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Morphoanatomical standardization and elemental analysis of Clerodendrum chinense (Osbeck) Mabberley (India)

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Abstract:
Clerodendrum chinense (Osbeck) Mabberley have many uses in Indian and Chinese ethnomedicine. In this study we aim to establish the pharmacognostic profile of C.chinensis leaves to assist in the standardization for quality, purity and for the identification of sample. The fresh and dried leaves were evaluated to determine the macro and micromorphological characters using light and Scanning Electron Microscope (SEM) The leaves were also subjected to elemental analysis by an Energy Dispersive X-ray Spectrometer (EDS or EDAX).Plant anatomy illustrated dicot histology. The leaves under LM are amphistomatic (anisocytic and diacytic), according to stomatal analysis .Several simpler multicellular covering trichomes and some glandular trichomes are found on the TS of midrib and TS of lamina shows dorsiventral leaf with few covering and glandular trichomes whereas numerous simple, multicellular covering trichomes were found in TS of petiole which was also found in SEM. The powdered drug showed the presence of several trichomes, stomata, fragment of mesophyll tissue, thin walled fibre with wide lumen and narrow lumen, vessels with spiral and annular thickenings, and prismatic crystals. The histochemical study of leaf and petiole showed the presence of cutin in epidermis, tannin deposition in hypodermis and lignin in xylem.The elemental analysis using EDS showed the presence of higher chlorine content but petiole contains more of potassium than chlorine. Various pharmacognostic characters observed in this study helps in botanical identification and standardization of Clerodendrum chinense (Osbeck) Mabberley in crude form.

Keywords: Ethnobotany, Clerodendrum chinensis, Histochemistry, Morphology, Elemental analysis

Introduction

Poultry Over a long period of time, there has been a sharp rise in the use of natural therapies for illness management. This can be ascribed to the significant role that plants have played in offering a vast array of affordable yet effective therapeutic solutions [1] . The World Health Organization states that describing a medicinal plant's macroscopic and microscopic characteristics is the first stage 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 throughout the world, and as a result, quality control, safety, and effectiveness of herbal medicines have taken on paramount importance [2]. Herbal markets offer therapeutic herbs in powdered or raw form, as well as extracts and fragments. The principal causes of adulteration in medicinal plants that will determine the requirements for quality control are differences and nomenclature systems in various geographical regions of the world. As the herbal industry works to develop new medicines, the two biggest problems it faces are the absence of regulations for medicinal plants and people's unscrupulous dealings. Herbal plant botanical descriptions are especially helpful for academic and pharmaceutical research to uncover new goods, as well as traditional practices [3].The future of medicinal plants is bright, as there are around 500,000 plants in the world, many of which have unexplored pharmacological properties that could significantly impact ongoing or upcoming research [4]. The present study focus to create an awareness about the plant *Clerodendrum chinense (Osbeck) Mabberley* (Family: Lamiaceae) popularly known as ‘Fragrant glory bower’ in English and ‘Madras malli’ in Tamil which is found abundantly in India is known for its wild growth, vegetative spread and as an ornamental plant but not much utilised for its therapeutic purpose [5,6].

Material and Methods

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.

Collection and Preparation for pharmacognostic studies
Healthy and disease-free plants of *Clerodendrum chinense* (Osbeck) Mabberley were collected from Kancheepuram district of TamilNadu. The collected specimens were identified and deposited in 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.

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

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

Microscopic Evaluation
The sample was kept for longer than 48 hours in fixative FAA. Using a sharp knife, the preserved specimens were divided into thin transverse sections, which were then dyed with toluidine blue [9,10]. Under bright field light, transverse sections were captured on camera using an Axiolab5 trinocular microscope coupled to a Zeiss Axiocam208 color digital camera. A scale bar was used to show magnifications.

Powder microscopy
A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains [11,12]. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.

Quantitative microscopy
Rectangular cut leaf pieces were boiled with saturated chloral hydrate solution until colourless and slides prepared for vein islets, vein termination. In order to prevent desiccation and facilitate the procurement of epidermis, new leaves were submerged in water for stomatal examination. Using a razor, the abaxial and adaxial surfaces were separated and put on a glass slide. They were then mounted in fluid Canada balsam and inspected under a microscope [13,14]. Using a light microscope (Labomed Lx 400) Model, the existence and absence of stomata on each epidermis, as well as their kind, distribution, and position in relation to other stomata and epidermal cells, as well as their stomatal index, were examined. The micrometer was used to perform micrometery.

Histochemical tests
Plant sections were treated using the following standard procedures:
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 conc. HCl, wherein dissolution of crystal and formation of needles of Calcium sulphate indicated the presence of calcium oxalate crystals
2. Fats, Fatty oils volatile oils and resins: About1 to 2 drops of Sudan-IV was added to the section and allowed to stand for a few minutes. Presence of fatty oil substances were indicated by orange red/pink/red colored globules, while red coloured irregular contents indicated resin.
3. Starch: A drop of 2% iodine water solution was added - blue colour indicated starch.
4. Tannin: A drop of alcoholic ferric chloride was added - bluish black coloured contents indicated tannin.
5. Mucilage: A drop of ruthenium red was added - pink to red colored contents indicated mucilage.

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 presence of lignin.
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 cell wall.
8. Alkaloids: A drop of Wagner's reagent was added - the presence of yellow to reddish brown colored contents confirmed alkaloids [15,16,17].

SEM and EDS Analysis
The dried leaves for SEM analysis of (1x1 mm) were mounted on the specimen stub and samples were analysed by Hi Resolution Scanning Electron Microscope(HRSEM) Thermoscientific Apreo S with a quantitative elemental mapping EDS attachment. When the leaf and petiole was bombarded by the electron beam of the SEM, electrons were ejected from the atoms on the specimens surface and microanalysis was carried out. The EDS consist of X-ray detector, a pulse processor and multiple channel analyser. The EDS Xray detector measures the number of X - rays emitted versus their energy. A spectrum of the energy versus relative counts of the detected X-rays is obtained and evaluated for the determinations of the elements [18,19].

Results

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]
5,4'-dihydroxy kaempferol-7-O-β rutinoside	Leaf	Anti hypertension, Furunculosis [25].
leucoseceptoside A	leaf	Anti hypertension [26].
Verbascoside	leaf	Colorectal cancer [27].
24β-methylcholesta- 5, 22E,25-trien-3β-ol	Whole plant	Antirheumatic [24].
Trans-β-elemenone	Leaves	Insecticidal activity against *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus* [28].
Eugenol	Leaves and Flowers	Toothache [29]

Macroscopy
Fresh leaves are dark green coloured, simple, opposite, broadly ovate to nearly cordate shaped, below velvet hairy especially on veins and with several large glands near base, margin sparsely irregularly toothed, tip acuminate; measuring 6 to 18 cm long and 5 to 20 cm wide; petiole 3 to 15 cm long; odour is characteristic and tastes bitter. Flowers are white to whitish pink, terminal, corymb like cyme inflorescence; calyx purple or red, campannulate, 5 lobed, measuring about 1 to 1.5 cm long; corolla pale pink, usually doubled by petaloid stamens; peduncle is short and woolly; bracts numerous, foliaceous and lance shaped, 1.5 to 3 cm long; odour is fragrant and tastes bitter (Fig. 1).

Fig. 1: Macroscopy of *Clerodendron chinensis*

Organoleptic Evaluation

The organoleptic characteristics of the petiole and leaf are listed in Table 2a, 2b. according to Carrillo-Galván et al the organoleptic properties of plants are crucial in establishing their medicinal status and are also utilized to characterize the alleviation of illness [30].

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

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

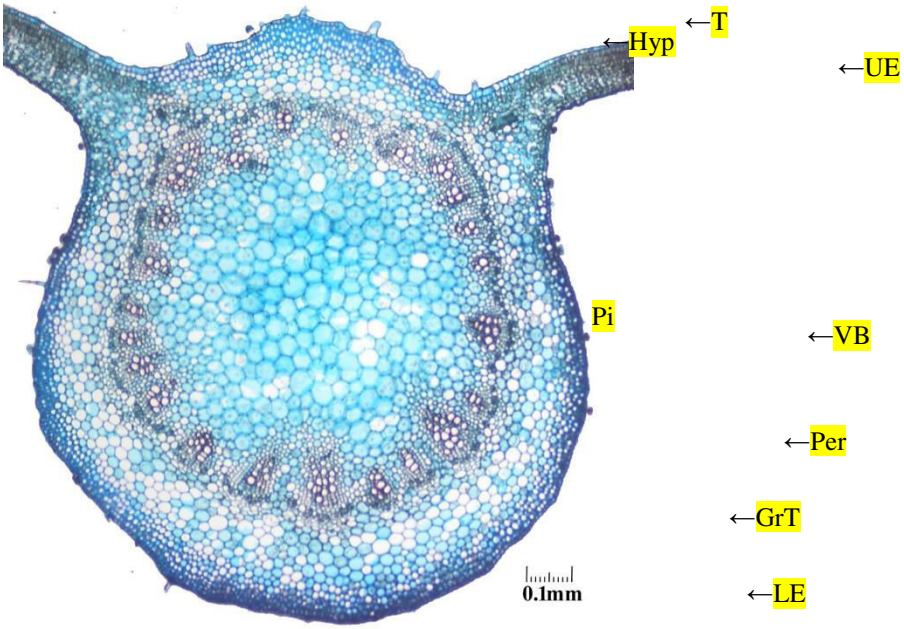
Parameter	Fresh	Dry
Color	Green	Brown
Size	Ave. Length= 15.0cm & Ave. width =15mm	Ave. Length= 15.5cm & Ave. width =1.0mm
Texture	Herbaceous	Herbaceous
Shape	Cylindrical	Cylindrical & shrunken
Odour	Indistinct	Indistinct
Taste	Slightly bitter	Slightly bitter

