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Original Research Article

PHARMACOGNOSTIC EVALUATION *OF LASIMORPHA SENEGALENSIS* SCHOTT LEAVES (ARACEAE)

GINIKACHUKWU MARYROSE OKOH[1]*, OBINNA SABASTIN ONUGWU[1], OBODIKE CHRISTOPHER EZUGWU[2]

1. Department of Pharmacognosy, Faculty of Pharmaceutical Science, Enugu State University of Science and Technology, Agbani, Nigeria.
2. Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Science, University of Nigeria Nuskka, Nigeria.

ABSTRACT

The leaves of *Lasimorpha senegalensis* Schott (Araceae) have long been utilized in conventional medicine to treat conditions like diabetes, inflammation, malaria, oxidative stress, and fever. However, its properties have not been standardized to prevent adulteration. The purpose of this research is to determine *Lasimorpha senegalensis* Schott's numerous pharmacognostic, physicochemical, and phytochemical parameters. Fresh leaf samples and dried leaf powder were examined macroscopically and microscopically. The physicochemical and phytochemical parameters were determined using the standard methods. Fresh leaves have a unique scent and are dark green in colour, leathery texture, and acute apex. In addition to lacking trichomes, they have calcium oxalate crystals, starch grains, xylem, phloem, epidermal cells, and collenchyma cells. There was no mucilage, but lignin, starch, cellulose, oil globules, and calcium oxalate crystals were found by chemomicroscopic examination. The physicochemical assessment showed 9.2 % moisture content, 8.4 % total ash value, 0.9 % acid insoluble ash value, 2.6 % water soluble ash value, 16.5 % water soluble extractive value, and 41.5 % alcohol soluble extractive value. The qualitative phytochemical test identified tannins, glycosides, alkaloids, terpenoids, phenols, and flavonoids, as well as trace amounts of steroids, saponins, and hydrogen cyanides. This study provided valuable insight into the pharmacognostic standardization of *L. senegalensis* leaves and can be used to create a monograph and prevent adulteration of this important leaf plant.

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INTRODUCTION

The process of standardizing crude drugs involves several important steps, including authenticating the drug's identity, determining its quality and purity, and identifying any potential adulteration. The most critical factors regarding medications are purity, safety, potency, and efficacy for consumers. Medicinal plants have become increasingly popular worldwide due to cultural and historical factors, but this has also led to safety, efficacy, and regulation concerns. To solve these

problems, the public and medical professionals must have access to trustworthy data regarding the efficacy and safety of medicinal plants[1]. One of the major challenges in this area is the lack of standardization techniques for herbal medicines. To promote safety, efficacy, and quality control, the World Health

***Corresponding author:** ginikachukwu.uzor@esut.edu.ng; +234-806 775 5972

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Organization encourages countries to develop safe and effective traditional remedies. By encouraging uniformity in the use of herbal remedies, the creation of monographs can help achieve this objective. To standardize crude drugs, their physicochemical, phytochemical, and pharmacognostic properties are essential to ensure the effectiveness and safety of herbal remedies[2].

Lasimorpha senegalensis Schott. a perennial plant belonging to the Araceae family, commonly referred to as swamp Arum. Locally, it is called Mpoto-iyi, Ope igo, and Minjirya in Igbo, Hausa, and Yoruba, respectively[3]. This plant has a short, thick, stoloniferous rhizome that supports a cluster of leaves. Each leaf consists of an erect, spiny petiole with a leaf blade in the shape of an arrow on top. The inflorescence is a purplish, cylindrical spadix enclosed by a spathe on a spiny, solitary peduncle emerging from the leaves[4]. They are Indigenous in Africa, growing abundantly in lakes, waterways, streams, and swampy woodlands[5]. The leaves are used by the indigenous people of Nsukka, Isi-uzo, and Nike in Enugu State to treat hepatitis and fever-producing illnesses such as malaria. The fruits are used as a component of several medications and are reportedly an effective treatment for urinary tract infections, dysentery, and other digestive disorders[4]. According to earlier research, the plant's leaf extract has different medicinal activities including antioxidant and hepatoprotective activities[6], and antibacterial activity[4]. The purpose of this study is to standardize the pharmacognostic characteristics of the leaves of *Lasimorpha senegalensis*. This will be accomplished through the application of various techniques, including microscopy, macroscopy, physicochemical analysis, phytochemical screening, and extractive values. These elements are important in assessing the drug's quality and can help create accurate monographs for its authentication and identification. Again, this data can also prove instrumental in incorporating the drug into the African Pharmacopoeia.

