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ANATOMICAL STUDIES OF *ELAEOCARPUS SERRATUS* LINN.

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Keywords:

Anatomical study, Rudraksh, calcium oxalate crystals, Lignified xylem fibers, Atarch grains

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ABSTRACT: The medicinal plant *Elaeocarpus serratus* Linn., (Elaeocarpaceae) has been utilized in traditional medicine for various ailments. However, upon reviewing the literature, it was found that no anatomical work had been conducted on this plant. Therefore, our present study aimed to determine the macroscopic and microscopic characteristics of fresh leaf and stem of *Elaeocarpus serratus*. The macroscopic study is a technique of qualitative evaluation based on the study of morphological and sensory profiles. Externally, the leaves simple, alternate, spiral, clustered at twig ends, petiole 1.2- 4cm long and glabrous, lamina is elliptic with an acuminate or obtuse apex, base acute, serrate margin and glabrous and it appears red when senescent, midrib slightly raised above the surface. Inflorescences are in racemes, with white flower petals. The microscopic study is a qualitative evaluation technique used to confirm the structural details of drugs. Internally, the leaf exhibits both abaxial and adaxial epidermis, granular trichomes are unicellular, unbranched, wide and straight with pointed tip, cortex with tannin contents and calcium oxalate crystals. The petiole shows a boat-shaped sectional view with dense amorphous inclusions surrounded by a rosette of parenchyma cells in ground tissue. The stem shows a fissured periderm, distinct growth rings, and lignified xylem fibers. The seed contains thick cellular endosperm with starch grains. Powder microscopy reveals the presence of cyclocytic and apostomatic type stomata, trichomes and prismatic crystals in the leaf. The stem contains thick-walled fibers, periderm tissue, fibriform-like sclereids and some vessel elements. The seed coat exhibits brachy sclereids, lignified walls, canal-like pits and a wide lumen.

INTRODUCTION: The medicinal plant, *Elaeocarpus serratus* Linn. belonging to the family Elaeocarpaceae (Rudraksha family) is widely distributed from Madagascar in the west through India, Southeast Asia, Malaysia, Southern China, and Japan, through Australia to New Zealand, Fiji, and Hawaii in the east with its approximately 350 species. The islands of Borneo and New Guinea have the greatest concentration of species.

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In India, 25 species have been reported which are widely distributed in the foothills of Himalayan and Western Ghats[1]. Several species of Elaeocarpus are known for broad therapeutic properties and have been used in traditional medicine for the treatment of several disorders [2, 3].

E. serratus was collected from the Upper Palani Hills of Western Ghats, India. Traditionally the plant is used to treat various ailments. They are used in the treatment of rheumatism and as an antidote to poison, diuretic and as a cardiovascular stimulant [4]. Leaves contain Myricitrin, Myricetin, Mearnsitrin and Ellagic acid. Fruits contain tannin and a large amount of plant acids [5, 6, 7]. Seeds are roasted and eaten [8]. The plant extract possessed antioxidant properties due to the presence of

myricitrin, anti-diabetic, anti-arthritic, anti-microbial, anti-inflammatory and anti-ulcer activity [9-16]. Huang *et al*. (2021) reported that leaf extract of *Elaeocarpus serratus* has the potential to treat anti-melanogenesis and can be used as skin-whitening ingredients [17].

Despite the modern techniques, the identification of plant drugs by pharmacognostical studies is more reliable for the proper identification of the plant. Hence, the present research was carried out on the macroscopical and microscopical features of *Elaeocarpus serratus*.

MATERIALS AND METHODS:

Collection and Authentication of the Plant Material: The plant of *Elaeocarpus serratus* L. for the proposed study was collected from the Upper Palani Hills of Western Ghats (Kodaikanal Forest Division), India and was authenticated by the Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and the herbarium of Voucher specimen number BSI/SRC/5/23/2011-12/Tech.454 has been deposited at the P.G. and Research Department of Botany, Vellalar College for Women, Erode (T.N), India (Plate 1).

PLATE 1: *ELAEOCARPUS SERRATUS* LINN. - HABIT

Macroscopical Studies: The morphological features of the various plant parts (leaf, fruit and seed) were analyzed with the aid of Gamble (2005) in the field and photographed under the original environment and evaluated botanically [18].

Microscopical Study of Fresh Plant Parts:

Preparation of Specimens: The fresh sample of different parts (leaf, fruit and seed) were cut into small pieces and fixed in FAA solution (Formalin 5ml + Glacial acetic acid 5ml + 70% Ethanol 90ml) as per the schedule given by Sass (1940) [19]. After fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol (TBA) as per the standard procedure. After complete dehydration, the specimens were embedded in paraffin wax.