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

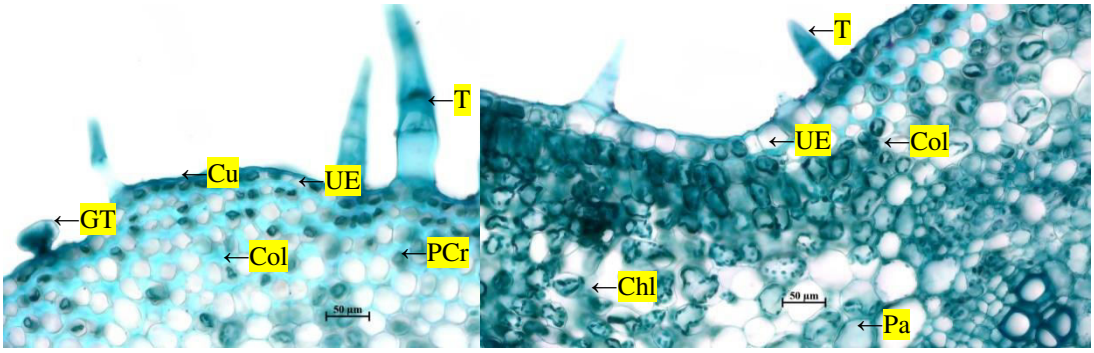
Microscopy
Leaf
TS of leaf shows upper slightly elevated and broadly convex lower midrib surface with lateral laminar extensions (Fig. 2).

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 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 (Fig. 2).

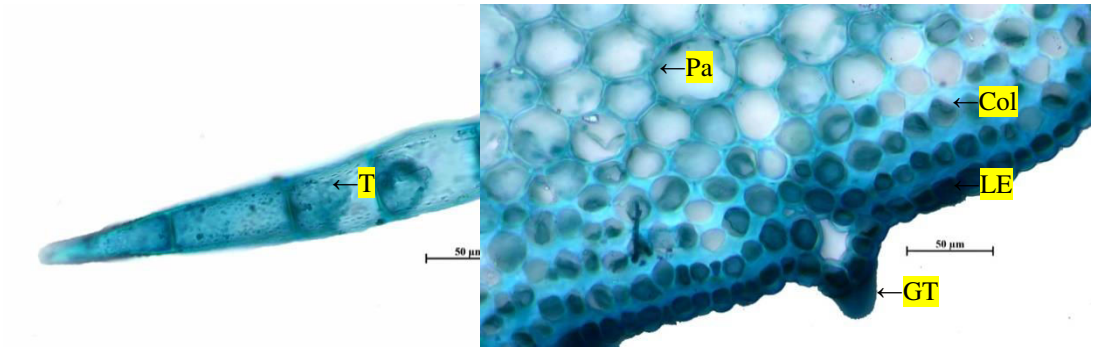
Lamina:
TS of lamina shows dorsiventral leaf with single layered upper and lower epidermis covered by thin cuticle and bears few covering and glandular trichomes; mesophyll tissue consists of upper double layered palisade cells followed by 6 to 7 rows of loosely arranged spongy parenchyma cells; veins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



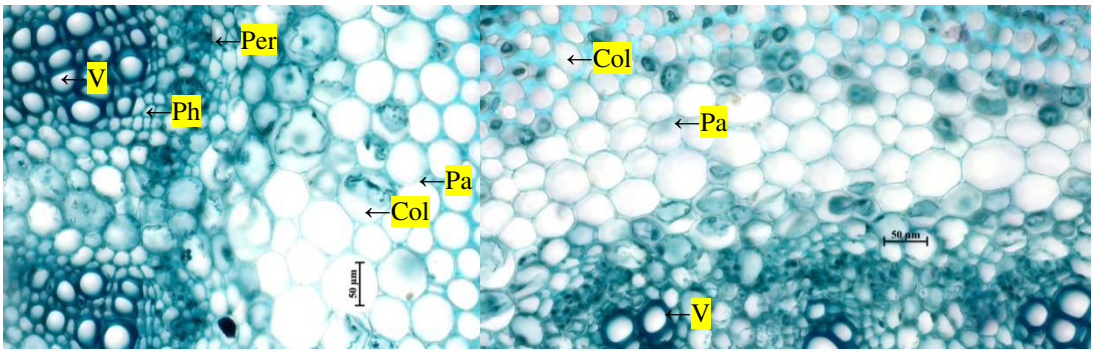
TS of lamina passing through midrib



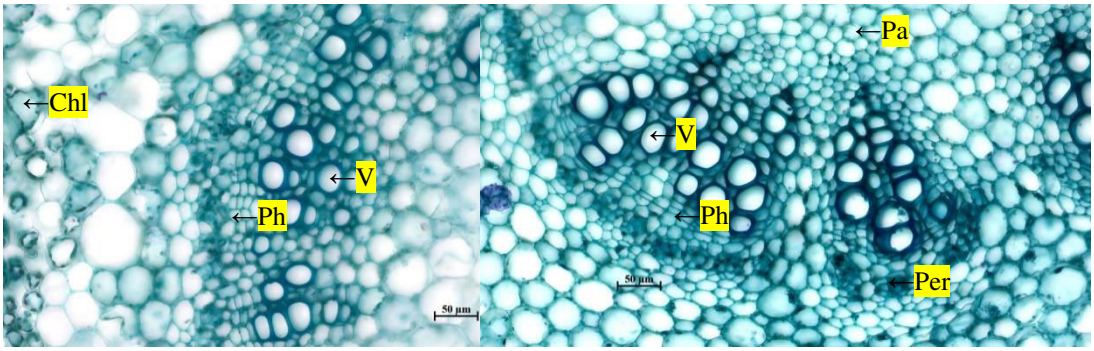
Midrib upper portion enlarged



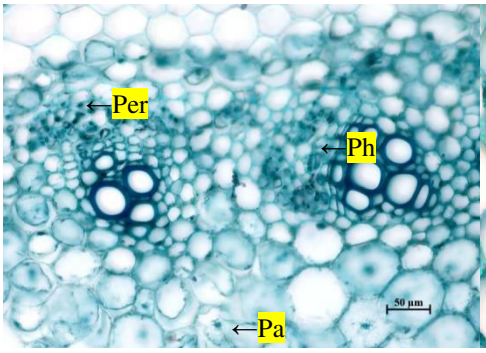
Single multicellular covering trichome Glandular trichome



