Microscopic authentication of Ayurvedic medicinal plant Thumari (Securinega leucopyrus [Wild.] Muell)

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Abstract:
Traditional healing arts are generally based on a single medicinal plant drug or multiple drugs. The formulae used in some Ayurvedic and Siddha drug preparations are taken even from palm – leaf manuscripts. In such circumstances, the medicinal plant used for the preparation of a drug should be authentic and genuine. The evaluation or standardization of a crude drug involves classical microscopic methods. For the better quality control and standardization processes, the present study attempts anatomical authentication of Ayurvedic plant Thumari. Transverse section of leaf shows adaxial palisade cells consists of circular chambers possess single large calcium oxalate druses. The petiole consists of thick and prominently papillate epidermal layer of cells. In root the xylem fibres are mostly gelatinous type having thin mucilaginous inner secondary walls. The investigations recorded in the present study provided the basis for microscopic authentication of Ayurvedic plant Thumari.
Key words: 1. Anatomy, 2. Authentication, 3. Ayurvedic medicine, 4. microscopy, 5. plant identification

Introduction:

Thumari *(Securinega leucopyrus* [Wild.] Muell) (Synonym: *Flueggea leucopyrus* Willd.) is an erect shrub and distributed in India, Pakistan, Sri Lanka and Myanmar. It belongs to the family Euphorbiaceae (as per APG IV, Phyllanthaceae). It is traditionally used in Ayurveda and Siddha system of medicine. It is commonly known as Katupila, Thumri in Sanskrit, Humari in Hindi, Velaipula in Tamil, Perinklavu in Malayalam, Shinavi in Gujarat and "Spinous fluggea" in English. Indigenous people use the leaf paste for the treatment of wound. Various medical literature of the Indian system of medicines describe the utility of this plant for the treatment of diseases related to Pitta, Shushka kasa, buring sensation, Tamaka Swasa, bowel complaints, kidney stones, artavadushti and general debility.[1-5]

In recent years, the demand for herbal drugs is exploding in the world trade market. This resulted in adulteration and substitution of original drugs. The identification of raw herbal drugs is also a major bottleneck in the drug development. To guarantee the efficiency, purity and quality of traditional medicinal plants require correct identification and authentication.[6] Botanical standardization methods are useful for identification and helpful to determine the purity and quality of herbal drugs.[7,8,9] Different Indian Pharmacopeias describes the significant microscopic methods for identification and authentication of fragmented herbal drugs.[10-19] The present study provided the microscopic authentication of Ayurvedic plant Thumari.Many clinical studies for the use of selected plant in wound healing prove its significant effects in treatment of different Dushta Vrana. In a clinical study, 45 years old male patient with non-healing Beurger's ulcer at lateral malleolus of left foot healed completely in four months. The blackish discoloration of left lower leg changes to normal.[20] The safety and efficacy of Thumari gel for the treatment of chronic non-healing diabetic wound was reported by Ghodela et al[21]. In another significant study on Thumari Taila, complete wound healing of diabetic foot ulcers in a 68-year-old female patient was achieved with unit healing time of 12.79 days/cm^3 in 60 days.[22] Thumari oil has Savarnikaran property and it was experimentally proved in the management of Stanagatvrana.[23] These case reports suggested the utility of Thumari and need for the present study.

Materials and Methods:

Plant materials:

The healthy plants collected from various locations in Tirunelveli district. The specimens were identified with reference to the Flora of Presidency of Madras.[24] and Flora of Tamil Nadu Carnatic[25]. Specimen voucher of the plants are kept at the Xavier's College Herbarium (XCH 26586) St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu.

Micropreparation:

Various organs such as leaf, petiole, young and old roots and stems were cut and fixed in FAA (5 ml formaldehyde solution + 5 ml glacial acetic acid + 90 ml 70% ethyl alcohol). After 24 hours of fixation, the samples were passed with graded series of tertiary butanol according to Sass.[26] For microtome sectioning, the samples were prepared into paraffin blocks and dewaxing by the technique given by Johansen.[27] The sections were stained with toluidine blue according to the standard technique.[28] Paradermal sections were prepared (segments taken parallel to the surface of the leaf) to study stomatal morphology, venation pattern and trichome distribution by utilizing Jeffrey's maceration liquid.[26] Microphotographs of various magnifications were reported with Nikon Labphoto 2 Microscopic unit. Descriptive terminology for the anatomical description are referred from standard literature.[29, 30]

Results:

Leaf Anatomy:

a) **Structure of midrib:**

Leaf consists of fairly prominent midrib and thick lamina. The midrib has single, semicircular vascular strand (Fig: 2a). The midrib is flat on the adaxial side and slightly conical on the abaxial side. The midrib is 30 µm thick. The adaxial epidermis in the region of the midrib comprises of smaller squarish thin walled cells. The abaxial epidermis contains prominently conical outer tangential walls of the epidermis. The vascular bundle is bounded by parenchymatous ground tissue which outspreads adaxially into thick pillar of bundle sheath extension.

