Original Research Article

Anatomical and Palynological Studied 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 occurred 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]. It occurs in tropical rain-forest such as guinea-Congolan African, certain species occur in the coastal. Forest anatomical and palynological characters play a relevant role in plant identification. For instance, anatomical characteristics are important for the formulation of phylogenetic and phonetic groups [2-6] The use of 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.

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 at the University of Port Harcourt herbarium and anatomical studies and palynological studies.

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	206/2017	Lat. 5.18° North Long. 7.71° East
2	Okon 002	Oku Iboku, Itu L. G. A., Akwa Ibom State	03/06/2017	Lat.5.24^0 North Long. 7.44^0 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

2.2 Stem, petiole, and midrib anatomical studies: Collected samples of the stem, petiole, and midrib of about 10cm in length and 6mm in diameter were cut and fixed in formalin acetic acid and alcohol (FAA) of 1ml: 1ml: 3ml respectively for 24hours. After this, samples

were passed through a series of different alcoholic concentrations of 50% and 70% [12] for 3hours and 24hours respectively. Samples were infiltrated, embedded in paraffin wax and a section was made. The transverse sections obtained were stained with Alcian blue, countered stained with Safranin, and mounted on glycerine. All slides were examined under a microscope.

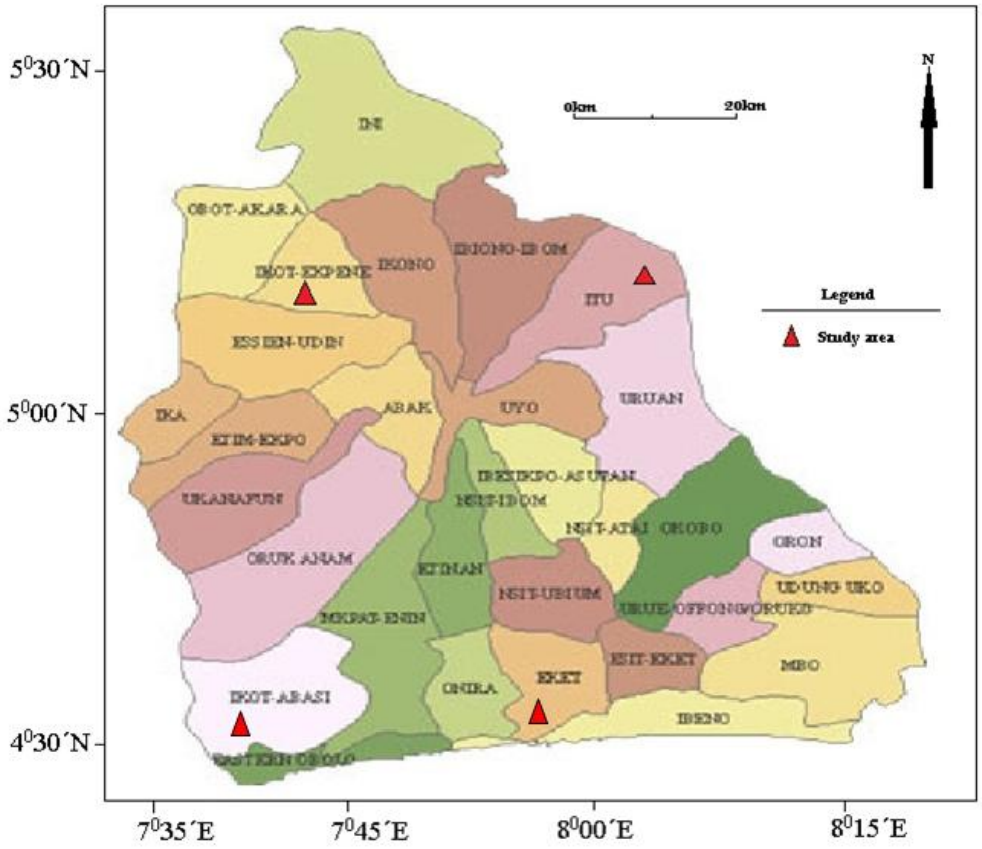


Figure 1: Map of Akwa Ibom State showing the Local Government Areas sampled.

