

**PHARMACOGNOSTICAL CHARACTERIZATION OF *PHYLA NODIFLORA* L. (VERBENACEAE):
A VALUABLE MEDICINAL PLANT**

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ABSTRACT

Objective: The objective of the present study was to evaluate the pharmacognostical and histochemical characters of *Phyla nodiflora* L. (Verbenaceae) an important medicinal plant.

Methods: Pharmacognostical characters such as macroscopic and anatomical characters of root, stem and leaf were studied. Physico-chemical characteristics of crude drugs of aerial parts and root were determined. To localize various phytochemicals, histochemical studies were also determined.

Results: Pharmacognostical studies helped in the identification and authentication of the plant material. Physico-chemical properties such as water soluble extractive value (21.44±0.009% w/w), alcohol soluble extractive (13.39±0.03% w/w), total ash (20.85±0.09% w/w), loss on drying (15.68±0.05% w/w) were calculated. Histochemical study indicated the presence of alkaloids, lignin, starch, proteins, lipids, phenolics, and tannins in the plant parts examined.

Conclusion: This study revealed the pharmacognostical properties and characteristics of *P. nodiflora*, an important medicinal plant. The presence of alkaloids, lignin, starch, proteins, lipids, phenolics, and tannins in the plant parts can be attributed to the cytotoxic, antimicrobial, antiviral, and antifungal properties of *P. nodiflora*.

Keywords: *Phyla nodiflora*, Microscopy, Pharmacognosy, Stomatal index, Histochemistry.

INTRODUCTION

Phyla nodiflora (L.) commonly known as poduthalai or frog fruit is a perennial herb, with prostrate stem and roots at the nodes which are scanty [1,2]. *P. nodiflora* (L.) is a medicinal plant, native of Brazil and United States. It is distributed in India, South and Central American countries, and tropical African territories [3]. The branches are slender, procumbent or ascending, with densely appressed strigillose and stems are much branched extending from 15 cm to 30 cm. The plant possesses a number of ethnobotanical uses [4]. The plant possesses cooling, diuretic, and febrifuge properties.

The chemical composition of the plant includes several flavones, glycosides, alkaloids, terpenoids, and essential oils [5,6]. Nodifloridin-A and nodifloridin-B are compounds isolated from *P. nodiflora* [7]. Previous phytochemical investigations on this plant have resulted in the isolation of several flavones, glycosides, including lippiflorin A and B, nodiflorin A and B, nodifloritin A and B, alkaloids, essential oils, resins, stigmasterol, β -sitosterol, sugars, mono, and diflavone sulfates of neptin, jaceosidin, hispidulin, and 6-hydroxy-luteolin [5,6,8,9]. A recent study has shown antidiabetic activity of γ -sitosterol isolated from *Lippia nodiflora* [10].

In Brazil and Central America, it is used as a menstrual disorder remedy [11]. In India, the plant has been used traditionally for the treatment of various health problems including respiratory disorders, gastrointestinal infections, diabetes, fungal infections, jaundice, and cardiovascular diseases [12,13]. In ayurvedic system of medicine, *P. nodiflora* is used as a cooling, aphrodisiac, astringent to the bowels, vulnerary, stomachic, anthelmintic, and alexiteric.

The plant possesses various medicinal properties and used to treat different ailments. Plant preparations are referred to as either

medicinal or herbal when used in promoting health beyond their use in nutrition. To evaluate the therapeutic efficacy of herbal drugs, some histological and histochemical techniques are of immense use. However, there is no report on the histochemical and pharmacognostical report on anatomical and other physico-chemical standards required for the quality control of crude drug. Hence, this study was planned to investigate the detailed pharmacognostical and histochemical aspects of *P. nodiflora*.

METHODS**Collection and identification of plant material**

P. nodiflora were collected from the medicinal plant garden at the Irula Tribal Women's Welfare Society, Chengalpattu, Tamil Nadu, India during the months of June to August, 2009.

Macroscopic evaluation

Fully matured, fresh, and healthy plant materials of *P. nodiflora* were used for macroscopic evaluation. Organoleptic characters such as color, size, odor, taste, and texture were also determined according to the method described by Wallis [14].

Microscopic evaluation**Specimen preparation**

Fresh plant parts, namely root, stem, petiole, leaf, and flower were collected and gently washed to remove any dust or dirt particles adhering to the plant tissues. They were then cut into small pieces and fixed in 5 mL of formalin, 5 mL of acetic acid, and 90 mL of 70% ethyl alcohol for 24 hrs. The materials were dehydrated with graded series of tertiary butyl alcohol (TBA). Infiltration of the specimens was carried out by gradual addition of paraffin wax until TBA solution attained supersaturation. The specimens were embedded into paraffin blocks for sectioning.

