

Taxonomic study on *Lindernia* All. (*Linderniaceae*) from some parts of South Eastern Nigeria

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Abstract

The morpho-anatomical characteristics of *Lindernia diffusa* var. *diffusa* in E. & P., *L. oliveriana* (Benth.) Wettst. and *L. crustacea* (L.) F. Muell.) were investigated with a view of providing additional line of evidence in the delimitation of the species in Nigeria. The specimens were subjected to microtomy. The anatomy, fruit morphology, leaf shape, number of fruits per node and, flower colour differed among the species. The species have two trichome types (glandular and non-glandular) with varying number of gland head and basal cells. The glandular trichomes have 4-7 gland heads and 4-8 basal cells while the non-glandular type-1 and 2 trichomes with 5-10 basal cells. Type-1 non-glandular trichome occurred in *L. crustacea* and *L. diffusa* var. *diffusa* while type-2 occurred in *L. oliveriana*. The palisade and spongy mesophylls are 1-layer and 3-5 layers respectively in *L. diffusa* var. *diffusa*, 1-2 layers and 4-6 layers in *L. oliveriana* and 2-3 layers and 3 layers in *L. crustacea*. The palisade and spongy mesophylls are poorly differentiated. Two to five vascular bundles at the basal and distal ends of the petioles were observed in the *Lindernia* species with the main vascular bundle forming circular-united or fused vascular bundles in *L. diffusa* var. *diffusa* and *L. crustacea* and arced or horizontally elongated vascular bundle separated by parenchymatous rays in *L. oliveriana*. In *L. diffusa* var. *diffusa*, the pith covered 55.99% of the stem, 24.27% in *L. crustacea* and 72.74% in *L. oliveriana*. These characters could be used to delimit the species studied.

Keywords: anatomy; mesophyll; morphology; palisade; spongy; trichome; vascular bundle

Introduction

The family Scrophulariaceae s.l. has been classified in modern phylogenetic study into different families one of which is Linderniaceae by different taxonomists. Also, this family (*Scrophulariaceae*) is considered problematic (Saikat, 2015). Traditionally, the genus *Lindernia* All. belongs to the family Scrophulariaceae Juss (Liang *et al.*, 2012). Rahmzabeh *et al.* (2005) grouped the genus *Lindernia* into the family Linderniaceae (Rechb.) Borsch, K. Müller, and Eb. Fisch., and was accepted by APG III (2009). Although the classification of this genus has been a challenge, the system employed by Pennell (1935) has been used and accepted by several authors (Li, 1950; 1961; Yamazaki, 1977; Tsoong and Ku, 1979). Fischer *et al.* (2013) proposed a new

circumscription of the genera, reducing *Lindernia* to ca. 30 species. In order to simplify the identification of the taxa and to avoid classification issues, we adopted a broader circumscription of *Lindernia sensu lato* Rahmanzadeh *et al.* (2005), also adopted by Souza and Giulietti (2009) and the List of Species of the Brazilian Flora (BFG 2015). This genus is pan tropical in distribution with its highest abundance in Asia, Africa, and America and contains approximately 100 species (Lewis, 2000; Liang *et al.*, 2012; Wannan, 2016). The morphology of the members of this genus has been described in Australia (Barker, 2018) and in India (Saranya and Anto, 2017). In West Africa, Hutchinson and Dalziel (1963) reported and described 8 species of the genus.

Lindernia All. is a hydrophytic genus, often regarded as a common weed and found in agricultural and disturbed sites and sometimes in damp places, roadsides, marshy or wetlands (Lansdown, 2011; Randall, 2012). Linderniaceae has great morphological diversity, from aquatic herbs to poikilohydric rupestrian subshrubs (André and Nara, 2016). The trado-medicinal potentials of *Lindernia* have not been explored probably because it is regarded as a weed in that region. In other parts of the world Petard (1972) in Whistler (1992) reported that *L. crustacea* is used in the treatment of umbilical infections, jaundice, boils and colic. In Brunei, a mixture of the powdered herb is mixed with rice water to relieve diarrhoea, vomiting and cholera. It is a very common herb in Chinese pharmacy (Flora of China, 2015; PROTA, 2015). Wannan (2013 and 2016) have described the floral parts of some members of this genus. Also, few works have been done on stem anatomy (Wannan, 2016). Although the anatomical studies of this genus have been performed, most are related to seeds (Santos, 2003; Martini *et al.* 2008), whereas the vegetative organs remain poorly investigated (Metcalf and Chalk, 1950; Nakayama *et al.*, 1996; Ferreira *et al.*, 2008).

In Nigeria, the genus is widely distributed and their morphological descriptions have been reported (Hutchinson and Dalziel, 1963). Scanty or no information is available on the anatomy of *Lindernia* species from Nigeria. The present study is geared towards providing comparative morphological and anatomical studies on three *Lindernia* species (*L. diffusa* var. *diffusa* in E. & P., *L. oliveriana* (Benth.) Wettst. and *L. crustacea* (L.) F. Muell.) with a view of providing anatomical information as evidence in the delimitation of the species in Nigeria.

Materials and Methods

Plant materials

Three *Lindernia* species namely *L. diffusa* var. *diffusa*, *L. oliveriana* and *L. crustacea* were collected from some parts of Nigeria (Table 1). The plants were authenticated and voucher specimens were deposited in the University of Port Harcourt Herbarium, Nigeria.

Morphological studies

Observations on the morphological characters (vegetative and floral) were made on the *Lindernia* species from Southern Nigeria. Photographs representing the different plant parts were taken to show some of the diagnostic characters. Other parameters (plant height, leaf shape, length, width etc.) were taken.

Microscopic analysis

For the anatomical study, fresh leaves, petioles, and stems were fixed in AA (1:3) of acetic acid (30%) and ethanol (70%). Fixation, embedding, sectioning, epidermal mechanical scrapping and staining were done according to the procedures by Johansen (1940) and Sass (1951) with suitable modification (Metcalf and Chalk, 1979; Ekeke and Agogbua, 2019). Stem, petiole and midrib transverse section of the specimens were made with a sliding microtome at 20-30 µm thickness and stained in 1% safranin O in 50% alcohol and 1% alcian green in 100 ml purified water with three drops of acetic acid. Sections of the leaf marginal and lamina were made from the middle and marginal parts of the leaf lamina using a sliding microtome. The sections were

observed under a microscope with clear microphotographs of fine sections taken using Leica WILD MPS 52 microscope fitted with Amscope digital camera.

Table 1. List of *Lindernia* species studied

S/N	Species name	Date of collection	Herbarium number	Name(s) of collector	Locality
1	<i>Lindernia diffusa</i> var. <i>diffusa</i> -in E. & P.	10/10/2019	UPH/V/1453	Ekeke, Chimezie	Obiga-Asa, Ukwu-West LGA, Abia State
2	<i>L. oliveriana</i> Dandy	19/11/2019	UPH/V/1452	Ekeke & Ogazie	Civil Eng. Building, University of Port Harcourt, Rivers State
3	<i>L. crustacea</i> (L.) F. Muell.	09/08/2019	UPH/V/1454	Ekeke & Ogazie	Behind Convocation Arena, University of Port Harcourt, Rivers State
4	<i>Lindernia diffusa</i> var. <i>diffusa</i> -in E. & P.	19/11/2019	UPH/V/1451	Ekeke & Ogazie	Agricultural Farm, University of Port Harcourt, Rivers State

Results

Morphological characters

Lindernia diffusa var. *diffusa*

Stem 10-15 cm tall, generally branched, decumbent or prostrate. Leaves are opposite, partly hairy to glabrous, 1.0-1.5 cm wide, 1.2-2.4 cm long, lamina ovate and petiole 0.2-0.3 cm long. Flower solitary, calyx tube including flower 0.7-1.0 cm long, white, and stalk 0.1-0.3 cm long. Fruit solitary, 0.2-0.3 cm wide, 0.8-1.1 cm long, and stalk 0.2-0.3 cm long (Figure 1).

