

Morpho-Anatomical Studies of *Gnetum africanum* Welw and *Gnetum buchholzianum* Engl. (Gnetaceae Lindley) in Nigeria

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Author's contribution

This work was carried out by author MGA. She designed the study, wrote the protocol and wrote the first draft of the manuscript. She also managed the literature searches, analyses of the study performed the spectroscopy analysis and managed the experimental process and identified the species of plant..

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ABSTRACT

A comparative study aimed at providing detailed morpho-anatomical information on two species of *Gnetum* found in Nigeria was carried out. The species are of high nutritive importance and taxonomic information on them is scanty. The species are *Gnetum africanum* Welw. and *Gnetum buchholzianum* Engl. These are the only species of *Gnetum* found in Nigeria in particular and Africa in general. *Gnetum*, belonging to the family Gnetaceae, a gymnosperm, is a tropical genus which occurs in Asia, Africa and South America. Members of this genus are mostly climbers, shrubs or small trees. The leaves are borne only on shoots of limited growth and are large, oval, entire, with net venation making the plant look like an angiosperm. Hand sections of the stem, leaf, and petiole were made and photographs taken with a Leitz Diaplan photomicroscope fitted with Leica WILD MPS 52 camera. Features of taxonomic value include presence of accessory transfusion tissue in the leaves, brachysclereids in the stem, leaf and petiole, druses and prisms in the petiole, astrosclereids in the

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stem and leaf. The morphological features of interest include variations in the shape, sizes and colour of leaves and character of the male reproductive parts.

In conclusion, it can be stated based on the result of this work that this two species of *Gnetum* are very closely related anatomically based on the fact that there was little variation in their stem, petiole and leaf anatomy. The work is however not conclusive. More investigations especially in cytogenetics and phytochemistry may provide relevant information to authenticate the classification of these species.

Keywords: *Gnetum africanum*; *G. buchholzianum*; stem; petiole; leaf; morphology; anatomy; sclereids.

1. INTRODUCTION

Gnetum is the sole genus of the family Gnetaceae. It belongs to the order Gnetales, which comprises other two genera namely: *Ephedra* and *Welwitschia*. It is predominantly a tropical evergreen genus of about 30-35 species of gymnosperms occurring in Asia, South America, Africa, specifically Nigeria, Cameroun, Gabon and Angola [1,2]. Members are dioecious, perennial forest climbers, lianes, shrubs or small trees. Members of Gnatales are the only group of gymnosperms that possess vessel elements in the wood, a characteristic found only in the angiosperms. Leaves are borne only in shoots of limited growth and are large, oval, and entire with net venation making the plant look eudicotyledonous. The leaves are oppositely arranged and decussate on the long shoots [3]. The reproductive organ, called the strobilus, are commonly branched, pendulous, and cat-kin, bearing numerous male and female flowers.

In Nigeria, only two species, *G. africanum*, and *G. buchholzianum*, are found, and they have as many names as there are ethnic groups that consume them. The Efik and Ibibio tribes in Akwa Ibom State call them 'afang'; and the Igbos calls them 'Okasi'. They are good for human consumption and for herbal medicine. The leaves are shredded and mixed with palm oil and salt and eaten as vegetable salad by the Igbos in Nigeria [2]. In Akwa Ibom State of Nigeria, the leaves are cooked in soups, in conjunction with other vegetables such as water leaf (*Talinum triangulare* (Jacquin) Willd.), okro (*Abelmoschus esculentus* (L.) Moench), or egusi melon (*Citrullus lanatus* (Thunb.) Mansf). The soup remains the most popular soup in this locality with the exception of 'edikang ikong soup' which is regarded as the soup of soups. The fruits and seeds are edible. They are roasted and consumed.

Because *Gnetum* is very rich in proteins and minerals, it plays a potential role in the fight

against malnutrition in poor rural areas with limited source of meat. Today, dishes based on the leaves of *Gnetum* are prominent on the menu list in many restaurants and special eateries in Europe and U.S.A. In herbal medicine, the leaves are used to treat enlarged spleen, sore throat, and as a cathartic. The species provide an arrow poison antidote. Chopped leaves are used as a dressing on furuncles to hasten maturation. The stem is used in preparations in order to ease childbirth. The plant is highly significant in traditional ceremonies in Nigeria [4-8]. The sclerenchyma deposition, formation and arrangement of vascular tissues, epidermal character differentiation, presence or absence of trichomes, and other anatomical characters have been reported and utilized at various systematic studies, or applied to resolve taxonomic problems [9-14]. This study is aimed at providing morphological and anatomical description of the two species of *Gnetum* for the purposes of taxonomic identification and classification.