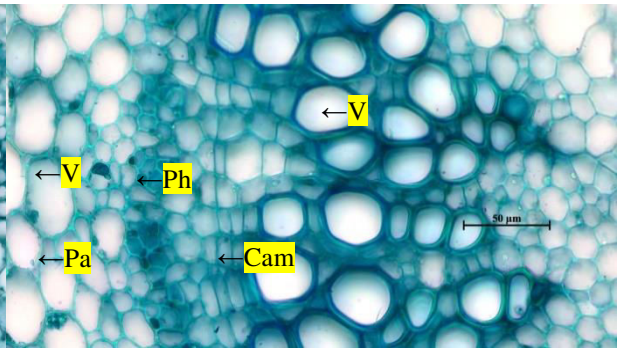
Cortex enlarged view



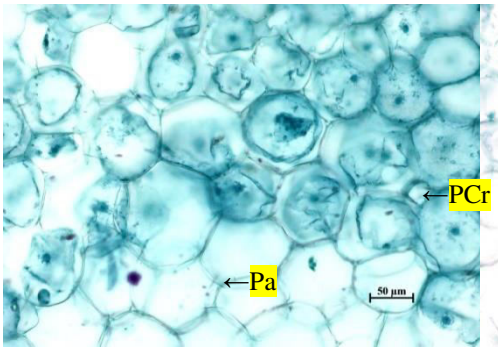
Enlarged vascular bundles

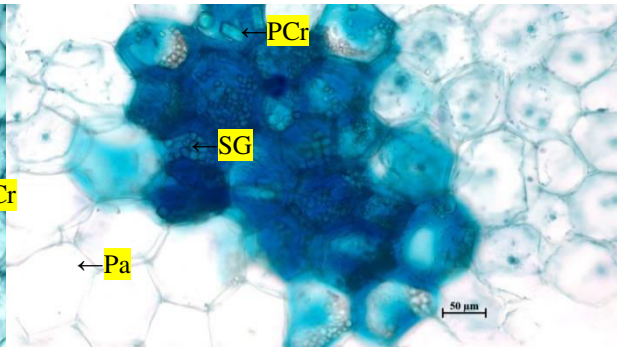


Vascular bundle

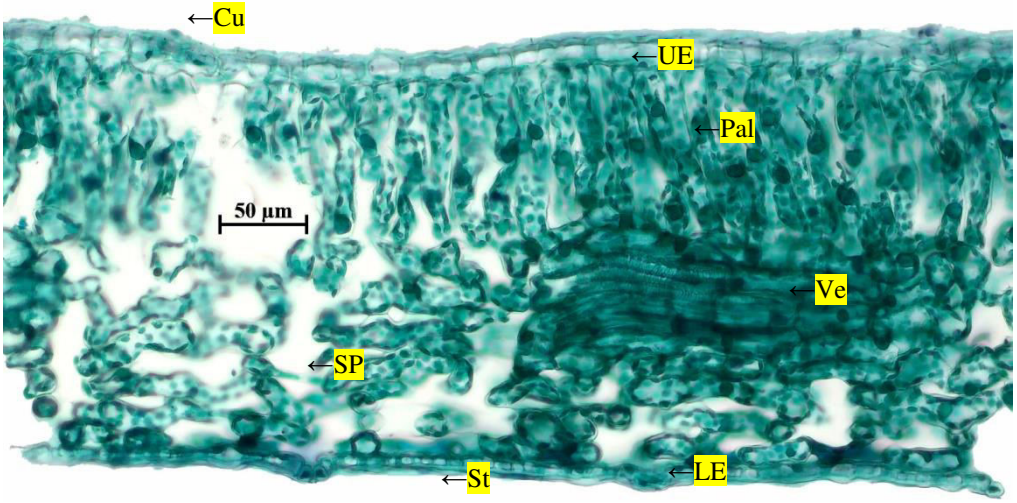


Xylem and phloem

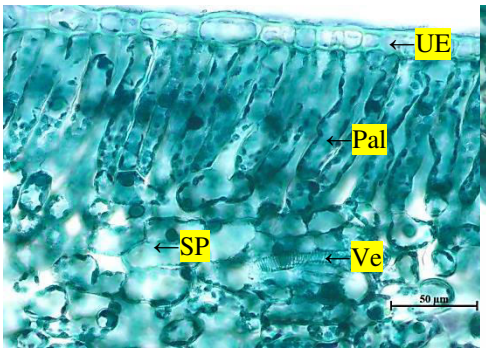


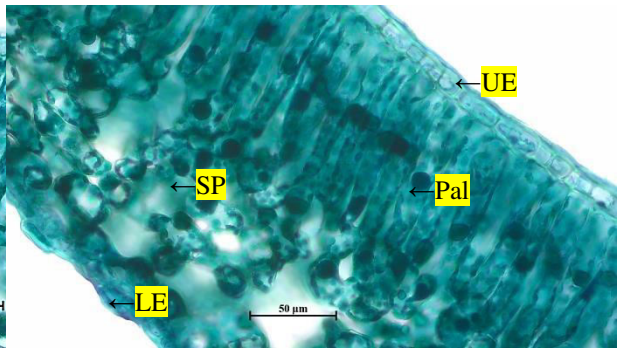


Enlarged view of pith

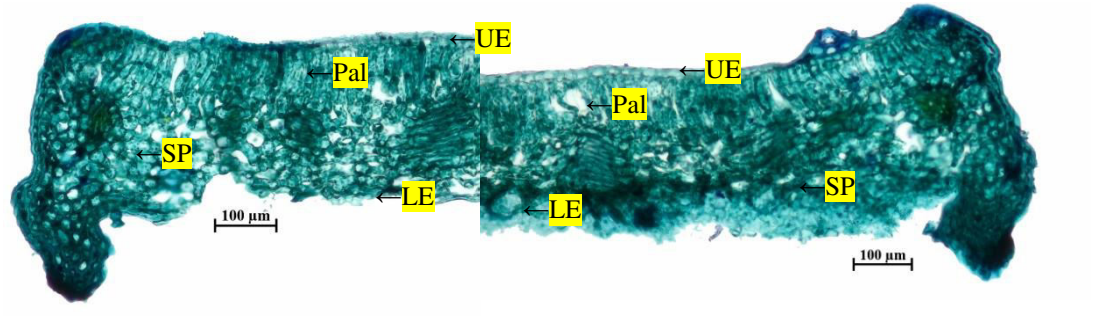


TS of lamina





Lamina enlarged



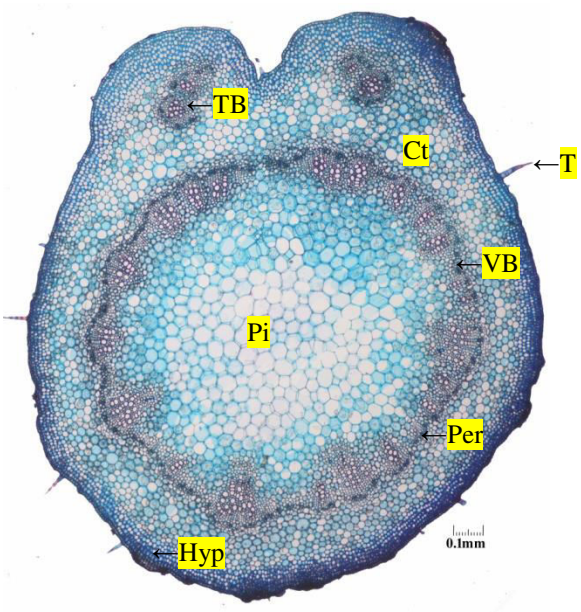
TS of leaf margin

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.

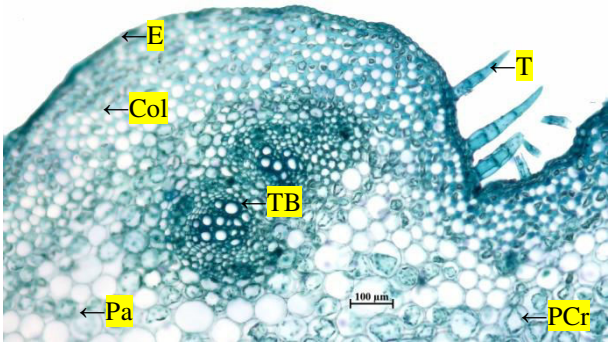
Fig. 2: T.S of *Clerodendrum chinense* leaf

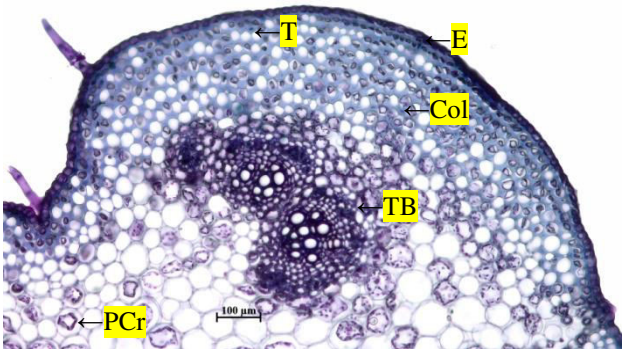
Petiole

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 (Fig. 3).