Lindernia oliveriana

Stem 10-25 cm tall, generally branched, decumbent or prostrate and rectangular. Leaves are opposite, partly hair to glabrous, 0.8-1.8 cm wide, 2.5-3.5 cm long, lamina ovate and petiole 0.8-1.4 cm long. Flower including calyx tube 1.2-1.5 cm long, blue with white and yellow patches or markings, solitary stalk 1.0-1.3 cm long, and 2-5 per node. Fruit 3-4 at each node, 0.2-0.4 cm wide, 1.2-1.6 cm long, and stalk 1.0-1.6 cm long (Figure 2).

Lindernia crustacea

Stem 10-20 cm tall, generally branched, decumbent or prostrate. Leaves are opposite, partly hairy to glabrous, 0.3-0.5 cm wide, 1.4-1.8 cm long, lamina ovate to elliptic and petiole 0.5-0.7 cm long. Flower including calyx tube 1.1-1.8 cm long, blue, solitary 2-4 per node and stalk 0.3-0.5 cm long. Fruit 2-3 at each node, 0.3-0.5 cm wide, 0.6-1.0 cm long, and stalk 1.4-3.0 cm long (Figure 3).



Figure 1. Morphological features of *L. diffusa*; (A) habit; (B) leaves and fruits

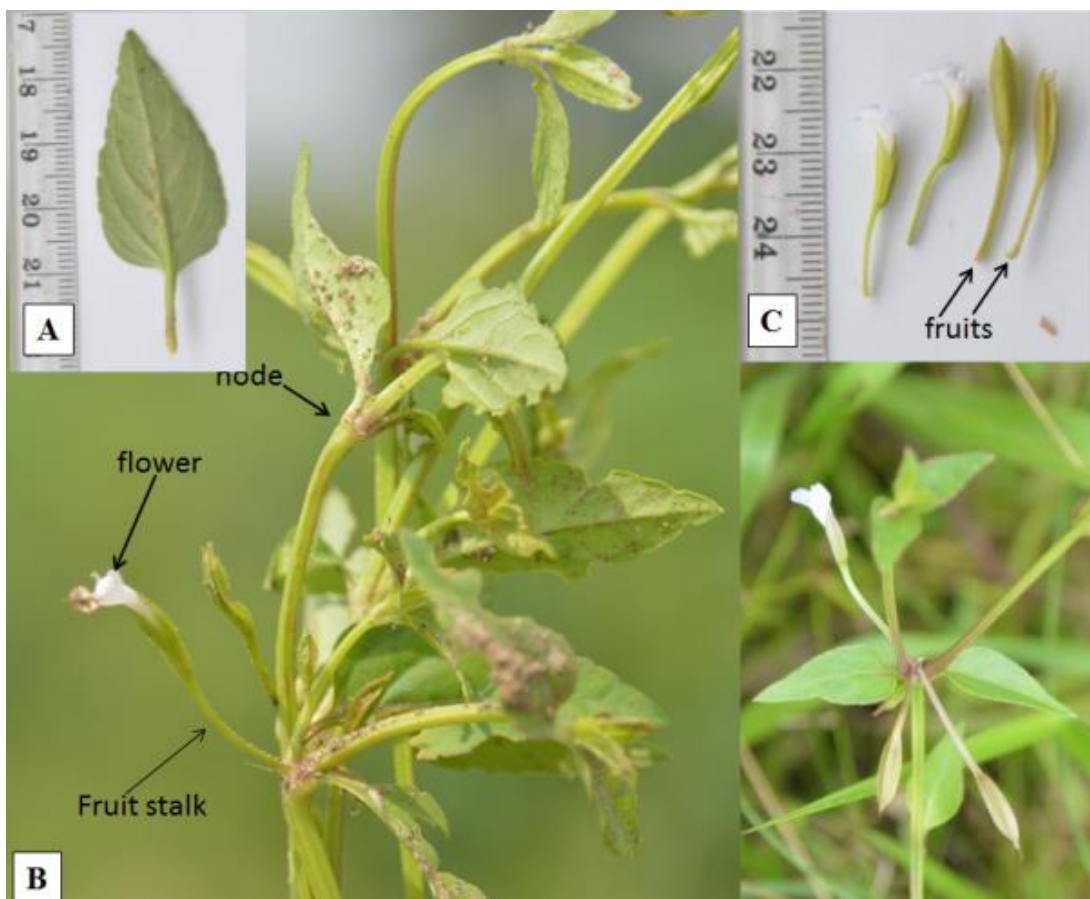


Figure 2. Morphological features of *L. oliveriana*; (A) leaf, (B) whole plant and (C) fruits and flowers

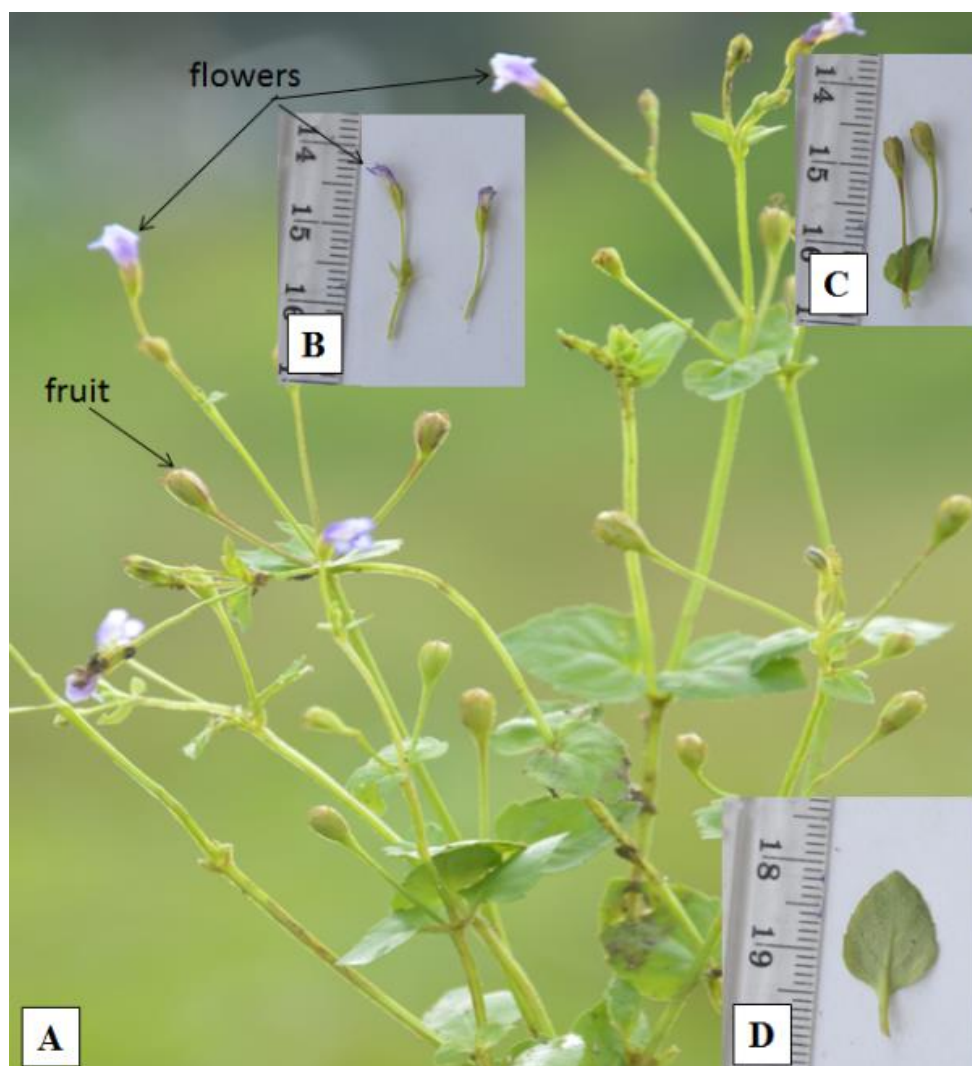


Figure 3. Morphological features of *L. crustacea*; (A) whole plant; (B) flowers; (C) fruits; (D) leaf

Anatomical characters

Lindernia crustacea

Epidermal characteristic. This species is amphistomatic, adaxial epidermis has tetracytic, anisocytic, and diacytic stomata (Figure 4A). The epidermal cells are irregular in shape with curved to wavy cell wall. The abaxial epidermis has anisocytic and isotricytic stomata, epidermal cells are irregular in shape with wavy or undulating cell wall (Figure 4B).

