Acta Biologica Marisiensis sciendo

LEAF AND PETIOLE MICRO-ANATOMICAL DIVERSITIES IN SOME SELECTED NIGERIAN SPECIES OF *COMBRETUM* LOEFL.: THE SIGNIFICANCE IN SPECIES IDENTIFICATION AT VEGETATIVE STATE

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Received: 21 April 2020; **Accepted:** 06 June 2020; **Published:** 30 June 2020

Abstract: Leaf and petiole samples of four *Combretum* Loefl. species which were identified in the Herbarium (IFE) were investigated anatomically in search of stable taxonomic micro-anatomical attributes to improve our knowledge of identification of members of the genus. Anatomical characters; in particular, upper and lower cuticles and epidermal structures, fibre structures, vascular architectures, petiolar outlines and trichome micro-morphology are good taxonomic tools to identify the taxa. The invariable uniseriate to multiseriate upper and lower epidermis; the absence of trichome in the petiole and the presence of branched trichome in the mid-rib region of *C. zenkeri* P. Beauv delimit the taxa. Variations in vascular architectures can be used to identify the taxa while some other anatomical features in the genus suggest great taxonomic affinities. However, the artificial key, which was constructed using stable taxonomic characters, is a reliable taxonomic tool for proper identification of the four species and which can as well be employed in separating each of the taxa from their close relatives. A detailed micro-anatomical study of leaf and petiole structures of the Nigerian *Combretum* species may provide an invaluable tool for determination and identification of the four taxa studied, thereby assisting in promoting quality assurance in the genus.

Keywords: Artificial key, *Combretum*, diagnostic, lamina, palisade parenchyma, petiole.

1. Introduction

The genus *Combretum* Loefl. is the largest in the family Combretaceae and the type genus of the family (Systma et al., 2004). The family constitutes one of the most specious families of plant in West Africa (Fontes and Guinko, 1995; Thiombiano, 2005), while sectional classification within the genus *Combretum* as first suggested by Engler and Diels (1899; 1900) was updated through the years by various authors and today comprises 46 sections. The genus *Combretum* comprises about 370 species of trees and shrubs, 300 of which are native to tropical and southern Africa, about five to Madagascar, twenty-five to tropical Asia and forty to tropical America.

However, Keay (1989) reported 25 species of the genus found in Nigeria. Despite extensive taxonomic, morphological and anatomical studies on *Combretum* in tropical, southern and West Africa (Stace, 1965; 1969; 1980a; 1980b; 1981; Exell, 1968; 1978; Verhoeven and Vander' Schijff, 1973; Oladipo et al., 2016; Akinsulire et al., 2018a; Akinsulire et al., 2020), there are still nomenclatural problems remaining. Some names are misapplied and the identity of some taxa in southern as well as western African herbaria is uncertain. Recently, the molecular work carried out by Maurin et al. (2010) dealt mostly with the subgeneric, sectional and generic delimitation of *Combretum* and has indicated that taxa boundaries need revising to reflect more accurately the phylogeny of *Combretum* and its allies.

Several species in the genus *Combretum* are used in African or Indian traditional medicine. Leaves of *C. glutinosum* Perr. ex. DC. are browsed by ruminants, and it is the preferred browse species for adult giraffes. Extracts from the bark, leaves and especially the roots produce a yellow dye. The yellowish wood is hard and extremely durable, and it is used in construction and general carpentry. It also makes good fuel wood and charcoal. Many medicinal uses have been reported using the roots, stems, leaves, bark and fruit. *Combretum* species are used in the treatment of influenza, rheumatism, intestinal worms, coughs, colic, impotence, haemorrhoids, constipation, anorexia, malaria, wounds and syphilis.

On the species' morphological examination, Akinsulire et al. (2018a) has though reported the various morphological means by which the species under study could be identified, but thorough investigation into the anatomy of leaves and petioles of *Combretum* species become imperative since plant anatomy deals with the structure, content and development of cells and tissues. It is of primary and great importance for all aspects of research in plant sciences such as morphogenesis, physiology, ecology, taxonomy, evolution, genetics, reproduction, etc. (Fahn, 1990). The macroscopic and microscopic description of a plant as employed in this study, is the first step towards establishing the identity and purity of the plant and should be carried out before any tests are undertaken (Anonymous, 1996). Correct botanical identity based on external morphology is possible when a complete plant is available (Jainab and Kensa, 2018) while anatomical characters as employed in this paper can as well help species identification when morphological features are indistinct (Cutler et al., 2008). According to Jayeola et al. (2009), identification using morphological evidences posed a threat, while scientific diagnosis calls for a very sound knowledge of anatomical structures.

Since very little or no studies have been conducted on leaf and petiole anatomy of these four species of *Combretum*, this paper therefore tends to providing useful taxonomic information to the plant identification problems especially when the reproductive morphological means of identification becomes unavailable. The systematic vegetative anatomy as carried out in this research is also aimed at relating structures particularly of vegetative organs of the genus *Combretum* to taxonomic identification and classification of the species in which the characters are exemplified. The representative species were selected for the study based on the confusion in their correct identities, paucity of information into the leaf and petiole anatomical status of each of the species and their conflicting taxonomic delimitations, their large ecological distribution, their importance in Pharmacopeia, wood fuel and other economic importance (Thiombiano, 2005). On the other hand, the research also serves a complementary purpose to previous investigations of Oladipo et al. (2016),

Akinsulire et al. (2018a) and Akinsulire et al. (2020) in the family Combretaceae.

