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Research Article

PETIOLE ANATOMY OF SOME SELECTED SPECIES OF THE FAMILY VERBENACEAE (LAMIALES) IN NIGERIA

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ABSTRACT
Petiole anatomical characteristics of Verbenaceae species occurring in Nigeria were studied using 28 species belonging to 10 genera with the aid of light microscopy. The taxonomically useful features of this vegetative structure are shape and outline which vary from furrowed, dorsiventrally flattened to non-furrowed, semi-circular, broadly and narrowly furrowed in transverse sections of individual species with the abaxial surface semi-circular and adaxial surface furrowed. In most taxa, the vascular structure is composed of collateral bundle along with marginal, dorsal and lateral traces or ribs. Free traces that are U-shaped form a ring, crescent arc and invaginated arc shapes were also observed within the family. The perivascular tissues are composed of collenchyma and parenchyma cells. Anatomical characters have provided features that are taxonomically useful in delineating individual species. The relative consistency of the vascular structure existing in other groups of taxa reveals the close genetic affinity existing between them.

Keywords: *Anatomy, Angiosperm, Morphology, Taxonomy*

INTRODUCTION
Many species of the family Verbenaceae are economically important as sources of timber, medicine, food and aesthetics (Bailey, 1949; Burkill, 1994). This wide range of uses indicates the morphological variation that exists within the family. There are *ca.* 32 genera and 840 species occurring in open and forested, xeric and mesic habitats in the family (O'Leary *et al.*, 2012). Out of these, thirteen genera and sixty-four species exist in Africa while only ten genera and sixty-four species have been recorded in Nigeria (Hutchinson and Dalziel, 1958).
The classification of members of the family has been questioned by various workers (Marx *et al*., 2010; Atkins, 2004) creating a need for re-evaluation of the morphological characters used (Hutchinson and Dalziel, 1958; Cronquist, 1981), or the use of molecular tools in establishing relationships (Marx *et al.* (2010) by previous workers. The various changes in taxonomic opinions have led to separation and transfer of taxa in the family Verbenaceae to Lamiaceae, Acanthaceae and Avicenniaceae (Thorn, 1992; Marx *et al*. 2010). However, many characters traditionally employed in the classification have not supported the relationship within the family, with traditional assumptions of character polarity in many cases shown to be wrong (O'Leary *et al*., 2012). Thus, the need for additional taxonomic characters is imperative; and absence of information on some taxa from some understudied regions such as Nigeria has militated against complete comprehension of the taxonomic affinities among the various taxa of the family.
Although, there are several accounts on the anatomical characters of the vegetative structures of the various species of Verbenaceae but the Nigerian species are rarely investigated (Mathew and Shah, 1987; Solereder, 1908; Inamdar, 1968; Inamdar and Patel, 1969). Petiole is an important vegetative structure capable of providing taxonomic characters that can aid species identification (Radford *et. al.*, 1974; Metcalfe and Chalk, 1950, 1979; Olowokudejo, 1987; Wilkinson, 1989). Therefore, anatomical structures of Verbenaceae species have been investigated and are presented.

MATERIALS AND METHODS

To do the research, the first step was to dry the fresh plant specimens from the field with plant press, then study the dried plant samples in the herbaria of the Forestry Research Institute of Nigeria, Ibadan (FHI), University of Ibadan, (UIH), University of Lagos (LUH), University of Nigeria, Nsukka (UNH) and Nnamdi Azikiwe University (NAUH). These abbreviations follow Holmgren *et al*. (1990). Field samples were also collected from several Forest Reserves and National Parks in Nigeria, notably Kainji Lake National Park, Niger State and Gashaka Gumti National Park, Taraba State and examined; the representatives of each species were deposited as vouchers at the University of Lagos Herbarium data of the samples examined are shown in Tables 1 and 2.

Table 1: Field collection used for the study

S/N	Species	Collectors	Location	Date	Herbarium number
1.	*Clerodendrum capitatum*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Ikire, Osun State.	10/8/2013	LUH 6194
2.	*C. splendens*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Challenge, Oyo State.	8/7/2013	LUH 6193
3.	*C. volubile*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Bodija, Oyo State	8/7/2013	LUH 6192
4.	*Duranta repens*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	University of lagos campus akoka, Lagos State.	8/7/2013	LUH 6197
5.	*Gmelina arborea*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Apata, Ibadan, Oyo State.	8/7/2013	LUH 6191
6.	*Lantana camara*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Eleyele, Ibadan, Oyo State.	10/8/2013	LUH 6188
7.	*Lippia multiflora*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Gashaka Gumti National Park, Taraba State.	8/7/2013	LUH 5560
8.	*Lippia sp.*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Kainji Lake National Park, Niger State.	8/7/2013	LUH 2980
9.	*Stachytarpheta angustifolia*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Ojo, Oyo State.	21/5/2013	LUH 6198
10.	*S. cayennensis*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Ido, Oyo State.	8/7/2013	LUH 6195
11.	*S. jamaiscensis*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Omi-adio, Oyo State.	8/7/2013	LUH 6196
12.	*Tectona grandis*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Jericho, Oyo State.	8/7/2013	LUH 6187
13.	*Vitex doniana*	Anorue, G.U., Kadiri, A. B., Olowokudejo, J.D.	Kainji Lake National Park, Niger State.	8/7/2013	LUH 6186

