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Comparative morphological and anatomical traits of *Adenia lobata* (Jacq) Engl. and *Adenia cissampeloides* (Planch. ex Benth.) Harms. (Passifloraceae)

Chimezie EKEKE*, Josephine U. AGOGBUA

*University of Port Harcourt, Faculty of Sciences, Department of Plant Science and Biotechnology, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria; ekeke.uche@uniport.edu.ng (*corresponding author); josephine.agogbua@uniport.edu.ng*

Abstract

Comparative morph-anatomical studies of leaf, stem, and tendril were carried out on two *Adenia* species to enhance the delimitation of the species. The *Adenia* species have alternate phyllotaxy, simple leaf, glands at the base of their petioles, hypostomatic, similar stomata types, solitary crystal, similar vascular bundle type, tanniferous cells, and dorsiventral leaves. Both species have tetracytic and anomocytic stomata in addition *A. lobata* had paracytic, and anisocytic stomata. Mucilaginous cells or cavities occurred in the petiole, leaf lamina, midrib, stem, and the tendril of *A. cissampeloides* but were only observed in the tendril and upper epidermis of *A. lobata.* The mucilaginous and tanniferous cells were more abundant in *A. cissampeloides* compared to *A. lobata* and could account for the reason why *A. cissampeloides* is used as fish poison. The sclereids in *A. cissampeloides* are surrounded by lignified cells while the ones in *A. lobata* do not have such feature. Also, xylem cells in the *A. cissampeloides* tendril are thicker compared to *A. lobata.* This could be attributed to the fact that the fruits in *A. cissampeloides* are directly attached to the tendril. The outline and number of vascular bundles in the midrib and petiole differed among the two species. In *A. lobata*, the ridge averaged 130 µm thick and 73.76 µm in *A. cissampeloides*. The analyzed characteristics are valuable in the delimitation of these *Adenia* species.

Keywords: *Adenia*; anatomy; glands; petiole; stomata; vascular bundles

Introduction

Passifloraceae is an angiosperm family with approximately 575 to 750 species in 17 to 27 genera which are lianas, climbers, shrubs, and trees and grow mainly in tropical and warm temperate regions (Feuillet and MacDougal, 2007; Mabberley, 2008; APG, 2009; Kishore and Himansu, 2016). *Adenia* Forssk. (80 – 100 species) and *Passiflora* L. (430 species) are the largest genera in this family (Hearn, 2007; Mabberley, 2008) and are mainly found in the tropical regions of the old world (Hearn, 2007). In West Africa, *Adenia* Forssk.

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comprised 10 species and five in Nigeria namely *A. lobata* (Jacq.) Engl., *A. cissampeloides* (Planch. ex Benth.) Harms., *A. gracilis* Harms, *A. mannii* (Mast.) Engl., and *A. venenata* Forsk. out of which, *A. Venenata* is a Savannah species (Hutchinson and Dalziel, 1954).

Adenia species are used for different economical purposes (Neuwinger, 2004; Ramachandran, 2007; Mabberley, 1997 in Emeji and Ibiam, 2018). *Adenia cissampeloides* and *Adenia wightiana* are eaten as vegetables (Neuwinger, 2004; Ramachandran, 2007), *A. wightiana* is used for the treatment of peptic ulcers (Kottaimuthu, 2008), and *A. cissampeloides* used as a fish poison, treatment of itching, ringworm, haemorrhoids, sores, and wounds (Mabberley, 1997; Emeji and Ibiam, 2018).

Anatomy is of primary importance for all aspects of research in plant sciences and contributes immensely to plant taxonomy (Metcalfe and Chalk, 1979; Mabberley, 2008; Presena and Pragasam, 2016; Ekeke *et al*., 2020). When a plant part is cut into pieces some of the anatomical characters such as tracheary elements, sclereids, crystals, silica bodies, stomata, fibres, starch grains, trichomes, leaf epidermal cells, etc., remain unaffected (Presena and Pragasam, 2016). Comparative anatomy of petiole, stem, midrib, and epidermal studies have been applied in enhancing the identification of different plant families, genera, and species (Noraini and Cutler, 2009; Baruah *et al*., 2017; Keshavarzi *et al*., 2012**;** Manning and Goldblatt, 2011).

Earlier research on Passifloraceae investigated a few (one to four) species of *Adenia.* Obaton (1960) studied only one species of *Adenia* while Janse van Vuuren (1970) studied four species of *Adenia.* Furthermore, Ayensu and Stern (1964) investigated 44 members of Passifloraceae but their work characterized only *A. lobata.* In recent times, Hearn (2009) examined 58 species of *Adenia* based on their 47 morphological and anatomical characters. Though Hearn (2009a) studied 58 species of *Adenia,* his work was mainly on the stem and root anatomy of this genus. In Passifloraceae, diffuse included phloem strands and successive cambia have been documented in *Adenia* (Hearn 2009a, b) and *Passiflora* (Kishore and Himansu, 2016). Hearn (2009) described the developmental anatomy of root and stem in 58 species of *Adenia* (Passifloraceae). Also, Presena and Pragasam (2016), comprehensively described the anatomy of *A.wightiana.* Though the stem and root anatomy of these species was described by (Hearn 2009a, b), the leaf characteristics such as petiole and midrib anatomy are yet to be described and there is scanty work on the leaf epidermal features of *Adenia* species.