Sectioning of specimen

The paraffin-embedded blocks were mounted on wooden stubs and microtome sections 8-14 μm [15] were cut using a Spencer 820 rotary microtome (American Optical Corporation, Buffalo, NY, USA). The resulting paraffin ribbons were stained with alcoholic safranin (0.5% w/v) and counterstained with fast green (0.25%, w/v) solution. After staining with safranin, the slides were dehydrated by employing a graded series of ethyl alcohol (30%, 50%, 70%, 90%, and absolute alcohol). The slides were stained with fast green in clove oil and xylol:alcohol (50:50). They were then passed through 100% xylol and finally mounted in distyrene plasticizer and xylene mountant [16]. The anatomical features were described as given in the standard anatomy books [17,18].

Quantitative microscopy

Quantitative microscopy was carried out and values were determined as per the procedure described by Wallis [19]. To study the stomatal morphology and pattern, paradermal sections were taken in addition to clearing of leaves with 5% NaOH. The cleared materials were washed thoroughly and stained with safranin. The stained sections were mounted on a clean slide using dilute glycerine. Epidermal peelings were partially macerated using Jefferey's maceration fluid in order to study trichome distribution [15].

Powder microscopy and fluorescence analysis

The powder was subjected to microscopical study after staining with safranin [20]. Finely ground plant powder was taken on a microscopic slide, and 1 or 2 drops of 0.1% phloroglucinol solution was added along with a drop of concentrated hydrochloric acid. It was mounted in glycerol and observed under a microscope. The characteristic features of the powder were noted.

Photomicrographs of different magnifications were taken with the help of a Nikon Eclipse E200 microscope. Magnifications of the figures are indicated by scale bars.

Fluorescence analysis was carried out as per the method described by Kokoski *et al.*; Chase and Pratt [21,22]. The powders of aerial parts and roots were treated with different solvents and reagents such as NaOH, HCl, H_2SO_4 , ammonia, hexane, chloroform, ethyl acetate, acetone, glacial acetic acid and methanol and observed under visible light and ultraviolet light of 365 nm.

Physico-chemical analysis

Physico-chemical characteristics such as total ash, water soluble ash, acid soluble ash and extractive values such as water and alcohol were determined to evaluate the quality and purity of the crude drug. Experiments were carried out by employing a standard method described as per WHO guidelines [23,24]. The determination of the extractive values of the powdered drug was carried out according to the procedure described by Mukherjee [25]. The experiments were performed in triplicates and the results were expressed as mean \pm standard error. The results were calculated and expressed in terms of percentage (w/w).

Histochemical studies

Specimen preparation and histochemical studies

Root, stem and leaf were used to localize various compounds. Freshly cut sections were used for histochemical analysis. The sections were stained in Dragendorff's reagent for localization of alkaloids, safranin for localization of lignin, Iodine potassium iodide (I_2KI) for localization of starch, Coomassie Brilliant Blue (CBB) R-250 for localization of proteins, Sudan III for localization of lipids, vanillin for phenolics and dimethoxybenzaldehyde (DMB) reagent for tannins. Standard histochemical localization procedures were used to localize the compounds [26-28].

RESULTS AND DISCUSSIONS

Macroscopic characters

The root system in *P. nodiflora* consists of tap roots which are cylindrical in nature measuring 30-35 cm in length and 0.2-0.5 cm in

diameter, brown in color (Table 1). The secondary and tertiary roots are fibrous measuring 7-10 cm in length and 1.0-1.5 mm in diameter. The nodal roots are smaller and unbranched. Stems are herbaceous and much branched, sub-quadrangular, 2.5-5 mm in diameter with roots at the nodes, more or less clothed, appressed by two armed white hairs. Leaves are simple, small, opposite, subsessile, 1-4 cm long and 0.5-2 cm broad. They are broad, obtuse, obovate, spatulate, cuneate at the base, deeply and sharply serrate toward the apex, appressed by two armed with white minute hairs on both adaxial and abaxial sides. Venation is reticulate. Flowers are small, sessile, densely packed in long pedunculate axillary spikes, mature ones measure 1.0-2.0 cm long and 0.4-0.5 cm broad, peduncles are 2.5-7.5 cm long, bracts broadly elliptic or obovate, cuneate at base, mucronate and glabrous. The calyx is membranous, bilobed, compressed, miter shaped, pubescent underneath with ordinary trichomes closely covering the fruit, the acuminate lobes projecting beyond it. The corolla is 2.5-3 mm long, white or pale pink, bilipped, upper lip erect and bifid, lower lip 3 lobed of which the middle lobe largest and falls off as a calyptra when the fruit ripens. The stamens are 4 in number, didynamous, anthers 2-celled dehiscent longitudinally and dorsifixed. The ovary is superior, bicarpellary with ovules in each cell solitary. The style short and stigma is oblique and subcapitate. Fruits are small 1.5-2 mm long, globose, oblong, splitting into two 1-seeded and plano-convex pyrenes. The seeds are exalbuminous about 1 mm in size. The twig of the plant bearing flower is depicted in Fig. 1.

