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Leaf epidermal and venation studies of some *Bridella* species (*Phyllanthaceae*)

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Abstract

Leaf anatomical characters are valuable tools for resolving taxonomic relationships among closely related plant species. The species studied here are *Bridelia atroviridis*, *Bridelia ferruginea* and *Bridelia micrantha*. *Bridelia* species have been collected within the campus of Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. This study examines the foliar epidermal features, transverse section anatomy, and venation patterns of *Bridelia* species to identify the diagnostic characters of taxonomic significance. Leaf epidermal, transverse section of leaf, and venation patterns have been studied using the standard microscopic techniques, with subsequent analysis of both qualitative and quantitative characters. All species have hypostomatic leaves with anisocytic stomata, uniseriate epidermis, thick non-striated cuticle, dorsiventral mesophyll, and collateral vascular bundles indicating generic similarity. However, interspecific variations have been observed in the epidermal cell size, stomata index and density, trichome distribution, ergastic substances, midrib tissue arrangement, and venation patterns. *Bridelia micrantha* is distinguished by eucamptodromous venation and larger areole area, while *B. ferruginea* records higher stomata index values. The combined foliar anatomical characters have proved effective for species delimitation within the genus *Bridelia*.

Keywords

anisocytic, *Bridelia*, druses, hypostomatic,styloid, trichome, venation pattern

Citation

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Introduction

Phyllanthaceae, the second-largest lineage within *Euphorbiaceae sensu lato*, comprised about 2000 species in 59 genera (Hoffmann & al. 2006). The name *Phyllanthaceae* was first published by Ivan Ivanovich Martynov in 1820 in his *Tekhno-botanico Slovar* and was later legitimized by Reveal & al. (2007). The Angiosperm Phylogeny Group (APG III 2009) recognized five distinct families within *Euphorbiaceae* s.l., namely *Euphorbiaceae sensu stricto*, *Pandaceae*, *Phyllanthaceae*, *Picrodendraceae*, and *Putranjivaceae*, based on both molecular and morphological evidence (Webster 1994). With few exceptions, *Phyllanthaceae* has been primarily pantropical in distribution (Stevens 2001), and its members include trees, shrubs, semi-succulents, annual herbs, and even free-floating species (Hoffmann & al. 2006). The distinctiveness of *Phyllanthaceae* has been supported by multiple lines of

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evidence, including seed coat anatomy (Meeuse 1990; Machado & al. 2005; Kapgate & al. 2017), seed protein and serological data (Vogel 1986; Jensen & al. 1994; Wudarck & al. 2004; Sahul & al. 2019; Rozar & al. 2025), chemotaxonomy (Kusriani & al. 2024; Hegnauer 1977; Beutler & al. 1996), phytochemistry (Maulydia & al. 2025), and molecular and morphological attributes (Hoffmann & al. 2006). More recently, Moawed & al. (2015) have further recommended the segregation of *Phyllanthaceae* from *Euphorbiaceae* s.l. based on micro and macro morphological traits, as well as vein architectural characters.

Genus *Bridelia* Willd. (*Phyllanthaceae*) belongs to the subfamily *Phyllanthoideae*, tribe Brideliae, and order Malpighiales. In Nigeria, Hutchinson & Dalziel (1952) have reported four species in the south and two in the north. Beyond Nigeria, *Bridelia* occurs widely in tropical and subtropical Africa and Asia (Ngueyem & al. 2009; Maroyi 2017). While plant anatomy has long been recognized as a reliable tool in taxonomy due to its stability and reduced environmental plasticity (Stace 1980), limited attention has been given to the foliar and wood anatomy of *Bridelia*. Earlier studies within *Phyllanthaceae*, such as comparative leaf micromorphology of the *Phyllanthus* species (Solihan & al. 2015), have revealed diagnostic traits, including epicuticular wax types, stomata distribution, and trichome morphology. However, such anatomical investigations of *Bridelia* remain scarce, leaving a gap in the knowledge of the potential of foliar and wood anatomical features for species delimitation within the genus.

This study, therefore, aims at examining the foliar anatomical characteristics of selected *Bridelia* species based on their availability in Southwest Nigeria: *Bridelia atroviridis* Müll. Arg., *Bridelia ferruginea* Benth. and *Bridelia micrantha* (Hochst.) Baill. By identifying and comparing the diagnostic anatomical features, the research seeks to improve taxonomic resolution of *Bridelia*, minimize misidentification arising from morphological similarities, and contribute to a broader effort of refining the classification of *Phyllanthaceae* in relation to *Euphorbiaceae* s.l.

Materials and method

Plant collection

The *Bridelia* species were collected within the campus of Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria (N 07° 31.252′ E 04° 31.307′). Herbarium samples were deposited at the IFE Herbarium for voucher numbers. Table 1 shows the location, GPS coordinates and voucher numbers of the species.