Midrib. Upper cuticle wavy and subcircular on the lower surface, vascular bundle collateral, from small arc 35 x 26 μm surrounded by parenchymatous cells (Figure 5A). Adaxial cortex 7 layers, 62 μm thick, epidermal cells 4-6 μm thick while the abaxial cortex consists 6 layers, 46 μm thick and epidermis 4-5 μm thick and the parenchymatous cortex oval in shape (Table 2).

Lamina. Palisade mesophyll 2-3 layers, 17-23 μm thick, cylindrical; spongy mesophyll 3 layers, 12-20 μm thick, loosely packed; vascular bundles embedded in the mesophyll (Figure 5B, Table 3), stomata on adaxial cuticle slightly elevated, lamina with many secretory cells, peltate trichome obvious on the adaxial and abaxial surfaces (Figure 5D). Leaf margin slightly bent adaxially, 21 μm thick with 6 cells (Figure 5E).

Petiole. A transverse section taken from the distal part (Figure 8A). Adaxial cuticle V-shaped, epidermis 4-10 μm thick; abaxial cuticle subcircular, epidermis 4-8 μm thick; rib trace three each on both wings. Adaxial cortex 5-6 layers, 20 μm thick; abaxial cortex 7-8 layers, 86 μm thick; vascular bundles collateral, 28 x 20 μm ,

surrounded crushed parenchymatous cells (Table 2). A transverse section taken from the basal part (Figure 8B). Adaxial cuticle sub-circular, 4-6 μm thick; abaxial cuticle curved, 3-4 μm thick; rib trace one-each on both wings (Fig. 8B). Adaxial cortex 8 layers, 64 μm thick; abaxial cortex 4-5 layers, 11 μm thick; vascular bundles collateral, 44 x 30 μm , surrounded by parenchymatous pericycle vessels separated by prerenchymatous rays (Table 2).

Stem. Four-angled with ridges with sclerenchymatous fibre on each ridge, parenchymatous cortex 6-7 layers, 47-63 μm thick; pith 32-43 μm wide covering 24.27% of the stem, vascular bundle ring 74-85 μm and ridges 12-18 μm thick (Table 4).

Fruit stalk. Fruit is polygonal with two protuberances, cortex consists of 4-5 layers, central vascular bundle circular, united and the vessels arranged in radial multiples (Figure 10D).

Table 2. Anatomical characteristics of petiole and midrib of *Lindernia* species studied

Species name	Plant part	Epidermal thickness (μm)		Cell ratio (L/W)		Size of Vb (μm)		TOC (μm)		Ratio (Ad/Ab)
		Ad	Ab	Ad	Ab	L	W	Ad	Ab	
<i>L. diffusa</i> var. <i>diffusa</i>	Petiole basal	5 – 14	5 – 7	1.15	1.13	45	18	60	24	2.5
	Petiole distal	4 – 6	6 – 10	1.02	0.64	39	21	51	14	3.64
	Midrib	6 – 8	6 – 9	0.70	0.86	33	12	38	16	2.38
<i>L. crustacea</i>	Petiole basal	4 – 6	3 – 4	1.04	0.89	44	30	64	11	5.82
	Petiole distal	4 – 10	4 – 8	0.91	0.86	51	28	20	86	0.23
	Midrib	4 – 6	4 – 5	0.81	0.83	35	26	62	46	1.35
<i>L. oliveriana</i>	Petiole basal	2 – 3	4 – 7	0.82	0.96	100	22	87	40	2.18
	Petiole distal	3 – 5	4 – 5	1.18	0.86	124	31	88	46	1.91
	Midrib	2 – 3	4 – 7	0.75	1.08	56	27	41	25	1.64

Note: Ad- adaxial surface, Ab-abaxial surface, L - length, W - width, TOC - thickness of cortex

Lindernia oliveriana

Epidermal characteristic. This species is amphistomatic, adaxial epidermis has tetracytic, isotricytic and anisocytic stomata (Figure 4C). The epidermal cells are irregular in shape with undulating cell wall. The abaxial epidermis has isotricytic stomata epidermal cells are polygonal in shape with straight or curved cell wall (Figure 4D).

Midrib. Upper cuticle curved/relatively flat, subcircular on the lower surface, vascular bundle with parenchymatous rays, arc 56 x 27 μm , collateral. Adaxial cortex 7-9 layers, 41 μm thick, epidermal cell 2-3 μm thick while the abaxial cortex had 5-7 layers, 25 μm thick, epidermis 4-7 μm thick and oval in shape (Figure 6A).

Lamina. 38-40 μm thick, palisade mesophyll 1-2 layered, 11-15 μm thick, cylindrical; spongy mesophyll 4-6 layers, 14-19 μm thick, loosely packed with air spaces (Figure 6C, Table 3), stomata on adaxial cuticle slightly elevated, lamina with many secretory cells, vascular bundles embedded in the mesophyll, peltate trichome obvious on the abaxial surface (Figure 6E). Leaf margin slightly bent adaxially, 33 μm . Adaxial epidermis 3-7 μm thick and epidermis 5-9 μm thick.

Petiole. A transverse section taken from the distal part (Figure 8C). Adaxial cuticle U-shaped, epidermis 3-5 μm thick; abaxial cuticle subcircular, epidermis 4-8 μm thick; rib trace two-each on both wings. Vascular bundle separated by parenchymatous fibres (forming about 5 traces). Adaxial cortex 8-10 layers, 88 μm thick, epidermis 3-5 μm thick; abaxial cortex 6-7 layers, 46 μm thick; vascular bundles collateral, arced, xylem separated by parenchymatous rays, 124 x 31 μm , surrounded by parenchymatous cells (Table 2). A transverse section is taken from the basal part (Figure 8D). Adaxial cuticle U or V-shaped, epidermis 2-3 μm thick; abaxial cuticle curved, 2-3 μm thick; rib trace one-each on both wings (Figure 8D). Adaxial cortex 7-8 layers, 87 μm thick; abaxial cortex 5-7 layers, 40 μm thick; vascular bundles collateral, arced, xylem separated by

parenchymatous rays, 100 x 22 μm , surrounded by parenchymatous pericycle vessels separated by parenchymatous rays (Table 2).

Stem. Four-angled with ridges, parenchymatous cortex 4-6 layers, 16-31 μm thick; pith 142-161 μm wide covering 72.74% of the stem, 6-vascular bundle in an open ring, 13-18 μm (Table 4, Figure 9B).