The main aim of the present study is to compare the morphological and anatomical features of the *A. lobata* (Jacq.) Engl. and *A. cissampeloides* (Planch. ex Benth.) with the view to enhancing the delimitation of the species.

Materials and Methods

Source of plant material

Fresh specimens of the species were obtained from the University of Port Harcourt Biodiversity Centre, Choba. They were then authenticated by the Herbarium Curator and deposited at the University of Port Harcourt Herbarium (UPH) as UPH/V/1460 for *A. cissampeloides* and UPH/V/1461 for *A. lobata.*

Morphological description

The overall morphology of the leaf, flowers, fruits, and seeds of the herbarium and fresh specimens was calculated using a meter rule and a hand lens. The photographs of the vegetative and other parts of the plant were taken and documented using Nikon D3200 digital camera.

Epidermal preparations

Epidermal peels were obtained from both the leaf surfaces using the method of Ayodele and Olowokudejo (2006) with little modifications. Fresh leaf specimens were peeled using forceps and fixed in 50%

ethanol for about 1-2 hrs to remove chlorophyll and make the peels transparent. The peels were washed in distilled water, stained in Safranin O, mounted in glycerine (slides), slides properly labeled, and stored in slide racks.

Anatomical preparation

Cut sections of petiole, stem, tendril, and midrib from matured leaves and stems 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 3 hours in each and embedded in wax. Freehand sections and thin sections were selected, de-waxed, stained with 1% Safranin O, counterstained with Alcian blue, mounted on slides, properly labeled, and stored in slide racks.

Microscopy

Twenty slides each for epidermal peels, petiole, midrib, lamina, tendril, and stem were examined under the trinocular research microscope and photomicrographs of sections were taken with Optika B-1000 FL LED fitted with Amcope digital camera. The thickness of the cells was measured with Amcope 3.7 version software.

Statistical analysis

The mean values and range of the data generated were determined using Microsoft Excel 2010.

Results

Morphology

Adenia lobata

Adenia lobata is a tall forest climber. Leaves widely ovate; broadly cordate or truncate at base, entire margin, acute to subacute at apex, cordate to fairly sagittate at the base (Figure 1A); 6.9 – 12.2 cm long, 3.3 – 8.2 cm broad; petiole half the length of the leaf length, with a pair of separate glands at the top (Figure 1B); Sepals united about 3/5 up, inner and edges of the tube covered with woolly hairs (Figure 1C), peduncles 0·5 – 1.0 cm, flowers about 1·5–2·5 cm. long; calyx campanulate, lobes lanceolate, acute; petals laciniate, and ripped fruits yellow; tendrils present and coil in the clockwise direction (Figure 1D).

Adenia cissampeloides

Adenia cissampeloides is a climber common in forest regrowth and margins (Figure 2A). Leaves very widely ovate to more or less rhomboid, entire or partly lobed, 4.0 – 9.4 cm long, 4.6 – 11.8 cm broad, acute to rounded at apex, truncate or widely cordate at base and conspicuously spotted (Figure 2B). Petiole with a gland at the top (Figure 2B), approximately twice the leaf length; Inflorescence has many flowers, with a fairly long peduncle 1.8 - 2.5 cm (Figure 2A); sepals free to the base (Figure 2C); capsule 6-angled, ovoid, averaging 2 cm. long; seeds black, coarsely pitted (Figure 2D).

