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STUDIES ON THE TAXONOMIC CHARACTERISTICS OF *Cythula prostrata* (L.) BLUME (AMARANTHACEAE)

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ABSTRACT

Cythula prostrata (L.) Blume is used in tradomedicine for calming and treating heart palpitation, and as animal feeds in Nigeria. It is a potential vegetable for health needs. This report highlights the morphological and anatomical properties, and phytochemical analysis of C. prostrata which belongs to the family Amaranthaceae. It is an annual trailing to semi erect, severally branched weed attaining up to a height of 55 ± 35 cm. The samples were fixed in standard fixative (Formaldehyde Glacial Acetic acid Alcohol in the ratio of 1:1:18) and standard methods were used for the epidermal, anatomical and phytochemical properties. The results revealed presence of anomocytic stomata which are amphistomatic, numerous specs of calcium oxalate crystals in epidermal cells, glandular and uniseriate trichomes. Alkaloids, tannins, saponins, flavonoids, Cardiac glycosides and steroids are observed present, except that flavonoid was absent in the crude extract but present in the ethanol extract. This information would assist in improving already existing knowledge of the plant.

Key words: anatomy, morphology, phytochemistry, stomata, taxonomy

INTRODUCTION

The family Amaranthaceae to which *Cythula prostrata* belongs has about 60 genera and 800 species. They are mostly distributed in tropical and temperate regions, believed to have originated from the south-western region of the United States, Latin America, or Africa (Basu *et al.,* 2014). The habit is made up of mostly herbs and few shrubs (Lewis *et al.*, 1983; Woodhouse, 1957). The Amaranth family is among the most species-rich lineage in the flowering plants in the order Caryophyllales, presently includes the goosefoot family, Chenopodiaceae. In other words, the enlarged family contains about 180 genera and 2,500 species. The genus *Cythula* has other species such as *C. cylindrica, C. uncinulata, C. pedicellata, C. achyranthoides, C. pobeguinii, C. cylindrica* var, *mannii* (Hutchinson and Dalziel, 1954). There are about 24 species including varietal forms of presently *C. protrata* known (WHO, 2024). *Cythula prostrata* is as a pan-tropical weed in cocoa or rubber plantations and cultivated fields (Akobundu and Agyakwa, 1998; PROTA, 2015). It grows well in light to dense shade from sea level up to 1650 m altitude (Chuakul *et al.*, 2001; PROTA, 2015). It is an annual or perennial herb rooting at nodes with ascending branches terminating in inflorescence. Stem is violet-red and leaves are twice as long as wide (Hutchinson and Dalziel, 1954; Sereena and Sreeja, 2014). The epidermis reveals mostly anomocytic and actinocytic stomata on the leaf surfaces. Calcium oxalate crystals are associated with members of the family Amaranthaceae (Metcalfe and Chalk, 1950; Ogundipe and Kadiri, 2012; Sonibare and Olatubosun, 2015).

The transverse section of stem is circular, hypodermis (1-3 layers) while cells of the cortex are angular (Sonibare and Olatubosun, 2015). It is used as medicine in combating the following diseases such as dysentery, rheumatism, and eye troubles and wounds in tropical Africa, Asia, and Australia (Burkill, 1995), including cytotoxicity against a cervical cancer cell line, at a concentration of 250 µg/mL (Sowemimo *et al.*, 2009). Secondary metabolites in *C. prostrata* include alkaloids in ethanol extract of leaf and stem bark but not in roots while terpenoids, tannins, saponins, flavonoids, glycosides and steroids were observed present in leaf, stem bark and root extracts (Ogu *et al*., 2012). The presence of terpenes or flavonoids or any steroid-like compound could be the main reason for the anti-inflammatory activity identified with the plant (Oladimeji *et al*., 2012; Oladimeji, 2012; Oladimeji and Usifoh, 2013; Oladimeji *et al*., 2015; Palmer and Ghosh, 1978). The increasing knowledge about the economic importance of *Cythula prostrata* has motivated this research on "Studies on the taxonomic characteristics of *Cythula prostrata* (L.) Blume (Amaranthaceae)".