MATERIALS AND METHODS

Materials

Ethanol (90 %), ethyl acetate, n-hexane, butanol, ferric chloride, million reagent, alpha-naphthanol, Dragendorff reagent, Benedict's solution, diluted hydrochloric acid, sodium hydroxide, ammonia, sulfuric acid, and detecting reagents are of analytical grade gotten from the Pharmacognosy Department's research lab at Enugu State University of Science and Technology, Agbani.

Plant collection and identification

Lasimorpha senegalensis fresh leaves were collected in September 2022 at Ako-Nike in Enugu-East Local Government Area, Enugu State located in the Southeast, Nigeria. Dr. Felix Nwafor of the University of Nigeria, Nsukka (UNN), Enugu State, Nigeria, Department of Pharmacognosy and Environmental Medicine, verified the plant. Voucher specimens PCG/UNN/0445 were placed in the Department's Herbarium for later use.

Extraction and Fractionation Methods

Fresh leaves of the plant were collected and cleaned under running water to get rid of dust and other debris and were allowed to air dry for 15 days. The dried plant was pulverized using a grinding machine, sieved to regulate the particle size, and then kept in a sterile, sealed amber bottle. The powdered leaves sample weighing 994.94 grams was macerated with 3000 ml of 90 % ethanol at room temperature for 72 hours. A rotary evaporator was utilized to recover the filtrate, and a water bath set at 40 degrees Celsius was used to concentrate it until it was completely dry. Before usage, the extract was kept in a refrigerator. By adsorbing the 40 g crude ethanol extract of *L. senegalensis* on 60 g of silica gel, the crude extract was fractionated. Organic solvents of increasing polarity such as n-hexane, ethyl acetate, and butanol were used as the mobile phase, to obtain the different fractions using column chromatography.

Macroscopic Evaluation

The fresh leaves' macroscopic characteristics studied are leaf size, shape, type of venation, colour, margin, leaf base, leaf apex, odor, and taste of the leaf [7]

Microscopic Evaluation

The clearing approach was used to prepare the foliar epidermis on both the adaxial (higher) and abaxial (lower) leaf surfaces. Using an industrial bleach that contained 3.5% hypochlorite sodium, the leaf samples were cleaned for eighteen hours. After gently scraping the leaf sample epidermal strips with forceps, they were put on a sanitized slide. The slide was stained with a solution of safranin and covered with a cover slip. Using a light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA), the slides were magnified 40, 100, and 400 times. The photomicrographs were taken using the Moticam 2.0 image system with software (Motic Carlsbad, CA, USA) connected to the microscope [8].

Quantitative Microscopic Evaluation

Using the method described by Obinna *et al*, quantitative leaf microscopy was performed on epidermal strips to measure the palisade ratio, stomata number, stomata index, vein islet number, and vein let termination number[8].

Chemomicroscopic Examination

The powder leaves sample was analyzed using standard methods to check for protein, cellulose, fatty oil, crystals of calcium oxalate, mucilage lignified walls, and starch grain[9].

Analytical Standard Determination

To assess the drug's quality and purity, an analytical constant of the leaves was established. With minor adjustments, the techniques of Evans [9] and Mustherjeee [10] were used to assess the ash values, extractive values, and moisture content.

Qualitative Phytochemical Screening

To test for glycosides, tannins, hydrogen cyanides, alkaloids, flavonoids, steroids, phenols, terpenoids, and saponins on the powdered leaves, qualitative phytochemical screening was done using standard methods [9] [11].

RESULTS

The Extractive Yield of *Lasimorpha senegalensis* Leaf

A total of 101.31 g of ethanol crude extract (ECE) was obtained after the extraction process. Using 40 g of the ECE in Vacuum Liquid Chromatography, ethyl acetate fraction as shown in **Table 1**, gave the highest percentage yield of 39.8 % which indicates that it extracted more phytochemical constituents than other solvents used.

