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Vegetative anatomy of five domesticated members of the genus *Cucurbita* (Linn.) in Southwestern Nigeria

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ABSTRACT

The vegetative anatomy of five domesticated species of the genus *Cucurbita* L. found in South Western Nigeria was studied. The aim was to establish some useful diagnostic features that may be employed in combination with other characters as intra or inter-specific or generic tools for their delimitation. The study revealed exciting features that are helpful in the identification of each species. The genus can be divided into three groups based on the shape of the midrib. Those with a U-shape are *C. pepo*, *C. argyrosperma* and *C. ficifolia*. *C. maximum* has a round/flat shape while *C. moschata* has V shape. Those with a U-shape can further be classed into those with a wide and narrow neck. *C. argyrosperma* has narrow neck with two vascular bundles, four layers of palisade tissues and 6 layers of abaxial epidermis. *C. pepo* has a wide neck with three bicollateral bundles and two layers of palisade tissues while *C. ficifolia* has two bundles and three layers of palisade tissues. Overlaps and similarities in the number of vascular bundles, and layers of tissues showed generic affinity among the members.

KEYWORDS: Affinity, Anatomy, Bicollateral bundles, Comparative, *Cucurbita*, Generic

INTRODUCTION

The Family Cucurbitaceae Juss. has 120 genera and about 800 species (Teppner, 2004) medium sized, predominantly tropical with the exception of a few members adapted to the temperate areas. The life form is varied; from lianas, annuals, perennials, herbaceous lianas and wood lianas (Teppner, 2004). In West Tropical Africa, the Family Cucurbitaceae consists of 23 genera and 41 species (Hutchinson & Dalziel, 1952). The Genus *Cucurbita* consists of 15 species of which 10 are wild and five cultivated. The most important and cultivated are *Cucurbita pepo* Linn., *Cucurbita moschata* Duchesne ex Lam Duchesne ex Poir, *Cucurbita maxima* Duchesne ex Poir, *Cucurbita argyrosperma* Huber and *Cucurbita ficifolia* Bouche (Teppner, 2004; Hadia *et al.*, 2008; Nontuthoko *et al.*, 2016). Two species were described in the Flora of West Tropical Africa (Hutchinson & Dalziel, 1952) namely *C. pepo* Linn. and *C. maxima* Duchesne; suggesting that the other species were introduced to West Africa much later.

Aruah *et al.* (2010) noted that *C. argyrosperma* has not been frequently mentioned in research because of its less cultivation. Saade and Montes (1994) described *C. argyrosperma* and

C. ficifolia as less relevant among the cultivated species. Nontuthoko *et al.* (2016) reported that the limited cultivation of *C. argyrosperma* resulted from the bitterness of the matured leafy shoot and the preference of farmers and consumers for species with better taste and it was selected for its seeds. This has led to its rarity and process of being threatened. Members of the Genus are important because the leaves, fruits, male flowers, tips of the vines and seeds are consumed as food. Also, the plant has been used to treat various diseases as an alternative medicine (Hadia *et al.*, 2008; Nontuthoko *et al.*, 2016; Nyabera *et al.*, 2019). Members of the Genus *Cucurbita* in Africa, play nutritional and economic roles (Hadia *et al.*, 2008).

Nyabera *et al.* (2019) noted that the most popular species; *C. pepo* and *C. maxima*, *C. moschata* and *C. ficifolia* show wide variations in their morphology. Nontuthoko *et al.* (2016) also reported that *Cucurbita* species vary widely, morphologically, genetically and agro-ecologically.

Decades of research on the Family Cucurbitaceae showed that there are lots of morphological variations among and within the genus and family. Aruah *et al.* (2010) noted that despite the fact that members of the Genus *Cucurbita* in Africa play nutritional

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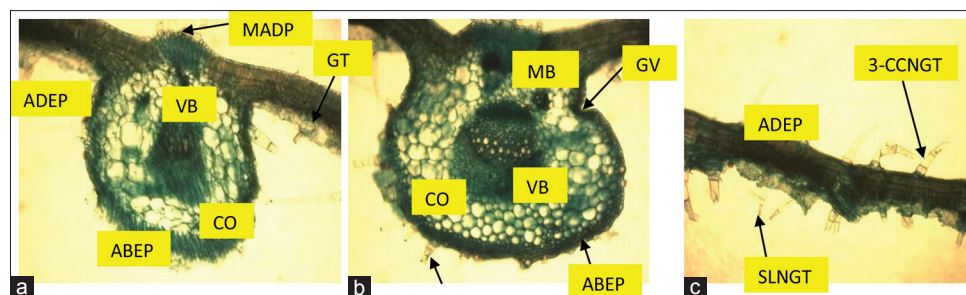


Figure 1: *C. pepo* showing: a) Midrib, b) Midrib showing medullary bundles and c) Leaf lamina 3-CCNGT-3-Celled Non Glandular trichome; ABEP-Abaxial epidermis; ADEP-Adaxial epidermis; CO-Collenchyma; GT-Glandular trichome; GV-Groove; MB-Medullary bundle; SLNGT-Spinelike Non Glandular trichome; VB-Vascular bundle

