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Original Article

Pharmacognostical Standardization of *Oldenlandia umbellata* L.-An Important Medicinal Plant

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ABSTRACT

Background: *Oldenlandia umbellata* is a notable medicinal plant traditionally used to treat respiratory diseases, with its roots producing a red-coloured anthraquinone-based dye. Ensuring clinical efficacy with *Oldenlandia umbellata* necessitates identifying the authentic drug and its standardization. **Objectives:** This study aimed to establish phyto-pharmacognostic standards for *Oldenlandia umbellata*. **Materials and Methods:** The standardization process encompassed macroscopic and microscopic examinations, organoleptic analysis, preliminary phytochemical evaluation, fluorescence studies, and HPTLC analysis. **Results:** Microscopic analysis of the roots revealed distinct features such as well-developed secondary growth, raphides and calcium oxalate crystals in the cortex, and lignified xylem fibres in the xylem tissue. Stem microscopy showed thin-walled and suberized phellem cells along with raphides. The leaf structure displayed a conjoint, collateral vascular bundle and paracytic stomata, with two subsidiary cells and raphides in the mesophyll. Physicochemical values were assessed, including total ash content (7.075%), water-soluble ash (1.725%), the alkalinity of water-soluble ash (0.35375 mL), and acid-insoluble ash (1.06%). Preliminary phytochemical analyses revealed the presence of secondary metabolites in two solvent systems. HPTLC analysis identified significant phytoconstituents with various R_f values. **Conclusion:** These phyto-pharmacognostic studies of *Oldenlandia umbellata* facilitate the differentiation of the plant sample from other botanical sources within the same genus and authenticate the original drug.

Keywords: Anatomy, *Oldenlandia umbellata*, Phyto-Pharmacognosy, Rubiaceae, Quality Assurance.

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INTRODUCTION

O. umbellata, also known as "Indian Madder," is notable for producing red dye from its roots. The primary coloring agents in the dye are anthraquinone derivatives, which include compounds like alizarin and purpurin. These compounds are known for their strong and lasting red color. This dye is used in various cultural practices, including textile dyeing, painting, and ceremonial uses.[1] Belonging to the Rubiaceae family, this plant is prominent in traditional medicine due to its hemostatic properties.[2] Its leaves and roots are effective expectorants for asthma, bronchitis, and bronchial catarrh.[2] An infusion of its leaves is used as a topical rinse to treat venomous bites.[3] Furthermore, in 2024, the Survey of Medicinal Plants Unit, NRIUMSD, Hyderabad, documented an ethnobotanical claim from the Nandyal Forest Division, Andhra Pradesh, stating that a decoction of the root is used as a febrifuge. In the Siddha system of medicine, the root bark is administered for tuberculosis treatment.[2] Pharmacological investigations have demonstrated its hepatoprotective and antioxidant,[4] antibacterial,[5] anti-inflammatory, and antipyretic properties.[6] A comprehensive phytochemical analysis of *O. umbellata* has revealed the presence of anthraquinone derivatives.[1,3]

Despite the extensive exploration of its pharmacological and ethnobotanical attributes, the dried form of *O. umbellata* poses challenges in its differentiation from other *Oldenlandia* species. The literature review indicates a need for more standardization studies. Given the significance of this species, there is a pressing need for its standardization. To address this gap, the current study undertook phyto-pharmacognostic investigations encompassing macroscopic, microscopic, phytochemical, physicochemical, and HPTLC parameters.

MATERIALS AND METHODS

The plant material comprising both the aerial and underground parts of *Oldenlandia umbellata* was collected from the healthy specimens found in the Talakona forest region of Tirupati District, Andhra Pradesh. The collected specimens were authenticated using available Herbaria-SVUB 1231, 1324, and 4520,

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housed within the Department of Botany at Sri Venkateswara University, Tirupati. A voucher specimen (No.: SVUB-5990) was meticulously documented and preserved in the Herbaria section of the Department of Botany at Sri Venkateswara University for future reference.[7-12]

Macroscopic study

The external morphology and macroscopic characteristics of *O. umbellata* were carefully documented with the aid of naked eye observation.[13-18]

Organoleptic properties

The crude drug's organoleptic characteristics, including aroma, texture, color, and taste, were methodically observed and recorded following established protocols.[7-10,18,19]

Anatomical evaluation

Root and leaf anatomical investigations were conducted employing standard procedures encompassing sectioning, maceration, and powder microscopy, as well as leaf cleaning and veining studies.[8-10,13-17]

Physicochemical studies

Using standard techniques, physicochemical data were analyzed and documented by calculating ash and extractive values in various solvent systems and measuring the weight loss upon drying.[8-10,13-17]

Fluorescence studies

Fluorescence studies involved treating the plant powder with various reagents and observing the color changes under a UV chamber to document the fluorescence of the powder drug.[8,9,14-18]

Preliminary phytochemical studies

Alcoholic and aqueous extracts of *O. umbellata* were prepared by cold maceration. Five grams of air-dried, coarsely powdered material was placed in a conical flask with 100 mL of the respective solvent. The mixture was shaken periodically for 6 hr and then left undisturbed for 18 hr. After filtration to minimize solvent loss, the filtrate was evaporated to dryness at 60°C using a digital water bath. The dried extracts were then qualitatively screened for secondary metabolites.[7-12,14-18]

Procedure for HPTLC analysis

HPTLC studies were conducted using the CAMAG Automatic TLC Sampler 4 (ATS4). Silica gel 60 F 254 TLC plates measuring 5 x 10 cm (manufactured by E. MERCK KGaA) served as the stationary phase. The mobile phase comprised toluene: ethyl acetate in a ratio of 6:4. Samples at concentrations of 4, 6, and 8 mcg/L were applied to the plate with an 8mm band length using the CAMAG 100 mcg/L sample syringe (Hamilton, Bonaduz, Switzerland) via an automated CAMAG TLC applicator Linomet 5 with N2 flow. For the development of HPTLC plates, a CAMAG twin trough glass tank measuring 20 x 10 cm was employed. Subsequently, the plate was scanned using the CAMAG scanner TLC scanner 3 at a scan speed of 10 mm/sec.[11-12,15-18]

RESULTS

Taxonomy of the plant

The plant described is an annual erect herb reaching 20-30 cm heights. Its leaves are arranged decussately or appear clustered, being linear in shape, measuring approximately 1.5 x 0.3 cm. They are rough to the touch (scabrous), occasionally parchment-like (chartaceous), with a single prominent vein running along the length. The base of the leaf is prolonged downward (decurrent), and the margin is revolute, ending in a sharp or pointed tip (acute to apiculate). The leaves are sessile, meaning they lack a stalk (Figures 1a and b).

The flowers are white, arranged in terminal or upper axillary clusters or on peduncles, with peduncles reaching up to 1 cm in length. The calyx is nearly truncate, about 1 mm long, and sparsely hairy on the outer surface, with four lanceolate lobes measuring around 1.5 mm each.

The corolla is pinkish-white, approximately 4 mm in diameter, and bell-shaped (campanulate), with four lanceolate lobes, each around 3.5 mm long. The lobes are fused in the middle and tapering to a sharp point (acute), sometimes bending inward (incurved) (Figure 1a and b).

There are four protruding stamens with filaments measuring about 1.2 mm and oblong anthers approximately 1 mm in size. The ovary is around 0.8 mm, with a style of 0.5 mm and a stigma divided into two linear lobes, partially enclosing the style (Figure 1b).

The fruit is a capsule, and the seeds have a reticulated surface pattern.

Flower and Fruiting: August - February.

Macroscopical Observations of the Root

The roots are brown in colour, with a few lateral roots branching from the central axis (Figure 1a). The outer layer of the root is not easily peelable. They emit a pleasant aroma and do not possess a distinct taste.

Microscopical Observations of the Root

The root structure exhibited significant thickening as a result of secondary growth. A Transverse Section (T.S.) revealed clearly differentiated tissue zones, including the periderm, cortex, and mature vascular tissues. The outermost layer consisted of a smooth periderm approximately 8 µm in thickness, comprising seven to nine layers of tabular, thin-walled, suberized phellem cells, a distinct meristematic phellogen layer, and one to two

layers of phelloderm. Beneath this, the cortex was relatively broad, measuring around 400 µm in diameter, and composed of compactly arranged polyhedral parenchyma cells with thin walls. Numerous calcium oxalate druses were observed scattered within the cortical cells (Figure 2a and 2b).

The secondary phloem formed a continuous narrow band characterized by radial arrangements of phloem elements and medullary rays. The secondary xylem appeared as a dense, compact cylindrical core about 700 µm in diameter. Xylem vessels were thin-walled, circular in outline, and distributed singly in a diffuse manner, with an average diameter of 25 µm. The xylem fibers were strongly lignified and thick-walled, whereas the rays consisted of a single layer of thin-walled cells (Figure 2a and 2b).

