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Stem and Leaf Anatomical Studies of Selected Species of Barlerieae and Ruellieae (Acanthaceae) from Yemen

(Kajian Anatomi Batang dan Daun Spesies Terpilih Barlerieae dan Ruellieae (Acanthaceae) dari Yemen)

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ABSTRACT

The anatomy of stems and leaves of selected species of tribes Barlerieae and Ruellieae (Acanthaceae) was studied to assess the taxonomic significance of anatomical characteristics in the two tribes. The anatomical characters such as the outline of stems, midribs, petioles, margins, shape of epidermal cells, vascular bundles, stomata, anticlinal cells, cystoliths, aerenchyma cells, layer of wax, layer of hypodermis were observed and analysed. Type of trichomes observed on the leaf surface using Scanning Electron Microscopy and light microscope provides information of taxonomic significance to both tribes and the taxa within them. The trichomes vary from non-glandular (unicellular, bicellular, multicellular, and multiradiate) to capitate glandular. Abundance of long-stalked trichomes was recorded only in Neuracanthus aculeatus. Cystoliths of different size and position are present in epidermis, parenchyma and collenchyma in addition to oil cells in some genera. The placement of Neuracanthus among the recognized tribes of Acanthaceae is assessed using anatomical characters.

Keywords: Acanthaceae; anatomy; Barlerieae; Neuracanthus; Ruellieae; Yemen

ABSTRAK

Anatomi batang dan daun bagi spesies terpilih daripada tribus Barlerieae dan Ruellieae (Acanthaceae) telah dikaji untuk menilai kesignifikanan ciri taksonomi dalam kedua-dua tribus yang tersebut. Beberapa sifat seperti bentuk umum luar batang, tulang daun, petiol, tepi daun, bentuk sel epidermis, berkas vaskular, stomata, sel antiklin, sistolit, aerenkima, lapisan lilin, lapisan hipodermis juga telah diperhati dan dianalisiskan. Jenis trikom atas permukaan daun yang dicerap di bawah mikroskop imbasan elektron dan mikroskop cahaya memberikan maklumat taksonomi yang signifikan untuk kedua-dua tribus dan juga takson yang berada di bawahnya. Trikom bervariasi daripada yang ringkas (bukan berkelenjar unisel, bisel, berbilang sel dan berbagai radiat) hingga berkelenjar berkepala. Kelimpahan trikom bertangkai hanya direkodkan pada Neuracanthus aculeatus. Sistolit pelbagai saiz dan kedudukan hadir dalam epidermis, parenkima dan kolenkima yang hadir bersama dengan sel minyak dalam beberapa genus. Perletakan Neuracanthus dalam tribus yang diiktirafkan dalam Acanthaceae turut dinilai menggunakan ciri anatomi.

Kata kunci: Acanthaceae; anatomi; Barlerieae; Neuracanthus; Ruellieae; Yemen

INTRODUCTION

Acanthaceae is a tropical plant family comprising of ca. 250 genera and 2,500-3,000 species world-wide (Scotland 1992; Vollesen 2008). It is characterized by its zygomorphic flowers with persistent 4-5 lobed calyx, gamopetalous corolla, cylindrical or ventricose floral

tubes, with 5 sub-equally spreading lobes or strongly 2-lipped. The four stamens are 4 didynamous or rarely 2, ovary conical or oblong-cylindrical, bicarpellate, syncarpous, with superior axile placentation and the fruits are capsules which are bivalved and 2-loculed (Clarke 1900; Lindau 1895; Wood 1997). Scotland and Vollesen (2000) and Vollesen (2008) classified the family into four subfamilies, viz. Nelsonioideae, Thunbergioideae, Ruellioideae, and Acanthoideae. The first two subfamilies are absent in Yemen and the latest recognized subfamily Ruellioideae is characterised by cystoliths in leaves, leaves and bracts are opposite, calyx 5-lobed, stamen 2-4 with 1- or 2-theca, ovules 2-28 per locule, and the retinaculae are hook-shaped.

The subfamily Ruellioideae is further divided into four tribes, namely Ruellieae, Whitfieldieae, Barlerieae, and Justicieae. The tribe Barlerieae is characterized by perennial herbs or shrubs, often spiny, stems 4-angled or subterete, leaves opposite, decussate, entire or spinose, calyx with 4-unequal segments, the outer 2 broader, longer and the inner 2 narrower and smaller. The double cystoliths are present in epidermal cells, the stamens are 2-4, with 2-thecous anthers, ovules 1-2 per locule, seeds 2-8 per fruit, with hygroscopic hairs (Ensermu et al. 2006; Hedren & Thulin 2006; Wood 1997; Wood et al. 1983). Within the tribe are 30-33 genera, and in Yemen the genus *Barleria* is represented by four sections, namely sect. Barleria, sect. Fissimura, sect. Prionitis and sect. Somalia (Al-Hakimi & Latiff 2017; Alkhulaidi 2013; Balkwill & Balkwill 1997; Darbyshire et al. 2019).

The tribe Ruellieae is characterized by plants whose habits are annual or perennial herbs with solitary stems, erect or prostrate to decumbent, glabrous to densely hairy and cystoliths are present in the leaves. The leaf margin is entire-crenate, the flowers are solitary or in pair, open at sunset then fall quickly after, corolla 5-lobed, stamen 4, sub-equal, didynamous, anthers 2, not tailed, thecae oblong-rounded; styles filiform, hairy or glabrous, capsules cylindrical to clavate; seeds 4-20 per fruit, ovate to orbicular, with dense hygroscopic white appressed hairs on the margin (Al-Hakimi & Latiff 2016; Balfour 1888; Clarke 1900; Forsskal 1775; Miller 2004; Vollesen 2008; Tripp 2007; Tripp et al. 2013; Wood 1997). *Ruellia* is generally considered to be the second largest genus in Acanthaceae after *Justicia* (Daniel 1984). However, there have been no phylogenetic studies within *Ruellia*, nor of relationships among genera comprising the tribe Ruellieae except those studies of Moylan et al. (2004), Tripp (2007) and Tripp et al. (2013).

Within Ruellieae, the genus *Dyschoriste* is distributed in the tropics and subtropics of the Americas, Africa, and Asia. It consists of perennial herbs, cystoliths present in the leaves, stem terete to angular, leaves opposite, bracts shorter than calyx, outer foliaceous, bracteoles 2, almost linear. The flowers are axillary, in cymes or spiciform terminal panicles, calyx 5-lobes, the lobes are triangular, acuminate with central vein. The corolla 5, with sub-equal lobes, 2-lipped, stamens 4 didynamous, anthers 2 thecous with spurs at the base, ellipsoid, parallel, stigma lobes minute. The fruits with 4 seeds, discoid or ovoid, covered by hygroscopic hair (Al-Hakimi & Latiff 2015; Chumchim et al. 2015; Hedrén & Thulin 2006; Vollesen 2008; Wood 1997). The genus has been treated in taxonomic and palynological treatments (Daniel 2013, 1995, 1984; Henrickson 1999; Long 1970; Vollesen 2008; Wasshausen 1998; Wasshausen & Wood 2003). Although it is one of the largest genera in Ruellieae, its anatomical, morphological and molecular phylogeny are less studied (Tripp et al. 2013).

Phaulopsis belongs to Ruellieae and is distinguished by quadrangular stems, leaves often anisophyllous, inflorescence on one side, bracts on main axis and foliaceous, the inflorescences are cymes, bracteates, calyx deeply divided into 5-segments, corolla contorted in buds, floral tube cylindrical, 2-lipped, densely hairy on lower lips; stamens 4, didynamous, inserted in throat, seeds 4 per fruit, discoid, densely covered with hygroscopic hairs (Manktelow 1996; Vollesen 2008).

Neuracanthus is one of the genera with interesting morphological and anatomical characters as the genus occurs in the arid environment. It is distinguished by its habit which is perennial herbs or subshrubs, cystoliths are present in the epidermal cells, the stems are woody and spiny. The leaves are usually crenate, sessile, undulate, and spine-tipped. The inflorescences are terminal and spicate, subtending, bracts linear, leaf-like or spinose in a few rows, imbricate, calyx 2-lipped, deeply separated with prominent 3-veins at upper part and 2-veins at lower part; corolla whitish-purple, 5-lobed, sub-equal, upper lobes shortly bifid, with short cylindrical tube, broad throat. The number of stamen is 4, didynamous inserted in the base of throat, styles glabrous with oblong lobes. Fruits unlobed and 4-seeded, seeds discoid, covered with hygroscopic hairs (Balfour 1888; Darbyshire et al. 2010; Ensermu et al. 2006; Hedrén & Thulin 2006; Miller 2004).

