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Original Research Article

Histological and Micromeritic Differences Between the Two Varieties of *Lonchocarpus sericeus* (Poir.) Kunth ex DC. (Fabaceae)

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Abstract

Purpose: *Lonchocarpus sericeus* (Poir.) kunth ex DC. (Fabaceae) is a tropical plant used in ethnomedicine as an analgesic, appetite stimulant, antibacterial and cytotoxic agent. This study aims to establish histological and micromeritic parameters as standards for the leaves of brown and white varieties.
Methods: The leaves of both varieties were collected, dried under shade and pulverized. Microscopic evaluations were done on both the fresh and powdered samples. Micromeritic properties were determined for the plant organs collected. Carr's index, angle of repose and Hausner's ratio were calculated. Chemomicroscopic and fluorescence evaluations were also done on the powders.
Results: The results obtained from the microscopy of the fresh leaves revealed hypostomatic stomatal distribution and anomocytic type of stomata for the brown variety and paracytic type of stomata for the white variety both on abaxial surfaces. The epidermal cell shapes for the abaxial and adaxial surfaces were both irregular. The stomatal number was found to be 36.9 ± 0.71 and 17.8 + 0.97 for the brown and white varieties respectively. The stomatal index was 8.13 %. and 7.45 % for the brown and white varieties respectively. The angle of repose for the brown and white varieties were 33.7 ± 0.72 and 40.05 ± 0.57 respectively. The chemomicroscopic result revealed that both leaves contained cellulose, mucilage, lignin, protein and starch.
Conclusion: The results obtained from this study can be used to distinguish between the two varieties, and could also serve as pharmacopeial standards for quality assurance of the medicinal plant as a herbal drug.

Keywords: *Lonchocarpus sericeus*, Carr's index, Hausner's ratio, micromeritic, histological

Introduction

The identification of ethnomedical important species [1] and varieties is fundamental to a successful usage of medicinal plant as a phytotherapeutic agent. Issues of adulteration has resulted in the loss of trust in herbal formulations. Non intentional adulteration of herbal drugs could occur as a result of faulty collection due to similarity in appearance and color of plants closely related to the authentic drug. A typical example is the substitution of *Mucuna pruriens* (L.) DC. (Fabaceae) with *Mucuna pruriens* var. utilis (Wall. ex Wight) L.H. Bailey (Fabaceae) marketed as the white variety [2].

Lonchocarpus sericeus (Poir.) Kunth ex DC. (Fabaceae) commonly named cube root is a tree of about 15 meters high [3,4] used in ethnomedicine for the treatment of convulsion, painful condition, arthritis, infection and as insect repellants [5,6]. Numerous reports have validated the usefulness of this plant as claimed in folkloric applications including anti-inflammatory, antimicrobial [7], analgesic [6],

antiplatelet activity [4], cytotoxic activity [8] and hepatoprotective effect [9].

However, this important legume exists in two varieties with similar morphological features. It is pertinent to distinguish histologically the differences between the two varieties of *L. sericeus* to avoid a situation of faulty or erroneous collection of the plant for research or therapeutic purposes. This study aims to establish histological and micromeritic parameters of the leaf to serve as a means of identifying the varieties.

Methods

Plant identification and collection

L. sericeus brown variety (LSB) and *L. sericeus* white variety (LSW) leaves were collected in the month of December 2021 at the Ibesikpo/Asutan Local Government Area in Akwa Ibom State and were identified by Professor Mrs. Margarete Bassey of the Department of Botany and Ecological Studies, Faculty of Sciences, University of Uyo, Nigeria and the herbarium specimens were deposited in the University of Uyo Herbarium with voucher specimen number UUH 3213 for the brown variety and UUH 3212 for the white variety for reference purpose.

Drying, pulverization and extraction of plant material

Foreign matters were removed from the *L. sericeus* organs collected and then air dried separately under shade for 7 days. The dried plant parts were pulverized, sieved through 350 µM and separately stored in an airtight container in a cool and dry place.

The powdered plant parts were extracted with methanol (99 %) through maceration for 72 hours and extracts obtained were concentrated in vacuum at 60ºC using rotary evaporator. The extracts were weighed, kept in labelled bottles and stored in the refrigerator at 4º C until required.

