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# PHOTOMICROGRAPHIC IDENTIFICATION AND AUTHENTICATION OF PLANT MATERIALS IN KALLADAIPPU KUDINEER

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## ABSTRACT
The incidence of nephrolithiasis is a major problem worldwide and it is increasing at a higher rate in the recent decade. It is otherwise called as kidney stone or Urolithiasis that depends on various factors like gender, diet, obesity and age. Apart from surgical procedures many Siddha formulations are used to treat this condition. *Kalladaippu Kudineer* is a drug formulation used in the traditional Siddha system of medicine for the treatment of kidney stone. Standardization of herbal medicines is very important criteria which include harvesting, drying, storage, transportation and processing that may affect the quality of herbal drugs. Correct identity and authentication of materials used are most important step in standardization procedure. In the present study a detailed microscopic evaluation of anatomical study herbal ingredients of Kalladaippu Kudineer was performed. Plant anatomical studies were done for the ingredients of kalladaippu kudineer *Tribulus terrestris*, *Aerva lanata* root, *Pavonia odorata* root, *Crateva religiosa* bark of the *Kalladaippu Kudineer*. The anatomical characteristic of the plant tissue parts were carefully collected and slides for each part were prepared with great care and later visualized using different magnifications of Nikon lab photo 2 microscopic Unit. This study had provided the in depth analysis of the of the herbal plant part used in the *Kalladaippu Kudineer* and thus it helps in the genuine identification of the plant part at a species level and it serves as a gold standard procedure when compared to the visualization through the naked eye.

**KEYWORDS:** Drug Standardization, Microscopy, *Kalladaippu Kudineer*, pharmacognostic techniques.

## INTRODUCTION
In the recent decade the ancient system of medicine is gaining importance in the community, this is due the adverse effect caused by the current system of modern medicines. The herbal plant has been reported to have different healing property and is of least side effect. The ancient literature have already given a detailed view of the herbal drugs there effective plant parts and there significant biological activity. Despite of the various beneficial activities all the drug formulation need to be documented and standardized for their effective use in the community.[1,2] The standardization of plant materials is an important step in the formulation of drug that can be achieved by detailed pharmacognostic studies.[3] Such studies will help in effective identification and authentication of the herbal plant parts that help in correct identification and quality assurance of the starting materials which ensures the reproducible quality of herbal medicine that will ensure its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics.[4]

The standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety. The major factors that have to be monitored are Macro and microscopic examination, Foreign organic matter, Ash values, Moisture content, Extractive values, Crude fibre, Qualitative chemical evaluation, Chromatographic examination, Quantitative chemical evaluation, Toxicological studies. The *Kalladaippu Kudineer(KK)* is believed to be a classical *Siddha* formulations that have longstanding literature evidences in the treatment of kidney stones though not scientifically evaluated. Nephrolithiasis is a global issue in the recent decade affecting geographical regions globally. Annually approximately it is prevalent in 3-5% and life time prevalence is approximately 15-25%.[5,6] High incidence rate is reported in Middle East (20-25%) due to hot

climate with increased chances of dehydration.[7] The main factor is that Nephrolithiasis tend to cause recurrence in most of patients. In India, 12% of our population prone to have kidney stones and out of which 50% may have renal damages. Approximate 2 million of Indian population is affected with nephrolithiasis every year and some parts of the country is named as the stone belt that includes, Gujarat, Maharashtra, Punjab, Rajasthan, Delhi, Haryana and part of North East side of the country.[8] They affect men more than women. It is estimated that renal colic (severe pain caused by a kidney stone) affects about 10-20% of men, and 3-5% of women and majority 80% of calcium oxalate stones.[9] Some ingredients of KK have been pharmacologically reported for their anti-lithiatic activities. The present study is to investigate the pharmacognostic properties mainly the macroscopic and microscopic investigation of the herbal drugs used in the formulation of *Kalladaippu Kudineer.*

## MATERIALS AND METHOD

### Sourcing of raw materials

The ingredients for the chosen drug Kalladaippu Kudineer (KK) were collected through plant collectors from various places during the month of August 2011 to September 2011. All the raw materials required for the preparation of KK were procured from the market.

