

Research Article

Comparative Study of Leaf Anatomy and Micromorphology of Selected *Justicia* Species from Peninsular Malaysia

Aidatul Aifa Mohd Tajudin¹, Che Nurul Aini Che Amri^{1,2*} and Rozilawati Shahari^{1,2}

¹Department of Plant Science, Kulliyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

²Sustainable Agriculture and Green Technology Research, Kulliyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

*Corresponding author: chenurulainicheamri@iium.edu.my

ABSTRACT

Justicia has been recognized as one of the largest genera in the Acanthaceae family with 600 species have been identified. Most researchers have problems identifying *Justicia* species especially if the samples are incomplete. Other than that, there is no comprehensive study on *Justicia* species, especially in Peninsular Malaysia. Therefore, a comprehensive study of leaf anatomy and micromorphology of three selected *Justicia* species which are *Justicia adathoda*, *Justicia gendarussa* and *Justicia procumbens* were conducted in order to assess the leaf anatomical and micromorphological variations that would be useful to identify the plant species. The methods used in this study involved several methods such as cross-section using a sliding microtome and observation under the scanning electron microscope (SEM) for the epidermis of abaxial and adaxial surfaces. Collenchyma, sclerenchyma and mucilage cells can be found either in the petiole or the midrib in all species studied. Amphistomatic stomata have also been recorded in all species examined. Meanwhile, several differences were discovered in this study, including the type of trichomes, the pattern of vascular bundles, the type of epicuticular wax structures, cuticle ornamentation and the presence of cystolith cells. In conclusion, the characteristics of some *Justicia* species in Peninsular Malaysia can be identified using leaf anatomy and micromorphology.

Key words: Acanthaceae, *Justicia*, Leaf anatomy, Leaf micromorphology

Article History

Accepted: 1 December 2022

First version online: 26 December 2022

Cite This Article:

Mohd Tajudin, A.A., Che Amri, C.N.A. & Shahari, R. 2022. Comparative study of leaf anatomy and micromorphology of selected *Justicia* species from peninsular Malaysia. Malaysian Applied Biology, 51(5): 221-228. <https://doi.org/10.55230/mabjournal.v51i5.2345>

Copyright

© 2022 Malaysian Society of Applied Biology

INTRODUCTION

Acanthaceae is one of the most attractive and distinctive plant family and this family is also known as one of the largest family in the world with roughly 346 genera and nearly 4,300 species which has been recorded in tropical and subtropical regions (Khan *et al.*, 2017). The habitat ranges from marshes and bays to severely arid conditions, but the majority of these plants prefer damp, shady environments (Kumar *et al.*, 2016). *Justicia* is the largest genus in this family, with 600 species, while *Avicennia* with at least eight ecologically important mangrove species (Petruzzello, 2013). According to Choopan and Grote (2015), cystoliths are one of the major characteristics in Acanthaceae that are visible with a magnifying lens as rod-shaped, especially in the epidermis surfaces of the leaves except in subfamily Nelsonioideae, Thunbergioidae and also in the tribe of Acanthaceae. Other than that, the presence of a distinct fruit type with seed borne on retinacula, also can be used to identify Acanthaceae species.

Acanthaceae species are widely recognized as potential medicinal plants and also useful as a traditional therapeutic remedy. According to Burkill (1985), even though the Acanthaceae family is not designated for economic purposes, 103 species from West Tropical Africa are considered valuable plants. The previous study reveals the medicinal, socio-cultural and other important uses of the Acanthaceae species such as *Acanthus montanus* (Nes.) T.

Anders in Mount Cameroon Region (Fongod *et al.*, 2013). In terms of medicinal value perception, for example, in Ayurveda, *J. gendarussa* is used to treat inflammation, bronchitis, vaginal discharges, eye problems, heartburn, and fever (Kavitha K. *et al.*, 2014).

Previous research by Nurul-Aini *et al.* (2018) found that identifying and classifying Acanthaceae species is difficult because of morphological resemblances with other species, especially those in the same genus. According to Alamgir (2017), while several plant species' external appearances are identical, morphological data is usually necessary to be supported by anatomical characteristics, especially when specimens are insufficient. Therefore, the leaf anatomy and micromorphology data of the three species studied can be used as research data to support the identification process and to assist taxonomic research, especially for incomplete samples (Khatijah & Ruzi, 2006). It is very important to ensure the correct specimen, especially that have been used as raw materials for medicinal purposes. As a result, this study was carried out to investigate the interspecific variations of leaf anatomy and micromorphological characteristics of three *Justicia* species from Peninsular Malaysia, which can be used to supplement taxonomic classification.

