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PHYTOCHEMICAL ANALYSIS AND PHARMACOGNOSTIC EVALUATION OF NILI-ROOT (*Indigofera tinctoria L.*)

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ABSTRACT
Nili (*Indigofera tinctoria* L.) is a shrub belonging to family Fabaceae and its root used in various indigenous systems of medicine against several diseases such as arthritis, fever, cough and cold, intestinal worms, stomach disorder and spleen disease. It is also used in the preparation of several Ayurvedic formulations such as Nilibhringadi Taila, Mahapancagavya Ghrta, Arvindasava and Triphaladi. The present paper provides a detailed account of the pharmacognostical evaluation of *Indigofera tinctoria* L. root. The study includes macro and microscopic characters, powder characteristics, HPTLC fingerprinting, preliminary phytochemical screening, physicochemical parameters. The information generated by this particulars study provides relevant pharmacognostical and physicochemical data needed for proper identification and authentication of Nili root.

KEYWORDS: *Indigofera tinctoria*, Pharmacognostic evaluation, HPTLC fingerprinting, Physico-chemical analysis, Preliminary phyto- chemical investigation.

INTRODUCTION
Indigofera tinctoria L. (Family Fabaceae) is an erect, much branched shrub, 1.5 to 2 meter high, stem and branches slender, terete dark or purplish brown and covered with very fine apprised grey hairs. Leaves imparipinnate, 5-10 cm long; petioles 12-25 mm long; leaflets 7-13, elliptic or oblong, obtuse or retuse, pubescent beneath. Racemes axillary, sub sessile, 4-12 cm long, many flowered. Flower lilac red. Pods 2-4 cm long, turgid, straight or slightly curved, 8-10 seeded. It is found throughout and widely cultivated in many parts of the country. It was cultivated on a large scale in many parts of north India for extracting the dye indigo from its leaves.[1]

Indigofera tinctoria L. is commonly known as Nili, and very useful in various indigenous systems of medicine. Its root and leaves used against several diseases such as arthritis, fever, cough and cold, intestinal worms, stomach disorder, spleen disease, epilepsy and other nervous disorders, sores, old ulcers, wounds, piles, blennorrhagia, urinary complaints, hepatitis, bronchitis, dropsy, eye disease, hair growth, heart ailments, kidney and liver disorders whooping cough.[2,3,4,6] It is also used in preparation of several Ayurvedic formulations such as Nilibhrrigadi Taila (for external use only), Mahapancagavya Ghrta, Arvindasava and Triphaladi.[7]

Despite the numerous medicinal uses attributed to this plant, there are no pharmacognostical studies on the root of this plant have so far been carried out. Hence, the present work deals with the morphological, anatomical evaluation, physicochemical constants and preliminary phytochemical screening and HPTLC fingerprint profile of *Indigofera tinctoria* L. which could serve as a valuable source of information and provide suitable standards for the further identification of this plant.

MATERIALS AND METHODS
Collection of specimens
The fresh plant root of *Nili was* collected from the Sati Anusuiya forest of, Chitrakoot of Satna district (M.P.) in the month of November. The voucher specimens were collected and placed in the herbarium of Department of Pharmacognosy, Ayurveda Sadan, Research Laboratory, Deendayal Research Institute Chitrakoot.

Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical, phytochemical and HPTLC studies.

Macroscopy
Macroscopic or organoleptic characters like appearance, colour, odour and taste were evaluated.

Microscopy
Fresh root section was cut by free hand sectioning and numerous sections examined microscopically. Photographs of the microscopical sections were captured with the help of Olympus trinocular research microscope CX- 21*l* with Digieye camera using Caliper plus version 4.2 software.[8-10]

Powder microscopy
The dried root was subjected to powdered and completely passes through 355 µm IS Sieve (old sieve number 44) and not less than 50% pass on through 180 µm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, pour out the water without loss of material. Mounted a small portion in glycerin, warmed a few mg with chloral hydrate solution, wash and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, about 1 g of powder warmed over water bath with 50% con. Nitric acid till brown fumes appear, cool and wash with water thoroughly and mount a small portion in glycerin and seen under microscope at 40 X 10X magnification of the trinocular research microscope.[11-13]