MATERIALS AND METHODS

Geographic Location

The *Cythula prostrata* used was collected fresh within the campus of the University of Port Harcourt (4 0 53' 30.12" N; 6 0 55' 39.6" E).

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Morphological Studies
The meter rule was used to ascertain morphological measurements of plant parts such as: plant height from the root-collar to the terminal bud, the leaf length from the leaf tip to the petiole base and the leaf width across the leaf lamina, from one margin to another at the widest region.

Epidermal Studies
Fresh foliar organs collected were peeled manually, and soaked in 90% alcohol solution for 5 to 10 minutes, 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 Study
The leaves, petioles, stems, flowers, fruits and roots harvested for the study, were fixed in FAA previously prepared in the ratio: 1:1:18 of 40 % formaldehyde, glacial acetic acid and 70 % alcohol for 2 to 48 hours following the methods of Johansen (1940) modified; Free hand sections were done as described by Wahua (2020). Slides with good sections were placed on the stage, viewed and photo-micro graphed using Leica WILD MPS 52 microscope camera on Leitz Dra plan microscope.

Phytochemical Study
The foliar organs, the leaves, of *C. prostrata* were sun dried for 72 h and later weighed. Fifty grams 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° C. Residue yields were noted and a portion used for the phytochemical investigation.

Test for alkaloids
Thereafter, 0.5 g of the plant extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath; 1ml of the filtrate was treated with few drops of Mayer's reagent and a second 1 ml portion was treated in same way with Dragendorff's reagent. The third 1 ml 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 used. One gram of the extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then treated twice with 10 ml of chloroform. The extracts were combined and concentrated to 5 ml. The chloroform extract was spotted on thin-layer plates. Four different solvent systems were used to develop each plant extract. The presence of alkaloids in the developed chromatograms was detected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. 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: 5 g of the pulverized sample was boiled in 5 ml of distilled water for 5 min. on water bath and filtered while still 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: 5 g of pulverized sample was boiled in 5 ml of distilled water for 5 min. in water bath and filtered while still hot. Then, 2 ml of 10% lead acetate was added to the filtrate and observed. Yellow precipitate indicated presence of flavonoids.

Test for tannins
Ferric chloride test ($FeCl_3$) 5 g of the prepared sample was boiled in 5 mls 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 carefully 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
About 0.5 g of the extract was dissolved in 2 ml of acetic anhydride and cooled in ice. Thereafter, 1 ml of sulphuric acid was added in drops until a colour change from violet to blue to green indicating presence of steroidal aglycones in the extract (Shoppe, 1964).

Test for Saponins

Frothing tests
Preliminary test following the method described by (Wall *et al.,* 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. Then, 0.5 g 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 agar. Complete haemolysis of red blood cells around the disc after about 6 hours was taken as further evidence of the presence saponins in sample.

RESULTS

Morphological Study

It is an annual to perennial pan tropical prostrate, trailing to semi erect, severally branched herb attaining up to a height of 55 ± 35 cm used as medicine; seen growing in humid regions, at low and medium altitudes especially lowlands rice and marshy regions. Plate 1 and Table1.

Epidermal Studies

The micro-morphological studies showed presence of trichomes and glandular ones in *C. prostrata*, mainly in the form of piloses. The stomata are basically anomocytic and amphistomatic, nucleated eepidermal cells and trichomes were present. Plates 2a and b.

Anatomical Studies

The transverse sections conducted showcased epidermis of one row of cells. The hypodermis is made of 2 to 3 rows of collenchyma, the general cortex has 7 to 10 rows of parenchyma and the pith is made of parenchyma entirely. Plate 3 a, b, c, d and Table 2.

Phytochemical Analysis

Alkaloids, tannins, saponins, cardiac glycosides, steroids are present except for flavonoids which showcased in ethanol extract but absent in the crude extract; the data are presented in Table 3.