2. MATERIALS AND METHODS

The two species of *Gnetum*, *G. africanum* and *G. buchholzianum* studied were collected fresh from different parts Akwa Ibom and Rivers States of Nigeria, as shown in Table 1. Collection of plant materials were made between February, 2013 and January, 2014. Voucher specimens of all the plant materials studied were deposited in the Ignatius Ajuru University of Education Herbarium. In all cases, both male and female plants with strobili were collected. Morphological studies involved visual observation of the vegetative and reproductive parts of the species. Samples were obtained from ten randomly selected plant materials of each species. Quantitative morphological studies included the measurement of the leaf length, leaf width, petiole length and width, and seed length and width, using a meter rule. Qualitative morphological plant features studied include the shape, base, surface, apex, margin, type, venation, phyllotaxy, and petioles of leaf, habit, seed arrangement, sizes and color,

and the positioning of the inflorescence-like strobili.

For anatomical studies, fresh stems, leaves, and petioles were fixed in Formal –Acetic-Alcohol (FAA) for 48 h, washed in several changes of distilled water, dehydrated through alcohol series (30, 50, 70, 95 and 100%), 2 h in each solution and embedded in wax. Transverse sections were made of the proximal, median, and basal portions of the stem and petioles of the two species. Longitudinal sections were made of the median portion of the leaves on each node. This was done for the three nodes in *G. africanum* and two nodes in *G. buchholzianum* that make up the short shoot. All the sections were obtained by free hand sectioning. Sections were deposited in watch glasses containing 1% safranin for one minute. The stained sections were dehydrated through alcohol series and mounted on clean slides in 25% glycerol. Photomicrographs of the anatomical sections were taken with a Leitz Diaplan photomicroscope fitted with Leica WILD MPS 52 camera.

3. RESULTS AND DISCUSSION

3.1 Morphology

Observations of the vegetative and reproductive parts of the species and quantitative measurements made are presented in Fig. 1.

Both species are understorey lianas and are similar but can only be distinguished by the

shape of the leaves and characters of the male reproductive parts. *G. buchholzianum* has thicker and darker green leaves, and the male catkins possess thick internodes which widens towards the apex, while *G. africanum* has relatively thinner and paler green leaves, and the male catkins possess slender internodes which are of equal breadth from the base to the apex.

The leaves of *G. buchholzianum* are broadly elliptical, about 9-17 cm by 4.5-9 cm, base slightly cuneate to rounded, apex abruptly acuminate, entire, simple, glabrous, darker green above, but a little paler beneath, opposite, exstipulate, petiolate, about 1.2 cm long. Inflorescence axillary or terminal on a short branch, often in groups, about 9cm long. Female inflorescence with turbinate internodes and 3 flowers at each node. Seeds are drupe-like, ellipsoid, apiculate, about 9-13 mm by 3.5-7.5 mm, enclosed in a fleshy envelope, with copious endosperm, orange-red when ripe. *G. africanum* has elliptic-oblong leaves, opposite, entire, simple, abruptly acuminate, 11-12 cm by 3.5- 4.5 cm, petiolate, about 1 cm long, and exstipulate. The male inflorescence has flowers that are evenly spaced and close together. Female inflorescence has flowers that possess closely fitted utricle, like an ovary. Seeds have copious endosperm.

So, both species of *Gnetum* were found to share similar morphological characteristics.

Table 1. Sources of *Gnetum* materials studied

Taxa	Collection & Accession No.	Date of Collection	Locality
<i>Gnetum africanum</i> Welw.	Ajuru 055	12/02/2013	Private residence, Abak Town, Akwa Ibom State, Nigeria
	Ajuru 056	25/03/2013	Rumuokoro main market, Port Harcourt, Rivers State, Nigeria
	Ajuru 067	02/06/2013	Rumuolumeni town market, Iwofe, Port Harcourt, Rivers State
	Ajuru 070	13/09/2014	Ozuaha village, Ikwerre LGA, Rivers State
<i>G. buchholzianum</i> Engl.	Ajuru 057	04/11/2013	Oron main market, Akwa Ibom State
	Ajuru 060	15/11/2013	Mile 3 market, Port Harcourt, Rivers State
	Ajuru 065	05/01/2014	Rumuokoro main market, Port Harcourt, Rivers State
	Ajuru 066	10/01/2014	Mile 1 market, Port Harcourt, Rivers State