Microscopic characters

Root

Microscopic method is one of the cheapest and simplest methods to start with establishing the correct identification of the source material [29]. Transverse section (TS) of root is circular in outline and



Fig. 1: *Phyla nodiflora* twig with a flower

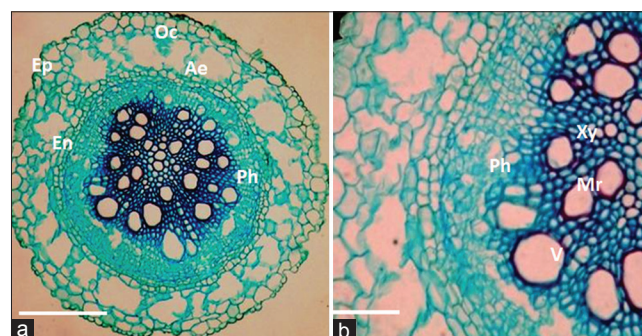


Fig. 2: Transverse section of root: (a) ground plan (bar 0.3 mm); (b) a portion enlarged (bar 0.1 mm) (Ae: Aerenchyma, En: Endodermis, Ep: Epidermis, Oc: Outer cortex, Ph: Phloem, V: Vessel, Mr: Medullary ray, Xy: Xylem)

shows epidermis, cortex, phloem, and xylem (Fig. 2a). The outermost epidermis is composed of small rectangular cells. Inner to the epidermis, the cortex is differentiated into two regions. The outer cortex is made up of two rows of thick-walled tangentially elongated parenchyma cells. The inner cortex consists of parenchymatous cells having 5-7 layers of almost spherical to oval thin-walled cells with large intercellular spaces and irregular cavities forming aerenchyma. Endodermis comprises a single layer of barrel shaped, closely packed cells with thickened radial walls. The phloem consists of 4-6 rows of cubical to polygonal thin-walled cells. Cambium is found between xylem and phloem and has 1 or 2 layers of narrow, thin-walled and rectangular cells.

The medullary rays are mostly uniseriate or sometimes bi or tri-seriate. The cells are thin-walled, polygonal to hexagonal and radially elongated in xylem and phloem region. Phloem consists of phloem parenchyma, sieve tubes and companion cells. Xylem consists of vessels, tracheids, and xylem parenchyma. Vessels are prominent, mostly solitary, rarely in groups of 2 or 3, large, spherical to oval in shape (Fig. 2b).

Stem

The TS of stem is nearly quadrangular in outline with 6 ridges and 6 furrows (Fig. 3a). Epidermis consists of rectangular tangentially elongated cells covered with a striated cuticle. Some of the epidermal cells elongate to form unicellular trichomes with two unequal arms. Glandular trichomes are also present and these are situated in a small depression. The outer cortex is about 3 or 4 cells deep in the furrows, made up of chlorenchymatous cells while those of ridges are composed of 5 or 6 rows of collenchyma cells. The inner cortex consists of 8-10 layers of parenchyma cells arranged with intercellular spaces. Pericycle is represented by discontinuous ring of 1 or 2 cell deep pericyclic fibers. The vasculature is represented by a complete ring of xylem lined on the outer side with phloem. The pith of the plant stem is formed from thin walled parenchymatous cells.

Medullary rays are mostly uniseriate or sometimes biseriata. The cells are radially elongated, oval to oblong or polygonal, parenchyma cells. Phloem is composed of phloem parenchyma, sieve tubes and companion cells. Xylem consists of vessels, tracheids, and xylem parenchyma. The vessels are prominent, solitary or in groups of two (Fig. 3b). The cross section of the stem also revealed the presence of trichomes (Fig. 3c) and spiral thickenings (Fig. 3d).

