

Anatomical character of *Aglaia cucullata* (Roxb.) Pellegr., a medicinal plant found in mangrove forest of Thailand

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Abstract

Aglaia cucullata is the sole member of the genus *Aglaia* that is regarded as a true mangrove species. The plant was discovered in the mangrove forest of Thailand's peninsular region. Anatomical structure has a significant impact on how a plant functions and adapts to its environment. The plant samples were collected from Tha Pom Klong Song Nam, Krabi province, a mangrove forest of Thailand. The materials were processed into permanent slides using a paraffin method, a sliding microtome technique and scanning electron microscopic technique. The anatomical characters of root, stem, petiole, petiolule, rachis, leaf and wood were investigated. The results revealed that the vascular system of the petiole and petiolule are heart-shaped, whereas the rachis appears concave shaped on both sides. These features are essential for taxonomic identification. Additionally, the multiseriate aseptate peltate trichomes discovered in this study are also exclusive to this genus. Anatomically, multiple palisade mesophyll, tanniferous cells and crystals were observed. The results of this study revealed that anatomical traits can be used to certify the species and to understand how the plant adapts its structure to survive in severe environment.

Keywords: *Aglaia cucullata*; Anatomical structure; Mangrove

1. Introduction

Meliaceae is a big family which comprises 49–50 genera with 620 species. They are found throughout the pantropical region and into the subtropical zone (Mabberley *et al.*, 2007; Mabberley, 2011). The largest genus in the Meliaceae family is *Aglaia* Lour. It comprises approximately 120 species, which are found from Southeast Asia, including Sri Lanka and India, to Australia and as far east as the island of Samoa (Pannell, 1992; Mabberley *et al.*, 2007; Muellner *et al.* 2008). However, there are only 32 species of *Aglaia* have been recorded in Thailand (Wongprasert, 2011). *Aglaia* distribution

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throughout Thailand, encompassing the Northern, North-Eastern, South-Western, South-Eastern, and Peninsular regions (no verb). This genus grows in a wide range of forests, including tropical evergreen rain forests, lowland evergreen forests, mixed deciduous forests, swamp forests, along ridges near sea or streams, limestone bedrock, sandstone bedrock, and granite bedrock (Wongprasert, 2011). However, only one species, *A. cucullata*, presents in mangrove forests (Heads, 2019).

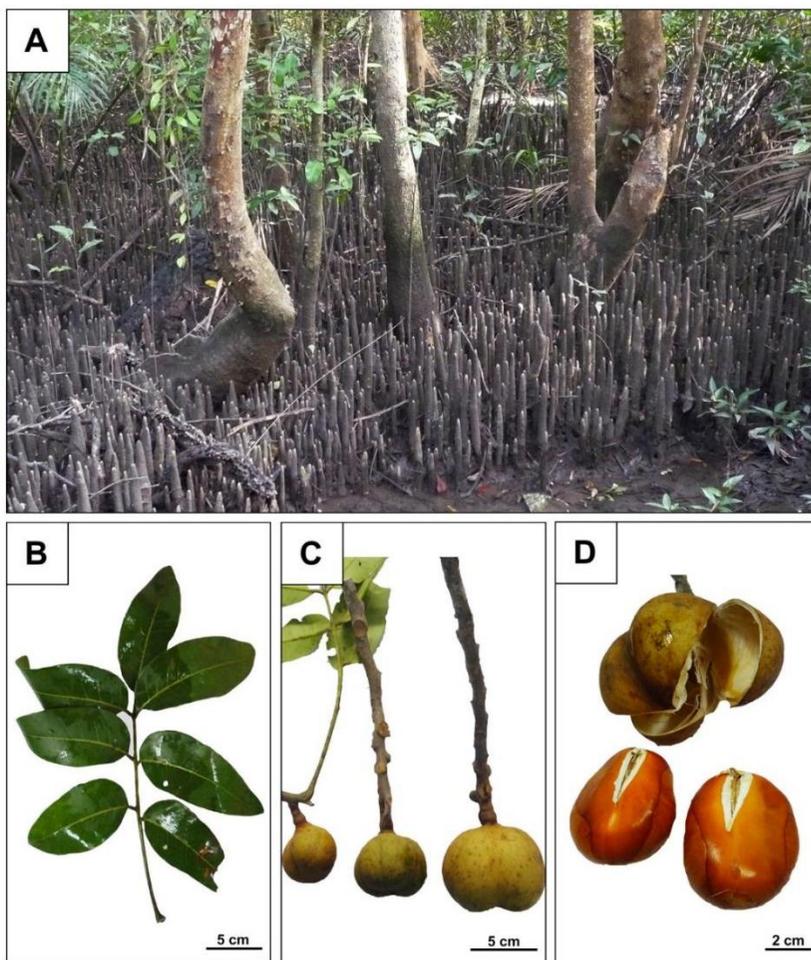


FIGURE 1. Morphological characters of *A. cucullata*; (A) The pneumatophores in mangrove forest (B) Imparipinnate compound leaves with 7 leaflets (C) Fruits with 2 locules (D) Seeds covered with orange aril

Aglaia cucullata (Roxb.) Pellegr. is a medium sized tree up to 20 m tall, with stellate hairs or peltate scales. The trunk is smooth with brown or pale orange, which peels in small brittle scales.