Sectioning: The paraffin-embedded specimens were sectioned with the help of a rotary microtome (thickness 10-12µm). De-waxing and staining of the sections were done by customary procedure [20]. The sections were stained with Toluidine blue as per the method published by O'Brian *et al*. (1964) [21].

Staining: Tannic Acid-Ferric Chloride counterstained with 0.5% alcoholic safranin. This schedule was found to be quite satisfactory for all young plant tissues in which the primary walls were stained. Alcoholic safranin (0.5%) counterstained with 0.25% fast green. This schedule gives good results for studying the histology of different tissues of the plant organs, especially the cell inclusions. Toludine Blue-O stain was prepared by dissolving 0.25gm of the stain in the mixture of benzoic acid 0.25gm, sodium benzoate 0.29gm and distilled water 200ml with a pH of 4.2- 4.4. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good and 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. After de-waxing, the slides were stained for 5-10 minutes and then dehydrated (Sass, 1940) [19].

Photomicrograph: All permanent slides, after staining were dehydrated by using graded series of Ethanol + Xylol and mounted in DPX. Photomicrographs were done on NIKON-Lab phot-2 microscope using Konica colour film (100 ASA). For normal observations, bright field was used. For the study of crystals and starch grains, the sections were photographed under polarized light. Magnifications of the figures are indicated by scale bars. Descriptive terms of various observations are as found in standard anatomy books [22].

Powder Microscopic Study: The leaf, fruit and seed were boiled with chloral hydrate for 5-10 minutes, and then stained with phloroglucinol and Toludiene and observed for the microscopic features under high power (40 x) [20, 21, 22].

RESULTS:

Macroscopic Evaluation: The macroscopical characteristics of the plant can serve as diagnostic parameters to provide the standards, identify the crude drug and avoid adulteration of drugs [23]. *Elaeocarpus serratus* is a tree that grows up to 18m tall in evergreen to semi-evergreen forests. Bark brownish, smooth; blaze orange-red. Leaves simple, alternate, spiral, clustered at twig ends; petiole 1.2-4cm long, swollen at both ends, glabrous; lamina elliptic, apex acuminate or obtuse, base acute, margin serrate, glabrous, red when senescent; midrib slightly raised above; secondary nerves 5-9 pairs, inflorescence racemes, flower white. Fruit drupe, oblong, ellipsoid or ovoid; containing a much tubercled, 1-seeded stone [14]. Plate 1.1, 1.2.

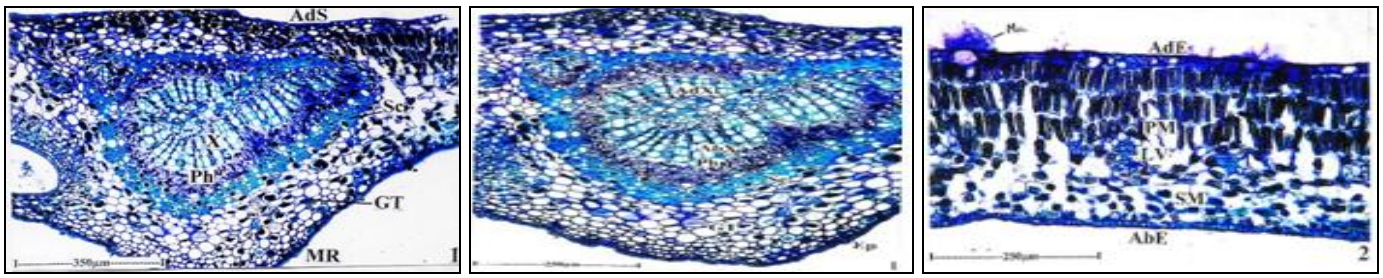


PLATE 2: T.S. OF MIDRIB OF THE LEAF 2.1. T.S. OF MIDRIB-ENLARGED PORTION 2.2. T.S. OF LAMINA OF THE LEAF
AbE: Abaxial Epidermis; AdE: Adaxial Epidermis; AdS: Adaxial Side; GT: Ground Tissue; Sc: Sclerenchyma; LV: Lateral Vein; MR: Midrib; PM: Palisade Mesophyll; SM: Spongy Mesophyll; X: Xylem; Ph: Pholem; Mu: Mucilage

Microscopic Evaluation:

