Journal of Tropical Agriculture 55 (2): 121-133, 2017

Leaf epidermal morphology and petiole anatomy of the genus *Anthocleista* Afzel. ex R.Br. (Gentianaceae)

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Received 31 October 2017; received in revised form 7 December 2017; accepted 12 December 2017

Abstract

Morphology of leaf epidermis and petiole transverse section in three species of *Anthocleista* was studied using a light microscope with a view to understanding the systematic significance of epidermal morphology and petiole anatomy within the genus. Interestingly, important taxonomic characters such as epistomata were observed in *A. vogelii* and amphistomata in *A. djalonensis* and *A. nobilis*. Scale trichomes were found in both epidermal layers of *A. djalonensis* and glandular trichome, which is multicellular and unicellular in *A. nobilis* and *A. djalonensis* respectively. Stomatal Index was highest at adaxial side of *A. nobilis* (10.14%), lowest at *A. djalonensis* (4.56%) and did not exist at all at the abaxial surface of *A. vogelii*. Common generic characteristics are polygonal cell shape, straight anticlinal wall, dendritic trichomes and petiole anatomy. The petiole is longest in *A. nobilis*, shortest in *A. djalonensis* and absent in *A. vogelii*. This work therefore, revealed that species within the genus differ in epidermal characteristics which could be used to delimit them although they share some similarities in cell geometry, petiole transverse section and anticlinal walls; all these are important taxonomic tools used in delimiting species in the genus.

Keywords: *Anthocleista*, Anticlinal wall, Dendritic trichomes, Morphology, Stomatal index

Introduction

The Genus *Anthocleista* Afzel. ex R. Br. (Gentianaceae) is a dicot genus consists of approximately fifty species worldwide and fourteen species in tropical Africa including Comoros, Madagascar and Mascarene Island. Members are mostly trees and shrubs, usually with woody stem (Palmer and Pitmer, 1972; Keay, 1989; Leeuwenberg and Leehourts, 1980; Struwe and Albert, 2002; Sonibare et al., 2007; Hyde, 2013). Leaves are opposite, simple, stipulate or estipulate. Flowers are hermaphroditic, usually actinomorphic with 2-4 celled superior ovaries. Fruit may be capsule, drupe or berry. Their leaves are glabrous, leathery and large and are often over 1 ft long in mature trees and up to 5 ft long in saplings. The base of the leaf stalk is dilated and sometimes more or less winged. The leaves of these species range from obovate to oblanceolate shapes and are clustered at the ends of the branchlets, mostly 6-18 inches long; and the flowers 1-3 inches long. The flower colour ranges from white to cream in *A. djalonensis* and *A. nobilis* while it is orange or fawn colour in *A. vogelii* (Leeuwenberg and Leehourts, 1980; Hutchinson and Dalziel, 1994; Burkill, 1995; Burkill et al., 1995; Edwin-Wosu and Ndukwu, 2012).

Species in this genus include:

i) *A. amplexicaulis* Baker
ii) *A. djalonesis* A. chev.
iii) *A. exelliana* Th. Monod
iv) *A. grandiflora*
v) *A. keniensis*
vi) *A. liebrechstiana*

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vii) A. madagascariensis
viii) A. nobilis G. Don
ix) A. procera
x) A. rhizophoroides Baker
xi) A. scandens
xii) A. schweinfurthii
xiii) A. vogelii
xiv) A. zambesiaca

The four species occurring in West Africa have the same vernacular names and are used by local practitioners for the same medicinal purposes (Dalziel, 1954; Leeuwenberg, 1961; Watt and Breyer-Brandwijk, 1962; Chapelle, 1976; Adjanohoun et al., 1979; Akubue et al., 1983; Abbiw, 1990; Burkill, 1995; Neuwinger, 2000; Togola et al., 2005; Ateufack et al., 2006; Sonibare et al., 2007; Mabberley, 2008; Antia et al., 2009). The description of their phytochemical evaluation by Sonibare et al. (2007) and other researchers was elaborate and from available reports, they share almost the same phytochemical contents and medicinal properties (Githens, 1949; Okorie, 1976; Bierer et al., 1995; Onocha and Okorie, 1995; Jensen and Schripsema, 2002; Onocha et al., 2003; Okoli & Iroegbu, 2004; Campaner dos Santos et al., 2005; Chah et al., 2006, Olowokudejo, 2008; Antia et al., 2009; Odeghe et al., 2012). Because of uncontrolled harvesting for use in local medicine, species are threatened by over exploitation in Mali and Burkina Faso. A concerted effort should, therefore, be undertaken to conserve the species (Leeuwenberg, 1961).

