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VASCULAR DIFFERENTIATION AND SIMILARITIES OF *Anthocleista* species COMMONLY FOUND IN PARTS OF SOUTHERN ECOLOGICAL ZONE OF NIGERIA

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Abstract

Vascular differentiation and similarities is a function of anatomical diagnosis of plant species. Various lines of taxonomic evidence have been drawn on the four Anthocleista species recorded in southern Nigeria; however, not much effort toward the anatomy of the four taxa has been made. Thus, the aim of this study was to determine the importance of their vascular similarities and variation in the taxonomy of the genus; with the objective of investigating the anatomy of the leaf, petiole, root and stem of the species and by implication access the suitability of the diagnostic features for possible taxonomic delimitation. The result has revealed similarities in the four studied anatomical indices (foliar, petiole, root and stem) among the species. However, there were distinct variations as follows: hypodermis, endodermis, and air sac in the foliar anatomical features; idioblast, spongy cell, intercellular spaces and vascular bundles in the petiolar anatomy; exodermis, sclereid, pericycle and vascular bundles in the root anatomy, while cortex, collenchyma and vascular bundles were variations in stem anatomy of the species. Therefore, with the variation based on distinguishable anatomical characters; the Anthocleista species can be segregated from each other on the basis of their quantitative anatomical parameters in diverse features of the anatomical indices. This consequently can be a panacea for ethnobotanical and economic valuation of the species based on its therapeutic potentials as antimicrobial agent, medicine, antioxidant, hypoglycemic agent, anticorrosion agent and environmental bioindicator.

Key Words: Anatomy, Foliar, Petiole, Stem, Root

Introduction

The genus *Anthocleista*, Afzel.ex.R.Br., commonly called cabbage tree is a medium size tropical Africa genus composed of medium, small trees or shrubs with soft white wood belonging to the family Loganiaceae (Keay, 1989; Edwin-Wosu *et al.*, 2015). It consists of 50 species, mainly native to tropical African

mainland, Madagascar and Mascarene Island. Six of the 50 species are commonly found and recorded in Nigeria (Keay, 1989). Phytogeographically four of the six species of common occurrence involving *Anthocleista nobilis, Anthocleista djalonesis, Anthocleista liebrechtsiana* and *Anthocleista vogelii* has been revealed in parts of southern Nigeria (Edwin-Wosu

and Omara-Achong, 2010; Edwin-Wosu et al., 2015). The four species of the southern Nigeria occur in various habitats (Edwin-Wosu and Omara-Achong, 2010). Several studies have noted these species for their economic significance in light of their ethnobotanical and ethnomedicinal value chain (Keay, 1989; Luter et al., 2012; Ayodele et al., 2013; Anyanwu et al., 2013; Ngbolua et al., 2014) as well as their bioactive constituents and diverse potentials for optimal utilization (Edwin-Wosu et al., 2017; Edwin-Wosu and Okafor, 2017). In search of understanding phylogeny of the genus relationship among the species, several similarities and variation have been recorded among the commonly found species across taxonomic lines evidence involving: phytoevolution and species composition of the genus (Keay, 1989; Backlund et al., 2000); phytogrossmorphological characterization (Sonibare et al., 2007; Chinwe and Maria, 2009; Edwin-Wosu et al., 2010; Edwin-Wosu, 2012; Edwin-Wosu et al., 2012; Edwin-Wosu and Ndukwu. 2012): phytogeography (Edwin-Wosu, and Omara-Achong, 2010; Edwin-Wosu et al., 2015); chemotaxonomy (Sonibare et al., 2007); histochemistry (Edwin-Wosu and Ndukwu, 2012); phytochemistry (Luter et al., 2012; Ngbolua et al., 2014); anatomy of Anthocleista djalonesis (Chinwe and Maria, 2009).

differentiation Vascular and similarities is a function of anatomical diagnosis of plant species. This corroborates the relationship of various aspect of function in anatomical characterization of plant as well as phylogenetic relatedness among taxa by influencing the comparative analysis of plant species in light of their variation and (Harvey similarities etal.,