Although the variation in the anatomical structures could provide diagnostic and taxonomic values, only a few studies on the anatomical features of Acanthaceae are available. Some earlier descriptions were provided

by Metcalfe and Chalk (1950), who stated that many anatomical features of Acanthaceae were similar to those of a typical dicotyledonous plant. However, there are unique features in some species related to hot/dry or arid environment, especially some characters in *Justicia* and *Neuracanthus* species. One of the major characteristics in Acanthaceae is the presence of cystoliths that are visible even under magnifying lens as rod-shaped crystals, especially in the epidermal surfaces of the leaves (McDade et al. 2008).

Caitlin (2010) also referred to some epidermal characteristics such as the occurrence and significance of cystoliths in Acanthaceae that are present in laminar and petiole epidermis. Scotland and Vollesen (2000) used cystoliths as one of the taxonomic characters to divide the subfamilies in Acanthaceae. Aoyama and Indriunas (2012) studied the leaf anatomy of *Justicia brandegeana,* and Al-Hakimi and Olawale (2013) studied some anatomical characters in *Anisotes trisulcus.* However, there is no detail or specific study on the anatomy of genera in Acanthaceae except those of Ahmad (1978) and Singh and Jain (1975) focusing on the type of trichomes in Acanthaceae.

The morphology and anatomy of the Acanthaceae from Yemen have not been studied previously especially in tribes Barlerieae and Ruellieae. Therefore, the present study aims to investigate the anatomical features of selected species of Ruellieae and Barlerieae including *Neuracanthus* to determine the various types of trichomes and their distribution on the leaf parts and evaluate the usefulness of these characters for systematic purposes.

Materials and Methods

Plant materials of 18 species belonging to Acanthaceae family were collected as fresh samples from different locations in Yemen (Table 1). The plant materials were fixed in the mixture of 95% alcohol and glacial acetic acid (3:1). The middle part of stems, petioles, midribs, lamina and margins were embedded in polystyrene and sectioned transversely on a sliding microtome (Leica Jung Histolide 200).

Transverse sections were made at 20-30 µm thick, depending on the texture of the specimens. Sections were pre-soaked in distilled water with a few drops of NaHCl (Clorox) for 5 min to clear the tissues. The sections were rinsed with distilled water 2-3 times and steeped in Safranin solution for approximately 5 min, rinsed with water, then stained for approximately 5-10 min in Alcian green (or Alcian blue). They were dehydrated in a series of alcohol concentrations starting from 50, 70, 95 to 100% (ca. 2 min each), 1-2 drops of concentrated HCl were added to the 70% treatment to change the colour of the leaves to purplish.

Finally, the samples were mounted on microscope slides in Euparal or Canada balsam as permanent medium, carefully covered with slide covers, and then kept in drying oven at 60 °C for a week. Epidermal peels were prepared by mechanical scraping. The samples were cut approximately 1 cm^2, and then the small parts were soaked in Jeffrey solution (10% nitric acid + 10 chromic acid; 1:1) at room temperature for 1-3 h, sometimes for a day. The solution was diluted with distilled water, until the mesophyll tissue could be separated easily from both epidermises. The samples were stained, dehydrated and mounted in the same way, and observed and viewed with light microscope (Olympus CH_20). Images were captured using a Leitz Diaplan microscope fitted with a video camera connected to a computer using analysis Docu software. Leaf specimens examined with the SEM were washed with phosphate buffer solution (PBS) three times and dehydrated through a series of acetone, and then critical-point dried, coated with gold and observed under FESEM, ZEISS Super A, 55VP with various magnifications (500–10,000×). Voucher specimens are kept at herbarium Universiti Kebangsaan Malaysia (UKMB).

Results and Discussion

Anatomical characters of 18 species, namely *Barleria acanthoides*, *B. aculeata*, *B. orbicularis*, *B. parviflora*, *B. ventricosa*, *B. proxima*, *B. prionitis*, *B. tetracantha,* and *B. bispinosa* belonging to Barlerieae tribe, *Ruellia patula*, *R. prostrata*, *R. grandiflora*, *R. paulayana*, *R. dioscoridis*, *R. insignis*, *Phaulopsis imbricata,* and *Dyschoriste nagchana* belonging to Ruellieae tribe in addition to *Neuracanthus aculeatus* were recorded (Table 1). Results showed that many characters can be used to differentiate the tribes and genera such as the outline of stems, midribs, petioles and margin, shape of anticlinal walls, layers of collenchyma and parenchyma cells in stems and petioles as well as vascular tissue in the petioles and midribs, and type of trichomes. In addition, the type and position of cystoliths were also investigated and analysed in current study.

TABLE 1. List of species and samples studied

No.	Species	Collection
1	*Barleria acanthoides*	Yemen, Taiz, Wadi Sala, 13.57°N, 44.04°E, 6 September 2010, Wahab A 391 (UKMB); Habshi Mountain, 13.49°N, 43.95°E, 17 September 2010, Anisa S 40 (UKMB); Hajda,13.58°N, 43.81°E, 18 November 2010, Wahab A 412 (UKMB).
2	*B. aculeata*	Yemen, Taiz, Wadi Daneghan, 7 km SEast of Hadibo, 6 March 1989, Miller 8643 (K); 4 km SEast of Hadiboh, 16 January 2011, Wahab A 432 (UKMB).
3	*B. bispinosa*	Yemen, Taiz, Habshi Mountain, 13.49°N, 43.95°E, 17 September 2012, Wahab A 476 (UKMB); Alhashma, 13.60°N, 43.99°E, 21 November 2012, Anisa S 79 (UKMB); Wadi Sala, 13.57°N, 55.04°E 6 September 2012, Anisa S 71(UKMB); Warazan, 13.41°N, E 44.24°E, 29 October2012, Anisa S 75 (UKMB).
4	*B. orbicularis*	Yemen, Taiz, Hajda, 13.58°N,43.81°E, 18 November 2011, Anisa S 62 (UKMB).
5	*B. parviflora*	Yemen, Taiz, Wadi Sala, 13.57°N, 44.04° E, 7 September 2010, Wahab A 393 (UKMB); Habshi Mountain, 13.49° N, 43.95°E, 19 September 2010, Anisa S 42 (UKMB); Warazan, N 13.41°, E44. 24°, 28 October 2012, Wahab A 479 (UKMB).
6	*B. prionitis*	Yemen, Taiz: Salah, Adanan Road, 28 June 1983, Gordon 122 (E); Wadi Sala, 13.57°N, 44.04°E. 6 September 2012, Wahab A 472 (UKMB); Jara Mountain, 13.59°N, 44.009°E, 28 August 2012, Anisa S 70 (UKMB); Warazan,13.41°E, 44.24°E, 28 October 2012, Anisa S 74 (UKMB).
7	*B. proxima*	Yemen, Taiz, Habeel Salman, near Taiz University, 13.56°N, 43.98°E, 3 November 2010, Anisa S 44 (UKMB); Habshi Mountain, 13.4976°N, E43.95°E, 17 September 2012, Wahab A 475(UKMB).
8	*B. tetracantha*	Yemen, Socotra, Dihshal, 20 km, SEast of Hadibo, 1 March 1989, Miller 8546 (K); Shauab, 13 January 2011, Wahab A 429 (UKMB).
9	*B. ventricosa*	Yemen, Taiz, Abadan, 13.51°N, 44.07°E, 30 November 2010, Wahab A 419, (UKMB); Sanaa, Shahara, 22 September 1978, Miller 188 (K).
10	*Dyschoriste nagchana*	Yemen, Taiz, Alsyani, 13.83◦ N, 43.18◦ E, 14 November 2011, Wahab A 468 (UKMB).
11	*Neuracanthus aculeatus*	Yemen, Socotra, Jebel Derafonte, 4 April 1967, Smith & Lavranose N 238 (K); Jebel Derafonte, 13 January2011, Anisa S 55 (UKMB).
12	*Phaulopsis imbricata*	Yemen, Taiz, Warazan, 28 October 2011, Wahab A 460 (UKMB); Gara mountain, 17 December 2010, Anisa S 53 (UKMB); Alsyani, 13.828◦ N, 43.178◦ E, 14 November 2011, Wahab A 467 (UKMB).
13	*Ruellia dioscoridis*	Yemen, Soqotra, Hadebu, 19 January 2011, Wahab A 434 (UKMB): Ridged plateau, 21 February 1989, Miller 8323 (K).
14	*R. grandiflora*	Yemen, Taiz, Salah, 16 November 2010, Anisa S 50 (UKMB); Gara mountain, 14 August 2010, Wahab A 377 (UKMB); Alhashma, 21 November 2010, Wahab A410 (UKMB); Alhabeel, 1 December 2010, Wahab A 422 (UKMB).
15	*R. insignis*	Yemen, Soqotra, Hadebu, Almashtal, 22 January 2011, Wahab A 436 (UKMB); Jebel Rughid, 8 February 1990, Miller 10349 (K).
16	*R. patula*	Yemen, Taiz, Agricultural Research and Extension Authority, 6 November 2010, Anisa S 45 (UKMB); Salah, 15 November 2010, Anisa S 49 (UKMB).
17	*R. paulayana*	Yemen, Socotra, N. Hadiboh, Dhemalu, 19 April 1985, Smith & Lavranos 429 (K); Hadiboh, near coastal and sandy hill, 15 January 2011, Wahab A 431 (UKMB)
18	*R. prostrata*	Yemenaiz, Agricultural Research and Extension Authority, 6 November 2010, Anisa S 46 (UKMB); Salah, 15 November 2010, Anisa S 48 (UKMB); Al-Selw mountain, 15 August 2010 Wahab A 380 (UKMB).

