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PHARMACOGNOSTIC INVESTIGATIONS AND PRELIMINARY PHYTOCHEMICAL STUDIES OF *INDIGOFERA TINCTORIA* LINN.

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Keywords:

Indigofera tinctoria, Macroscopy, Microscopy Phytochemical evaluation

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ABSTRACT: Objective: To study detailed pharmacognostic profile and preliminary phytochemical investigation of the leaves of *Indigofera tinctoria* Linn. commonly known as 'indigo' belongs to the family Fabaceae. It is distributed through the hotter parts of India. Medicinal uses include the juice of the leaves as a prophylactic against hydrophobia, and as a decoction for blennorrhagia; plant extract as the treatment for epilepsy, nervous disorders, bronchitis, and as an ointment for sores, old ulcers, and hemorrhoids; and roots for hepatitis, scorpion bites, and urinary complaint. **Methods:** Leaf of *I. tinctoria* Linn. was studied by macroscopical, microscopical, quantitative microscopy, physicochemical, phytochemical analysis of leaf powder of the plant and other methods for standardization recommended by WHO. **Results:** Macroscopically the leaves with 9-13 leaflets and broken pieces of rachis, opposite, oblanceolate with very short mucronate tip, pale greenish black, mucronate apex, cuneate base, smooth texture, characteristic odor and taste. Leaflets 1-2.5 cm long and 0.3-1.2 cm wide. Microscopically, the leaflet appears wavy in T.S. view; prominent adaxial and abaxial thickening, the vascular bundle is simple and wide, angular thick-walled xylem elements, the thick arc of phloem. Sclerenchymatous bands occur on the adaxial side, cylindrical with thick walls epidermal cells. Small, collateral vascular strand, anamocytic stomata, thin-walled lignified sclerenchyma cells are wide on the adaxial side. Preliminary phytochemical studies of the powder showed the presence of flavonoids, glycosides, tannins, terpenoids, mucilage, and saponins. **Conclusion:** The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations and applications.

INTRODUCTION: Medicinal plants are playing a very active role in traditional medicines for the treatment of various ailments [1]. However, a key obstacle, which has hindered the promotion in the use of alternative medicines in the developed countries, is no evidence of documentation and absence of stringent quality control measures.

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There is a need for the record of all the research work carried out on traditional medicines in the form of documentation. With this drawback, it becomes extremely important to make surety about the standardization of the plant and parts of the plant to be used as a medicine.

In the process of standardization, we can use different techniques and methodology to achieve our goal in a stepwise manner like pharmacognostic and phytochemical studies. These steps and processes are helpful in identification and standardization of the plant material. Correct characterization and quality assurance of starting material is an essential step to ensure reproducible

quality of herbal medicine, which will help us to justify its safety and efficacy [2, 3, 4, 5]. The World Health Organization (WHO) estimates that more than 80% of the populations in developing countries rely on traditional medicine for their primary health care [6]. The value of ethnomedicine and traditional pharmacology is these days achieving great appreciation in modern medicine, as the search for new potential medicinal plants is frequently based on an ethnomedicinal basis *I. tinctoria* Linn. is a leguminous plant which is widespread across tropical regions around the globe, as it had been cultivated and highly valued for centuries as the main source of indigo dye, leading to its common names 'true indigo' and 'common indigo' before commercial synthetic indigo production came into use in 1897 and reduced the world's total plant-derived indigo production to 4% by 1914.

Medicinal uses include the juice of the leaves as a prophylactic against hydrophobia, and as a decoction for blennorrhagia; plant extract as a treatment for epilepsy, nervous disorders, bronchitis, and as an ointment for sores, old ulcers, and hemorrhoids; and roots for hepatitis, scorpion bites, and urinary complaints. Pharmacognostic studies on leaves are not adequate necessitating the present investigation. The current work aims to contribute to solving the problems of controversial drugs prevalent in Ayurveda besides helping in laying down Pharmacopoeial standards. Therefore, keeping above view in mind various macroscopic, histological and physiochemical and quantitative microscopical studies and preliminary phytochemical investigation of leaves of *I. tinctoria* Linn. was carried out in the present study.

