpose [8]. The aim of the present research was to identify and characterise flavonoids by using High performance thin layer chromatography (HPTLC) and Fourier Transform Infrared (FT-IR) spectroscopic technique. The whole plant photograph of *Indigofera aspalathoides* was shown in Figure 1.

MATERIAL AND METHODS

Plant material and Extraction

Fresh whole plant of *Indigofera aspalathoides* was collected from Madurai district of Tamil Nadu in the month of November 2011. The plant was identified and authenticated at 'The Rapinat Herbarium and Centre for Molecular Systematics', St.Joseph's College, Tiruchirappalli, Tamil Nadu. The samples of *Indigofera aspalathoides* leaves alone & its Aerial parts (leaves, stems and seeds) were shade dried and powdered separately and stored in a air-tight containers at room temperature (30± 2ºC). The powder was treated with petroleum ether (60-80ºC) for dewaxing and removal of chlorophyll. Later, it was packed (150g) in a Soxhlet apparatus and subjected to continuous hot percolation for 8 hours using 450ml of n-hexane, chloroform, ethyl acetate and ethanol (95%v/v) as solvent respectively. The extracts were concentrated under vacuum, dried and stored in the desiccators for further analysis.

Fig. 1: Whole Plant of *Indigofera aspalathoides*

Phytochemical analysis

The extracted material was used for the phytochemical analysis. 1 gram of various extracts of *Indigofera aspalathoides* leaves and its aerial parts were dissolved separately in 100 ml of appropriate solvent (i.e. mother solvent) to prepare a stock solution of 1%w/v and then subjected to phytochemical screening of alkaloids, amino acids, carbohydrates, flavonoids, glycosides, tannins and phenolic compounds [9-15].

FT-IR analysis

FT-IR spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants. Moreover, FT-IR spectroscopy is an established time-saving method to characterize and identify the functional groups [16]. The extracts were analyzed to determine their functional groups using FT-IR Spectroscopy (Shimadzu IR Prestige-21, FTIR-84005) using KBr as rock salt. The extract was mixed with a disc using hydraulic press and mould. The mixture on the disc was inserted in the path of the IR beam and held in position.

Preparation of Stock solutions for HPTLC

Preparation of Quercetin Standard solution

A stock solution of standard Quercetin (1mg/mL) was prepared by transferring 10 mg Quercetin, accurately weighed, in to 10mL volumetric flask, dissolving in 5mL methanol. It was then sonicated for 10 minutes and the final volume of the solution was made up to 10mL with methanol to get stock solution 1mg/mL.

Preparation of Sample solution

Accurately weighed 20.5mg & 51.3mg of alcoholic extracts of leaves and aerial parts of *Indigofera aspalathoides* were transferred to a 10mL volumetric flask separately and dissolved in 8mL of methanol. It was then sonicated for 10 minutes and the contents of the flasks were filtered through Whatman No.1 paper (Merck, Mumbai, India). The final volume of the solutions made up to 10mL to get stock solutions containing 2.05 mg/mL & 5.13 mg/mL respectively.

Instrumentation and Chromatographic conditions

HPTLC was performed on a precoated silica gel 60 F_{254} (E.Merck, Mumbai, India). Standard solution of quercetin and sample solutions were applied to the plates as bands 8.0mm wide, 30.0mm apart and 10.0mm from the bottom edge of the same chromatographic plate by the use of a Linomet 5 sample applicator. Ascending development to a distance 8cm was performed as room temperature (28±2ºC), with toluene, ethyl acetate, acetic acid, methanol in the ratio of 2.5:7.0:0.25:0.25 as mobile phase, in a Camag glass twin-trough chamber previously saturated with mobile vapour for 20 min. After development the plates were dried with a hair drier and then scanned at 254 nm with Camag TLC scanner 3 and observe the plate under UV light at 254 & 366 nm using Camag Reprostar 3 [17].

RESULTS AND DISCUSSION

Phytochemical Screening of *Indigofera aspalathoides*

Phytochemical studies revealed the presence of alkaloids, flavonoids, glycosides, tannins, phenol, terpenoids and steroids in various extracts of *Indigofera aspalathoides* leaves and aerial parts. Summary of the screening results are listed in Table 1.

