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Research Article

PHARMACOGNOSTIC STANDARDIZATION AND HPTLC FINGERPRINTING OF *JALAPIPPALI (Phyla Nodiflora)*

Aakriti Asthana[1]*, Monika Tyagi[2], Arun Kumar[3]
*[1]PG Scholar, [2]Assistant Professor, Dept. of Dravyaguna, A & U Tibbia College & Hospital, Karol Bagh, Delhi.
[3]Project Associate II (Plant Taxonomy) RRDR, Dept. of Dravyaguna, All India Institute of Ayurveda, New Delhi.

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ABSTRACT
Phyla nodiflora is a medicinal herb widely used in traditional and Ayurvedic medicine for various therapeutic purposes. The present study was undertaken to establish its pharmacognostical, phytochemical, and HPTLC profile for proper identification, authentication, and standardization. Detailed macroscopic and microscopic studies of the root, stem, and leaf were carried out along with powder microscopy. Preliminary phytochemical screening of alcoholic and aqueous extracts was performed using standard qualitative methods. HPTLC analysis was conducted on silica gel 60 F_{254} plates using toluene:ethyl acetate:formic acid (5:4:1) as the mobile phase, and chromatograms were scanned at 254nm and 366nm. Microscopic evaluation revealed characteristic anatomical features including epidermal layers, vascular arrangement, cortex, medullary structures, and trichomes. Powder microscopy showed diagnostic features such as epidermal fragments, vessel elements, fibers, stomata, and trichomes. Phytochemical analysis revealed the presence of glycosides, flavonoids, terpenoids, and steroids in both extracts, while saponins were detected only in the aqueous extract. HPTLC profiling demonstrated multiple peaks, indicating diverse phytoconstituents. A major peak at Rf 0.34–0.36 suggested flavonoid compounds, while higher Rf peaks indicated possible phytosterols. The study establishes standard reference parameters for authentication and quality control of *Phyla nodiflora*.

INTRODUCTION
Phyla nodiflora (Linn.) Greene, also known as *Jalapippali* in Ayurveda and commonly referred to as frog fruit or turkey tangle, is a small perennial, creeping herb belonging to the family Verbenaceae. It is naturally distributed across tropical and subtropical regions of the world and is abundantly found in many parts of India[1]. The plant typically grows in moist and marshy environments, such as along riverbanks, pond margins, drainage lines, irrigated fields, and open wastelands, where it forms a dense mat-like ground cover[2]. Its ability to thrive under full sunlight, partial shade, and varied soil conditions makes it an ecologically adaptable species [3].

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Traditionally, *Phyla nodiflora* holds significant importance in Ayurveda due to its *Pitta-shāmaka, Shothahara* (anti-inflammatory), *Vranaropaka* (wound healing) and *Krimighna* (antimicrobial) properties[4]. It is used in managing skin diseases, fevers, respiratory complaints, digestive disorders, urinary conditions, and inflammatory ailments[5]. Folk practices also utilise the plant for cooling, soothing, and topical therapeutic applications. The herb is known to contain a diverse range of phytoconstituents such as flavonoids, terpenoids, phenolics, alkaloids and essential oils which contribute to its broad spectrum of pharmacological effects[6].

With increasing interest in medicinal plants and the growing need for quality assurance, a detailed pharmacognostical assessment of *Phyla nodiflora* becomes essential. Such evaluation helps in confirming its identity, identifying diagnostic features, and establishing standard parameters for its safe and effective use in herbal formulations[7]. This article presents a comprehensive overview of the

macroscopic, microscopic, physicochemical, and phytochemical characteristics of *Phyla nodiflora* to support its authentication and standardisation[8].

METHODS

Collection and identification of plant material

Fully matured, fresh, and healthy plant materials of *P. nodiflora* were collected from the herbal garden of Ayurvedic and Unani Tibbia College and Hospital, Karol Bagh, New Delhi, during July to September, 2025. Herbarium specimen was prepared for identification and authentication of drug. The plant sample were taxonomically identified and authenticated by BGIR- Botanical Garden of Indian Republic, BSI (Botanical Survey of India), Sector 38A, Gautam Budh Nagar, Noida with Authentication no. BSI/BGIR/1/TECH./2025/277 at. dated: 19-11-2025.

Plant Sample Processing

The whole and powdered drug samples were stored in airtight, light-resistant containers under ambient conditions in accordance with the standards of the Ayurvedic Pharmacopoeia of India[1]. Finely sieved (60# mesh) and coarsely powdered samples were used for powder microscopy, physicochemical evaluation, phytochemical screening, and chromatographic studies following standard procedures.

Macroscopy of Plant Material

The plant was evaluated for morphological characteristics such as structure, shape, size, and texture using unaided vision and a simple microscope (Olympus OIC DM). Organoleptic properties including colour, odour, and taste were assessed systematically through sensory evaluation of the whole plant powder.[1,9]

Sectioning of specimen

The root, stem and leaf sample was pre-soaked in water and transversely sectioned using a sharp diamond-edged blade. Temporary mounts of the sections were prepared following standard procedures and examined under a digital trinocular compound microscope (Olympus CX21i fitted with a Magcam DC14 camera). Diagnostic features were observed and photomicrographs were recorded[1,9].

Powder microscopy of whole plant

Approximately 2gm of finely dried powder, sieved through 80# mesh, was prepared as wet mounts using 50% glycerine and as dry mounts by the smearing method for uniform distribution. The preparations were examined under a Zeiss AxioCam ICc5 microscope at 10× and 40× magnifications, and photomicrographs were recorded to document the diagnostic microscopic features.