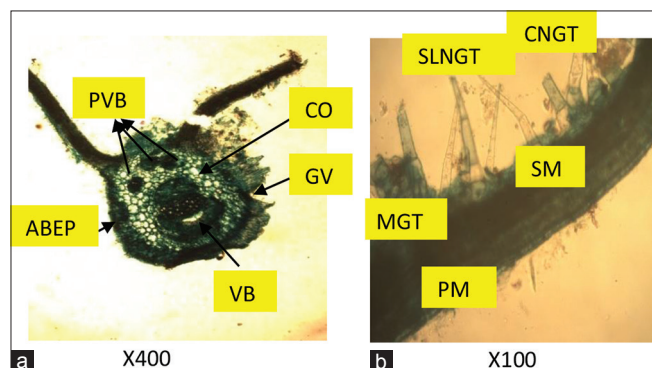


Figure 2: *C. maxima* showing: a) Midrib and b) Leaf lamina showing trichomes ABEP-Abaxial epidermis; CO-Collenchyma; CNGT-Conical non glandular trichome; GV-Groove; MGT-Multicellular glandular trichome; PM-Palisade mesophyll; PVB-Proximal vascular bundle; SLNGT-Spinelike Non Glandular trichome; SM-Spongy mesophyll; VB-Vascular bundle

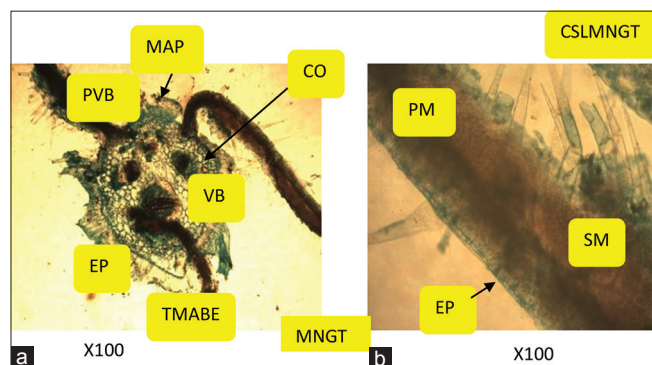


Figure 3: *C. mochatata* showing: a) Midrib and b) Leaf lamina showing trichomes CO-Collenchyma; CSLMNGT-Conical, spine like multicellular non glandular trichome; EP-Epidermis; MAP-Median adaxial protrusion; PM-Palisade mesophyll; PVB-Proximal vascular bundle; SM-Spongy mesophyll; TMABE-Tapered median abaxial epidermis; VB-Vascular bundle

and economic roles (Hadia *et al.*, 2008) they have not benefited from research efforts because they are considered as neglected crops. Saade and Montes (1994) described members of the genus as neglected crops. Previous studies were based on species of the family Cucurbitaceae that most times do not belong to the same genus. Research activities have also been concentrated

on the three commonly cultivated species. According to Aruah *et al.* (2010), authors have used different descriptions of the Cucurbits resulting in incongruent classification of the *Cucurbita* species.

The taxonomic challenges on proper identification of the cultivated members coupled with the paucity of information must be resolved and clarified. The uniqueness of this study is that this is the first time the five cultivated species are compared together. Therefore this study seeks to use anatomical features to delineate members and give better insight into the anatomy of the domesticated members of the Genus *Cucurbita* found in South-western Nigeria. The study compared the leaf, stem and root anatomy of the Genus *Cucurbita* in the study area.

MATERIALS AND METHODS

Plant Collection and Establishment

Fruits of *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita maxima*, *Cucurbita argyrosperma* and *Cucurbita ficifolia* were collected from South - western Nigeria which lies on Latitude 6°-9° N and Longitude 3°-9° E. The matured fruits of the species were cut open to remove the seeds. The seeds were not planted immediately but were sun dried to prevent spoilage and mould attack.

Establishment

Plants were established on the research farm at Adeyemi Federal University of Education, Ondo, Ondo State, Nigeria. The experimental plots were cleared manually with cutlass and hoes. The land was cleared and tilled. Short ridges of *Panicum maximum* and *Andropogon* were removed with hoes and shovels. Ridges were made with shovels. Seeds were planted on the ridges made. Seeds were not treated with pesticides and seeds of each species were soaked in water overnight prior to planting by hand on 4th of April 2021. Seed were spaced at 20 cm in rows which were 25 cm apart. Plants were rain fed and no fertilizer was applied. At maturity, i.e. at flowering, vegetative parts were harvested and preserved in 70% ethanol until when required.