### Identification and authentication of raw materials

All the herbal ingredients for the drug formulations were identified and authenticated by Dr. Sasikala Ethirajulu, Research Officer Scientist II (Pharmacognosy), Siddha Central Research Institute, Arumbakkam Chennai and plant anatomy photo micrographs were done by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, West Tambaram, Chennai. Voucher specimen was prepared and preserved at the drug standardization laboratory in the Department of Siddha of Tamil Nadu Dr. MGR Medical University, Chennai.

### Preparation of specimens

The plant specimens were collected with care by selecting the healthy plants and their normal parts. The required samples were cut and removed from the plant and fixed in FAA (Formaldehyde - 5ml + Acetic acid - 5ml + 70% Ethyl alcohol - 90ml). After 24 h of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule given by Sass (1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60$^0$C) until TBA (Tert-butanol) solution attained super saturation. The specimens were then cast into paraffin blocks.

### Sectioning of plant materials

The paraffin-embedded specimens were sectioned with the help of Rotary Microtome. Dewaxing of the sections was by customary procedure. The sections were stained with toluidine blue (polychromatic stain) results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies, etc., Wherever necessary, sections were also stained with safranin and Fast-green and IKI (for starch).

To study the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) the leaf part were cleared with 5% sodium hydroxide and epidermal peeling was done by partial maceration employing Jeffrey's maceration fluid. Glycerine-mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared using NaOH and mounted in glycerine medium after staining. Different cell components were studied and measured.

### Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical features are followed as given in the standard Anatomy books.

## RESULTS AND DISCUSSION

### Herbal dugs of Kalladaippu Kudineer

All the herbal drug used in the preparation of Kalladaippu Kudineer namely *Tribulus terrestris, Aerva lanata* root, *Pavonia odorata* root, *Crateva religiosa* (Figure 1) bark were collected and authenticated by physical examination with the naked eye before performing the detailed anatomical study in Table 1.

