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Research Article

MORPHO-ANATOMICAL PROFILE OF ELAEOCARPUS TUBERCULATUS ROXB

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ABSTRACT

Objective: The anatomical studies of *Elaeocarpus tuberculatus* Roxb. (*Elaeocarpaceae*), an indigenous medicinal plant in the Indian pharmacopoeia.

Methods: Fresh leaf and stem samples of *E. tuberculatus* were scrutinized macroscopic and microscopically by standard procedures.

Results: Macroscopically, the leaves are simple, long, coriaceous, and rusty below, base obtuse, margin serrate, apex obtuse and petiole 3 cm long. Microscopically, the leaf showed the presence of four prominent collateral bundles. Paracytic stomata were distributed in the abaxial epidermis whereas the adaxial epidermis was apostomatic. The petiole was four-angled including wing bundles. The stem exhibited fissured periderm, wide secondary phloem, secondary xylem with incipient growth ring and abundant calcium oxalate crystals. Powdered microscopic study of the leaf revealed the presence of long whip-like epidermal trichomes, crystals, vessel elements showing pits and fiber-like sclereids. The stem powder showed thick-walled fibers with wide lumen, narrow fibers and a sclerenchyma cell showing wide pits.

Conclusion: The results of the study can serve as a valuable source of information and provide suitable standards for the identification of *E. tuberculatus* in future investigations and applications.

Keywords: *Elaeocarpus tuberculatus*, Leaves, Trichome, Stomata, Crystal, Sclereids.

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INTRODUCTION

Plants consist of various phytoconstituents which exert therapeutic properties [1]. In the past few years, alternatives to conventional medicine like herbal therapy have increased in popularity due to their lesser side effects [2]. Furthermore, accurate plant identification and authentication play crucial roles in ensuring the safety and efficacy of herbal treatments. *Elaeocarpus tuberculatus* Roxb. is a majestic tree about 80 feet high and 7 feet in girth distributed from South and East Asia through Malaysia to Australia and the Pacific Islands. In India, the species is widely available in the Western Ghats particularly common in Nilgiri, Palni, and Annamalai hills. Decoction of the stem bark is used as a remedy for rheumatism, indigestion, and biliousness. Rudraksa beads or seeds are used as a treatment for rheumatism, typhoid fever, and epilepsy, controls heartbeat, stress, anxiety, depression, palpitation [3]. Various species of *Elaeocarpus* have been known to possess antimicrobial [4,5], anti-arthritic [6,7], anti-diabetic activities [8,9], anti-inflammatory and anti-ulcer activity [10]. Survey of the literature revealed that no anatomical studies have been carried out in this plant. Hence, the present investigation was undertaken to study the morphological and anatomical characters of *E. tuberculatus* which serves as a basis of establishing the correct identity of this plant.

METHODS

Plant material

The leaf and stem of *E. tuberculatus* Roxb. were collected from the Upper Palani Hills of Western Ghats (Kodaikanal Forest Division), Tamil Nadu, India, and were authenticated at Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and the herbarium of Voucher specimen number BSI/SRC/5/23/2011-12/Tech.239 has been deposited at the Department of Botany, Vellalar College for Women, Erode (Tamil Nadu), India, for future reference.

Macroscopic study

The fresh aerial plant parts of *E. tuberculatus* Roxb. were studied individually for its morphological characters [11] in the field and photographed under the original environment and evaluated botanically.

Microscopic study

Preparation of specimens

Care was taken to select healthy plant parts of *E. tuberculatus*. The fresh sample of different parts (leaf and stem) was cut into small pieces and fixed in FAA solution (Formalin-5 mL + Glacial acetic acid-5 mL + 70% Ethyl Alcohol-90 mL) as per the schedule given by Sass (1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58–60°C) tertiary butyl alcohol (TBA) until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin-embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10–12 µm. De-waxing of the sections was done by customary procedure [12]. The sections were stained with toluidine blue as per the method published by O'Brien *et al*. [13].

