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Leaf epidermal and petiole anatomical features as taxonomic characters in some *Vernonia* species in Nigeria

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Abstract

Foliar and petiole micro-morphological characteristics of some members of the genus *Vernonia* namely; *Vernonia amygdalina* Del. (bitter variety), *Vernonia amygdalina* Del. (non-bitter variety), *Vernonia cinerea* (L) Less., *Vernonia galamensis* (Cass.) Less., and *Vernonia adoensis* Sch. Bip. in Jos, Nigeria was investigated to provide additional micro-morphological characters to support existing taxonomic information regarding the species. Epidermal peels of fresh leaves were made and transverse sections of petioles were cut using a rotary microtome. Staining was done using Safranin and slides observed using a light microscope. Quantitative characters were measured and subjected to Duncan Multiple Range Test. Stomatal distribution was amphistomatic except for *V. galamensis* and *V. cinerea* with hypostomatic distribution; stomata type was mostly anomocytic except *V. galamensis* with paracytic stomata. Stomata index varied among taxa with *V. adoensis* and *V. amygdalina* (bitter variety) having the highest (10.84 %) and lowest (0.67%) respectively. Anticlinal cell wall patterns were straight to slightly undulate while wavy anticlinal cell wall pattern was diagnostic to *V. cinerea*. Trichome types observed were glandular and multicellular uniseriate with highest and lowest trichome indices recorded in *V. adoensis* (2.80%) and *V. galamensis* (0.02%) respectively. Petiole outline was convex to concave, epidermis was uniseriate, trichome multicellular uniseriate and vascular bundles bicollateral. Variations observed in the stomatal complex, epidermal cell complex, petiole outline, petiole vasculature type, trichome and stomata indices could be employed for species identification and delimitation.

Keywords: Stomata, Epidermis, Taxonomy, *Vernonia*, Bicollateral, Anatomical.

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INTRODUCTION

The use of anatomical data has been long recognized in plant taxonomy since variations within taxa is usually reflected in anatomical features. Data from gross morphology, wood anatomy, and foliar epidermal morphology have proved valuable in plant identification and classification (Kadiri *et al*., 2006). One of the noteworthy taxonomic practices is foliar epidermis, and characters such as trichomes, stomatal features and anticlinal cell wall patterns have been used in taxonomic studies of a number of families (AbdulRahaman *et al*., 2014). The significance of petiole anatomical characters in understanding the relationship within plant taxa has also been studied and reported to be useful in separating plants of different species, genera and families (Metcalfe and Chalk, 1979, Kamel and Loutfy, 2001). Its usefulness as a supportive tool in plant taxonomy has recently increased and progress has been made in using this data for plant classification (Ogundare and Saheed, 2012, Bercu and Popoviciu, 2014).

The genus *Vernonia* contains more than 500 species distributed in North and South America, tropical Africa especially in Nigeria, Zimbabwe and South Africa and it is domesticated in parts of West Africa (Farombi, 2003, Kemka-Evans *et al*., 2017). Some *Vernonia* species are consumed as vegetables while others are used in indigenous medicine. For instance, the roots and leaves of *Vernonia amygdalina* are used in treatment of malaria, infertility, diabetes, gastrointestinal problems and sexually transmitted diseases (Ene and Atawodi, 2012, Adedapo *et al*., 2014). The roots and leaves of *Vernonia cinerea* are also used for treating nerve disorder, kidney disease, and as a potent analgesic (Syed *et al*., 2011, Thiagarajan, 2014). A decoction of *Vernonia cinerea* is used in treatment of urinary tract infection, cough, stomach ache and diarrhea (Farombi and Owoeye, 2011). In Nigeria, the leaves of *V. amygdalina* induces fertility in women (Adedapo *et al*., 2014), and in Ghana, it is consumed for breast milk enhancement in nursing mothers (Kankara *et al*., 2015)

Researchers have attempted the taxonomy of the genus *Vernonia* and some of these studies focused on the foliar morphology (Oladele, 1990, Adedeji and Jewoola, 2008, Nwakanma *et al*., 2018). However, a comparative study on the petiole anatomy of species of this genus have not received much attention and data is still insufficient. Therefore, this study describes the epidermal morphology and petiole anatomical structures of four species of *Vernonia* and the non-bitter variety, with the aim of providing additional micro-morphological characters to support existing taxonomic information regarding the species.

