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Justicia gendarussa (BURM F.) (WILLOW-LEAVED *Justicia*) FROM PENINSULAR MALAYSIA: LEAF ANATOMY AND MICROMORPHOLOGY

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ABSTRACT

Acanthaceae or the *Acanthus* family has been commonly used by local peoples as traditional medicines, especially in Peninsular Malaysia. *Justicia gendarussa*, also known as 'daun rusa' or 'genda rusa,' treats inflammatory disorders, asthma, hepatic injuries, arthritis, and digestion problems. Many taxonomists nowadays struggle to identify Acanthaceae species due to morphological similarities and incomplete parts during the identification process. Other than that, there has been no comprehensive study of *Justicia gendarussa* leaf anatomy to date. Therefore, this study aims to describe the leaf's anatomical and micromorphological characteristics that would be useful to identify this plant species. The leaf anatomical study involved several methods such as cross-section using a sliding microtome and observation under a light microscope. While the leaf micromorphology method involved the observation under a scanning electron microscope (SEM) of the epidermis of abaxial and adaxial surfaces. The result of this study showed the presence of collenchyma, sclerenchyma, and mucilage cells in the petiole and midrib. The patterns of petiole and midrib vascular bundles were described as the main vascular bundle (opened system with continuous rings of the vascular bundle) with two additional vascular bundles located the above right and left of the main vascular bundle near each wing. The cystolith cells are present in the epidermis and parenchyma cortex while glandular and non-glandular trichomes were recorded in the species studied. In conclusion, comprehensive data for the leaf anatomical and micromorphological characteristics can be used to supplement data in the identification of *Justicia gendarussa.*

Key words: Acanthaceae, *Justicia gendarussa*, leaf anatomy, and micromorphology, Willow-leaved *Justicia*

INTRODUCTION

Acanthaceae is a large family with 4300 species divided into 346 genera and widely distributed in Europe, Pennsylvania, Japan, India, New Holland, IndoMalaysia, Asia, Africa, Brazil, and also Central America (Sharma, 2016). Most Acanthaceae species can be found in moist and shady habitats either in the wastelands of the forest, as well in grassy areas. Acanthaceae species also can be recognized either as shrubs and annual or perennial herbs, except for woody climbers in *Thunbergia species* (Dieter, 2004). *Justicia* L. is known as one of the genera under Justicieae, the largest tribes of Acanthaceae which comprise 2000 species spread across 100 genera (Alcantara *et al*., 2022). *Justicia gendarussa* Burm F. is an evergreen shade-loving plant that has been recorded mostly in damp environments. It is widespread throughout India, Sri Lanka, and Malaysia and is thought to be native to China (Paval *et al*., 2009). This species is also commonly known as 'Daun rusa' or 'genda rusa' and is traditionally used in alternative treatment for local people. Jaijesh *et al*. (2009a) reported *Justicia gendarussa* as one of the medicinal plants that are commonly used in traditional Indian and Chinese medicine. This plant consists of cystoliths which is one of the main characteristics to differentiate the family of Acanthaceae from other families (Patil & Patil, 2011). Cystoliths are classified as silicified bodies that are sometimes not encrusted and can be found in the axis and thus the leaf of numerous species (Metcalfe & Chalk, 1965).

Previous research by Nirmalraj *et al*. (2015) discovered *Justicia gendarussa* as one of the potential medicinal plants that can be useful for medicinal purposes. It had been shown that the decoction of the leaves and tender shoots were diaphoretic (causes perspiration) and is used to treat chronic rheumatism (Mateshwari & Disha, 2020). Whereas the oil extracted from the leaves is effective for eczema (Sangeetha *et al*., 2014). The current study recorded that *Justicia gendarussa* ethanolic extract demonstrated significant anti-arthritic activity comparable to aspirin was observed (Jaijesh *et al*., 2009b).

