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Histological evaluation of *Calotropis gigantean* (L.) R. Br. – Leaf, Root, Stem

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Abstract
The present study was aimed to evaluate the histological parameters of different part of *Calotropis gigantea*. *Calotropis gigantea* (L.) R. Br. belongs to the family Asclepiadaceae is a well known medicinal shrub commonly known as milkweed and has been used in Unani, Ayurvedic and Siddha system of medicine for years. On large scale this plant is found in India, China and Malaysia. Also it is distributed in almost all over the world. Traditionally all the parts of the plant have been used as medicine, as well as an important ingredient in a number of Unani formulations. The plant is also commonly used in Ayurvedic system of medicine for healing various ailments. However the present study was aimed to evaluate the histological parameters to determine the identity, purity and strength of the plant for quality control purpose. The investigations of the study show the presence of madullary rays, xylem fibers, vessels, parenchyma cells, phloem, narrow fibers, wide fibers, lignified fibers and stomata.

Keywords: Asclepiadaceae, histological, *Calotropis gigantea* (L.) R. Br.

Introduction
Calotropis gigantea (L.) R. Br. is a big shrub, it is looking like a small tree. Sports, clusters of waxy flowers that are either white or lavender in colour found. Each flower consists of five pointed petals and a small, elegant "crown" rising from the center which holds the stamens [1]. *Calotropis gigantean* (family – Asclepiadaceae) frequently known as Madar in Hindi is a perennial herb with a long history of use in traditional medicines. *Calotropis gigantean* is used as a traditional medicinal plant. The plant root shows nootropic activity in methanolic extract. It can be also used in many disease like anxiogenic, expectorant, antihelmintic, sedative, leprosy, ulceration, cough, ringworm of the scalp, piles, explosion on the body, asthma etc [2]. Plant has many unique properties as the plant can survive in any type of soil. It is a drought tolerant plant also. It is not consumed by grazing animals. Along with the medicinal property it possess fungicidal and insecticidal properties [3].

Plate 1: *Calotropis gigantea* (L.) R. Br. Plant

The literature review reveals that powdered root used in asthma, bronchitis, and dyspepsia. Flowers of *C. gigantea* have been found to have hepatoprotective activity against carbon-tetrachloride-induced liver damage in mice [4]. The leaves are useful in the treatment of paralysis, arthralgia, swellings, and intermittent fevers. The leaves have been found to have sedative and anxiolytic effect [5]. The flowers are bitter, digestive, astringent, stomachic, anthelmintic, and tonic. The alcoholic extract of the flowers of *C. gigantea* was found to possess analgesic activity in chemical and thermal models in mice [6]. The traditional practitioners use the leaf extract for the treatment of inflammatory painful conditions and rheumatic pain. The root extract is used for the treating inflammation and as an analgesic by many folk tribes of state of West Bengal, India [7]. Therefore in the present study, standardization of leaf, root and stem of *Calotropis gigantea* (L.) R. Br. has been done for evaluation of histological parameters to determine the identity, purity and strength of the plant for quality control purpose.

Materials and Methods

Collection of Plant material and authentication

For the study, whole parts of the plant of *Calotropis gigantea* were collected from the local market and town, Kolkata, West Bengal. The plant is identified and authenticated by Dr. V. Sampath Kumar, Botanical Survey of India, Government of India, Howrah, West Bengal and a voucher specimen was also deposited for future reference.

Microscopic analysis

Microscopic examinations of different parts of the plant were studied according to the standard method [8,9]. Transverse sections of lamina, stem and root (thin and thick) were prepared and stained with saffranin and fast green as per procedure. Same procedure was followed for powder microscopy [10, 11]. The microphotographs were taken by bright field microscope with digital camera.