Table 1: Phytochemical screening of *Indigofera aspalathoides* leaves (L) &Aerial parts (AP)

S. No.	Phytochemical and Test	HEX		CHL		EA		ETH	
		L	AP	L	AP	L	AP	L	AP
1	**Test for alkaloids**								
	(a) Dragendroff's test	+	+	+	+	+	+	+	+
	(b) Mayer's test	+	+	+	+	+	+	+	+
2	**Test for amino acids**								
	(a) Ninhydrin test	-	-	-	-	-	-	-	-
	(b) Cysteine test	-	-	-	-	-	-	-	-
3	**Test for carbohydrates**								
	(a) Barfoed's test	-	-	-	-	-	-	-	-
	(b) Fehling's test	-	-	-	-	-	-	-	-
	(c) Benedict's test	-	-	-	-	-	-	-	-
4	**Test for flavonoids**								
	(a) Shinoda's test	+	+	+	+	+	+	+	+
	(b) Ammonia test	+	+	+	+	+	+	+	+
	(c) Aluminium test	+	+	+	+	+	+	+	+
5	**Test for glycosides**								
	(a) Killer-Killani test	+	+	+	+	+	+	+	+
	(b) Baljet test	+	+	+	+	+	+	+	+
6	**Test for Tannins & phenol**								
	(a) Lead Acetate test	+	+	+	+	+	+	+	+
	(b) Ferric Chloride test	+	+	+	+	+	+	+	+
	(c) Ammonia test	+	+	+	+	+	+	+	+
7	**Test for Terpenoids and steroids**								
	(a) Salkowski test	+	+	+	+	+	+	+	+
	(b) Liebermann's test	+	+	+	+	+	+	+	+

HEX: n-Hexane; CHL: Chloroform; EA: Ethyl acetate; ETH: Ethanol; (+): Present; (-): Absent

FT-IR analysis

FT-IR spectra of investigated *Indigofera aspalathoides* ethanolic extract of leaves & aerial parts are shown on Figure 2 and the spectral assignments are given in Table 2. Inspection of the spectra revealed the presence of following peaks at wave number 3410.75 cm^{-1}, 3380.87 cm^{-1} representing O-H stretching vibration of Phenols; peaks at wave number 2929.26 cm^{-1}, 2932.79 cm^{-1} representing C-H stretching and 1616.73 cm^{-1}, 1618.66 cm^{-1} for C=O group of ketones. The peaks at 1382.11 cm^{-1}, 1381.65 cm^{-1} represents in plane O-H bending of Phenols and 1072.07 cm^{-1}, 1075.81 cm^{-1} for C-O group. The peaks at 697.56 cm^{-1}, 661.05 cm^{-1} represents out of plane C-H bending. The sharp absorption peaks at 1616.73 cm^{-1} (Leave Extract) and 1618.66 cm^{-1} (Aerial part Extract) are assigned to C=O stretching vibration in carbonyl compounds which may be characterized by the presence of high content of flavonoids and terpenoids. On the overall spectra indicated the presence of hydroxyl, carboxyl, carbonyl and ketone groups.

HPTLC analysis

The HPTLC procedure was identified the flavonoid group present in both sample extracts (T2&T3), when compare to Quercetin standard (T1). The mobile phase consisting of Toluene: Ethyl acetate: Acetic acid: Methanol (2.5:7.0:0.25:0.25) gave a visible band (Rf 0.74) for sample solution corresponding to Quercetin standard solution. The TLC plate was visualized under UV light at 254nm and 366nm. Photograph of a TLC plate after chromatography of Quercetin standard and the ethanolic extracts of leaves and aerial parts of

Indigofera asphalathoides were shown in Figure 3. The identity of the Quercetin bands (flavonoids) in sample chromatogram was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution and by comparing retention factors of Quercetin from sample and standard solutions. The peak corresponding to Quercetin from the sample solution had same retention factor (Rf 0.74) as that from Quercetin standard (Figure 4).

Table 2: Wave number and interpretation FT-IR data of *Indigofera aspalathoides*

S. No.	Wave Number (cm^{-1})		Bond	Functional Group
	Leaves Extract	Aerial Parts Extract		
1.	3410.75	3380.87	O-H stretching	Phenol
2.	2929.26	2932.79	C-H stretching	Aromatic
3.	1616.73	1618.66	C=O	Ketone
4.	1382.11	1381.65	O-H bend	Phenol
5.	1072.07	1075.81	C-O stretching	Alcohol
6.	697.56	661.05	Out of plane C-H bending	Aromatic

