



THE ANATOMICAL CHARACTERISTICS OF *Ficus auriculata* LOUR. AND THEIR IMPLICATIONS FOR TAXONOMIC CLASSIFICATION WITHIN THE MORACEAE FAMILY

Che Nurul Aini Che Amri^{1,2*}, Rozilawati Shahari^{1,2}, Noor Syaheera Mohd Yunus^{1,2},
Wan Nurul Najihah Binti Wan Zainuzzaman¹, Mohd Razik Midin^{1,2}, Mohd Fauzihan
Karim^{1,2}

¹Department of Plant Science, Kulliyah of Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia

²Sustainable Agriculture and Green Technology Research Unit, Kulliyah of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

ABSTRACT

Ficus species is a source of human food worldwide due to its nutrients and medicinal potential. The present study aimed to determine the leaf anatomical and micromorphological characteristics that can be used to identify and classify *Ficus auriculata*. The method used in the anatomical study was sectioning of petioles, midrib, lamina, and margin using a sliding microtome and observation under a light microscope. Whereas the method used in micromorphological study was observing the adaxial and abaxial epidermal layers under a scanning electron microscope (SEM). The leaf anatomical characteristics that can be used to identify plant species are such as the presence of mucilage cells, hypodermis cells, type of cystoliths, the epidermal cells of the petiole and midrib, stomata type, cuticle layer, sclerenchyma cells and cuticular striation. In addition, the number of parenchyma cell layers, number of collenchyma cell layers, mesophyll cell, type of crystal oxalate, type of trichomes, location of idioblast tannin, type of marginal outline, and type of wax have been also recorded in this study. The information obtained from this study such as three types of trichomes were identified, the presence of idioblast tannin, mucilage canals at parenchyma cortex, presence of cystolith, solitary crystals and druses at the parenchyma cortex as well as the occurrence of granules and crustose waxes at the epidermal surface can be used for the identification of *Ficus auriculata*.

Keywords: Leaf anatomy, *Ficus auriculata*

ABSTRAK

Ficus merupakan tumbuhan yang menjadi sumber makanan manusia di seluruh dunia kerana khasiat dan potensi perubatannya. Kajian ini bertujuan untuk mengenal pasti dan menyenaraikan ciri-ciri anatomi dan mikromorfologi daun yang boleh digunakan untuk mengenalpasti dan mengelaskan spesies *Ficus auriculata*. Kaedah yang digunakan dalam kajian anatomi ialah hirisan petiol, tulang daun, lamina, dan tepi daun menggunakan mikrotom gelongsor dan pemerhatian di bawah mikroskop cahaya. Manakala, kaedah yang digunakan dalam kajian mikromorfologi dilakukan dengan memerhati lapisan epidermis adaksial dan abaksial di bawah mikroskop imbasan elektron (SEM). Ciri anatomi daun yang digunakan untuk pengecaman spesies kajian ialah seperti kehadiran sel musilaj, sel hipodermis, jenis sistolit, sel epidermis petiol dan tulang daun, jenis stomata, lapisan kutikel, sel sklerenkima, dan jaluran kutikel. Ciri tambahan seperti bilangan lapisan parenkima, bilangan lapisan kolenkima, sel mesofil, jenis kristal oksalat, jenis trikoma, lokasi tanin idioblast, jenis tepi daun, dan jenis lilin juga telah direkodkan. Maklumat yang diperolehi daripada kajian ini seperti tiga jenis trikoma telah dikenalpasti, kehadiran tanin, salur musilaj pada korteks parenkima, kehadiran sel sistolit, kehadiran kristal pada parenkima korteks serta lilin jenis berlakunya granula dan krutos pada permukaan epidermis boleh digunakan untuk mengenal pasti *Ficus auriculata*.

