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Original Article

Investigation of Pharmacognostic and Phytochemical Profiling of *Cordia macleodii* Hook.

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ABSTRACT

Aim: *Cordia macleodii* Hook., a medium-sized polygamous tree native to parts of India, holds significant promise in traditional medicine. Despite its ethnobotanical relevance, comprehensive pharmacognostic and phytochemical data are limited, making standardization and quality assurance a challenge. This study aims to establish a detailed pharmacognostic profile and perform phytochemical screening of *C. macleodii* bark and leaf to support its identification, authentication, and potential inclusion in therapeutic formulations. **Materials and Methods:** Macroscopic and microscopic examinations of the bark, petiole, and leaf tissues were conducted using standard botanical techniques and photomicrography. Physicochemical parameters, such as ash values and extractive yields, were measured. Fluorescence analysis and preliminary phytochemical screening were performed using conventional reagents. **Results:** HPTLC was employed to generate a densitometric fingerprint profile and to quantify β-sitosterol as a biomarker compound. The plant exhibited distinct morphological and anatomical features, including tomentose leaves, bicollateral vascular bundles, and prismatic calcium oxalate crystals. Physicochemical studies revealed significant water and methanol solubility. HPTLC analysis confirmed the presence of β-sitosterol (R_f 0.59) in methanol extract at 600 nm. Phytochemical tests showed varied presence of saponins, tannins, reducing sugars, and glycosides across different extracts. The integrated pharmacognostic and phytochemical data provide essential diagnostic markers for *C. macleodii*, enhancing its standardization and quality control. **Conclusion:** These findings support their safe and effective inclusion in phytotherapeutic applications and set the stage for further pharmacological investigations.

Keywords: β-sitosterol, *Cordia macleodii*, Fluorescence Analysis, Phytoconstituents, Stomata.

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INTRODUCTION

Medicinal plants have long served as a cornerstone in traditional systems of medicine, notably Ayurveda, Siddha, and Unani, where their pharmacognostic characteristics contribute substantially to therapeutic efficacy and safety (Saxena, 1995). Among them, *Cordia macleodii* Hook. (family Boraginaceae), commonly known as Dahiman or Lasura in various Indian dialects, represents a lesser explored but pharmaceutically significant species. Found predominantly in the tropical and sub-tropical zones of India, *C. macleodii* is a deciduous tree traditionally recognized for its utility in treating respiratory ailments, gastrointestinal disorders, and inflammatory conditions (Dubey *et al.*, 2008; Acharya *et al.*, 2008).

The correct identification and standardization of herbal drugs are foundational to developing safe, reproducible phytotherapeutic products. However, variations due to geographical, environmental, and collection parameters often confound the integrity of medicinal plant materials. This inconsistency is particularly problematic for under-documented species like *C. macleodii*, where taxonomic ambiguities and limited pharmacognostic data impede both academic research and clinical application. The plant's mention in regional ethnobotanical literature suggests its promising potential, yet systematic botanical validation and biochemical fingerprinting remain fragmented or anecdotal (Kirtikar *et al.*, 2003).

Pharmacognostic studies-including macroscopic and microscopic analysis of plant organs-offer crucial insights into species authentication and structural biomarkers. Features such as trichomes, medullary rays, lignified fibres, and calcium oxalate crystals contribute to the anatomical identity of medicinal bark and leaf tissues (Evans, 2005). These characteristics not only aid in distinguishing genuine plant material from adulterants but also establish a scientific basis for developing regulatory monographs (Anonymous, 1999). Furthermore, microscopic profiling plays

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a critical role in standardizing crude drug formulations in the context of industrial-scale preparation.

Equally imperative is the phytochemical profiling of plant constituents using both classical and modern approaches. Preliminary screening methods can detect the presence of key bioactive groups such as flavonoids, saponins, alkaloids, and glycosides, while sophisticated chromatographic tools like High Performance Thin Layer Chromatography (HPTLC) enable quantitative fingerprinting and compound validation (Chase *et al*., 1949). In this context, β-sitosterol-a plant-derived sterol known for its anti-inflammatory and immunomodulatory effects-has emerged as a meaningful marker for several herbal species, including *C. macleodii*.

