Comparative foliar micro-morphological study of six cucurbitaceous species

ABSTRACT

A character comparison of the leaf epidermis and petioles of six species belonging to six different genera in the family Cucurbitaceae was conducted with light microscope. Taxonomically useful differences and similarities were recorded across the taxa; and based on this taxa affinity was understood. The useful characters of the leaf epidermis include stomata, trichomes and the epidermal cells. Across the species, paracytic, anomocytic and anisocytic stomatal types were found. All the species are pubescent and trichomes can be unicellular, multicellular and uniseriate, epidermal cell shape and anticlinal wall patterns are also useful but the quantitative data greatly overlap. However, the most outstanding character of the petioles is the number of vascular bundles plates; the number varies from 7-14 across the species but *Luffa cylindrica* and *Momordica cissoides* appear closest with the vascular bundles plates not being above eight in number.

Keywords: Citrus L.; Foliar characters; Nigeria; Petiole anatomy; Taxonomy.

INTRODUCTION

The family Cucurbitaceae are generally known as cucurbits; they are well diverse in the tropical and sub-tropical regions with the Southeast Asia, West Africa, Madagascar, and Mexico as the recognized hotspots in the world [19]. The members are generally edible; not fully exploited though, and they grow in all continents of the world. The family is composed of about 130 genera and 800 species [5]. They are well represented in Nigeria [4, 14].

Comment [w1]: is

The species are mostly prostrate or climbing herbaceous annuals or perennials, they may be variably pubescent and sometimes with tuberous root stock. The leaves are petiolate, exstipulate, alternate and usually palmately veined, simple or sedately compound; often with extra-floral nectarines [6]. The tendrils are lateral to the petiole base, usually 1 to 4 at each node, branched, simple bifid or multi-fid with sensitive or non-spiraling base. They are further characterized anatomically by commonly having angled stems with bicollateral vascular bundles often arranged in two concentric rings.

The anatomical characterization of plants is not affected by environmental changes. Anatomical knowledge has been utilized to delimit species, genera and families in plants. It is widely used in systematic identification, placing anomalous groups in a satisfactory position in classification and explaining patterns of relationship that may have not been clearly expressed in morphological features [20].

The plant morphology and anatomy have been used to delimit the species of Cucurbitaceae and distinguish them from other angiosperms taxa [1].The leaf anatomy has been taxonomically useful in achieving taxa distinction [7]. The focus six species are medicinally useful and they form important components of herbal recipes in Nigeria. Given the nature of condition of the herbal samples available for procurement in the Nigerian herbal markets, the leaves of these species are in fragments. Therefore, because of the regular inclusion of these plants in herbal preparations for the treatment of common ailments in Nigeria such as fever, constipation and insomnia, we undertook this study comparable foliar micro-morphological assessment of the species with a view to document their distinguishing anatomical characteristics for ease of identification even when the leaves are mixed up or fragmentary.

MATERIALS AND METHODS

Time frame: This research was carried out in the University of Lagos, 2017

Sample collections and Plant materials:

The herbarium specimens of five different species of *Lagenaria breviflora* (Jeffrey(Benth.)Rob) FHI0041583, *Luffa cylindrical* (M. Roem) FHI004390, *Momordica cissoides* (Planch ex Benth) FHI100042, *Citrullus colocynthis* (Lin) FHI39007, *Telfairia occidentalis* (Hook.f.) FH0064444 were obtained from the Forestry Research Institute of Nigeria Ibadan (FHI) and fresh specimens *Cucurbita pepo* (Lin) FHI0047290 collected from fields were used for the study.

