

Original Research Article

Anatomical and Palynological Study of *Lasianthera africana* P. Beauv (Icacinaceae)

ABSTRACT

This study examined *Lasianthera africana* P. Beauv based on, anatomical and palynological parameters. The anatomical studies were carried out with freehand sectioning. The species is hypostomatic, the epidermal cells are ~~are~~ irregular, with undulating anticlinal walls. The stomata types are anomocytic, and anisocytic. The midrib has bicollateral vascular bundles and the petiolar vascular bundle is arced with rib traces on both sides of the wings. Calcium oxalate and tannin occur ~~red~~ in the lamina, midrib, petiole and stem. The pollen grains are smooth, intectate, with thick exine, with tricolporate aperture, and with an equatorial diameter of 21.5-25.8 µm. These features are diagnostic and confirm the placement of this species in Icacinaceae.

Keywords: Calcium oxalate, *Lasianthera africana*, taxonomy, pollen grain.

1. INTRODUCTION

Lasianthera africana, natively called "Editan" in Akwa Ibom state is a perennial shrub of about 4m in height [1] pls other diagnostic features.. It was the type species of the *Lasianthera* in Apocynaceae (pls cite flore d'Oware). It occurs in tropical rain-forest and coastal vegetation such as of Guineo-Congolian African, that includes..(Nigeria..pls add citation). certain species occur in the coastal. Forest anatomical and palynological characters play a relevant role in plant identification. For instance, a anatomical characteristics are important for the formulation of phylogenetic and phonetic groups [2-6] The use of

Comentado [A01]: Its important talk about external morphology. Are there characters that help put L. Africana in Icacinaceae and get out of Stemonuraceae? You should include published results in introduction and discussion. *Lasianthera* is morphologically less representative. *Lasianthera* has, for example, fused petals and an African distribution. Moreover, *Stemonurus* is one of two genera with more than a handful species as well as a rather wide southeast Asian and Malesian distribution. Jesper Kårehed 2001

Comentado [A02]: There is nothing about in the text...What means? L. Africana is placed in Stemonuraceae. If the aim of the paper is replace this species in Icacinaceae you must provide taxonomic history, data, tables showing it is an Icacinaceae

Comentado [A03]: Needs deep modification
1 *Lasianthera* is a motipic genera of Stemonuraceae, L. Africana is the type species
Diagnostic features of species
2 Occurrence area (it is confuse)..Guineo-Congolian is a biogeographic term..pls explain
3 Taxomic history of L Africana with changes of family..citing papers, since the description as Apocynaceae until the placed in Stemonuraceae
4 Why it was placed in this family, what carcters are discordanting with Stemonuraceae and concordant with Icacinaceae.
5 Importance of anatomy/palynology to circumscribe families focusing Icacinaceae family/genera
6 aim of study..is verify if the actual classification agree with anatomical and palynological features of Stemonuraceae or indicates that should be replaced in Icacinaceae

Comentado [A04]: Where is this place? Which country? Many people don't know states of Africa..pls add a map

Comentado [A05]: The references are in alphabetic order..I could not check the citations.

information of anatomy ~~such as pollen~~, petiole, stem, and leaf in taxonomic delimitation however restricted, has been documented [7-10]. Estimable taxonomic evidence has been acquired from the pollen, leaf, stem, epidermis, and stomata. Some of these anatomical characteristics are so diagnostic that they are now usually utilized in routine plant identification, rather than being confined to a used in phylogeny or classification or the identification of fragment of a plant [11]. The relevance of palynological information has been stressed by several researchers in the family Cruciferae. The pollen exine ornamental plays important role in the delimitation of some closely related taxa in Cruciferae [9]. This study is to re-examine *L. africana* **taxonomy** concerning anatomical and palynological data obtained in Akwa-Ibom State, Nigeria.

Comentado [A06]: There is not any reference to taxonomic history of the species..

2. MATERIALS AND METHODS

2.1 Source of plant materials: The samples were gotten from different wild and cultivated locations in Akwa Ibom State, Nigeria (Table 1 and Figure 1). The samples were ~~authenticated~~ identified? at the University of Port Harcourt herbarium (abbreviation following index herbariorum), ~~and~~ Anatomical studies and palynological studies were done in xx and xx laboratories of Port Harcourt University.