Staining

For anatomical studies, the following staining schedules were followed: Toluidine blue stain was prepared by dissolving 0.25 g of the stain in the mixture of benzoic acid 0.25 g, sodium benzoate 0.29 g and distilled water 200 mL with pH of 4.2–4.4. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color 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). (IKI-lugol's iodine is a brown solution that turns black in the presence of starch). For studying stomata morphology, venation pattern, and trichome distribution, paradermal sections (sections

taken parallel to the surface of leaf) as well as clearing of the leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid [14] were employed. Glycerin-mounted temporary preparations were made for macerated materials. Powdered materials of different parts (leaf and stem) were cleared with NaOH and mounted in a glycerin medium after staining. Different cell components were studied and measured.

Photomicrographs

All permanent slides, after staining were dehydrated using graded series of Ethanol + Xylol and mounted in DPX. Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo-2 microscopic using Konica color film (100ASA). For normal observations, bright field was used. For the study of crystals, starch grains, and lignified cells, polarized light was used since these structures have a birefringent property and appear bright against the dark background. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical features were taken from the standard anatomy books [15].

Microscopic study of powder plant parts

The leaf and stem were boiled with chloral hydrate for 5–10 min, and then stained with phloroglucinol, and toludiene and observed for the microscopic features under high power (×40) [12-15].

RESULTS AND DISCUSSION

Macroscopic characteristics

Macroscopically, the fresh leaf of *E. tuberculatus* is 20 × 9 cm and petiole 3 cm in length, simple, coriaceous, rusty below, base obtuse, margin serrate, apex obtuse, and green in color. Inflorescence is pendulous, massive, and rusty. Racemes up to 14 cm, below the foliage, 15-flowered; peduncle 2 cm. Flowers 2 cm wide, nodding; pedicel 2 cm. Sepals 5, lanceolate, margin recurved. Petals 5, cream, obovate; apical frills 20. Disk 10-angled. Stamens 60, in 3 whorls. Ovary globose, 2-celled; ovules 8 per cell. Fruits are massive, persisting into the next season. Fruit is drupe ellipsoid, laterally compressed; seed 1, ellipsoid [9] (Plate 1).

Microscopical characteristics

Leaf (Figs. 1 and 2)

The leaf consists of a thick and broad midrib which is wavy in outline (Fig. 1a). The midrib is more projecting on the abaxial side and slightly raised on the adaxial side. The midrib measures 1.1 µm thick and the adaxial part is 550 µm wide; the abaxial part is nearly 1 µm wide. The midrib has multistranded complex vascular system. The abaxial part is lobed with thick ridges and wide furrows. The epidermal layer of the midrib is thick; the cells are small and darkly stained. The ground tissue is parenchymatous wherein the cells are circular to angular, thick-walled and compact. A thick dark layer of crushed cells is seen in the median part of the ground tissue. The vascular strands are organized into a wide bowl-shaped abaxial part, a slightly curved adaxial horizontal plate, and a medullary band of bundles (Fig. 1b).

Abaxial bowl-shaped line of bundles consists of several discrete xylem strands with convex outer ends. Each strand includes 4–6 short parallel rows of xylem elements. On the convex outer ends are located large expanded masses of phloem. The phloem masses comprise dense small phloem elements with radial rows of dilated, hyaline parenchyma cells. These radial rows of parenchyma cells clear the vascular bundles into independent units. Adaxial horizontal plates of bundles are in a continuous undulate band comprising of thick layer of phloem located on the outer surface of the xylem strand (Fig. 2b). Medullary system consists of four prominent collateral bundles with adaxial bands of xylem and wide abaxial zone of phloem elements and dilated parenchymatous cells.