Microscopic Characteristics of the Young Stem

A transverse section (T.S.) of the young stem reveals a nearly circular contour, with distinguishable tissue zones such as the periderm, cortex, and regions showing signs of secondary growth (Figures 3a-3c). Remnants of the epidermis can occasionally be identified in certain areas. The periderm is uniformly distributed around the stem and comprises three to four layers of thin-walled, suberized, tabular phellem cells. The phellogen layer is not distinctly visible, and the entire peridermal region is about 100 µm thick.

Beneath the periderm, a narrow cortical region is present, consisting of approximately six layers of horizontally oriented, elongated parenchyma cells with thin walls and minimal intercellular spaces. This cortical zone measures nearly 100 µm in thickness. The central pith region is relatively large and composed of compact, thin-walled parenchymatous cells. Evidence of secondary growth is observed within the central vascular zone, including a small, circular cavity at the core of the pith (Figures 3a and 3b). The secondary phloem encircles the xylem in a continuous ring and contains uniseriate radial rows of ray cells along with sieve elements. Additionally, vessels and fibers are present in the phloem. The xylem features wide, circular vessels with thickened walls, well-developed broad rays, and highly lignified, thick-walled xylem fibers (Figure 3c).

Macroscopic Characteristics of the Mature Stem

The mature stem reaches a thickness of approximately 2-4 cm and exhibits a coarse texture with shallow fissures on its surface. The outer layer is not peelable and does not possess any noticeable taste or aroma.

Microscopic Structure

In Transverse Section (T.S.), the mature stem displays well-defined tissue zones, including the periderm, cortex, and developed secondary vascular tissues (Figure 4a). The periderm, measuring around 200-250 µm in thickness, is composed of six to seven layers of thin-walled, suberized, tabular phellem cells, along with two to three layers of phelloderm (Figure 4b).

The cortical region is compact and uniform, approximately 150 µm wide, made up of tangentially elongated, radially flattened

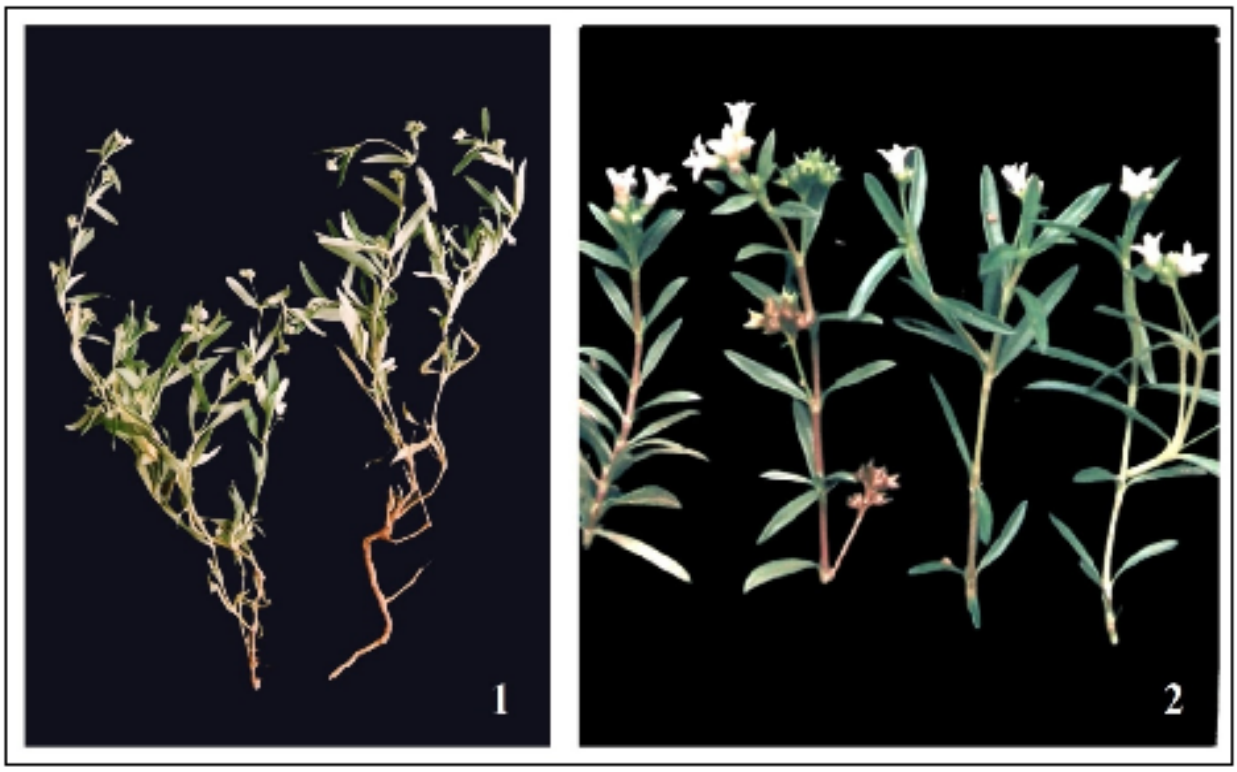

Figure 1: *Oldenlandia umbellata* 1a: Whole plant along with roots; 1b: Plant with umbellate inflorescence.

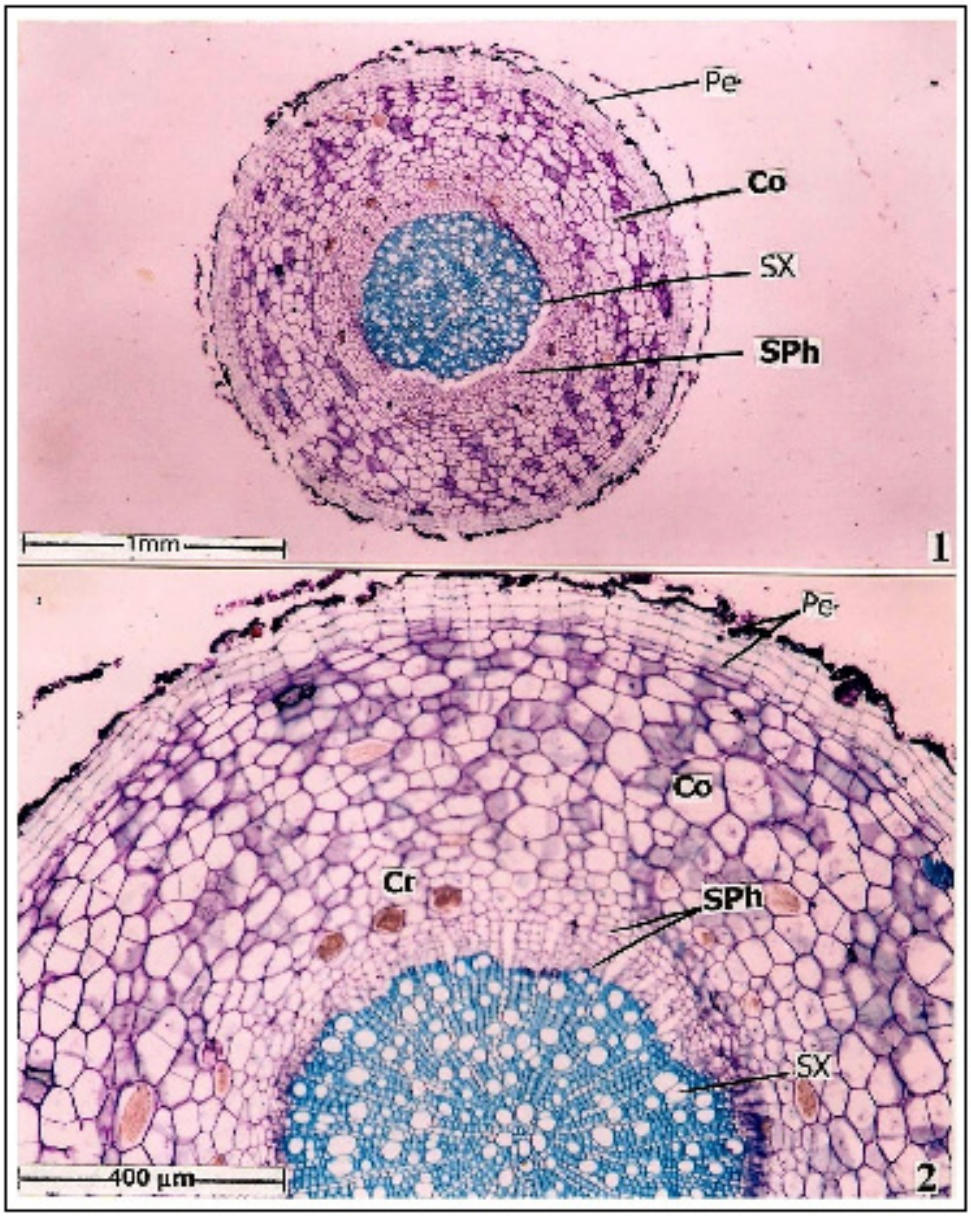

Figure 2: Microscopical characters of Root; 2a: T.S. of root - Entire view; 2b: T.S. of root - A sector enlarged; Co: Cortex; Cr: Crystals; SPh: Secondary phloem; SX: Secondary xylem.