Nine species were recorded in West Tropical African region (Burkill, 1995; Oduoye, 2013) out of which four are reported in Nigeria. Three of these species, *A. djalonensis, A. vogelii* and *A. nobilis,* are abundant and widespread in the South Western region. They are usually small trees or scrambling shrubs with soft white wood (Sonibare et al., 2007). Menninger (1967) reported that nearly all have vicious forking thorns, adding that at a certain stage in their development, they lose their bark and leaves, but retain their thorns on the trunk and come to resemble a thorny naked pole. However, wider literature suggests that the thorns are not always present (Leeuwenberg, 1961, 1983, 1992; Leeuwenberg and Leehourts, 1980; De Ruijter, 2007)

Anthocleista is faced with problems of classification. One of the historic problems with classifying this genus under Loganiaceae family was that most taxon assigned to this family had rather generalized or plesiomorphic traits (Leeuwenberg and Leehourts, 1980; Mabberley, 2008). The genus had in the past been classified in the Potaliaceae and in the Loganiaceae but data from phylogenetic studies in 1990 and subsequently, the Angiosperm Phylogeny Group (Angiosperm Phylogeny Group, 2003) has now placed the genus in the Gentianaceae.

Due to its possession of supermerous corollas and seminal parts, *Anthocleista* was believed to have diverged from the gentian flora as it appears to lack post genital fusion of carpels which is typical to gentians. So it was concluded that *Anthocleista* is a tropical woody genus with showy flowers and fleshy or leathery berries, whereas most gentians are smaller herbs or shrubs with dry capsular fruits (Struwe and Albert, 2002). The core objective of this study was to examine morphological characteristics of the species *vis a vis* their leaf epidermis and anatomy of their petiole in order to re-evaluate the taxonomic significance of structures observed. The study also assisted in reviewing available taxonomic data of the species and in re-evaluating key structures responsible for species characterization.

Materials and Methods

Mature and fresh leaves of *Anthocleista* species were collected from trees (Table 1; Plate 1-3). *A. djalonensis* and *A. nobilis* were collected from different locations in Abeokuta while *A. vogelii* was collected from the University of Lagos, south western Nigeria (Table 1). The three species were deposited in Lagos University Herbarium for verification.

Table 1. Sources of species used for the study

Species	Location	Collector	Coordinates
A.djalonensis	Vice Chancellor's Lodge, Federal University of Agriculture (FUNAAB), Abeokuta, Nigeria	Muhali Jimoh	Lat. 7º12¹58.66º N Long. 3º26¹32.91ºE
A. nobilis	TejumolaMajekodunmi Close, Ibara GRA, Abeokuta, Nigeria	Muhali Jimoh	Lat. 7º07¹24.81ºN Long. 3º21¹31.43ºE
A.vogelii	Behind Faculty of Science, University of Lagos(UNILAG), Akoka, Nigeria	Muhali Jimoh	Lat. 6º30¹58.92ºN Long. 3º23¹57.32ºE

Plate 1. A. djalonensis

Plate 2. A. nobilis

Plate 3. A. vogelii

Epidermal preparation and petiole transverse section

About 3cm^2 portions of the leaf were cut off with a razor blade and leaf pieces were boiled in water for 7-10 mins and then irrigated in concentrated Hydrogen Tetraoxonitrate (V) acid (HNO_3) for 8-10 hrs in covered specimen bottle to macerate the mesophyll (Kadiri and Olowokudejo, 2010; Kotresha and Seetharam, 2000).

The appearance of bubbles around treated leaf indicated tissue disintegration and the epidermises were subsequently transferred into cold water inside Petri dishes for separation using forceps and mounting needle. The tissue debris was removed from the epidermises and washed in several changes of water with a soft brush. Few drops of ethanol

were added to dehydrate the cells or to harden it and stained with 3-4 drops of Safranin-O before mounting in glycerine with outer surfaces uppermost on a glass slide. The treated cells were covered with glass coverslips and ringed with a lacquer to prevent dehydration and exposure to the atmosphere. This epidermal preparation method follows the method employed by Clark (1973) and modified by Kadiri and Olowokudejo (2010).

For petioles, about 0.1-0.2 mm sections were carefully sliced with a razor blade and soaked in sodium hypochlorite for 5minutes to bleach them, then transferred to Petri dishes containing distilled water. The preparations were dehydrated on a glass slide for about 3 minutes by adding few drops of ethanol and then stained in Safranin-O for five minutes before being mounted in glycerin on glass slides. The slides were ringed with varnish to cover them (Kadiri and Olowokudejo, 2010; Illoh and Inyang, 1998).

Thereafter, the slides were labeled and examined with a Zeiss Light Microscope; model Axio Lab A.1 at different magnifications (x4, x10, x40 and x100). Photomicrographic images of examined cell features were taken digitally with a Motic image plus version 2.0 ml embedded on the light microscope. This work was carried out in the Botany Research Laboratory, University of Lagos, Akoka, Nigeria.

Macromorphological studies

Leaves of the three species were cross-examined physically to obtain macromorphological characteristics of the species and compared critically *vis a vis* leaf length, leaf apex type, leaf base form, presence or absence of petiole and its length and the nature of petiole base at the point of attachment to the stem. This was carried out with the aid of a calibrated ruler and measurements were taken in centimeters.

Micromorphological examinations

Morphological study of the leaf epidermal layers was carried out to observe both qualitative and quantitative characters of the adaxial and abaxial surfaces (Figure 1-9). Qualitative characters included; stomatal type, epidermal type, trichomes, cell geometry, anticlinal wall pattern. The quantitative characters examined were epidermal cell wall thickness, stomatal length, epidermal cell length, number of stomata per field of view and number of epidermal cells per field of view. With the aid of micrometer eyepiece, quantitative characters were measured and mean values recorded for comparative analysis.