2. Materials and methods

2.1 Study area and target species

This research was carried out in southern Nigeria. Healthy and matured plant samples of the four *Combretum* species namely: *C. platypterum* Hutch. and Dals, *C. zenkeri* Engl. and Diels., *C. racemosum* P. Beauv. and *Combretum dolichopetalum* (syn. *C. comosum* var. *dolichopetalum* (Engl. and Diels) Jongkind), were collected during regular field trips to various parts and localities in Ondo, Osun and Edo States all in southern Nigeria, while all sites of collection were geo-referenced using a GPS device (Garmin nuvi 2597LMT) (**Table 1**). Samples were identified by standard reference text (Panshin and DeZeeuw, 1964; Hutchinson and Dalziel, 1964-72) and were later used in obtaining corresponding botanical names in the Herbarium (IFE). The leaves were harvested from all parts of each of the plants, which were totally exposed to the sun and none of the plants were in shadow area. One to four accessions were collected for each of the species from different localities (**Table 1**), while ten matured leaves from different parts of each of the accessions where harvested for leaf and petiole micro-anatomical investigations.

2.2 Leaf and Petiole Anatomy

As reported by Akinsulire et al. (2018a) that the four members of the genus *Combretum* considered in this paper are subsessile, consequently, only the transverse sections of petioles of the four taxa were studied, together with their leaves. Leaf anatomy was studied using sizeable portions from the leaves which were taken from the standard median portion from each sample, while petiole anatomy of the taxa was investigated by selecting matured petiole samples from the accessions. Transverse sections of all leaf and petiole samples were obtained as still fresh, embedded in paraffin and sectioned using Reichert Microtome (Reichert Austria Nr. 367 019) and at a thickness of 8 to 10 µM. The sections were later processed, stained in Safranin O solution for 3 to 5 minutes, rinsed with 4 to 5 changes of water to remove excess stain and counterstained in Toluidine Blue solution for 3 to 5 minutes. The sections were again rinsed thoroughly with 4 to 5 changes of water and treated in series of ethanol dilution-50%, 70%, 80%, 90% and 100% to enhance dehydration process. The dehydrated sections were then transferred into absolute xylene to remove any remaining trace of water and ethanol. These made the sections clearer and prevented cloudiness of the slides. Sections were therefore mounted in 25% glycerol containing thymol crystals (to prevent fungal attack) on a clean glass slide, and covered with microscope cover slip, for light microscopy (Sonibare et al., 2014; Ogundare and Saheed 2015; Akinsulire et al., 2018b; Jainab and Kensa, 2018; Priya and Hari, 2018).

2.3 Light Microscopy

For each species, micro-anatomical parameters such as thickness of upper cuticle, thickness of upper epidermis, thickness of palisade layer, thickness of spongy layer, thickness of lower epidermis as well as thickness of lower cuticle and other micro-anatomical characteristics were investigated and documented both quantitatively and qualitatively while thirty replicates (n = 30) were taken for each of the quantitative anatomical character (including trichomes) investigated. Descriptive terminologies were also carried out while list of microscopic features were also made.

All slides were observed (at magnification of X40) using light microscope (Leica Galen

III) equipped with calibrated ocular eyepieces (square and linear micrometres) to enable the measurement of leaf and petiole cell parameters and to enable recording cell and tissues dimensions.

Photomicrographs of all anatomical and diagnostic features were made with the aid of Accu-scope Trinocular Microscope (Accu-scope 33001 LED Trinocular Microscope fitted with 3.2 MP CMOS Digital Camera).

2.4 Statistics

All quantitative parameters were measured using ocular micrometer and the measurements were converted to microns using the stage micrometre and ocular constant with respect to the objective with which the measurements were taken. The data were computed and were analyzed using One Way Analysis of Variance (ANOVA), while means were separated using Tukey's Honestly Significant Difference (HSD). Multivariate Statistical Analysis, Principal Components Analysis (PCA) and Cluster Analyses were also carried out on the data using Paleontological Statistics Software Package for Education and Data Analysis (Hammer et al., 2001).

2.5 Dichotomous Key

An artificial key for the identification of members of the genus was constructed using taxonomic information generated from the leaf and petiole micro-anatomical investigations.

Table 1. Sites of collection and coordinates of the four species of *Combretum*

Species/Accession	Site of Collection	Collector(s)	Coordinate
C. platypterum	International Secondary School, Road 7, OAU, Ile-Ife, Osun State, Nigeria	B.E Omomoh and O.P Akinsulire	N07˚31.205′ E004˚32.086′
C. platypterum	Imole-Ayo Street, Ajagbale Road, Oka – Ondo, Ondo State, Nigeria	O.P Akinsulire	N07˚4.769′ E004˚49.9642′
C. platypterum	Beside Asikolaye lodge, Apata II, Ile-Ife, Osun State, Nigeria	O.P Akinsulire	N07˚27.364′ E004˚33.512′
C. platypterum	Prince Olu-Adegbite Street, Oka-Ondo, Ondo State, Nigeria	O.P Akinsulire	N07˚3.9667′ E004˚50.25
C. zenkeri	International Secondary School, Road 7, OAU, Ile-Ife, Osun State, Nigeria	B.E Omomoh and O.P Akinsulire	N07˚31.238′ E004˚31.958′
C. zenkeri	Beside New Bukka, OAU, Ile-Ife, Osun State, Nigeria.	O.P Akinsulire	N07˚31.027′ E004˚30.811′
C. racemosum	Biological Garden, OAU, Ile-Ife, Osun State, Nigeria	B.E Omomoh and O.P Akinsulire	N07˚31.351′ E004˚31.410′
C. racemosum	Awo Community, Ejigbo Road, Ede, Osun State, Nigeria	B.E Omomoh and O.T Oladipo	N07˚46.265′ E004˚24.320′
C. racemosum	Adeyemi College of Education Road, Lipakala, Ondo, Ondo State, Nigeria	O.P Akinsulire	N07˚4.7206′ E004˚49.4362′
C. dolichopetalum	Uromi Town, Edo State, Nigeria	O.T Oladipo, B.E Omomoh and A.J Akinloye	N07˚46.26′ E004˚24.32′