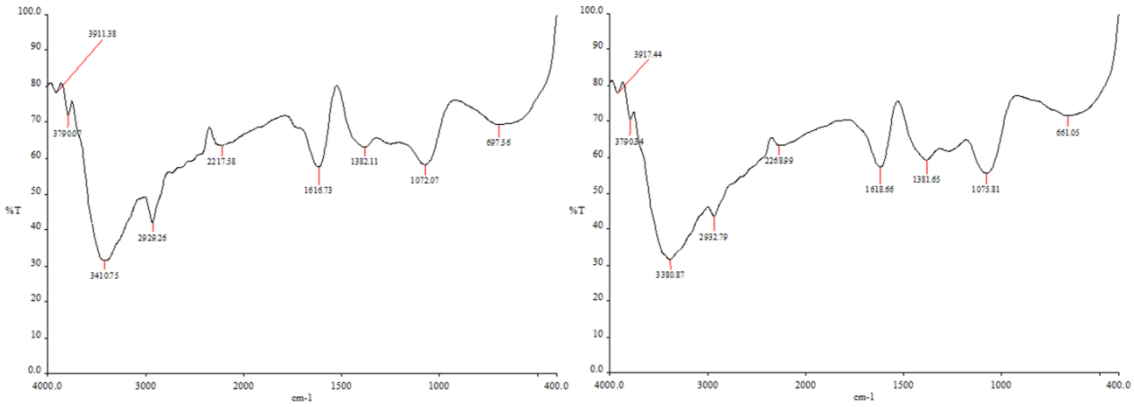

Fig. 2: FT-IR spectrum for ethanolic extract of *Indigofera aspalathoides* leaves and aerial parts

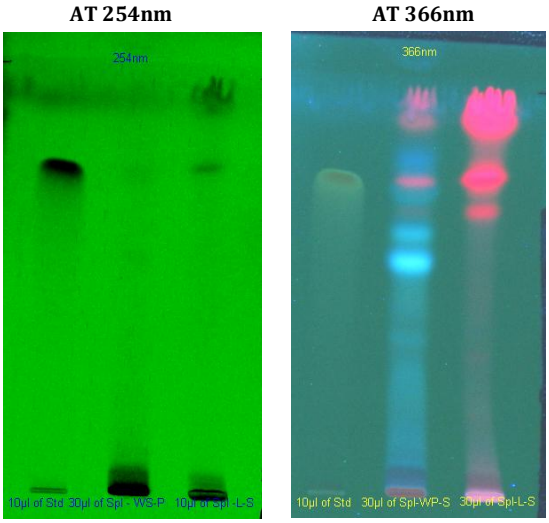

Fig. 3: HPTLC Fingerprinting Profile of Ethanolic Extract of *Indigofera asphalathoides*

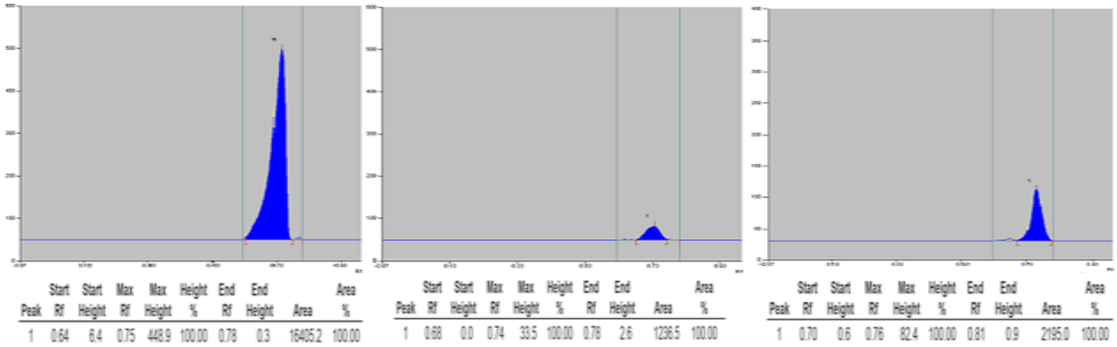

Fig. 4: HPTLC chromatogram of ethanolic extract of *Indigofera asphalathoides*

CONCULSION

This study revealed the identification of flavonoids in ethanolic extract of *Indigofera asphalathoides* leaves and its aerial parts. The presence of flavonoid in the ethanolic extract of *Indigofera asphalathoides* leaves and its aerial parts was further confirmed by HPTLC and FT-IR spectroscopy. The analysis carried out on this plant shows that the plant rich in secondary metabolites (Flavonoids) which could be explored as potential drug in phytomedicine.

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