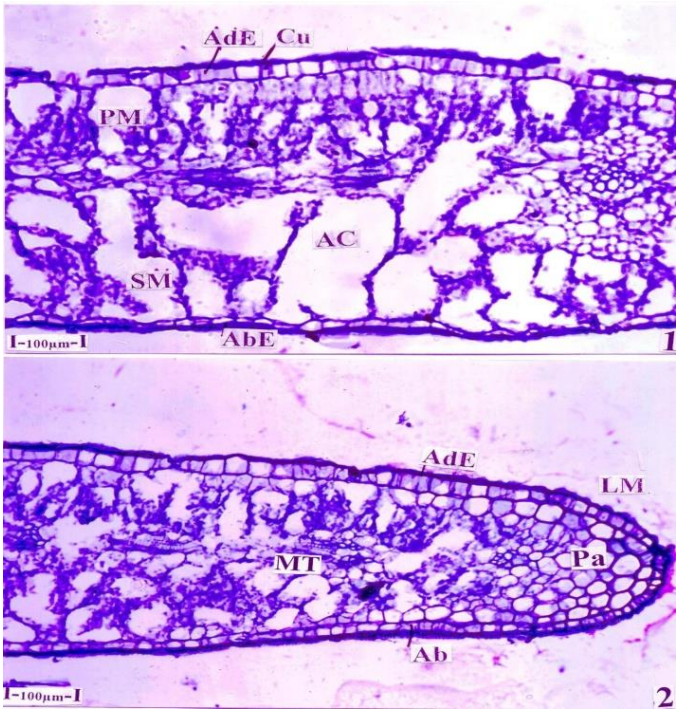
Results and Discussion

Observation for Microscopic Analysis

1. Leaf

The leaf is dorsiventral with thick smooth and even lamina (Fig-1. 1, 2) and very thick planoconvex Midrib (Fig. 3). The lamina is 530µm thick. The adaxial epidermal layer consists of thick, vertically oblong thin walled cells with thick cuticle. The cells are 40µm thick. The abaxial epidermis has thin tabular epidermal cells with prominent cuticle (Fig. 1.1). The lamina is amphistomatic (Stomata occur on both upper and lower epidermis). The mesophyll tissue is differentiated into one or two layers of adaxial, vertically cylindrical compact palisade cells. The spongy mesophyll is wider and consists of large air-chambers divided by partition filaments of spongy parenchyma cells.

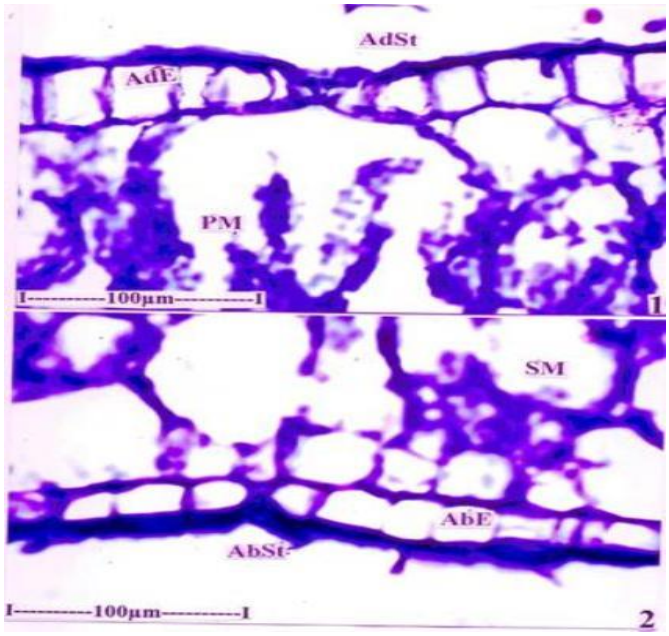
Leaf-Margin (Fig. 1.2) – The marginal part of the lamina is semicircular and slightly conical. It is 300µm thick. The epidermal cells of the marginal part are smaller, but thick walled with prominent cuticle. The mesophyll tissue in the marginal position consists undifferentiated, compact mass of fairly thick walled angular, parenchyma cells (Fig. 1.2).



Abbreviation: Ab – Adaxial Side, AbE – Abaxial Epidermis, AdE – Adaxial Epidermis, AC – Air chambers, Cu – Cuticle, LM – Leaf Margin, MT – Mesophyll Tissue, PM – Palisade Mesophyll, Pa – Parenchymatous tissue, SM – Spongy Mesophyll.

Fig 1: T.S of Lamina (Fig. 1.1 – T.S of lamina, Fig. 1.2 – T.S of lamina through leaf margin).

The stomata on the adaxial epidermis are located along the surface of lamina. The stoma has two semicircular guard cells and larger subsidiary cells (Fig. 2.1). The abaxial stoma has reduced guard cells and occur beneath the level of the epidermal cells (Fig. 2.2).

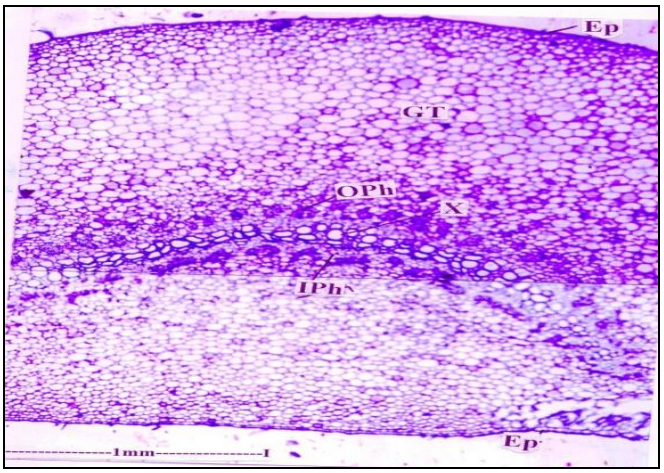


Abbreviation: AbE – Abaxial Epidermis, AbSt – Abaxial Stoma; AdE – Adaxial Epidermis; AdSt – Adaxial Stoma, PM – Palisade Mesophyll, SM – Spongy Mesophyll.