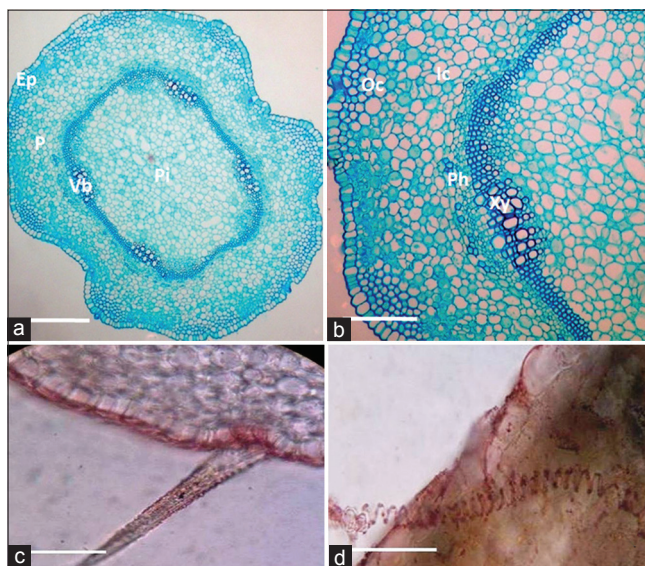


Fig. 3: Transverse section of stem: (a) ground plan (bar 0.3 mm); (b) a portion enlarged (bar 0.1 mm) (Ep: Epidermis, Ic: Inner cortex, Oc: Outer cortex, P: Parenchyma cells, Ph: Phloem, Pi: Pith, V: Vessel, Vb: Vascular bundle, Xy: Xylem)

Leaf

Petiole

The epidermis is made up of small vertically elongated parenchyma cells. Glandular trichomes are seen in small depressions. It is followed by 2 or 3 layers of collenchymatous cells in the adaxial and abaxial side and 3-5 layered in the wings region. These cells are circular to oval, isodiametric with angular thickenings. Inner to collenchyma, the ground tissue consists of thin-walled circular-oval parenchymatous cells having distinct intercellular spaces.

A shallow crescentic, collateral vascular bundle is situated in the center. Two small accessory bundles are situated in the wing region, one on either side of the median vascular bundle (Fig. 4a and b).

Midrib

The TS of midrib shows a small protuberance on the adaxial side and convexity on the abaxial cell. Adaxial and abaxial epidermis consists of thin-walled, compact, oval to oblong cells covered with a thin cuticle. Some of the epidermal cells possess unicellular, two armed non-glandular or glandular trichomes. The glandular hairs consist of the one-celled stalk and 1 or 2 celled globose thick-walled head protruding out of the epidermis. Epidermis is followed by 2 or 3 layers of collenchyma cells. The ground tissue consists of 6-8 layers of thin-walled, round to oval parenchyma cells with intercellular spaces (Fig. 5a and b).

Lamina

TS of lamina shows dorsiventral structure. The adaxial and abaxial epidermis consists of round to oval, thin-walled, compactly arranged cells covered with a thin cuticle. Some of the epidermal cells elongate to form trichomes on both surfaces. The palisade tissues are compact, made up of 2 or 3 layered and columnar closely packed cells. Spongy parenchyma is made up of oval to oblong, large, thin-walled cells (Fig. 6).

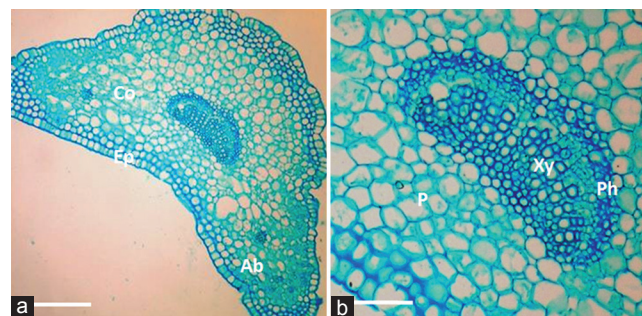


Fig. 4: Transverse section of petiole: (a) ground plan (bar 0.3 mm); (b) a portion enlarged (bar 0.1 mm) (Co: Collenchymatous cells, Esp: Epidermis, Ab: Accessory bundle, P: Parenchyma, Xy: Xylem, Ph: Phloem)

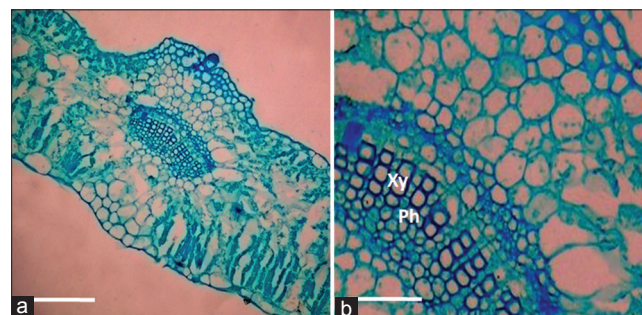


Fig. 5: Transverse section of Midrib: (a) ground plan (bar 0.3 mm); (b) a portion enlarged (bar 0.1 mm), (Ph: Phloem, Xy: Xylem)

Flower

Longitudinal section (LS) of flower bud shows sepal with a single layered epidermis and loosely packed parenchymatous ground tissue traversed by a row of vascular strands. The petal shows upper and lower epidermis and parenchymatous ground tissue. LS of stamen shows single layered upper and lower epidermis followed by 3-5 layers of rounded to oval and large parenchyma cells. The anther shows pollen chambers, which contain a few rounded pollen grains. The gynoecium shows superior ovary, style short, and stigma oblique and subcapitate.