Lamina (Fig. 3)

The lamina is distinctly dorsiventral. Epidermal trichomes are sparsely seen on the abaxial surface (Fig. 3a). The trichomes are unicellular, unbranched, thick-walled, straight, or curved. The trichomes are 250 µm long 10 µm thick. The lamina is 250 µm thick. The adaxial epidermal layer includes wide, thick-walled vertically oblong rectangular cells (Fig. 3b). The palisade zone consists of three or four horizontal rows of columnar cells; the total height of the palisade zone is 150 µm. The spongy parenchyma consists of 5 or 6 layers of spherical cells arranged in vertical chains forming wide air spaces. Venation Pattern (Fig. 4): The veins are thick and form dense reticulate venation. The vein islets are narrow, formed by thick straight vein boundaries. The vein terminations are thick, stumpy, straight or curved, simple or curved (Fig. 4a and b).

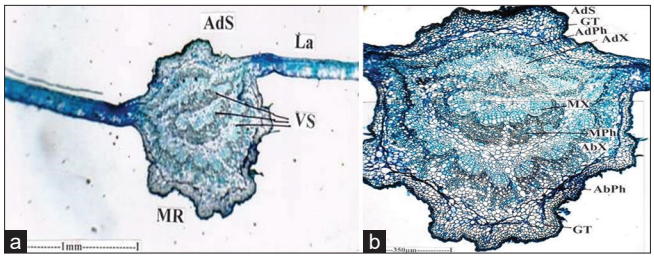

Fig. 1: (a) T.S. of leaf through midrib, (b) T.S. of midrib enlarged. Abx: Abaxial xylem, Abph: Abaxial phloem, Ads: Adaxial side, Adph: Adaxial phloem, Adx: Adaxial xylem, GT: Ground tissue, MR: Midrib, La: Lamina, MX: Medullary xylem, VS: Vascular strands, TS: Transverse section

Plate 1: *Elaeocarpus tuberculatus Roxb.* - Habit

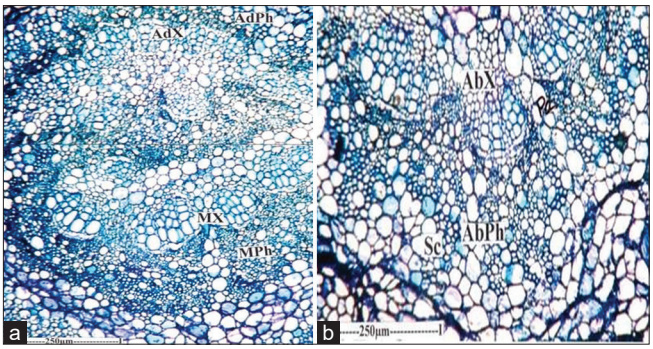

Fig. 2: (a) T.S. of midrib upper vascular system enlarged, (b) Lower vascular system enlarged. Abx: Abaxial xylem, Abph: Abaxial phloem, Adph: Adaxial phloem, Adx: Adaxial xylem, Sc: Sclerenchyma, Pa: Parenchyma, MPh: Medullary phloem, MX: Meddulary xylem, TS: Transverse section

Epidermal cells and stomata (Fig. 5)

The abaxial epidermis shows dense distribution of stomata. The epidermal cells are, thick-walled and the anticlinal walls are straight (Fig. 5a). The stomata are paracytic type; the stoma has two subsidiary cells, lying parallel to the guard cells. The stoma is broadly elliptical and the stomatal pore is wide. The stomata are 15–20 × 8 µm in size (Fig. 5b). The adaxial epidermis is apostomatic (Fig. 5c). The epidermal cells are small and have thick straight and anticlinal walls. The cells are polygonal in shape.