MATERIALS AND METHODS

Sample collection.

Freshly collected specimens of *Vernonia* were collected in Jos, Plateau state specifically in Federal college of Forestry and Shere hills (Latitudes ^{0}93 N and 9^{0}95 N and longitudes 8^{0}89 E and 8^{0}93 E). The plants were identified, authenticated and voucher specimens deposited at the Herbarium of the Department of Plant Science and Biotechnology, University of Jos, Plateau state, Nigeria. The voucher numbers for the identified specimens are *Vernonia amygdalina* Del. (Non-bitter variety)- JUHN21000334, *Vernonia cinerea* (L.) Less.- JUHN21000330, *Vernonia amygdalina* Del. (Bitter variety)- JUHN21000331, *Vernonia galamensis* (Cass.) Less- JUHN21000332, *Vernonia adoensis* Sch.Bip.- JUHN21000333

Sample Preparation and Analysis

The epidermal peels of both the adaxial and abaxial surfaces of the leaves of each plant were obtained. Specifically, the leaf materials were soaked in concentrated trioxonitrate (v) acid (HNO_3) in a glass petri dish, covered and allowed to stand for 3 hours. The epidermises were separated with forceps, rinsed in distilled water, stained in Safranin solution and mounted in 5% diluted glycerol (Metcalfe and Chalk, 1978). The parameters of the stomata observed were stomatal distribution, stomatal index, stomatal length and width. Epidermal cell length and width were measured. The characters were on the basis of 25 sample size and the measurement for each character was replicated 10 times. The epidermal characteristics were described using Metcalfe and Chalk (1978). Parameters of the trichome determined include trichome type, trichome index, trichome length and width. Stomata and trichome indices were calculated using the formulae of Stace (1965).

$$\text{Stomata index (S.I)} = \frac{S}{E+S} \times 100$$

$$\text{Trichome index (T.I)} = \frac{T}{E+T} \times 100$$

Where,
S=Stomata number per unit area
E=Epidermal cell number per unit area
T=Trichome number per unit area

For the petiole anatomy of the species, transverse sections from the median regions for each of the species were obtained using a rotary microtome. The sections were stained in 10 % aqueous Safranin solution and mounted in 5% diluted glycerol Specimen slides were observed using light microscopy and photomicrographs were captured under magnification of X400 and X100 using Olympus microscope with attached Celestron digital camera. The identification and description of tissue and cell followed Fahn (1997). The quantitative data was subjected to Analysis of Variance and Duncan Multiple Range Test (Duncan, 1955, Bailey, 1976).

Figure 1 (A-E): Morphology of the studied Vernonia species- A: *V. adoensis,* B: *V. galamensis*, C: *V. amygdalina* (Non bitter variety), D: *V. amygdalina* (Bitter variety), E: *V. cinerea*

RESULTS

Detailed epidermal morphology and petiole anatomy of the studied taxa are presented below. The qualitative and quantitative characters of the epidermises and petioles are presented in Tables 1 and 2, accompanying photomicrographs in Figures 2-6.

V. adoensis

Foliar epidermis

Epidermal cells were polygonal and anticlinal wall was straight on the abaxial surface (Fig. 2A); mean length and width of cells up to 1.92 µm and 2.00 µm respectively. Stomata anomocytic, stomata index 10.8 %, mean stomata length and width up to 1.24 µm and 0.50 µm respectively. Multicellular uniseriate trichome present, trichome index 0.10 %, mean trichome length and

width 12.24 µm and 2.70 µm respectively. However, on the adaxial surface (Fig. 2B), epidermal cells were polygonal with straight anticlinal walls; mean length and width of cells up 1.96 µm and 2.08 µm respectively. Stomata anomocytic, stomata index 0.59 %, mean stomata length and width 1.16 µm and 6.02 µm respectively. Glandular and multicellular uniseriate trichomes present, trichome index 0.02 %, mean trichome length and width were 11.88 µm and 6.02 µm respectively (Table 1).