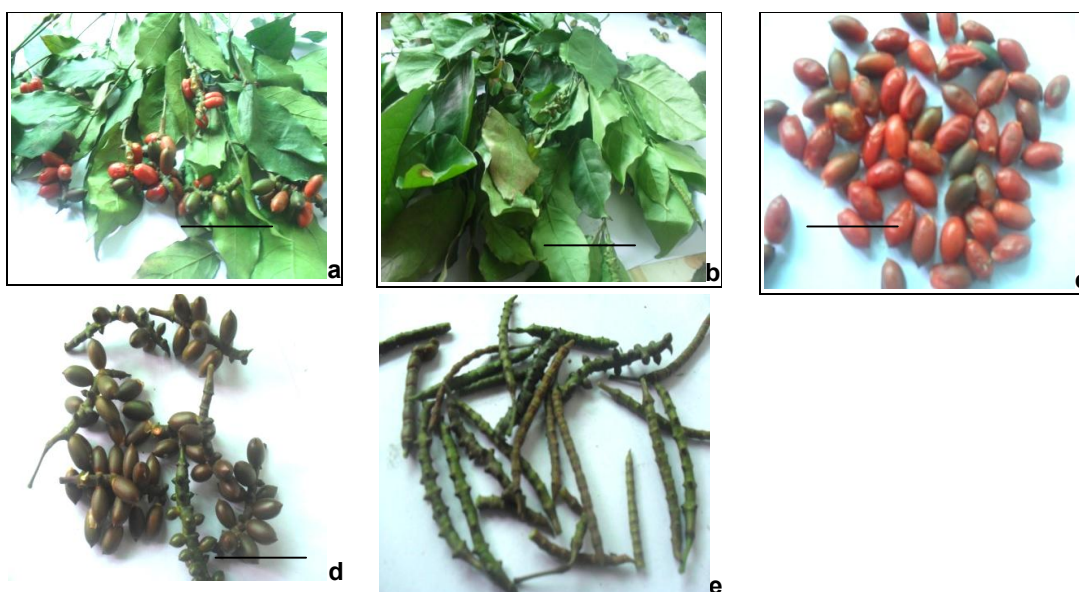


Fig. 1. Showing the morphological characteristics of the two species: a- Leaves and fruits of *G. africanum*; b- Leaves of *G. buchholzianum*; c- Ripe seeds of *G. africanum*; d- Unripe seeds of *G. buchholzianum*; e- The male catkins in *G. africanum*), (Bar: 100 μ m)

3.2 Anatomy

Observations of the anatomical features of all the species made are presented in Plates 6-21

3.2.1 Stem anatomy (transverse section)

3.2.1.1 The male plants

The proximal internode sections in the two species showed a superficial covering layer, the epidermis, enclosing a primary cortex. Few rows of cells directly beneath the epidermal layers are differentiated into a hypodermis consisting of sclerenchyma and collenchyma cells. In the cortex are embedded stone cells occurring either singly, in twos or threes. An endodermal layer is obvious enclosing the phloem tissue beneath the pericycle and the xylem tissue already showing signs of secondary thickening. Vascular tissues are in bundles forming a ring enclosing the pith. Medullary rays are also present. Metaxylem has a lot of vessels. The protoxylem is encroaching on the pith area which is filled with thin walled parenchyma cells, some of which have been squashed.

The median internode section in *G. africanum* showed more secondary tissues produced especially in the xylem. The medullary rays were also obvious. The metaxylem is made of fibres,

tracheids, and vessels. The pith is also filled with parenchyma cells near the periphery while the central portion is filled with thin walled cells, some of which have been squashed.

The basal internode sections of both species showed that the endodermis is no longer obvious, but the cambial ring and medullary rays are conspicuous (Fig. 2).