Physicochemical Evaluation

Physicochemical evaluation of the whole plant of *Phyla nodiflora*, including loss on drying, ash values, and extractive values, was carried out according to the methods prescribed in the Ayurvedic Pharmacopoeia of India and WHO guidelines. Solvent extractability was assessed using ethanol and water through conventional extraction methods[1,9].

Determination of Moisture Content (Loss on Drying)

This procedure was carried out to determine the amount of volatile matter, mainly moisture content, present in the drug. Approximately 5gm of powdered *P. nodiflora* was taken separately in three Petri dishes to obtain the average value. The samples were dried at 105°C for 5 hours, cooled, and weighed. Drying was continued at the same temperature with repeated weighing at one-hour intervals until a constant weight was achieved. The percentage loss on drying was then calculated with reference to the air-dried drug using the standard formula

Weight of the empty petri dish = W 1gm

Weight of the drug sample = X gm

Weight of the petri dish with drug before drying (W3) = (W1+ X)

Weight of petri dish after drying = W2gm

Loss on drying in % = [W3-W2/X] × 100

Determination of Extractive Values

Determination of Alcohol-Soluble Extractive

Approx 10gm each of powder of *P.nodiflora* were weighed and macerated with 100ml of ethanol. It was then kept in a closed flask for twenty-four hours, shaken frequently during first six hours and then allowed to stand for rest of the eighteen hours. It was then filtered rapidly. Precautions were taken against loss of solvent. Then filtrate was evaporated to dryness in a tarred flat-bottomed shallow petri dish, and dried at 105°C, to constant weight and weighed. The percentage of alcohol soluble extractive with reference to the airdried drug was calculated using the formula.

Weight of the drug material = X gm

Weight of the empty petri dish = W1 gm

Weight of the petri dish with dried extract = W2 gm

Percentage of extractive value = [W2-W1/X] × 100

Determination of Water-soluble Extractive

The procedure was carried out in the same manner as for the alcohol-soluble extractive determination, using distilled water in place of ethanol.

Determination of Ash Values

Approximately 2gm of *P.nodiflora* powder was accurately weighed into a pre-weighed silica crucible and incinerated at the required temperature. 500-600°C until free from carbon. It was then cooled under

desiccator and weighed. The ash content was determined as a percentage of the air-dried drug using the standard formula.

Wt. of Empty Silica Crucible = A1 gm

Wt. of Sample (X) = X gm

Wt. of the Crucible with Ash = A2 gm

Percentage of Total Ash = [A2 - A1/X] × 100

Determination of Acid-Insoluble Ash (AIA)

Each crucible containing the total ash of *P.nodiflora* was treated with 25ml of dilute HCl. Then these crucibles were kept in heating mantle until they start to boil after which the solution was filtered using the ashless filter paper (Whatman 41). The insoluble matter was collected on the filter paper and washed with hot water until the filtrate was neutral. After collecting the insoluble matter on filter paper, it was transferred to the same crucible, dried in the muffle furnace, and subjected to ignition until a stable weight was obtained. The residue was cooled in a desiccator for 30 minutes, weighed immediately, and the percentage of acid-insoluble ash relative to the air-dried sample was calculated using the standard formula.

Wt. of drug sample = X gm

Wt. of Crucible = G1 gm

Wt. of Crucible with insoluble Ash = G2 gm

Wt. of insoluble ash (G3) = G2-G1

Percentage of acid insoluble ash = G3/X × 100

Determination of pH range

Approx. 3gm each of powder of *P.nodiflora* were weighed and immersed in 30ml of distilled-water. It was then kept in a closed flask and was shaken frequently for five hours and then allowed to stand overnight. It was then filtered into another beaker and the pH of the formulation was determined using a pH paper by noticing the colour change.

Preliminary Phytochemical Analysis

Approximately 5g of powdered *P.nodiflora* was taken in conical flask, and solvents (ethanol and distilled water) were added in a 1:10 ratio (50ml per sample). The samples were subjected to rotary shaking for six hours, allowed to settle, and subsequently passed through Whatman filter paper to yield the extracts. The filtrate was taken for screening of phytochemicals by using standard chemical tests [10,11].

Test for Alkaloids

Dragendroff's Test– 2ml alcoholic/aqueous extract of powder of *P.nodiflora* were taken separately in the test tubes and few drops of diluted hydrochloric acid (dil. HCl) were added to them respectively. The solution formed was then filtered and 1ml of Dragendroff's reagent (potassium bismuth iodide solution) was added to each. Formation of orange precipitate indicates the presence of alkaloids.

Test for Saponins

Foam Test– 2.5ml alcoholic/aqueous extract of powder of *P.nodiflora* were taken separately in the test tubes and 10ml distilled water was added to them. The mixture was shaken thoroughly and allowed to stand for two minutes. Formation of honeycomb- like froth indicated the presence of saponins and visa-versa.

Test for Tannins

Ferric Chloride Test– 1ml alcoholic/aqueous extract of powder of *P.nodiflora* were taken separately in the test tubes and 5ml distilled water was added to them. Then, few drops of 10% $FeCl_3$ solution were added to the respective test tubes. A greenish-black or bluish-black precipitate appears, indicating tannins are present.

Test for Glycosides

Keller-Killiani test– 1ml alcoholic/aqueous extract of powder of *P.nodiflora* were taken separately in the test tubes after which the extracts were dissolved in approximately 1ml of glacial acetic acid, 10% ferric chloride solution, and concentrated sulfuric acid. A reddish-brown colour ring at the junction of two layers indicated the presence of glycosides.

Test for Flavonoids

Alkaline Reagent Test- In separate test tubes containing 1ml of alcoholic/aqueous extract of *P.nodiflora*, a few drops of 20% sodium hydroxide solution were added, resulting in the formation of a yellow precipitate. A few drops of dilute hydrochloric acid make the solution colourless, thereby confirming flavonoid content.