Transverse Section of the Leaf Blade

Transverse sections of the leaf blade and midrib were cut from the median portion of the materials available using

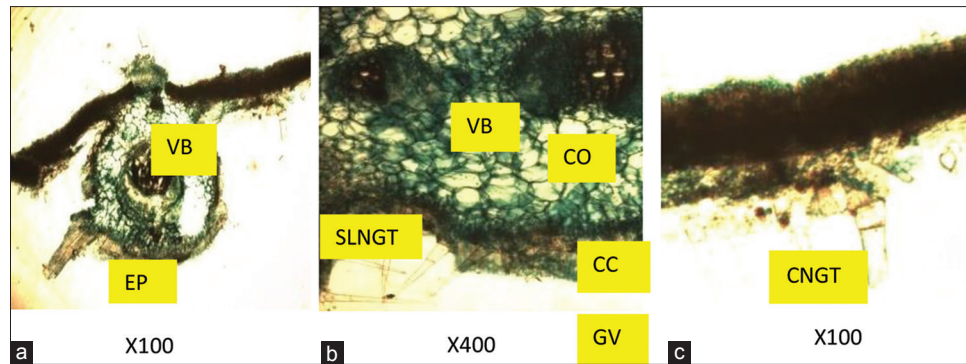


Figure 4: *C. argyrosperma* showing: a) Midrib, b) Vascular bundle and spine like non glandular trichome and c) leaf lamina showing conical trichomes CC-Cylindrical cells; CO-Collenchyma; CNGT-Conical non glandular trichome; EP-Epidermis; GV-Groove; MAP-Median adaxial protrusion; SLNGT-Spine like non glandular trichome; VB-Vascular bundle

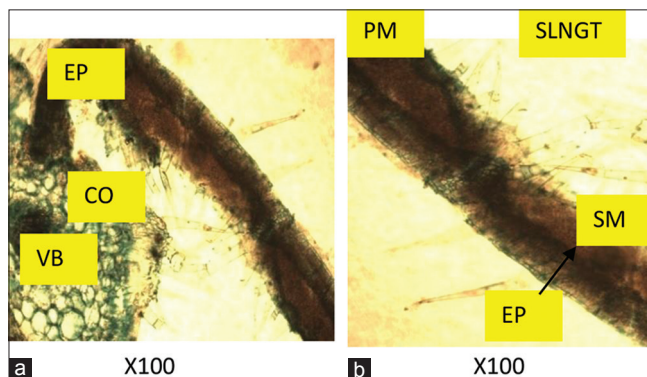


Figure 5: *C. ficifolia* showing: a) Midrib and b) Vascular bundle and spine like non glandular trichome CO-Collenchyma; EP-Epidermis; SLNGT- Spine like non glandular trichome; VB- Vascular bundle

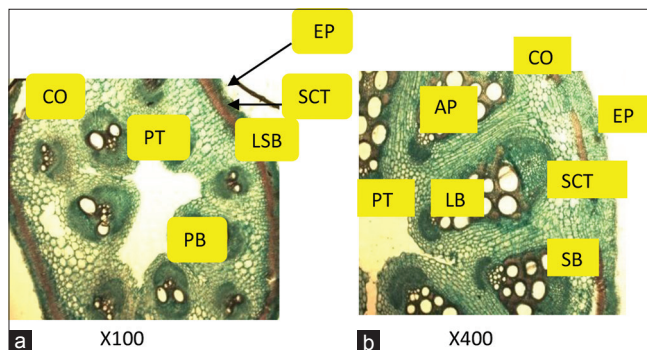


Figure 6: *C. pepo* showing: a) Transverse section of the Stem and b) Transverse section of the Root AP- Axial parenchyma; CO-Collenchyma; EP-Epidermis; LB-Large bundle; LSB-Lower strata bundle; PB-Paired bundle; PT-Pith; SCT-Sclerenchymatous tissue; SB-Small bundle

Reichert sliding microtome at 8-15 μm . Sections were stained in Safranin O for 3 minutes, rinsed in water to remove excess stain and then counterstained in Alcian blue for 3 minutes. These were rinsed in water to remove excess stains and then treated to serial grades of alcohol and mounted in dilute glycerol for anatomical studies. Photomicrographs were taken with the aid of 3013 ACCU-SCOPE Trinocular Microscope with Digital Camera.

Transverse Sections of the Stem and Root

Transverse sections of mature stems and roots were cut at 8-15 μm on Reichert sliding microtome. Some sections were made free-hand for preliminary studies. Each section was stored in 50% alcohol for anatomical studies. Sections were stained in Safranin O for 3 minutes, rinsed in water to remove excess stain and then counterstained in Alcian blue for 3 minutes. These were rinsed in water to remove excess stain and then treated to serial grades of alcohol and mounted in dilute glycerol for anatomical studies. Photomicrographs were taken with the aid of 3013 ACCU-SCOPE Trinocular Microscope with Digital Camera. Microscopic measurements were made with the aid of an ocular micrometer inserted in the eyepiece of the Leica binocular microscope.

RESULT AND DISCUSSION

The Midrib Outline

A summary of the midrib anatomy is in Table 1. The midrib outline of the species of the genus *Cucurbita* can be classified into three groups based on the shape of the midrib, namely those with U-shape. These are *C. pepo*, *C. argyrosperma* and *C. ficifolia* (Figures 1a, b, 4a and 5a). Grooves are present on the epidermis of *C. pepo* (Figure 1c) but absent in the other two species. *C. maxima* have an oval/round shape (Figure 2a) while *C. moschata*, is tapered at the median abaxial portion to form a V-shape (Figure 3c).

