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Micromorphological and Phytochemical Studies on *Cleome rutidosperma* Linn.

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KO and CE designed the study, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to determine the micromorphological structure and quantify the phytochemical components of *Cleome rutidosperma* Linn. The study revealed the presence of five stomatal types (anomocytic, staurocytic, tetracytic, anisocytic and isotricytic) and polar contiguous stomata on both abaxial and adaxial surface of the leaf of *C. rutidosperma*. The shapes of the adaxial epidermal cells are relatively regular while the abaxial epidermal cells are irregular. There are fewer stomata on the adaxial epidermal surface than the abaxial surface. The range of the stomatal index (SI) on the adaxial and abaxial surfaces is between 3.85-20.0 and 63.64-84.21 respectively. *Cleome rutidosperma* has eglandular trichomes which occur on the leaf surfaces and stem of the plant; these trichomes are disrriate or trisariate with multicellular base. The petiole has five U-shaped free open collateral vascular bundles. The midrib is U-shaped adaxially and contains 5-free vascular bundles which formed a semicircle. The adaxial and abaxial epidermis have single layer of cell with 4-5 and 4-6 layers of parenchymatous cells, respectively. The upper parenchyma comprised 3-4 layers of cell while lower parenchyma has 4-7 layers of cells. The fruit stalk comprised 7-vascular bundles in a concentric ring. The cuticle is undulated with one-layer of epidermis and 3-4 layers of parenchymatous cells. The stem is pentagonal with 18-20 vascular

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bundles in a concentric ring. The epidermis has 1-layer of cell, collenchymatous cells 1-3 layers and parenchyma cells 1-4 layers but up to 6-10 layers the angles or protruded ends. The quantitative analysis of the phytochemicals showed that the *C. rutidosperma* contains 1.64% alkaloid, 2.84% flavonoid, 0.145% tannin, 3.25% saponin, 30 mg/kg cyanogenic glycoside, 48.8 mg/g oxalate while phytate was not detected. These micromorphological information recorded in this work can be used to distinguish *C. rutidosperma* from other members of the genus and are therefore of taxonomic value. Also, the presence of these phytochemicals could account for its use by the local communities in south-east Nigeria for the treatment of ear infection and other illnesses.

Keywords: Anatomy; Cleome; phytochemical; stomatal index; trichome.

1. INTRODUCTION

Cleome rutidosperma Linn. belongs to the family Cleomeceae. It is native to Nigeria and has been reported to be among the 50 species of *Cleome* occurring in Africa [1]. A plant with spreading and prostrate branches and leaves divided in 3 leaflets. Flowers are small, violet-blue, 5 mm long, borne on long stalks from the leaf axil of the flowering stems. Fruit is cylindrical and curved, about 5 cm long, with numerous round, reddish-brown to black seeds, 1.5 mm diameter with barred surfaces. *C. rutidosperma* is an annual species reproducing solely by seed. In Africa, within its native distribution range, flowering and fruiting plants of *C. rutidosperma* can be found throughout the year, although most abundantly in the rainy season [2]. It is a common weed with economic impacts in a wide range of crops, where its scrambling habit smothers and stunts young crop plants. It is an environmental weed in disturbed ground, roadsides, gardens, and abandoned land as well in natural and semi-natural coastal forest where it has the potential to outcompete native vegetation [3,4]. *C. rutidosperma* spreads by seeds. Seed dispersal is myrmecochorous, ants being attracted to the seeds by the fatty elaiosome[5]. In Malaysia, *C. rutidosperma* is planted around field edges as part of insect control programs [2].