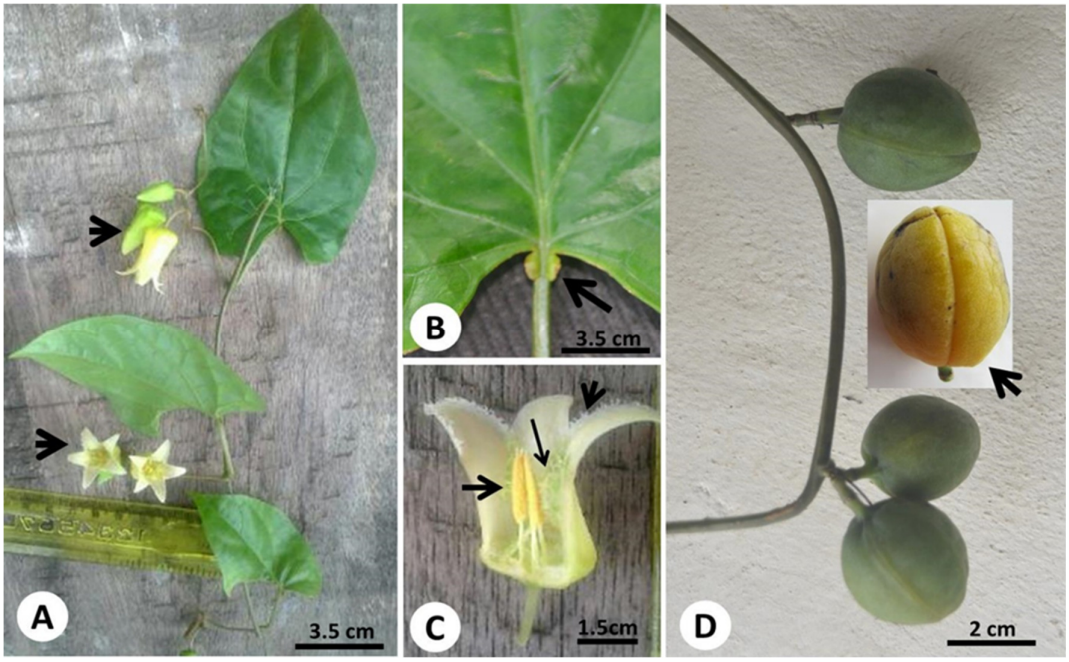

Figure 1(A-D): *A. lobata* (A) Plant habit (arrows show flower buds), (B) Leaf (arrow shows glands), (C) Flower arrows show styles and hairs, and (D) ripped and unripped fruits (arrow shows ripped fruit)

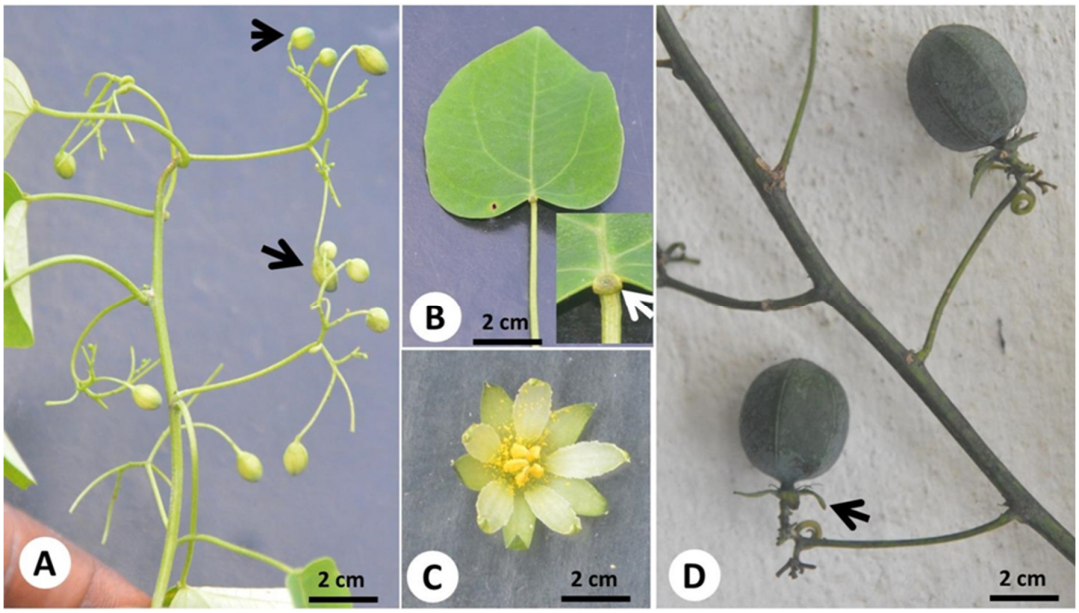

Figure 2(A-D): *A. cissampeloides* (A) Plant habit (arrows show flower buds), (B) Leaf (arrow shows glands), (C) Flower, and (D) fruits (arrow shows persisted sepals)

Micromorphology and anatomy

Adenia cissampeloides

Epidermis

Hypostomatic, abaxial surface comprised of tetracytic and anomocytic stomata (Figure 3A), oval to elliptic, and measuring 17.73 × 11.61 µm. Both abaxial and adaxial cells are polygonal in shape with curved anticlinal walls (Figures 3A and 3B).