Table 1: Location of areas of Sample Collection

S/N	Collection No.	Location	Date	Geographical coordinates
1	Okon 001	Aya, Ikot Ekpene, Akwa Ibom State	20/6/2017	Lat. 5.18° North Long. 7.71° East
2	Okon 002	Oku Iboku, Itu L. G. A., Akwa Ibom State	03/06/2017	Lat.5.24° North Long. 7.44° East
3	Okon 003	Ibiono	04/06/2017	Lat. 5°12'25.34" North Long. 7.53'35.12" East
4	Okon 004	Ikot Abasi	06/06/2017	Lat.4°36'20.92" North Long. 7°37'27.84" East

finger on both hands and making angular tear across the lamina of the exposing epidermal peels of both surfaces. The translucent epidermal peels of about 4mm× 5mm were cut off and fixed in a separate bottle containing 95% ethanol for 24hours for a clear epidermis, and these samples were rinsed with distilled water and stained with safranin. The stained samples were mounted on a slide containing a drop of glycerine. Photomicrographs were taken from Leiz-Habolux-12-microscope filled with a Wild-MPS camera.

2.4 Palynological studies: Fresh mature flower buds were fixed in ethyl alcohol (96% ethanol) to separate the other flower parts which could be separated inside distilled water. Flower buds were transferred into test tubes; the pollens were dried in a thermostat and wetted with an acetolysis mixture (acetic anhydride and concentrated sulphuric acid in a 9:1 ratio) repeatedly. After this, the test tubes were placed together with granules and acetolysis mixture in a 70°C water bath (for 5seconds). The granules (pollen) were centrifuged and cleaned with distilled water several times. The pollens were placed on a slide that contains a drop of glycerine and observed using a microscope.

Comentado [A09]: Add citations

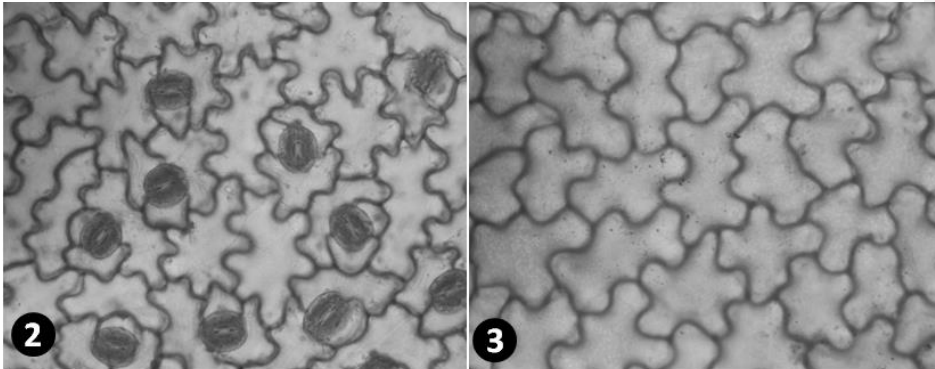
3. RESULTS

~~Results of the anatomical studies of the stem, petiole, and leaf including pollen morphology of *L. africana* are shown on (Figs. 2 – 7).~~

3.1 3.1 Leaf blade

3.1.2 Epidermal featuresparadermic section: The leaf of this plant species is hypostomatic (stomata are only on the abaxial and surfaces). The abaxial and adaxial cells are irregular with undulating anticlinal walls (Fig. 2 and 3). The stomata on the abaxial leaf surfaces are anisocytic, and anomocytic. The predominant stomata type is the anisocytic type