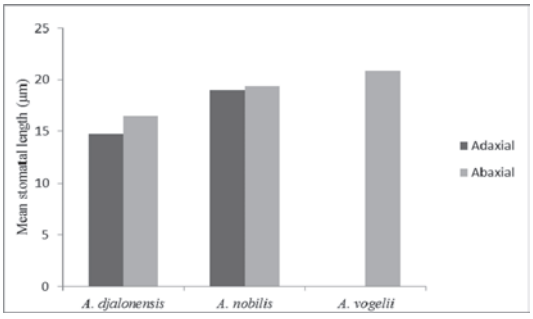

Figure 1. Leaf stomatal length *Anthocleista* spp.

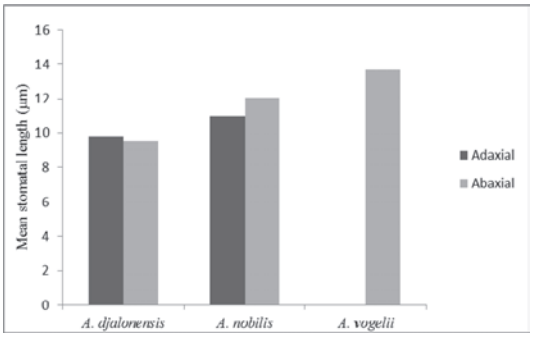

Figure 2. Leaf stomatal width *Anthocleista* spp.

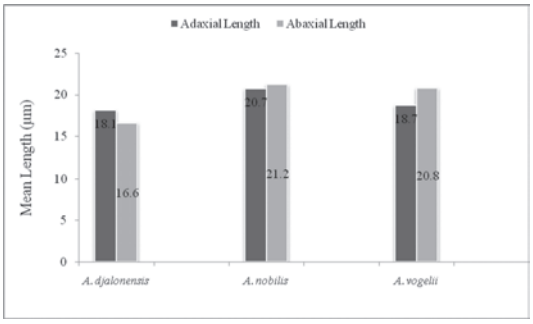

Figure 3. Comparative mean leaf epidermal cell length *Anthocleista* spp.

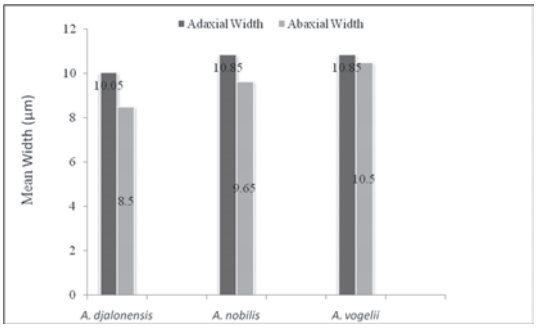

Figure 4. Comparative mean leaf epidermal cell width *Anthocleista* spp.

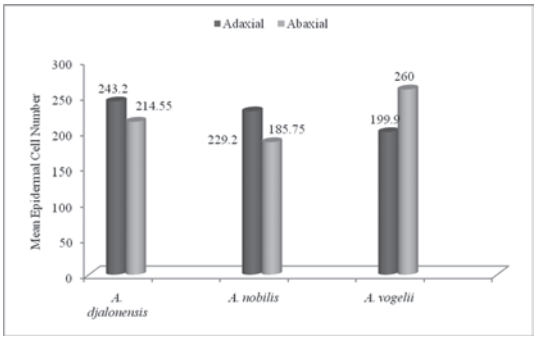

Figure 5. Comparative leaf epidermal cell numbers of *Anthocleista* spp.

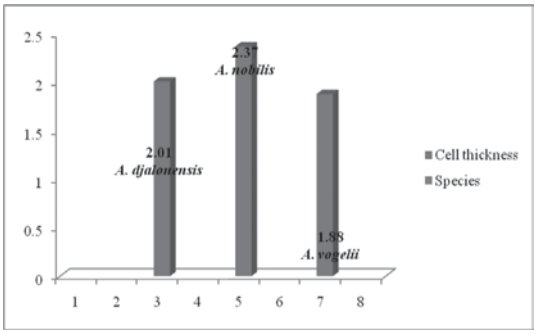

Figure 6. Epidermal cell thickness of *Anthocleista* spp.