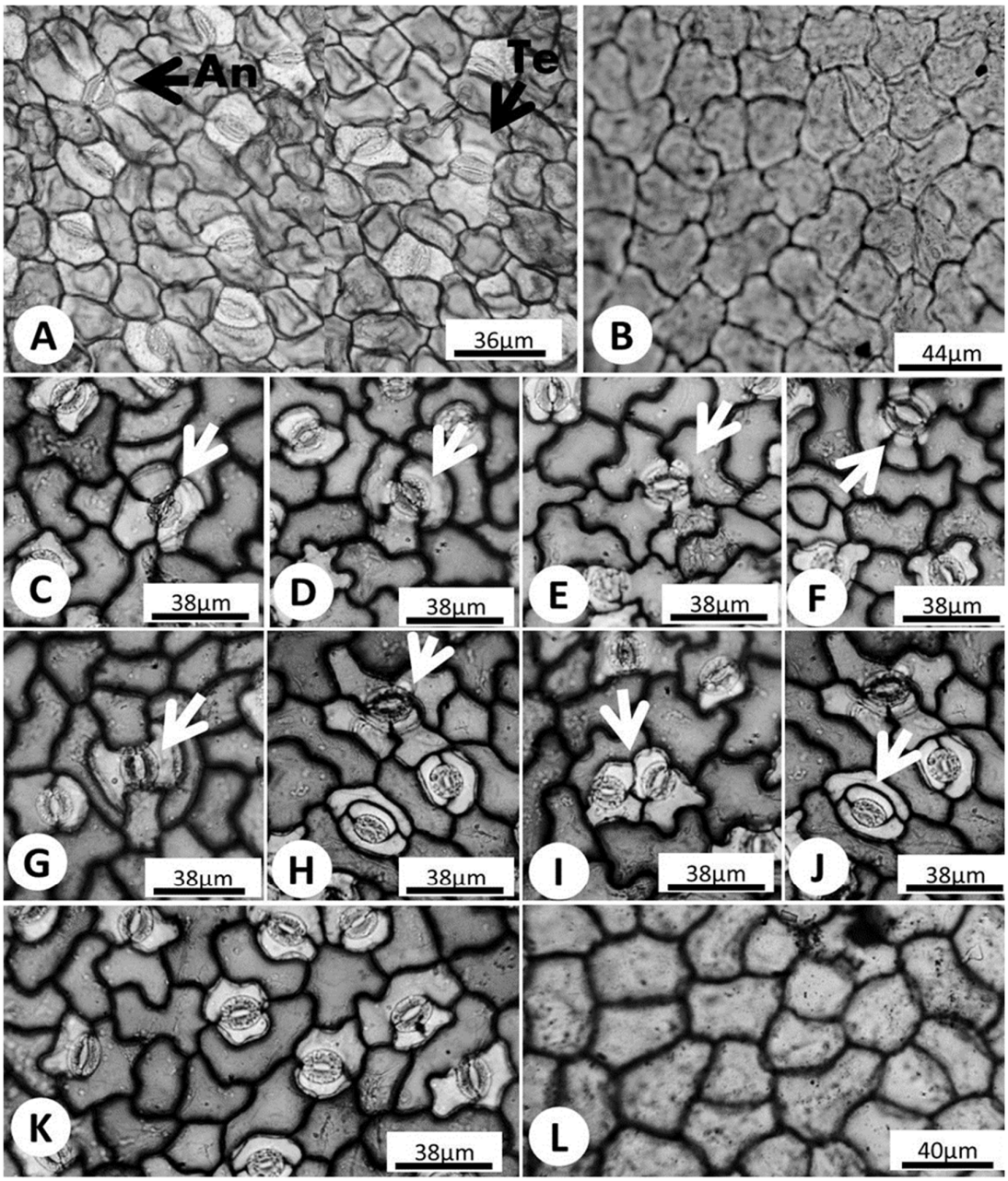

Figure 3. Epidermal and stomatal types in *Adenia* species studied; (A) Abaxial surface of *A. cissampeloides,* (B) Adaxial surface of *A. cissampeloides*, (C – K) Abaxial surface of *A. lobata* and (L) Adaxial surface of *A. lobata*

An = anomocytic stoma; Te = tetracytic stoma

Petiole

Plano-convex, adaxial cuticle wavy with two furrows. The vascular system consists of 6-open circular bundles with 2-rib traces and adaxial collenchymatous cells containing tanniferous cavities (Figure 4A); cortical and ground parenchymatous cells comprised mucilaginous cells and solitary crystals (Figure 4B).