Preparation of samples for light microscopy:

For leaf epidermal preparation, 5 leaf samples per specimen were examined and 2-6 specimens were studied per species, a representative sample per species is presented in Table 1. Small pieces of each leaf sample (5-8mm^2) taken from a standard central position, usually midway between the apex and the base of the lamina, were soaked in concentrated trioxonitrate (v) acid in capped specimen bottles for about 30min to 72hrs, depending on the nature of the leaf. Specimens that were not well macerated within this period were transferred to water bath at 60^{o}C for 60 minutes and the epidermises were separated using a pair of forceps and dissecting needle. Any mesophyll tissue adhering to the membrane was removed using ultra-soft artist brush and then rinsed in sufficient water. Each epidermal membrane was transferred into 50% ethyl alcohol for 2 minutes in order to harden the cells, stained with Safranin O for 5 minutes and freshly prepared glycerine was used as the mountant. Images of the features were examined under the microscope and carefully evaluated both qualitatively and quantitatively [5, 12, 10].

For petiolar anatomy, dried herbarium specimens were initially soaked in boiling in water so as to rehydrate the tissues and fixed in FAA for 12 hours. Petiole sections of all taxa were obtained using free-hand sectioning method as suggested by Pandey [13]. Transverse sections were taken from the middle part of fully grown petioles and portions taken and stained with Safranin O and then mounted on

glycerin. Cover slips were placed on the slides and ringed with nail polish. The prepared specimens were evaluated under a Zeiss microscope with a digital camera attached to a computer.