Petiole anatomy

Petiole outline was convex on abaxial surface and concave on adaxial surface. Epidermis was uniseriate having multicellular uniseriate trichomes. Collenchyma oval to angular, 5-8 layered and parenchyma oval, 7-15 layered (Table 2). Vascular bundle bicollateral forming an arc with about 9 bundles. Crystals and druses were randomly distributed on the cortex. Petiolar vasculature type was closed (Fig. 2C-D).

V. galamensis

Foliar epidermis

Epidermal cells were pentagonal to hexagonal and anticlinal wall was straight on the abaxial surface (Fig. 3B); mean length and width of cells up to 3.64 µm and 2.62 µm respectively. Stomata paracytic, stomata index 2.96 %, mean stomata length and width up to 2.46 µm and 1.01 µm respectively. Glandular and multicellular uniseriate trichomes present, trichome index 2.28 %, mean trichome length and width 11.86 µm and 4.06 µm respectively. However, on the adaxial surface (Fig. 3A), epidermal cells were pentagonal to hexagonal with undulating anticlinal walls; mean length and width of cells up 3.32 µm and 2.03 µm respectively. Multicellular uniseriate trichome present, trichome index 0.02 %, mean trichome length and width were 10.74 µm and 3.07 µm respectively (Table 1). Stomata are absent

Petiole anatomy

Petiole outline was convex on both adaxial and abaxial surfaces. Epidermis was uniseriate having multicellular uniseriate trichomes. Collenchyma angular, 2-3 layered and parenchyma oval to angular, 5-6 layered (Table 2). Vascular bundle bicollateral, C shape with about 6 bundles. Crystals and druses were randomly distributed on the cortex and pith. Petiolar vasculature type was open (Fig. 3C-D).

***V. amygdalina* (Non-bitter variety)**

Foliar epidermis

Epidermal cells were polygonal and anticlinal wall was straight on the abaxial surface (Fig. 4A); mean length and width of cells up to 2.26 µm and 1.88 µm respectively. Stomata anomocytic, stomata index 2.40 %, mean stomata length and width up to 1.64 µm and 0.70 µm respectively. Glandular and multicellular uniseriate trichomes present, trichome index 1.31 %, mean trichome length and width 6.16 µm and 1.07 µm respectively. However, on the adaxial surface (Fig. 4B), epidermal cells were polygonal with straight anticlinal walls; mean length and width of cells up 1.58 µm and 1.24 µm respectively. Stomata anomocytic, stomata index 2.33 %, mean stomata length and width 1.56 µm and 0.72 µm respectively. Glandular trichome present, trichome index 0.91 %, mean trichome length and width were 18.02 µm and 2.06 µm respectively (Table 1)

Petiole anatomy

Petiole outline was convex on the abaxial surface and concave on adaxial surface. Epidermis was uniseriate having multicellular uniseriate trichomes. Collenchyma oval to angular, 3-4 layered and parenchyma oval to angular, 5-7 layered (Table 2). Vascular bundle bicollateral, C shape with about 6 bundles. Crystals and druses were randomly distributed on the cortex. Petiolar vasculature type was closed (Fig. 4C-D).

***V. amygdalina* (Bitter variety)**

Foliar epidermis

Epidermal cells were polygonal and anticlinal wall was straight on the abaxial surface (Fig 5B); mean length and width of cells up to 1.88 µm and 1.36 µm respectively. Stomata anomocytic, stomata index 0.67 %, mean stomata length and width up to 1.46 µm and 0.09 µm respectively. Multicellular uniseriate trichome present, trichome index 1.14 %, mean trichome length and width 4.94 µm and 1.44 µm respectively. However, on the adaxial surface (Fig. 5A), epidermal cells were polygonal with straight

anticlinal walls; mean length and width of cells up to 1.76 µm and 1.58 µm respectively. Stomata anomocytic, stomata index 0.70 %, mean stomata length and width 1.68 µm and 0.88 µm respectively. Multicellular uniseriate trichome present, trichome index 0.60 %, mean trichome length and width were 6.62 µm and 2.14 µm respectively (Table 1).

Petiole anatomy

Petiole outline was convex on the adaxial surface and concave on abaxial surface. Epidermis was uniseriate having multicellular uniseriate trichomes. Collenchyma oval to angular, 4-5 layered and parenchyma oval, 9-10 layered (Table 2). Vascular bundle bicollateral, C shape with about 5 bundles. Crystals and druses were randomly distributed on the cortex. Petiolar vasculature type was closed (Fig. 5C-D).