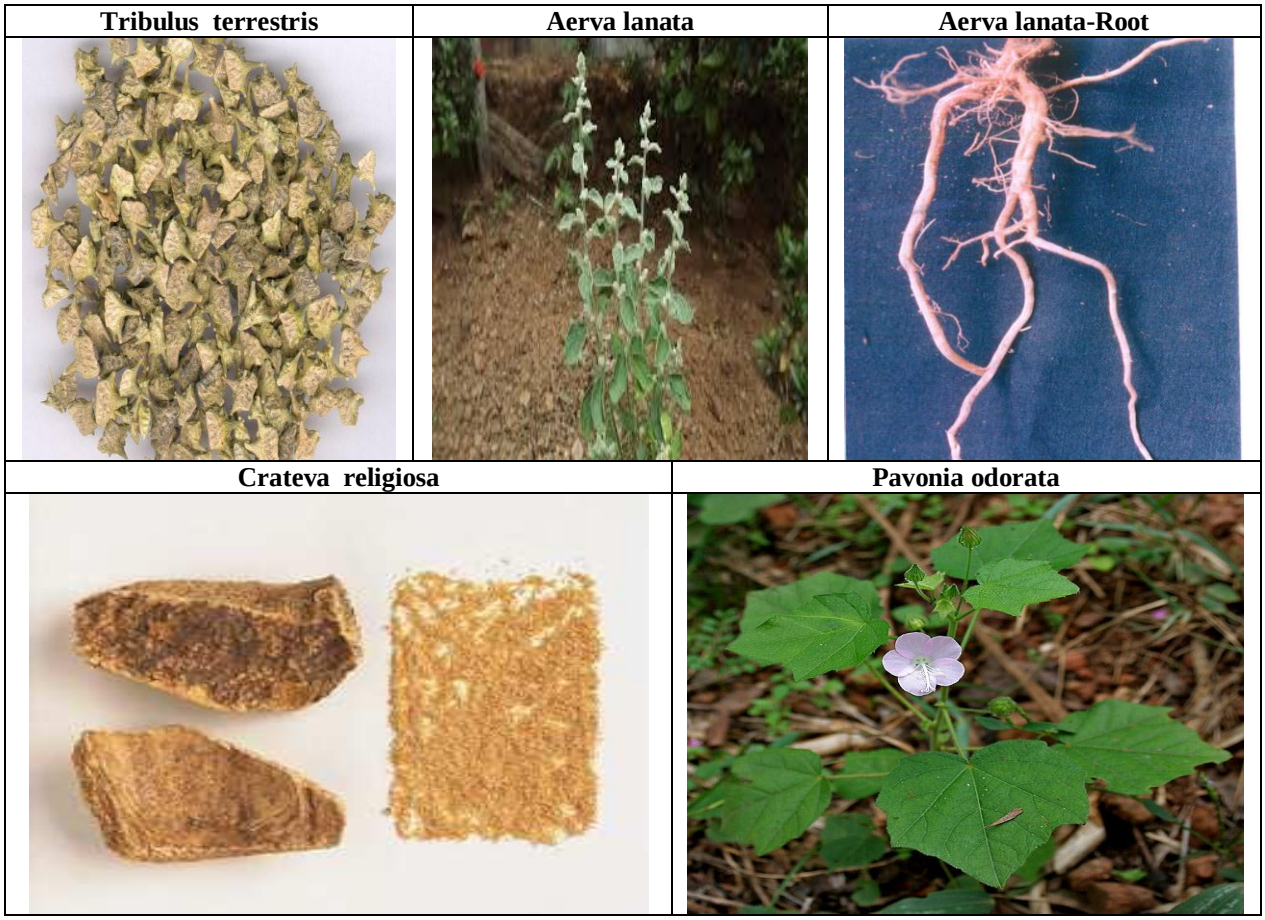


**Figure (1): Physical examination of ingredients of *Kalladaippu Kudineer.***

**Table (1): List of raw materials and their source of Kalladaippu Kudineer.**

| Sl.No | Ingredient | Source/ place of collection | Period | Part used |
|---|---|---|---|---|
| **1** | *Tribulus terrestris* | Namakkal district | Aug 2011 | Fruits |
| **2** | *Aerva lanata* | Namakkal district | Aug 2011 | Root |
| **3** | *Crateva religiosa* | Market sample from Ramasamy Chetty & Sons, Chennai | Sep 2011 | Bark |
| **4** | *Pavonia odorata* | Salem district | Aug 2011 | Root |

**Plant anatomical studies**

Plant anatomical studies were done for *Tribulus terrestris*, *Aerva lanata* root, *Pavonia odorata* root, *Crateva religiosa* bark.

**Morphology of *Tribulus terristris L***

**Family**: Zygophyllaceae

It is an annual herbal plant with a long, slender, branched tap-root with a greenish-red stems up to 2 m long, branched, radiating from a central axis and covered with fine hairs. The leaves are 3-7 cm long, in opposite pairs with one pair slightly smaller than the other. Each leaf has a oblong-lanceolate leaflets, each leaflet being 5 to 15 mm long and 3 to 5 mm wide. The fruit is a schizocarp, which develops from a five loculed ovary. There are five ovules in locule situated on axile placentation. The fruit is pentagonal in outline with five cocci which are hard and woody. Each coccus has three long thick parallel spines.

**Microscopic examination of *Tribulus terristris L* fruit**

The schizocarp bears three pairs of horn shaped spines which are in opposite poles Figure (2). The spines are tapering and pointed at the tip. They are 2.5 mm long and 350 µm thick at the base and in between the spines, there is a thick hemispherical outgrowth. In between the opposite pair of spines, there are three conical ridged structures, the central outgrowth is more prominent. The diameter of the fruit along the ridges is 2 cm. The spines have a thin epidermal layer with small less prominent squarish vertically elongated cells. There is a thick vertical row of vascular elements running along the median part of the spine.