Kata kunci: Anatomi daun, *Ficus auriculata*

*Corresponding author:

Che Nurul Aini Che Amri

Kulliyah of Science,

International Islamic University Malaysia

Email: chenurulainicheamri@iium.edu.my

Introduction

According to Francis (2004), Moraceae is a tropical tree family with 37 genera and around 1100 species. Moraceae is distinguishable from other families by its milky or occasionally watery latex. Meanwhile, most plants in this family have unisexual flowers and alternate, simple, entire, and pinnately veined leaves. Francis (2004) agreed that the timber and bark of most plants in the Moraceae family have medicinal value, but the most medicinal plant organ is their fruit. The fruits usually are drupaceous and embedded in a fleshy receptacle, producing a syncarp, whilst the seeds are either big without endosperm or tiny with endosperm, depending on the plant type.

Among 37 genera in the Moraceae family, the *Ficus* genus is the most popular due to its benefit. *Ficus* has also considered one of the biggest flowering plants in the tropical and semi-tropical temperate zones. The plants in this genus vary from evergreen trees, shrubs, herbs, creepers, epiphytes, semi-epiphytes, and rheophytes. According to Herre et al. (2008), other than being used as a source of medicines and foods, *Ficus* species are also used as ornamental trees, lac hosts, fodder, fuel, hedges, and enclosures.

Ficus auriculata is also known as the Elephant ear fig tree because of its enormous leaves. In India, it is known as Timla, Gular, Tirmal, Timal, Timbal, Tremal, and Trimmal. The plants are mostly found in temperate, tropical, and subtropical climates, with elevations ranging from 1800 to 2600 meters. It is native to Asia, specifically India, Nepal, China, Bhutan, Pakistan, Thailand, Vietnam, Myanmar, and Malaysia (Tamtan et al., 2021).

The plant is a perennial shrub that can grow between four to ten meters tall, with greyish-brown rough bark and aerial roots (Figure 1). Its young leaves are initially red, transitioning to dark green as they mature. The leaves are simple, oval or ovate in shape, measuring 5-12.5 cm in

width and 5-8 cm in length, with petioles 1.2-5 cm long and stipules 1.5-2 cm. The fruit is pear-shaped, has 8-12 longitudinal ridges, is 3-5 cm in diameter, and contains embedded achenes. When ripe, the fruit turns dark red.

Ficus auriculata is an ethnomedicinal plant used in traditional folk medicine to treat a variety of human maladies. Tamuly et al. (2014) discovered that stem barks of *F. auriculata* had been used to treat diarrhea, dysentery, cuts, and wounds since ancient times and continue to be so today. This is because the bark, leaves, and fruit extracts contain phytochemicals such as carbohydrates, saponins, alkaloids, resins, glycosides, phytosterols, diterpenes, phenols, tannins, proteins, amino acids, and flavonoids. These phytochemical compounds cause the fruits, leaves, and bark of *F. auriculata* to have antioxidant, antibacterial, hepatoprotective, toxicity activity, anticancerous, antidiabetic, hyperlipidemic, hyperglycemic, and anti-inflammatory properties as proved in studies conducted by Kumari et al. (2008) and Tamtan et al. (2021). Aside from that, the leaf extract of *F. auriculata* plays a vital role in Glutathione levels in cardiac tissue (Tamtan et al., 2021). According to Damy et al. (2009), glutathione is essential for cell survival, and glutathione deficiency is frequently associated with heart failure.

Taxonomy research is necessary for identifying and classifying plant species. One of the most critical parts in plant identification is the anatomical part. Incomplete data can lead to contradictory and ineffective plant identification and classification. Data validity cannot be established solely based on taxonomic data. *Ficus* species, a plant consumed by people all over the world due to its nutrients and medicinal potential, has undergone extensive research on medicinal values, including biological activities and bioactive contents. Although studies on the medicinal properties of *Ficus* have been extensively studied, research on the anatomy and

micromorphology of this genus is still very little. Thus, this study explains their anatomical and micromorphological features, which can assist taxonomists and botanists in identifying and classifying plants by providing additional information for *F. auriculata*.