Wood is pale yellowish-brown to orange-brown, with white latex. Pneumatophores are conical shape up to 60 cm tall (Figure 1A). Imparipinnate compound leaves, which arranged subopposite or alternate, have 5-9 leaflets, their leaflets are sickle-shaped with indumentum (Figure 1B). Flowers are thyrses, which arrange in axillary leaf and branch. Florets include 3 yellowish petals with 6 protruding anthers. Ovary superior, with 3 locules, each locule has 1 ovule. Fruits are reddish yellow sub-globose up to 7 cm diameter, which are loculicidal capsules with 1 to 3 locules (Figure 1C). The locule has 1 seed, which is enclosed with red aril (Figure 1D) (Mabberley *et al.*, 1995; Giesen *et al.*, 2006; Boonyawetchewin and Buasali, 2011; Mabberley, 2011; Wongprasert, 2011; Heads, 2019). (We can keep or leaf this phase)

Aglaia cucullata (Roxb.) Pellegr. (syn. *Amoora cucullata* Roxb.) is a medium sized tree up to 20 m tall (Figure1A-1D). The botanical applications of this species *Aglaia cucullata* (syn. *Amoora cucullata* Roxb.) include medicinal and wood purposes. The plant produces bioactive components, which are diterpenoids (Ahmed *et al.*, 2010), triterpenoids (e.g., fridelin, betulinic acid) (Rahman *et al.*, 2005), alkaloids (e.g., putrescine bisamide, cucullamide) (Abdelfattah *et al.*, 2010; Ahmed *et al.*, 2010), flavonoids, glycosides, steroids (e.g., stigmasterol, beta-sitosterol) and rocaglamide derivatives (Chumkaew *et al.*, 2006; Ahmed *et al.*, 2010; Xu *et al.*, 2019). Their bioactive potentials influence cytotoxic activity, antibacterial activity, and antifungal activity;. Moreover, their phytochemical components have been utilized to treat inflammation, marrow, and diarrhea (Basak *et al.*, 1996; Chumkaew *et al.*, 2006; Rahman and Rashid, 2009; Ahmed *et al.*, 2010; Pervin *et al.*, 2016; Xu *et al.*, 2019). *Aglaia* timber is very useful for uses include construction, furniture, flooring and boat construction as well as fuel wood (Mabberley *et al.*, 1995; Giesen *et al.*, 2006; Khaopakro *et al.*, 2015).

In mangrove forests, plant morphology and anatomy play significant roles in adaptation to stressful environments., although very little information is known about them. This study concentrated on the anatomical features of the root, stem, petiole, rachis, petiolule, and leaf blade of *A. cucullata* found in mangrove forest of Thailand. The collected The results may support species identification and understanding the plant adaptations.

2. Methods

2.1 Paraffin method

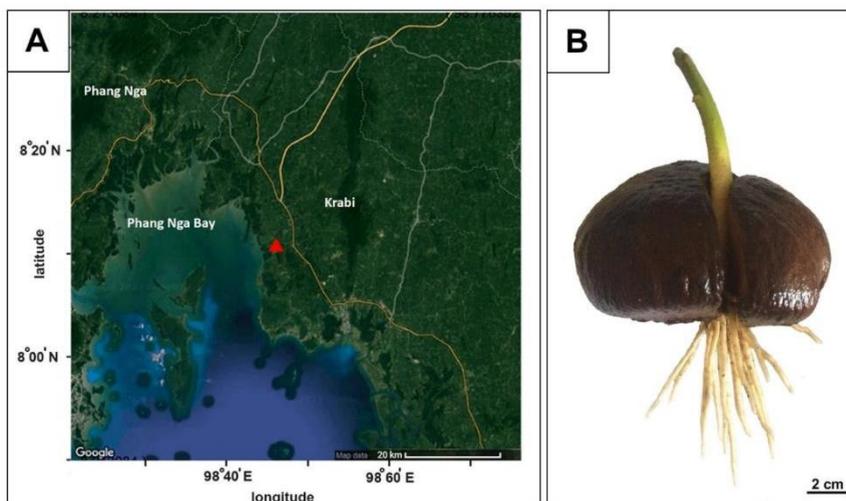


FIGURE 2. Material collection of *A. cucullata*; (A) Tha Pom Klong Song Nam mangrove forest on google earth (B) Germinating seed

The samples were collected in Tha Pom Klong Song Nam mangrove forest, Krabi province, Thailand (08°12'N, 98°46'E) (Figure 2A). Six mature seeds were collected and germinated in pots. The six replicates of roots at 2 cm from root tip, that germinated from seed (Figure 2B), stems at 5 cm below shoot tip and mature leaves at 5th node from the apex. The samples were preserved in formalin acetic alcohol (FAA) 50% solution. The paraffin method was used to make permanent slides (Johansen, 1940; Kermanee, 2008). The specimens were dehydrated using a tertiary butyl alcohol series, infiltrated in liquid paraffin, embedded in paraplast. The samples were sectioned into 10–15 micron thicknesses using a rotary microtome (Leica; RM 2165, Germany). The sections were stained with safranin-T and fast green CFC combination, the slides were mounted with permount. The slides were observed under a compound light microscope (Zeiss; Axioskop 2, Germany) equipped with a camera (Zeiss; AxioCam MRc, Germany). The data were analyzed by a Zen 2 program.