TRANSVERSE SECTION OF STEMS

Both Barlerieae and Ruellieae tribes showed variations in stem outline. It is squarish in Barlerieae whereas it varies from subcircular to squarish with 2-grooved or squarish with multi-grooved in Ruellieae. Within Ruellieae the stem outline observed is subcircular in *R. insignis,* subcircular with multi-grooves in *R. dioscoridis,* squarish with 2-grooved in *R. grandiflora*, *R. patula,* and *R. prostrata,* squarish with 2-grooved and prominently angular in *D. nagchana* and *P. imbricata,* and circular shape in *N. aculeatus* (Figure 1, Table 2). There are two types of trichomes on epidermal cells, namely e-glandular trichome which has unicellular, bicellular, multicellular and multiradiate trichomes provided with 3-4 basal cells, and glandular trichome which is sessile and capitate. Most of the trichomes are covered with striated or echinate cuticles and the others have smooth cuticle.

The layer of parenchyma cells varies from 6 to 10 layers in Barlerieae whereas 5-15 layers in Ruellieae (Table 2). The layer of collenchyma also varies from 3 to 7 in both tribes Barlerieae and Ruellieae. Epidermis consists of one layer of cells in most of the species of Barlerieae and Ruellieae except in *R. insignis* that has 2-3 layers of hypodermis. Cystoliths are distributed in the cells of epidermis in most species of Barlerieae and Ruellieae. Moreover, the presence of cystoliths in collenchyma cells in *R. patula, R. grandiflora,* and *R. paulayana* and in the pith of *D. nagchana* and *P. imbricata* is a significant character in Ruellieae tribe (Figure 1 & Table 2).

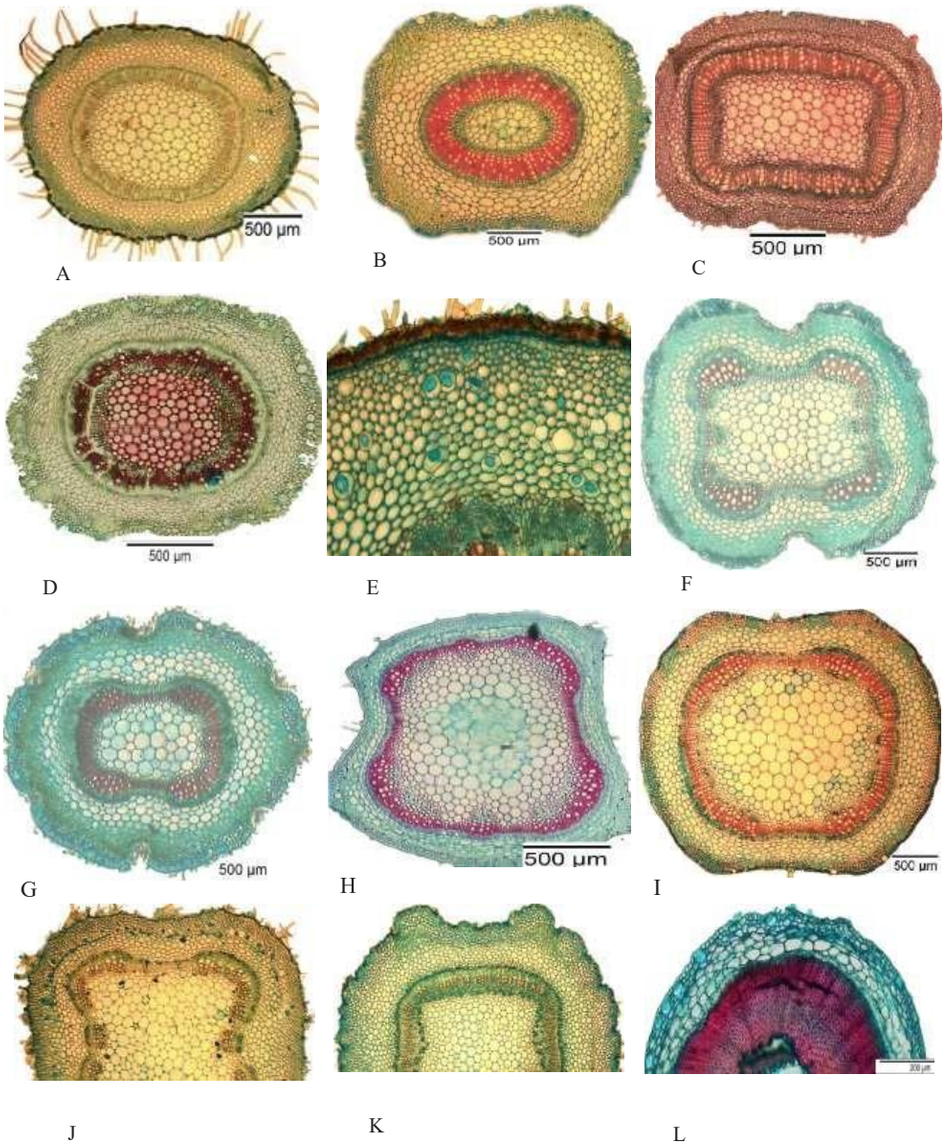

FIGURE 1. Anatomical characters of stems. A: Subcircular outline in *Barleria acanthoides,* B: Uneven squarish outline in *B. prionitis*, C: Squarish outline in *B. parviflora* and *B. bispinosa,* D: Squarish shape in *B. aculeata,* E: Part of stem in *Ruellia insignis,* F: Uneven squarish outline in *R. patula,* G: Subcircular outline in *R. dioscoridis.* H: Uneven squarish outline in *Phaulopsis imbricata*, I: Uneven squarish outline in *Dyschoriste nagchana.* J: Part of stem of *R. grandiflora,* K: Part of stem of *Barleria proxima,* and L: Part of circular stem of *Neuracanthus aculeatus*

TABLE 2. Anatomical characters of stems in Barlerieae and Ruellieae

Character species	Outline type	Collenchyma	Parenchyma	Type, shape of vascular bundle	Cystoliths Size/ position	Trichomes
Barleria acanthoides	subcircular	7	10	closed, squarish	51 µm / epidermis	+
B. aculeata	uneven squarish	4	10	closed, squarish	92 µm / epidermis and collenchyma	+
B. bispinosa	squarish	6	7	closed, squarish	89 µm / epidermis	+
B. orbicularis	squarish	4	7	closed, squarish	60 µm /epidermis	+
B. parviflora	squarish	4	7	closed, squarish	90 µm /epidermis	+
B. prionitis	squarish	5	7–8	closed, squarish	30 µm / epidermis	+
B. proxima	squarish, 2 deep curved	3	6	closed, rounded	60 µm / epidermis	+
B. tetracantha	squarish, 2deep curved	4	7–8	closed, squarish	50 µm / epidermis	+
B. ventricosa	squarish, 2 curved	4	7	closed, squarish	26 µm / epidermis	+
Dyschoriste nagchana	squarish, 4 prominent angles	7	8	closed, squarish	33 µm / parenchyma	+
Neuracanthus aculeatus	circular	4	5	closed, circular	16–28 µm / epidermis	+
Phaulopsis imbricata	squarish, 4 prominent angles	5	5	closed, squarish	87 µm /epidermis	+
Ruellia patula	squarish with 2-deep-grooved	5	5	closed, squarish 4 projection	41–99 µm / epidermis & parenchyma	+
Ruellia prostrata	squarish and 2-grooved	5	5	closed, squarish, 4 projection	18–26 µm / parenchyma	+
R. grandiflora	squarish with 2-deep-grooved	7	11	closed, squarish, 4 projection	18–43 µm / parenchyma	+
R. dioscoridis	irregular square, with-grooved	3	10	close, squarish 4 projection	62 µm / parenchyma	+
R. insignis	subcircular	3	15	close, squarish	42 µm / parenchyma & collenchyma	+
R. paulayana	squarish, 2-grooved	6	12	close, squarish 4 projection	65 µm / parenchyma	+