Figure 1. A) Trees of *F. auriculata*.



Figure 1. B) Fruits of *F. auriculata*.

Materials and Method

Ficus auriculata samples were collected from International Islamic University Malaysia (IIUM) in Kuantan, Pahang. Information of the collected samples in triplicates was recorded by using sample

description tag including the morphological description. The samples were pressed using wood pressers and dried at 55°C in an oven for two weeks duration. The dried samples were mounted on herbarium vouchers after two weeks by knitting the dried samples with needles and cotton thread. The herbarium specimens were labeled and kept in the IIUM Kuantan herbarium room for future reference. To eliminate dirt, the freshly obtained fresh leaf specimens were washed with tap water. The plant specimens were then sliced into three sections mainly apex, middle, and base, all of which are connected to the petiole. The plant specimens were then kept in glass jars with fixative solution (a combination of 70% ethanol and 30% acetic acid in a 1:3 ratio) (Johansen, 1940). After that, the glass jars are placed in sample storage.

To investigate the anatomical features of *F. auriculata*, three sections of the leaves, the petiole, midrib, and the margin were chosen. These components were cut using a sliding microtome supported with polystyrene (Choat & Schmitz, 2011). The polystyrene was trimmed beforehand to suit the specimens. The slicing was done using a disposable knife at a thickness of 40-60 μm . The sliced specimens were then submerged in a petri dish filled with water in order to remove the specimens from the polystyrene. The sliced parts were then placed in a petri dish filled with distilled water and Clorox. The specimens were stained using Safranin and Alcian Blue then dehydrated in a series of alcohol (50 %, 70 %, 90 % & 100 %) solutions mounted on a glass slide with a few drops of alcohol 100% to flatten the mounted specimen. Euparal was used to seal the slide, which was then covered with a coverslip. The slides were dried in an oven with a temperature of 50°C for two weeks.

The selected plant materials were taken from the herbarium dried sample for the leaf micromorphology study. The leaves samples were cut into 1 cm^2 piece

(leaves samples were cut at the lamina area) and mounted on a mounting holder that positioned with gold using a plated machine. The sample were then observed under scanning electron microscope under magnifications of 100x, 500x, and 1000x. Vital characteristics such as wax structure, trichome, and stomata arrangement were observed.

Results and Discussion

Cross Section of Petiole

Vascular tissue: Closed vascular system with non-continuous rings together with fifteen medullary vascular bundles located at the parenchyma pith (Figure 2A). **Sclerenchyma cell:** Clusters of sclerenchyma cells present at the vascular tissues (Figure 2B). **Parenchyma cell:** 4-14 layers of parenchyma cells (Figure 2A). **Collenchyma cell:** 12-20 layers of collenchyma cells present under the epidermis of abaxial and adaxial (Figure 1C). **Idioblast tannin:** Present in parenchyma cortex and pith parenchyma (Figure 2H). **Mucilage cell/canals:** mucilage cells present in parenchyma cortex and mucilage canals present in pith parenchyma. **Crystal oxalate:** solitary crystals and druses present at the parenchyma cortex and pith parenchyma (Figure 2B). **Trichomes:** Simple unicellular trichome (short, pointed end); present at the epidermis of abaxial and adaxial (Figure 2A).

Cross Section of Midrib

Vascular tissue: Closed vascular system with continuous rings together with ten medullary vascular bundles located at the pith parenchyma (Figure 2D); present of intraxylary phloem at pith parenchyma. **Sclerenchyma cell:** Clusters of sclerenchyma cells present at the vascular tissues (Figure 2E). **Parenchyma cell:** 10-14 layers of parenchyma cells (Figure 2D). **Collenchyma cell:** 6-7 layers of collenchyma cells under the epidermis of

abaxial and adaxial (Figure 2F). **Idioblast tannin:** Present. **Mucilage cell/canal:** Mucilage cells present in parenchyma cortex and mucilage canals present in pith parenchyma. **Crystal oxalate:** Solitary crystal present at the parenchyma cortex and pith parenchyma. **Trichome:** Simple unicellular trichome (short, pointed end) and Simple unicellular trichome (long, pointed end); present at the epidermis of abaxial and adaxial (Figure 2F).