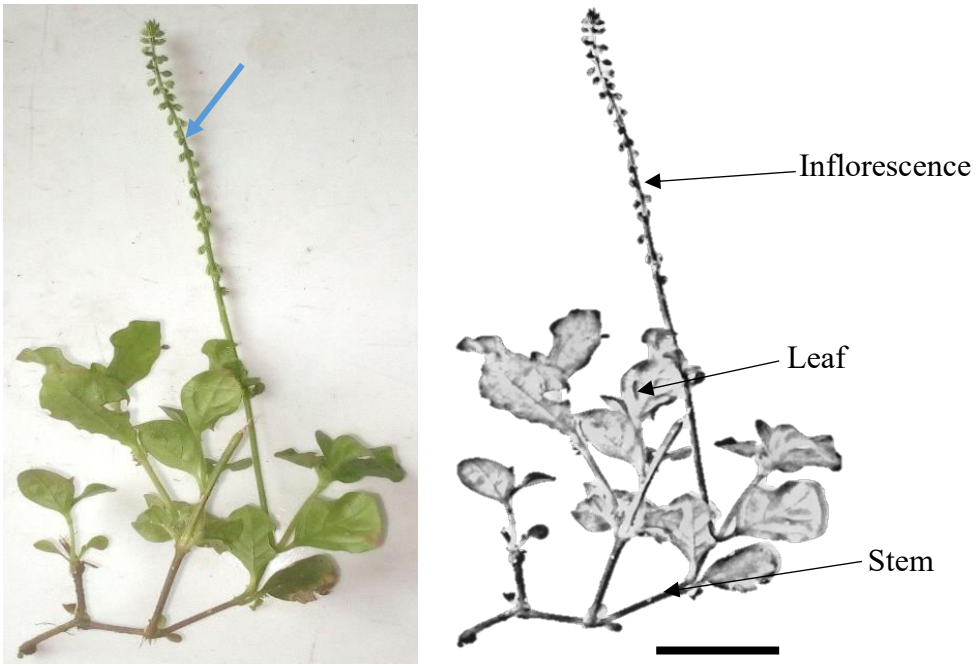

Plate 1: *Cythula prostrata* (L.) Blume
Arrow showcased racemose inflorescence. Scale bar represents 45 cm.

Table 1: Morphological properties of *Cythula prostrata*

Characters	*C. prostrata*.
Habitat	Terrestrial
Growth form and nature of branching	Broadleaf, branching profusely, grows up to 55 ± 35 cm high
Stem	The stem is stout, angular with dense pilose.
Leaves shape	Elliptical, rhombic-obovate or rhombic- oblong, acute at both ends, or cuneate at base, petiolate, trichomes present and opposite phyllotaxy
Length of 1st leaves	5.5 ± 2.5 cm
Width of 1st leaves	3.5 ± 1.5 cm
Inflorescences	Slender, elongated, Spike terminal, solitary or in group of threes, and flowers are grouped in small dense glomerules at the top, separated at base.
Flowers	The floral glomerules are sub-globose and ovoid. In groups of 3-5, where one is perfect and others are not; flower clusters in raceme, hermaphrodite. Bracts ovate, membranous, often spiny. Tepals 5, nearly equal and membranous. Stamens 5; anther 2-loculed, oblong; filaments connate into a short cup at base, alternating with Utricles included in persistent perianth, globose, ellipsoid, or obovoid, membranous, indehiscent. Seeds oblong or ellipsoid. bracts and bracteoles similar, lanceolate, acuminate, pubescent; acute, hooked awn.
Sepals	3 membranous whitish sepals, the anterior 2 welded.
Stamens	Style is simple, Stamens 5, filaments united into a membranous truncated cup and staminodes membranous which alternate with stamens.
Pistils	Style in slender suspending stigma which is rounded and capitellate, the ovary is ovoid.
Fruit	Spinous indehiscent capsule
Seed	Seed is shiny, brown, and lenticular ovoid structure.