Dubuisson et al., 2011). Anatomy of plant is investigated at cellular level and often involves the sectioning of tissues and microscopy of various parts of plant (Okoli, 1987). Morphological features are of great taxonomic importance but cannot always be used solely in grouping plants to particular taxa (Stace, 1980). It was suggested that anatomical features in plants might be more important than morphological features systematically hence the features are less susceptible to environmental change (Stace, 1991). The incorporation of anatomical data with findings from studies of gross morphology, cytology and palynology etc. enables those making revision classification of plants to produce more natural system (Enwin-Wosu, et al., 2012). The features have earlier been found to be very important tools in taxonomic essence (Stace, 1980). It has been useful in the classification of species and genera in plant kingdom (Brazier, 1968); been a very functional and of strong diagnostic value in resolving identification problems, classification, characterization and delimitation of species (Akinloye et al., 2012; Oladipo and Illoh, 2012). It has also been used to trace evolutionary relationship among plant species (Oladipo and Oyaniran, 2013). Comparative anatomical studies have been carried out on several other species (Wickremasinghe Harah, 2006; Oladipo et al., 2016; Mudasiru et al., 2016). Inspite of the economic values of the Anthocleista species and the various aforementioned lines of taxonomic evidence drawn on the four Anthocleista species recorded in southern Nigeria; no serious attention has been paid to the anatomy of the four Nigeria taxa. This prompted the need for this study with the aim of investigating the

anatomy of the leaf, petiole, stem and root of the species with a view to determining the importance of their vascular similarities and variation in the taxonomy of the genus and by implication to access the suitability of the diagnostic features for possible taxonomic delimitation.

Materials and Methods

Sample Collection and Anatomical Study

The sample collection of species of the *Anthocleista* genus was based on accessibility and availability of the species. Using a hand-held Geographic Positioning System (GPS – Garmin Dakota 10 Model) the georeferencing of the sampled point was carried out. All the samples were identified and authenticated at the University of Port Harcourt Herbarium (UPH) by the Curator and voucher specimen deposited at the Herbarium (Table 1).

Fresh specimen of the leaf, petiole, root and stem of collected samples were fixed in FAA 1:1:18 (1 part formalin, 1 part glacial acetic acid, 18 parts of 70% ethanol (v/v)) for 48 hours (Johansen, 1940). The specimens were washed in two changes of distilled water to get rid of the fixative and passed through graded ethanol series in the order 30%, 50%, 70% and 95% (each for two hours) and finally absolute ethanol (overnight) (Okoli, 1987). Transverse sections of the leaf, petiole, root and stem were made using a

sliding sledge microtome at a thin section thickness of 1µ by making horizontal cuts close to the surface. The first sections were discarded; subsequent sections placed in water, held in a glass Petri dish. The sections (leaf and petiole) were stained with 1% methyl blue, root and stem with 1% safranin and methyl blue (counter staining), the sections were rinsed with distilled water to get rid of excess stain and then placed on a slide with a drop of glycerine and covered with a cover slip. Photomicrographs of the prepared slides were taken with the aid of photomicrographic apparatus microscope with built – in-camera optics (Amescope digital camera mounted on a celesteron binocular microscope). Photomicrograph was taken from good for preparations tissue and cells identification and description in tandem with Metcalfe and Chalk (1989) and Katethrine (1958).

Results

The result of the spatial geographical distribution and herbarium voucher sample deposit are presented in Tables 1 and 2. The anatomical result summary (Tables 3 - 6) and interpretation of study observations on the leaf, petiole, root and stem of the species are presented below, while the photomicrographs of the sections as shown are presented in Plates 1-16.

Table 1: GPS Co-ordinates of the Sample collected

Species	Latitude	Longitude	Altitude
Anthocleista vogelii Planch	6°55'45"N	4°53'38"E	13m
Anthocleista nobilis G. Don	6°55'45"N	4°53'37"E	11m
Anthocleista liebrechtsiana De Wild and Th. Dur	6°55'39"N	4°53'43"E	11m
Anthocleista djalonensis A. Chev	6°55'56"N	4°53'43"E	16m

Table 2: Herbarium Number of Voucher Deposit

S/No	Species	Accession No	UPH No
1.	Anthocleista vogelii Planch	001	V/1208
2	Anthocleista nobilis G. Don	002	V/1209
3.	Anthocleista liebrechtsiana De Wild and Th. Dur	003	V/1210
3.	Anthocleista djalonensis A. Chev	004	V/1211