Test for Carbohydrates

Molisch's Test- About 1ml of the aqueous/alcoholic extract of *P.nodiflora* was taken in a test tube, to which 2–3 drops of Molisch's reagent were added. Subsequently, concentrated sulphuric acid was carefully poured along the side of the tube to form a separate layer. The formation of a violet ring at the interface indicated the presence of carbohydrates.

Test for Steroids

Salkowski test– 2ml alcoholic/aqueous extract of powder of *P.nodiflora*, were taken separately in the test tubes to which 2ml of chloroform along with equivalent quantity (2ml) of conc. H_2SO_4 was added, respectively. The formation of the pink/red ring indicated the presence of steroids.

Fingerprint Analysis by High-Performance Thin Layer Chromatography (HPTLC)

The filtrate was concentrated to 1gm per 10ml and then subjected to HPTLC profiling. The extract (4µL) was spotted on a pre-coated silica gel GF 60 254

aluminium plate using a Camag Linomat V sample applicator equipped with a 100L Hamilton syringe. The plate was developed in a pre-saturated twin trough chamber using the mobile phase as Toluene, Ethyl Acetate, Formic Acid (5:4:1) (v/v/v) to a distance of 90 mm, dried for 5 min in ambient air. After development, a densitometric scan was done with a Camag TLC scanner III in reflectance absorbance mode at UV detection wavelengths of 254nm and 366nm using Win CATS Software (V 1.2.1. Camag).

Macroscopic characters

Root– Brown, fibrous, branched roots, 2–10cm long and 1.0–1.5mm in diameter; nodal roots smaller, unbranched, 0.5–1.0cm long. **Stem** – Branched, slightly four-angled stem, 1–2mm in diameter, rooting at nodes and covered with appressed two-armed white hairs; internodes 5.0–9.0cm long. **Leaf**– Opposite, nearly sessile, spathulate leaves, 1.5–3.7×1–2cm, with cuneate base, serrated margins, and fine two-armed hairs on both surfaces. **Flower**– Sessile flowers densely arranged on pedunculate axillary spikes (1.0–2.0cm long). Corolla white to pale pink, bilabiate, 2.5–3.0mm long; stamens four, didynamous; ovary superior, bicarpellary. **Fruit**– Small globose to oblong fruits, 1.5–2.0mm long, splitting into two one-seeded pyrenes; seeds exalbuminous, about 1mm long. [Table 1, Fig 1]

RESULTS

Table 1: Organoleptic features of *Phyla nodiflora* (whole plant) powder

S.No.	Character	Phyla nodiflora (whole plant) Powder
1.	*Shabda* (sound)	-
2.	*Sparsha* (touch)	Smooth
3.	*Roopa* (colour)	Light greenish yellow
4.	*Rasa* (taste)	*Tikta* (bitter) – *Kashaya* (astringent)
5.	*Gandha* (odor)	None

Table 2: Physicochemical observations of *Phyla nodiflora* (whole plant)

S.No.	Test	Observations
1.	Foreign matter	1.37%
2.	Extractive value (alcohol soluble)	17.54%
3.	Extractive value (water-soluble)	9.16%
4.	Total ash	12.11%
5.	Acid insoluble ash	4.02%
6.	Moisture content (LOD)	9.72%
7.	pH value	6.1

Table 3: Phytochemical Observations of Phyla nodiflora (whole plant)

S.No.	Phytochemical	Alcohol extract	Aqueous extract
1.	Alkaloids- Dragendroff's Test	-	-
2.	Saponins- Foam test	-	+
3.	Tannins- FeCl3 test	-	-
4.	Glycosides- Keller–Killiani test	+	+
5.	Flavonoids- Alkaline Reagent Test	+	+
6.	Carbohydrates- Molisch's test	-	-
7.	Steroids- Salkowski Test	+	+

Note: (-): Absent, (+): Present

Fig. 1 *Jala pippali* (*Phyla nodiflora*)

Microscopic characters

Transverse section of stem

The transverse section of the stem of *Phyla nodiflora* observed at 10× magnification shows a single-layered upper epidermis lined with a thin cuticle**,** providing protection against desiccation. Beneath it, the cortex is composed of several layers of parenchymatous cells forming the ground tissue, which serves both storage and mechanical functions. The centrally placed vascular bundle consists of xylem, oriented towards the pith, responsible for the conduction of water and minerals, and phloem, positioned externally, aiding in the translocation of organic solutes. At 40× magnification, the stem exhibits two distinct trichome types: two-armed simple trichomes**,** which serve as mechanical defence structures, and glandular trichomes**,** which secrete secondary metabolites involved in protection and ecological adaptation. (Fig.2 A, B, C, D, F).