Leaf: Microscopic evaluation allows a more detailed examination of a drug and it could be used to identify the drug by its known histological characters of plant origin. The leaf is dorsiventral and has a thick plano convex midrib (700µm and 900µm wide). The epidermal layer of the midrib consists of cells which are small, elliptical and thick-walled, parenchymatous ground tissue with tannin distributed in most of the cells (Plate 2). The vascular system includes abaxial and adaxial strands which are just opposite with their xylem. Phloem occurs on the outer boundary of the xylem. The entire vascular strands are surrounded by a thick sclerenchyma sheath (Plate 2.1.). The lamina is even and smooth (300µm thick). The adaxial epidermis is fairly thick, the cells being squarish and the cell lumen is often filled with mucilage. The abaxial epidermis is thin and the cells are small and circular. Palisade cells are in three rows; they are cylindrical and possess tannin contents. The spongy parenchyma cells are in 6 to 7 layers; they are small and lobed, loosely arranged (Plate 2.3.) with wide air chambers. Calcium oxalate crystals are abundant in the leaf mesophyll and veins. In the mesophyll, the crystals are druses **(**30µm in diameter) (Plate 3.1, 3.2). Along the veins, the crystals are prismatic type and are up to 20µm long. (Plate 3.3). They occur in parenchyma cells that cover the veins. The major lateral veins and vein lets are thick and straight. The vein-islets are wide, polygonal in outline and have distinct vein boundaries (Plate 4.1, 4.2). The vein-terminations are mostly repeatedly branched forming a dendroid outline within the islets (Plate 4.3).

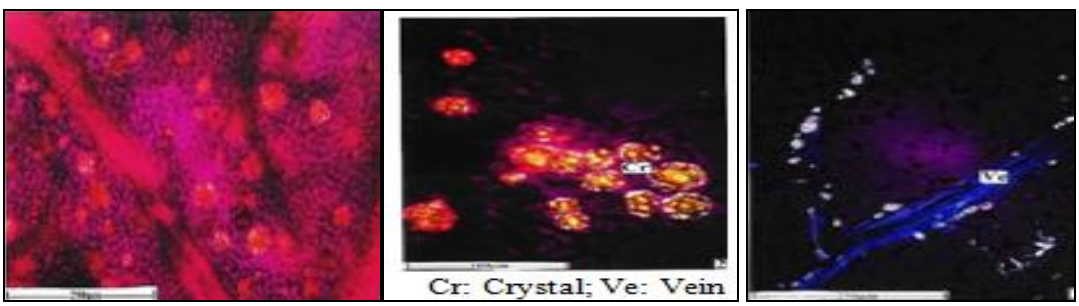
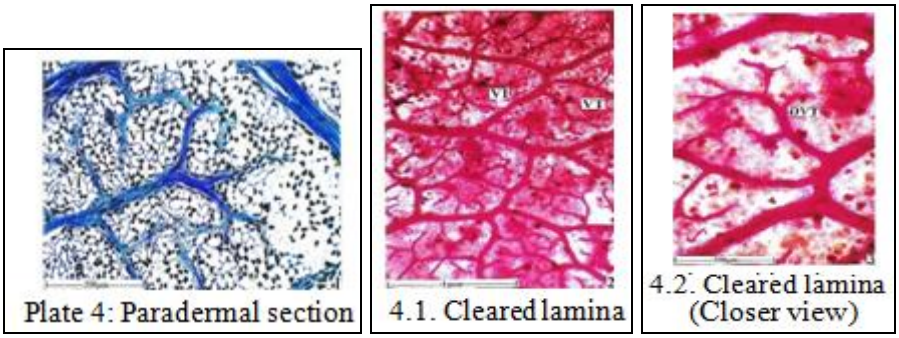


PLATE 3. 1, 2, 3: CRYSTAL DISTRIBUTION OF THE LAMINA AND VEINS



DVT: Dendroid Vein Termination; VI: Vein Islet; VT: Vein Termination

PLATE 4: VENATION PATTERN IN LEAF

Petiole: The basal part of the petiole is boat-shaped in sectional view, with two adaxial-lateral short thick wings and a flat adaxial side and 130µm thick and 140µm wide (Plate 5). Some of the cells have dense amorphous inclusions which are surrounded by a rosette of parenchyma cells. The vascular strands are thick and wide, triangular in outline and conjoint, collateral and closed. There are several, closely arranged parallel lines of xylem elements, each line possessing 6 or more cells. Phloem occurs in a thick sheath enclosing the xylem. This is a circular, small single bundle placed within the wing. The wing bundle is also collateral, comprising a cluster of xylem and a cap of phloem. The proximal (upper) end of the petiole is similar to the lower end in structure and outline. The triangular vascular cylinder is cleaved narrowly at the adaxial end of the strand (Plate 5.1).