Macroscopic Evaluation of *Lasimorpha senegalensis* Leaf

According to Table 2 of this study, the macroscopic characteristics of *L. senegalensis* leaves include an entire border, net venation, an acute apex, a dark green colour, and a distinctive scent. The live picture of the plant as shown in **Figure 1** has a very long petiole (75 - 80 cm) with broad leaves and are mostly abundant in swampy and water-logged areas.

Microscopic Evaluation of *Lasimorpha senegalensis* Leaf

The leaf stomata are paracytic and amphistomatic, meaning they exist on both the bottom and top surfaces, as illustrated in Figures 2 and 3. The microscopic characteristics of the leaf section reported in Table 3 revealed a polygonal epidermal layer on both the upper and below surface of the leaf. Other cell inclusions such as calcium oxalate, oil globules, starch grain, and lignified tissues were all present as indicated in **Table 4.** The photomicroscopy of these cell inclusions is also represented in **Figure 4-7.** The absence of trichomes and mucilage was also observed in the leaf. Figure 8 depicts the transverse section of the leaf, which reveals vascular bundles, spongy and palisade mesophyll, and two epidermal layers.

Phytochemical Evaluation of *Lasimorpha senegalensis* Leaf

Table 5 shows the outcome of the qualitative phytochemical analysis of the fractions and crude extract of ethanol. The crude extract and n-butanol fraction of *L. senegalensis* contains all the phytochemical constituents tested. The n-hexane fraction was positive to only saponin test while the Ethylacetate fraction tested negative to only tannins and glycoside.

Analytical Evaluation of *Lasimorpha senegalensis* Leaf

As seen in Table 6, the moisture content, ash value, and extractive values are among the physicochemical properties assessed. L. senegalensis had a moisture content of 9.2% and a total ash value of 8.4%. Extractive values for alcohol and water were also measured; alcohol yielded the highest value at 41.5%.

DISCUSSION

The evaluation of medicinal plants must consider several crucial factors, including precise identification, quality control, and the establishment of pharmacognostic standards. According to the World Health Organization (WHO), the identification and degree of purity of a medicinal plant are first determined by macroscopic and microscopic examination before any tests are conducted on it [12]. Macroscopic examination of *L. senegalensis* leaves indicated that they have an entire border, net venation, an acute apex, dark green colour, and a distinct fragrance. As a result, for accurate identification and authenticity, macroscopic analysis of medicinal plants should be performed to confirm their identity and purity to avoid unintentional adulteration during plant collection and distinguish them from other plants. The study investigated stomata, epidermal cells, oil globules, calcium oxalate, and lignified tissues. The leaf lacked both trichomes and mucilage.

According to [13], the presence or absence of are commonly employed in taxonomy to distinguish between families, genera, and species. Microscopy of the powdered leaf of the investigated plant may reveal its diagnostic traits.

Conventional phytochemical testing remains the most cost-effective and resource-efficient choice for preliminary screening [14]. Preliminary phytochemical analysis of crude and n-butanol revealed higher levels of flavonoids, alkaloids, tannins, phenols, terpenoids, and glycosides

in larger amounts, while saponins, hydrogen cyanides, and steroids were detected in the lowest amount as equally reported [6]. Over the years, medicinal leaves plants have been scientifically shown to contain phytochemical substances, making them useful in traditional treatment. According to Paliwal *et al*[15], the therapeutic properties of medicinal plants may be attributable to secondary metabolites found in them.

Standardization and quality control of plant-based medicine rely primarily on physicochemical properties such as moisture content, ash levels, and extractive values. The loss on drying test is a typical method for determining moisture levels in powdered samples. To avoid the growth of yeast, bacteria, or fungi during storage, drugs should be kept at low moisture levels. *L. senegalensis*'s percentage moisture content indicates a lower likelihood of microbial hydrolysis and degradation because it is within the generally accepted range of 8–14%. According to [16], too much moisture in crude medications can cause significant components to break down and microbes to develop, particularly when the medications are being stored.