2.2 Sliding microtome method

Wood samples were cut into a block of 1×1×3 cm at three surfaces (transverse, tangential and radial longitudinal surfaces). After that a sliding microtome (Leica; SM 20120 R, Germany) was used to section the samples at 20 μm thickness. The sections were stained with 1% safranin T for 12 h. The excess staining was washed away with water and then dehydrated with ethyl alcohol series (30%, 50%, 70%, 95% and 100%), transferred to a mixture of absolute ethanol and xylene (1:1) finally immersed in pure xylene at least 6 h. The wood sections were mounted onto a microscopic slide with permount.

2.3 Scanning electron microscopic (SEM) method

Wood samples derived from a sliding microtome section (100 µm thickness), stem and leaf sections were affixed on a stub with a double-sided carbon tape and coated with gold particles using a sputter coater gold particles machine (Quorum; SC7620, England). The specimens were observed under a scanning electron microscope (FEI; Quanta 45, Czech Republic).

2.4 Data analysis

The wood anatomical characters were analyzed and described following the IAWA list of microscopic features for hardwood identification (IAWA Committee, 1989). The qualitative and quantitative data were analyzed and measured under a light microscope (Zeiss; Axioskop 2, Germany) assembled with a camera (Zeiss; AxioCam MRc, Germany) and Zen 2 program. The quantitative data were measured at least 50 replications of each feature. These features consist of vessel density, vessel area percentage, vessel diameter, vessel element length, fiber diameter, fiber lumen diameter, fiber length, fiber wall thickness, ray spacing, percentage of ray area, ray width and ray height. The data were exported to evaluation including the average and standard deviation.

3. Results and Discussion

3.1 Root

There is a large of epidermis which tangential wall is thick. Cortex contains multilayer of parenchyma and air spaces were are observed in cortex (Figure 3A). A large of endodermis presents beneath the cortex. Tanniferous cells are abundance in this layer. There are 2-4 layers of pericycle. A phloem zone presents under pericycle. Multiple grounds of primary xylem occur around pith. There are 2-5 layers of cambium present between phloem and xylem. Pith contains parenchyma which some cells deposit tannin (Figure 3B).

3.2 Stem

There is a layer of epidermis which all cells accumulate tannin (Figure 3C). Multiseriate aseptate peltate trichomes were observed on stem epidermis under scanning electron microscope analysis (Figure 3E, 3F). A layer of hypodermis occurs under epidermis. Cortex contains parenchyma which some cells accumulate tannin. Ergastic crystals were observed in outer cortical parenchyma (Figure 3D). A ring of phloem and xylem surround is divided by vascular cambium. Pith is in the center of stem which contains parenchyma.

