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# Research Article
# Leaf Anatomical Studies of *Alchornea* Sw., *Mallotus* Lour. and *Macaranga* Thou. (Euphorbiaceae) from Some Parts of Nigeria

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## Abstract

**Background and Objective:** The members of the genera *Alchornea*, *Mallotus* and *Macaranga* from Euphorbiaceae are closely related and difficult to differentiate. The leaf anatomy of these genera was studied to determine the interspecific and inter-generic variation among these taxa. **Materials and Methods:** Epidermal peels of the leaves were made with forceps. The petiole and midribs were fixed in FAA, dehydrated, sectioned, stained, observed and photo-micrographs taken with a trinocular research microscope (Amscope T340B) fitted with Amscope digital camera. **Results:** These genera share some similar characteristics such as paracytic stomata, trichome types, vascular bundle type and arrangement, petiole and midrib outlines, layers of palisade and spongy mesophylls, crystal (druses) distribution, etc., in common. Although some of these characteristics overlap among these taxa some of them such as trichome type, stomata type and distribution, crystal (druses) distribution, midrib and petiole vascular bundle pattern, etc., could be diagnostic at species and generic levels. We observed the peltate glandular hairs only on the adaxial surface of *Mallotus oppositifolius* and *Mallotus subulatus* but not in the other genera (*Alchornea* and *Macaranga*) studied. This character made this genus distinct from *Alchornea* and *Macaranga.* **Conclusion:** Trichome types, stomata types, petiole and midrib vascular bundle patterns and crystal (druses) distribution were diagnostic at species and generic levels and could be used to differentiate these taxa.

Key words: *Alchornea*, Euphorbiaceae, *Macaranga*, *Mallotus*, paracytic stomata, trichomes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Euphorbiaceae is a large, diverse and complex angiosperm family with 334-340 genera[1,2] and over 8,000-9,000 species[2,3], found mainly in the tropical regions of the world[2]. The presence of *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg. and *Manihot esculenta* Crantz in this family made it one of the world's important plant families[4].

The taxonomy of Euphorbiaceae has been investigated and documented[4-8], while The Angiosperm Phylogeny Group[9] divided the family into four groups namely: Euphorbiaceae *Sensu stricto*, comprising the subfamilies with uniovulate ovary locules (Euphorbiaceae, Crotonoideae and Acalyphoideae), Phyllanthaceae, including the Phyllanthoideae (biovulateovary locules), Picrodendraceae, including the Oldfieldioideae (biovulate ovary locules) and Putranjivaceae (biovulate ovary locules, comprising Drypetes Vahl and Putranjiva Wall.). Beyond these studies, other phylogenetically taxonomic changes have been made using molecular data[2,3,10,11]. These changes gave rise to subfamilies and differed from the traditional classification and revision proposed by Webster[1]. The efforts of these researchers based on economic botany, anatomy, phytochemistry and molecular systematic, catalyzed the splitting of the family into four to five subfamilies[1,2,9-12]. Despite these efforts, there are still considerable gaps in the knowledge of this family, especially the morphology[7], therefore[13], highlighted that comprehensive morphological and anatomical studies involving many genera in Euphorbiaceae are needed to come up with a better classification for this family.

*Macaranga* Thouars consists of about 200 species worldwide, 24 in Malaysia[13] and nine in Nigeria[8]. Members of this genus have a symbiotic relationship with ants[14-16] and are mainly distributed in the primary lowland forest[8,14,17]. Based on leaf morphological characters, the genus has been divided into six to eight sections[18,19]. *Macaranga* species are often confused with *Mallotus*, because they are closely related and are referred to as sister genera[10,13,20] and share some leaf anatomical features in common[4]. Although these taxa are closely related, Norfaizal[13] stated that some authors have distinguished *Macaranga* from *Mallotus* using the presence of red latex and its association with ants and these characteristics are not observed on herbarium specimens and necessitated the need for other means of identifying them[4]. Also, Wurdack *et al.*[10] based on the molecular phylogenetic study of Euphorbiaceae *Sensu stricto* (s.s.) demonstrated that *Macaranga*, *Mallotus* and *Trewia* form a well-supported clade. In tropical Africa and Madagascar, *Mallotus* species are represented by two species namely: *M. oppositifolius* (Geiseler) Müll. Arg. (*M. oppositifolius* var. *glabratus* Müll. Arg. and *M. oppositifolius* var. *pubescens* Pax.) and *M. subulatus* Müll. Arg.[6,8,21]. Kadiri *et al.*[8] used epidermal characteristics to identify these two *Mallotus* species.

Also, *Alchornea* Sw., is a sister genus to *Mallotus* and comprised four species (*A. cordifolia* (Schum. and Thonn.) Müll. Arg., *A. floribunda* Müll. Arg. *A. hirtella* Benth. and *A. laxiflora* (Benth.) Pax and K. Hoffman) in West Africa and three (*A. cordifolia* (Schum. and Thonn.) Müll. Arg., *A. floribunda* Müll. Arg. and *A. laxiflora* (Benth.) Pax and K. Hoffman) in Nigeria[17]. Taxonomic studies have been carried out on *Alchornea* and its relative genera however, the generic limit is somewhat ambiguous and has made some species be removed from *Alchornea*[21] or moved into this genus[22]. These species are morphologically similar and are difficult to identify and the presence of lignans and tannins in the genus may be of chemotaxonomic value[23]. Due to this morphological similarity, some authors such as Sam *et al.*[24] have misidentified *Alchornea laxiflora* for *Mallotus* species. Also, most of the works on African and Nigerian species of *Alchornea*, *Mallotus* and *Macaranga* have been on the individual genera and not comparing these genera.

There are scanty works mainly on the leaf anatomy and micro-morphology of *Alchornea* and *Macaranga*. Based on the close relationship among these genera, this work aims to compare the anatomical characteristics of the leaves of *Alchornea*, *Mallotus* and *Macaranga* species found in some parts of Nigeria to understand the intergeneric similarities and variations among these species, which will contribute to the systematics of these taxa.

## MATERIALS AND METHODS

**Study area/plant materials:** The plant materials were collected from some parts of Rivers State, Abia State, Delta State and Anambara State (Table 1). They were authenticated by the Curator in the Department of Plant Science and Biotechnology Herbarium (UPH), processed and voucher specimens were deposited in the Herbarium for reference purposes. Fresh leaves were removed from the plants, washed with distilled water and used for anatomical and micro-morphological analysis. This research was conducted in the Plant Taxonomy and Biosystematics Research laboratory, Department of Plant Science and Biotechnology, University of Port Harcourt between March, 2019-December, 2021.

**Microscopic analysis:** Fresh leaves and petioles were harvested and fixed in FAA (1:1:3) of formalin (40%), acetic acid (30%) and ethanol (70%). Fixation, embedding,

Table 1: List of voucher specimens studied

| Species name | Locality | Date of collection | Collector (s) name | Herbarium numbers |
|---|---|---|---|---|
| *Mallotus subulatus* | Omambara, Anambara State | 11/03/2018 | Ekeke, C. and others | UPH/V/1022 |
| *Macaranga hurifolia* | Ebendo Flowstation, Delta State | 28/04/2018 | Ekeke, C. and others | UPH/V/1031 |
| *Alchornea cordifolia* | Agric farm, University of Port Harcourt, Rivers State | 10/08/2019 | Ekeke, C. and Ogazie, C.A. | UPH/V/1041 |
| *Alchornea laxiflora* | IITA station Onne, Rivers State | 25/08/2021 | Ekeke, C. and others | UPH/V/1056 |
| *Mallotus oppositifolius* | Obiga-Asa, Abia State | 10/12/2014 | Ekeke, C. | UPH/V/0910 |
| *Macaranga monandra* | IITA station Onne, Rivers State | 30/05/2019 | Ekeke, C. and Ogazie, C.A. | UPH/V/0923 |
| *Macaranga heudelotii* | Botanic Garden, Uniport, Rivers State | 29/05/2019 | Ekeke, C. and Ogazie, C.A. | UPH/V/0912 |
| *Macaranga barteri* | IITA station Onne, Rivers State | 29/05/2019 | Ekeke, C. and others | UPH/V/0935 |

sectioning, epidermal mechanical scraping and staining were done according to the procedures with suitable modification[25]. The petioles (basal parts) were hand sectioned and stained in 1% Safranin O and counter in 1% Alcian blue. The slides were then mounted in glycerogelatin and sealed with transparent nail polish[25]. Twenty good sections were selected and observed using a research microscope and clear photo-micrographs taken with a trinocular research microscope (T340B) fitted with an Amscope digital camera. The images were processed using the Analysis Document Software imaging system. The vascular bundle arrangement in the petiole and midrib was classified according to Ekeke and Ogazie[26].

