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COMPARATIVE LEAF ANATOMY AND MICROMORPHOLOGY OF *ASYSTASIA GANGETICA* T.ANDERSON SUBSP. *MICRANTHA* (NEES) ENSERMU AND *RHINACANTHUS NASUTUS* (L.) KURZ (JUSTICIINAE, ACANTHACEAE) FROM PENINSULAR MALAYSIA

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ABSTRACT
AMRI, C. N. A. C., ZAKARIA, S. M., SHAHARI, R., TAJUDIN, A. A. B. M., TALIP, N., RAHMAN, M. R. A. & SIAM, N. A. 2023. Comparative leaf anatomy and micromorphology of *Asystasia gangetica* T.Anderson subsp. *micrantha* (Nees) Ensermu and *Rhinacanthus nasutus* (L.) Kurz (Acanthaceae) from Peninsular Malaysia. *Reinwardtia* 22(2): 79–89. — Acanthaceae family has been used traditionally for medicinal purposes, especially amongst the native communities in Peninsular Malaysia. Nowadays, many taxonomists have difficulties in the identification of the Acanthaceae species due to its morphological similarities and when there is an incomplete part of plants obtained from the field sampling. But until now, there is no comprehensive study that has been documented especially on the Acanthaceae family, specifically for *A. gangetica* subsp. *micrantha* and *R. nasutus*. To avoid incorrect species identification, a systematic study that involved the leaf anatomy and micromorphology parts is being used for the identification and classification of plants in the Acanthaceae. Therefore, the main objective of this present study is to identify the leaf anatomical and micromorphological characteristics that can be used in plant identification and for supportive data in plant classification. The leaf anatomical and micromorphological studies that are conducted on species studied involve several procedures such as cross-section using a sliding microtome, and observation under a light microscope and scanning electron microscope. The anatomical and micromorphological characteristics observed that have been used to identify each species studied include patterns of petiole and midrib vascular bundles, leaf margin, leaf lamina, presence of cuticular striae, and the presence of trichomes. The results of this study showed that the cystolith cells can be found only in midrib of *A. gangetica* subsp. *micrantha* while it also recorded in petiole, midrib, and the leaf lamina of *R. nasutus*. Observation under the light microscope revealed nine types of trichomes in *R. nasutus* meanwhile seven trichomes were recorded in *A. gangetica* subsp. *micrantha.* Other than that, the present of cuticular striae only recorded at the abaxial epidermis of *A. gangetica* subsp. *micrantha.* In conclusion, results showed that anatomical and micromorphological characteristics have taxonomic significance that can be used in the identification and classification, especially at the species level.

Key words: Acanthaceae, *Asystasia gangetica* subsp. *micrantha*, cystolith, *Rhinacanthus nasutus*.

ABSTRAK

AMRI, C. N. A. C., ZAKARIA, S. M., SHAHARI, R., TAJUDIN, A. A. B. M., TALIP, N., RAHMAN, M. R. A. & SIAM, N. A. 2023. Perbandingan anatomi dan mikromorfologi daun *Asystasia gangetica* T.Anderson subsp. *micrantha* (Nees) Ensermu dan *Rhinacanthus nasutus* (L.) Kurz (Acanthaceae) dari Semenanjung Malaysia. *Reinwardtia* 22 (2): 79–89. — Suku Acanthaceae telah digunakan dalam pengobatan tradisional, terutama bagi masyarakat asli di Semenanjung Malaysia. Sampai dengan saat ini, ahli taksonomi mengalami kesulitan terutama dalam determinasi jenis suku Acanthaceae karena ciri morfologi yang hampir sama terutama untuk spesimen yang tidak lengkap saat pengambilan sampel di lapangan. Namun hingga saat ini, kajian anatomi daun yang komprehensif khususnya *A. gangetica* subsp. *micrantha* dan *R. nasutus* masih sangat terbatas. Dalam rangka menghindari kesalahan dalam determinasi jenis, kajian sistematik khususnya anatomi dan mikromorfologi daun telah digunakan sebagai data tambahan bagi determinasi dan pengelompokkan jenis-jenis Acanthaceae. Kajian ciri anatomi menggunakan metode dengan irisan mikrotom geser (ciri anatomi tangkai, helaian, tulang dan tepi daun), sedangkan kajian ciri mikromorfologi menggunakan metode dengan melihat secara langsung permukaan epidermis abaksial dan adaksial daun di bawah mikroskop pemindaian elektron. Ciri anatomi dan mikromorfologi yang telah direkam adalah ciri berkas pengangkut pada tangkai dan tulang daun, tepi dan helaian daun, keberadaan kutikula serta keberadaan trikom pada setiap jenis yang diteliti. Hasil penelitian menunjukkan keberadaan sel sistolit hanya pada tulang tengah *A. gangetica* subsp. *micrantha*, tangkai, tulang, dan helaian daun *R. nasutus*. Hasil pengamatan di bawah mikroskop menunjukkan sembilan jenis trikom pada *R. nasutus* dan tujuh jenis trikom pada *A. gangetica* subsp. *micrantha*. Selain itu juga, kehadiran kutikel hanya dijumpai pada permukaan epidermis abaksial *A. gangetica* subsp. *micrantha.* Hasil penelitian menunjukkan bahwa ciri anatomi dan mikromorfologi daun dapat digunakan sebagai data tambahan khususnya untuk determinasi jenis yang diteliti.