TRANSVERSE SECTION OF PETIOLES AND MIDRIBS

Inamdar et al. (1990) studied the cystoliths of Acanthaceae and Scotland and Vollesen (2000) used the presence of cystoliths to differentiate between the two subfamilies of Acanthaceae: subfamily Ruellioideae has different shape and size of cystoliths whereas subfamily Acanthoideae lacks cystoliths. Recently, Choopan and Grote (2015) studied the cystoliths in the leaves of *Pseuderanthemum* (Acanthaceae) in Thailand, and they found that all cystoliths are found in the epidermis, both lithocysts and crystals ranging from 65 to 300 µm in length. Current study found that the size of cystoliths varies even within the same species. Small size of cystoliths (27 µm) were observed in *B. acanthoides* and large size (172 µm) of cystoliths were observed in *R. insignis*. Type of cystoliths differs from solitary type in

Ruellieae tribe and *Neuracanthus* to double type in most species of Barlerieae.

The occurrence, type, and location of calcium oxalate crystals in the leaves were first studied by Genua and Hillson (1985). In this study, calcium oxalate crystals are observed to be secreted in epidermis or parenchyma tissue with different size occurring as a common character in most of the species of *Ruellia* and *Barleria*. They are mostly elongated, narrow or broad, pointed or blunt at one or both ends. Oil cells were also recorded in some species of Barlerieae and Ruellieae.

Petiole adaxial surface with concave to convex in outline is present in Barlerieae except *B. acanthoides* that showed straight adaxial surface whereas all the genera within Ruellieae showed the presence of uneven shape adaxial surface. Adaxial surface in midrib also varies from humped, convex, and concave in Barlerieae and Ruellieae except *B. aculeata* that has straight shape (Tables 3 & 4). Both the rhaphid and aerenchyma cells are present in Ruellieae, rhaphides are present in the petiole of *P. imbricata* and aerenchyma are present as ground tissue of petiole in *R. patula* and *R. prostrata* (Figure 2(g), 2(h) & Table 3).

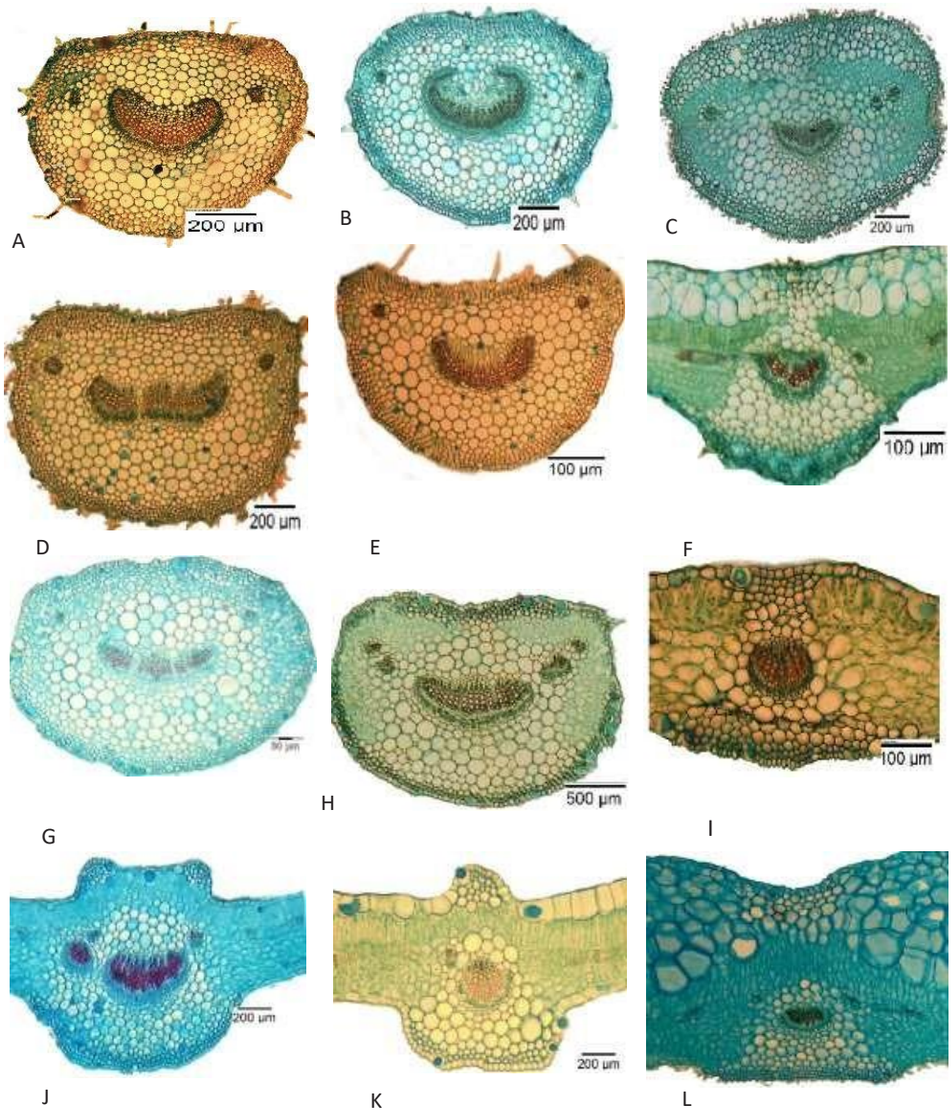

FIGURE 2. Anatomical characters of petioles and midribs. A: Petiole with straight adaxial surface in *Barleria acanthoides,* B: Curved ends of vascular bundles of petiole in *B. ventricosa,* C: Straight adaxial surface in petioles of *Ruellia paulayana*, D: Petiole of *R. grandiflora*, E: Petiole of *Dyschoriste nagchana*, F-L: TS of midrib, F: Slightly straight adaxial surface in *Barleria,* G: Irregular adaxial adaxial surface and aerenchyma cells in ground tissue of *Ruellia prostrata*, H: Aerenchyma cells in ground tissue of *Ruellia patula*, I: slightly straight adaxial surface in *Barleria aculeata,* J: Concave adaxial surface in *Ruellia prostrata,* K: Midrib of *Ruellia patula*, and L: Slightly concave adaxial surface in *Ruellia paulayana*

The vascular tissue present is of an open type, arc-shaped in most species of Ruellieae and Barlerieae whereas U-shaped is present only in *B. bispinosa,* and as an extending ends of vascular bundle in *B. ventricosa* (Table 3 & Figure 2(b)). The vascular bundle has 5-18 rows of xylems except in *P. imbricata* and *R. grandiflora* which have more than 20 rows of xylem (Figure 2). The adaxial surface of midribs varies in Barlerieae, straight in *B. aculeata*, concave-shaped in *B. bispinosa,* convex in *B. proxima* and *B. tetracantha,* humped in the rest of species such as *B. acanthoides, B. parviflora, B. ventricosa, B. orbicularis,* and *B. prionitis.* In *Ruellia,* the adaxial surface of midribs is concave in *R. grandiflora* and *R. insignis*, convex-shaped in *R. dioscoridis* and *R. paulayana,* and it is humped in *R. patula* and *R. prostrata*, as well as in *D. nagchana, N. aculeatus,* and *P. imbricata* (Table 3).