## RESULTS

**Epidermal description:** The result of the epidermal features of the different taxa studied are presented in Fig. 1. The shape of the adaxial and abaxial epidermal cells varied slightly among the genera studied: Irregular in *A. cordifolia*, *A. laxiflora*, *M. barteri*, *M. hurifolia*, *M. monandra* and *M. heudelotii* (Fig. 1a-l), rectangular to polygonal in *M. oppositifolius* and *M. subulatus* (Fig. 1m-p). The anticlinal walls are wavy in *A. cordifolia*, *A. laxiflora*, *M. barteri*, *M. hurifolia*, *M. monandra* and *M. heudelotii* (Fig. 1a-l) but straight or curved in *M. oppositifolius* and *M. subulatus* (Fig. 1m-p, Table 2). *Alchornea cordifolia*, *M. oppositifolius*, *M. subulatus*, *M. monandra* and *M. heudelotii* are amphistomatic while *A. laxiflora*, *M. barteri* and *M. hurifolia* are hypostomatic. The dominant stomata type among the taxa is paracytic stomata and was observed in all the species studied. In addition, anisocytic stomata were observed in the adaxial surface of *A. cordifolia* and *M. oppositifolius*, abaxial surface of *A. laxiflora* and *M. oppositifolius*, anomocytic stomata on the abaxial surface of *A. cordifolia* and contiguous stomata in the abaxial surface of *M. monandra* and *M. heudelotii*.

In the taxa investigated, the stomatal index varied from 0-2.63 in the adaxial surface to 19.05-32.0 on the abaxial surface. The mean epidermal size (length$\times$width) on the adaxial epidermis varied from 46.07$\times$28.32 µm in *A. laxiflora* to 17.79$\times$10.32 µm in *A. cordifolia* while in the abaxial epidermis it varied from 38.38$\times$18.70 µm in *Macaranga monandra* to 22.86$\times$10.58 µm in *Mallotus subulatus* (Table 2).

**Trichome types:** Generally, the different trichome types observed in this include glandular and non-glandular types (Fig. 2). These trichomes were classified into the non-glandular multibranched, multiarmed trichomes, non-glandular T-shaped and non-glandular cylindrical trichomes (Fig. 2a-c) and the glandular peltate, disc-shaped multicellular trichomes (Fig. 2c-d). The adaxial leaf surface of *M. oppositifolius* and *M. subulatus* have glandular peltate trichomes, the abaxial surface of *M. barteri*, *M. hurifolia* and *M. heudelotii*, disc-shaped multicellular glandular in the abaxial surface of *M. oppositifolius* and *M. subulatus*. *Alchornea cordifolia* has non-glandular stellate, T-shaped and multiarmed trichomes (Fig. 2), *M. oppositifolius*, *M. subulatus* and *M. heudelotii* non-glandular cylindrical trichomes (Table 2).

**Lamina:** All the taxa studied have a 1-layer epidermis except *M. monandra* with 1-2-layers of the epidermis (Fig. 3a-k). Palisade mesophyll is 1-layer in *A. laxiflora*, *M. oppositifolius* and *M. heudelotii*, 2-layers in *A. cordifolia* and *M. barteri* and 1-2-layers in *M. subulatus*, *M. hurifolia* and *M. monandra.* Calcium oxalate crystals (druses) occurred between the upper epidermis and palisade mesophyll in *Mallotus subulatus* and *Macaranga monandra*, in the palisade and spongy mesophylls of *A. cordifolia*, between the upper epidermis, palisade and spongy mesophylls of *M. oppositifolius*, in the palisade mesophyll of *M. hurifolia*, in the palisade and adaxial epidermis of *M. monandra* but absent in *A. laxiflora*, *M. heudelotii* and *M. barteri*. The spongy mesophylls generally have 1-6 layers of cells in the taxa studied. *A. cordifolia*, *A. laxiflora* and *M. subulatus* have 4-layer cells, *M. oppositifolius* 3-6 layers, *M. barteri* and *M. heudelotii* 5-6 layers, *M. monandra* 3-4 layers and *M. hurifolia* 4-5 layers. The spongy parenchyma cells are loosely packed and contain a bundle sheath surrounded with thick-walled, interrupted fibre in it.

Table 2: Epidermal and lamina characteristics

| Plant parts | *A. cordifolia* | *A. laxiflora* | *M. oppositifolius* | *M. subulatus* | *M. barteri* | *M. hurifolia* | *M. monandra* | *M. heudelotii* |
|---|---|---|---|---|---|---|---|---|
| **Adaxial epidermis** | | | | | | | | |
| Shape | Irregular | Irregular | Rectangular to polygonal | Polygonal | Irregular | Irregular | Irregular | Irregular |
| Anticlinal wall | Wavy | Straight/wavy | Straight/curved | Straight/curved | Wavy | Wavy | Wavy | Wavy |
| Size (μm) | 17.79±3.41×10.32±1.74 | 46.07±8.99×28.32±3.67 | 24.39±4.95×11.25±2.39 | 23.88±4.19×12.49±2.28 | 29.25±2.96×18.59±2.84 | 29.29±5.03×18.27±3.23 | 42.45±5.95×24.76±5.82 | 36.93±4.96×22.85±3.63 |
| Stomata type | Pa, ani and tetra | Absent | Pa and Ani | Pa | Absent | Absent | Pa mainly close to veins | Pa mainly close to veins |
| Trichome | Absent | Absent | Glandular peltate | Glandular peltate | Absent | Absent | Absent | Absent |
| Stomatal index | 2.63 | 0 | 0.95 | 1.0 | 0 | 0 | 2.44 | 1.75 |
| **Abaxial epidermis** | | | | | | | | |
| Shape | Irregular | Irregular | Rectangular to polygonal | Pentagonal to polygonal | Irregular | Irregular | Irregular | Irregular |
| Anticlinal wall | Wavy | Wavy | Straight/curved | Straight/curved | Wavy | Wavy | Wavy | Wavy |
| Size (μm) (Mean±STD) | 24.13±2.59×12.58±2.19 | 35.65±6.94×19.16±4.02 | 35.03±4.69×19.51±3.21 | 22.86±5.67×10.58±2.09 | 27.79±5.19×15.07±3.58 | 28.95±5.77×12.89±3.34 | 38.38±9.99×18.70±7.82 | 35.64±8.07×18.03±5.26 |
| Stomata type | Pa and Ano | Pa and ani | Pa and ani | Paracytic | Paracytic | Paracytic | Pa and contiguous | Pa and contiguous |
| Trichome | Non-glandular stellate and T-shaped | Absent | Disc-shaped MG and simple non-glandular cylindrical | Disc-shaped MG and simple non-glandular | Glandular peltate | Glandular peltate | Simple non-glandular | Peltate glandular |
| Stomatal index | 29.63 | 25.0 | 20.0 | 24.24 | 19.05 | 26.67 | 21.43 | 32.0 |
| **Transverse section (T/S) of Lamina** | | | | | | | | |
| Thickness (μm) | 139.87±5.17 | 153.52±2.79 | 109.89±6.07 | 112.38±2.86 | 160.60±1.91 | 122.28±5.32 | 208.81±1.93 | 156.37±9.60 |
| AD epidermis | 1-layer | 1-layer | 1-layer | 1-layer | 1-layer | 1-layer | 1-2 layers | 1-layer |
| Druse occurrence | PM and SM | Absent | Absent | PM | Absent | Absent | AD epidermis and PM | PM |
| PM thickness | 36.33±2.53 | 69.17±2.37 | 35.39±1.87 | 42.01±3.06 | 77.21±4.71 | 44.53±2.79 | 55.35±2.79 | 43.89±5.88 |
| SM thickness | 69.65±3.20 | 56.27±5.57 | 44.69±4.37 | 43.39±3.23 | 59.46±3.83 | 51.21±11.66 | 114.64±5.97 | 72.64±7.04 |
| PM/SM ratio | 0.52±0.04 | 1.24±0.16 | 0.80±0.10 | 0.97±0.09 | 1.30±0.11 | 0.88±0.15 | 0.48±0.05 | 0.61±0.06 |
| PM layers | 2 | 1 | 1 | 1-2 | 2 | 1-2 | 1-2 | 1 |
| SM layers | 4 | 4 | 3-6 | 4 | 5-6 | 4-5 | 3-4 | 5-6 |
| Papillae | Absent | Absent | Short/not pronounced | Short/not pronounced | Long | Long | Short/not pronounced | Short/not pronounced |
| Crypt | Absent | Absent | Present | Present | Present | Present | Present | Present |

