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A pharmacobotanical study of two medicinal species of Fabaceae

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PEER REVIEW

Peer reviewer

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Comments

It is a good paper reporting the anatomical and pharmacognostic features of two Nigerian medicinal plants, thus ensuring the correct identification of both plants when collected in fragmentary forms and screened for subsequent drug development.
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ABSTRACT

Objective: To carry out a pharmacobotanical study of *Lonchocarpus cyanescens* (Schum & Thonn) Benth (*L. cyanescens*) and *Leptoderris micrantha* Dunn (*L. micrantha*) which are two key medicinal plants from the family Fabaceae.

Methods: The epidermal peel was obtained by soaking the leaf in concentrated nitric acid (HNO_3) in a petri dish. Both surfaces were carefully mounted on clean glass slides and dehydrated by ethyl alcohol, and stained with safaranin O for 2 min. Transverse sections of plant leaf were obtained by free hand sectioning. Phytochemical screening for various constituents was carried out on the powdered leaves. Other parameters such as, moisture content, ash value, acid insoluble ash, water-soluble ash, water and alcohol extractive values were obtained by standard techniques.

Results: The distinctive features of the species include: the presence of stomata on both surfaces of *L. cyanescens* and the absence in *L. micrantha*. Presence of larger epidermal cells in both upper and lower surfaces of *L. cyanescens* [(35.25±1.64)×(31.25±2.36), (43.0±2.63)×(39.5±5.11)] respectively compared to *L. micrantha*. Glandular multicellular trichomes are present in *L. micrantha* but absent in *L. cyanescens*. Numerous trichomes surround the transverse section of the leaf of *L. micrantha* but absent in *L. cyanescens*. Preliminary phytochemical screening showed that both species contain secondary metabolites such as alkaloids, anthraquinones, cardiac glycosides, tannins, saponins, steroids and flavonoids.

Conclusions: The microscopic and phytochemical data provided in this study are useful for the standardization of the medicinal plants.

KEYWORDS

Leaf epidermis, Trichomes, Stomata, Anatomy, Medicinal plants, Nigeria

1. Introduction

Medicinal plants have great applications in folk medicine within the African region; there is heavy reliance on them in alleviating various disease conditions with the consequent risk of gradual but imminent extinction[1-5]. Many plants are investigated for the development of phytocompounds useful in drug development[6]. There is no doubt that these natural compounds from plants had contributed positively to the health care delivery system in many rural communities in Africa[7]. However, a review of literature revealed that there are still seemingly insurmountable problems of mis-identification in many medicinal plant groups. This has increased the quest for search for other efficient methods for the identification of medicinal plants and herbal medicines[8-10].

Thus, with the increasing trend in the use of medicinal plants, botanicals or herbal preparations particularly in

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developing countries where orthodox medical care is neither readily accessible nor affordable, it is only proper to ascertain quality control standards in the herbal products from these plants before they are subjected to human utilization[11]. The two medicinal plants treated in this study are important in African ethno medicine. The two plants have been mentioned in our recent ethnobotanical survey as part of the plants used in treating psychosis in Nigeria[12,13]. Other medicinal uses of *Leptoderris micrantha* (*L. micrantha*) include its use for dropsy, swellings, oedema, gout, and pulmonary troubles[14]. The root of *Lonchocarpus cyanescens* (*L. cyanescens*) is used to teat arthritis and root and stem decoction is given to females after childbirth and also used to treat hernia[15].

Anatomical and micromorphological characteristics of leaves have played an important role in plant taxonomy, especially of particular groups at generic and specific levels. Studies in this field have attracted the attention of plant morphologists and systematists to resolve taxonomic conflicts in different groups of plant[16,17]. Although many studies have been conducted in this area in other plants for the purpose of correct identification of plant[18-22], little is known about the anatomy and micromorphology of the medicinal plants under study. Apart from a sketchy description of *L. micrantha*, little or no work is available on its pharmacognostic features. The same is true for *L. cyanescens*.