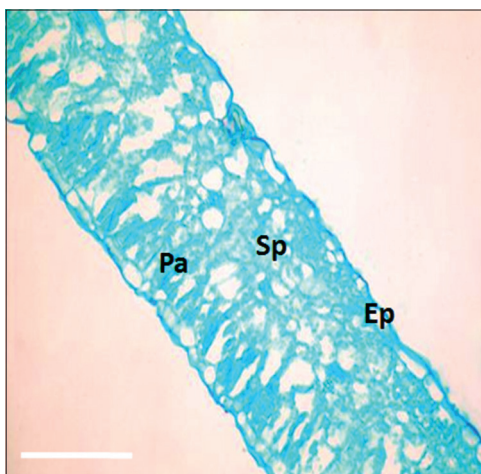


Fig. 6: Transverse section of lamina: Lamina (bar 0.1 mm) (Pa: Palisade tissue, Sp: Spongy parenchyma, Ep: Epidermis)

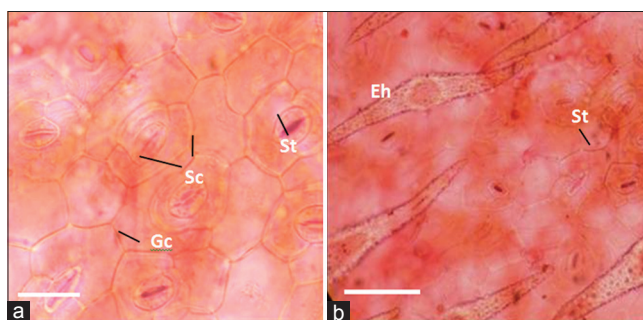


Fig. 7: Epidermis in surface view: (a) Adaxial epidermis with stomata (bar 0.5 mm); (b) with epidermal hairs (bar 0.5 mm) (Eh: Epidermal hair, Gc: Guard cell, Sc: Subsidiary cell, St: Stomata)

Quantitative microscopy

The adaxial (upper) and abaxial (lower) epidermis are made up of polygonal cells in surface view. The margins of these epidermal cells do not show any waviness. They are perforated by stomata but the frequency is less in adaxial epidermis. Diacytic type of stomata (cross-celled), previously known as caryophyllaceous is present. The stoma is accompanied by two subsidiary cells whose common wall is at right angles to the guard cells. Stomata are more in adaxial (20-30 mm²) surface (Fig. 7a and b) when compared to abaxial (8-11 mm²) surface. Palisade ratio for upper epidermis was found to be 6-11 and for the lower epidermis it was determined to be 8-13. The vein islet number was determined to be 3-4 mm². The leaf constants, i.e. stomatal index, palisade ratio, and vein islet numbers are presented in Table 2.

Powder microscopy and fluorescence analysis

The powder appeared pale green in color with no characteristic odor and taste. Glandular and non-glandular trichomes were noted. Glandular hairs consist of one or two-celled, globose, thick-walled head, and one-celled stalk. Non-glandular trichomes were unicellular, with 2 arms (Fig. 8a). When viewed under the microscope, it revealed the presence of fibers (Fig. 8b), parenchyma cells, palisade cells, fragments of leaf with paracytic stomata, mucilage cells, calcium oxalate crystals (Fig. 8c), epidermal cells, cortex cells and vessels.

Table 1: Organoleptic characters of *P. nodiflora*

Part used	Character	Observation			
		Color	Texture	Odor	Taste
Root	Fresh	Brown	Fracture fibrous	No characteristic	No taste
	Dried	Dark brown	Thick hard	No characteristic	No taste
Stem	Fresh	Green	Soft fibrous	Characteristic	Bitter
	Dried	Brown	Hard	No odor	Tasteless
Leaf	Fresh	Green	Soft	Characteristic (castor)	Bitter
	Dried	Yellow	Hard	No odor	No taste

P. nodiflora: Phyla nodiflora

Table 2: Quantitative microscopical parameters of the leaf of *P. nodiflora*

Leaf constants	Values obtained
Stomatal index for adaxial epidermis (upper)	20-30/mm ²
Stomatal index in abaxial epidermis (lower)	8-11/mm ²
Palisade ratio in adaxial epidermis	6-11
Palisade ratio in abaxial epidermis	8-13
Vein islet number	3-4/mm ²