Fruit/fruit stalk. Fruit has five long projections and 201 μm in diameter (Figure 10B). Fruit with two ridges/projections, cortex 4-6 layers, central vascular bundle circular, united, covering about 20.0% (Figure 10E).

Table 3. Anatomical characteristics of lamina of *Lindernia* species studied

Parameter (s)		<i>L. diffusa</i> var. <i>diffusa</i>	<i>L. crustacea</i>	<i>L. oliveriana</i>
Thickness		41 – 49	41 – 46	38 – 40
Thickness of palisade mesophyll		8 – 15	17 – 23	11 – 15
Layers of palisade mesophyll		1	2 – 3	1 – 2
Thickness of spongy mesophyll		9 – 31	12 – 20	14 – 19
Layers of spongy mesophyll		3 – 5	3	3 – 4
Ratio (palisade/ spongy)		0.72	1.32	1.19
Thickness of leaf margin		15 – 16	21 – 34	24 – 30
Epidermal cell size	Adaxial	3 – 4	2	3 – 7
	Abaxial	5 – 7	4 – 6	5 – 9
Epidermal cell ratio (length/width)	Adaxial	1.06	1.80	1.22
	Abaxial	1.51	1.30	2.19
Trichome	Glandular	Gland head 4-5, base 4-8 cells	Gland head 4, base 4-7 cells	Gland head 4, base 4-7 cells
	Non-glandular	Type-1, base 8-9 cells	Type-1, base 5-6 cells	Type-2, base 7-10 cells

Table 4. Anatomical characteristics of stem of *Lindernia* species studied

Parameter (s)	<i>L. diffusa</i> var. <i>diffusa</i>	<i>L. crustacea</i>	<i>L. oliveriana</i>
Diameter (μm)	189 - 197	212 – 227	264 – 285
Parenchyma/cortex thickness (μm)	18 – 25	47 – 63	16 – 31
Pith thickness (μm)	65 – 82	32 – 43	142 – 161
Epidermal width (μm)	3 – 5	2 – 5	2 – 4
Epidermal length (μm)	3 – 6	2 – 4	1 – 3
Epidermal ratio	1.06	1.25	0.69
Ridge (μm)	12 – 16	12 – 18	13 – 20
Sclerenchyma (μm)	10 – 12	12 – 14	13 – 18
Diameter of vascular bundle ring (μm)	98 – 100	74 – 85	174 – 195
Area covered by pith (%)	55.99	24.27	72.74

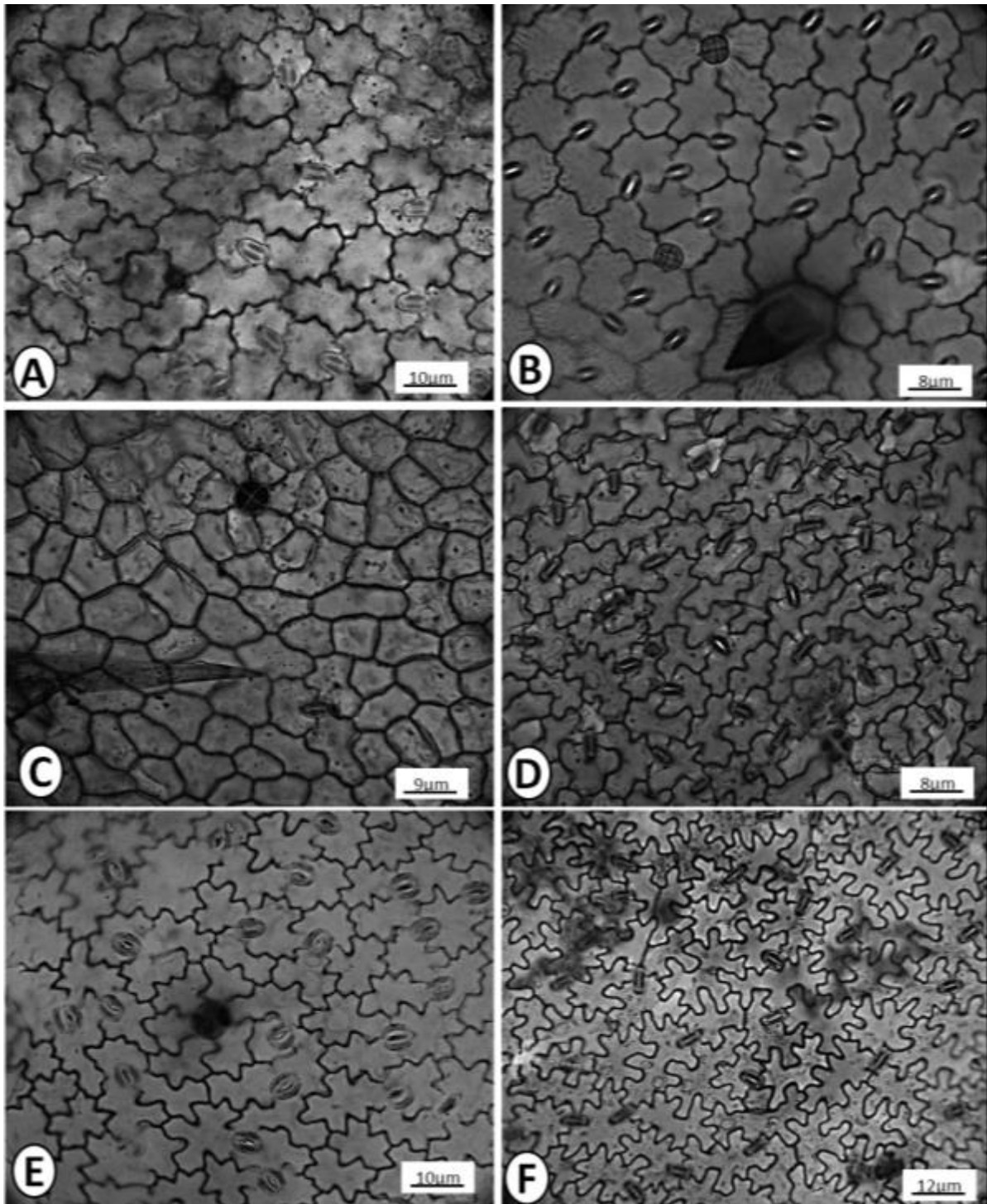


Figure 4. Epidermal characteristics of the *Lindernia* species studied; (A) abaxial surface of *L. crustacea*; (B) adaxial surface of *L. crustacea*; (C) abaxial surface of *L. oliveriana*; (D) adaxial surface of *L. oliveriana*; (E) abaxial surface of *L. diffusa* var. *diffusa*; (F) adaxial surface of *L. diffusa* var. *diffusa*