Table 3: Summary of Foliar Anatomical Features

S/N	Foliar Features	Species			
		A. vogelii	A. nobilis	A. liebretchsiana	A. djalonensis
1	Epidermis	+ (1 cell thick)	+ (2 cell thick)	+ (2 cell thick)	+ (2 cell thick)
2	Hypodermis				+ (multiple)
3	Collenchyma	+ (2 cell thick)	+ (1 cell thick)	+ (1 cell thick)	
4	Palisade cell	+ (2 cell thick)	+ (1 cell thick)	+ (1 cell thick)	+ (3 cell thick)
5	Spongy cell	+ (irregular mass)	+ (irregular mass)	+ (irregular mass)	+ (irregular mass)
6	Vascular bundle	+ crescent			+ crescent
		+ bicollateral	+ bicollateral	+ bicollateral	+ bicollateral
			+ medullary		
7	Endodermis				+ (1 cell thick)
8	Stellate idioblast		+ (several)	+ (several)	
9	Air sac			+	

NOTE: + = Presence of features; -- = Absence of features

Table 4: Summary of Petioliar Anatomical Features

S/N	Petiole Features	Species			
		A. vogelii	A. nobilis	A. liebretchsiana	A. djalonensis
1	Epidermis	+ (1 cell thick)	+ (1 cell thick)	+ (1 cell thick)	+ (1 cell thick)
2	Collenchyma	+ (3 cell thick)	+ (3 cell thick)	+ (3 cell thick)	+ (3 cell thick)
3	Sclerenchyma	+ (1 cell thick)	+ (1 cell thick)	+ (1 cell thick)	+ (1 cell thick)
4	Idioblast	+ (stellate, simple, complex)	+ (stellate, simple, complex)	+ (stellate)	
5	Spongy cell				+ (irregular mass)
6	Intercellular spaces				+
7	Vascular bundle	+ bicollateral	+ bicollateral	+ bicollateral	+ bicollateral
					+ medullary

NOTE: + = Presence of features; -- = Absence of features

Table 5: Summary of Root Anatomical Features

S/N	Root Features	Species				
		A. vogelii	A. nobilis	A. liebretchsiana	A. djalonensis	
1	Epidermis	+ (2 cell thick)	+ (2 cell thick)	+ (2 cell thick)	+ (2 cell thick)	
2	Exodermis		+	+		
3	Cortex	+	+	+	+	
4	Air chamber	+ (moderate)	+ (abundance)	+ (abundance)	+ (moderate)	
5	Sclereid		+	+ (several)		
6	Endodermis	+	+	+	+	
7	Pericycle	+			+	
8	Vascular bundle	+(11)	+ (12)	+ (12)	+ (7)	
9	Pseudopith	+	+	+	+	

NOTE: + = Presence of features; -- = Absence of features

Table 6: Summary of Stem Anatomical Features

S/N	Stem Features	Species			
		A. vogelii	A. nobilis	A. liebrechtsiana	A. djalonensis
1	Epidermis	+ (2 cell thick)	+ (2 cell thick)	+ (1 cell thick)	+ (2 cell thick)
2	Cortex				+
3	Astrosclereid	+ (few)	+ (abundance)	+ (abundance)	+ (several)
4	Collenchyma	+ (6 cell thick)	+ (4 cell thick)	+ (8 cell thick)	+ (9 cell thick)
5	Endodermis	+	+	+	+
8	Vascular bundle	+ (26)	+ (28)	+ (38)	+ (22)
9	Pseudopith	+	+	+	+

NOTE: + = Presence of features; -- = Absence of features

Leaf -Midrib Anatomy

The transvers section of the leaf midrib anatomy has revealed Anthocleista vogelii Planch (Plate 1) with single layer of epidermal cells, single layer of collenchymatous cells and two layers of mesophyll elongated palisade irregular layers of spongy mesophyll, crescent shaped vascular bundles, several bicollateral vascular bundles arranged in a concentric ring with eight medullary vascular bundles. Anthocleista nobilis G.Don (Plate 2) recorded two layer of upper epidermis, a single layer of collenchymatous cells, a single layer of elongated palisade mesophyll and spongy mesophyll cells, several idioblastic sclereids, several bicollateral vascular bundles and fourteen medullary bundles.

While Anthocleista liebrechtsiana De Wild and Th. Dur (Plate 3) has recorded two layer of epidermal cells, single layer of collenchymatous cells, single thick layer of elongated palisade mesophyll and shaped irregularly thick spongy mesophyll, stellate shaped idioblast in the spongy mesophyll tissue, presence of air sacs (Lacunae) in the leaf lamina and vascular bundles; Anthocleista dialonesis A.Chev leaf (Plate 4) recorded two thick layer of epidermis, multiple hypodermis, thick layer of elongated palisade mesophyll parenchyma, layer of irregular spongy mesophyll parenchyma cell, several crescent shaped and bicollateral vascular bundles with single layer of endodermis.

