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Morphoanatomical and Elemental Analysis of *Clerodendrum chinense* (Osbeck) Mabberley

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Abstract

Background: *Clerodendrum chinense* (Osbeck) Mabberley (*Lamiaceae*) is an underexplored medicinal plant traditionally used in various indigenous systems of medicine. Despite its therapeutic relevance, detailed pharmacognostic and microscopic data required for proper authentication and quality control of the raw drug remain limited. **Aim:** The present study aimed to establish comprehensive pharmacognostic standards for *C. chinense* through detailed anatomical, microscopic, histochemical, and surface micromorphological investigations, along with ethnobotanical documentation. **Methods:** Fresh plant materials were subjected to macroscopic and organoleptic evaluation. Anatomical studies were carried out using Light Microscopy (LM) on transverse sections of the leaf, midrib, and petiole. Powder microscopy and histochemical tests were performed to localise cellular constituents. Surface micromorphology and elemental composition were analysed using Scanning Electron Microscopy (SEM) coupled with Energy-Dispersive Spectroscopy (EDS). **Results:** Anatomical examination revealed typical dicotyledonous features. Leaves were amphistomatic, showing both diacytic and anisocytic stomata. The midrib exhibited several simple multicellular covering trichomes with a few glandular trichomes, while the lamina showed a dorsiventral structure with sparse trichomes. The petiole contained numerous multicellular covering trichomes. Powder microscopy showed diagnostic elements such as trichomes, stomata, mesophyll fragments, fibres, vessels with spiral and annular thickenings, and prismatic crystals. Histochemical analysis confirmed the presence of cutin in the epidermis, tannins in the hypodermis, and lignin in the xylem. **Conclusion:** The study establishes reliable diagnostic parameters for the identification and standardisation of *C. chinense*.

Major Findings: These findings support the development of a herbal monograph and enable accurate authentication of the raw drug, thereby strengthening regulatory quality control.

Keywords: *Clerodendrum chinensis,* Ethnobotany, Histology, Morphology

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1. Introduction

Over a long period, there has been a sharp rise in the use of natural therapies for illness management. This can be attributed to the significant role that plants have played in offering a vast array of affordable yet effective therapeutic solutions[1]. The World Health Organisation states that describing a medicinal plant's macroscopic and microscopic characteristics is the first step in determining its identity and level of purity and should be done before conducting any additional testing. The use of traditional medicine has increased dramatically worldwide, and as a result, the quality control, safety, and effectiveness of herbal medicines have become paramount[2].

Herbal markets offer therapeutic herbs in powdered or raw form, as well as extracts and fragments. The primary causes of adulteration in medicinal plants, which determine the requirements for quality control,

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are differences in nomenclature systems across various geographical regions of the world. As the herbal industry works to develop new medicines, the two biggest challenges it faces are the absence of regulations for medicinal plants and unethical practices. Botanical descriptions of herbal plants are especially helpful for academic and pharmaceutical research in uncovering new products, as well as for traditional practices[3].

The future of medicinal plants is promising, as there are around 500,000 plant species in the world, many of which have unexplored pharmacological properties that could significantly impact ongoing or future research[4]. The present study focuses on creating awareness about the plant *Clerodendrum chinense* (Osbeck) Mabberley (Family: *Lamiaceae*), popularly known as 'Fragrant Glory Bower' in English and '*Madras Malli*' in Tamil. Found abundantly in India, it is known for its wild growth, vegetative spread, and ornamental value, yet it remains underutilised for therapeutic purposes[5,6].

2. Materials and Methods

2.1 Review of Ethnobotanical Uses

Google Scholar, Web of Science, Springer Link, Wiley, and the Technology Journal Database were used to compile the data on the plant's ethnobotanical usage.

2.2 Collection and Preparation for Pharmacognostic Studies

Healthy and disease-free plants of *Clerodendrum chinense* (Osbeck) Mabberley were collected from the Kancheepuram district of Tamil Nadu. The collected specimens were identified and deposited in the Department of Pharmacognosy, Siddha Central Research Institute (SCRI), Ministry of Ayush, Government of India, Chennai, under voucher number C17082301C. Throughout the current investigation, very careful consideration was given to the plant collections. The leaf specimen was cleaned, rinsed, divided, and allowed to dry at room temperature for 15 to 20 days in the shade. After that, the specimens were crushed using a pestle and mortar and kept dry in an airtight vessel to fend off moisture.

2.3 Organoleptic Evaluation

It refers to the evaluation of plant material by colour, taste, odour, shape, texture, etc. Fresh, dried leaves and petiole of *C. chinense* were considered for macroscopical evaluation[7].

2.4 Macroscopic Evaluation

Fresh and healthy leaves of the plant *C. chinense* were assessed for their external characteristics[8].

2.5 Microscopic Evaluation

The sample was kept in fixative FAA for more than 48 hours. Using a sharp knife, the preserved specimens were cut into thin transverse sections and stained with toluidine blue[9,10]. The transverse sections were then examined under bright-field light and photographed using an Axiolab5 trinocular microscope equipped with a Zeiss Axiocam208 colour digital camera. A scale bar was included to indicate magnification.

2.6 Powder Microscopy

A small amount of the powdered sample was mounted on a microscope slide with a drop of 50% glycerol after being cleared with a saturated solution of chloral hydrate. The sample was then treated with iodine solution to confirm the presence of starch grains[11,12]. The microscopic characteristics were examined using a Nikon ECLIPSE E200 trinocular microscope equipped with a Zeiss ERc5s digital camera under bright-field light. Photomicrographs of the diagnostic features were captured and documented.

2.7 Quantitative Microscopy

Rectangular-cut leaf pieces were boiled in a saturated chloral hydrate solution until they became colourless, and slides were prepared to examine vein islets and vein terminations. To prevent desiccation and facilitate the isolation of the epidermis, fresh leaves were submerged in water for stomatal examination. Using a razor, the abaxial and adaxial surfaces were carefully separated and placed on a glass slide. They were then mounted in fluid Canada balsam and examined under a microscope[13,14]. A light microscope (Labomed Lx 400 model) was used to analyse the presence or absence of stomata on each epidermis, as well as their type, distribution, and position relative to other stomata and epidermal cells. The stomatal index was also determined. Micrometry was performed using a micrometre.

2.8 Histochemical Tests

Plant sections were treated using the following standard procedures:

2.8.1 Crystals

The section was mounted in water, and one end of the cover slip was irrigated with acetic acid. While looking through the microscope, the water within the cover slip was replaced using a piece of filter paper at the opposite end of the cover slip.

- Formation of air bubbles indicated Calcium carbonate crystals
- If no air bubbles were formed, the experiment was repeated with a conc. HCl, wherein dissolution of the crystal and formation of Calcium sulphate indicated the presence of calcium oxalate crystals.

2.8.2 Fats, Fatty Oils, Volatile Oils and Resins

About1 to 2 drops of Sudan-IV were added to the section and allowed to stand for a few minutes. Presence of fatty oil substances was indicated by orange-red/ pink/red colored globules, while red coloured irregular contents indicated resin.

2.8.3 Starch

A drop of 2% iodine water solution was added - blue colour indicated starch.

2.8.4 Tannin

A drop of alcoholic ferric chloride was added - bluish black coloured contents indicated tannin.

2.8.5 Mucilage

A drop of ruthenium red was added - pink to red colored contents indicated mucilage.

2.8.6 Lignified Cell Walls

A drop of phloroglucinol was added to the section and allowed to stand for about 2 min or until almost dry. A drop of 50% HCl was added and observed over a cover-glass - cell walls stained pink to cherry red, indicating the presence of lignin.

2.8.7 Suberized or Cuticular Cell Walls

A drop of Sudan red III was added and allowed to stand for a few minutes, warmed gently if necessary - cell walls-stained orange-red or red indicated suberin or cutin deposition over the cell wall[15-17].

2.9 SEM and EDS Analysis

The dried leaves (1x1 mm) for SEM analysis were mounted on a specimen stub and examined using a High-Resolution Scanning Electron Microscope (HRSEM) Thermo Scientific Apreo S, equipped with a quantitative elemental mapping EDS attachment. When the leaf and petiole were bombarded by the electron beam of the SEM, electrons were ejected from the atoms on the specimen's surface, enabling microanalysis. The EDS system consists of an X-ray detector, a pulse processor, and a multichannel analyser. The EDS X-ray detector measures the number of X-rays emitted in relation to their energy. A spectrum of energy versus the relative counts of detected X-rays is generated and analysed to determine the elemental composition[18,19].

3. Results

3.1 Ethnobotanical Uses

Ethnobotanical uses of *Clerodendrum chinensis* are given in Table 1.