3.2.1.2 The female plants

In both species, the female plants was similar to the male plants in all the internodes, except that sclereids occur more in the female plants than in the male plants (Fig. 3)

3.2.2 The petiole (transverse section)

3.2.2.1 The male plants

The proximal nodes in the two species showed a conspicuous groove on the adaxial surface, which is absent in the female plants. The epidermis is enclosed by a thick cuticle. The broad cortex is composed of two distinct types of cells: sclerified parenchyma cells with small intercellular air spaces, then large thin walled parenchyma cells with prominent air spaces. Numerous sclereids are scattered throughout. The cells are also filled with druses and prisms.

There are four vascular bundles in the two species, made up of xylem which consist of vessels arranged in a single crescent shape with the concave side of the crescent facing the upper

epidermis and the convex side the lower epidermis. Brachysclereids occur between some bundles. The sclereids increase in occurrence towards the base of the short shoot (Fig. 4).

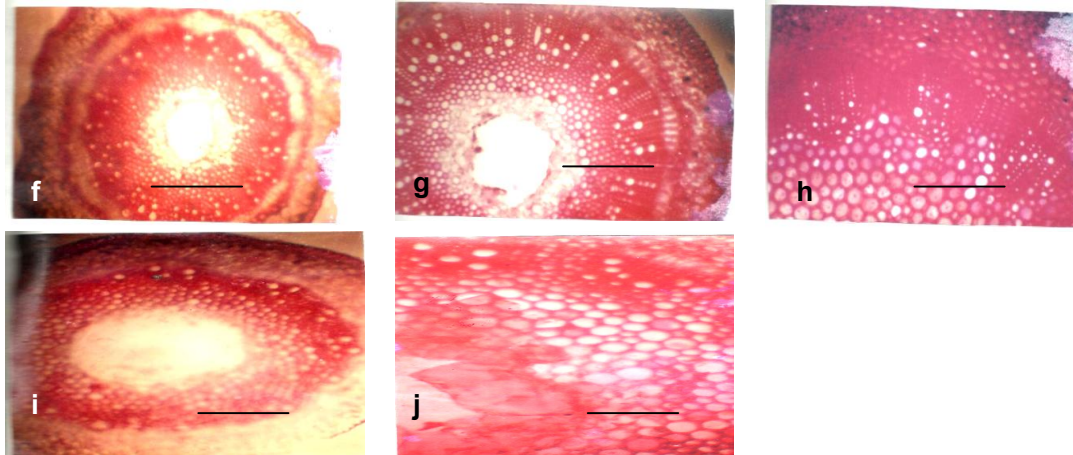


Fig. 2. Showing the stem anatomy in the two species, f- first internode of the male plant of *G. africanum*; g- second internode of the male plant of *G. africanum*; h- third internode of the male plant of *G. africanum*; i- first internode of the male plant of *G. buchholzianum*; j- second internode of the male plant of *G. buchholzianum* (Bar: 100 μ m)

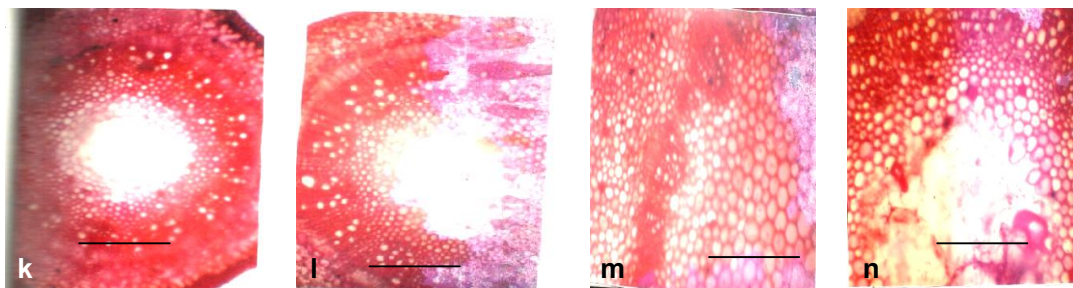


Fig. 3. Showing stem anatomy of the female plants, k- First internode of the female plant of *G. africanum*; l- second internode of the female plant of *G. africanum*; m- third internode of the female plant of *G. africanum*; n- the female plant of *G. buchholzianum* (Bar: 100 μ m)