The epidermal cells are 2-4 layered adaxially and 3-4 layers abaxially. In all the species the median adaxial surface of the midrib is protruded with trichomes. The abaxial epidermis of *C. pepo* and *C. maxima* are grooved (Figure 1b) but in *C. maxima* trichomes are buried in the groove (Figure 2a). This suggests an affinity between *C. pepo* and *C. maxima*.

The cortex is multiseriate with angular collenchyma cells. Vascular bundles are bicollateral in all members but the number varies in the species; *C. pepo* - 3 (Figure 1a); *C. maxima* - 4 (Figure 2a); *C. moschata* - 4 (Figure 3a); *C. argyrosperma* - 2 bundles (Figure 4a) and *C. ficifolia* - 2 (Figure 5a).

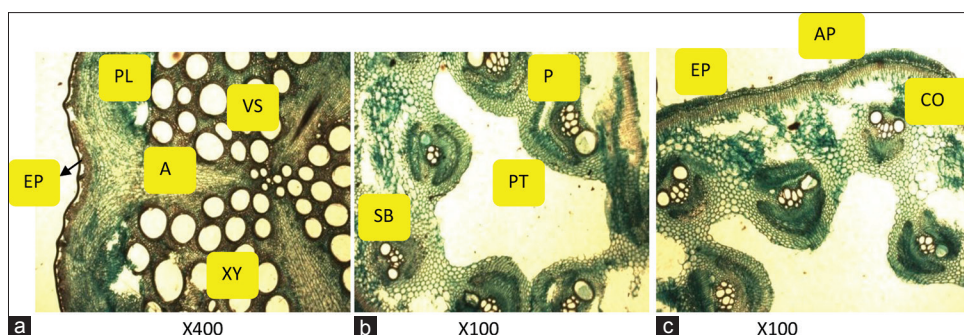


Figure 7: *C. maxima* showing: a) Transverse section of the Stem, b and c) Transverse section of the Root
AP-Axial parenchyma; CO-Collenchyma; EP-Epidermis; LB-Large bundle; SSB-Single lower strata bundle; PAR-Parenchyma; PB-Paired bundle; PI-Phloem; PT-Pith; SB- Small bundle; VS-Vessel; XY-Xylem

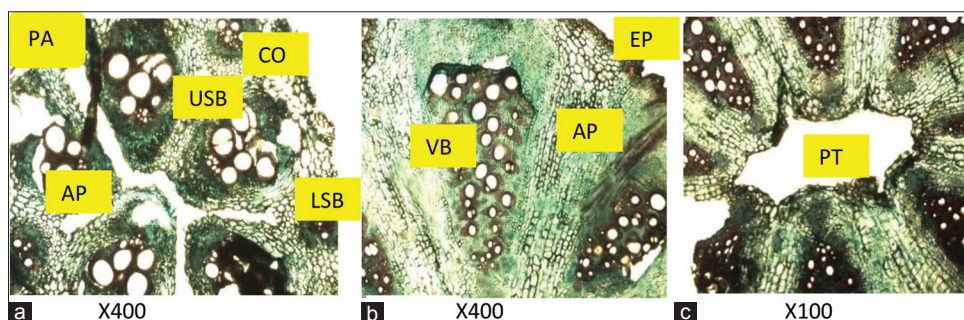


Figure 8: *C. moschata* showing: a) Transverse section of the Stem and b) Transverse section of the Root
AP-Axial parenchyma; CO-Collenchyma; EP-Epidermis; LSB-Lower strata bundle; PT-Pith; USB-Upper strata bundle; VB-Vascular bundle

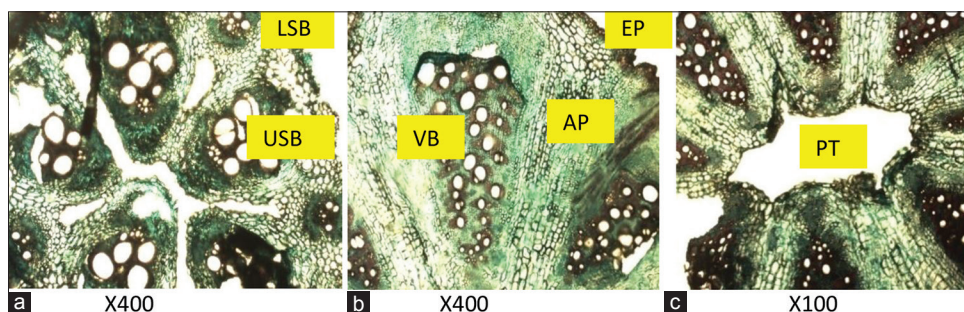


Figure 9: *C. argyrosperma* showing: a) Transverse section of the Stem, b and c) Transverse section of the root
AP-Axial parenchyma; EP-Epidermis; LSB-Lower strata bundle; PT-Pith; USB-Upper strata bundle; VB-Vascular bundle

