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MICRO-ANATOMICAL CHARACTERIZATION OF AFRICAN NATIVE MONOTYPIC GENERA - *ANOGEISSUS* (DC.) GUILL AND *QUISQUALIS* LINN (COMBRETACEAE)

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Abstract: This study was designed to explore the micro-anatomical characteristics of leaf and petiole of two unstudied West African native monotypic genera - *Anogeissus* and *Qusqualis* in Combretaceae with a view to characterizing the taxa, providing useful research-based information for identification. The samples were prepared following standard procedures. The light microscopic study of the transverse sections of the leaves in *A. leiocarpus* revealed a thick upper and lower cuticle while the cuticle on both leaf surfaces in *Q. indica* remain thin. The midrib vascular bundle of the leaf in *Q. indica* is characterized by arc-shape and starch grains were observed in the parenchyma cells of the ground tissue. The uniseriate rows of lamina epidermis were oval, squared, rectangular or polygonal in *Q. indica* while it was rectangular or slightly oval in *A. leiocarpus*. Vascular bundle in the leaves and petioles of both taxa were collateral. Simple, slender and short unicellular non-glandular trichomes were also observed in the leaf and petiole micro-anatomy of both taxa. It was concluded that the micro-anatomical features of the leaves and petioles are important parameters of characterization, used in the identification of the studied taxa.

Keywords: *Anogeissus leiocarpus*, Combretaceae, Ground tissue, Monotypic, Palisade, *Quisqualis indica.*

1. Introduction

The genus *Anogeissus* and the genus *Quisqualis* belong to the subtribe Combretinae in the family Combretaceae (Maurin et al., 2010) and according to Mann et al., (2009), *Anogeissus* is a genus of trees which are native to South Asia, the Arabian Peninsula, and Africa. The genus has been reported to consist of eight species. Of these, five are native to South Asia, two are endemic to the Southern Arabian Peninsula, and only one species is native to Africa namely- *Anogeissus leiocarpus* (Hutchinson and Dalziel, 1958). Previous researches of Andary et al., (2005) and USDA, (2010) revealed that "in Africa, *A. leiocarpus* spreads over a large range of ecosystems, from dry savannah to wet forest borders, in wooded grassland and bushland, and on riverbanks in Ethiopia, Sudan, Cameroon, Congo-Kinshasa, Benin, Cote d'Ivoire, Gambia, Ghana, Guinea, Mali, Niger, Senegal, and Nigeria."

The leaves of *Anogeissus* serve several economic importances. It can be used as a yellow dye in ancestral Bogolan textile techniques in Mali and Burkina-Faso (Heuze et al., 2016). According to Andary et al., (2005), "the wood makes an excellent fuel and yields

good charcoal. Similarly, the barks, leaves and roots have ethno-medicinal properties (antimicrobial and anthelmintic activity) as they are usually taken as decoctions or aqueous extracts (Andary et al., 2005). Derivatives of ellagic acids ("anogelline") extracted from the bark have been shown to delay the degradation of collagen and the tree is grown commercially since 2000 for the production of cosmetics in the Koro region of Burkina Faso (Jansen and Cardon, 2005). "

The inner bark is used as chewing sticks in Nigeria while extracts of the bark show antibacterial properties. Reported as being very sensitive to fire, *A. leiocarpus* can serve reforestation purposes (Andary et al., 2005). The leaves of this taxa are rich in tannin: they contain ellagic, gallic and gentisic acids, derivatives of gallic and ellagic acid, and several flavonoids (derivatives of quercetin and kaempferol) that have been investigated to be very useful for dyeing (Andary et al., 2005), but that may have deleterious effects on nutritive value. The leaf have also been reported as having a relatively low protein content of about 12-1;4% (Fall Toure, 1991), and the leaf/stem ratio is also quite low (0.4) (Yahaya et al., 2000).

The genus *Quisqualis*, established by Linnaeus (1762), is an extremely shrubby vine or liana, found in thickets or secondary forests of Philippines, India, Malaysia, and Africa. It is a climbing, mesophytic to xerophytic, or helophytic genus (Akinsulire et al., 2018a). "Lawson (1871) and Brandis (1898) separated *Quisqualis* from the genus *Combretum* on the basis of such characteristics as its elongated, tubular, upper hypanthium which is however, subterete throughout and not constricted towards the base - a character-state absent in typical *Combretum*." According to Hutchinson and Dalziel (1958), the genus consists of three species with only one species native to West Africa - *Quisqualis indica*, also called Rangoon creeper.