Kata kunci: Acanthaceae, *Asystasia gangetica* subsp. *micrantha, Rhinacanthus nasutus,* sistolit.

INTRODUCTION

The Acanthaceae family, which is known as a large pan-tropical family is mainly herbaceous and shrubs. However, some of the members are climbers or liana, whilst a few species are woody plants (Metcalfe & Chalk, 1965; Scotland *et al.,* 1995). Acanthaceae family belongs to the order Lamiales consists of at least 3000 species in some 250 genera with the centre of distribution in Indo-Malaysia, Africa (including Madagascar), Northern South America, Central America, and Mexico; with thirty-five genera are native or naturalized in Peninsular Malaysia (Keng, 2003).

Mc Dade *et al.* (2008) divided Acanthaceae into four families: Acanthoideae, Andrographideae, Nelsonioideae, and Thunbergioideae. Whislt Vollesen (2008) later elevated the tribe Ruellieae to the subfamily Ruellioideae. New findings by Schwarzbach & Mc Dade (2008) and Borg (2008) have suggested that the genus *Avicennia* has a sister relationship with Acanthaceae through the floral characteristics that have been shared between *Avicennia* and Thunbergioideae. Stevens (2017) and Manzitto-Tripp *et al.* (2021) recognized the updated classification and the placement of *Avicennia* into Acanthaceae as in Angiosperm Phylogeny Website (APweb) by which the classification of Acanthaceae is divided into four subfamilies (Nelsonioideae, Acanthoideae, Thunbergioidae, and Avicennioideae).

Asystasia gangetica subsp. *micrantha* is recognized as weed and locally known as Chinese violet or rumput Israel in Malaysia. This plant species is native in Africa, India, and Sri Lanka (Hsu *et al.,* 2005). *Asystasia*, define as inconsistency that refers to the characteristics of corolla which is remarkable trait of uncommon characters for Acanthaceae family. Meanwhile, the origin of the word "gangetica" is from the Ganges River. This species also has been used as cover plant in orchards because it can prevent the growth of noxious weed and soil erosion (Gopal *et al*., 2013). This weed plant also are very useful in ethnobotanical study which can treat rheumatism, stomach pain, heart problems, and asthma (Hamid *et al*., 2011).

Rhinacanthus nasutus, commonly known as snake jasmine, *pokok kepala sari* or *ubat kurap*. This plant is native to India and widely distributed in Malaysia, Laos, Indonesia, Thailand, Myanmar, and Vietnam. It is subshrubs or perennial herbs that grow up to 1.5 m tall. The capsule is pubescent with gland-tipped trichomes, and the seeds are papillose (Chia-chi *et al.,* 2011). Munavvar *et al.* (2004) mentioned that the leaf of *R. nasutus* is commonly used in traditional medicine preparations to treat various skin problems. For instance, the leaves of *R. nasutus* are pounded with benzoin and sulphur and applied externally to the area infected by ringworm. In Thailand, *R. nasutus* is traditionally used to treat various cancers such as colon (Kupradinum *et al.,* 2009), and cervical and liver cancers (Rojanapo *et al*., 1990).

Besides, the Acanthaceae is also recognized with the occurrence of cystoliths that are visible with the magnifying lenses as rod-shaped, especially in the epidermis surfaces of the leaves. Hence, it is very significant to have an additional

tool to provide information about the inner part of the plants. Previous study by Maisarah *et al.* (2020) reported four main types of cystoliths cells based upon 41 taxa studied in Acanthaceae. Four types of cystoliths identified as Type 1 (solitary cystolith with rounded-shaped), Type 2 (solitary cystolith with blunt-end), Type 3 (solitary cystolith elongated with one end pointed), and Type 4 (solitary cystolith elongated with end pointed both). It was also observed that the absence of cystoliths occurred in 13 taxa out of 41 taxa in Acanthaceae from genera of *Acanthus*, *Avicennia*, S*taurogyne*, and *Thunbergia*, thus recognized the significance of cystoliths in the identification and classification of Acanthaceae species.