Table 2: Exsiccate data of field and herbarium specimens used for the study

S/N	Species	Location	Collectors	Date	Herbarium Number
1.	*C. aculeatum*	Oyo state	H.V. Levy.	10.06.30	FHI 10851
2.	*C. bulchholzii*	Oyo state	J. Lowe.	1. 11. 89	UIH 21435
4.	*C. dusenii*	Edo state	J. Lowe.	4.4.76	UIH 16958
5.	*C. formicarium*	Taraba state	O.O. Oyebanji & A. Akinsoji.	1.7.2016	LUH 7144
6.	*C. glabrum*	Oyo state	T.K. Odewo.	4.6.47	FHI 21316
7.	*C. polycephalum*	Ogun state	O.O. Oyebanji.	22.7.2013	LUH 5930
9.	*C. thomsonae*	Yenegoa province	J. Lowe.	28.5.75	UIH 16295
10.	*C. tomentellum*	Plateau state	J.A.D. Jackson.	11.7.67	FHI 17700
11.	*C. umbellatum*	Ogun state	T.K. Odewo	11.7.2013	UIH 10029
12.	*C. violacium*	Ogun state	T.K. Odewo & O.O. Oyebanji.	11.7.2013	LUH 5029
16.	*G. hystrix*	Ekiti state	B.O. Oyejola	18.3.62	UIH 12830
18.	*L. rhodensiensis*	Osun state	J. Lowe.	2.5.78	UIH17882
19.	*Lippia chavalieri*	Kaduna state	S.O. Oyewole	18.12.72	UIH 13838
22.	*Phyla nodiflora*	Sokoto state	A.B. Kadiri.	2.5.2011	LUH 3834
23.	*Petrea volubilis*	Oyo state	B.O. Daramola	2.1.2010	LUH 2980

Petiole Preparation

Five specimens per species were studied. Thin sections of the petioles were obtained from the median of the first node because of the limited variation observed between it and, distal and proximal sections. The sections were first boiled in water while fresh samples were used directly. They were soaked in 50% sodium hypochlorite solution [commercial bleach] for 20-30 minutes to bleach so as to enhance clarity. The transparent sections were placed on the slides after cleansing in several changes of water. A few drops of concentrated ethyl alcohol were added in order to harden the tissue; then samples were stained in 1% aqueous Safranin O for 1 minute and countered stained with 1% Toluidine blue for 2 minutes. Excess stain was washed off with a few drops of concentrated ethyl alcohol. The stained sections were mounted in glass slide and covered with cover slips and the edges were ringed with nail polish to avoid dehydration. All slides were examined on an Olympus microscope carrying a camera attached to a computer (O'Brien *et al*., 1964; Kadiri 2006), with slight modifications. Both light micrographs and camera lucida drawings are employed to reveal the various features of the observed specimens.

RESULTS AND DISCUSSION

The results of the investigation are presented in Table 3 and described for each species hereunder.

***Clerodendrum aculeatum* (Linn) Griseb:**

The petiole shape of *C. aculeatum* is shallowly furrowed on the adaxial surface while it is semi-circular on the abaxial surface. Vascular bundle is made up of angular collenchyma cells with polygonal parenchyma cells found outside and inside the pith. Vascular bundle consists of open collateral bundles, centrally located and U-shaped. The vascular pattern is composed of many vascular bundles forming a ring with 2 marginal traces. Trichomes are present only on the adaxial surface while they are absent on the abaxial surface of the epidermis (Table 3, Figure 1).