The present investigation seeks to integrate morphological, anatomical, physicochemical, and phytochemical evaluations of *C. macleodii* to establish a comprehensive quality control framework. This work follows guidelines suggested by the Ayurvedic Pharmacopoeia of India and incorporates multi-layered scientific analysis to facilitate the systematic authentication of this medicinal resource. By bridging ethnomedicinal knowledge with empirical validation, the study not only substantiates the therapeutic relevance of *C. macleodii* but also contributes to expanding the corpus of pharmacognostic literature for emerging Ayurvedic botanicals.

MATERIALS AND METHODS

Leaves and bark of *C. macleodii* Hook. were used as the material.

Collection of sample

Flora of Orissa was crucial in confirming the plant's identity (Saxena, 1995). The scholar personally collected the plant's leaves in November 2008 from its native habitat, and the Dravyaguna department of IPGT and RA has maintained a voucher specimen. Shade drying was done after the leaves were washed. Pulverised and passed through 80 mesh, the leaves were subsequently stored in an airtight container.

HPTLC studies

To create a unique fingerprint profile that can be used for quality assessment and standardisation, a densitometric HPTLC analysis was carried out. The sample was prepared to 80 mm in a Camag glass twin-trough chamber (20 cm×10 cm) that was saturated with mobile phase vapour for 20 min. The mobile phase used was toluene: ethyl acetate: formic acid (80: 20: 5 v/v). The β-sitosterol (R_f 0.59) was measured at the same time using a CAMAG TLC Scanner model-3 using Wincats version 3.2.1 software after the plates were fully dried in a hot oven at 110°C for 15 min after removal from the chamber. Slit width of 5 x 0.45 nm, frequency of lambda max-600 nm, and absorption-reflection scan mode were the scan conditions that were utilised.

Preservation of wet sample

A solution was produced using glacier-generated acetic acid, alcohol, formalin, and distilled water to preserve the material (Chopra *et al*., 1956).

Microscopic and macroscopic evaluation

To identify different components, thin pieces of the petiole, leaf, and midrib part were obtained using the maceration method. These sections were subsequently treated with fluoroglucinol, HCl, and iodine (Johansen *et al*., 1940). Using a Canon digital camera mounted to a Zeiss microscope, photomicrographs were captured.

Phytochemical evaluation

Following the protocol established by the Ayurvedic Pharmacopoeia of India, the physicochemical and preliminary phytochemical analyses were performed on the dried sample (Kirtikar *et al*., 1935).

RESULTS

Morphology

The plant is a small tree reaching a height of 9-12 M with a trunk diameter of 50-60 cm. Its bark features white tomentose branchlets and a reddish-green inner layer that exudes a substance upon injury, with an overall thickness of 12-15 mm. The leaves are wide-ovate, measuring approximately 20-25 × 15-18 cm, with a glossy dark green dorsal surface and a lighter, hair-covered ventral side. They are fully serrated, sharply acuminate or sometimes obtuse, slightly rugose on the upper surface, and bear numerous white cystoliths. Each leaf is 3-5 nerves from or near the base, which is typically deeply cordate. The petioles measure 3.7-7.5 cm in length, and the leaves are either opposed or extra-axillary. The yellowish-white, polygamous male flowers lack stigma and style but contain a rudimentary ovary. They are subsessile and form dense paniculate cymes at the apices and axils, covered in a tomentose texture. The calyx is obconic and ribbed, around 8 mm long, with short, obtuse lobes that are thickly tomentose. The corolla has a tube longer than its width, with spathulate-oblong, obtuse, veined lobes measuring 1.6 cm long and 8 × 2.5-3 mm in size. Typically, six stamens are present, with hairy-based filaments. The male anthers are notably large, while those of the female are significantly smaller. The fruit is a drupe, positioned atop the calyx, and is subglobose, yellowish, slightly tomentose, and apiculate. The calyx itself is broadly campanulate, with toothed or lobed margins (Figure 1).

A brief taxonomic description of the plant

Having greyish brown bark, this medium-sized polygamous tree reaches a height of approximately 12 M. The leaves are alternate and have a cordate-ovate shape with three to five veins. They are permanently tomentose beneath and seldom subopposite. At

maturity, the leaves are 12.5 cm in diameter, and the petiole is 2.5-5 cm long. The inflorescence corymbs are short and tomentose. The calyx is about 1.2 cm long and tubular clavate, densely tomentose. It is ripped upwards or, on smaller scales, not ribbed. The corolla lobes can be as long as 1.6 cm and as narrow as 1 cm. Persistent calyx widely funnel-shaped or subcompanulate; fruit acutely conical until almost mature.

