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Chemotaxonomic Characteristics of *Tapinanthus bangwensis* (Engl. & K. Krause) Danser of Loranthaceae Collected from University of Port Harcourt, Rivers State, Nigeria

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ABSTRACT: *Tapinanthus bangwensis* commonly known as mistletoe, is a hemi parasitic shrub of Loranthaceae, and the native range is West Tropical Africa to Chad; used in tradomedicine to remedy various ailments in Nigeria. Hence, the objective of this paper was to investigate the chemotaxonomic characteristics of *Tapinanthus bangwensis* (Engl. & K. Krause) Danser of Loranthaceae collected from University of Port Harcourt, Nigeria using appropriate standard methods. Results of anatomy revealed 4 to 5 rows of collenchyma at hypodermis, parenchyma domiciled in the ground tissues, vascular sclerenchyma present especially in vascular system. In the phytochemistry, alkaloids, tannins, saponins, phenolics and flavonoids were present while terpenoids absent. These information would assist for further delimitation of the species.

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Mistletoe belongs to Loranthaceae of the order Santalales composed of 75 genera and 900 species (Judd *et al.*, 2002). It is speculated to have originated from Eastern Asia to Australia. Loranthaceae has 3 genera terrestrial and 72 genera aerial branched parasites (Wilson *et al.*, 2006). Grow on gymnosperms and angiosperms and are tropical (Dambele *et al*., 1994). Members of Loranthaceae (mistletoes) are parasitic plants known for their destructive nature to their host plants (Room, 1973). Six genera are found in Nigeria: *Tapinanthus*, *Agelanthus*, *Loranthus*, *Globimetula*, *Phragmanthera* and *Englerina*. Recent revision of the family in Nigeria documented fifteen species (Hussain and Karatela, 1989). Mistletoe is a general term for woody shoot parasites in several plant families, especially Loranthaceae and Viscaceae (Parker and Riches, 1993). Research in to the cuticular epithelium was done by Wilson and Calvin (2003). The comparative anatomical features of the family Loranthaceae in Nigeria is scanty except for the work of Bako *et al.* (2003) on the anatomy of *Tapinanthus dodoneifolius* and Ibrahim *et al*. (2009, 2013), who investigated the taxonomic significance of epidermal and phytochemical characters of the leaves of *Agelanthus dodoneifolius* in relation to their hosts and taxonomic significance of leaf epidermal characters of the family Loranthaceae in Nigeria, revealed further the presence of amphistomatic leaf type, polygonal cell shape, straight to curved anticlinal wall and pericytic stomata were features common to all the species, alongside trichomes and trichome bases were restricted to the genus *Phragmanthera*, striations were absent in *Agelanthus brunneus* (Engl.) Balle & Halle, *Englerina gabonensis* (Engl.) Balle and *Tapinanthus*

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cordifolius Polh. & Wiens with the occurrence of smallest cells in *Tapinanthus bangwensis* (Engl. & K. Krause) Danser. Wahab *et al*. (2010) made known the presence of tannin, saponin, flavonoid, alkaloid leaf extract in mistletoe. *Tapinanthus*, a semi-parasitic evergreen plant, is actually an obligate parasite, obtaining part of its food from the host plant. It is generally used as anticancer agents (Grossarth-Maticek and Ziegler, 2007) and in the management of diabetes mellitus (Kafaru, 1993; Obatomi *et al*., 1994; Osadebe *et al.*, 2004) as well as antihypertensive agent (Kafaru, 1993). Kafaru (1993) described the mistletoe as "an all-purpose herb" because of its rich folkloric uses. Some of these uses have been reported (Obatomi *et al*., 1994; Osadebe *et al*., 2004). Bassey *et al.* (2012) revealed that the leaves of the plant contained an appreciable amount of fiber, carbohydrate, protein and mineral elements. There were several reports on the phytochemical and antimicrobial properties of African mistletoe *Loranthus micranthus* (Osadebe and Ukweze, 2004). Phlobatannins, alkaloids, anthraquinones as well as cardiac and steroidal glycosides have also been reported (Wahab *et al*., 2010). Mistletoe plants have morphological resemblances which create difficulty in identification except during flowering: *Phragmanthera* has yellow flowers, *Loranthus* and *Tapinanthus* have red flowers (Begho *et al*., 2007); and they have high therapeutic effect in curing several diseases hence necessitating use of taxonomic lines of evidence. The objectives therefore focused on the investigation of chemotaxonomic characteristics of *Tapinanthus bangwensis* (Engl. & K. Krause) Danser of Loranthaceae collected from University of Port Harcourt, Rivers State, Nigeria.

MATERIALS AND METHODS

Sample Collection: The *Tapinanthus bangwensis* used for this research was collected from Delta Park Campus, University of Port Harcourt, Rivers State, Nigeria.

Morphological Properties: The meter ruler was used for measurement involving plant height from the root-collar to the terminal bud. The leaf length from the leaf tip to the petiole base, leaf width across leaf lamina, from one margin to another at the widest region. The root system, haustarium is embedded in host plant.