This present study thereby involved the investigation of the leaf anatomy and micromorphological characteristics of *A. gangetica* subsp. *micrantha* and *R. nasutus* in Peninsular Malaysia that possessed taxonomic significance and can be used as additional data to avoid misidentification of the species.

MATERIALS AND METHODS

Fresh leaf samples of *A. gangetica* subsp. *micrantha* and *R. nasutus* were collected at several open areas such as in Nilai, Negeri Sembilan, Selangor, and Perak. Five replicates were used throughout this research. Voucher specimens were deposited at Universiti Kebangsaan Malaysia Herbarium (UKMB). Fresh specimens were fixed in 3:1 AA Solutions (70% Alcohol: 30% Acetic Acid). In this study, four parts were observed including petiole, midrib, lamina, and marginal parts. These parts were sliced by using a sliding microtome composed of a fixed sample holder known as polystyrene. The slicing was made by using a disposable knife (Leica 818) at a range of thickness of 10–40 µm. The cut parts were immersed into a petri dish filled with bleach 'Clorox' solution for 10 minutes until the original pigment at the specimen vanished. Next, the sliced specimens were washed three times with distilled water to remove any residue left. The staining process involved two stages of staining solution which are alcian blue and safranin. Then, the specimens obtained from the sliding microtome were undergone a dehydration process in a series of alcohol and mounted in Euparal. All slides were covered with coverslips and kept in the oven for two weeks at about 60°C for drying purposes. Anatomical images were captured using a video (3CCD) camera attached to a Leitz Diaplan microscope using Cell^B software. Suitable modifications in terms of fixation and embedding followed the method by Johansen (1940) and Saas (1958). For the micromorphology study, the scanning electron microscopy (SEM) method was applied. The specimens were taken from a dried sample of the herbarium in which 1 cm^2 lamina portions of leaf sample were cut and mounted on a mounting holder. The specimens were then coated with gold by using a sputter-coated machine. The observation of micromorphological characteristics was done under Scanning Electron Microscope Zeiss Model Evo 50.

RESULTS

Figure 1 shows the characteristics of leaf anatomy and micromorphology of *A. gangetica* subsp. *micrantha.* The descriptions of the leaf anatomical and micromorphological characteristics for *A. gangetica* subsp. *micrantha* are summarized as below:

Petiole. Adaxial outline: concave with V-wide shape and two ear-like at the left and right side of the petiole outline. Abaxial outline: ¾ round shape. Vascular tissue: main vascular tissue (opened system with non-continuous ring of vascular bundle) with four additional vascular bundles are situated at the above left and right side of the main vascular bundle. Parenchyma cells: *ca.* 7 –9 layers of parenchyma cells. Sclerenchyma cells: clusters of sclerenchyma cells present at the vascular bundles. Collenchyma cells: 4–9 layers present under the epidermis of abaxial and adaxial surfaces. Mucilage cell: Present at the parenchyma cortex. Trichomes: peltate glandular trichomes (terminal unicellular), simple unicellular trichomes (short, pointed end), multicellular trichomes (long, pointed end) and multicellular trichomes (long, tapered end) present at the epidermis of abaxial and adaxial surfaces (Fig. 1A).

Midrib. Adaxial outline: slightly concave hump with ½ inverted rectangle shape. Abaxial outline: ½ round shape. Vascular tissue: main vascular tissue (opened system with non-continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle. Sclerenchyma cells: clusters of sclerenchyma cells present at the vascular bundles. Collenchyma cells: 2–4 layers present under the epidermis of abaxial and adaxial surfaces. Mucilage cell: Present at the parenchyma cortex. Cystolith cells: rounded, solitary cystolith cells present at the epidermis of abaxial and adaxial surface. Trichomes: peltate glandular trichome (terminal multicellular), simple unicellular trichomes (short, pointed end), multicellular trichome (short, pointed end) and multicellular trichome (short, tapered end), present at the epidermis of abaxial and adaxial surfaces (Fig. 1B).

Leaf Margin. Outline: slightly tapered, 40º recurved downwards to the abaxial side (Fig. 1C).