Transverse section of Leaf

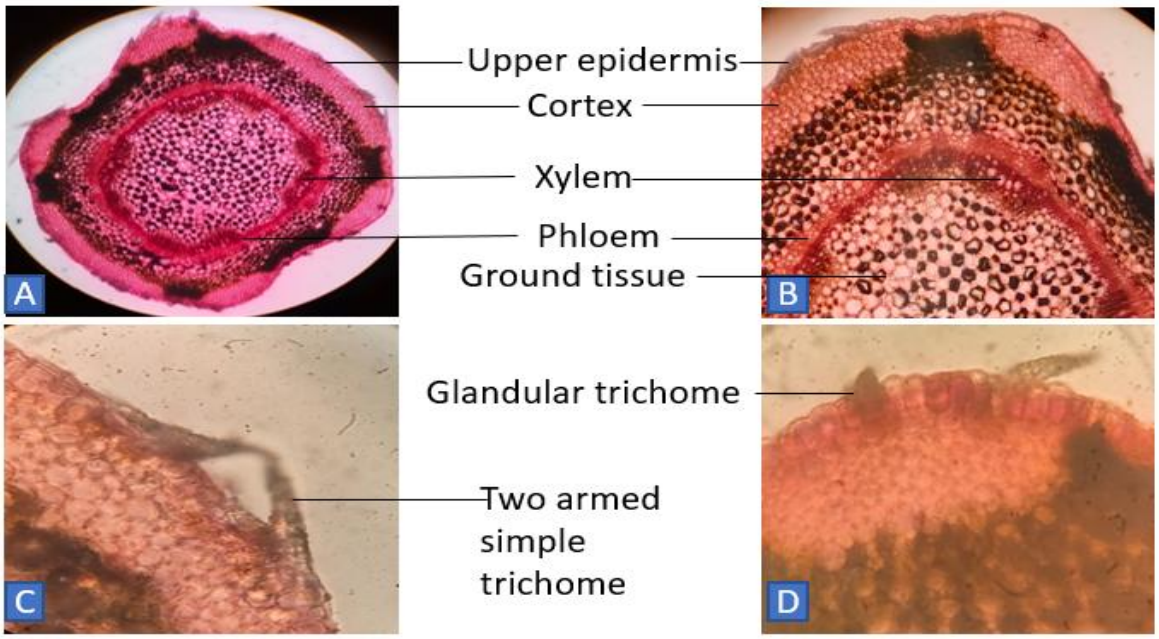
The leaf T.S. at 40× reveals an upper and lower epidermis with a protective cuticle and numerous glandular trichomes**.** The mesophyll is composed of chlorenchyma cells that facilitate photosynthesis and gaseous exchange. A centrally located vascular bundle ensures efficient transport of water and assimilates. (Fig.2 E)

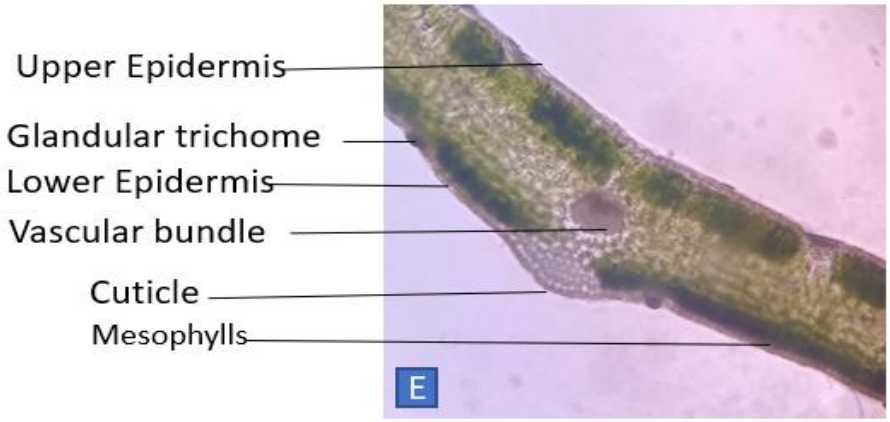
Transverse section of Root

The root T.S. at 40× shows an outer epidermis bearing abundant root hairs**,** increasing absorptive efficiency. The cortex consists of parenchyma, while the endodermis with a Casparian strip regulates ion entry. The central stele contains xylem, phloem, and a pericycle involved in lateral root formation. (Fig.2 G)

Powder Microscopy of whole plant

The safranin-stained powder of *Phyla nodiflora* examined under 40× magnification shows a variety of characteristic diagnostic structures. Numerous unicellular trichomes (Fig. 2A) appear as elongated, thin-walled projections contributing to surface protection by reducing herbivory and limiting pathogen entry. Simple trichomes (Fig. 2B) and abundant two-armed simple trichomes (Fig. 2C, D) are also present, the latter showing bifurcated arms that function in mechanical defense and reduction of water loss as well as aiding in trapping small particulate matter and minimizing transpiration under stress. Fragments of phloem parenchyma cells (Fig. 2E) are observed as thin-walled, isodiametric cells involved in storage within the vascular tissue and assisting in lateral transport of metabolites. Several groups of fibres (Fig. 2F), including sclerenchymatous fibres (Fig. 2H), are present; these are thick-walled, lignified elements that provide rigidity and structural strength to the plant and offer resistance against mechanical injury and bending stress.