Anatomical evaluation of LSB and LSW leaf

Qualitative microscopical examination

A fully grown, well-expanded leaf was sliced along the midline. To enable microscopic examinations of the epidermis on both the adaxial and abaxial sides, the sliced portion was set up on a glass slide. The sample was gently scraped with a sharp razor blade to remove the loose skin and acquire the necessary epidermis after being cleaned with water. Later, sodium hypochlorite was used to clean the epidermal peels.

The epidermis was stained with Safranin-O solution for 5 minutes after which excess stain was washed off with water before being mounted using 10% glycerol. The stained samples were examined using a binocular microscope. An Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera was used to take photomicrographs of preparations. Magnification for measurements was x10, and for photomicrographs it was x40 [10].

Quantitative microscopical examination

Using standard techniques, measurements of the leaf constants, such as stomatal length and breadth, guard cell length and breadth, stomatal number, stomatal index, epidermal cell length and width, number of epidermal cells, and thickness of epidermal cells, were taken. Data for 10 randomly selected microscopic views were presented as mean ± standard error of mean (SEM) for all readings taken using a calibrated ocular micrometre [11].

Analysis of LSB and LSW leaf powders

The bulk density, tap density, angle of repose, Hausner's ratio, and Carr's index of the pulverized and sieved powdered leaves were measured using standard methods [10,12,13]. In order to explore the chemomicroscopic aspects of the powders, including cellulose, mucilage, lignin, starch, protein, oils, and calcium oxalate crystals, conventional chemomicroscopic methods were used [10,14]. The fluorescence examination of the powders was performed using the standard technique.

Statistical analysis

GraphPad prism version 6.01 was utilized in this study and results were presented using the descriptive statistics mean and SEM.

Results

Figure 1 revealed that the adaxial surfaces of both varieties have irregular epidermal cell wall pattern in common. Figure 2 displays the abaxial surface of both varieties showing anomocytic type of stomata for the brown variety while paracytic type of stomata was seen in the white variety. The cell walls were observed to be undulated and anticlinal in nature. Figure 3 reveals unicellular or covering trichomes on the abaxial surfaces of both varieties.

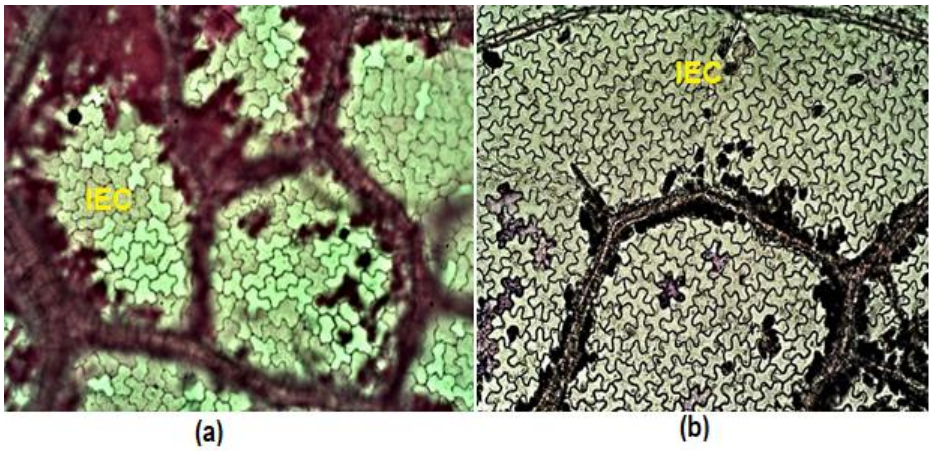

Figure 1: Adaxial surface of the leaf of LSB (**a**) and LSW (**b**) showing irregular epidermal cells (IEC) ×40