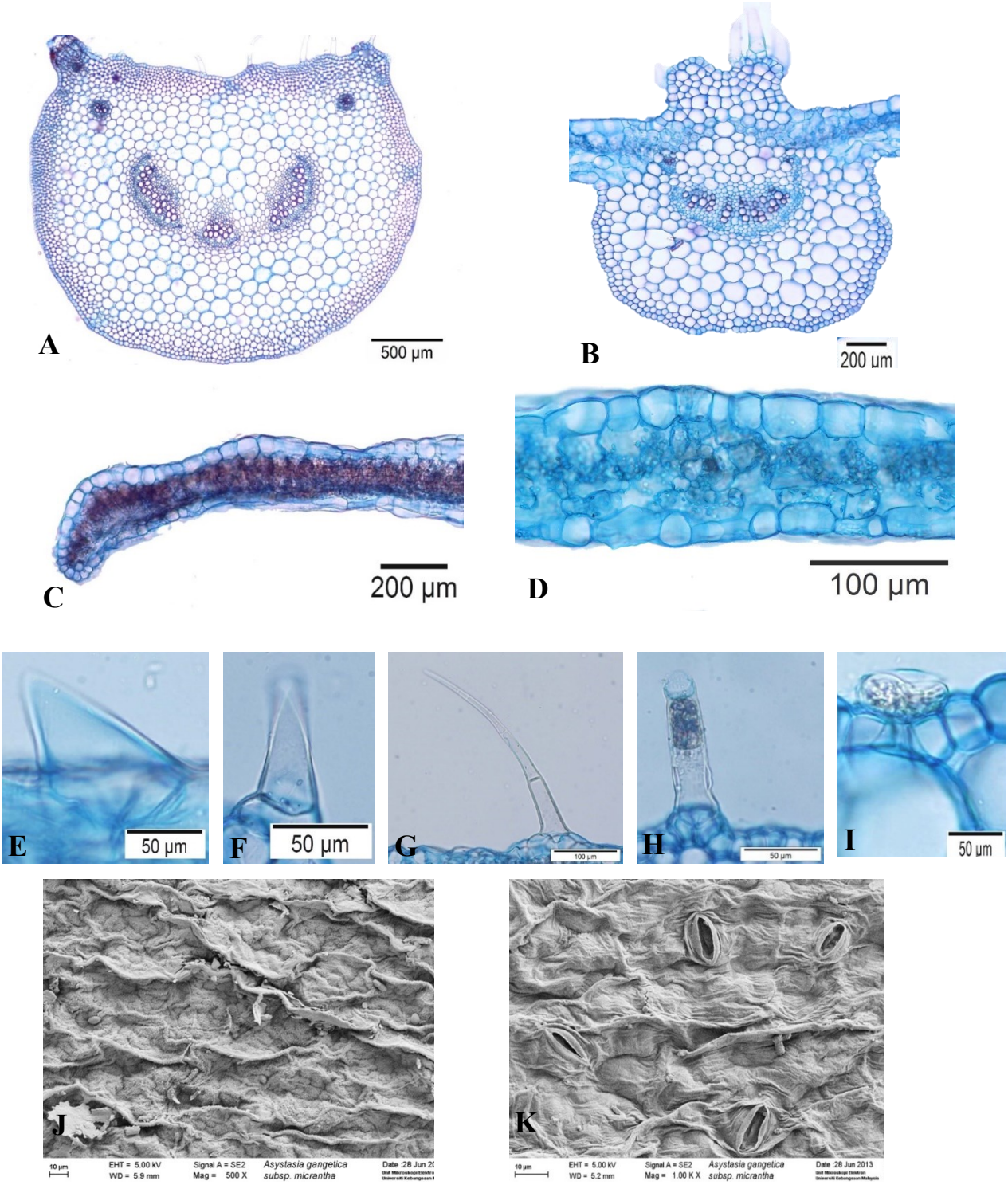

Fig. 1. *Asystasia gangetica* subsp. *micrantha*: A. Petiole cross-section. B. Midrib cross-section. C-D. Lamina and margin cross-section. E. Simple unicellular trichome (short, pointed end). F. Simple multicellular trichome (short, tapered end). G. Simple multicellular trichome (long, pointed end). H. Simple multicellular trichome (short, tapered end). I. Peltate glandular trichome. J. Adaxial surface. K. Abaxial surface. Scale: J & K 10 µm.