Therefore, in view of the wide medicinal applications of the two plants under investigation, the present study was undertaken in order to document information on micromorphological features of the plants which would help in their identification and authentication as well as provide basic pharmacognostical data required for a herbal pharmacopoeial compilation.

Thus, this paper reports on the macro and micro characters of *L. cyanescens* and *L. micrantha* leaves, their specific physical and chemical standards which may be useful as quality control parameters in the Nigerian herbal pharmacopoeia.

2. Materials and methods

2.1. Plant collection and authentication

L. cyanescens was collected at the main site of Faculty of Veterinary Medicine, University of Ibadan, Nigeria. while *L. micrantha* was collected at Iddo local Government along Eruwa road in Ibadan, Nigeria. The specimens were identified and authenticated by Mr. O. A. Osiyemi at the Forest Herbarium, Ibadan (FHI), Nigeria where vouchers were also deposited under FHI number 109689 for *L. cyanescens* and FHI number for *L. micrantha*, respectively.

2.2. Gross morphology

Morphological studies were carried out by observation of plant parts on the field with naked eyes and with the use of a hand lens where necessary.

2.3. Surface tissue preparation

For epidermal studies, Shultze's method of maceration with improved technique[23] was followed. Leaves were taken in petri dishes, covered with 4 mL of concentrated nitric acid and kept under sun for 30 min. Adaxial and abaxial epidermal peels obtained with the use of camel hair brush were rinsed in distilled water severally, bleached with one to two drops of chloral hydrate for 30 seconds. To remove chlorophyll, stained in 1 % aqueous Safranin O solution for 3 min and mounted in dilute glycerol for observation.

2.4. Transverse section

Leaf transverse sections were made with free hand sectioning using surgical scapel. Briefly, scalpel was used to cut 3 cm×4 cm of the plants through the midrib region. The small portion was inserted into a 3 cm×4 cm section of unripe pawpaw to enhance easy cut. The transverse sections obtained were cleared using sodium hypochlorite 3.85% M/V and stained with Safranin O reagent and rinsed with 70% alcohol. Glycerol was added as mountant. The tissue distribution through the midrib was examined under low power magnification and was photographed with compound microscope fitted with a digital camera.

2.5. Phytochemical screening

Fresh plant leaves were air-dried, powered with a blender and stored in cellophane bags for phytochemical screening. Phytochemical screening on the powdered leaves for various constituents such as alkaloids, anthraquinone, cardiac glycoside, glycoside, tannins, terpenoids, saponins and steriods flavonoids, phlobatannins etc. was done using the standard procedure[24].

2.6. Moisture content

The moisture content of the drugs (air dried plants) was determined. Briefly, powdered drug (2 g) was transferred into a china dish and distributed evenly to a depth not exceeding 10 mm. The loaded plate was heated at 105 °C in hot air oven and weighed at different time intervals until a constant weight was obtained. The difference in weight after drying and initial weight is the moisture content. Same experiment was repeated six times for precision and percent moisture for the sample was calculated.

2.7. Total ash value

Powdered drug (2 g) was weighted accurately into a tarred silica crucible and incinerated at 450 °C in muffle furnace until free from carbon. The crucible was cooled to room temperature and weighed. Percentage of ash was calculated with reference to air dried substance.

$$\% \text{ Ash value} = \frac{\text{Weight of residual ash}}{\text{Original weight of sample}} \times 100$$

2.8. Acid insoluble ash

Ash obtained from total ash was boiled with 25 mL of 2 N HCl for few minutes and filtered through an ashless filter paper. The filter paper was transferred into a tarred silica crucible and incinerated at 650 °C in muffle furnace until free from carbon. The crucible was cooled and weighted. Percentage of acid insoluble ash was calculated with reference to air dried substance as:

$$\% \text{ Acid insoluble ash} = \frac{\text{Weight of residual ash}}{\text{Weight of original sample}} \times 100$$

2.9. Water soluble ash

Ash obtained from total ash was boiled with 25 mL of distilled water for few minutes and filtered through an ashless filter paper. The filter paper was transferred into a tarred silica crucible and incinerated at 450 °C in muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of water soluble ash was calculated with reference to air dried substance.