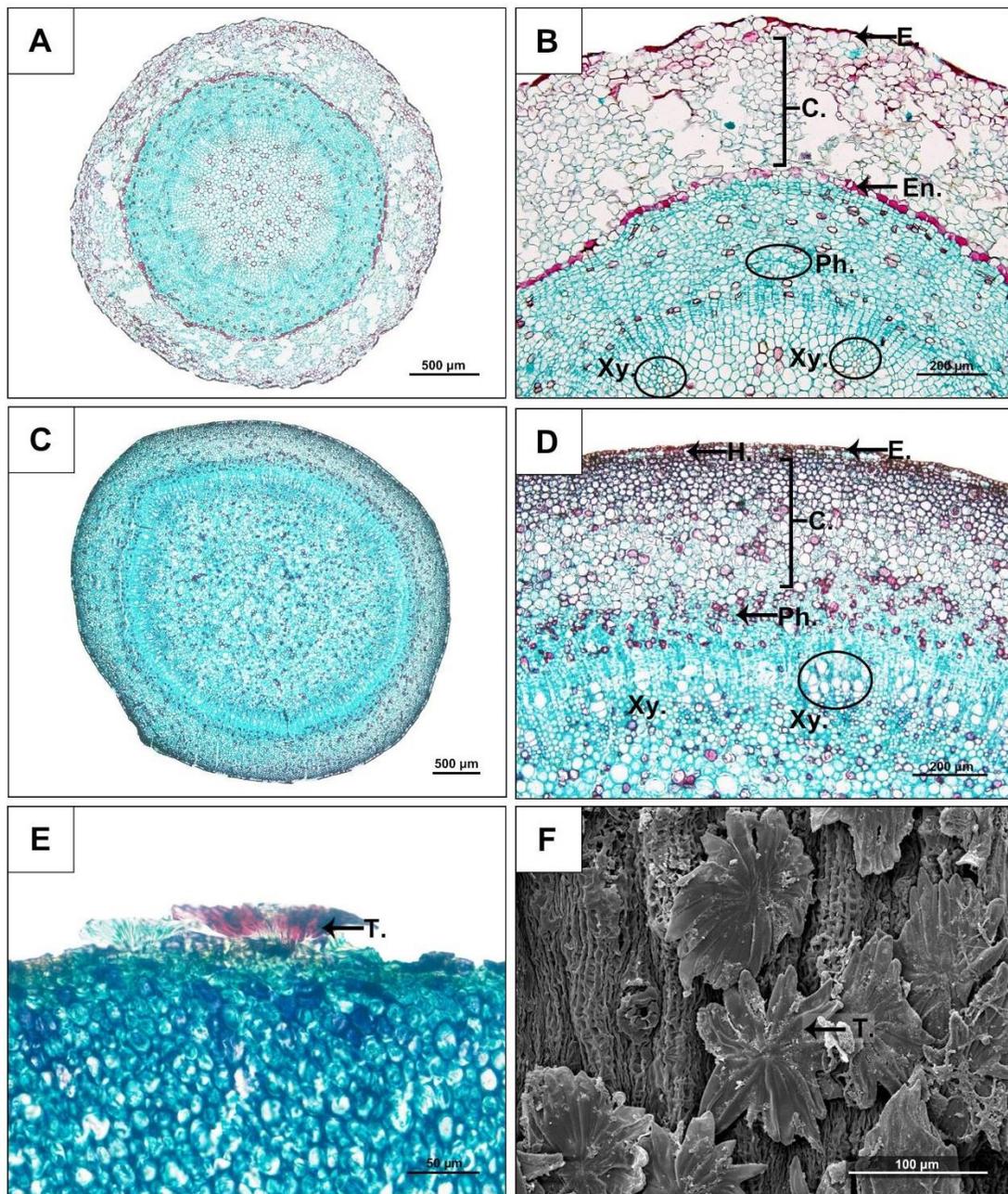


FIGURE 3. Anatomical characters of *A. cucullata*; (A) Root (B) Stele of root (C) Stem (D) Epidermis, cortex, phloem, xylem and pith of stem (E) Multiseriate aseptate peltate trichome (F) Multiseriate aseptate peltate trichomes under SEM (C. = cortex, E. = epidermis, En. = endodermis, H. = hypodermis, Ph. = phloem, T. = trichome, Xy. = xylem)