Table 1. Ethnobotanical profile of *C. chinensis*

Phytoconstituents	Parts used	Folklore medicinal uses
Linalol	Flowers	To cure headache[20]
5-*O*-β-glucopyranosyl-harpagide (iridioid Glycoside)	Leaves	Anti-pyretic and Anti-inflammatory[21,22]
Kaemferol, Acteoside	Whole plant, leaves	Anticancer[23]
24α-ethyl-5α-cholest-22E en-3β-ol	Roots	Antirheumatic, Anti Asthmatic[24]
24β-methylcholesta- 5, 22E,25-trien-3β-ol	Whole plant	Antirheumatic[24]
5,4′-dihydroxy kaempferol-7-O-β rutinoside	Leaf	Anti-hypertension, Furunculosis[25]
leucoseceptoside A	leaf	Anti-hypertension[26]
Verbascoside	leaf	Colorectal cancer[27]
Trans-β-elemenone	Leaves	Insecticidal activity against *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus*[28]
Eugenol	Leaves and flowers	Toothache[29]

3.2 Macroscopy

Fresh leaves are dark green, simple, and opposite, with a broad ovate to nearly cordate shape. The underside is velvety hairy, especially along the veins, and features several large glands near the base. The margins are sparsely and irregularly toothed, with an acuminate tip. Leaves measure between 6 and 18 cm in length and 5 and 20 cm in width. The petiole ranges from 3 to 15 cm long. The leaves have a characteristic odour and a bitter taste.

Flowers are white to whitish pink, arranged in a terminal, comb-like cyme inflorescence. The calyx is purple or red, campanulate, and five-lobed, measuring approximately 1 to 1.5 cm in length. The corolla is pale pink, typically double due to petaloid stamens. The peduncle is short and woolly. Bracts are numerous, foliaceous, and lance-shaped, measuring 1.5 to 3 cm in length. The flowers have a fragrant odour and a bitter taste (Figure 1).

3.3 Organoleptic Evaluation

The organoleptic characteristics of the petiole and leaf are listed in Tables 2a and 2b. According to *Carrillo-Galván et al.,* the organoleptic properties of plants are crucial in establishing their medicinal status and are also utilised to characterise the alleviation of illness[30].

3.4 Microscopy

3.4.1 Leaf

The Transverse Section (TS) of the leaf shows an upper surface that is slightly elevated and a lower midrib surface that is broadly convex, with lateral laminar extensions (Figure 2).

3.4.2 Midrib

TS of midrib shows single layered epidermis covered by cuticle and bears several simple, multicellular covering trichomes and some glandular trichomes; 4 to 5 layers of collenchymatous hypodermis is present followed by 7 to 8 layers of parenchymatous ground tissue with few prismatic crystals; a ring of about 25 vascular bundles present capped by discontinuous patches of pericyclic ring; vascular bundles are conjoint, collateral and closed with endarch xylem; phloem is arranged facing



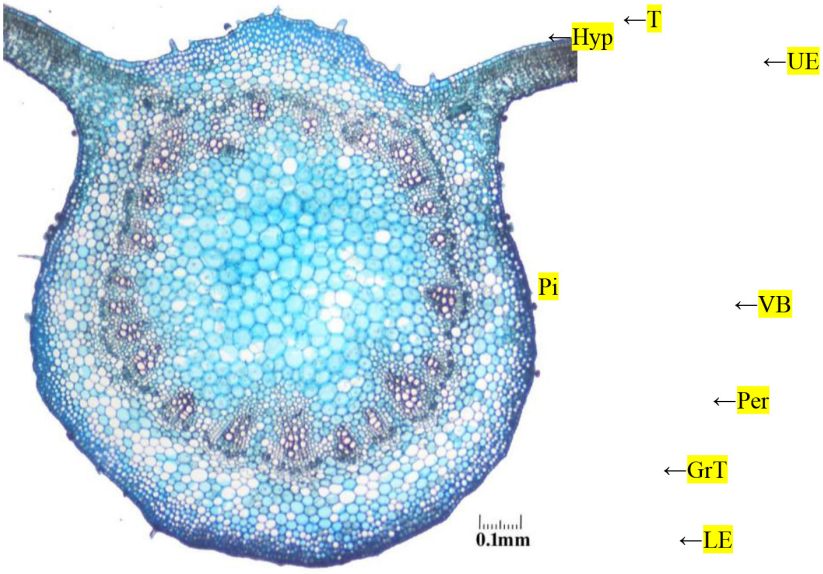
Figure 1. Macroscopy of *Clerodendron chinensis.*

Table 2a. Organoleptic features of the leaf of *C. chinensis*

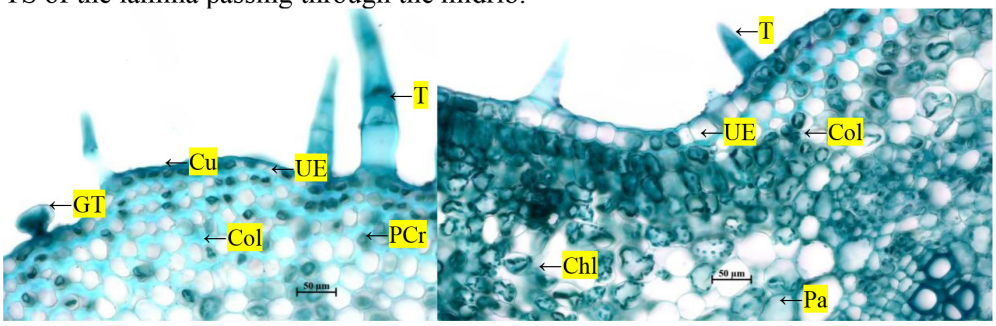
Parameter	Fresh	Dry
Color	Upper surface dark green and lower surface light green.	Greenish Brown
Size	Ave. Length= 18.0cm and Ave. width =5cm	Ave. Length= 16.0cm and Ave. width =4.5cm
Texture	Herbaceous	Herbaceous
Shape	Cordate	Cordate
Odour	Indistinct	Indistinct
Taste	Bitter	Slightly bitter

Table 2b. Organoleptic features of the petiole of *C. chinensis*

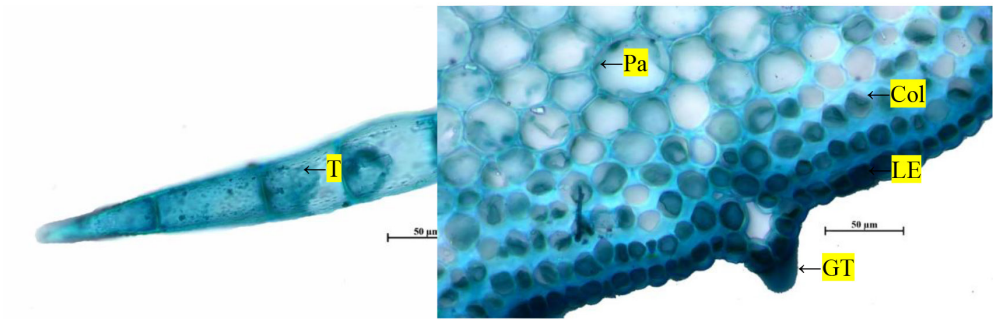
Parameter	Fresh	Dry
Color	Green	Brown
Size	Ave. Length= 15.0cm and Ave. width =15mm	Ave. Length= 15.5cm and Ave. width =1.0mm
Texture	Herbaceous	Herbaceous
Shape	Cylindrical	Cylindrical and shrunken
Odour	Indistinct	Indistinct
Taste	Slightly bitter	Slightly bitter



TS of the lamina passing through the midrib.

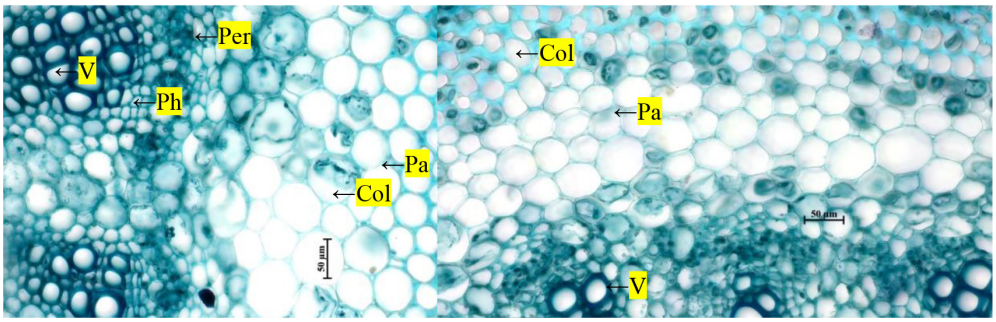


The midrib upper portion is enlarged.