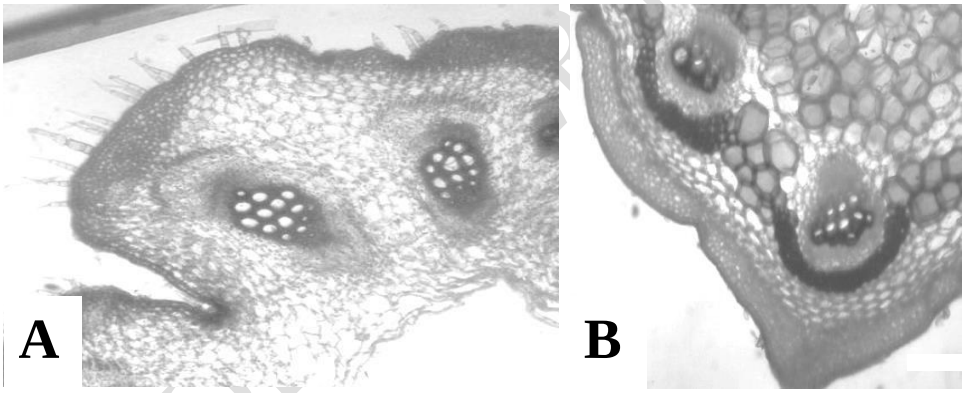
RESULTS

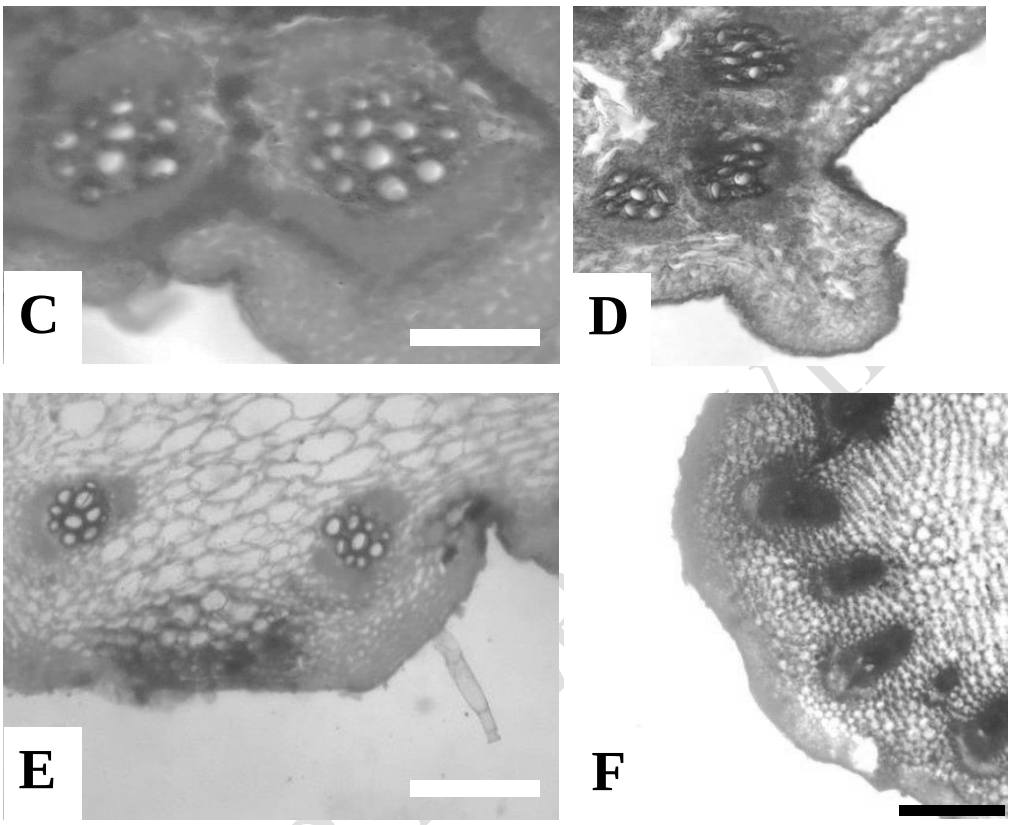
Summary of the findings are presented in Plate 1, Plate 2, Plate 3 while Table 1 and 2 show all the features encountered in the studied taxa. In the six species studied, non-glandular or glandular, uniserate, multicellular or rarely unicellular trichomes were recorded. Some are short stalked with the base consisting of one or more cells while others are long stalked with base containing one or two to many epidermal cells. The unicellular trichomes which are prickle-like are present only in *Telfairia occidentalis*. The multicellular type which may be short, with bulbous base was found in *Lagenaria breviflora*; long trichomes with smooth to thin walls were encountered in *Momordica cissoides* on the abaxial surface. Epidermal cells are usually irregular or polygonal in shape with the latter type being common on the lower epidermis of all species taxa. Quantitatively, the mean epidermal cell lenth ranges from 11.33**µm** to 11.66**µm** in *Momordica cissoides* , 11.03**µm to** 11.72**µm** in *C. colocynthis*, 10.12**µm** to 10.94**µm** in *T. occidentalis*, 10.8µm to 11.23**µm** in *L. cylindrical*. Some species have more epidermal cells on the adaxial surface than abaxial surface, and vice versa in others (Table 2).

The paracytic, anomocytic and anisocytic stomata types are present in the studied species. Paracytic type was found in all the species except *L. cylindrica* where anisocytic was recorded. Mean stomatal number per microscope field varies from **8** in *C. pepo* to **5** in *Telfairia occidentalis*.