OAU: Obafemi Awolowo University

3. Results

Qualitative results are presented below with reference to corresponding figures and photomicrographs while a list of important qualitative leaf and petiole micro-anatomical characters are presented in **Table 2**, as **Table 3** shows the species' significant differences in cells and tissues dimensions of the leaves and petioles with Tukey's (HSD). **Table 4** provides the information regarding the Principal Components Analyses (PCA) Factor Loadings.

Table 2. Qualitative leaf and petiole anatomical characters of four species of *Combretum*

Species Characters	***C. platypterum***	***C. zenkeri***	***C. racemosum***	***C. dolichopetalum***
Leaf Anatomy (Lamina)				
Leaf	Bifacial	Bifacial	Bifacial	Bifacial
Upper Cuticle	Thin	Thin	Thick	Thin
Upper Epidermis	Uniseriate	Uniseriate / Biseriate	Uniseriate	Uniseriate
Palisade Mesophyll	1-layered	1-layered	1-layered	1-layered
Lower Epidermis	Uniseriate	Uniseriate / Biseriate / Multiseriate	Uniseriate	Uniseriate
Lower Cuticle	Thick	Thin	Thin	Thin
Trichome/ Trichome Type	-	+ (SUNT, BTR)	-	-
Leaf Anatomy (Midrib)				
Fibre	Absent	Absent	Present	Absent
Vascular Bundle	Collateral	Collateral	Collateral	Collateral
Vascular Architecture	Falcate	Falcate	Falcate	Falcate
Petiole Anatomy				
Outline	Oval/ Grooved	Triangular/ Grooved	Oval/ Grooved	Oval/ Grooved
Vascular Bundle	Collateral	Collateral	Collateral	Collateral
Vascular Architecture	Falcate / Open adaxial	Triangular/ Dissected	Circular/ Open adaxial	Oval/ Dissected
Trichome Presence / Type	+ (SUNT)	-	+ (SUNT)	+ (SUNT)

SUNT=Simple Unicellular non-glandular Trichome; BRT=Branched Trichome, + = Presence of a Character, - = Absence of a Character, Bi/Multiser=Biseriate/Multiseriate

3.1. *Combretum platypterum* (**Fig. 1A** and **B**; **Fig. 2A** and **B**)

The transverse section of the leaf was examined and it was revealed that leaf in *C. platypterum* is bifacial with thin upper cuticle and uniseriate epidermis (**Fig. 1A**). The cells of the upper epidermis are majorly rectangular or square, with straight or slightly undulating periclinal walls (**Fig. 1A**). Palisade mesophyll cells are 1-layered and are highly chlorophyllous. The cells are cylindrical in shape and are compact in arrangement, occurring on the adaxial region (**Fig. 1A**). Spongy mesophyll layer is distinguishable from the palisade cells, the cells are largely irregular in shape and are arranged with small to large intercellular air spaces, the cells are generally circular, oval, polygonal or spindle-shaped (**Fig. 1A**).