V. cinerea

Foliar epidermis

Epidermal cells were irregular and anticlinal wall was wavy on the abaxial surface (Fig. 6B); mean length and width of cells up to 3.46 µm and 3.03 µm respectively. Stomata anomocytic, stomata index 9.58 %, mean stomata length and width up to 1.54 µm and 0.66 µm respectively. Glandular trichome present, trichome index 1.23 %, mean trichome length and width 6.86 µm and 3.18 µm respectively. However, on the adaxial surface (Fig. 6A), epidermal cells were irregular with wavy anticlinal walls; mean length and width of cells up 3.82 µm and 4.22 µm respectively. Stomata was absent on the adaxial surface. Multicellular uniseriate trichome present, trichome index 0.97 %, mean trichome length and width were 24.64 µm and 4.22 µm respectively (Table 1).

Petiole anatomy

Petiole outline was convex on the abaxial surface and concave on adaxial surface. Epidermis was uniseriate having multicellular uniseriate trichomes. Collenchyma oval to angular, 2-3 layered and parenchyma oval, 4-5 layered (Table 2). Vascular bundle bicollateral, ark shape with about 5 bundles. Druses were randomly distributed on the cortex. Petiolar vasculature type was open (Fig. 6C-D).

DISCUSSION

Anatomical characters have been employed in the classification of plant taxa and its usefulness in the delimitation and separation of species is reported relevant. Foliar epidermal anatomy is one of the most noteworthy taxonomic characters from the systematic point of view and several delimitations of taxa have been done based on leaf epidermal characters (Aworinde *et al*., 2014, Ekeke and Agbagwa, 2015, Bello *et al*., 2017, Raza *et al.,* 2020). Mbagwu and Edeoga (2006) opined that characters such as stomata type, distribution and size, epidermal cell shape, trichome type and distribution are tools useful in the identification and classification of many angiosperms. These anatomical characters are reported not to be altered or affected by the environmental factors.

Diagnostic anatomical characters useful in identification and delimitation of these species were observed in this study. Generally, the appearance of stomata on the leaf surfaces varied among the studied species. The presence of hypostomatic feature in *V. cinerea* and *V. galamensis* contradicts earlier reports by Nwakanma *et al.* (2018) and Asuzu (2020) who reported amphistomatic feature in the two species. Further, the presence of paracyctic stomata type in *V. galamensis* also contradicts the observations of Asuzu (2020) that reported only anomocytic stomata in the species studied. However, this feature distinguishes *V. galamensis* from others with anomocyctic stomata type. Hetherington and Woodward (2003) opined that stomata types cannot be influenced by the environment as they are genetically determined and are therefore good taxonomic tool. Further, the number of stomata was more on the abaxial surface than the adaxial surface. Consequently, the stomata index was higher on the abaxial surfaces of the taxa studied. There was an overlap in stomata size (length and width) of the taxa which limits its usefulness as a diagnostic feature. This agrees with Davies and Heywood (1963) who reported that stomata size is too variable to be considered a diagnostic character.

The presence of irregular cells and wavy anticlinal wall pattern on both surfaces of *V. cinerea* distinguished it from others with polygonal shaped cells and slightly undulate to straight anticlinal wall pattern.

Table 1: Leaf epidermal characteristics of the *Vernonia* species studied

Features	Surfaces	*V. adoensis*	*V. galamensis*	*V. amygdalina* (Non bitter variety)	*V. amygdalina* (Bitter variety)	*V. cinerea*
Stomata length	Adaxial	1.16±00.05[a]	-	1.56±00.10[a]	1.68±0.13[a]	-
	Abaxial	1.24±00.04[a]	2.46±00.07[b]	1.64±00.09[a]	1.46±01.13[a]	1.54±00.09[a]
Stomata width	Adaxial	0.46±00.31[a]	-	0.72±00.04[a]	0.88±00.10[b]	-
	Abaxial	0.50±00.33[a]	1.01±00.05[b]	0.74±00.13[a]	0.09±00.10[a]	0.66±00.06[a]
Stomata index	Adaxial	0.59[a]	-	2.33[b]	0.70[a]	-
	Abaxial	10.84[d]	2.96[b]	2.40[b]	0.67[a]	9.58[c]
Trichome index	Adaxial	0.02[a]	0.02[a]	0.91[c]	0.60[b]	0.97[a]
	Abaxial	0.10[a]	2.28[b]	1.31[a]	1.41[c]	1.23[a]
Stomata type	Adaxial	Anomocytic	-	Anomocytic	Anomocytic	-
	Abaxial	Anomocytic	Paracytic	Anomocytic	Anomocytic	Anomocytic
Anticlinal wall	Adaxial	Straight	Undulate	Straight	Straight	Wavy
	Abaxial	Straight	Straight	Straight	Straight	Wavy