Leaf epidermal studies

The scraping technique described by Metcalfe (1960) and modified by Arogundade & Adedeji (2016) was used in the preparation of the epidermal peels, where the desired epidermis was obtained by scraping off the unrequired layer and the mesophyll. Both abaxial and adaxial epidermal peels were done by positioning the desired surface face down on a glass slide and all tissue above the required epidermis was scraped off with a sharp blade until the required epidermis was obtained. The epidermal peels were stained by safranin O, and subsequently mounted in diluted glycerin solution. Stomata density was estimated by counting the number of stomata per square micrometer across different regions of the epidermal surface. For each specimen, stomata and epidermal areas were calculated as the product of length and width, with 25 measurements taken per feature from 10 randomly selected leaves. Stomata index which is the percentage proportion of the stomata to the epidermal cells of a leaf was calculated according to the formula:

$$S.I = \frac{S}{S + E} \times 100$$

SI: Stomata index

S: Number of stomata per unit area

E: Total number of subsidiary cells and regular epidermal cells in a given unit area.

Transverse section of the leaf

Transverse sections of the leaf of each *Bridelia* species were cut using a Reichert sliding microtome. These sections were then stained for 5 minutes with safranin O, rinsed, and counter-stained for 3–5 minutes with alcian blue to enhance the contrast of the cells. The sections were then cleaned and subjected to various ethanol dilutions of 50%, 70%, 80%, and 90% for dehydration and differentiation. They were afterwards mounted in diluted glycerol solution and observed under a microscope.

Venation studies

The method followed by Olatunji (1983): Mature samples of each *Bridelia* species were collected from the median portion of well-expanded leaves. To remove chlorophyll, the leaf samples were boiled in 90% ethyl alcohol until decolorized. The decolorized tissues were further cleared by immersing them in a 5% solution of domestic bleaching agent and then thoroughly rinsed in water. The cleaned leaf portions were preserved in 50% ethanol, stained with 1% aqueous safranin O, and mounted in 25% glycerol on clean glass slides for microscopic examination. A total of 25 areoles were measured per species, using 10 randomly selected leaves.

Statistical analysis

Data generated from the parameters of leaf epidermal and venation studies were subjected to Duncan Multiple Range Test (DMRT) for mean separation to show differences between the *Bridelia* species

Table 1. Location, GPS coordinates and voucher numbers of the species of genus *Bridelia*

S/N	Specis	Location	GPS coordinates	Voucher specimen number
1.	*Bridelia micrantha*	Along Conference Centre, O.A.U., Ile-Ife, Osun State	N07°31.331′ E004°31.932′	IFE 18205
2.	*Bridelia atroviridis*	Behind Moremi Hall, O.A.U., Ile-Ife, Osun State	N07°31.097′ E004°31.180′	IFE 18206
3.	*Bridelia ferruginea*	Along Road 8, Hill 2, O.A.U., Ile-Ife, Osun State	N07°31.834′ E004°31.623′	IFE 18207

Results

Leaf epidermal studies

A. Bridelia atroviridis

On the adaxial surface, the anticlinal walls are non-pitted, ranging from straight to undulating, with epidermal cells varying from polygonal to irregular in shape and possessing smooth periclinal walls. The epidermal cell area ranges from 375 μm^2 to 731.25 μm^2, with a mean value of 519.0 μm^2. Multicellular non-glandular trichomes are present, while stomata and ergastic substances are absent (Plate 1A; Table 2-3).

On the abaxial surface, the anticlinal walls are also non-pitted, straight to undulating, with irregularly shaped epidermal cells and smooth periclinal walls. The epidermal cell area ranges from 125 μm^2 to 262.5 μm^2, with a mean value of 221.25 μm^2. Stomata are present, indicating a hypostomatic condition. The stomata type observed is anisocytic, with elliptic to circular stomatal shapes. Stomata size ranges from 125 μm^2 to 306.25 μm^2, with a mean value of 201.75 μm^2, while the stomata index ranges from 7.65% to 18.67%, with a mean value of 10.75%. Multicellular, non-glandular trichomes are also present. Druses have been observed as ergastic substances (Plate 2 C-D; Table 2-3)

B. Bridelia ferruginea

On the adaxial surface, anticlinal walls are non-pitted, ranging from straight to slightly wavy, with epidermal cells of polygonal shape and possessing smooth periclinal walls. The epidermal cell area ranges from 437.5 μm^2 to 1512.5 μm^2; with a mean value of 1178.0 μm^2. Stomata and ergastic substances are absent. Trichome base is present (Plate 1 B-C; Table 2-3).