The relevance of *Q. indica* in traditional medicine cannot be overemphasized (Sahu et al., 2012). Decoction of the roots, seeds or fruits can be used as anti-helmintic to expel parasitic worms or for alleviating diarrhoea. Fruit decoction can also be used for gargling against toothache as well as combating nephritis while the leaves can be used to relieve pain caused by fever. The roots are used to treat rheumatism. *Quisqualis* are also used as a cough cure. Decoction of boiled leaves is used to relieve flatulent distention of the abdomen. Leaves and fruits are reported to be anthelmintic and also used for nephritis. Dried seeds are preferred for deworming and roasted seeds for diarrhoea and fever. Seeds can be given with honey as electuary, and the preparation used for the expulsion of parasitic worms in children. Seeds are also vermifuge; destroying intestinal worms in children. Seeds macerated in oil are applied for parasitic skin diseases. Seeds are also used for diarrhoea and leucorrhoea discharge in female children (Sahu et al., 2012).

Some research works have been carried out on these two African monotypic genera – *Anogeissus* and *Quisqualis*, which include the work of Oladipo et al. (2016) on the species' wood anatomy, and Akinsulire et al. (2018a) on their vegetative and reproductive morphology, but investigating the leaf and petiole micro-anatomy of the taxa becomes imperative. *Quisqualis* have been known for its taxonomic complexity (Jordaan et al., 2011), while several literature surveys showed a few or no review which correlates the data of the micro-anatomy of the transverse sections of the leaves and petioles of both genera together, hence this study. The choice of the two plant species was also owing to the fact that the two taxa are the only West African monotypic genera in the family Combretaceae and yet unstudied.

2. Materials and methods

Three apparently healthy *Quisqualis indica* samples were collected from three different locations within Obafemi Awolowo University, Ile-Ife (OAU) campus, Osun state, while due to its distribution; the sample of *Anogeissus leiocarpus* was collected at the University of Ibadan campus, Oyo state. All collection sites were geo-referenced using a GPS device (Garmin nuvi 2597LMT) (**Table 1**). Ten mature leaves were harvested from each of the accessions for leaf and petiole micro-anatomical investigations and were preserved in formalin acetic-alcohol (FAA). For leaf micro-anatomy, each of the leaves and midribs was cut at approximately halfway between the base and the apex, while due to the subsessile natures of the petiole (Akinsulire et al., 2018a), transverse sections of the median portions were considered. All leaf and petiole samples were sectioned using Reichert Sliding Microtome (Reichert Austria Nr. 367 019) and at a thickness of 8 to 10 microns. The sections were stained with Safranin O for 3 to 5 minutes, rinsed with 4 to 5 changes of water to remove excess stain and counterstained with Toluidine Blue for 3 to 5 minutes as well. The sections were then rinsed thoroughly with 4 to 5 changes of water and treated in series of ethanol dilutions (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 slide. Sections were afterwards mounted in 25% glycerol containing thymol crystals to prevent fungal attack on a clean glass slide for light microscopy (Sonibare et al., 2014; Akinsulire et al., 2018b; Jainab and Kensa, 2018; Priya and Hari, 2018).

2.1. Light Microscopy

Quantitative leaf anatomical parameters such as thickness of upper and lower cuticle, thickness of upper and lower epidermis, length of palisade layers all at 40 measurements per parameter (n = 40), as well as qualitative leaf and petiole micro-anatomical characters such as nature of spongy layers, nature of ground tissues, types of trichomes were all observed and documented using light microscope (Leica Galen III). All quantitative parameters were taken with the aid of ocular micrometre. Photomicrographs of the transverse sections of the leaves and petioles were made with the aid of "Accu-scope Trinocular Microscope (Accu-scope 33001 LED Trinocular Microscope fixed with 3.2 MP CMOS Digital Camera)."

2.2. Data Analysis

In order to shed light on the dimensions of cells and tissues of the two taxa, "quantitative data generated in the study were subjected to One Way Analysis of Variance (ANOVA)."