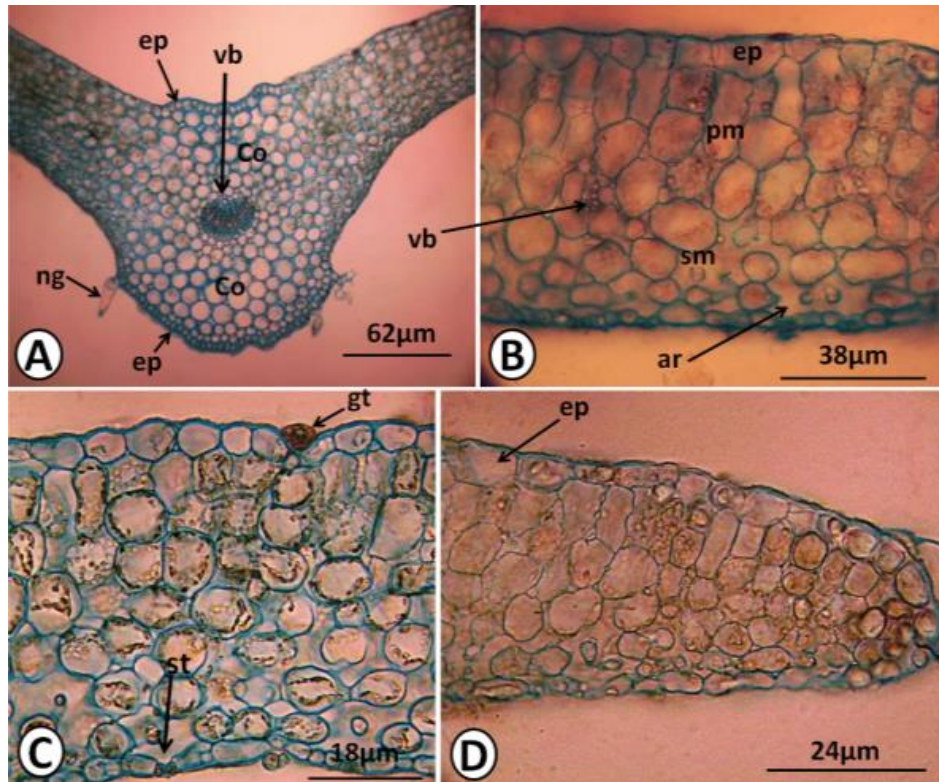


Figure 5. Transverse section of midrib and leaf lamina of *L. crustacea*; (A) midrib; (B and C) leaf lamina; (D) leaf margin (ep-epidermis, ar-air spaces, gt-glandular trichome, Co-cortex, st-stomata, vb-vascular bundle, pm-palisade mesophyll, sm-spongy mesophyll, ng-non-glandular trichome)

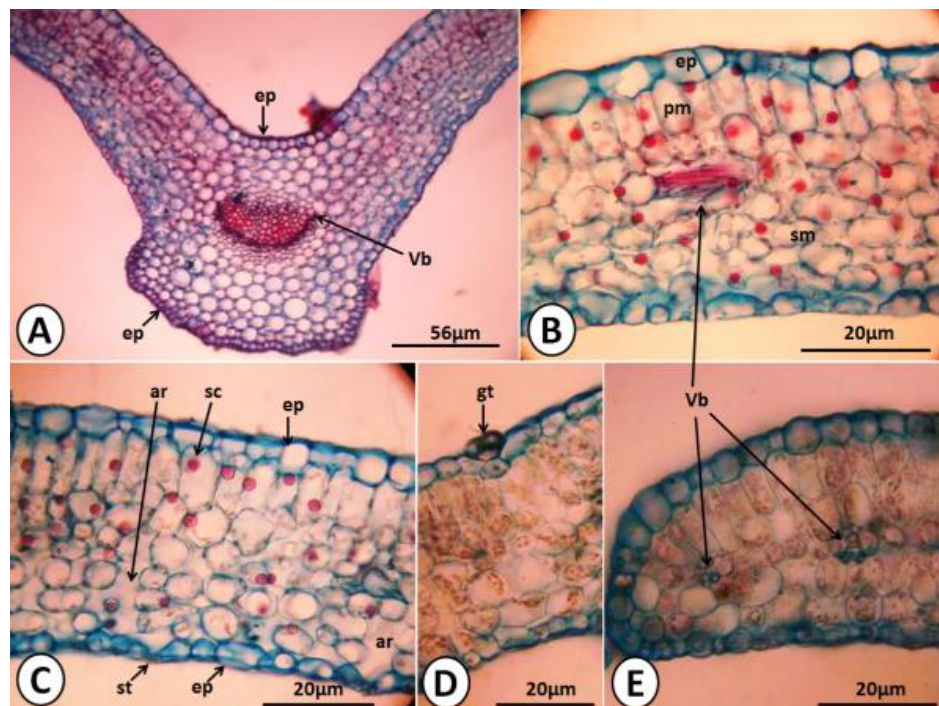


Figure 6. Transverse section of midrib and leaf lamina of *L. oliveriana* (A) midrib, (B, C and D) lamina and (E) leaf margin (ep-epidermis, ar-air spaces, gt-glandular trichome, Co-cortex, st-stomata, vb-vascular bundle, pm-palisade mesophyll, sm-spongy mesophyll, sc-secretory cell)

Lindernia diffusa var. *diffusa*

Epidermal characteristics. This species is amphistomatic, adaxial epidermis has tetracytic, anisocytic, and rarely diacytic (Figure 4E). The epidermal cells are irregular in shape with curved to wavy cell walls. The abaxial epidermis has anisocytic, isotricytic and contiguous stomata, epidermal cells are irregular in shape with wavy or undulating cell wall (Figure 4F).

Midrib. Upper cuticle wavy and subcircular on the lower surface, vascular bundle collateral, from small arc 33 x 12 µm surrounded by parenchymatous cells. Adaxial cortex 4-5 layers, 38 µm thick, epidermal cells 6-8 µm thick while the abaxial cortex had 2-3 layers, 16 µm thick and 6-9 µm thick and parenchymatous cortex oval in shape (Figure 7A).

Lamina. 41-49 µm thick, Palisade mesophyll 1 layer, 8-15 µm thick, cylindrical; spongy mesophyll 3-5 layers, 9-31 µm thick, loosely packed, vascular bundles embedded in the mesophyll (Figure 7B, Table 3), stomata on adaxial cuticle slightly elevated, peltate trichome obvious on the adaxial and abaxial surfaces (Figure 7D). Leaf margin slightly bent adaxially, 16 µm thick with 6 cells (Figure 7E). Adaxial epidermis 3-4 µm thick and abaxial epidermis 5-7 µm thick

Petiole. A transverse section taken from the distal part (Figure 8E). Adaxial cuticle curved, epidermis 4-6 µm thick; abaxial cuticle subcircular, epidermis 6-10 µm thick; rib trace one-each on both wings. Adaxial cortex 7 layers, 51 µm thick; abaxial cortex 4 layers, 14 µm thick; vascular bundles collateral, oval in shape, 39 x 21 µm, surrounded crushed parenchymatous cells (Table 2). A transverse section is taken from the basal part (Figure 8F). Adaxial cuticle V-shaped, epidermis 5-14 µm thick; abaxial cuticle curved, 5-7 µm thick; rib trace two-each on both wings (Figure 8F). Adaxial cortex 5-6 layers, 60 µm thick; abaxial cortex 2-3 layers, 24 µm thick; vascular bundles collateral, oval in shape, 45 x 18 µm, surrounded by parenchymatous pericycle vessels separated by parenchymatous rays (Table 2).

Stem. Four-angled with ridges and sclerenchymatous fibre on each ridge, parenchymatous cortex 3-4 layers, 18-25 µm thick; pith 65-82 µm wide covering 55.99% of the stem, vascular bundle ring 74-85 µm and ridges 12-16 µm thick (Table 4, Figures 9C and D).

Fruit/fruit stalk: Fruit with 5 projections and 280 µm in diameter (Figure 10C). Fruit stalk rectangular with four protuberances, cortex consists of 3-5 layers, central vascular bundle circular, relatively separate and partly joined by small ring by parenchymatous fibre (Figure 10F), vascular bundle covered 24.36% of the fruit stalk.

Trichome types. Among the species studied, we recorded two main trichome types namely glandular and non-glandular trichomes (Figure 11, Table 3). The glandular trichomes have peltate head ie. 4-gland heads (Figures 11A-11E), 5-gland heads (Figures 11F-11H), 6-gland heads (Figures 11I and 11J) and 7-gland heads (Figure 11K). The number of the cells at the base of the trichomes varied from 3-7 and 5-10 cell in glandular and non-glandular trichomes respectively. Two morphological types of non-glandular trichomes were observed: the type-1 (Figures 11L and 11M) found in *L. diffusa* var. *diffusa* and *L. crustacea* while the type-2 (Figures 11N - 11Q) found in *L. oliveriana*.