The vascular strand comprises of dense and dense compact xylem elements which are circular thick walled and lignified. Phloem occurs on the lower part of the xylem strands. The phloem elements are very narrow, darkly strained and compact. The vascular bundle is 80 x 100 µm in size.

b) Structure of the Lamina:

The lamina is 160 µm thick. It includes wide and thick adaxial epidermal layer of cells with fairly thick walls. The abaxial epidermis comprises of broad cells with conical outer tangential walls (Fig: 2b). The mesophyll tissue contains an adaxial band of 2 layers of cylindrical compact cells. There is lower band of palisade cells which includes 2 layers of columnar cells.

In the median part of the lamina occur 2 or 3 layers of spherical or slightly lobed hyaline spongy parenchyma cells. Some of the adaxial palisade cells become highly dilated into circular, chambered called idioblasts. These chambers possess single large calcium oxalate druses. Small druses also occur in the median spongy parenchyma cells.

c) Structure of the leaf margin:

The marginal part of the lamina is bluntly conical and the basic structure is similar to median part of the lamina. The marginal part of the lamina consists of wide adaxial epidermal cells with thick cuticle. The abaxial epidermal cells are broadly conical. The mesophyll tissue includes adaxial and abaxial palisade layer and median spongy parenchyma. The leaf margin is 130 µm thick.

d) Structure of the Venation Pattern:

Venation of the lamina is densely reticulate. Veins forming the vein islets are uniformly thin (Fig: 2c). The vein islets are variously shaped and have district vein boundaries. The vein terminations are either simple or unbranched. Branched vein islets are farming two equal or unequal ends of the terminations.

e) Structure of the Epidermal Cells and Stomata:

In surface view, paradermal sections of the lamina are polygonal in outline with thick straight anticlinal walls. The adaxial epidermis is apostomatic (Fig: 2d). The abaxial epidermal cells are comparatively smaller in size with thick straight anticlinal walls. The epidermis is stomatiferous. The stomata are mostly paracytic with subsidiary cells occurring on both side and parallel to the guard cells. The guard cells are elliptical measuring 10 X 20 µm in size.

f) Structure of the Petiole:

The petiole is semicircular in cross sectional view measuring 60 µm in thickness. The petiole consists of thick and prominently papillate epidermal layer of cells. The ground tissue is homogenous and parenchymatous. The centre of the petiole occurs fairly prominent semicircular vascular strand (Fig: 3a).

The vascular strand is collateral comprising upper strand of xylem elements and lower strand of phloem elements. Xylem elements occur in several parallel rows. The elements are angular, wide, and thick walled. The phloem elements are in small and isolated clusters.

Stem Anatomy:

g) Structure of the stem:

Both young and mature stems were studied. The young stem measures 1.7 mm thick. It is circular in outline and includes thin and fairly prominent periderm, thin layer of secondary phloem and thick hollow cylinder of secondary xylem. The pith is wide and circular (Fig: 3b). The periderm is sub-superficial and occurs as their continuous layer of 5 or 6 cells in thickness. The epidermis and cortical region are broken forming wide fissures.

The inner part of the cortex comprises of a thin discontinuous layer of darkly stained sclereids. In the thick stem, the periderm consists of distinct layer of phellogen which has given rise to outer wide, squarish phellem cells and inner narrow phelloderm cells. Secondary phloem occurs discontinuous prominent masses around the xylem cylinder. Secondary phloem consists of rectangular size elements with prominent laterally situated companion cells.

h) Structure of the Secondary Xylem:

Secondary xylem of thick stem consists of inner zone of several radial chains of vessels and outer of solitary diffuse and wider vessels. The outer zone of the xylem cylinder actually represents secondary xylem of the stem (Fig: 3c).

Secondary xylem consists of wide, thin walled elliptical or circular vessels. The vessels are up to 30 µm diameter. Xylem fibre is arranged in compact radial lines. The cells are highly thick walled with wide lumen. Some of the fibre has lignified secondary walls. Fibres in some other region have gelatinous secondary walls. The xylem rays are thin straight. The cells are squarish or radially oblong and thick walled.

i) Structure of the Root:

In cross sectional outline the root appears circular with well developed vascular system and fairly thick bark. The root is 650 µm thick. The root comprises of prominent superficial zone of periderm comprising 4 or 5 layers of wide, tabular, thin walled and suberized phloem cells. The cortical zone is narrow occurs is this layer encircling the xylem cylinder.