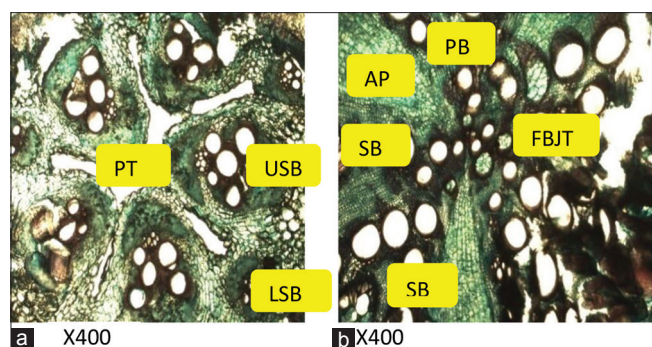


Figure 10: *C. ficifolia* showing: a) Transverse section of the Stem and b) Transverse section of the root
AP-Axial parenchyma; FBJT-Four bundles joined together; LSB-Lower strata bundle; PB-Paired bundles; PT-Pith; SB-Single bundle; USB-Upper strata bundle; VB-Vascular bundle

Lamina Anatomy

The Mesophyll

A summary of the lamina anatomy is in Table 2. Lamina anatomy showed that the members of the genus studied have uniseriate epidermis. The lower epidermis of the lamina is biseriata in *C. pepo* (Figure 1c); uniseriate in *C. maxima* (Figure 2b) and *C. moschata* (Figure 3a), in *C. ficifolia* it is bi-triseriate (Figure 5b) but in *C. argyrosperma* it is 6 layered (Figures 4b and c). Mesophyll is chlorenchymatous and well differentiated. Palisade tissue layers are: *C. pepo*- 2 (Figure 1b); *C. maxima*- 2 (Figure 2b); *C. moschata*- 2 (Figure 3b); *C. argyrosperma*- 4 (Figure 4b) and *C. ficifolia*- 3 (Figure 5b). Spongy tissues are generally multiseriate. *C. pepo*- 2 layered; *C. maxima*- 3-4 layered; *C. moschata*- 4-6 layered; *C. argyrosperma*- 3-4 layered

Table 1: Summary of the Midrib outline of the Five domesticated species of *Cucurbita*

Characters/ Features	Adaxial surface				
	Species <i>C. pepo</i>	<i>C. maxima</i>	<i>C. moschata</i>	<i>C. argyrosperma</i>	<i>C. ficifolia</i>
Shape	Convex. Oval to almost round with two ribs upper median portion protruded	Convex. Oval to almost round with two ribs upper median portion protruded	Convex. Oval to almost round with two ribs upper median portion protruded	Convex. Oval to almost round with two ribs upper median portion protruded	Convex. Oval to almost round with two ribs upper median portion protruded
Epidermis	2 layered rectangular cells	3-4 layered rectangular cells	Uniseriate cylindrical cells.	2 layered rectangular cells	2-3 layered. Made up of rectangular and cylindrical cells
Cortex	4-5 layers of Compactly arranged angular collenchyma cells.	3-8 layers of compactly arranged angular collenchyma cells.	3-4 layers of compactly arranged angular collenchyma cells.	3-8 layers of Compactly arranged angular collenchyma cells.	4-5 layers of compactly arranged angular collenchyma cells.
Vascular bundles	One bicollateral bundle occurring centrally plus 1-2 medullary bundles	4 bicollateral bundles: One centrally placed with 3 smaller bundles proximally placed	4 bicollateral bundles; one centrally placed and 3 smaller ones lies proximally	One bicollateral bundle	2 bicollateral bundle; one big bundle and a smaller bundle on top.
Trichomes	non-glandular; may be one celled or 2-5 celled; conical shaped;	1 celled non glandular	2-3 celled non- glandular trichomes, multicellular spine like, 2-3 celled conical non-glandular trichomes	Conical shaped 2-3 celled non glandular	1-3 conical non-glandular trichomes; some spine like with 3-6 cells.s
Characters/ Features	Abaxial surface				
	Species <i>C. pepo</i>	<i>C. maxima</i>	<i>C. moschata</i>	<i>C. argyrosperma</i>	<i>C. ficifolia</i>
Shape	Convex. grooved towards the lower end of the abaxial surface U-shaped with wide neck (Figure 1a & b)	Convex. grooved towards the lower end of the abaxial surface. Round or oval shaped (Figure 2a)	Convex. Abaxial surface tapered at the median portion. V-shaped with wide neck (Figure 3a)	Convex. U-shaped with narrow neck (Figure 4a)	Convex. U-shaped with wide neck (Figure 5a)
Epidermis	2 layers of cylindrical or atimes rectangular cells	2-3 layers of cylindrical cells. Trichomes buried in the groove.	2- layers of cylindrical cells	One layer of cylindrical cells	2 layers of cylindrical cells
Trichomes	Non glandular; glandular trichomes multicellular trichomes	Non glandular multicellular trichomes; some have tapered like spines with 3-6 cells	Non glandular trichomes; 3-6 celled spine like with tapered end and conical trichomes 2-3 celled.	Non glandular trichomes; 2-3 celled conical cells.	Non glandular multicellular trichomes; some have tapered like spines with 3-6 cells; some are 2 celled with round haed; some are conical.