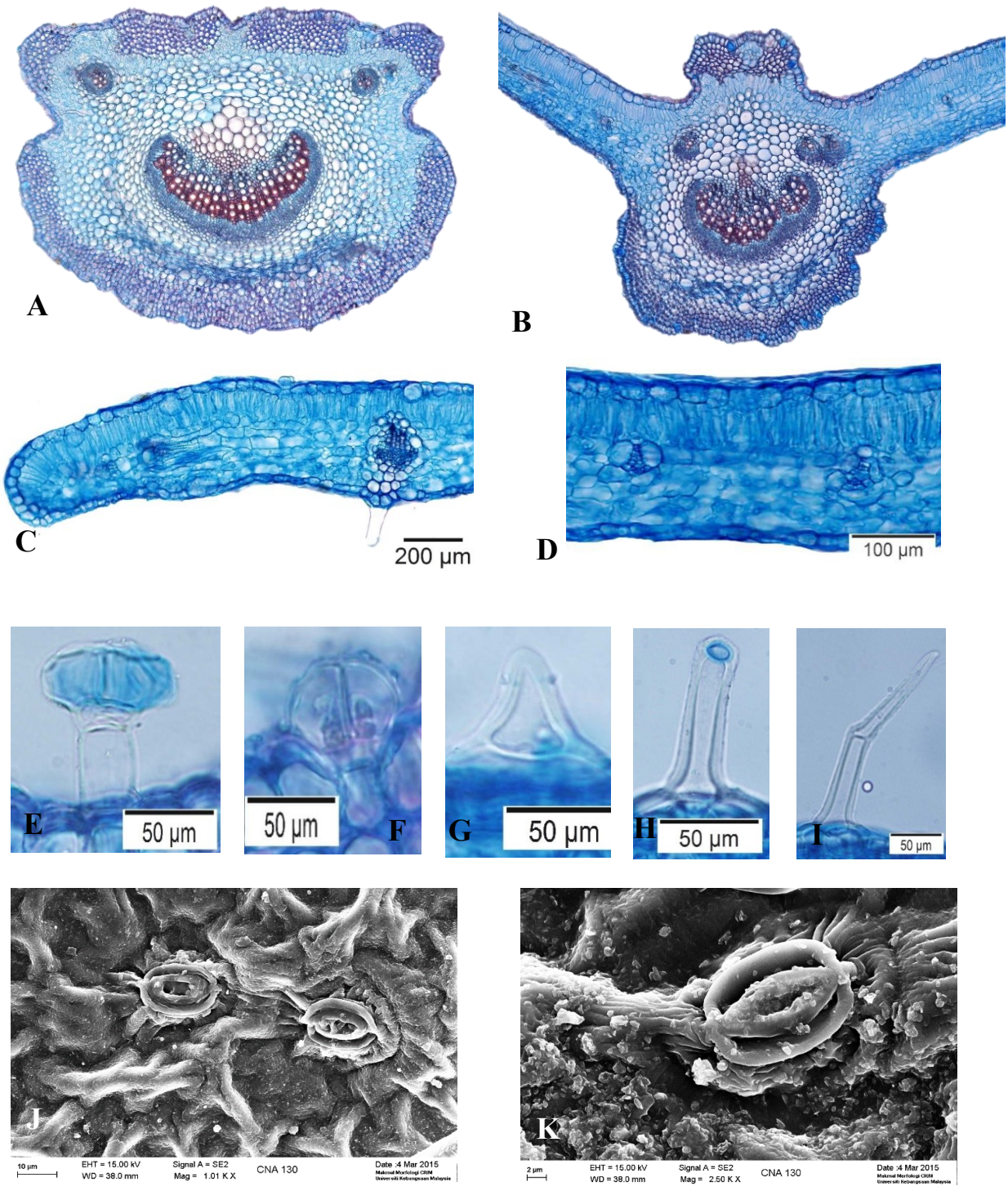

Fig. 2. *Rhinacanthus nasutus*. A. Petiole cross-section. B. Midrib cross-section. C-D. Lamina and margin cross-section. E. Capitate glandular trichome. F. Peltate glandular trichome. G. Simple multicellular trichome (short, tapered end). H. Simple multicellular trichome (long, tapered end). I. Simple multicellular trichome (long, pointed end). J. Adaxial surface. K. Abaxial surface. Scale: J. 10 µm. K. 2 µm.

Leaf Lamina. Cuticular layer: relatively thin. Adaxial and abaxial epidermis: single layer with height: width ratio – 1:1 and 1:2. Chlorenchyma cells: mesophyll palisade: two layers filling ½ part of the height of leaf lamina. Spongy mesophyll: 2–3 layers of spongy mesophyll. Vascular bundles: simple vascular bundles. Parenchyma cells: single layer encircling each vascular bundle. Trichome: peltate glandular trichome (terminal multicellular), simple unicellular trichomes (short, pointed end), multicellular trichome (short, pointed end) and multicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces (Fig. 1D).

Leaf Epidermis. Epicuticular waxes: films, granules, flakes, and crustose present at the epidermis of adaxial surface, verrucate and buttress-like wax present at the epidermis of abaxial surface. Cuticular striation: anticlinal and periclinal wall can not be differentiated on both sides, cuticular striae present at the epidermis of abaxial. Stomata: diacytic, superficial, elliptic shape. Stomata size range: H × W (15.79–18.95 μm) × (10.98–13.07 μm). Trichome: Peltate glandular trichome (terminal multicellular) present at the adaxial surface only while peltate glandular trichome (terminal unicellular) and multicellular trichomes (long, pointed end, echinate ornamentation) present at both sides (Figs. 1J & 1K) .

