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Pharmacognostic characterization and phytochemical screening of different parts of *Indigofera viscosa* Lam

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Abstract
Pharmacognosy studies can provide reliable crude drug information of the plant/parts. These information in turn can be used for quality control of the genuiness of material for herbal drug preparation. The present study was conducted to examine the pharmacognostic features and phytochemical screening of various parts of *I. viscosa*. In the results, macroscopic and organoleptic studies provided the morphological characters and sensory features of the plant parts. Sectioning and leaf constants results exhibited complete anatomical nature of leaf, stem and root of the plant. Fluorescence analysis showed the colour illumination of the compounds by the treatment of various chemicals. Physicochemical properties exhibited the percentage of moisture content, and determination swelling (gum & mucilage) index and foaming (saponin) index. Phytochemical screening showed the presence of alkaloids, flavonoids, tannins, steroids, triterpenoids and saponins in different parts. Thin layer chromatography results provided the fingerprint information of the compound flavonoids. The aggregated data of the present research will be helpful in the standardization of *I. viscosa*. Further studies are needed to characterize the bioactive phytochemicals present in the plant.

Keywords: *Indigofera viscosa*, pharmacognosy, extract, secondary metabolites, TLC

Introduction
The use of plant parts for medicinal purposes is considered as herbal medicines. Such medicines have been widely used since immemorial days in developing and developed countries and are minimum in side effects [1]. Plant based medicines are initially utilized as crude drugs in the form of tinctures, teas, poultices, powders and other form of formulations [2]. In the present context, the traditional system of herbal medicine is widely accepted. The World Health Organization (WHO) reported that 80% of the world population, especially people of developing countries using herbal medicines for some aspects of their primary healthcare benefits [3, 4]. Many plant species have limited distribution in their natural habitats [5], unavailability of such medicinal plants in time led to arbitrary adulteration and substitution [6, 7]. Adulteration or substitution can be terminated by standardization. The process of standardization includes comprehensive data of the entire field of study, from birth of a plant to its clinical applications, it ensures the quality and purity of the herbal drugs. Standardization of herbal medicines can be achieved by pharmacognosy and phytochemical studies. Pharmacognosy is the study of medicinal material derived from natural sources. It is a simple and reliable tool, by which the complete information of the crude drugs can be obtained [8] and is the study of physical, chemical, biochemical and biological properties of the crude drugs.
Plants contain an amazing diversity of chemical constituents, and are grouped as primary and secondary metabolites based on their role in the plants. Secondary metabolites are produced for continue to survival of the plant in its specific environment. Secondary metabolites including different class of compounds such as alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, anthraquinones, etc. [9], they have the ability to cure or prevent various diseases. Several research reports have been proved that secondary metabolites are medicinally active components, they possess numerous therapeutic actions such as antimicrobial, antioxidant, anti-inflammatory, anticancer, antiviral, wound healing, hepatoprotective, antimalarial and so on.
Fabaceae (= Leguminosae) family consist of huge number of economically valuable species. Most of the species of this family are used in folk as well as traditional system of medicines. In addition, several investigations have been conducted to discover the phytochemical and pharmacological properties of *Indigofera* species. However, some of the species of the genus scientifically not validated for their phytochemical and pharmacological properties and also for pharmacognostic studies. *Indigofera viscosa* Lam. [synonym: *Indigofera colutea* (Burm.f.) Merr.] is commonly known as 'sticky Indigo' found in various parts of Tamil Nadu. This plant is used to treat stomach aches [10, 11], diarrhea [12], boils [13], cuts, bruises and scabies [14] by local healers at different parts of the world. Although, *Indigofera viscosa* is underexplored in the field of pharmacognosy and phytochemical studies. Based on these information, the present investigation was aimed to document the pharmacognostic features and secondary metabolite screening of various parts of *I. viscosa*.

Materials and Methods
Collection and preparation of plant materials
Healthy flowered twigs of *Indigofera viscosa* was collected from Thondamuthur region, Coimbatore district of Tamil Nadu, and the specimen was authenticated by Botanical

Survey of India (BSI), Coimbatore. The voucher specimen was conserved in our laboratory. Fresh leaf, stem and root of the plant was collected separately and washed with tap water for removal of dirt and debris and allowed to dry in shade condition. Dried samples were milled to a coarse powder by using mortar and pestle, then the powders were stored in sterile bottles and kept at air free room temperature.