Table 2: Summary of leaf lamina anatomy of the five domesticated species of *Cucurbita*

Characters/ Features	Species				
	<i>C. pepo</i>	<i>C. maxima</i>	<i>C. moschata</i>	<i>C. argyrosperma</i>	<i>C. ficifolia</i>
Adaxial Epidermis	Uniseriate with thin cuticle	Uniseriate with thin cuticle	Uniseriate with thin cuticle	Uniseriate with thin cuticle	Uniseriate with thin cuticle
Trichomes on the adaxial epidermis	Non-glandular Trichomes 2-6 celled tapered at the end	Non-glandular Trichomes 2-6 celled tapered at the end	Non-glandular Trichomes 2-6 celled tapered at the end; one celled glandular trichome	Glandular trichome with round head; 2-3 celled	
Palidase mesophyll	2 layers of compactly arranged elongated cells	2 layers of compactly arranged elongated cells	3 layers of compactly arranged elongated cells	4 layers of compactly arranged elongated cells	3 layers of compactly arranged elongated cells
Spongy mesophyll	3 layers of loosely arranged elongated cells	3-4 layers of loosely arranged elongated cells	3 layers of loosely arranged cells	3-4 layers of loosely arranged cells	3-4 layers of loosely arranged elongated cells
Abaxial epidermis	biseriate	uniseriate	Uniseriate	Multiseriate 6- layers	2-3 layered
Trichomes on abaxial epidermis	Conical 2-celled non glandular trichomes; spine like 3-8 celled non glandular. Trichomes are on raised portions of the epidermis	Conical 2-celled non glandular trichomes; spine like 3-8 celled non glandular trichomes; trichomes with compound foot; multi-celled glandular trichomes (Figure 2b)	Conical shaped non glandular trichomes; 2-3 celled. Multi celled trichomes tapered at the end. (Figure 3b)	Conical shaped non glandular trichomes; 2-6 celled.	Conical shaped non glandular trichomes; 2-3 celled. Multi celled trichomes tapered at the end

and *C. ficifolia*-3-4 layers. Mohammed and Guma (2015) noted that *C. moschata* has 2-6 layers of spongy mesophyll.

Trichomes are characteristics of the family Cucurbitaceae and they are found in the epidermis of the leaves, stem and

Table 3: Summary of the stem anatomy of the five domesticated species of the Genus *Cucurbita*

Characters/features	Species				
	<i>C. pepo</i>	<i>C. maxima</i>	<i>C. moschata</i>	<i>C. argyrosperma</i>	<i>C. ficifolia</i>
Epidermis	Uniseriate with thin cuticle	Uniseriate with thin cuticle	Uniseriate with thin cuticle	Uniseriate with thin cuticle	Uniseriate with thin cuticle
Sclerenchymatous tissue	Continuous	Continuous	Continuous	Continuous	Continuous
Cortex	6 layers of Angular collenchyma	3-4 layers of Angular collenchyma	4-6 layers Angular collenchyma	5-6 layers of Angular collenchyma	6 layers of Angular collenchyma
Vascular bundles	10 bicollateral bundles. Two rings of bundles; 2 paired bundles and 2 single bundles on the upper ring plus 5 bundles on the lower ring	3 collateral bundles arranged in a ring	10 bicollateral bundles. Two rings of bundles; 5 bundles on the upper ring plus 5 bundles on the lower ring	10 bicollateral bundles. Two rings of bundles; 5 bundles on the upper ring plus 5 bundles on the lower ring	10 bicollateral bundles. Two rings of bundles; 5 bundles on the upper ring plus 5 bundles on the lower ring
Vessels	Elliptic to oval; 7-9 in number	Elliptic to oval; 16-21 in number	Elliptic to oval; inner vessels, 12-14 while outer bundles have vessels 6-10 in number	Elliptic to oval; 8-10	Elliptic to oval; 12-15 in number in the upper ring while in the lower ring vessel number is 5-8
Pith trichomes	present Non-glandular and glandular	Absent Non-glandular and glandular	Partially present Non-glandular and glandular	Partially present Non-glandular and glandular	Partially present Non-glandular and glandular

Table 4: Summary of the root anatomy of the five domesticated species of the Genus *Cucurbita*