MATERIALS AND METHODS:

Collection and Authentication: *I. tinctoria* Linn. leaf was collected, from in and around Palakkad, Kerala, India and authenticated by a Taxonomist and the authenticated specimen is deposited in the Department of Pharmacognosy, Sanjo College of Pharmaceutical studies, Palakkad. Authentication specimen number is SCPS/P.COG/008/2018 the fresh leaves were kept for shade drying.

The dried specimen was powdered using a mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an airtight container.

Pharmacognostic Standardization: Organoleptic characters such as shape, size, color, odor, the taste of leaf was determined. Microscopic studies were carried out by preparing a thin hand section of the leaf with chloral hydrate solution, stained with phloroglucinol- hydrochloric acid (1:1) and mounted in glycerine [10]. Histochemical studies and powder microscopy were carried out to know about the inclusions and detailed anatomical characters of the material [7].

Quantitative Microscopy and Physico-chemical Evaluations: The vein islet number, vein terminal number, stomatal number, stomatal index were determined on fresh leaves using standard procedure [8, 9, 10]. The parameters were done to evaluate the proceedings of total ash; water-soluble ash; acid insoluble ash and sulfated ash were calculated as per Indian Pharmacopoeia [11]. Extracts of the powdered leaf were prepared with different solvents for the study of extractive value. Fluorescence analysis was also carried out for the powder and extract as per standard procedure [12].

Extraction of Plant Material: For preliminary phytochemical analysis, the extract was prepared by weighing 1 kg of the dried powdered leaf was subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, benzene, chloroform, ethanol and aqueous. The extracts were filtered in each step using Whatman filters paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45 ºC) and pressure. The presence or absence of the primary and secondary phyto-constituents was detected by usual prescribed methods [13, 14].

Powder Analysis: Preliminary analysis of the powder of the leaf powder of *I. tinctoria* Linn. with different chemical reagents was carried out microscopically [15, 16].

RESULTS:

Macroscopical Characters of the Leaf: [17] The plant occurs in the form of leaflets and broken pieces of rachis, leaflets 1-2.5 cm long and 0.3-1.2 cm wide, opposite, membranous, rounded and apiculate, oblanceolate with very short mucronate

tip green but drying greenish black. Mucronate apex, cuneate base, smooth texture, characteristic odor, and bitter taste. Leaf was 1.65 cm length and 1.34 cm width.

FIG. 1: DORSAL VIEW OF THE LEAF

FIG. 2: VENTRAL VIEW OF THE LEAF

Anatomy of Leaf:

Leaf: The leaflets appear waxy in the transactional view. The midrib and lateral view are prominent with adaxial and abaxial thickening **Fig. 3** and **4**. The midrib is 150 µm thick and 180 µm wide. The adaxial epidermis of the midrib consists of vertically elongated rectangular or horizontally cylindrical thin-walled cells.

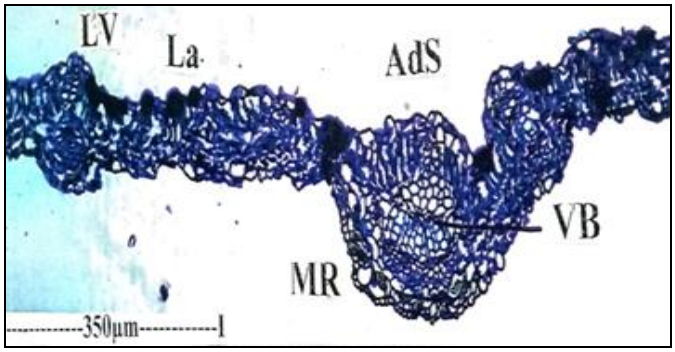

FIG. 3: T.S OF THE LEAFLET THROUGH MIDRIB

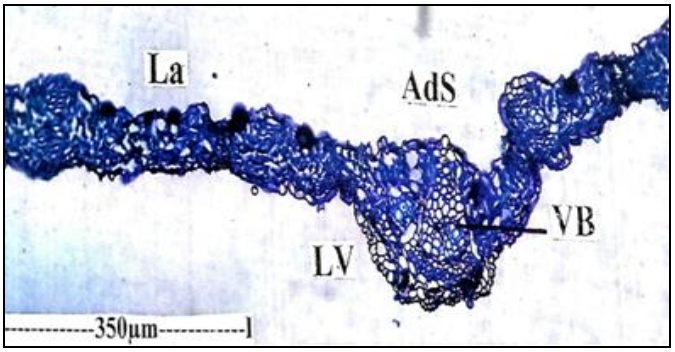

FIG. 4: T.S. OF THE LEAFLET THROUGH A LATERAL VEIN

LV-Lateral vein, La – Lamina, Ads – Adaxial side, VB- Vascular bundle, MR – Midrib