Figure 2 shows the characteristics of leaf anatomy and micromorphology of *R. nasutus*. The descriptions of the leaf anatomical and micromorphological characteristics for *R. nasutus* are summarized as below:

Petiole. Adaxial outline: straight with two ear-like at the left and right side of the petiole outline. Abaxial outline: ¾ oblong shape. Vascular tissue: main vascular tissue (opened system with continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle. Parenchyma cells: *ca.* 9–12 layers of parenchyma cells. Sclerenchyma cells: clusters of sclerenchyma cells present at the vascular bundles. Collenchyma cells: 3–10 layers present under the epidermis of abaxial and adaxial surfaces. Mucilage cell: Present at the parenchyma cortex. Cystolith cells: rounded, solitary cystolith cells present at the epidermis of abaxial and adaxial surface. Trichomes: capitate glandular trichomes (terminal multicellular, rounded), peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end), and unicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces (Fig. 2A).

Midrib. Adaxial outline: convex with slightly hump. Abaxial outline: U shaped. Vascular tissue: main vascular tissue (opened system with continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle. Sclerenchyma cells: clusters of sclerenchyma cells present at the vascular bundles. Collenchyma cells: 3–10 layers present under the epidermis of abaxial and adaxial surfaces. Mucilage cell: Present at the parenchyma cortex. Cystolith cells: rounded, solitary cystolith cells present at the epidermis of abaxial and adaxial surface. Trichomes: capitate glandular trichomes with multicellular stalk, peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end), unicellular trichomes (long, tapered end), and unicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces (Fig. 2B).

Leaf Margin. Outline: slightly tapered, 30–45º recurved downwards to the abaxial side (Fig. 2C).

Leaf Lamina. Cuticular layer: relatively thin. Adaxial and abaxial epidermis: single layer with height: width ratio – 1:1 and 1:2. Chlorenchyma cells: mesophyll palisade: two layers filling ½ part of the height of leaf lamina. Spongy mesophyll: 5–6 layers of spongy mesophyll. Vascular bundles: simple vascular bundles. Parenchyma cells: single layer encircling each vascular bundle. Cystolith cells: rounded, solitary cystolith cells present at the epidermis of adaxial surface. Trichome: peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (long, tapered end), unicellular trichomes (long, pointed end), and multicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces (Fig. 2D).

Leaf Epidermis. Epicuticular waxes: granules, crustose present at the epidermis of adaxial surface, verrucate and granules present at the epidermis of abaxial surface. Cuticular striation: anticlinal and periclinal wall can be differentiated directly on both sides. Stomata: diacytic, superficial, elliptic shape. Stomata size range: H × W (16.55–20.51 μm) × (12.12–15.75 μm). Trichome: Peltate glandular trichome present at both sides while multicellular trichomes (long, pointed end, echinate ornamentation) present at the abaxial surface only (Figs. 2J & 2K).

DISCUSSION

The result of this study showed the significance of leaf anatomical and micromorphological characteristics that can be useful in identification of species studied. The results of this study showed that the cystolith cells can be found only in midrib of *A. gangetica* subsp. *micrantha* while it also recorded in petiole, midrib, and the leaf lamina of *R. na-*

Table 1. Summary of leaf anatomical and micromorphological variation of species studied