Vb: Vascular bundle, PT: Pith thickness, ET: Cortical thickness, ADE: Adaxial epidermis, ABE: Abaxial epidermis, PM: Palisade mesophyll, SM: Spongy mesophyll, MG: Multicellular glandular, Pa: Paracytic, Ani: Anisocytic and Ano: Anomocytic

Table 3: Petiole and midrib characteristics

| Plant parts | *A. cordifolia* | *A. laxiflora* | *M. oppositifolius* | *M. subulatus* | *M. barteri* | *M. hurifolia* | *M. monandra* | *M. heudelotii* |
|---|---|---|---|---|---|---|---|---|
| **Petiole** | | | | | | | | |
| Shape | Oval | Planoconvex | Planoconvex | Oval | Oval/circular | Oval/circular | Oval/circular | Oval/circular/plano-convex |
| Trichome type/abundance | Non-glandular cylindrical (++) | Non-glandular cylindrical (+) | Non-glandular cylindrical(+) | Absent | Absent | Non-glandular cylindrical (+) | Non-glandular cylindrical (+) | Non-glandular cylindrical (+) |
| Vascular bundle | Closed circle of 13-14 vbs and a medullary bundle | Partly open/closed circle of 14-15 vbs and a medullary bundle | Open circle of 12 vbs and a medullary bundle | Open circle of 16-18 vbs and a medullary bundle | Two open circles 14 vbs and a medullary bundle | Open circle of 9 vbs and 2-medullary bundles | Open circle of 8-9 vbs and a medullary bundle | Open circle of 9 vbs and 2-medullary |
| Secretory cells/cavities | + (cortex) | + (cortex/pith) | + (cortex) | + (pith/cortex) | +++ (pith/cortex) | ++ Pith/cortex) | ++(pith/cortex) | (Pith/cortex) |
| Cortex parenchyma | 8-9 layers | 7-8 layers | 11-14 layers | 13-18 layers | 5-9 layers | 7-8 layers | 5-7 layers | 15-20 layers |
| Druses/mucilage | +/- | -/+ | ++/- | Pith/cortex (++/++) | Cortex (++/++) | Cortex (++) | Cortex/pith | Absent, Cortex/pith |
| **Midrib** | | | | | | | | |
| Adaxial cortical layer | 6-11 | 6-10 | 12-16 | 3-8 | 9-13 | 3-8 | 3-8 | 4-8 |
| Abaxial cortical layer | 6-14 | 5-8 | 10-14 | 9-18 | 7-9 | 2-4 | 7-10 | 4-8 |
| Abaxial outline | Convex | Convex | Convex | Convex | Convex/wavy | Convex/wavy | Convex/wavy | Convex/wavy |
| Vascular bundles shape | 3/4 cressent | 1/3 cressent | 1/3 cressent | 1/2 cressent | Open circle | 3/4 crescent | 3/4 crescent | 3/4 crescent |
| Adaxial plate | Present | Present (2 co-joined adaxial bundles) | Present | Present | Absent | Present | Present | Present |
| Rib traces | 2 (1 on each side) | 2 (1 on each side) | 6 (3 on each side) | 6 (3 on each side) | Absent | Absent | Absent | Absent |
| Medullary bundle | 2 | absent | Absent | Absent | 5 | Absent | 1 | 2 |
| Vessels | Radial multiples of 2-5, partly in tangential pairs | Radial multiples of 4-6, partly in tangential pairs | Radial multiples of 2-5 cells | Radial multiples of 4-7, tangential multiples of 2-4 | Radial multiples of 3-6, partly in tangential pairs | Radial multiples of 2-4 | Radial multiples of 2-4, partly in tangential pairs | Radial multiples of 2-3, partly in tangential pairs |
| Occurrence of secretory cells/mucilage | Cortex | Phloem and cortex PA | Phloem and cortex PA | Cortex and cortex | Pith, cortex and phloem | Pith/cortex | Pith, cortex | Pith/cortex (+) |
| Druses | Cortex (+) | Absent | Cortex PA and pith | Pith and cortex PA | Cortex PA | Cortex PA | Absent | Cortex PA |
| Ad surface (hairs) | Non-glandular (+) | Absent | Non-glandular (+) | Multi-armed | Glabrous | Non-glandular | Glandular and non-glandular (+) | non-glandular (+) |

PA: Parenchyma

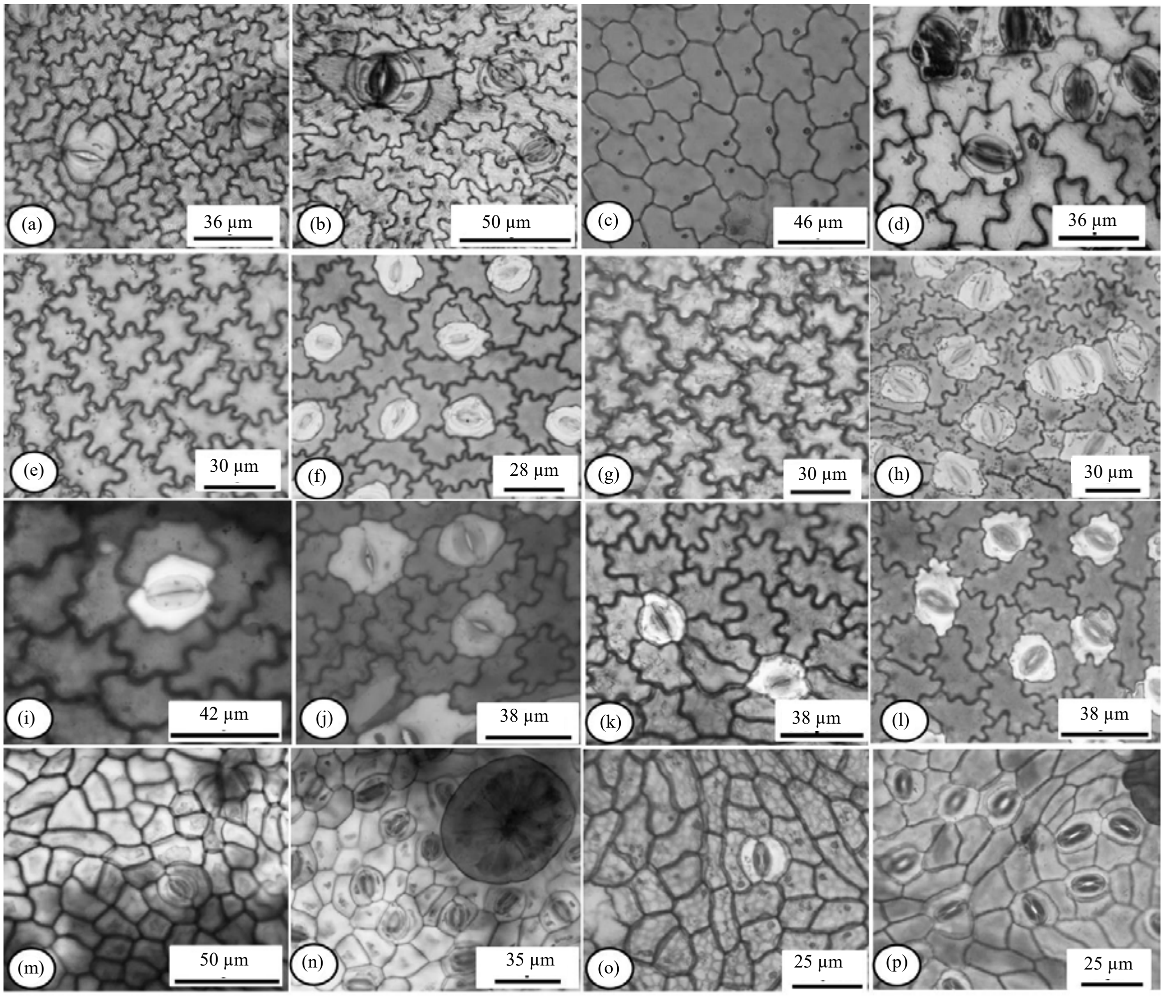


Fig. 1(a-p): Epidermal peels of the taxa studied, (a) *A. cordifolia* adaxial, (b) *A. cordifolia* abaxial surface, (c) *A. laxiflora* adaxial surface, (d) *A. laxiflora* abaxial surface, (e) *M. barteri* adaxial surface, (f) *M. barteri* abaxial surface, (g) *M. hurifolia* adaxial surface, (h) *M. hurifolia* abaxial surface, (i) *M. monandra* adaxial surface, (j) *M. monandra* abaxial surface, (k) *M. heudelotii* adaxial surface and (l) *M. heudelotii* abaxial surface, (m) *M. oppositifolius* adaxial surface, (n) *M. oppositifolius* abaxial surface, (o) *M. subulatus* abaxial surface and (p) *M. subulatus* abaxial surface