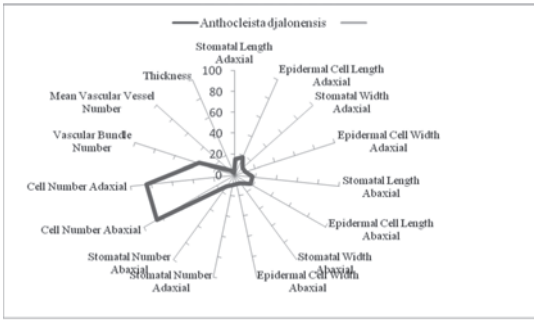

Figure 7. Polygraph mean plot for leaf epidermal characters in *A. djalonensis*

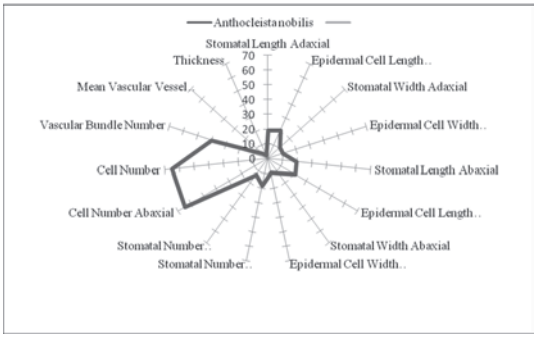

Figure 8. Polygraph mean plot for leaf epidermal characters *A. nobilis*

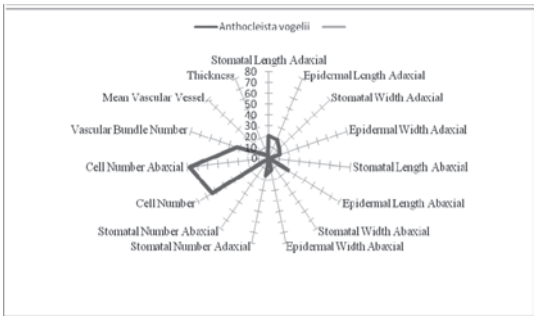

Figure 9. Polygraph mean plot for leaf epidermal characters *A. vogelii*

Character analysis

Twenty epidermal cells were counted and selected randomly and their lengths, widths and wall thickness were measured. For stomata, five cells were selected randomly and measured to obtain their lengths and width using micrometer eyepiece while the number in the field was counted in ten views per species.

Petiole anatomy

The internal structure of the petiole was examined to compare arrangement of the vascular bundle and association of tissues in the three species. A number of vascular bundles were counted per field of view as well as mean vessel number per vascular bundle.

Table 2. Comparative quantitative macromorphological features of *Anthocleista*

Species	Macromorphological characteristics					Mean ±SD
Anthocleista djalonensis						
Leaf blade length (cm)	15.3	17.6	14.9	16.8	18.4	16.6 ±1.33
Leaf blade width (cm)	10.4	11.1	9.8	11.3	11.7	10.86±0.68
Petiole length (cm)	6.2	7.3	6.5	6.7	7.1	6.76±0.40
Petiole base (cm)	2.2	1.8	1.6	1.9	1.7	1.84±0.21
Anthocleista nobilis						
Leaf blade length (cm)	23.2	29.5	30.6	38.2	45.6	33.42±7.73
Leaf blade width (cm)	12.9	15.2	16.2	22.4	18.6	17.06±3.23
Petiole length (cm)	14.9	14.3	12.8	13.3	12.6	13.58±0.88
Petiole base (cm)	2.7	2.9	2.6	2.1	3.5	2.76±0.45
Anthocleista vogelii						
Leaf blade length (cm)	28.7	21.2	27.4	24.3	25	25.32±2.60
Leaf blade width (cm)	13.4	12.9	16	18.2	17.8	15.66±2.18
Petiole length (cm)	0.9	0.8	0.8	0.7	1.1	0.86±0.14
Petiole base (cm)	1.9	2.3	2.2	2	2.5	2.18±0.21

SD= standard deviation

Results and Discussion

This study was carried out to investigate characters of taxonomic importance that are common to all or distinguish the three species from one another. Tables 2-7 present morphological features observed in the leaves and a transverse section of the petiole. Macromorphological examination showed that *A. djalonensis, A. vogelii* and *A. nobilis* are closely related. They all have spines and white flowers but *A. vogelii* is practically stalk less while *A. djalonensis* and *A. nobilis* are distinctly stalked. In all the species, leaves are opposite, simple and entire. The petiole is auricled and about 6-7cm long in *A. djalonensis*. Leaf apex of *A. djalonensis* is rounded with the cordate base; mature leaf blade is about 15-18 cm long, 10-12 cm broad. Leaf base is cuneate, apex rounded in *A. vogelii.* Mature leaf length is about 32-36 cm and is 17-22 cm broad. In *A. nobilis,* leaf blade is usually 23-47 cm long, 13-22 cm broad; rounded or bluntly pointed at the apex, tapering gradually to a cuneate or oblique base; light green abaxial and dark green adaxial surfaces crowded at the apices of the branchlets. The petiole is 13-15 cm long in *A. nobilis* and dilated at the base for the three species.