Pharmacognostic studies

Macroscopic features

The macroscopic investigation of various parts of *I. viscosa* was recorded by various parameters such as colour, size, shape, texture and special features [15].

Organoleptic features

The organoleptic evaluation of leaf, stem and root samples were carried out to document the colour, odour and taste of the parts [16].

Sectioning and Anatomy

Transverse sections of the plant parts were prepared through free hand sectioning with the help of razor blade, stained by safranin and mounted by glycerine with the help of usual microtechniques [9].

Leaf constants

The stomatal number, stomatal index, vein-islet number, vein termination number and palisade ratio were recorded and tabulated by using standard procedures [17]. Anatomical nature and leaf constants features were photographed in different magnifications by using compound binocular microscope.

Fluorescence analysis

Fluorescence analysis of crude powders [18] and extracts [19] were carried out by the standard procedures.

Physicochemical properties

Evaluation of moisture content [8], swelling index [20] and foaming index [8] of the powders were measured by standard methods.

Phytochemical studies

Extraction of plant materials

100g of each (part) powder was separately extracted by using soxhlet apparatus by the adaptation of successive extraction method [21]. In order to low polar to high polar petroleum ether, dichloromethane, ethanol and water solvents were used for extraction, each extract was run for 48 hours. Each time before employing to higher polarity solvent, the marc was dried then used for extraction. The extracts were stored in sterile glass containers at room temperature.

Phytochemical screening

Secondary metabolites such as alkaloids [22], flavonoids [9], tannins [23], steroids [24], triterpenoids [24], saponins [25] and glycosides [26] were tested for their presence in the various extracts.

Thin layer chromatography (TLC) for Flavonoids

Ethanol extract of leaf, stem and root were subjected to TLC separation of flavonoids by the procedure as described by Harborne [27]. Clean rectangular glass plates were coated (2mm thickness) by required amount of silica gel-G_{254} slurry mixed with distilled water and allowed to dry at 100°C for 1 hour. The extracts were spotted (4mm diameter) on one edge of the plate from 2cm distance of bottom of the plate. Then the plate was dipped into mobile solvent (chloroform-90ml: methanol-10ml) containing chamber and closed with lid and kept undisturbed. The plate was removed from chamber once the mobile solvent running near to the opposite end of the plate. After that the plate was allowed to dry and sprayed with 1% ethanolic Aluminium chloride ($AlCl_3$) solution for the detection of flavonoids. After that the *Rf* value of the compound was calculated by the following equation,

$$Rf \text{ value} = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

Results and Discussion

Pharmacognostic evaluation

Macroscopic features

I. viscosa can well grow in hill slopes, cultivation fields, sand or sandy loam soils, riverine floodplains and roadsides (plate 1). The plant is erect and spreading annual/short-lived perennial herb, up to 10–90cm tall. Stems spreading, white appressed or spreading biramous hairs present in most parts and erect multicellular glandular hairs present which are up to 1.2 (rarely 2–3) mm long. Stipules linear-lanceolate, spreading, up to 4mm long. Leaves compound, leaf-rachis up to 7cm long. Leaflets 9–11 or rarely up to 15, elliptic-oblong, up to 14×4mm, glandular hairs present on margins and also on both upper and lower surfaces of the leaf blades. Petiole is longer than the basal leaflets. Inflorescence are racemes, 1–4cm long with narrowly triangular bracts. Flowers 7–20, 4mm long, the peduncle up to 7–12mm long, pedicels 0.51mm long. Sepals 5, deeply divided, 3–4mm long, obovate, hairy on the back. Petals 5, red-pink, white pubescent outside; keel petals 3.5×11.5 mm, hairy at lower margin. Stamens 10 arranged in 9+1; staminal tube 3–4mm long. Style 1.2–1.6mm long, slightly curved, sparsely strigose, sloping upwards at about 45°, ovules 8–11. Pods spreading, straight, up to 18–23mm long, more or less densely covered with erect multicellular glandular hairs which are not above in 0.6mm long. Seeds 8–14 per pod, 11.2×1mm, shortly cylindrical to quadrangular, slightly wrinkled, endocarp spotted. The flowering & fruiting usually occurred in the month of October to February.