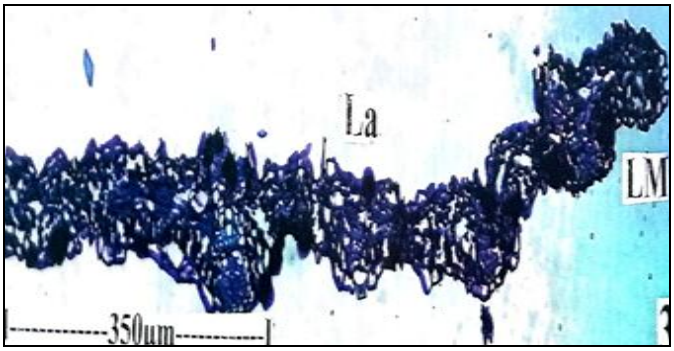

FIG. 5: T.S. OF LEAFLET-LAMINA MARGINAL PART

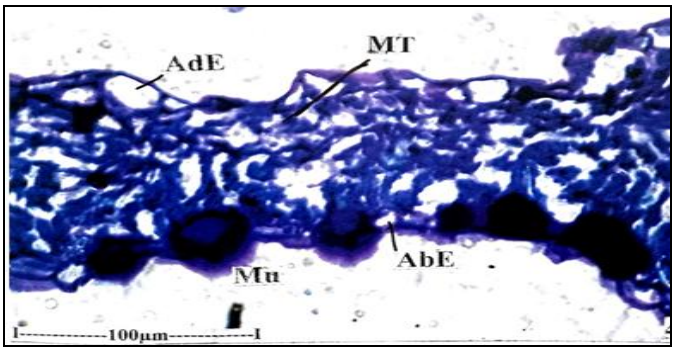

FIG. 6: T.S. OF THE LAMINA

La –Lamina, MU –Mucilage, MT- Mesophyll tissue, LM – Leaf margin, AdE – Adaxial epidermis, AbE- Abaxial epidermis

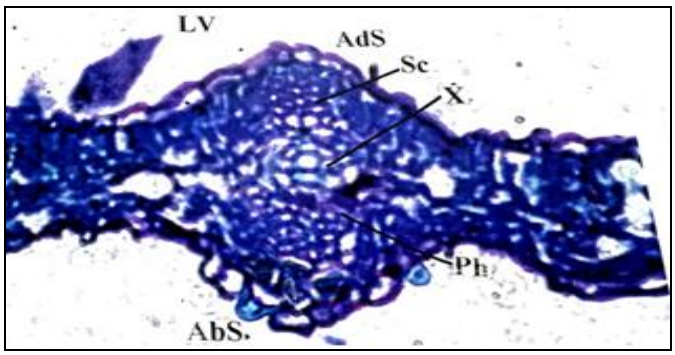

FIG. 7: T.S. OF LATERAL VEIN ENLARGED

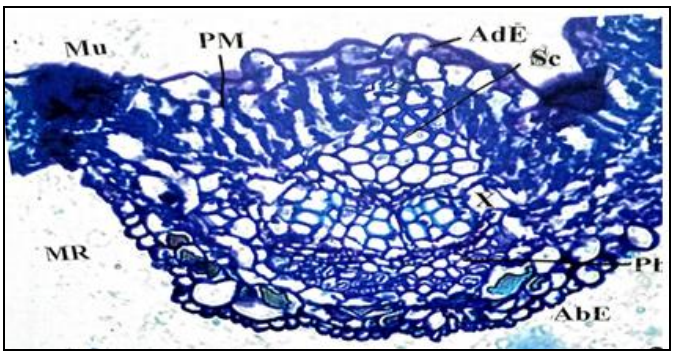

FIG. 8: T.S. OF MIDRIB ENLARGED

Sc- Sclerenchyma, X – Xylem, Ph – Phloem

The abaxial epidermis is there with papillate small thick-walled cells. The vascular bundle is single and wide. It consists of a few compact radial rows of wide angular thick-walled xylem elements and a thick arc of phloem elements on the lower end of the xylem. Sclerenchymatous bands occur on the adaxial side there is a wide and thick conical mass of sclerenchyma cells which extend up to the adaxial epidermis. The adaxial sclerenchyma cells are wide, thick-walled, and lignified.