In the epidermal and stomatal cell structures, the three species exhibited polygonal cell shape on both layers (Plates 4a-6b). Some triangular cell shapes

Table 3. Summarized qualitative epidermal and stomatal features of *Anthocleista* species

Species	Nature	Stomatal Type	Cell Geometry	Anticlinal Wall Pattern	Trichomes Types
A.djalonensis Adaxial	*Amphistomatic*	Cyclocytic Triangular	Polygonal/	Straight/Bend	Dendritic/tubular/ unbranched/Scale
Abaxial		Cyclocytic	Polygonal	Straight/Bend	Dendritic/tubular/ unbranched/Scale
*A.nobilis*Adaxial	*Amphistomatic*	Anomocytic	Polygonal	Straight/Bend	Dendritic/multicellular glandular
Adaxial		Actinocytic	Polygonal	Straight/Bend	Scale/ Dendritic
*A. vogelii*Adaxial	*Epistomatic*	Paracytic	Polygonal	Straight	Dendritic
Adaxial		Nil	Polygonal	Straight	Unicellular Glandular

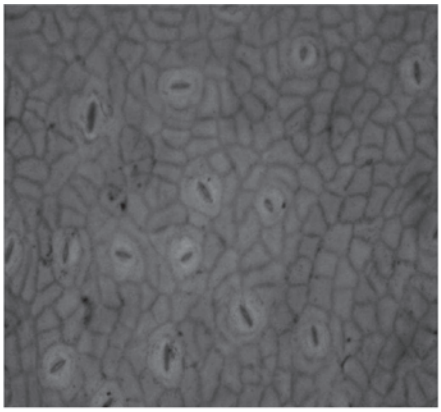

Plate 4a. Epidermal tissue in *A. djalonensis* (abaxial) Mag X10

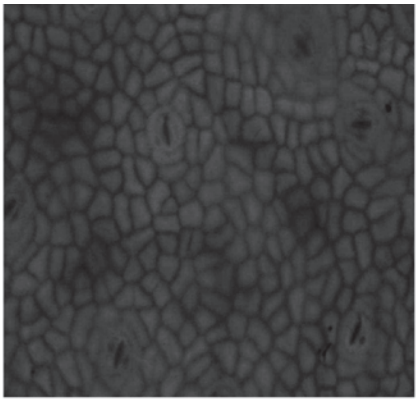

Plate 4b. Epidermal tissue in *A. djalonensis* (abaxial) Mag X10

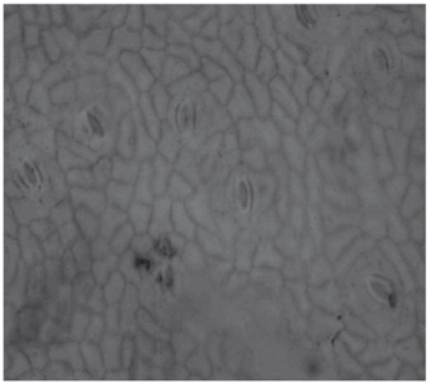

Plate 5a. Epidermal tissue in *A. nobilis* (abaxial) Mag X10

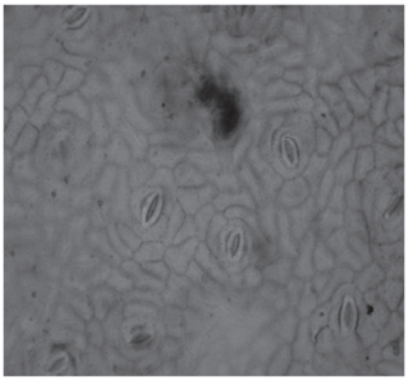

Plate 5b. Epidermal tissue in *A.nobilis* (adaxial) Mag X10

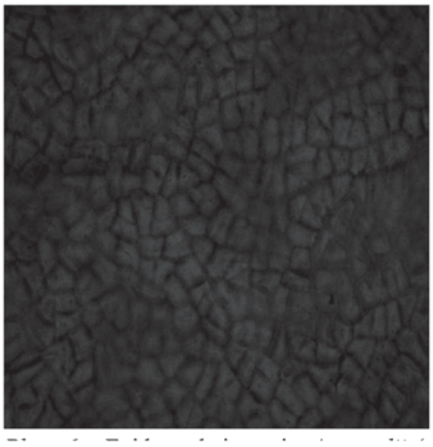

Plate 6a. Epidermal tissue in *A. vogelii* (abaxial) Mag X10

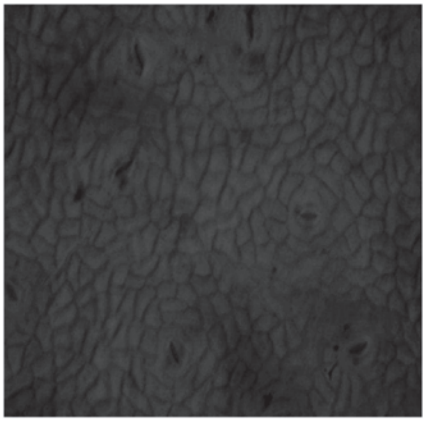

Plate 6b. Epidermal tissue in *A. vogelii* (adaxial) Mag X10

Table 4. Summarized quantitative leaf epidermal and stomatal characters of the species of *Anthocleista*

