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

(Akanomi 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 capitiate 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, herba 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 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 bertepak. 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 diiktiraf 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...
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 lip; 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 polysyrene 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 cm², 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,0). 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.
| 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 Hadibo, 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 October 2012, 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°, E 44.24°, 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, Dihaish, 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, Shara, 22 September 1978, Miller 188 (K). |
| 10  | *Dyschoriste nagchan* | 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 January 2011, 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, Socotra, 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, Socotra, 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*        | Yemen, 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. hispinosa*, 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 & 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, 2 deep 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 PETOLES AND MIDRIHS**

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

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|c|c|}
\hline
Species / Characters & Adaxial & Parenchyma no. & Collenchyma no. & V.B. type & V.B. shape & Cystoliths & Oil cells & Trichomes \\
\hline
\textit{Barleria} acanthoides & straight & 19 & 4 & open & arch-shape & + (27μm) & + & + \\
\textit{B. aculeata} & convex & 19 & 3-5 & open & arch-shape & + (31μm) & + & + \\
\textit{B. bispinosa} & concave & 11 & 2-4 & open & U-shape & + (46μm) & - & + \\
\textit{B. orbicularis} & convex & 12 & 3 & open & arch-shape & + (28μm) & - & + \\
\textit{B. parviflora} & convex & 12 & 3 & open & arch-shape & + (49μm) & - & + \\
\textit{B. proxima} & convex & 16 & 3-5 & open & arch-shape & + (42μm) & + & + \\
\textit{B. prionitis} & convex & 15 & 2-4 & open & arch-shape & + (32μm) & + & + \\
\textit{B. tetracantha} & convex & 11 & 2 & open & arch-shape & + (36μm) & - & + \\
\textit{B. ventricosa} & concave & 11 & 4 & open & arch-shape + extended end & + (25μm) & + & + \\
\textit{Dyschoriste} nagachana & uneven & 17 & 3 & open & arch-shape & + (33μm) & - & + \\
\textit{Neuracanthus} aculeatus & straight & 14 & 3 & open & arch-shape & + (72μm) & - & + \\
\textit{Phaulopsis} imbricata & uneven & 20 & 4-5 & open & arch-shape & + (112μm) & + & + \\
\textit{Ruellia} patula & uneven & 17 & 3+ aerenchyma & open & arch-shape & + (77μm) & + & + \\
\textit{R. prostrata} & uneven & 18 & 3+ aerenchyma & open & arch-shape & + (37μm) & + & + \\
\textit{R. grandiflora} & uneven & 17 & 5 & open & arch-shape & + (31μm) & + & + \\
\textit{R. dioscoridis} & uneven & 8 & 3 & open & arch-shape & + (62μm) & - & + \\
\textit{R. insignis} & uneven & 15 & 3 & open & arch-shape & + (172μm) & - & + \\
\textit{R. paulayana} & uneven & 12 & 5 & open & arch-shape & + (73μm) & - & + \\
\hline
\end{tabular}
\caption{Anatomical characters of petioles in Barlerieae and Ruellieae. V.B.=Vascular Bundle, = + present, and - = absent}
\end{table}
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: 6-10 armed multitrate 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(c), 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: Eglanular trichome, glandular trichomes and stomata in *B. aculeata*, H: Dense stomata and trichomes in *B. bispinosa*, I: Eglanular 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 | glandular trichome | Margin shape | Stomata type | Anticlinal wall |
|--------------------|-----------|-----------------|---------------|-----------|-------------------------|-------------------|-------------|--------------|----------------|
| Barleria-acanthoides | 1         | 1               | 5             | +         | Unicellular conical filiform (191 μm) | +                 | uneven      | diacytic     | straight       |
| B. aculeata        | 2–3,      | 1               | 3             | +         | Unicellular (23 μm) and conical filiform (327 μm) | +                 | rounded     | diacytic     | straight       |
|                   | hypodermis|                 |               |           |                         |                   |             |              |                |
| B. bispinosa       | 1         | 1               | 3             | +         | Unicellular conical filiform (278 μm) | +                 | rounded     | diacytic     | straight       |
| B. ventricosa      | 1         | 1               | 3             | +         | Unicellular conical filiform (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       |
| Species              | E-glandular Trichomes       | Adaxial surface of petiole | Stem Outline       | Echinate trichome | E-glandular trichomes (size) | Glandular type                                                                 |
|----------------------|-----------------------------|-----------------------------|--------------------|-------------------|------------------------------|--------------------------------------------------------------------------------|
| **Barleria**         |                             |                             |                    |                   |                              |                                                                                |
| *Barleria*           | Unicellular, circular base and square base showed in *B. aculeata* | Straight and convex and concave | Squarish and subcircular (*B. acanthis*) | Present | 32 μm-1.13 mm | Sessile, glandular and capitate, glandular in *B. bispinos* |
| *Dyschoriste nagchana* | Multicellular                | Uneven                      | Squarish with 4 prominent angles | Present | 152 μm       | Sessile, glandular            |
| *Neuracanthus aculeatus* | Multicellular                | Uneven                      | Circular straight | Absent            | Long and hard to measure     | Absent                                                                          |
| *Phaulopsis imbricata* | Multicellular                | Uneven                      | Squarish with 4 prominent angles | Present | 304 μm       | Sessile, glandular            |
| *Ruellia*            | Multicellular                | Uneven                      | Squarish with 2-deep grooved | 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, biccular, multiccular, 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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