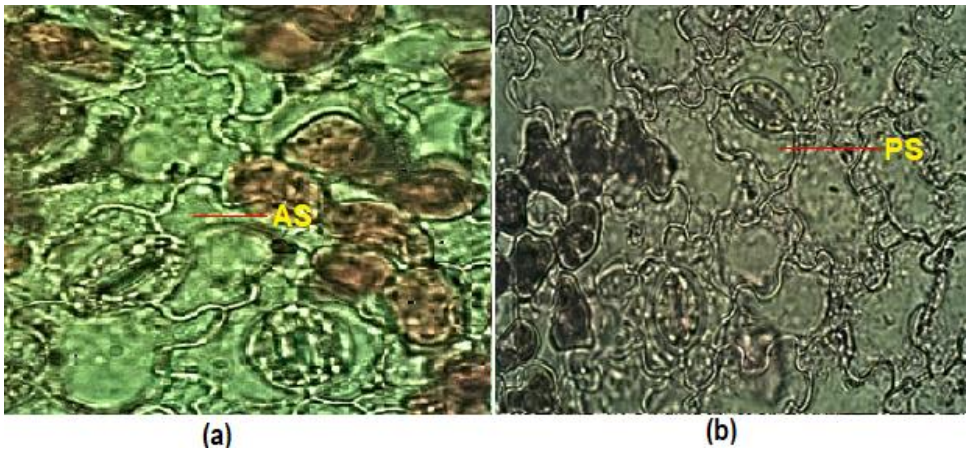

Figure 2: Abaxial surface of the leaf of LSB (**a**) and LSW **(b**) showing anomocytic (AS) and parasitic (PS) stomata respectively with epidermal cells ×40

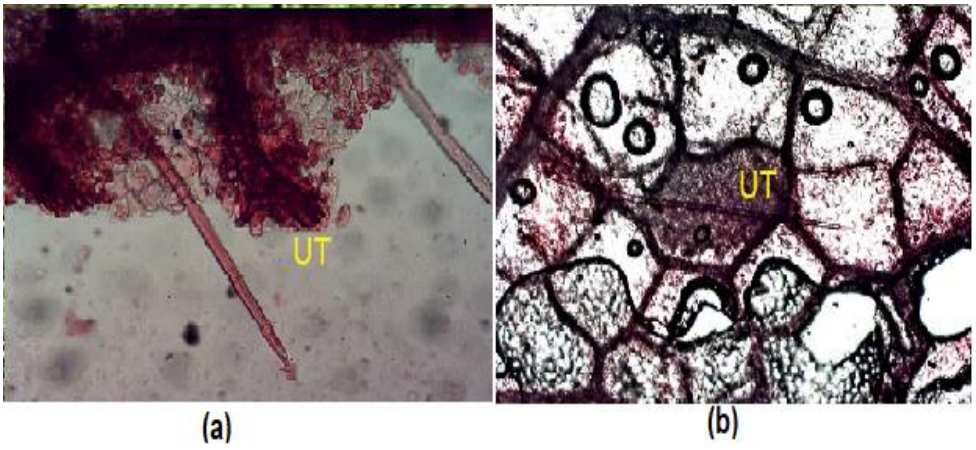

Figure 3: Abaxial surface of the leaf of LSB (**a**) and LSW (**b**) showing unicellular trichomes (UT) ×40

Table 1 shows that higher values were obtained for epidermal cell number (465 ± 1.38) and cell wall thickness (2.87 ± 0.16) for the white variety while lower values were obtained for the epidermal cell length (37.06 ± 1.57) and cell wall thickness (1.79 ± 0.04) for the brown variety. The brown variety's epidermal cell width (15.01 ± 1.12) is of higher value than the white variety with an epidermal cell width of 13.6 ± 1.63.

Table 1: Quantitative and qualitative micromorphological features of the leaf of two varieties of *L. sericeus*

Parameters	**LSB - Adaxial**	**LSW - Adaxial**
Epidermal cell shape	Irregular	Irregular
Epidermal layer number	298(350 ± 9.62)377	225(319 ± 13.11)348
Epidermal cell length (µm)	32.32(37.06 ± 1.57)48.98	43.83(46.5 ± 1.38)57.27
Epidermal cell width (µm)	10.08(15.01 ± 1.12)19.14	9.44(13.06 ± 1.63)23.68
Epidermis cell wall thickness (µm)	1.60(1.79 ± 0.04)1.99	1.96(2.87 ± 0.16)3.61
Anticlinal wall pattern	Undulate and sinuous	Undulate and sinuous
Stomata distribution	Hypostomatic	Hypostomatic

Values are expressed as Mean ± SEM. n=10

Table 2 compares the values obtained for LSB and LSW. Higher values were obtained for epidermal cell number (416 ± 3.74), cell wall thickness (2.77 ± 0.09), stomata pore length (7.12 ± 0.44), stomata width (11.84 ± 0.35) and stomata number (369 ± 0.71) for the brown variety while lower values were gotten for epidermal cell number (221.00 ± 2.27), cell wall thickness (209.00 ± 0.12), stomata pore length (6.92 ± 0.42), stomata width (11.34 ± 0.44) and stomata number (17.80 ± 0.97). Also, higher values were obtained for the trichome length (373 ± 4.9), trichome width (7.27 ± 0.15), the width of epidermal cell (16.87 ± 1.08), guard cell length (10.2 ± 0.46), guard cell width (5.12 ± 0.34) with lower values in stomata length (19.8 ± 0.81) and stomata width (11.3 ± 0.44) for the abaxial surface of the white variety while lower values where gotten for trichome length (334.22 ± 3.74), trichomes width (6.23 ± 0.188), the width of epidermal length (16.47 ± 1.42), guard cell length (9.35 ± 0.34), guard cell width (4.37 ± 0.25) and higher value of stomata length (21.83 ± 0.14), stomata width (11.84 ± 0.35) for the abaxial surface of the brown variety.