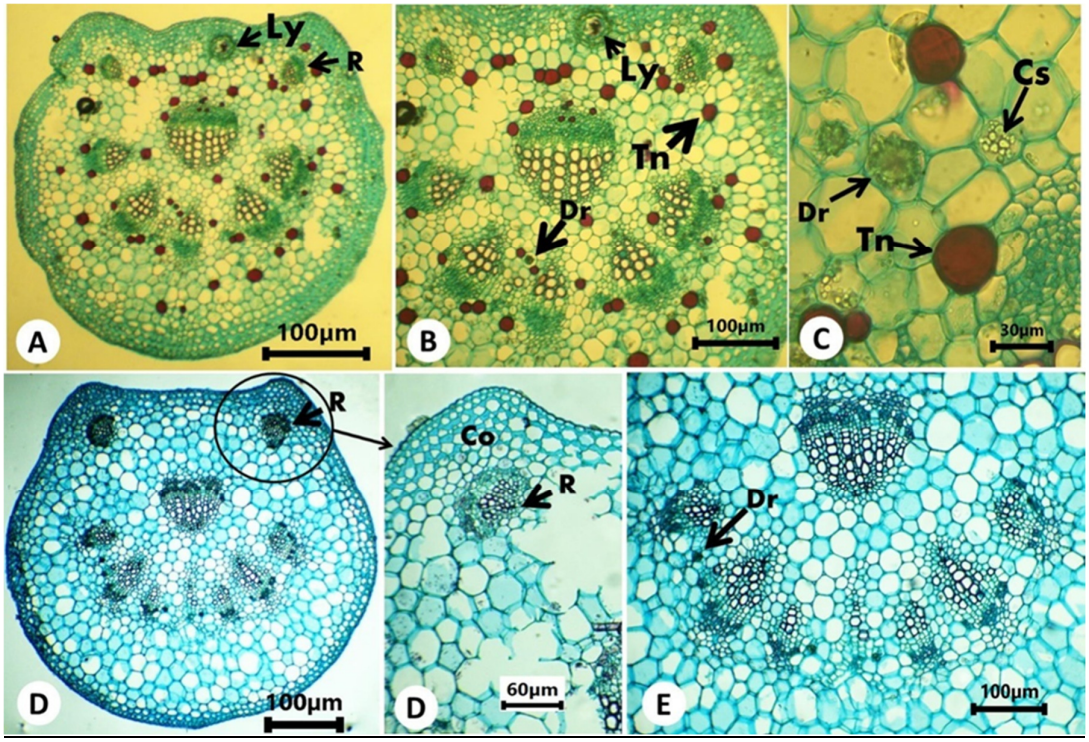

Figure 4. Transverse sections of petiole of *Adenia*species (A – C) *A. cissampeloides* and (D – E) *A. lobata*
R = rib trace, Dr = solitary crystal, Tn = tanniferous cell, Ly = lysigenous cavity, Cs = crystal sand

Midrib

Boat-shaped, the vascular system comprised 5 vascular bundles (4-abaxially co-joined ones and 1-adaxially inverted ones) (Figure 5A), and an adaxial cuticle elevated to about 74 µm high (with 6-layers of collenchymatous cells) (Figure 5B) and solitary crystals (Figure 5C).

Lamina

Dorsiventral, 146.72 – 184.57 (159.55) µm thick and abaxial surface covered with papillae; palisade mesophyll cells closely packed, 30.11 – 33.98 (31.65) µm thick with series of sclereids vertically arranged or embedded in the palisade mesophyll cells; spongy mesophyll cells loosely packed 107.03 – 127.06 (113.54) µm thick forming a network of intercellular spaces with the sclereids horizontally embedded in the spongy cell; bundle sheaths are surrounded series of parenchymatous cells and tanniferous cavities present in the palisade and spongy mesophylls (Figure 5H). The ratio of the average leaf thickness to palisade thickness is 5.04 while the average spongy/palisade thickness is 3.59.

Stem

Contains lysigenous cells and phloem fibre bundles in the cortex parenchyma, tanniferous cells measuring 25.02 ± 5.57 µm and solitary crystal (19.95 ± 1.99 µm) in the ground tissues (Figures 6A – 6D), and vessels measuring 32.92 ± 8.14 µm.

Tendril

The tendril contains sclereids encapsulated by a phloem fibre bundle (Figure 6E), a thick layer of xylem cells (Figure 6F), tanniferous cells, and patches of phloem in the xylem (Figure 6G).