Nurul-Aini *et al*. (2018) stated that the

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identification and classification of Acanthaceae species are quite challenging because of the similar morphological characteristics with other species, especially species under the same genus. As a result, leaf anatomy and micromorphology data can be used as research data to support the identification process and to assist the systematic study, particularly for incomplete samples (the absence of floral & fruiting materials) (Khatijah & Ruzi 2006). It is very important to ensure the correct specimen, especially that have been used as raw materials for medicinal purposes. Thereby, this study was conducted to investigate and list out the comprehensive leaf anatomy and micromorphological characteristics of *Justicia gendarussa* which can be useful as additional data in the systematic classification of Acanthaceae species.

MATERIALS AND METHODS

Fresh samples leave of *Justicia gendarussa* were collected at Glasshouse and Nursery Complex, International Islamic University Malaysia, Kuantan, Pahang. Three to five replicates were used throughout this research. These plant specimens are mounted on a sheet of high-quality paper, a label is affixed at the lower right corner of the sheet and the samples were pressed by a wooden presser and appropriately tied. Then, the plant specimens were labeled and placed in an oven for two weeks duration at 55 °C. The completed voucher specimens were deposited at Herbarium IIUM Kuantan. For the sectioning method, part of the petioles, midribs, leaf lamina, and margin were sectioned using a sliding microtome in a variety of thicknesses (15-40 m) and stained with Safranin and Methylene blue. The specimens obtained from the sliding microtome were undergone a dehydration process in a series of alcohol (50%, 70%, 95%, & 100%) after the staining process and were mounted in Euparal. All slides were covered with coverslips and dried in the oven for two weeks at around 55°C. Anatomical images were captured using Leica LAS EZ software and a video (3CCD) camera attached to a Leitz Diaplan microscope. The histology method in terms of fixation and embedding was modified based on Johansen and Saas (1940). For the leaf micromorphology study, the samples were taken from the dried sample of the herbarium. Then, the samples were cut at the lamina area in 1 cm^2 measurement and mounted on a mounting holder. The samples were coated with gold by using a sputter-coated machine and finally observed under a scanning electron microscope Zeiss Model Evo 50. The micromorphological features such as the structure of waxes, cuticular sculpturing, trichomes, and stomata were observed under magnification of 100x, 500x, 1000x, and 2000x.

RESULTS

The characteristics of leaf anatomy and micromorphology for *Justicia gendarussa* are summarized below:

Petiole (Figure 1a)

Epidermal cell: Adaxial epidermis: One layer with a ratio of height: width (2:1 & 1:1). Abaxial epidermis: One layer with a ratio of height: width (1:2 & 2:1). **Vascular tissue**: Main vascular bundle (opened system with continuous rings of the vascular bundle) with two additional vascular bundles located at the above right and left of the main vascular bundle near each wing. **Sclerenchyma cell**: clusters of sclerenchyma cells present surrounding phloem tissue of the main vascular bundle and two additional vascular bundles. **Parenchyma cell**: Adaxial surface; ca. 4-6 layers of parenchyma cells. Abaxial surface; ca. 7-9 layers of parenchyma cells. **Collenchyma**: Present at the epidermis of the adaxial and abaxial surface. **Mucilage cell**: present at parenchyma cortex. **Cystolith**: Present at the epidermis and parenchyma cortex. **Trichome**: Glandular trichome.

Midrib (Figure 1b)

Adaxial epidermis: One layer with a ratio of height: to width (1:2) (Figure 1e). **Abaxial epidermis**: One layer with a ratio of height: to width (1:2,1:1,2:1). **Vascular tissue**: Main vascular bundle **(**opened-system with a continuous ring of the vascular bundle) with two additional vascular bundles located at the above left and right of the main vascular bundle. **Sclerenchyma cell**: clusters of sclerenchyma cells present surrounding phloem tissue of the main vascular bundle and two additional vascular bundles. **Parenchyma cell**: Adaxial surface; ca. 5-7 layers of parenchyma cells. Abaxial surface; ca. 3-7 layers of parenchyma cells. **Collenchyma**: present under the epidermis of adaxial and abaxial. **Mucilage cell**: present at parenchyma cortex. **Lithocyst**: present at the epidermis of adaxial and abaxial (Figure 1g). **Cystolith**: present at the parenchyma cortex. **Trichome:** Nil.