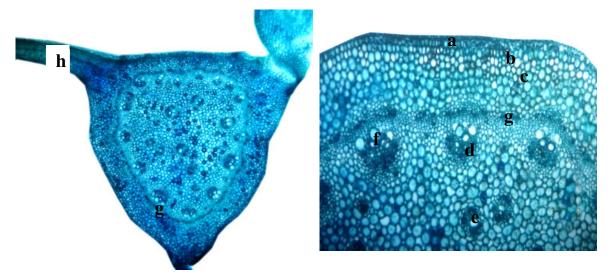


Plate 1: Transverse section of the midrib of *Anthocleista vogelii*. Planch (x10) a: Epidermis; b: Palisade mesophyll; c: Spongy mesophyll; d: Crescent vascular bundle; e: Bicollateral vascular bundle (Phleom); f: Vascular bundle (Xylem); g: Endodermis

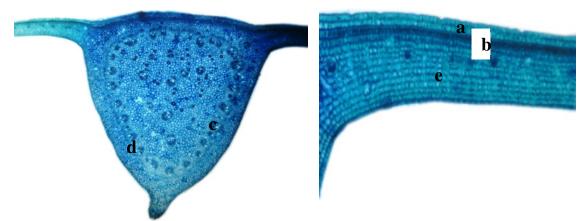


Plate 2: Transverse sections of the leaf / midrib of *Anthocleista nobilis* G.Don. (x10) a: Epidermis; b: Palisade mesophyll; c: Vascular bundles; d: Endodermis; e: Collenchyma

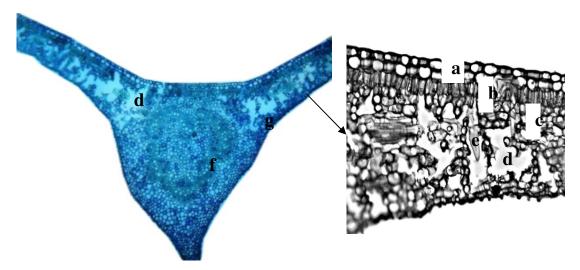


Plate 3: Transverse section of the Leaf / Midrib of *Anthocleista liebrechtsiana* De Wild and Th. Dur (x10) a: Epidermis; b: Palisade mesophyll parenchyma; c: irregular spongy mesophyll cell; d: intercellular air space; e: Idioblatic sclereids; f: Vascular bundle; and g: Collenchyma

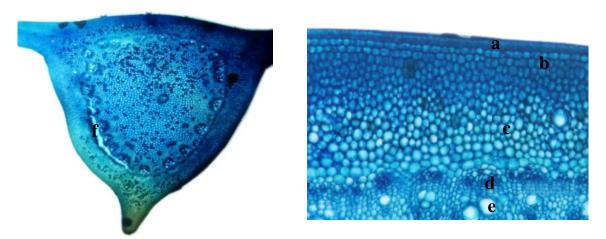


Plate 4: Transverse sections of the Leaf/Midrib of *Anthocleista djalonesis* A.Chev (x10) a: Epidermis; b: Hypodermis; c: irregular spongy mesophyll parenchyma; d: vascular bundle (phloem) e: vascular bundle (xylem); and f: endodermis

Petiole Anatomy

The petiole anatomy from the various species has indicated Anthocleista vogelii Planch (Plate 5) with its heart shape, recorded single layer of thick epidermal cells, 2-3 layers of collenchymatous cells, a single layer of sclerenchymatous cell, and several bicollateral vascular bundles. It has numerous idioblast, some with stellate shape, others with simple or complex branching. Anthocleista nobilis G.Don (Plate 6) had single layer of epidermis, two to three layers of collenchymatous cells, one layer of sclerenchyma cell and several bicollateral vascular bundles. sclerenchymatous idioblast are present, some with stellate shape, others with simple or complex

branching. While Anthocleista liebrechtsiana De Wild and Th. Dur (Plate 7) transverse section recorded single layer epidermal cell, 3 layers collenchymatous cells, single layer of sclerenchymatous cell, numerous stellate shape idioblast and several bicollateral vascular bundles; Anthocleista djalonesis A.Chev (Plate 8) with single layer of epidermal cell, 3 layers cells, collenchymatous a layer of sclerenchyma cells, spongy parenchyma cells containing a lot of intercellular spaces. Vascular bundles are amphicribral bicollateral arranged in a concentric ring. There are seven medullary vascular within bundles scattered the pith representing vein traces.