Cross Section of Lamina

Cuticles: Thick layer of cuticle present on abaxial and adaxial epidermis. **Hypodermis cells:** Present. **Chlorenchyma cells:** Palisade cells present in ca. 2-3 layers equal to 1/2 of leaf thickness, each cell five to six times higher than wide. Spongy mesophyll cells in ca 5-7 layers, rounded, with intercellular spaces (Figure 3A). **Cystolith:** Present (Figure 3A). **Crystal oxalate:** Druses present in chlorenchyma cells. **Trichomes:** Simple multicellular trichome (short, pointed end) and simple multicellular trichome (short, blunted end); present at the epidermis of abaxial and adaxial.

Cross Section of Margin

Outline of leaf margin: Truncate with pointed end, recurved 90° towards abaxial epidermis, the size decreases gradually towards edge of margin, hypodermis, and vascular bundles absence at edge of margin (Figure 3B & C). **Trichomes:** Absent.

Leaf Epidermis Micromorphological Characteristics

Abaxial wax: granules and crustose. **Adaxial wax:** Granules and Crustose. **Adaxial cuticular sculpturing:** Epidermal cells outline is distinguishable, anticlinal walls slightly raised into ridges and periclinal walls sunken; striae present on adaxial surfaces (Figure 3 D&E). **Abaxial cuticular sculpturing:** Epidermal cells outline is indistinguishable, anticlinal and periclinal walls cannot be differentiated due to the presence of high density of trichomes

and stomata (Figure 3 F-H). **Stomata:** Hypostomatic; only present on abaxial epidermis. epidermal and subsidiary cells cannot be differentiated, strip of cuticular wax present, guard cells pair is elliptic Stomata size; abaxial: W=6.82 μ m, H=10.36 μ m (width: min= 6.74 μ m, max=6.90 μ m; height: min=8.63 μ m, max=12.08 μ m). **Trichome:** Simple multicellular trichome (long, pointed end) (densely scattered on the abaxial surface) (Figure 3I).

The findings of the study highlighted the importance of leaf anatomical characteristics in identifying *F. auriculata*. According to Cutler et al. (2008), mucilage cells contain mucilage, gum, or similar carbohydrate substance and can be found distributed throughout the parenchyma. The mucilage in leaves may assist the leaves to maintain water capacity when soil water deficiencies arise; hence, it aids in the storage of food and water (Tosif et al., 2021). In short, the mucilage in plants can be said to aid in the storage of water and food, seed germination, and the thickening of membranes. Mucilage cells may be easily distinguished from other cells in the petiole and midrib cross-sections because they are distinct and oversized. In this finding, mucilage cells were found in the parenchyma cortex of plant species investigated.

The hypodermis is a layer of cells that lies directly under the epidermis and is made up of sclerenchyma or collenchyma. It appears between the epidermis and palisade tissue. The hypodermis is typically a water-storing tissue. Based on the findings of this study, the hypodermal cell exists in *F. auriculata* which is a layer of hypodermal cells is detected underneath the epidermal cells of adaxial lamina parts. Bercu and Popoviciu (2014) also concluded that the thick cuticle thicknesses, a multiple epidermis, and hypodermis are probably anatomical features of adaptation to xerophytic environments since *Ficus* species also considered as a xerophytic plant. Hypodermal cells are most common

in xerophytic plants because they are adaptations for living in environments with minimal liquid water (Cutler et al., 2008).