Macroscopic characteristics of bark

Bark that has matured and dried is a greyish brown tint; it is 8-15 cm long, 1-2 mm thick, and has a rough exterior. The bark is curled or channelled. Surface fibres on the inside; white when new, drying to a brownish hue. Coarse, splintery, fibrous, fractured texture. Absence of distinctive flavour and aroma.

Microscopic characteristics of bark

The outermost 8-12 layers of cork are tangentially elongated and organised radially in a cross-section of the bark. Two or three cell broad cork cambium layers later, secondary cortex or phelloderm. The phelloderm is parenchymatous and contains tannin-containing cells, clusters of fibres, prismatic crystals of calcium oxalate, and small groups of stone cells. Medullary rays divide the expansive secondary phloem into narrower sections that include companion cells, axially running bands of thick-walled lignified fibres, phloem parenchyma, and sieve tubes. Additionally, multiseriate medullary rays cross the major phloem, which becomes progressively larger as it approaches the periphery (Figure 2). Bone scans are unconventional, with a width of 4-6 cells and a height of 18-24 cells.

HPTLC

Figure 3 showing HPTLC chromatogram of *Cordia macleodii* bark sample with β-Sitosterol.

Physico-chemical and fluorescence studies

Overall, it has 10.55 percent ash, 0.43% acid insoluble ash, 0.75% hexane soluble extractive, 3.80% alcohol soluble extractive, 26.80% water soluble extractive, 1.89% sugar, 9.32 percent starch, and 0.05% tannins. The following percentages of solubility were obtained by successive solvent extraction using the Soxhlet apparatus: 6.20% for hexane, 3.42% for chloroform, 2.46% for acetone, 13.48% for methanol, and 14.34% for water (Table 1).

A homogeniser was used to powder the air-dried barks, and the resulting powder was deemed a medicine. Under both natural light and ultraviolet light (254 nm), the bark powder in various solvents was studied.

The previously mentioned study shows that macroscopic characteristics of bark make it easy to distinguish between different types of bark. For example, dried bark is often greyish brown in colour, curled to channelled in shape, and shatter splintery fibrous. Crystals, stone cells, heterogeneity, multi-seriate medullary rays, and resin cells can all be seen in the bark under a microscope.

It is possible to observe physico-chemical parameters, such as total ash, acid insoluble ash, extractive solubility in hexane, alcohol, and water. When looking for inorganic substances like silicate ions, total ash and acid-resistant ashes are good indicators to look for. Sugar, starch, tannin, and hexane/alcohol/water soluble extractives were also identified, serving as markers for the total solvent soluble component. Hexane, Chloroform, Acetone, Ethanol, and Water are polarity indicators for the total soluble component, as determined by successive solvent extractions carried out by the soxhlet apparatus. As a chemical marker, the