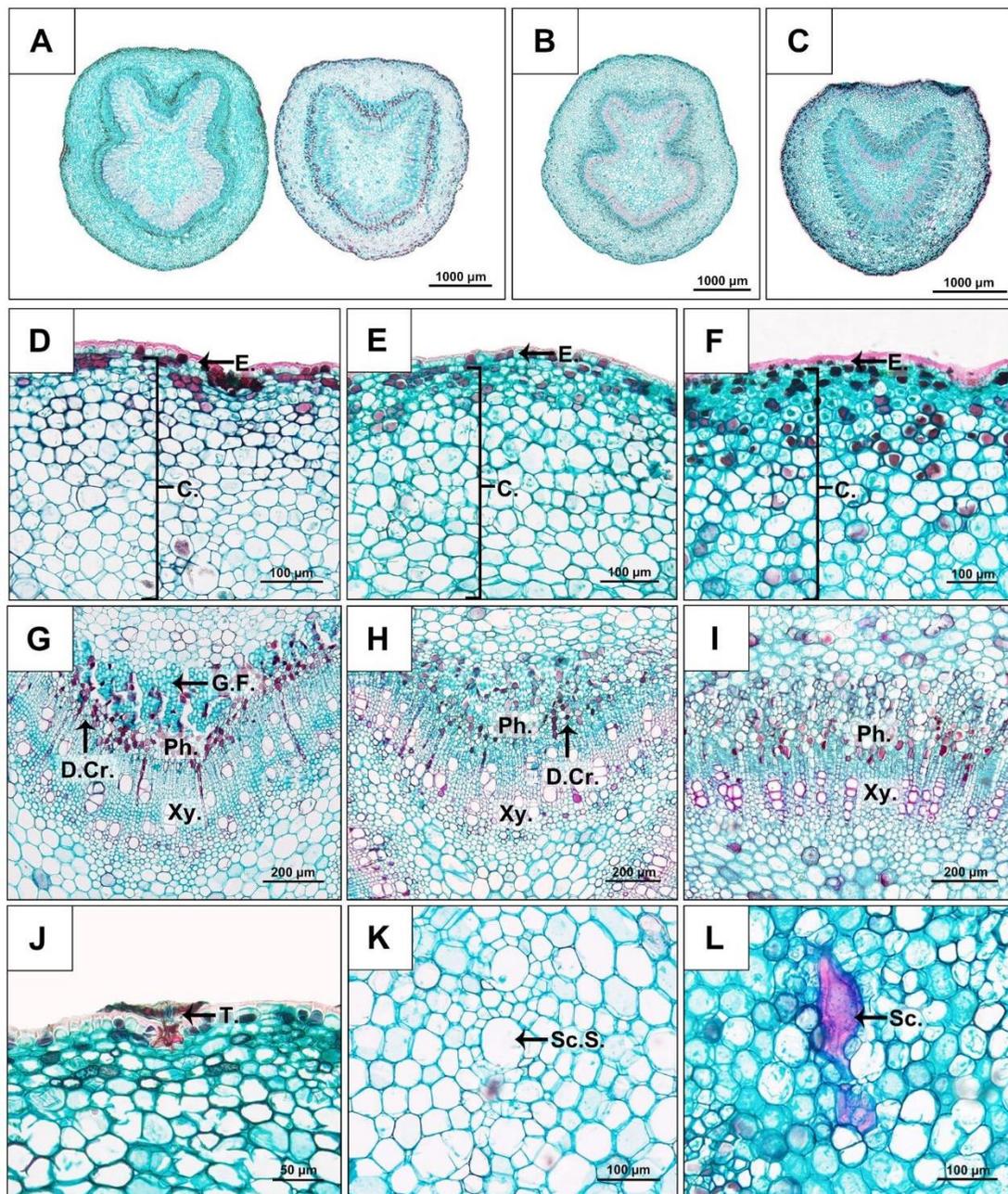


FIGURE 4. Anatomical characters of *A. cucullata*; (A) Petiole (B) Rachis (C) Petiolule (D) Epidermis and cortex of petiole (E) Epidermis and cortex of rachis (F) Epidermis and cortex of petiolule (G) Stele of petiole (H) Stele of rachis (I) Stele of petiolule (J) Trichome on petiole epidermis (K) Schizogenous secretory in rachis pith (L) Sclereid in petiolule pith (C. = cortex, D.Cr. = druse crystal, E. = epidermis, G.F.= gelatinous fiber, Ph. = phloem, Sc. = Sclereid, Sc.S. = schizogenous secretory, T. = trichome, Xy. = xylem)

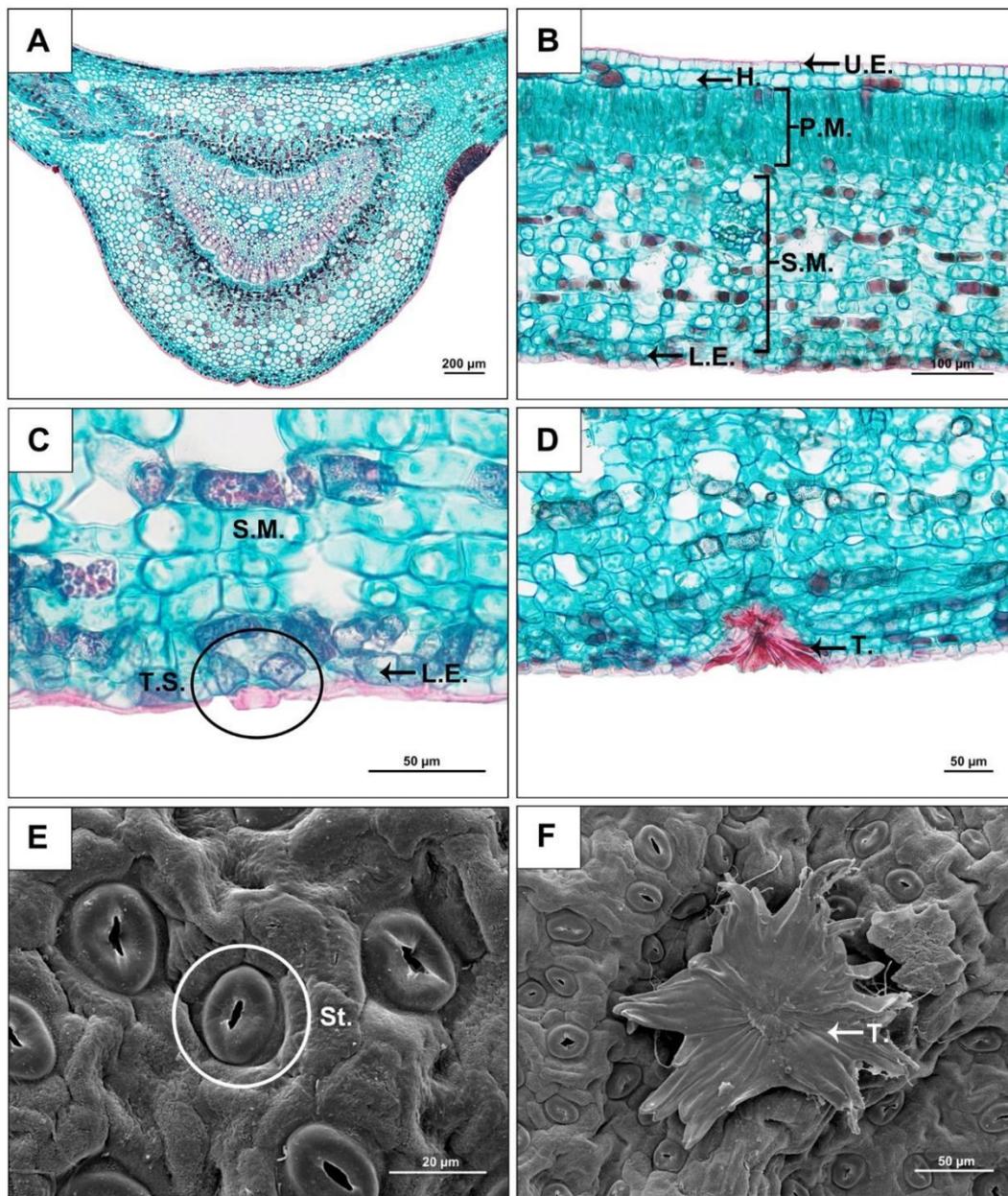


FIGURE 5. Leaf blade anatomy of *A. cucullata* and leaf surface under SEM; (A) Midrib (B) Leaf blade (C) Lower epidermis with typical stoma (D) Lower epidermis with multiseriate aseptate peltate trichome (E) Stomata on lower epidermis under SEM (F) Multiseriate aseptate peltate trichome on lower epidermis under SEM (H = hypodermis, L.E. = lower epidermis, P.M. = palisade mesophyll, S.M. = spongy mesophyll, St. = stoma, T. = trichome, T.S. = typical stoma, U.E. = upper epidermis,)