The young shoots and leaves of *C. rutidosperma* are eaten as a cooked vegetable or added to soup in most part of Africa and Asia [6,7]. In Ghana, Gabon and DR Congo, the leaf sap is applied to cure earache and deafness. In Ghana, the leaf extract of *C*. *rutidosperma* is used to treat irritated skin and in Nigeria it is used to treat convulsions [1]. The plant is used as antimalarial by traditional healers in Cameroon. The plant is used in the treatment of paralysis, epilepsy, convulsion and spasm [8]. The plant is frequently used in traditional medicine [7,9]. Ethno-botanical survey conducted in Ogoni, Nigeria claimed that the extract from the leaf of *C. rutidosperma* calms irritation and itching in the ear, this experience has made the indigene of Ogoni to describe *C. rutidosperma* as "hospital too far" because of the relieve it provides. A similar claim was also reported in India that *C. rutidosperma* relieve ear pain and skin diseases [8].

Preliminary phytochemical screening of the aqueous extract of *C. rutidosperma* showed the presence of tannins, saponins, flavonoids and carbohydrates [10]. Similar phytochemical screening carried out on the seed flours of *C*. *rutidosperma* revealed the presence of alkaloids, steroids, tannins, flavonoids, cardiac glycosides, pentose, and reducing sugars [11]. This promising plant, *C. rutidosperma,* if well researched will clear the doubt of its inherent potential in curing most ailments. This study focuses on the micromorphological and quantification of some of the phytochemical component of *C. rutidosperma* otherwise considered by many as weed. The result of the study will broaden the taxonomic knowledge base and probably provide information for the pharmaceutical industries to improve upon.

2. MATERIALS AND METHODS

2.1 Sources Plant Material

Fresh plant materials of *C. rutidosperma* used for this study were collected from University of Port Harcourt Park, properly identified, authenticated and deposited in the University of Port Harcourt Herbarium with reference number UPH/V/1254.

2.2 Anatomical Studies

The central portions of the matured leaf were peeled, stained with 1% safranin O and in slides with glycerin. Specimens for anatomical analysis were obtained fresh from matured plants and fixed in FAA for 12 hrs. They were transferred to 50% and 70% ethanol and kept at room

temperature until required. The petiole, midrib and fruit stalk were hand sectioned using sharp razor blades [12,13]. The sections were stained in 1% Safranin red for two minutes, counter stained Alcian blue and mounted on a slide. Thereafter, the slides viewed and photographed with LeitzDiaplan photomicroscope fitted with Leica WILD MPS 52 camera. The terminology used in respect to stomatal complex followed Malvey [14].

2.3 Determination of Tannin

The percentage composition of tannin in the plants was determined using the methods of AOAC (1980) with some modifications. Folin-Denis reagent and saturated sodium carbonate were prepared in accordance with the procedure to analyze the tannin content. Standard solution of tannic acid was freshly prepared by dissolving 10 mg of tannic acid in 100 mL water. A series of tannic acid standards were prepared in the range of 0-2.5 mL, aliquots in 25 mL volumetric flasks, then added to 1.25 mL Folin-Denis reagent and 2.5 mL sodium carbonate solution. The mixture was made up to the volume and the colour was measured after 30 min at 760 nm using a spectrophotometer (Perkin Elmer). The samples were prepared by boiling 1 g of their dried powder in 80 mL of water for 30 min. The samples were cooled, transferred into a 100 mL volumetric flask and diluted to mark. The solution was filtered to get a clear filtrate and analyzed as in the standard. Tannin content was determined by a tannic acid standard curve and expressed as milligrams of Tannic Acid Equivalence (TAE) per 100 g of dried sample [15].

$$Solubletannins(\%) = \frac{\text{C (mg)} \times \text{extract volume (mL)}}{10 \times \text{aliquot (mL)} \times \text{sample wt (g)}}$$

2.4 Determination of Alkaloid

5 g of the sample was weighed into 250 mL beaker and 200 mL of 10% Acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue was dried and weighed [16].

$$\text{Alkaloid (\%)} = \frac{\text{(A - B)}}{\text{Weight of sample used (g)}} \times 100$$

Where:

A = weight of filter and residue and
B = weight of empty filter paper

2.5 Determination of Flavonoid by Bohm and Kocipal-Abyazan (1994)

10 g of the plant sample was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

$$\text{Flavonoid (\%)} = \frac{\text{(A - B)}}{\text{Weight of sample used (g)}} \times 100$$

