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Morpho-anatomical and histochemical markers supporting taxonomic delimitation of the endemic western ghats species *Syzygium laetum* (Buch.-Ham.) Gandhi

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Abstract
Syzygium laetum (Buch.-Ham.) Gandhi is an endemic species in India's Western Ghats. It was characterized morphologically, anatomically, and histochemically for its taxonomic authentication. Field-collected specimens were examined macroscopically, and thin sections of different parts were prepared. The tree is approx. 10 m tall, with smooth grey-white bark and opposite elliptic-oblong glabrous leaves. Flowers are solitary or in small clusters, red or lemon-yellow, with numerous stamens, and fruits are ovoid berries. Leaves show a thick midrib, dorsiventral lamina, bicollateral bundles, and abaxial paracytic stomata. The stem has a corky periderm, sclerenchymatous cortex, and crystal-rich tissues. Bark is tannin-rich, and the wood is diffuse-porous with short vessels, paratracheal parenchyma, and uni- to biseriate rays. Collectively, these morpho-anatomical and histochemical traits serve as diagnostic markers for *S. laetum*, highlighting its ecological and medicinal relevance.

Keywords: *Syzygium laetum*, western ghats, endemic, morphology, anatomy, histochemical

Introduction
Syzygium species inhabit a variety of environments that vary depending on forest topography and succession (Gamage *et al*., 2003) [5]. The genus *Syzygium* is known for its applications in the food, pharmaceutical, cosmetic, and agricultural industries (Nigam & Nigam, 2012) [9]. It is the world's most diverse tree genus in terms of species richness. Analyses of phylogenomic data suggest that the genus *Syzygium* originated from the Australia-New Guinea region, subsequently dispersing east toward the Pacific and west toward India and Africa, where episodes of rapid speciation produced the poorly resolved branches observed in phylogenies (Low *et al*., 2022) [7].
Due to their high efficacy, affordability, and accessibility, plants in the genus *Syzygium* (Myrtaceae) are the leading suppliers of phytochemicals and nutraceuticals and are used to treat a wide range of illnesses (Snehalatha & Rasmi, 2022) [16]. Representing the family Myrtaceae, the genus *Syzygium* Gaertner is composed of about 1200 species in the Old World tropics, with 45 species documented in the Western Ghats region of India (Shareef *et al*., 2012; Rameshkumar *et al*., 2015) [10]. *Syzygium laetum* (Buch.-Ham.) Gandhi is a Western Ghats endemic, typically occurring along riverine ecosystems (Nadar & Sellappan, 2025) [8]. It is extensively found in evergreen forests in the Western Ghats and is native to the Southern Western Ghats (Irulandi *et al*., 2016) [6].
The taxonomic categorization of *Syzygium*, like that of other Myrtaceae genera, has been one of the primary problems that plant taxonomists are now dealing with (Retamales *et al*., 2014; Abdulrahman *et al*., 2018) [11, 1]. Morpho-anatomical studies of *Syzygium* provide key structural markers for accurate species identification and taxonomic authentication (Bhavani *et al*., 2023) [3]. They also support quality control, prevent adulteration, and form a basis for further pharmacological and phytochemical research. To the best of our knowledge, there is limited literature available on *Syzygium laetum*. The present study was carried out to examine the morpho-anatomical and histochemical characteristics of *Syzygium laetum,* which will provide a detailed description for taxonomic and identification purposes.

Materials and Methods
Plant collection and identification: *Syzygium laetum* (Buch.-Ham.) Gandhi was collected from the Nelliyampathy hills in the Nemmara forest division, Palakkad, Kerala, India.

Morpho-anatomical studies

Both the naked eye and a magnifying lens were used to study and carefully record the morphological characteristics of *S. laetum*. The stomatal morphology was investigated through the use of leaf maceration and epidermal peeling methods. A Leica RM2235 rotary microtome was used to cut the paraffin-fixed leaf, stem, flower, and bark samples into sections that were 10-12 µm thick. The plant sections in this experiment were stained with toluidine blue (Sass, 1940) [12]. Using a Leica DM 750 HD digital microscope, images were captured.