Physico-chemical parameters
Physico-chemical parameters such as moisture content (loss on drying at 105^0C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and water soluble ash were calculated.[14-15]

Preliminary phytochemical studies
Preliminary tests were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavanoids, tannins, resins, carbohydrates, proteins and saponins.[16]

High Performance Thin Layer Chromatography (HPTLC)
For HPTLC, the powdered Root 2 gm of sample was extracted with 50 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F_{254} (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of *Hexane: Ethyl acetate (6:4* v\v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, TLC plates was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with 50% *Vanillin- sulphuric* reagent) at 254nm, 366nm and day light with Win cat software and R_f values noted.[17-18]

RESULTS AND DISCUSSION

Macroscopy
The root is pale-yellow to light yellowish brown in colour and odour not distinct and taste slightly bitter. Root mostly available in pieces, cylindrical, hard, woody,0.1cm to 2cm thick, surface nearly smooth except for a few scattered lenticles. (Fig. 1 & 2).

Microscopy
Root transfer section shows a narrow zone of cork consisting of 3-8 layers of tangentially elongated, rectangular, thin walled cells with lenticels. A narrow zone of secondary cortex consisting of polygonal to rectangular thin walled cells, thick walled and lignified with wide lumen group of fibres. Secondary phloem composed of usual elements. Vessels solitary or 2-4 in groups having simple pits, fibres present in the form of alternating bands of parenchyma. Medullary rays 1-4 cells wide, prismatic crystals of calcium oxalate present in secondary cortex, phloem and xylem parenchyma. Starch grains simple, round to oval present in cortex, phloem, xylem parenchyma and medullary rays (Fig. 3).

Powder microscopy
The powder colour is light yellowish brown, not distinct odour and slightly bitter taste. Under microscope examined powder showed prismatic crystals of calcium oxalate, cork cells sectional view and surface view, simple round to oval starch grains measuring 2-10µ in diameter, simple pitted vessels and tracheids parenchyma, radialy cut medullary rays and lignified thick walled with wide lumen fibres measuring 10-16µ in diameter. (Fig. 4).

Fig. 1 Plant.

Fig.2 Dried pieces of root.

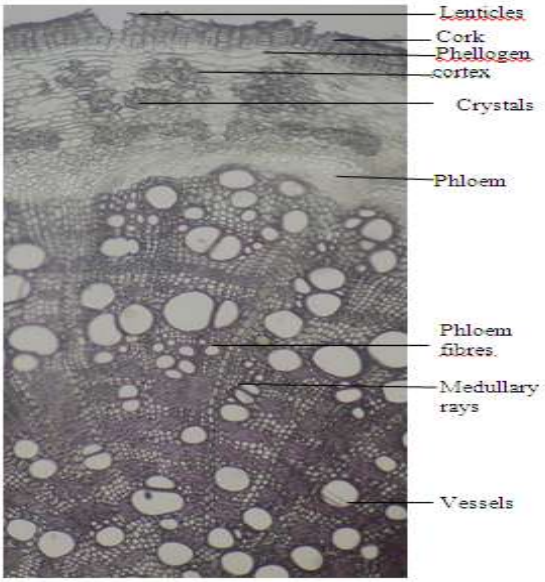


Fig. 3 (TS Root).

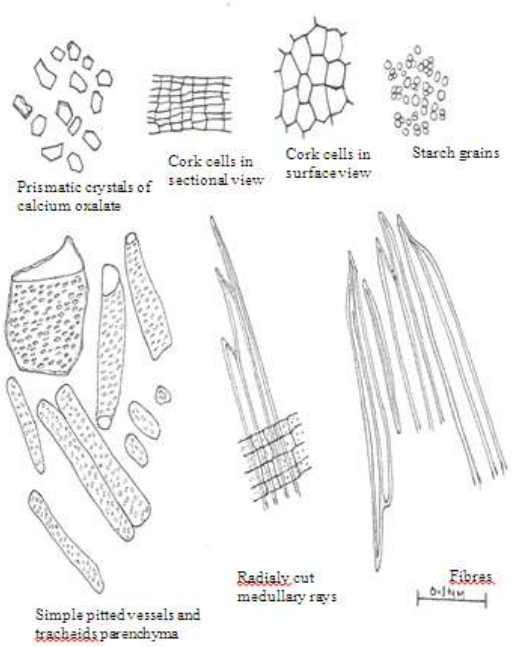
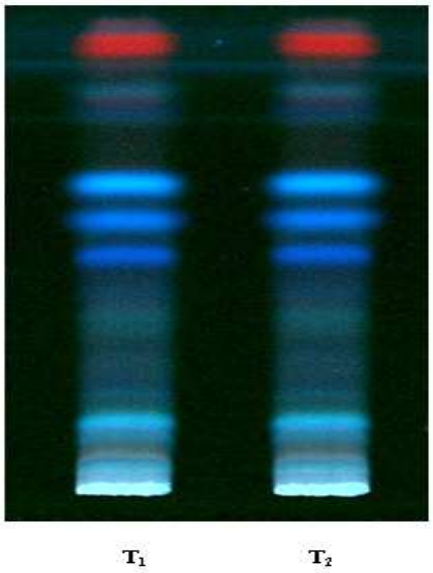