2.10. Alcohol-soluble extractive value

About 5 g of the powdered drug was accurately weighed into 250 mL stoppered conical flask. A hundred milliliters of 90% ethanol was added and tightened firmly. The flask was shaken using a mechanical shaker for 6 h, and then allowed to stand for 18 h. The extract was filtered quickly (by suction filtration). The weight of a clean, heated and cooled flat-bottomed evaporating dish was accurately determined. Tweenty milliliters of the filtrate was evaporated to dryness. The residue was dried to constant weight at 105 °C in an oven and the final weight determined. The alcohol soluble extractive value was calculated thus:

$$\% \text{ Alcohol soluble extractive value} = \frac{\text{Weight of residue in 20 mL}}{20} \times 100$$

2.11. Water-soluble extractive values

About 5 g of powdered drug was treated with 100 mL water at in a stoppered flask with frequent shaking during first 6 h using electrical shaker and allowed to stand for 24 h. Temperature was maintained at 45 °C during entire process. Extract was filtered and 10 mL of filtrate was evaporated in a tarred dish at 105 °C and weighed. Water soluble extractive value was calculated as:

$$\% \text{ Water soluble extractive values} = \frac{\text{Weight of residue in 20 mL}}{20} \times 100$$

3. Results

3.1. Macroscopic characteristics

The two medicinal plants: *L. cyanescens* and *L. micrantha* differ in their morphological features. In *L. cyanescens* leaf shape is lanceolate, margin is entire, surface is glabrous, midrib is prominent with papery texture. In *L. micrantha*, leaf is oblanceolate in shape, margin is entire, apex is acute, surface is glabrous, petiolate with sessile flower and flat, fruit is broadly elliptic.

3.2. Epidermis

Leaf epidermal studies of *L. micrantha* and *L. cyanescens* were carried out in search of anatomical characters of taxonomic value that may contribute to the proper identification of the plants. Qualitative and quantitative features of the epidermal morphology of the plants are recorded in Table 1. The epidermal cells on the adaxial surface of *L. micrantha* have straight anticlinal walls and unicellular trichomes (Figures 1 A and B), while on the adaxial surface of *L. cyanescens*, epidermal cells have wavy/undulating anticlinal walls (Figures 1 C and D).

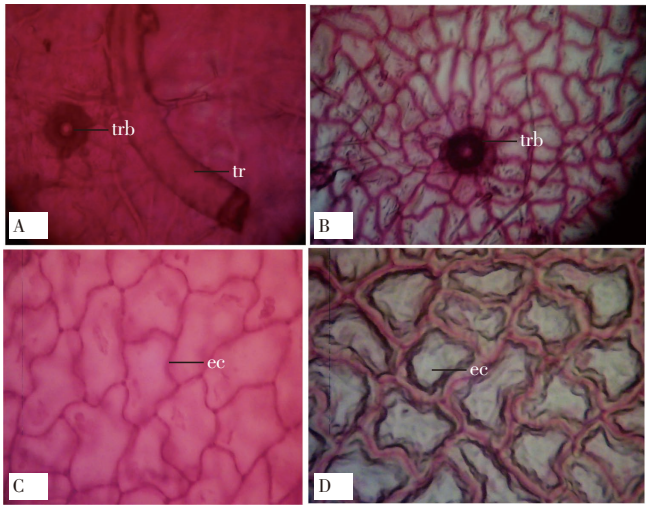

Figure 1. Adaxial epidermis of *L. micrantha* and *L. cyanescens*. (400×)
A&B: Adaxial epidermis of *L. micrantha*; C&D: Adaxial epidermis of *L. cyanescens*. trb: Trichome base; tr: Trichome; ec: Epidermal cell.