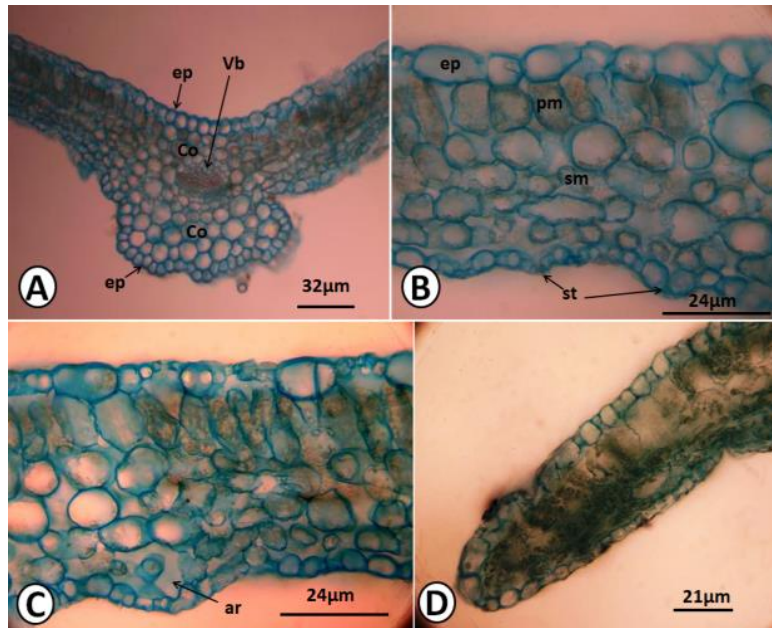


Figure 7. Transverse section of midrib and leaf lamina of *L. diffusa* var. *diffusa* '(A) midrib, (B and C) lamina and (D) leaf margin (ep-epidermis, ar-air spaces, gt-glandular trichome, co-cortex, st-stomata,vb-vascular bundle, pm-palisade mesophyll, sm-spongy mesophyll)

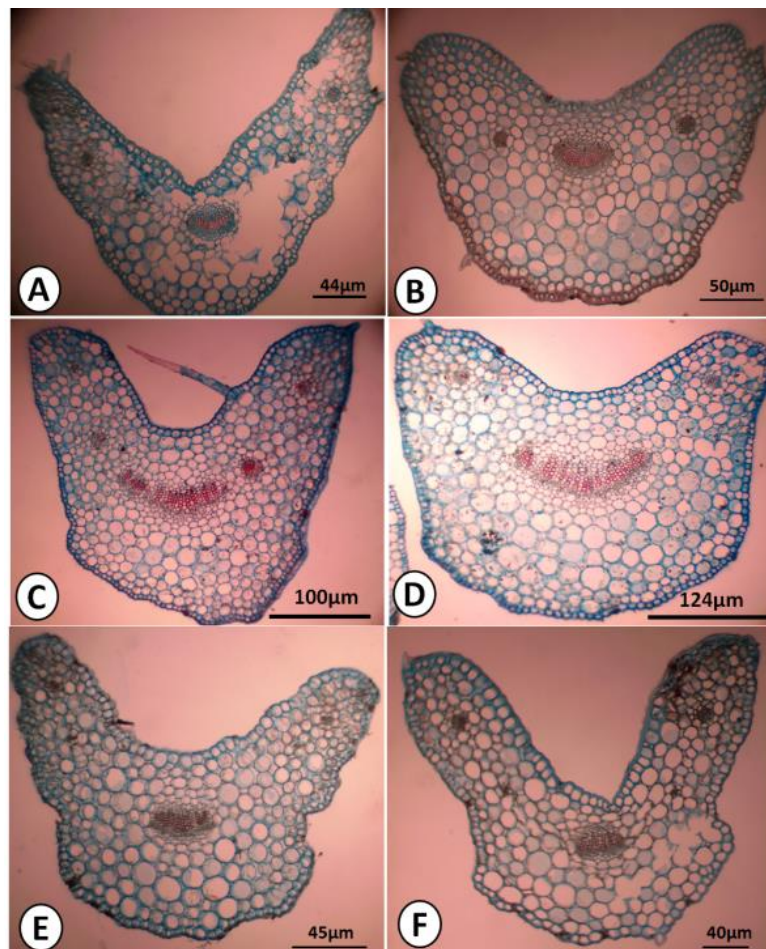


Figure 8. Transverse section of the basal and distal portions of petiole in *Lindernia* species studied; (A) and (B) *L. crustacea*; (C) and (D) *L. oliveriana*; (E) and (F) *L. diffusa* var. *diffusa*