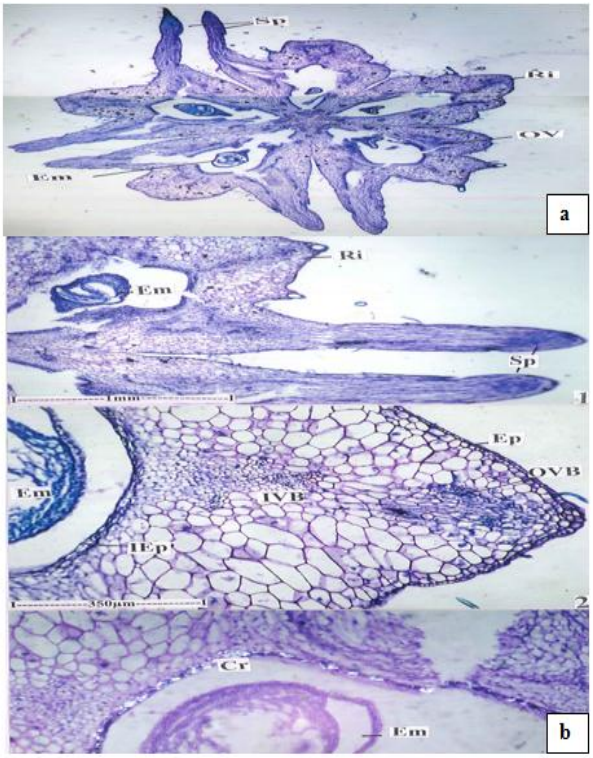


**Figure (2): T.S. view of *Tribulus terristris L* fruit.**

a. The entire view of the fruit of *Tribulus terrestris* were Em -embryo; OV-Ovary; Ri-Ridge; Sp-Spine
b. The view of the carpel and its embryo of *Tribulus terrestris* fruit- a ridge of carpel and a pair of spines, enlarged Ridge- enlarged

(Em- Embryo; Ep- Epidermis; Cr-Crystal; IEP-Inner epidermis; IVB-Inner vascular bundle; OVB- Outer vascular bundle; Ri-Ridge; Sp-Spin).

The ridges are 800 µm thick and 950 µm wide. The ground tissue of the ridges includes large, polygonal, thin-walled compact parenchyma cells and the innermost layer, enclosing the ovule is the inner epidermis. There are two vascular strands in the side, one being within the conical edge and the other being in the inner part. There is a single seed (at maturity of the fruit) in each carpel. Calcium oxalate prismatic crystals are seen located in the inner epidermal layer of the carpel.

**Morphology of *Aerva lanata***

**Family:** Amaranthaceae

*A. lanata* is a branched plant with white to pale pink spikes of clusters of flowers that is 1 to 1.5 inches long. The herb is a common habitat throughout India, Ceylon, Arabia, Tropical Africa, Java, Philippines. *Aerva lanata Linn*. is a branched hardy woody herb with 30 to 80 cm height, the main stem is short but stout and woody at the base and rise to form 4 to 10 or more elongate hairy branches. *A. lanata* can be differentiated from other species on the basis of morphological features such as, perianth lobes position of the spikes on the plant, phyllotaxy of the leaves and habitat of the plant species.

**Microscopic examination of *Aerva lanata* root**

Roots of varying thickness were taken for the study. All the roots have distinct periderm of varying thickness, narrow cortex and secondary growth of unusual type in Figure (3).

In a thin root, the epidermis is broken at several places exposing the inner tissues. Periderm is seen beneath the broken and intact regions of the epidermis. It consists of about four layers of thin-walled suberised cells arranged in radial files. The cortex is narrow and is only two or three layered. The xylem cylinder is dense and circular comprising of few solitary scattered vessels with varying diameter. A second outer cylinder is the xylem-phloem that starts to develop in the outer region of the first formed central cylinder. In the fairly thick roots, the second ring of xylem and phloem is well developed. The second cylinder has 6 or 7 clusters of wide vessels alternating with the segments of fibres. A third cylinder is seen originating from cortical tissue lying outside the secondary cylinder. The second and third cylinder may be a broken circular ring or may be in the form of arcs which lie in the opposite places of the first formed central cylinder. Thus there are several successive cylinders of vascular tissues. The number of cylinders depends upon the thickness of the root. This type of origin of several successive cambia from the cortical zone is said to be an anomalous of secondary growth. Each cylinder consists of discontinuous clusters or radial short rows of vessels and dense fibres. The outer border of the ring occurs in the secondary phloem whose vessels are circular, narrow and thick walled.