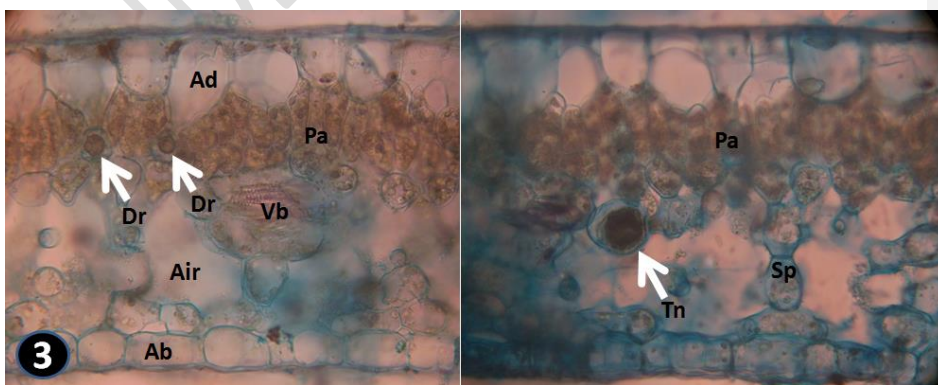
while the other two are few and the anomocytic stomata occurred mainly on the leaf veins. The stomatal guard cells are oval.



Figures 2 – 3: Epidermal peels paradermic sections of *L. africana* (1) abaxial epidermis and (2) adaxial epidermis showing..

3.2- Leaf blade Lamina: The cross-section of *L. africana* leaf lamina showed a layer of oval and anticlinal elongated adaxial epidermal cells with cylindrical and periclinal elongated abaxial epidermal cells (Fig. 4). The palisade mesophyll comprised 2 – 3-layers of palisade chlorenchyma, followed by while the spongy mesophyll-chlorenchyma has with 3 – 4-layers with intercellular air spaces.

Comentado [AO10]: You should provide better and standardized anatomical description since epidermis until vascular bundles for petiole, leaf blade, midrib and stem

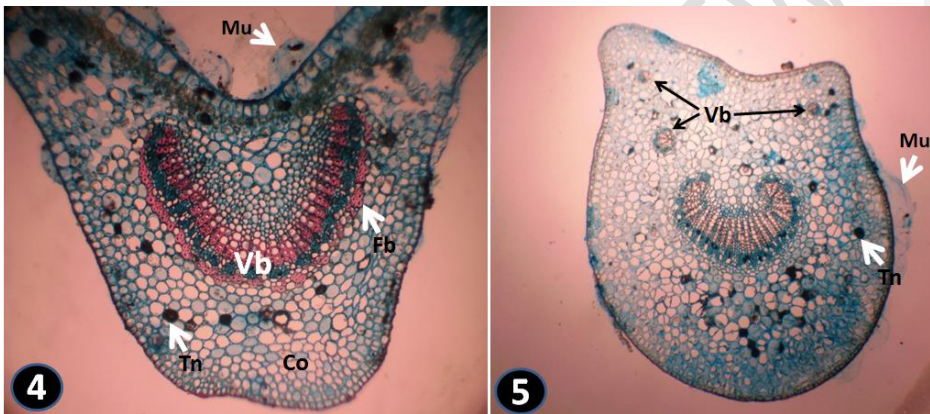


Figures 3: Transverse section of *L. africana* leaf lamina, Vb –vascular bundles, Ad – adaxial

Comentado [AO11]: Pls add scale and if it is possible an image of the druse with greater magnification

epidermis, Tn – tannin, Ab – abaxial epidermis, Sp – spongy ~~chlorenchyma~~ ~~mesophyll~~, Pa – palisade ~~mesophyll~~ ~~chlorenchyma~~, Dr – druses, Air – intercellular air spaces

3.3 Leaf Midrib: ~~*L. africana* midrib~~ transverse section showed a V-shaped adaxial outline and a circular or U-shaped abaxial outline (Fig. 4). The vascular bundle is collateral, U- or V-shaped, with a continuous layer of fibre or sclerenchymatous cells of the outer region. The adaxial cortex has 5 – 13-layers of parenchymatous cell while the abaxial cortex has 7 – 12-layers.



Figures 4 – 5: Transverse section of *L. africana* (4) midrib and (5) petiole: Vb –vascular bundles, Co – cortex, Ad – adaxial epidermis, Tn – tannin, Fb – fibre, Mu – mucilage.