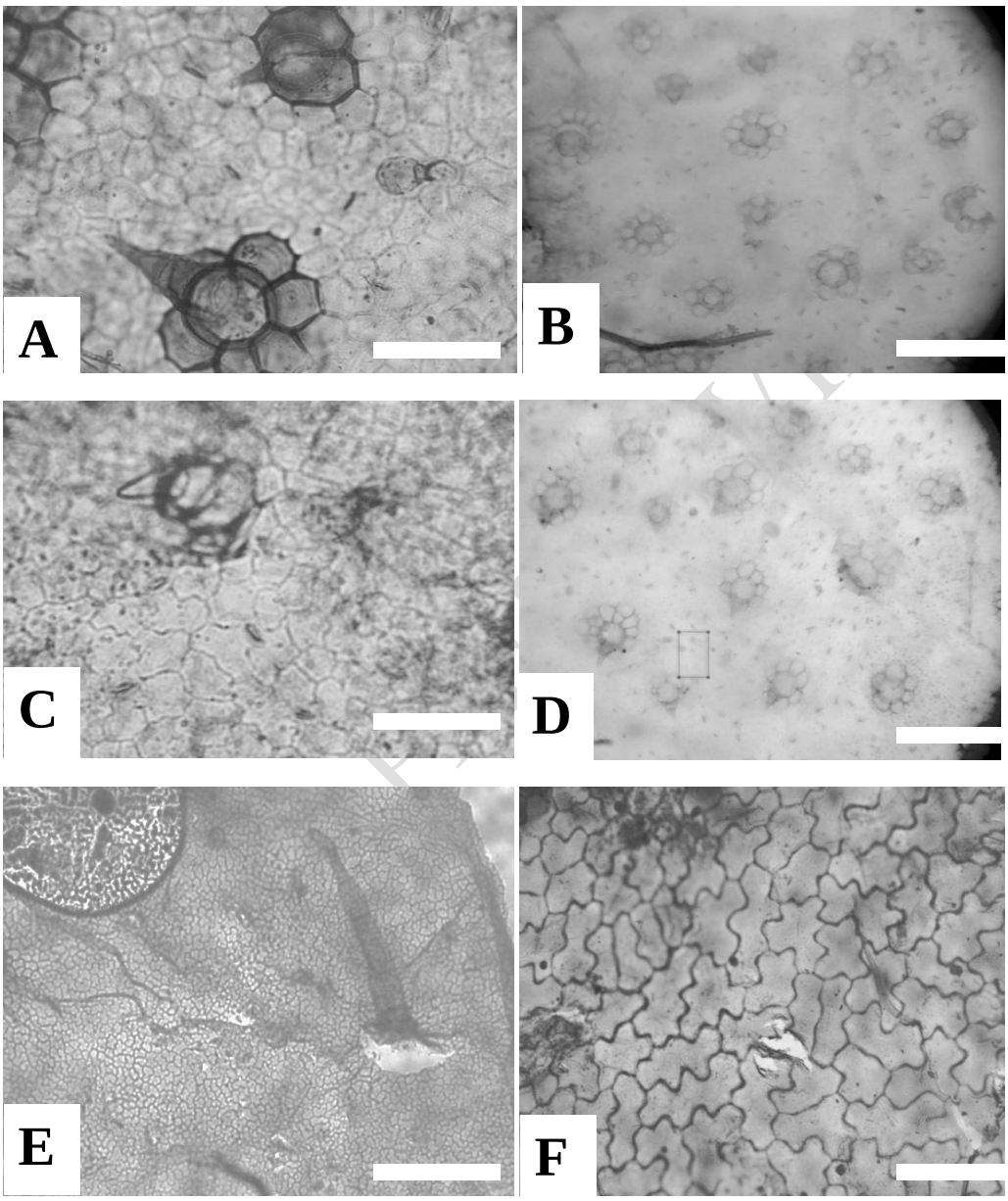
The petioles of all the species have cortical cells in the right order, namely collenchyma, sclerenchyma and parenchyma respectively. Parenchyma has the highest number cell layer while others are 2-4 layered. The petiole surface is pubescent in all the species. But the vascular bundle plates varied from

species to species. In *Cucurbita pepo*, they can be up to 10; *Lageneria braviflora* up to 9; *Luffa cylindrica* and *Momordica cissoides* both have up to 8; *Citrullus colocythes* has up to 7; while in up to 14 were recorded in *Telfairia occidentalis*.