The abaxial surface of *L. micrantha* has straight to curve walls and peltate trichomes. No stomata were observed on either of the surfaces in this species (Figures 2 A and B). Epidermal cells are smooth to slightly wavy on the abaxial surface of *L. cyanescens* (Figure 2C). Both surfaces have many stomata, paracytic and anisocytic on the adaxial and abaxial surfaces, respectively (Figure 2D). However, fewer stomata and trichomes were seen on the adaxial surface on *L. cyanescens* compared to the numerous seen on its abaxial surface. Multicellular

Table 1
Combined qualitative and quantitative features of the epidermal morphology of *L. micrantha* and *L. cyanescens*.

Taxa	Leaf surface	Stomatal type	Epidermal cell shape	Anticlinal wall pattern	Trichome type	Trichome length (μm)	Trichome width (μm)	Epidermal cell length (μm)	Epidermal cell width (μm)	Stomatal length (μm)	Stomatal width (μm)
L. micrantha	Adaxial	Absent	Polygonal	Straight	Unicellular	232.5±29.5	26.0±5.8	27.0±1.7	21.0±1.7	Absent	Absent
	Abaxial	Absent	Polygonal/Irregular	Straight/Curved	Glandular, Peltate	338.8±47.3	19.3±1.5	25.0±1.4	22.5±1.6	Absent	Absent
L. cyanescens	Adaxial	Paracytic	Irregular	Undulate	Unicellular	253.0±57.2	18.0±3.2	35.1±1.6	31.1±2.4	–	–
	Abaxial	Anomocytic	Irregular	Undulate	Multicellular, Uniseriate	102.3±4.1	12.0±0.7	43.0±2.6	39.5±5.1	15.0±1.1	12.8±1.1

uniseriate trichomes were observed on the abaxial surface. Mucilage was observed on the abaxial epidermal cells of both plants.

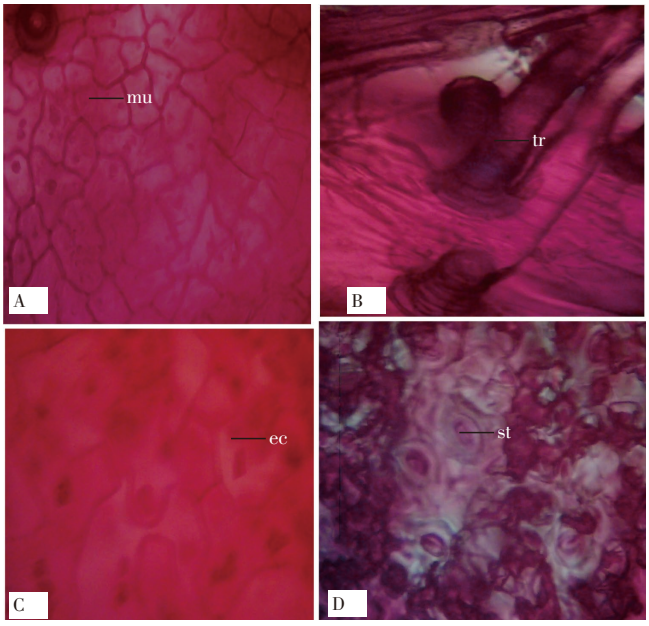

Figure 2. Abaxial epidermis of *L. micrantha* and *L. cyanescens*. (400×)
A & B: Abaxial epidermis of *L. micrantha*; C & D: Abaxial epidermis of *L. cyanescens* showing smooth to slight wavy epidermal wall and anisocytic stomata. mu: Mucilage; tr: Trichome; ec: Epidermal cell; st: Stomata.