Fig 2: T.S. of leaf. (Fig. 2.1 – T.S of leaf showing adaxial Epidermis with stoma. Fig 2.2 T.S of leaf with abaxial stoma.

2. Midrib

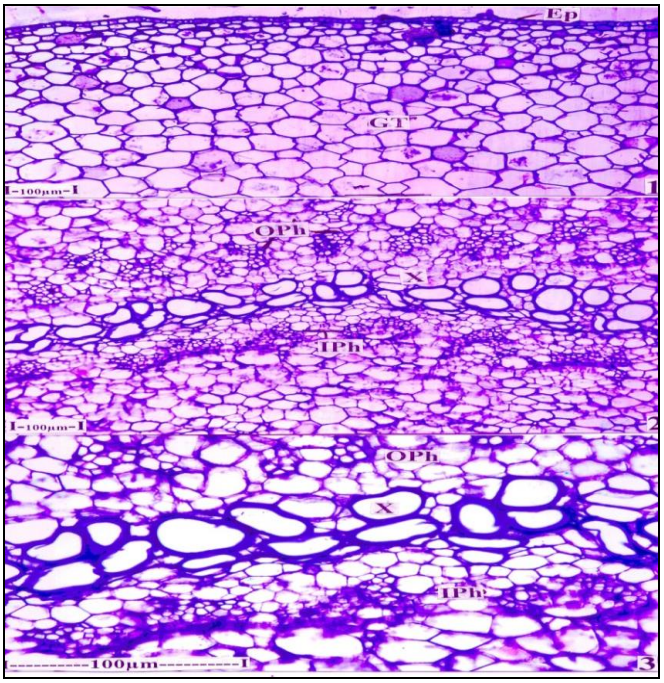
Midrib of the leaf is planoconvex in sectional view. The adaxial side is flat and abaxial side is slightly convex (Fig. 3.1). The midrib is 2.85mm thick. It consists of thin epidermis wide homogeneous ground tissue and thin and wide are of vascular strand (Fig. 3).



Abbreviation: Ep – Epidermis, GT – Ground Tissue, IPh – Inner Phloem, OPh – Outer Phloem, X-Xylem.

Fig 3: T.S of Midrib

The epidermis layer is thin comprising small squarish slightly thick walled cells (Fig. 4). The ground tissue consists of large, angular thin walled compact parenchyma cells (Fig. 4.1). The vascular strand is a thin wide shallow core of xylem elements and inner and outer several units of phloem elements (Fig. 4.2). The xylem elements are two layered. They are circular, elliptical or uneven in outline. The cells have thick, lignified walls and wide lumen. Along the outer and inner regions of the xylem – are occur large discrete masses of phloem elements. The phloem elements are prominent, wide and darkly stained (Fig. 4.3).

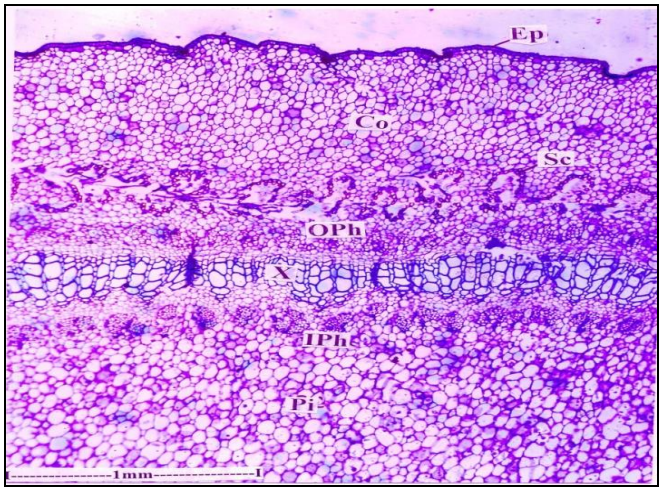


Abbreviation: Ep – Epidermis ; GT – Ground Tissue; IPh – Inner Phloem ; OPh – outer Phloem. X – Xylem.

Fig 4: T. S of Midrib; (Fig. 4.1 – Abaxial Ground Tissue of the Midrib. Fig. 4.2 – Middle portion showing outer and inner phloem and Middle xylem. Fig.4 3 – Xylem and phloem tissues enlarged).

3. Stem

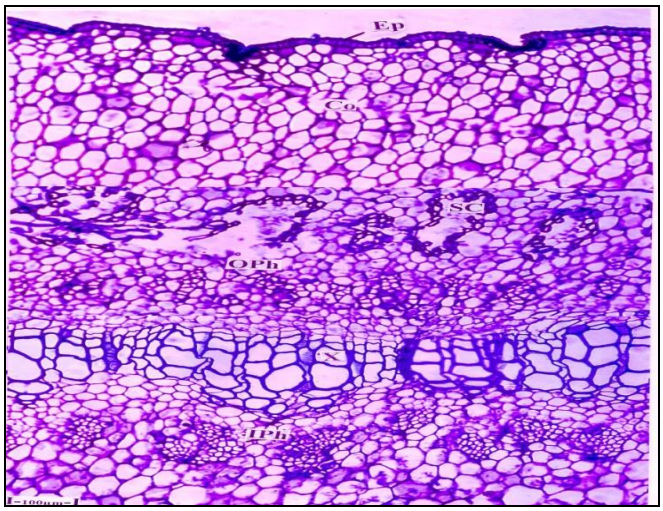
The stem is circular in sectional view with slight undulate outline (Fig. 5). It consists of thin epidermis, wide cortex, thin bicollateral hollow vascular cylinder and wide pith.



