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Comparative Foliar Micro-Morphological Study of Six *Cucurbitaceous Species*

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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.

INTRODUCTION

The family *Cucurbitaceae* is 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. 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 and they are well represented in Nigeria [1-5].

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, and alternate and usually palmately veined, simple or sedately compound; often with extra-floral nectarines. 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 [6-8].

According to experts, 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 [9,10]. The plant morphology and anatomy have been used to delimit the species of *Cucurbitaceae* and distinguish them from other angiosperms taxa and in particular, leaf anatomy has been taxonomically useful in achieving taxa distinction. 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 [11-15]. 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 [16-20].

MATERIALS AND METHODS

The herbarium specimens of five different species of *Lagenaria breviflora* FHI0041583, Luffa *cylindrical* FHI004390, *Momordica cissoides* FHI100042, Citrullus *colocynthis* (Lin) FHI39007, *Telfairia occidentalis* 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.

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 30 min to 72 hrs, depending on the nature of the leaf. Specimens that were not well macerated within this period were transferred to water bath at 60°C for 60 min 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 min in order to harden the cells, stained with Safranin O for 5min 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. The adopted approach and terminologies used follow team of expert [21-25].

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 hr. Petiole sections of all taxa were obtained using free-hand sectioning method as suggested by the experts [26-28]. 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 [29,30].

RESULTS AND DISCUSSION

Summary of the findings are presented in Figures 1-3 while Table 1 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 [31-33]. 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 [34,35]. 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*, 10.8 µm 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 1).

The paracytic, anomocytic and anisocytic stomata types are present in the studied species [36,37]. 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 [38-40]. 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* (Figures 1-3) (Tables 1,2).

Figure 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*

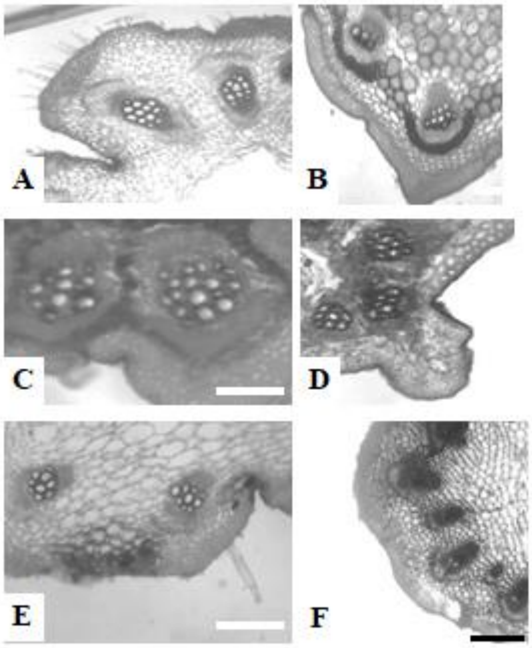

Figure 2. Photomicrograph of leaf surface of (a) *Lagenaria breviflora* adaxial showing trichomes with cone base. (b) abaxial of *L.breviflora*.(c) abaxial of *C. colocynthis*. (d) adaxial of *C. colocynthis* with presence of both trichomes and trichomes base. (e,f) *Momordica cissoides* abaxial surface showing a trichomes and adaxial.

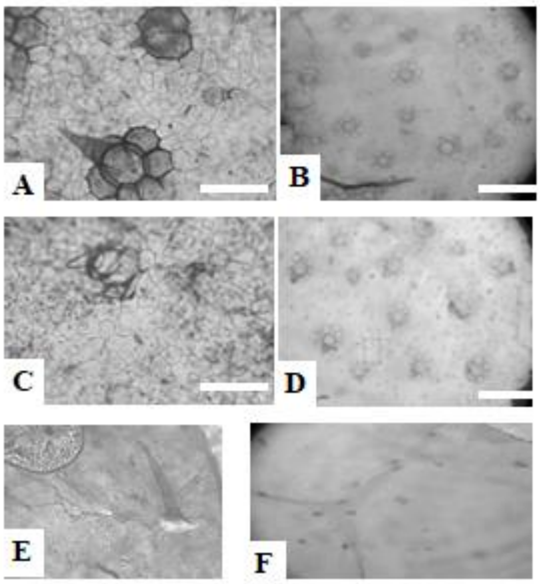

Figure 3. Photomicrographs of leaf surfaces in *cucurbitaceae*. (a) showing the adaxial of *Telfairia occidentalis* (b) abaxial of *Telfairia occidentalis*.(c) Adaxial of *Luffa cylindrical* showing long trichomes with base. (d) Abaxial of *Luffa*

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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.

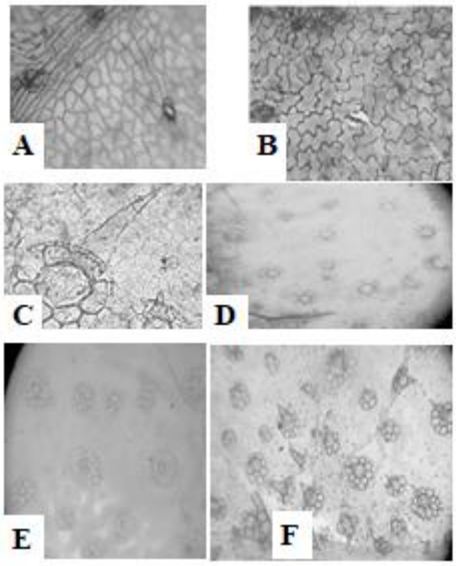

Table 1. Summary of the anatomical features of the Petiole of the studied species.

Plant part	**Species**						
Petiole	**Features**	***Cucurbita* Pepo**	***Lageneria braviflora***	***Luffa cylindrica***	***Momordica cissoids***	***Citrillus colocythes***	***Telfiaria occidentalis***
	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	3 cell layers	2 cell layers	3-4cell layers	3-4 cell layers	2 cell layers	1-2 cell layers
	Parenchyma	Present	1-2 layers	1-2 layers	Present	1-3layers	Present
	Collenchyma	2 cell layers	1-2 cell layers	2 layers	1-2 layers k	1-2-cellayers	2 cell layers

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

	Species					
Features	***Cucurbita Pepo***	***Lagenaria breviflora***	***Luffa cylindrical***	***Momordica cissoids***	***Citrullus colocythis***	***Telfiaria occidentalis***
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

The taxonomic relevance of anatomical characters of six phylogentically related species in *Cucurbitaceae* is substantiated with features of leaf epidermis and petioles [41-45]. 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 [46-50]. This is consistent with the different types of glandular and eglandular trichomes that have been studied and described in cucurbits. 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. 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 [51-54].

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 trachoma is 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.

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