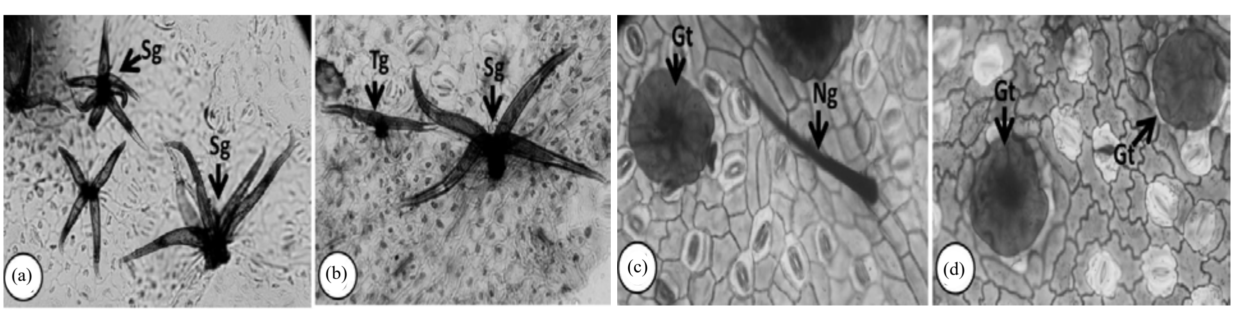


Fig. 2(a-d): Glandular and non-glandular trichomes, (a) Sg-non-glandular multibranched or multiarmed trichomes, (b) Tg-non-glandular T-shaped trichome and Sg-non-glandular multibranched or multiarmed trichomes, (c) Gt-glandular peltate, disc-shaped multicellular trichomes and Ng-non-glandular cylindrical trichome and (d) Gt-glandular peltate and disc-shaped multicellular trichomes

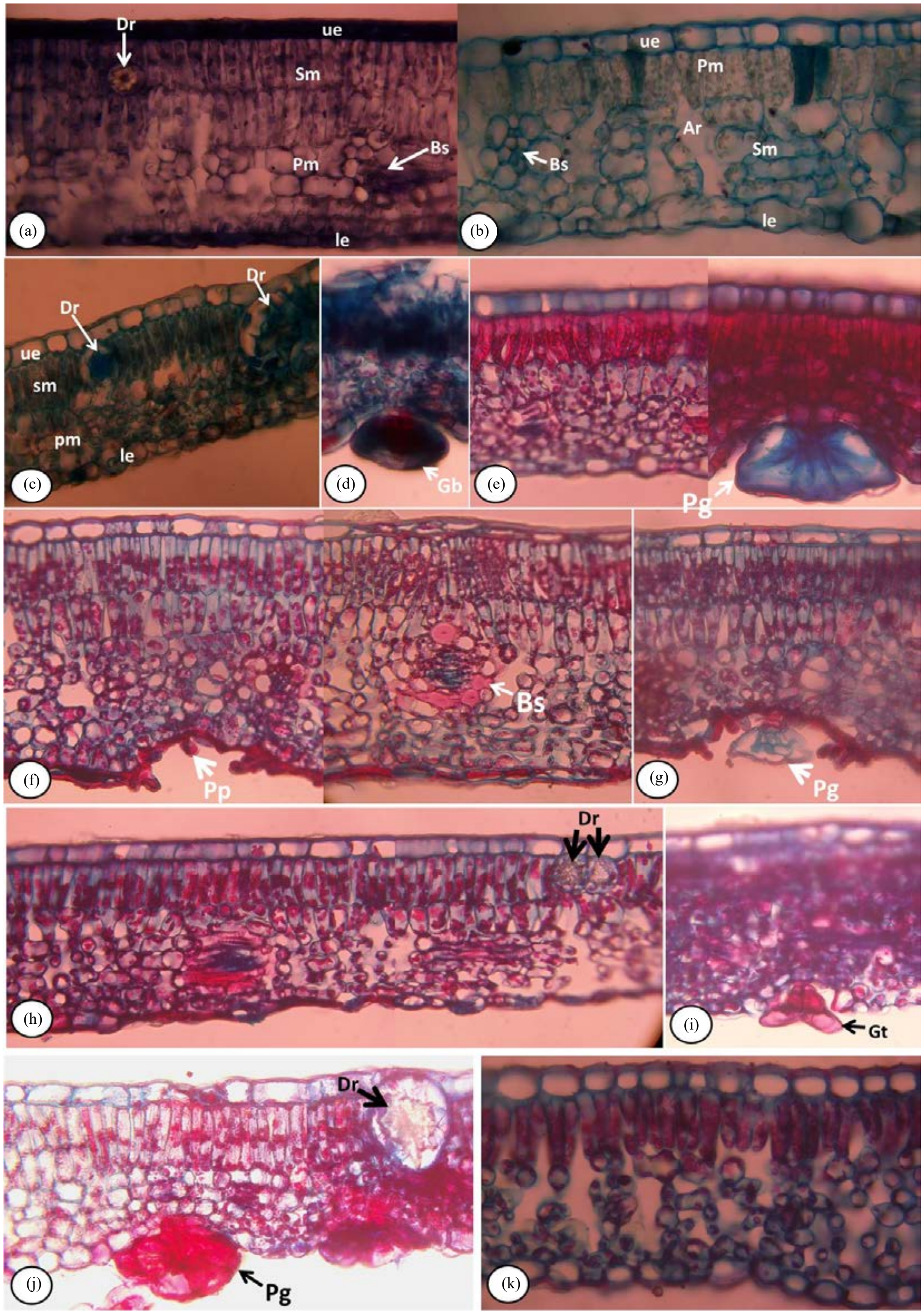


Fig. 3(a-k): Transverse section of the lamina of taxa studied, (a) *A. cordifolia*, (b) *A. laxiflora*, (c-d) *M. oppositifolius* glandular hair in a crypt, (e) *M. monandra* glandular hair in a crypt, (f-g) *M. barteri* glandular hair in a crypt, (h-i) *M. hurifolia* glandular hair in a crypt, (j) M. subulatus glandular hair in a crypt and druse in palisade cell and closely packed spongy cells and (k) *M. heudelotii* with loosely packed spongy cells

E: Epidermis, Dr: Druse, pm: Palisade mesophyll, le: Abaxial epidermis, sm: Spongy mesophyll, Pg: Peltate glandular trichome, Ar: Air space, Bs: Bundle sheath, ue: Adaxial epidermis, Pp: Papillae and Gt: Glandular peltate tufted hair