*Legend: Measurements= Mean±SE

Mean values with the same letter (s) along each horizontal array do not differ significantly at $p < 0.05$.

Table 2: Qualitative anatomical characters of the petiole of *Vernonia* species studied

	Taxa				
Characters	*V. adoensis*	*V. galamensis*	*V. amygdalina* (Non bitter variety)	*V. amygdalina* (Bitter variety)	*V. cinerea*
Adaxial petiole outline	Concave	Convex	Concave	Convex	Concave
Epidermis layer	Single	Single	Single	Single	Single
Parenchyma	Oval	Oval/Angular	Oval/Angular	Oval	Oval
Collenchyma	Oval/Angular	Angular	Oval/Angular	Oval/Angular	Oval/Angular
Trichome type	Multicellular uniseriate	Multicellular uniseriate	Multicellular uniseriate	Multicellular uniseriate	Multicellular uniseriate
PVT	Closed	Open	Closed	Closed	Open
Vascular bundles	Bicollateral	Bicollateral	Bicollateral	Bicollateral	Bicollateral
Crystals	Present	Present	Present	Present	Absent
Druses	Present	Present	Present	Present	Present

*PVT=Petiolar vasculature type

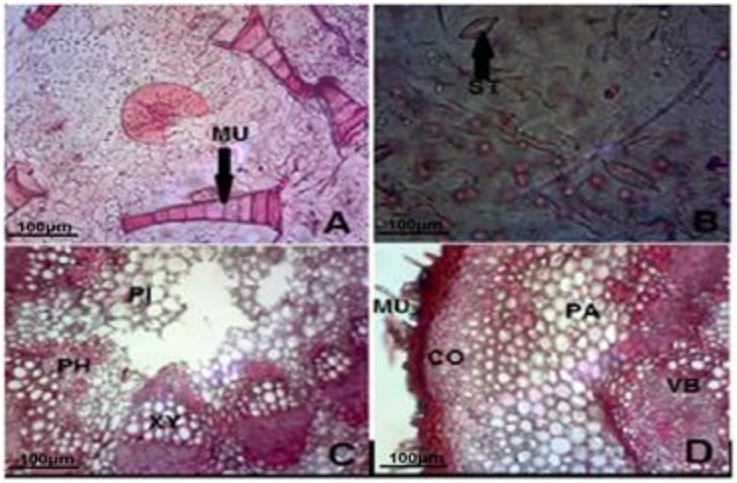

Figure 2(A-D): Epidermal and petiole anatomical features of *V. adoensis*. MU-multicellular uniseriate trichome, ST-stomata, PI-pith, PH-phloem, XY-xylem, CO-collenchyma, PA-parenchyma, VB-vascular bundle. Mg: A-D= X100

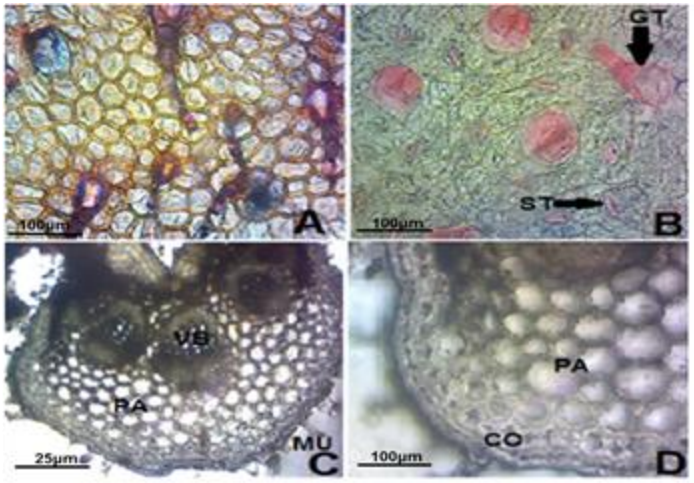

Figure 3(A-D)-Epidermal and petiole anatomical features of *V. galamensis*. MU-multicellular uniseriate trichome, ST-stomata, GT-glandular trichome, CO-collenchyma, PA-parenchyma, VB-vascular bundle. Mg: A-C= X100, D= X400