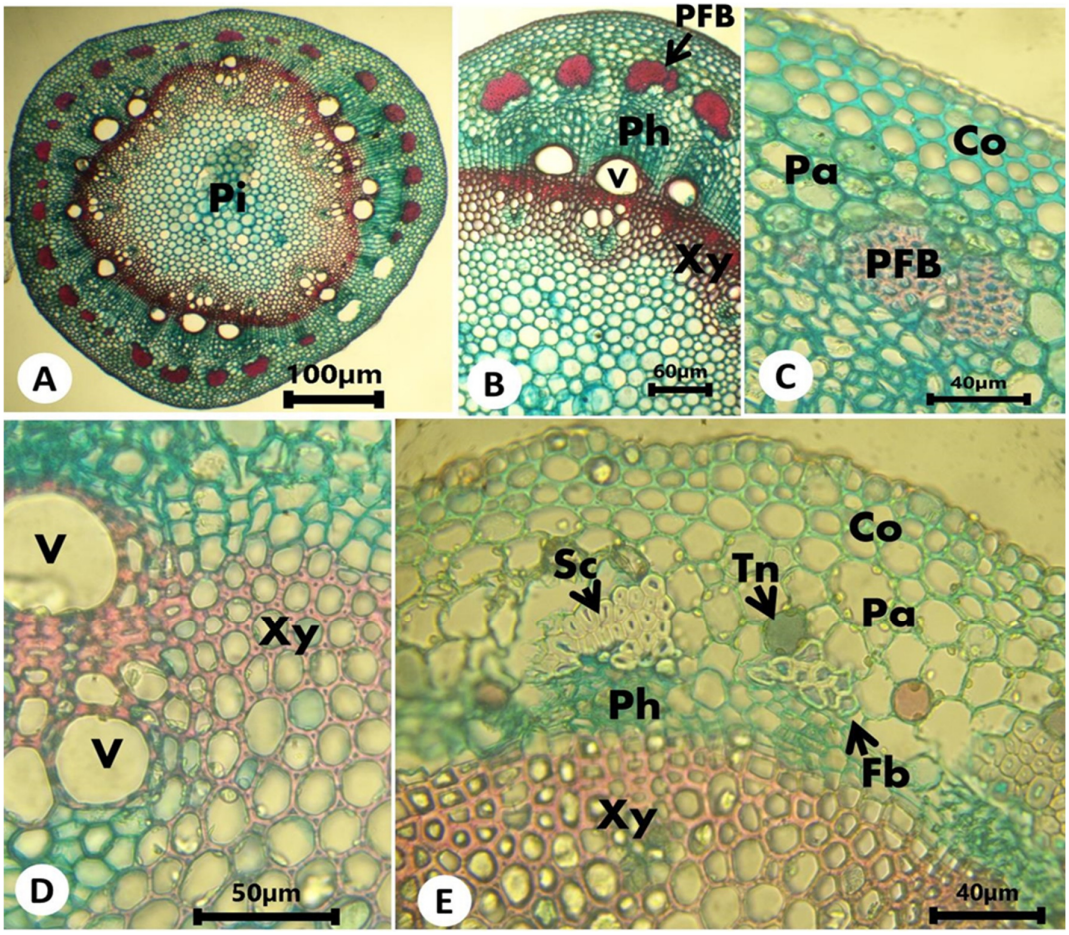

Figure 5. Transverse sections of midrib and lamina of *Adenia* species studied: (A-C) midrib of *A. cissampeloides*, (D – F) midrib of *A. lobata,* (G) Midrib of *A. lobata* (arrow shows stoma), (H) Lamina of *A. cissampeloides*, (I) lamina of *A. lobata*

P = palisade mesophyll, Bs = Bundle sheath, S = spongy mesophyll, Dr = druse crystal, Pl = papillae, Sc = sclereid, Co = collenchyma, Pa = parenchyma, Dr = druse crystal, Ta = Tannin sack, St – stoma.

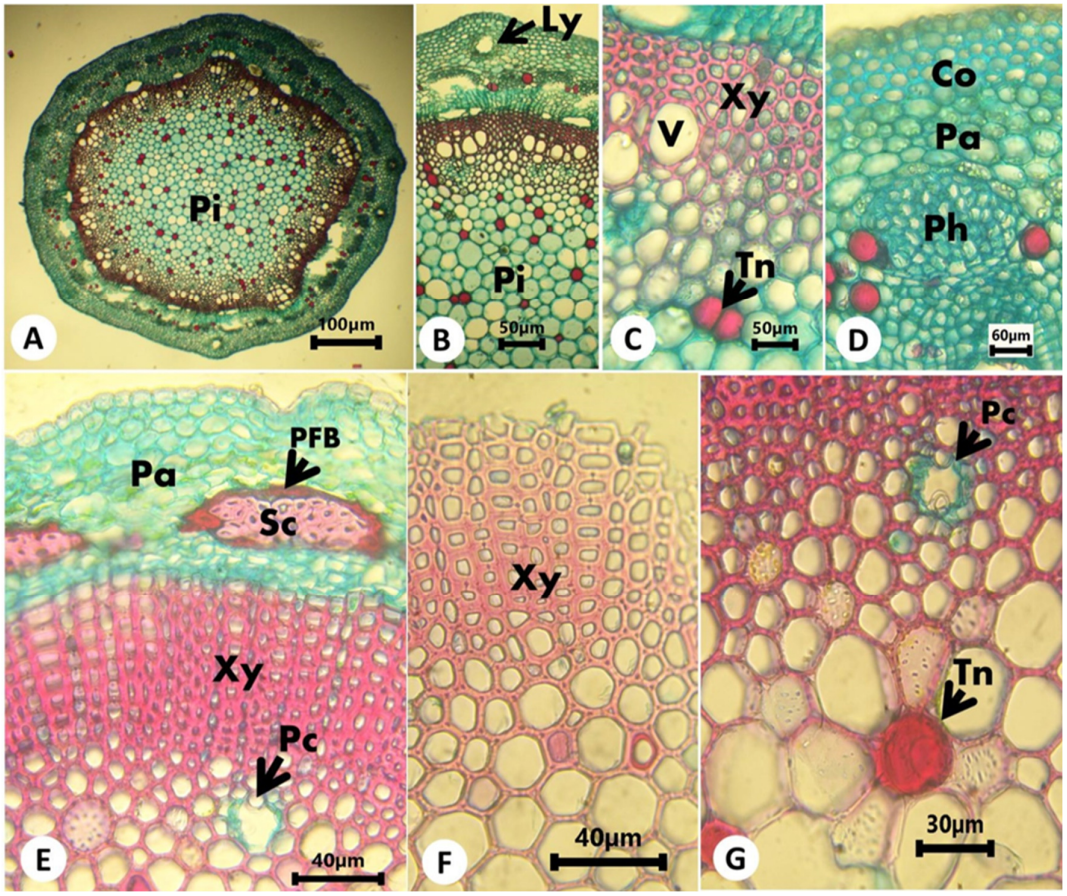

Figure 6. Transverse sections of Stem (A – D) and tendril (E – G) of *A. cissampeliodes*
Pi = pith, Xy = xylem, Ph = phloem, Fb = fibre, G = lysigenous cell, Pa = cortex parenchyma, Co = collenchyma, V = vessel, sd = tanniferous cell.