3. Results and discussion

3.1. *Anogeissus leiocarpus* - Micro-anatomy

The transverse section of the leaf revealed a very thick upper cuticle measuring 16.00±0.42 µm; uniseriate upper epidermis of mainly rectangular or slightly oval cells with slightly undulating periclinal walls and a mean length of 22.00±0.96 µm. Palisade mesophyll is one-layered, cylindrical, compact and occasionally in slanting rows. Mean length of palisade mesophyll cell was 62.50±0.69 µm (**Table 2**). Hence, studies by Okeke et al. (2015) reported uniseriate epidermis and one to three layers of palisade cells in the transverse sections of leaf in the genus *Stachyterpheta*, and opined that these features could not proffer any taxonomic relevance to the delimitation of the species.

Meanwhile, Akinsulire et al. (2020) have used leaf and petiole micro-anatomical parameters in the identification of the members of the genus *Combretum* (Combretaceae) and their significance explained. Spongy mesophyll layer is fairly parenchymatous; the cells are polygonal or irregular with intercellular airspaces. Lower epidermal cells are uniseriate and rectangular in shape with undulating periclinal wall while the mean length was 22.64±0.84 µm. Akinsulire et al. (2018b) reported similar features in the leaf lamina of some members in Combretaceae. Similarly, the lower cuticle of this species is thick and measured 14.80±0.28 µm (**Fig. 1A**).

One taxonomic character worthy of note is the possession of simple and slender unicellular trichome on the epidermal region of the lamina (**Fig. 1A**). However, Akinsulire et al. (2018b) had reported simple unicellular trichomes in some species of *Terminalia* (Magnoliopsida: Combretaceae), stating its usefulness in plant identification. The midrib of the leaf shows a thick cuticle that is generally darkly stained with a uniseriate row of epidermal cells, followed by 3 to 5 layers of parenchyma. The vascular bundle in the midrib in this species is collateral. Adenegan-Alakinde and Jayeola (2015) had earlier reported collateral vascular bundles in the lamina midrib of some members of the genus *Rhizophora* Linn. in Nigeria and its usefulness in plants identification. The shape of the vascular bundle in the midrib region is arched or crescentiform while short and slender simple unicellular trichomes are also present on the epidermis.

In the transverse section of the petiole (median), a very thick and darkly stained cuticle (**Fig. 1C**) and a uniseriate or biseriate row of epidermis and hypodermis can be used to characterize this monotypic genus. The cells are also round or polygonal in shape. Types and thickness of cuticle, epidermal cell row and nature of hypodermis have been employed by Priya and Hari (2018) in the identification of *Garcinia indica*. The collateral vascular bundle in the petiole of this species, which resolved into a prominent central arc or semi-ovoid vascular strand, capped on the outside by one to three layers of sclerenchyma is a useful tool for characterization in this plant group. Similar petiole anatomical features have previously been employed by different researchers (Akinsulire et al., 2018b; Priya and Hari, 2018) in the taxonomy of several plant groups. The three-winged petiole outline in this species is a spot character, diagnostic for this taxon (**Fig. 1C**).

Table 1. Sites of collection and coordinates of the studied monotypic genera of Combretaceae

Genus	Species/ accession	Location	Geographical coordinate
Anogeissus	*A. leiocarpus*	University of Ibadan, Ibadan, Oyo State, Nigeria.	N07˚23.47′, E003˚55.00′
Quisqualis	*Q. indica*	Department of Food Science & Technology, Obafemi Awolowo University, Ile-Ife (OAU), Nigeria.	N07˚31.191′E004˚31.634′
	Q. indica	Adjacent Alumni Hall, Obafemi Awolowo University, Ile-Ife, Nigeria.	N07˚31.278′E004˚30.978′
	Q. indica	Moremi Hall, Obafemi Awolowo University, Ile-Ife (OAU), Nigeria.	N07˚31.172′E004˚31.286’

Short, slender simple unicellular non-glandular trichomes were observed on the epidermis of the petiole. The taxonomic importance of trichomes has been highlighted by some researchers which include Akinsulire et al. (2018b) on the genus *Terminalia* as well as Adedeji et al. (2007) on some members of the family Solanaceae. Starch grains were also present between the vascular area and the hypodermis of the petiole (**Fig. 1D**).