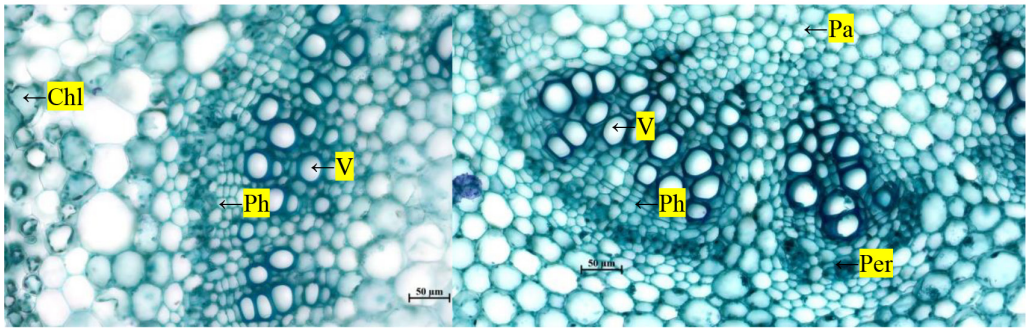


Single multicellular covering trichome

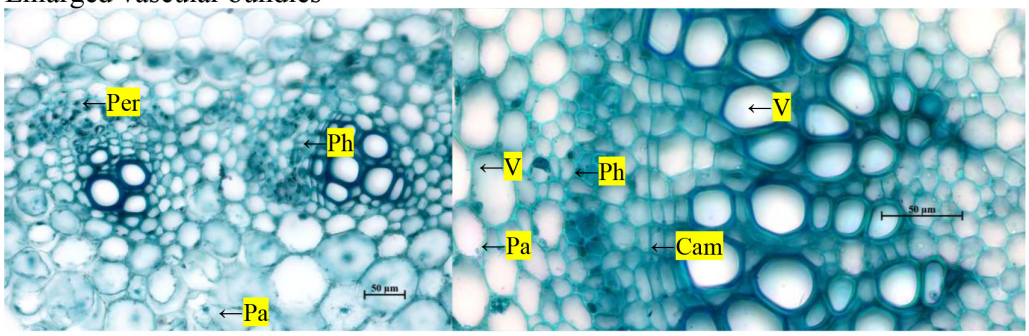
Glandular trichome



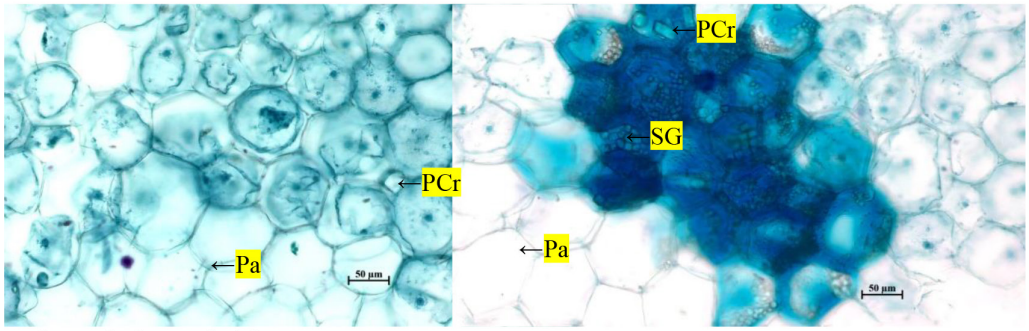
Cortex enlarged view



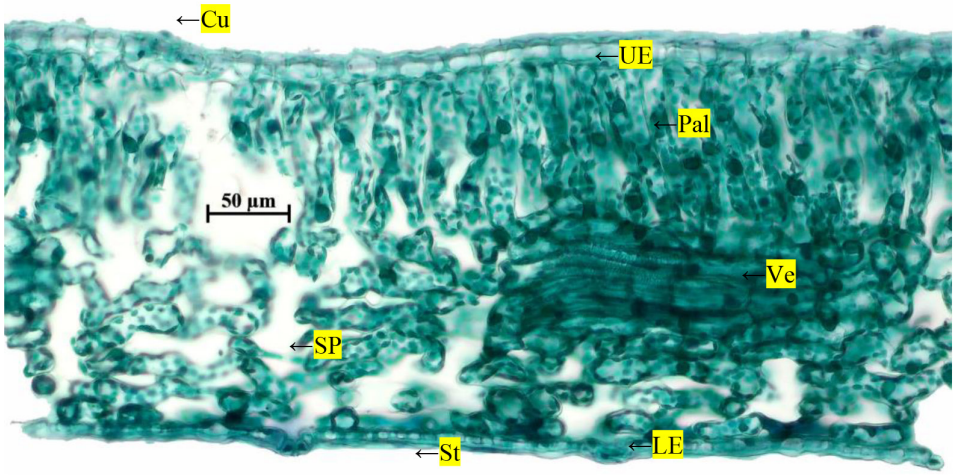
Enlarged vascular bundles



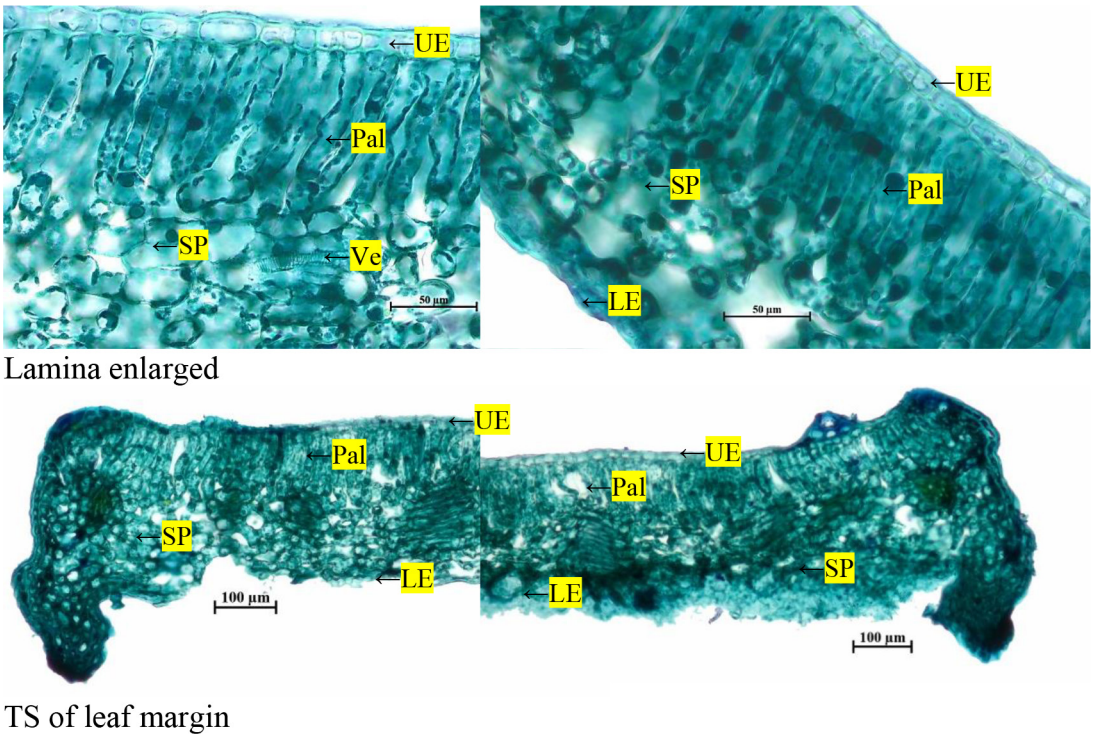
Vascular bundle Xylem and phloem



Enlarged view of pith



TS of the lamina



Cam - cambium; **Chl -** chlorenchyma; **Col** - collenchyma; **Ct** - cortex; **GrT** - ground tissue; **GT** - glandular trichome; **Hy** - hypodermis; **LE** - lower epidermis; **Pa** - parenchyma; **Pal -** palisade; **PCr** - prismatic crystal; **Per** - pericycle; **Ph** - phloem; **Pi** - pith; **SG** - starch grains; **SP** - spongy parenchyma; **St** - stomata; **T** -trichome; **UE** - upper epidermis; **V** - vessel; **VB** - vascular bundle.

Figure 2. T.S of *Clerodendrum chinense* leaf.

towards outside and xylem towards inner side; xylem and phloem is formed of normal vascular elements; broad parenchymatous pith is present at the centre with plenty of starch grains and few prismatic crystals (Figure 2).

3.4.3 Lamina

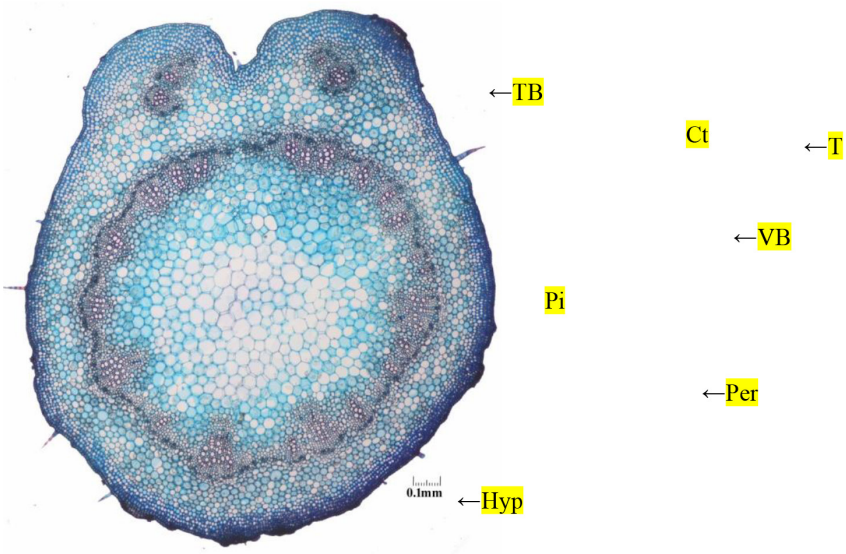
TS of lamina shows dorsiventral leaf with single layered upper and lower epidermis covered by thin cuticle and bears few covering and glandular trichomes; mesophyll tissue consists of upper double layered palisade cells followed by 6 to 7 rows of loosely arranged spongy parenchyma cells; veins are found traversing through the mesophyll tissue; compactly arranged mesophyll tissue is found towards the leaf margin (Figure 2).