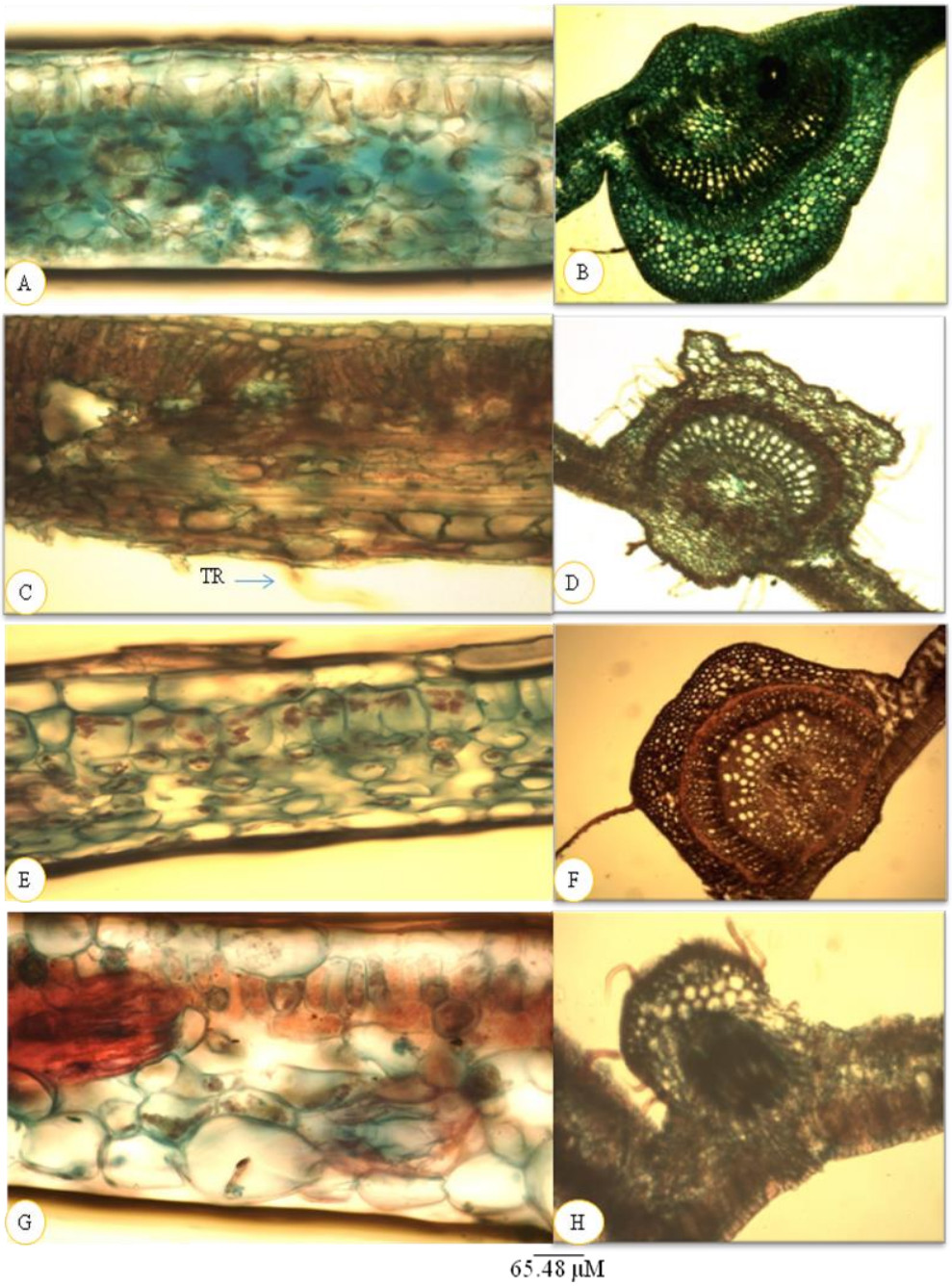

Fig. 1. Leaf anatomy of four species of *Combretum*
A and B = Photomicrographs of lamina and midrib of *C. platypterum*
C and D = Photomicrographs of lamina and midrib of *C. zenkeri*
E and F = Photomicrographs of lamina and midrib of *C. racemosum*
G and H = Photomicrographs of lamina and midrib of *C. dolichopetalum*

Lower epidermis is uniseriate, the cells are rectangular with straight or undulating periclinal walls, lower cuticle is thick (**Fig. 1A**). In the midrib region (**Fig. 1B**), the epidermis is darkly stained and composed of uniseriate row of cells, the vascular bundle is collateral and the vascular architecture is falcate (**Fig. 1B**). Simple, unicellular, non-glandular and unbranched trichomes present on the midrib region but sparsely distributed, trichome density is 0 to1 mm^2 (**Fig. 1B**).

Table 3. Dimensions of cells and tissues of the leaves in four *Combretum* species with Tukey's Honestly Significant Difference (HSD ^{a})

Species	*C. platypterum*	*C. zenkeri*	*C. racemosum*	*C. dolichopetalum*
TUC (µM)	12.00±0.56^{a}	12.00±0.56^{a}	18.00±0.56^{b}	12.25±0.56^{a}
TUE (µM)	34.00±1.12^{a}	52.00±3.21^{c}	45.00±1.15^{b}	44.00±1.12^{b}
TPL (µM)	55.50±1.02^{b}	38.63±0.82^{a}	67.13±1.27^{c}	54.75±0.65^{b}
TSL (µM)	110.00±2.18^{d}	46.35±0.98^{a}	100.69±1.91^{c}	65.70±0.78^{b}
TLE (µM)	24.00±1.12^{a}	59.50±2.35^{c}	24.50±1.14^{a}	34.50±1.14^{b}
TLC (µM)	20.00±0.00^{c}	14.75±0.99^{b}	12.00±0.56^{a}	10.00±0.00^{a}

Values are expressed as mean of thirty replicates (n=30) ± S.E.M (Harmonic mean sample size-30.00)

Values in each row with different superscripts are significantly different ($p<0.05$)

TUC=Thickness of Upper Cuticle; TUE=Thickness of Upper Epidermis; TPL=Thickness of Palisade Layer; TSL=Thickness of Spongy Layer; TLE=Thickness of Lower Epidermis; TLC=Thickness of Lower Cuticle; µM=Micrometer

Table 4. Principal Components Analysis (PCA) factor loadings of some important leaf anatomical characters of species of *Combretum*

	PCA 1	**PCA 2**	**PCA 3**	**PCA 4**
Upper Cuticle Thickness	0.518	0.754	0.404	-
Upper Epidermis Thickness	-	0.567	-	-
Palisade Layer Thickness	0.895	0.437	-	-
Spongy Layer Thickness	0.974	-	-	0.801
Lower Epidermis Thickness	-	-	-	-
Lower Cuticle Thickness	-	-	0.424	-

Petiole (**Fig. 2A and B**): The petiole is oval in sectional outline (**Fig. 2A**) with no wings and slightly grooved. The ground tissues form the outer and the central portions, consisting of thin-walled polygonal parenchymatous cells (**Fig. 2B**). The vascular bundle is collateral and single, surrounded by a thin layer of phloem and a single parenchymatous bundle sheath. The xylem elements are in compact parallel lines with four to six cells in each row (**Fig. 2B**). The vascular system of the petiole is central and the vascular ring is falcate (**Fig. 2A**), embedded in rounded or polygonal conjunctive tissues. Simple unicellular, non-glandular trichomes are present but are sparse in distribution, 0 to 2 mm^2 (**Fig. 2B**).