MATERIALS AND METHODS

The three selected species which are *J. adathoda*, *J. gendarussa* and *J. procumbens* were collected at Sungai Besar, Kedah and Bangi, Selangor respectively. Three to five replicates were used throughout this research. These plant specimens have been mounted on a sheet of high-quality paper, a label has been affixed to the lower right corner of the sheet, and the samples have been pressed with a wooden presser and appropriately tied. Then, the plant specimens were labeled and placed into an oven for two weeks duration at 55 °C. The finished voucher specimens were stored in the Herbarium IUM Kuantan. The leaf anatomical study involved cross sectioning with a sliding microtome, the dehydration process involving a series of alcohol, the staining process by Safranin and Alcian Blue and lastly drying process in the oven for two weeks at around 55 °C. The anatomical characters were acquired using Leica LAS EZ software. The histology method in terms of fixation and embedding was modified based on Johansen (1940). For the scanning electron microscope method, the herbarium samples were taken. Then, the lamina area in 1 cm² measurement was cut and mounted on a mounting stud. The samples were sputtered with gold before being examined under a Zeiss Model Evo 50 scanning electron microscope. Under magnifications of 100x, 500x,

1000x, and 2000x, micromorphological features such as wax structure, cuticular sculpturing, trichomes, and stomata were observed.

RESULTS

Findings of this study including the leaf anatomical and micromorphological features for the three species were described below:

A. *Justicia adathoda*

Vascular tissue of petiole cross section (Figure 1a): Type 1. **Cystolith:** Present at the collenchyma cells and parenchyma cortex of petiole. **Trichome (Figure 1f):** Simple multicellular trichomes with pointed ends. **Vascular tissue of midrib cross section (Figure 1b):** Type 2. **Cystolith (Figure 1e):** Present at the collenchyma cell and parenchyma cortex of midrib. **Cuticular layer:** relatively thin. **Chlorenchyma cells:** mesophyll palisade in one layer filling 1/3 part of the leaf thickness of lamina. Spongy mesophyll in four to five layers of spongy mesophyll filling 2/3 part of the leaf thickness of lamina. **Outline of leaf margin (Figure 1d):** rounded, 15° recurved downwards towards the abaxial epidermis, the size uniform towards the edge of the margin. **Parenchyma cell:** cluster of parenchyma cells present at the end of the leaf margin. **Wax:** Film-like layer and granules found on adaxial and abaxial surfaces. **Adaxial cuticular sculpturing (Figure 1g):** Epidermal cells outline is distinguishable, periclinal wall raised into ridges and anticlinal wall sunken. **Abaxial cuticular sculpturing (Figure 1h):** Epidermal cells outline is distinguishable, anticlinal wall sunken and periclinal wall raised into ridges. **Stomata:** Elliptic shaped stomata, amphistomatic. **Trichome:** Peltate trichome found on adaxial surface. Simple multicellular trichome (long with blunt end) and glandular peltate trichome are found on abaxial surface.

B. *Justicia gendarussa*

Vascular tissue petiole cross section (Figure 2a): Type 1. **Cystolith (Figure 2e):** Present at the epidermis of the cell. **Trichome:** Glandular peltate trichome. **Vascular tissue midrib cross section (Figure 2b):** Type 1. **Outline of leaf margin (Figure 2d):** Rounded with U-shaped outline, 30-45° recurved downwards to the abaxial epidermis, the size decreases gradually towards margin. **Cuticular layer:** relatively thin. **Chlorenchyma cells:** mesophyll palisade in one to three layers occupying 1/3 part of the leaf thickness of lamina. Spongy mesophyll in five to six layers of spongy mesophyll filling 2/3-part of the leaf thickness of lamina. **Wax:** Film-layer, crust of wax and granules