Petiole (Figs. 6 and 7)

The petiole is four-angled in sectional view. It is 2.2 µm thick. It consists of the outer boundary of a thick dark zone (Fig. 6a). This dark zone consists of a dense mat of short tanniniferous trichomes. The ground tissue is homogenous and parenchymatous. The cells are circular, thick-walled, and compact (Fig. 6b). The vascular system is a complex multi-strand type. It includes wing bundles, central circle of medullary bundles, and abaxial semicircular bundles. The wing bundles are a group of three strands, the bundles semicircular surrounded by a thick layer of sclerenchyma (Fig. 6b). In the central portion is a single circular bundle, enclosed by a wide ring of several bundles. Beneath the central core of bundles is a shallow row of bundles followed by another arc of abaxial bundles (Figs. 6a, 7a and b). All the bundles are collateral with parallel lines of xylem and thick conical masses of phloem.

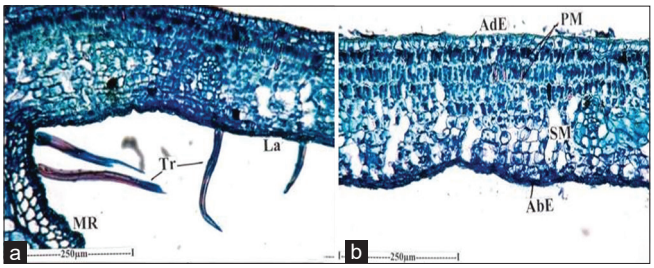

Fig. 3: (a) T.S. of lamina with trichomes, (b) T.S. of lamina. AbE: Abaxial epidermis, AdE: Adaxial epidermis, Tr: Trichome, La: Lamina, MR: Midrib, SM: Spongy mesophyll, PM: Palisade mesophyll, TS: Transverse section

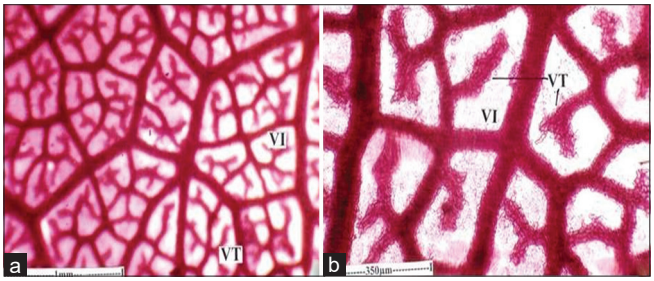

Fig. 4: (a and b) Venation system showing vein islet and vein termination. VI: Vein islet, VT: Vein termination

Stem (Figs. 8-10)

The stem exhibits the following zones: Periderm is superficial zone of 100 µm thick. It is fissured at several places and exfoliating thick pieces of periderm. The cells of the periderm are thick-walled, small, suberized, and tubular in shape (Fig. 8a and b). The cortical zone is heterogeneous. The outer cortex is parenchymatous with tangential files of cells and the inner cortex is a wide and sclerenchyma cells mixed with parenchymatous cells. Secondary phloem (Fig. 9a) is a wide and continuous cylinder comprising of radial compact files of sieve elements and parenchymatous cells. The sieve elements are wide and angular in outline. There are thick lines of phloem rays. The secondary xylem exhibits incipient growth ring (Fig. 8a). The xylem includes solitary or short multiples of three or four vessels. The vessels are 40–80 µm wide. Xylem rays are thick and run straight excepting the regions of the vessel where they bend and continue (Fig. 9b). Xylem fibers are thick-walled and lignified, the lumen being wide. Calcium oxalate crystals of druses are abundant (Fig. 10). In the petiole the crystals are located in the phloem tissue (Fig. 10a). In the stem, they are found in regular parallel lines and within the phloem ray cells (Fig. 10b).

Powder microscopic characteristics (Figs. 11-13)

The macerated preparation shows the following elements: The long whip-like epidermal trichomes are frequently seen. They are unicellular, thick-walled and unbranched. Sometimes the crystals are seen adhering over the trichomes (Fig. 11a and b). Cylindrical vessel elements are very common (Fig. 12a and b). They have short or long tails. The perforation is simple, oblique. Pits on the lateral walls are circular, densely crowded, and alternate. The vessel elements are 200–450 µm long.

iii) Wide and narrow fibers are dominant cells in the stem powder. The wide fibers have thick walls and wide lumen (Fig. 13a). They are 500 µm long and 100 µm wide. The narrow fibers are thick-walled with reduced lumen. They are 750 µm long and 5 µm thick. Fiber-like sclereids are rarely seen. They have thick walls with wide canal-like pits (Fig. 13b and c).