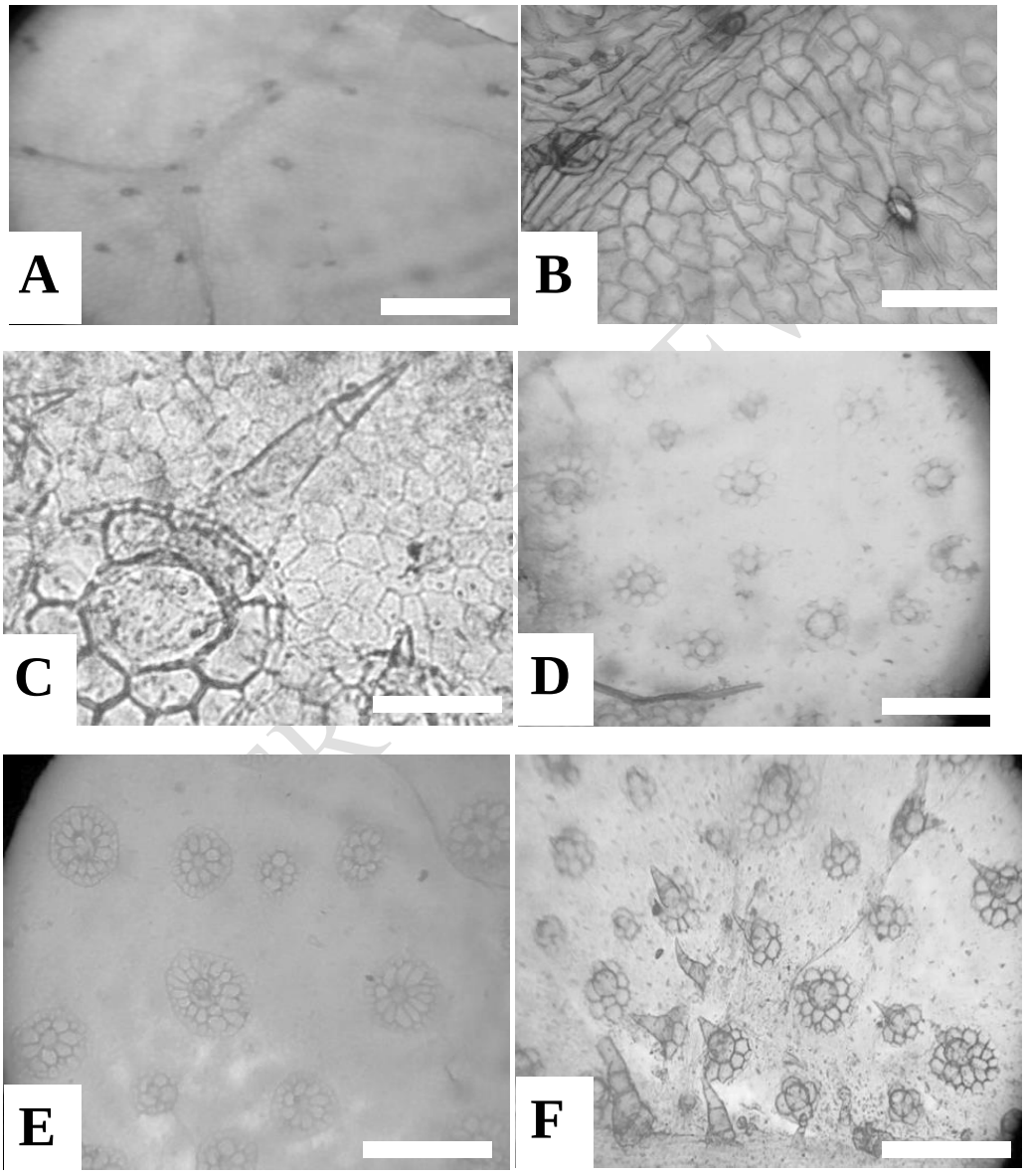


Plates 1: Photomicrographs of petiole in the family cucurbitaceae. (a) *C.pepo* (b) *Lagenaria breviflora*.(c) *L. cylindrica*.(d) *Momordica cissoides*.(e) *C. colocynthis*.(f) *Telfairia occidentalis*



Plates 2: Photomicrograph of leaf surface of (a) *Lagenaria breviflora* adaxial showing trichomes with cone base. (b) abaxial of *L.breviflora.*(c) abaxial of *C. colocynthis*. (d) adaxial of *C. colocynthis* with

presence of both trichomes and trichomes base. (e-f) *Momordica cissoides* abaxial surface showing a trichomes and adaxial.