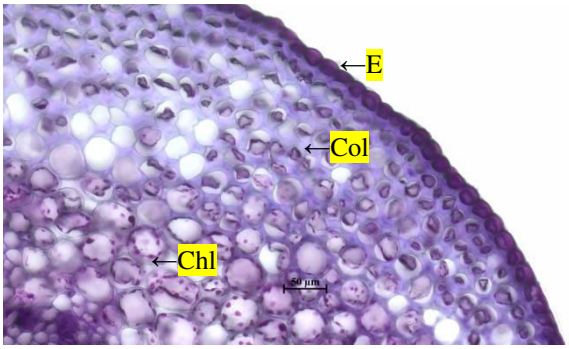


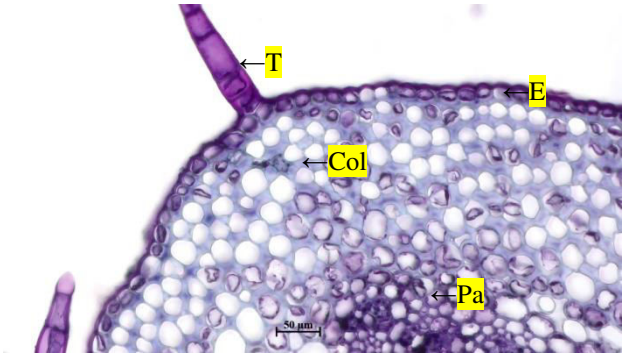
TS of petiole



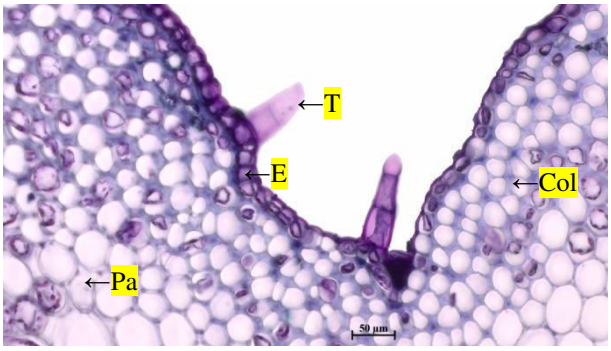


Wing region enlarged

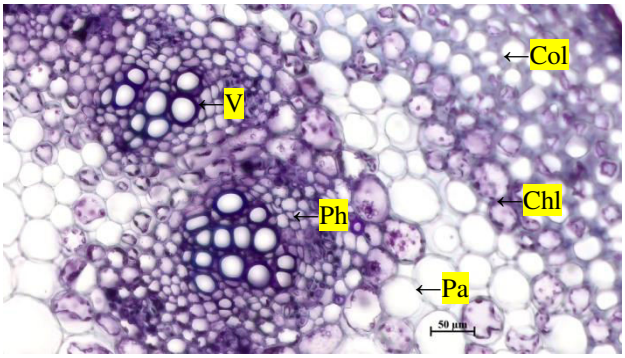




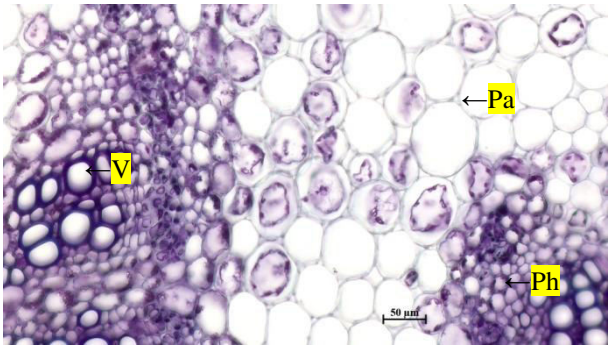
Outer region enlarged



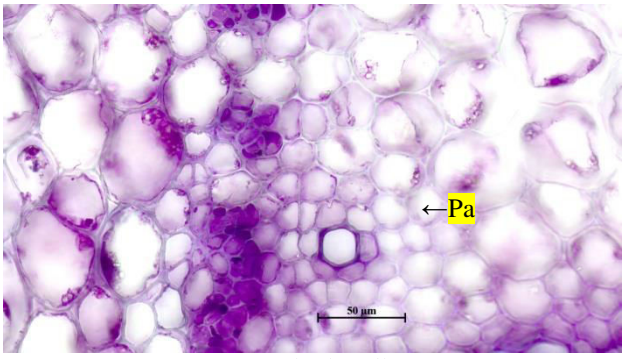
Upper ridge portion enlarged



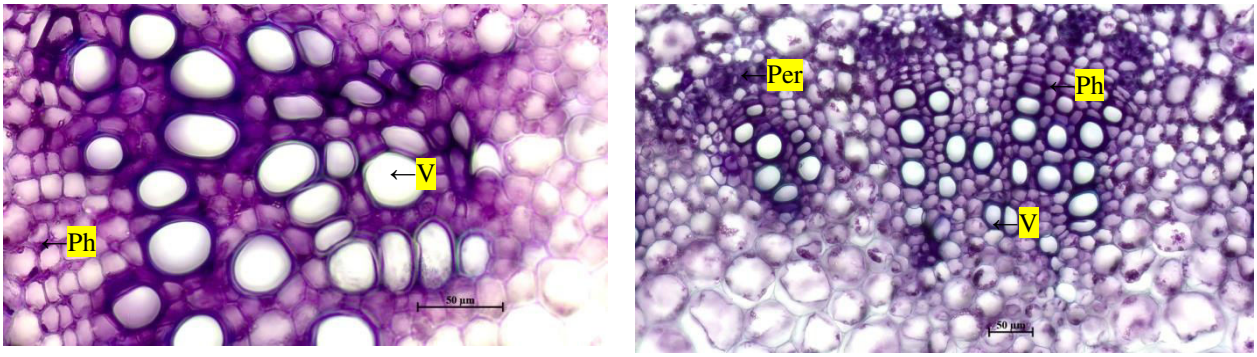
Cortex and trace bundles



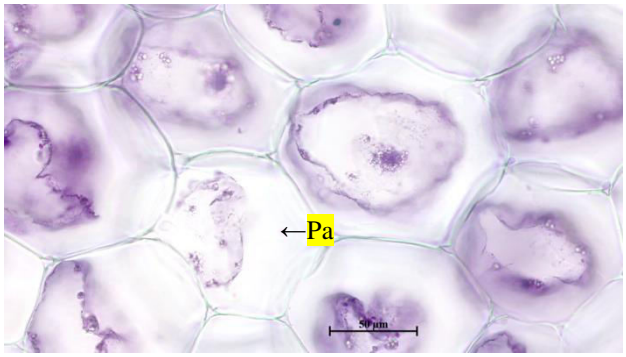
Trace bundle



Vascular bundle



Enlarged view of vascular bundles



Enlarged view of pith region

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.

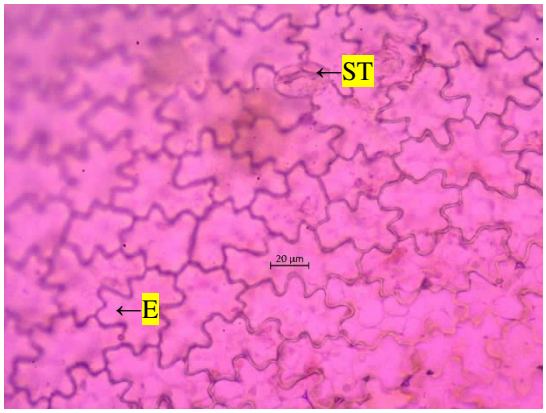
Fig. 3: T.S of *Clerodendrum chinense* petiole

Quantitative microscopy

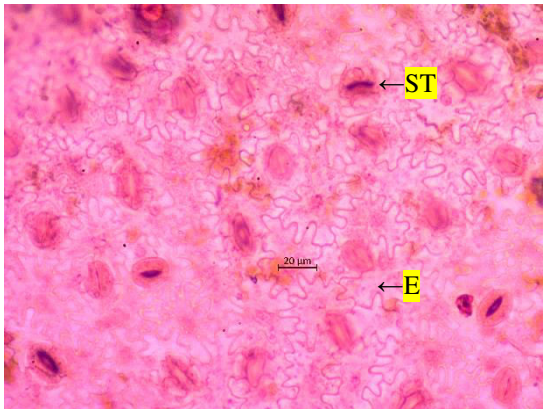
The quantitative parameters obtained during microscopic observation of epidermal peelings of leaves were recorded in Table 3. The leaves are amphistomatic and showed anomocytic and diacytic stomata; comparatively very less number of stomatal distribution was observed in upper epidermis (Fig. 4).

Table 3. Quantitative microscopy of *Clerodendrum chinense*

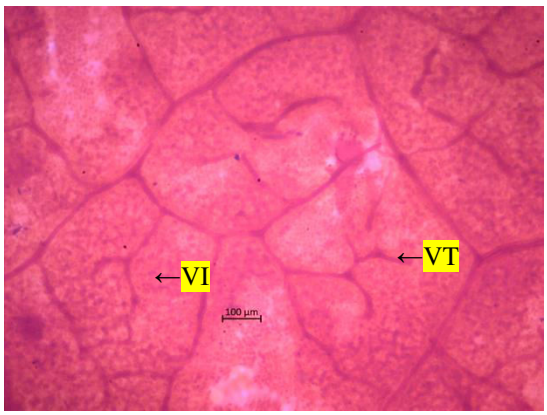
Parameters	**Upper epidermis (/mm^2)**	**Lower epidermis(/mm^2)**
Epidermal number	350 - 365	390 - 410
Stomatal Number	2 - 5	160 - 175
Stomatal Index	1	29 - 30
Palisade ratio	7 - 10	
Vein islets	10 - 12	
Vein termination	18 - 20	



Upper epidermis



Lower epidermis



Vein islet and termination

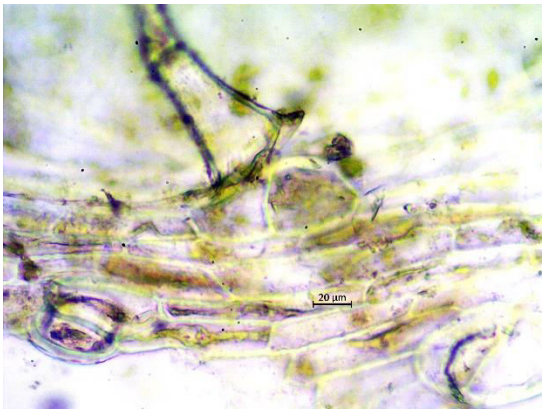
ST- Stomata; E- Epidermal cells; VI- Vein Islet; VT-Vein Termination

Fig. 4: Quantitative microscopy of *Clerodendrum chinense* leaf

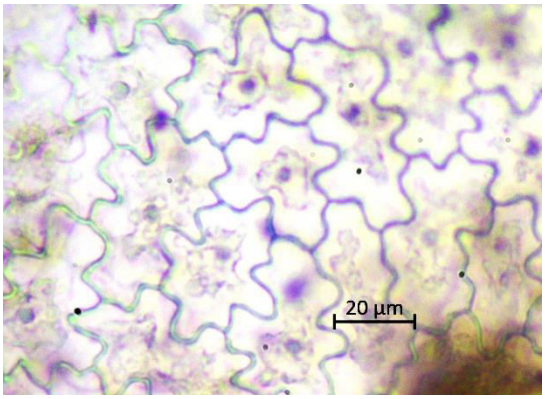
Powder microscopy

Leaf

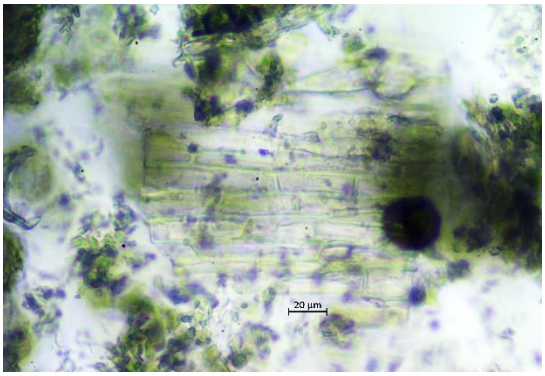
The powder is yellowish green in colour with no characteristic odor with slightly bitter taste and shows fragment of epidermis with simple covering multicellular trichomes and stomata, upper epidermis in surface view, petiole epidermis, fragment of mesophyll tissue, thin walled fibre with wide lumen, thick walled fibre with narrow lumen, vessels with spiral and annular thickenings, and prismatic crystals (Fig. 5).