3.4.4 Petiole

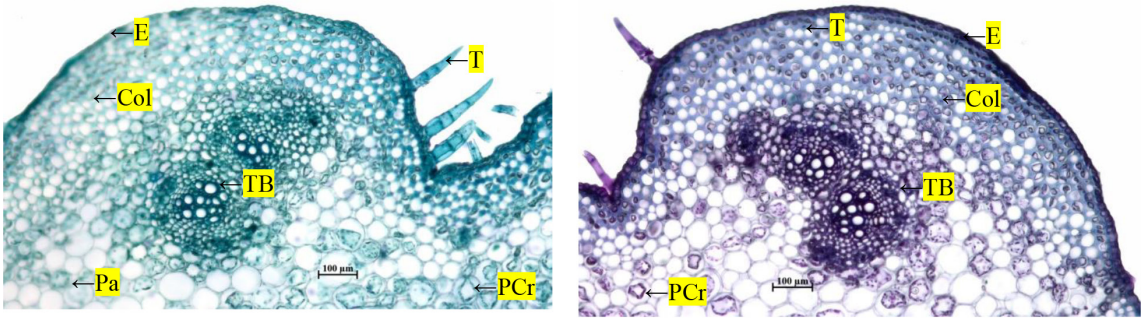
TS of petiole is nearly oval in outline with a notch at the apex with slightly wing like lateral projections and convex lower surface; outer layer is formed of single row of circular to oval shaped epidermal cells covered by thick cuticle and bears numerous simple, multicellular covering trichomes; cortex consists of 6 to 7 layers of collenchymatous cells followed by 13 to 14 layers of parenchyma cells of which first 2 to 3 layers are chlorenchymatous; few prismatic crystals are found scattered throughout the cortical cells; inner to the cortex a ring of about 20 vascular bundles present capped by discontinuous patches of pericyclic ring; vascular bundles are conjoint, collateral and closed with endarch xylem; phloem is found below the pericycle followed by xylem elements; xylem and phloem is formed of normal vascular elements; two trace bundles are found in the upper cortical region below the each wing; broad parenchymatous pith is present at the centre (Figure 3).

3.5 Quantitative Microscopy

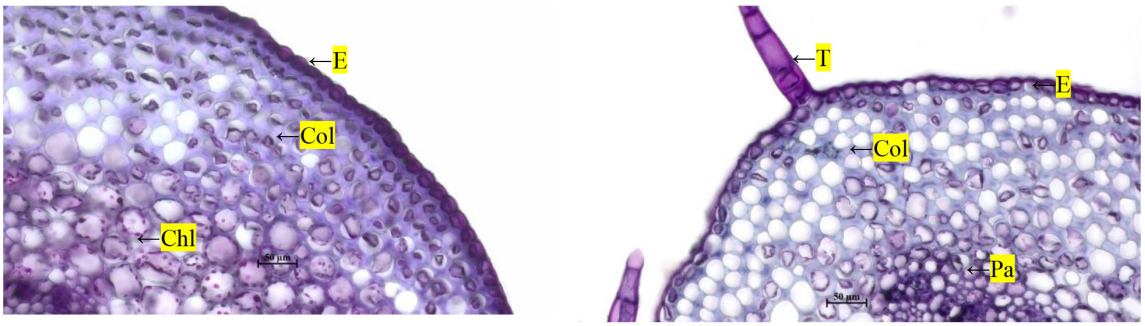
The quantitative parameters obtained during microscopic observation of epidermal peelings of leaves were recorded in Table 3. The leaves are



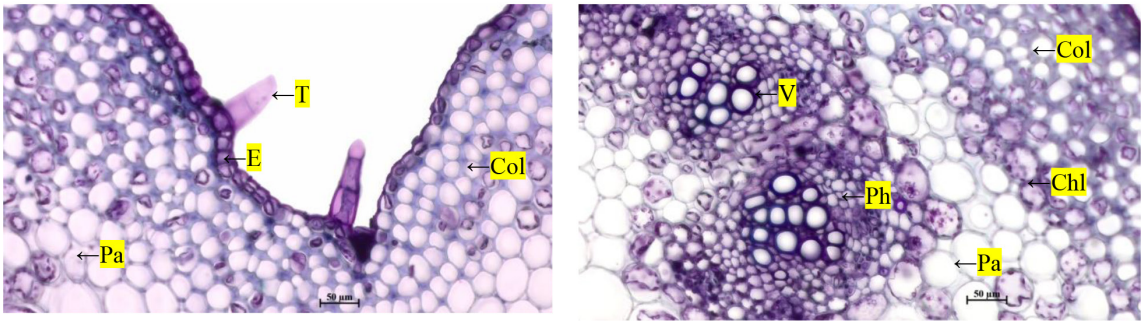
TS of petiole

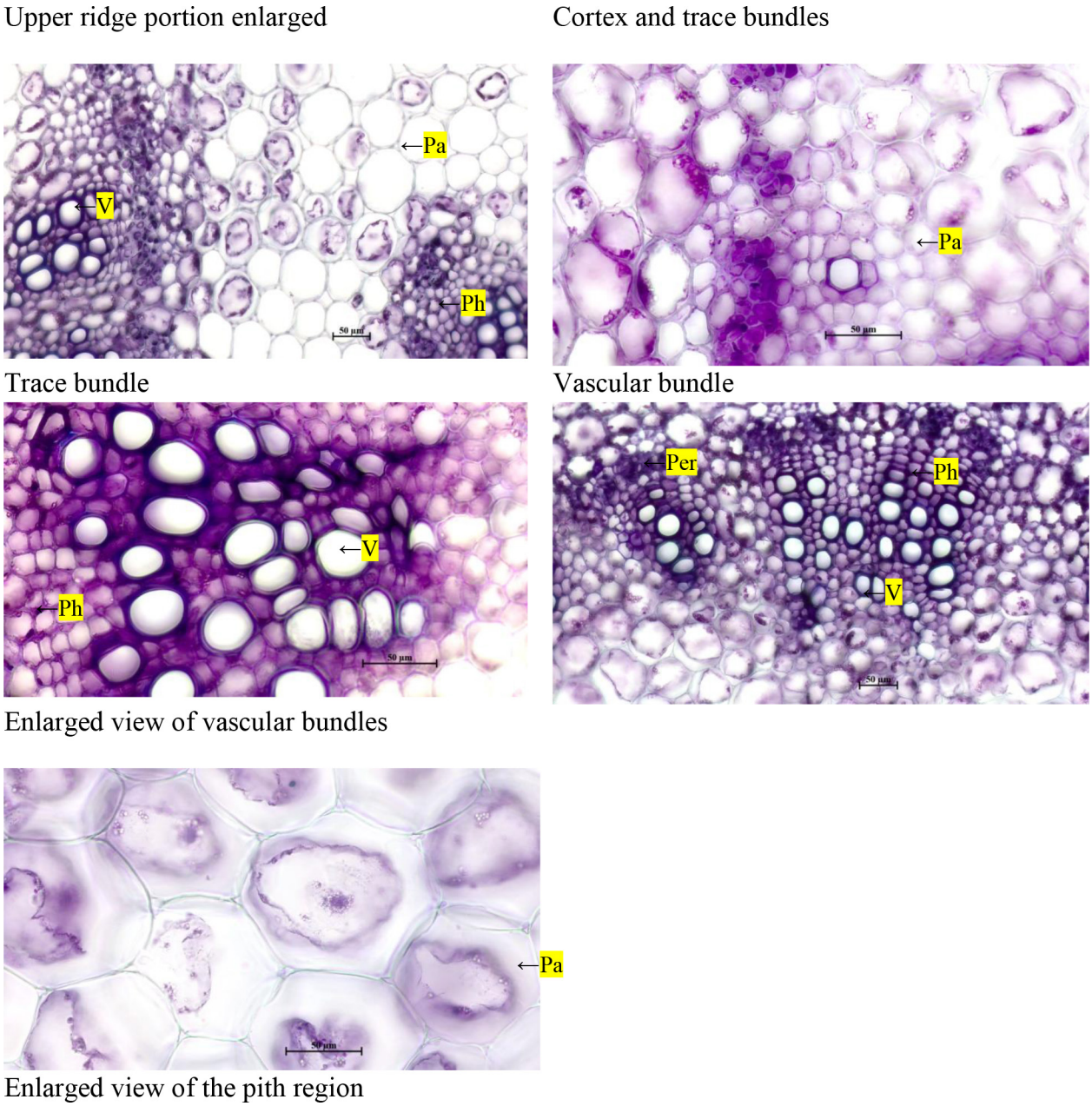


Wing region enlarged



Outer region enlarged





Chl - chlorenchyma; **Col** - collenchyma; **Ct** - cortex; **E** - epidermis; **Hy** - hypodermis; **Pa** - parenchyma; **PCr** - prismatic crystal; **Per** -pericycle; **Ph** - phloem; **Pi** - pith; **T** - trichome; **TB** - trace bundle; **V** - vessel; **VB** - vascular bundle.