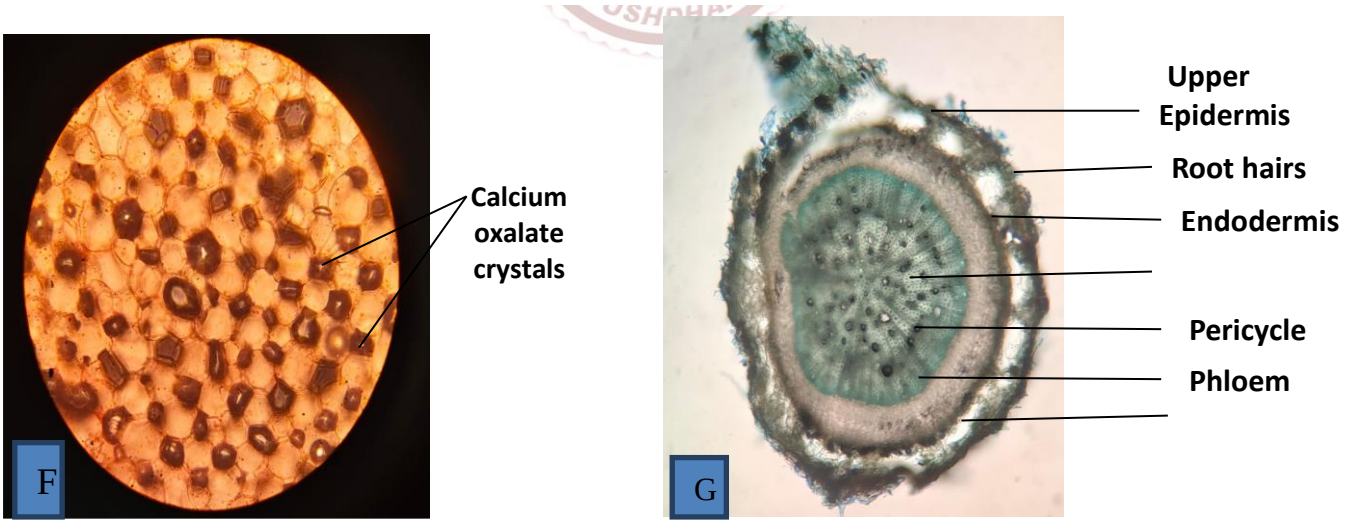
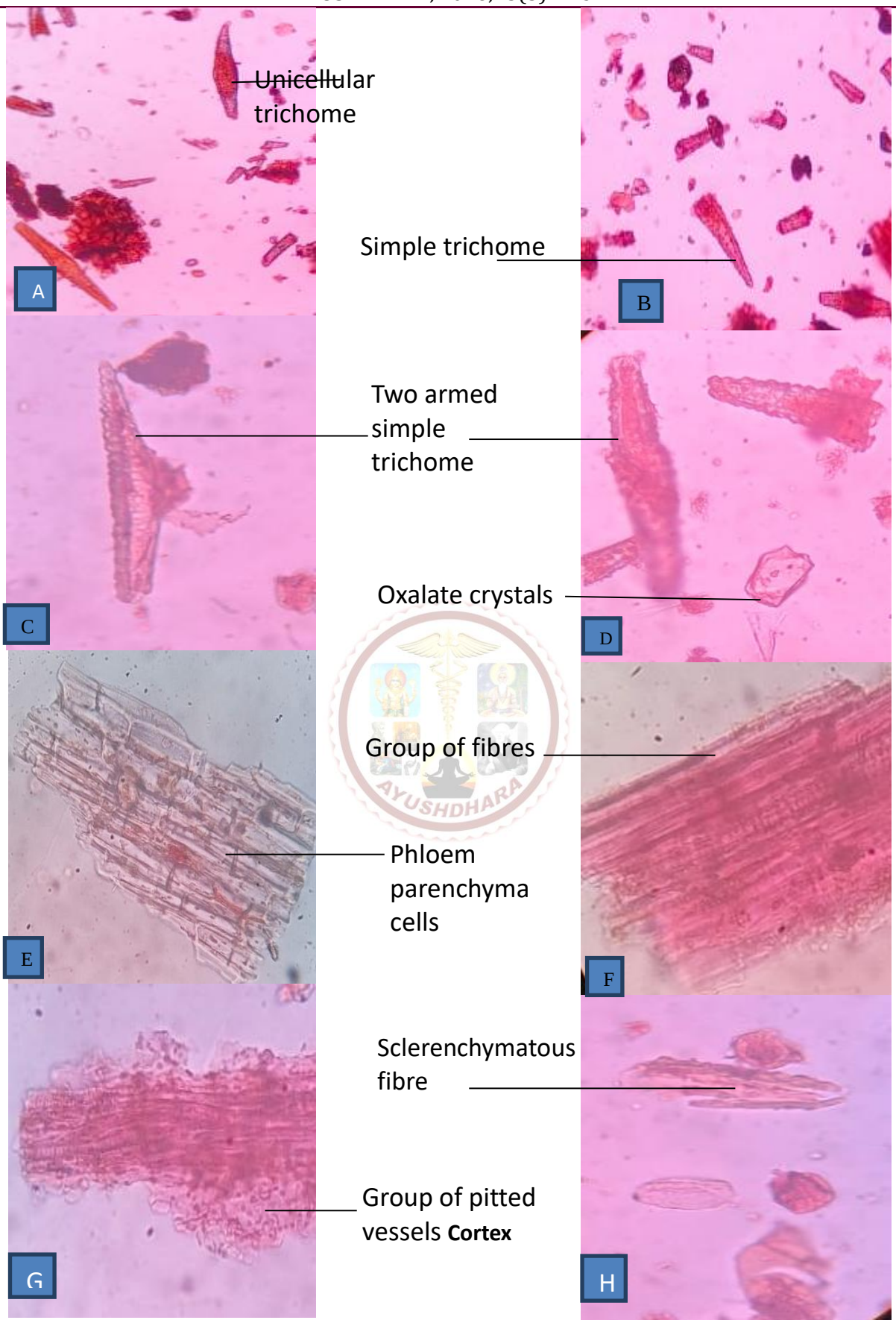


Figure 2: *Phyla nodiflora;* Transfer section of stem showing at 10x; **A & B**: Upper epidermis, Cortex, Xylem, Phloem, Ground tissue, T. S. of Stem showing at 40x **C & D**. Two-armed simple trichome and Glandular trichome, T.S. of Leaf showing at 40x **E**. Upper Epidermis, Glandular trichome, Lower Epidermis, Vascular bundle, Cuticle, Mesophylls, T.S. of Root showing at 40x **G.** Upper Epidermis, Root hairs, Endodermis, Xylem, Pericycle, Phloem, Cortex. **F.** Shows calcium oxalate crystals in T.S of stem.