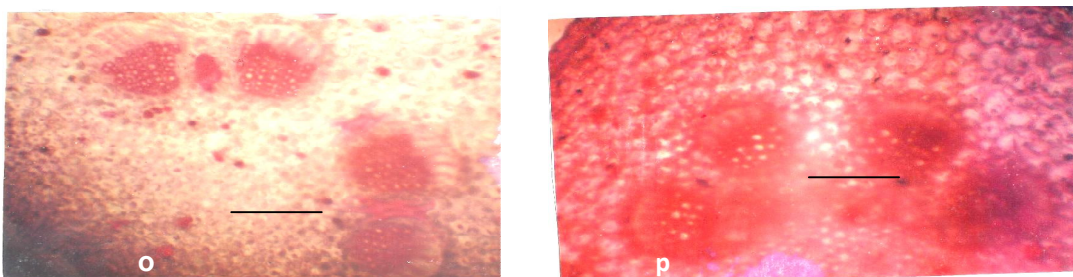


Fig. 4. Showing the petiole anatomy of the two species, o- the male plant of *G. africanum*; p- The male plant of *G. buchholzianum* (Bar: 100 μ m)

3.2.2.2 The female plants

The petiole anatomy of the female plants on the whole is similar to that of the male although more sclereids were observed (Fig. 5).

3.2.3 Leaf Anatomy (Longitudinal Section of Lamina)

3.2.3.1 The male plants

The epidermal layer in the two species are covered by a thick cuticle. Beneath the epidermis is a hypodermal layer of collenchyma cells. The

mesophyll consists of palisade and spongy mesophyll enclosing a transfusion tissue. Sclerenchyma cells surround the vascular bundles as a bundle sheath. There are two vascular tissues, the xylem occurs towards the abaxial surface. Brachysclereids were also observed (Fig. 6).

3.2.3.2 The female plants

The sections of the female plants were similar to the male leaf, except that there were three vascular bundles. Astrosclereids were also present (Fig. 7).

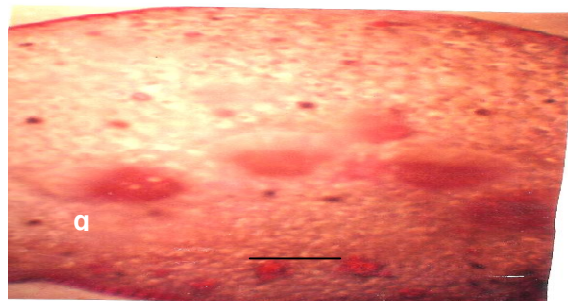


Fig. 5. q- Petiole anatomy of the female plant of *G. africanum* (Bar: 100 μ m)

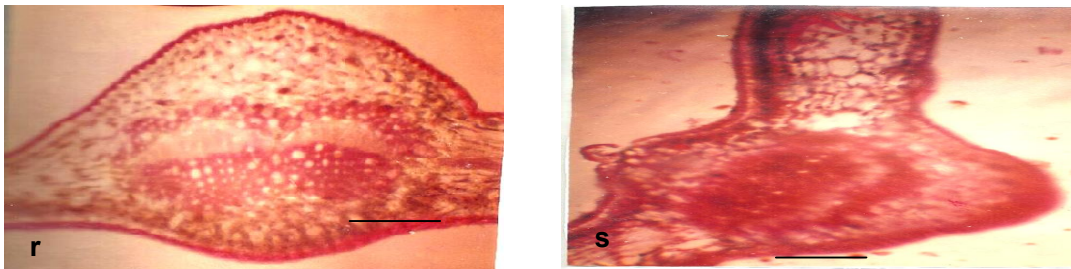


Fig. 6. Showing the leaf anatomy of the two species, r- Leaf anatomy of the male plant of *G. africanum*; s- Leaf anatomy of the male plant of *G. buchholzianum* (Bar: 100 μ m)

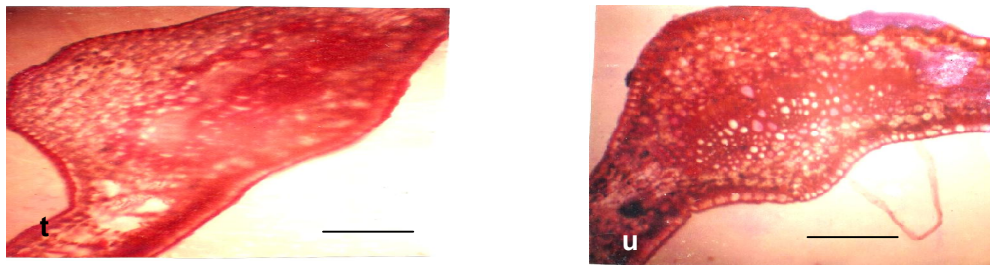


Fig. 7. Showing the leaf anatomy of the female plants of the two species, t- leaf anatomy of the female plant of *G. africanum*; u- leaf anatomy of the female plant of *G. buchholzianum* (Bar: 100 μ m)