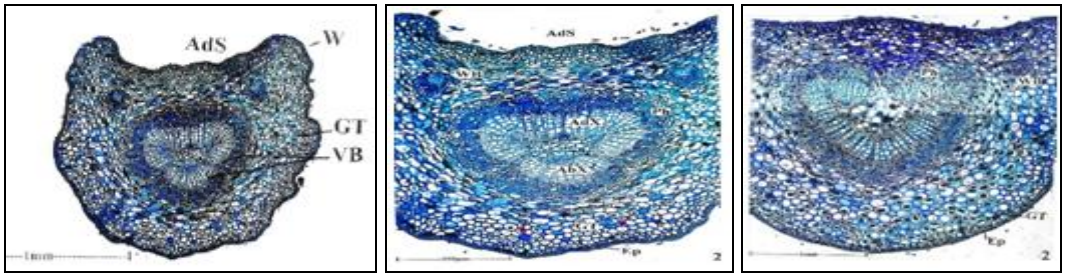


PLATE 5: T.S. OF THE PETIOLE 5.1. ENLARGED PORTION OF T.S. OF BASAL PART 5.2. T.S. OF UPPER PART. AbX: Abaxial xylem; AdX: Adaxial xylem; AdS: Adaxial side; Ep: Epidermis; GT: Ground tissue; Ph: Pholem; X: Xylem; VB: Vascular Bundle; VS: Vascular Strand; W: Wing; WB: Wing Bundle

Stem: The stem consists of 2.6mm thick and fissured periderm. The periderm consists of narrow and thin-walled tubular cells and 70µm thick. There is a broad parenchymatous cortex comprising elliptic cells with a dense accumulation of tannin (250µm wide). The cortex is followed by a zone of dense sclereids which is gradually transformed into a phloem zone (Plate 6 - 6.1, 6.2). The phloem zone consists of mixed masses of sclerenchyma and radial fiber of sieve elements. The secondary xylem exhibits a distinct growth ring, which is demarcated by a line of thick-walled cells at the beginning of the growth (Plate 6.3, 6.4). The vessels are angular, thick-walled and occur mostly in radial multiples of 2-6 cells. Xylem rays are fairly prominent; the ray cells are wide, thick-walled and straight (Plate 6.5). Xylem fibres are thick-walled, lignified and occur in radial rows.

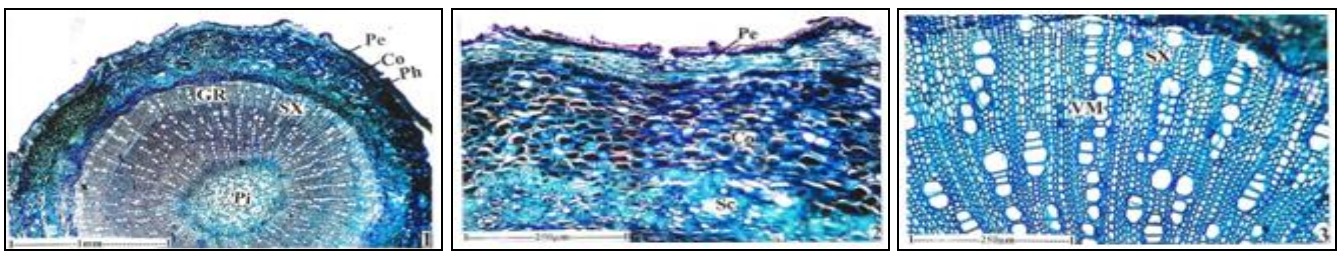
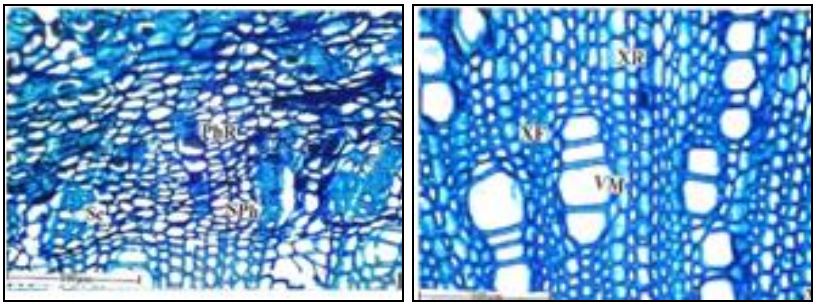


PLATE 6: 6.1. ENTIRE VIEW OF T.S. OF STEM 6.2. PERIDERM AND CORTEX 6.3. ENLARGED PORTION OF SECONDARY XYLEM. Co: Cortex; GR: Growth Ring: Pi: Pith; Ph: Pholem; Sc: Sclerenchyma; Pe: Periderm; VM: Vessel Multiples



6.4. ENLARGED PORTION OF SECONDARY PHLOEM 6.5. ENLARGED PORTION OF SECONDARY XYLEM. PhR: Pholem Ray; Sph: Secondary Pholem; Sc: Sclerenchyma; VM: Vessel Multiples; XR: Xylem Ray; XF: Xylem Fibre

Fruit: The fruit is a drupe with an epicarp, mesocarp and endocarp. Epicarp is thin and membranous. The epidermal cells are small and less prominent and the cuticle is thin (Plate 7.1, 7.2). The mesocarp is thick, fleshy and parenchymatous cells are thin-walled angular and compact and cells possess dense deposition of tannin. Amid tannin-bearing cells, there are groups of tannin-free cells (Plate 7.3). The tanniniferous and tannin-free cells are random in distribution. The mesocarp tissue is small with vascular strands possessing four or five xylem elements and about six phloem elements, associated with partially encircling sclereids (Plate 7.4). They are also sclereid clusters that are not associated with any vascular elements (Plate 7.5).