Plates 3: Photomicrographs of leaf surfaces in cucurbitaceae. (a) showing the adaxial of *Telfairia occidentalis* (b) abaxial of *Telfairia occidentalis.*(c) Adaxial of *Luffa cylindrical* showing long trichomes with base. (d) Abaxial of *Luffa cylindrical* (e). Photomicrograph of leaf surfaces on *Cucrbita pepo.*showing the abaxial of *C. pepo* with trichome base. (f) Showing the adaxial of *C. pepo* with thin trichomes.

Table 1. Summary of the anatomical features of the Petiole of the studied species.

Plant part		Species					
	Features	***Cucurbita Pepo***	***Lageneria braviflora***	***Luffa cylindrica***	***Momordica cissoids***	***Citrillus colocythes***	***Telfiaria occidentalis***
Petiole	Number of vascular bundles	10 plates	9 plates	7 plates	8 plates	7 plates	13 plates
	Trichomes Presen/absent	Present	Absent	Present	Absent	Present	Absent
	Trichomes type	Unicellular multiserate	Absent	Uniserate		Uniserate	Absent
	Sclerenchyma	3cell layers	2-cell layers	3-4cell layers	3-4cell layers	2-cell layers	1-2cell layers
	Parenchyma	Present	1-2 layers	1-2 layers	Present	1-3layers	Present
	Collenchyma	2-cell layers	1-2cell layers	2-layers	1-2layers k	1-2-cellayers	2-cell layers

Table 2. Summary of leaf epidermal of the studied species.

Plant part		Species					
	Features	***Cucurbita Pepo***	***Lagenaria breviflora***	***Luffa cylindrical***	***Momordica cissoids***	***Citrullus colocythis***	***Telfiaria occidentalis***
Leaf	Trichomes type	Multicellular	Unisrate	Uniserate	Multicellular	Uniserate	Unicellular multiserate
	Cell shape	Polygonal	Polygonal	Polygonal	Polygonal	Polygonal	Regular
	Crystal/cell inclusion	Present	Present	Absent	Present	Absent	Present

DISCUSSION

The taxonomic relevance of anatomical characters of six phylogentically related species in Cucurbitaceae is substantiated with features of leaf epidermis and petioles [15]. The trichomes found in the six studied species are uniseriate eglandular (modified basal cell) form. Glandular trichomes with a 4-celled head were identified in *Cucurbita pepo* while only unicellular multiserate trichomes were observed in *Telfairia occidentalis*. Comparatively, unicellular multiserate trichomes were found in both species but the ones in *Cucurbita pepo* are short and thick, while the ones found in *Luffa cylidrica* are long and thin. This is consistent with the different types of glandular and eglandular trichomes that have been studied and described in cucurbits [4, 11]. Variations were also observed in the shape of the epidermal cells. The abaxial cells are irregular, wavy or crenulated while the adaxial cells are more regular in shape. The measurable characters such as epidermal cell size and trichome length overlap significantly, this reflect the infra-familiar closeness of the studied species and their distinct grouping in the family Cucurbitaceae. Measurable characters have been employed by other workers for taxonomic interpretations [9, 11]. However, in the petioles, the ground tissues conform to the existence order but the vascular bundle plates number varied from 7-14 across the species. The variation can be combined with other data for species delimitation and understanding of affinity in the family. *Luffa cylindrica* and *Momordica cissoides* do not have more than 8 vascular bundle plates whereas other species do. Based on these features, an indented dichotomous key is prepared for delimiting the six species.

CONCLUSION

Morphology of plants is an important factor used in making useful taxonomic conclusion about plants but it cannot be solely used. Anatomical feature is also of great importance in taxonomy since they are less affected by environmental factors. In this study, the vascular system of the petiole, the presence of different types of trichome are all diagnostic. The diagnostic features of the petioles of the six species in different genera belonging to the family Cucurbitaceae include the presence of bicollateral vascular bundles and arrangement of the vascular bundles in the rows. However, these features can be used in combination with one another and other separate characters for enhanced identification of the species.