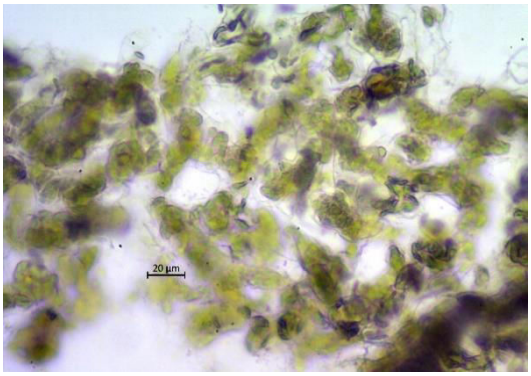
Epidermis with stomata and trichomes



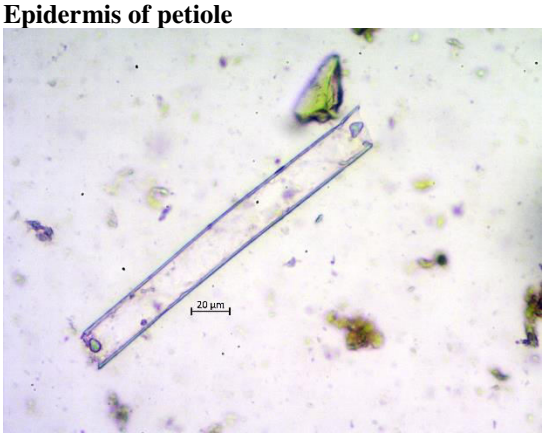
Surface view of upper epidermis



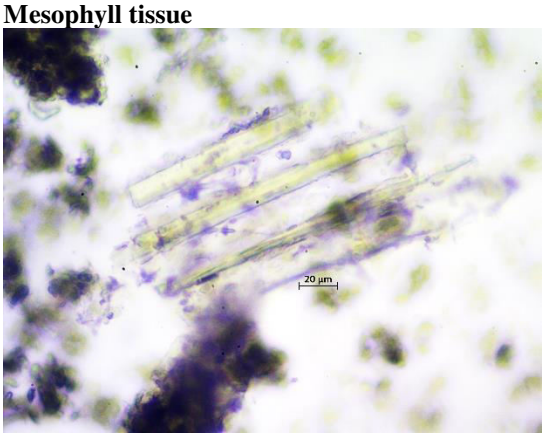
Epidermis of petiole



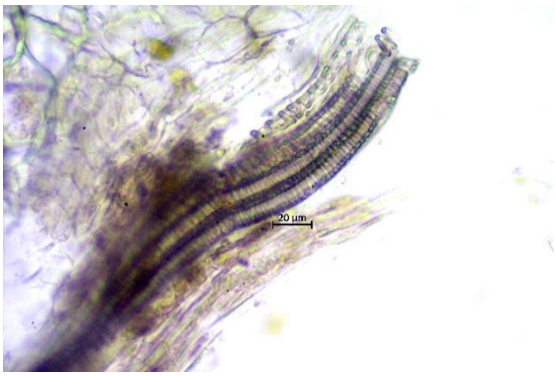
Mesophyll tissue



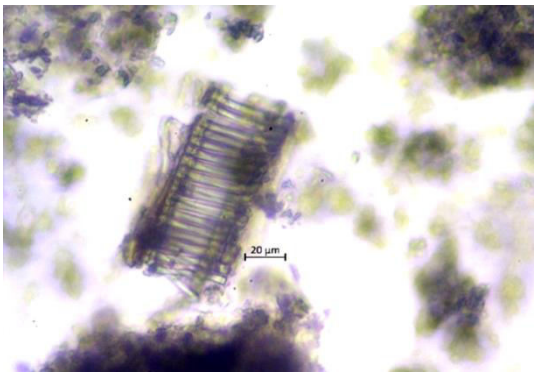
Thin-walled fibre with wide lumen



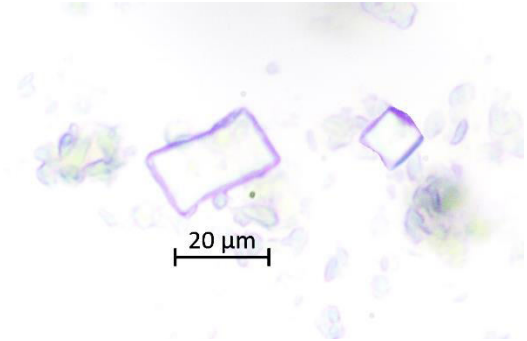
Thick-walled fibre with narrow lumen



Spiral vessels



Annular vessel



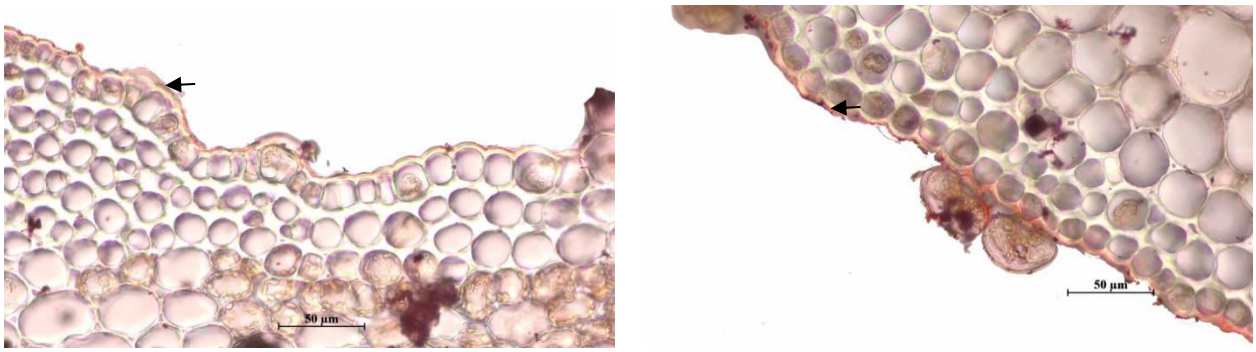
Prismatic crystals

Fig. 5: Powder microscopy of *Clerodendrum chinense* leaf

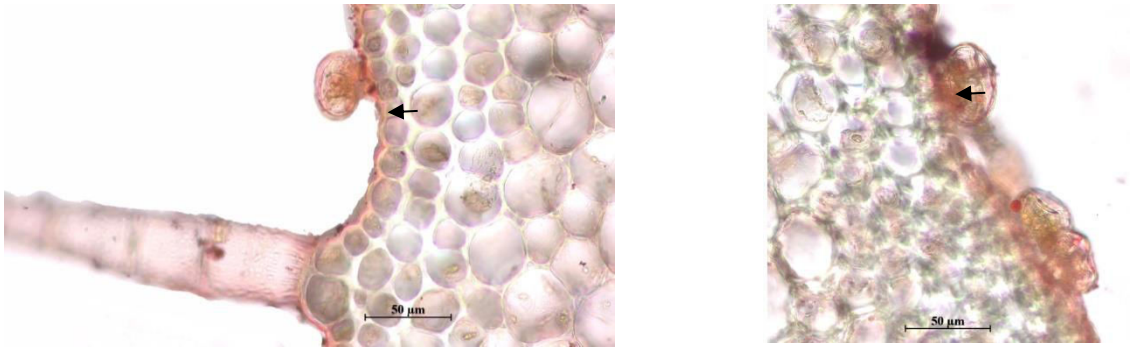
Histochemistry

Leaf

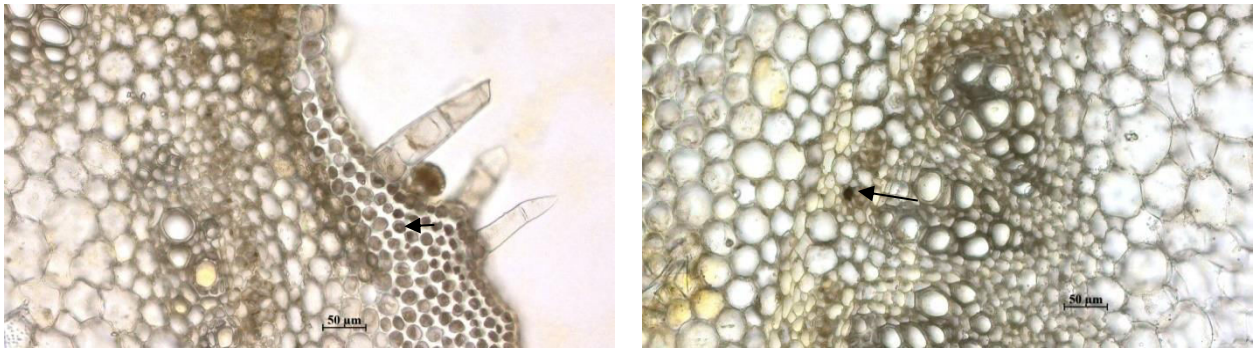
The histochemical study showed the presence of cutin and oil in epidermis, tannin depositions in hypodermis and vascular region, alkaloids in hypodermis, cortex and pith, lignin in xylem, mucilage in hypodermis and starch grains in pith parenchyma (Fig. 6).