Anatomical characteristics	*Asystasia gangetica* subsp. *micrantha*	*Rhinacanthus nasutus*
Petiole	Adaxial outline: concave with V-wide shape and two ear-like at the left and right side of the petiole outline; abaxial outline: ¾ round shaped.	Straight with two ear-like at the left and right side of the petiole outline; abaxial outline: ¾ oblong shaped.
	Main vascular tissue (opened system with non-continuous ring of vascular bundle) with four additional vascular bundles are situated at the above left and right side of the main vascular bundle.	Main vascular tissue (opened system with continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle.
	Peltate glandular trichomes (terminal unicellular), simple unicellular trichomes (short, pointed end), multicellular trichomes (long, pointed end) and multicellular trichomes (long, tapered end) present at the epidermis of abaxial and adaxial surfaces.	Capitate glandular trichomes (terminal multicellular, rounded), peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end) and unicellular trichomes (long, pointed end) present at the epidermis of abaxial and adaxial surfaces
Midrib	Adaxial outline: slightly concave hump with ½ inverted rectangle shape; abaxial outline: ½ round shape.	Adaxial outline: convex with slightly hump; abaxial outline: u shaped.
	Main vascular tissue (opened system with non-continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle.	Main vascular tissue (opened system with continuous ring of vascular bundle) with two additional vascular bundles are situated at the above left and right side of the main vascular bundle.
	Peltate glandular trichome, simple unicellular trichomes (short, pointed end), multicellular trichome (short, pointed end) and multicellular trichome (short, tapered end)	Capitate glandular trichomes with multicellular stalk, peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end), unicellular trichomes (long, tapered end), and unicellular trichomes (long, pointed end)
Leaf margin	Slightly tapered, 40º recurved downwards to the abaxial side	Slightly tapered, 30–45º recurved downwards to the abaxial side
Leaf lamina	Spongy mesophyll: 2–3 layers of spongy mesophyll. Peltate glandular trichome, simple unicellular trichomes (short, pointed end), multicellular trichome (short, pointed end) and multicellular trichomes (long, pointed end)	Spongy mesophyll: 5–6 layers of spongy mesophyll. Peltate glandular trichome, simple unicellular trichomes (short, tapered end), unicellular trichome (long, tapered end), unicellular trichomes (long, pointed end), and multicellular trichomes (long, pointed end)
Leaf epidermis	Epicuticular waxes: films, granules, flakes, and crustose present at the epidermis of adaxial surface, verrucate and buttress-like wax present at the epidermis of abaxial surface.	Epicuticular waxes: granules, crustose present at the epidermis of adaxial surface, verrucate and granules present at the epidermis of abaxial surface.
	Anticlinal and periclinal wall cannot be differentiated on both sides, cuticular striae present at the epidermis of abaxial.	Anticlinal and periclinal wall can be differentiated directly on both sides.
	Peltate glandular trichome present at the adaxial surface only while peltate glandular trichome (terminal unicellular) and multicellular trichomes (long, pointed end, echinate ornamentation) present at both sides	Peltate glandular trichome present at both sides while multicellular trichomes (long, pointed end, echinate ornamentation) present at the abaxial surface only

sutus. The occurance of cystolith cells in all species studied supported previous research by Metcalfe & Chalk (1965), Nurul-Aini *et al.* (2018), and Maisarah *et al.* (2020) which recorded the presence of cystoliths cells in Acanthaceae species. The type of cystoliths also varied even within the same species either in petiole, midrib, or lamina. The previous research that has been done by Metcalfe & Chalk (1965) stated that the presence of cystolith cells is known as one of the important characteristics that can be used to identify and classify certain plant families such as Acanthaceae, Moraceae, Urticaceae, and Boraginaceae.

Hare (1942) reported that the vascular tissue system has significant value in plant taxonomy. Previous studies on three species of the genus *Shorea* by Rojo (1987) support the research findings by Hare (1942) by which the pattern of vascular tissue on the petiole part can be used to distinguish the three species of *Shorea.* Furthermore, a study by Ruzi *et al.* (2009) on the genus *Dipterocarpus* (family Dipterocarpaceae) showed the arrangement of vascular tissue has taxonomic value, especially in the classification of species at the genus level. Additionally, Nurul-Aini *et al.* (2013) also reported on the presence of high variation of vascular tissue patterns in the genus *Microcos* that can be used in species classification. Apart, the results of this study also showed that the type of vascular bundles in the midrib and petiole can be used to identify species studied.

O'Neill (2010) stated that the thickness of collenchyma cells makes the stems to be strong for protection against the wind. Nurul-Aini *et al.* (2018) recognized the presence of collenchyma cells in the petiole and midrib of several Acanthaceae species such as *Acanthus ebracteatus*, *Andrographis paniculata*, and *Chroesthes longifolia* which is very useful, especially in the identification of Acanthaceae family. Three to ten layers of collenchyma cells have been recorded in *R. nasutus*, meanwhile four to nine layers of collenchyma cells occurred in *A. gangetica* subsp. *micrantha.* Thus, supporting the previous research that has been done by Verdam *et al.* (2012) and Nurul-Aini *et al.* (2018). According to Leroux (2012), collenchyma cells are highly dynamic compared with sclerenchyma cells, whereby the collenchyma cells become more rigid due to changes in cell wall deposition or may undergo sclerification through lignification of newly deposited cell wall materials.

Furthermore, the results also showed that the mucilage cells were present either in the petiole, lamina or even in the midrib of all species studied. A study on the Shoreae tribe by Noraini (2006) reported the presence of mucilage cells or canals as a common feature of the tribe. Previously, Bass & Gregory (1985) explained that the presence of mucilage cells is a diagnostic characteristic of some plant species. Metcalfe & Chalk (1950) also recognized the presence of mucilage and oil cells in Lauraceae, thereby this feature giving additional data to characterize *R. nasutus*. Noraini *et al.* (2005) mentioned that even though the leaf margin is rarely used in the systematic study of plants, the results on leaf anatomy of the genus *Alpinia* from the family Zingiberaceae showed that the leaf margin has taxonomic value in the identification and classification of species. Results of the leaf lamina showed that *R. nasutus* have slightly tapered, 30–45º recurved downwards to the abaxial side. However, *A. gangetica* subsp. *micrantha* have slightly tapered with 40º recurved downwards to the abaxial side.