Where;

A = weight of flask and sample residue and
B = weight of empty flask

2.6 Determination of Phytate

0.1 g of the plant was extracted with 100 mL of 0.5 *M* HCl for 2 hours and filtered. 15 mL of the aliquot was neutralized with 0.5 *M* NaOH, made slightly acidic with 0.17 *M* HCl and diluted to 50 mL with distilled water. 4 mL of 0.25% w/v $FeCl_3$ (0.25 g in 100 mL) added to 10 mL aliquot in centrifuge tube, heated for 15 minutes at 100℃, cooled, centrifuged and supernatant discarded. The residue was washed with 0.5 *M* and then 0.17 *M* HCl, centrifuged and the liquid discarded. 2 mL water was added to residue and heated for few minutes. Thereafter, 2 mL of 0.5 *M* NaOH was added, heated for 15 minutes and filtered into Kjeldahl flask. Washed with hot water and retained washings in the flask. 0.5 mL conc. H_2SO4 was added and boiled until the fumes turns white. HNO_3-$HClO_4$ mixture (5 mL HNO_3 : 1 mL 60% $HClO_4$) was added, digested and the sample diluted to 50 ml. 0-15 mL of working standard was pipette into 50 mL volumetric flask to give a standard range of 0 to 0.03 mg P. 1-5 mL sample was taken and P determined using colorimetric method. At this point, the standards and samples were treated in the same way. The flasks were diluted until flasks were about two-thirds full. 2 mL ammonium molybdate and 2 mL stannous chloride reagents were added, mixed, diluted to volume and allowed to stand for 30 minutes and absorbance measured at 700 nm optical density. A calibration curve was prepared

from the standards and used to determine P (mg) in the sample aliquot [15].

$$\text{Phytate (\%)} = \frac{(P\ (mg) \times A \times B \times C)}{10 \times D \times E \times F \times \text{sample wt (g)}} \times 100$$

Where:

P (mg) = phytate obtained from the graph
A = digest solution (ml); B = neutral solution (ml); C = acid extractant (ml); D = aliquot for (ml); E = aliquot for digestion (ml) and F = aliquot for neutral (ml)

2.7 Determination of Oxalate

100 mL of distilled water was added to 5 g of the plant sample, heated for 1 hour and allowed to cool. The mixture was made up to 100 mL with distilled water. To 25 mL of the aliquot and 25 mL of oxalic acid (oxalate standard) in separate flasks were acidified with 20 mL of 2 M H_2SO_4, heated to 70°C and allowed to cool. The solutions were titrated with 0.02 M $KMnO_4$.

2.8 Determination of Cyanogenic Glycoside

5 g of the plant sample was weighed into a clean distillation flask, 20 mL distilled water was added and the sample was allowed to stand overnight for proper hydrolysis to be attended. The sample was distilled into 20 mL NaOH containing 0.5 g crystals. The distillate was titrated with 0.02 *N* $AgNO_3$ in the presence of 0.2 mL of 5% KI and 1 mL of NH_4OH to a permanent turbidity.

Calculation: 1 mL 0.02 *N* $AgNO_3$ = 1.08 mg HCN (Ag equiv. to 2 CN)

2.9 Determination of Saponin by Soxhlet Extraction Method

2 g of the plant sample was inserted into a filter paper and was placed into a soxhlet extractor. The extractor was pre-weighed dried distillation flask. Then the methanol was introduced into the distillation via the condenser end attached to the soxhlet extractor. The set-up was held in place with a retort stand clamp. Cooled water jet was allowed to flow into the condenser and the heated solvent refluxed. The saponin in the solvent chamber was extracted in the process of continuous refluxing. When the saponin was observably extracted completely from the sample, the condenser and the extractor were disconnected. The solvent was evaporated to concentrate the saponin. The flask was dried in the air oven to constant weight and re-weighed to obtain the saponin weight.