Species	Surface	Stomatal cells: Length (µm) Min (Mean±SD) Max	Width (µm) Min (Mean±SD) Max	Stomatal Number Min (Mean±SD) Max	Stomatal Index (%)	Epidermal cells: Length (µm)Min (Mean±SD) (Max	Width(µm)Min Mean±SD) Max	Cell NumberMin (Mean±SD) Max
A.djalonensis	adaxial	11.0(14.70±2.49) 19.0	7.0(9.80±2.04) 14.0	8.0(10.10±1.74) 13.0	4.56	11.0(18.10±3.49) 19.0	7.0(10.05±1.82) 13.0	208.0(243.20±16.88) 263.0
	abaxial	15.0(16.50±1.24) 18.0	8.0(9.55±2.46) 19.0	10.0(13.60±2.88) 18.0	5.96	15.0(16.60±2.68) 19.0	6.0(8.50±1.57) 11.0	178.0(214.55±16.66) 241.0
A. nobilis	adaxial	17.0(19.00±1.97) 22.0	8.0(11.00±1.59) 14.0	14.0(18.40±2.28) 23.0	10.14	17.0(20.70±3.20) 25.0	7.0(10.85±2.56) 18.0	180.0(229.20±22.87) 259.0
	abaxial	16.0(19.40±1.57) 22.0	10.0(12.05±1.15) 14.0	11.0(13.40±1.85) 17.0	7.63	15.0(21.20±5.40) 31.0	6.0(9.65±2.06) 13.0	148.0(185.75±19.86) 226.0
A. vogelii	adaxial	19.0(20.85±1.39) 23.0	12.0(13.70±1.08) 15.0	11.0(15.95±1.85) 18.0	9.05	12.0(18.70±3.60) 24.0	8.0(10.85±1.69) 14.0	155.0(199.90±31.15) 241.0
	abaxial	Nil	Nil	Nil	0	15.0(20.80±2.91) 24.0	8.0(10.45±1.73) 13.0	218.0(269.00±26.72) 304.0

SD= standard deviation

were observed, especially in *A. djalonensis* (adaxial). Anticlinal wall pattern is straight in all the species, but bent walls were seen, especially in cells surrounding guard cells (Table 2). The abaxial surface of *A. vogelii* has the greatest number of epidermal cells (260) due to absence of stomatal followed by *A. djalonensis* (adaxial; 243), *A. nobilis* (adaxial; 229), *A. djalonensis* (abaxial; 215), *A. vogelii* (adaxial; 200) and *A. nobilis* (abaxial; 186). This is presented in Table 4.

Presence of scale was also observed on both surfaces of *A. djalonensis* and *A. nobilis* but absent in *A. vogelii*. Unicellular glandular trichomes were observed in the abaxial surface of *A. vogelii*. This peculiar feature was not seen at its adaxial surface as only unicellular dendritic trichomes were observed. At both surfaces of *A. djalonensis*, dendritic, tubular, and unbranched and prominent scale trichomes were found. For *A. nobilis*, trichomes were scaly and dendritic. Also, typical multicellular glandular trichomes were observed at the adaxial surface of *A. nobilis* (Table 2). While *A. vogelii* was observed to be epistomatic, *A. djalonensis* and *A. nobilis* were amphistomatic. The mean stomatal number (18.4) was highest in *A. nobilis* (adaxial) followed by *A. vogelii* (adaxial; 15.95), *A. djalonensis* (abaxial, 13.6), *A. nobilis* (abaxial, 13.4), *A. djalonensis* (adaxial, 10.1) while no stomata was recorded in *A. vogelii* (abaxial)

Table 5. Quantitative stomatal character of *Anthocleista spp.*

Species	Stomatal cells: Adaxial Length Mean (µm)	SD	Adaxial Width Mean (µm)	SD	Abaxial Length Mean (µm)	SD	Abaxial Width Mean (µm)	SD	Mean Stomatal Number Adaxial	Abaxial
Anthocleista djalonensis	14.7	2.49	9.8	2.04	16.5	1.24	9.55	2.46	10.1	13.6
Anthocleista nobilis	19	1.97	11	1.59	19.4	1.57	12.05	1.15	18.4	13.4
Anthocleista vogelii	20.85	1.39	13.7	1.08	0	0	0	0	15.95	0

SD= Standard Deviation

Table 6. Quantitative leaf epidermal character of *Anthocleista* spp.

Species	Epidermal cells: Adaxial Length Mean (µm)	SD	Adaxial Width Mean (µm)	SD	Abaxial Length Mean (µm)	SD	Abaxial Width Mean (µm)	SD	Mean Cell Number (X40) Adaxial	Abaxial	Thickness Mean	SD
Anthocleista djalonensis	18.1	3.49	10.05	1.82	16.6	2.68	8.5	1.57	243.2	214.55	2.01	0.43
Anthocleista nobilis	20.7	3.2	10.85	2.56	21.2	5.4	9.65	10.06	229.2	185.75	2.37	0.62
Anthocleista vogelii	18.7	3.6	10.85	1.17	20.8	2.91	10.5	1.82	199.9	260	1.88	0.44