Adenia lobata

Epidermis

Hypostomatic, abaxial surface comprised of paracytic, anisocytic, tetracytic, and anomocytic stomata, irregularly shaped with undulating anticlinal walls (Figures 3C–3K), oval to elliptic, and measuring 18.27 × 15.89 µm. Adaxial epidermal cells are polygonal in shape with curved or straight anticlinal walls (Figure 3L).

Petiole

Plano-convex and adaxial cuticle flat. The vascular system consists of 8-open circular bundles with 2-rib traces (Figures 4C and 4D), and solitary crystals enshrined between the vascular bundles (Figure 4D).

Midrib

Boat-shaped, the vascular system comprised 3 vascular bundles in a triangular orientation (Figure 5D), an adaxial cuticle elevated to about 130 µm high (with 8 – 9-layers of collenchymatous cells) (Figure 5E), and collenchyma 2 – 3-layers thick (Figure 5F).

Lamina

Dorsiventral, thickness 38.06 – 43.55 (40.58) µm, tanniferous cells confined to the adaxial epidermis, and solitary crystals in the palisade mesophyll (Figure 5I); palisade mesophyll cells closely packed, 8.03 – 12.05

(10.37) µm thick; spongy mesophyll cells closely packed 15.28 – 22.26 (19.57) µm thick (Figure 5I). The ratio of the average leaf thickness to palisade thickness is 3.97 while the average spongy/palisade thickness is 1.91.

Stem

Collenchyma and cortex distinguishable contain patches of phloem fibre bundles (38.88 ± 15.02 µm), vessels 39.16 ± 8.06 µm in tangential diameter, and interrupted cambia growth with include phloems (Figures 7A – 7D).

Tendril

Xylem is not very thick, and patches of sclereids, fibre, and tanniferous cells (Figure 7E).

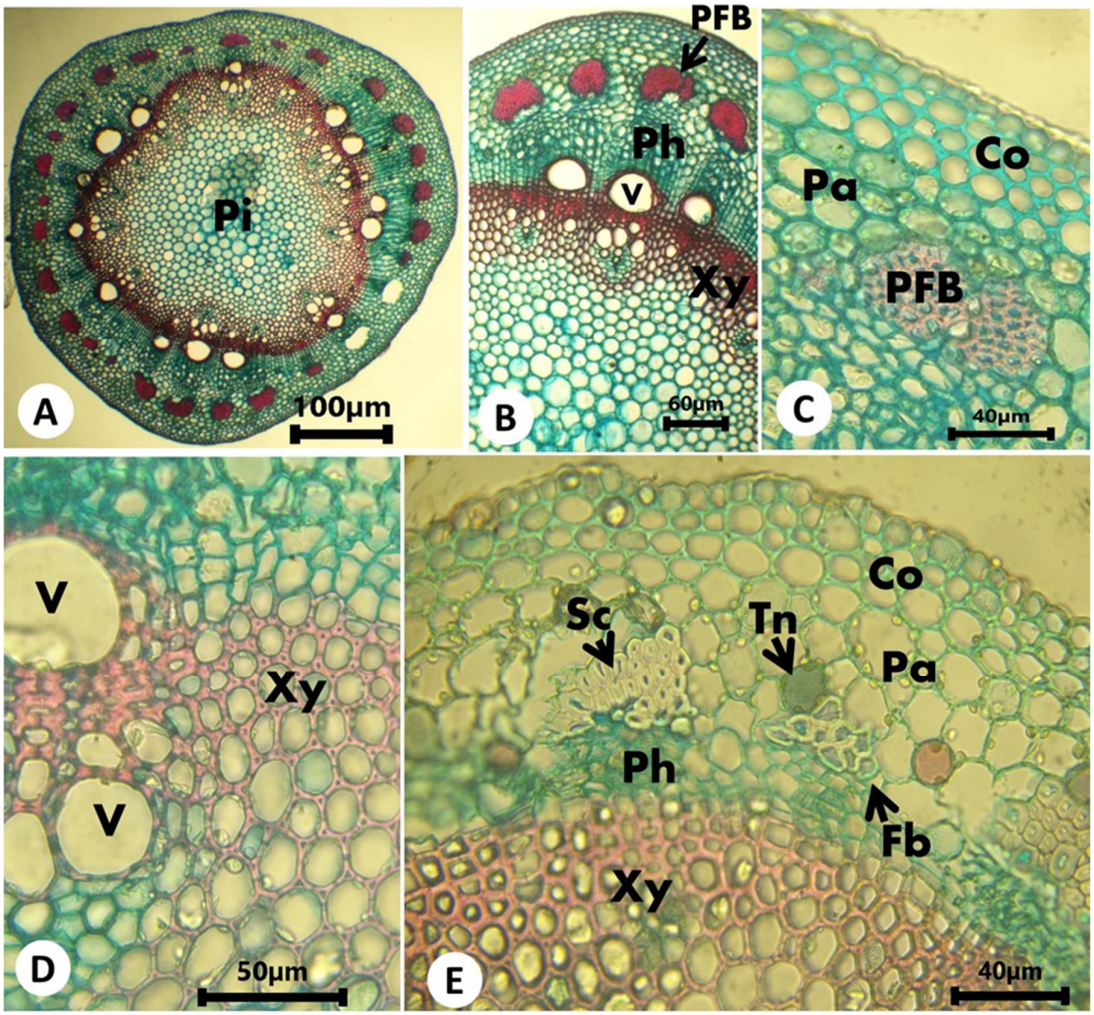

Figure 7. Transverse sections of Stem (A – D) and tendril (E) of *A. lobata*

Pi = pith, Xy = xylem, Ph = phloem, Fb = fibre, G = secretory gland, Pa = cortex parenchyma, Co = collenchyma, V = vessel, sd = duct.