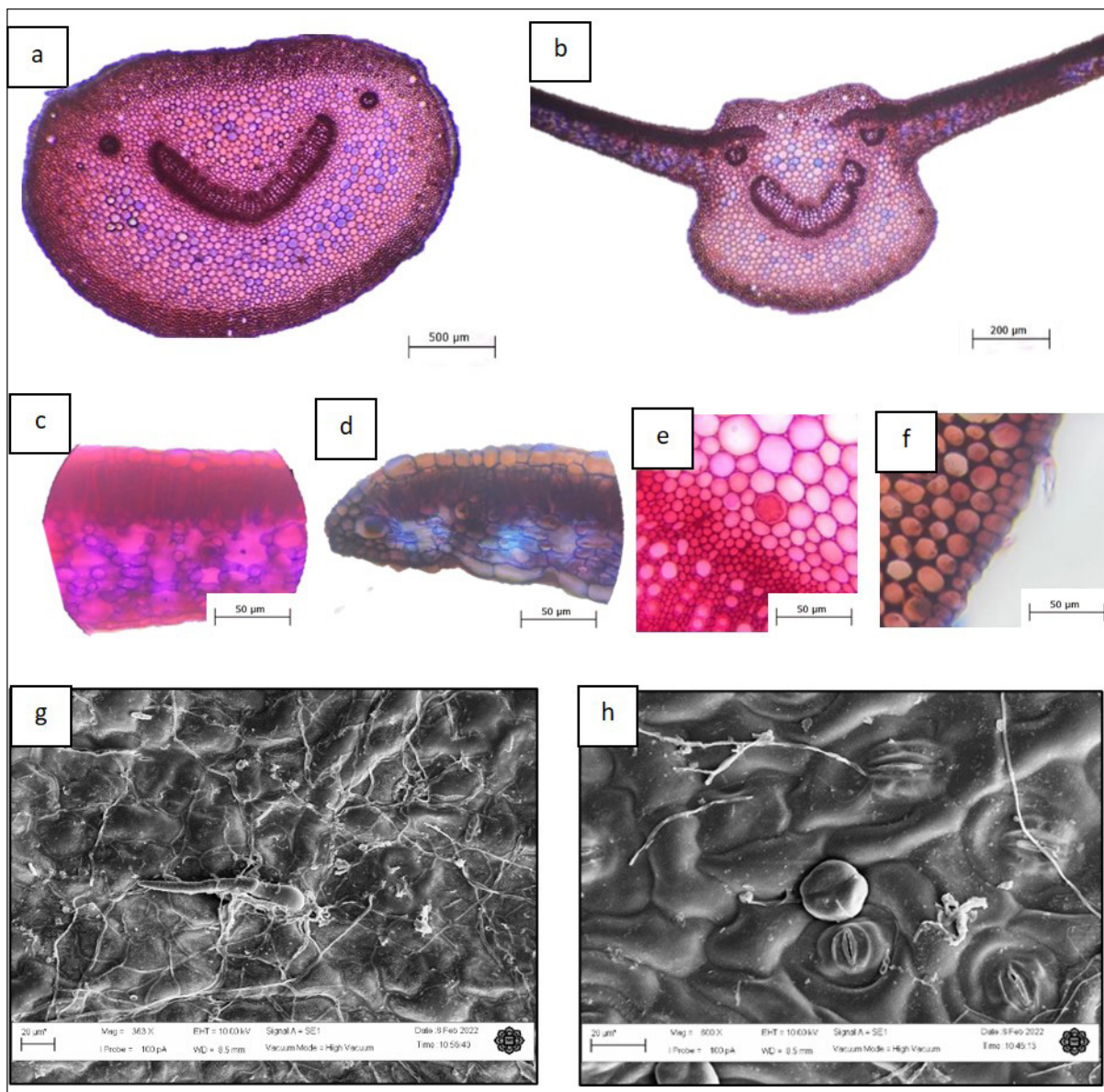


Fig. 1. *Justicia adathoda*: a) Petiole cross section. b) Midrib cross section. c-d) Lamina and margin cross section. e) Cystolith. f) Simple multicellular trichome. g) Adaxial surface. h) Abaxial surface. Scale: a) 500 µm. b) 200 µm. c-f) 50 µm. g-h) 20 µm.