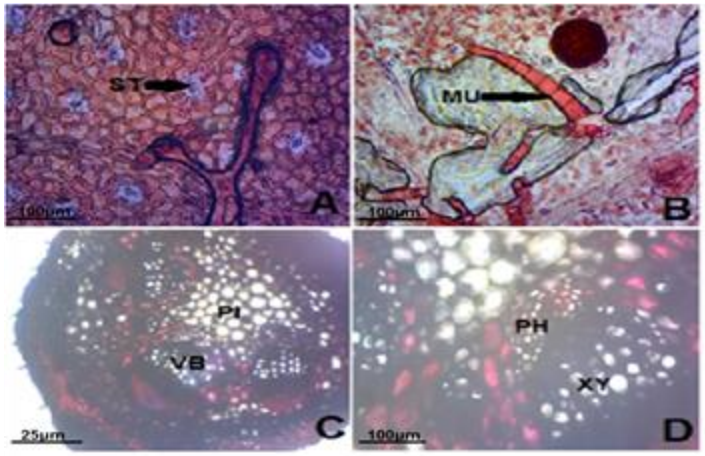

Figure 4(A-D)-Epidermal and petiole anatomical features of *V. amygdalina* (Non bitter variety). Mu-multicellular uniseriate trichome, ST-stomata, PI-pith, PH-phloem, XY-xylem, VB-vascular bundle. Mg: A-D= X100

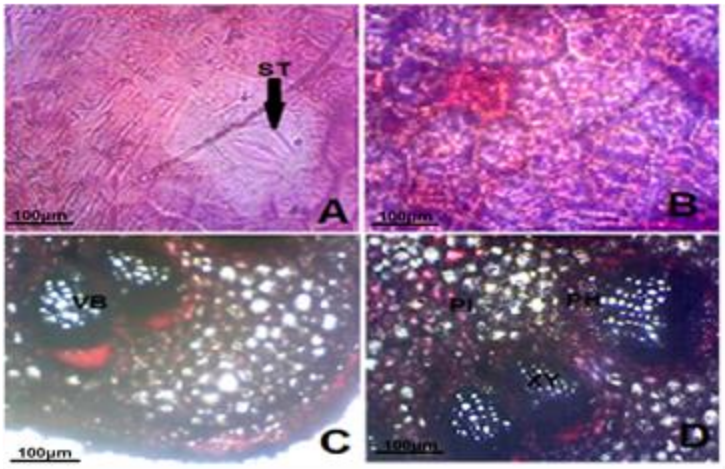

Figure 5(A-D)-Epidermal and petiole anatomical features of *V. amygdalina* (Bitter variety). ST-stomata, PI-pith, PH-phloem, XY-xylem, VB-vascular bundle. Mg: A-B= X400, C-D= X100

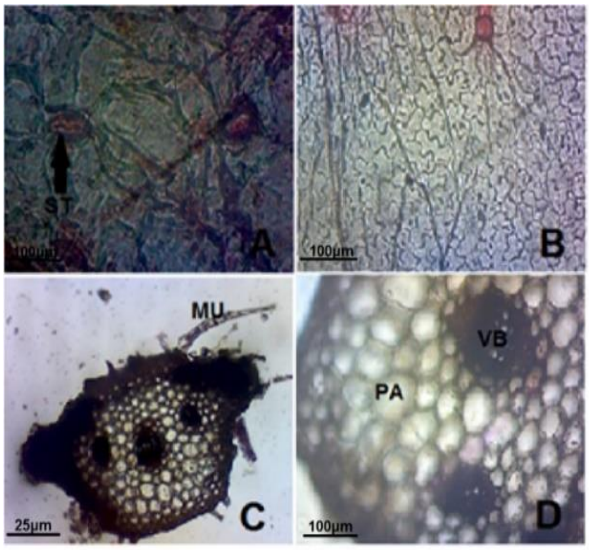

Figure 6(A-D)-Epidermal and petiole anatomical features of *V. cinerea*. Mu-multicellular uniseriate trichome, PA-parenchyma, VB-vascular bundle. Mg: A-C= X100, D= X400