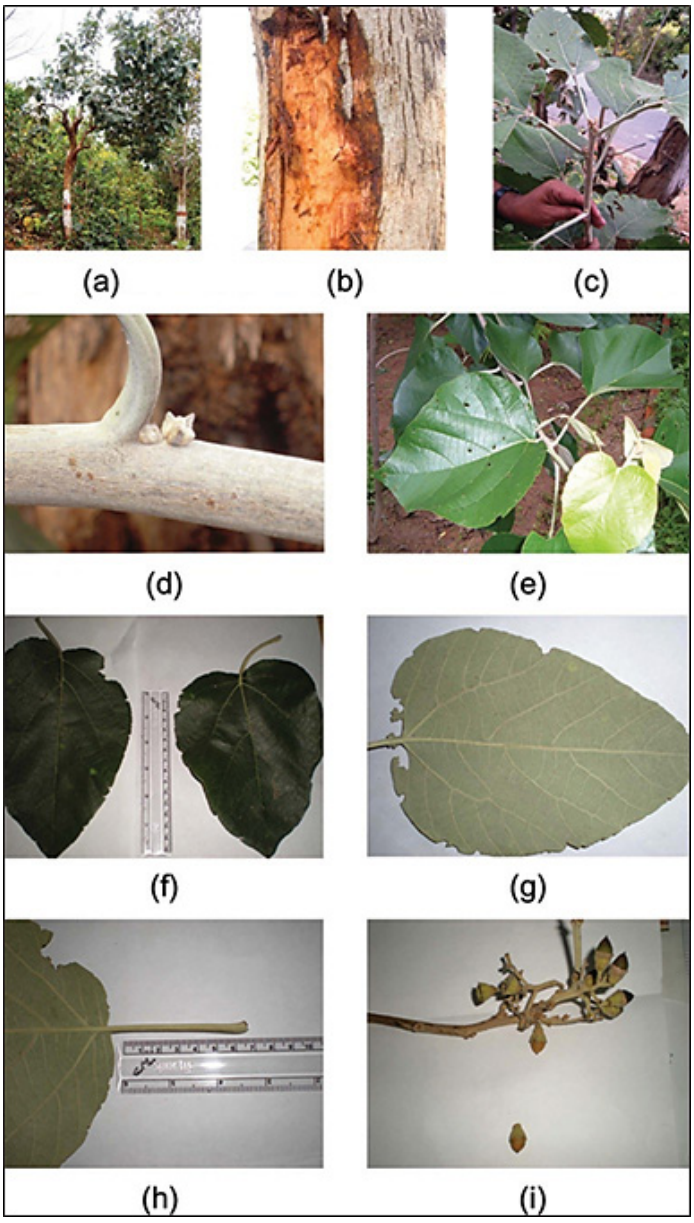


Figure 1: Morphology of the plant *Cordia macleodii* Hook. (a) Whole plant, (b) bark, (c) arrangement of leaves, (d) branchlet, (e) a branch of tree, (f) Individual leaf, (g) individual leaf - ventral side, (h) petiole, (i) Branchlet with fruits.

Table 1: Fluorescence analysis of the bark powder of *C. macleodii*.

Sl. No.	Treatment	Daylight	UV light (254 mm)
1.	Powder as such	Creamish brown	Light greenish brown
2.	Powder+1N aq. NaOH	Dark orangish brown	Dark greenish black
3.	Powder+1N ALC. NaOH	Dark yellowish brown	Greenish black
4.	Powder+1N HCl	Yellowish brown	Fluorescent green
5.	Powder+NH_3	Yellowish Green	Dark green
6.	Powder+I_2	Light Yellow	Greenish Black
7.	Powder+$FeCl_3$	Blackish Green	Black
8.	Powder+Acetic acid	Yellowish Brown	Greenish Black
9.	Powder+1N HNO_3	Yellowish orange	Blackish Green

Table 2: Phytochemical screening of the successive solvent extractives of *C. macleodii* bark.

Extractive	Triterpenoides	Saponins	Flavonoids	Tannins	Reducing sugar	Glycosides	Alkaloids
Hexane	+	-	-	-	-	-	-
Chloroform	+	-	-	-	-	-	-
Acetone	-	-	-	-	+	-	-
Methanol	-	-	-	-	+	-	-
Water	-	+	-	+	+	+	+

methanol extract yields 0.447% of β-sitosterol on quantitative HPTLC examination.

Microscopic study

TS of petiole

In outline, the TS of the petiole resembles an urn. The concentric bottom region takes up most of the section, while the elevated upper part has a narrowly channelled depression in the middle and is elevated on both sides (Figures 4a - 4b).

Epidermis and trichomes

An epidermis covering the petiole's surface is covered with many epidermal projections, including glandular and non-glandular trichomes (Figure 4c).

Collenchyma

There is a collenchymatous area with regions of chlorenchyma underneath the epidermis (Figure 4d).

Vascular bundle

The entire central area is occupied by a huge, spherical vascular bundle that resembles a stem. Underneath the higher elevations, you can also see two auxiliary vascular bundles. The xylem's outer and inner surfaces are covered with bicollateral phloem patches, which are encased in a parenchymatous bundles sheath. The top subsidary vascular bundles are bicollateral, similarly arranged, and encased in parenchymatous bundle sheaths. They also have central pith (Figure 4e).

Medullary rays

Radially arranged medulla rays pass through the spaces between the vascular bundles, which are xylem and phloem (Figure 4f).

Parenchyma

A mixture of brownish, dark brownish, and reddish-brown substances is also found inside most of the parenchyma cells. Additionally, brown materials are commonly found embedded with prismatic calcium oxalate crystals. Certain parenchyma cells within the bundle sheath also contain simple starch granules.