Table 2. Qualitative and quantitative characteristics of cells and tissues of two monotypic genera of Combretaceae. Quantitative values are expressed as mean ± standard error (n = 40)

Characters	***A. leiocarpus***	***Q. indica***
Thickness of upper cuticle (µm)	16.00±0.42	12.48±0.16
Nature of upper and lower epidermis	Uniseriate	Uniseriate
Shapes of upper epidermal cells	Rectangular/oval	Rectangular/squared/ polygonal/ oval
Periclinal walls on upper epidermis	Slightly undulating	Straight/undulating
Thickness of upper epidermis (µm)	22.00±0.96	36.52±0.86
Layer of palisade cells	One	One
Thickness of palisade mesophyll (µm)	62.50±0.69	68.14±1.90
Shape of lower epidermal cells	Rectangular	Rectangular/oval/squared
Periclinal walls on lower epidermis	Undulating	Undulating
Thickness of lower epidermis (µm)	22.64±0.84	34.48±0.65
Thickness of lower cuticle (µm)	14.80±0.28µm	12.06±0.08
Type of trichome on the epidermis (lamina, midrib and petiole)	Slender simple unicellular	Short, slender simple unicellular
Type of vascular bundle (midrib)	Collateral	Collateral
Shape of vascular bundle (midrib)	Arched or crescentiform	arc-shaped
Starch grains in the pith (midrib)	Absent	Present
Petiole outline	Three-winged	Arched and furrowed
Petiole epidermis and hypodermis	Uniseriate/biseriate	Uniseriate
Type of trichome on petiole epidermis	Short, slender simple unicellular	Short, slender simple unicellular

3.2. *Quisqualis indica* - Micro-anatomy

The transverse section of the leaves is characterized by a thinner upper and lower cuticle with dimensions 12.48±0.16 µm and 12.06±0.08 µm respectively. The upper epidermis composed of uniseriate row of large epidermal cells that are rectangular, squared or polygonal, occasionally oval with straight to undulating periclinal wall; upper epidermal cells are uniseriate measuring 36.52±0.86 µm while the lower cells are rectangular, oval or squared with a mean length of 34.48±0.65 µm (**Fig. 2A**). Palisade mesophyll is 1-layered and cylindrical, compact and highly pigmented with a mean length of 68.14±1.90 µm. Spongy mesophyll cells are irregular, parenchymatous and fairly compact with moderate intercellular airspaces. The transverse section of the midrib of this species is characterized by a thick and darkly stained cuticle and a uniseriate row of epidermis, followed by a mass of 5 to 7 layers of thin walled parenchyma (**Fig. 2B**).

The vascular bundle is collateral and arc-shaped. Similar feature was reported by Akinsulire et al. (2018b) in some *Terminalia* species in Combretaceae to which *Quisqualis indica* belongs. Another defining characteristic in *Q. indica* is the parenchymatous pith which shows the presence of starch grains as simple short unicellular trichomes were also observed in the midrib epidermal region.