Abbreviation: Co – Cortex; Ep – Epidermis; IPh – Inner Phloem; OPh – Outer Phloem; Pi – Pith; X-Xylem.

Fig 5: T.S. of Stem – A Sector

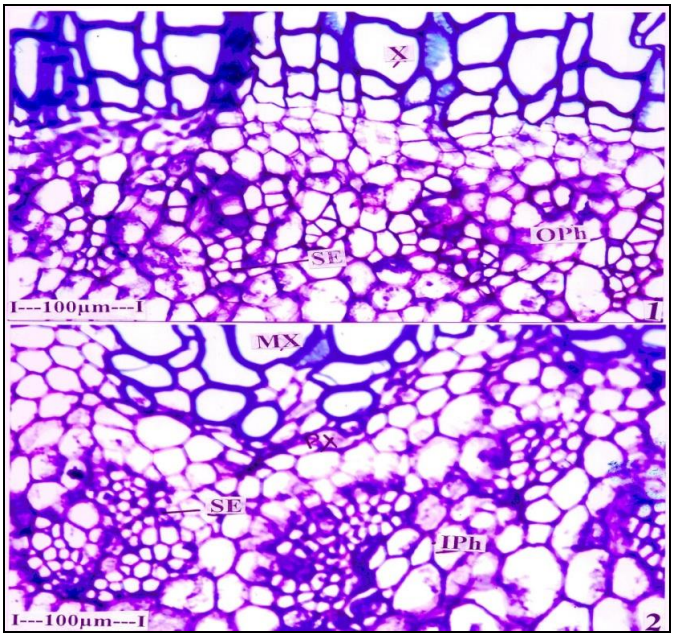
The epidermal layer consists of narrow, radially oblong thick walled parenchyma cells with thick cuticle (Fig. 6). The cortical zone is 850µm thick. The cortical cells are wide, angular thin walled compact parenchyma cells (Fig. 5; 6). Along the inner boundary of the cortex occurs discontinuous rings of sclerenchyma cells. These sclerenchyma rings are uneven in outline. The cells are small, angular and have thick lignified walls (Fig. 6).



Abbreviation: Ep – Epidermis; Co-cortex; IPh - Inner –Phloem; OPh – Outer Phloem; X-Xylem, Sc – Sclerenchyma.

Fig 6: T.S. of stem – A sector enlarged.

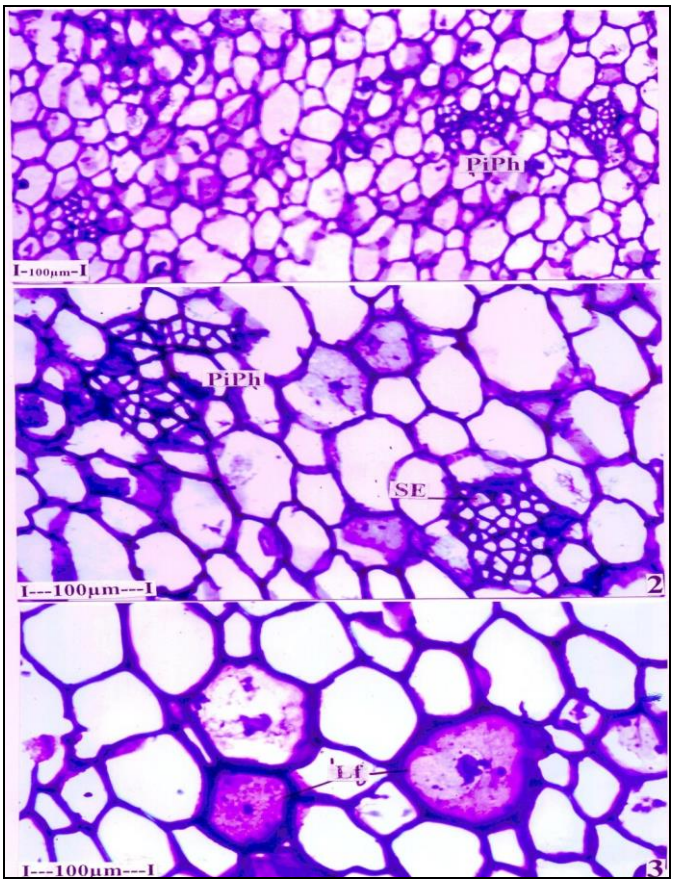
The vascular cylinder consists of a thin continuous ring of xylem elements. The elements are arranged short radial lines. The metaxylem elements are wide, radially oblong or squarish with thick lignified walls (Fig. 6; 7.1). The cells are 50µm in diameter. Along the outer zone of the xylem cylinder occur large clusters of outer phloem; the phloem elements are wide, thick walled and darkly stained (Fig. 4.3; 7.1). Inner to the xylem cylinder and adjacent to the protoxylem elements are large circular units of phloem elements. These inner phloem elements are also wide, thick walled and darkly stained (Fig. 7.2).