Plate 1: Macro-morphological features of Indigofera viscosa lam. A - Habit and a twing insert; B - inflorescence; C - Pods

Macroscopic features of a plant could be useful to differentiate the desired plant species/plant parts from morphologically similar species/materials that could occur as potential adulterants [28]. This is the simple and useful technique in the field of taxonomy for the species identification. However, which is an important parameter in pharmacognostic analysis.

Organoleptic studies

Plants exhibit different colours, odours and tastes depending upon the nature of phytochemicals which they possess [29]. Organoleptic characterization of *I. viscosa* was evaluated by the sensory organs such as eye, tongue, nose and skin to identify the general features of the plant and the results were presented in table 1. Bitter and sweet taste; characteristic odour; green, pink and brown shaded colours were observed in the plant parts. Similar organoleptic studies were conducted on some species of *Indigofera* [30, 31, 32]. This study is an important parameter in the process of standardization of herbal drugs.

Table 1: Organoleptic studies

Organoleptic Characters	Leaf	Stem	Root
Colour	Green	Green to Pink	Light Brown
Odour	Characteristic odour	Odourless	Characteristic odour
Taste	Slightly bitter	Bitter	Bitter + sweet

Sectioning and Anatomy

The stem section showed thin and single layered epidermis and is not clear to visible. Lengthy multicellular trichomes were present throughout the epidermis, which are glandular with sticky head. Cortex comprises of 7-10 layers, it include elliptical chlorenchyma, parenchyma and sclereid cells, which are varying in size. 20-30 prominent vascular cylinders arranged in a circle. Phloem cells are compressed and not clear. Xylem tissue include narrow and thick walled vessels and thin walled lignified fibres and tracheids. Pith cells are circular, thin walled and compact. Oil droplets frequently distributed in the pith and cortical cells. Tiny starch grains present in pith cells (plate 2).

Root is circular in sectional view and it's outer part consists of 2-3 layer of unclear periderm cells. The cortex filled by 5-10 layer of irregular elliptical parenchyma cells. Scattered sclereids distributed throughout the cortex. Phloem cells are small and not clear. The xylem layer is big, circular and prominent, it occupied half of the whole section. Secondary xylem placed near by the phloem, and primary xylem occurred in the middle of the root. Xylem includes narrow and densely arranged vessels, fibres and tracheids. 12-15 medullary rays were present between the xylem cells. Pith is absent. Starch grains and resin ducts were observed in cortex. Tannins abundantly stored at intercellular space of cortical cells and medullary ray cells (plate 3).

The leaf cross section showed that the midrib is situated in the median part of the leaf. The epidermis consists of single layered hemispherical cells. Papillated outer tangential wall present in the epidermal cells. Palisade parenchyma and spongy parenchyma cells were observed. The mesophyll tissue consists 3-5 layer of collenchyma. The vascular bundle is small, circular and prominent. The xylem consists of 3-4 row of angular thick walled cells. The phloem is compact and unclear. Glandular and non-glandular trichomes were observed on the epidermal layers. Paracytic stomata was observed in both the epidermises. Tannins and crystals were distributed in the lamina region. In petiole, the epidermis was single layered and covered with thin cuticle. Hypodermis consists of 3-4 layer of chlorenchyma cells. Below that, parenchyma cells are arranged loosely with larger intercellular spaces. Three vascular bundles are roughly arranged in a ring (plate 4).