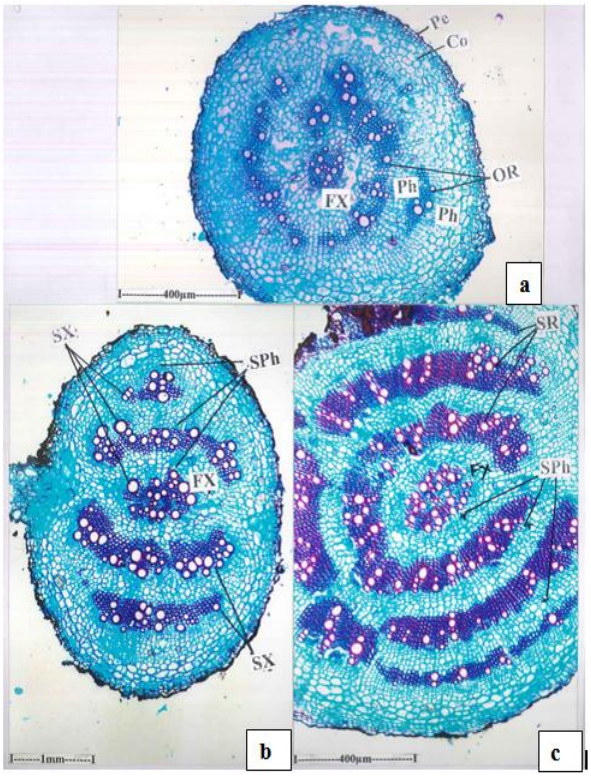


**Figure (3): T.S. view of *Aerva lanata* root.**

a. TS of root with two central masses of first formed vascular tissues.
b. Central mass and two brackets of successive vascular tissues
c. Three successive rings of xylem-phloem

(Co-cortex; FX-first formed xylem; OR- outer ring of vascular cylinders; Pe-periderm; Ph- phloem; SPH-secondary phloem; SR- successive ring of secondary xylem-phloem; SX- secondary xylem).

**Morphology of *Crateva religiosa***
**Family: Caparaceae**
Crataeva religiosa (Varuna/Mother Tincture) is a medium-sized tree that grows up to 10 metres long that grows well along the streams. The bark of the tree has 2-3 mm longitudinal wrinkles. The bark is smooth and greyish-white in colour with bisexual and creamy white flower. It has tri-foliate leaves that alternate and ex-stipulate. The branches have white patches with purple or yellow tinges with oval leaflets, and there flowers have fragrance.

**Microscopic examination of *Crateva religiosa* bark**
The sections of stem bark were obtained by usual techniques. The bark is 4.6 mm thick. The surface is shallowly fissured; otherwise the bark surface is smooth and even the periderm of the bark is 250-µm thick. The periderm includes outer part of scaled off pieces of dry scales and inner intact part. The intact periderm consists of about six layers of phellem and inner several layers of phelloderm. The phellem cells are dead suberised cells, while the phelloderm cells are living with storage materials. The phelloderm cells occur in regular compact radial rows. The outermost layer of the phellem contains fairly large calcium oxalate prismatic crystals displayed in Figure (4).

The secondary phloem is the wider and major part of the bark. It is differentiated into two regions Outer collapsed phloem and the inner non collapsed phloem The collapsed phloem is wider than other regions of the bark. It includes thick dark tangential blocks of crushed and compressed sieve elements; dilated wavy phloem rays and enlarged parenchyma cells. Large irregular masses of sclerenchyma elements are seen scattered in the outer part of the collapsed phloem. While the non collapsed intact phloem is narrow and occurs in between the collapsed phloem and cambial zone. It consists of well preserved, sieve elements, narrow phloem rays and undilated parenchyma cells.

TLS (Tangential longitudinal section) of the phloem: In longitudinal sections, the phloem rays, sieve tubes and parenchyma cells were studied. The phloem rays are in levels of arrangement. They are multi seriate rarely biseriate; the rays have more than two rows of cells. The rays are homocellular, comprising all similar type of cells. The multi seriate rays are 200-400 µm in height and the width is 100 µm. The biseriate rays are 100 µm in height and 50 µm in thickness. The sieve tubes are 200 µm in height and 30 µm wide. The sieve plate is oblique. The axial parenchyma is transversely septate forming strand parenchyma.