The 8.4% total ash value of *L. senegalensis* leaves further demonstrated the level of care used in plant preparation. An acid-insoluble ash test was also conducted to determine if the plant extract included calcium oxalate or silica [12]. According to Anumudu et al. [18], the high concentration of calcium oxalate crystal in the leaf may be the cause of the result's 0.9% acid-insoluble ash content. A substantial percentage of the

total ash elements were soluble in water, as indicated by the water-soluble ash of 2.6% that was obtained. Another crucial

Table 1: Yields from *L. senegalensis* Schott. leaves' crude extract and fractions.

S/N	Solvent	Sample weight (g)	Extract/Fraction weight (g)	Percentage yield
1	Ethanol	994.94 (PS)	101.31	10.18
2	n-hexane	40 (ECE)	3.83	9.6
3	Ethylacetate	40 (ECE)	15.91	39.8
4	n-butanol	40 (ECE)	11.41	28.5

Key: PS = powdered sample, ECE = ethanol crude extract.

Figure 1: Live picture of *Lasimorpha senegalensis* Schott. Plant

Table 2: Macroscopic features of *Lasimorpha senegalensis* Schott leaves

S/N	Parameters	Features
1	Type	Simple leaf (lobed)
2	Odor	Characteristic smell
3	Color	Dark green
4	Shape	Sagittate
5	Margin	Entire
6	Texture	Leathery
7	Leaf size	45 cm long and 36 cm wide
8	Leaf base	Auriculate
9	Leaf apex	Acute
10	Base of lamina	Symmetric
11	Venation	Reticulate

Table 3: Microscopic features of the leaf of *Lasimorpha senegalensis* Schott

Parameters	Features
Epidermal cell	The upper and lower sides of polygonal epidermal cells have straight anticlinal cell walls.
Stomata type	The leaf has paracytic stomata (two subsidiary cells run parallel to the guard cells), which are amphistomatic (stomata appear on both the upper and lower surfaces).
Trichome	Absent
Stomata number (p.f.v.)	Adaxial: 2.75 ± 0.25; Abaxial: 11.75 ± 0.85
Stomata density (mm^{-2})	Adaxial: 16.18 ± 1.47; Abaxial: 69.12 ± 5.02
Stomata length (µm)	Adaxial: 40.09 ± 0.34; Abaxial: 34.21 ± 1.05
Stomata width (µm)	Adaxial: 16.85 ± 0.44; Abaxial: 14.48 ± 0.54
Stomata size (μm^2)	Adaxial: 675.39 ± 19.31; Abaxial: 495.28 ± 24.35
Stomata index (%)	Adaxial: 5.65 ± 0.53; Abaxial: 22.56 ± 1.37

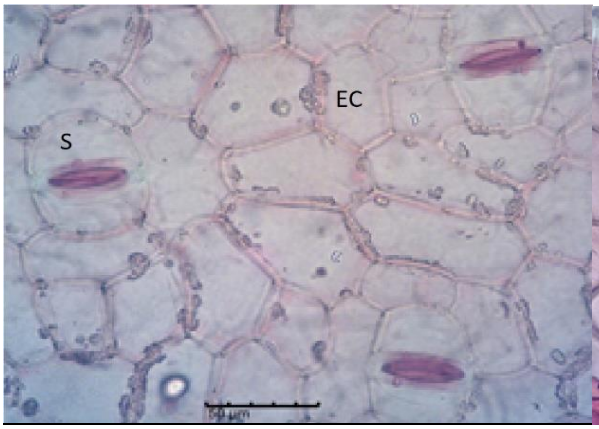

Figure 2: Photomicrograph of Lasimorpha senegalensis leaf adaxial surface displaying EC (epidermal cells) and S (stomata) (x400)