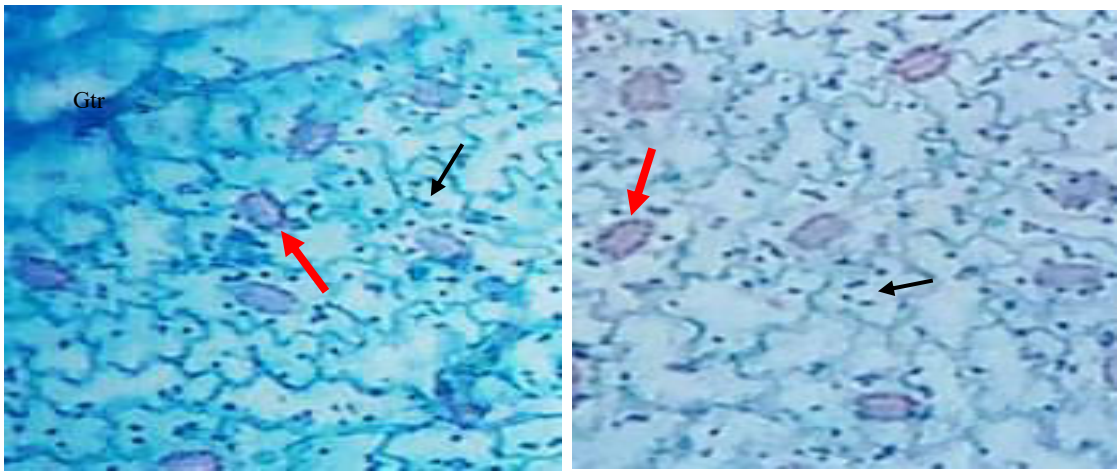

Plate 2a: *Cythula prostrata* Adaxial foliar epidermis; **2b:** *C. prostrata* Abaxial epidermis. Red arrow revealed stomata on both sides. Also observed were contiguous cells. Gtr -Glandular trichomes as shown on plate 2a. Black arrow showcased numerous black specs of calcium oxal.