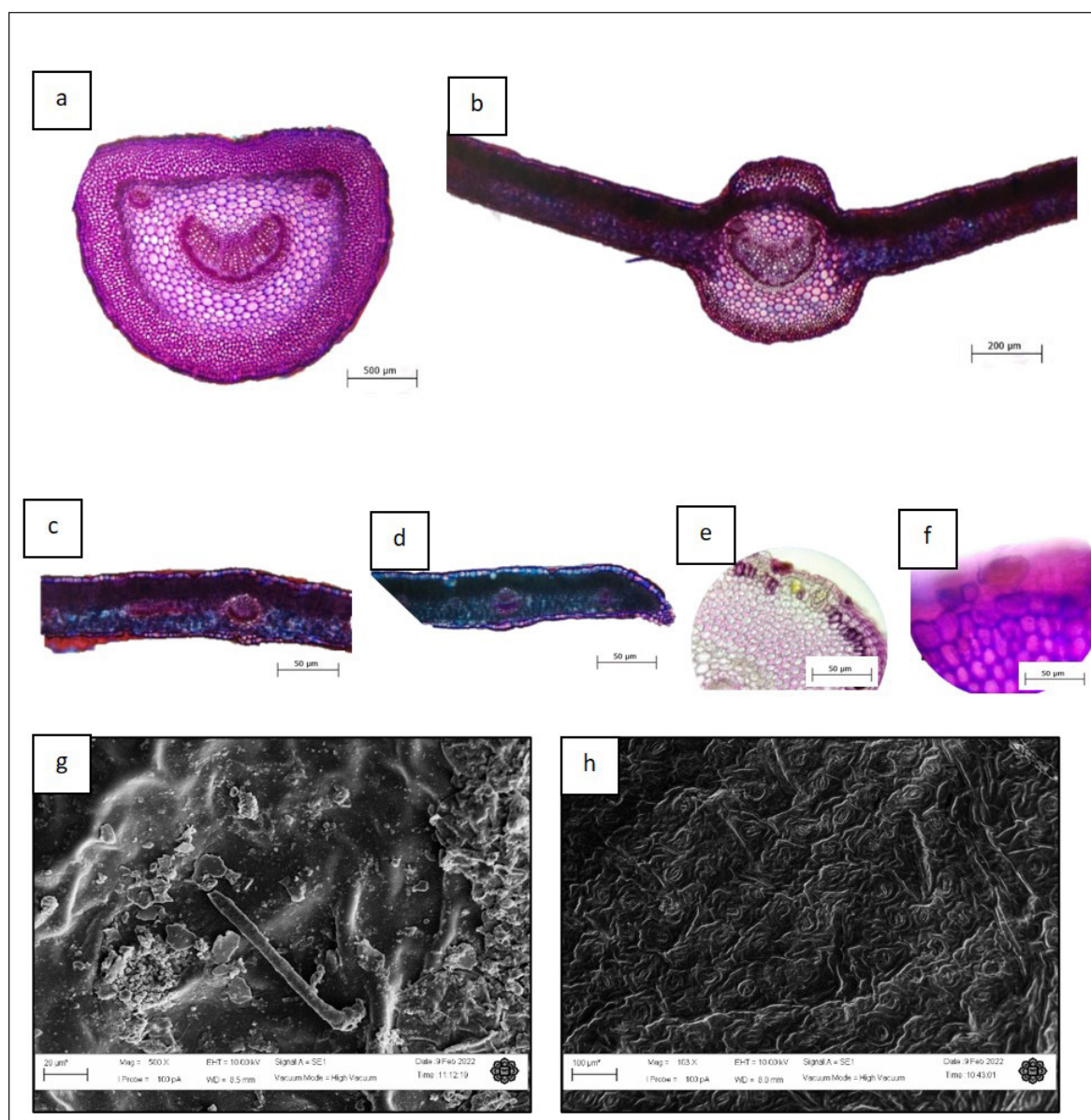


Fig. 2. *Justicia gendarussa*: a) Petiole cross section. b) Midrib cross section. c-d) Lamina and margin cross section. e) Cystolith f) Glandular trichome. g) Adaxial surface. h) Abaxial surface. Scale: a) 500 µm. b) 200 µm. c-f) 50 µm. g-h) 20 µm.

present on adaxial and abaxial surfaces. **Adaxial cuticular sculpturing (Figure 2g):** Epidermal cells outline is distinguishable, anticlinal wall raised into ridges and periclinal wall sunken. **Abaxial cuticular sculpturing (Figure 2h):** Epidermal cells outline is distinguishable, anticlinal wall sunken and periclinal wall raised into ridges. **Stomata:** Elliptic shaped stomata, amphistomatic. **Trichome:** Glandular peltate trichome found on adaxial surface. Simple multicellular trichome (long with point end, echinate ornamentation) found on abaxial surface.

C. *Justicia procumbens*

Vascular tissue of petiole cross section (Figure 3a): Type 1. **Cystolith (Figure 3e):** Present at the epidermis of the cell and parenchyma cortex. **Trichome (Figure 3f):** Simple multicellular trichomes with point end and simple unicellular trichome with blunt end. **Vascular tissue of midrib cross section (Figure 3b):** Type 1. **Trichome:** Simple unicellular trichome with blunt end. **Outline of leaf margin (Figure 3d):** Rounded with U-shaped outline, 30-45° recurved downwards to