Table 1: Organoleptic Characteristics.

Colour	Appearance	Odour	Taste
Pale green	Coarse powder	No Characteristic smell	No Characteristic taste

Table 2: Histochemical Tests.

Drug	Reagents	Test for	Reaction	Results
Section	Iodine solution	Starch	Blue colour	+
Section	Ferric chloride solution	Tannin	Black	+
Section	Sudan III solution	Oil globules	No effervescence	-
Section	Phloroglucinol + dil. HCl + Alcohol	Lignin	Magenta	+
Section	Conc. HCl	Crystals	No effervescence	+

+ = Present; - = Absent.

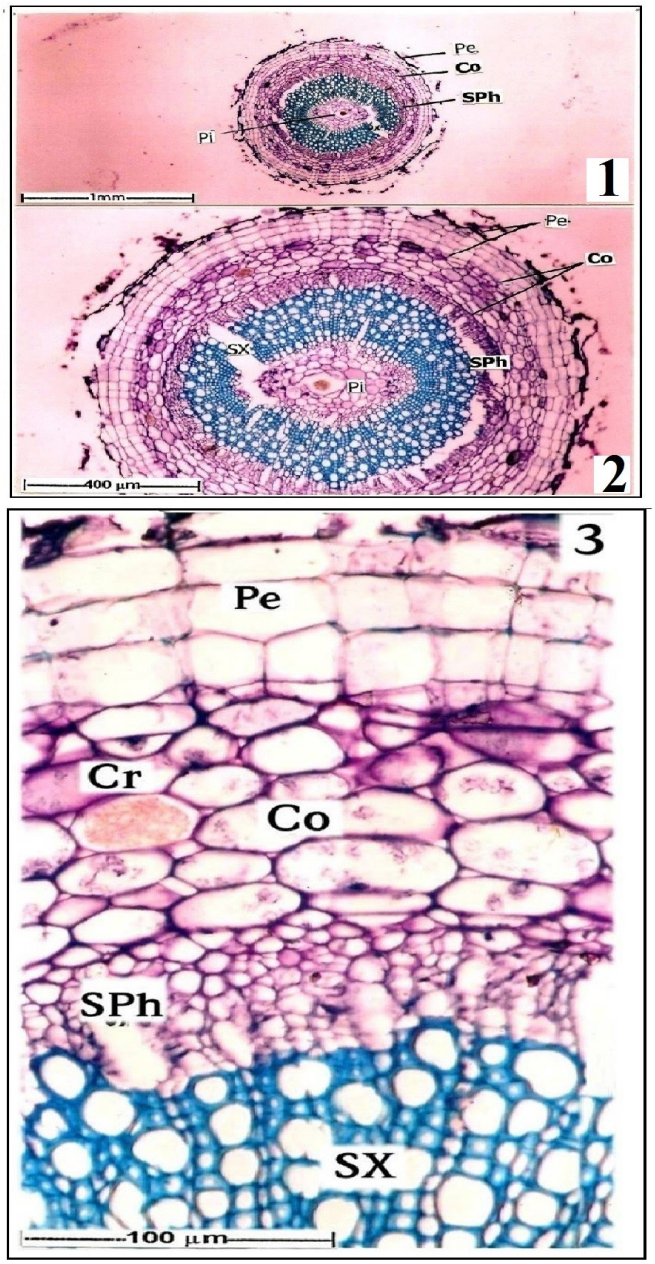

Figure 3: Microscopical characters of young stem; 3a: T.S. of stem - Entire view; 3b: T.S. of stem - Enlarged view; 3c: T.S. of stem - A sector enlarged; Co: Cortex; Cr: Crystals; Pe: Periderm; Pi: Pith; SPh: Secondary phloem; SX: Secondary xylem.

parenchyma cells with thin walls and minimal intercellular spaces.

The secondary phloem is prominently developed, forming a continuous ring about 300 µm in width. It features uniseriate medullary rays that gradually broaden outward, with sieve elements arranged in one to three rows and interspersed among phloem parenchyma cells (Figure 4b).

The secondary xylem is densely packed and structurally robust, composed of conspicuous vessels and fibers. The vessels are thick-walled, solitary, circular, and scattered throughout the xylem region. Growth rings are absent. The xylem fibers are heavily lignified and thick-walled. The xylem region spans approximately 700 µm in thickness.

The central pith is formed by thin-walled parenchymatous cells that are circular to oval in shape with limited intercellular space. In certain regions, these cells undergo breakdown, resulting in the formation of a distinct central cavity.

Stem - Macerate

The stem macerate showed the following elements.

Vessel elements (Figures 5a-5c)

The vessels observed were elongated and cylindrical in shape, featuring simple, horizontal, or slightly oblique perforation plates. These elements exhibit short tails at one end or are tailless. Circular lateral pits were abundant and alternated along the vessel walls. The vessel elements measured between 240-380 µm in length and 40-45 µm in width.

Xylem fibres

The vessel elements were thick-walled, elongated, and tapered at the ends. Some exhibited a narrow lumen (Figure 5a), while others displayed a wide lumen (Figure 5b). Lateral wall pits were not evident. The vessel elements measured 350-540 µm in length and were approximately 20 µm thick in the middle.

The macroscopical character of the Leaf

The leaves are sessile, measuring 0.5-1.6 cm in length and 0.2-0.4 cm in width, with a linear-lanceolate shape. They have a decurrent base, revolute margins, an acute apex, a scabrous texture, and a single prominent vein. The stipules feature several bristles and have a triangular base.

Microscopical characters

The leaf's Transverse Section (T.S.) appears even, with a distinct midrib, measuring 275 µm in thickness (Figure 6b).

The leaf's upper (adaxial) epidermis consists of thin-walled, radially elongated cells, while the lower (abaxial) epidermis has thin-walled rectangular cells. The mesophyll is distinctly divided into a single layer of palisade cells and three to four layers of spongy parenchyma. In the midrib region, the lower side contains several layers of compact spongy parenchyma, whereas the upper side has a single layer of parenchyma. The vascular bundle is small, conjoint, and collateral, with a few xylem elements and a narrow arc of phloem (Figure 6b).

Venation pattern (Figure 7a)

The lateral veins of the leaf are prominently thick, in contrast to the finer veinlets. The vein islets are clearly visible, appearing as horizontally elongated and arranged in parallel. Within some