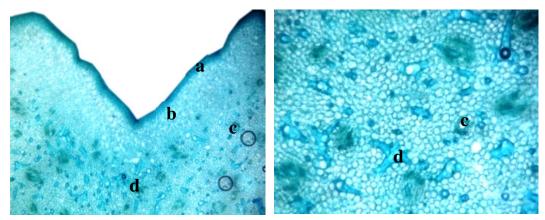
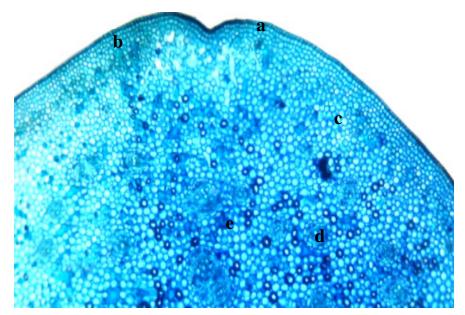
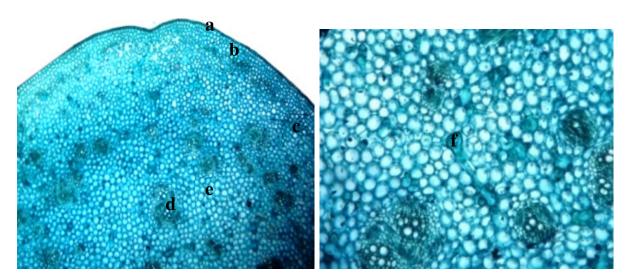


Plate 5: Transverse sections of the petiole of *Anthocleista vogelii* Planch (x10). a: Epidermis; b: Collenchyma; c: Vascular bundle; d: Sclerenchymatous idioblast



Plate, 6: Transverse section of the petiole of *Anthocleista nobilis* G.Don x10, a: Epidermis; b: Collenchyma; c: Sclerenchyma; d: Vascular bundle; e: Sclerenchymatous idioblast



Plate, 7: Transverse section of the petiole of *Anthocleista liebrechtsiana* De Wild and Th. Dur (X10). a: Epidermis; b: Collenchyma; c: Vascular bundle; d: Medullary bundle; e: Sclerenchyma: f: Sclerenchymatuos idioblast

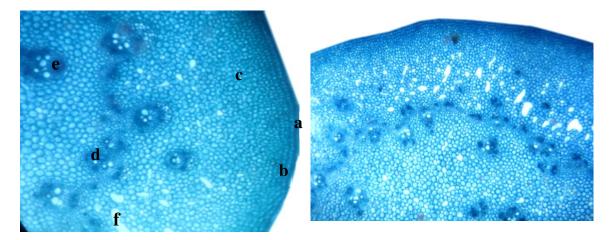


Plate 8: Transverse sections of the petiole of *Anthocleista djalonesis* A.Chev x10. a: Epidermis; b: Collenchyma; c: Sclerenchyma; d: Vascular bundle; e: Medullary bundle; f: Intercellular space

Root and Stem Anatomy

The transverse section of the root and stem anatomy has revealed the internal structure of the four species studied (Plates 9 - 16). The root of *Anthocleista* vogelii. Planch (Plate 9) recorded two layers of epidermis, cortex, several air chambers, endodermis, pericycle, several

vascular bundles and pseudopith. *Anthocleista nobilis*. G. Don (Plate 10) revealed two layers of epidermis, a layer of exodermis, cortex, several air chambers, endodermis, several vascular bundles, and several astrosclereid; While *Anthocleista liebrechtsiana*. De Wild and Th. Dur., (Plate 11) recorded two layers of

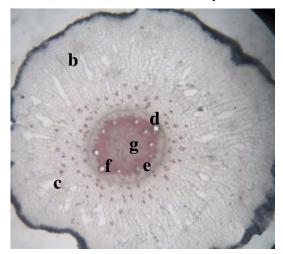


Plate 9: Transverse section of the Root of *A. vogelii*. (x10). a: Epidermal cell; b: Cortex; c: Air chamber; d: Endodermis; e: Pericycle; f: Vascular bundles; g: Pseudopith

epidermis, exodermis, several air chambers, cortex, several sclereid, endodermis, 12 vascular bundles and pseudopith; *A. djalonensis.* A. Chev (Plate 12) had two layers of epidermis, cortex with the presence of air chamber, endodermis, pericycle, seven vascular bundles and pseudopith.