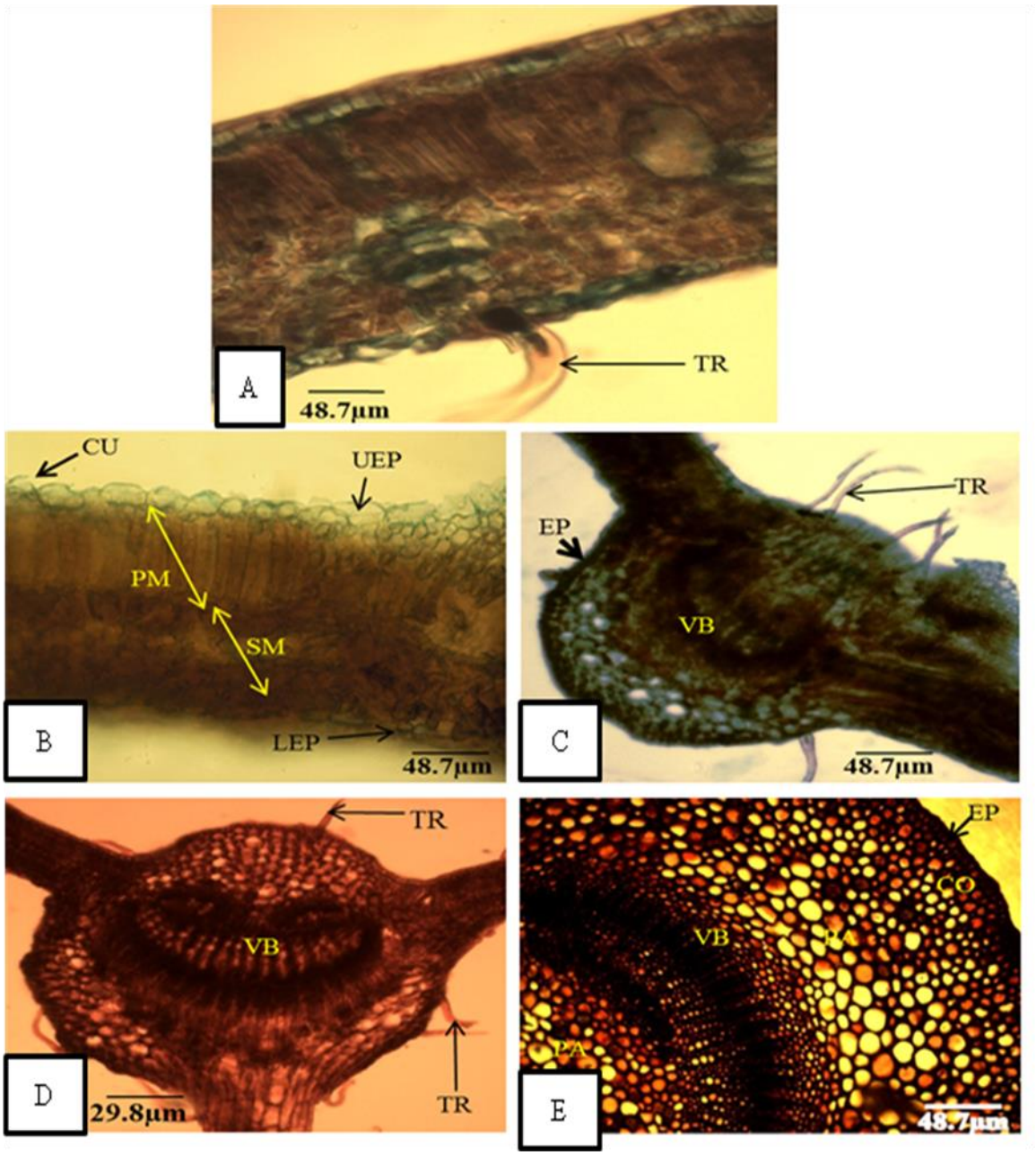

Fig. 1. Micro-anatomy of the transverse sections of the leaf and petiole of *Anogeissus leiocarpus* A and B = Leaf (Lamina), C = Leaf (Midrib), D = Petiole, E = Petiole (Transect)
Abbreviations: CU = Cuticle, **UEP =** Upper Epidermis, **LEP** = Lower Epidermis, **CO =** Collenchyma, **PA =** Parenchyma, **SM =** Spongy Mesophyll, **TR =** Trichome, **PM =** Palisade Mesophyll, **PH =** Phloem, **µm** = Micrometre, **VB =** Vascular Bundle

The adaxial outline of the petiole (median) is arched and furrowed (**Fig. 2C**). The epidermis of the petiole is thick with uniseriate row of cells while the hypodermis is also uniseriate. The vascular bundle in the petiole is collateral (**Fig. 2C**) while the vascular architecture is lunar-shaped as reported in some other members in Combretaceae (Akinsulire et al., 2018b). Outside to the vascular area were thin-walled 6 to 7-layered parenchyma cells which houses sparsely distributed starch grains (**Fig. 2D**) while 2 to 3 layers of angular collenchyma cells were also observed below the hypodermis.