The xylem cylinder is very thick and solid occupying the major portion of the root (Fig: 3d). The vessels are diffusely distributed and they are either solitary or short radial multiples vessels. The vessels are upto 30 µm diameter. The xylem fibres are mostly gelatinous type having thin mucilaginous inner secondary walls. Xylem rays are thin and straight. The ray cells are thick walled and either lignified or mucilaginous. Starch grains are abundant in the lignified xylem fibres.

j) Powder microscopy:

In powder preparation of the leaf and stem the following inclusions are seen.

Fragmentary lamina:

Small pieces of lamina are seen in the powder and exhibit distinct vein islets and vein terminations. The terminations are long unbranched or branched one.

Periderm cells:

Broken segments of periderm cells are seen in the powder. They appear in surface view. The cells of the periderm are in compact, regular parallel lines. The cells are square shaped and thick walled. The periderm cells are 50 x 30 µm in diameter.

Crystals:

Calcium oxalate crystals of prismatic type are seen within the lumen of the fibres. It is an unusual feature and the crystals are prismatic type which range from polyhedral to rectangular shape (Fig: 3e).

Fibres:

Xylem fibres are abundant in the powder. The fibres are either narrow or wide. Both narrow and wide fibres are long with tapering ends. The wide fibre has thin walls and wide lumen. The fibres are 450-650 µm long.

Vessel elements:

The vessel elements are also long, narrow and cylindrical. They have wide, elliptical and oblique end wall perforations. Pits are abundant in the lateral walls. They are multiseriate and alternate. The pits are elliptical in outline. The vessel elements are 250 µm long and 20 µm wide (Fig: 3f).

Discussion:

Some of the root drugs, leaf drugs and compound formulations have been attributed efficacy towards the immunity of the human body system. These drugs incidentally possess peculiar anatomical features. Shatavari *(Asparagus racemosus)* characterized by the presence of acicular crystals and raphides and Amla (*Emblica officinalis)* is detected by the presence of crystal masses. Guduchi *(Tinospora cordifolia)* is diagnosed by simple starch grains of varied size and shape with central hilum encircled by concentric rings. Bhumyamalaki *(Phyllanthus amarus) is* identified by the presence of calcium oxalate crystals and fibers with pointed end. Brahmi/gotu kola *(Centella asiatica)* is identified by its rosette type of crystals.[31]

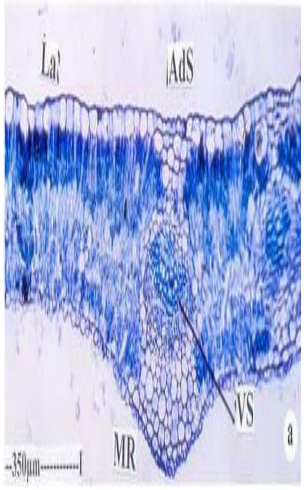
Standardization and maintenance of quality should start from the correct identification and botanical authentication of plant material. Macroscopic and microscopic characters of the leaves of *Alangium salvifolium* and histochemical tests revealed the occurrence of calcium oxalate crystals, starch and tannins in the ground tissue.[32] Microscopical, morphological and quantitative data of authentic samples of *Melaleuca leucodendron*.[33] *Berberis asiatica* is a very common substitute to "Daru Haridra" that is *B. aristata,* which is used in the Ayurvedic system of Medicine and therefore a study was carried out to identify the diagnostic features of *B. asiatica* root.[34]

Conclusion:

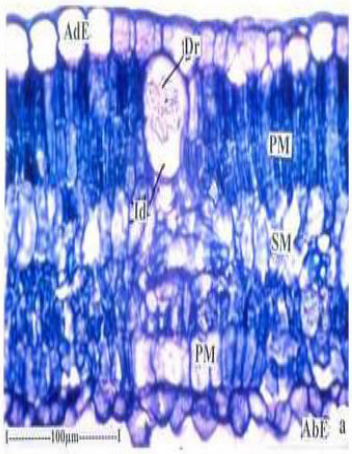
Microscopical study has highlighted the arrangement of different tissue system and helps to resolve botanical identity of the chosen plant specimen. The study of powder microscopy, crystals, starch grains, and lignified cells is of indispensible value in the identification of the pure powdered drug.