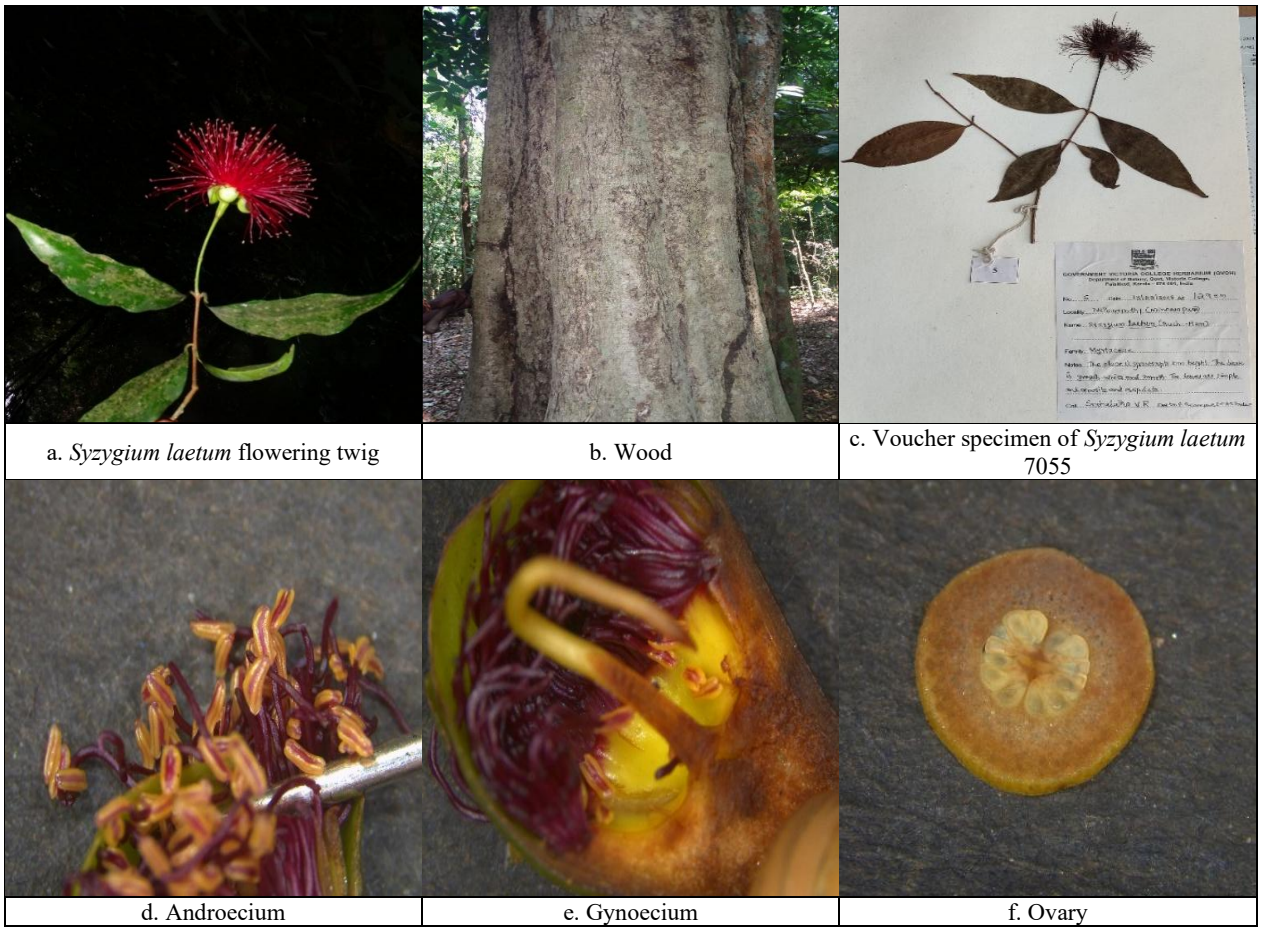
Histochemical studies

After collecting and carefully cleaning the fresh leaves, petiole, stem, bark, and wood of *S. laetum* with distilled water, thin slices of the thicker tissues were made using a Leica RM2235 plant microtome. To identify certain cell components, these sections were stained histochemically. In order to identify lignified cells, the sections were treated with phloroglucinol and then hydrochloric acid, which caused the tissues to become pink to red. Sudan Red staining allowed for the identification of fixed oils by causing oil droplets to become orange to reddish-pink. Iodine-potassium iodide solution was used for observing the color of the starch and aleurone granules. Staining using ferric chloride, which resulted in a bluish or greyish-black color, verified the existence of tannins. A digital microscope called the Leica DM 750 HD was used to record microscopic observations (Tandon & Sharma, 2017) [18].