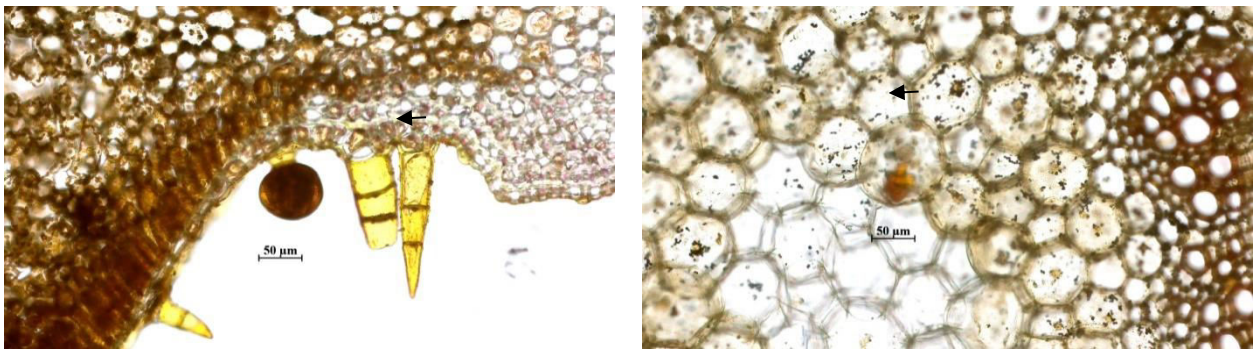
Cutin in epidermis



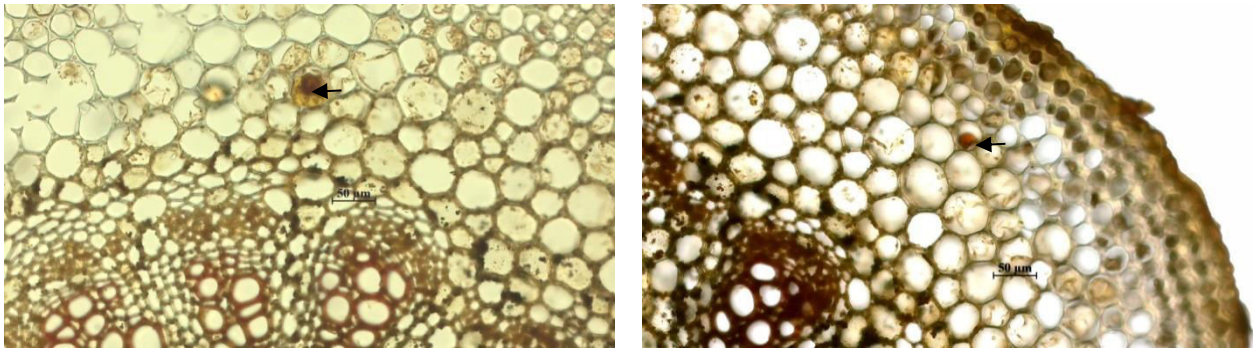
Cutin in epidermis **Cutin and oil in epidermis**



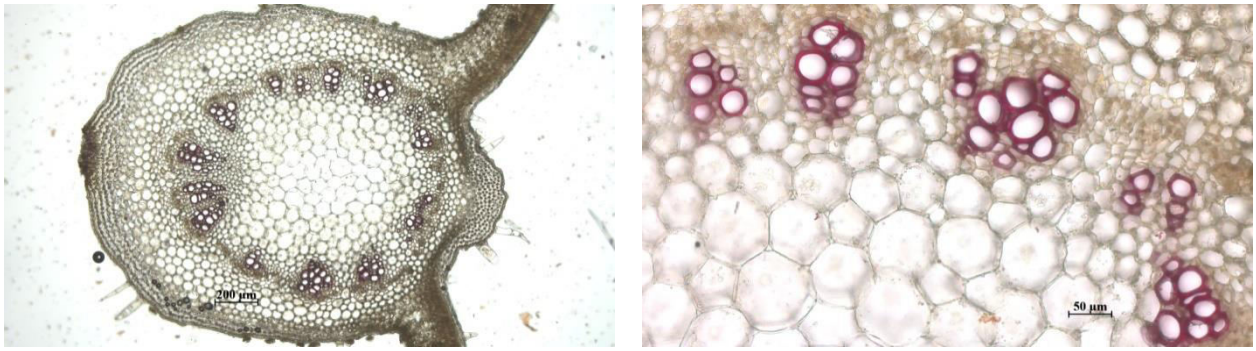
Tannin in hypodermis **Tannin in vascular region**



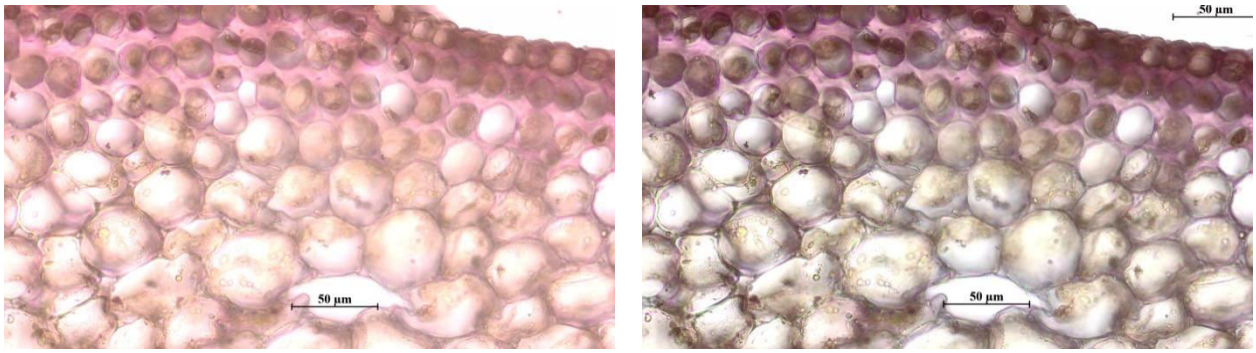
Alkaloid in hypodermis **Alkaloid in pith**