Leaf margin (Figure 1c)

Outline: Rounded with a U-shaped outline, 30-45° recurved downwards to the abaxial epidermis, the size decreases gradually towards the margin. **Collenchyma**: 2-3 layers of collenchyma cells present at the end of the margin. **Parenchyma cell**: a cluster of parenchyma cells present at the end of the leaf margin. **Trichome**: Nil.

Leaf lamina (Figure 1d)

Cuticular layer: relatively thin. **Adaxial epidermis**: single layer with height: width ratio – 1:2. **Abaxial epidermis**: single layer with height: width ratio – 1:2. **Chlorenchyma cells**: mesophyll palisade: ca. 1-3 layers filling 1/3 part of the height of leaf lamina. Spongy mesophyll: 5-6 layers of spongy mesophyll filling 2/3 part of the height of the leaf lamina. **Vascular bundles**: simple vascular bundles. **Parenchyma cells**: Nil. **Trichome**: Nil.

Leaf epidermis (Figure 1i & 1j)

Wax: Film-layer and granules present on adaxial and abaxial surfaces. **Adaxial cuticular sculpturing**: Epidermal cells outline is distinguishable, anticlinal wall raised into ridges, and periclinal wall sunken. **Abaxial cuticular sculpturing**: Epidermal cells

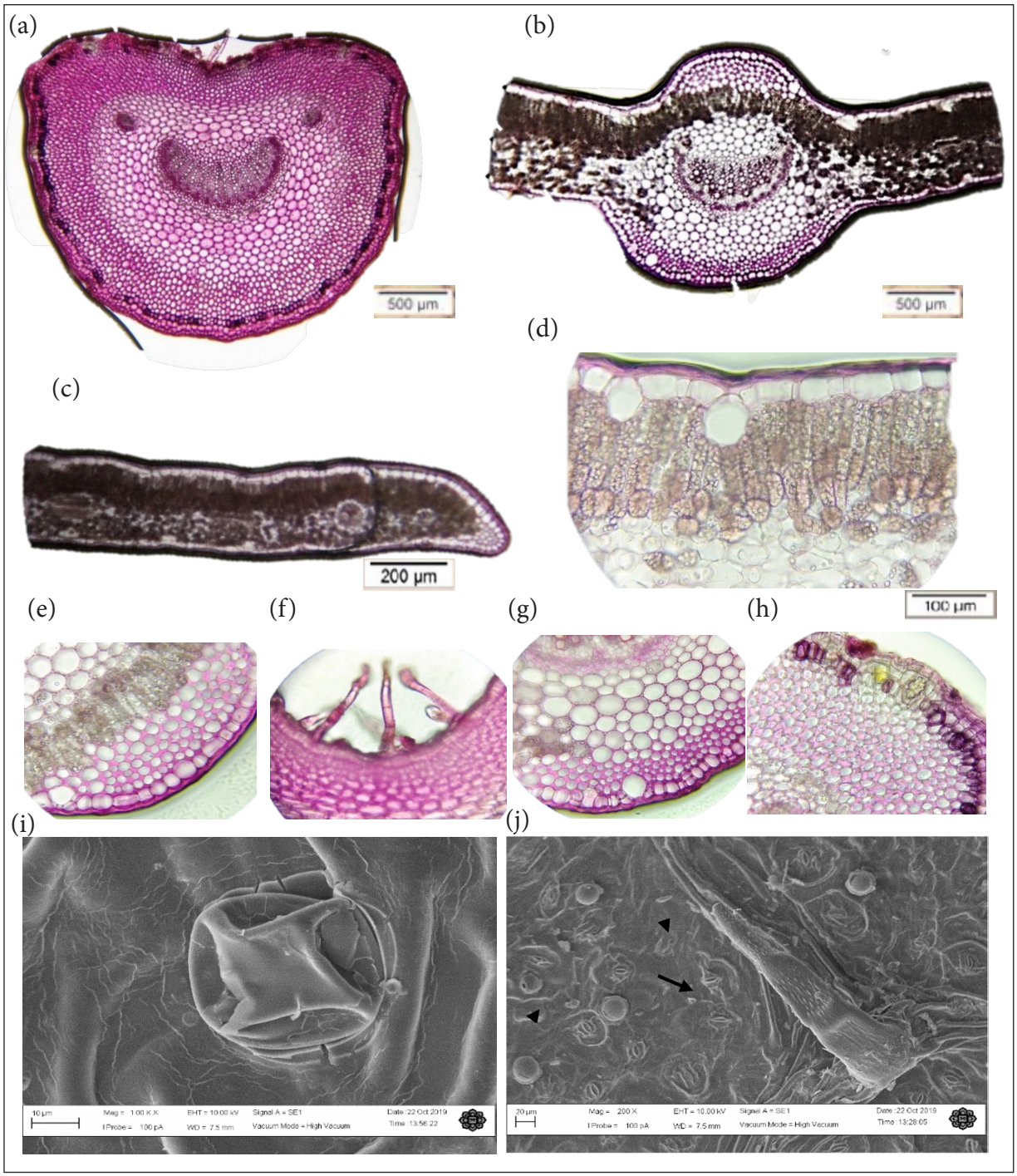

Fig. 1. *Justicia gendarussa*: a) Petiole cross section. b) Midrib cross-section. c-d) Margin and lamina cross-section. e) Epidermal cell. f) Simple multicellular trichomes (long with a blunt end, echinate ornamentation) are found on the abaxial surface. g) Lithocyts. H) Cystolith. I) Glandular trichome on the adaxial surface. j) Simple multicellular trichomes, stomata, and waxes on the abaxial epidermis. Scale: a) 500 µm. b) 500 µm. c) 200 µm. d) 100 µm. e-f) 50 µm. g-h) 50 µm. i) 10 µm. j) 20 µm.

outline is distinguishable, anticlinal wall sunken and periclinal wall raised into ridges. **Stomata**: Elliptic-shaped stomata, homostomatic. **Trichome**: Glandular peltate trichome found on the adaxial surface (Figure 1i). Simple multicellular trichome (long with point end, echinate ornamentation) found on abaxial surface (Figure 1j).