3.2. *Combretum zenkeri* (**Fig. 1C** and **D**; **Fig. 2C** and **D**)

It was examined in the transverse section of the leaf of *C. zenkeri* that the leaf is bifacial with thin upper cuticle (**Fig. 1C**). Upper epidermis is mostly biseriated, occasionally uniseriated and laterally compressed with straight to undulating periclinal walls and the cells are majorly rectangular or square.

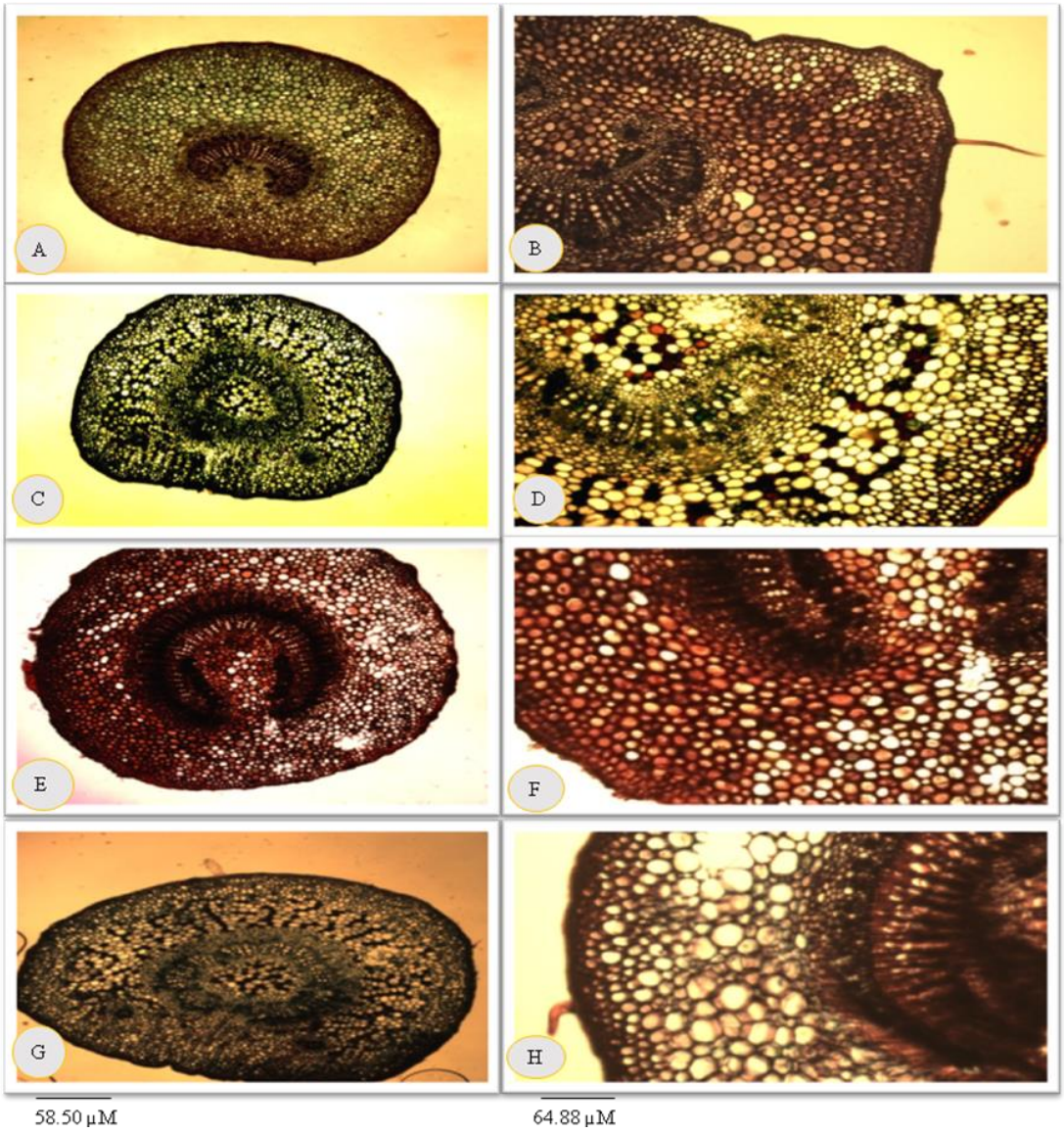

Fig. 2. Petiole anatomy of four species of *Combretum*
A and B = Photomicrographs of petiole of *C. platypterum*
C and D = Photomicrographs of petiole of *C. zenkeri*
E and F = Photomicrographs of petiole of *C. racemosum*
G and H = Photomicrographs of petiole of *C. dolichopetalum*

Palisade mesophyll cells are 1-layered, cylindrical and compactly arranged, occurring in slanting rows and occurred on the adaxial region (**Fig. 1C**). Spongy mesophyll cells are largely irregular in shape and arranged with small to large intercellular air spaces, spongy mesophyll cells are either polygonal or spindle-shaped, lower epidermis is uniseriated, biseriated and multiseriated, the cells are rectangular or polygonal in shape, lower cuticle is thin, simple unicellular trichomes attached to the lower epidermal region (**Fig. 1C**). Anatomical examination of the midrib region (**Fig. 1D**) revealed the darkly stained epidermal cells to compose of small barrel-shaped cells, vascular bundle is collateral, vascular architecture is falcate.

