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COMPARATIVE MORPHOANATOMICAL STUDIES OF SOUTH EASTERN NIGERIAN REPRESENTATIVES OF *OLDENLANDIA* L. (RUBIACEAE)

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ABSTRACT

Leaf and stem anatomical structure of the four *Oldenlandia* L. (*O. affinis* (Roem. & Schult.) DC., *O. corymbosa* L., *O. herbacea* (Linn.) Roxb., and *O. lancifolia* (Schumach) DC.) from some parts viz. Ogbokor (Edo State), Obiga-Asa (Abia State), IITA staion Onne (Rivers State), and Agricultural farm Uniport (Rivers State) Nigeria were examined by light microscopy. The epidermal cells are pentagonal to polygonal with straight, curved or wavy anticlinal walls, and paracytic stomata. All the species have dorsiventral leaf with the leaf vein vascular bundles embedded in the spongy mesophyll. The midribs vascular bundles form an arc enclosed by parenchymatous endodermal cells. *O. herabcea* is amphistomatic while the other species are hypostomatic. Raphide bundles were seen only in the lamina of *O. corymbosa.* Tuft hair is absent in *O. herbacea* but occurred on the adaxial leaf surfaces of *O. affinis*, *O. corymbosa*, and *O. diffusa*. The stem of *O. diffusa* is terete while other species have quadrangular stem. Papillae occurred on the adaxial epidermis of *O. affinis* and *O. corymbosa.* The stem pith thickness (PT)/cortical thickness (ET) ratio varied among the species. Notable diagnostic features in these species include the PT/ET ratio, layers of cortex in the stem, occurrence of tuft hairs on the leaf veins and surface, presence or absence of raphides and papillose, layer of abaxial and adaxial cortex in the midrib, and amphistomatatic or hypostomatic leaf.

INTRODUCTION

The Rubiaceae is one of the largest West African flowering plant family and comprised of 91 genera including *Oldenlandia* L. (Hutchinson and Dalziel, 1954). *Oldenlandia* L. is pantropical in distribution with about 240 species worldwide (David, 2008; Inge *et al.*, 2009), and 12 speceis in West Africa out of which 10 occurs in Nigeria (Hutchinson and Dalziel, 1954). Among the Nigerian species, *Oldenlandia lancifolia* (Schumach) DC., *O. corymbosa* L., *O. affinis* (Roem. & Schult.) DC., and *O. herbacea* (Linn.) Roxb. occur as weeds of cultivated farms, open lawns, and and roadsides (Hutchinson and Dalziel, 1954; Akobundu *et al.*, 1998, 2016; Ekeke and Ogazie, 2017; Ekeke *et al*, 2019). A number of these species have ethnomedicinal uses (Chen et al., 1992; Ezeabara and Egwuoba, 2016; Yeon *et al.*, 2017). *Oldenlandia corymbosa* L.has anti-inflammatory and anti-bacterial properties (Chang *et al.*, 1986). It is also used in treatment or preventing stomach ache (Lin *et al.*, 2002). *O. herbacea* (Linn.) Roxb. is useful in treatment of elephantiasis, fever, dyspepsia, flatulence, colic, asthma,

bronchitis, ulcers and hydrocele Ezeabara and Egwuoba (2016), while *O. diffusa* extracts has anti-tumor effects (Yeon et al., 2017).

Though existing reports have shown that anatomical and epidermal characteristics contribute emmensely to the taxonomy of angiosperms and Rubiaceae in particular (Metcalfe & Chalk, 1972, 1979; Herman *et al.,* 1986; Kocsis and Borhidi, 2003; Patil and Patil, 2012; Mownika *et al.,* 2020; Tarsila *et al.,* 2021). Few existing reports on the genus *Oldenlandia* show that the genus has conflicting generic delimitation, due to different floral syndromes (Mariela *et al.,* 2016). Furthermore, anatomy and micro-morphology of Nigerian *Oldenlandia* species are yet to investigated. Therefore, the anatomical and micromorphological characters of the leaves and stems of *O. affinis, O. corymbosa*, *O. herbacea*, and *O. lancifolia* from selected sites in South-Eastern Nigeria were investigated and described to better understand the interspecific variation among these species, and contribute to the systematics of the genus.