3.3 Petiole, Rachis and Petiolule

Petiole is an oval shape with a heart shape bundle. There is a layer of epidermis which has with thick tangential wall. Tannins are deposited in some epidermal cells (Figure 4D). Multiseriate aseptate peltate trichomes were found on epidermis (Figure 4J). Cortex contains multilayers of parenchyma. The vascular systems are cylindrical with adaxial V-shaped notch and cylindrical with three V-shaped notches (Figure 4A). A ring of phloem surrounds a xylem ring. Groups of gelatinous fiber have been found in the phloem. Vascular cambium presents between the phloem and xylem (Figure 4G). Pith is in the center which contains parenchyma. Sclereids were occasionally found in cortex and pith (Figure 4L). Druse crystal were found in phloem (Figure 4G) and prismatic crystals were found in pith. Tanniferous cells are common in all parts of petiole. Schizogenous secretory structures were observed in cortex and pith (Figure 4K).

Anatomical characters of rachis and petiolule are similar to petiole (Figure 4E, 4F, 4H, 4I) except vascular bundle shape. The vascular ring of rachis is cylindrical with three V-shaped notches (Figure 4B), while petiolule is cylindrical with adaxial V-shaped notch (Figure 4C).

3.4 Leaf

Leaf is bifacial. Stomata and multiseriate aseptate peltate trichomes were observed on abaxial surface (Figure 5C, 5D, 5E, 5F). Stomata are cyclocytic. There is a layer of epidermis on both adaxial and abaxial surfaces. Cutinized layer was noticed on outside wall of epidermis. A layer of hypodermis presents under the upper epidermis. There are 2-3 layers of palisade mesophyll. A number of air spaces were found in spongy mesophyll (Figure 5B). Midrib has a large convex shape bundle and two small rounded bundles (Figure 5A). Tanniferous cells are common in all parts of leaf except epidermis. Druse crystals were found in phloem.

3.5 Wood

Wood is yellow-brown color with fine texture and odorless. Growth ring boundaries are indistinct (Figure 6A). The anatomy shows that the wood is diffuse-porous with solitary and multiple (2-4) pores. Vessels are circular to oval shapes with simple perforation. Vessel diameters are 77.02-121.51 μm and 216.15- 521.13 μm long. Vessel density is 15.9 ± 3.36 cells/ mm^2 . Vessel covering area are 10.93% of transverse surface. Colored deposits were observed in the lumen of vessels (Figure 6B). Libriform septate fibers were usually observed. The fiber diameters are 11.71- 21.90 μm and 754.68- 1372.59 μm long. Fiber walls are very thin with about 4.25 ± 0.66 μm in thickness. Rays are multiseriate with 2-3 cells wide, which contain several rows of procumbent cell and two rows of upright cell (Kribs III) (Figure 6D). Rays are 27.48 ± 3.61 μm wide and 297.03 ± 72.54 μm high (ok). Ray covering area is 15.9% in

tangential longitudinal surface. Axial parenchyma are is aliform paratracheal, confluent paratracheal and banded apotracheal to banded paratracheal. Prismatic crystals were are observed in axial parenchyma (Figure 6C). Tannin deposition were is noticed in ray cells and axial parenchyma (Figure 6D).