P. nodiflora: Phyla nodiflora

Table 3: Fluorescence analysis of crude extracts of *P. nodiflora*

Chemical treatments/ solvents used	Aerial parts		Roots	
	Visible light	UV light (365 nm)	Visible light	UV light (365 nm)
Powder as such	Green	Green	Brown	Brown
Powder+water	Brownish yellow	Yellowish green	Yellowish brown	Brownish yellow
Powder+1M sodium hydroxide	Brown	Orange	Brownish black	Black
Powder+1N hydrochloric acid	Brown	Yellow	Green	Brownish black
Powder+1N sulphuric acid	Black	Brownish black	Brown	Dark brown
Powder+dilute ammonia	Orange	Orange brown	Brown	Dark brown
Powder+hexane	Green	Greenish yellow	Brown	Brownish black
Powder+chloroform	Green	Dark green	Yellowish green	Green
Powder+ethyl acetate	Green	Dark green	Green	Yellowish green
Powder+acetone	Green	Yellowish green	Brown	Brownish black
Powder+glacial acetic acid	Green	Dark green	Brown	Dark brown
Powder+methanol	Yellowish green	Green	Brown	Brownish black

P. nodiflora: Phyla nodiflora

The fluorescence characteristic of the powdered drug was studied by observing under visible light and UV light with different chemical reagents. The data of the fluorescence analysis is tabulated in Table 3. Some of the substances present in plant will fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products which do not visibly fluoresce in daylight [30]. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. This is an important parameter for pharmacognostic evaluation of crude drugs which are often determined qualitatively in this way [31].

Physico-chemical analysis

The results of the physico-chemical analysis of the crude drug of *P. nodiflora* are tabulated in Table 4. The value for loss on drying at 105°C of aerial parts was 15.68±0.05 and for roots it was 12.64±0.00. Lower percentage loss on drying will be better because fungal contamination will be less due to lesser moisture. This value also indicates that the drug is safe as an antibiotic. The results corroborate with earlier findings in the plant of the same family *Clerodendrum inerme* and *Gmelina arborea* Roxb [32,33].

The results showed that total ash content of the aerial parts was 20.85±0.09% (w/w), acid insoluble ash was 0.80±0.01%, and water soluble ash was 7.24±0.006%. The water extractive value of aerial parts was 21.44±0.009% and alcohol extractive value was 13.39±0.003%.

Table 4: Physico-chemical analysis of *P. nodiflora*

Parameters	Aerial parts (values in [%w/w])	Root (values in [%w/w]) ^a
Loss on drying at 105°C	15.68±0.05	12.64±0.00
Total ash	20.85±0.09	25.74±0.07
Water soluble ash	7.24±0.006	8.05±0.03
Acid insoluble ash	0.80±0.01	0.43±0.01
Water extractive value	21.44±0.009	22.23±0.02
Alcohol extractive value	13.39±0.003	11.75±0.01

P. nodiflora: *Phyla nodiflora*, *: significant at 5% level

Whereas, the total ash content of roots was 25.74±0.07%, acid insoluble ash was found to be 0.43±0.01% and water soluble ash was found to be 8.05±0.03%. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign matter such as metallic salts or silica [34,35]. Water soluble ash was more in roots when compared to aerial parts. On the other hand, presence of acid insoluble ash was more in aerial parts when compared to roots. The water extractive value of roots was 22.23±0.02% and alcohol extractive value 11.75±0.01% was recorded (Table 4). Acid insoluble ash value indicates the presence of higher bicarbonates which has high diuretic activity. If water soluble extractive value was found to be more, it indicates that it contains more polar phytoconstituents than non-polar phytoconstituents. Acid insoluble ash value indicates the presence of higher bicarbonates which has high diuretic activity. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not [36].

Histochemical studies

TS of the fresh plant material were taken and histochemical reactions were carried out using different stains. Results obtained are presented in Table 5. Histochemical study indicated the presence of alkaloids, lignin, starch, proteins, lipids, phenolics, and tannins in the plant parts examined.

Cortex, xylem, phloem, and pith of root section stained red in color which indicated the presence of lignin and vascular tissue possesses more lignin than other regions. In stem and root outer cortex, xylem and phloem stained red in color. In leaves, outer and inner cortex, xylem and phloem stained red. The presence of starch was noticed by bluish green color when treated with potassium iodide solution. Cortex region, xylem, phloem and pith of stem, outer and inner cortex of root and leaf stained bluish green color indicating the presence of starch, and cortex region contained more starch than other regions. Proteins stained blue in color when stained with CBB stain. Vascular tissues of stem, cortex of root, and leaf stained blue in color. Fresh sections treated with Sudan III stained brown in color. Phloem tissues