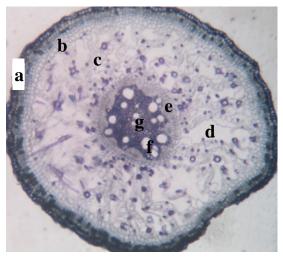


Plate 10: Transverse section of *A. nobilis*. Root (x10). a: Epidermis; b: Exodermis; c: Cortex; d: Air chamber; e: Endodermis; f: Vascular bundles; g: Pseudopith

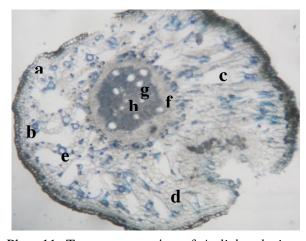


Plate 11: Transverse section of *A. liebrechtsiana* Root (x10). a: Epidermis; b: Exodermis; c: Air space; d: Cortex; e: Sclereid; f: Endodermis; g: Vascular bundles; h: Pseudopith

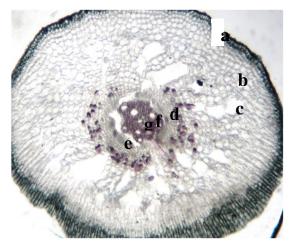


Plate 12: Transverse section of *A. djalonensis* Root (x10). a: Epidermis; b: Cortex; c: Air chamber; d: Endodermis; e: Pericycle; f: vascular bundles; g: Pseudopith

The stem anatomy of *Anthocleista vogelii*. Planch (Plate 13) revealed layers of epidermis, layers of collenchyma, endodermis, several vascular buddles, and few sclereid. *Anthocleista nobilis*. G. Don (Plate 14) recorded pseudopith, several sclereid, vascular bundles, endodermis, collenchyma cells and epidermal cells. *Anthocleista liebrechtsiana*. De Wild and

Th. Dur (Plate 15) had a layer of epidermis, layers of collenchyma, endodermis, several vascular buddles, several astrosclereid and pseudopith. *Anthocleista djalonensis*, A. Chev (Plate 16) has revealed a thick layer of epidermis, cortex, several astrosclereid, 9 layers of collenchyma, endodermis, several vascular buddle, and pseudopith.

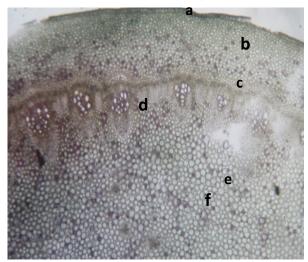


Plate 13: Transverse sections of *A. vogelii* stem (x40), a: Epidermal cells; b: Collenchyma cells; c: Endodermis; d: Vascular bundle; e: Pseudopith; f: Sclereid

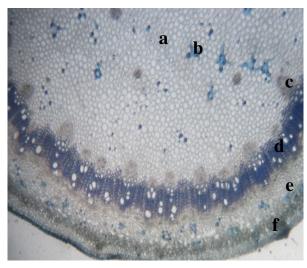


Plate 14: Transverse sections of *A. nobilis* stem (x40). a: Pseudopith; b: Sclereid; c: Vascular bundle; d: Endodermis; e: Collenchyma cells; f: Epidermal cells



Plate 15: Transverse sections of *A. liebrechtsiana* stem (x40), a: Epidermis; b: Collenchyma; c: Endodermis; d: Vascular bundle; e: Sclereid; f: Pseudopith

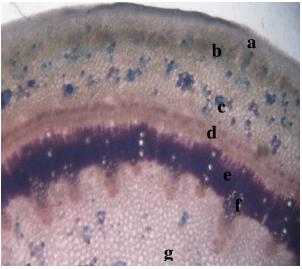


Plate 16: Transverse sections of *A. djalonensis* stem (x40), a: Epidermis; b: Cortex; c: Scleried; d: Collenchyma; e: Endodermis; f: Vascular bundles; g: Pseudopith