Characters/features	Species				
	<i>C. pepo</i>	<i>C. maxima</i>	<i>C. moschata</i>	<i>C. argyrosperma</i>	<i>C. ficifolia</i>
Epidermis	Uniseriate with thin cuticle	Uniseriate with thin cuticle	Uniseriate with thin cuticle	Uniseriate with thin cuticle	Uniseriate with thin cuticle
Sclerenchymatous tissue	Interrupted at intervals	Continuous	Continuous	Continuous	Continuous
Cortex	6-8 layers of Angular collenchyma	6-8 layers of Angular collenchyma	5-6 layers of Angular collenchyma	6-layers of Angular collenchyma	6-layers of Angular collenchyma
Vascular bundles	10 bicollateral bundles. Two rows of bundles; 5 bundles on the upper row plus 5 bundles on the lower row. The smaller bundles alternate with the larger bundles	10 bicollateral bundles. Two rows of bundles; 5 bundles on the upper row plus 5 bundles on the lower row. The smaller bundles alternate with the larger bundles	8 bicollateral bundles- each bundle occurred singly.	8 bicollateral bundles	8 collateral bundles- 4 bundles joined together; 2paired bundles and 2 single ones.
Vessels	Elliptic to oval; 11-24 in number	Elliptic to oval; 8-11 in number	Elliptic to oval; 20-37 in number	Elliptic to oval; 20-29 in number	Elliptic to oval; 7-13 in number
Pith trichomes	Pith present Absent	Pith large 2-3 celled glandular trichomes	present Absent	present Absent	Absent Absent

roots. Trichomes in the Genus *Cucurbita* study showed that trichomes are majorly glandular and non-glandular. Non-glandular trichomes are multi-celled usually between 2-8 celled (Figures 1-5), conical or tapered at the end (spine-like). Glandular trichomes have rounded ends. In addition to this *C. maxima* has multicellular glandular trichomes that are 25 celled (Figure 2c). Another unique feature of *C. maxima* lamina epidermis is the alternating of trichomes with a single foot with trichomes with a compound foot.

Stem and Root Anatomy

A summary of the stem anatomy is in Table 3. Stem epidermis is uniseriate in all species studied with thin

cuticle. Sclerenchymatous tissue is continuous. Cortex is made of collenchyma cells; *C. pepo* - 6 layered (Figure 6a); *C. maxima* - 3-4 layered (Figure 7a); *C. moschata* - 4-6 layered (Figure 8a); *C. argyrosperma* - 5-6 layered (Figure 9a) bundles and *C. ficifolia* - 6 layered (Figure 10a). Ajuru and Okoli (2013) reported that *C. moschata* has 5-6 layers of collenchyma cells.

The numbers of vascular bundles of the stem are as follows: *C. pepo* - 10 (Figure 6a); *C. maxima* - 3 and collateral (Figure 7a); *C. moschata* - 10 (Figure 8a); *C. argyrosperma* - 10 (Figure 9a) and *C. ficifolia* - 10 (Figure 10a). Bundles are bicollateral except in *C. maxima* which is collateral. Bundles are in rings usually two levels; one above and the other below.

Vessels are elliptic to oval, solitary in all the members but vary in number from one species to the other; *C. pepo* - 7-9; *C. maxima* - 16-21; *C. moschata* - 6-10; *C. argyrosperma* - 8-10 and *C. ficifolia* - 12-15. Pith is absent in *C. maxima* (Figure 7a) but present in the other four species.

A summary of the root anatomy is in Table 4. The root epidermis showed uniseriate rectangular to cylindrical cells in all members of the genus *Cucurbita* studied. Sclerenchymatous tissue is continuous in all species except *C. pepo* where it is interrupted at intervals (Figure 6b). Cortex is made of collenchyma cells; *C. pepo* - 6-8 layered (Figure 6a); *C. maxima* - 6-8 layered (Figure 7a); *C. moschata* - 5-6 layered (Figure 8a); *C. argyrosperma* - 6 layered (Figure 9a) and *C. ficifolia* - 6 layered (Figure 10a).

The root vascular bundles are bicollateral in *C. pepo* - 10 (Figure 6b); *C. maxima* - 10 (Figure 7b); *C. moschata* - 8 (Figure 8b); *C. argyrosperma* - 8 (Figure 9b) however in *C. ficifolia* there are 8 collateral bundles (Figure 10b). Bundles are in rings usually one tiered in four species except *C. maxima* where there are two tiers of rings of bundles (Figure 7c). Vessels are elliptic to oval, solitary in all the members but vary in number from one species to the other; *C. pepo* - 11-14 (Figure 6b); *C. maxima* - 8-11 (Figure 7b); *C. moschata* - 20-37 (Figure 8b); *C. argyrosperma* - 8-10 (Figure 9b) and *C. ficifolia* - 7-13 (Figure 10b). Pith is present in the species.

DISCUSSIONS

The search for taxonomic features useful for delineating members of the family Cucurbitaceae has been on for a long time. The uses of anatomical features as taxonomic tools are well-defined in literatures. Agbagwa and Ndukwu (2005) delineated the three must-cultivated species of *Cucurbita* namely, *C. pepo*, *C. maxima* and *C. moschata* based on the number of petiolar bundles. This study showed that anatomically, there are features that indicate that the five members of the Genus *Cucurbita* have a generic affinity. These are bicollateral bundles in the leaves, the bundles of the stems and the roots are arranged in rings which may be one-tiered or double-tiered. The collenchyma of the stem and the root is angular and multiseriate. Vascular bundles of the stem are separated by axial parenchyma cells. Vessels are elliptic to oval, solitary but varied in number. Sclerenchyma tissue is present in the cortical area of the stem and the root, which may be continuous or interrupted. Trichomes in the Genus *Cucurbita* studied showed that they are majorly glandular and non-glandular.