Comentado [AO12]: Pls add scale

3.4 Petiole: ~~*L. africana* petiolar~~ transverse section showed a semicircular vascular bundle with rib traces on both sides of the main vascular bundle (Fig. 5). The adaxial outline is concave while the abaxial outline is circular. The cortex is made up of mainly multiseriate parenchymatous cells, which are mainly oval but partly semicircular.

3.5 Stem: ~~*L. africana* stem transversal~~ section is oval with two protrusions and uniseriate epidermis supported by about 8 – 12-layers of the parenchymatous cortex. The narrow pith consists of angular to isodiametric cells ~~and is narrow~~ (Fig. 6).

3.6 Calcium oxalate, mucilage, and tannin distribution: The occurrence and distribution of calcium oxalate crystal and tannin varied in the different parts of the plant. Also, ~~only~~ druse crystals ~~occurred in *L. africana* and~~ were observed in the palisade mesophyll (Fig. 3). Mucilage was observed in the petiole and midrib (Figs. 4 and 5). Tannin occurred in the midrib, petiole, and stem. In the midrib, it was observed in the ~~epidermal cells (Fig. 4), in adaxial palisade clorenchyma?, and in abaxial cortical cells, and adaxial palisade, and epidermal cells (Fig. 4), in the cortical and~~ In the petiole tannin was observed in phloem cells of the petiole (Fig. 5). In the stem and it was observed in the phloem cells and in the cells of the pith and phloem cells in the stem (Fig. 6B). ~~The vessels are solitary, imperforate tracheary, and diffused~~

Comentado [AO13]: Which parts?

Comentado [AO14]: Vessels of stem or leaf? Pls replace this information

Comentado [AO15]: I think its better add this information for each part of the plant..leaf blade, leaf midrib, petiole and stem

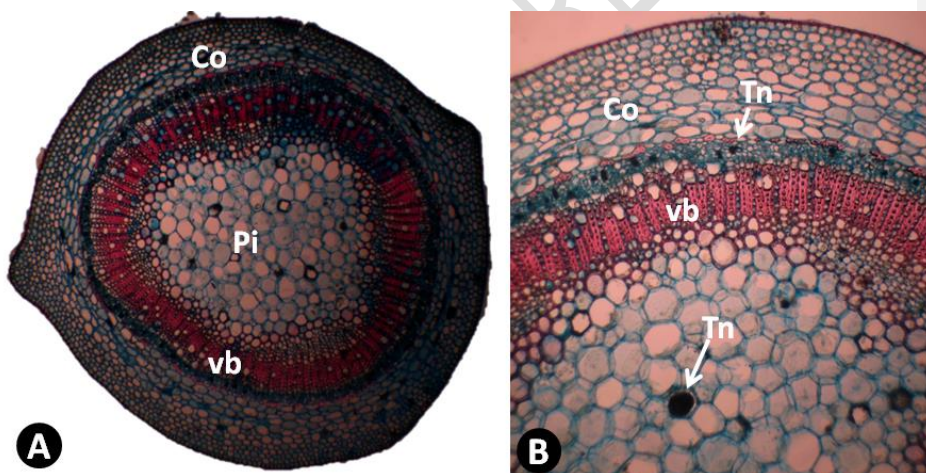


Figure 6: Stem anatomical features of *L. africana*: Pi – pith, Vb – vascular bundles, Tn – tannin, and Co – cortex.

3.7 Pollen morphology: ~~The morphology of the pollen of *L. africana*~~The exine showed a smooth, intectate and thin exine. The aperture type is tricolporate. ~~These species discharge their pollen by a non-explosive mechanism. The pollination is either by insects or wind.~~

Comentado [AO16]: You must include SEM images to describe exine

Comentado [AO17]: This is not your result

From the equatorial view, the species are oblate in shape, while from the polar view is

circular. The surface pattern is reticulate. The walls of the pollen are sculptured. The grains are present as a monad. The equatorial diameter is 21.5 - 25.8µm (Fig. 7).