Inamdar (1967) stated that the characteristics of trichomes can be used for species delimitation. Trichomes are the physical structure that is present in plants (Levin, 1929) and can be used as one of the characteristics to identify certain plant species (Metcalfe & Chalk, 1979). Observation under the light microscope revealed nine types of trichomes in *R. nasutus* (Figs. 2E–2I) which are capitate glandular trichomes with multicellular stalk, capitate glandular trichomes (terminal multicellular, rounded), peltate glandular trichome (terminal multicellular), simple unicellular trichomes (short, tapered end), unicellular trichome (short, pointed end), unicellular trichomes (long, tapered end), unicellular trichomes (long, pointed end), multicellular trichomes (long, pointed end), and multicellular trichomes (long, pointed end, echinate ornamentation). Meanwhile seven trichomes were recorded in *A. gangetica* subsp. *micrantha* which are peltate glandular trichomes (terminal unicellular), peltate glandular trichomes (terminal multicellular), simple unicellular trichomes (short, pointed end), multicellular trichomes (long, pointed end), multicellular trichomes (long, tapered end), multicellular trichomes (short, pointed end), and multicellular trichomes (long, pointed end, echinate ornamentation) (Figs. 1E–1I). Zainab Sholehah *et al.* (2022) also mentioned the systematic significance based on the type of trichomes, especially to resolve the taxonomic conflicts of the species. Even previous research by Amirul-Aiman *et al.* (2014) agreed that the presence and types of trichomes have systematic value as in some petals of Acanthaceae species.

Besides, Barthlott (1990) recorded the presence of epicuticular wax in the cuticle layer which has significant variation as well as high taxonomic value in the angiosperm, gymnosperm, pteridophyte, and bryophyte groups. The results of a study by Nurhanim *et al.* (2014) showed that variation in wax types is significant and useful in the species identification and classification for *Schoutenia*. Results showed three types of wax present in *R. nasutus* which are granules, verrucate and crustose layers, meanwhile, in *A. gangetica* subsp. *micrantha*, types of wax such as films, granules, flakes, and crustose present at the epidermis of adaxial surface, verrucate, and buttress-like wax

present at the epidermis of abaxial surface. Thus, giving good criteria to recognize each species studied. A previous study conducted by Patil & Patil (2011) on 22 species of the family Acanthaceae reported the presence of hypostoma-tic and diacytic stomata. Besides, preliminary studies by Metcalfe & Chalk (1965) also recorded the presence of diacytic type stomata either on both surfaces of the epidermis (amphistomatic) or on the apical epidermal layer only (hypostomatic), thereby giving evidence of the presence of amphistomatic and diacytic type of stomata in all species studied. Barthlott (1981) mentioned that morphological characteristics of the leaf surface, such as the presence or absence of cuticular ornamentations, can be used to classify genus and species level. Result of this study showed the present of cuticular striae only at the abaxial epidermis of *A. gangetica* subsp. *micrantha*. Therefore, the results of this study support previous studies where the characteristics of stomata have significant value in the classification of species studied.

The dichotomous key that has been constructed from the present study thus gives evidence on the significance of leaf anatomy and micromorphology characteristics as supportive data in the identification and classification of species studied.

1 Peltate glandular trichome (terminal unicellular) and (terminal multicellular); absence of capitate glandular trichomes; presence of cuticular striae at the abaxial epidermis; adaxial outline: midrib slightly concave hump with ½ inverted rectangle shape; abaxial outline: ½ round shape; verrucate and buttress-like wax present at the epidermis of abaxial surface……. *A. gangetica* subsp. *micrantha*

1 Peltate glandular trichome (terminal multicellular); presence of capitate glandular trichomes with multicellular stalk, capitate glandular trichomes (terminal multicellular, rounded); absence of cuticular striae at the abaxial epidermis; midrib adaxial outline: convex with slightly hump; abaxial outline: U shaped; verrucate and granules present at the epidermis of abaxial surface……..… *R. nasutus*

CONCLUSION

Given that *A. gangetica* subsp. *micrantha* and *R. nasutus* is well known as a medicinal plant, systematic evidence obtained from the study of leaf anatomy and micromorphology characteristics is useful in providing significant distinctive characters for plant identification and classification. The results of this present study reported potential important leaf anatomy and micromorphological characteristics of species studied. Thus, the findings of this study suggest that the characteristics such as patterns of petiole and midrib vascular bundles, the presence of cuticular striae, type of stomata, types of trichomes and types of epicuticular waxes can be used to identify each species studied. As such, the anatomical and micromorphological tools are proven to be important tools in the systematic study.

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