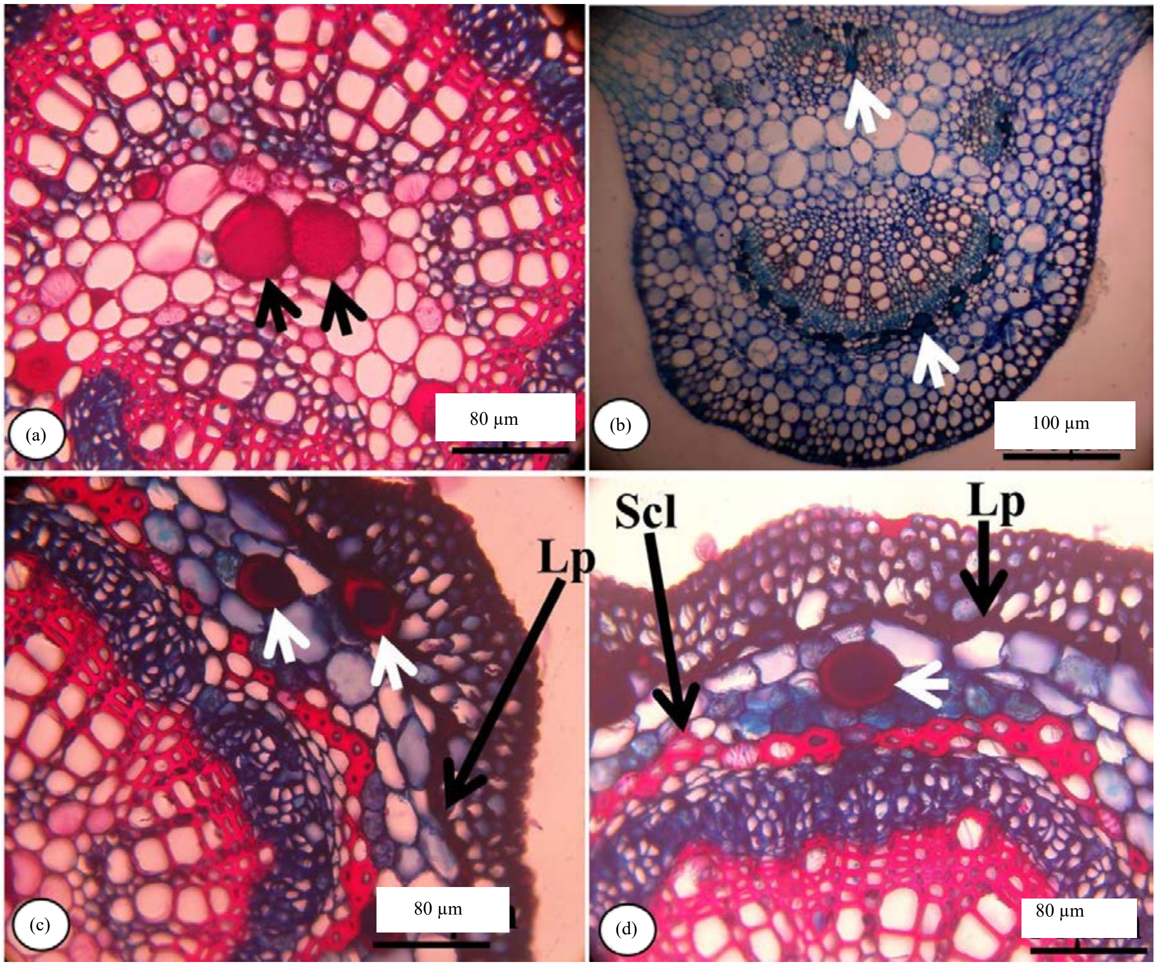


Fig. 4(a-d): Localization of secretory ducts in the plant tissues of the taxa studied, (a) Secretory ducts in the pith, (b) Secretory ducts in the phloem cells, (c and d) Secretory ducts in the cortex parenchyma
Scl: Sclerenchymatous fibre and Lp: Lignified parenchyma

The lignified parenchymatous cells and sclerenchymatous fibre are found in the midribs of *M. barteri*, *M. monandra*, *M. hurifolia* and *M. heudelotii* (Fig. 4a-d). Patches of sclerenchymatous fibre are observed in *M. barteri* while *M. monandra*, *M. hurifolia* and *M. heudelotii* have continuous layers of sclerenchymatous fibre (Fig. 4d). These features are absent in *A. cordifolia*, *A. laxiflora*, *Mallotus oppositifolius* and *M. subulatus*.

**Petiole:** The outline of the petioles is almost the same and varied from circular to oval. The abaxial and adaxial outlines varied slightly among the studied species: Concave-convex outline in *A. cordifolia* (Fig. 5a), *M. barteri* (Fig. 5b) *M. hurifolia* (Fig. 5c) *M. monandra* (Fig. 5d) and *M. heudelotii* (Fig. 5e) and plano-convex in *A. laxiflora* (Fig. 5f), *Mallotus oppositifolius* (Fig. 5g) and *M. subulatus* (Fig. 5h). Most of the species have lots of secretory cells which when stained appeared blue or red (Fig. 5i). The petiole vascular bundle number and orientation varied among the analyzed species: A closed circle of 13-14 vascular bundles with a medullary bundle in *A. cordifolia* (Fig. 5a), a partly open/closed circle of 14-15 vascular bundles with a medullary bundle in *A. laxiflora* (Fig. 5f), open circle of 12 vascular bundles with a medullary bundle in *M. oppositifolius* (Fig. 5g) open circle of 16-18 vascular bundles with a medullary bundle in *M. subulatus* (Fig. 5h), two open circles of 14 vascular bundles with a medullary in *M. barteri* (Fig. 5b), open circle of 9 vascular bundles with two medullary bundles in *M. hurifolia* (Fig. 5c) and *M. heudelotii* (Fig. 5e) and an open circle of 8-9 vascular bundles with a medullary in *M. monandra* (Fig. 5d).

Calcium oxalate crystals (druses) and mucilage occur in the parenchymatous cortex and pith in *Macaranga barteri*, *M. monandra*, *M. heudelotii*, *Mallotus subulatus* and