A cystolith is a particular protrusion of the cell wall formed with calcium carbonate. According to Cutler et al. (2008), cystolith is a common feature of several plants of the *Ficus* genus, a genus in the Moraceae family. As a result, this trait may be used to distinguish plants in the Moraceae family from those in other families. Cystoliths are specialized structures, and the enlarged structures are called lithocysts, where both are seen in *F. carica* (Bercu & Popoviciu, 2014). Gal et al. (2012) described that the cystoliths act as internal light scatterers, distributing light flux more uniformly inside the leaf. Cystolith formation begins as the inner cell wall expands towards the cell interior. While the receding cell membrane confines the mineral, this extracellular protrusion grows and mineralizes simultaneously. The cystolith found in this study support the findings of Bercu and Popoviciu (2014), proving that the plant in the genus *Ficus* possesses cystolith. On the other hand, calcium carbonate crystals are found as cystoliths in a few families, including Moraceae, Urticaceae, and Acanthaceae. This character also can be considered as a valuable character in the taxonomic study, especially to characterize the subfamilies and genus of Acanthaceae (Zakaria et al., 2020; Mohd Tajudin et al. 2022).

The observation under Scanning Electron Microscope (SEM) revealed the stomatal type of species studied is known as hypostomatic (only has stomata on the underside), although the form and condition of stomata are unknown due to epidermal and subsidiary cells cannot be differentiated. Based on the study reported by Khan et al. (2011), *F. auriculata* have a paracytic stomatal type that hypostomatic characterizes. In addition, the cuticular membrane, is known as an extracellular structure composed of cutin and waxes. It works as a barrier against desiccation and external environmental forces in general. It

is often defined as a thick extracellular waxy coating covering the epidermis's outermost layer. Carrillo-López and Yahia (2019) define the cuticular membrane by its resistance to water loss by transpiration, pathogen invasion, and chemical penetration. Thus, the present findings established the existence of thick layers on both the abaxial and adaxial epidermis of *F. auriculata*.

Fibers and sclereids are the two primary forms of sclerenchyma cells. Fibers are elongated cells found in stems, roots, and vascular bundles in leaves. As in the case of asparagus, fibers provide fibrousness. Sclereids come in various morphologies (spherical, oval, or cylindrical) and may be found in various plant tissues, including the periderm, cortex, pith, xylem, phloem, leaves, and fruits. According to Carrillo-López and Yahia (2019), this type of cell is responsible for the hardness of the shell of nuts, the coat of numerous seeds, and the stone of drupes (cherries and plums). Sclereids, which are found distributed in the parenchymatous tissue of various fruits, such as pears and quinces, can lend a gritty feel to them. In this study, the results showed that sclerenchyma cells were observed in the vascular bundles of species studied thus support previous findings by Fan et al. (2019) which mentioned certain *Ficus* species have sclerenchyma cell distribution.

The abaxial cuticular sculpturing based on electron microscope for *F. auriculata* cannot differentiate. Plus, anticlinal and periclinal walls cannot be differentiated due to a high density of trichomes. Meanwhile, the adaxial cuticular sculpturing, epidermal cells outline is distinguishable for both species, anticlinal walls slightly raised into ridges and periclinal walls sunken; striae present on adaxial surfaces. According to Pott et al. (2007), most striae arise at the papillae's terminals on stomatal subsidiary cells. Striae also contribute to the non-smoothness of the cuticle region, which aids in plant protection.

Cross-section of petioles and midrib of *F. auriculata*, revealing the existence of parenchyma cells. The results of the study show that the layers of *F. auriculata* are 4-14 layers on the petiole and 10-14 layers on the midrib. More layers of parenchyma cells in both species analyzed may be related to increased photosynthesis and transpiration. Other than that, cross-sections of the petiole and midrib of *F. auriculata*, demonstrated the presence of collenchyma cells beneath the epidermal layer. The results of this study show that 12-20 layers of collenchyma are found in the petiole species studied while 6-7 layers are recorded on the midrib cross-section. Collenchyma cells are defined as cells that offer support, structure, mechanical strength, and flexibility to the petiole, leaf veins, and young stem of the plants, allowing for easy bending without breakage (Carrillo-López & Yahia, 2019). It appears to be well adapted to support leaf and stem growth as it grows harder or more complex in older plant parts than in younger ones and may turn into sclerenchyma by the deposition of lignified secondary walls (Evert, 2006).