Results

Macroscopic Characteristics

Syzygium laetum (Buch.-Ham.) Gandhi is a medium-sized tree reaching up to 10 m in height, with smooth, greyish-white bark and slender, terete branchlets. The leaves are opposite, simple, and glabrous with an elliptic to ovate-oblong form, entire margins, and an acuminate or obtusely acuminate apex. The leaves bear petioles of 5-10 mm and display intercostate-reticulate venation. The bisexual flowers occur solitarily or in cymose clusters of 2-5, either axillary or terminal. Flowers are crimson or lemon yellow, distinguished by numerous stamens (2-3 cm) of crimson to yellow hue. The persistent pedicel measures 2-5 cm, and the calyx tube is subcylindrical (1.5-2 cm). Petals are orbicular, punctate, and glandular with distinct venation. The ovary is inferior, bicarpellary, and two-celled, containing multiple ovules. Fruits are ovoid berries with reflexed calyx lobes forming a crown (Fig 1).



a. *Syzygium laetum* flowering twig | b. Wood | c. Voucher specimen of *Syzygium laetum* 7055

d. Androecium | e. Gynoecium | f. Ovary

Fig 1: Photographs showing different parts of *Syzygium laetum*

Microscopic studies

Anatomical and microscopic features of various vegetative and reproductive parts of *Syzygium laetum* is shown in Fig 2. Histological features of different plant parts of *S. laetum* is shown in Fig 3.

Leaf

The leaf shows a prominent plano-convex midrib and thick, smooth lamina. The midrib measures about 750 µm in thickness and 1 mm in width. Both upper and lower epidermis have small, thick-walled, squarish cells. The ground tissue is made of compact parenchyma with several circular secretory cavities filled with dark brown resinous material. The vascular system is broad and bowl-shaped, bicollateral, and composed of compact, thick-walled xylem elements and phloem on both sides.

The lamina (370 µm thick) is dorsiventral with two adaxial layers of palisade cells and about eight layers of spongy

parenchyma below. The adaxial epidermis has rectangular cells with thick cuticles, while the abaxial epidermis has smaller, thick-walled cells. The lamina also contains small vascular bundles and numerous circular secretory cavities (100 µm wide) filled with tannin.

The adaxial epidermis has thick-walled, wavy cells without stomata, whereas the abaxial side is densely stomatiferous with paracytic stomata. Guard cells are 30×20 µm and elliptical with narrow pores.

The leaf margin is conical and thick, with a strong cuticle and small vascular strands. The venation is densely reticulate with free-ending veinlets. The petiole is semicircular (12 mm thick, 15 mm wide), composed of compact parenchyma and wide air chambers on the adaxial side. It contains bicollateral, arc-shaped vascular strands and many calcium oxalate druses and resin-filled idioblasts.

Stem

The stem has a thin periderm of 4-5 phellem layers, a phellogen, and a narrow phelloderm. The cortex includes thick sclerenchyma bands and parenchyma. The secondary phloem forms continuous radial rows with calcium oxalate crystals, while the xylem (200 µm thick) has thick-walled vessels, fibres, and tangential fibre bands. The centre contains a large pith surrounded by medullary phloem and sclerenchyma.

Flower

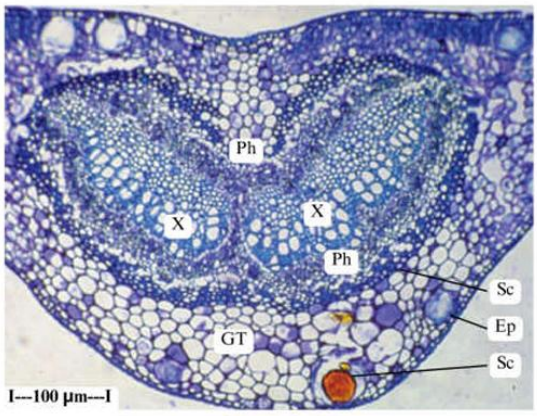
Perianth lobes are folded radially with outer ridges and smooth inner surfaces. The mesophyll has an outer palisade and inner spongy parenchyma. Small vascular bundles are present in each lobe. The style is made of parenchyma with central vascular tissue having both xylem and phloem strands.