Simple, unicellular, non-glandular trichome present, trichome density is 0 to 6 mm^2, slightly branched trichome also present but very scanty, density is 0 to 1 mm^2 (**Fig. 1D**).

Petiole (**Fig. 2C** and **D**): The petiole is slightly triangular or oval in sectional outline (**Fig. 2C**) with no wings; the epidermal cells are small and darkly stained. The ground tissues consist of about five to seven thick walled cells that are compact in arrangement; other ground tissues also form the outer and the central portions consisting of thin-walled polygonal parenchymatous cells. The vascular bundle is collateral (**Fig. 2D**). The xylem elements are in compact parallel lines with five to seven cells in each row. The vascular system of the petiole is central while the vascular ring is continuous and in the form of a prominent triangular-shape and which is dissected adaxially and embedded in rounded or polygonal conjunctive tissues (**Fig. 2C**). Simple unicellular non-glandular trichomes are present, 0 to 2 mm^2 (**Fig. 2D**).

3.3 *Combretum racemosum* (**Fig. 1E** and **F**; **Fig. 2E** and **F**)

Transverse section of the leaf shows that leaf in *C. racemosum* is bifacial and it was noted that the upper cuticle is thick while the lower cuticle is thin (**Fig. 1E**). The adaxial and abaxial epidermal cells are periclinally elongated. Both surfaces compose of uniseriate rows of cells (**Fig. 1E**), palisade mesophyll cells are 1-layered; cells are highly chlorophyllous and are slightly elongated with cylindrical and compactly arranged cells (**Fig. 1E**). Spongy mesophyll cells are largely irregular in shape and are arranged with large intercellular air spaces, the highly chlorophyllous spongy cells are circular, oval or polygonal in shape (**Fig. 1E**). In the mid-vein region (**Fig. 1F**), epidermal cells are darkly stained, vascular bundle is collateral, vascular architecture is largely falcate, simple unicellular non-glandular trichomes are present on the midrib, trichome density is 1 to 2 mm^2 (**Fig. 1F**).

Petiole (**Fig. 2E** and **F**): The petiole is slightly circular or oval in sectional outline (**Fig. 2E**) with no wings; the epidermal cells are small and darkly stained. The ground tissues consist of about five to seven thick walled cells that are compact in arrangement (**Fig. 2F**); other ground tissues form the outer and the central portions consisting of thin-walled polygonal parenchymatous cells. The vascular bundle is collateral, vascular architecture is single arched, surrounded by a thin layer of phloem and a single parenchymatous bundle sheath (**Fig. 2F**). The xylem elements are in compact parallel lines with four to eight cells in each row. The vascular system of the petiole is central while the vascular ring is continuous and in the form of a prominent circular shape with an open adaxial (**Fig. 2E**), embedded in rounded or polygonal conjunctive tissues. Simple unicellular non-glandular trichome present, trichomes are sparsely distributed, density is 0 to 2 mm^2 (**Fig. 2F**).

3.4 *Combretum dolichopetalum* (**Fig. 1G** and **H**; **Fig. 2G** and **H**)

Cross-sectional examination of the leaf shows that its upper and lower cuticles are thin (**Fig. 1G**), while upper and lower epidermal cells are largely rectangular in shape and occur in uniseriate rows. The cells of upper and lower epidermis are periclinally elongated (**Fig. 1G**), Palisade mesophyll cells are1-layered and occur on the adaxial region (**Fig. 1G**). The layer composed of compactly arranged cells that are generally cylindrical in shape, containing a large mass of chloroplasts (**Fig. 1G**). Spongy mesophyll cells are largely irregular in shape with small intercellular air spaces (**Fig. 1G**). In the mid-vein region (**Fig. 1H**), the barrel shaped or slightly rectangular

epidermal cells are darkly stained and are uniseriated. Vascular bundle is collateral (**Fig. 1H**), vascular architecture is falcate; structure is almost closed but narrowly opens out adaxially (**Fig. 1H**).

Petiole (**Fig. 2G** and **H**): in cross-sectional outline, the petiole has a broadly oval shape (**Fig. 2G**) with even and smooth outline except on the abaxial region which is somewhat flattened and slightly arched, or slightly ridged and grooved (**Fig. 2G**). The epidermis is uniseriate and darkly stained, consisting of small sized barrel-shaped cells with outer thick walls (**Fig. 2H**). The cortex consists of outer angular collenchyma of three to five layers and inner parenchyma, the parenchyma are rounded or polygonal in shape and are four to eight layered (**Fig. 2H**). The vascular bundle is collateral while the pith comprises of rounded to polygonal parenchyma cells. Vascular ring is slightly oval but dissected and discontinuous with two to three solitary vascular bundles together with the main bundle all along the same plane (**Fig. 2G**), simple unicellular non-glandular trichome present, density 0 to 1 mm^2 (**Fig. 2H**).