Discussion

The anatomical features of the Anthocleista species studied are classificatory and diagnostic. The study revealed both intraspecific and interspecific similarities and variation which can be employed in the identification and delimitation of the species studied. The role of anatomical data in taxonomy has been recognized, since the variation within a species, genus or family is usually reflected in anatomical features. It was suggested that anatomical features in plant might be more important than morphological features, hence the former can be less or non-influenced by environmental changes (Stace, 1991). studies have comparatively Several revealed considerable significance of foliar anatomy in taxonomy (Cutler 1984; Afolayan and Meyer 1995; Chinwe and Maria, 2009)

The foliar anatomical features are generally similar for all the species, which is in tan- dem with the assertion by Metcalfe and Chalk (1959) to include such structures as epidermis, hypodermis, collenchyma, palisade cell, spongy cell, and bicollateral, medullary and crescent vascular bundles. Others are endodermis, sclerechymatous idioplast and air sac. The similarities observed among the species reflect phylogenetic relatedness of the taxa, and this was compared with that of Hutchinson (1959) who based his system of classification on phylogenetic line. This corroborate the assertion by Dutta (2002) that the major purpose of classification is to arrange plants in such a way as to give an idea of their phylogenetic relationships (i.e., the sequence of their origin and evolution from simpler, earlier and more primitives types to more complex, recent advanced types during different periods of their growth).

However, there were interspecific variations in quantitative and qualitative character among the species. Anthocleista djalonesis has revealed some differences in both quantitative and qualitative attributes of foliar anatomical characters with multiple layers of epidermis (hypodermis), presence of thickened endodermis and three-layer thickened palisade cell. This agrees with similar report on A. djalonesis by Chinwe and Maria (2009). Anthocleista dialonesis is mesophytic in habitat adaptation and a secondary terrestrial forest species (Edwin-Wosu et al., 2015). Thicken leaves well-developed epidermal cuticle, hypodermal layer, well developed palisade tissue and highly arranged spongy tissues that characterize plants in habitat of mesophytic condition similar to high light intensity have been reported (Mendes et al. 2001; Cai and Song 2001; Rocas et al. 2001). This can be the bases for its lack of sclerenchymtous idioblast, but with many intercellular spaces, well developed cuticle to aid water loss prevention

Numerous sclerenchymatous idioblast and air sacs seen in *A. liebrectsiana* depict its preference for semi-aquatic (wetland) habitat. Other variations among the species are reported in Table 3. This can be attributed to differences in habitat adaptation of various species (Edwin-Wosu and Omara-Achong 2010; Edwin-Wosu *et al.*, 2015; Mudasiru *et al.*, 2016).

The petiole anatomical characters though revealed similarity in the features among species, there are differences in light of the absence and presence of idioblast, spongy cells, intercellular spaces and medullary vascular bundles as exemplified in Table 4. This corroborates Muhali and James (2017), Chinwe and Maria (2009) whose study on A.

djalonensis has revealed the presence of idioblast, medullary vascular bundles, among other structures earlier revealed in Metcalfe and Chalk (1959). The root anatomical features of Anthocleista also revealed in qualitative and quantitative terms similarity in epidermal layer, cortex, air chamber and endodermis among the species, while exodermis and sclereid were only revealed in A. nobilis and A. liebrectsiana with 11 vascular bundles and 7 vascular bundles respectively in A. vogelii and A. djalonensis as variation among the taxa. The stem anatomical features has also revealed similarity and distinction among the taxa with the cortex shown only in A. djalonensis, 6,4,8 and 9 cell thickness of collenchyma in A.vogelii, A. nobilis, A. liebrectsiana and A. djalonensis respectively and vascular bundles in quantitative variation among species. This corroborates the findings of Chinwe and Maria (2009). The present study supports the finding of other workers (Jayeola et al., 2009; Kadiri and Ayodele, 2010; Oladipo and Illoh, 2012) who reported similar variation on plant species distinguishable based on The complete anatomical characters. Anthocleista species can be segregated from each other in the basis of their quantitative anatomical parameters a diverse dimension of foliar character.

Conclusion

The role of anatomical data in taxonomy has been recognized since variation within a species, genus or family is usually reflected in anatomical features. Comparative anatomy has shown to be of considerable significance in taxonomy, thus the variation and similarity observed in qualitative and quantitative features of the anatomy of these plants can be of immense significance in the taxonomic

delimitation of these *Anthocleista* species and equally add to the existing taxonomic information on these taxa.

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