Figure 3. T.S of *Clerodendrum chinense* petiole.

Table 3. Quantitative microscopy of *Clerodendrum chinense*

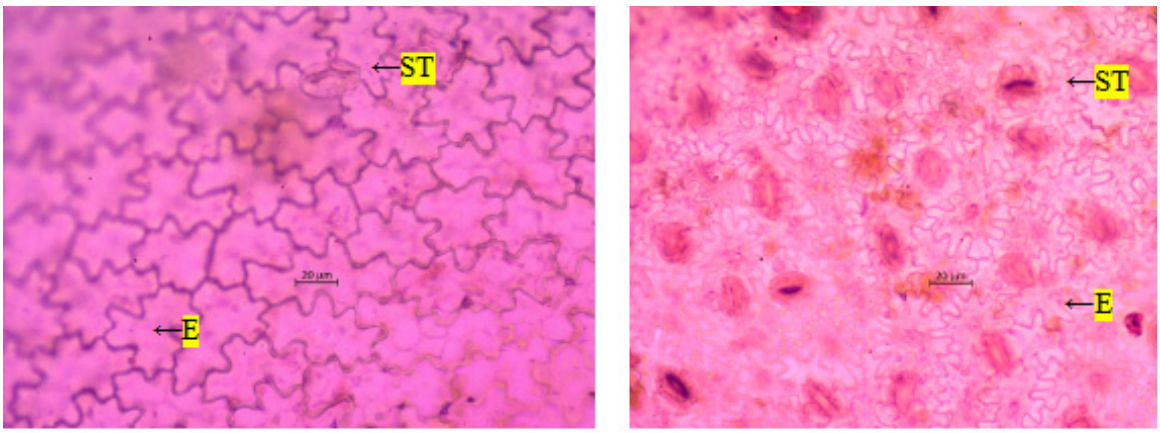
Parameters	Upper epidermis (/mm^2)	Lower epidermis(/mm^2)
Epidermal number	350 - 365	390 - 410
Stomatal Number	2 - 5	160 - 175
Stomatal Index	1	29 - 30
Palisade ratio	7 - 10	
Vein islets	10 - 12	
Vein termination	18 - 20	

amphistomatic and showed anomocytic and diacytic stomata; a comparatively very small number of stomata was observed in the upper epidermis (Figure 4).

3.6 Powder Microscopy

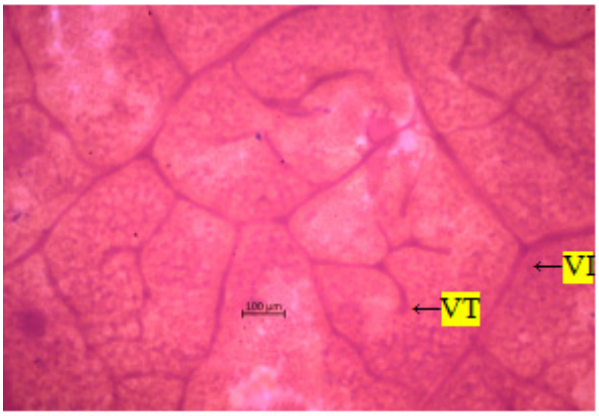
3.6.1 Leaf

The powder is yellowish green in colour with no characteristic odor with slightly bitter taste and



Upper epidermis

Lower epidermis



Vein islet and termination

ST- Stomata; **E**- Epidermal cells; **VI**- Vein Islet; **VT**-Vein Termination

Figure 4. Quantitative microscopy of *Clerodendrum chinense* leaf.

shows fragment of epidermis with simple covering multicellular trichomes and stomata, upper epidermis in surface view, petiole epidermis, fragment of mesophyll tissue, thin walled fibre with wide lumen, thick walled fibre with narrow lumen, vessels with spiral and annular thickenings, and prismatic crystals (Figure 5).

3.7 Histochemistry

3.7.1 Leaf

The histochemical study showed the presence of cutin and oil in epidermis, tannin depositions in hypodermis and vascular region, cortex and pith, lignin in xylem, mucilage in hypodermis and starch grains in pith parenchyma (Figure 6).

3.7.2 Petiole

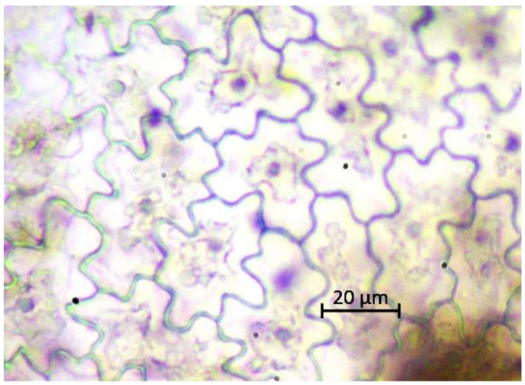
The histochemical study showed the presence of cutin in epidermis, tannin deposition in hypodermis, cortex and phloem, mucilage in hypodermal and cortical region, lignin in xylem, oil globules in cortex; starch was absent (Figure 7).

3.8 SEM and EDS Analysis

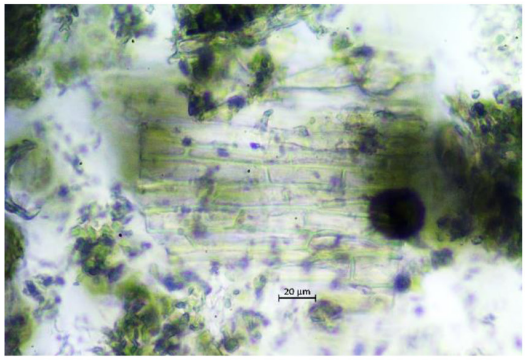
SEM showed the multicellular covering trichomes and some glandular trichomes on the midrib, and the veins show prominent multicellular covering trichomes. The lamina of the leaf has few covering and glandular trichomes. The stomatal type is amphistomatic (anomocytic and diacytic). The petiole