Epidermal studies: Fresh foliar organs harvested were peeled chemically using nitric acid and neutralized in 70 % alcohol, thereafter stained with Safranin O, rinsed with distilled water and counter stained with Alcian blue for 5 minutes in each, rinsed again and mounted in aqueous glycerol solution placed on glass slide with coverslip following the method of Cutler (1978). Slides with good sections were placed on the stage, viewed and photo-micro graphed using Android Sony Camera on Monocular microscope.

Anatomical Characteristics: The samples were fixed in Formaldehyde Glacial Acetic acid 70% Alcohol in the ratio 1:1:18 following the methods of Johansen (1940). Free hand section was done following the method of Wahua (2020). Thereafter, photo micrographs were taken from good slides.

Phytochemical Properties: The leaves of *Tapinanthus bangwensis* were sun dried for 72 hours and later weighed. Fifty grammes (50 g) of the dried leaves were macerated in 96 % ethanol with a pestle and a mortar. The extract was filtered and then evaporated to dryness using a rotary evaporator set at 45^0 C. Residue yields were noted and a portion used for the phytochemical studies.

Test for Alkaloids: In this regard, 0.5g of the extract was stirred with 5ml of 1% aqueous HCL on a water bath 1ml of the filtrate was treated with few drops of Mayer's reagent and a second 1ml portion was treated in same way with Dragendorff's reagent. The third 1ml was treated with Wagner's reagent. Turbidity or precipitation with these reagents was taken as preliminary evidence for the presence of alkaloids (Harborne, 1973; Trease and Evans, 1989). A modified thin-layer chromatography (TLC) method as described by Farnsworth and Euer (1962) was also adopted. A positive reaction on the chromatograms (indicated by an orange or darker colored spot against a pale yellow background) was used as confirmatory evidence for the presence of alkaloid.

Test for flavonoids

Shinoda reduction test: 5g of the pulverized sample was boiled in 5ml of distilled water for 5 minutes on water bath and filtered while hot. Magnesium (Mg) was added to the filtrate and few drops of conc.H_2SO_4 were carefully introduced into the mixture. The formation of orange, red, crimson or magenta was taken as evidence of preliminary presence of flavonoid.

Lead acetate test: 5g of pulverized sample was boiled in 5ml of distilled water for 5 minutes in water bath and filtered while hot. 2ml of 10% lead acetate was added to the filtrate and observed. Yellow precipitate indicated presence of flavonoids.

Test for tannins

Ferric chloride test ($FeCl_3$): Here, 5g of the prepared sample was boiled in 5mls of distilled water for 5 minutes on water bath. This was filtered while hot. 1ml

of 5% $FeCl_3$ was added to the filtrate and observed. Blue-black, green or blue-green precipitate was taken as tannins present in the sample (Trease and Evans, 1989)

Test for cardiac glycosides
Lieberman's test: 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled in ice. One milliliter (1ml) of Sulphuric acid was added in drops until a color change from violet to blue to green indicating that steroidal aglycones were present in the extract (Shoppee, 1964).

Test for Saponins: Frothing tests preliminary following the method described by (Wall and Eddy, 1952) was observed. The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was observed as screening test for saponins. 0.5g of the plant extract was shaken with water in a test tube. Frothing which continued on warming was taken as preliminary evidence that saponins were present in the sample. The disc was then washed in ether, dried and placed on a 7% blood nutrient agar. Complete haemolysis of red blood cells around the disc after about 6 hours was taken as further evidence of saponins presence in sample.

RESULTS AND DISCUSSION

Morphological Properties: *Tapinanthus bangwensis* was collected fresh within University of Port Harcourt Campus. Plate 1.

Plate 1: *Tapinanthus bangwensis* growing on Orange tree as host plant.
Arrow showed Mistletoe with wider leaves. Scale bar represents 80cm

Epidermal Properties: The stomata is paracytic and amphistomatic in nature. The rest of epidermal cells are nucleated and in various stages of cell cycle, irregular in arrangement and shape. Plate 2.

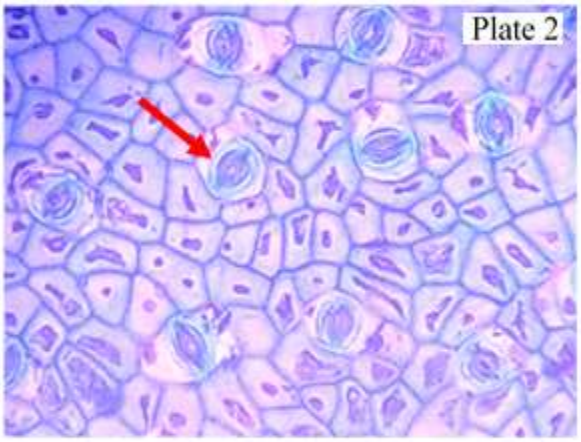

Plate 2: *Tapinanthus bangwensis* Abaxial Epidermis. Arrow showed paracytic stoma