4. Discussions

Anatomical line of evidence has always been used in the classification and identification of different plant taxa (Metcalfe and Chalk, 1979; Aguoru and Okoli, 2008; 2012; Ajuru and Okoli, 2013; Ekeke and Mensah, 2015; Agogbua et al., 2015; Ekeke et al., 2015; Ekeke and Agbawa, 2016; Ekeke et al., 2016; Oladipo et al., 2016; Ekeke et al., 2017; Akinsulire *et al.*, 2018a; Akinsulire et al., 2018b; Akinsulire et al., 2018c; Akinsulire et al., 2020). Close affinities based on investigated leaf and petiole anatomical characters were however observed in the four *Combretum* species. It should be noted that collateral vascular bundles observed in the leaves midrib (**Fig. 1B, D, F, H**) and petioles in the four species (**Fig. 2B, D, F, H**) suggest a generic character, useful in the identification of the studied genus.

Other generic features include 1-layered palisade cells (**Fig. 1A, C, E, G**) on the lamina. Meanwhile, simple unicellular non-glandular trichomes on the midribs are classificatory and as well diagnostic for each of *C. platypterum*, *C racemosum* and *C. dolichopetalum* (**Fig. 1B, F, H**) which can as well be employed in species identification purpose. The lamina and midrib anatomy of the four species also showed some clear variations in the natures and arrangements of upper and lower epidermal cells (**Table 2**), thickness of both upper and lower cuticles (**Table 3**), as well as vascular architectures of the midrib (**Fig. 1B, D, F, H; Table 2**) which are of great taxonomic significance and are reported in this plant group for the very first time.

In the lamina, the uniseriate or superimposed biseriate/multiseriate upper and lower epidermis as well as branched trichome in addition to seemingly generic simple unicellular trichomes found in *C. zenkeri* (**Fig. 1C**) is diagnostic and are good taxonomic tools which can be employed in the identification of the taxa as the epidermis in both surfaces in other three species remain uniseriated, while their midrib trichomes remain only simple unicellular (**Fig. 1A, E, G**), hence useful in grouping the taxa.

In the vascular area of the midrib, falcate vascular architecture seemed to be generic and can be used for grouping the taxa (**Fig. 1B, D, F, H**; **Table 2**). On the petiole, triangular and grooved petiole outline and the absence of trichome are diagnostic for *C. zenkeri* (**Fig. 2C**) while other three species can be classified on the basis of their oval and grooved petiolar outlines as well as their possession of trichomes (**Fig. 2A, D, E**; **Table 2**).