According to Tütüncü Konyar et al. (2014), even though the shape, size, and composition of crystals vary between species, they will be categorized into five major classes based on their morphology: prism, druses, styloid, raphides, and crystal sand. Numerous physical, chemical, and biological elements, including light, temperature, pH, ion concentration, and herbivory, have been shown to influence the location, size, and other aspects of crystals in plants (Tütüncü Konyar et al., 2014). This study discovered calcium oxalate crystals as solitary crystals and druses in the parenchyma cortex and pith of petiole and midrib cross-sections of *F. auriculata*. According to Sharawy (2004), the presence of calcium oxalate crystals in the vegetative leaf of the Moraceae plants is ubiquitous.

Idioblast tannins are specialized cells that contain tannins. Based on this study, idioblast tannin were identified in petiole part of *F. auriculata*. Idioblast tannins protect plants from dehydration, rotting, and insect damage. Tannins are present in cells of the parenchyma and collenchyma. According to Berg et al. (2006), idioblast tannins are abundant in the Moraceae family, particularly in the *Ficus* genus. In addition, Khan (2011) and Shakir and Bagi (2016) discovered that most *Ficus* species, including *F. auriculata*, had non-glandular trichomes. Khan (2011), however, discovered that *F. auriculata* only had unicellular trichomes. Other studies notice distinct trichomes in *Ficus* species might be due to the plant's varied habitats and adaption processes. Trichomes were seen on the species investigated in this study on the adaxial and abaxial surfaces which is *F. auriculata* has simple unicellular trichomes (short, pointed end), simple unicellular trichome (long, pointed end) and simple multicellular trichome (short, blunted end).

This study also revealed that the abaxial and adaxial waxes of *F. auriculata* are granules and crustose. According to Chessa et al. (1992), epicuticular wax also has a significant function in decreasing fungal invasion and mechanical abrasion and in resisting harsh climatic conditions such as cold and pollution. However, the changes in the structure, amount, and composition of the surface wax layer vary during the different stages of the plant. Besides that, wax is frequently impacted by temperature, sunshine, humidity, and wind.

As a result of these considerations, it is debatable whether the type of wax may be utilized as a critical characteristic in the anatomical studies.

Conclusion

Plant species can be identified and classified using anatomical and micromorphological research data. Plant anatomy and micromorphology can reveal common and variation traits that help identify plant species, especially for plants with similar morphology. Results showed the presence of idioblast tannin and mucilage canals at parenchyma cortex, presence of cystoliths, solitary crystals and druses at the parenchyma cortex as well as the occurrence of granules and crustose waxes at the epidermal surface can be useful as an additional data for identification of species studied. Other than that, three types of trichomes were identified which are simple unicellular trichomes (short, pointed end), simple unicellular trichome (long, pointed end) and simple multicellular trichome (short, blunted end). In conclusion, the combination of leaf anatomy and micromorphological characteristics can be useful in identification and classification of *F. auriculata*.