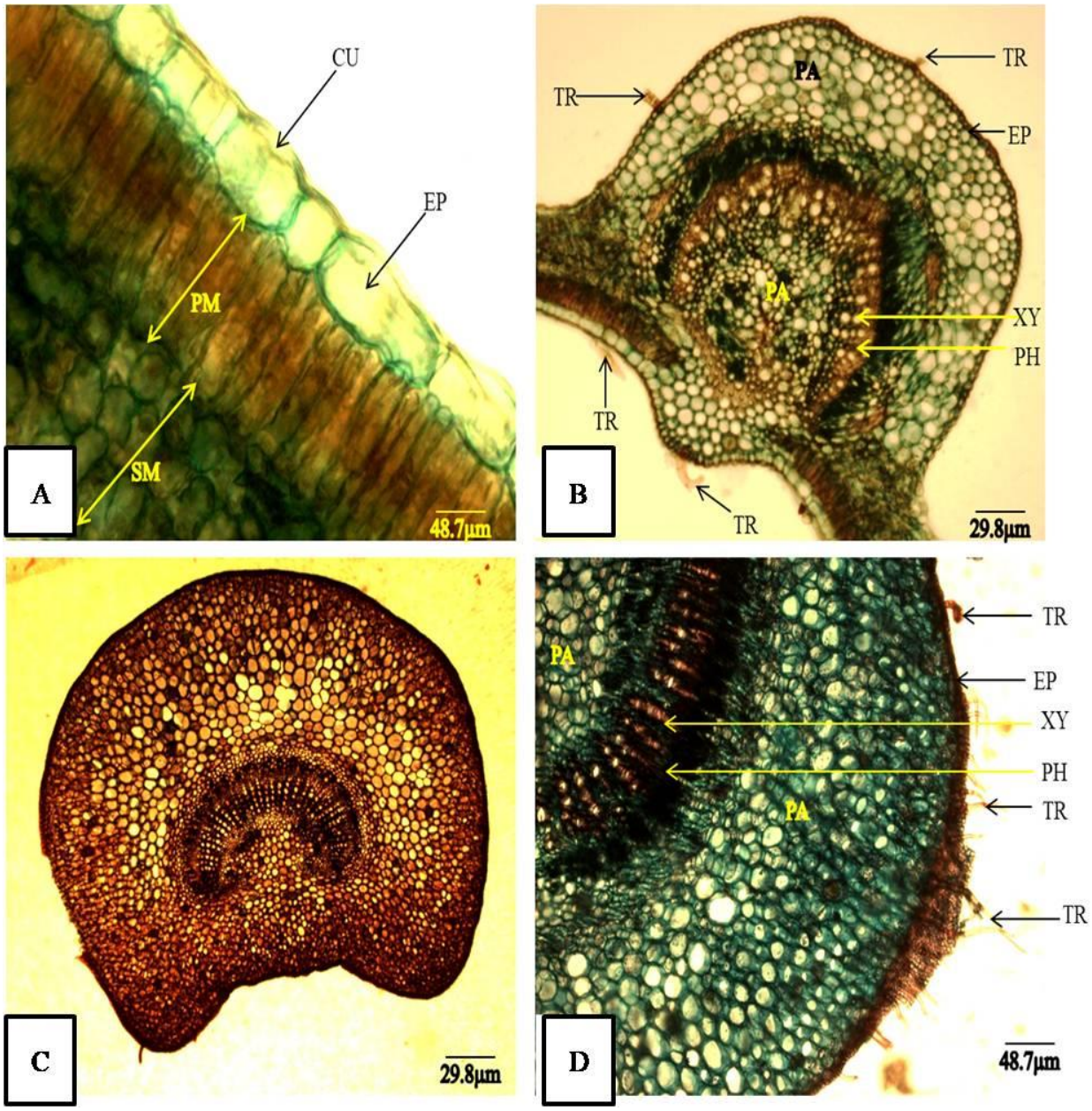

Fig. 2. Micro-anatomy of the transverse sections of the leaf and petiole of *Quisqualis indica*
A = Leaf (Lamina), B = Leaf (Midrib), C = Petiole, E = Petiole (Transect)
Abbreviations: CU = Cuticle, **EP** = Epidermis, **PA** = Parenchyma, **SM** = Spongy Mesophyll, **TR** = Trichome, **PM** = Palisade Mesophyll, **XY** = Xylem, **PH** = Phloem, **µm** = Micrometre

4. Conclusions

This study has provided information on the micro-anatomy of the transverse sections of the leaf and petiole of the two monotypic genera thereby enhancing their identification. It is however recommended that molecular investigation into the family Combretaceae generally be conducted to enhance the taxonomy of the family.

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Conflict of interest

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

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