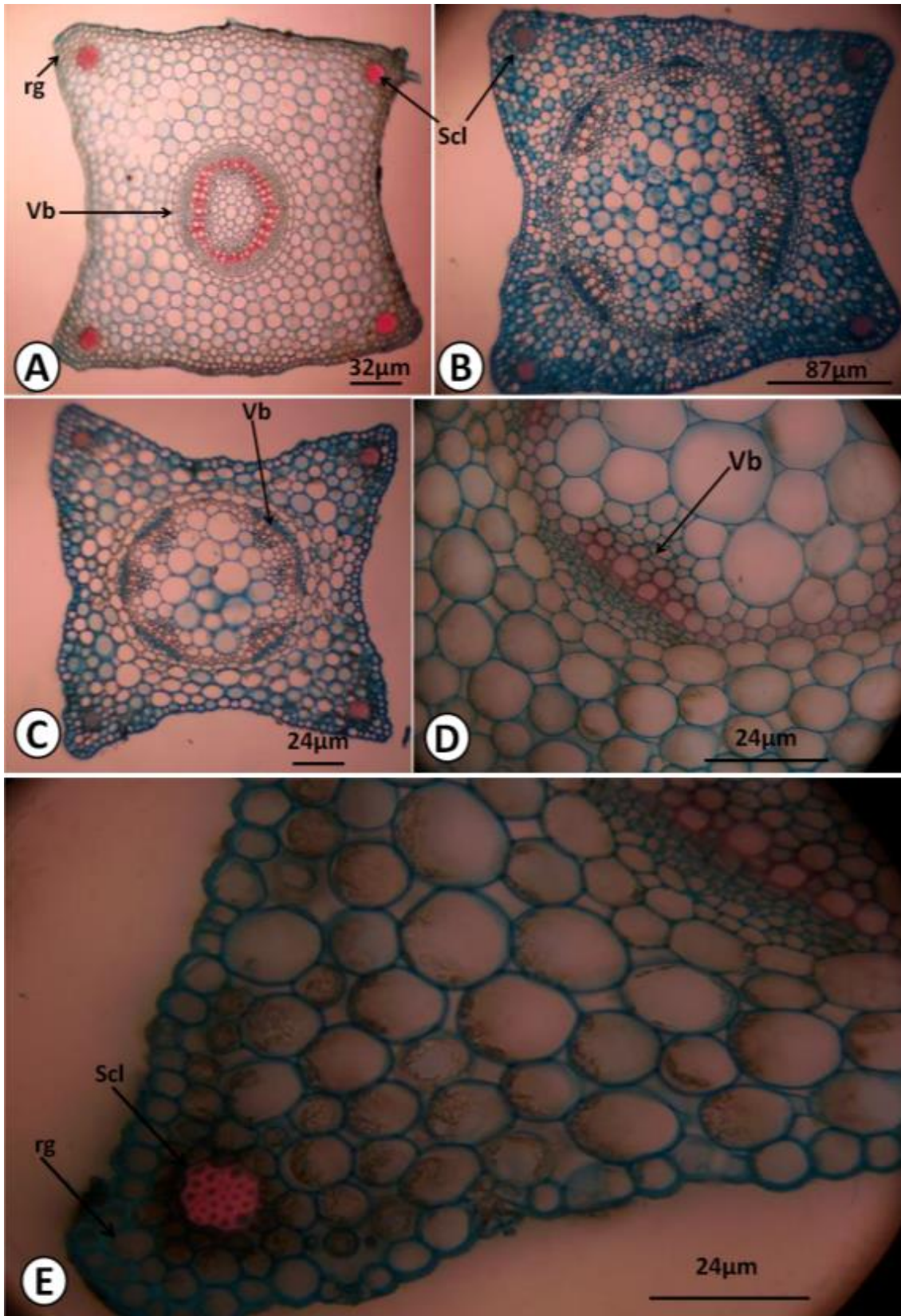


Figure 9. Stem anatomy of the *Lindernia* species studied; (A) *L. crustacea*; (B) *L. oliveriana*; (C and D) *L. diffusa* var. *diffusa*; (D) and (E) projection/ridge
 Note: scl - sclerenchymatous cell; vb - vascular bundle; rg - ridge

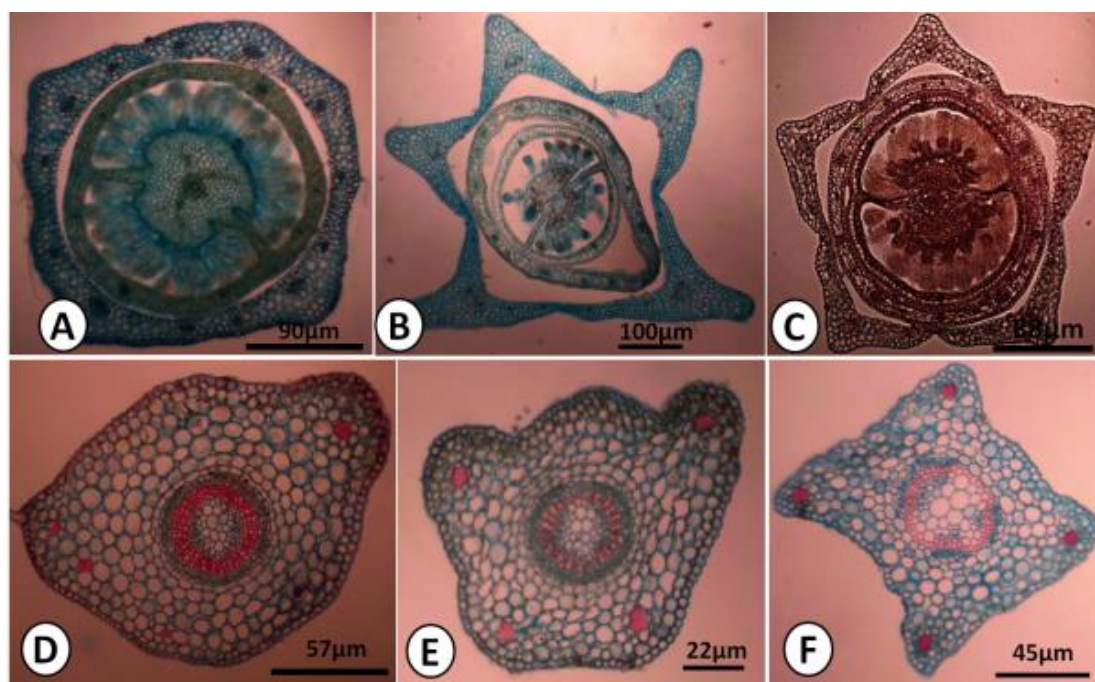


Figure 10. Transverse section of the flower and fruit stalk in *Lindernia* species studied; (A) and (D) *L. diffusa* var. *crustacea*; (B) and (E) *L. oliveriana*; (C) and (F) *L. diffusa* var. *difussa*

Discussion

This work seeks to provide additional information that will aid in the identification of *Lindernia*. In the other part of the world works have been carried out on the morphology (Mukerjee, 1945; Mirashi, 1956; Andre and Nara, 2016; Saranya and Anto, 2017; Barker, 2018), anatomy (Saeidi-Mehrvarz, 2004; Makbul *et al.*, 2006; Ayse *et al.*, 2007; Karima *et al.*, 2014; Saikat, 2015; Saranya and Anto, 2017), palynology (Ayse *et al.*, 2007) and, molecular biology (Olmstead *et al.*, 2001; Oxelmem *et al.*, 2005) of Scrophulariaceae. Most of these authors suggested reclassification of the members of this family based on their findings. However, among the Nigerian species of *Lindernia* except for morphological description by Hutchinson and Dalziel (1954), there is so far no work on the anatomy of members of this genus. We observed that among the taxa studied the stem anatomy including percentage cover of the pith in the stem, shape of the fruit shape of the fruit stalk were diagnostic.

Mukerjee (1945) has enumerated the morphological attributes of twenty-five species of *Lindernia* in India. He noted that members of this genus are mainly hydrophytes and grow in marshy and damp places. Also, Marishi (1956), Barker (2018), Saranya and Anto (2017), Balkrishna *et al.* (2017), and Andre and Nara (2016) reported that many members of this genus are annual herbs with erect, sub-erect or prostrate stems with several branches, slender, glabrous, sparsely hairy or hairy Wannan (2016) and Yi-Shuo *et al.* (2012). Our findings on these species corroborate with these reports. Furthermore, the fruit morphology (length of fruit and stalk), leaf shape, number of fruits per node and, flower colour differed among the species.

Among the genus *Scrophularia* L. (Scrophulariaceae) anomocytic stomata has been reported as the dominant stomata type (Makbul *et al.*, 2006) and amphistomatic in *Odontites* Ludwig (Saeidi-Mahrvarz, 2004). Also, the epidermal cells of some members of Scrophulariaceae are polygonal or irregular in shape with undulating or straight walls (Saeidi-Mahrvarz, 2004; Karima *et al.*, 2014). Our result of the epidermal characteristics is similar to the previous reports on Linderniaceae.