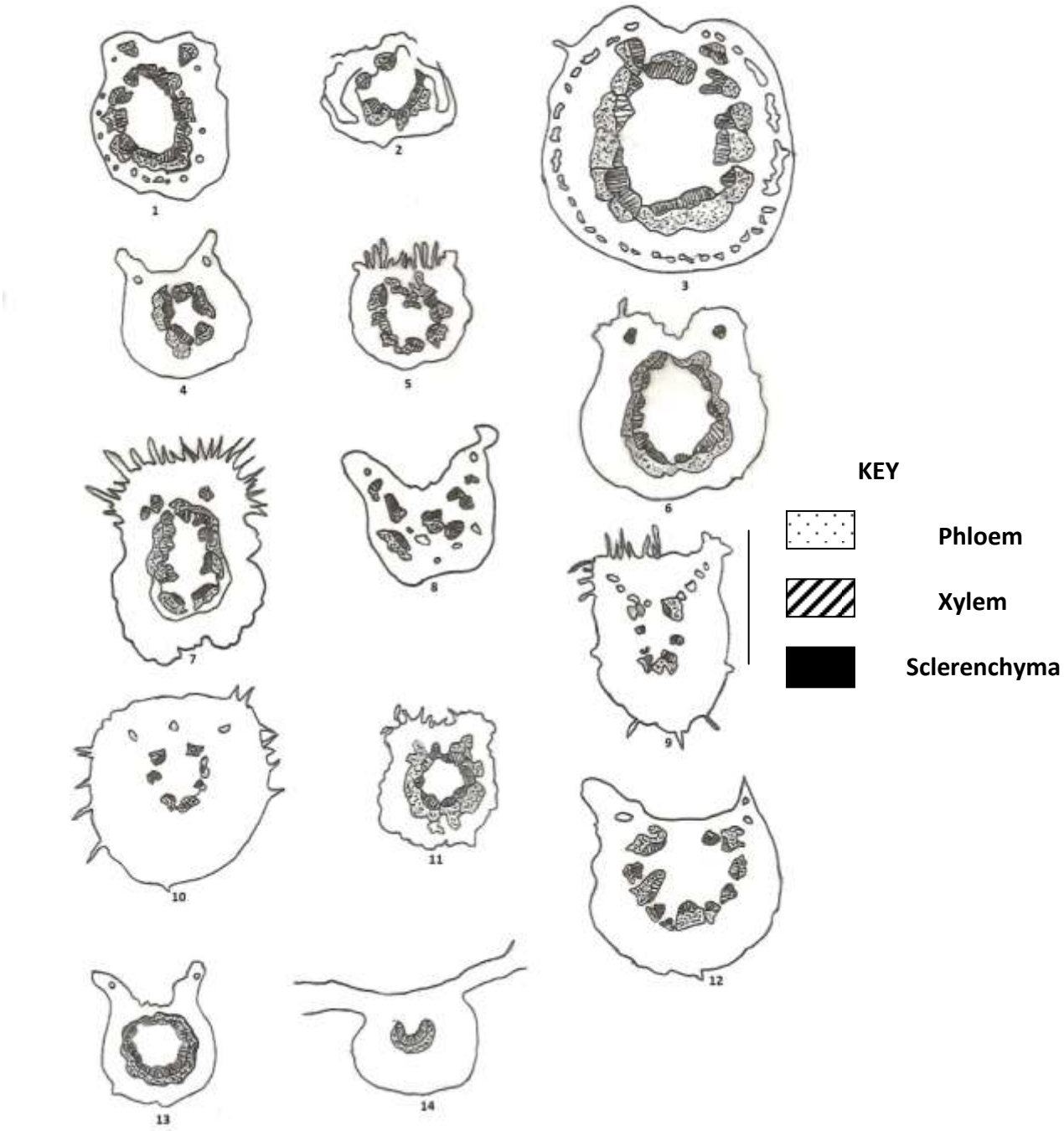


Figure 1-14: Transverse Sections of Petioles of Verbenaceae Species In Nigeria. *Clerodendrum aculeatum, C. buchholzii, C. capitatum, C. dusenii, C. formicarium, C. glabrum, C. polycephalum, C. splendens, C. thomsonae, C. tomentellum, C. umbellatum, C. violacium, C. volubile* and *Duranta repens. Scale bar = 25µm.*

Table 3: Petiole Features of Verbenaceae species in Nigeria

S.N.	Species	Petiole shape	Vascular bundle shape	Trichome		Vascular bundle pattern	Vascular bundle position	Rib traces			Petiole shape Adaxial surface	Petiole shape Abaxial surface
				AD	AB			D	L	M		
1.	*C. aculeatum*	Furrowed	U-shape	+	-	Free bundles in rings	Central	-	-	2	Shallowly furrowed	Semi-circular
2.	*C. bulchholzii*	Furrowed	Scattered	-	-	Free bundles in rings	Central	--	-	-	Broadly furrowed	Semi-circular
3.	*C. capitatum*	Furrowed	Circular in rings	+	-	Free bundles in rings	Central	-	-	2	Narrowly furrowed	Circular
4.	*C. dusenii*	Furrowed	U-shape	-	-	Collateral free bundles	Central	-	-	2	Broadly furrowed	Semi-circular
5.	*C. formicarium*	Non-furrowed	U-shape	+	-	U-shape free bundles	Central	-		2	Multiply flattened	Circular
6.	*C. glabrum*	Furrowed	U-shape	+	-	U-shape with medullary cylinder	Central	-	-	2	Narrowly furrowed	Semi-circular
7.	*C. polycephalum*	Furrowed	U-shape	+	-	U-shape fused collateral bundles in a ring	Central	-	-	3	Multiply and narrowly furrowed	Doubly furrowed
8.	*C. splendens*	Furrowed	Collateral scattered	-	-	Free scattered not in rings	Central	-	-	2	Broadly, deeply furrowed	Shallowly furrowed
9.	*C. thomsonae*	Furrowed	U-shape	+	+	U-shape free bundles in a ring	Central	-		8	Multiply Furrowed	Semi-circular
10.	*C. tomentellum*	Non-furrowed	U-shape, invaginated ends	+	+	Fused bundles in a ring	Central	-	-	2	Circular	Circular