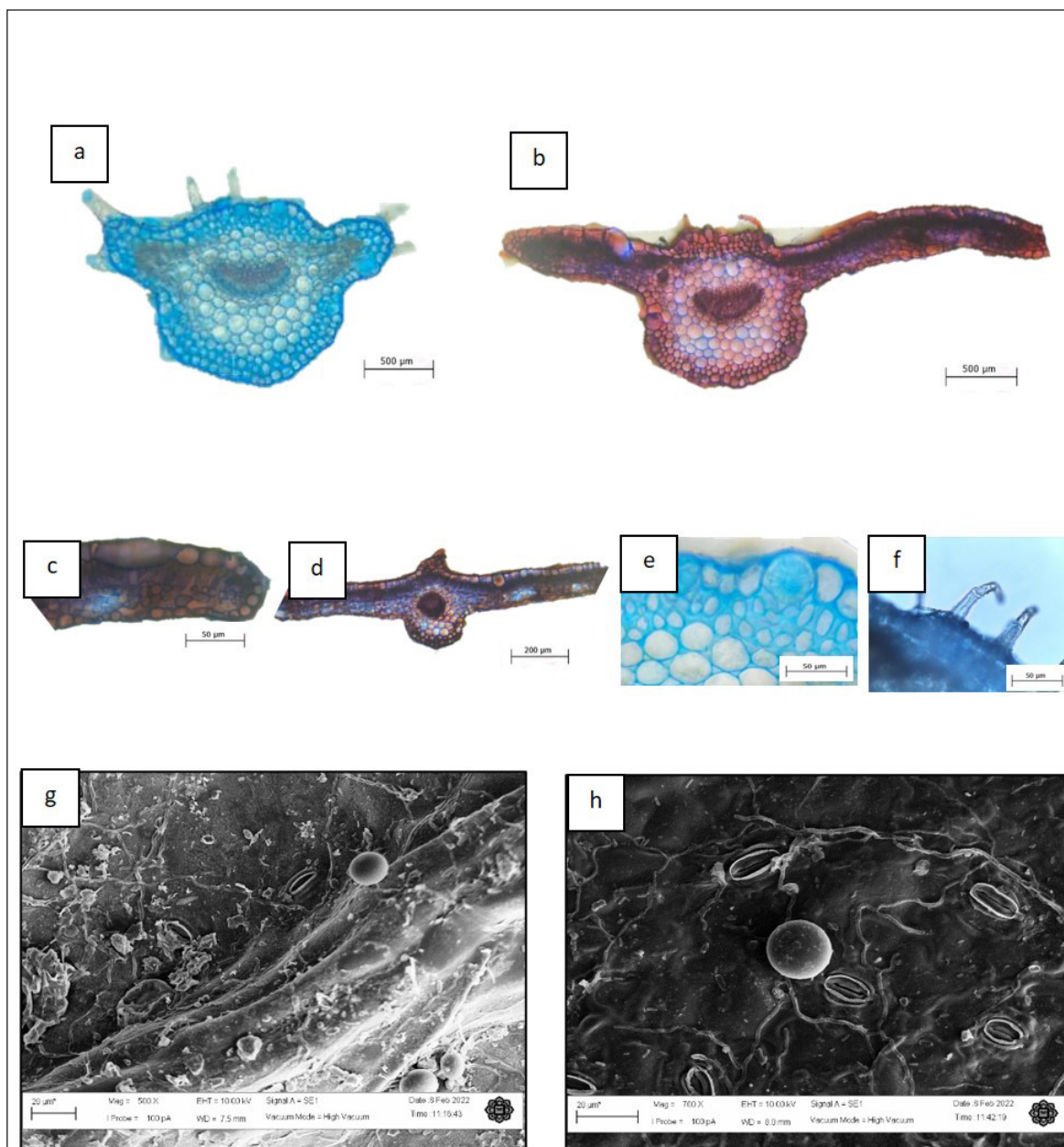


Fig. 3. *Justicia procumbens*: a) Petiole cross section. b) Midrib cross section. c-d) Lamina and margin cross section. e) Cystolith. f) Simple multicellular trichome. g) Adaxial surface. h) Abaxial surface. Scale: a & b) 500 µm. c) 50 µm. d) 200 µm. e-f) 50 µm. g-h) 20 µm.

the abaxial epidermis, and the size decreases gradually towards margin. **Cuticular layer:** relatively thin. **Chlorenchyma cells:** mesophyll palisade in one to two layers occupying 1/2 part of the leaf thickness of lamina. Spongy mesophyll in five to six layers of spongy mesophyll occupying 1/2 part of the height of leaf thickness of lamina. **Wax:** Film-like layer, crust of wax and granules found on adaxial and abaxial surfaces. **Adaxial cuticular sculpturing (Figure 3g):** Anticlinal wall and periclinal wall cannot be distinguishable. **Abaxial cuticular sculpturing (Figure 3h):** Epidermal cells outline is distinguishable, periclinal wall raised into ridges and anticlinal wall sunken. **Stomata:** Elliptic shaped stomata, amphistomatic. **Trichome:** Glandular peltate trichome found on adaxial and abaxial surface. Simple multicellular trichome with blunt end on abaxial surface.