This agrees with the observations of Aworinde *et al.* (2013) and Asuzu (2020) who reported similar epidermal cell shapes and anticlinal wall patterns. The use of anticlinal wall pattern was also employed by Adedeji *et al*. (2007) as a taxonomic tool for separating some species in the family Solanaceae. Furthermore, there was an overlap in the size of the epidermal cells limiting its usefulness in delimiting the studied taxa.

A range of petiole anatomical characters was observed and some of these characters are diagnostic and important for the delimitation of these *Vernonia* species. The presence of uniseriate epidermis, multicellular uniseriate trichomes, oval to angular collenchyma cells and bicollateral vascular bundles in the petioles of all the taxa studied could serve as diagnostic features for the genus and this corroborates the report of Asuzu (2020) who observed similar characters in the *Vernonia* species studied. There are however differences in petiole outline, petiolar vasculature type, number of vascular bundles, number of layers of parenchyma and collenchyma cells which could be employed for species identification. The number of vascular bundles ranges from 5 to 9 with *V. adoensis* having the highest number of vascular bundles. The petiolar vasculature type was open in *V. cinerea* and *V. galamensis* and closed in the other three taxa. The differences in these features have been reported by Akinnubi *et al.* (2013) to be useful in delimitation and classification of some species in the family Asteraceae.

Furthermore, though the foliar and petiole anatomy of *V. amygdalina* (Bitter variety) and *V. amygdalina* (non-bitter variety) shared similar characters, there however exist striking differences in stomata index, trichome type, petiole outline and number of vascular bundles. An artificial key to the studied *Vernonia* species using leaf epidermal and petiole anatomical characters is provided in support of existing identification keys.

1a. Leaf amphistomatic, petiolar vascular type closed, collenchyma layers 3-8 ……2
2a. stomata index 0.59-10.48 %, trichome glandular and multicellular

uniseriate on adaxial surface……..
……..…..***V. adoensis***
2b. stomata index 0.67-2.40 %, trichome glandular on adaxial surface……3
3a. stomata length 1.00 to 2.60 µm, epidermal cell length 1.4 to 2.4 µm, adaxial petiole outline convex, vascular bundle 5, Trichome index 0.60-1.41%……..
……..…**V. *amygdalina* (bitter)**
3b. stomata length 1.2 to 2.2 µm, epidermal cell length 1.2to 2.0 µm, adaxial petiole outline concave, vascular bundle 6, Trichome index 0.91-
1.31%……..
……..***V. amygdalina* (non-bitter)**
1b. Leaf hypostomatic, petiolar vascular type open, collenchyma layers 2-3……..4
4a. stomata type paracytic, stomata index 2.96%, epidermal cell shape polygonal, anticlinal wall undulate to straight…… ***V. galamensis***
4b. stomata type anomocytic, stomata index 9.58%, epidermal cell shape irregular, anticlinal wall wavy…….. ***V. cinerea***

CONCLUSION

The importance of foliar and petiole anatomical characters in some *Vernonia* species in Nigeria

was shown in this investigation. Taxonomic similarities and dissimilarities among the species and diagnostic characters important for species identification and delimitation were shown. These diagnostic characters include stomata size, stomata index, stomata types, trichome index, petiole outline, petiole vasculature type and epidermal cell shapes, which varied among the species of this genus. Although, this study agrees with some of the existing taxonomic data on the foliar epidermis and petiole anatomy of this genus, it also shows characters not previously reported, thereby serving as additional data to already existing taxonomic information on these species.

Conflict of interest

Authors have no conflict of interest to declare.

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AUTHORS' CONTRIBUTIONS

Author OEO designed the study, participated in the bench work and wrote the first draft of the manuscripts. Author SGA carried out field work, bench work and performed the statistical analysis. Author OAO also performed part of the statistical analysis and reviewed the manuscripts. The three authors read and approved the final manuscript.

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