Abbreviation: IPh – Inner Phloem; MX – Metaxylem; OPh – outer phloem; Px – Protoxylem; DE – Sieve Elements; X-Xylem.

Fig 7: T.S of Stem – Fig 7.1 - Xylem cylinder with outer phloem; Fig 7.2 -Protoxylem side of the xylem with inner phloem.

The pith is wide and parenchymatous. Diffusely distributed in the pith region are several small strands of pith phloem or medullary phloem (Fig. 8.1, 2). Some of the pith cells function laticifers or the latex secreting cells. The laticifers are circular in outline, thick walled and possess dark latex (Fig. 8.3).

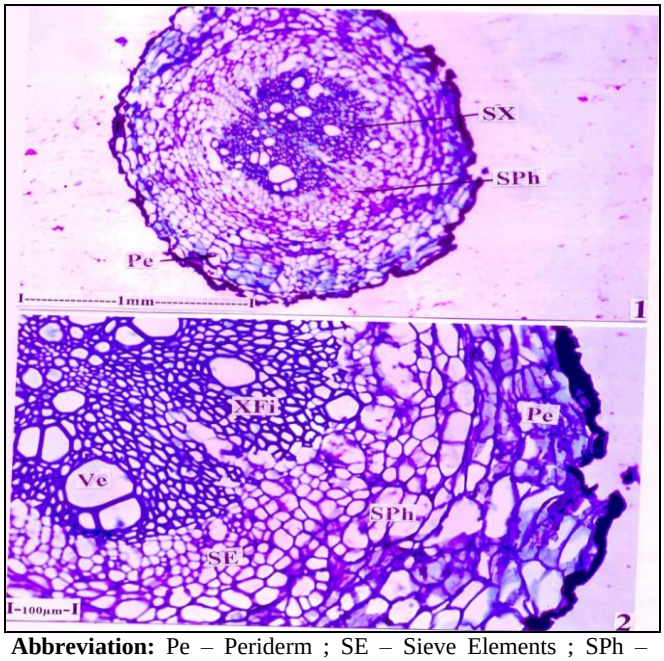


Abbreviation: Lf – Laticifers; Piph – Pith Phloem, SE – Sieve – Elements.

Fig 8: T.S of Stem – Fig. 8.1 -Pith (Medullary) Phloem strand; Fig 8.2 - Pith phloem stands – enlarged. Fig 8.3 - Laticifers in the pith).

4. Root

Both thin and thick roots were studied. The thin root is circular measuring 1.5mm in diameter (Fig. 9.1). The root consists of a thick, dark crushed outer layer of epidermis followed by four or five layers of less distinct periderm cells. There is fairly thick cortical zone where the cells are tangentially elongated and compact. (Fig. 9.2). The vascular cylinder consists of a central, circular solid cylinder of secondary xylem, surrounded by a thick layer of secondary phloem. The secondary xylem consists of a few, diffusely distributed wide and narrow vessels and dense ground tissue of secondary xylem fileses (Fig. 9.2)

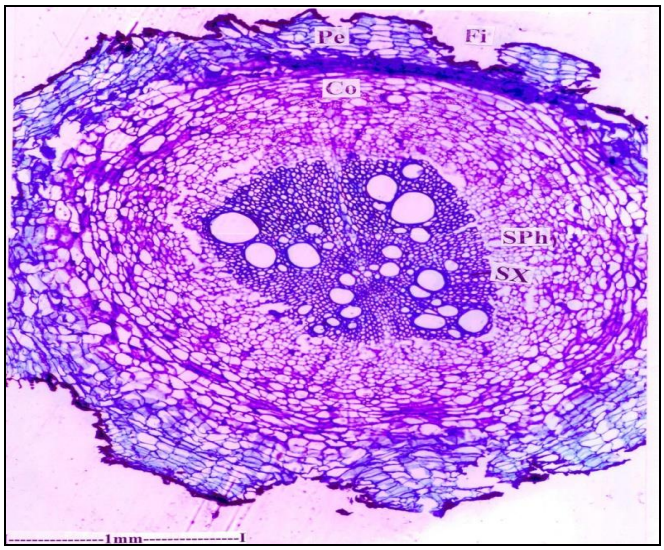


Abbreviation: Pe – Periderm ; SE – Sieve Elements ; SPh – Secondary Phloem ; SX – Secondary Xylem ; Ve – Vessels ; XFi – Xylem Fibres.