Bark

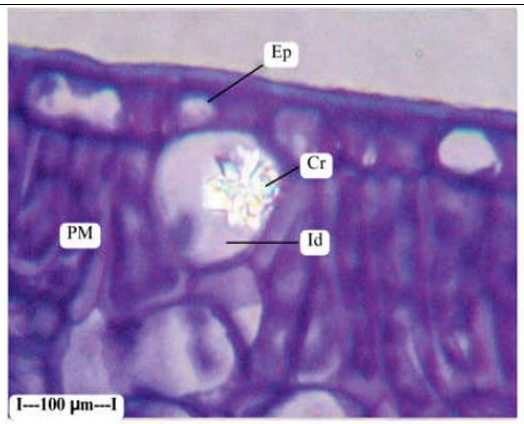
The bark has a thick periderm (over 650 µm) of alternating squarish and rectangular cells. The cortex is compact with tannin-rich cells, followed by secondary phloem with dilated rays and collapsed sieve elements. Tangential sections show spindle-shaped tannin-filled rays, while radial sections reveal heterocellular rays made of procumbent and upright cells. Phloem parenchyma cells contain raphides of calcium oxalate.

Wood

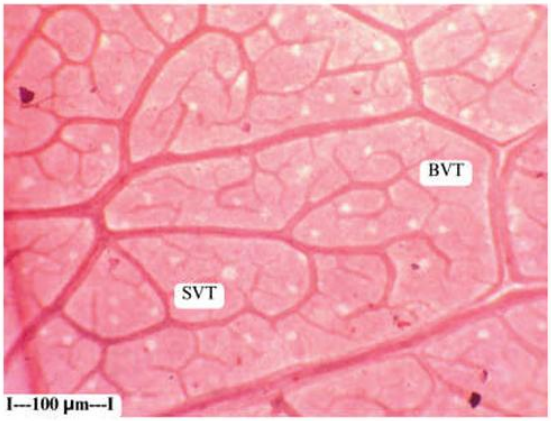
The wood is diffuse-porous with vessels, fibres, parenchyma, and rays. Vessels are angular (70-80 µm wide), solitary or in short radial multiples. Paratracheal banded parenchyma surrounds the vessels. Rays are uni- or biseriate, made of upright and procumbent cells. Fibres are thick-walled and lignified. In tangential view, vessels show oblique end walls and bordered pits; radial sections display horizontal rays and vertical fibre strands, indicating compact, well-organized xylem tissue.



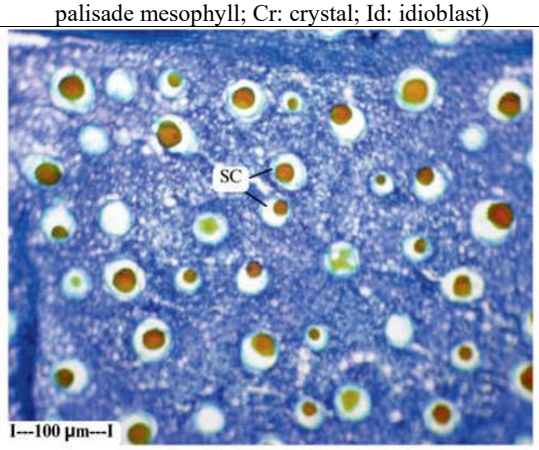
a) Vascular strands of midrib enlarged (Ph: phloem; X: xylem; Sc: sclerenchyma; Ep: Epidermis; GT: ground tissue)



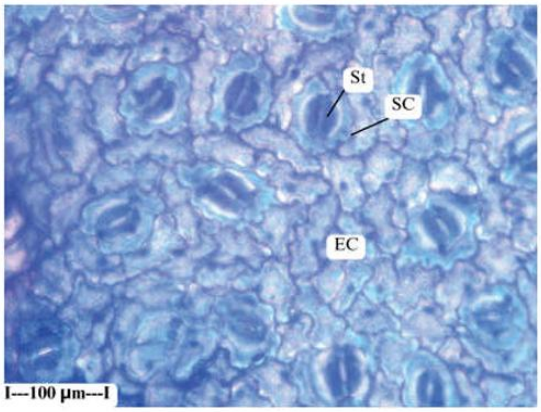
b) Druse type of crystal in the idioblast of the leaf (Ep: epidermis; PM: palisade mesophyll; Cr: crystal; Id: idioblast)