Fig. 4 Powder microscopy.

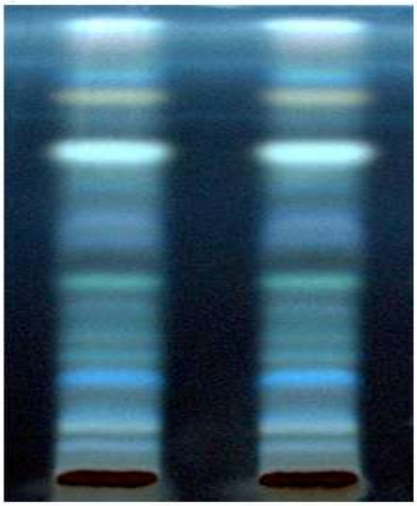
Table 1- R_f Values in test solution of Nili root.

R_f values	Root test solution of *Indigofera tinctoria*		
	366nm(before derivatization)	366nm (after derivatization)	UV light (after derivatization)
R_f1	0.10(brown)	0.10(whitish brown)	0.10(brown)
R_f2	0.55 (blue)	0.20 (blue)	0.50(yellow)
R_f3	0.60 (blue)	0.65(brownish white)	0.65(black)
R_f4	0.65 (sky blue)	0. 70(brown)	0.70(brown)
R_f5	0.80(red)	0.85(whitish pink)	0.80(dark brown)



T_1 T_2

Fig. 5, 366nm, BD.



T_1 T_2

Fig. 6, 366nm, AD.

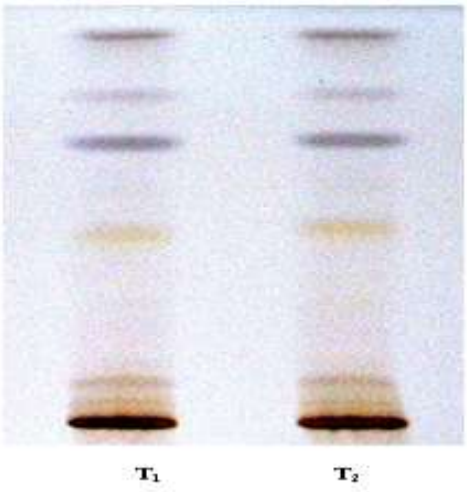


Fig. 6, 366nm, AD.

Physico-chemical analysis

The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physico-chemical results of the drug are loss on drying at 105^0C 7.5%, ethanol soluble extractive value 12%, water soluble extractive value15%, total ash value 5% and acid-insoluble ash value 0.60%.

Preliminary phytochemical studies

Qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. The screening exhibited presence of saponin, alkaloids, tannin and resin.

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the ethanolic extract two spots of the sample extracts applied in the TLC plate. Major spots R_f values with colour were recorded under 366nm, after derivatization 366nm and UV light. Chromatogram profile and R_f values are given (Fig. 5, 6, 7 & Table 1).

CONCLUSION

The pharmacognostic characters and phytochemical values reported in this work may play a major role in setting some diagnostic indices for identification and preparation of a monogrsaph of the plant, which might broaden its pharmacological, botanical and economical importance. With the help of this referential information, a researcher can easily reject the fake and adulterated plant products which are deviated from the above mentioned characters and select the correct herbal specimen for further investigations.

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