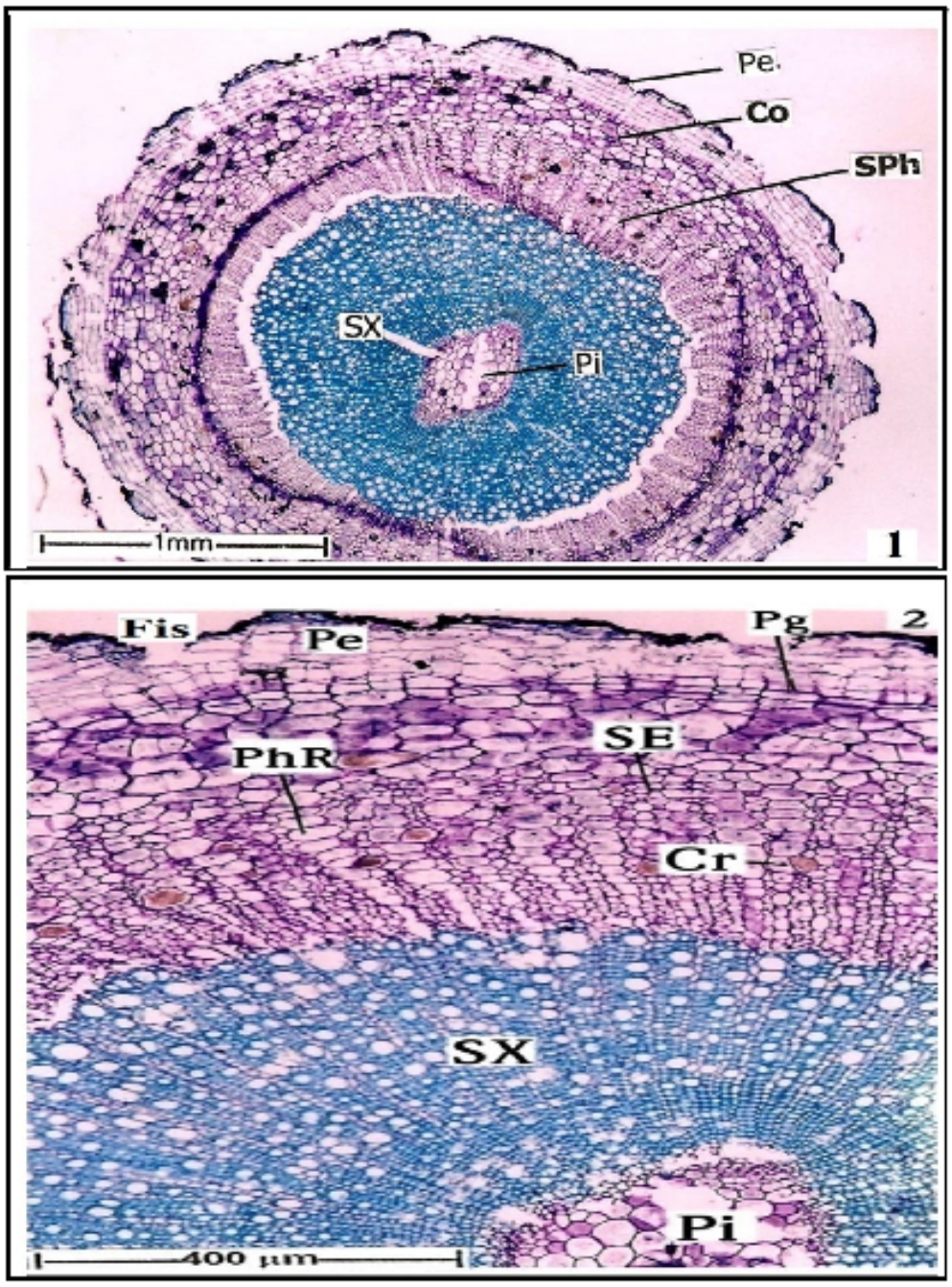

Figure 4: Microscopical characters of mature stem; 4a. T.S. of stem - Entire view; 4b T.S. of stem - A sector enlarged; Co: Cortex; Cr: Crystals; Fis: Fissures; Pe: Periderm; Pg: Phellogen; PhR: Phloem rays; Pi: Pith; SPh: Secondary phloem; SE: Sclerenchyma; SX: Secondary xylem.

Table 3: Ash Values.

Total ash (%)	Water soluble ash (%)	Alkalinity of water-soluble ash (mL)	Acid insoluble ash (%)
7.075	1.725	0.35375	1.06

Table 4: Extractive Values.

Alcohol soluble extract (% w/w)	Water soluble extract (% w/w)	Hexane soluble extract (% w/w)	Chloroform soluble extract (% w/w)
0.975	1.2	0.4083	0.913

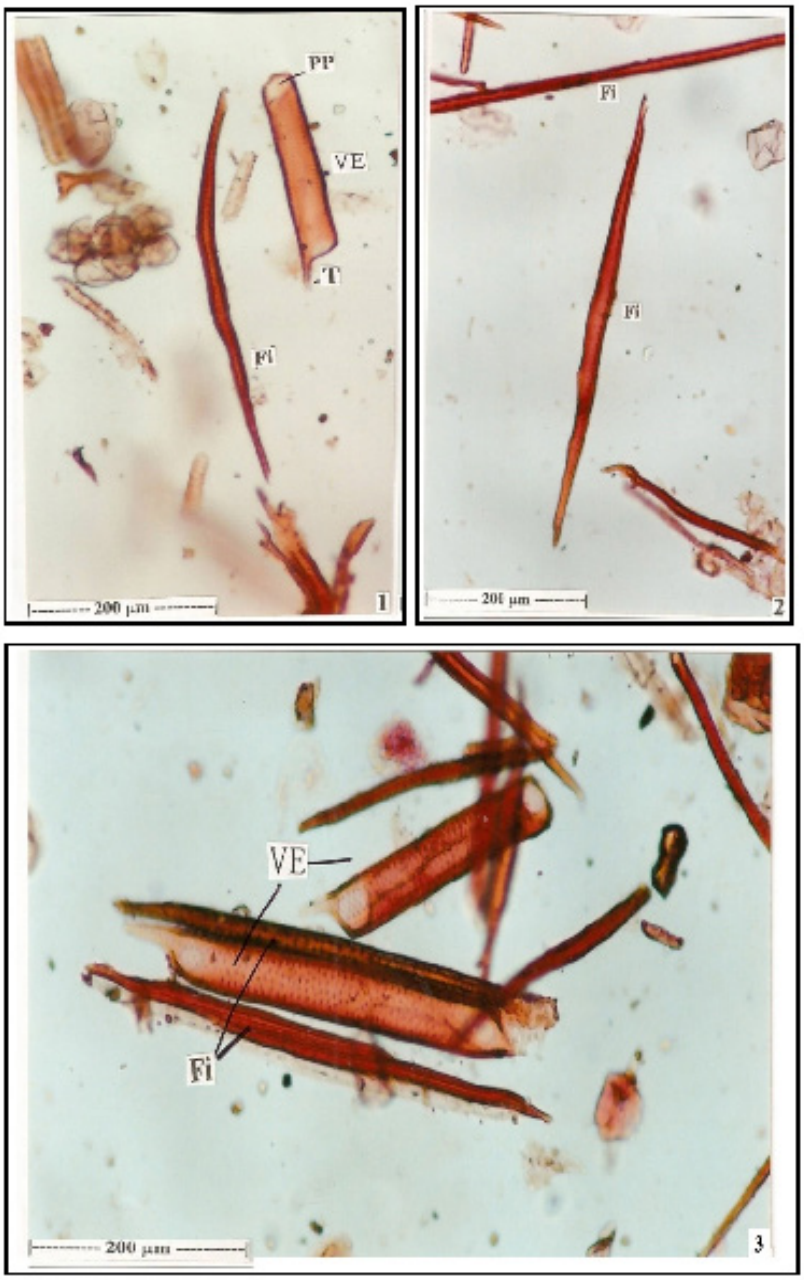

Figure 5: 5a-5c Stem macerate; Fi - Fibres; T - Tail; PP - Perforation plate; VE - Vessel elements.

islets, simple, slender, and straight vein endings can occasionally be seen.

Stomatal morphology (Figure 7b)

Stomata were exclusively present on the abaxial side of the leaf, with a density of 36 stomata per square millimetre. These stomata consistently exhibited a paracytic arrangement characterized by the presence of two subsidiary cells.

Crystal distribution (Figures 8a and 8c)

Two forms of calcium oxalate crystals were identified in the ground tissue. Raphides (needle-like structures) were commonly found in the mesophyll (measuring up to 220 µm), as well as in the cortical regions of both root and stem (about 120 µm). In addition to these, druse crystals were also frequently seen in the cortical tissues of the root and stem (Figure 8a and 8b).

Leaf - Macerate (Figures 9 and 10)

Leaf maceration revealed several anatomical features. The upper epidermis displayed paracytic stomata, each flanked by two parallel subsidiary cells, with surrounding amoeboid epidermal cells having wavy anticlinal walls. In contrast, the lower epidermal cells were hexagonal, arranged in orderly layers with thick, straight walls, and measured between 90-130 µm, though some appeared elliptic or angular. Numerous trichomes were observed on the epidermis, noted for their distinct shape-short and broad

at the base, tapering to a pointed tip-with thick walls and a finely spiny (echinate) texture. These structures measured 1-20 µm wide at the base and about 10 µm at the apex.

Physicochemical Evaluation

Organoleptic Properties

The powdered plant material was assessed based on its sensory attributes, including taste, odor, and texture. Observations were systematically recorded and are presented in Table 1.

Histochemical Analysis

To assess the localization and chemical nature of constituents within the plant tissue, fresh sections were treated with specific chemical reagents:

- Iodine solution.
- Ferric chloride solution.
- Phloroglucinol.
- Dilute hydrochloric acid and alcohol.
- Sudan III solution.
- Concentrated hydrochloric acid.

These treatments induced characteristic color reactions, which were carefully observed and documented for interpretative analysis (Table 2).

Physicochemical Parameters

The powdered drug was subjected to standard physicochemical evaluations, including extractive values and different ash contents. These parameters help determine the levels of inorganic matter, such as soil and silica, that may be present along with the pharmacologically active components. The findings are summarized in Tables 3 and 4.

Table 5: Qualitative Phytochemical Screening of Methanol and Aqueous Extracts of Whole plant.