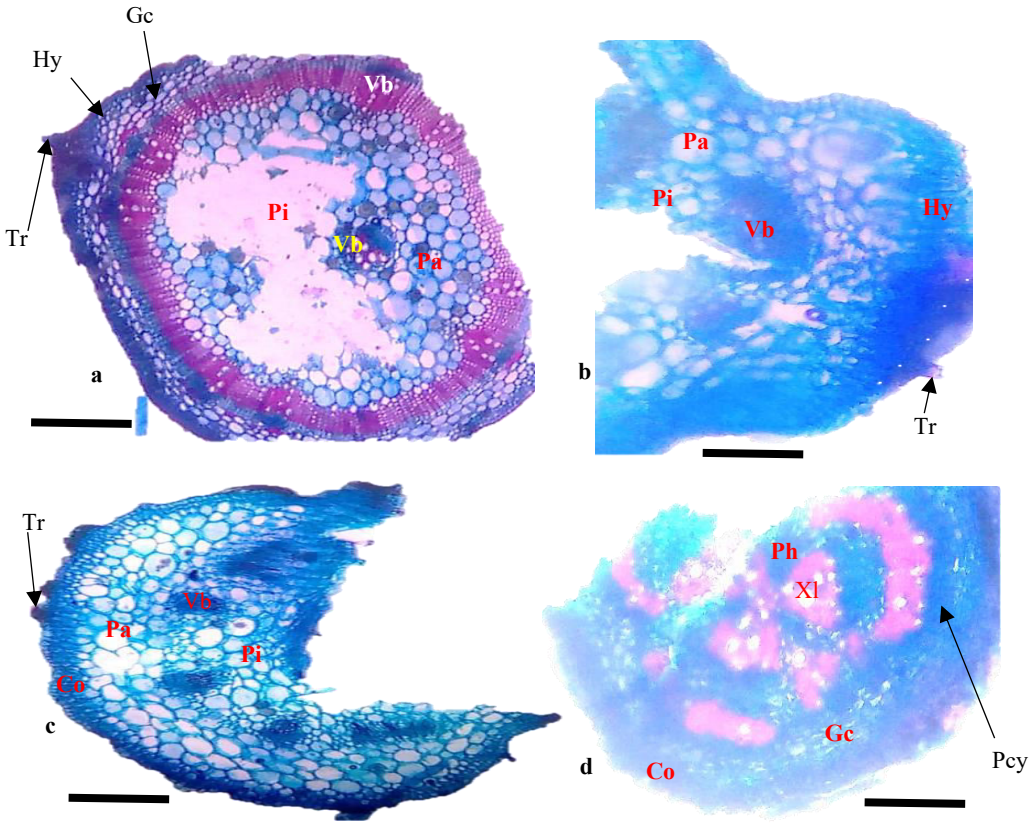

Plate 3a: *Cythula prostrata* stem; **3b;** *C. prostrata* mid-rib; **3c:** *C. prostrata* petiole and **3d:** *C. prostrata* root. All in transverse sections (T.S.), Hy - Hypodermis; Gc - General cortex; Vb -vascular bundles; Pi -pith, Tr - trichome (pilose); Pa - parenchyma; Xl -xylary rays. Pcy - Pericycle; Co – collenchyma; Ph - Phloem. Scale bar represents 2 mm

Table 2: Anatomical properties of *Cythula prostrata*

Features	*C. prostrata*
Hairy nature of stem	Long visible hairs present
Epidermis of stem, petiole and stem	Single roll of cells
Hypodermis	4 to 5 layers of cells of collenchyma
General cortex	5 to 7 rolls of cells parenchyma.
Pericycle of roots	3 to 4 layers of compact cells
Root pith	Pith obliterated

Table 3: Phytochemical composition of *Cythula prostrata* (L.) Blume

Secondary metabolites	Crude Extract	Ethanol Extract
Alkaloid	+	+
Tannins	+	+
Saponins	+	+
Flavonoids	-	+
Cardiac glycosides	+	+
Steroids	+	+

Key: Present +, Absent –

DISCUSSION

The *Cythula prostrata* described conformed to those of Hutchinson and Dalziel (1954) and Sereena and Sreeja (2014), used in tradomedicnie to compact heart palpitation and related cardiac problems, and as animal feed and may be a potential vegetable for health needs in Nigeria. The epidermal study revealed anomocytic stomata which are amphistomatic as also supported by Sonibare and Olatubosun (2015). The epidermal cells have numerous specs of calcium oxalate crystals which is in accordance to the works of Metcalfe and Chalk (1950), Ogundipe and Kadiri, (2012), and Sonibare and Olatubosun (2015) on members of the family Amaranthaceae. Glandular and linear to hook-shaped uniseriate trichomes observed in both foliar surfaces. Apart from the ringed vascular system of the stem, there were vascular bundles in the region of ground tissues (the pith). The parenchyma distribution in pith are the largest in size in stem, whereas, the petiole and mid-rib those in the general cortex also have large sized parenchyma. In the phytochemical analysis, alkaloids, tannins, saponins, flavonoids, cardiac glycosides and steroids were present in crude and ethanol extracts as also supported by the findings of Ogu *et al*. (2012) in which terpenoids, tannins, saponins, flavonoids, glycosides and steroids were observed present in leaf, stem bark and root extracts. Flavonoids was only absent in the crude extract.

CONCLUSION

Cythula prostrata is used in tradomedicine to compact heart palpitation and related cardiac problems, and as animal feed and may be a potential vegetable for health needs in Nigeria. The epidermal cells have numerous specs of calcium oxalate crystals, glandular and linear to hook-shaped uniseriate trichomes in the leaves. Vascular bundles showcased in pith. The phytochemical ingredients are the reasons for its therapeutic properties. Suggested areas of further work may include DNA sequencing, pollen morphology and vein islet structure.

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