Fig: 1 a) Habit b) Flower c) Flowering twig d) Fruits setting on the twig

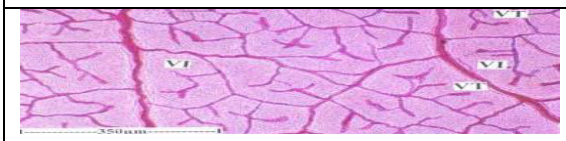
Fig 2: Anatomy of leaf



a) T.S. of leaf through midrib (10 x view)



b) T.S. of Lamina (10 x view)



c) Vein islets and vein termination (40 x view)

d) Paradermal section of abaxial epidermis (40 x view)

La- Lamina: MR – Midrib: VS – Vascular Strand: Ads – Adaxial side: AdE – Adaxial Epidermis: Dr – Druses: PM – Palisade Meophyll: SM – Spongy Mesophyll: AbE – Abaxial Epidermis: Id – Idioplast: VI – Vein Islets: VT – Vein Termination: EC – Epidermal cell: St – Stomata: SC – Subsidiary Cell

Fig 3: Anatomy of petiole, stem and root

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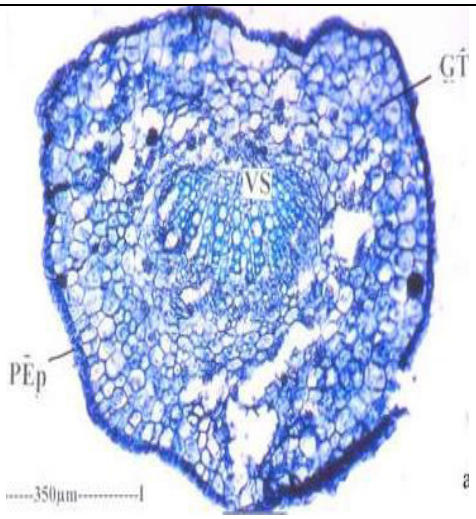
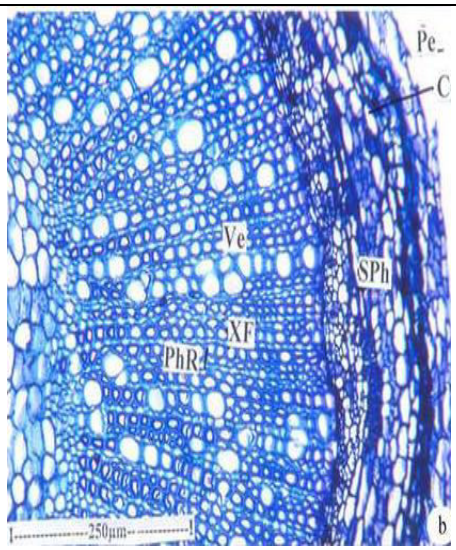
<tr><td> 
</td><td> 
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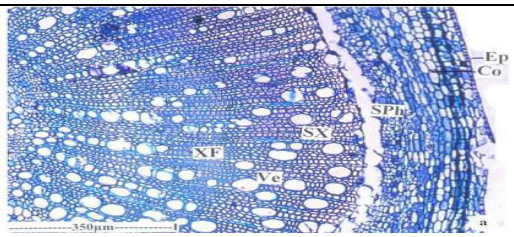
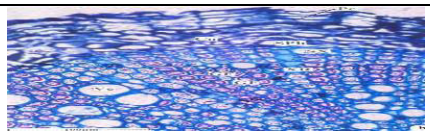
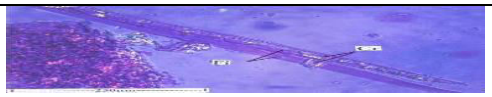
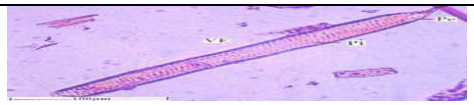
<tr><td>a) Ground plan of the petiole (10 x view)</td><td>b) T.S. young stem – a portion enlarged (40 x view)</td></tr>
<tr><td> 
</td><td>  </td></tr>
<tr><td>c) T.S. mature stem – a portion enlarged (40 x view)</td><td>d) T.S of root – a portion enlarged (40 x view)</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>e) Xylem fibre with calcium oxalate crystal embedded in the lumen (60 x view)</td><td>f) A vessel with simple end wall perforation (40 x view)</td></tr>
<tr><td colspan="2">GT – Ground Tissue: Pep – Papillate Epidermal cells: Ve – Vessel: XF – Xylem fibre: SPh- Secondary Phloem: PhR- Phloem Ray: Pe- Periderm: Co – Cortex: Ep – Epidermis: GF – Gelatinous fibre: Cr- Crystal: Pi- Pit</td></tr>
</table>

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Conflicts of Interest: The authors declare they have no conflict of interests.

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