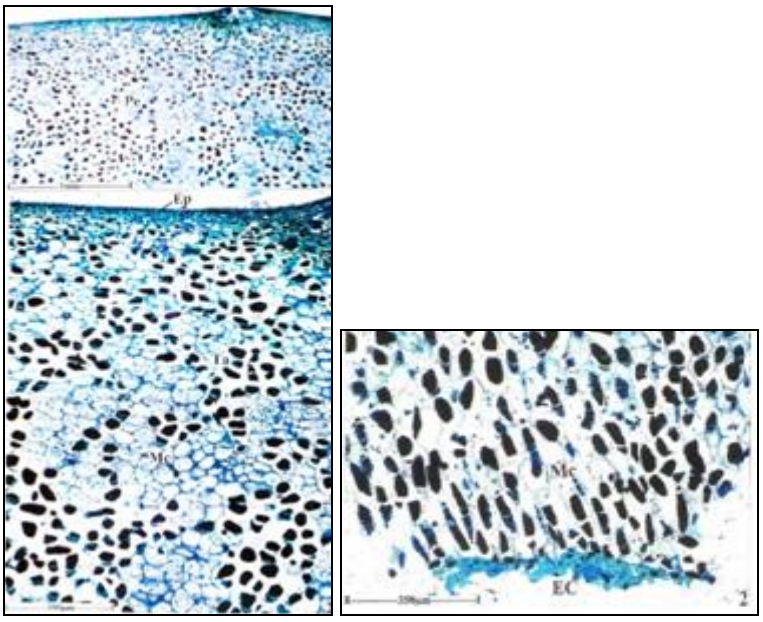


PLATE 7: T.S. OF PERICARP PLATE 77.1. T.S. OF INNER ZONE OF PERICARP

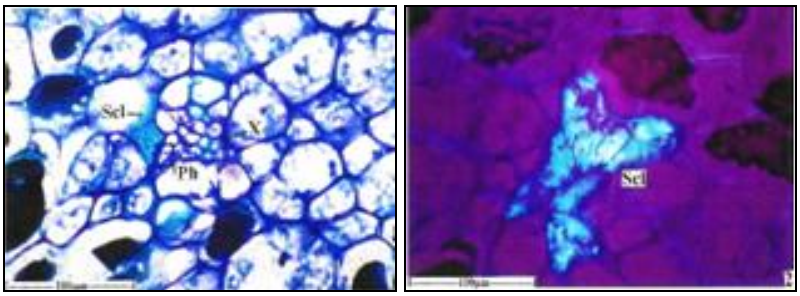


PLATE 77.2. T.S. OF MESOCARP SHOWING VASCULAR STRAND PLATE 77.3. SCLERIDS IN THE MESOCARP AS SEEN UNDER POLARIZED LIGHT. Ep: Epicarp; Mc: Mesocarp; Pe: Pericarp; Ta: Tannin; Ec: Endocarp; Ph: Pholem; Scl: Sclerids; X: Xylem

Seed: The endosperm is cellular and a thick segment comprising vertical rows of parenchymatous cells. The surface layer of the endosperm appears to have an epidermal layer;

inner small squares of parenchymatous cells arranged in compact parallel lines (Plate 8). Towards the inner position, the cells become slightly longer and are in most conspicuously featured parallel lines (Plate 8.1). The conspicuous feature of the endosperm cells is the presence of spherical bodies of smaller and larger sizes (Plate 8.2). The chemical nature of the spherical bodies is not known. Calcium oxalate crystals are frequent in the outer zone of the endosperm (Plate 9.3). The crystals are similar to druses, comprising of thin needles radiating the central dark circular body which is an ergastic substance. This type of crystal is known as rosettes (15µm in diameter) and is only one in a cell (Plate 8.4). Starch grains are 25µm in diameter and also sparsely seen in the endosperm. The grains are circular and the hilum is in the center (Plate 8.5).