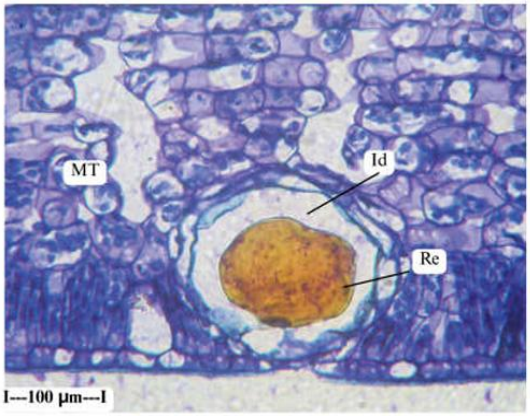
c) Venation pattern of the lamina showing vein islets, simple, and branched vein terminations (SVT: simple vein termination; BVT: branched vein termination)



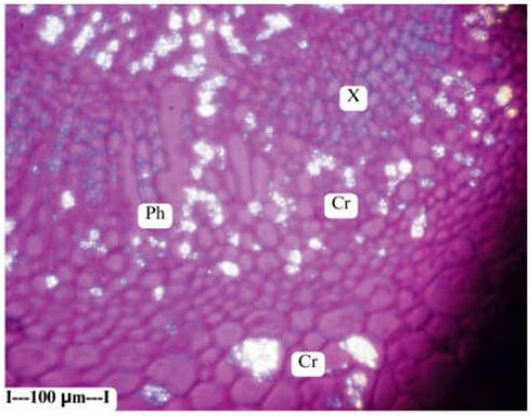
d) Surface vein of the lamina showing numerous crowded circular secretory cavities with darkly stained secretory bodies (SC: secretory cavity)



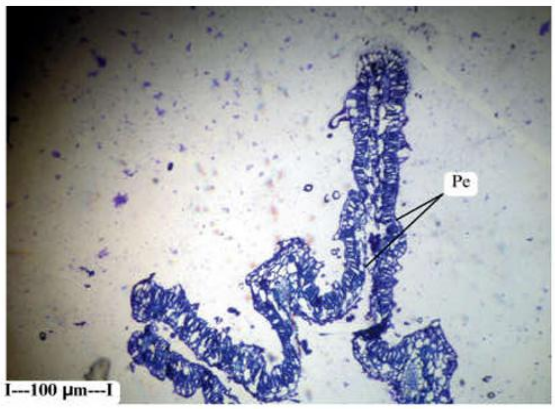
e) Abaxial epidermis showing paracytic stomata (St: stomata; SC: subsidiary cells; EC: epidermal cells)



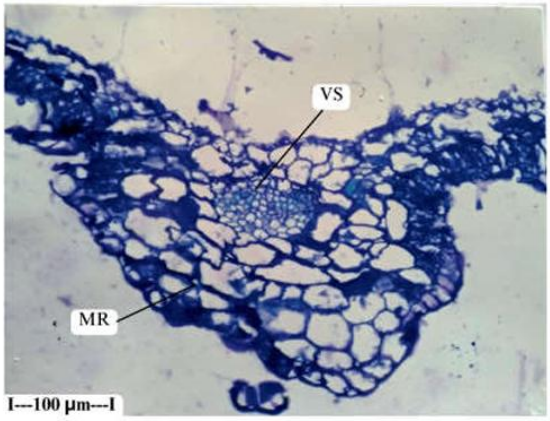
f) T.S. of a leaf showing resin-containing idioblast (MT: mesophyll tissue; Id: idioblast; Re: resin)



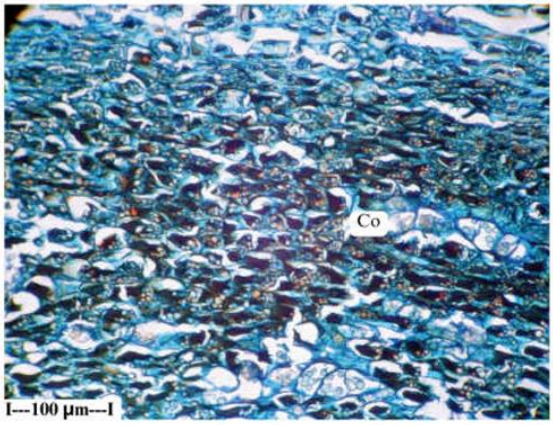
g) T.S. of petiole showing crystal distribution (X: xylem; Ph: phloem; Cr: crystal)