DISCUSSION

This study reported on the importance of leaf anatomical and micromorphological characteristics in identifying the three selected species which are *J. adathoda*, *J. gendarussa*, and *J. procumbens*. The different shapes and types of cystolith cells can be useful in the identification of selected family and plant species (Maisarah et al. 2020). Cystolith cells can be found in many parts of the plant, particularly the leaves, which also include xylem and phloem rays (Mauseth 1988). Nurul-Aini et al. (2018) and Maisarah et al. (2020) reported that the variation of cystolith cells can be used in differentiating species in Acanthaceae. Results showed the presence of solitary cystoliths rounded-shaped in all species studied either in the epidermis layer, collenchyma cells, or parenchyma cortex. Hence, this research is supported by Pierantoni et al. (2018) who mentioned that cystolith deposition can occur either in the abaxial or adaxial epidermis, but in some circumstances, it may extend into the mesophyll area.

The results of this study revealed variations in the pattern of the vascular bundle in the petiole and midrib that can be used for the identification of species studied. Ghazalli et al. (2021) explained the taxonomic importance of midrib and petiole vascular bundle in species identification of *Mitragyna speciosa* with supported by previous research on Rubiaceae family by Metcalfe and Chalk (1950). The pattern of vascular bundles that have been successfully identified in the petiole of each species studied is characterised as Type 1 with opened system in U shape (continuous ring of a vascular bundle) with the presence of two additional vascular bundles located at the right and the left wing of the petiole. In addition, this study also observed the midrib of vascular bundle arrangements in *J. gendarussa* and *J. procumbens* which are identified as the main vascular bundle

with an opened system (continuous ring of a vascular bundle) and also with the presence of two additional vascular bundles that located at the right and left wing of the midrib. Meanwhile, Type 2 with the main vascular bundle (an opened system in a U-shape with a non-continuous ring of the vascular bundle) and the occurrence of two additional vascular bundles located at the right and the left wing of the midrib can be found in *J. adathoda*. In his description of the fundamental principles of petiole anatomy, Candolle (1879) distinguished two vascular bundle systems known as the open system and the close system.

Dickison (2000) stated that sclerenchyma cells comprise various cell types with thickened secondary walls lignified when mature. Sclerenchyma cells mature with the surrounding tissues and also give more stable support when compared to collenchyma cells (Lopez and Barclay, 2017). Rojo (1987) pointed out that some leaf anatomical characters such as the petiole vascular patterns can be used in the classification and identification of selected Philippine dipterocarps when associated with other characters, such as the presence of sclerenchyma cells around the vascular bundle and also the number of resins. All the species examined showed the presence of sclerenchyma cells surrounding the phloem tissue of the main vascular bundle and two additional vascular bundles in the petiole and midrib. Noraini and Cutler (2009) also reported that the presence of sclerenchyma cells surrounding the primary and secondary vascular bundles in lamina can be used to identify selected *Parashorea* species.

Singh and Jain (1975) recorded 40 types of trichomes with 19 types of glandular trichomes and 21 types of simple non-glandular trichomes in 41 taxa of the Acanthaceae family. Meanwhile, Nurul-Aini et al. (2018) revealed 12 types of trichomes in selected *Staurogyne* species of Acanthaceae. The results of this study showed that the presence of trichomes can be used to differentiate *Justicia* species. There are five types of trichomes that have been recorded in this study which are; glandular peltate trichomes, simple unicellular trichomes with a blunt end, simple multicellular trichomes with a point end, simple multicellular trichomes with a blunt end and simple multicellular trichomes with point end (echinate ornamentation). Previous research by Verdam et al. (2012) proposed that trichomes can be useful in the identification of *Justicia* species even either in microscopic view or in powder form.

The present study also managed to record the occurrence of stomata on both adaxial and abaxial surfaces (amphistomatic) with type of diacytic stomata in three species examined. Thus, it can be useful as common characters to identify *Justicia* species. Besides, the findings of

the present research have been also supported by Nurul-Aini *et al.* (2014) and Noor-syaheera (2015) on the presence of amphistomatic leaves among selected *Justicia* species of Acanthaceae in Peninsular Malaysia. Whang *et al.* (2004) discovered the taxonomic importance of epidermal surface analysis in terms of species identification for Pinaceae species. All species studied recorded two types of waxes which are film like layer and granules while crust of wax was only reported in *J. gendarussa* and *J. procumbens*. Variation between species can be observed through several characters such as type of wax and trichome, cuticular ornamentation pattern and can be used as an alternative feature as a tool to differentiate species in *Pentace* genus as mentioned by Noraini *et al.* (2022). Therefore, this character is important and might be a good criterion for delineating plant taxa, and also to classify plants up until the genus level.

CONCLUSION

In conclusion, the findings of this research demonstrated that leaf anatomical and micromorphological characteristics are important tools and also additional data to differentiate plant species in the Acanthaceae family. Three common characteristics were identified in this study which are the presence of mucilage cells, sclerenchyma and collenchyma cells in all species examined.