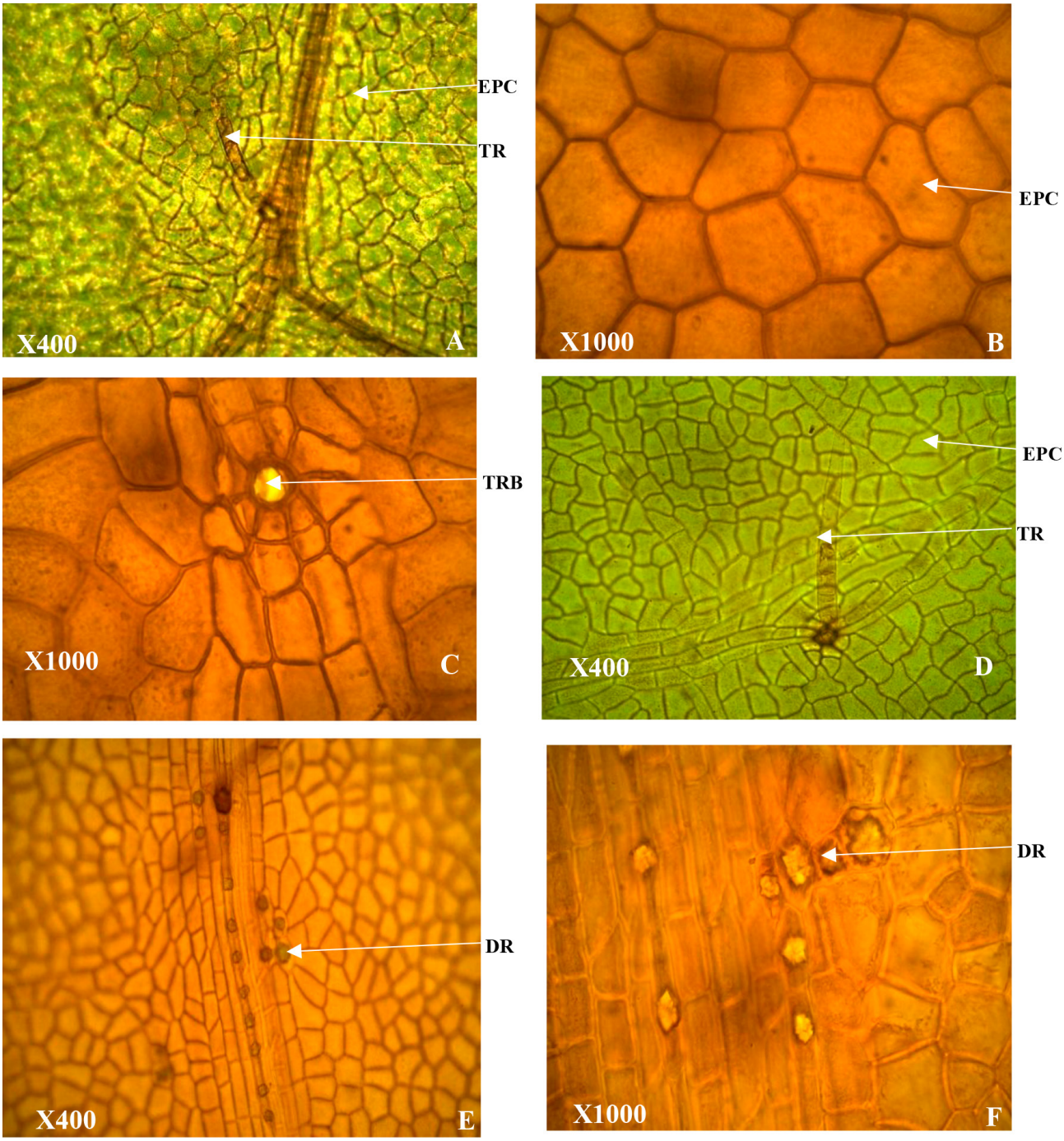
On the abaxial surface, anticlinal walls are non-pitted, ranging from straight to undulating, with polygonal to irregular shapes of the epidermal cells and smooth periclinal walls. The epidermal cell area ranges from 200 μm^2 to 975 μm^2, with a mean value of 356.0 μm^2. Stomata are present, indicating a hypostomatic condition. The stomata type observed is anisocytic, with elliptic shape. Stomata size ranges from 150 μm^2 to 337.5 μm^2, with a mean value of 280.75 μm^2, and stomata index ranges from 14.61% to 26.25%, with a mean value of 15.23%. Multicellular non-glandular trichomes. Ergastic substances are absent (Plate 2 A-B; Table 2-3).

c) *Bridelia micrantha*

On the adaxial surface, anticlinal walls are non-pitted, ranging from straight to slightly wavy, with a polygonal to irregular shape of the epidermal cells and smooth periclinal walls. The epidermal cell area ranges from 300 μm^2 to 900 μm^2; with a mean value of 565.0 μm^2. Stomata are absent. Trichome is multicellular and non-glandular. Druses have been observed as ergastic substances (Plate 1 D-F; Table 2-3).