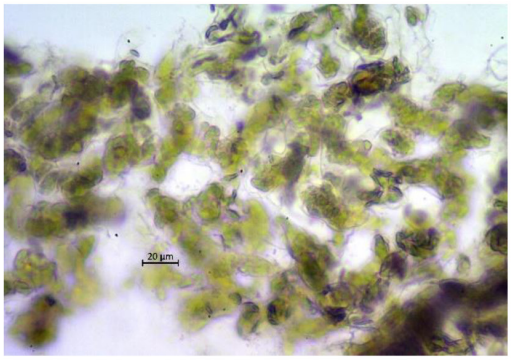
Epidermis with stomata and trichomes



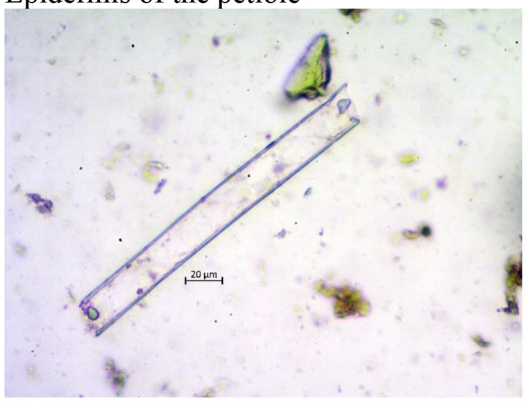
Surface view of upper epidermis



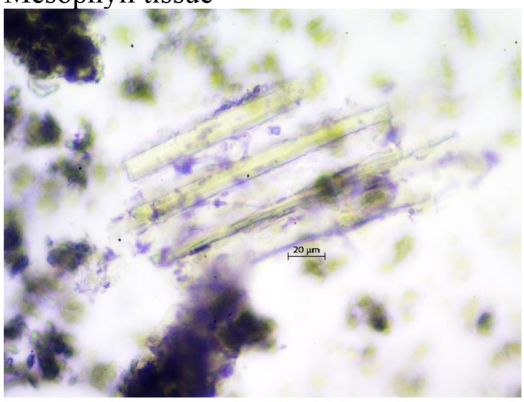
Epidermis of the petiole



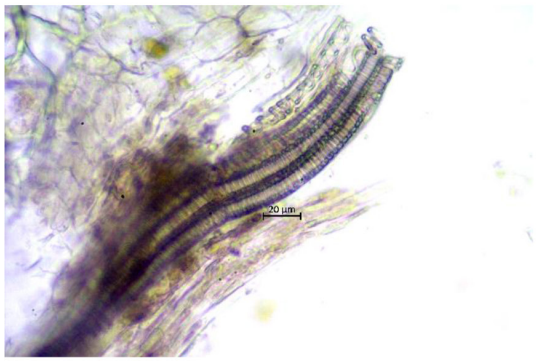
Mesophyll tissue



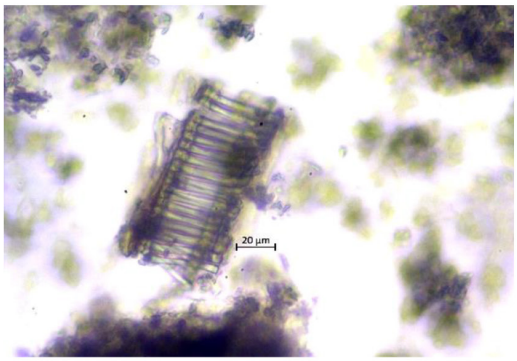
Thin-walled fibre with wide lumen



Thick-walled fibre with narrow lumen



Spiral vessels



Annular vessel

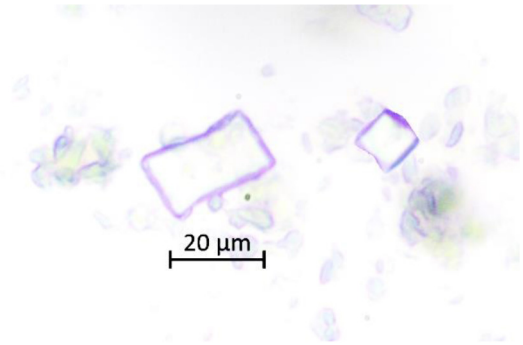


Figure 5. Powder microscopy of *Clerodendrum chinense* leaf.

shows numerous covering trichomes (Figures 8, 9 and 10).

In EDS analysis apart from carbon and oxygen the leaf of *C. chinensis* contains inorganic elements like Mg, Si, Cl, K, Ca, Al, S, were present in which the Chlorine occupies the maximum weight percent (Table 4a, Figure 11) thus the leaves can be an effective tool to fight against infections, to maintain the pH of the body fluids and blood pressure. On the otherhand the petiole contains the same elements, but the potassium content is more than chloride (Table 4b, Figure 12). Hence, the petioles can be used in the correction of imbalance of acid-base metabolism in humans and also may be useful in the maintenance of electrical excitability of nerves and muscles[31].

4. Discussion

The present investigation provides comprehensive pharmacognostic, microscopic, histochemical, and elemental data essential for the authentication and standardisation of *Clerodendrum chinensis*. The ethnobotanical profile corroborates the traditional use of various plant parts for ailments such as headache, inflammation, hypertension, cancer, and infectious diseases, which can be attributed to the presence of bioactive phytoconstituents, including iridoid glycosides, flavonoids, phenylpropanoids, and terpenoids. Macroscopic and organoleptic characters such as cordate leaves, bitter taste, and indistinct odour serve as preliminary diagnostic features. Detailed microscopic studies revealed

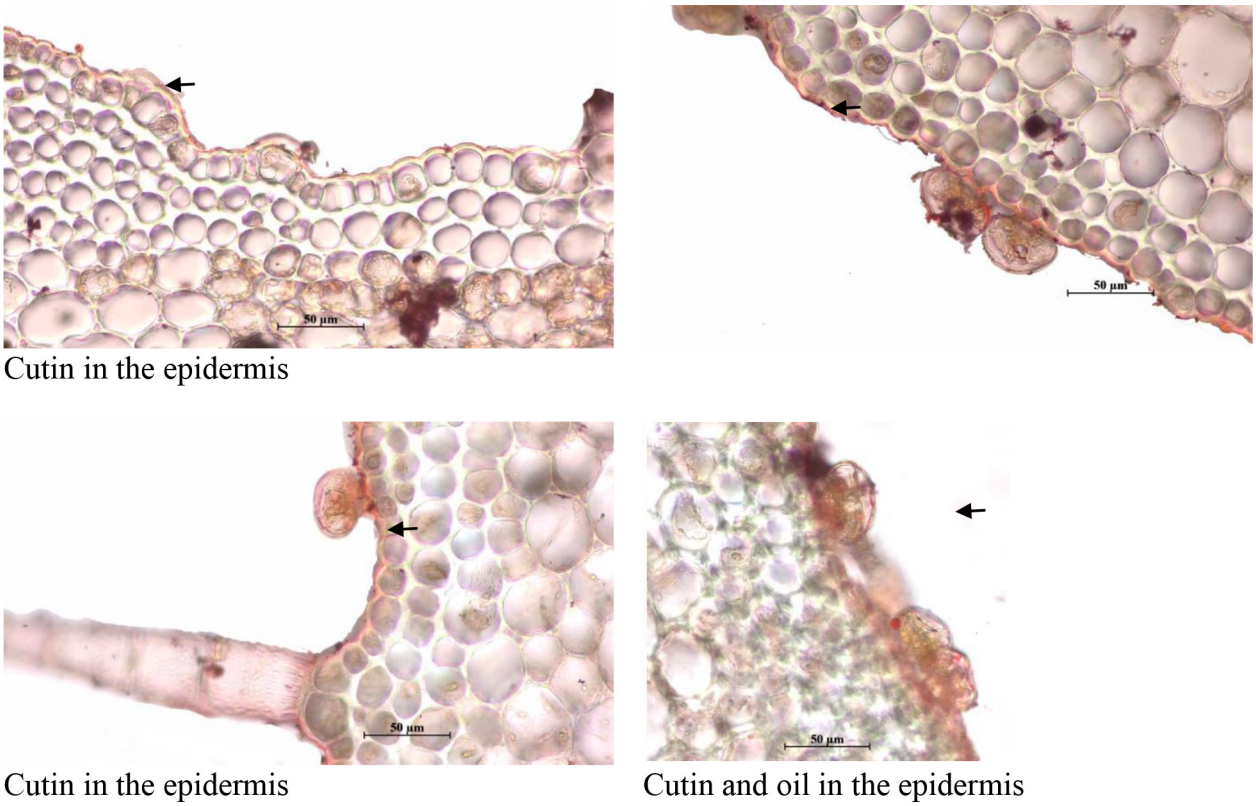
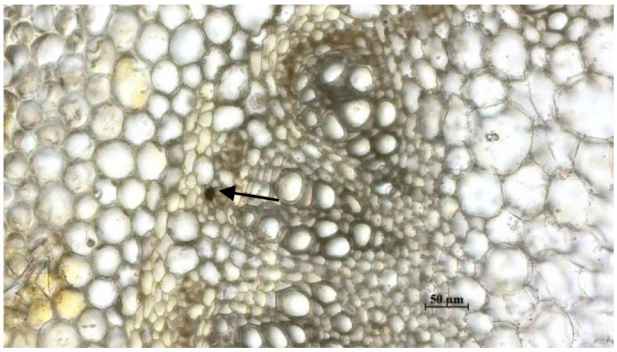


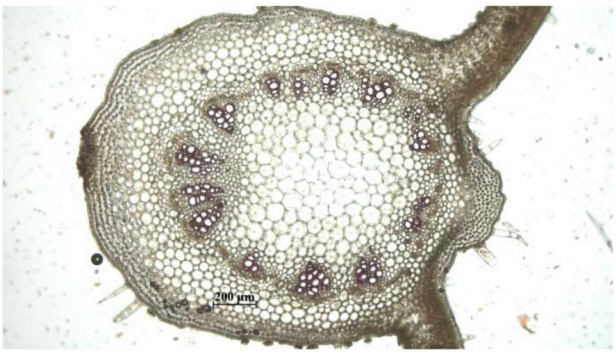
Figure 6. Histochemistry of *Clerodendrum chinense* leaf.