Unicellular trichome
A
Simple trichome
B
Two armed simple trichome
C
Oxalate crystals
D
Group of fibres
Phloem parenchyma cells
E
F
Sclerenchymatous fibre
Group of pitted vessels Cortex
G
H

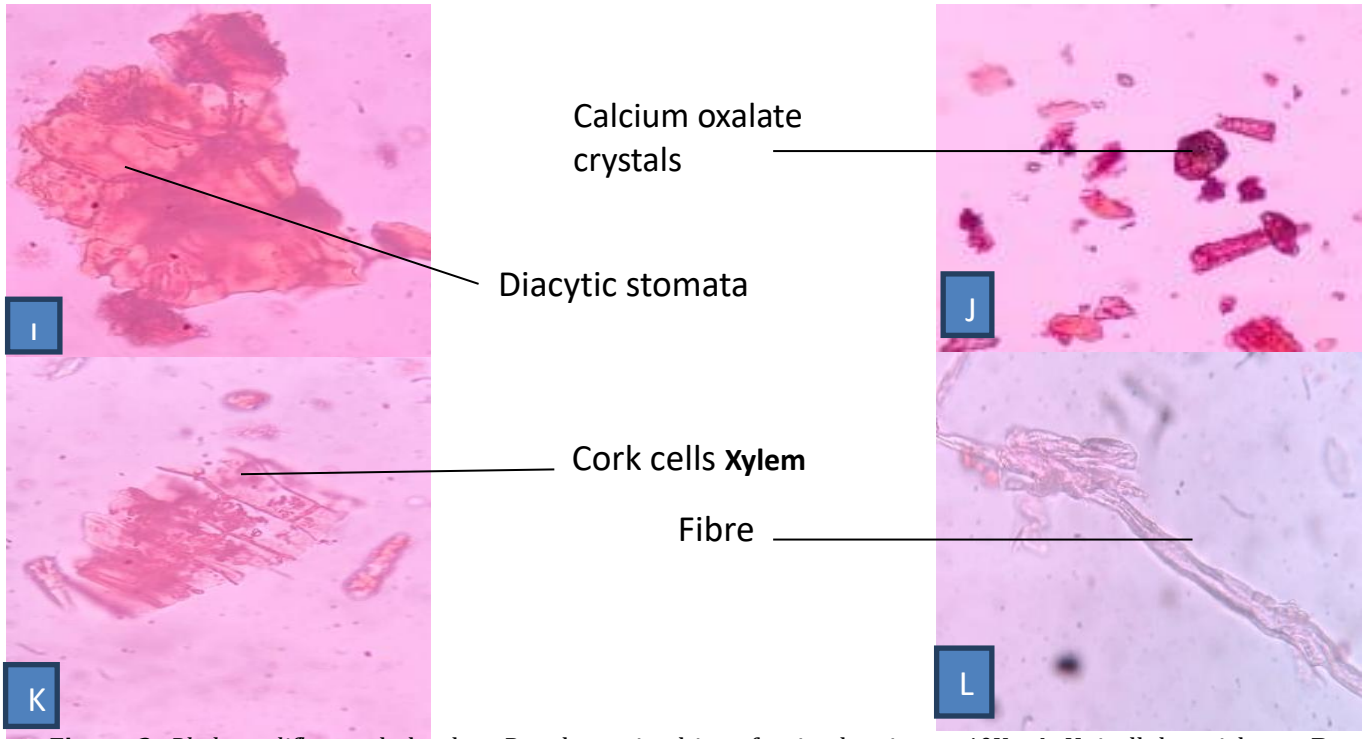


Figure 3: *Phyla nodiflora*; whole plant Powder stained in safranin showing at 40X ; **A**. Unicellular trichome **B.** Simple trichome **C & D.** Two armed simple trichome **E.** Phloem parenchyma cells **F.** Group of fibres **G.** Group of pitted vessels **H.** Sclerenchymatous fibre **I.** Diacytic stomata **J.** Calcium oxalate crystals **K.** Cork cells **L.** Fibre

The powder contains clusters of pitted vessels (Fig. 2G), identifiable by bordered pits characteristic of xylem elements essential for water conduction and maintaining hydraulic continuity during transpiration. Diacytic stomata (Fig. 2I) are also observed, distinguished by two subsidiary cells arranged perpendicular to the guard cells, reflecting the epidermal features of the leaf and enabling efficient gas exchange and transpiration regulation**.** Abundant calcium oxalate crystals (Fig. 2J) occur as diagnostic crystalline inclusions, serving defensive and physiological roles such as deterring herbivores, regulating calcium levels, and aiding in tissue rigidity. Fragments of cork cells (Fig. 2K) with suberized walls are present, representing peridermal tissue that offers protection against desiccation, pathogens, and environmental stress. Additionally, isolated fibres (Fig. 2L) of varying lengths and thicknesses appear throughout the powder providing additional support and contributing to the characteristic texture of the powdered drug.

HPTLC Analysis of *Phyla nodiflora*

HPTLC analysis of *Phyla nodiflora* extract was performed using pre-coated silica gel 60 F_{254} plates. The sample was applied using Linomat 5 applicator in different concentrations (2–7µL). The mobile phase consisted of toluene: ethyl acetate: formic acid (5:4:1). The plates were developed in a saturated chamber for 20 minutes and scanned at 254nm and 366nm using a TLC scanner. The HPTLC fingerprint profile of *Phyla nodiflora* extract revealed the presence of multiple phytoconstituents when analyzed at 254nm and 366nm.

At 254nm, the chromatogram showed a prominent peak at Rf value around 0.34–0.36, indicating the presence of a major phytoconstituent. Additional peaks were observed at Rf values approximately 0.46 and 0.79–0.80, suggesting the presence of other secondary metabolites. In higher concentrations (4–7 µL), multiple peaks were distinctly resolved, confirming the chemical complexity of the extract.

At 366nm, the chromatogram exhibited fluorescent peaks, with significant bands observed in the regions of Rf 0.35–0.42 and 0.70–0.85, indicating the presence of UV-active compounds.