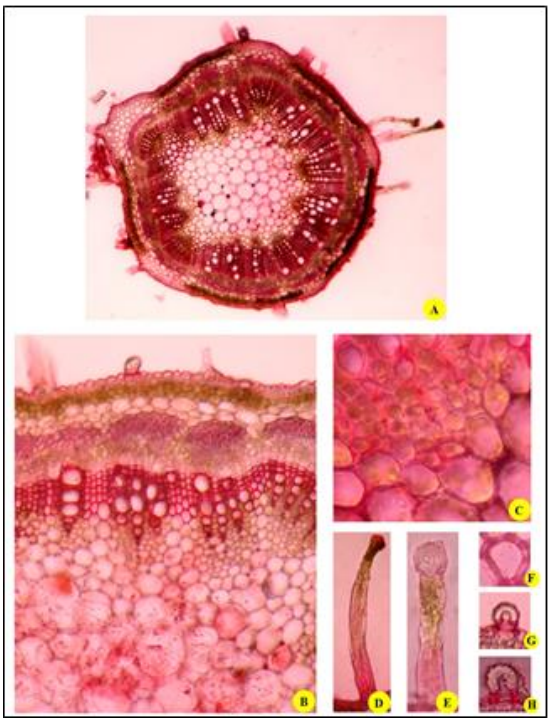

Plate 2: Anatomical features of stem A - CS of stem; B - portion enlarged; C - oil droplets in pith; D, E – Glandular trichomes; F – Strach grains; G, H – Origins of trichomes

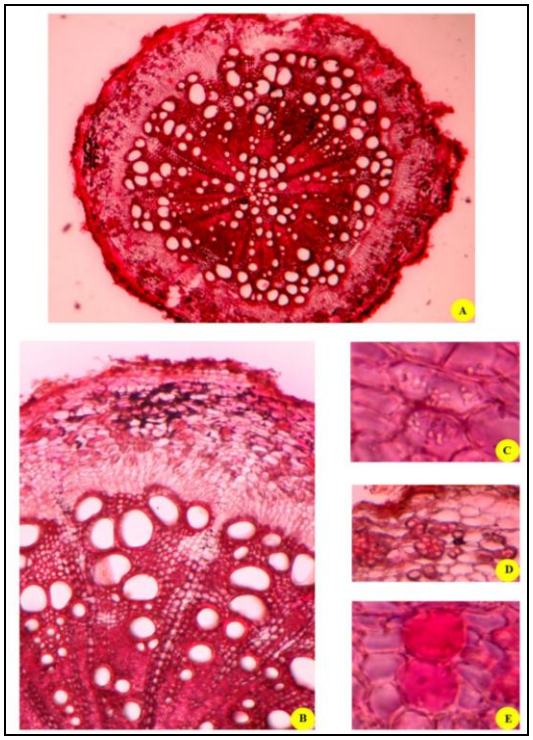

Plate 3: Anatomical features of root A - CS of root; B – A protion enlarged; C – Starch grains; D – Scatteref sclereids; E – resin Ducks

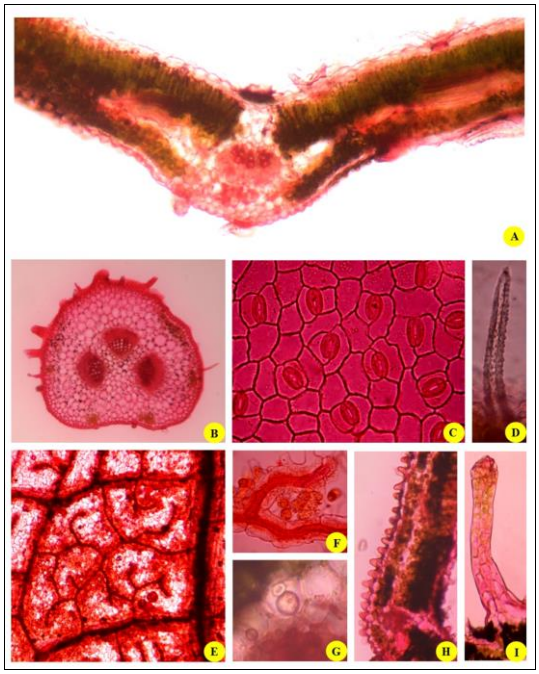

Plate 4: Anatomical features of leaf A – CS of midrib; B – CS of petiole; C – paracytic stomata; D – non-glandular trichome; E – vein islets; F – vein ending; G – crystals; H – Papillated leaf epidermis; I - glandular trichome

The present study showed oil droplets and starch grains in cortex of *I. viscosa* stem. But, which are not reported in previous study conducted in stems of 8 *Indigofera* species [33]. The present study also noted the starch grains and resin ducts in cortex of *I. viscosa* root, the same features haven't reported in roots of *I. aspalathoides* [34], *I. hirsuta* [35] and *I. linnaei* [36]. Trichomes are not observed in the leaves of some *Indigofera* species [37]. But, in our study, lengthy multicellular-glandular and unicellular-nonglandular trichomes were abundantly present in leaf epidermis and lot of crystals also noted in the leaves.