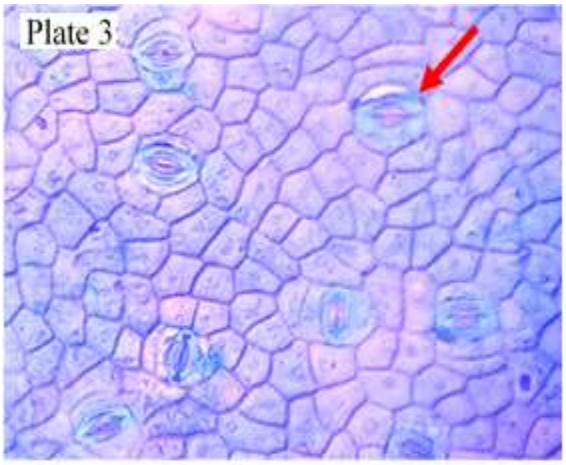

Plate 3: *Tapinanthus bangwensis* Adaxial Epidermis. Arrow showed paracytic stoma

Anatomical Properties: The mid-rib, petiole, node and root anatomies showed open vascular bundles, hypodermis predominated with collenchyma, general cortex and pith made of parenchyma and node is trilacunar. Vascular bundles bicollateral. Plates 4, 5 and 6. Phytochemical Studies (Quantitative): The following phytochemicals were found present in *Tapinanthus bangwensis*: Table 1.

Table 1: *Tapinanthus bangwensis* phytochemical information

Phytochemicals	% per Quantity of Sample
Tannin	0.30% per 0.1g
Alkaloid	6% per 5g
Flavonoid	4.02% per 10g
Saponins	27.80% per 20g
Glycoside	0.05% per 5g

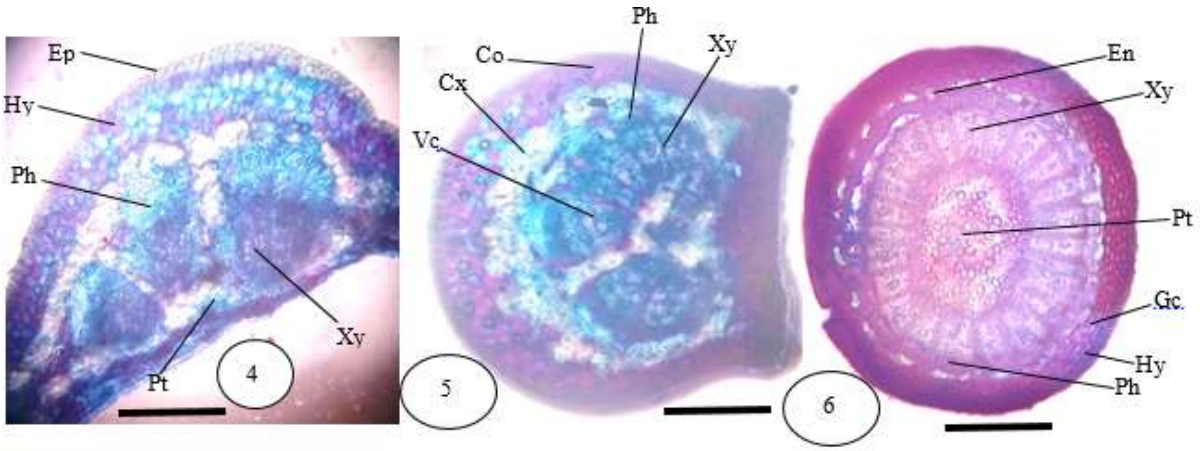

Plate 4: *Tapinanthus bangwensis* Mid rib Anatomy; **Plate 5**: *T. bangwensis* Petiole Anatomy; **Plate 6:** *T. bangwensis* Stem Anatomy; Ep – Epidermis, Hy – Hypodermis, Co –collenchyma Cx – Cortex; Ph – Phloem, Xy – Xylem, Pt –Pith; En –Endodermis, Gc – General cortex. Scale bar represent 2 mm.

Tapinanthus bangwensis described in this research conformed to those of Begho *et al*. (2007). *T. bangwensis* apart from the medicinal value, may be a very good source for the processes involving saponification. Hence a good material for soap making due to the high content of saponins, as also support by Ido *et al*. (2016). The mid-rib, petiole, node and root anatomies showed open vascular bundles, hypodermis predominated with collenchyma, general cortex and pith made of parenchyma, which also conformed to the leaf and stem anatomies of Ido *et al*. (2016). Node is trinocular. Epidermal study revealed paracytic and amphistomatic in agreement to the work of Ibrahim *et al*. (2009, 2013) with branched multiseriate trichomes. Tannin, saponin, flavonoid, alkaloid and glycoside were observed present as also demonstrated by Wahab *et al*. (2010).

Conclusion: Mistletoe is a hemi parasite, which occasionally kill the host plant. The roots, haustoria are difficult to get since they are embedded within the host plant tissues. It is now obvious fact that mistletoes, *T. bangwensis*, are paracytic and amphistomatic. The node is trilacunar. The presence of secondary metabolites in mistletoes, is the reason for their use in remedying several diseases and served as food for animals.

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