Name of the Compound	Alkaloids	Flavonoids	Terpenoids	Steroids	Tannins	Phenols	Anthraquinones	Coumarins	Quinones
Aqueous extract	+	-	-	-	+	+	+	+	+
Methanolic extract	+	+	+	-	+	+	+	+	+

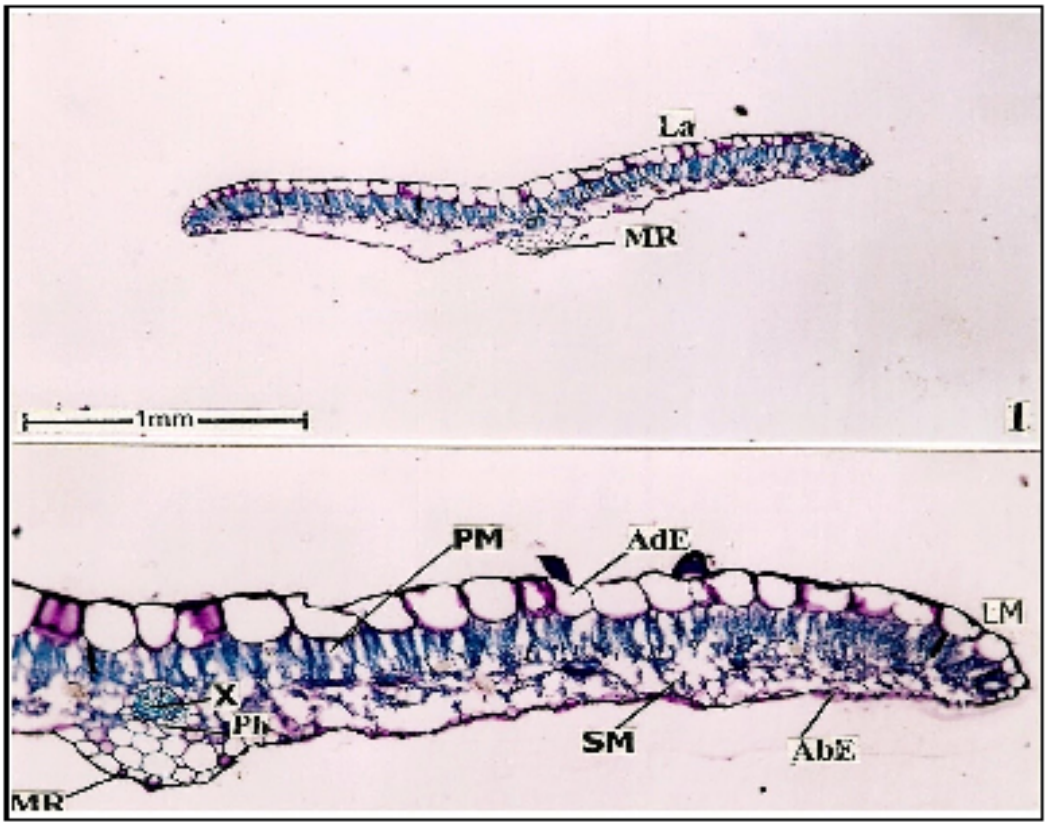

Figure 6: Microscopical characters of leaf; 6a. T.S. of Leaf - Entire view; 6b. T.S. of Leaf - A sector enlarged; AbE: Abaxial epidermis; AdE: Adaxial epidermis; La: Lamina; LM: Leaf mesophyll; MR: Midrib; PM: Palisade mesophyll; SM: Sponcgy mesophyll.

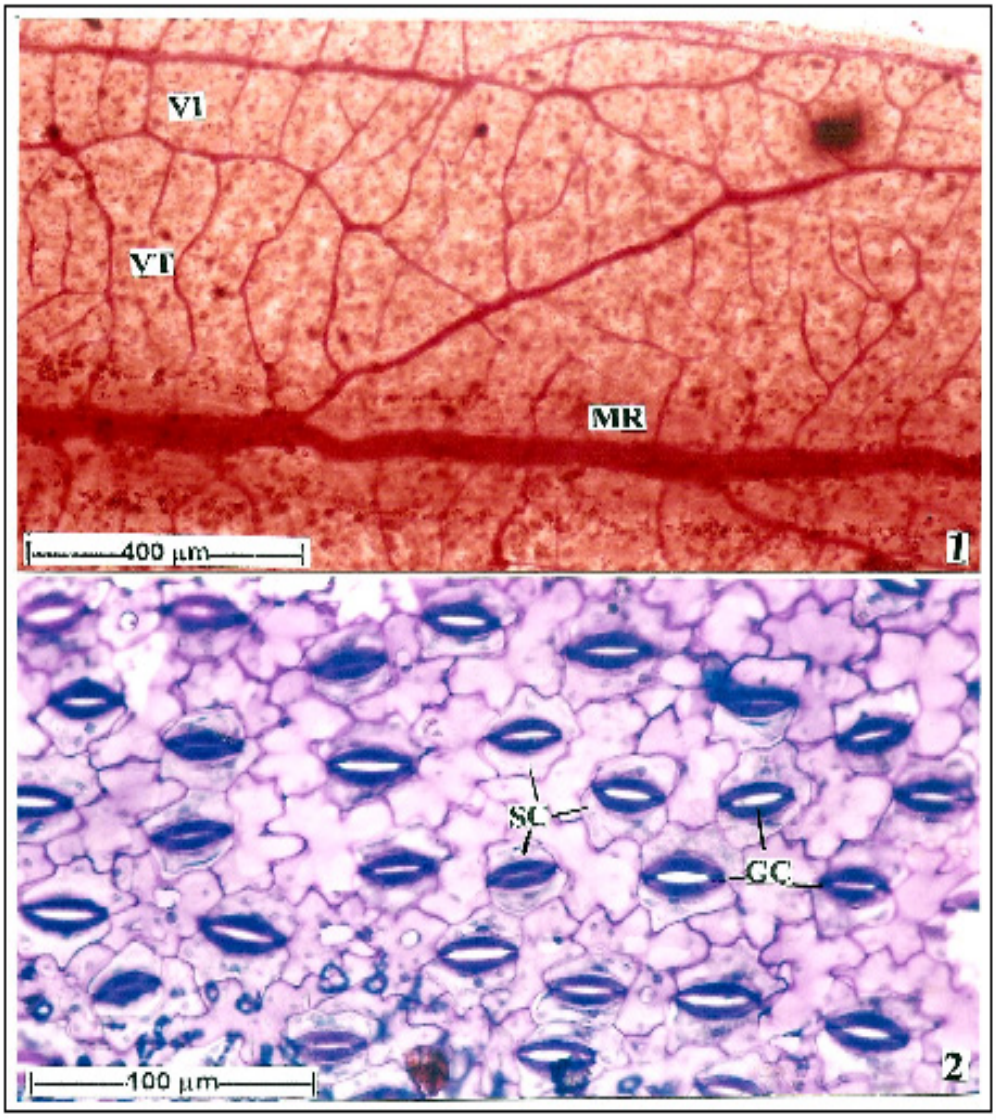

Figure 7: Venation pattern and stomatal morphology; 7a: Leaf showing vein-islets and vein-terminations; 7b: Stomatal morphology; VI: Vein-Islets; VT: Vein-Termination; MR: Midrib; SC: Subsidiary cells; GC: Guard cells.

Table 6: Fluorescence analysis for powdered drug.

Experiments	Visible/Daylight	UV Light	
		254 nm	365 nm
Drug powder	Pale green	Green	Brown
Drug powder + 1 N NaOH (aq.)	Brown	Yellowish green	Green
Drug powder + 1 N NaOH (alc.)	Brown	Yellowish green	Green
Drug powder + 1 N HCl	Brown	Black	Black
Drug powder + 50% H_2SO_4	Reddish brown	Yellowish green	Yellowish green
Drug powder + 50% HNO_3	Orange	Green	Green
Drug powder + Picric acid	Green	Yellowish green	Yellowish green
Drug powder + Acetic acid	Brown	Green	Green
Drug powder + Ferric chloride	Dark olive (green)	Green	Black
Drug powder + HNO_3 + NH_3	Reddish orange precipitate	Yellowish green	Green

Preliminary Phytochemical Screening

Methanolic and aqueous extracts of the whole plant were prepared to investigate the presence of key secondary metabolites. Standard qualitative tests were employed to detect phytoconstituents such as alkaloids, flavonoids, tannins, saponins, glycosides, and others. The screening results are presented in Table 5.

Fluorescence Characteristics

Fluorescence analysis under Ultraviolet (UV) light is a valuable tool for the detection of specific plant constituents and can aid in the authentication of crude drugs. The powdered samples were observed under UV light at 254 nm and 366 nm, as well as under visible light, both in untreated form and after treatment with various chemical reagents. Fluorescence behavior was noted and compiled in Tables 6 and 7.

High-Performance Thin-Layer Chromatography (HPTLC) Profiling (Figures 11-14)

Methanol extracts from both the leaf and root of *O. umbellata* were applied to silica gel "G" TLC plates. The chromatographic separation was performed using a mobile phase comprising Toluene: Ethyl acetate: Methanol in the ratio of 7:2:1. Following development, the chromatograms were visualized under UV light at 366 nm, 254 nm, and in visible light (Figures 11 and 13). The