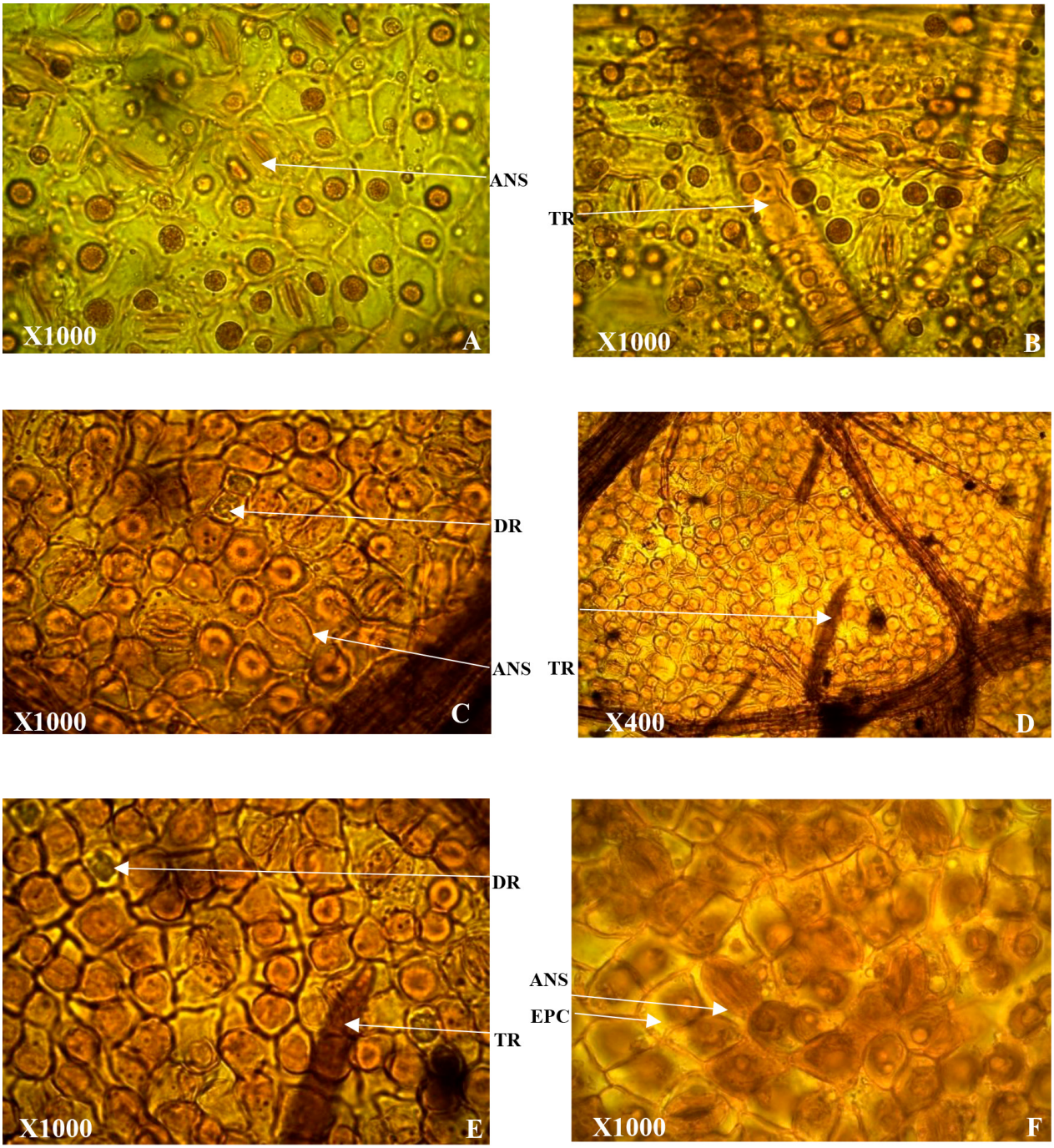
On the abaxial surface, the anticlinal walls are non-pitted, ranging from straight to slightly undulating, with a polygonal to irregular shape of the epidermal cells and smooth periclinal walls. The epidermal cell area ranges from 343.75 μm^2 to 1187.5 μm^2; with a mean value of 939.0 μm^2. Stomata are present, indicating a hypostomatic condition. The stomata type observed is anisocytic, elliptic in shape. Stomata size ranges from 125 μm^2- 337.5 μm^2; the mean value is 180.25 μm^2, and the stomata index ranges between 8.94%-16.09%, with a mean value of 10.0%. Trichomes are multicellular and non-glandular. Druses have been observed as ergastic substances (Plate 2 E-F; Table 2-3).

Plate 1. Photomicrograhs showing the adaxial surfaces of the *Bridelia* species
A: Epidermal cell and trichome (*B. atroviridis*); **B-C:** Epidermal cell and trichome base (*B. ferruginea*); **D-F:** Epidermal cell, trichome and druses (*B. micrantha*).



Legend: DR- druses, TR- trichome, EPC- epidermal cell, TRB- trichome base.

Plate 2. Photomicrograhs showing the abaxial surfaces of the *Bridelia* species
A-B: Stomata type and trichome (*B. ferruginea*); **C-D:** Druses, stomata type and trichome (*B. atroviridis*); **E-F:** Druses, epidermal cell, stomata type and trichome (*B. micrantha*).



Legend: TR- trichome, ANS- anisocytic stomata, EPC- epidermal cell, DR- druses.