Calculation:

$$\text{Saponin (\%)} = \frac{(A - B)}{\text{Weight of sample extracted (g)}} \times 100$$

Where:

A = weight of flask and extract and
B = weight of empty flask

3. RESULTS AND DISCUSSION

3.1 Epidermal Characteristics

Five stomatal types and polar contiguous stomata were observed in both abaxial and adaxial surface of the leaf of this species. These include anomocytic (Figs. 1a-d), staurocytic (Figs. 1e-f), tetracytic (Figs. 1g-i), anisocytic (Figs. 1j-k) and isotricytic (Figs. 1l-m) and polar contiguous (Fig. 1n) stomata types. The shapes of the adaxial epidermal cells are relatively regular (Figs. 1o-r) while the abaxial epidermal cells are irregular (Figs. 1t-v). There are fewer stomata on the adaxial epidermal surface than the abaxial surface. The range (mean±standard deviation) of the stomatal index (SI) on the adaxial and abaxial surfaces is 3.85-20.0 (11.61±5.25) and 63.64 - 84.21 (76.22±6.78) respectively. This species has eglandula rtrichomes which occur on the leaf surfaces, stem and other organs of the plant. These trichomes are disrriate or trisariate with multicellular base (Figs. 2a-b). The result of the epidermal characteristics is in contrast with the findings of Edeoga et al. [17] but supports the works of Metcalfe and Chalk [18,19], Jelani et al. [20] and Jansen [1], In their studies, Edeoga et al. [17] reported stomatal index of 2.78±0.88 and 17.85±1.76 on upper and lower epidermis respectively and the absence of multicellular and glandular in the species. On the other hand, Metcalfe and Chalk noted the presence of glandular trichomes among the *Cleome* species [18]. In the leaves of *C. viscosa,* multicellular trichomes were observed, these trichomes were large and long with a club shaped head and glandular basal end. These glandular hairs have earlier been described and this feature separates it from other *Cleome* species [1,19]. Jelani et al. have described foliar epidermal cells, stomatal cells and trichome complexes in some Indian species of *Cleome* (*C. asperaK*oen. ex DC. *C. chelidonii* L.f., *C. feline* L.f., *C. monophylla*

L.,*C. gynandra* L., *C. tenella* L.f. and *C. viscose* L) and noted six trichome types (uniseriatecapitate, uniseriate cylindrical, biseriatecapitate, multiseriatecapitate, multiseriateclavate and multiseriate conical trichomes) [20].

3.2 Petiole/Midrib/Fruit Stalk/Stem

The petiole comprised five (5) U-shaped free open collateral vascular bundles. The adaxial surface has 1-layer of epidermis and 4-5-layers of parenchymatous cells while the abaxial epidermis comprised 1-layer of cell and 4-6-layers of parenchymatous cells (Fig. 2c). The midrib is U-shaped adaxially and contains 5-free vascular bundles which formed an arc or semicircle. The adaxial (upper) and abaxial (lower) epidermis have single layer of cell. The upper parenchyma comprised 3-4 layers of cell while lower parenchyma has 4-7 layers of cells (Fig. 2d). The fruit stalk comprised 7-vascular bundles in a concentric ring. The cuticle is undulated with one-layer of epidermis and 3-4 layers of parenchymatous cells (Fig. 2e). The stem is 5-angled (pentagonal) with 18-20 vascular bundles in a concentric ring. The epidermis has 1-layer of cell, collenchymatous cells 1-3-layers and parenchyma cells 1-4 layers but up to 6-10 layers the angles or protruded ends (Figs. 2f-g). The adaxial and abaxial epidermal cell in the petiole, midrib and stem have 1-layer of cell and is in conformation with the previous report of Edeoga et al. [17] who reported the presence of single layer of cell in the epidermis of *C. rutidosperma.* Also, Metcalfe and Chalk noted that the shape of the vascular bundles in the midrib is arced with convex surface towards the lower surface [18]. In the petiole, the vascular bundles is shallow arced with about 14 separate bundles in *Cleome spinosa*and the presence of isolated strand of fibre in the pericycle of the stem. This same observation was made in the *C. rutidosperma* studied and confirms that the species is a member of Cleomaceae.