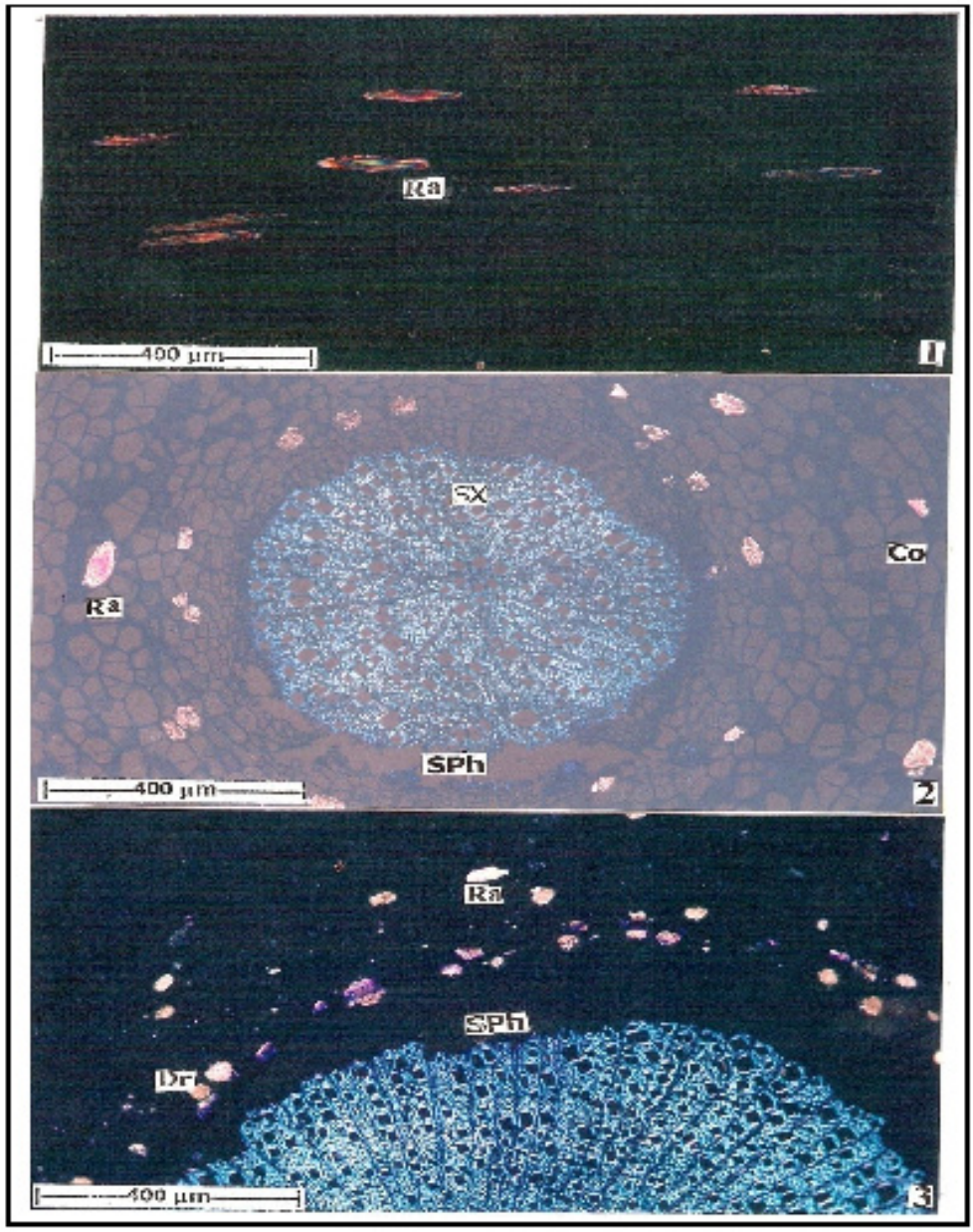

Figure 8: Brightfield images; 8a. Raphides or bundles of thin, pointed needles of calcium oxalate crystals are fairly common in the mesophyll of leaf; 8b: Cortical tissues of the root; 8c: Cortical tissues of the stem; Ra: Raphides; Co: Cortex; Dr: Druces; SPh: Secondary phloem; SX: Secondary xylem.

Table 7: Fluorescence analysis for extracts.

Extract	Treatment	Observation
Alcohol (ethanol)	Daylight	Canary yellowish
	Short UV	Fluorescent green
	Long UV	Off white
Water	Daylight	Brown
	Short UV	Brown
	Long UV	Brown
Hexane	Daylight	Pale yellow
	Short UV	Pale yellow
	Long UV	Light cream
Chloroform	Daylight	Peppermint
	Short UV	Off white
	Long UV	Mid cream

Table 8: Peak list and densitogram of leaf powder of *Oldenlandia umbellata* at 366 nm UV light with R_f values of the spots.

Peak no	Y-Pos	Area	Area (%)	Height	R_f values
1	10.3	946.02	75.5	462.95	0.02
2	22.7	28.89	2.3	14.23	0.19
3	26.3	7.44	0.6	3.74	0.24
4	34.4	35.81	2.9	13.62	0.36
5	38.4	8.36	0.7	2.96	0.41
6	60.6	40.98	3.3	9.73	0.73
7	73.3	4.59	0.4	2.35	0.91
8	79.5	181.44	14.5	45.84	0.99

Table 9: Peak list and densitogram of root powder of *Oldenlandia umbellata* at UV 366 nm with R_f values of the spots.

Peak no	Y-Pos	Area	Area (%)	Height	R_f values
1	10.3	2383.68	50.5	1050.93	0.00
2	15.4	264.51	5.6	129.56	0.08
3	20.3	76.24	1.6	37.92	0.15
4	25.4	1171.34	24.8	463.87	0.22
5	28.3	190.15	4.0	122.26	0.26
6	39.0	325.95	6.9	101.77	0.41
7	50.0	20.14	0.4	5.52	0.57
8	55.0	5.07	0.1	2.77	0.64
9	61.5	228.67	4.8	72.95	0.74
10	72.5	50.75	1.1	26.35	0.89

Retention factor (R_f) values and corresponding spot colors were noted and are documented in Tables 8 and 9. Densitograms were also generated for further analysis (Figures 12 and 14).

In the leaf extract, eight distinct bands appeared under 366 nm UV light with the following R_f values and colors: 0.02 (blue), 0.19, 0.24, 0.36 (red), 0.41 (blue), 0.73 (blue), 0.91, and 0.99 (red). Under 254 nm UV light, three spots (R_f: 0.02, 0.19, 0.24) were visible in light yellow, while iodine vapor treatment revealed two light yellow spots at R_f 0.02 and 0.19 (Figure 11).

For the root extract, nine primary spots were detected under 366 nm UV light, with R_f values and colors as follows: 0.02 (brown), 0.22, 0.27 (red), 0.43 (blue), 0.57, 0.75, 0.82, 0.91, and 0.99 (red). At 254 nm, five spots (R_f: 0.02, 0.27, 0.75, 0.91, 0.99) showed light green coloration. Under iodine vapor, eight spots were observed,

including light yellow at R_f 0.02 and 0.22, blue at 0.27, 0.91, and 0.99, and light yellow at 0.57, 0.75, and 0.82 (Figure 13).

DISCUSSION

Most of the global population-estimated at around 80%-continues to depend on traditional medicine due to its perceived safety, widespread availability, and low cost.[19-23] Despite this reliance, the herbal industry is increasingly challenged by the issue of adulteration. Historical records show that ancient healers developed and refined herbal remedies through empirical methods, with their formulations recorded in various regional languages.[22-26] Decoding these traditional texts to accurately identify raw plant materials remains complex.

Additionally, discrepancies in nomenclature among present-day scholars, traditional healers, and practitioners from diverse systems of medicine have led to confusion. A single medicinal plant may be known by multiple names, each linked to different therapeutic applications. Conversely, one vernacular name may be applied to different botanical species due to their overlapping pharmacological properties.[8,9,16-18]

An illustrative example comes from Unani medicine: 'Ushba-e-Hindi' is a single-drug formulation employed in the treatment of gastrointestinal issues, diarrhea, and blood disorders. While *Hemidesmus indicus* is the authentic botanical source, commercial samples frequently contain roots of *Decalepis hamiltonii*, which closely resemble *H. indicus* in both Aroma and morphology. Such instances highlight the critical need for precise