TS of the leaf through the midrib

Through the midrib, the T.S. of the leaf has a dorsiventral outline. Table 2 shows phytochemical screening of plant extract.

Epidermis

One layer makes up the leaf's top epidermis. The cuticle is thick, and the cells are rectangular. The lowest part of the lamina has relatively smaller epidermal cells.

Trichomes

On both the top and bottom layers of skin, you can see trichomes that are either glandular or non-glandular in nature. The trichomes that fail to develop glands are either multicellular or uniseriate and have a bulbous base. The glandular trichomes often have a bulbous head that is composed of a single cell. There are some enormous glandular trichomes with multicellular crowns and a single cell stalk. The midrib's lower half has a circular shape with a central vascular bundle, while its upper half has a little rise

in the centre. Collenchyma patches occupy the tissues underlying the upper elevation.

The plant's greenery

Lower stellate parenchymatous tissues with air gaps and upper axially elongated narrow palisade cells make up the mesophyll. The mesophyll is nearly filled with parenchymatous tissues that resemble stars.

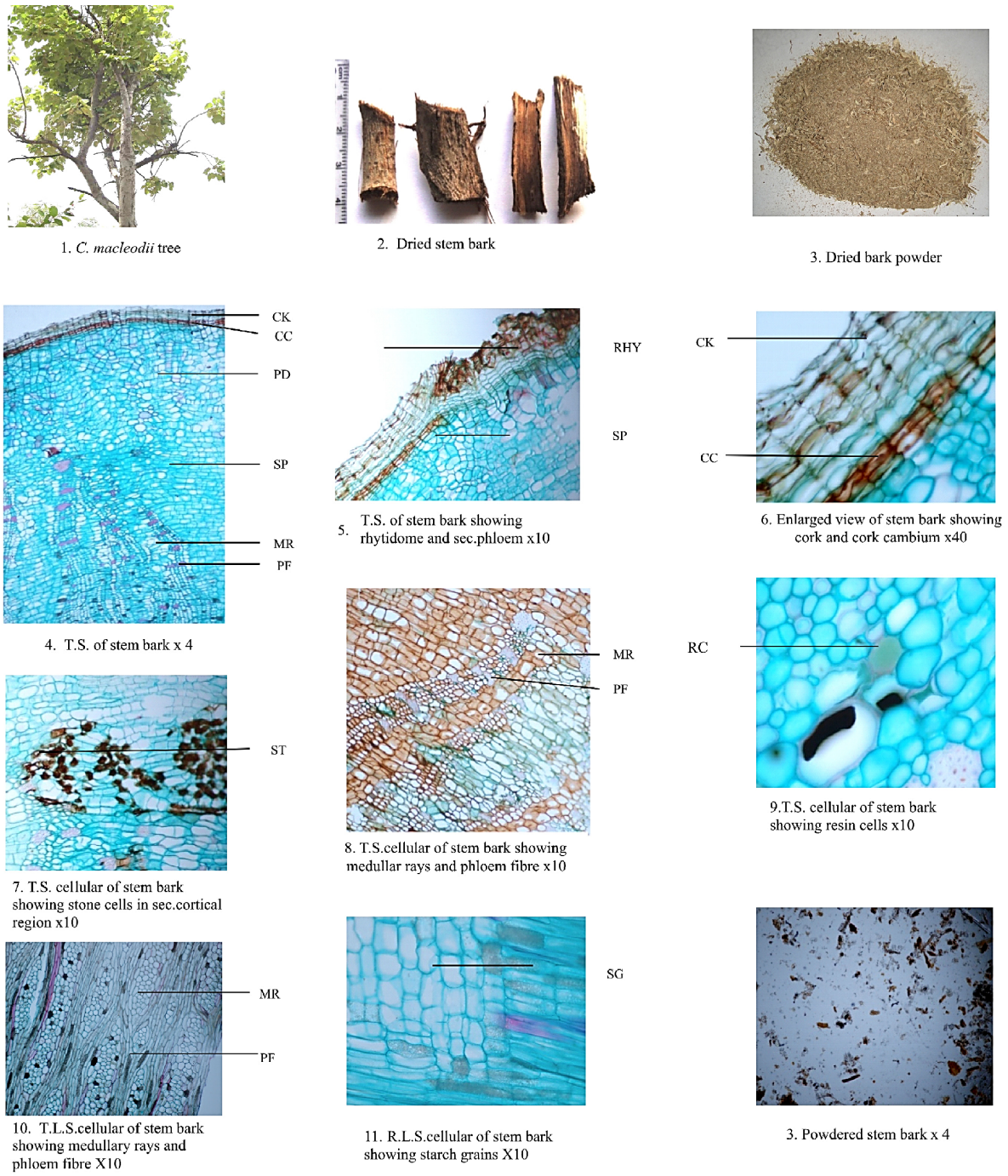
Vascular bundle

Crystals

Also prevalent in this area are prismatic crystals of calcium oxalate.

Stomata

In surface view, the cells of the epidermis of the leaf have a wavy outline, and most of the stomata are located on the underside of the leaf, with just a small number visible on the upper side. Most of