TABLE 3. Anatomical characters of petioles in Barlerieae and Ruellieae. V.B.=Vascular Bundle, = + present, and - = absent

Species / Characters	Adaxial	Parenchyma no.	Collenchyma no.	V.B. type	V.B. shape	Cystoliths	Oil cells	Trichomes
Barleria acanthoides	straight	19	4	open	arch-shape	+ (27µm)	+	+
B. aculeata	convex	19	3–5	open	arch-shape	+ (31µm)	+	+
B. bispinosa	concave	11	2–4	open	U-shape	+ (46µm)	-	+
B. orbicularis	convex	12	3	open	arch-shape	+ (28µm)	-	+
B. parviflora	convex	12	3	open	arch-shape	+ (49µm)	-	+
B. proxima	convex	16	3–5	open	arch- shape	+ (42µm)	+	+
B. prionitis	convex	15	2–4	open	arch-shape	+ (32µm)	+	+
B. tetracantha	convex	11	2	open	arch-shape	+ (36µm)	-	+
B. ventricosa	concave	11	4	open	arch-shape + extended end	+ (25µm)	+	+
Dyschoriste nagachana	uneven	17	3	open	arch-shape	+ (33µm)	-	+
Neuracanthus aculeatus	straight	14	3	open	arch-shape	+ (72µm)	-	+
Phaulopsis imbricata	uneven	20	4–5	open	arch-shape	+ (112µm)	+	+
Ruellia patula	uneven	17	3+ aerenchyma	open	arch-shape	+ (77µm)	+	+
R. prostrata	uneven	18	3+ aerenchyma	open	arch-shape	+ (37µm)	+	+
R. grandiflora	uneven	17	5	open	arch-shape	+ (31µm)	+	+
R. dioscoridis	uneven	8	3	open	arch-shape	+ (62µm)	-	+
R. insignis	uneven	15	3	open	arch-shape	+ (172µm)	-	+
R. paulayana	uneven	12	5	open	arch-shape	+ (73µm)	-	+

TABLE 4. Anatomical characters of midribs in Ruellieae and Barlerieae species

Characters / Species	Adaxial wall	Abaxial wall	Parenchyma in tissue	Collenchyma	Vascular bundle shape	Cystoliths	Oil cells
Barleria acanthoides	humped	rounded	12	4	arch-shape	+	+
B. aculeata	straight	rounded	18	4	arch-shape	+	+
B. bispinosa	concave	uneven rounded	10	4	U shape	+	+
B. parviflora	humped	rounded	8	4	arch-shape	+	+
B. orbicularis	humped	rounded	10	4	arch-shape	+	-
B. ventricosa	humped	rounded	10	4	arch-shape with extended ends	+	-
B. proxima	convex	rounded	13	4	arch-shape	+	+
B. prionitis	humped	rounded	15	4	arch-shape	+	
B. tetracantha	convex	rounded	10	4	arch-shape	+	-
Dyschoriste nagchana	humped	rounded	9	3	arch-shape	+	
Neuracanthus aculeatus	humped	rounded	3	11	arch-shape	+	-
Phaulopsis imbricata	humped	rounded	2	16	arch-shape	+	-
Ruellia patula	humped	rounded	3	8	arch shape	+	-
R. prostrata	humped	rounded	3	12	arch shape	+	-
R. grandiflora	concave	rounded	4	12	arch shape	+	-
R. dioscoridis	convex	rounded	3	10	arch shape	+	+
R. insignis	concave	rounded	4	12	arch-shape	+	
R. paulayana	convex	rounded	4	12	arch shape	+	+

TRANSVERSE SECTION OF LAMINA AND MARGIN

The leaf is one of the most important vegetative organs which anatomy can be successfully exploited for systematic purposes (Cutler et al. 2008). In this study, the lamina, anticlinal walls, midribs, and margins were found to be useful in taxonomy. There are a few studies on the anatomy of Acanthaceae except *Anisotes trisulcus* (Al-Hakimi & Olawale 2013; Al-Rahaily 2000; Balkwill & Norris 1988). Recently, Amri et al. (2018) described the leaf characters of selected Acanthaceae species and recorded the presence of cystoliths and hypodermal cells, and they explained that the presence of hypodermal layers in some species of Acanthaceae is related with their environmental factors.

Metcalfe and Chalk (1950) stated that stomata are diacytic and confined to the lower epidermal surface of the leaf. In the current study, however, both the upper and lower epidermis cells showed the presence of diacytic stomata (Figure 3(l)), disagreeing with Metcalfe and Chalk (1950). This study concentrated on anatomical characters in lamina and margin that may be useful in the identification of some genera within Barlerieae and Ruellieae tribes. The abundance of cystoliths was observed in most species of Ruellieae and Barlerieae. Double cystoliths can be a useful character in *Barleria* species differentiating it from solitary, elongated, elliptical or rounded cystoliths as observed in *Ruellia*, *D. nagchana*, *P. imbricata,* and *N. aculeatus* (Figure 3(d), 3(e), 3(f), 3(i), 3(k)).

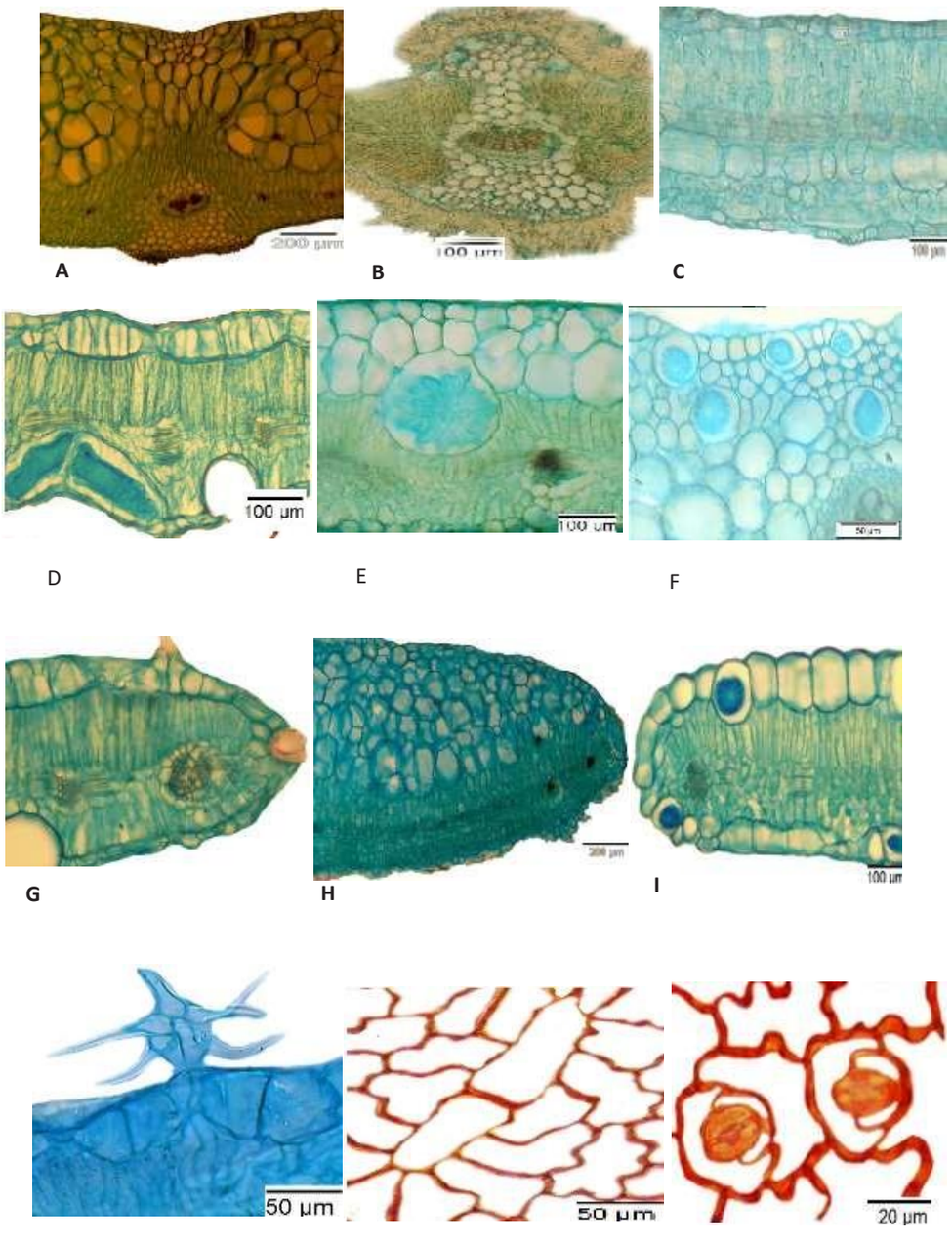

FIGURE 3. Anatomical characters of leaves under LM. A: 3-4 layers of hypodermis, cystoliths cells in the midribs of *R. paulayana*, B: Concave adaxial surface covered by dense trichomes in *Neuracanthus aculeatus,* C: Lamina of *Dyschoriste nagchana,* D: Double cystoliths in *Barleria acanthoides*, E: Layer of epidermis and irregular cystoliths in *Barleria aculeata,* F: Different size of cystoliths in the lamina of *B. bispinosa*, G: Leaf margin in *Barleria acanthoides*, H: Margin of *R. paulayana*, I: straight rounded margin in *R. patula*, J: 6-10 armed multiradiate trichomes in *B. grandiflora*, K: double cystoliths in *Barleria* under LM, and L: shape of stomata under LM