Ethno-medically, the leaves and stem of *E. tuberculatus* plant were used by local people in the treatment of various ailment conditions without standardization. The World Health Organization norms an examination to determine the sensory, macroscopic, and microscopic characteristics is the first step towards establishing the identity and the degree of purity of medicinal plant materials and should be carried out before any further tests are undertaken [16,17]. In the present study on *E. tuberculatus*, a detailed analysis was made on the anatomical features of this taxon.

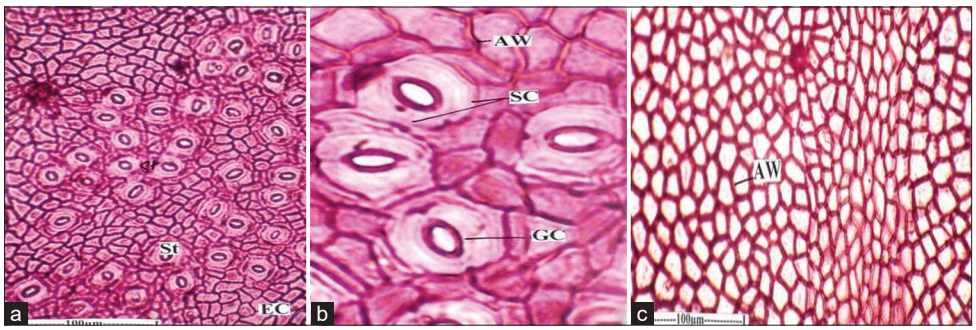

Fig. 5: Paradermal section of leaf, (a) Abaxial epidermis showing stomata, (b) Stomata enlarged, (c) Adaxial epidermis. AW: Anticlinal wall, GC: Guard cell, EC: Epidermal cell, St: Stomata, SC: Subsidiary cell

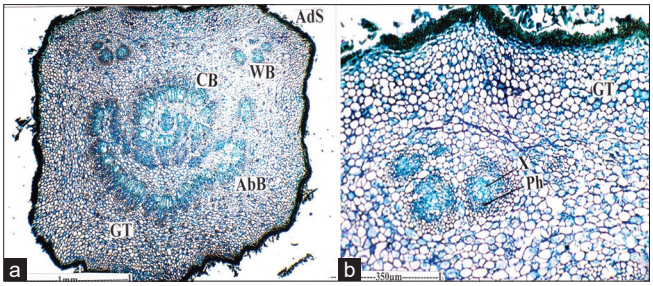

Fig. 6: (a) T.S. of petiole entire view, (b) Wing bundles of the petiole. AbB: Abaxial bundle, Ads: Adaxial side, CB: Central bundle, WB: Wing bundles, GT: Ground tissue, Ph: Phloem, X: Xylem

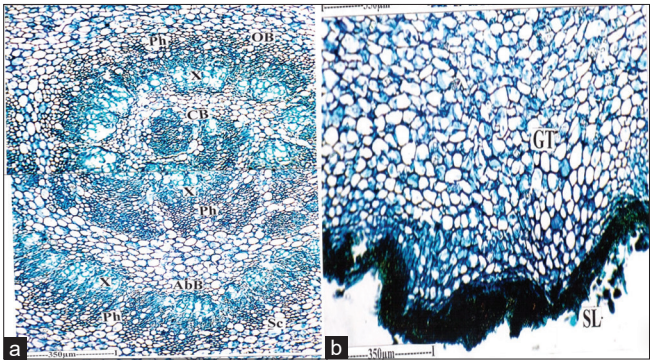

Fig. 7: (a) Central portion of the petiole enlarged, (b) Lower ground tissue of the petiole. AbB: Abaxial bundle, CB: Central bundle, GT: Ground tissue, OB: Outer bundle, Ph: Phloem, X: Xylem