MATERIALS AND METHOD

Sample collection and study area

Samples of *Oldenlandia* species were collected from four sites in South-Eastern Nigeria were identified, processed, catalogued and deposited in the University of Port Harcourt Herbarium (Table 1). The anatomical analysis was carried out in the Plant Biosystematics and Taxonomy Research Laboratory, Department of Plant and Biotechnology, Faculty of Science, University of Port-Harcourt between December 2019 and April 2021.

Table 1: List of voucher specimens of *Oldenlandia* species studied

species name	Locality/coordinate	Date of collection	Collector(s) name	Herbarium number
O. affinis	Ogbokor, Edo State	11/01/2019	Ekeke, C.	??
O. corymbosa	Obiga-Asa, Abia State	23/04/2019	Ekeke, C.	??
O. lancifolia	Agric farm, University of Port Harcourt, Rivers State	10/08/2019	Ekeke, C.	??
O. herbacea	IITA staion Onne, Rivers State	25/08/2021	Ekeke, C. and Ogazie, C. A.	??

Epidermal studies

Fresh leaf materials for epidermal studies were collected from plants growing in the wild. The adaxial and abaxial epidermal surfaces were peeled, fixed in 30% eathanol, stained with 1% safranin or alcian blue, rinsed with distilled water to remove excess stain, mounted in a drops of pure glycerine on clean glass slides, coverslips placed over the peels, and sealed with nail varnish to prevent dehydration (Ndukwu & Okoli 1992). Twenty good slides were selected,

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observed, and microphotographed using a trinocular research microscope (T340B) fitted with Amcope digital camera. The epidermal features are described according to Van Cotthem (1970), Metcalfe and Chalk (1979), and Fatemeh (2007).

Anatomical studies

Cut sections of the midrib, and stem from matured plant were fixed in FAA (formaldehyde: glacial acetic acid: ethanol in the ratio of 1:1:18 parts of 70% ethanol v/v) for at least 24 hours. These materials were washed in several changes of distilled water, dehydrated through alcohol series (30%, 50%, 70%, and 100%) solution for two hours in each and embedded in wax. Freehand sections were made from the stem and leaf and the thin sections selected, de-waxed, stained with 1% Safranin O, counterstained with Alcian blue, mounted on slides, and photographed with a trinocular research microscope (T340B) fitted with Amcope digital camera.

Statistical analysis

The mean values of the different parts of the plant tissues examined and measured were determined using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Oldenlandia species are presented in Figs. 1 – 11 and Table 2. Generally, the epidermal cells are pentagonal to polygonal with straight, curved or wavy anticlinal walls, and paracytic stomata (Figs. 1-9). Also, all the species studied have dorsiventral leaf with the leaf vein vascular bundles (bundle sheaths) embedded in the spongy mesophyll (Fig. 10). The vascular bundles in the midribs fromed an arc that is enclosed by parenchymatous endodermal cells (Table 2). *Oldenlandia herabcea* is amphistomatic while the other species are hypostomatic. This feature distinguishes this species from other ones studied. Hypostomatic leaves, paracytic stomata, dorsiventral mesophyll, and collateral bundles are common in Rubiaceae (Metcalfe & Chalk, 1950; Robbrecht (1988), and have been specifically reported in *Morinda citrifolia* L. (Mownika *et al.*, 2020), *Psychotria* species (Tarsila *et al.*, 2021), and *Cordiera sessilis* (Vell.) Kuntze (Teixeira et al., 2016). Also, parallelocytic stomata have been observed in some species of *Psychotria* (Gomes et al. 1995; Vieira et al. 1995; Quinteiro et al. 2006).