Table 2: Quantitative and qualitative micromorphological features of the leaf of two varieties of *L. sericeus*

Parameters	**LSB - Abaxial**	**LSW - Abaxial**
Epidermal cell shape	Irregular	Irregular
Epidermal layer number	393 (416.6±3.74) 424	211 (221±2.27) 231
Epidermal cell length (µm)	20.32 (28.04±1.63) 36.94	32.94 (42±3.1) 55.19
Epidermal cell width (µm)	10.71 (16.47±1.42) 23.49	13.35(16.87±1.082)23.84
Epidermis cell wall thickness (µm)	1.96 (2.27±0.09) 2.48	1.24(2.09±0.12)2.48
Stomata type	Anomocytic	Paracytic
Anticlinal wall pattern	Undulate sinuous	Undulate sinuous
Stomata distribution	Hypostomatic	Hypostomatic
Stomata pore length (µm) (X10)	5.32 (7.12±0.44) 9.15	5.44(6.92±0.42)9.32
Stomata pore width (µm) (X10)	2.18 (2.95±0.14) 3.31	2.07(3.22±0.20)3.92
Stomata width (µm)	10.70 (11.84 ± 0.35) 14.05	10.26(11.3 ± 0.44) 13.38
Stomata length (µm)	21.07 (21.83 ± 0.14) 22.34	17.02(19.8 ± 0.81)23.46
Stomata number	33 (36.9±0.71) 41	14(17.8±0.97)22
Stomata index (%)	8.13	7.45
Trichome type	Unicellular trichome	Unicellular trichome
Trichome length (µm)	311.91(334.22±3.50)344.80	345.57(373.22±4.90)391.71
Trichome width (µm)	5.54(6.23±0.188)7.01	6.67(7.27±0.15)7.94
Length of guard cell (µm)	8.69(9.35±0.34)10.84	9.43(10.2±0.46)13.26
Width of guard cell (µm)	3.28(4.37±0.25)5.47	3.87(5.12±0.34)7.23

Values are expressed as Mean ± SEM. n=10

Table 3 shows the micromeritic evaluation of the leaf powders of the two varieties. Bulk and tapped densities were observed to be 44.66 ± 0.816 and 31.33 ± 0.816 respectively for the brown variety while bulk and tapped densities of the white variety were 36.00 ± 0.70 and 26.33 ± 0.81 respectively. Hausner's ratio and Carr's index of 1.42 ± 0.03 and 30.56 ± 1.14 were obtained respectively for the brown variety, while that of the white variety were 1.37 ± 0.02

and 28.00 ± 1.20 respectively. The angle of repose for the brown and white varieties was 33.70 ± 0.72 and 40.05 ± 0.057. Table 4 displays the chemomicroscopy analysis results of the powdered leaf of both varieties. The presence of mucilage, cellulose, lignin, starch, and protein were observed in the samples. Table 5 displays the fluorescence analysis results of LSB and LSW. The presence of secondary metabolites such as anthocyanins, phenolics, and flavonoids based on the emission of light with different wavelengths were observed.

Table 3: Micromeritic properties of the leaf of two varieties of *L. sericeus*

Micromeritic	LSB	LSW
Bulk Volume (mL)	44.66 ± 0.816	36.00 ± 0.70
Tapped Volume (mL)	31.33 ± 0.816	26.33 ± 0.81
Bulk density (mL)	0.21 ± 0.003	0.27 ± 0.004
Tapped density (g/mL)	0.31 ± 0.004	0.38 ± 0.012
Flow rate (g/s)	0.32 ± 0.07	0.24 ± 0.02
Angle of repose (degrees)	33.7 ± 0.72	40.05 ± 0.57
Carr's index (%)	30.56 ± 1.14	28.00 ± 1.20
Hausner's ratio	1.42 ± 0.03	1.37 ± 0.02