The use of the midrib shape in the family Asteraceae was identified by Ekeke and Mensah (2015). In this case, the shape of the midrib of the Genus *Cucurbita* is a diagnostic tool for the genus. The genus can be divided into three groups based on the shape of the midrib. Those with a U-shape are *C. pepo*, *C. argyrosperma* and *C. ficifolia*. *C. maximum* has a round/flat shape while *C. moschata* has V shape. Those with a U-shape can further be classed into those with wide and narrow necks. Maksoud and Nassar (2013) reported that there are 3 bicollateral bundles in the midrib of *C. maxima* while Ajuru and Okoli (2013) observed 4 bundles in the midrib of

C. moschata and Mohammed and Guma (2015) reported that the midrib of *C. moschata* has 7 bundles. There were overlaps in the number of bundles observed in the present study. The similarities are more than the differences observed among the members. This study confirmed the report of Agbagwa and Ndukwu (2005), Ajuru and Okoli (2013) and Mohammed and Guma (2015) that cultivated species of *Cucurbita* in Nigeria show marked similarities and overlaps in the number of layers of cells, tissues, distribution and differentiation. The number of palisade and spongy tissues is within the range cited by Ajuru and Okoli (2013) and Mohammed and Guma (2015) for *C. pepo*; *C. maxima* and *C. moschata*. This study provided anatomical information on *C. argyrosperma* and *C. ficifolia*.

Trichomes are characteristics of the family Cucurbitaceae and they are found in the epidermis of the leaves, stem and roots. Trichomes in the Genus *Cucurbita* study showed that trichomes are majorly glandular and non-glandular. Non-glandular trichomes are multi-celled usually between 2-8 celled, conical or tapered at the end (spine-like). Glandular trichomes have rounded ends. Mohammed and Guma (2015) noted that there are two basic types of hairs in the Family Cucurbitaceae namely; glandular and non-glandular. It was further noted that non-glandular trichomes are multicellular with various shapes. In addition to this *C. maxima* has multicellular glandular trichomes that are 25 celled. Another unique feature of *C. maxima* lamina epidermis is the alternating of trichomes with a single foot with trichomes with the compound foot. The occurrence of three types of trichomes in *C. maxima* was also reported by Sutherland and Hallett (1993).

Stem anatomy of four species showed two levels of ring arrangement except *C. maxima* which has only one tier of ring. Mohammed and Guma (2015) reported that one ring of vascular bundle in *C. moschata* while Ajuru and Okoli (2013) reported that in *C. moschata* there were 12 bundles arranged in two tiers. Variations in the number of strata or tiers of vascular bundles and sclerenchymatous tissues have been reported (Okeke *et al.*, 2015). This study showed that the stem of *C. maxima* has three collateral bundles which could have distinguished the species from others that have 10 bicollateral bundles in two tiers of ring; however it cannot be used as a taxonomic feature because Yasuda (1903) reported that in the Genus *Cucurbita*, the vascular bundles of the inner ring are collateral but at a later stage, they become bicollateral. Eight collateral bundles were observed in the root anatomy of *C. ficifolia*, but bundles were 8 in number and bicollateral in *C. moschata* and *C. argyrosperma*.

The anatomy of the leaves, stems and roots of the five species show great overlap and similarities this could have resulted from the large number of cultivars reported in the genus especially *C. pepo*.

CONCLUSION

The study revealed exciting features that are helpful in the identification of each species. The shape of the midrib is a taxonomic tool and can be used with other anatomical features of the genus in separating the species. The genus can be divided into three groups based on the shape of the midrib. Those with a U-shape are *C. pepo*, *C. argyrosperma* and *C. ficifolia*. *C. maximum*

has a round/flat shape while *C. moschata* has V shape. Those with a U-shape can further be classed into those with a wide and narrow neck. *C. argyrosperma* has a narrow neck with two vascular bundles, four layers of palisade tissues and 6 layers of abaxial epidermis. *C. pepo* has a wide neck with three bicollateral bundles and two layers of palisade tissues while *C. ficifolia* has two bundles and three layers of palisade tissues. *C. maxima* has multicellular glandular trichomes that are 25 celled. Another unique feature of *C. maxima* lamina epidermis is the alternating of trichomes with a single foot with trichomes with a compound foot. The grouping of the domesticated five species into three groups based on the shape of the midrib tallies with that of Teppner (2004) with slight modifications. However, more studies such as biomolecular markings may be done to shed more light on the genus.

AUTHORS' CONTRIBUTION

Concept, collection, establishment of *Cucurbita* species and harvesting was done by Adejoke O. Baderinwa-Adejumo. Anatomical studies, and interpretation, writing of the manuscript was done by Taiwo A. Adenegan-Alakinde. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest related to this article.

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