Determination of Leaf constants

Determination of leaf constants is an important quantitative parameter in the evaluation of crude herbal drugs [38]. The quantitative results of the leaves of *I. viscosa* was showed in table 2.

Table 2: Determination of leaf constants

Leaf constants		Determination / sq. mm		
		I. viscosa		*I. tinctoria* [39]
		Range	Mean	Mean
Stomatal number	*Upper epidermis*	17-23	19.6	16.16
	Lower epidermis	26-30	27.6	28.66
Stomatal index	*Upper epidermis*	14.81–16.94	16.59	6.31
	Lower epidermis	18.27–21.34	19.69	8.70
Vein islet number		11–15	13.33	8.0
Vein termination number		7–10	8.66	6.0
Palisade ratio		1 : 2		Not reported

The present results are compared with previous study [39] conducted on true Indigo (*Indigofera tinctoria*). The leaf constants are unique for each species and are fingerprint characters of the plants. As showed in table 2, the stomatal number of *I. viscosa* and *I. tinctoria* was almost similar. But the stomatal index, vein islet number and vein termination number of both the species were distinguishable from each other. This observation reveals structural variation among the leaves of two closely related species. Thus the leaf constant parameters of the present study will be helpful to identify the *I. viscosa* from its closely related species.

Fluorescence analysis

Fluorescence analysis of various powders and extracts of *I. viscosa* was carried out under visible and ultraviolet lights. The powders were treated with various chemicals, solvents and reagents, but the crude extracts were not treated by them. The results are depicted in table 3 and table 4.

Recently, fluorescence analysis of *Indigofera* genus has scientifically validated by various researchers [32, 35, 39, 40, 41]. Those previous studies showed significant fluorescent illumination in the powders as well as extracts of the studied plant parts, which are distinctive characters of the concerned species. For illustration, NaOH treated stem powders showed brown colour in visible light and green colour in ultraviolet light in *I. aspalathoides* [40]. The ethanol stem extract exhibited green colour in visible light and brown colour in ultraviolet light in *I. tirunelvelica* [32]. The ethanol leaf extract showed green colour in visible light and dark green colour in ultraviolet light in *I. tinctoria* [39]. In contrast, the present study showed brown colour in visible light and greenish brown colour in ultraviolet light in NaOH treated stem powder of *Indigofera viscosa*. The ethanol extract of *I. viscosa* stem exhibited pale yellow colour in visible light and light green colour in ultraviolet light. Ethanol leaf extract of *I. viscosa* exhibited yellowish brown colour in visible light and green colour in ultraviolet light. In florescence analysis, the colour of the drug is mainly based on the phytochemicals present in the tissue concerned [42]. Therefore, fluorescence analysis is one of the most important parameter in pharmacognostic studies.

Determination of Physiochemical properties

Estimation of physicochemical properties are important parameters in the identification of adulterants and improper handling of drugs [43]. Table 5 showed the physicochemical parameters of *I. viscosa*. The moisture content of leaf, stem and root was 5.79 ±0.64, 4.96 ±1.02 and 5.14 ±0.91 percent respectively. Stem has little bit of low moisture content than other parts. Root powder showed high swelling index (0.9 ±0.05ml) followed by stem and leaf powders. The foaming index was significant (less than 100) in root powder but which is not significant in both leaf and stem powders. Thus concluded that the root possess good volume of swelling and foaming index.

Different values of moisture content observed in *Indigofera* species such as *I. aspalathoides* [40], *I. barberi* [41], *I. tirunelvelica* [32], *I. hirsuta* [35] and *I. tinctoria* [39]. The moisture content of leaf, stem and root of *I. aspalathoides* was found to be 53.80, 64.12 and 49.4 percent respectively [40]. In another study, *Indigofera barberi* leaves showed significant (less than 100) results in foaming index and 1.2% in the swelling index [44]. The previous studies related to physicochemical properties of *Indigofera* genus demonstrated that the moisture content, foaming index and swelling index of *Indigofera* species vary to each other. Interestingly, *I. viscosa* thus possess significant variation in physicochemical properties, which in turn could be useful for the identification of the species.