Cystoliths are arranged randomly in the petioles, midribs and lamina of Barlerieae and Ruellieae but parallel in midribs and lamina of *N. aculeatus*. Figure 3 shows one layer of epidermis of lamina in most of the *Ruellia* species except *R. paulayana, R. dioscoridis* and *B. aculeatus* with additional cells of hypodermis that could be a good character to differentiate them from those species with 2-5 layers of hypodermis arranged under epidermis cells (Figure 3(a), 3(e), 3(h) & Table 5). *Barleria aculeata, R. paulayana,* and *R. dioscoridis* have somewhat different anatomical characters that may be implied by the ecological adaptation of these species occurring on the arid Socotra Island, an off-shore island far from Yemen mainland. Additional character observed in *R. insignis* is the leaf surface covered by layers of wax (Figure 4(k), 4(l)). The anticlinal walls are straight to sinuous in most genera of Barlerieae and Ruellieae except *R. grandiflora* which has wavy anticlinal walls (Table 5). Singh and Jain (1975) described and illustrated the types of trichomes of Acanthaceae. In current study, the

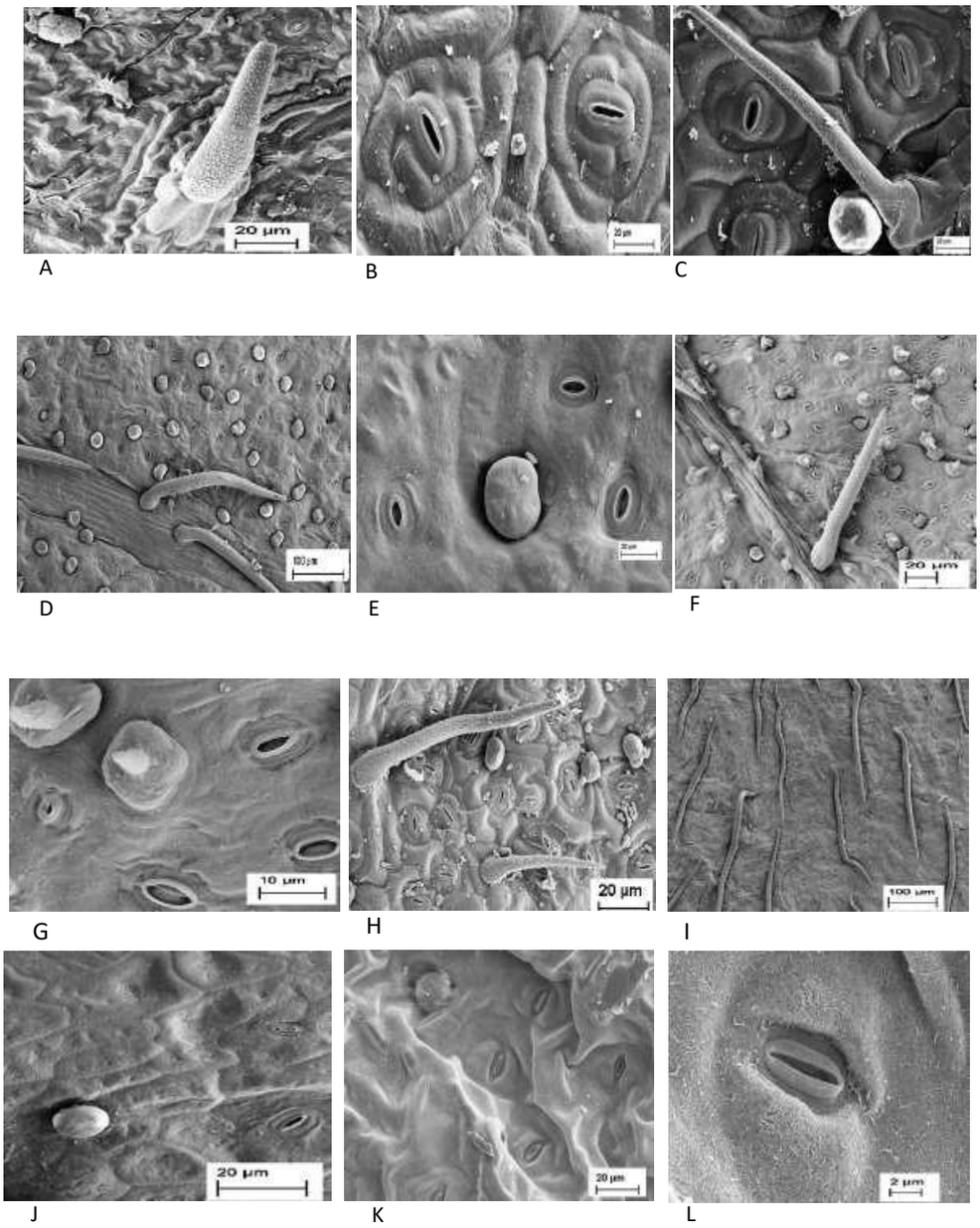

FIGURE 4. Anatomical characters of leaves under SEM.. A: Trichomes *in Dyschoriste nagchana,* B: Stomata and anticlinal walls in *Barleria acanthoides*, C: Trichomes in *B. acanthoides,* D&E: Stomata and glandular trichomes in *B. prionites,* F&G: Eglandular trichome, glandular trichomes and stomata in *B. aculeata*, H: Dense stomata and trichomes in *B. bispinosa*, I: Eglandular trichomes in *B. orbicularis,* J: Anticlinal walls and trichomes in *B. parviflora*, and K&L: Stomata and wax covered the surface of *B. tetracantha*

epidermal cells have two types of trichomes - glandular and e-glandular trichomes. The glandular trichomes are sessile, or with very short stalks and capitate. The e-glandular trichomes are represented by unicellular, bicellular or multicellular and multiradiate trichomes. Within Barlerieae, unicellular and bicellular trichomes with conical shape and capitate were observed in all the *Barleria* species except *B. aculeata* which has trichomes supported by smooth and squarish cellular basal cells (Figure 4(g)).

Within Ruellieae, sessile glandular trichomes were observed in all the species studied except *R. insignis* which lacks of glandular trichomes. Results showed that *R. grandiflora* can be identified based on the type of trichomes with 6-10 armed multiradiate trichomes of 346 µm long (Figure 5(g), 5(h)). Multicellular trichomes are also found in *P. imbricata* and *D. nagchana*. In addition to that, *R. paulayana* and *R. dioscoridis* are distinguished by the abundance of uniseriate bicellular trichomes, and