The palisade zone extends up to the lateral sides of the sclerenchyma mass **Fig. 8**. The lateral vein is somewhat similar to the midrib **Fig. 7**. The vein is conically projecting both on the adaxial and abaxial sides. The epidermal cells on both adaxial and abaxial sides are vertically cylindrical or horizontal with thick walls. There is a small, collateral vascular strand comprising a group of wide thick-walled xylem elements and a small cluster of phloem elements. Thick and wide masses of sclerenchyma cells are situated on the adaxial and abaxial ends of the vascular strand **Fig. 7**. The lateral vein is 120 µm thick and 70 µm wide.

Lamina: The lamina consists of the thick and wavy epidermal layer of thin-walled cells. Some of the epidermal cells are dilated and filled with large mass of mucilage **Fig. 6**. The mesophyll consists of an adaxial layer of single vertically elongated palisade cells and 2 to 3 layer of loped and loosely arranged spongy parenchyma cells.

The thickness of the lamina is 70 µm. The marginal part of the lamina is semicircular without any modification of the lamina. It is comparable to the remaining part of the lamina **Fig. 5**.

Powder Microscopy: Powder characteristics revealed the presence of epidermal cells with starch grains, covering trichomes, epidermal cells with trichomes, Anamocytic stomata, vessel elements.

Quantitative Microscopy: The quantitative microscopy such as vein-islet number, vein-terminal number, stomatal number, and stomatal index were determined, and the results were tabulated **Table 1**.

Physicochemical Features: The powdered drug was evaluated for its physicochemical parameters like total ash values, acid-insoluble ash, water-soluble ash, and loss on drying, and the results were tabulated **Table 2**.

TABLE 1: QUANTITATIVE EVALUATION OF THE CRUDE DRUG OF THE LEAF OF *I. TINCTORIA* LINN.

S. no.	Plant constants	Values
1	Vein islet no.	8/sq mm
2	Vein termination no.	6/sq mm
3	Stomatal number (upper)	16.16
4	Stomatal number (lower)	28.66
5	Stomatal index (upper)	6.311
6	Stomatal index (lower)	8.703

TABLE 2: PHYSICOCHEMICAL EVALUATION OF THE CRUDE DRUG OF LEAF OF *I. TINCTORIA* LINN.

S. no.	Physical Evaluation	%w/w
1	Total ash	5.52
2	Acid-insoluble ash	4.61
3	Water soluble ash	3.58
4	Loss on drying	0.3

Fluorescence Analysis of the Extracts: The extracts were prepared as per their polarity in hot successive extraction technique, and they were treated with reagents, and the color changes were observed under Ultra Violet light, and the results were tabulated **Table 3**.

TABLE 3: FLUORESCENCE ANALYSIS OF LEAF OF *I. TINCTORIA* LINN.

S. no.	Sample	Colour in Day Light	Colour in UV Light
1	Petroleum ether extract	Pale green	Dark green
2	Benzene extract	Green	Light green
3	Chloroform extract	Brownish green	Green
4	Ethanol extract	Green	Dark Green
5	Aqueous extract	Brownish green	Yellowish green

Extractive Values: The extracts were prepared according to the polarity, and they were concentrated, and their values were calculated concerning air-dried drug, and the results were tabulated **Table 4**.

TABLE 4: EXTRACTIVE VALUES OF THE LEAF OF WITH *I. TINCTORIA* LINN. DIFFERENT SOLVENTS

S. no.	Sample	Extractability (%)
1	Petroleum ether extract	3
2	Benzene extract	3.2
3	Chloroform extract	2.4
4	Ethanol extract	2.2
5	Aqueous extract	20

Preliminary Phytochemical Analysis: The leaf powder and various extracts such as petroleum ether extract, benzene extract, chloroform extract,

ethanol extract, and aqueous extract were subjected to preliminary phytochemical screening for their presence or absence of the constituents, and the results were tabulated **Table 5**.