SD= Standard Deviation

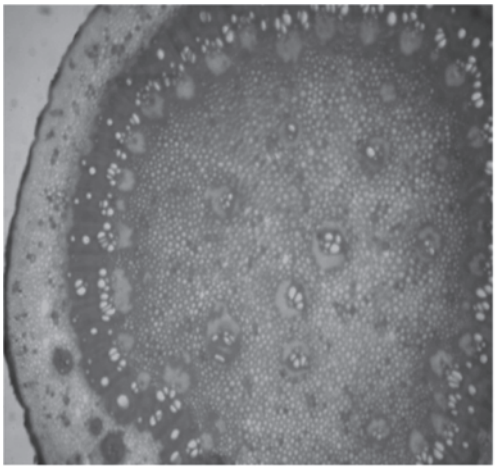

Plate 7a. Petiole of *A. nobilis*(X4)

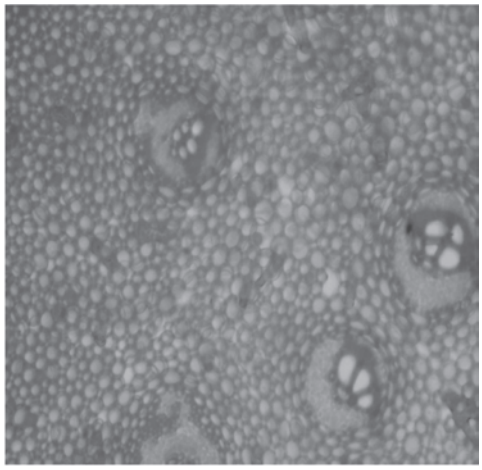

Plate7b. Petiole of *A. nobilis*(X10)

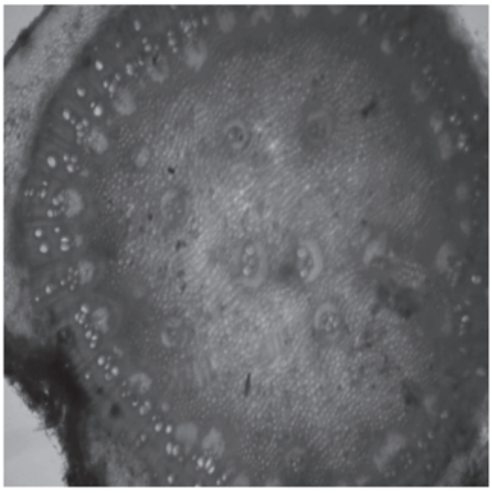

Plate 8a. Petiole of *A. vogelii* (X4)

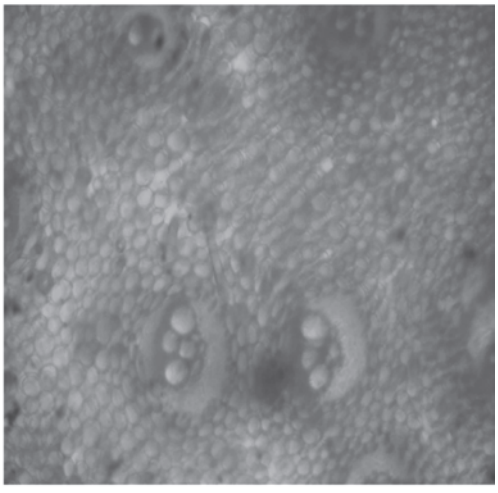

Plate 8b. Petiole of *A. vogelii* (X10)

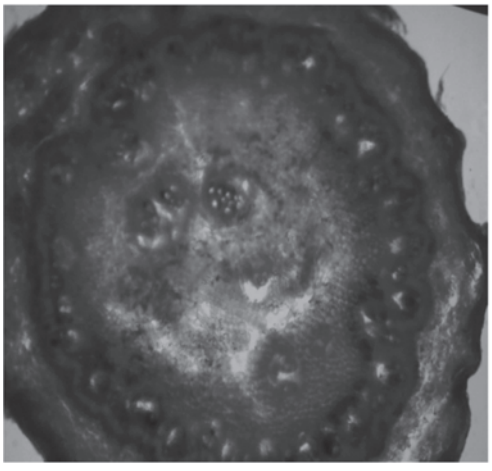

Plate 9a. Petiole of *A. djalonensis* (X4)

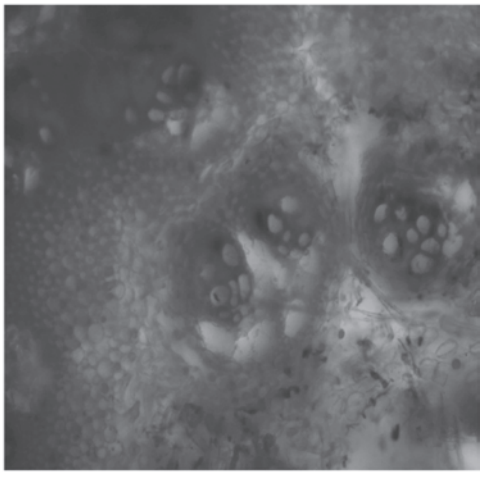

Plate 9b. Petiole of *A. djalonensis* (X10)

Table 7. Showing Comparative Quantitative Petiole Features

Species	Comparative Quantitative Petiole Features		
	Vascular bundle	Vascular bundle number	Mean number of vessel elements
Anthocleista djalonensis	Scattered	36	6.4
Anthocleista nobilis	Scattered	36	5.4
Anthocleista vogelii	Scattered	36	5.2

(Table 3). Longest stomata were recorded on the adaxial surface of *A. djalonensis* (20.85 µm length; 13.7 µm width), followed by *A. nobilis* (19 µm length; 11 µm width), and *A. djalonensis* (14.7 µm).