11.	*C. umbellatum*	Furrowed	Circular in rings	+	-	Fused collateral bundles in a ring	Central	-	-	2	Multiply furrowed	Multiply furrowed
12.	*C. violacium*	Furrowed	U-shape	-	-	Free collateral bundles in a ring	Central	-	-	3	Broadly furrowed	Semi-circular
13.	*C. volubile*	Furrowed	Circular in rings	-	-	Collateral ring of bundles	Central	-	-	2	Narrowly furrowed	Semi-circular
14.	*D. repens*	Non-furrowed	Crescent arc	-	-	Open without traces	Central	-	-	-	Dorsiventrally flattened	Semi-circular
15.	*G. arborea*	Furrowed	Circular in rings	-	-	Free bundles in rings	Central	-	-	2	Broadly furrowed	Semi-circular
16.	*G. hystrix*	Furrowed	Arc shaped	-	-	Arc shaped	Central	-	-	2	Broadly and multiply-furrowed	Semi-circular
17.	*L. camara*	Furrowed	Shallow crescent arc shape	+	-	Semi-arc with a slight invaginated end	Central	-	-	2	Broadly and multiply-furrowed	Semi-circular
18.	*L. rhodensiensis*	Furrowed	Shallow crescent arc shape	-	-	Deep arc without invaginated ends	Central	-	-	2	Broadly and multiply-furrowed	Semi-circular
19.	*Lippia chavalieri*	Furrowed	Broad crescent arc, slightly invaginated ends	-	-	An arc with invaginated ends	Central	2	-	2	Shallowly furrowed	Semi-circular
20.	*L. multiflora*	Furrowed	Broad crescent arc without	-	-	An arc without	Central	2	-	-	Multiply-furrowed	Semi-circular

			invaginated ends			invaginated ends						
21.	*Lippia sp.*	Non-furrowed	Closed arc like heart-shape	+	+	Closed arc with deep invaginated end	Central	-	3	-	Circular	Semi-circular
22.	*Phyla nodiflora*	Furrowed	Semi-crescent shape	-	-	Semi-flat arc	Central	-	-	5	Deeply furrowed	Semi-circular
23.	*Petrea volubilis*	Non-furrowed	Closed arc	+	-	Medullated vascular cylinder	Central	-	2	-	Flat	Circular
24.	*Stachytarpheta angustifolia*	Non-furrowed	Simple arc shape	+	-	Open arc	Central	-	-	2	Dorsiventrally flattened	Semi-circular
25.	*S. cayennensis*	Furrowed	Semi-arc shape	+	-	Open arc slightly invaginated end	Central	-	2	-	Narrowly and multiply furrowed	Circular
26.	*S. jamaicensis*	Furrowed	Crescent arc shape with invaginated ends	+	-	Open arc with rib traces	Central	-	2	-	Broadly furrowed	Semi-circular
27.	*Tectona grandis*	Furrowed	Circular in rings	-	-	Collateral forming medullated cylinder	Central	-	-	-	Shallowly furrowed	Semi-circular
28.	*Vitex doniana*	Furrowed	Free traces in medullary bundle	-	-	Free traces in concentric rings	Central	-	-	-	Narrowly furrowed	circular

- = Absent; + = Present; D = Dorsal traces; L = Lateral traces; M= Marginal rib traces; AD= Adaxial; AB = Abaxial

***Clerodendrum bulchholzii* Gürke**:
Trichomes are absent on both surfaces of the epidermis. Petiole shape on the adaxial surface is broadly furrowed while on the abaxial surface, petiole shape is semi-circular. The vascular bundle is a collateral bundle with polygonal parenchymal cells found around and in-between them. The vascular pattern is a free bundle without dorsal, lateral and marginal traces. Vascular bundle is usually positioned at the center of the petiole (Table 3, Figure 2).

***Clerodendrum capitatum* Willd**:
The petiole shape observed in *C. capitatum*, is narrowly furrowed on the adaxial surface but circular on abaxial surface of the epidermis. Vascular bundle pattern is a free bundle that forms a ring while the vascular bundle shape is collateral and circular with 2 marginal traces. The location of the vascular bundle is positioned at the center. Trichome is found on the adaxial but absent on abaxial surface. Free polygonal parenchymal cells make up the pith in this species (Table 3, Figure 3).

***Clerodendrum dusenii* Gürke:**
The shape of the vascular bundle of this species are somewhat scattered with 2 marginal traces while the vascular pattern is free collateral bundles. Trichome is found only on the adaxial surface but are absent on the abaxial surface of the epidermis. The vascular bundle is centrally placed and perivascular tissues such as collenchyma and parenchymal cells were observed. On the adaxial surface, petiole shape is broadly furrowed while the on the abaxial surface, it is semi-circular in shape (Table 3, Figure 4).

***Clerodendrum formicarium* Gürke:**
Petiole shape is multiply furrowed on the adaxial surface but circular on the abaxial surface of the epidermis. The vascular bundle is located at the center of the petiole with 2 marginal traces located on either side of the adaxial surface. The vascular pattern of the petiole is a scattered one while trichome is restricted to the adaxial surface alone but absent on the abaxial surface of the epidermis (Table 3, Figure 5).

***Clerodendrum glabrum* E. May:**
The perivascular tissue found in *C. glabrum* consists of both collenchyma and parenchymal cells with the pith composed only of loosely packed parenchymal cells. Petiole shape is furrowed. On the adaxial surface, the petiole shape is narrowly furrowed while on the abaxial the petiole shape is semi-circular. Central vascular bundle is U-shaped that forms a medullary cylinder with 2 marginal traces while vascular pattern is U-shaped. Trichome is restricted to the adaxial surface but absent on abaxial surface of the epidermis (Table 3, Figure 6).