3.3 Phytochemical Constituent of *C. rutidosperma*

The study revealed that *C. rutidosperma* contains the following phytochemicals in these proportions: alkaloid (1.64%), flavonoid (2.84%), tannins (0.145%), saponins (3.25%), cyanogenic glycoside (30 mg/kg), oxalate (48.8 mg/g) while the phytate was not detected. The available

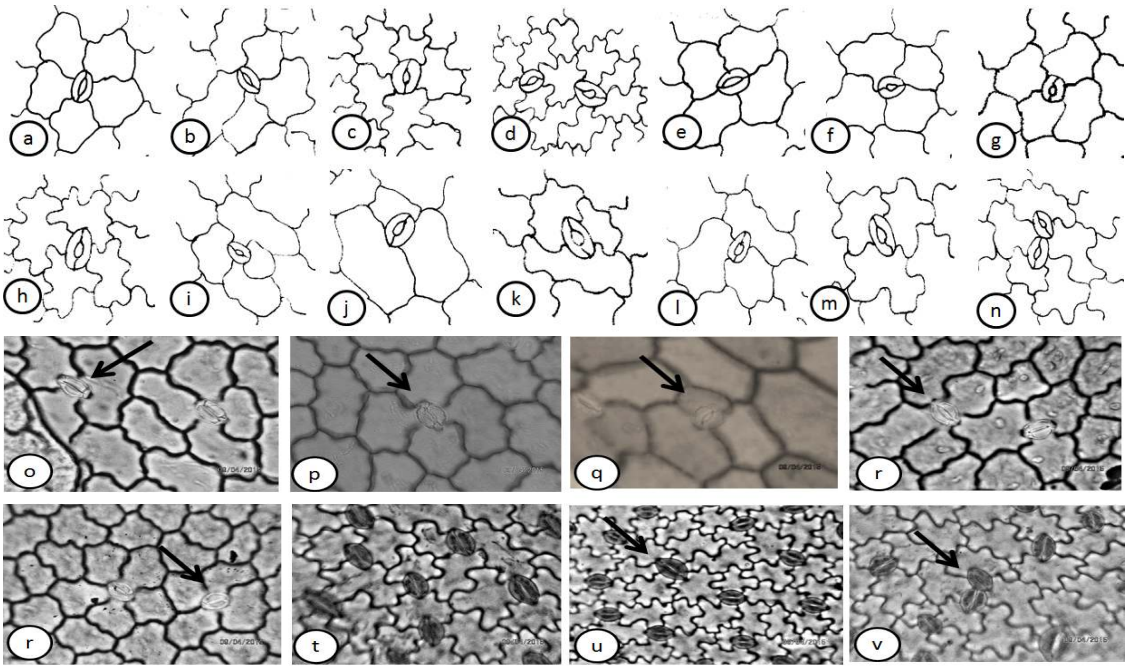

Fig. 1. Stomatal and epidermal characteristics of *Cleome rutidosperma* x124: (a-d) anomocytic; (e-f) staurocytic; (g-i) tetracytic; (j-k) anisocytic; (l-m) isotricytic; (n)polar contiguous stomata; (o-r) upper epidermal cells arrows show different stomatal types and (t-v) lower epidermal cells arrows show different stomatal types