2.3 Epidermal peels: The fresh leaves of both abaxial (lower) and adaxial (upper) part of *L. africana* were obtained by holding portions of the leaf between the left thumb and index 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

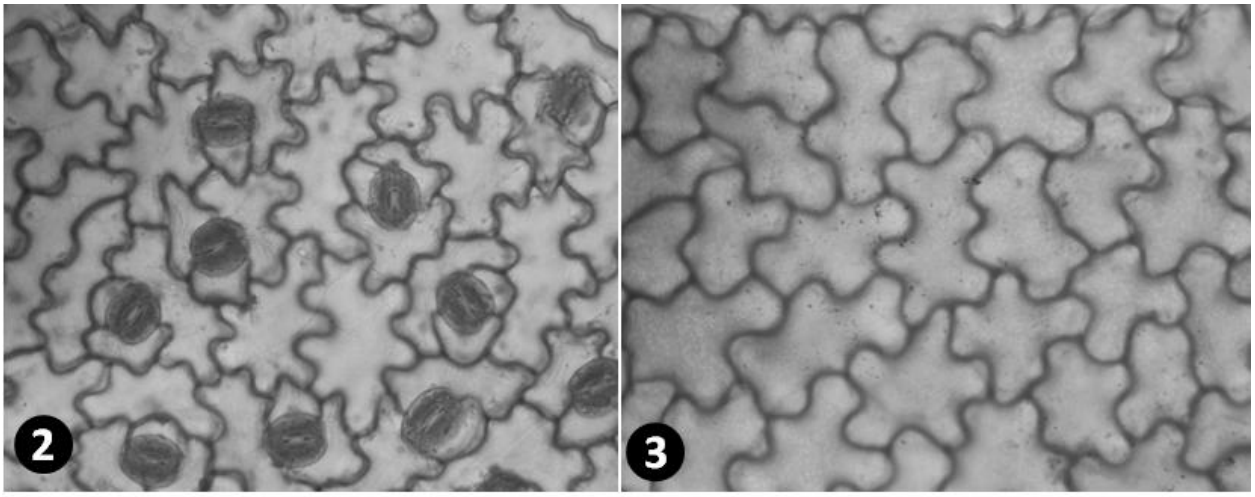
samples were mounted on a slide containing a drop of glycerine. Photomicrographs were taken from Leiz-Habolux-12-microscope filled with a Wild-MPS camera.

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^{o}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.

3. RESULTS

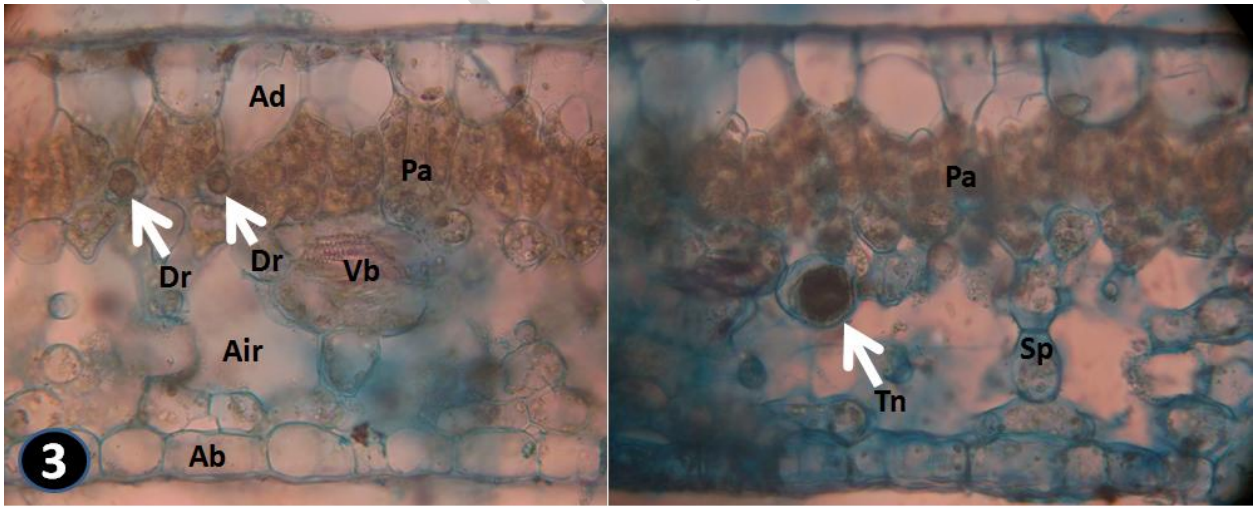
Results of the anatomical studies of ~~the~~ stem, petiole, and leaf including pollen morphology of *L. africana* are shown on (Figs. 2 – 7).