The major peak at Rf ~0.34–0.36, consistently observed across all tracks, may be attributed to phenolic or flavonoid-type phytoconstituents. Previous phytochemical studies on *Phyla nodiflora* have reported the presence of flavonoids such as luteolin and apigenin, which could contribute to the observed chromatographic profile.

The peaks observed at higher Rf values (~0.75–0.80) may be attributed to non-polar phytoconstituents such as phytosterols. *Phyla nodiflora* is reported to contain β-sitosterol, and these peaks may possibly correspond to its presence; however,

further confirmation using standard compounds is required. Overall, the HPTLC analysis confirms the presence of multiple bioactive constituents, and the developed fingerprint profile can be used as a reference for identification and quality control of *Phyla nodiflora*.

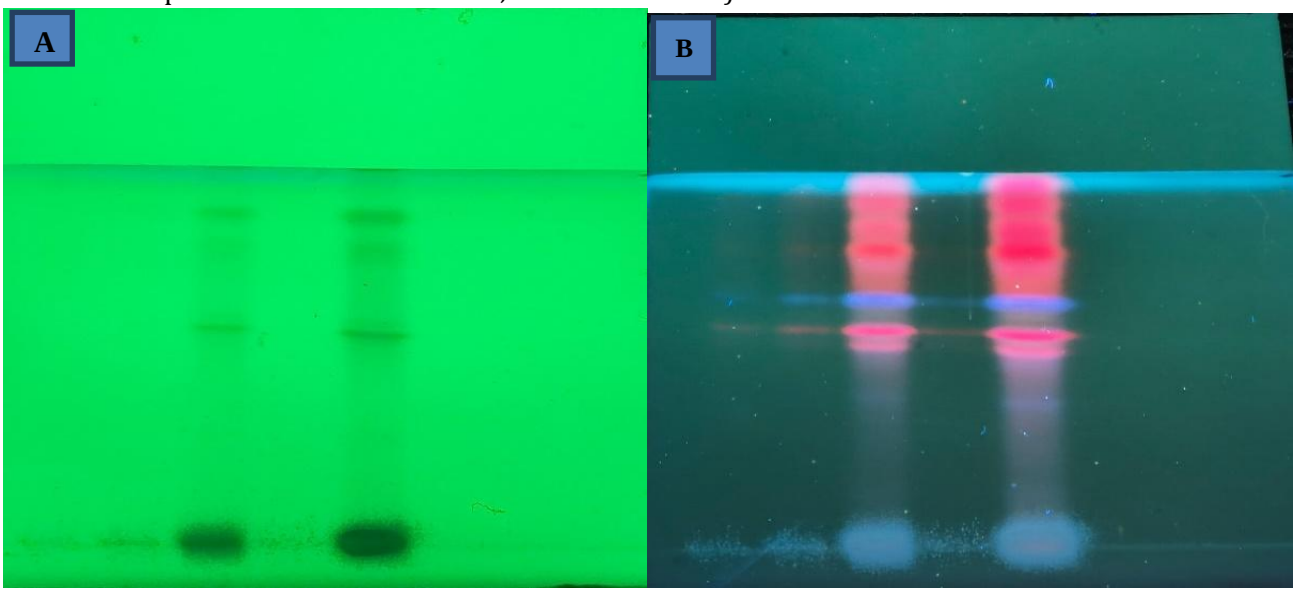


Figure 4: HPTLC chromatogram of *Phyla nodiflora* extract developed using Toluene: ethyl acetate: formic acid (5:4:1, v/v/v) as the mobile phase. (A) Plate viewed under UV 254 nm showing major UV-quenching zones, primarily at R_f ~0.36. (B) Plate viewed under UV 366 nm displaying a complex fluorescent profile with distinct pink, violet, and blue bands representing diverse secondary metabolites.

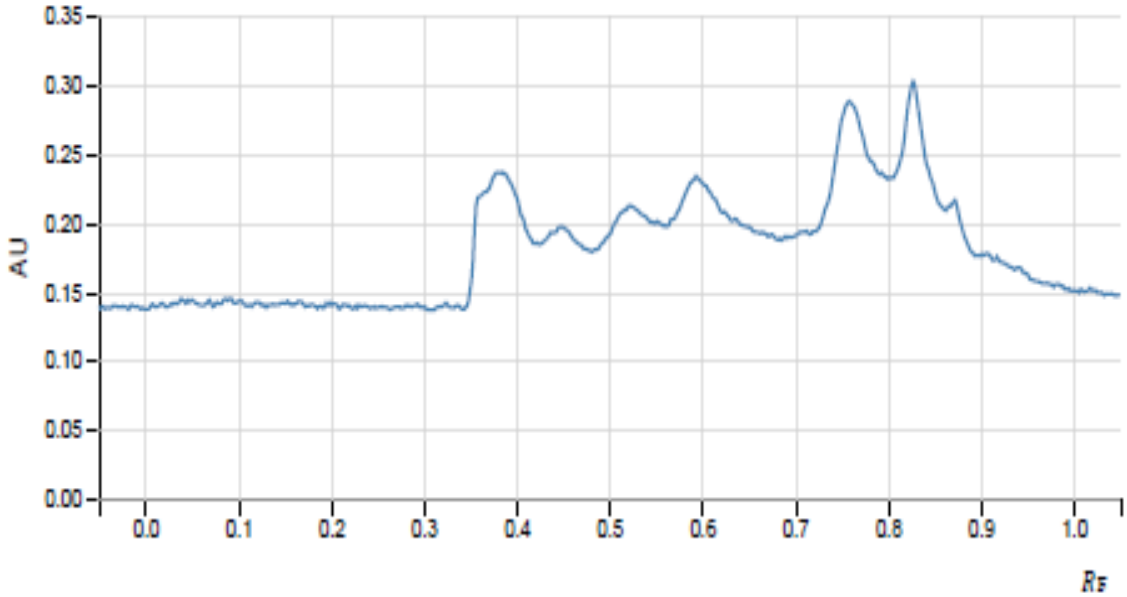


Figure 5: HPTLC densitogram of *Phyla nodiflora* (4.0 µL application) recorded at 366 nm. The profile showcases superior resolution with six distinct peaks identified at Rf values of 0.37, 0.44, 0.52, 0.59, 0.75, and 0.82. Peak 6 (Rf 0.82) and Peak 5 (Rf 0.75) represent the most prominent fluorescent constituents in the extract.