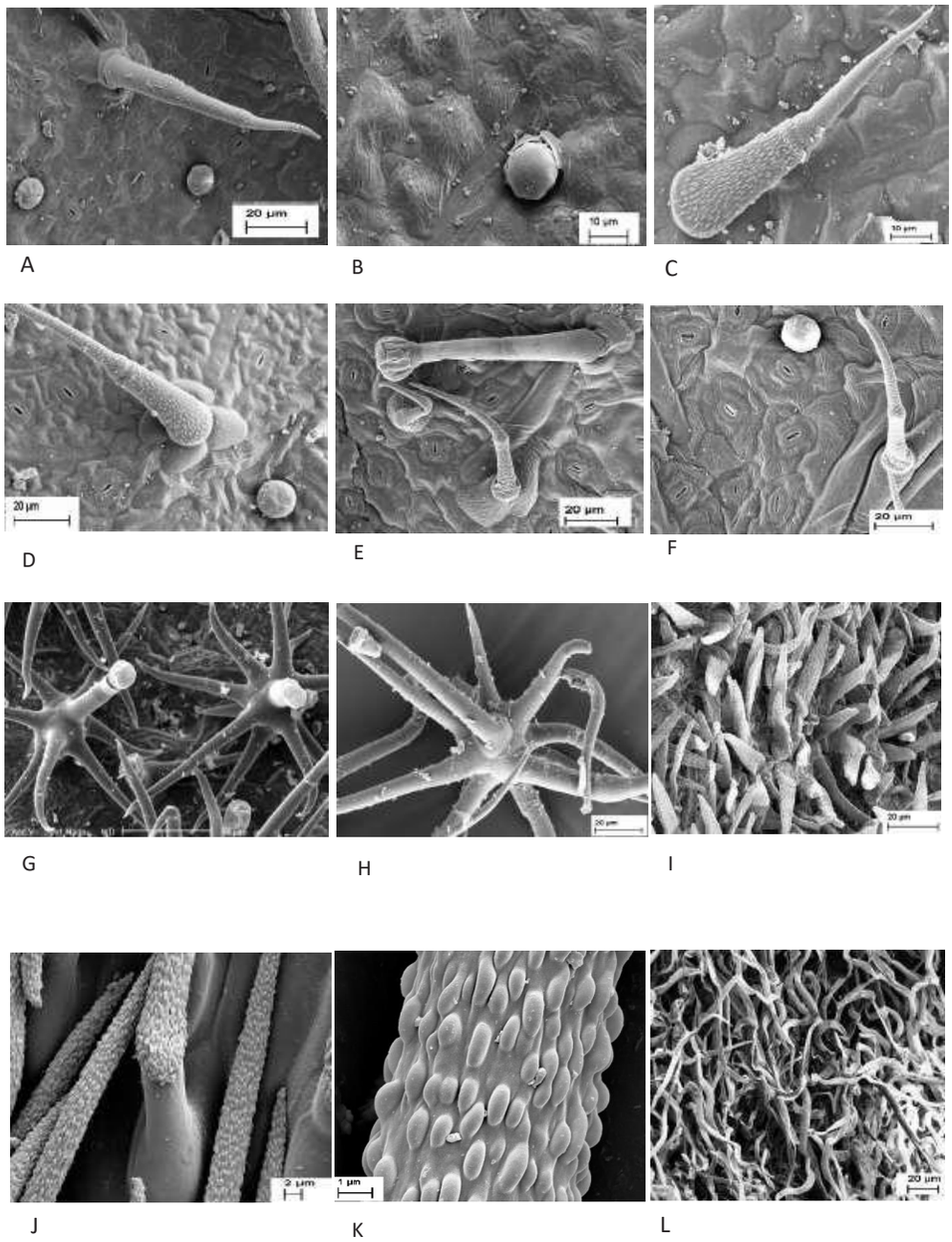

FIGURE 5. Anatomical characters of leaves under SEM. A&B: Anticlinal walls and trichomes in *Phaulopsis imbricata*, C: Anticlinal walls and trichomes in *Ruellia prostrata*, D: Glandular and base of non-glandular trichomes in *Ruellia prostrata,* E-F: Different trichome types in *R. patula,* G&H: Multiradiate trichomes in *R. grandiflora*, I: E-glandular trichomes in *R. insignis*, J&K: Trichomes and upper cells covered by echinate ornamentation in *R. paulayana,* and L: Long and dense trichomes in *Neuracanthus*

the lower cells of trichomes are smooth and upper cells are covered by echinate ornamentation (Figure 5(j), 5(k)). On the other hand, the whole trichomes are covered by echinate ornamentation in the rest of species (Figure 5(i)). Capitate glandular trichomes were only observed in *R. patula, R. grandiflora* and *B. bispinosa* (Figure 5(e), 5(f)).

The tomentose indumentum, long unicellular trichomes were observed only on the midribs, lamina and margin of *N. aculeatus*. The size of trichomes varies from 30-90 µm in *B. aculeata, B. proxima*, and *B. tetracantha*, 683 µm in *B. orbicularis* to 1.13 mm in *B. parviflora*. All the Ruellia species, *D. nagchana* and *P. imbricata* have trichome size of 57-304 µm whereas *N. aculeatus* has very long trichomes which cover the whole surfaces and the exact measurement was hard to take due to the matted nature of the tomentose trichomes (Figure 5(l) & Table 6).

Our anatomical study on *N. aculeatus* seem to be in agreement with the molecular phylogenetic results of McDade et al. (2008) who showed that the genus *Neuracanthus* is strongly monophyly, and they proposed the placement of this genus as sister to Barlerieae, Andrographideae and Whitfieldieae as it was weakly supported by both Bayesian and parsimony analyses. The current anatomical study showed that *N. aculeatus* is different from both Barlerieae and Ruellieae tribes, and it may be placed in its own tribe - Neuracanthieae.

TABLE 5. Lamina and margin characters and stomata types

Characters/ Species	Epidermis	Palisade layers	Spongy layers	Cystoliths	Non-glandular trichomes Shape/Size	glandular trichome	Margin shape	Stomata type	Anticlinal wall
Barleria-acanthoides	1	1	5	+	Unicellular conical filiform (191 µm)	+	uneven rounded	diacytic	straight
B. aculeata	2–3, hypodermis	1	3	+	Unicellular (23 µm) and conical filiform (327 µm)	+	rounded	diacytic	straight
B. bispinosa	1	1	3	+	Unicellular conical filiform (278 µm)	+ capitate	rounded	diacytic	straight
B. ventricosa	1	1	3	+	Unicellular conical (474 µm)	+	rounded	diacytic	sinuous
B. orbicularis	1	1	3	+	Unicellular conical filiform (683 µm)	+	rounded	diacytic	sinuous
B. parviflora	1	1	3	+	Unicellular conical filiform (1.13 mm)	+	rounded, downward	diacytic	sinuous
B. proxima	1	1	3	+	Unicellular (90 µm)	+	rounded	diacytic	straight
B. prionitis	1	1	3	+	Unicellular conical filiform(317 µm)	+	rounded	diacytic	straight
B. tetracantha	1	1	4-5	+	Unicellular conical filiform (32 µm)	+	rounded	diacytic	straight

Dyschoriste nagchana	1	1	5	+	Uniseriate bicelled (152 µm) and multicellular	+	rounded	diacytic	sinuous
Neuracanthus aculeatus	1	1	4	+	Uniseriate,	-	rounded	diacytic	straight
Phaulopsis imbricata	1	1	4	+	multicellular (304 µm)	+	rounded	diacytic	sinuous
Ruellia patula	1	1	4	+	Unicellular conical filiform (84 µm) and (3 bases)	+ capitate	rounded	diacytic	straight
R. prostrata	1	1	3-4	+	Uniseriate bicelled (151 µm) (4 bases)	+	rounded	diacytic	sinuous
R. grandiflora	1	1	4	+	6-10 armed multiradiate (244 µm)	+ capitate	rounded	diacytic	wavy
R. dioscoridis	2 hypodermis	1	4	+	Uniseriate bicelled (239 µm)	+	rounded	diacytic	straight
R. insignis	1	1	4	+	bicelled and unicellular conical (57 µm)	-	rounded	diacytic	straight
R. paulayana	2-3 hypodermis	2	4	+	Uniseriate bicelled (206 µm)	+	rounded	diacytic	straight

TABLE 6. Anatomical characters in Ruellieae and Barlerieae genera

Characters / Species	Stem Outline	Adaxial surface of petiole	E-glandular Trichomes	Echinate trichome	E-glandular trichomes (size)	Glandular type
Barleria	Squarish and subcircular (*B. acanthoides*)	Straight and convex and concave	Unicellular, circular base and square base showed in *B. aculeata*	Present	32 µm-1.13 mm	Sessile, glandular and capitate, glandular in *B. bispinosa*
Dyschoriste nagchana	Squarish with 4 prominent angles	uneven	multicellular	Present	152 µm	Sessile, glandular
Neuracanthus aculeatus	Circular	straight	tomentose indumentum unicellular trichomes	Absent	Long and hard to measure	Absent
Phaulopsis imbricata	Squarish with 4 prominent angles	uneven	multicellular	Present	304 µm	Sessile, glandular
Ruellia	Squarish with 2-deep grooved	uneven	6-10 armed multiradiate and Unicellular conical and bicelled with 4 bases, 3 bases or circular shape	Present except in *R. grandiflora*	57-244 µm	Sessile, glandular and capitate, glandular in *R. grandiflora* and *R. patula*

Conclusion

The results of this study showed that a combination of anatomical characters can be used to differentiate the species and genera within Barlerieae and Ruellieae tribes. Although descriptions of the morphology of Acanthaceae species from Yemen are available, information on anatomical characters of the genera and species is still lacking. This study has attempted to evaluate the taxonomic value and significance of the stem and leaf anatomy in 18 species of Barlerieae and Ruellieae in addition to one *Neuracanthus* species as a part of wider studies of the family Acanthaceae in Yemen. The variation in stem outline, adaxial surface of the petioles and midribs in addition to anticlinal walls of lamina among genera were also very useful for the identification of the genera and provide much useful information for the reassessment of taxonomic relationships among species and genera of Ruellieae and Barlerieae. Different anatomical characters were investigated such as double cystoliths that are observed in all the species of Barlerieae. Trichomes are present in different sizes, types and structure from unicellular, bicellular, multicellular, multiradiate, capitate, and sessile glandular trichomes. This study has showed that the genus *Neuracanthus* does not share similar leaf anatomical characteristics with the other taxa in Barlerieae and Ruellieae tribes. Some leaf anatomical characters may be used to distinguish *Neuracanthus* species from the other species in the Barlerieae and Ruellieae tribes, such as circular outline stems, dense long trichomes covering the whole leaf surface, the presence of cystoliths on the along of adaxial and abaxial of lamina. Thus, the placement of *Neuracanthus* into the Barlerieae has not been supported but probably may be placed on its own tribe Neuracanthieae.