Table 3: Fluorescence analysis of powders

Reagents with Powder	Leaf		Stem		Root	
	Visible Light	UV Light	Visible Light	UV Light	Visible Light	UV Light
Powder alone	Dark Green	Green	Light Green	Light Green	Pale Brown	Pale Green
Powder + Cold water	Light Green	Light Green	Light Green	Green	Light Brown	Light Green
Powder + Hot water	Light Green	Green	Light green	Green	Light Brown	Light Green
Powder + 70% Ethanol	Dark Green	Bluish Green	Green	Bright Green	Light Brown	Light Green
Powder + 70% Methanol	Light Pale Green	Bright Green	Green	Dark Green	Brown	Green
Powder + 5% NaOH	Dark Brown	Yellowish Brown	Brown	Greenish Brown	Bright Brown	Green
Powder + 10% HCl	Light Brown	Dark Brown	Pale Brown	Light Green	Light Brown	Light Green
Powder + conc. HCl	Dark Green	Dark Brown	Dark Green	Dark Green	Brown	Dark Green
Powder + conc. H_2SO_4	Reddish Brown	Dark Brown	Dark Brown	Dark Brown	Dark Brown	Dark Brown
Powder + conc. HNO_3	Yellowish Orange	Orange	Orangish Brown	Light Green	Orangish Brown	Light Green
Powder + Saturated Picric acid	Yellowish Green	Dark Green	Light Green	Greenish Yellow	Yellow	Yellow
Powder + Acetic acid	Brown	Dark Brown	Light Brown	Green	Brown	Green

Table 4: Fluorescence analysis of various extracts

Extracts	Leaf		Stem		Root	
	Visible light	UV Light	Visible light	UV Light	Visible light	UV Light
Petroleum ether	Dark green	Yellowish brown	Light green	Bright green	Pale green	Light green
Dichlomethane	Yellowish Green	Green	Brownish yellow	Light brown	Pale brown	Colourless
Ethanol	Yellowish brown	Green	Pale yellow	Light green	Pale yellow	Colourless
Water	Yellow	Yellowish brown	Reddish green	Greenish red	Colourless	Colourless

Table 5: Determination of Physiochemical properties

Physicochemical properties	Plant parts		
	Leaf	Stem	Root
Moisture content (% w/w)	5.79 ±0.64	4.96 ±1.02	5.14 ±0.91
Swelling index (ml)	0.2 ±0.08	0.5 ±0.21	0.9 ±0.05
Foaming index	Not significant	Not significant	Significant

Phytochemical studies

Secondary metabolite screening

Secondary metabolite screening of *I. viscosa* was performed in different parts (table 6). The results showed the presence of all the tested compounds in leaf and stem extracts. However, tannins, steroids and glycosides were completely absent in root extracts. Alkaloids, tannins, steroids, triterpenoids and saponins were detected in leaf extracts. Whereas, flavonoids, tannins, steroids and triterpenoids were present in stem extracts. Flavonoids and triterpenoids were detected in root extracts. Repeated detection of compounds in various parts of *I. viscosa* may be the result of abundant occurrence of the compounds in the whole plant.

Table 6: Secondary metabolite screening of various extracts

Phytochemicals	Leaf				Stem				Root			
	P	D	E	W	P	D	E	W	P	D	E	W
Alkaloids	_	_	++	+	_	_	+	_	_	_	+	_
Flavonoids	_	_	+	_	+	_	+	+	_	+	+	+
Tannins	_	_	+	+	_	_	+	+	_	_	_	_
Steroids	+	+	_	_	+	+	_	+	_	_	_	_
Triterpenoids	_	_	++	+	+	_	+	+	+	+	+	+
Saponins	_	_	+	+	_	_	_	+	_	_	_	+
Glycosides	+	_	_	_	+	_	_	_	_	_	_	_