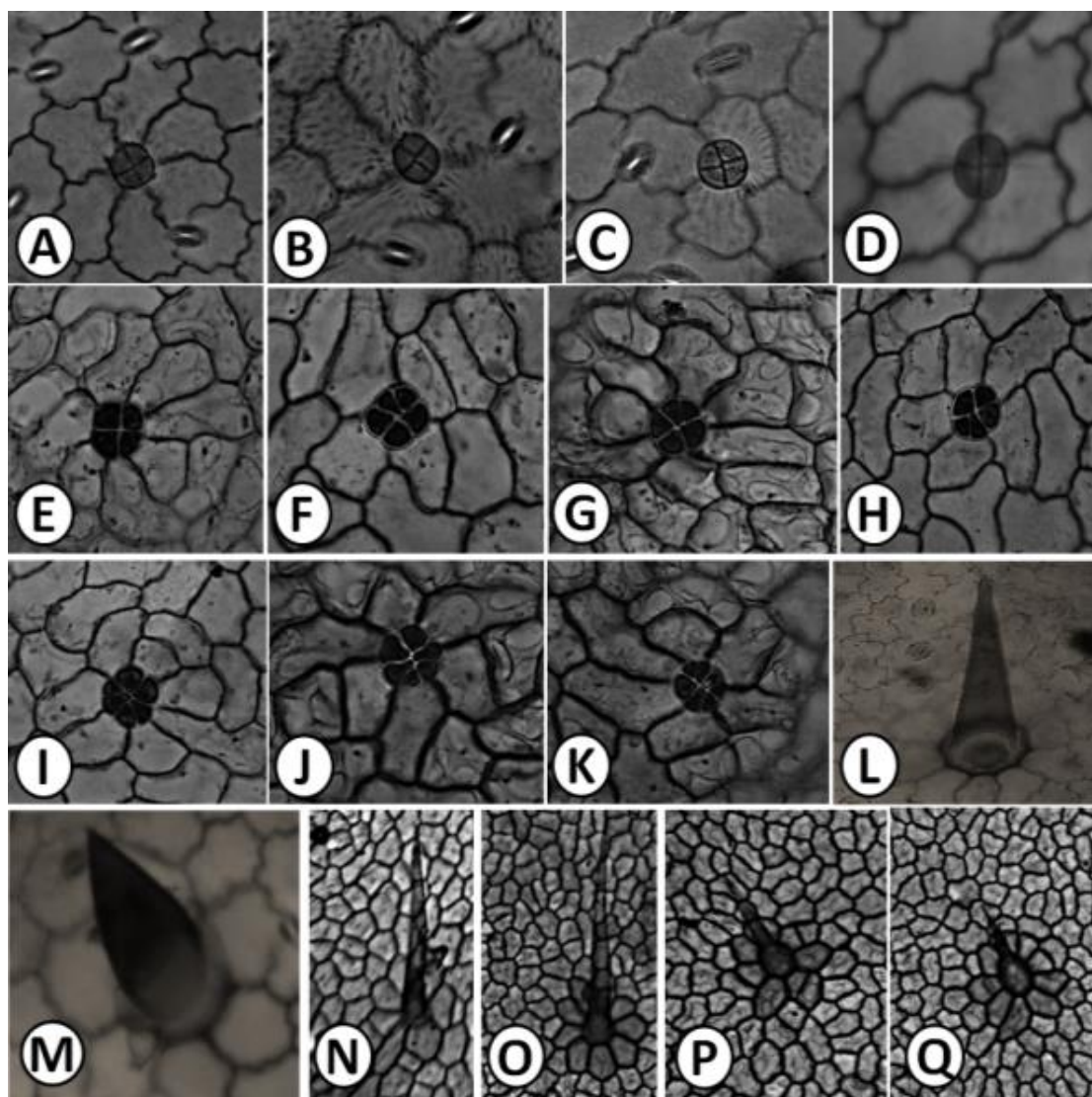


Figure 11. Trichome types in *Lindernia* species studied; (A-K) glandular trichomes; (L-Q) non-glandular trichomes

We have observed two different trichome types (glandular and non-glandular) in the taxa studied with varying number of gland head and basal cells. Glandular trichomes measuring 0.2-1.0 mm long with multicellular uniseriate stalk have been reported in the family Linderniaceae. Presence or absence and relative length on the vegetative part and inflorescence of these trichomes can be diagnostic at species or infraspecific level (Barker, 2018). Also, Barker (2018) observed that other tiny trichomes that terminated with four-celled glands, 15-30 μm in diameter occur across this family and are diagnostic. He further reported antrorse narrow deltoid single-celled trichome on the leaf, bract and sepal margins of Linderniaceae. Simple, unbranched, unicellular and multicellular glandular and non-glandular trichomes are one of the features of Scrophulariaceae (Saeidi-Mahrvarz, 2004; Makbul *et al.*, 2006; Ayse *et al.*, 2007; Karima *et al.*, 2014). Similar trichomes were observed in this study and their variation could be used to differentiate the species studied. The gland head in *L. crustacea* is 4 and basal cells 4-7, glandhead in *L. diffusa* var. *diffusa* is 4-5 and basal cells 4-8 and gland head in *L. oliveriana* 4-7 and basal cells 4-7. Also, type-1 non-glandular occurred in *L. crustacea* and *L. diffusa* var. *diffusa* and basal cells 5-6 and 8-9 respectively while type-2 occurred in *L. oliveriana* with basal cells 7-10.

Well-developed and differentiated or poorly differentiated spongy and palisade mesophyll occur in some members of Scrophulariaceae. Saeidi-Mahrvarz (2004) reported spongy mesophyll of 2-3 layers, 17-100 μm thick, palisade mesophyll of 2-6 layers, 50-250 μm thick with intercellular spaces and midrib of 185-350 μm thick in *Odontites* Ludwig. Also, spongy mesophyll of 2-6 layers and palisade mesophyll of 1-6 layers in *Scrophularia* L. (Makbul *et al.*, 2006) and spongy mesophyll of 2-4 layers and palisade mesophyll of 2-4 layers but not differentiated in *Veronica* L. (Ayse *et al.*, 2007). In this present study we recorded palisade mesophyll 1-layer, 8-15 μm thick, spongy mesophyll of 3-5 layers, 9-31 μm thick in *L. diffusa* var. *diffusa*, palisade mesophyll 1-2 layers, 11-15 μm thick, spongy mesophyll of 4-6 layers, 14-19 μm thick in *L. oliveriana* and palisade mesophyll 2-3 layers, 17-23 μm thick, spongy mesophyll of 3 layers, 12-20 μm thick in *L. crustacea*. The palisade and spongy mesophylls are poorly differentiated as reported by Ayse *et al.* (2007) in *Veronica* L.

In our study, we recorded 2-5 vascular bundles at the basal and distal ends of the petioles in *Lindernia* with the main vascular bundle forming circular-united or fused vascular bundles in *L. diffusa* var. *diffusa* and *L. crustacea* and arced or horizontally elongated vascular bundle separated by parenchymatous rays in *L. oliveriana*. Previous reports have shown 3 vascular bundles, arced, oval, rounded or horizontally elongated in *Scoparia dulcis*, *Majus pumulis*, *Mecardonia chemidroides*, *Bacopa monnieri*, *Antirrhinum majus* and *Scutellaria* sp. (Saikat, 2015).

We observed weak secondary growth in the same portion of stem in *L. oliveriana* and *L. crustacea* but well-developed secondary growth in *L. diffusa* var. *diffusa*. Also, this feature has been opined to be associated with some members of Scrophulariaceae (Saeidi-Mahrvarz, 2004). Furthermore, the stem of all the species studied have projections (ridges) with sclerenchymatous cells, a complete cambium ring and a parenchymatous central pith. In *L. diffusa* var. *diffusa*, the pith covered 55.99% (65 - 82 μm) of the stem, 24.27% (32 - 43 μm) in *L. crustacea* and 72.74% (142 - 161 μm) in *L. oliveriana*. Also, the diameter of the central varied; 98 - 100 μm in *L. diffusa* var. *diffusa*, 74 - 85 μm in *L. crustacea* and 174 - 195 μm in *L. oliveriana*. Stem projections or ridges with sclerenchymatous cells have been reported among *Scrophularia* (Scrophulariaceae) (Makbul *et al.*, 2006) and *Lindernia* (Linderniaceae) (Mirashi, 1956; Saranya and Anto, 2017). Also, percentage area occupied by cortex, xylem, and pith in the stem have reported among some members of the *Scrophularia* (Makbul *et al.*, 2006), and *Veronica* (Ayse *et al.*, 2007) to have diagnostic values. In the stem of *Lindernia*, varying layers of cortex 3-6 have been reported (Saranya and Anto, 2017). Other authors have also observed 6-8 layers of the cortex in *Scrophularia* (Makbul *et al.*, 2006) and 4-8 layers in *Veronica* (Ayse *et al.*, 2007) both in Scrophulariaceae. Our results are in consonance with the previous reports on the member of this family.

The anatomy of the *Lindernia* species in Nigeria is reported here for the first time. The anatomical characters varied among the species and could be used to delimit the species.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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