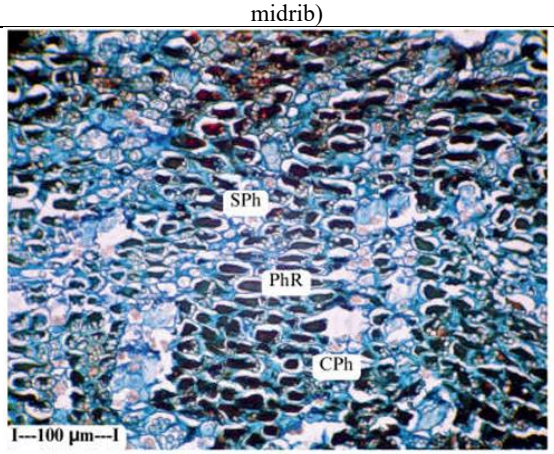
h) T.S. of a flower showing folded petal lobes (Pe: petal)



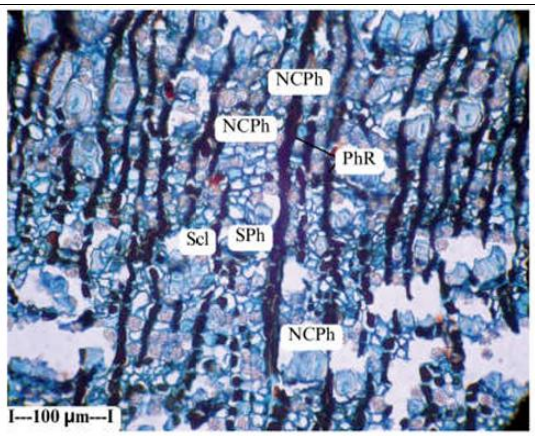
i) T.S. of the style and midrib of the petal (VS: vascular strand; MR: midrib)



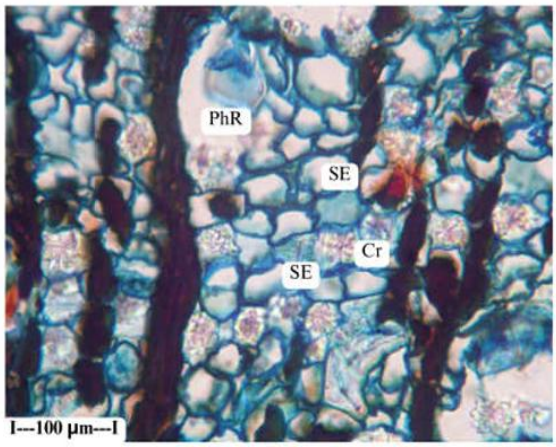
j) T.S. of bark cortical zone (Co: cortex)



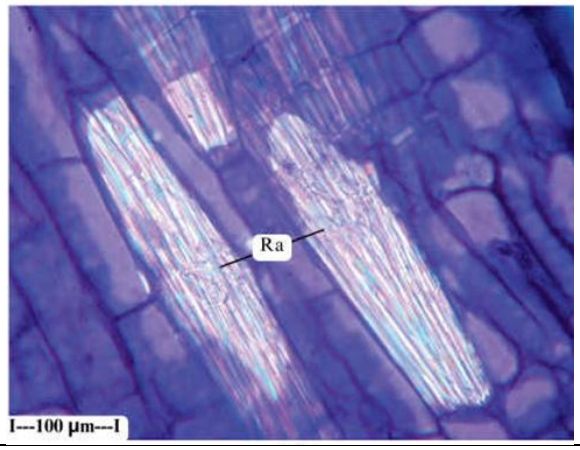
k) Collapsed phloem zone (SPh: secondary phloem; PhR: phloem ray; CPh: collapsed phloem)