Discussion

Generally, the two *Adenia* species (*A. cissampeloides* and *A. lobata*) have alternate phyllotaxy, simple leaf, glands at the base of their petioles, hypostomatic, and dorsiventral leaves, and similar stomata types, solitary crystal, similar vascular bundle type, and tanniferous cells. Despite these similarities, morphologically, the shape of the flowers, the number of glands at the top of the petiole, leaf shape, and fruit size could be used to distinguish the species. These morphological attributes we observed are in line with the report of Hutchinson and Dalziel (1954). Furthermore, there are variations among these characters that could be used to identify them. For instance, *A. cissampeloides* had tetracytic and anomocytic stomata while *A. lobata* had paracytic, anisocytic, and stomata in pairs in addition to tetracytic and anomocytic stomata. Metcalfe and Chalk (1972) found isobilateral and dorsiventral leaves, mostly anomocytic and partly paracytic stomata in Passifloraceae. They observed that the stomata are restricted to the abaxial epidermis. Though isobilateral leaves were reported in *Adenia* L., *Passiflora,* and other members of Passifloraceae (Metcalfe and Chalk,1972) we observed only dorsiventral leaves were observed in *A. lobata* and *A. cissampeloides.* Our observation of stomata types partly conflicted with Metcalfe and Chalk (1972) because, in addition to anomocytic and paracytic stomata, we reported tetracytic and anisocytic in *A. lobata.*

Adenia cissampeloides has mucilaginous cells or cavities in the petiole, leaf lamina, midrib, stem, and the tendril a feature that we only observed in the tendril of *A. lobata.* Also, tanniferous cavities of cells occurred in the petiole, lamina, midrib, and stem of *A. cissampeloides.* On the other hand, tenniferous cells were only observed in the leaf upper epidermis of *A. lobata.* The mucilaginous and tanniferous cells were more abundant in *A. cissampeloides* compared to *A. lobata* and could account for the reason why *A. cissampeloides* is used as fish poison (Mabberley, 1997; Emeji and Ibiam, 2018).

The anatomical features of the tendril in both species differed slightly. The sclereids in *A. cissampeloides* are embedded or surrounded by lignified cells or fibre while the ones in *A. lobata* do not have such feature. Also, xylem cells in the *A. cissampeloides* tendrils are thicker compared to *A. lobata.* This could be attributed to the fact that the fruits in *A. cissampeloides* are directly attached to the tendril therefore, the thick xylem cells support bearing the fruits. Similar observations have been reported among the family Cucurbitaceae Juss. were the tendril that supports the bearing and anchoring of fruits are thicker compared to ones that do not have such responsibility (Ekeke *et al*., 2015).

The midrib and petiole characters such as the outlines, number of vascular bundles, and thickness or height of the adaxial collenchyma (the ridge of the adaxial surface of the midrib). In *A. lobata*, the ridge was pronounced (averaged 130 µm) thick or high while in *A. cissampeloides* it averaged 73.76 µm high. Furthermore, the number of vascular bundles in the petiole and midrib of *A. lobata* are 8 and 3 respectively while in *A. cissampeloides* are 6 and 5 respectively. Both species have two rib traces and adaxially inverted vascular bundles. The adaxial outline was flat in *A. lobata* and undulating in *A. cissampeloides.* Ekeke and Ogazie (2020) and Ekeke *et al*. (2016) demonstrated that variation in petiolar and midrib outline, vascular bundle number, and arrangement could be used to enhance the identification of Asteraceae from Nigeria. Also, Matias *et al.* (2007) used petiolar anatomy to enhance the delimitation of members of *Echinodorus* Richard occurring in north-eastern Brazil. In their study, they reported that the outline of midrib and petiole and vascular bundle characteristics (number and arrangement) were important features that can delimit the genus. In the same way, Arogundade and Adedeji (2017) and Baruah *et al*. (2017) found the foliar and petiole anatomy as a diagnostic feature in some members of the *Dieffenbachia* Schott (Araceae) and *Smilax* (Smilacaceae). Our study corroborates these previous reports.

Conclusions

The data generated from the work showed some outstanding differences in the morphological and anatomical characteristics of *A. lobata* and *A. cissampeloides*. Thicker midrib ridge, paracytic and anisocytic stomata, and a pair of petiolar glands in *A. lobata;* abundance of mucilaginous and tanniferous cells, sclereids surrounded by lignified cells, and thicker layers of fibre in tendril of *A. cissampeloides*. The findings of this work, therefore, contribute to and enhance the delimitation of the two species.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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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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