The presence of a superficial layer, the epidermis, is as reported by [9] on the general anatomy of stems in plants. The report made by [15] that the pith is usually destroyed during secondary growth and that this destruction occurs only in the internodes of plants was confirmed in *Gnetum*. The presence of an epidermal layer and a sclerified hypodermis has also been reported by [15] in Pine needles. The mesophyll which is differentiated into the palisade and spongy mesophyll is found as reported in *Cycas*, a gymnosperm, by [15]. Druses and prism crystals observed in this work are confirmed by [15] in *G. gnemon* and *G. indicum* respectively.

The groove in the petiole of the male plants, and the number of vascular bundles in the leaves, stand out as the only difference between the male and female plants of both species.

Taxonomic information on this highly nutritive and medicinal genus is scanty with genus considerably involved in cross-border trade across Africa, and the resource base been seriously threatened by unsustainable harvesting methods and the gradual disappearance of the forests in which they thrive. Therefore, the results of this study will help in filling the gap in the systematics of this genus, and enhance their sustainability through conservation, genetic improvement, and *ex-situ* management.

4. CONCLUSION

Based on the result of this work, these two species of *Gnetum* are very closely related morphologically and anatomically based on the fact that there was little variation in their stem, petiole, and leaf anatomy. The work is however not conclusive. More investigations especially in cytogenetics and phytochemistry may provide relevant information to authenticate the classification of these species.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Dutta AC. Botany for degree students, 5th Ed. Oxford University Press, New Delhi. 1979;681-685.
2. Eyo ES, Mohme, Abel JJ. Chemical composition and amino acid content of *Gnetum africanum*, *Heinsia crinata* and *Piper guineensis*, Nigerian Journal of Nutritional Science. 1983; 4(1):57-62.
3. Hutchinson J, Dalziel JM. Flora of West Tropical Africa 2. Crown agents for Oversea Government and Administration, London. 1966;330.
4. Hutchinson J, Dalziel JM. Flora of West Tropical Africa. Crown agents, London. 1954;220.
5. Burkill HM. The useful plants of west tropical Africa, ed. 21. Families A-D, Royal Bot. Gardens, Kew. 1985;960.
6. Carlquist S, Robinson AA. Wood and bark anatomy of the African species of *Gnetum*, Botanical Journal of the Linnaen Society. 1995;18(2):123-137.
7. Okafor JC. Strategies for enhancement of utilization potential of edible woody forest species of South-Eastern Nigeria. In: Maesen LGH, editor. The Biodiversity of African Plants: Proceedings. Kluwer Academic Publishers. 1996;684-695.
8. Hmialoundama F. Tropical forests, people and food: Biocultural interactions and Applications to development. In: Hladik C, editor. Man and the Biosphere Series. 1993;13:177-181.
9. Metcalfe CR, Chalk L. Anatomy of the dicotyledons, 2nd Edn. Clarendon Press, Oxford. 1979;276.
10. Stace CA. Plant taxonomy and Biosystematics. Edward Arnold (Publishers) Limited, London. 1980;113-129.
11. Ndukwu BC, Okoli BE. Studies on Nigerian *Cucurbita moschata*. Nigerian Journal of Botany. 1992;5:18-26.
12. Ajuru MG. Anatomical studies of some species of the genus *Amaranthus* L. in Nigeria. Journal of Science and Technology. 2012;5(8):407- 419.
13. Ajuru MG, Okoli BE. Comparative Vegetative Anatomy of some Species of the Family Cucurbitaceae Juss. in Nigeria. Research Journal of Botany. 2013;8(1): 15-23.
14. Ajuru MG, Okoli BE. Morphological and epidermal studies on certain species of *Napoleona* P. Beauv.

(Lecythidaceae) in Nigeria. International Journal of Modern Botany. 2012;2(4):115-119. 15. Esau K, Plant Anatomy. John Wiley and Sons Inc., New York. 1965;767.

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