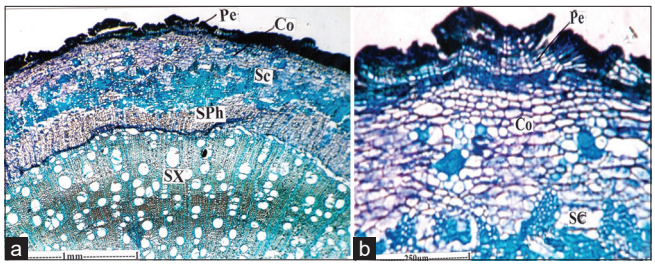

Fig. 8: (a) T.S. of stem portion enlarged, (b) Periderm and cortex enlarged. CO: Cortex, Pe: Periderm, Sc: Sclerenchyma, Sph: Secondary phloem, SX: Secondary xylem

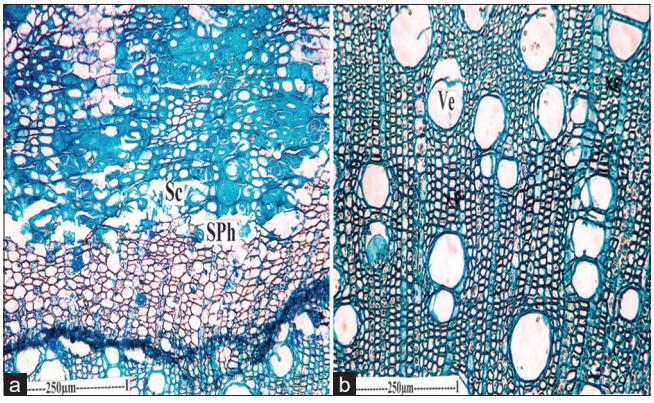

Fig. 9: (a) Sclerenchyma zone and secondary phloem of stem, (b) Secondary xylem of the stem. Sc: Sclerenchyma, Sph: Secondary phloem, Ve: Vessel, XF: Xylem fiber, XR: Xylem ray

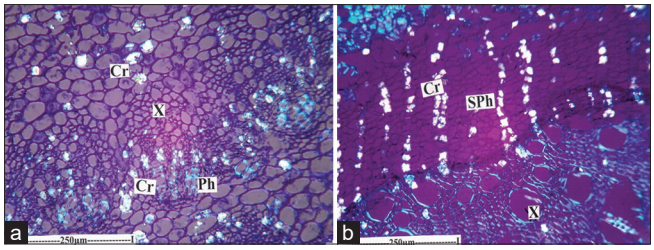

Fig. 10: Crystal distribution, (a) Phloem tissue of the petiole, (b) Phloem tissue of the stem. Cr: Crystals, Ph: Phloem, Sph: Secondary phloem, X: Xylem

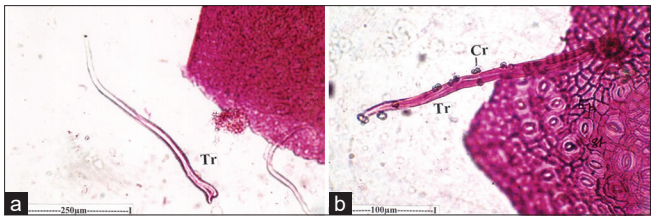

Fig. 11: Microscopic study – leaf powder, (a) Epidermal trichome, (b) Abaxial epidermis, bearing trichome. Cr: Crystals, Tr: Trichome