1. *C. macleodii* tree
2. Dried stem bark
3. Dried bark powder
4. T.S. of stem bark x 4
5. T.S. of stem bark showing rhytidome and sec.phloem x10
6. Enlarged view of stem bark showing cork and cork cambium x40
7. T.S. cellular of stem bark showing stone cells in sec.cortical region x10
8. T.S.cellular of stem bark showing medullar rays and phloem fibre x10
9. T.S. cellular of stem bark showing resin cells x10
10. T.L.S.cellular of stem bark showing medullary rays and phloem fibre X10
11. R.L.S.cellular of stem bark showing starch grains X10
3. Powdered stem bark x 4

Figure 2: Macro and microscopic features of the stem bark of *Cordia macleodii* CK, CC, SP, RHY, MR, PF, ST, RC, and SG all pertain to the stem bark.

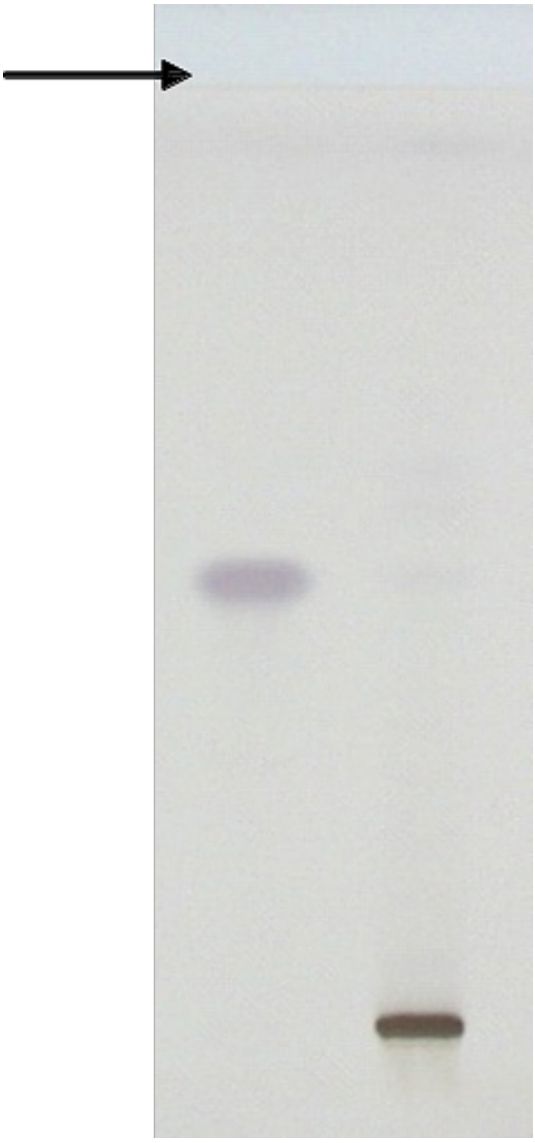


Figure 3: HPTLC profile of C. macleodii bark under 600 nm along with β-Sitosterol (STD).

the stomata are ranunculaceous, although there are a couple that are cruciferous (anisocytic and anomocytic). Covering trichomes make it nearly impossible to distinguish stomata in older leaves. Young leaves make it easy to distinguish between stomata.

DISCUSSION

The present study provides an exhaustive pharmacognostic and phytochemical characterization of *Cordia macleodii* Hook., a species that possesses significant ethnomedicinal interest. Macroscopic, microscopic, physicochemical and chromatographic analyses jointly form a solid foundation for botanical identification and quality control.