Table 2. Summary of important qualitative foliar epidermal features of the adaxial and abaxial surfaces of the *Bridelia* species

Species	Surface	Epidermal cell shape	Anticlinal wall pattern	Stomata shape	Stomata type	Other features
Bridelia atroviridis	Adaxial	Polygonal to irregular	Straight to undulating	Nil	Nil	Multicellular non-glandular trichomes
	Abaxial	Irregular	Straight to undulating	Elliptic	Anisocytic	Druses, multicellular non-glandular trichomes
Bridelia ferruginea	Adaxial	Polygonal	Straight to slightly wavy	Nil	Nil	Trichome base
	Abaxial	Polygonal to irregular	Straight to undulating	Elliptic	Anisocytic	Multicellular non-glandular trichomes
Bridelia micrantha	Adaxial	Polygonal to irregular	Straight to slightly wavy	Nil	Nil	Druses, multicellar non-glandular trichomes
	Abaxial	Polygonal to irregular	Straight to slightly undulating	Elliptic	Anisocytic	Multicellular non-glandular trichomes

Table 3. Summary of important quantitative foliar epidermal features of the adaxial and abaxial surfaces of the *Bridelia* species

Species	Surface	Stomata area (μm²)	Stomata index (%)	Stomata density (mm²)	Epidermal cell area (μm²)	Areole area (μm²)
Bridelia atroviridis	Adaxial	Nil	Nil	Nil	519.0±21.83^{a}	10149.8±4681.65^{a}
	Abaxial	201.7±9.31^{a}	10.75±0.32^{a}	7.87±0.28^{a}	221.25±11.0^{a}	
Bridelia ferruginea	Adaxial	Nil	Nil	Nil	1178.0±71.93^{b}	5181.75±437.78^{a}
	Abaxial	280.75±9.34^{b}	15.23±0.30^{a}	8 .14±0.29^{b}	356.0±29.67^{a}	
Bridelia micrantha	Adaxial	Nil	Nil	Nil	565.0±38.28^{a}	12467±865.33^{a}
	Abaxial	180.25±10.39^{a}	10.0±0.24^{a}	5.10±0.17^{a}	939.0±335.50^{b}	

******Means with the same superscript along the same column are not significantly different at p ≤ 0.05.

Transverse section of the leaves

a) Bridelia atroviridis

Both the adaxial and abaxial surfaces have a uniseriate epidermis covered by a thick, non-striated and undulating cuticle. The mesophyll is differentiated into palisade and spongy tissues. The palisade mesophyll consists of a single layer of tightly packed, cylindrical, elongated cells, whereas the spongy mesophyll comprises 1–2 layers of loosely arranged cells enclosing conspicuous intercellular spaces. The midrib cortex consists of 7–8 layers of parenchyma cells and 2–3 layers of sclerenchyma cells. The vascular bundle is irregular in shape and collateral. Multicellular, non-glandular trichomes are present on the abaxial surface of the midrib. Druses have been observed as ergastic substances (Plate 3 A-D; Table 4).