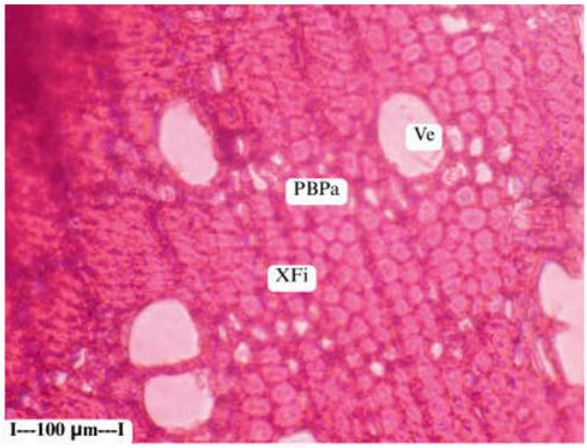
l) Non-collapsed phloem (NCPh: non-collapsed phloem; PhR: phloem ray; Scl: sclereids; SPh: secondary phloem)



m) T.S. of phloem showing sieve elements (PhR: phloem rays: SE: sieve elements; Cr: crystal)

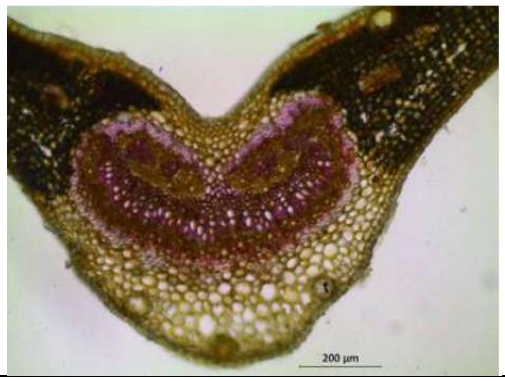


n) R.L.S. of phloem showing raphide bundles (Ra: raphides)

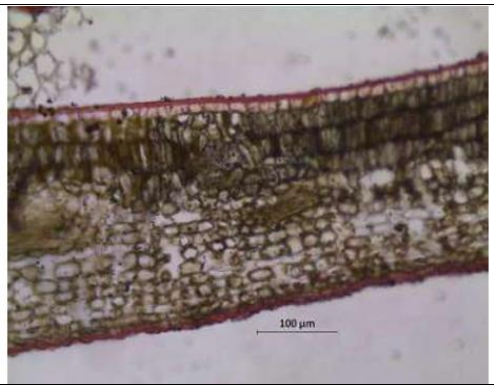


o) T.S. of wood showing vessels and paratracheal banded parenchyma (Ve: vessels; PBPa: paratracheal banded parenchyma; XFi: xylem fibres)

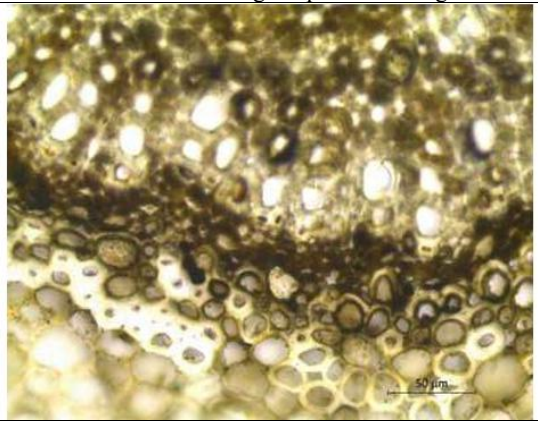
Fig 2: Anatomical structures of different plant parts of *Syzygium laetum*



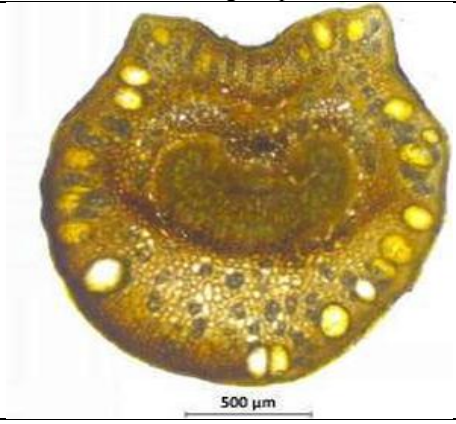
a) Histochemical studies showing the presence of lignin in the leaves