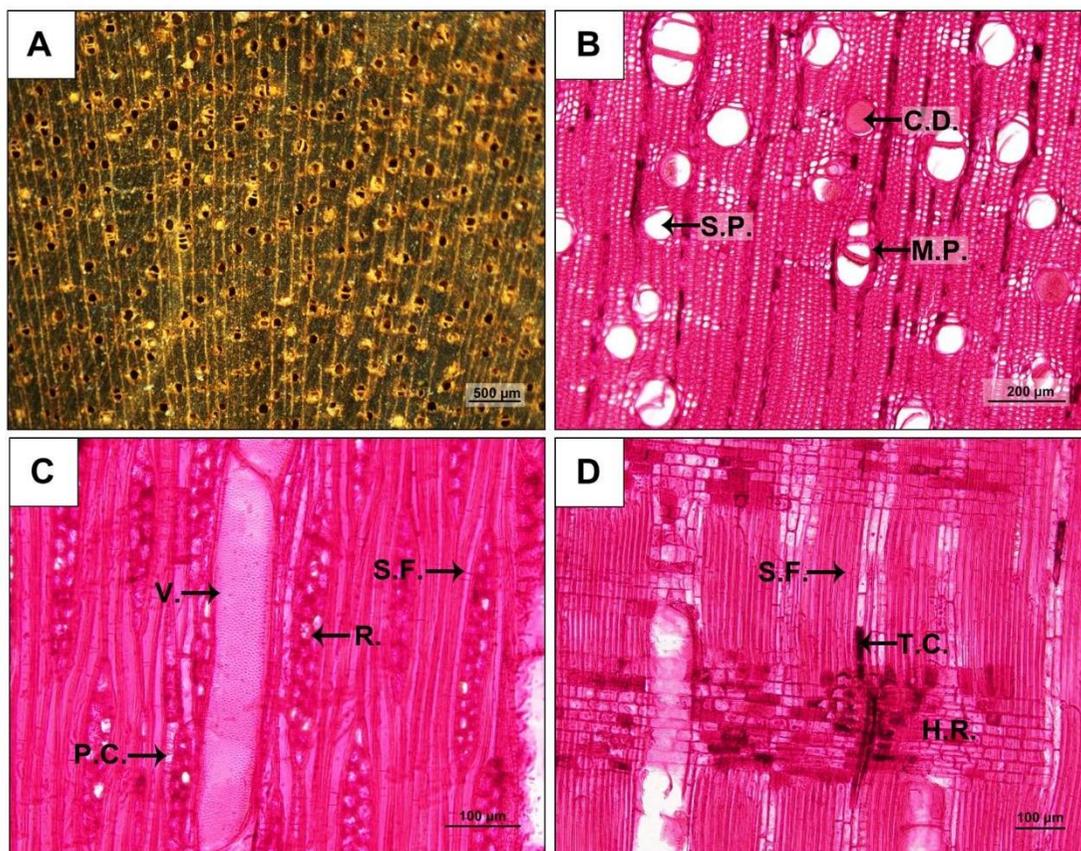


FIGURE 6. Wood characters of *A. cucullata*; (A) Transverse surface showing diffuse-porous and growth ring boundaries (B) Transverse surface showing solitary pores, multiple pores and deposit in vessel (C) Tangential surface showing septate fibers, prismatic crystals and ray cells (D) Radial long surface showing heterogenous rays and tanniferous cells (C.D. = color deposit, H.R. = heterogenous ray, M.P. = multiple pores, P.C. = prismatic crystals, R. = ray cells, S.F. = septate fibers, S.P. = solitary pores, T.C. = tanniferous cells, V. = vessel element)

3.6 Discussion

The mesophyll of *A. cucullata* presents palisade and spongy parenchyma differentiation. The dorsiventral mesophyll of *A. cucullata* shows a Meliaceae family characteristic which is similar to those found in *Azadirachta indica*, *Melia azedarach*, *Munronia pinnata*, *Toona ciliata*, *Xylocarpus granatum* and *X. moluccensis* (Abdel-Hameed, 2014; Dharmadasa *et al.*, 2014; Rodrigues *et al.*, 2016; Chorchuhirun *et al.*, 2020). However, the palisade parenchyma of *Aglaia cucullata*, the only one mangrove plant has 2-3 layers which is similar to those found in *Xylocarpus granatum* and *X. Moluccensis* but different from the others. The palisade parenchyma of *Azadirachta indica*, *Melia azedarach*, *Munronia pinnata* and *Toona ciliata* contains just one layer.

The mesophyll of *A. cucullata* presents palisade and spongy parenchyma differentiation. The dorsiventral mesophyll is a Meliaceae family characteristic that has been identified in *Azadirachta indica*, *Melia azedarach*, *Munronia pinnata*, *Toona ciliata*, *Xylocarpus granatum* and *X. moluccensis* (Abdel-Hameed, 2014; Dharmadasa *et al.*, 2014; Rodrigues *et al.*, 2016; Chorchuhirun *et al.*, 2020). The palisade parenchyma of *Azadirachta indica*, *Melia azedarach*, *Munronia pinnata* and *Toona ciliata* contains just one layer, however *Aglaia cucullata*, a mangrove plant similar to *Xylocarpus granatum* and *X. moluccensis*, has 2-3 layers as well.

The characteristic of *A. cucullata* midrib is composed of collateral vascular bundles that create a continuous vascular ring with phloem on outside and xylem on the inner side, similar to *Xylocarpus granatum* and *Toona ciliata* (Rodrigues *et al.*, 2016; Chorchuhirun *et al.*, 2020). The midrib of *A. cucullata* differs from the two species previously mentioned by having two small vascular bundles on its adaxial surface, however *Cordia sessilis* midrib in the Rubiaceae family also shares this feature (Teixeira *et al.*, 2016).

Druse crystals were observed in the phloem tissue inside the midrib of *A. cucullata*. These crystals have also been described in other Meliaceae species, including *Munronia pinnata*, *Toona ciliata*, *Xylocarpus granatum* and *X. moluccensis* (Dharmadasa *et al.*, 2014; Rodrigues *et al.*, 2016; Chorchuhirun *et al.*, 2020), which are mostly present in phloem tissue.