A dichotomous key for identifying the species based on the anatomical and micro-morphological features is as follows:

1a. Leaf veins glabrous; papillose and raphides absent, midrib curved or arced, adaxial epidermal cells 6.22 – 9.6 µm long; lamina 33.75 – 39.26 µm thick; ……2

1b. Leaf veins hairy (with tuft hairs); papillose and raphides present, midrib circular, curved or arced, adaxial epidermal cells 10.41 – 11.02 µm long; lamina 28.38 – 29.53 µm thick; ……3

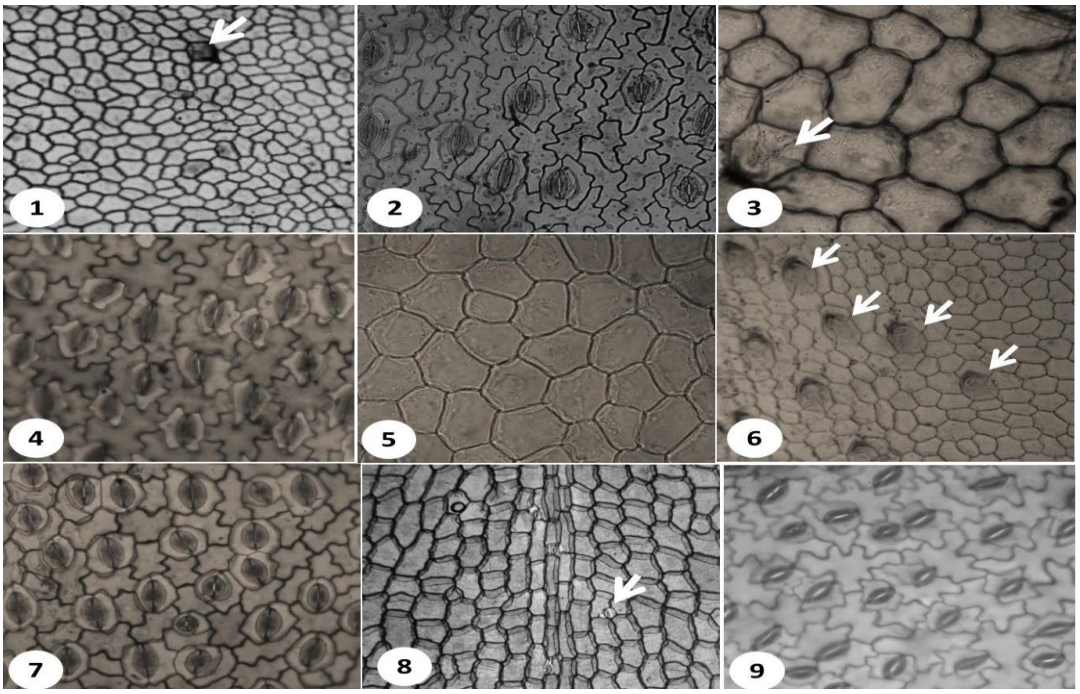
2a. Leaf surface and edge glabrous, amphistomatic; midrib adaxial cortex 1-layer, abaxial cortex 3-layers; palisade mesophyll 1-layer, spongy mesophyll 3-layers; stem cortical cells 3-7 layers, PT/ET ratio 5 ……. *O. herbacea*

2b Leaf surface and edge hairy, hypostomatic; midrib adaxial cortex 3-4-layer, abaxial cortex 4-5-layers; palisade mesophyll 1-2-layer, spongy mesophyll 4-layers; stem cortical cells 5-6-layers, PT/ET ratio 8.97 …… *O. diffusa*

3a Midrib 99.87 µm thick, adaxial cortex 3-4-layers, abaxial cortex 4-6-layers; vascular bundle arc 44.26 µm long; lamina: adaxial epidermis 10.06 µm thick, abaxial epidermis 4.03 µm thick; leaf edge pointed, raphides absent, palisade mesophyll 2-3-layer, spongy mesophyll 3-5-layers; stem cortical cells 3-8-layers, PT/ET ratio 5.04 …… *O. affinis*

3b Midrib 62.31 µm thick, adaxial cortex 2-3-layers, abaxial cortex 3-layers; vascular bundle arc 20.73 µm long; lamina: adaxial epidermis 7.5 µm thick, abaxial epidermis 2.84 µm thick; leaf edge curved, raphides present, palisade mesophyll 1-layer, spongy mesophyll 4-5-layers; stem cortical cells 5-10-layers, PT/ET ratio 3.05 ……. *O. corymbosa.*

Tuft hairs is absent in *O. herbacea* but present on the adaxial leaf surface of *O. affinis* (Fig. 1), *O. corymbosa* (Fig. 3), and *O. diffusa* (Fig. 6). Also, the stem of *O. diffusa* is oval or circular (Fig. 11C) while the remaining have quadrangular stem. Among Rubiaceae, the stems are circular or polygonal with collenchymatous ribs, well-defined endodermis with pericycle devoid of sclerenchyma (Metchalfe and Chalk, 1972). According to Kocsis and Borhidi (2003), the nature of epidermal cells, non-glandular hairs, cortical parenchyma and vascular tissue in the petioles of the genera *Rondeletia*, *Javorkaea*, *Rogiera* in Rubiaceae family seems to have a taxonomic value at generic level. In our study, this` feature was diagnostic.