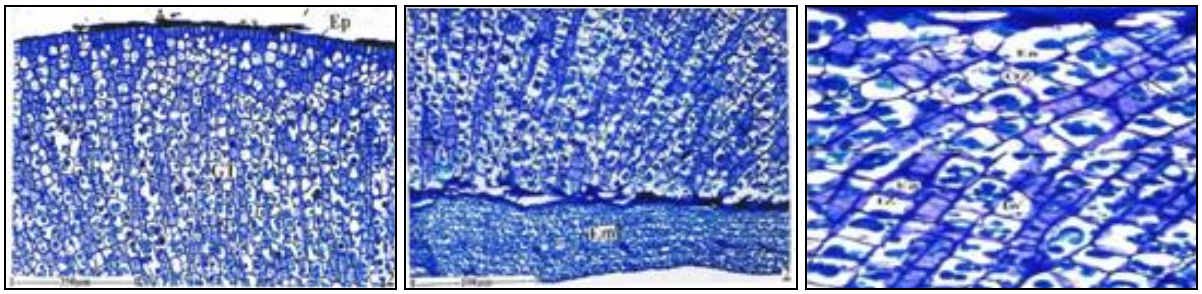


PLATE 8: T.S. OF SEED - CELLULAR ENDOSPERM 8.1. ENLARGED PORTION 8.2. ENDOSPERM SHOWING CELL INCLUSIONS. Col: Collenchyma; Ep: Epicarp; En: Endosperm; GT: Ground Tissue; Em: Embryo; In: Inclusion; Iz: Inner zone; Oz: Outer zone

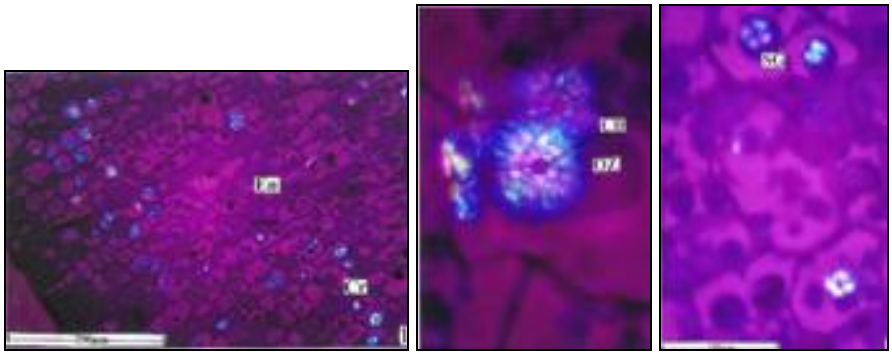


PLATE: 8.4.5. CRYSTAL DISTRIBUTION IN THE ENDOSPERM 8.6. STARCH GRAINS IN THE ENDOSPERM CB: Central Body; En: Endosperm; Oz: Outer zone Cr: Crystal; SG: Starch Grain

Powder Microscopy: Microscopic observations of the leaf powder showed thin and small fragments of epidermal peelings consisting of polygonal cells with thick anticlinal walls. The stomata are cyclocytic type of stomata in abaxial peeling of the epidermis and the ad axial epidermal peeling was apostomatic (Plate 9.1, 9.2).

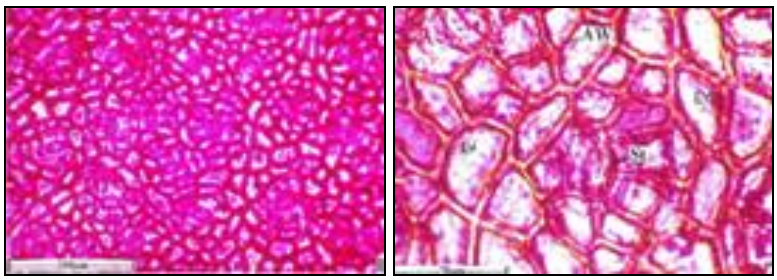


PLATE: 9.1. ABAXIAL EPIDERMIS OF LEAF 9.2. ABAXIAL EPIDERMIS OF LEAF SHOWING STOMATA. AW: Anticlinal wall; Ec : Epidermal cell; St: Stomata

Minute prismatic crystals and unicellular unbranched, wide and straight with pointed tip of trichomes having granular inclusions are seen on the epidermal cells (Plate 10.1,10.2,10.3). The stem powder includes thick-walled fibres with wide lumen that are 600µm long and 40µm wide (Plate 11). The narrow fibres are uniform in thickness and tapering at the ends (780µm long and 10µm thick)

(Plate 11.1). Thick pieces of periderm tissue consist of rectangular or squarish cells arranged in compact parallel rows and their walls are thick (Plate 11.2). Long, fibriform (fibre-like) sclereids are frequently seen. They are long, narrow and pointed at the tip, the cell walls are thick and possess wide canal-like simple pits (Plate 11.3). The vessel elements are 600µm long, wide and cylindrical. They have wide, circular multiseriate, alternate-bordered pits. The perforation plate is simple, circular and oblique. Some of the vessel elements have long pointed tails (Plate 11.4, 11.5). Fruit powder consists of thin-walled parenchymatous cells of different shapes and sizes and has no inclusions (Plate 12). The seed coat powder includes only brachy sclereids of varying shapes and sizes (Plate 13.1, 13.2). They have thick lignified walls, canal-like pits and wide lumen.