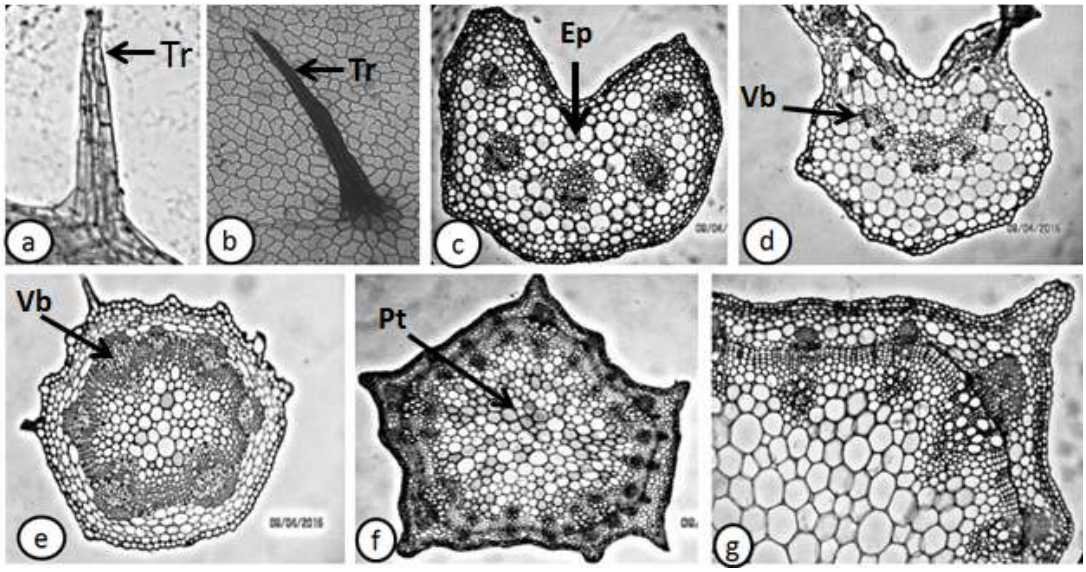

Fig. 2. Micro-anatomical characteristics of *Cleome rutidosperma* x40 (a-b) Trichome types; (c) Transverse section of petiole; (d) Transverse section of midrib; (e) Transverse section of fruit stalk and (f-g) Transverse section of stem; Abbreviations: Tr = trichome, Ep = epidermal cells, Vb = vascular bundle and Pt = pith

percentage alkaloid, flavonoid, saponin obtained from this result were high while the tannin content was low when compared with the work of Edeoga et al. [21]. This quantitative assessment result disagrees with the work of Edeoga et al. who had earlier reported that the percentage of crude phytochemical constituents of *C. rutidosperma* were as follows: alkaloid (0.34±0.1%), tannin (15.25±0.11%), saponin (2.0±0.11%), and flavonoid (0.34±0.20%) [21]. However, this result confirms the presence of these phytochemicals in *C. rutidosperma* which agrees with the qualitative screening of *C. rutidosperma* carried out by other researchers [21-23]. Preliminary phytochemical screening of *C. rutidosperma* revealed the presence of saponins, alkaloids, flavonoids, cardiac glycosides, tannins and carbohydrates according to Arhoghro et al. [22]. These chemicals are present in the stem, leaf and root of *C. rutidosperma* while some are absent in other *Cleome* species like *C. viscose* [21,22,24,25]. The use of *C. rutidosperma* roots for wound healing activities as claimed in the folklore literature was justified by Mondal and Suresh [23]. According to Pier-Giogio, many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions [26]. Flavonoids may help provide protection against diseases like injury, cancer, aging, atherosclerosis, ischemic, inflammation, neurodegenerative diseases by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of human body [27,28]. The ethno-botanical survey of *Cleome rutidosperma* showed that the plant can be use to stop itching in the ear. This observation could be attributed to the presence of some secondary metabolites inherent in the plant.

4. CONCLUSION

The micromorphological information (stomata type, shape of the epidermal cells, trichome types, shapes of the fruit stalk, petiole, stem and midrib, including number vascular bundle and of layers parachymatous cells) recorded in this work can be used to distinguish *C. rutidosperma* from other members of the genus and are therefore of taxonomic value. Also, the presence of alkaloid, flavonoid, tannin, saponin, cyanogenic glycoside and oxalate could account for its use by the local communities in south-east Nigeria for the treatment of ear infection and other illnesses. There is therefore need to intensify efforts towards extracting these phytochemicals for proper medicinal and pharmacological uses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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