Table 5: Histochemical analysis of the root, stem, and leaf of *P. nodiflora*

Metabolites	Stain	Color	Regions localized		
			Root	Stem	Leaf
Alkaloid	Dragendorff's	Pink	Outer cortex, inner cortex, xylem, phloem	Outer cortex, xylem, phloem	Cortex, xylem, phloem
Lignin	Safranin	Red	Outer cortex, xylem, phloem	Outer cortex, xylem, phloem	Outer and inner cortex, xylem, phloem
Starch	I ₂ KI reagent	Purple	Outer and inner cortex	Outer cortex, inner cortex xylem, phloem, pith	Outer and inner cortex, xylem, phloem, pith
Protein	CBB reagent	Blue	Outer and inner cortex	Xylem, phloem	Outer and inner cortex
Lipid	Sudan III	Brown	Epidermis, vascular region	Outer cortex, xylem, phloem, pith	Epidermis, vascular region
Phenolics	Vanillin	Red	Outer and inner cortex	Outer and inner cortex	Cortex
Tannins	Ferric sulfate	Blue	Vascular region	Vascular region	Cortex

P. nodiflora: *Phyla nodiflora*, CBB: Coomassie Brilliant Blue

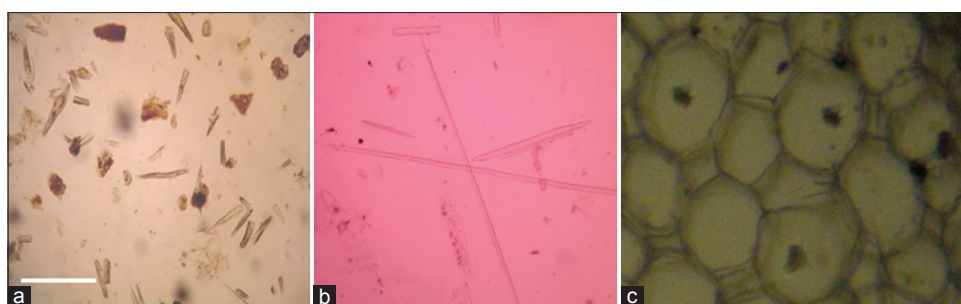


Fig. 8: Powder microscopy: (a) Non-glandular trichomes (bar 0.1 mm); (b) libriform fibres (bar 0.3 mm); (c) calcium oxalate crystals (bar 0.5 mm)

contained more lipids than other tissues. In the stem, outer cortex, vascular tissues and pith contained lipids and more lipids were localized in the vascular region. In roots and leaves, the epidermis and vascular regions contain more lipids.

Sections stained with Dragendorff's reagent turned pink in color which indicated the presence of alkaloids. Alkaloids are natural product that contains heterocyclic nitrogen atoms, are basic in character. Outer cortex region, xylem and phloem of stem and root showed the presence of alkaloids. In leaves, the cortex, xylem and phloem showed the presence of alkaloids. Sections treated with vanillin turned red color which indicated the presence of phenolics. The cortex region of stem and root showed the presence of phenolics. The presence of tannins was noticed with purplish red color when treated with DMB reagent. The vascular region of root and stem and cortex region of leaf showed the presence of tannins.

CONCLUSION

In this paper, the information on the botanical description, traditional uses, phytochemistry, and pharmacology of *P. nodiflora*, a medicinal plant distributed in India, Sri Lanka, South and Central America, and Tropical Africa. However, it is imperative that more clinical and pharmacological studies should be conducted to investigate the unexploited potential of this plant. Anatomical and morphological parameters discussed here provide useful information in regard to the correct evaluation and authentication of the plant. Physico-chemical analysis and phytochemical screening add to its quality control and quality assurance, and serves as a standard monograph for identification and substantiation of the crude drug.