3.3. Leaf transverse section

Transverse sections of leaf blades of *L. cyanescens* and *L. micrantha* are shown in Figures 3 A and B. The transverse section of *L. cyanescens* appeared concave on the abaxial and convex on the adaxial surface. Cuticle is moderately thick and the vascular bundle is arc-shaped. The xylem vessel is lignified as it picks up stain with safranin reagent. The transverse section of *L. micrantha* has numerous unicellular trichomes, covered with moderately thick cuticle. The vascular bundle is arc shaped with xylem inside and phloem outside. Variations were found in shapes and numbers of vascular bundles, trichomes and presence of mucilage. Also, numerous peltate trichomes were seen in *L. micrantha* while these were absent in *L. cyanescens*.

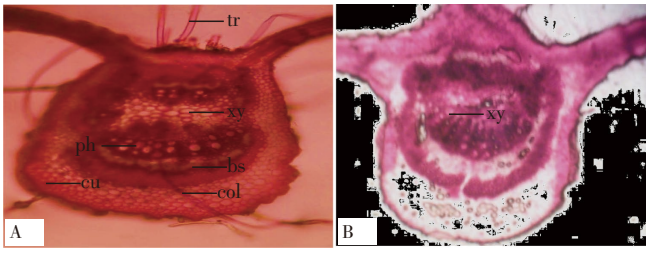

Figure 3. Transverse section of the leaf through the midrib regions of *L. micrantha* and *L. cyanescens*. (100×)
A: *L. micrantha*; B: *L. cyanescens*. tr: Trichome; xy: Xylem; bs: Bundle sheath; col: Collenchyma; cu: Cuticle.

3.4. Physicochemical constant and phytochemical data

The physicochemical constants recorded for the two medicinal plants: *L. micrantha* and *L. cyanescens* include: moisture content, total ash, acid insoluble ash, water insoluble ash, water extractive value, acid extractive value as presented in Table 2. Phytochemical data appeared in Table 3.

Table 2
The analytical values in respect of physicochemical constant for *L. micrantha* and *L. cyanescens*.

Physico-chemical constants	Mean analytical values	
	L. micrantha	*L. cyanescens*
Moisture content	6.500±6.500	14.000±2.800
Total ash	8.000±0.000	14.500±2.100
Acid insoluble ash	4.500±0.700	7.500±0.700
Water insoluble ash	10.000±1.400	7.750±1.100
Water extractive value	0.146±0.020	0.121±0.001
Acid extractive value	0.450±0.100	0.550±0.100

Table 3
Phytochemical screening results of the powdered leaves of *L. micrantha* and *L. cyanescens*.

Phytoconstituents	*L. micrantha*	*L. cyanescens*
Alkaloids	+	+
Anthraquinones	+	+
Cardiac glycosides	+	+
Tannins	+	+
Saponins	+	+
Steroids	+	+
Flavonoids	+	+
Phlobatannins	−	−

+: Present; −: Absent.

4. Discussion

The variations observed in the epidermal morphology of *L. micrantha* and *L. cyanescens* from the family Fabaceae could be used to distinguish the species. Anatomical features are widely used in systematics for identification, for placing anomalous groups in satisfactory position in classification and for indicating patterns of relationship that may have been observed by superficial convergence in morphological features[25]. Non-glandular unicellular trichomes on both adaxial and abaxial surfaces are considered interesting and the density of hairs was more abundant on the abaxial surface for both plants. The high density of thick and coated hairs probably serves to reduce the rate of transpiration in plants and this buttresses the importance of trichomes in taxonomy as a diagnostic tool[26].

The adaxial and the abaxial surfaces of both plant species have comparable sizes of epidermal cells. Few stomata and trichomes were seen on the adaxial surface on *L. cyanescens* compared to the numerous seen on its abaxial surface. Stomata were not observed on the adaxial and abaxial surfaces of *L. micrantha* although there are numerous trichomes on both surfaces. Thus, stomata character and trichomes can aid classification and identification of these plants.

The two medicinal plants appear to be rich in secondary metabolite, widely used in traditional medicine to manage or cure various ailments. Phytochemical screening showed that both species contain metabolites such as alkaloids, anthraquinones, cardiac glycosides, tannins, saponins, steroids and flavonoids. The presence of the various secondary metabolites may support the folkloric claims on the medicinal

potential of these plants as anti-inflammatory, antispasmodic, analgesic and antidiuretic.