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References

Ahmad, K.J. 1978. Epidermal hairs of Acanthaceae. *Blumea: Biodiversity, Evolution and Biogeography of Plants* 24(1): 101-117.

Al-Hakimi, A.S. & Latiff. A. 2017. A revision of *Barleria* L. (Acanthaceae: Ruellioideae: Barlerieae) of Yemen. *The Malayan Nature Journal* 69(3): 259-272.

Al-Hakimi, A.S. & Latiff, A. 2016. A revision of *Ruellia* L. (Ruellioideae: Acanthaceae) of Yemen. *The Malayan Nature Journal* 68(3): 53-61.

Al-Hakimi, A.S. & Latiff, A. 2015. Pollen and seed morphology of *Ruellia* L., *Phaulopsis* Willd. and *Dyschoriste* Nees (Acanthaceae: Ruellioideae: Ruellieae) of Yemen. *Plant Systematics and Evolution* 301(1): 1-13.

Al-Hakimi, A.S. & Olawale, F.A. 2013. Morphological, palynological and anatomical descriptions of a medicinal shrub. *Anisotes trisulcus* L. *Malaysian Journal of Microscopy* 9(1): 184-186.

Alkhulaidi, A.A. 2013. *Flora of Yemen*. The Sustainable Natural Resource Management Project (SBRMP II) EPA and UNDP.1-5. Republic of Yemen.

Al-Rahaily, A.J. 2000. Pharmacognostic studies on the leaf of *Anisotes trisulcus* (Forssk.) Nees. *Pakistan Journal of Biological Science* 3(9): 1427-1430.

Amri, N.A., Tajudin, N.S., Shahari, R., Azmi, F.M., Talip, N. & Latiff, A. 2018. Comparative leaf anatomy of selected medicinal plants in Acanthaceae. *International Medical Journal Malaysia* 17(2): 17-23.

Aoyama, E.M. & Indriunas, A. 2012. Leaf anatomy of *Justicia brandegeana* Wassh. & L.B. Sm. (Acanthaceae). *Communications in Plant Sciences* 2(3-4): 37-39.

Balfour, I.B. 1888. Botany of Socotra. *Transactions of the Royal Society of Edinburgh* 31: 1-446.

Balkwill, K. & Norris, F.G. 1988. Classification of the Acanthaceae. A southern African perspective. *Monographs Systematic Botany Missouri Botanical Garden* 25: 503-516.

Balkwill, M.J. & Balkwill, K. 1997. Delimitation and infra-generic classification of *Barleria* (Acanthaceae). *Kew Bulletin* 52(3): 535-573.

Caitlin, S.O. 2010. Anatomy of the shrimp plant, *Justicia brandegean* (Acanthaceae). *Studies by Undergraduate Researchers at Guelph* 3(2): 41-47.

Choopan, T. & Grote, P.J. 2015. Cystoliths in the leaves of the genus *Pseuderanthemum* (Acanthaceae) in Thailand. *NU International Journal of Science* 12(2): 13-20.

Chumchim, N., McDade, L.A. & Fisher, A.E. 2015. Phylogeny of *Dyschoriste* (Acanthaceae). *Aliso* 33(2): 77-89.

Clarke, C.B. 1900. Acanthaceae. In *Flora of Tropical Africa.* London: W.T. Reeves & Sons Pty. Ltd. 5: 1-261.

Cutler, D.F., Botha, T. & Stevenson, D.W. 2008. *Plant Anatomy. An Applied Approach.* Hoboken: Blackwell. pp. 70-120.

Daniel, T.F. 2013. Taxonomic, distributional, and nomenclatural notes on North American Acanthaceae. *Memoirs of the New York Botanical Garden* 108: 85-114.

Daniel, T.F. 1995. Acanthaceae. In *Flora of Chiapas.* 4th ed. San Francisco: California Academy of Sciences pp. 1-158.

Daniel, T.F. 1984. The Acanthaceae of the south-western United States. *Desert Plant* 5: 162-179.

Darbyshire, I., Tripp, E.A. & Chase, F.M. 2019. A taxonomic revision of Acanthaceae tribe Barlerieae in Angola and Namibia. *Kew Bulletin* 74(1): 1-85.

Darbyshire, I., Vollesen, K. & Ensermu, K. 2010. Acanthaceae. In *Flora of Tropical East Africa*. Kew: Royal Botanic Garden pp. 287-755.

Ensermu, K., Edwards, S. & Persson, E. 2006. *Flora of Eritrea and Ethiopia*. Sweden: Uppsala University.

Forsskal, P. 1775. *Flora Aegyptiaco-Arabica*. Hauniae, Moller, Kjobenhavn, Sweden.

Genua, J.M. & Hillson, C.J. 1985. The occurrence, type and location of calcium oxalate crystals in the leaves of fourteen species of Araceae. *Annals of Botany* 56(3): 351-361.

Hedrén, M. & Thulin, M. 2006. Acanthaceae. In *Flora of Somalia.* Kew: Royal Botanic Gardens 3: 374-454.

Henrickson, J. 1999. Studies in the genus *Dyschoriste* (Acanthaceae): Plants of northern Mexico, Texas to Arizona. *Lundellia* 2: 72-99.

Inamdar, J.A., Chaudhari, G.S. & Rao, T.R. 1990. Studies on the cystoliths of Acanthaceae. *Feddes Repertorium* 101(7-8): 417-424.

Lindau, G. 1895. Acanthaceae. In *Die natürlichen Pfl anzenfamilien*. Germany: Leipzig, Engelmann pp. 274-353.

Long, R.W. 1970. Genera of Acanthaceae in the southeastern United States. *Journal of the Arnold Arboretum* 51(3): 257-309.

Manktelow, M. 1996. Phaulopsis (Acanthaceae) - a monograph. Uppsala University. PhD Thesis. (Published). Sweden: Almqvist & Wiksell International.

McDade, L.A., Daniel, T.F. & Kiel, C.A. 2008. Toward a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae *s.l.* (Lamiales). *American Journal of Botany* 95(9): 1136-1152.

Metcalfe, C.R. & Chalk, L. 1950. *Anatomy of the Dicotyledons*. London, Oxford: Clarendon Press 1: 1014-1023.

Miller, A.G. 2004. *Ethnoflora of the Soqotra Archipelago*. Edinburgh: Royal Botanic Garden pp. 400-414.

Moylan, E.C., Bennett, J.R., Carine, M.A., Olmstead, R.G. & Scotland, R.W. 2004. Phylogenetic relationships among *Strobilanthes s.l.* (Acanthaceae): Evidence from ITS nrDNA, trnL-F cpDNA, and morphology. *American Journal of Botany* 91(5): 724-735.

Scotland, R.W. & Vollesen, K. 2000. Classification of Acanthaceae. *Kew Bulletin* 5: 513-580.

Scotland, R.W. 1992. Systematics, similarity and Acanthaceae pollen morphology. *Botanical Journal of the Linnean Society* 109(4): 529-541.

Singh, V. & Jain, D.K. 1975. Trichomes in Acanthaceae: 1. general structure. *Journal of the Indian Botanical Society* 54(1-2): 116-127.

Tripp, E.A. 2007. Evolution relationships within the species rich genus *Ruellia* (Acanthaceae). *Systematic Botany* 32(3): 628-649.

Tripp, E.A., Daniel, T.F., Fatimah, S. & McDade, L.A. 2013. Phylogenetic relationships within Ruellieae (Acanthaceae) and a revised classification. *International Journal of Plant Sciences* 174(1): 97-137.

Vollesen, K. 2008. Acanthaceae. In *Flora of Tropical East Africa*. Kew: Royal Botanic Gardens pp. 1-285.

Wasshausen, D.C. 1998. Acanthaceae of the southeastern United States. *Castanea* 63: 99-116.

Wasshausen, D.C. & Wood, J.R.I. 2003. The genus *Dyschoriste* (Acanthaceae) in Bolivia and Argentina. *Brittonia* 55(1): 10-18.

Wood, J.R.I. 1997. *A Hand Book of the Yemen Flora*. Kew: Royal Botanic Garden pp. 267-277.

Wood, J.R.I., Hillcoat, D. & Brummitt, R.K. 1983. Notes on the types of some names of Arabian Acanthaceae in the Forsskal Herbarium. *Kew Bulletin* 38: 429-456.

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