Fig 9: T.S. of thin Root, Fig. 9.1 – T.S of thin Root – entire view. Fig. 9.2 – T.S of thin Root – A sector enlarged.

Thick-root (Fig 10)

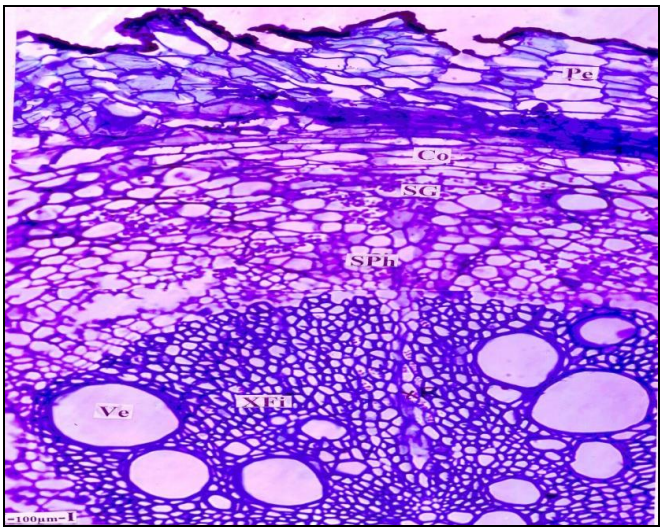
The thick root is 3mm thick. It consists of wide deeply fissured periderm, narrow cortex and central secondary xylem cylinder surrounded by secondary phloem (Fig. 10).



Abbreviation: Co-cortex; Fi – Fissues; Pe – Periderm; SPh – Secodnary Phloem; Sx – Secondary Xylem.

Fig 10: T.S of thick root – ground plan.

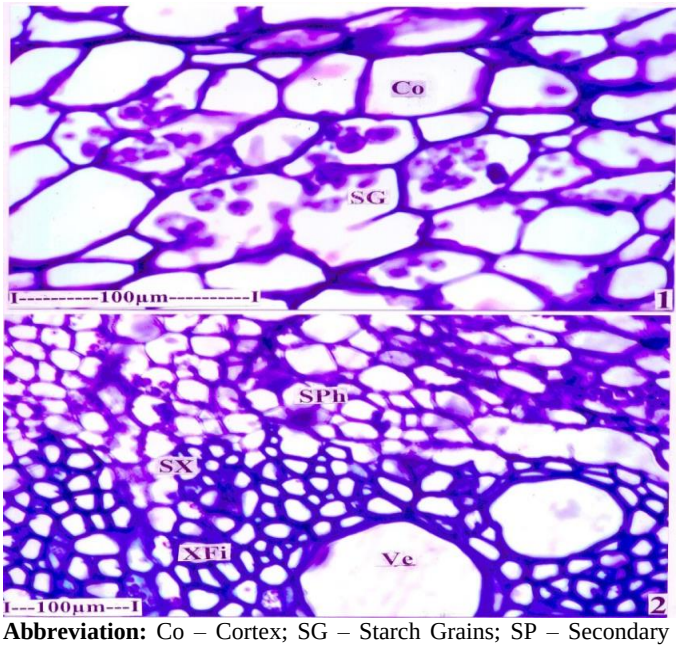
The epidermal layer is crushed into thick dark surface layer. The periderm is 250µm thick. It consists of about seven layers of thick, radial files thin walled cells (Fig. 11). The cortical zone includes about six layers of tangentially compressed thin walled cells (Fig. 11).



Abbreviation: Co – Cortex; Pe – Periderm; SG – Starch Grains; SPh – Secondary Phloem; Ve – Vessel; XFi – Xylem Fibres ; XR – Xylem Ray.

Fig 11: T.S of thick root – a Sector – enlarged.

Some of the inner cortical cells possess large spherical starch grains (Fig. 12.1). Secondary phloem is in the form of a wide sheath around the secondary xylem. It consists of small nests of sieve elements and large polyhedral parenchyma cells (Fig. 12.2). Secondary xylem has a small central core of a few narrow vessels and four radial segments which possess wide, circular thick walled vessels (Fig. 10, 11). The wide vessels are 200µm in diameter. Xylem-rays are fairly wide which extend up to the secondary phloem. Xylem fileses form the danse ground tissue of the secondary xylem. The fibres have thick lignified walls and wide lumen.