Physical constant parameters and quantitative evaluation such as moisture content, total ash value, acid insoluble ash, water insoluble ash, water and acid extractive values which form part of the parameters evaluated in standardizing herbal medicines, were also determined in this study. Low moisture content is reported to indicate less chances of microbial degradation of plant drugs during storage[27]. This is in line with our observations in this study, the moisture content value of *L. cyanescens* and *L. micrantha* are respectively, (14.0±2.8) %, w/w and (6.0±6.5)%, w/w. The general requirement of moisture content in crude drug is that, it should not be more than 14%[28], thus the values obtained in this study are within the accepted range. A standard that is expressed as "not more than" value is recommended for total ash and acid insoluble ash values while water insoluble ash is expressed as "not less than" value. Based on the results obtained for *L. cyanescens* and *L. micrantha*, the total ash should not be more than 14.5±2.1 and 8.0±0.0 respectively with the acid insoluble ash values of not more than 7.5±0.7 and 4.5±0.7 respectively. The water insoluble ash should not be less than 7.75±1.1 and 10±1.4 respectively. For water extractive and acid extractive values which are also expressed as "not less than", the results obtained indicate that *L. cyanescens* and *L. micrantha* should not be less than 0.121 ±0.001 and 0.146±0.020 respectively for water extractive values and 0.55±0.10 and 0.45±0.10 respectively, for acid extractive values.

Anatomical studies on the two medicinal plants revealed sharp generic variations in sizes and types of stomata, shapes of epidermal cells on the adaxial and abaxial surfaces, sizes and types of trichomes, all of which could be employed in species identification. Correct identification of medicinally important species is necessary for their sustainable and effective utilization as well as for detecting adulteration of plant drugs[29,30]. Thus, micromorphological character variations observed in our study had provided the basis for the identification of these plants highlighting their taxonomic relationships in line with report on other plants[31-33]. Leaf epidermal and anatomical features such as stomata, trichomes and other markers have proved useful and of great taxonomic significance[34-36]. In our study of the micromorphological features and phytochemical screening of the leaves of *L. micrantha* and *L. cyanescens*, we are able to draw the following conclusions: Firstly, stomata are absent on both adaxial and abaxial surfaces of *L. micrantha*. Secondly, the leaves of both plants contain alkaloids, anthraquinones, cardiac glycosides, tannins, saponins, steroids, flavonoids which may be responsible for the therapeutic effects of the two plant species.

The morpho-anatomical features observed in the two medicinal plants are sufficiently distinctive and may be used at specific level for delimitation of plants. The different features peculiar to each of the medicinal plants provide the basis for its detailed assessment and form part of the catalogue of characters that may be employed in proper identification of these plants and as quality control standards which can be used for the compilation of herbal pharmacopoeial.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

A major problem in traditional medicine is the misidentification of drug plants particularly when these are bought directly from the local markets. There is therefore the need to use other characters apart from the macrocharacters to ascertain the authenticity of such crude drug samples.

Research frontiers

The study provides information on the microcharacters and pharmacognostic features of *L. cyanescens* (Schum & Thonn.) Benth. and *L. micrantha* Dunn.

Related reports

Some related researches are Taxonomic importance of anatomical and micromorphological features in plants (Kahraman and Celep, 2010, Saheed and Illoh, 2010), and Plants are investigated for the development of phytocompounds useful in drug development (Cornara *et al.*, 2013).

Innovations and breakthroughs

Additional information on useful characters for the authentication of samples of the two important medicinal plants.

Applications

It provided some means of authentication of crude drug samples for new drug development.

Peer review

It is a good paper reporting the anatomical and pharmacognostic features of two Nigerian medicinal plants thus ensuring the correct identification of both plants when collected in fragmentary forms and screened for subsequent drug development.

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