3.1 Epidermal features: 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 of *L. africana* (1) abaxial epidermis and (2) adaxial epidermis

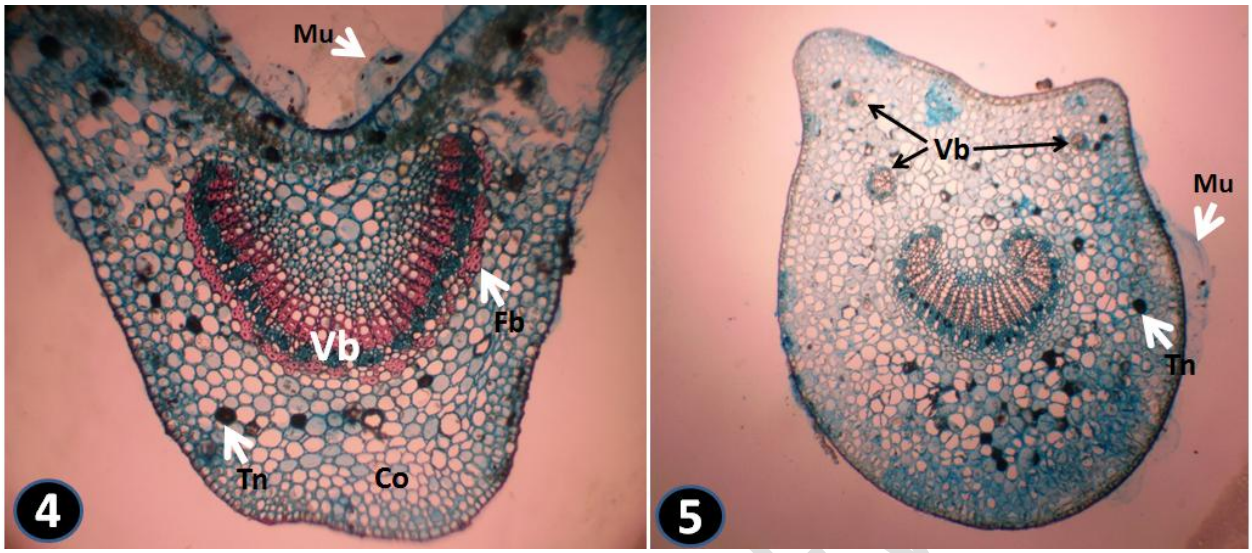
3.2 Lamina: The cross-section of *L. africana* leaf lamina showed a layer of oval and anticlinal elongated adaxial epidermal cells with cylindrical and periclinal elongated abaxial epidermal cells (Fig. 4). The palisade mesophyll comprised 2 – 3-layers while the spongy mesophyll has 3 – 4-layers with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



Figures 3: Transverse section of *L. africana* leaf lamina, Vb –vascular bundles, Ad – adaxial epidermis, Tn – tannin, Ab – abaxial epidermis, Sp – spongy mesophyll, Pa – palisade mesophyll, Dr – druses, Air – intercellular air spaces

3.3 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.

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 section is oval with two protrusions and uniseriate epidermis supported by about 8 – 12-layers of the parenchymatous cortex. The 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 abaxial cortical cells and

adaxial palisade and epidermal cells(Fig. 4), in the cortical and phloem cells of the petiole (Fig. 5), and pith and phloem cells in the stem (Fig. 6B). The vessels are solitary, imperforate tracheary, and diffused