The morphological observations including tomentose leaves, serrated margins and cordate leaf bases also conform to the past ethnobotanical description of the plant. The microscopic features such as bicollateral vascular bundles, prismatic calcium oxalate crystals, and multiseriate medullary rays were identified as diagnostic characters of *C. macleodii* against the probable adulterants.

Medicinal properties of these species are also likely due to the presence of polar bioactive constituents as implied by their physicochemical parameters, namely high water and methanol solubility. The plant's therapeutic potential is also supported with existing anti-inflammatory and immunomodulatory properties of the whole plant and at the other hand the compound β-sitosterol at R_f 0.59 which is identified by HPTLC fingerprinting.

Phytochemical screening showed differential presence of secondary metabolites (phyto-constituents) in a variety of metabolome through polar-nonpolar solvent extraction. The water extract displayed the richest profile containing pharmacologically important saponins, tannins, glycosides, and alkaloids. These results are relevant with the Ayurvedic Pharmacopoeia's focus on multi-solvent extraction to obtain thorough profiling.

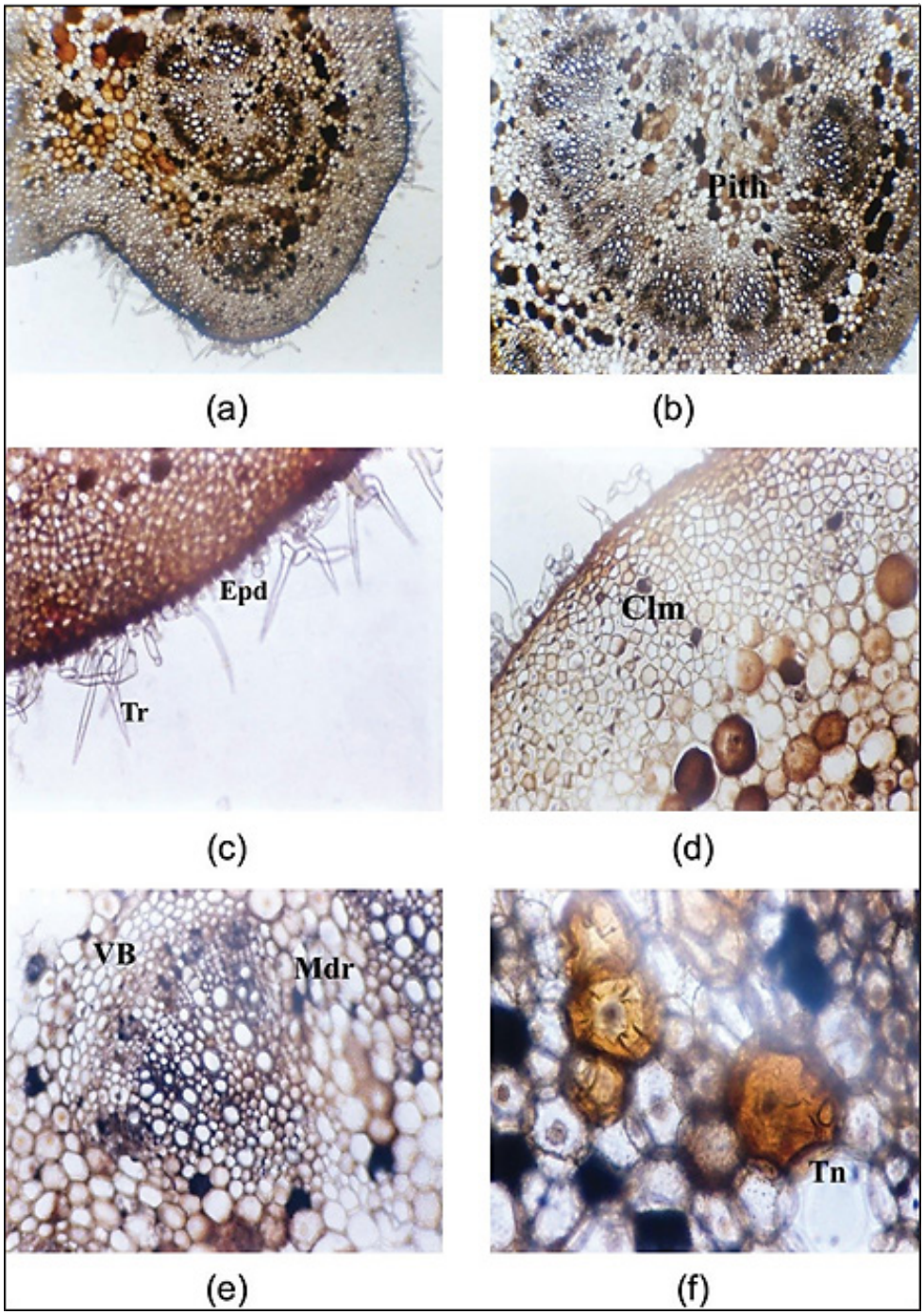


Figure 4: Photographs showing Transverse section of petiole (a) outline of transverse section of petiole (Magnification ×3.5) Epd- Epidermis Tr- Trichomes Clm- Collenchyma Tn- Tannin content Mdr- Medullary rays VB- Vascular bundle. (2) Central portion of lower region showing pith (Magnification ×3.5). (c) Photograph showing epidermis with trichomes (Magnification ×10). (d) Collenchyma (Magnification ×10). (e) A portion of vascular bundle (Magnification ×10). (f) Yellowish brown (tannin) content (Magnification ×20).

This suggests that *C. macleodii* may possess unique anatomical and chemical traits as compared to other members of the family, justifying its inclusion in herbal preparations. High β-sitosterol presence serves as an objective marker to enhance regulatory feasibility of this dietary supplement and facilitate future pharmacodynamics and pharmacokinetics studies. Ultimately, this research serves to connect traditional knowledge with empirical validation and provides a valuable reference point for pharmacological, toxicological and formulation-based research going forward.

CONCLUSION

The comprehensive pharmacognostic and phytochemical investigation of *Cordia macleodii* Hook. underscores its therapeutic potential and lays a foundational framework for