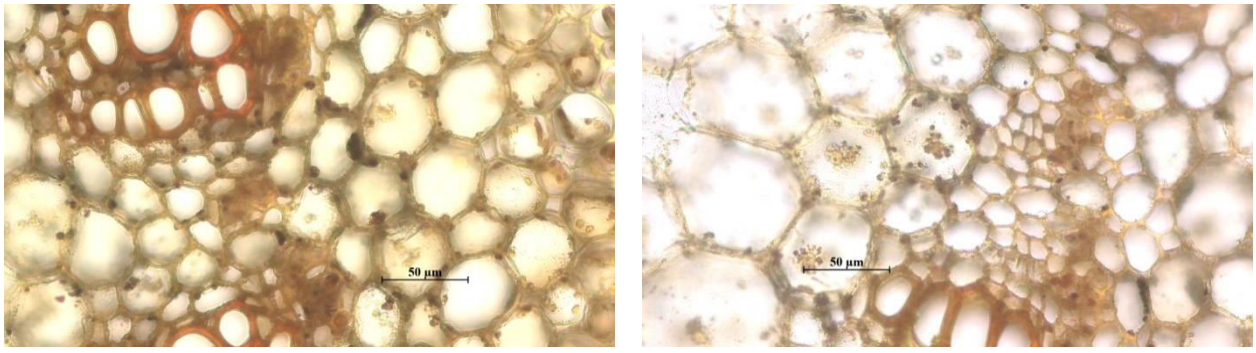
Alkaloid in cortex



Lignin in xylem



Mucilage in hypodermis

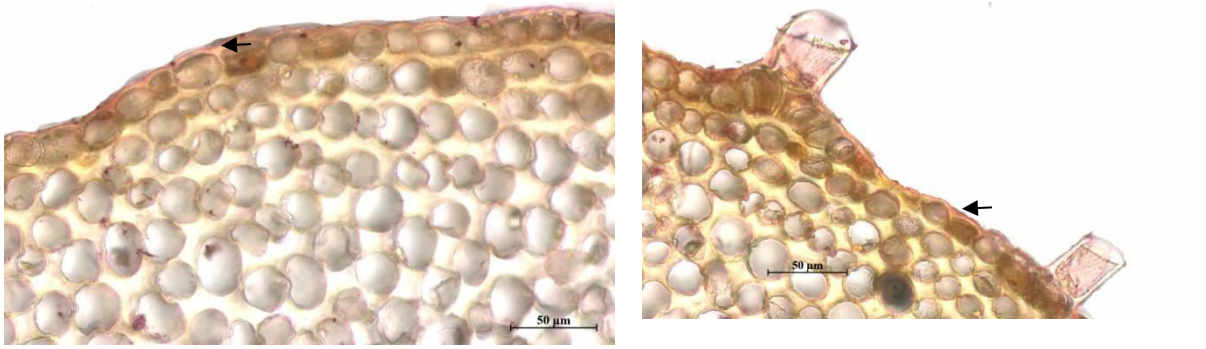


Starch grains in pith parenchyma

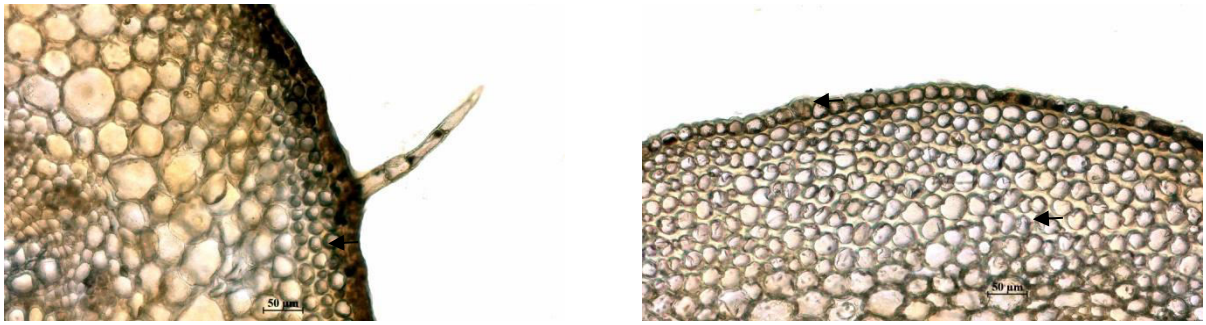
Fig. 6: Histochemistry of C*lerodendrum chinense* leaf

Petiole

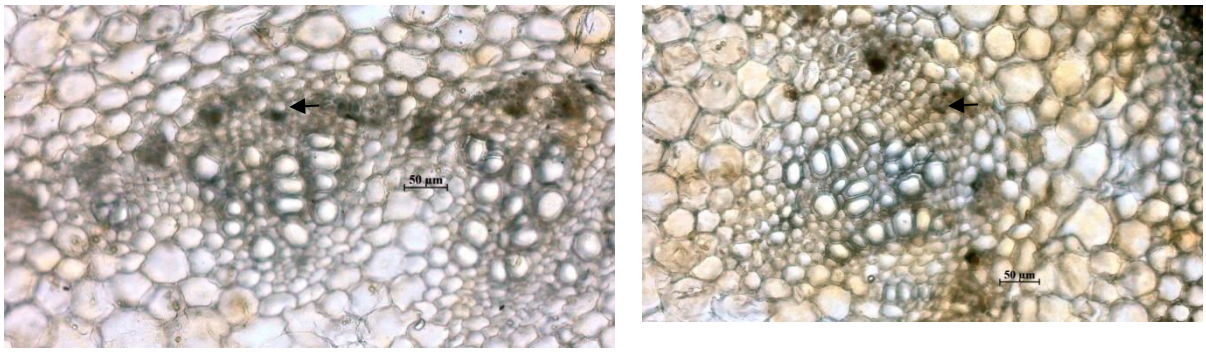
The histochemical study showed the presence of cutin in epidermis, tannin deposition in hypodermis, cortex and phloem, alkaloids in the cortical parenchyma; mucilage in hypodermal and cortical region, lignin in xylem, oil globules in cortex; starch was absent (Fig.7).



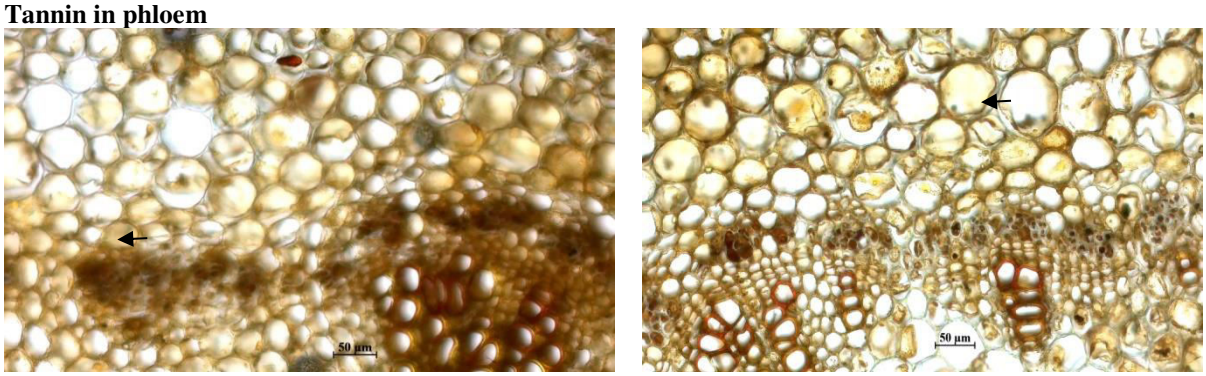
Cutin in epidermis



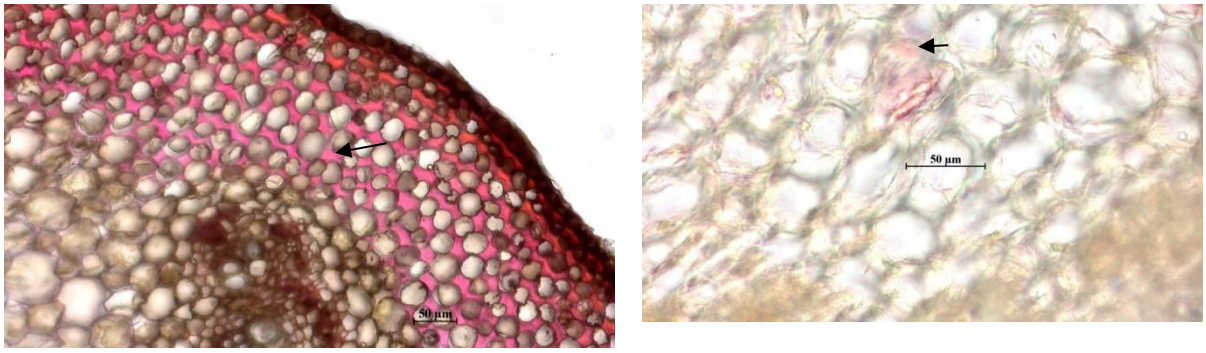
Tannin in hypodermis **Tannin in cortex**



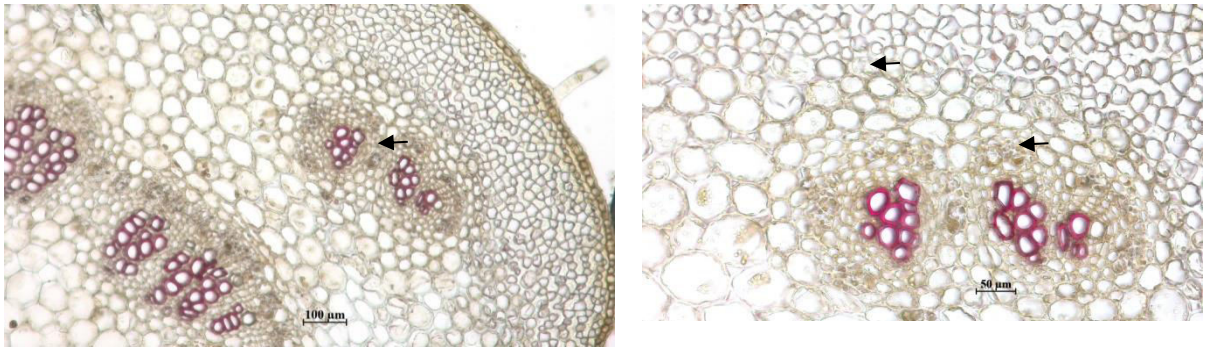
Tannin in phloem



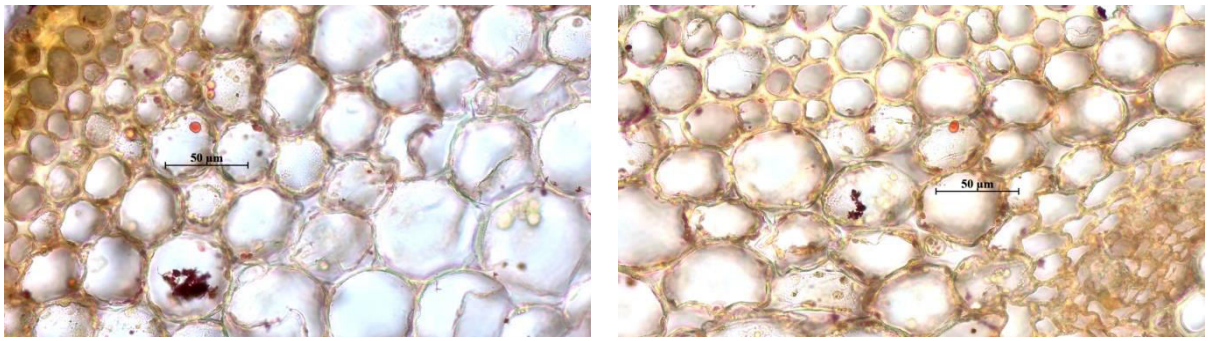
Alkaloid in cortical parenchyma



Mucilage in cortex



Lignin in xylem



Oil globules in cortex

Fig. 7: Histochemistry of *Clerodendrum chinense* petiole

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 leaf have few covering and glandular trichomes. The stomatal type is amphistomatic (anomocytic and diacytic). The petiole shows a numerous covering trichomes [Fig. 8,9,10].