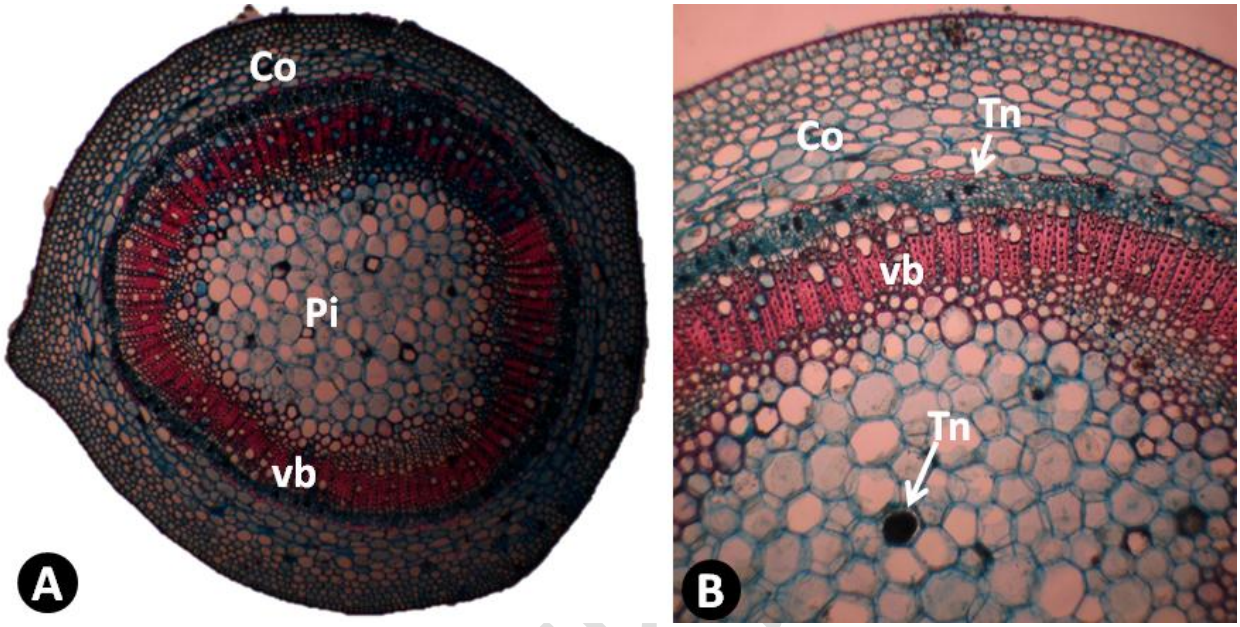


Figure 6: Stem anatomical features of *L. africana*: Pi – pith, Vb – vascular bundles, Tn – tannin, and Co – cortex.

3.7 Pollen morphology: The morphology of the pollen of *L. africana* showed a smooth, intectate 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. 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).

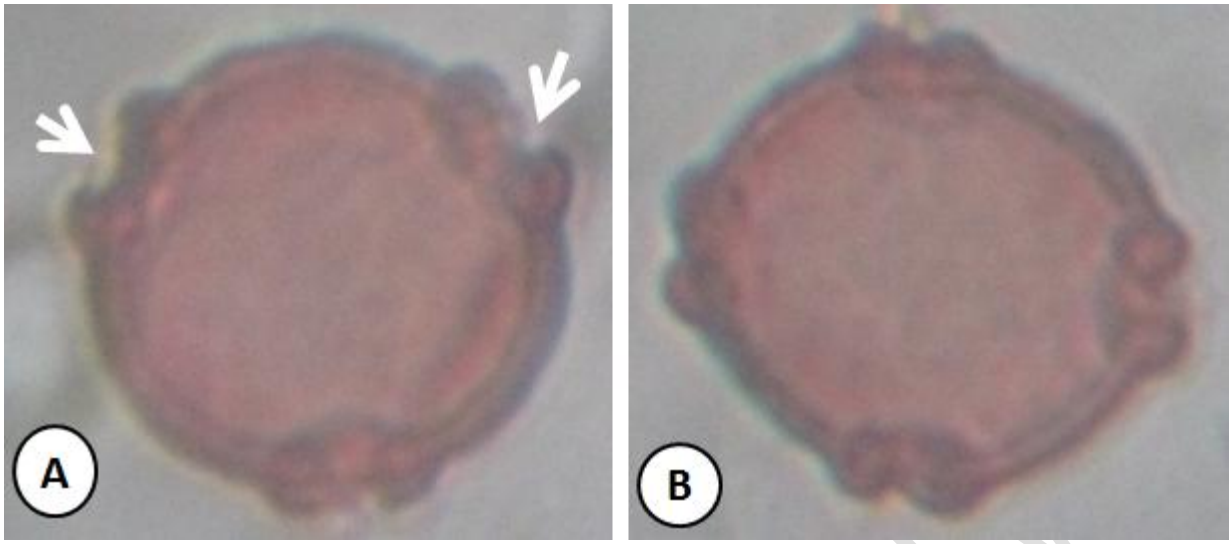


Figure 7: Pollen of *L. africana* (A) Polar view and (B) equatorial view, arrows show aperture

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 *L. africana* from some parts of Akwa Ibom State we recorded anomocytic, and anisocytic stomata, with the anisocytic type being more frequent. All the species studied are hypostomatic and the shape of the epidermal cells are irregular with undulating anticlinal walls. Our findings corroborate the previous reports on this species.

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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