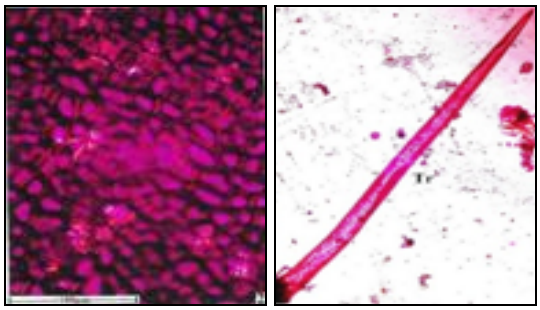


PLATE: 10.1. CRYSTALS IN THE ADAXIAL EPIDERMIS 10.2. A SINGLE EPIDERMAL TRICHOME OF LEAF. Cr: Crystal; Tr: Trichome

PLATE: 11. STEM - WIDE FIBRE 11.1. NARROW FIBRE 11.2. STEM POWDER SHOWING PERIDERM CELLS

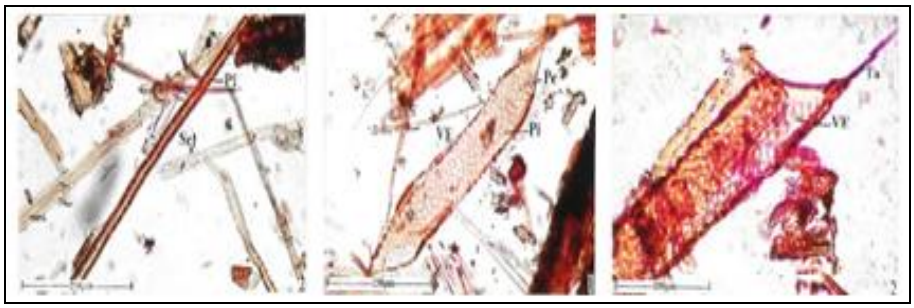


PLATE: 11.3. SCLEREID PLATE: 11.4. VESSEL ELEMENT PLATE: 11.5. VESSEL ELEMENT WITH LONG TAIL

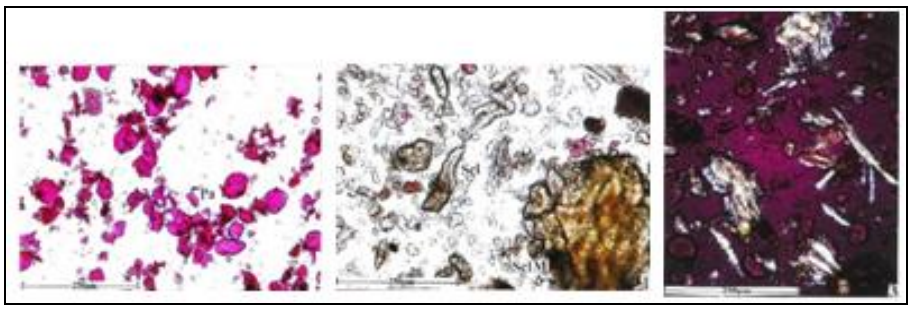


PLATE: 12. PERICARP PLATE 13.1. SEED COAT SHOWING SCLERIDS 13.2. SCLERIDS AS SEEN UNDER POLARIZED LIGHT

DISCUSSION: Anatomical features of plants have been considered highly dependable guidelines for the diagnosis of fragmentary plant [24, 25, 26]. In the present study, the leaves showed that the cyclocytic type of stomata in the abaxial side and the adaxial side were apostomatic. Similarly, Vijayan *et al.* (2010) investigated the leaf of *Elaeocarpus blascoi* and reported the presence of cyclocytic stomata on the abaxial epidermis and the adaxial epidermis were apostomatic and trichomes and calcium oxalate crystals [27]. In contrast, Yuvarani *et al* (2022) observed paracytic stomata in three species of *Elaeocarpus* such as *E. angustifolius, E. tuberculatus* and *E. variabilis*, respectively [28].