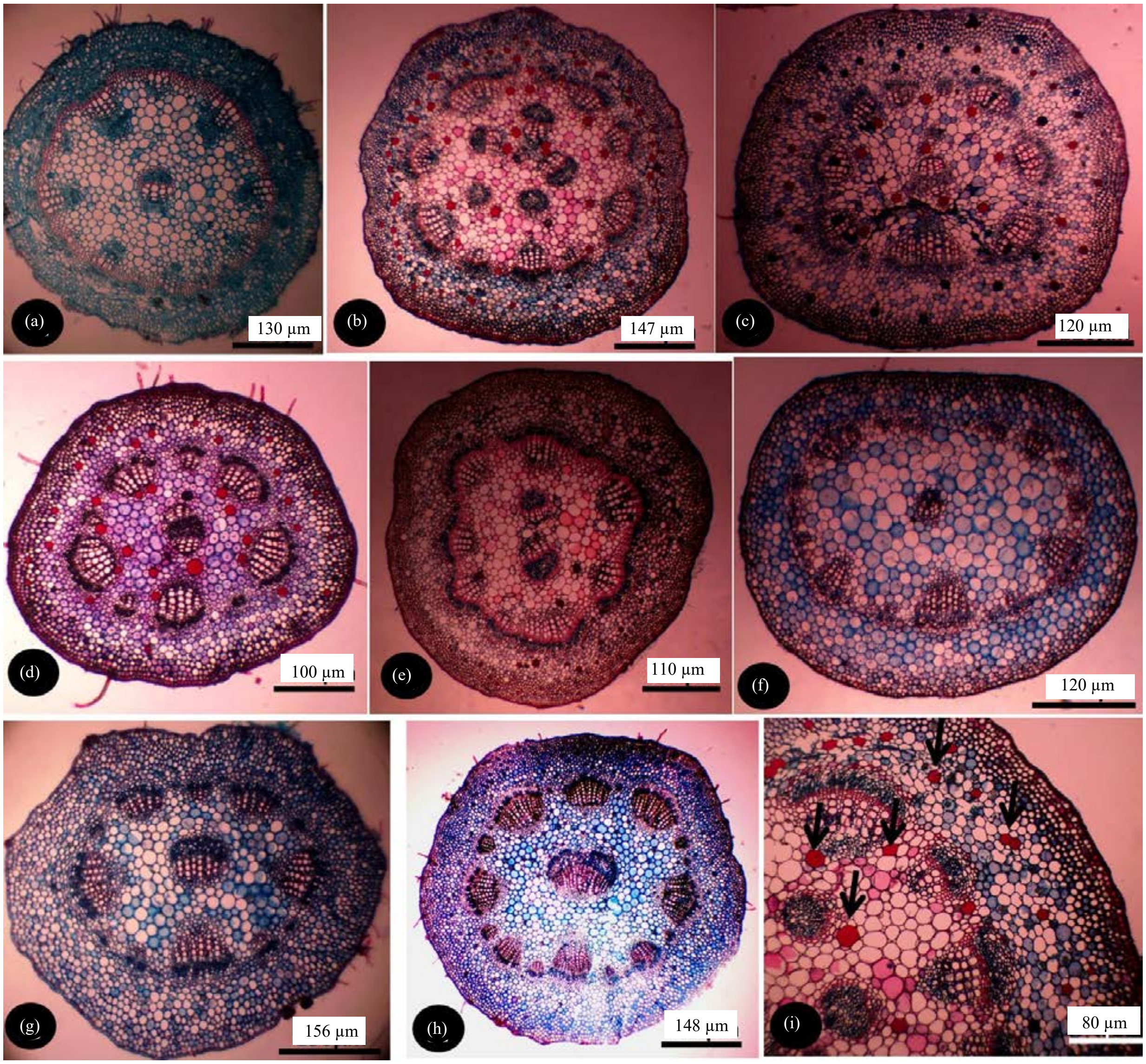


Fig. 5(a-i): Transverse sections petiole of the taxa studied, (a) *A. cordifolia*, (b) *M. barteri*, (c) *M. hurifolia*, (d) *M. monandra*, (e) *M. heudelotii*, (f) *A. laxiflora*, (g) *M. oppositifolius*, (h) *M. subulatus* and (i) *M. barteri*-arrows show secretory cavities

*M. oppositifolius*, druses in the parenchymatous cortex of *A. cordifolia* but absent in *A. laxiflora.* Also, mucilage was observed in the phloem cells in *A. laxiflora.* Fibre (sclerenchymatous) cells were present or absent in the outer regions of the vascular bundle, occurred in patches in *Macaranga barteri* and *M. heudelotii*, formed a continuous layer in *Mallotus subulatus* and were absent in the remaining species.

**Midrib:** The shape of the midribs in the species investigated are fairly the same with varying abaxial outlines (Fig. 6). A convex abaxial outline in *A. cordifolia* (Fig. 6a) *A. laxiflora* (Fig. 6b) *Mallotus oppositifolius* (Fig. 6c) and *M. subulatus* (Fig. 6d) and wavy in *Macaranga monandra* (Fig. 6e) *M. barteri* (Fig. 6f) *M. hurifolia* (Fig. 6g) and *M. heudelotii* (Fig. 6h). *M. bateri* contains secretory cells in the parenchymatous cortex and the pith (Fig. 6i). The vascular bundle formed 3/4 crescent and adaxial plate in *M. hurifolia* with 2-rib traces and 2-medullary phloem in *A. cordifolia*, 1-medullary phloem in *M. monandra* and 2-medullary phloems in *M. heudelotii*. The vascular bundle formed 1/3 crescent: 2 co-joined adaxial bundles and 2-rib traces in *A. laxiflora* and an adaxial plate with 6-rib traces (3 on each side of the crescent) in *M. oppositifolius*. The vascular bundle formed 1/2 crescent with an adaxial plate and 6-rib traces (3 on each side of the crescent) in *M. subulatus*, while the vascular bundle in


Fig. 6(a-i): Transverse sections midrib of the taxa studied, (a) *A. cordifolia*, (b) *A. laxiflora*, (c) *M. oppositifolius*, (d) *M. subulatus*, (e) *M. monandra*, (f) *M. barteri*, (g) *M. hurifolia*, (h) *M. heudelotii* and (i) *M. barteri*

*M. barteri* are five in an open circle with disjoined invaginated ends with 5 medullary phloem cells. The vessels in the midrib occurred in radial multiples of 2-7 and partly in tangential pairs (Table 3).

The layers of the parenchymatous cortex varied among the taxa: The adaxial has 6-11-layers in *Alchornea cordifolia* and *A. laxiflora*, 3-8-layers in *Mallotus subulatus*, *Macaranga hurifolia* and *M. heudelotii*, 12-16-layers in *Mallotus oppositifolius*, 9-13-layers in *Macaranga barteri* (Table 3). The abaxial cortex has 6-14-layers in *A. cordifolia*, 10-14-layers in *M. oppositifolius*, 4-8-layers in *A. laxiflora* and *M. heudelotii*, 7-10-layers in *M. barteri* and *M. monandra*, 9-18-layers in *Mallotus subulatus* and 2-4-layers in *Macaranga hurifolia.*

**Cortical characteristic:** Adaxial cortex among the taxa varied from 6-11 layers in *A. cordifolia* and *A. laxiflora*, 3-8 layers in *Mallotus subulatus*, *Macaranga hurifolia*, *M. monandra* and *M. heudelotii*, 12-16 layers in *Mallotus oppositifolius* and 9-13 layers in *Macaranga barteri* (Table 3). The abaxial cortex has 6-14 layers in *A. cordifolia*, 10-14 layers in *M. oppositifolius,* 4-8 layers in *A. laxiflora* and *M. heudelotii*, 7-10 layers in *M. barteri* and *M. monandra*, 9-18 layers in *M. subulatus* and 2-4 layers *M. hurifolia.*

## DISCUSSION

Generally, these genera we studied share some similar characteristics such as paracytic stomata, trichome types, vascular bundle type and arrangement, petiole and midrib outlines, layers of palisade and spongy mesophylls, crystal (druses) distribution, etc., in common. Although some of these characteristics overlap among the taxa some of them such as trichome type, stomata type and distribution, crystal (druses) distribution, etc., could be diagnostic at species and generic levels. Generally, the findings of our work greatly agreed with previous works conducted on, *Macaranga*[12,13,27], *Mallotus*[4-6,8,28,29] and *Alchornea*[21,30-32].

Existing reports showed that *Alchornea*, *Mallotus* and *Macaranga* have different hair (trichome) types such as short and long simple unicellular trichomes, peltate non-glandular hairs, peltate-stellate hairs with central glandular cell, globular to disc-shaped glandular hairs, stellate hairs with multicellular stalk and stellate tufted hairs[4,8]. The tufted hairs are more regular in *Mallotus* but are absent in *Macaranga.* They can occur as star-shaped (stellately tufted) or non-stellate hairs[4]. We observed the peltate glandular hairs only on the adaxial surface of *Mallotus oppositifolius* and *Mallotus subulatus* but not in the other genera (*Alchornea* and *Macaranga*) studied. A previous study noted glandular hairs as the most informative character among *Mallotus*, *Blumeodendron* and *Hancea*[4]. In the same way, our study showed that the peltate glandular hair could be used to distinguish *Mallotus* from *Alchornea* and *Macaranga*. In some members of these genera, the abundance of the capitate hairs vary and could be sometimes covered by the tufted hairs[8]. We did not observe capitate hairs in *Mallotus.* This character made this genus distinct from *Alchornea* and *Macaranga* and is in line with the report of Pečnikar *et al.*[4], who investigated *Mallotus*, *Blumeodendron* and *Hancea* and stated that capitate glandular hairs are restricted to the genus *Hancea* and not observed in *Mallotus.* The glandular peltate hairs are common among the *Macaranga* and *Mallotus*[4,8]. This hair type was not observed in *Alchornea* species we studied and further confirms the closeness of *Macaranga* and *Mallotus* as previously stated[10,13,22]. This character could be used to delimitate *Alchornea* from these two genera studied.