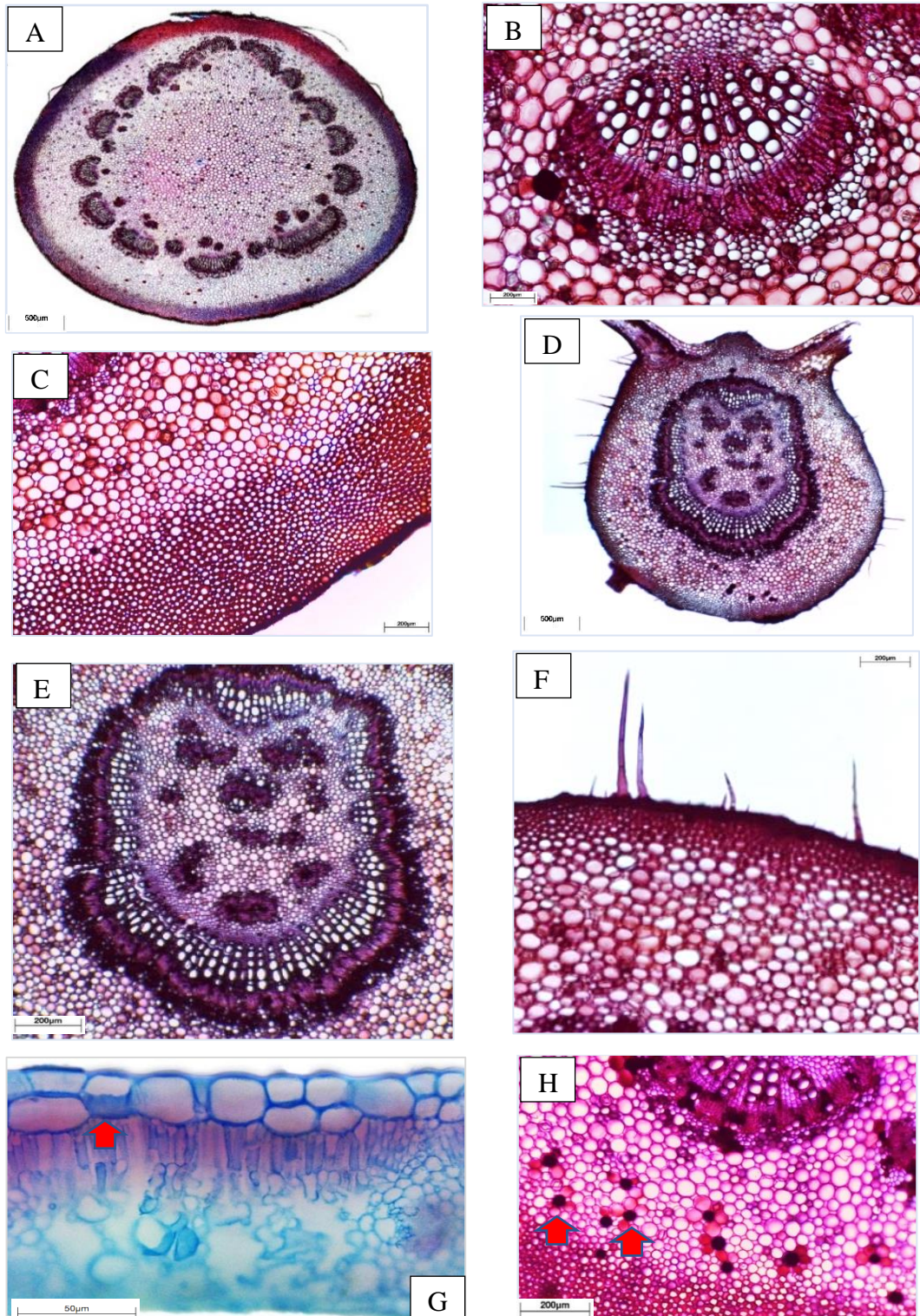


Figure 2: *Ficus auriculata*. A&B) Cross Section of Petiole and vascular bundle. C) Layers of collenchyma. D&E) Cross Section of Midrib. F) Trichomes. G&H) Cross Section of lamina; Cystolith (red arrow). I) Idioblast tannin (red arrow). Scale: A,D) 500 µm. B,C,E,F,G,H,I) 200 µm.