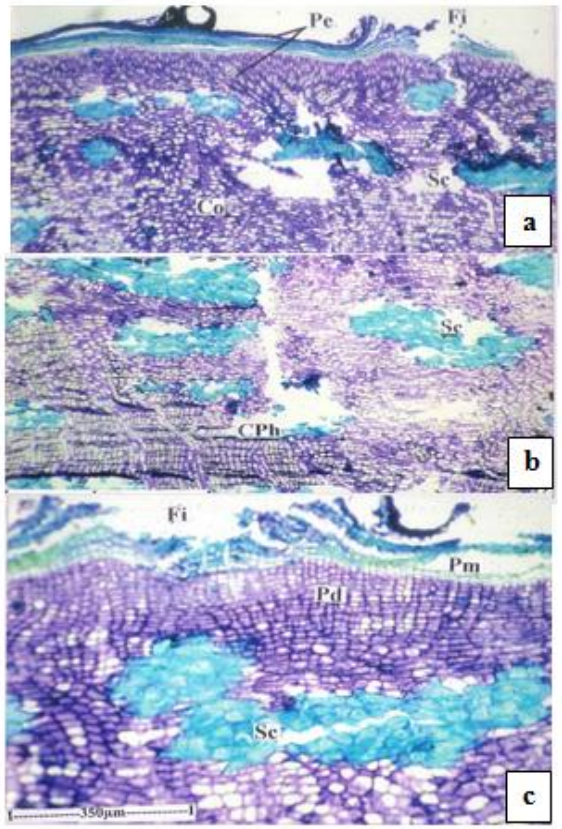


**Figure (4): T.S. view of *Crateva religiosa* bark.**
a. Middle collapsed phloem zone
(co-cortex; cph- collapsed phloem, Fi- fissure; Neph-non collapsed phloem; Pe- periderm; Sc- sclerenchyma)
b. TS of bark Outer periderm portion
(cph- collapsed phloem; DR- dilated rays; Fi- fissure; Pd-phelloderm; Pm-phellem; Sc- sclerenchyma).
c. Prismatic crystals in the phellem cells, inner collapsed phloem and dilated rays
(cph-collapsed phloem;cr-crystals; DR-dilated rays; Pa-parenchyma; Pd- phelloderm; Pm- phellem; PhR-phloem rays)

**Morphology of *Pavonia odorata***
**Family: Malvaceae**
*P. Odorataisan* annual branching plant with an approximate height of 45-90 cm, stems are viscously covered with pubescence and short hairs. Leaves are 2.5-7.5 cm long, 3-5 lobed, ovate in shape, the lower ones are entire, stellate-hairy on both surfaces; lower petioles are longer than the blades. The flower is pink in colour, twice longer than the sepal cup. The fruits are spherical with smooth mericarps and roots are brown, rough with diverse sizes. Fragrant Swamp Mallow is the habitat of India, Pakistan, Burma, Srilanka and East Tropical Africa.

**Microscopic characters of *Pavonia odorata* root**

Thick root Figure (5) with wide secondary xylem cylinder was studied. The root consists of periderm, cortex, secondary phloem and secondary xylem. Periderm is superficial and continuous around the root. These are narrow, irregular, shallow fissures on the periderm. The periderm consists of about six layers of phellem cells which are irregular radial rows; their walls are thick and suberised. The periderm is 80 µm thick. Inner to the periderm, occur a narrow region of two or three layers of cortical cells. The cells are thick walled and densely filled with mucilage. The secondary phloem is 450 µm thick. It includes outer region of collapsed cells and cells with large druses of calcium oxalate.

There are also thick masses of fibres diffusely distributed with phloem tissue. The Secondary xylem is thick and dense cylinder occupying a major portion of the root. It exhibits fairly distinct growth rings which are of ring porous type. The growth ring boundaries are demarcated by narrow, thick walled solitary vessels. The vessels are circular, fairly thick walled, mostly solitary, less frequently multiples of two and are 20-60 µm in diameter. The xylem fibers are thick walled and lignified. The cell lumen is wide. The xylem rays are narrowly elliptical and elongated; their walls are also lignified.