Figures 1 – 9: Epidermal peels of *Oldenlandia* species studied. (1 and 2) *O. affinis,* (3 and 4) *O. corymbosa,* (5-7) *O. diffusa,* (7) **(9)** Arrows show short hairs (tuft hairs), (8 and 9) *O. herbacea*.

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Small projections on the external periclinal walls of epidermal cells (papillae) found in the leaf lamina, stem, and flowers (Vieira et al. (1992; Davies and Turner, 2004) are of significant taxonomic value in angiosperms (Judd *et al.*, 2008), and have been reported in some genera in Rubiaceae (Vieira et al., 1992, 2001; Tarsila et al., 2021). According to Vieira et al. (1992), papillae were present on the abaxial epidermis of *Psychotria leiocarpa* Cham. & Schltdl but absent in *P. nuda* (Cham. & Schltdl.) Wawra. Also, Tarsila et al. (2021), observed papillae only on the adaxial epidermal cells of *P. hoffmannseggiana* (Willd. ex Schult.) Mull.Arg. and *P. deflexa* DC. Vieira et al., (1992) and Tarsila et al. (2021) noted that the presence or absence of papillae on the epidermal surface of *Psychotria* is diagnostic and could be used to separate members of this genus. In our study, we observed papillae only on the adaxial epidermis of *O. affinis* and *O. corymbosa.* This character distinguished these species from the species studied and support that this character could be used in the systematics of *Oldenlandia* L.

Lersten (1974) and Metcalfe & Chalk (1985) observed that raphide bundles are common feature in the subfamily Rubioideae. Vieira *et al.* (1992), Gomes *et al.* (1995), Quinteiro *et al*. (2006), and Tarsila et al. (2021), stated that druses, raphide bundles, styloid, prismatic, and sand-like are frequent *Psychotria* (Rubiaceae) and *Cordiera sessilis* (Teixeira et al., 2016). Furthermore, Tarsila et al. (2021) observed that raphide bundles, were found in the parenchyma in the petiole, midrib, and the leaf blade in the genus *Psychotria* except in the leaf blades of *Psychotria hoffmannseggiana* and *P. deflexa* while prismatic crystals were found in the leaf blade and cortex of the midrib of *Psychotria glaziovii* and *P. leiocarpa* and in the midrib of *P. hoffmannseggiana*. This character was reported to be diagnostic in this genus (Tarsila *et al.*, 2021). The species under study were devoid of prismatic crystals with raphide bundles in the lamina of *O. corymbosa* only. The presence of raphide bundles in this species suggests that this might be an important diagnostic character for the genus.

The organization of the vascular system have a higher taxonomic value in Rubiaceae (Martínez-Cabrera *et al.* (2009), and could be arranged in U, O or V-shapes (Kocsis *et al.*, 2004). Our study showed arced vascular bundle in the midrib of all the *Oldenlandia* species studied with the length of the arc varying among the species. The structural organization of vascular bundle in the petiole of some genera in Rubiaceae such as *C. sessilis* (Teixeira *et al.*, 2016), *Genipa americana* (Erbano and Duarte, 2010), and *Gardenia spathulifolia* (Metcalfe and Chalk, 1950) are arced with accessory lateral bundles. The presence of accessory lateral bundles is not of taxonomic importance in Rubiaceae (Kocsis *et al.*, 2004). According to Metcalfe and Chalk (1950), Martínez-Cabrera *et al.* (2009) and Moraes *et al.* (2011), the petiole has a considerable taxonomic importance because it is not heavily influenced by environmental changes. The shape of the vascular bundles in the midrib of *Oldenlandia* species are arced and is similar to the shape in the

petiole of *C. sessilis* (Teixeira *et al.,* 2016), *Palicourea longepedunculata* (Pereira *et al*., 2003), *Psychotria viridis*, (Quinteiro *et al.,* 2006), and *Rondeletia* (Kocsis *et al*., 2004). However, we did not record accessory lateral bundles in the midrib of the *Oldenlandia* species studied. The shape of the vascular bundles in the midrib was not diagnostic but the size varied among the species and could be of systematic value.