REFERENCES

- Alamgir, A.N.M. 2017. Pharmacognostical Botany: Classification of Medicinal and Aromatic Plants (MAPs), Botanical Taxonomy, Morphology, and Anatomy of Drug Plants. In: Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1. Progress in Drug Research. K.D. Rainsford. (Ed.). Springer, Cham, Switzerland. pp. 177–293. https://doi.org/10.1007/978-3-319-63862-1_6
- Burkill, H.M. 1985. Entry for *Lasiurus hirsutus* (Forssk.) Boiss. [family Poaceae]. In: The Useful Plants of West Tropical Africa. H.M. Burkill (Ed.). 2nd Ed. Royal Botanic Gardens, Kew, UK. pp. 254-257.
- Candolle, C.D.E. 1879. Anatomie compare des feuilles chez quelques familles de Dicotyledones. Mémoires de la Société de Physique et d'Histoire Naturelle de Genève, 26: 427-480.
- Choopan, T. & Grote, P.J. 2015. Cystoliths in the leaves of the genus *Pseuderanthemum* (Acanthaceae) in Thailand. International Journal of Science, 12(2): 13-20.
- Dickison, W.C. 2000. Integrative Plant Anatomy. New York. Harcourt Academic Press, pp 499-502.
- Fongod, A., Bibiche, M., Veranso-Libalah, & Marie, C. 2013. Ethnobotany of Acanthaceae in the Mount Cameroon region. Journal of Medicinal Plants Research, 7(38): 2859-2866.
- Ghazalli, M.N., Md Sah, M.S., Mat, M., Awang, K., Jaafar, M.A., Mirad, R., Zaini, A.Z., Nordin, A.R.M., Rusli, N.M., Mohamad, S.S. & Dalee, A.S.M. 2021. Leaf anatomy and micromorphology characteristics of Ketum [*Mitragyna speciosa* (Korth.) Havil.] (Rubiaceae). Tropical Life Sciences Research, 32(1): 107–117. <https://doi.org/10.21315/tlsr2021.32.1.7>
- Johansen, D.A. 1940. Plant Microtechnique. McGraw Hill Book Company, New York.
- Kavitha, K., Sangeetha, S., Sujatha, K. & Umamaheswari, S. 2014. Phytochemical and pharmacological profile of *Justicia gendarussa* Burm f. – Review. Journal of Pharmacy Research, 8: 990-997.
- Khan, I., Jan, S.A., Shinwari, Z.K., Ali, M., Khan, Y. & Kumar, T. 2017. Ethnobotany and medicinal uses of folklore medicinal plants belonging to family Acanthaceae: An updated review. MOJ Biology and Medicine, 1(2): 34–38. <https://doi.org/10.15406/mojbm.2017.01.00009>
- Khatijah, H. & Ruzi, A.R. 2006. Anatomical Atlas of Malaysian Medicinal Plants. Volume 2. UKM Press, Bangi.

A number of diagnostic characters are also reported in this study. Besides that, the species studied showed several types of trichomes but only *J. procumbens* possessed simple unicellular trichome, blunt end that are found on abaxial surface. Furthermore, the vascular bundle system also plays an important role in the identification of species, especially in the midrib vascular bundle arrangements of species studied. In conclusion, leaf anatomy and micromorphology characteristics of selected *Justicia* species are useful for the identification of species in Peninsular Malaysia. This may provide additional information for species classification.

ACKNOWLEDGEMENTS

The authors are appreciative of the Department of Plant Science, Kulliyah of Science, International Islamic University of Malaysia, Kuantan, Pahang and Scanning Electron Microscope (SEM) services at Kulliyah of Medicine. Not to forget, special thanks were dedicated to the Ministry of Higher Education (MOHE) and FRGS/1/2019/STG03/UIAM/03/2 for the financial support during this research period.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