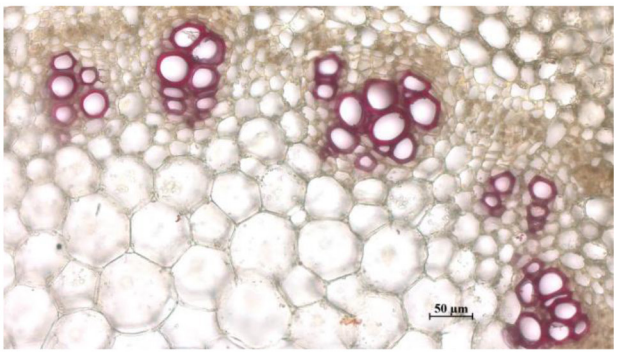
Tannin in the hypodermis

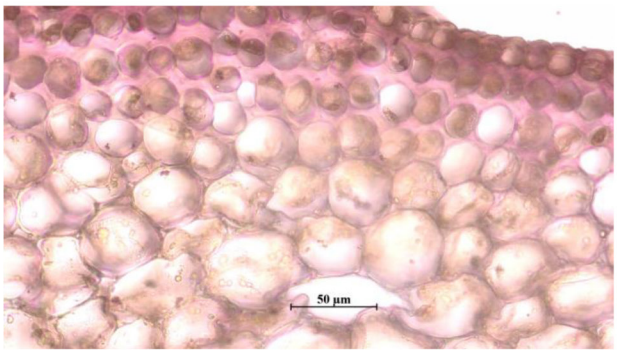


Tannin in the vascular region

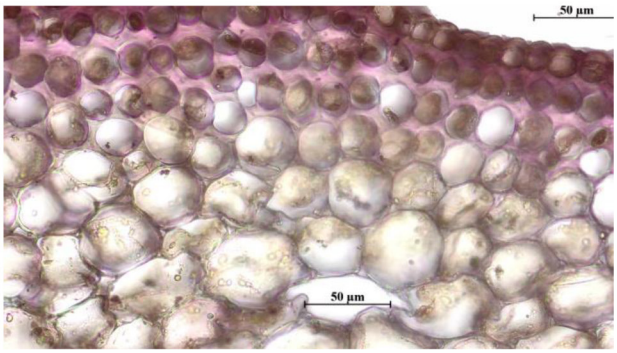


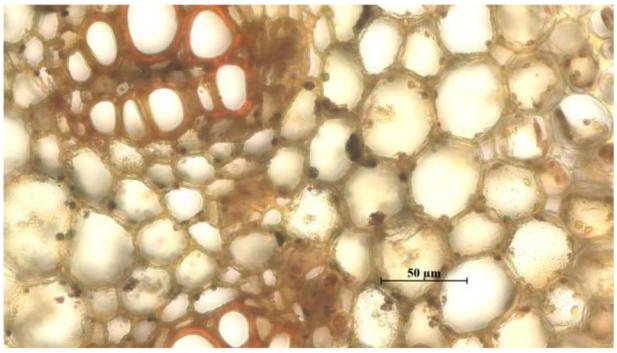
Lignin in xylem





Mucilage in the hypodermis





Starch grains in pith parenchyma

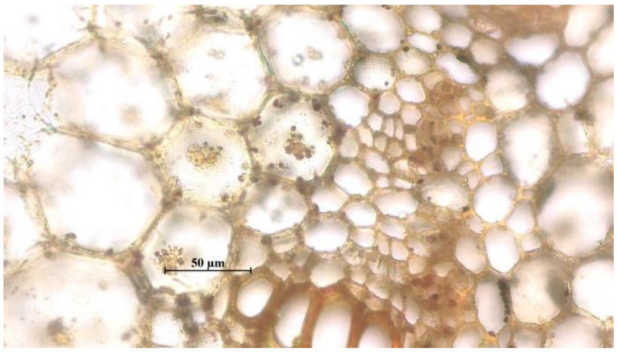


Figure 6. Continued...

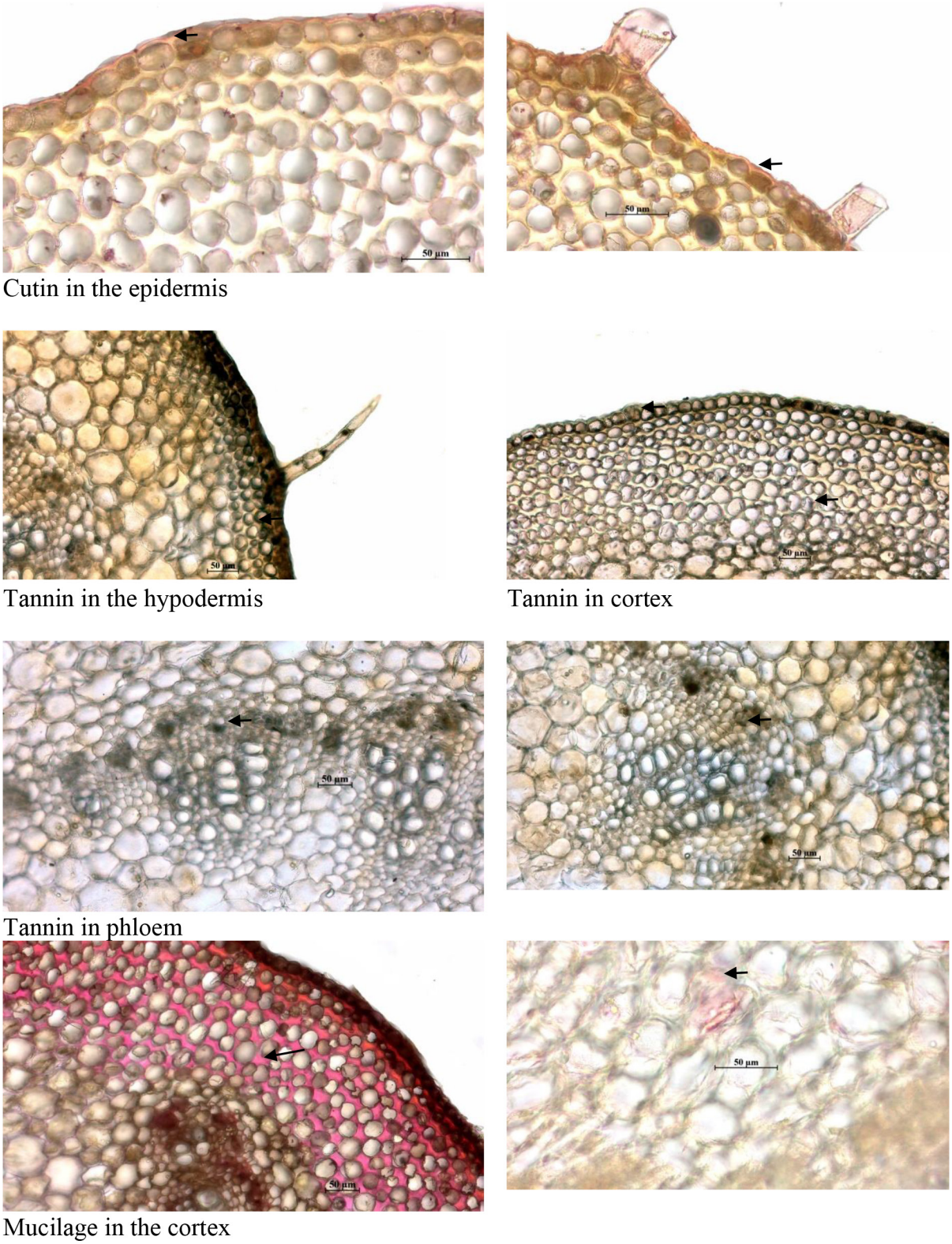
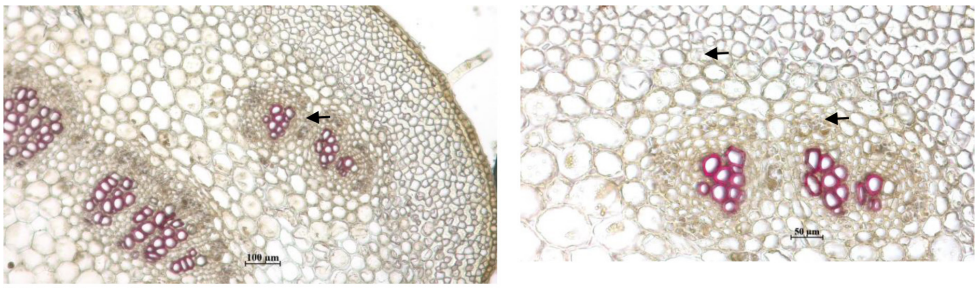
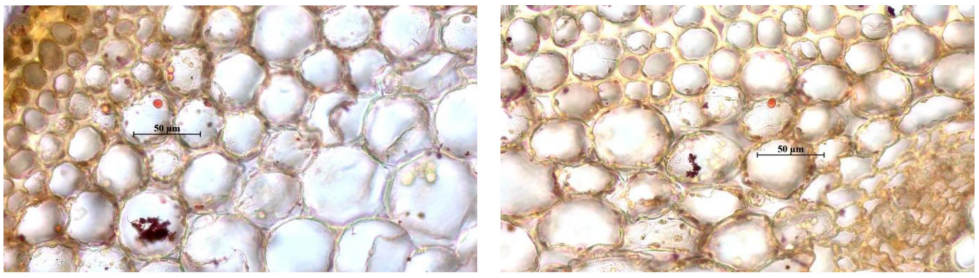


Figure 7. Histochemistry of *Clerodendrum chinense* petiole.



Lignin in xylem



Oil globules in the cortex

Figure 7. Continued...

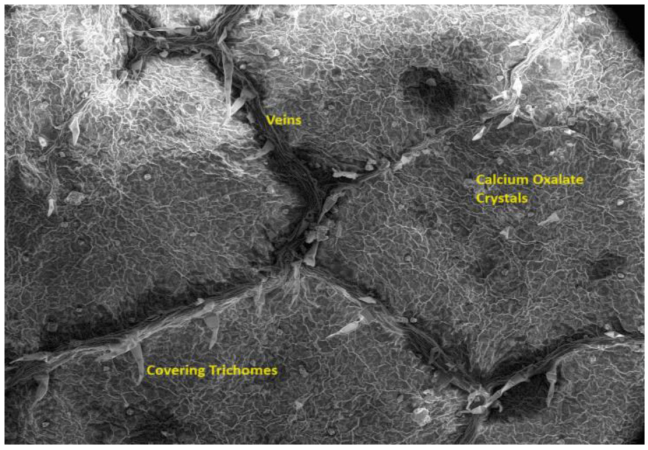


Figure 8. *C. chinensis* Leaf Lamina.