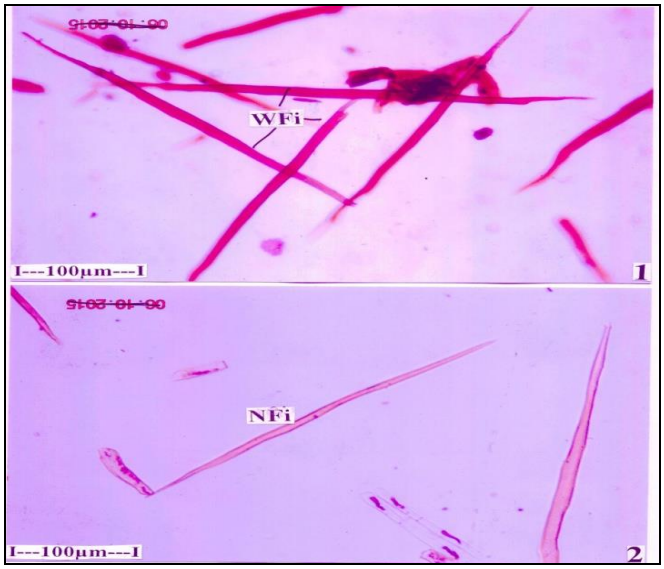
Abbreviation: Co – Cortex; SG – Starch Grains; SP – Secondary Phloem; SX – Secondary Xylem; Ve – Vessel; XFi – Xylem Fibres.

Fig 12: Fig. 12.1 - Thick Root – Cortical cells possessing Stanch – grains. Fig 12.2 - Thick Root – Secondary Phloem and Secondary Xylem – enlarged.

Powder Microscopic results

Powder preparation of the plant shows fibres, vessel elements and laticifers.

1. Fibres: There are two types of fibres in the powder. Some are wide and others are narrow. The wide fibres are more abundant than the narrow fibres. The wide fibres have thin walls and wide human (Fig. 13.1; 14.1, 2). They are 350µm long and 15µm thick. The narrow fibres have thick walls and narrow lumen. (Fig 13.2). They are 360µm long and 8µm thick.

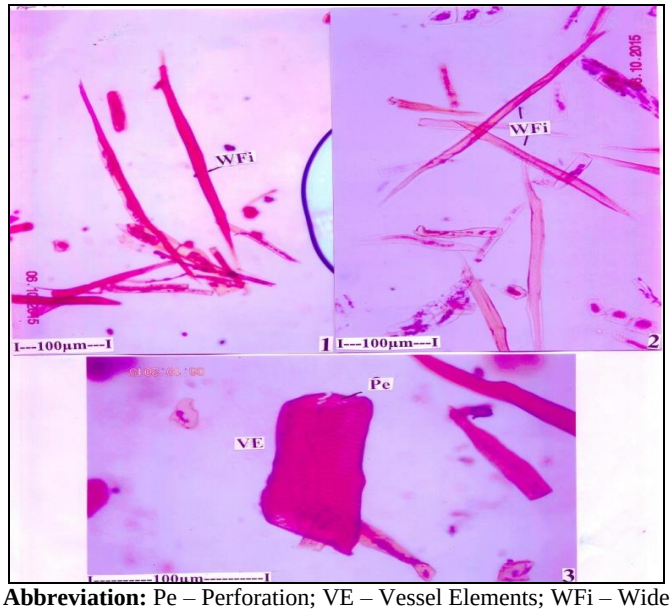


Abbreviation: NFi – Narrow Fibre; WFi – wide Fibres.

Fig 13: Powder Microscopic Observation: Fig. 13.1 - Wide Fibres; Fig. 13.2 - Narrow Fibres.

2. Vessel elements (Fig. 14.3 ; 15.1)

Vessel elements are less frequent in the powder. They are short, wide and cylindrical cells. The end wall perforations are elliptical are either horizontal or oblique in orientation (Fig. 14.3). Pits on the lateral walls are elliptical and multiseriate. The vessel elements are 120µm long and 40µm wide.

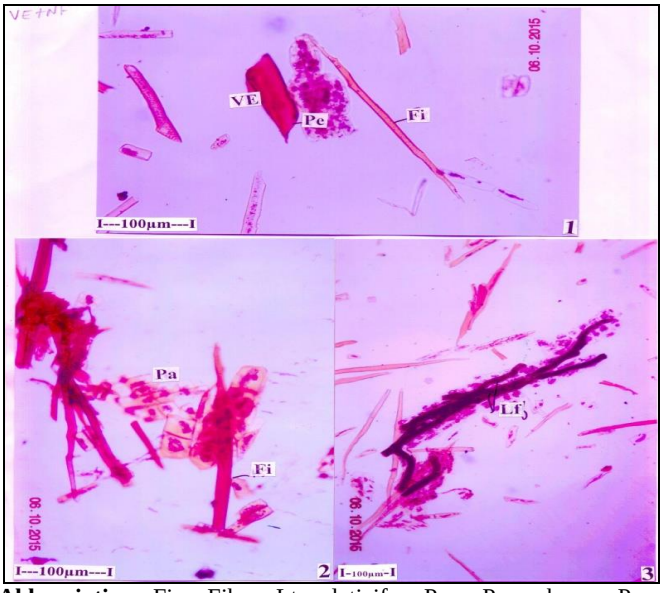


Abbreviation: Pe – Perforation; VE – Vessel Elements; WFi – Wide Fibres.

Fig 14: Fig. 14.1, Fig 14.2 – Wide Fibres. Fig. 14.3 – Vessel element.