***Clerodendrum polycephalum* Bark:**
Adaxial surface shape of the petiole of this species is multiply and narrowly furrowed but doubly-furrowed on the abaxial surface of the epidermis. The vascular shape is U-shaped collateral bundle that form a ring positioned at the center of the petiole with 3 marginal traces found at the adaxial side of the epidermis. The vasculation pattern is U-shaped fused bundles. Petiole outline of this species is furrowed. Trichome is found on the adaxial but absent on the abaxial surface of the epidermis (Table 3, Figure 7).

***Clerodendrum splendens* G. Don:**
Petiole shape of this species is broadly and deeply furrowed on the adaxial surface while the abaxial surface is shallowly furrowed. The shape of the vascular bundles is collateral and scattered with 2 marginal traces. Trichome is absent on both surfaces of the epidermis. Pattern of arrangement of the vascular bundle is free but scattered and not in rings (Table 3, Figure 8).

***Clerodendrum thomsonae* Balf.f:**
In *C. thomsonae* the vascular bundle is central having 2 pairs of marginal traces. On the adaxial surface, petiole shape is multiply-furrowed while on the abaxial surface, the petiole shape is semi-circular. Vasculation pattern of this species is free U-shaped bundles that form a ring as well as the vascular bundle shape. Parenchymal cells are oval, polygonal and loosely packed present within and without the pith.

Angular collenchyma cells are also present outside the pith below the parenchymal cells. Trichome is present on both surfaces of the epidermis (Table 3, Figure 9).

***Clerodendrum tomentellum* Hutch & Dalz:**

The transverse section of this petiole is circular on the adaxial surface and circular abaxial surface. Petiole outline is non-furrowed. Vascular bundle is collateral, open and centrally positioned with 3 lateral traces observed on the adaxial surface located. Vascular shape is U-shaped slightly invaginated at the ends while vasculation pattern is fused bundles with invaginated ends. Trichome is visible all over the petiole surface (Table 3, Figure 10).

***Clerodendrum umbellatum* Poir:**

Petiole is multiply furrowed on both adaxial and abaxial surfaces. Vascular bundles are situated at the center with 2 traces each, present at the margins. Vasculation pattern of the petiole is fused collateral bundles that form a ring. Trichome is present on the adaxial surface but absent abaxial surface (Table 3, Figure 11).

***Clerodendrum violacium* Gürke:**

Petiole vascular bundle is a free collateral bundle that form an arc with invaginated ends. The vascular shape is U-shape while pattern of vascular bundle distribution is an arc with invaginated ends. Adaxial surface of the petiole is broadly-furrowed with 2 marginal traces while the abaxial surface is semi-circular. Trichome is copiously absent on both surfaces of the epidermis (Table 3, Figure 12).

***Clerodendrum volubile* P. Beauv:**

Petiole shape is narrowly-furrowed adaxially and semi-circular abaxially. Vascular bundle is collateral ring of bundles and positioned at the center with 2 marginal traces while the vascular pattern is a medullated cylinder. Trichome is unarguably absent on both surfaces of epidermis. Perivascular tissues are present with polygonal loosely packed parenchymal cells found in the pith and around the vascular bundles with angular collenchyma present near the epidermal layer (Table 3, Figure 13).