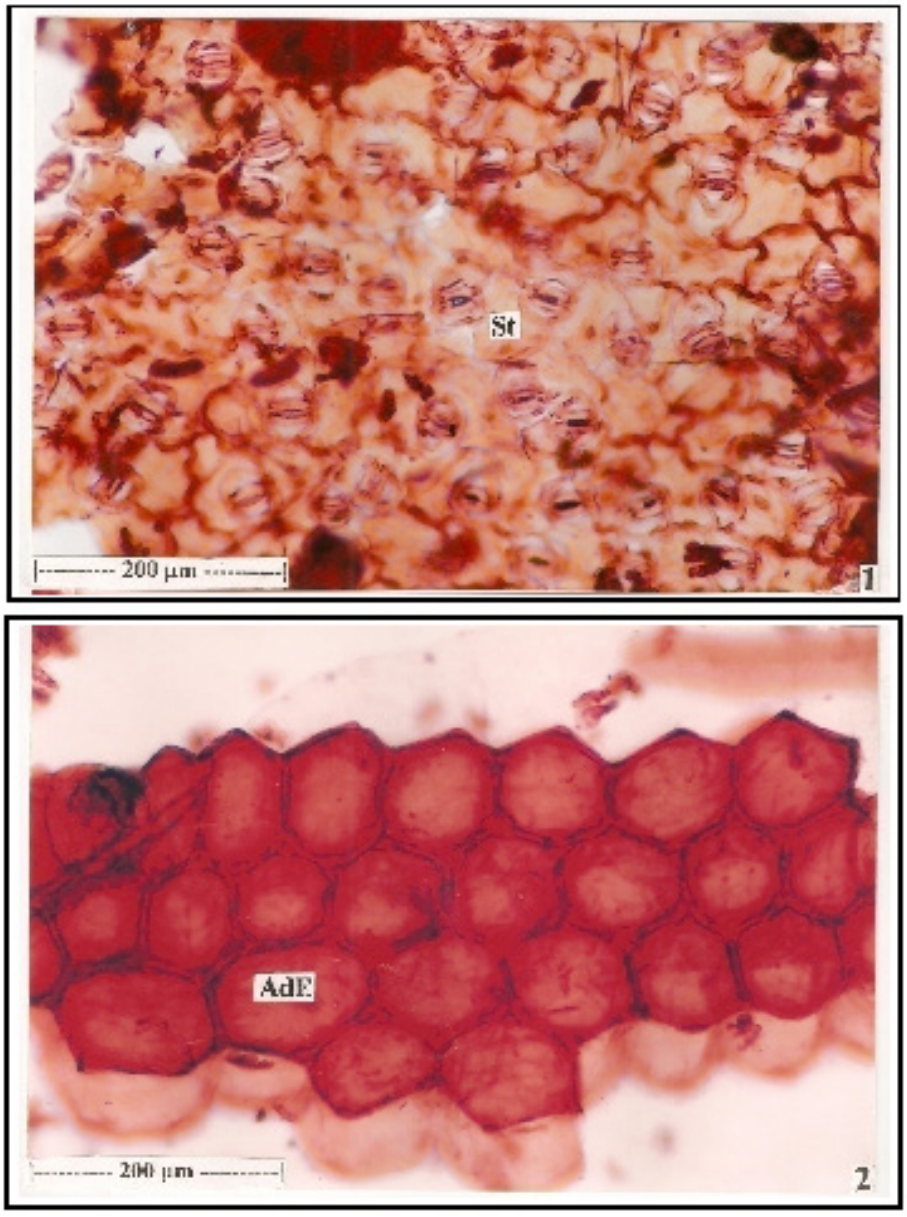

Figure 9: Leaf macerate; 9a: Abaxial epidermal fragments; 9b: Adaxial epidermis with Hexagonal epidermal cells; St - Stomata; AdE - Adaxial Epidermis.

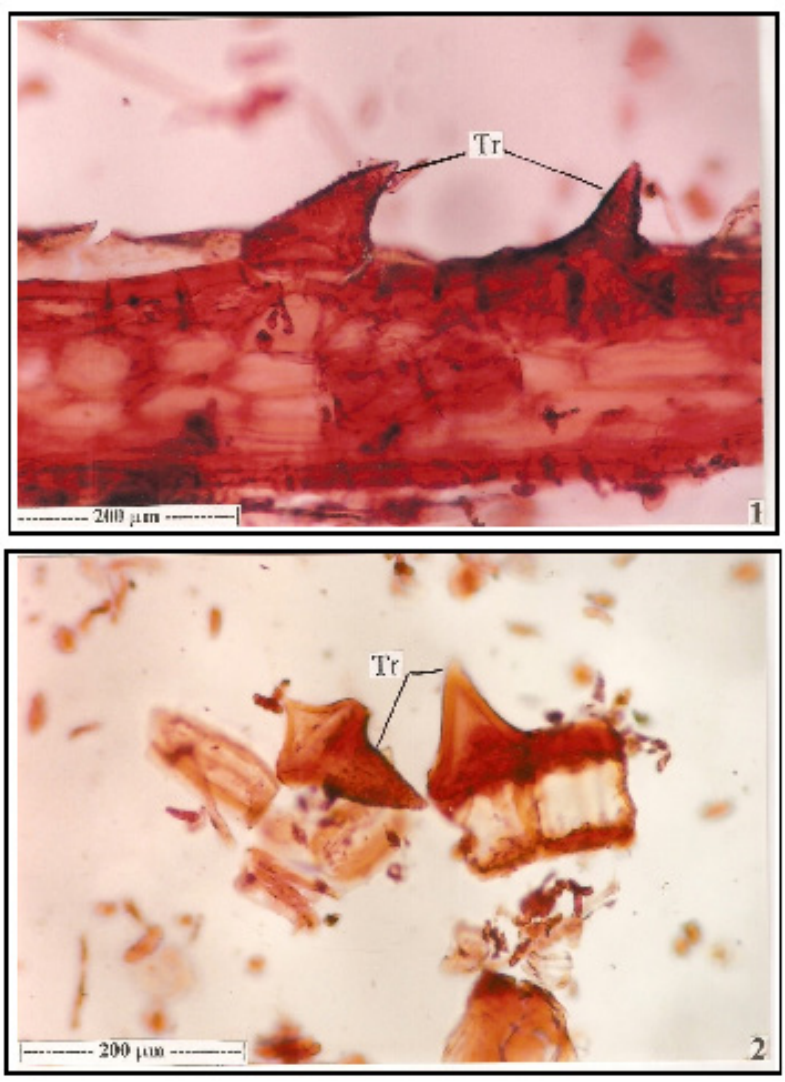

Figure 10: Leaf Macerate; 10a: Epidermal trichomes; 10b: Trichomes; Tr: Trichome.

botanical identification. However, the challenge extends beyond identification to ensuring the quality, efficacy, and safety of herbal raw materials. Pharmacognostic studies offer a foundational approach to resolving these issues by providing morphological, anatomical, and chemical parameters for proper authentication and standardization.

Among the medicinally significant plants, *Oldenlandia umbellata* has been reported in India, Sri Lanka, and Myanmar. Although well-documented in traditional systems, its dried form is often difficult to distinguish from other members of the *Oldenlandia* and *Hedyotis* genera.[27,28] To resolve this taxonomic ambiguity, the present investigation focused on detailed pharmacognostic evaluation.

The study successfully identified unique diagnostic features that can differentiate *O. umbellata* from morphologically similar species such as *Oldenlandia corymbosa, O. diffusa*, and *Hedyotis scandens*.[27-30] Furthermore, qualitative phytochemical screening revealed that *O. umbellata* shares certain phytoconstituents with unrelated species like *Glossocardia bosvallea, Mollugo nudicaulis, Sida cordifolia*, and *Cardiospermum canescens*. Nevertheless, High-Performance Thin-Layer Chromatography (HPTLC) profiling demonstrated clear differences, affirming the chemical uniqueness of *O. umbellata*.[8,9,16-18]

Methanolic extracts of the plant yielded more abundant secondary metabolites than aqueous extracts, emphasizing the critical role of solvent choice in phytochemical investigations. HPTLC proved to be an effective method for the standardization of herbal raw materials, offering detailed fingerprint profiles that can quantify specific constituents and detect potential contaminants. The observed R_f values, band patterns, and densitometric data were distinctive to *Oldenlandia umbellata*, reinforcing its identity and distinguishing it from closely related species.

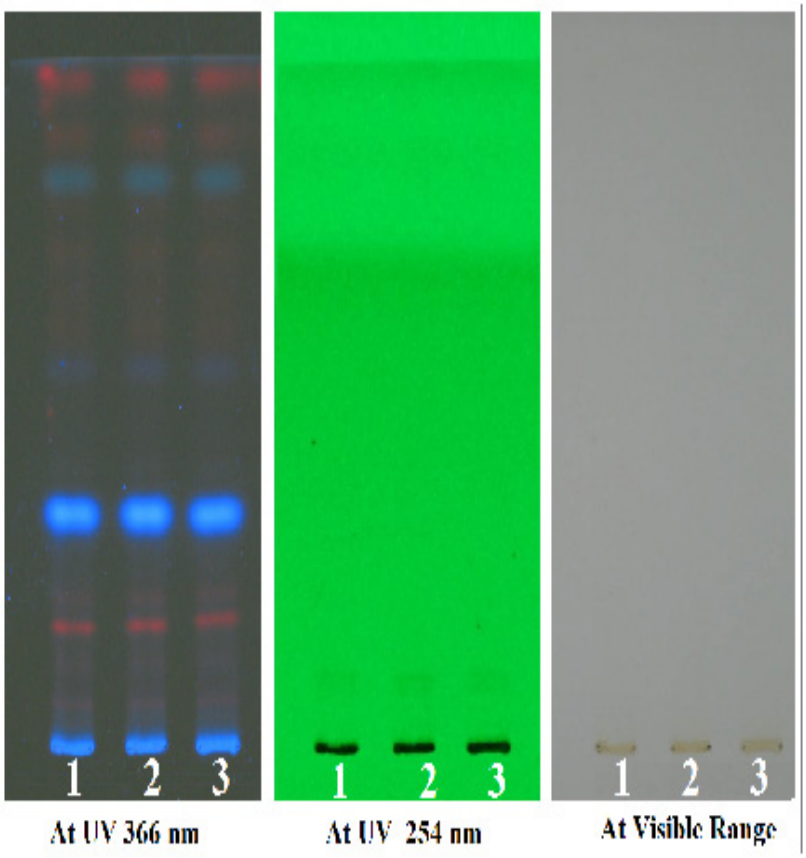

Figure 11: TLC Chromatogram of the leaf powder.