DISCUSSION

The outcome of this study reported the significance of leaf anatomical and micromorphological characteristics in the identification of *Justicia gendarussa.* Cystoliths are the removal of calcium carbonate found in different parts of plants. Except for *Avicennia, Staurogyne*, and *Thunbergia*, the presence of cystoliths has been used to identify and classify the Acanthaceae family (Maisarah, 2022). Normally, the cystoliths are present in different shapes, sizes, and also colors either in the lamina, petiole, the surface of the leaf epidermis and also in ground tissue. In some taxa, either both ends of the cystolith are obtuse or acute, while in others, only one end of the cystolith is obtuse or acute. They were observed either in single or double (Patil & Patil, 2011). The presence of cystolith cells is very useful in Acanthaceae families in the identification and classification of genus and species (Nurul-Aini *et al*., 2018). In *Justicia gendarussa*, the cystoliths are present either in double form or obtuse end shape (Figure 1h).

The pattern of vascular bundle in the petiole and midrib of *Justicia gendarussa* was observed. Noraini *et al*. (2016) explained the taxonomic importance of petiole vascular bundle in species identification and classification of species in Dipterocarpaceae. The pattern of vascular bundles that have been identified in the petiole and midrib for *Justicia gendarussa* is opened system, continuous rings of a vascular bundle with two additional vascular bundles located above the right and left wings of the main vascular bundle. Candolle's early research, which included his first comprehensive study of petiole anatomy, clarified several fundamental concepts about vascular bundles. He distinguished between two types of vascular systems: the 'opened system' and the 'closed system' (Nurul-Aini *et al*., 2018).