***Duranta repens* Linn:**

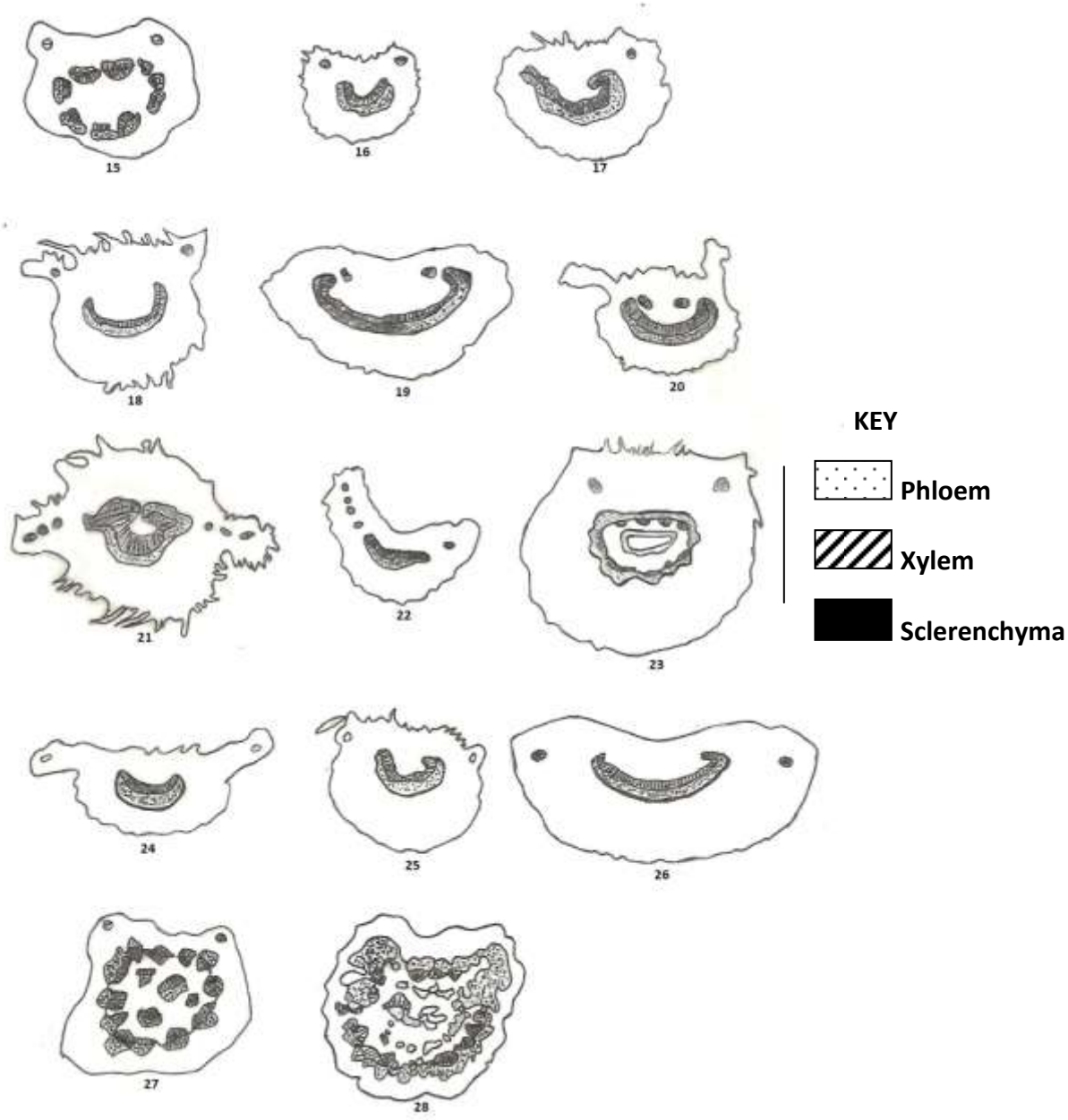
Petiole shape is dorsiventrally flattened on the adaxial surface but semi-circular on the abaxial surface. Centrally positioned vascular bundle is present without traces. Vascular bundle shape is a crescent arc with vascular pattern open without traces. Trichome is obviously absent on both surfaces of the epidermis (Table 3, Figure 14).

***Gmelina arborea* Roxb**

Petiole shape in *G. arborea* is broadly furrowed on the adaxial surface but semi-circular on the abaxial surface. Large closely packed parenchymal cells make up the pith. Collenchyma cells are also present but absent in the pith. Vasculation pattern is free bundles that forms a ring. Vascular bundle shape is circular with 2 marginal traces which are centrally situated. Trichomes are absent on both surfaces of the epidermis (Table 3, Figure 15).

***Gmelina hystrix* Schult:**

G. hystrix petiole is furrowed and composed of both collenchyma and parenchymal cells with the later found to be the only constituent of the pith. Vascular bundle is broadly and multiply-furrowed on the adaxial with semi-circular abaxial surface. The vascular bundle is located at the center having a shape of an arc shape. Pattern of vasculation is arc forming an arc shape. Trichomes are absent on both epidermal surfaces (Table 3, Figure 16).



Figures 15-28: Transverse sections of petioles of Verbenaceae species in Nigeria. *Gmelina arborea, G. hystrix, Lantana camara, L. rhodensiensis, Lippia chavalieri, L. multiflora, L. sp, Phyla nodiflora, Petrea volubile, Stachytarpheta angustifolia, S. cayennensis, C. jamaiscensis, Tectona grandis, Vitex doniana. Scale bar= 25µm.*

***Lantana camara* Linn:**
Lantana camara has a broadly and multiply furrowed petiole shape on the adaxial surface while on the abaxial surface, it is semi-circular. Collenchyma and Parenchymal cells are present with either polygonal, oval or circular shapes with the parenchymal cells observed in both the cortex and the pith. Vascular bundle shape in *L. camara* is a semi-arc with slight invaginated end and situated at the centre with 2 marginal traces while the vascular pattern is an arc. Trichome is only observed on the adaxial side of the epidermis but absent on the abaxial side of the epidermis (Table 3, Figure 17).

***Lantana rhodensiensis* Moldenke:**
The vascular system in this species is a deep arc with marginal ribs while the vascular bundle is centrally located, the vascular pattern is an arc. Perivascular tissues are present. Trichomes are found on both the adaxial surfaces of the epidermis. Petiole outline on the adaxial surface is broadly and multiply furrowed without dorsal and lateral traces while it is semi-circular on the abaxial surface of the epidermis (Table 3, Figure 18).

***Lippia chavalieri* Moldenke:**
The petiole shape is furrowed, Perivascular tissues are conspicuously present. Petiole shape is shallowly furrowed on adaxial surface but semi-circular on the abaxial surface. The vascular shape is broad crescent arc that is slightly invaginated with 2 dorsal traces. The pattern of vasculation is an arc with invaginated ends. Trichome is absent on both surfaces of the epidermis and vascular bundle position is central (Table 3, Figure 19).