On the stem, petiole, rachis, petiolule and lower surface of the *A. cucullata* leaves, there are multiseriate aseptate peltate trichomes. The trichomes compose of 20–35 body cells, and their basal ends juxtapose to create a thin foot that is embedded in the epidermis. The peltate body of the trichome is almost spherical, with arms that are expanded towards the center and taper to a serrate border at the distal end. The arms are free at the edge and are attached at the center for all or half part of their length, but the trichomes are more complicate with aseptate stellate scales, which have

the free arms (Rao and Ramayya, 1982; Pannell, 1992). For example, *A. odoratissima* and *A. basiphylla* have a mixture of stellate hairs and peltate trichomes (Pannell, 1992).

According to Metcalfe and Chalk (1950), the existence of specific types of trichome usually distinguishes between species, genera, or even whole families. The characteristic indumentum of peltate scales or stellate hairs that identifies *Aglaia* morphology from the majority genera of family Meliaceae, however simple hairs are never present on the plant's vegetative parts (Pannell, 1992; Muellner *et al.*, 2005; Mabberley, 2011). Several members of the Meliaceae family have trichomes of various types, including filiform unicellular trichomes (e.g., *Munronia pinnata*, *Azadirachta indica*, and *Melia azedarach*), bifurcate trichomes (e.g., *M. pinnata*), stellate trichomes (e.g., *Astrotrichilia* spp., *Azadirachta indica*, *Lepidotrichilia* spp., *Pterorhachis* spp. and *Trichilia* spp.), glandular sessile trichomes (e.g., *M. pinnata*, *A. indica* and *M. azedarach*) and glandular stalked trichomes (e.g., *M. pinnata*) (Clark, 1990; Muellner *et al.*, 2005; Abdel-Hameed, 2014; Dharmadasa *et al.*, 2013; 2014; Adejoke *et al.*, 2021). The trichomes are very useful in taxonomy, ecology and evolutionary investigations (Gomes and Neves, 2009). In terms of plant physiology, the presence of trichomes supplies protection against variables such as high light intensity, heat and water loss (Werker, 2000; Bieras and Sajo, 2009; Teixeira *et al.*, 2016). Additionally, the peltate trichomes also were discovered in *Olea europaea* leaf (Oleaceae family). The trichomes were easily identified under light microscope and in a powder being separated from the leaf (Long *et al.*, 2010). *O. europaea* peltate trichomes, which contain flavonoids and other phenolic chemicals, provide an abundance of capacity as a diagnostic characteristic in pharmacognosy (Liakoura *et al.*, 1997; Liakopoulos *et al.*, 2006; Long *et al.*, 2010).

Petiole, rachis, and petiolule of *A. cucullata* all represented secretory ducts in the cortex and pith layers. Additionally, similar traits have been discovered in *Azadirachta indica* (Abdel-Hameed, 2014) and *Munronia pinnata* (Dharmadasa *et al.*, 2014). Since the members of Meliaceae family include significant chemical substances such limonoids, terpenoids, alkaloids, flavonoids, and phenolic compounds (Yadav *et al.*, 2015; Xu *et al.*, 2019; Teerapongpaisan *et al.*, 2020). Therefore, the plants require the secretory ducts for keeping significant substances. According to reports, phenolic compounds are crucial to the functioning of plants (Croteau *et al.*, 2000), that supports plants in adapting to environmental factors such as drought, high light intensity, and soil condition, as well as biotic factors such as bacteria, fungus, and herbivores (Waterman and Mole, 1994; Ribeiro *et al.*, 2007; Teixeira *et al.*, 2016). An example of plant protection against biotic forces; the extract of *A. cucullata*

was able to prevent the growth of 13 bacterial and 3 fungal strains (Rahman and Rashid, 2009; Pervin *et al.*, 2016).

The vascular bundle of the Meliaceae petiole commonly has a cylindrical or crescent form. Furthermore, the heart-shaped vascular bundle of petiole was discovered in *Munronia pinnata* (Dharmadasa *et al.*, 2014) and in *A. cucullata*, however the vascular bundle of *A. cucullata* is more comparable to the vascular bundle of *Hevea brasiliensis* petiolule (Euphorbiaceae family) (Tadavi and Bhadane, 2014). Since vascular bundle patterns are not influenced by environmental factors (Metcalfe and Chalk, 1950; Martínez -Cabrera *et al.*, 2009; Moraes *et al.* 2011), they are crucial for taxonomic identification (e.g., family Meliaceae, Euphorbiaceae) (Howards, 1970; Bhadane, 2006; Tadavi and Bhadane, 2014).

Similar to the research findings of Khaopakro *et al.* (2015), *A. cucullata* wood exhibits the characteristics including a distinct growth ring, diffuse porous vessels, simple perforation plates, septate fibers, paratracheal axial parenchyma in aliform or confluent forms, heterogenous rays type III, and prismatic crystals in axial parenchyma cells. In contrast, the quantitative characteristics show little variation in the diameter, width, and length of vessels, fibers, and ray parenchyma.

4. Conclusion

The anatomy of *A. cucullata* may be identified by the vascular system characteristics in the petiole, petiolule, and rachis, as well as the trichome features that are unique to this genus. In addition to anatomical characteristics that can be utilized to distinguish species, it is also used to explain how plants have adapted to their surroundings. For instance, the existence of secretory ducts to assist the storage of phytochemicals required for plants as well as the presence of many palisade layers, which is comparable to other mangrove plants in this family.

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