Table 2: Anatomical characteristics of the *Oldenlandia* species studied

Plant Part	Taxa			
	O. affinis	***O. corymbosa***	***O. lancifolia***	***O. herbacea***
Adaxial epidermis				
Shape	Pentagonal to hexagonal	Pentagonal to polygonal	Rectangular to pentagonal	Pentagonal to hexagonal
Anticlinal wall	Straight/curved	Straight/curved	Straight/curved	Straight/curved
Average size (µm)	10.79 × 6.05	11.41 × 7.79	10.09 × 6.31	14.40 ×
Stomata	Absent	Absent	Absent	**Present on the veins**
Surface	Partly hairy	Partly hairy	Partly hairy	**Glabrous**
Abaxial epidermis				
Shape	Irregular	Irregular	**Polygonal to irregular**	Irregular
Anticlinal wall	Wavy	Wavy	Wavy	Wavy
Average size (µm)	11.02 × 4.03	10.41 × 4.75	6.22 × 3.13	9.60 × 5.25
Stomatal index				
Stomata type	Paracytic	Paracytic	Paracytic	Paracytic
Stomata shape	Elliptic	Elliptic	**Oval to elliptic**	Elliptic
Midrib				
Shape	**Circular**	Curved/arc	Curved/arc	Curved/arc
Thickness (µm)	99.87	62.31	63.35	66.74
Adaxial cortex	3 - 4 layers	2 - 3 layers	3 - 4 layers	1-layer
Adaxial cortex	4 - 6 layers	3 - layers	4 - 5 layers	3 - layers
Vb arc (µm)	44.26 × 26.77	20.73 × 14.60	29.33 × 14.50	25.42 × 15.90
Vb shape	Arc	Arc	Arc	Arc
Lamina				
Thickness (µm)	29.53	28.38	33.75	39.26
ADE thickness	10.06	7.5	7.06	10.34
ABE thickness	**4.03**	2.84	**1.77**	2.65
Leaf veins	Hairy	Hairy	Glabrous	Glabrous
Edge	**Pointed/**	Curved/	Curved/hairy	**Curved/**

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	glabrous	glabrous		**glabrous**
Papillose	Present	Present	Absent	Absent
Raphides	Absent	**Present**	Absent	Absent
MS thickness	**14.28**	**17.03**	**23.62**	**27.36**
PMS layers	2 - 3	1	1 - 2	1
SMS layers	**3 - 5**	**4 - 5**	**4**	**3**
Stem				
Shape	Quadrangular	Quadrangular	**Oval/circular**	Quadrangular
Epidermis	1-layer	1-layer	1-layer	1-layer
Cortex	3 -8 layers	5 - 10 layers	5 - 6 layers	3 - 7 layers
PT/ET	**5.04**	**3.05**	**8.97**	**5**

Note: Vb – vascular bundle, PT – pith thickness, ET – cortical thickness, MS – mesophyll, ADE – adaxial epidermis, ABE – abaxial epidermis, PMS – palisade mesophyll, SMS – spongy mesophyll.