In the petiole anatomy, the three species showed a high level of resemblance in the configuration of different tissues forming the petiole (Plates 7a-9b). The transverse sections clearly revealed scattered vascular tissues in the parenchymal cells of the central cylinder, vascular bundles numbering 8-10, each containing 5-9 tracheids in the xylem. Tannin is present and abundant in the three species and is reddish in colour. Apart from the scattered vascular bundle in the central cylinder, a circular ring was formed around the parenchyma cells by another set of bundles (Plates 7a-9b).

The problem of classification is historic with *Anthocleista* spp. (Leeuwenberg and Leehourts, 1980; Mabberley, 2008). As a result, the genus was placed in different families such as Loganaceae, Potaliaceae and Gentianaceae in the past on the account of plesiomorphic traits, the most recent being Gentianaceae on account of the phylogenetic studies proposed by the Angiosperm Phylogeny Group. This necessitated the need for critical studies of other parts of the plant, particularly the aerial parts, to unravel more characters of taxonomic significance in order to achieve a robust character database for the plant species and to make the delineation seamless.

This study showed that there are some variations in leaf morphological characters of *Anthocleista* species. Though they share many characters in common, some characters vary considerably between and within species of this genus. The distribution of the species in the West African region has been described by Burkill (1995) and their biosystematic studies have been discussed by Edwin-Wosu and Ndukwu (2012). Also, their phytochemical analysis (though out of the scope of this work) has been investigated and widely communicated by Akubue et al. (1983), Haslam (1981) and Neuwinger (2000) Sonibare et al. (2007) discussed in detail, a chemotaxonomic approach to the alkane content of the three species under study.

In the three species studied, elongated trichomes which differ in form, size, length, and density, depending on the species were observed. Stace (1965) suggested that hairs are constant in a species when present and showed a constant range of form and distribution useful in diagnosis, while Metcalfe and Chalk (1979) on the other hand reported that the length, size and trichome density are more liable to vary with the environment. *A. nobilis* and *A. djalonensis* for example, have glandular trichomes while the scale and stellate trichomes were prominent in both epidermal surfaces of *A. djalonensis*. Dendritic trichome is common to the three species, suggesting a morphological relationship.

Observations further revealed different types of stomata among species studied. This could be used as a key taxonomic factor in delimiting the species. *A. vogelii* was observed to have paracytic stomata at the adaxial surface only (epistomatic), *A. djalonensis* possesses cyclocytic stomata at both surfaces while *A. nobilis* differs by possessing anomocytic and actinocytic stomata at the adaxial and abaxial surfaces respectively (Prabhakar, 2004).

This finding, however, agrees with Edwin-Wosu and Ndukwu (2012) on stomatal variation in *Anthocleista* and may be used as a key factor in delimiting the species, though Metcalfe and Chalk (1979) stated that stomata of more than one type occur together sometimes on the same leaf surface or stomata on the upper and lower surfaces may differ. Therefore, not all stomata present on a leaf are good example of a single type and a fairly large number of stomata should be considered to determine the prevalent type (Cutler, 1969).

The epidermal cells are polygonal in shape with straight anticlinal wall pattern (Oduoye, 2013). Stace (1965) considered this feature peculiar to the environment of low humidity but *A. nobilis* used in this study was collected from a high humid location. The variations seen in the epidermal cell number and size between and within the species may be influenced by external environmental factors (Metcalfe and Chalk, 1979) suggesting that values recorded are susceptible to changes.

Worthy of note is the level of resemblance observed in petiole transverse section of the three species. The placement of vascular bundle in the pith, the presence of tannin and tissue arrangement were the same and the cells looked similar.

Therefore, it is pertinent to understand that diagnostic morphological and anatomical characters of species of *Anthocleista* are not the same for all the species. So, the need to rely on characters of high taxonomic value the status of which has been established such as those enumerated by Oduoye (2013) through principal component analysis of nine species of *Anthocleista* cannot be over emphasized.

The genus *Anthocleista* differs morphologically with regard to epidermal characters, such as cell size, cell number, wall thickness, stomata type, distribution, and index. They exhibit striking similarities in petiole transverse section, cell geometry, and anticlinal walls. These characters can, therefore, be used to either differentiate between and within specific levels or distinguish them from other genera.

Based on results obtained from this study, the artificial indented key to the species is presented as below.

1. Leaf Epistomatic
   1a. Paracytic stomata, unicellular glandular trichomes, sessile leaves, ……*A. vogelii*
2. Leaf amphistomatic
   2a. Cyclocytic stomata, scale trichomes (abaxial and adaxial), petiole (6-7cm) long……*A. djalonensis*
   2b. Anomocytic stomata (adaxial), Actinocytic (abaxial), trichomes; multicellular glandular, scale (abaxial), petiole (13-15cm), stomatal index (10.14%)…....*A. nobilis*

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