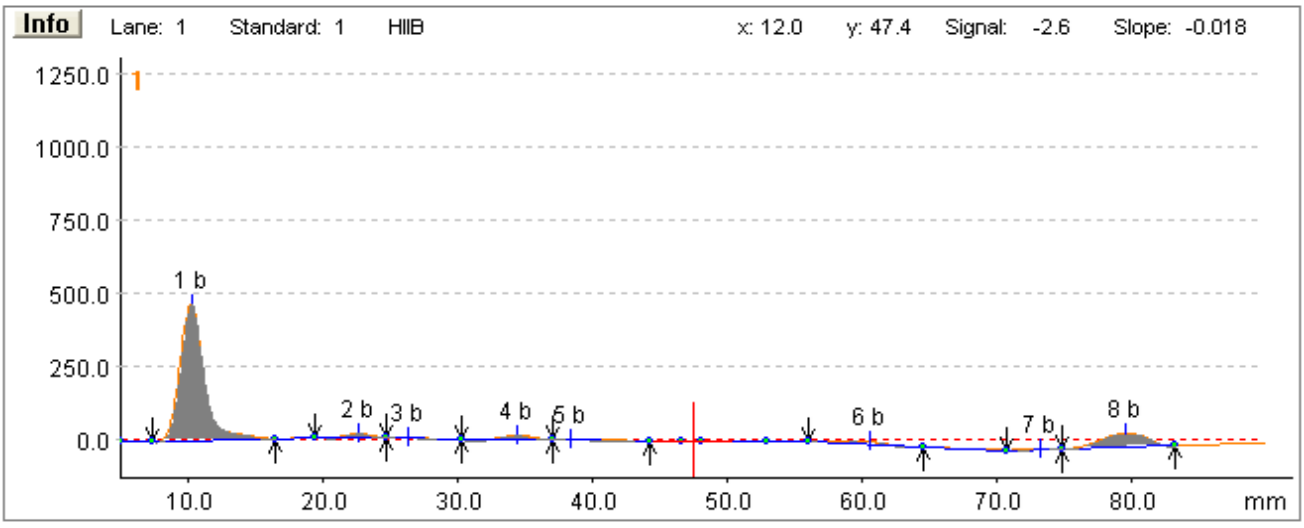

Figure 12: Densitogram showing the separation of peaks of leaf powder.

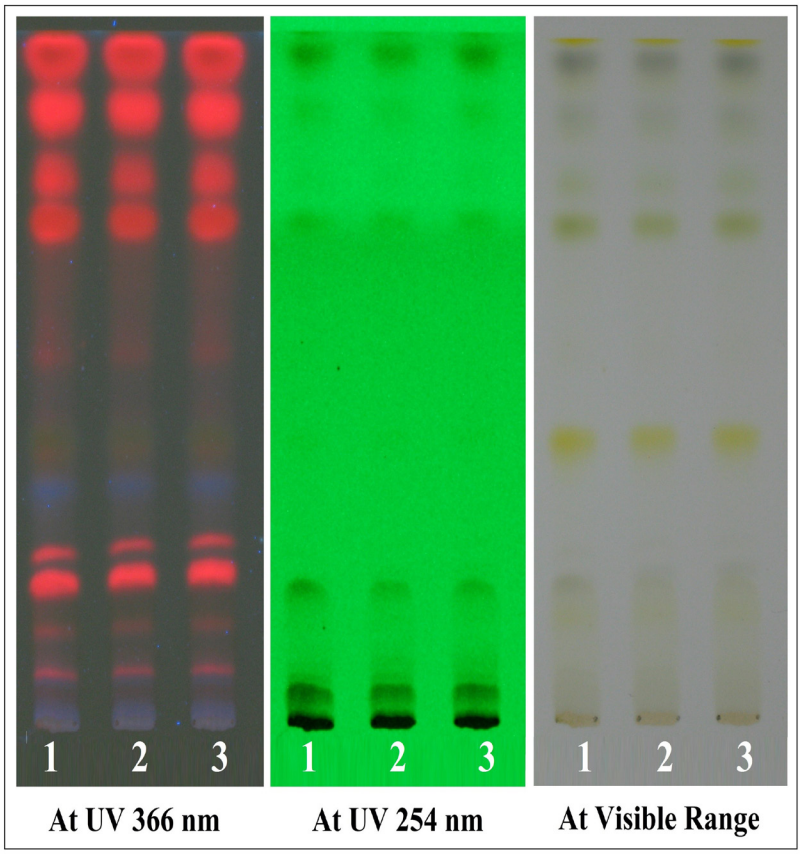

Figure 13: TLC chromatogram of the root powder.

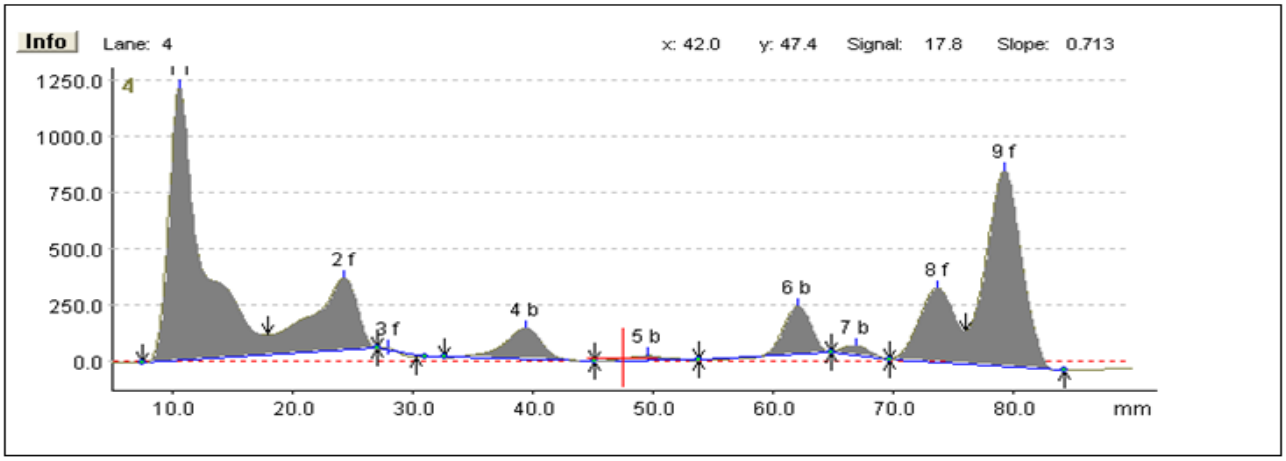

Figure 14: Densitogram showing the separation of peaks of root powder.

CONCLUSION

The present study, phyto-pharmacognostic standards on *Oldenlandia umbellata*, established detailed and specific data on macro-microscopical, physicochemical and chromatographical parameters. The R_f values and densitograms documented from HPTLC analysis would be used to identify and differentiate the authentic plant source from the adulterants. Further, this data could be utilized to evaluate pharmacopoeial measures in drug quality assurance in the pharmaceutical industry.

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ABBREVIATIONS

AbE: Abaxial epidermis; **AdE:** Adaxial epidermis; **Co:** Cortex; **Cr:** Crystals; **Dr:** Druces; **Fi:** Fibres; **Fis:** Fissures; **GC:** Guard cells; **La:** Lamina; **LM:** Leaf mesophyll; **MR:** Midrib; **Pe:** Periderm; **Pg:** Phellogen; **Pi:** Pith; **PhR:** Phloem rays; **PM:** Palisade mesophyll; **PP:** Perforation plate; **Ra:** Raphides; **SC:** Subsidiary cells; **SE:** Sclerenchyma; **SM:** Spongy mesophyll; **SPh:** Secondary phloem; **St:** Stomata; **SX:** Secondary xylem; **T:** Tail; **TLC:** Thin Layer Chromatography; **Tr:** Trichome; **UV:** Ultraviolet; **VE:** Vessel elements; **VI:** Vein-islets; **VT:** Vein For Figures 1–10, sub-figures are numbered 1–3 but referred to as (a)–(c) in the text.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The study conducted a detailed pharmacognostic and phytochemical investigation of Oldenlandia umbellata to establish reliable identification features and ensure quality control. Morphologically, the plant is a small, erect herb with linear, sessile leaves and white flowers. Microscopically, its root, stem, and leaf showed clear tissue differentiation, presence of calcium oxalate crystals, paracytic stomata, and distinctive xylem structures.

Physicochemical and histochemical analyses confirmed the presence of key phytoconstituents such as alkaloids, flavonoids, tannins, and saponins. The HPTLC profiling of methanolic extracts revealed unique chemical fingerprints, a novel aspect of the research that aids in distinguishing O. umbellata from similar species.

The study's findings emphasize the critical role of proper authentication in preventing adulteration in traditional medicine. By highlighting the value of integrated pharmacognostic tools, the study not only ensures the quality and safety of herbal drugs but also paves the way for their standardization.

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