The epidermal cells in *Macaranga* species varied from wavy to sinuous. They have mainly paracytic stomata but staurocytic stomata have been reported in *Macaranga javanica* (Blume) Müll. Arg. Also, members of this genus (*M. gigantea* and *M. hypoleuca*) have simple and unicellular glandular trichomes on the abaxial leaf surface. In the lamina of this genus, the palisade mesophyll has a layer of the cell of varying sizes with poorly developed hypodermis[13]. Papillae are found in the adaxial surface of *M. hulletii* and the abaxial surface of *M. javanica*[13]*.* Furthermore, these papilla holes or crypts contain glandular trichomes in *M. javanica* and could be a diagnostic character to distinguish *M. javanica* from other members of this genus[13]*.* In the taxa that we studied, we observed some differences and similarities. For instance, crypts without papillose but having glandular trichome was observed in *Mallotus oppositifolius* while *M. subulatus*, *Macaranga barteri*, *M. hurifolia*, *M. monandra* and *M. heudelotii* have crypts with papillae. In contrast, *A. cordifolia* and *A. laxiflora* did not have crypts. All the species studied have bundle sheaths embedded in the mesophyll but with sclerenchymatous fibre occurring only in *Macaranga monandra*, *M. barteri*, *M. hurifolia* and *Mallotus subulatus.* These features confirm the inter-generic relationship among these taxa as reported by the previous authors[10,13,20,23,29]. The adaxial outline of the transverse section of midribs in *Macaranga* species is U-shaped or circular while the abaxial outline is semi-circular or flat. Also, the vascular bundle tissues in the midrib are closed in *M. javanica*, *M. tanarius*, *M. gigantea* and *M. hypoleuca* but open in *M. hulletii.* The bundle sheaths are sclerenchymatous with strands of fibres incompletely encircling the complex. The petiolar outline in the genus is circular in the majority of the species however, it is oval in *M. tanarius*[13]. The vascular bundles formed closed rings with medullary bundle sheaths at the centre while crystals (druses) occur in the parenchyma and phloem tissues of midribs and petioles. Ghazalli *et al.*[13] observed these crystals in *M. javanica*, *M. tanarius*, *M. gigantea* and *M. hulletii* but not *M. hypoleuca*. Although Ghazalli *et al.*[13] reported paracytic stomata as the dominant stomata type in *Macaranga* and the tribe Acalypha however, staurocytic stomata occur in *M. javanica*[13] and anomocytic type is more common in Euphorbiaceae species[13,27].

In our study, all the *Mallotus* species investigated are amphistomatic and we recorded paracytic and anisocytic stomata in the abaxial and adaxial leaf surfaces of *M. oppositifolius* while *M. subulatus* has only paracytic stomata. Also, the stomatal index on both surfaces was higher in *M. subulatus.* Previously, in the genus *Mallotus*, some authors have studied and described them using their leaf characteristics[4,6,8]. The majority of the species in this genus have paracytic stomata and amphistomatic with stomata on the adaxial surface being restricted to the midribs and big veins[6,8] while few are hypostomatic[4]. Furthermore, the epidermal cells on both surfaces of the leaf are irregularly shaped with straight to curved anticlinal walls[8]. Kadiri *et al.*[8] and Sierra and Welzen[29] used the presence of brachyparacytic stomata and abaxially restricted disc-shaped multicellular glandular trichomes to distinguish *M. oppositifolius* var. *glabratus* from *M. oppositifolius* var. *pubescens* and the presence of hemibranch paracytic stomata and cuticular striations to differentiate *M. subulatus* from *M. oppositifolius*. This suggests that the stomatal complex could be used to differentiate *Mallotus* species. Globose to disc-shaped glandular hairs are mostly restricted to the lower leaf surface and the inflorescences[5] and the presence of extrafloral nectaries in other parts of the plants[6]. The occurrence of glandular hairs in depressions or crypts surrounded or partly covered by papillae is a major characteristic of *Mallotus*[4-6]. Similar observation was also made on the *Mallotus* species studied.

The palisade mesophyll has 1-2 layers in *Mallotus apetala* but 2-layers in *M. stewardii*, *M. barbatus* and *M. japonica.* Closely packed in *M. apetala*, *M. barbatus* and *M. japonica* but loosely packed with air spaces in *M. stewardii*[4]. In our study, we observed a layer of palisade and 3-4 layers of spongy parenchyma in *M. oppositifolius* while *M. subulatus* 1-2 layers of palisade and 4-layers of spongy parenchyma. The thickness of palisade parenchyma (42.01$\pm$3.06 μm) in *M. subulatus* was higher than that of *M. oppositifolius* (35.39$\pm$1.87 μm). Furthermore, crystals (druses) were present in the palisade mesophyll of *M. subulatus* but absent in *M. oppositifolius.* Sierra and Welzen[29] in their study on *Mallotus* from Southeast Asia reported druses in the palisade and spongy mesophylls layers including the ground (pith) tissues of members of this genus. Also, Sierra *et al.*[5] reported the presence of large and minute druses in the mesophyll of *M. blumeanus*. Our findings corroborate these previous reports.

In our study, the number and the orientation of the vascular bundles in the transverse section in the petioles and the midribs varied considerably. This character can be used to differentiate these species at genus and species levels. The petiole of *Macaranga hurifolia* and *M. heudelotii* have two rib traces while the species viz., *Alchornea cordifolia*, *A. laxiflora*, *Macaranga barteri*, *M. monandra*, *Mallotus oppositifolius* and *M. subulatus* have one rib trace on each side of the petiole. *Macaranga hurifolia* and *M. heudelotii* have two medullary bundles while other species have only one. Furthermore in the midrib, the rib traces are present in *A. cordifolia*, *A. laxiflora*, *M. oppositifolius* and *M. subulatus* but absent in all the *Macaranga* species studied. The *Alchornea* species have one rib trace each on both sides of the main vascular bundle while the *Mallotus* species have three each on both sides of the vascular bundle arc. The adaxial vascular bundle plate in *Alchornea laxiflora* has two co-joined bundles. This kind of observation has been previously documented to have contributed to the taxonomy of Asteraceae[26], Lauraceae[33], Myrtaceae[34], Smilacaceae[35], Lamiaceae[36], Fabaceae[37], Malvacea[38] and Dipterocarpaceae[39]. Petioles with separate vascular cylinders and two medullary bundles and a few thin-walled fibre caps or without supporting sclerenchymatous fibre are major characteristics of *Mallotus*[4-6]. Also, their midrib could be flat or elevated adaxially. The adaxial and abaxial arc could have some or a few lateral bundles surrounded by a thin interrupted fibre sheath in *M. blumeanus*[4] and grooved adaxially with 9 separate vascular cylinders supported by a continuous sheath of fibres with medullary or central bundles in *M. cauliflorus*[5].

In the present study, the most important diagnostics features are the occurrence of different trichome types, shaped epidermal cells, stomata types and distribution, arrangement and number of vascular bundles in midrib and petiole vascular pattern, sclerenchymatous ring in the cortex and secretory ducts in the pith and parenchymatous cortex. These feature are fairly different among the taxa and can be used to differentiate the different members of *Mallotus*, *Alchornea* and *Macaranga* studied. Though we worked on a limited number of specimens, the trichome types, stomata types, petiole and midrib vascular bundle patterns and crystal (druses) distribution were diagnostic at species and generic levels and could be used to differentiate these taxa.

## CONCLUSION

This study investigated the importance of leaf anatomical features of some members of *Mallotus*, *Alchornea* and *Macaranga* and their taxonomic implications. From our findings, the epidermal features such as trichome types and distribution, stomata types, the number of vascular bundles and arrangement in the petiole and midrib are important diagnostic characteristics in *Mallotus*, *Alchornea* and *Macaranga*.

## SIGNIFICANCE STATEMENT

This study provided information on some leaf anatomical features of some members of the genera: *Alchornea* and *Macaranga* from Nigeria which was lacking. It also compared the generic relationship among *Alchornea*, *Mallotus* and *Macaranga* based on these leaf characters and therefore, bridges the gap that resulted in the misidentification of some members of this species by other researchers. This study will further enhance the use of leaf anatomical characters in the identification of members of the Euphorbiaceae family especially *Alchornea*, *Macaranga* and *Mallotus*.