REFERENCES

- Manjunath BL. The Wealth of India. New Delhi: CSIR; 1962. p. 142-3.
- Jayaweera DM. Medicinal Plants used in Ceylon, Part IV. Colombo, Sri Lanka: National Science Council of Sri Lanka; 1982. p. 274-5.
- Terblanche FC, Kornelius G. Essential oil constituents of the genus *Lippia* (Verbenaceae). A literature review. *J Essent Oil Res* 1996;8:471-85.
- Kirtikar KR, Basu BD. Indian Medicinal Plants. Reprint Edition. New Delhi, India: Publications and Information Directorate, CSIR; 1975. p. 986-7.
- Francisco A, Barbaran T, Harborne BJ, Self R. Twelve 6 - oxygenated flavone sulphates from *Lippia nodiflora* and *L. Canescens*. *Phytochemistry* 1987;26(8):2281-4.
- Forestieri AM, Monforte MT, Ragusa S, Trovato A, Lauk L. Anti-inflammatory, analgesic and antipyretic activity in rodents of plant used in African medicine. *Phytother Res* 1996;10:100-6.
- Pal Singh I, Bharate SB. Phloroglucinol compounds of natural origin. *Nat Prod Rep* 2006;23(4):558-91.
- Basu AK, Chakraborti P, Sanyal PK. Nodifloretin – A new flavone from *Lippia nodiflora*. *J Indian Chem Soc* 1969;46(3):271-2.
- Nair AG, Ramesh P, Nagarajan S, Subramanian S. New flavones glycosides from *Lippia nodiflora*. *Indian J Chem* 1973;2:1316-7.
- Balamurugan R, Duraipandiyar V, Ignacimuthu S. Antidiabetic activity of γ - sitosterol isolated from *Lippia nodiflora* L. In streptozotocin induced diabetic rats. *Eur J Pharmacol* 2011;667(1-3):410-8.
- Morton JF. Atlas of Medicinal Plants of Middle America. Springfield, Illinois, USA: CC Thomas; 1981. p. 745-50.
- Hooker JD. Flora of British India. Vol. 4. Ashford, Kent, London: Reeve East House Book Publisher; 1885.
- Kirtikar KR. The Indian Medicinal Plants, Part-II. Allahabad: Office Bhuwaneswari Asrama Bahadur Gang; 1918. p. 986-7.
- Wallis TE. Textbook of Pharmacognosy. 5th ed. New Delhi, India: CBS Publishers and Distributors; 1985. p. 652.
- Sass JE. Elements of Botanical Microtechnique. New York: McGraw Hill Book Co; 1940. p. 222.
- Johansen D. Plant Micro Techniques. New York: McGraw Hill Publication; 1940.
- Esau K. Plant Anatomy. New York: John Wiley and Sons; 1964a.
- Esau K. Anatomy of Seeds. New York: John Wiley and Sons; 1964b.
- Wallis TE. Textbook of Pharmacognosy. 5th ed. London: J and A Churchill Limited; 1997.
- Wallis TE. Textbook of Pharmacognosy. 15th ed. London: T A Churchill Limited; 1967. p. 571-5.
- Kokoski CJ, Kokoski RJ, Slama FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. *J Am Pharm Assoc Am Pharm Assoc (Baltim)* 1958;47(10):715-7.
- Chase CR Jr, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J Am Pharm Assoc Am Pharm Assoc* 1949;38(6):324-31.
- Anonymous. Quality Control Methods for Medicinal Plant Materials. Geneva: World Health Organisation; 1998. p. 25-30.
- Indian Pharmacopoeia. Government of India. Ministry of Health and Family Welfare. New Delhi: Controller of Publications; 1996. p. A53-5.
- Mukherjee PK. Quality Control of Herbal Drugs. 1st ed. New Delhi: Business Horizons; 2002. p. 546-9.
- Jensen WA. Botanical Histochemistry: Principles and Practice. San Francisco: W.H. Freeman and Co; 1962.
- Harborne JB. In: Jackman H, editor. Phytochemical Methods. London: Butterworths; 1973. p. 70.
- Pearse AG. Histochemistry Theoretical and Applied. 4th ed. Vol. 2. Edinburgh, UK: Churchill Livingstone; 1980.
- Singh S, Manchawal L, Chauhan MG. Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. With special emphasis on microscopic technique. *J Pharmacogn Phytother* 2010;2(5):71-5.
- Kumar D, Kumar K, Kumar S, Kumar T, Kumar A, Prakash O. Pharmacognostic evaluation of leaf and root bark of *Holoptelea integrifolia* Roxb. *Asian Pac J Trop Biomed* 2012;2(3):169-75.
- Ansari SH. Essentials of Pharmacognosy. 1st ed. New Delhi: Birla Publications Pvt. Ltd; 2006.
- Devi VG, Vijayan C, John A, Gopikumar K. Pharmacognostic and antioxidant studies on *Clerodendrum inerme* and identification of Ursolic acid as marker compound. *Int J Pharm Pharm Sci* 2012;4(2):145-8.
- Acharya NS, Acharya SR, Shah MB, Santani DD. Development of pharmacognostical parameters and estimation of beta-sitosterol using HPTLC in roots of *Gmelina arborea* Roxb. *Pharmacog J* 2012;4(30):1-9.
- World Health Organization. Quality Control Methods for Medicinal Plant Materials. WHO/PHARM/92.559; 1998. p. 4-46.
- Anonymous. Ministry of health and family welfare. Indian Pharmacopoeia. Vol. 2. Govt of India, New Delhi: Controller of Publications; 1996. p. A - 53-4, A-95, A-97, A-109.
- Tatiya A, Surana S, Bhavsar S, Patil D, Patil Y. Pharmacognostic and preliminary phytochemical investigation of *Eulophia Herbacea* Lindl. Tubers (*Orchidaceae*). *Asian Pac J Trop Dis* 2012;2 Suppl 1:S50-5.