- Lopez, F.B. & Barclay, G.F. 2017. Plant anatomy and physiology. In: Pharmacognosy: Fundamentals, Applications and Strategies. S. Badal and R. Delgoda, (Eds.). Academic Press, USA. pp. 45-60. <https://doi.org/10.1016/B978-0-12-802104-0.00004-4>
- Maisarah, Z., Nurul-Aini, C.A.C., Noraini, T., Latiff, A., Amirul-Aiman, A.J., Rozilawati, S., Nur-Shuhada, T. & Ruzi, A.R. 2020. The variation of cystoliths and its taxonomic significance in Acanthaceae of Peninsular Malaysia. *Malaysian Applied Biology*, 49(5): 25–31. <https://doi.org/10.55230/mabjournal.v49i5.1634>
- Mauseth, J.D. 1988. *Plant anatomy*. California. Benjamin, Cummings.
- Metcalfe, C.R. & L. Chalk. 1950. *Anatomy of the dicotyledons*. Oxford. Clarendon Press.
- Noor-Syaheera, M.Y., Noraini, T., Radhiah, A.K. & Nurul-Aini, C.A.C. 2015. Leaf anatomical characteristics of *Avicennia* L. and some selected taxa in Acanthaceae. *Malayan Nature Journal*, 67(1): 81-94.
- Noraini, T. & Cutler, D.F. 2009. Leaf anatomical and micromorphological characters of some Malaysian *Parashorea* (Dipterocarpaceae). *Journal of Tropical Forest Science*, 21(2): 156–167.
- Noraini, T., Nurul-Aini, C.A.C., Nurhanim, M.N., Nabilah, M., Mohd-Norfaizal, G., Fitri, Z. & Hamidun, B. 2022. Taxonomic significance of leaf epidermis micromorphological characteristics of *Pentace* L. (Malvaceae s.l) in Malaysia. *Malaysian Journal of Microscopy*, 18(1): 192-201.
- Noraini, T., Ruzi, A.R., Ismail, B.S., Ummu Hani, B., Salwa, S. & Azi-Azeyanty, J. 2016. Petiole vascular bundles and its taxonomic value in the tribe Dipterocarpeae (Dipterocarpaceae). *Sains Malaysiana*, 45(2): 247–253.
- Nurul-Aini, C.A.C., Nurul-Atiqah, M.M. & Rozilawati, S. 2019. Leaf anatomy and micromorphology of selected plant species in coastal area of Kuantan, Pahang, Malaysia. *Science Heritage Journal (GWS)*, 3(2): 22-25. <https://doi.org/10.26480/gws.02.2019.22.25>
- Nurul-Aini, C.A.C., Noraini, T., Latiff, A., Amirul-Aiman, A.J., Ruzi, A.R. & Idris, S. 2014. Taxonomic significance of leaf micromorphology in some selected taxa of Acanthaceae (Peninsular Malaysia). *AIP Conference Proceedings*, 1614 (1): 727-733. <https://doi.org/10.1063/1.4895291>
- Nurul-Aini, C.A.C., Nur Shuhada, T., Rozilawati, S., Fatin, M.A., Noraini, T. & Latiff, A. 2018. Comparative leaf anatomy of selected medicinal plants in Acanthaceae. *IIUM Medical Journal Malaysia*, 17(2): 17-24. <https://doi.org/10.31436/imjm.v17i2.944>
- Petrizzello, M. 2013. *Avicennia*. *Encyclopedia Britannica*. <https://www.britannica.com/plant/Avicennia> (accessed 09.29.2022).
- Pierantoni, M., Tenne, R., Rephael, B., Brumfeld, V., Cateren, A.V., Kupczik, K., Oron, D., Addadi, L. & Weiner, S. 2018. Mineral deposits in *Ficus* leaves: Morphologies and locations in relation to function. *Plant Physiology*, 176: 1751-1763. <https://doi.org/10.1104/pp.17.01516>
- Rojo, J.P. 1987. Petiole anatomy and infrageneric in interspecific relationship Philippines *Shorea* (Dipterocarpaceae). In: Kostermans, A.J.G.H. *Proceedings of the Third Round Table Conference on Dipterocarps*. S. Appanah, and J. M. Turnbull (Eds.). Biotrop, Bogor, Indonesia. pp. 569-601.
- Saas, J.E. 1958. *Botanical Microtechnique*. 3rd Ed. Iowa State University, USA. <https://doi.org/10.31274/isudp.25>
- Sharma, A. & Kumar, A. 2016. Acanthaceae: Taxonomy and uses in traditional medicinal system. *World Journal of Pharmaceutical Research*, 5(7): 403-412.
- Singh, V. & Jain, D.K. 1975. Trichomes in Acanthaceae I. General structure. *Journal of the Indian Botanical Society*, 54: 116–127.
- Verdam, M., Ohana, D., Araújo, M., Guilhon-Simplicio, F., Mendonça, M. & Pereira, M. 2012. Morphology and anatomy of *Justicia acuminatissima* leaves. *Revista Brasileira de Farmacognosia*, 22: 1212-1218. <https://doi.org/10.1590/S0102-695X2012005000129>
- Whang, S.S., Kim, K. & Hill, R.S. 2004. Cuticle micromorphology of leaves of *Pinus* (Pinaceae) from North America. *Botanical Journal of Linnean Society*, 144(3):303–320. <https://doi.org/10.1111/j.1095-8339.2003.00245.x>