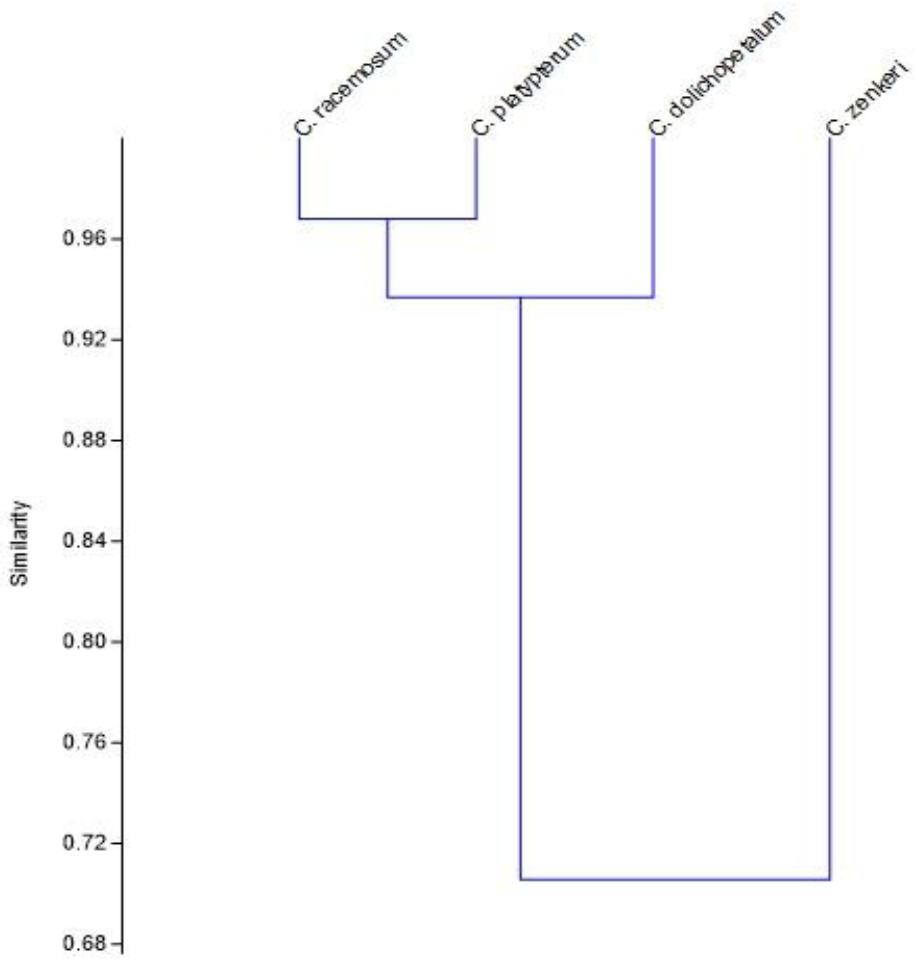

Fig. 3. Cluster analysis (Dendogram) of the *Combretum* species

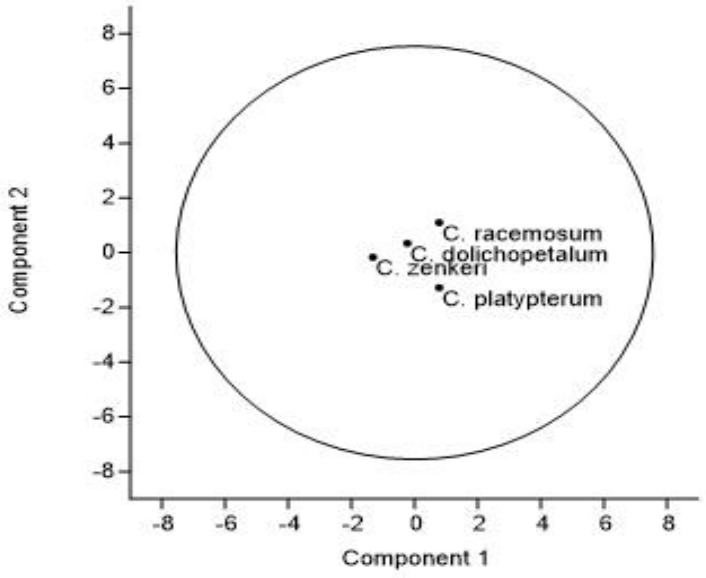

Fig. 4. Scatter plot of four *Combretum* species showing relationships and distances

This revelation partly agrees with recent investigations carried out by Oladipo et al. (2016) using information from wood anatomy in classifying the *Combretum* species under study, but strongly agrees with Akinsulire et al. (2018a) who as well classified members of this same genus using vegetative and reproductive morphological information. Each of the petiolar vascular architectures is distinct per species (**Table 2**) as it is falcate in *C. platypterum*, triangular in *C. zenkeri*, circular in *C. racemosum* and oval in *C. dolichopetalum*, and is therefore useful taxonomic character useful in identifying each of the species.

Considering the quantitative illustrations and analyses (**Table 3**), *C. racemosum* was revealed to have the thickest upper cuticle and palisade layer among the four taxa, *C. platypterum* possesses the thickest spongy mesophyll layer and lowest cuticle thickness (Table 2), while the highest level of thickness of upper and lower epidermis was documented in *C. zenkeri* (**Table 2**), hence diagnostic for each of the four species respectively, and are good taxonomic tools useful in species identification.

Quantitatively, the relationship between members of the genus and anatomical means by which individual members of the genus could be separately identified were also revealed as significant differences were observed in each of the anatomical characters considered (**Table 3**). However, the dendogram (**Fig. 3**) shows that *C. racemosum* and *C. platypterum*, which formed a clade and were clustered to the highest level, have greater similarities, and thus more closely related while *C. dolichopetalum* and *C. zenkeri* must have shared common ancestry with *C. racemosum* and *C. platypterum*, or both belong in the same haplogroup, hence their generic classification (Hutchinson and Dalziel, 1954).

The scatter plot (**Fig. 4**) revealed *C. racemosum*, and *C. dolichopetalum* to have more generic affinity to *C. zenkeri* than to *C. platypterum*, which is also a good systematic tool for the identification of members of the genus. The PCA factor loadings of the leaf quantitative anatomical characters revealed that characters like thickness of upper cuticle, thickness of upper epidermis, thickness of palisade layer and thickness of spongy layer are all important in separating the four *Combretum* species studied as they had higher loadings (**Table 4**). Moreover, principal components 1, 2 and 4 were important in bringing out the variations in the taxa as other components were increasingly uninformative.

A taxonomic (dichotomous) key based on leaf and petiole micro-anatomical characters of the species of *Combretum* examined in this research, and which is of great taxonomic usefulness in species identification and in separating the species even from their close relatives, is presented below:

1a. Upper epidermal cuticle thick, lower epidermal cuticle thin……..*C. racemosum*

1b. Upper epidermal cuticle thin, lower epidermal cuticle thick

2a. Upper epidermis uniseriate, biseriate or multiseriate…….*C. zenkeri*

2b. Upper epidermis uniseriate

3a.Vascular architecture falcate/open on adaxial side.....…*C. platypterum*

3b.Vascular architecture oval/ dissected/closed on adaxial side……...*C. dolichopetalum*

Conclusions

Investigations into the leaf and petiole anatomy of the species revealed great taxonomic affinity and a range of dissimilarities within the genus as well as unveiling diagnostic characters that can serve delimitation purposes, thereby enhancing the species identification. Considering their anatomical resemblances as even evident in

previous researches into the species' morphology and wood anatomy, the results strengthen the fact that these species belong in the same haplogroup and should be maintained as separate species placed in the same genus. Thus, leaf and petiole anatomical characters as investigated in this study should be taken as important lines of evidence in the identification and classification of these species. The close anatomical affinities observed in these investigations revealed that the species are closely related and therefore, their placement in one genus (Hutchinson and Dalziel, 1954) is strongly supported.

Acknowledgement

We acknowledge the Department of Botany, Obafemi Awolowo University, Ile-Ife (OAU), for providing equipment and technical support required to carry out this research. We also appreciate Dr A.J Akinloye and Mr Abiodun Omole, both in the Plant Anatomy and Taxonomy Laboratory, Department of Botany, (OAU), for assistance regarding slide preparation. Anonymous reviewers are also greatly appreciated for providing valuable and constructive comments on the manuscript.

Dedication

This work is dedicated to Late (Chief) Gabriel Ibhanesebhor, the former curator of IFE Herbarium who played a highly significant role in the morphological identification of the *Combretum* species.

Conflict of interest

The authors have declared that there is no conflict of interest.

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