botanical standardization. The integration of macroscopic, microscopic, and physicochemical data-combined with fluorescence analysis and HPTLC fingerprinting-offers robust diagnostic markers for authentication and quality control. The presence of β-sitosterol, alongside distinct anatomical features such as bicollateral vascular bundles and prismatic calcium oxalate crystals, further strengthens its candidacy for inclusion in phytotherapeutic formulations. These findings not only validate the ethnomedicinal relevance of *C. macleodii* but also contribute essential data for developing regulatory monographs and future pharmacological evaluations. Ultimately, this study bridges traditional botanical knowledge with empirical scientific rigor, reinforcing the plant's significance in contemporary herbal medicine.

ACKNOWLEDGEMENT

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ABBREVIATIONS

HPTLC: High Performance Thin Layer Chromatography; **UV:** Ultraviolet; **TLC:** Thin Layer Chromatography; **R_f:** Retention factor; **NaOH:** Sodium Hydroxide; **HCl:** Hydrochloric Acid; **NH_3:** Ammonia; **I_2:** Iodine; **$FeCl_3$:** Ferric Chloride; **HNO_3:** Nitric Acid; **ISM&H:** Indian Systems of Medicine and Homoeopathy; **CSIR:** Council of Scientific and Industrial Research; **TS:** Transverse Section; **VB:** Vascular Bundle; **Epd:** Epidermis; **Tr:** Trichomes; **Clm:** Collenchyma; **Tn:** Tannin Content; **Mdr:** Medullary Rays; **PF:** Phloem Fibres; **ST:** Sieve Tubes; **RC:** Resin Cells; **SG:** Stone Cells; **STD:** Standard (Reference Compound).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

KA- writing original draft, JK-conceptualization.

SUMMARY

The study presents a comprehensive pharmacognostic and phytochemical evaluation of *Cordia macleodii* Hook., a traditionally used Indian medicinal tree. It details macroscopic and microscopic features of bark and leaves, including tomentose surfaces, bicollateral vascular bundles, and calcium oxalate crystals. Physicochemical tests revealed high water and methanol solubility, while HPTLC confirmed β-sitosterol as a key biomarker. Fluorescence and phytochemical screening identified saponins, tannins, sugars, and glycosides. These findings support the plant's authentication, standardization, and potential inclusion in phytotherapeutic formulations, bridging ethnomedicinal relevance with scientific validation.

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