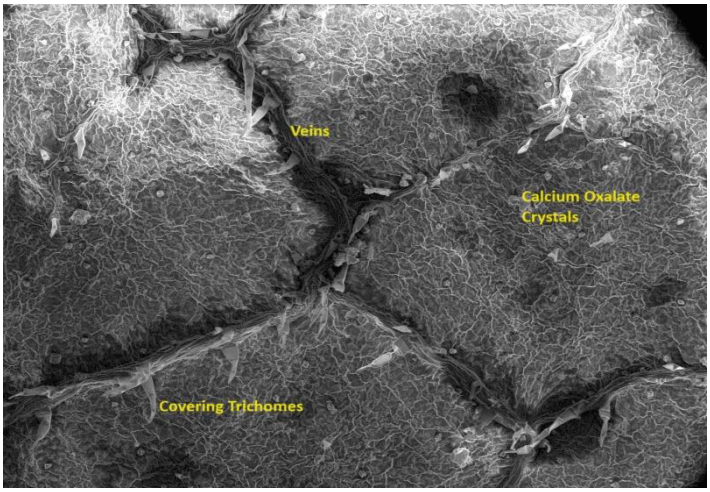


Fig. 8: *C.chinensis* Leaf Lamina

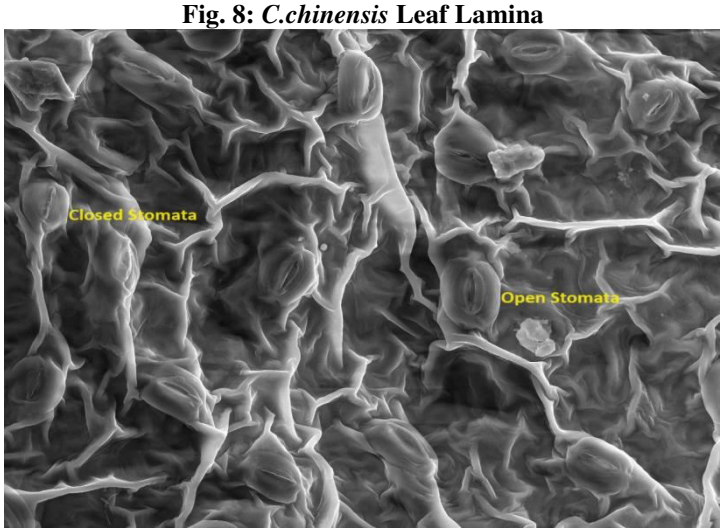


Fig. 9: Stomata of *C.chinensis* leaf

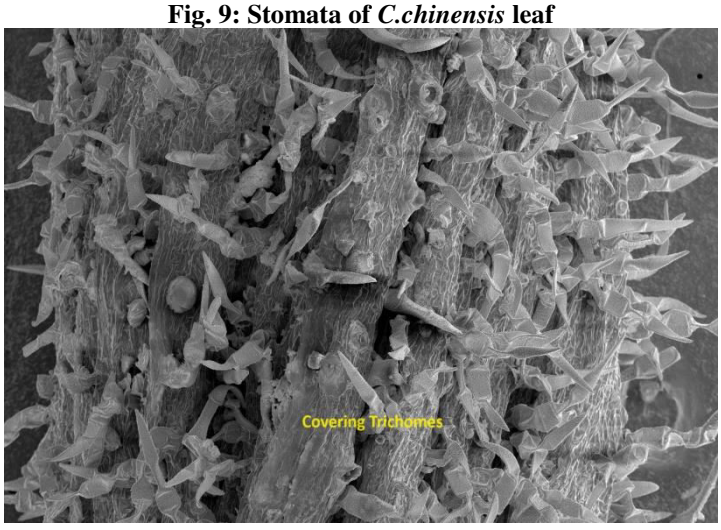


Fig. 10: Petiole of *C.chinensis*

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, Fig.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].

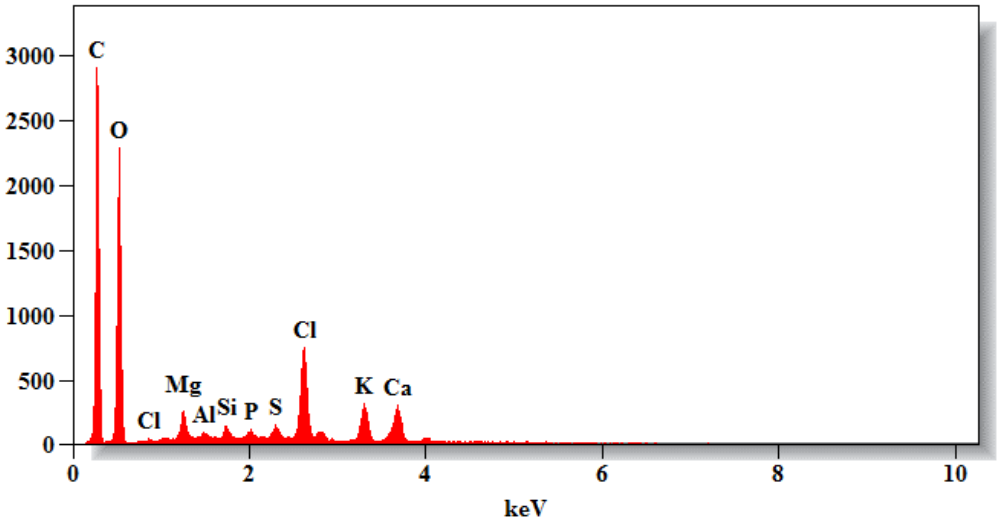


Fig. 11: EDS of *C.chinensis* leaf

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		

Table 4a. Quantitative Results for: *C.chinensis* Leaf

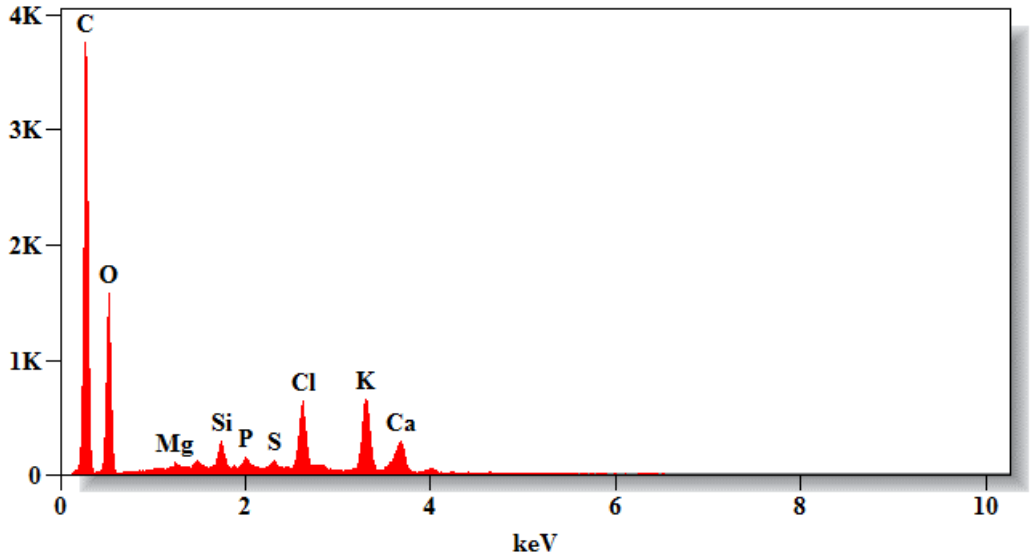


Fig. 12: EDS of *C.chinensis* petiole

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		

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

Discussion

This study offers some fresh insights. Chinese locals have long used *C.chinensis* to cure a range of illnesses. 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 very challenging task because of complex nature of chemical constituents. Microscopical evaluation is simplest and reliable tool for correct identification of herbs as well as small fragment of crude drugs or powderd 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 prioritized 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.

Declarations

Ethical approval and consent to participate: Not applicable.
Availability of data: The data used in this work are available.
Consent to publication: Not applicable.
Conflict of interest: The authors declare that there is no conflict of interest.
Funding: No research grant is involved in this research.
Authors' contributions: M, SB designed the study; M conducted the main statistical analysis, M wrote the manuscript, P revised the data analysis and the manuscript; all authors read, corrected, and approved the manuscript.

Conflicts of Interest
The authors declare no conflicts of interest.

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