In addition, Devesh *et al.*, (2013) stated the paracytic stomata in the lower epidermis of leaf of *Elaeocarus* sphericus [29]. In the present study, the vascular strands of *Elaeocarpus serratus* were triangular in outline and are delimited by a thick sclerenchyma sheath was also observed by Yuvarani *et al.* (2022) [28] in other species of *Elaeocarpus angustifolius*, *Elaeocarpus tuberculatus* and *Elaeocarpus variabilis*. Priya Shaival *et al.* (2012) and Bharti (2010) observed the pharmacognostical characteristics of the leaves of *Elaeocarpus ganitrus* and reported the presence of prisms of calcium oxalate in the leaf mesophyll and midrib [30, 31].

The vascular bundles were reported to be conjoint, collateral and closed. This finding was similar to the present study. Calcium oxalate crystals constitute one of the major ergastic storage products in plant tissues. Generally, the frequency, distribution and size of crystals are used as diagnostic characters in microscopical and powder studies of herbal drugs. An extensive survey of crystals in plants is mostly related to the taxonomic studies of plants [32, 33, 34]. Franceschi and Nakata (2005) described genetic factors play a crucial role in determining the morphology and distribution of crystals, leading to the development of distinct morphotypes within species [35]. Additionally, the significance of the arrangement of crystals in taxonomy and phylogenetics has been emphasized previously by Horner *et al*. (2012) [36]. In the present research, the anatomy of the *Elaeocarpus serratus* stem showed a fissured periderm which was consistent with the previous report in *Elaeocarpus tuberculatus* and *Elaeocarpus munronii* [37]. Metcalf and Chalf (1950) stated that axial parenchyma, a distinctive character of wood families was absent in genus Elaeocarpus [24]. Interestingly, *Elaeocarpus serratus* belongs to woody species no axial parenchyma was noticed in the stem. A broad parenchymatous cortex comprising of elliptic cells with a dense accumulation of tannin is seen in *Elaeocarpus serratus*. Contrasting observations in *Elaeocarpus munronii* where myriad contents of reddish brown colour cells indicating the presence of terpenoids were reported [37]. Furthermore, Yuvarani *et al.* (2022) reported few collenchymatous hypodermis with druse crystals seen in *Elaeocarpus* species including *Elaeocarpus angustifolius, Elaeocarpus tuberculatus* and *Elaeocarpus variabilis* which reveals the anatomical variations within the *Elaeocarpus genus* [28].

In the current research, the regular arrangements of epicarp, mesocarp and endocarp were observed in the fruit of *Elaeocarpus serratus* which aligns with the structural characteristics also observed in *Elaeocarpus angustifolius* fruit. Further, a small vascular bundle in the mesocarp region is witnessed in *Elaeocarpus angustifolius* analogous to our present study which together indicates the conserved features [38].

In the present investigation, the presence of elongated cells towards the inner position in parallel lines suggests a distinct structural pattern within the endosperm of *Elaeocarpus serratus.* Additionally, the presence of spherical bodies of varying sizes in the endosperm cells is a noteworthy feature. These characteristics resemblance for the presence of ruminate endosperm. These features align with the findings of Gagul Janet *et al*. (2018) who stated that the genus *Elaeocarpus* is known to possess ruminate endosperm [39]. Although the fruits of *Elaeocarpus serratus* exhibited evidence of ruminate endosperm, the lack of published data on this specific trait in this genus prevents diagnostic assignments at this time. Hence, this gap certainly prompts future investigations. Microscopic observations of leaf, stem, fruit and seed coat powders of *Elaeocarpus serratus* revealed distinctive features. Anatomy of the leaf showed cyclocytic type of stomata on the abaxial side and apostomata on the adaxial side, further, the

microscopic study of leaf powder also exhibited cyclocytic stomata on the abaxial peelings and apostomatic epidermis on the adaxial peelings. Similarly, a microscopic powder study of the leaf of *Elaeocarpus ganitrus* revealed the presence of anomocytic stomata [31]. These are the parameters used for identifying and distinguishing the drug from its substitutes and adulterants [36]. Stem powder exhibited that the fibres, periderm tissue, pits and fibres which were similar to those of *Elaeocarpus angustifolius* [40]. The presence of parenchymatous cells in fruits of *Elaeocarpus serratus* is parallel to the results of *Elaeocarpus angustifolius* [38]. In the present study, the seed powder described the presence of cellular endosperm, tannins, brachysclereids, calcium oxalate were also reported by Singh *et al*. (2010) in *Elaeocarpus* ganitrus[26]. In medicinal plants, trichome and crystal characters have been reported to act as biomarkers to identify the plant even in the raw material or powder form [41, 42, 43].

CONCLUSION: In conclusion, the comprehensive anatomical evaluation of *Elaeocarpus serratus* undertaken in this study provides valuable insights into its macroscopic and microscopic features, contributing to its botanical identification and potential pharmacological applications.

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CONFLICTS OF INTEREST: Nil

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