Sclerenchyma cells have thickened lignified walls that protect the plant and make them strong and waterproof. Lopez *et al*. (2017) stated that the sclerenchyma matures with the surrounding tissues and provides more stable support compared with the collenchyma cells. In addition, there are two main types of sclerenchyma cells which are fibers and sclereids (Armando *et al*., 2019). All plant cells including the Acanthaceae family initially have only a primary wall made mainly of cellulose. As sclerenchyma matures, a secondary wall with a high amount of lignin is deposited inside the primary wall. The secondary wall is very thick and highly lignified than the primary wall and gives great rigidity and hardness to the cell and tissue. They also function primarily in mechanical support and water conduction. The sclerenchyma cell is dead at maturity as the protoplast usually dies during development (Thomas *et al*., 2009). In this study, clusters of sclerenchyma cells present surrounding phloem tissue of the main vascular bundle and two additional vascular bundles in the petiole and midrib (Figure 1a & 1b).

There two types of trichomes were recorded in this study which is glandular trichome and non-glandular trichome. Trichomes are found on the plant surface and also on lichens and algae which it is specialized structures that function in stress resistance, such as excessive light or temperature, insect and pathogen defense, and water loss reduction (Yao-Ma *et al*., 2016). They are diverse in appearance and usually shaped as hair-like structures (Joanna *et al*., 2011), but they can also express as scales, buds, and papillae are examples of multicellular structures that range in size from very small unicellular structures to very large multicellular structures. One type is the glandular trichome, which is normally multicellular and contains one or more glandular cells capable of producing, storing, emitting, or exuding specialized compounds into the environment or onto the epidermal surface (Michael, 2019). Glandular trichomes are well-known as a source of essential oils and resins, which are widely used in the agricultural, pharmaceutical, and cosmetic industries (Kortbeek *et al*., 2016). Jia *et al*. (2013) also agreed that glandular trichomes are significant in plant growth and development. While non-glandular trichomes are classified as simple multicellular trichomes (long with point end, echinate ornamentation) and have also been recorded in this study. While the glandular trichomes found in this species were identified as glandular peltate trichomes found on the adaxial surface of leaf lamina.

There are several types of stomata found in plant species, including anomocytic, anisocytic, paracytic, diacytic, actinocytic, cyclocytic, and tetracytic (Willem, 1970). Homostomatic with the type of diacytic stomata present in this species studied. The present study also managed to record the presence of stomata on both adaxial and abaxial surfaces (amphistomatic) in *Justicia gendarussa*. The stomata's primary function is to exchange gases between the plant and the surrounding environment. Each stoma opens into a substomatal chamber or respiratory cavity to help with this function. The other function is the evaporation of water which also takes place through the stomata (Kirkham, 2014). The stomata will remain closed in dark conditions or with no light or night day and open in the daytime when there is light (Kirkham, 2014).

Waxes are long-chain acid esters and long-chain primary alcohol esters. It is commonly referred to as epicuticular waxes in plant surface lipids because it is made up of a diverse range of chemical compounds (Wilhelm, 1997). The types of epicuticular wax structures found in this study which are films, and very thin coverings that express the obligate outermost border of the cuticle, are barely visible in the SEM and do not show cracks after drying. Second is granules that are irregular in shape, mostly phenotypically normal, and frequently rounded crystalloids that are sometimes hollow (Wilhelm *et al*., 2017). The epicuticular wax is very important as it can exhibit great micromorphological diversity.

CONCLUSION

The result of this study showed that it is possible to use leaf anatomy and micromorphology characteristics as additional data to identify plant species in the Acanthaceae family. This research described many characteristics of the studied species that can be used to identify the *Justicia gendarussa* which are the presence of cystolith, stomata and trichomes types, anticlinal wall patterns, type of epicuticular wax structures, cell size, and vascular bundle patterns in petiole and midrib.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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