* P – Petroleum ether; D – Dichloromethane; E – Ethanol; W – Water

'+' indicate presence of compounds; '–' indicate absence of compounds

In leaf, ethanol extract showed the presence more diverse compounds. But in stem, petroleum ether and ethanol extracts showed diverse compounds; in root, ethanol and water extracts showed maximum compounds than other extracts. Here solvent polarity plays a key role in increasing metabolite solubility. Ethanol extract of all the parts showed the presence of maximum number of compounds. Subsequently TLC analysis of the present study was performed in ethanol extracts. A lot of investigations reported that the secondary metabolites be useful in the prevention of several diseases. For examples, alkaloids possess potent anti-inflammatory activity [45], flavonoids prevent the oxidative cell damage and are strong anticancer agents [46] and the steroids possess anti-cancer property [47]. Parvatikar and Madagi [48] confirmed the presence of flavonoids, steroids, tannins and triterpenoids in ethanol extract of *Indigofera hochestetteri*. Alkaloids, phenols, saponins and glycosides were detected in ethanol extract of *Indigofera barberi* leaves [44]. In the present study also phytochemical screening of various extracts of *I. viscosa* revealed variety of pharmacologically active secondary metabolites similar to the above referred investigations.

Thin layer chromatographic analysis

TLC analysis is a suitable method for monitoring the identity and purity of the plant secondary metabolites. It is also useful for detection of adulterants/substituents [49]. The TLC analysis for flavonoids was done in ethanol extracted samples of *I. viscosa* and the results were depicted in plate 5. After running the TLC plate in mobile solvent system (chloroform-90ml: methanol-10ml), grey, brown and green bands were observed on the plate. After spraying with 1% ethanolic $AlCl_3$ reagent on the plate, a distinct yellow band was observed in stem and root samples, which confirm the separation of flavonoids but not detected in leaf sample. The *Rf* value was calculated as 0.73 and 0.71 in stem and root samples respectively. Laitonjam and Wangkheirakpam [50] and Renukadevi and Sultana [51] separated flavonoids through TLC in *I. tinctoria*. Renukadevi and Sultana [51] noted the *Rf* value as 0.63 for flavonoids in *I. tinctoria*. Moreover, the TLC separation for flavonoids in *Indigofera colutea* (=*I. viscosa*) has already been reported [52]. The present study is in contrast with previous studies conducted

on *Indigofera* species with respect to mobile solvent and spraying reagent used.

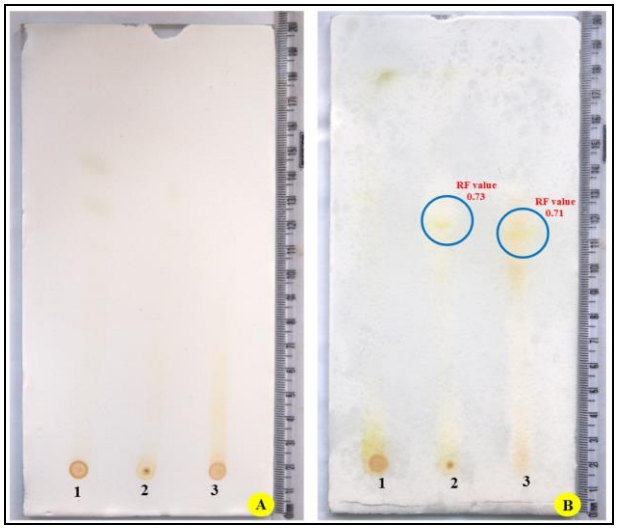

Plate 5: Thin Layer chromatography for flavonoids A – before spray of $AgCl_3$; B – After spray of $AgCl_3$ 1 – leaf; 2 – Stem; 3 – Root

Conclusion

The pharmacognostic analysis of *I. viscosa* could be an important study of the species, which will be helpful to distinguish the species from its allied ones in the process of standardization. The phytochemical screening results will be helpful in the isolation and characterization of chemical components present in the extracts and would further be valuable in ascertain the actual value of the compounds. Presence of variety of bioactive compounds in various parts of *I. viscosa* conclude that the therapeutic values of the plant exploited by ethnic communities was reasonable. The TLC results may serve as a characteristic fingerprint for the compound flavonoids. Based on the above findings, in future, other pharmacognostic studies (ash value, foreign matter, extractive value, etc.) should be done on the plant. Other phytochemical studies (column chromatography, LCMS, NMR) are needed for structural elucidation of the bioactive compounds. Various bioactivity studies should be done on the plant to find out the complete medicinal properties of the species.

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Competing Interests

Authors have declared that no competing interests exist.

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