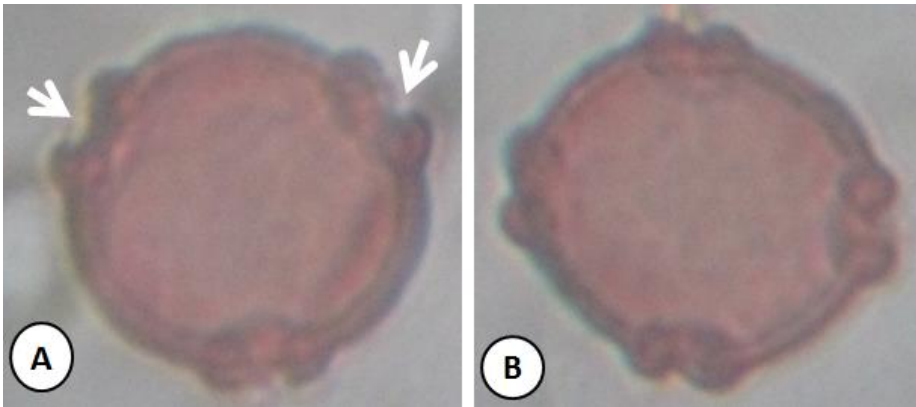


Figure 7: Pollen of *L. africana* (A) Polar view and (B) equatorial view, arrows show aperture

4. DISCUSSION

Morphological features and leaf epidermal characters are of taxonomic importance and have been commonly used for the classification of taxa in different plant families [13-15].

The members of Icacinaceae have been reported to have different stomata types such as paracytic and diacytic in *Icacina* [16], anisocytic, anomocytic, cyclocytic, and paracytic in Icacinaceae outside Malesia [17], anomocytic and cyclocytic in the genera *Apodytes*, *Cassinopsis*, and *Pyrenacantha* [18], and anisocytic in *L. africana* [18]. Also, in the genus *L. africana*, [19] reported the anticlinal walls in the forest variants are undulating while anticlinal walls in the variants that grow in the riverine areas are straight to wavy. In our study, ~~on specimens of~~ *L. africana* from some parts of Akwa Ibom State ~~we recorded~~ showed anomocytic, and anisocytic stomata, with anisocytic type being more frequent. All the specimens studied are hypostomatic and the shape of the epidermal cells are irregular

Comentado [AO18]: How is the stomata in Stemonuraceae?
This character helps to recognize *L. africana* as Icacinaceae?

with undulating anticlinal walls. Our findings corroborate the previous reports on this species (citation of previous reports).

The differences in shape, size, aperture, polar unit, symmetry, and wall sculpture of pollen

have been utilized by numerous authors in the delimitation of various taxa [20]. ~~These~~

~~pollen features are also of taxonomic value. Olowokudejo and Nyananyo [21] also use the~~

~~seed coat morphology and other palynological characters of *Talinum* and *Calandrinia* to~~

~~produce a more acceptable classification among these taxa. Edeoga and Ikem [22] also~~

~~proved that *Boerhavia coccinea* is characterized by tricolporate pollen grains while~~

~~*Boerhavia erecta* and *Boerhavia diffusa* have alcopate pollen grains. This means that *B.*~~

~~*coccinea* could be distinguished from other collections of *Boerhavia* in Nigeria based on~~

~~pollen features. Nyananyo [23] and Mbagwu and Edeoga [24] have utilized pollen features~~

~~to confirm true evidence of the relationship among certain groups of flowering plants in~~

~~Nigeria.~~—The result of palynological studies showed that the morphology of the pollen of *L.*

africana is tectate, with thick exine, tricolporate aperture, and the equatorial diameter is

21.5-25.8 μm . ~~These species discharge their pollen by a non-explosive mechanism. The~~

~~pollination is either by insects or wind. Results from these lines of evidence confirm the~~

placement of this species in Icacinaceae.

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Comentado [AO19]: You must focusing the discussion on Icacinaceae x Stemonuraceae members..how are the pollen grains in the families? Why your results indicate that *L. africana* should be placed in Icacinaceae? Why don't you cite palynological studies including pollen of these families? Example Schori 2010, Dahl 1952 (that study *L. africana* as Icacinaceae member..)

Comentado [AO20]: Seed coat not is a palynological character

Comentado [AO21]: Has Stemonuraceae members an explosive mechanism..?

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Comentado [AO22]: It is the base of your paper..the aim is discuss why L. Africana was removed of Icacinaceae and why it should remains.

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