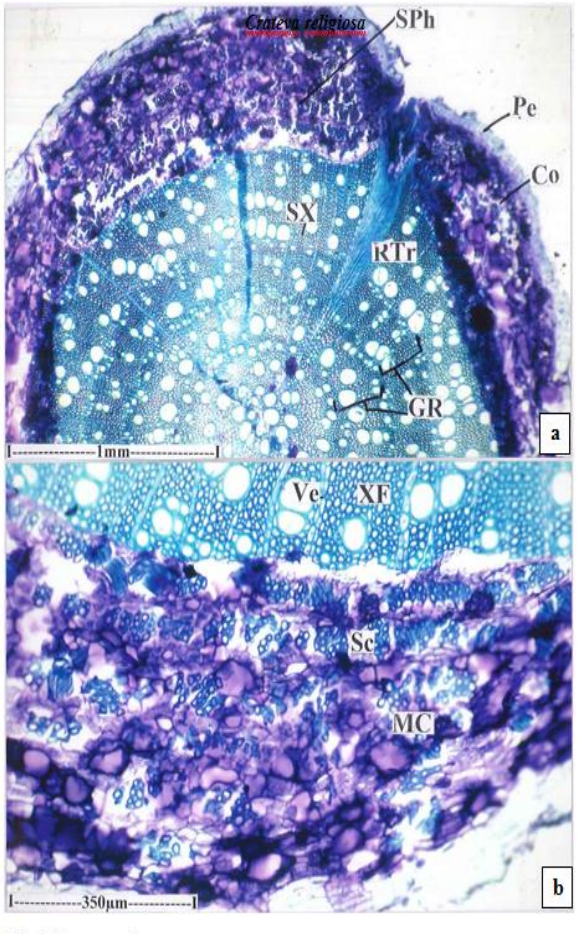


**Figure (5): T.S. view of *Pavonia odorata* root.**

a. The half root portion
b. T.S of secondary phloem with dense accumulation of mucilage
(co-cortex; GR-growth ring; MC-mucilage cells; Pe-periderm; Sc- sclerenchyma; Sph- secondary phloem; Sx- secondary xylem; Ve-vessel; XF- xylem fibres

The herbal drugs used in this Kalladaippu Kudineer are *Tribulus terrestris, Aerva lanata, Creteve religiosa and Pavonia odorata*. The herbal drug with aqueous extract of *T. terrestris* is helpful in restoring the phosphate level in the body and thus reducing the risk of stone formation and it is believed to have a role in causing diuresis, process of dissolving the preformed stones, and increased the excretion of urea, creatinine, and uric acid and would result in the normalization of kidney functions.[10,11] When raw materials like plant drugs and salts are sourced from the traders either whole and specific parts used, the purity and genuineness of the drug have to be evaluated before proceeding to further studies. In drug standardization macroscopic and microscopic evolution are the important criteria. Quality control of herbal drugs has traditionally been solely based on their physical appearance alone and in recent times microscopic evaluation is indispensable in the initial method in identification of herbs, as well as, small fragments of crude or powdered herbs that can effectively detect foreign matter or adulterants. Though it seems to be obvious, it is of prime importance, especially when different parts of the same plant are to be used for different treatments.[12]

In the present work the herbal drugs were collected and authenticated both physically and anatomically because while collecting the plants from the field the physical specific or external features identification may not be sufficient for authentication. A similar authentication study was performed for the root of *Plumbago Roseus* which is used in the treatment of colic inflammations bronchitis, helminthiasis, hemorrhoids, elephantiasis, hepatosplenomegaly, amenorrhea, odontalgia, piles, and diabetes.[13]

## CONCLUSION

The potency and quality of the finished product is dependent on the individual herbal ingredients used. Visual identification yields only a macroscopic identification and authentication for lookalike materials needs microscopic identification. It is very important step to authenticate the herbal plant at a species level and it is an important procedure to be followed during drug standardization. This present study had shed a light into the importance of the microscopic identification of herbal plants used in Kalladaippu kudineer at a species level.

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