3. Laticifers (Fig. 15.3): Long, narrow tubular laticifers are frequently seen in the powder. They are non-articulate and anastomosing type (non septate and branched). The laticifers are darkly stained with dense latex contents.

4. Parenchyma cells (Fig. 15.2): Squarish, thin walled parenchyma cells are common in the powder. The cells have thin walls and dark cell contents. The cells are 30 x 30µm in size.

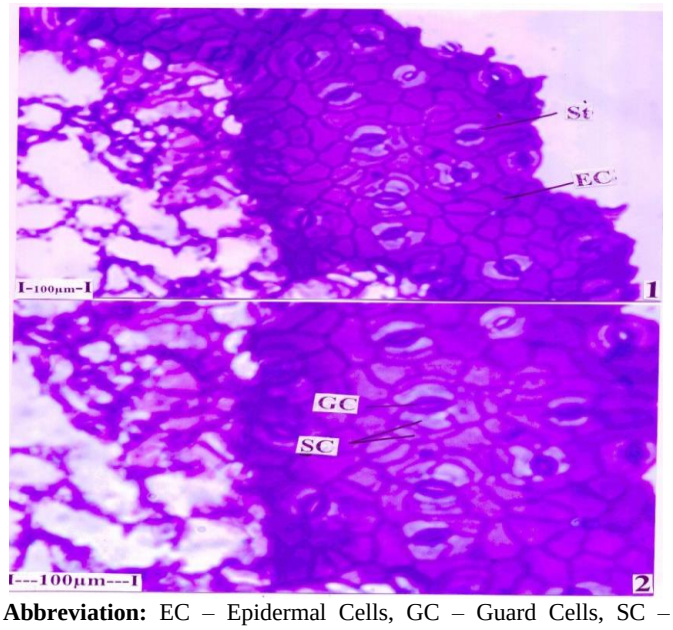


Abbreviation: Fi – Fibre; Lt – laticifer; Pa – Parenshyma; Pe – Perforation; VE – Vessel Element.

Fig 15: Fig. 15.1 - Vessel element and a fibre. Fig. 15.2 - Parendeyma cells and a fibre. Fig. 15.3 - Laticifer.

Stomatal morphology

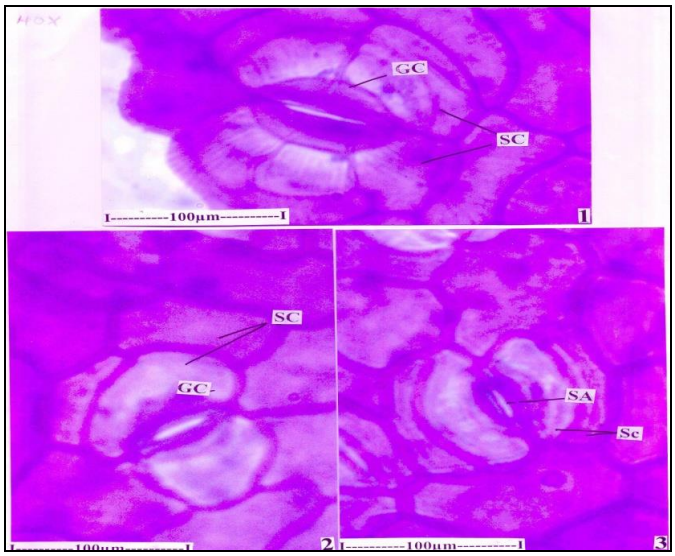
Lamina was subjected to paradermal section and the sections were viewed in surface view. The stomata were seen only on the abaxial side (Fig. 16.1, 2). The stomata are dense and diffuse in distribution. The guard cells are narrowly elliptical with district stomatal apertuse. The guard cells are 20 x 30µm in size.



Abbreviation: EC – Epidermal Cells, GC – Guard Cells, SC – Subsidiary Cells, St – Stoma.

Fig 16: 1, 2: Paradermal sections of lamina showing stomata and epidermal cells in surface view.

The stomata are basically paracytic type. In a stoma, there may be two subsidiary cells, one on either side of the guard cells and parallel to them. (Fig. 16.1, 2 ; 17.2). As a variation, there are two pairs of subsidiary cells, one pair on either side of the guard cells. (Fig. 17.3). Some of the stomata are cyclocytic type, where, these is a circle of 5 or 6 subsidiary cells around a stoma 17.1). The epidermal cells are polygonal in outline; their anticlinal walls are fairly thick and straight.



Abbreviation: GC – Guard cells; SA – Stomatal Aperture; SC – Subsidiary cells.

Fig 17: 1, 2, 3 – Stomatal types (1- Cyclocytic type; 2 – Single pair of unequal subsidiary cells, 3 – Two pairs of subsidiary cells.

Conclusion

In the present investigation, a detailed study of histological parameters was carried out and which could be helpful in better authentication of various parts of *Calotropis gigantea* (L.) R. Br. The finding of present study will also serve the reference material in the preparation of monograph of this plant. Therefore present study may help to various pharma industries to determine the identity, purity and strength of the plant.

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