TABLE 5: PRELIMINARY PHYTOCHEMICAL TESTS FOR DRUG POWDER AND VARIOUS EXTRACTS OF LEAF OF *I. TINCTORIA* LINN.

S. no.	Test	Drug powder	Petroleum ether extract	Benzene extract	Chloroform extract	Ethanol extract	Aqueous extract
1.	Sterols	-	+	-	-	-	-
2.	Terpenoids	+	-	-	-	+	+
3.	Carbohydrates	+	-	-	-	+	+
4.	Flavanoids	+	-	-	-	+	+
5.	Proteins	-	-	-	-	-	-
6.	Alkaloids	-	-	-	-	-	-
7.	Glycosides	+	-	-	-	+	+
8.	Saponins	+	-	-	-	-	-
9.	Tannins	+	-	-	-	+	+
10.	Mucilages	-	-	-	-	+	+
11.	Volatile oil	+	-	-	-	+	+

+ indicates positive reaction, - indicates negative reaction.

DISCUSSION: Our study has focused on examining pharmacognostic and preliminary phytochemical studies of leaves of *I. tinctoria* Linn. Normalization of the macroscopic and microscopic characteristics of the *I. tinctoria* drug remains essential in other to identify and avoid falsification. Microscopically, the leaf showed the presence of palisade, mesophyll, covering trichome, adaxial epidermis, adaxial epidermis, anamocytic stomata, mucilage, sclerenchyma, vascular strand are the diagnostic features noted from the anatomical study.

Organoleptic characteristics are important in drugs because they play a role in the detection of adulterated or substituted drugs [18]. Thus leaves pale greenish black in color, characteristic odor, bitter taste. The powdery appearance of the crushed leaves, with a coarse texture. The micrograph performed on the powder has highlighted several characteristic elements namely: epidermal cells with starch granules, covering trichomes, epidermal cells with trichomes, anamocytic stomata, vessel elements are diagnostic substances for drugs of plant origin. These diagnostic elements are consistent with botanical standards and WHO guidelines [19, 20].

The study of physicochemical parameters such as moisture content and ash values are useful as it determines the physiological and non-physiological state of ash, this will help to determine the possibility of microbial growth and lastly contaminant or impurities. The moisture content of the drug studied had a rate of 0.3 ± 0.1, which is below 10%. This result complies with the standards established by the International Pharmacopoeia, because this water content rate, prevent oxidation reactions, fermentation, and give less chance to microbial growth and contamination in drugs [21].

Therefore, for the proper conservation of drugs made from the leaves of *I. tinctoria*, it would be desirable to use those whose water content is less than or equal to 10%. The determination of total ash gave us a rate of 5.52 ± 0.03. This value indicates the level of minerals in drugs. Insoluble ash in hydrochloric acid gave a rate of 4.61 ± 0.02. Indeed, the ash insoluble in hydrochloric acid tells us about the contamination of the drug by siliceous elements [22]. This result is in agreement with Srikanth *et al*., [23] who found the rate of 0.97% and 0.5% respectively. The maximum extractive value was found in aqueous (20%), followed by ethanol (7.2%), benzene (3.20%), chloroform (2.4%). All the extracts of the drug were subjected to different tests for detecting the presence of various phytoconstituents present in the drug, which revealed the presence of flavonoids, glycosides, saponin, tannins, terpenoids, and mucilage.

Preliminary phytochemical analysis indicated a high percentage of quercetin and flavonoids, and this may be one of the reasons behind the pharmacological activity of the plant. These parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for

future identification and authentication of genuine plant material. Though *I. tinctoria* is a highly reputed drug used in Ayurveda. Barring the anatomical details and preliminary phytochemical screening, rest of the pharmacognostical parameters gives us a clue that it can be cashed economically as well to improve the standard of health in the developing countries.

CONCLUSION: WHO has emphasized the need to ensure quality control of the raw materials used for Ayurvedic medicines by using modern techniques and by applying suitable parameters and standards. In the present study various standardization parameters such as macroscopy, microscopy (histochemical and powder), physicochemical standards, preliminary phyto-chemical investigation, which are being reported for the first time in this plant and could be helpful in authentication and preparation of a suitable monograph for the proper identification of *I. tinctoria* Linn. for the future.

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CONFLICT OF INTEREST: Nil

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