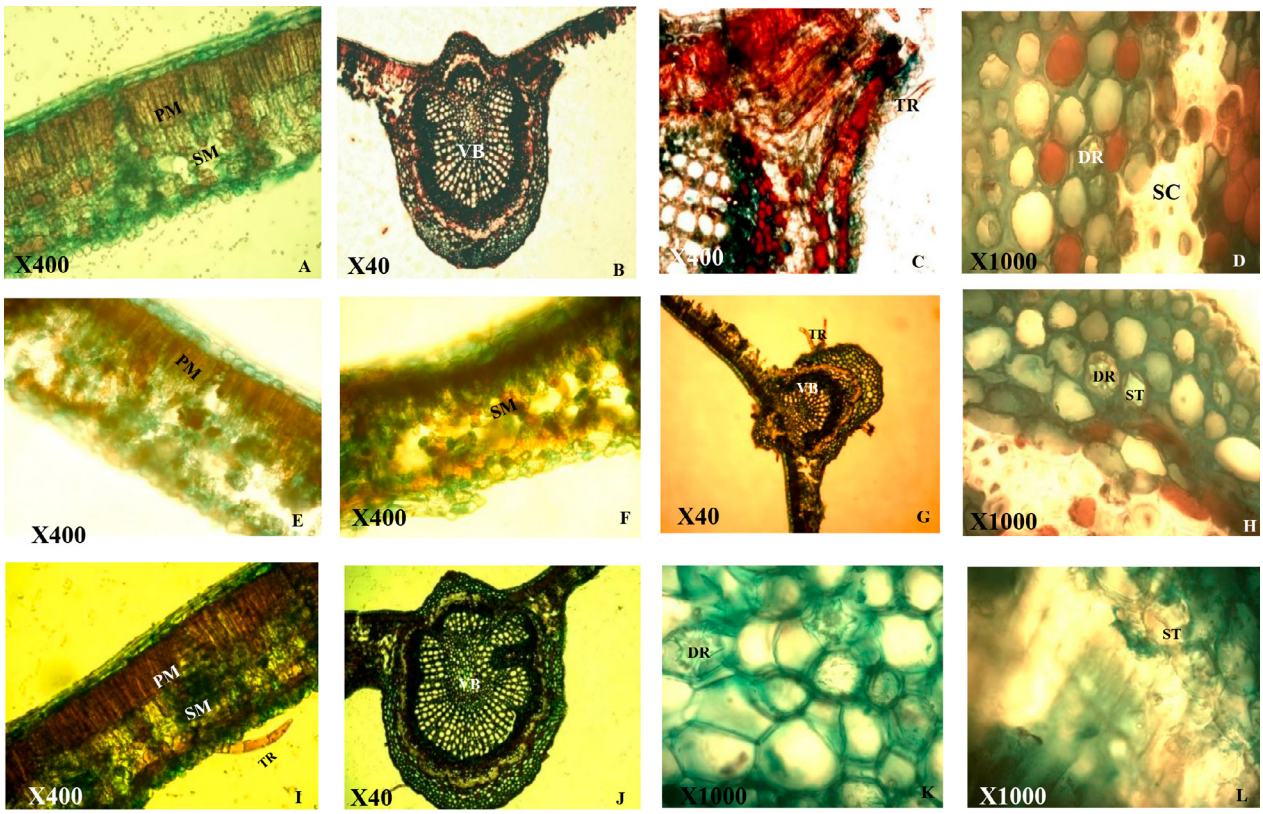
b) Bridelia ferruginea

Both the adaxial and abaxial surfaces have a uniseriate epidermis covered by a thick, non-striated and undulating cuticle. The mesophyll is separated into palisade and spongy mesophyll cells. The palisade mesophyll comprises a single layer of tightly packed, cylindrical elongated cells, whereas the spongy mesophyll comprises 1-2 layers of loosely arranged cells enclosing conspicuous intercellular spaces. The midrib cortex consists of 5-7 layers of parenchyma cells and 2-4 layers of sclerenchyma cells. The vascular bundle is irregular in shape and collateral. Multicellular trichomes are scanty on the adaxial surface and numerous on the abaxial surface of the midrib. Druses and styloid crystals have been observed as ergastic substances (Plate 3 E-H; Table 4).

c) Bridelia micrantha

Both adaxial and abaxial surface have uniseriate epidermis covered by thick, non-striated and undulating cuticle. The mesophyll is separated into palisade and spongy mesophyll layers. The palisade mesophyll comprises a single layer made up of tightly packed, cylindrical elongated cells, whereas the spongy mesophyll has 1-2 layers of loosely arranged cells enclosing conspicuous intercellular spaces. The midrib cortex consists of 6-8 layers of parenchyma cells and 2-5 layers of sclerenchyma cells. The vascular bundle is irregular in shape and collateral. Both adaxial and abaxial surfaces have multicellular non-glandular trichomes. Druses and styloid crystals have been observed as ergastic substances (Plate 3 I-L; Table 4).

Plate 3. Transverse section of the leaf of *Bridelia* species.
A-D: leaf lamina, midrib, trichome and druses (*B. atroviridis*); **E-H:** leaf lamina, midrib, trichome and druses and styloid crystals (*B. ferruginea*); **I-L:** leaf lamina, trichome, midrib, druses and styloid crystals (*B. micrantha*).



Legend: VB- vascular bundle, TR- trichome, PM- palisade mesophyll, SM- spongy mesophyll, DR- druses, ST- styloid crystals.

Table 4. Summary of the transverse section (TS) of leaves of the *Bridelia* species

Species	Cuticle	Epidermis	Mesophyll layer		Midrib cortex		Vascular bundle/ shape	Ergastic substance	Trichome
			PM	SM	PA	SC			
B. atroviridis	Thin, non-striated and undulating	Uniseriate	1	1-2	7-8	2-3	Collateral/ irregular	Druses	Multicellular non-glandular
B. ferruginea	Thin, non-striated and undulating	Uniseriate	1	1-2	5-7	2-4	Collateral/ irregular	Druses. Styloid	Multicellular non-glandular
B. micrantha	Thin, non-striated and undulating	Uniseriate	1	1-2	6-8	2-5	Collateral/ irregular	Druses. Styloid	Multicellular non-glandular

Legend: PM- palisade mesophyll, SM- spongy mesophyll, PA-parenchyma, SC-sclerenchyma.