***Lippia multiflora* Moldenke:**
Petiole shape is furrowed in *L. multiflora*. On the adaxial surface the petiole shape is multiply furrowed while on the abaxial surface is semi-circular and vascular shape is central, having a deep crescent shape having 2 dorsal traces without invaginated ends. Pattern of vasculation is an arc. The perivascular tissues are present. Trichome is absent on adaxial surface but present on abaxial surface of the epidermis (Table 3, Figure 20).

***Lippia sp*:**
In this species, the petiole shape is non-furrowed. On the adaxial surface of the epidermis, the shape is circular while on the abaxial side of the epidermis, it is semi-circular. Trichomes are present on both surfaces but more dominant on the abaxial surface of the epidermis. Central vascular bundle exists with deep invaginated heart-shape. Vascular pattern is an arc with deep invaginated ends. Three lateral traces are present with perivascular tissues well represented as collenchyma and parenchymal cells (Table 3, Figure 21).

***Phyla nodiflora* (L) Greene:**
The petiole shape is furrowed in *P. nodiflora.* On the adaxial surface deeply-furrowed while on the abaxial surface the shape is semi-circular. The vascular bundle is centrally positioned and semi-crescent shape with 5 marginal traces. Perivascular tissues are well represented and vascular pattern is semi-flat arc. Trichome is absent on both surfaces (Table 3, Figure 22).

***Petrea volubilis* Linn:**
Here, the petiole shape is non-furrowed and flat on the adaxial surface while on the abaxial surface, the petiole shape is circular. The vascular bundle is a closed medullated cylinder with 2 lateral traces occupying the center of the petiole. Vasculation pattern is central and trichome is only seen on the adaxial surface but absent on the abaxial surface of the epidermis (Table 3, Figure 23).

***Stachytarpheta angustifolia* (Mill.) Vahl**:
The petiole of this species is non-furrowed; petiole shape is dorsiventrally flattened adaxially while it is semi-circular on the abaxial surface. The vascular bundle shape is a simple arc centrally placed with 2 marginal traces. Vascular pattern is open arc. Trichome is only seen on adaxial surface but absent on abaxial surface (Table 3, Figure 24).

***Stachytarpheta cayennensis* (L.C.Rich.) Schau:**
S. cayennensis have adaxial petiole shape is narrowly and multiply furrowed while on the abaxial it is semi-circular. The vasculation pattern is an open arc but slightly invaginated at the ends. The shape of the vascular bundle is semi-arc shape with 2 lateral traces. Trichome is situated adaxially but absent abaxially (Table 3, Figure 25).
Stachytarpheta jamaicensis* (Linn.) Vahl*:
The petiole of *S. jamaicensis* is furrowed. On the adaxial surface it is shallowly furrowed while on the abaxial surface it is semi-circular. The vascular bundle shape is crescent arc shape that is centrally positioned with vascular pattern an open arc with rib traces. Trichome is copiously absent both surfaces of the epidermis (Table 3, Figure 26).
***Tectona grandis* Linn:**
Vasculation pattern is many traces in a ring while the vascular bundles shape is collateral forming a medullated cylinder. Trichome is absent on both surfaces. The adaxial petiole shape is shallowly furrowed but abaxial petiole shape is semi-circular. Perivascular tissues are visibly seen. Vascular bundle shape is collateral circular bundles that form a medullated cylinder without rib traces. Vascular bundles are centrally positioned which is characteristic of the family (Table 3, Figure 27).
***Vitex doniana* Sweet:**
Perivascular tissues are present. Vascular bundle shape is free traces that form medullary bundles without marginal traces. Vascular pattern of the petiole is free traces in concentric rings. Trichomes are absent on both surfaces of the epidermis. Petiole shape on the adaxial surface is narrowly furrowed while on the abaxial surface it is circular. The vascular bundle positioned at the center of the cortex (Table 3, Figure 28).
The petiole shape differs considerably among the investigated species. Perivascular tissues such as collenchyma, parenchyma and sclerenchyma cells are present in all species studied. Collateral vascular bundles, furrowed petiole shapes and centrally positioned vascular bundles are unique and common features observed in species examined in this family. All species in all genera studied have furrowed petiole shape except *C. formicarium, C. tomentellum*, *Duranta repens*, *Lippia sp*, *Petrea volubilis* and *S. jamaiscensis* whose petioles are non-furrowed. Vascular bundle type is another feature that has been used by previous authors to solve anatomical problems using petiole anatomy. In this family, vascular bundle shape of the petioles ranges from closed arc shape found only in *Petrea volubilis* and *Lippia sp.* among other species; circular shape forming rings in *Clerodendrum capitatum*, *C. umbellatum*, *C. volubile*, *Gmelina arborea* and *Tectona grandis*; heart-shaped types found only in *Lippia sp*; shallow crescent arc type in *Lantana camara* and *L. rhodensiensis*; semi-crescent arc found only in *Phyla nodiflora* among other species; scattered vascular bundle type was observed only *C. buchholzii* and *C. splendens*; U-shaped type found seen in *C. aculeatum*, *C. dusenii*, *C. formicarium*, *C. glabrum* *C. polycephalum*, *C. thomsonae*, *C. tomentellum* and *C. violacium;* free traces with medullary bundle were observed only in *Vitex doniana* (Table 3). The number, presence and absence of marginal ribs found in petioles of each species vary from 2 marginal ribs in all species except in *C. polycephalum* and *C. thomsonae* with 3 and 8 marginal ribs respectively. Lateral traces was also considered in distinguishing species. All species investigated in this study lack lateral traces except *Petrea volubilis* with 3 lateral traces, *Stachytarpheta angustifolia*, *S. cayennensis* and *S. jamaicensis* with 2 lateral traces (Table 3). Presence and absence of trichomes is another diagnostic feature employed in this study in delimiting the species in Verbenaceae for instance, trichome was present on the adaxial surface of all species except in *Clerodendrum splendens*, *C. thomsonae*, *C. tomentellum*, *C. volubile*, *Lippia multiflora* and *Lippia sp*.
The account recorded from this study is significant in the delineation of some species of this family. Okeke *et al.* (2015) reported that the petiole of the three species representatives of genus *Stachytarpheta* namely, *S. angustifolia, S. cayennensis* and *S. jamaiscensis* has little or no taxonomic significance in the description of the species, however, this study have shown that there are significant differences in the