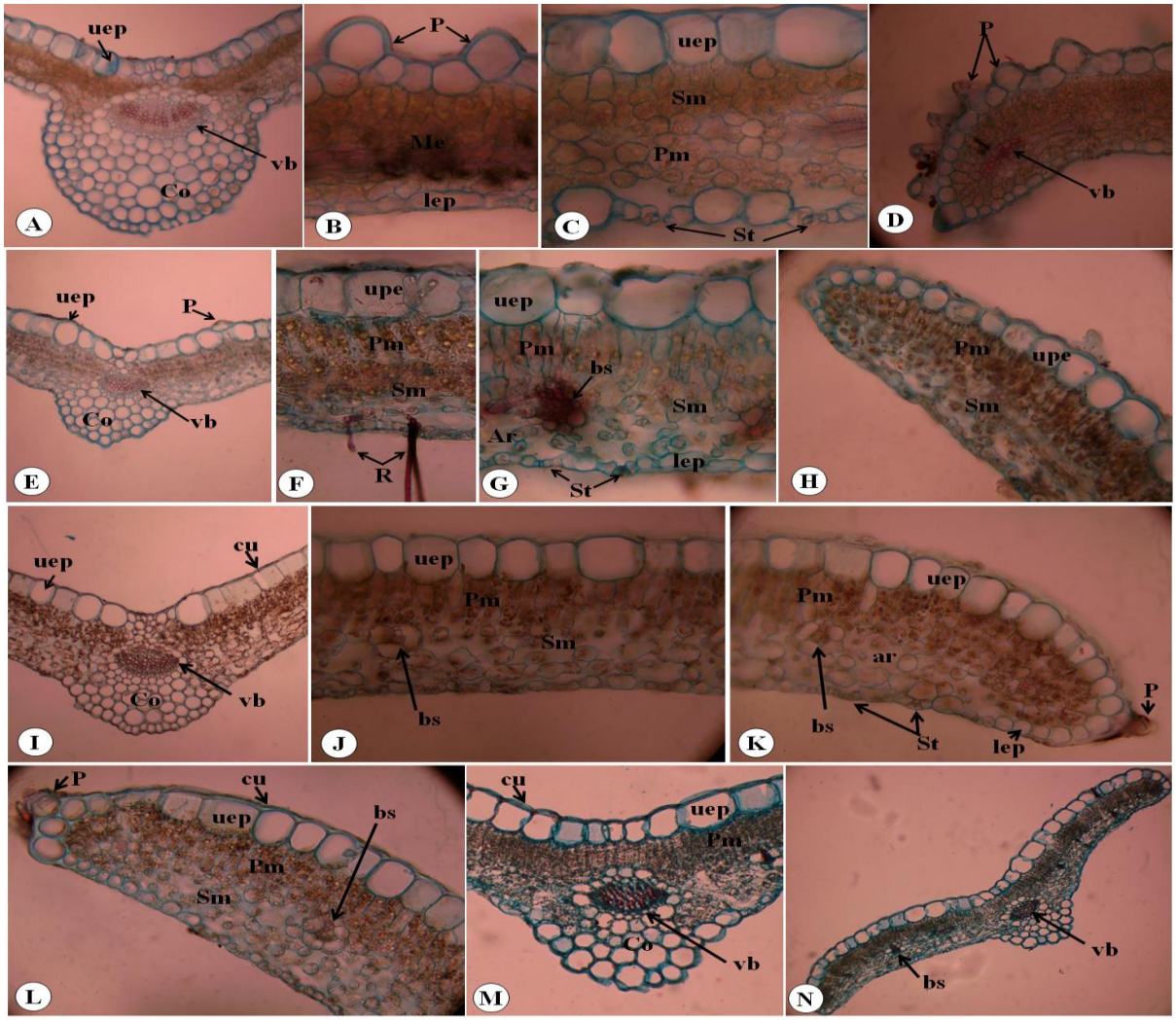

Figure 10: Midrib, lamina and leaf edge of *Oldenlandia* species: (A – D) *O. affinis*, (A) midrib, (B and C) lamina, (D) leaf margin; (E – H) *O. corymbosa*, (E) midrib, (F and G) lamina, (H) leaf margin; (I – K) *O. diffusa*, (I) midrib, (J) lamina, (K and L) leaf margin; (M – N) *O. herbacea* (M) midrib and (N) lamina and leaf edge; vb – vascular bundle, Co – cortex, upe – upper epidermis, lpe – lower epidermis, Pm – palisade mesophyll, Sp – spongy mesophyll, St – stomata, cu – cuticle, bs – bundle sheath and P – papillae.