Venation study

a) *Bridelia atroviridis*

The higher order of venation originates from a single primary vein, which falls within the pinnate primary vein group. Secondary venation is craspedodromous, with the secondary veins extending to and terminating at the leaf margin. Vein spacing is uniform and vein angle is acute. The shape of the areoles ranges from polygonal to irregular. Areoles vary in size (length and breadth). The area of areoles ranges from 2400 μm^2 to 121 800 μm^2, with a mean value of 10 149.8 μm^2. Veinlet endings range from 0-2 and may be singly diverged, forked or bifurcated (Plate 4 A; Table 5).

b) *Bridelia ferruginea*

The primary vein category is pinnate (with a single primary vein serving as the origin for the higher order of venation). Secondary venation is craspedodromous, with the secondary veins extending to and terminating at the leaf margin. Vein spacing is uniform and vein angle is acute. Shape of the areoles is polygonal. Areoles vary in size (length and breadth). The area of areoles ranges from 2731 μm^2 to 10 837.5 μm^2, with a mean value of 5181.75 μm^2. Veinlet endings range from 0-2 and may be singly diverged, forked or bifurcated (Plate 4 B; Table 5).

c) *Bridelia micrantha*

The primary vein category is pinnate (a single primary vein serving the origin for the higher order of venation). Secondary venation is eucamptodromous, characterized by secondary veins that do not terminate at the leaf margin. Vein spacing is uniform and vein angle is right. Shape of the areoles vary from polygonal to irregular. Areoles vary in size (length and breadth). The area of areoles ranges from 2906 μm^2 to 14 781.25 μm^2, with a mean value of 12 467.3 μm^2. Veinlet endings range from 0-2 and may be singly diverged, forked or bifurcated (Plate 4 C; Table 5)

Discussion

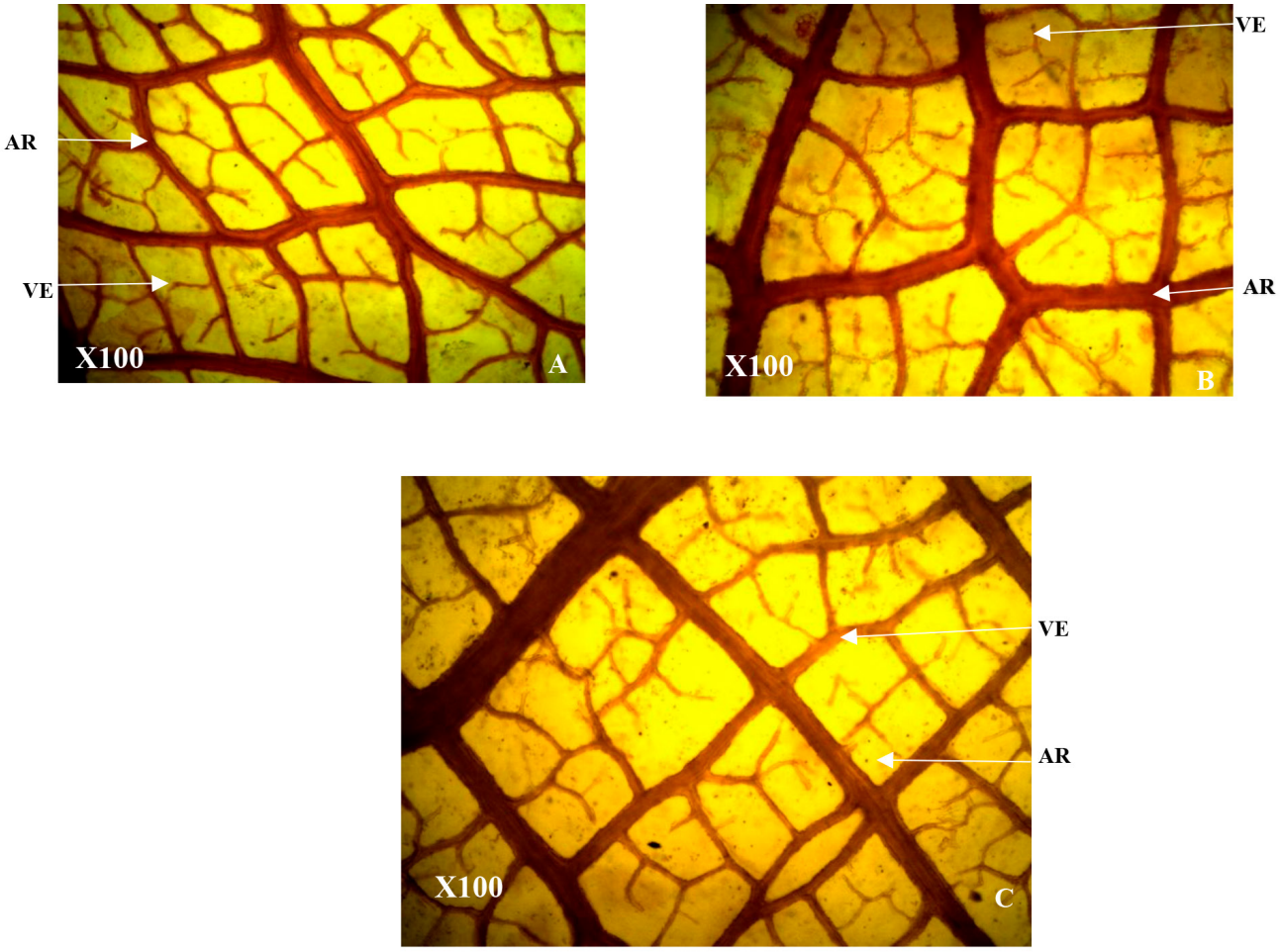
Plant anatomy has long been employed as a reliable tool in resolving identification problems in plant taxonomy. Numerous taxonomic challenges among plant groups have been clarified using anatomical characters such as epidermal cell shape, stomata type, anticlinal wall pattern, stomata index, and presence of cell inclusions, as these features are relatively stable and less influenced by environmental factors (Stace 1984; Gill & Keratela 1985; Edeoga 1991; Ogundipe & Wujek 2004; Olowokudejo & Ayodele 2006; Shokefun & al. 2014; Uka & al. 2014).