b) Histochemical studies showing the presence of oil in the leaves

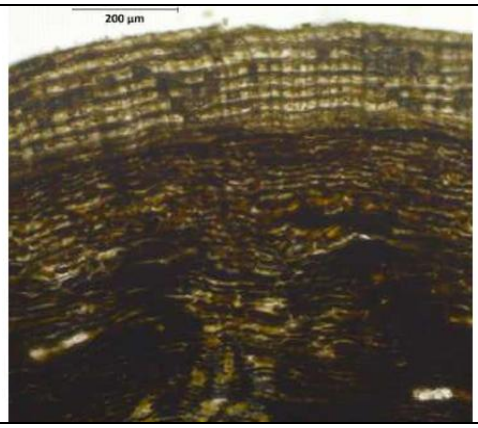


c) Histochemical studies showing the presence of tannin in the leaves



d) Histochemical studies showing the presence of starch in the petiole

e) Histochemical studies showing the presence of lignin in the stem



f) Histochemical studies showing the presence of tannin in the bark

Fig 3: Histological features of different plant parts of *Syzygium laetum*

Discussion

The functional traits of plants often reflect their ecological habitats (Zhu *et al*., 2015) [20]. Recent events have highlighted the significance of the association between plant structure and function, given that the physics of plant structure ultimately regulates key metabolic and physiological processes (Brodribb, 2009; Singh & Misra, 2015) [4, 14]. *Syzygium laetum* is a medium-sized tree distinguished by its smooth greyish-white bark, opposite elliptic leaves, and showy crimson or lemon-yellow bisexual flowers. Its long pedicels, prominent crimson stamens, and ovoid berry fruits with reflexed calyx lobes are distinctive features. According to Irulandi *et al*. (2016) [6], *S. laetum* is a medium-sized tree that can reach a height of 7 meters. Previous morpho-anatomical and histochemical investigations of *Syzygium* have primarily focused on the leaf and bark.

According to Soh and Parnell (2011) [17], subgeneric classification within *Syzygium* can be supported by a combination of anatomical traits, including stomatal type, crystal morphology and distribution, and midrib vascular organization. Anatomical and micromorphological traits of leaves are generally shared by some species within the *Syzygieae* tribe (Retamales *et al*., 2014) [11]. In the present study, leaf anatomy of *S. laetum* exhibited a thick midrib, dorsiventral lamina with distinct palisade and spongy tissues, well-defined secretory cavities, bicollateral vascular bundles, abaxial paracytic stomata, conical margins, and a semicircular petiole containing calcium oxalate druses and resin idioblasts. These structural characteristics not only aid in taxonomic identification but may also relate to the plant's physiological adaptations. Furthermore, Rameshkumar *et al*. (2015) [10] reported that the leaf essential oils of *S. laetum* are rich in sesquiterpenoids, particularly (Z,E)-α-farnesene and (E)-nerolidol, compounds that may contribute to its ecological and pharmacological significance.

The present study revealed that the bark of *S. laetum* possesses a thick periderm, a tannin-rich cortex, well-developed phloem rays, and calcium oxalate crystals within the parenchyma cells. Consistent with these structural characteristics, Irulandi *et al*. (2016) [6] reported that methanolic, ethyl acetate, and acetone extracts of *S. laetum* bark contain a wide range of secondary metabolites, including alkaloids, coumarins, flavonoids, phenols, saponins, terpenoids, tannins, and steroids. The methanolic extract exhibited strong antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, whereas the acetone extract showed comparable inhibition against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, and *Bacillus subtilis*.

The management of biodiversity has been severely hampered by the frequent misidentification of *Syzygium* in ecological assessments (Abdulrahman, 2021) [2]. Overall, characterization by anatomy and histochemistry offers strong evidence for quality control and can be used to distinguish between taxa that are difficult to understand (Smitha & Anto, 2023) [15]. It is possible to accurately recognize *Syzygium* species using both qualitative and quantitative anatomical features (Wangkhem *et al*., 2020) [19].

Conclusion

The present study provides information on the morpho-anatomical and histochemical profile of *Syzygium laetum*, highlighting its diagnostic features. Key features include a dorsiventral leaf with palisade and multiple mesophyll layers, paracytic stomata, and resiniferous cavities, bicollateral vascular bundles, a corky stem periderm, a tannin-rich cortex, and diffuse-porous wood with solitary vessels and thick fibers. Histochemical tests confirmed lignin in cell walls, lipid oils in secretory tissues, starch grains, and abundant tannins, which together serve as robust taxonomic markers. Overall, the combined morpho-anatomical and histochemical characteristics establish a taxonomic characteristic for *S. laetum*, facilitating accurate species identification and underscoring its ecological and pharmacological significance.

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