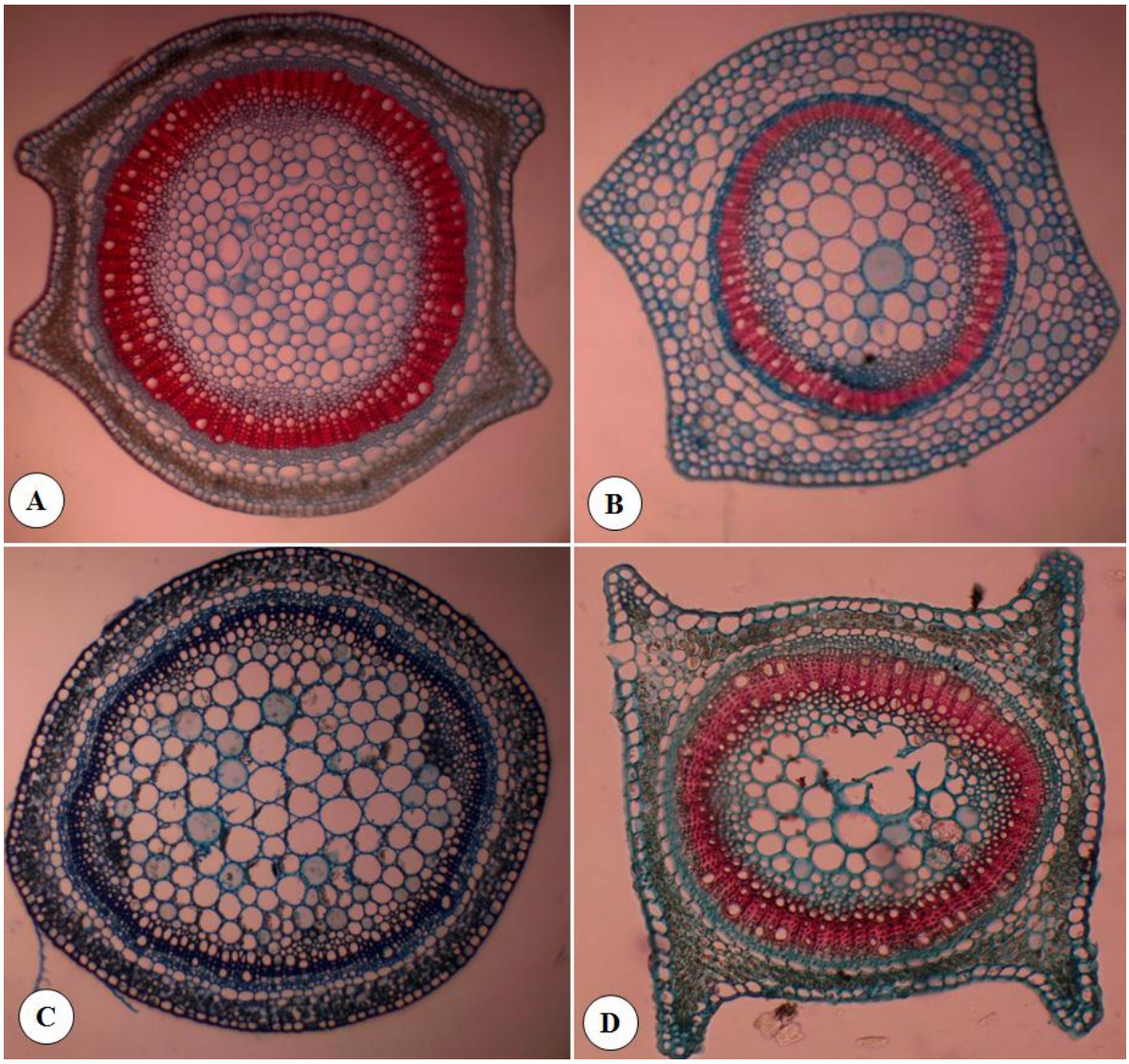

Figure 10: Transverse sections of *Oldenlandia* stems (A) *O. affinis*, (B) *O. corymbosa*, (C) *O. diffusa* and (D) *O. herbacea.*

Tarsila *et al.* (2021) when considering the data compiled on *Psychotria* species (Rubiaceae), concluded that the anatomy and micromorphology of the leaves of the interspecific groups and species studied could be used as diagnostic characters for the genus. In our study, notable diagnostic features are the PT/ET ratio, layers of cortex in the stem, nature of the leaf edge, occurrence of the hairs on the leaf veins and surface, presence or absence of raphides and papillose, layer of abaxial and adaxial cortex in the midrib, and amphistomatatic or hypostomatic leaf.

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