## REFERENCES


1. Webster, G.L., 1994. Synopsis of the genera and suprageneric taxa of Euphorbiaceae. Ann. Mo. Bot. Gard., 81: 33-144.
2. Wurdack, K.J., P. Hoffmann, R. Samuel, A. de Bruijn, M. van der Bank and M.W. Chase, 2004. Molecular phylogenetic analysis of Phyllanthaceae (Phyllanthoideae pro parte, Euphorbiaceae sensu lato) using plastid *RBCL* DNA sequences. Am. J. Bot., 91: 1882-1900.
3. Radcliffe-Smith, A., 2001. Genera Euphorbiacearum. Royal Botanic Gardens, UK, ISBN: 9781842460221, Pages: 455.
4. Pečnikar, Ž.F., K.K.J. Kulju, S.E.C. Sierra, P. Baas and P.C. van Welzen,, 2012. Leaf anatomy of *Mallotus* and the related genera *Blumeodendron* and *Hancea* (Euphorbiaceae sensu stricto). Bot. J. Linn. Soc., 169: 645-676.
5. Sierra, S.E.C., K.K.M. Kulju, J.F. Veldkamp and P.C. van Welzen, 2007. Resurrection of *Hancea* and lectotypification of *Adisca* (Euphorbiaceae). Blumea-Biodivers. Evol. Biogeogr. Plants, 52: 361-366.
6. Sierra, S.E.C., K.K.M. Kulju, Ž. Fišer, M. Aparicio and P.C. van Welzen, 2010. The phylogeny of *Mallotus* s.str. (Euphorbiaceae) inferred from DNA sequence and morphological data. Taxon, 59: 101-116.
7. de S. Secco, R., I. Cordeiro, L. de Senna-Vale, M.F. de Sales and L.R. de Lima *et al*., 2012. An overview of recent taxonomic studies on Euphorbiaceae s.l. in Brazil. Rodriguésia, 63: 227-242.
8. Kadiri, A.B., A.E. Ayodele and J.D. Olowokudejo, 2019. Leaf epidermis of the African genus *Mallotus* Lour. (Euphorbiaceae) section *Rottleropsis* Müll. Arg. and its systematic value. Webbia, J. Plant Taxon. Geogr., 74: 71-80.
9. The Angiosperm Phylogeny Group, 2003. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc., 141: 399-436.
10. Wurdack, K.J., P. Hoffmann and M.W. Chase, 2005. Molecular phylogenetic analysis of uniovulate Euphorbiaceae (Euphorbiaceae sensu stricto) using plastid RBCL and TRNL-F DNA sequences. Am. J. Bot., 92: 1397-1420.
11. The Angiosperm Phylogeny Group, 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc., 161: 105-121.
12. Webster, G.L., 1987. The saga of the spurges: A review of classification and relationships of the Euphorbiales. Bot. J. Linn. Soc., 94: 3-46.
13. Ghazalli, M.N., K. Hussin and M.R.A. Rahman, 2012. Leaf anatomical study of five *Macaranga* species (Euphorbiaceae). J. Trop. Agric. Food Sci., 40: 289-296.
14. Maschwitz, U., B. Fiala and K. Dumpert, 2004. An unusual myrmecophytic *Macaranga* association, occurring in a disjunct area in the monsoon zone of South-East Asia: Phenology and the description of a new ant species. Ecotropica, 10: 33-49.
15. Quek, S.P., S.J. Davies, T. Itino and N.E. Pierce, 2004. Codiversification in an ant-plant mutualism: Stem texture and the evolution of host use in Crematogaster (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). Evolution, 58: 554-570.
16. Linsenmair, K.E., M. Heil, W.M. Kaiser, B. Fiala, T. Koch and W. Boland, 2001. Adaptations to biotic and abiotic stress: Macaranga ant plants optimize investment in biotic defence. J. Exp. Bot., 52: 2057-2065.
17. Davies, S.J., 2002. Ethnobotany of *Macaranga* (Euphorbiaceae) among the kedayan of Brunei Darussala. Harvard Pap. Bot., 7: 7-12.
18. Davies S.J., 2001. Systematics of *Macaranga* Sects. *Pachystemon* and *Pruinosae* (Euphorbiaceae). Harvard Pap. Bot., 6: 371-448.
19. Kulju, K.K.M., S.E.C. Sierra and P.C. van Welzen, 2007. Re-shaping *Mallotus* [Part 2]: Inclusion of *Neotrewia*, Octospermum and *Trewia* in *Mallotus* s.s. (Euphorbiaceae s.s.). Blumea-Biodivers. Evol. Biogeogr. Plant, 52: 115-136.
20. Kulju, K.K.M., S.E.C. Sierra, S.G.A. Draisma, R. Samuel and P.C. van Welzen, 2007. Molecular phylogeny of *Macaranga*, *Mallotus* and related genera (Euphorbiaceae s.s.): Insights from plastid and nuclear DNA sequence data. Am. J. Bot., 94: 1726-1743.
21. Murillo-Aldana, J., 2018. Novelties in the genus *Alchornea* (Euphorbiaceae). Phytotaxa, 375: 203-213.
22. Govaerts, R., D.G. Frodin and A. Radcliffe-Smith, 2000. World Checklist and Bibliography of Euphorbiaceae (with Pandaceae). Royal Botanic Gardens, Kew, UK, ISBN: 9781900347846, Pages: 1621.
23. Cui, G.Y. and R.X. Tan, 2004. Lignans and tannins from *Alchornea davidii* (Euphorbiaceae) and their chemotaxonomic significance. Biochem. Syst. Ecol., 32: 99-102.
24. Sam, S.M., C. Wahua and E.K. Oruwari, 2018. Comparative chemotaxonomic studies on sroti (*Mallotus oppositifolius* (Geiseler)) mull and velvet bush willow (*Mallotus alternifolius*) Merr. Bayero J. Pure Appl. Sci., 11: 162-167.
25. Ekeke, C. and J.U. Agogbua, 2019. Comparative morphological and anatomical studies of *Boerhavia* (*Nyctaginaceae*) species in Nigeria. Phytologia Balcan., 25: 29-38.
26. Ekeke, C. and C.A. Ogazie, 2020. Systematic significance of petiole anatomical characteristics in some members of Asteraceae from some parts of Nigeria. Singapore J. Sci. Res., 10: 387-399.
27. Ishida, A., K. Yazaki and A.L. Hoe, 2005. Ontogenetic transition of leaf physiology and anatomy from seedlings to mature trees of a rain forest pioneer tree, *Macaranga gigantea*. Tree Physiol., 25: 513-522.
28. Hussin, K.H., B.A. Wahab and C.P. Teh, 1996. Comparative leaf anatomical studies of some *Mallotus* Lour. (Euphorbiaceae) species. Bot. J. Linn. Soc., 122: 137-153.

29. Sierra, S.E.C. and P.C. Van Welzen, 2005. A taxonomic revision of *Mallotus* section *Mallotus* (Euphorbiaceae) in Malesia. Blumea Biodivers. Evol. Biogeogr. Plants, 50: 249-274.
30. de S. Secco, R. and A.M. Giulietti, 2004. Synopsis of the species of *Alchornea* (Euphorbiaceae, Acalyphoideae) in Argentina. Darwiniana, 42: 315-331.
31. Webster, G.L., 2013. Euphorbiaceae. In: The Families and Genera of Vascular Plants, Kubitzki, k. (Eds.), Springer Berlin Heidelberg, Germany, pp: 51-216.
32. van Welzen, P.C. and L.J. Bulalacao, 2007. The genus *Alchornea* (Euphorbiaceae) in the Malay Archipelago and Thailand. Syst. Bot., 32: 803-818.
33. Kamel, E.A. and M.H.A. Loutfy, 2001. The significance of cuticular features, petiole anatomy and SDS-PAGE in the taxonomy of the lauraceae. Pak. J. Biol. Sci., 4: 1094-1100.
34. Al-Edany, T.Y. and S.A.A.M. Al-Saadi, 2012. Taxonomic significance of anatomical characters in some species of the family Myrtaceae. Am. J. Plant Sci., 3: 572-581.
35. Baruah, S., D. Baro and S.K. Borthakur, 2017. Petiole anatomy of Indian species of the genera *Smilax* L. and *Heterosmilax* Kunth. (Smilacaceae). Ann. Plant Sci., 6: 1690-1693.
36. Azimi, R.A.A., Z. Jamzad, F. Sefidkon, B.K.G. Reza, 2006. The potential value of phytochemical and micromorphological characters in taxonomic treatment of genus *Vitex* L. (Lamiaceae). Iran. J. Bot., 12: 15-35.
37. Zoric, L., L. Merkulov, J. Lukovic and P. Boza, 2012. Comparative analysis of qualitative anatomical characters of *Trifolium* L. (Fabaceae) and their taxonomic implications: Preliminary results. Plant Syst. Evol., 298: 205-219.
38. Bovini, M.G. and J.F.A. Baumgratz, 2016. Taxonomic revision of *Wissadula* (Malvoideae, Malvaceae) in Brazil. Phytotaxa, 243: 201-234.
39. Noraini, T., A.R. Ruzi, B.S. Ismail, B.U. Hani, S. Sahimi and J.A. Azeyanty, 2016. Petiole vascular bundles and its taxonomic value in the tribe dipterocarpeae (Dipterocarpaceae). Sains Malays., 45: 247-253.