petiole and vascular bundle shapes of the three species and has proven that each species possess peculiar anatomical characters that are diagnostic, for instance, the shape, vascular bundles shape and trichome distributions were observed to be different in the three species of genus *Stachytarpheta*; in *S. angustifolia*, the petiole shape is dorsiventrally flattened adaxially while it is narrowly and multiply furrowed in *S. cayennensis* and broadly furrowed in *S. jamaiscensis.* In terms of their respective vascular bundle shapes, a significant difference was observed in *Lippia sp* whose vascular bundle shape is arched like the shape of the human heart thereby separating it from the other two species of genus *Lippia* namely, *Lippia chavalieri* and *L. multiflora* with simple crescent arc shape. Many workers have employed anatomical approach to solve taxonomic problems. These include the work of Kadiri and Olowokudejo (2010) on foliar epidermal morphology in West African species of genus *Ludwigia* (Onagraceae); Olowokudejo and Nyananyo (1990) on epidermal morphology of *Khaya* of family Meliaceae; Olowokudejo (1987) on the value of petiole anatomy in genus *Biscutella* of the family Cruciferae; Olowokudejo (1993) on epidermal morphology of *Jatropha* species (Euphobiaceae) in West Africa; Ogundipe and Wujek (2004) on foliar anatomy of twelve genera of Bignoniaceae (Lamiales).

The petiole anatomy showed both intra- and inter-specific variations which are important in the categorization and delineation of the species in this family. The vasculation pattern and tissue constitution are not uniform in all species with few exceptions, specifically in the shape, vasculation pattern as well as the presence and absence of trichomes. However, the differences observed in the petiole shape of the adaxial surface and types of vascular bundles are useful for the separation of genera petiole. Most epidermises are adaxially furrowed and made up of polygonal collenchyma cells in cortex while parenchyma cells alone make up the pith. This is characteristic of the family. Anatomical differences occur in the vascular bundles of various species. The distinction in shape and type of vascular bundles is of taxonomic importance since it divided the species into ten different groups, that is, broad crescent arc shape, bundle fused forming a siphonostele (found in *Petrea volubilis*), circular forming rings, free traces in medullary bundle, heart-shape (found in *Lippia sp*.), scattered vascular bundle (found in *Clerodendrum buchholzii* and *C. splendens*), U- shape (in *Clerodendrum aculeatum, C. dusenii, C. formicarium, C. glabrum, C. polycephalum, C. glabrum, C. thomsonae, C. tomentellum, C. violacium*), semi-crescent shape and shallowly crescent shape (found in *Lantana camara* and *L. rhodensiensis*). Vascular bundles are either joined in free circular rings, as a cylinder or arranged in an arc. Two major types of vascular bundles occur in the species investigated: open collateral bundles and amphicribal bundles. Amphicribal bundle was found only in *Tectona grandis* and *Vitex doniana* in agreement to the work of Mathew and Shah (1987). *Lippia sp.* and *Petrea volubilis* are distinguished from other species because of the shape of their vascular bundles. *Lippia sp* has a deeply invaginated heart-shaped vascular bundle while that of *Petrea volubilis* is a closed arc with medullated cylinder. These separate the *Petrea volubilis* and *Lippia sp* from other species. The presence or absence of trichomes in the epidermis of the petioles is invaluable taxonomically. The species can be grouped into three, such as, those that possess trichomes at the adaxial surface alone, those that have trichomes at both abaxial and adaxial surfaces and those that lack trichome on either surface of the epidermis. The vascular bundle presents the most taxonomically important anatomical characters that have been observed in other plant groups (Mathew and Shah, 1987; Olowokudejo, 1987; Olowokudejo, 1993; Akçin *et al*., 2011; Okeke *et al*., 2015).

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