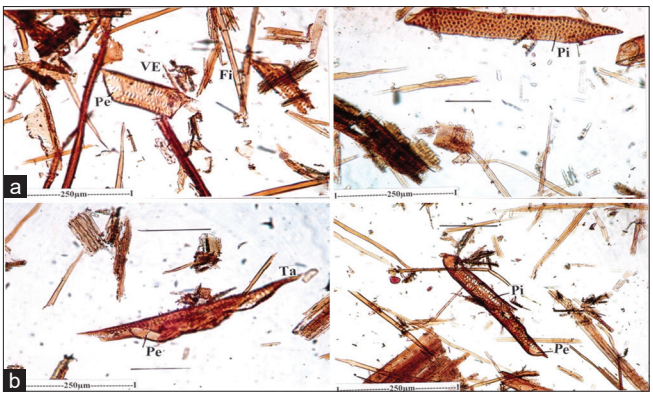

Fig. 12: Microscopic study – stem powder, (a) A vessel element showing pits, (b) A vessel element showing perforation. Pe: Perforation, Pi: Pits

Anatomical study of leaves and stem of *E. tuberculatus* is carried out for the 1st time. In the present investigations, *E. tuberculatus* exhibited paracytic stomata whereas *Elaeocarpus munronii* showed anomocytic stomata [18]. Midrib region of *E. tuberculatus* leaf showed prominent projection on abaxial side and slightly raised on adaxial side whereas it maintains broad conical shape in abaxial side and flat adaxial side in *Elaeocarpus serratus* leaf [19]. *E. tuberculatus* possessed a thick dark layer of crushed cells in the ground tissue. In contrast, deposition of tannins was observed in the palisade layer of *E. serratus* [19]. Meanwhile, myriad contents of reddish brown color cells showing the presence of terpenoids were reported in *E. munronii* [18]. One of the main ergastic storage products in plant tissues is calcium oxalate crystals. In powder and microscopical examinations of herbal medicines, the size, frequency, and distribution of crystals are typically utilized as diagnostic characteristics. A thorough examination of plant crystals is mostly connected to taxonomy research on plants [20-22]. Shaival *et al.* [23] reported the presence of prisms of calcium oxalate in both leaf mesophyll and midrib of *Elaeocarpus ganitrus.* Furthermore, in the case of *E. munronii* calcium oxalate crystals and resin canals were spotted in most of the mesophyll cells [18] while in our present study, *E. tuberculatus* is distinct from others by having an abundance of calcium oxalate druses thereby highlighting their unique anatomical characters.

Fig. 13: (a) Wide fiber, (b) Narrow fiber, (c) A sclerenchyma cell showing wide. Pi: Pit, Tw: Thick wall, Lu: Lumen, NF: Narrow fiber, WF: Wide fiber

In present investigations, *E. tuberculatus* showcased four prominent multistranded vascular bundles but in contrast, Devi [18] spotted one vasculature in *E. munronii*. In this current research, *E. tuberculatus* showed petiole with stained epidermis and homogenous parenchymatous ground tissue with dense mat of short tanniniferous trichomes was observed. In this study, *E. tuberculatus* stem showed fissured periderm, convincingly *E. munronii* stem also showed the fissured periderm, this similar characters may highlights the distinct feature of this genus [18].

In this present study *E. tuberculatus*, distinctive long whip-like unicellular trichomes and crystals were observed through powder microscopic studies. This finding aligns with previous studies conducted by Vijayan and Rajasekaran [24] in *Elaeocarpus blascoi*. Wide and narrow fibers are dominant and rarely the presence of fiber-like sclerids with thick walls is noticed is *E. tuberculatus* which is not reported in others species of *Elaeocarpus.* In summary, the morpho-anatomical investigations of *E. tuberculatus* revealed notable features which highlight the importance of species precise investigations within the genus in terms of the pharmacognostical realm.

CONCLUSION

In conclusion, these parameters which are being reported for the first time could be useful in setting some diagnostic indices for the identification and preparation of monograph of *E. tuberculatus*.

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