Values are expressed as Mean ± SEM. n=10

Table 4: Chemomicroscopic evaluation of the leaf of two varieties of *L. sericeus*

Constituents	LSB	LSW
Mucilage test	+	+
Lignin test	+	+
Starch test	+	+
Calcium oxalate	-	-
Protein test	+	+
Cellulose test	+	+

Table 5: Fluorescence analysis of the leaf of two varieties of *L. sericeus*

Extract	Physical observation (visible light)	UV-253.7 nm Colour	UV-365 nm Colour
Water			
LSB	Light brown	Grey	Light green
LSW	Pale brown	Grey	Light green
Methanol			
LSB	Light green	Light yellow	Orange-yellow
LSW	Light-green	Yellow	Light yellow
Ethyl acetate			
LSB	Green	Yellow	Red
LSW	Green	Pink	Deep pink
N-Hexane			
LSB	Dirty green	Yellowish-brown	Red
LSW	Dirty-green	Light brown	Deep pink
$FeCl_3$			
LSB	Brown	Purple	Maroon
LSW	Brown	Light purple	Maroon

Discussion

L. sericeus is an important medicinal plant with economic and ethnomedical importance. The two varieties of this plant have been observed to elicit different biological activities with a couple of other effects which they share in common. Sometimes, wrongful collection may not give the right biological effect as reported by traditional medicinal practitioners (TMP).

Micromorphological evaluation of the two varieties have shown that the plants have some basic histological features in common such as irregular epidermal cells (Figure 1), hypostomatic distribution of stomata, unicellular trichome (Figure 3) and undulated anticlinal cell wall pattern. Anomocytic type of stomata (Figure 3) was observed in the brown variety while paracytic type of stomata was seen in the

white variety. The qualitative parameters studied on the abaxial surface of LSB and LSW has lend credence to data that can be useful in the delineation of the two varieties during identification and authentication of the plants. However, quantitative microscopy has shown dissimilarities in some features that could be used to differentiate the two varieties as well.

Higher values were gotten for epidermal cell number (416 ± 3.74), cell wall thickness (2.77 ± 0.09), stomata pore length (7.12 ± 0.44), stomata width (11.84 ± 0.35) and stomata number (369 ± 0.71) for the brown variety while lower values were gotten for epidermal cell number (221.00 ± 2.27), cell wall thickness (209.00 ± 0.12), stomata pore length (6.92 ± 0.42), stomata width (11.34 ± 0.44) and stomata number (17.80 ± 0.97) (Table 2).

It is in the literature that epidermal features can serve as markers for the identification and authentication of medicinal plants [15]. The cited literature further affirms that these differences as documented by the study can be used to identify and authenticate the two varieties of *L. sericeus.*

Hausner's and Carr's index is an indication of the powder's flowability and characteristics. Hausner's ratio of less than 1.25 is an indication of a good flow property and vice versa. From the research, both indices were observed to be greater than the limits which suggest that the powders of both plants have poor flow profile (Table 3). It has been reported in the literature that factors such as moisture content, particle size and shape, and even storage period could be responsible for the poor flow observed [11].

The inter-particle friction and obstruction of movement in granular materials are fundamentally characterized by the angle of repose. The degree of friction and the likelihood of granular flows are reflected by this essential geometric characteristic [16].

Powders from both varieties were observed to have a poor flow profile. This could be due to the presence of fibre or moisture content that may increase particle size agglutination, thus increasing the particle size friction. This is an important consideration in the development of solid dosage forms, where the flow property is used for physical, mechanical and chemical processes.

Chemomicroscopy analysis of the powdered leaf of both varieties revealed the presence of mucilage, cellulose, lignin, starch, and protein as shown in Table 4. This shows the leaf could be used as a bulk laxative due to the presence of cellulose and high fibre content.

Fluorescence analysis as shown in Table 5 has revealed the presence of secondary metabolites such as anthocyanins, phenolics, and flavonoids based on the emission of light with different wavelengths based on the colour that fluoresces. Slight differences were observed which could be due to the expression of different compounds in the varieties.

Conclusion

The results obtained from this study have provided information that can be useful in the identification and authentication of the two varieties for correct collection and could also serve as standards for the quality control of herbal product formulation from these plants.

Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The study was conceived, designed and written by DA. JO conducted the study under supervision. IJ analyzed the samples. EO collated and analyzed the data. ECO assisted in the design of the study.

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