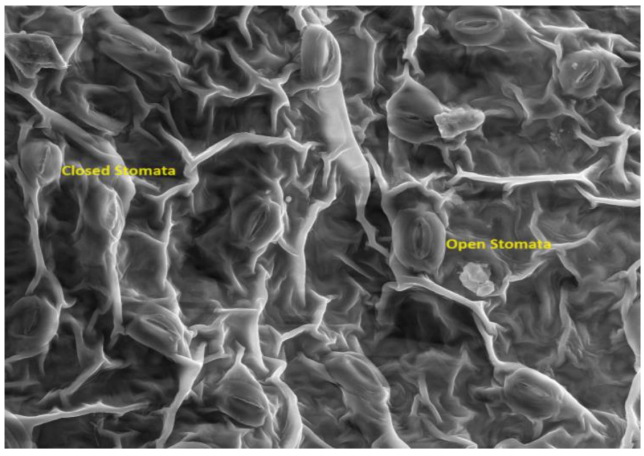


Figure 9. Stomata of *C. chinensis* leaf.

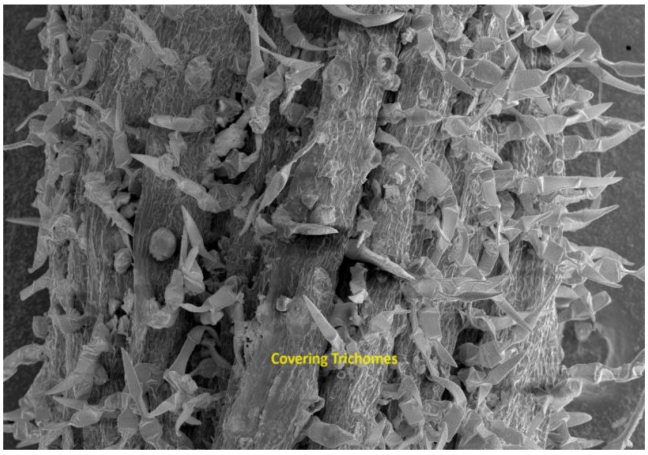


Figure 10. Petiole of *C. chinensis.*

Table 4a. Quantitative Results of *C. chinensis* Leaf

Element	Net Counts	Weight %	Atom %	Atom % Error	Formula
C	15258	34.08	43.44	± 0.34	C
O	12797	53.19	50.91	± 0.47	O
Mg	1503	1.35	0.85	± 0.04	Mg
Al	220	0.15	0.09	± 0.02	Al
Si	768	0.47	0.26	± 0.02	Si
P	563	0.31	0.15	± 0.01	P
S	1159	0.61	0.29	± 0.03	S
Cl	7628	4.81	2.08	± 0.04	Cl
K	3168	2.36	0.92	± 0.02	K
Ca	3248	2.67	1.02	± 0.02	Ca
Total		100.00	100.00		

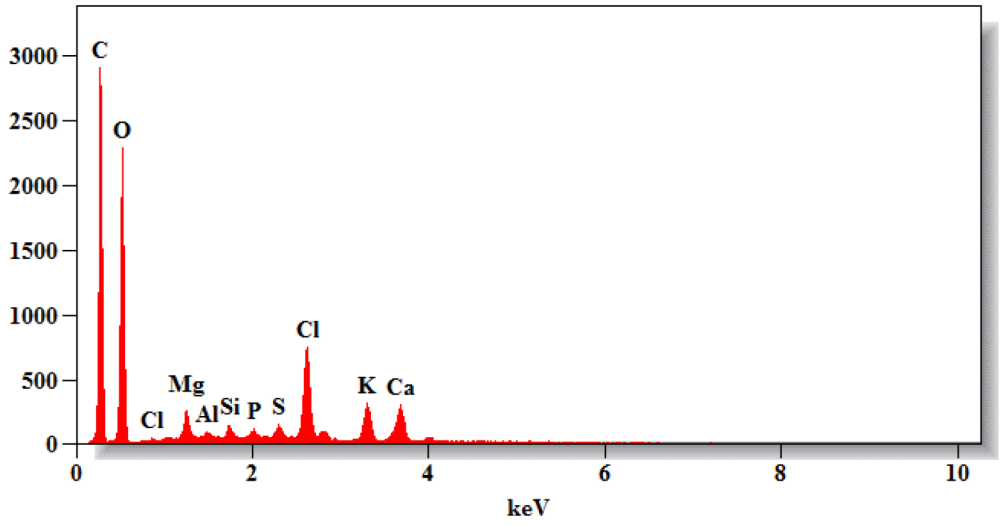


Figure 11. EDS of *C. chinensis* leaf.

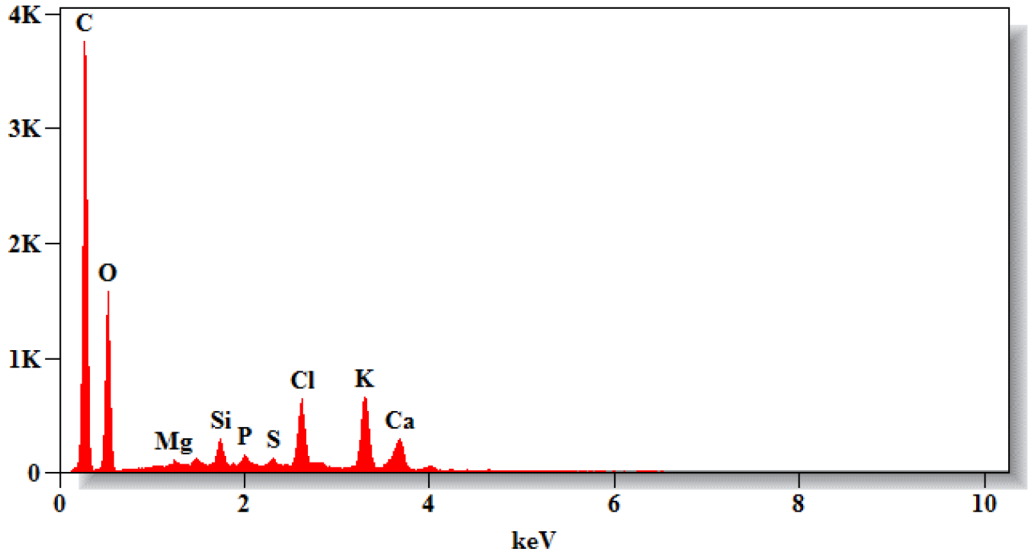


Figure 12. EDS of *C. chinensis* petiole.

Table 4b. Quantitative Results for *C. chinensis* petiole

Element	Net Counts	Weight %	Atom %	Atom % Error	Formula
C	23418	41.34	51.82	± 0.37	C
O	10050	45.21	42.55	± 0.50	O
Mg	400	0.31	0.19	± 0.02	Mg
Si	2207	1.19	0.64	± 0.02	Si
P	993	0.49	0.24	± 0.01	P
S	738	0.35	0.16	± 0.01	S
Cl	6386	3.61	1.53	± 0.02	Cl
K	7451	4.97	1.92	± 0.04	K
Ca	3363	2.53	0.95	± 0.04	Ca
Total		100.00	100.00		

characteristic dicot anatomy, amphistomatic leaves with anomocytic and diacytic stomata, abundant multicellular covering trichomes, and distinct vascular bundle arrangements, which are valuable markers for identification. Powder microscopy further supports drug authentication through diagnostic cellular elements. Histochemical localisation of cutin, tannins, lignin, mucilage, oils, and starch indicates structural integrity and therapeutic potential. SEM observations confirmed surface micromorphology, while EDS analysis revealed essential inorganic elements, suggesting a possible role in physiological regulation and antimicrobial defence.

5. Conclusion

This study offers some fresh insights. Chinese locals have long used *C. chinensis* to cure a range of illnesses. A pharmacognostic study is the initial step to confirm the identity and to assess the quality and purity of the crude drug. Quality control of crude drugs is a very challenging task because of the complex nature of chemical constituents. Microscopical evaluation is the simplest and most reliable tool for the correct identification of herbs as well as small fragments of crude drugs or powdered drugs and detection of adulterants and substituents[32]. Based on its pharmacognostic analysis, we confirm that this is the first thorough investigation from India. The current study was conducted to identify the parameters that could be useful in determining the validity of this medicinally robust plant, as no prior research has been done on it. Cultivation of *C. chinensis* should be prioritised in the nation to address the need for pharmaceuticals as well as local needs, given the country's projected growth in demand. Since it is a very significant plant in terms of medicine, valuable information should be provided to the various pharmaceutical companies so that they can effectively prepare for the treatment of various illnesses.

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Author Contribution

M. Mubeen conceived and designed the project. Dr. S. Prema drafted and made substantial improvements on the manuscript. Dr. S. Sathik Basha reviewed and made the modifications on the manuscript draft. All the authors have read and approved the final manuscript.