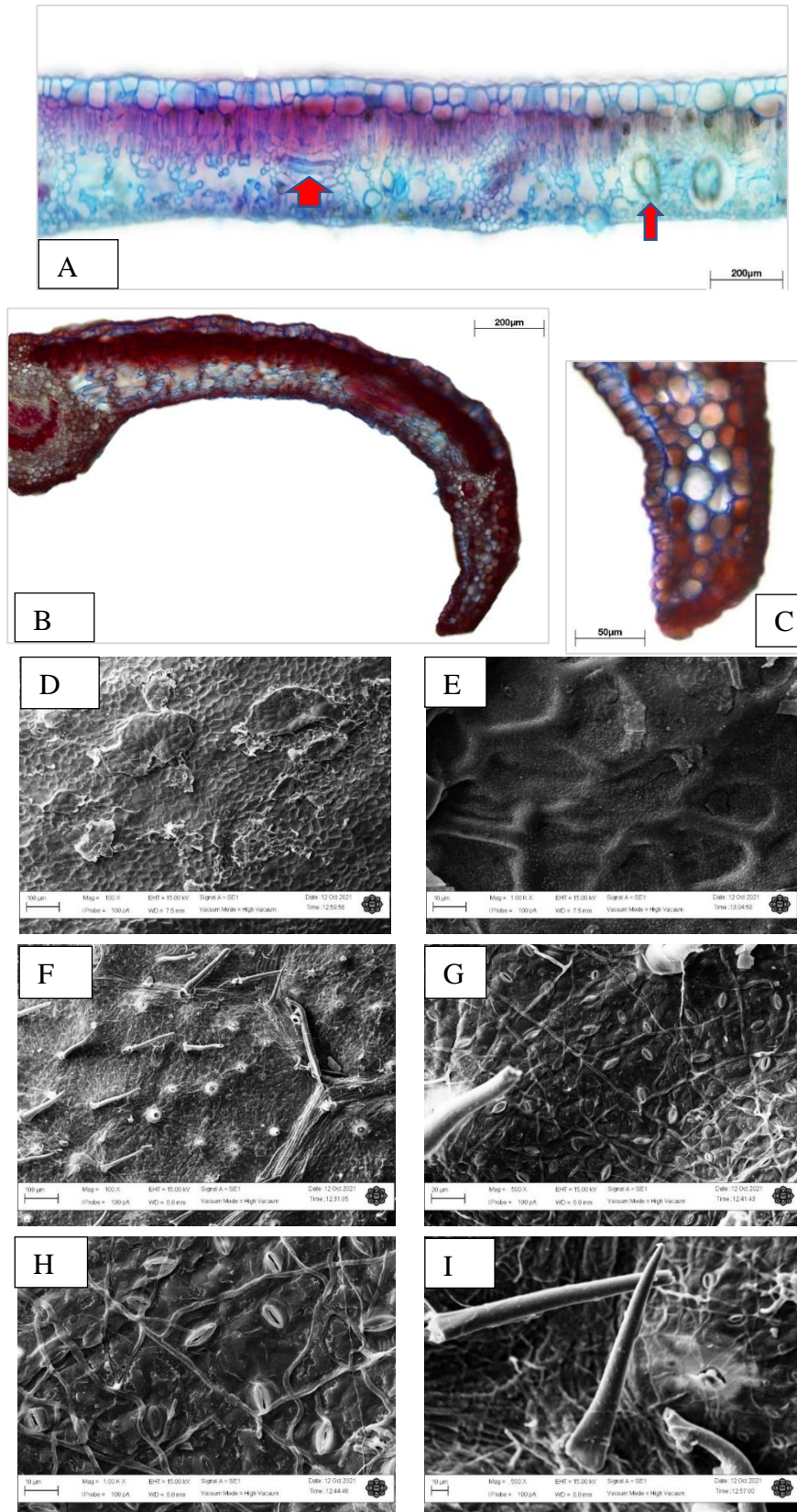


Figure 3: *Ficus auriculata*. A) Cross Section of Lamina. B&C) Cross Section of Margin. D-E) The adaxial epidermis showing cuticular striation. F-H) The abaxial epidermis showing cuticular striation. I) Trichomes.

Scale: A&B) 200 μm . D&F) 100 μm . C) 50 μm . E,G,H,I) 20 μm .

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