Comment [w2]: the presence of different types of trichome is

REFERENCES

1. Agbagwa I O, Ndukwu B C. The value of morphological anatomical features in the systematics of *Cucurbita* L. (Cucurbitaceae) species in Nigeria. African Journal of Biotechnology. 2004; **3**: 541-546.
2. Edeoga H O, Okoli B E. Midrib anatomy and systematics in dioscoreae. Journal of Economic Taxonomy of Botany. 200; **23:**1 –5.
3. Hutchinson J, Dalziel J M. Flora of West Tropical Africa. Crown Agents for Government, London. 1958; 205-207 pp.
4. Inamdar J A, Gangadhara M. Studies on the trichomes of some Euphorbiaceae. *Feddes Repertorium.* 1977; **88**: 1033-111.
5. Jeffery C. A new system of Cucurbitaceae. Botanical Gazzete. 2005; **90**: 332-335.
6. Jeffrey C. A review of Cucurbitaceae. Botany of Journal Linn Society. 1980; **81**: 233-2479.
7. Kadiri A B. Foliar epidermal morphology of the medicinal genus *Momordica* Linn. (Cucurbitaceae) in Nigeria. Nigerian Journal of Science. 2006; **37**(1): 25-53.

Comment [w3]: correct year

Comment [w4]: delete

8. Kolb D, Muller M. Light, conventional and environmental scanning electron microscopy of the trichomes of *Cucurbita pepo* subsp. *pepo* var. *styriaca* and histochemistry of glandular secretory products. Annals of Botany. 2004; **94**: 515-526.
9. Mauset J D. Plant Anatomy. Menlo Park: Benjamin Cummings. 1988; 568pp.
10. Mbagwu F N, Edeoga H O. Anatomical studies on the root of some *Vigna savi* species (Leguminosae-Papilionoideae) Agricultural Journal. 2006; **1**(1):.8-10.
11. Metcalfe C R, Chalk L. Anatomy of the dicotyledons. Vol 1 and 2 Clarendon Press. Oxford. 1950; 1067-1074pp.
12. Okoli B E, Ndukwu B C. Studies on Nigerian Curcurbita moschata. Nigerial Journal. 1992; **65**(9):15
13. Okoli B E. Anatomical studies in the leaf and probract of *Telfairia* Hooker (Cucurbitaceae). *Feddes Repert*. 1987; **98**: 231-236.
14. Okoli B E. *Fluted Pumpkin, Telfairia: The Under-Exploited Golden Treasure* (With notes on other under-exploited *Cucurbits*). University of Port Harcourt Press: Port Harcourt, Nigeria. 2013;654pp
15. Okoli B E. Anatomical studies in the leaf and probract of *Telferia* Hooker (Curcurbitaceae). *Feddes Repert*. 1987; **98**: 231-236.
16. Olowokudejo J D. Taxonomic significance of leaf indumentum characteristics of the genus *Biscutella* (*Cruciferae*). Folio Geobotanica et phytotaxonomic Praha. 1992; **27**: 1-19.
17. Pandey B P. A Book Text of Botany. Angiosperm Taxonomy, Anatomy 47. Embryology and Economic Botany. New Delhi-India. S. Chand And Co. Ltd. 2006; 208pp
18. Payne W W. Stomatal patterns in embryophytes: Their evolution, ontogeny and Inter prefatio. *Taxon*. 1979; **28**:117-132.

Comment [w5]: complete page numbers.

19. Schaefe H, Renne S S. "Phylogenetic Relationships in the Order Cucurbitales and a New Classification of the Gourd Family (Cucurbitaceae). *Taxon.* 2011; **60**(1): 122–138.

20. Stace C A. *Plant Taxonomy and Biosystematics*: Edward Amold Publishers Ltd.: London. 1980.