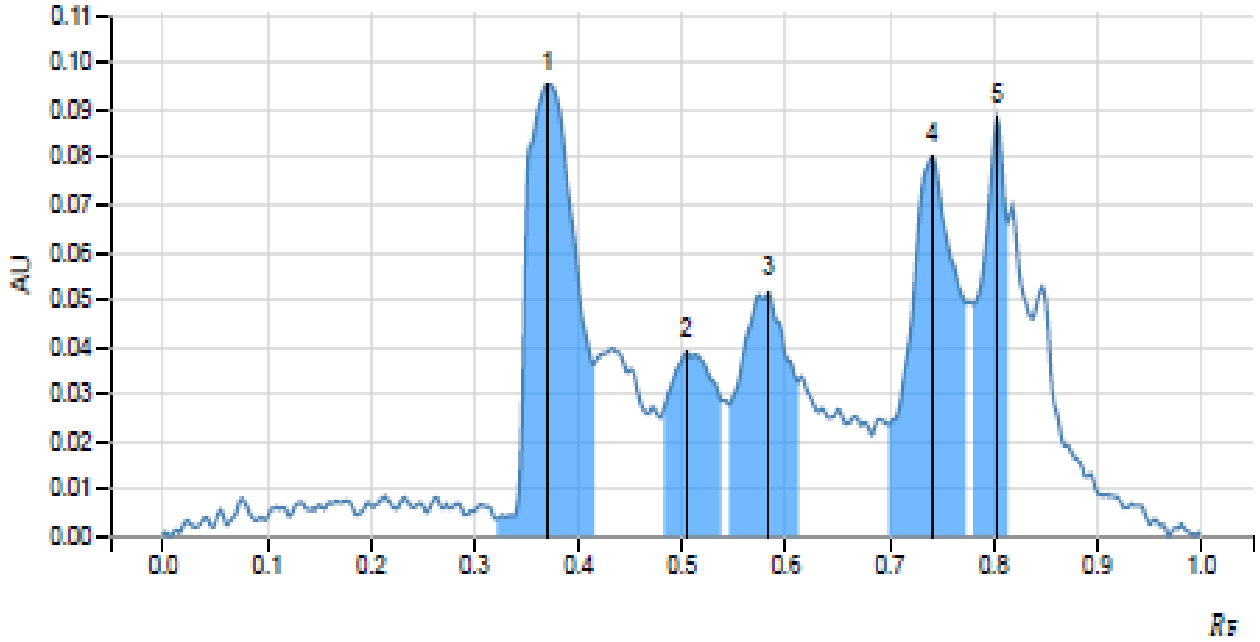


Figure 6: 3D densitogram overlay of Phyla nodiflora extract at varying application volumes (2.0µL to 7.0µL) recorded at 254nm. The overlay demonstrates excellent method precision and reproducibility, with peak heights and areas for the major constituent (Rf ~0.36) showing a concentration-dependent increase across all sample tracks.

CONCLUSION

The present pharmacognostical study of *Phyla nodiflora* provides comprehensive diagnostic features of the crude drug through detailed examination of the stem, root, and leaf, supported by powder microscopy, phytochemical screening, and HPTLC analysis. Anatomical characters such as vascular arrangement, epidermal layers, cortex, medullary structures, and trichome patterns serve as reliable markers for identification and authentication of the plant material. Powder microscopy further confirmed characteristic features including epidermal fragments, trichomes, vessel elements, fibers, and stomata.

Preliminary phytochemical analysis of the alcoholic and aqueous extracts revealed the presence of several bioactive constituents. Glycosides, flavonoids, terpenoids, and steroids were detected in both extracts, while saponins were observed only in the aqueous extract. Alkaloids, tannins, and carbohydrates were absent in both extracts. These findings indicate the rich phytochemical nature of *Phyla nodiflora* and support its medicinal significance.

HPTLC profiling further established the chemical fingerprint of *Phyla nodiflora* extract using silica gel 60 F_{254} plates with toluene:ethyl acetate:formic acid (5:4:1) as the mobile phase. Chromatographic analysis at 254nm and 366nm revealed multiple well-resolved peaks, confirming the presence of diverse phytoconstituents. A major peak consistently observed at Rf 0.34–0.36 may correspond to phenolic or flavonoid compounds such as luteolin and apigenin, while peaks at higher Rf values (~0.75–0.80) may indicate non-polar constituents including phytosterols like β-sitosterol. Fluorescent bands observed at 366 nm further suggested the presence of UV-active bioactive compounds.

Collectively, these macroscopic, microscopic, phytochemical, and HPTLC parameters establish a characteristic diagnostic profile for *Phyla nodiflora*, providing valuable baseline data for authentication, standardization, and quality control, and supporting its safe use in herbal and Ayurvedic formulations.

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***Address for correspondence**
Dr. Aakriti Asthana
PG Scholar,
Dept. of Dravyaguna,
A & U Tibbia College & Hospital,
Karol Bagh, Delhi.
Email: draakriti.asthana98@gmail.com