The hypostomatic nature observed in all *Bridelia* species, with stomata restricted to the abaxial surface, agrees with the earlier reports for related taxa in *Phyllanthaceae*, where hypostomatous type is considered an adaptive feature for reducing water loss in tropical environments (Metcalfe & Chalk 1979, 1989; Wilkinson 1979). Consistent presence of anisocytic stomata in all species strength-

Table 5. Summary of the venation pattern of the studied species of genus *Bridelia*

Species	Vein category		Vein spacing/ angle	Areole shape	Veinlet endings
	1^{o} veins	2^{o} veins			
B. atroviridis	Pinnate	Craspedodromous	Uniform/acute	Polygonal to irregular shape	0-2
B. ferruginea	Pinnate	Craspedodromous	Uniform/acute	Polygonal to irregular shape	0-2
B. micrantha	Pinnate	Eucamptodromous	Uniform/acute	Polygonal to irregular shape	0-2

Plate 4. Venation pattern of the studied species of genus *Bridelia*.
A: areole and veinlet endings (*B. atroviridis*); **B:** areole and veinlet endings (*B. ferruginea*); **C:** areole and veinlet endings (*B. micrantha*).



Legend: AR- areole, VE- veinlet endings.

ens their diagnostic relevance at generic level, given that that stomata type is commonly documented among other members of the family (Ogundipe & Olatunji 1991; Edeoga & al. 2006). Quantitative epidermal traits, however, provide clearer interspecific distinctions. *Bridelia ferruginea* exhibits significantly larger epidermal cell areas and higher stomata index values, compared to *B. atroviridis* and *B. micrantha*. These variations in the stomata index and size are known to reflect physiological adaptations and have been widely used as reliable taxonomic markers at species level (Stace 1984; Kadiri & Ayodele 2003). A comparatively lower stomata density and index recorded in *B. micrantha* indicate differences in transpiration control and ecological adaptation.

However, trichome occurrence also shows diagnostic relevance. Although all species have possessed multicellular non-glandular trichomes, their distribution and abundance have varied between surfaces and species. The presence of trichome bases on the adaxial surface of *B. ferruginea* distinguishes it from the other taxa and has been reported as a taxonomically informative character for species delimitation within *Euphorbiaceae* s.l. (Metcalfe & Chalk 1950; Evert 2006). The occurrence of druses as ergastic substances in *B. atroviridis* and *B. micrantha*, and their absence in *B. ferruginea* epidermis, has further strengthened their taxonomic value. Ergastic substances, particularly druses and styloid crystals, have been known to be genetically controlled and taxonomically stable characters (Franceschi & Nakata 2005).

The transverse section of the leaves revealed a high degree of anatomical uniformity among the species, notably, uniseriate epidermis, thick non-striated cuticle and dorsiventral mesophyll organization. These features were typical of mesophytic dicotyledonous leaves and consistent with earlier anatomical descriptions of the *Bridelia* species (Ogundipe & Akinrinlade 1999; Gill & al. 2012). Despite this general similarity, quantitative variations were observed in the number of parenchyma and sclerenchyma layers within the midrib cortex. *Bridelia atroviridis* possessed a thicker parenchymatous cortex, compared to *B. ferruginea*, while *B. micrantha* showed the highest sclerenchyma layer range. Such differences were significant, as vascular and mechanical tissues have been shown to aid species separation in closely related taxa (Esau 1977; Carlquist 2001).

The primary vein in all *Bridelia* species is pinnate, which confirms their placement within the dicotyledons. However, the secondary vein category has clearly separated eucamptodromous *B. micrantha* from the other species, while *B. atroviridis* and *B. ferruginea* have been craspedodromous. This is a character of high taxonomic significance (Hickey 1973; Ellis & al. 2009). Areole size and shape have also varied markedly among the species, with *B. micrantha* recording the largest mean areole area, followed by *B. atroviridis*, while *B. ferruginea* has had the smallest. Such quantitative venation characters have been shown to be consistent and reliable in species identification, especially when used alongside epidermal and anatomical data (Dilcher 1974; Kadiri 2005).

Conclusion

The examined *Bridelia* species have exhibited distinctive anatomical features that clearly differentiate them from the members of *Euphorbiaceae* described earlier by some authors. The observed anatomical characters in the *Bridelia* species correspond more closely to the diagnostic features of the family *Phyllanthaceae*.

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Authors' contribution

Both authors have conceptualized the study. The first author collected and analyzed the data and wrote the original draft of the manuscript. The second author has supervised the research, reviewed and edited the manuscript.

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