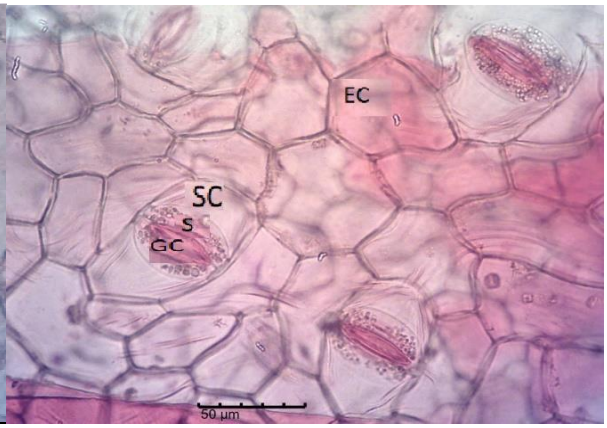

Figure 3: A photomicrograph of the Lasimorpha senegalensis leaf's abaxial surface reveals that EC stands for epidermal cells, GC for guard cells, S for stomata, and SC for subsidiary cells. (x400)

Table 4: Chemomicroscopy of the Powered leaves of *L. senegalensis.*

Parameter	Result
Starch grains	+
Lignified tissues	+
Calcium oxalates	+ (Prism-shaped)
Tannin	+
Cellulose	+
Gum/Mucilage	-
Protein	+
Oil	+

Key: + poisitive, - negative.

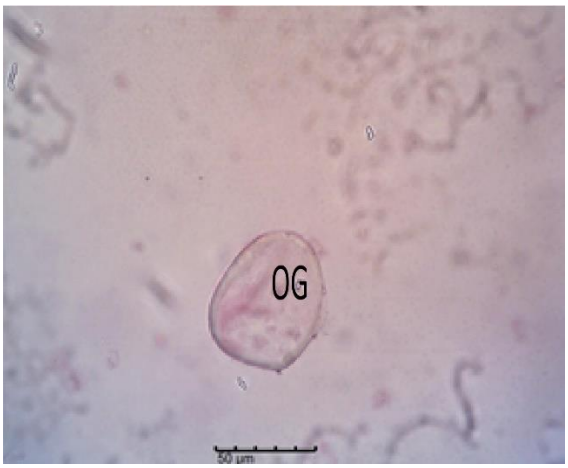

Figure 4: Powder microscopy of the leaf of *L. senegalensis* showing og=oil gland cell (x400)

Figure 5: Powder microscopy of the leaf of *L. senegalensis* showing f=fiber elements (x400)

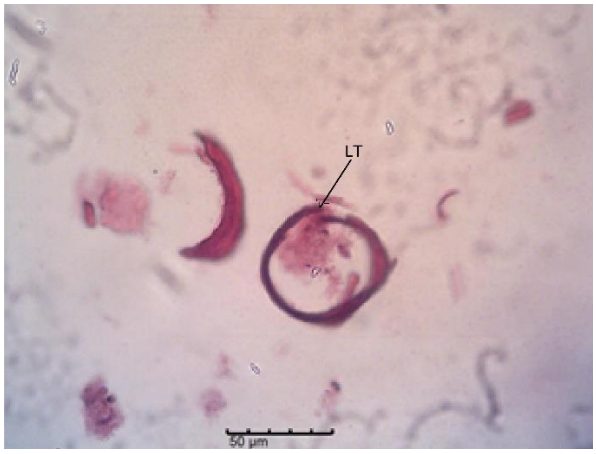

Figure 6: Powder microscopy of the leaf of *L. senegalensis* showing LT=lignified tissues (x400)

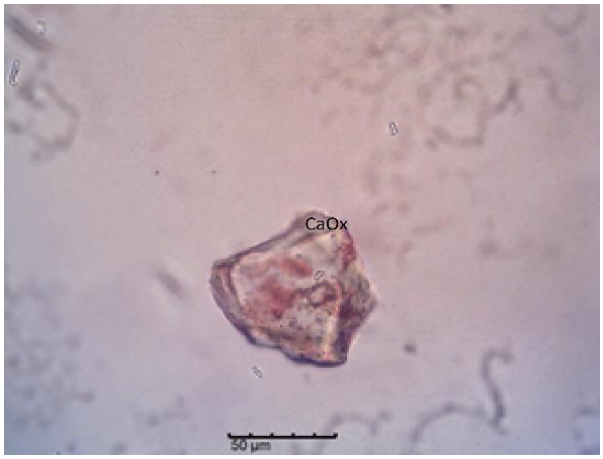

Figure 7: Powder microscopy of the leaf of *L. senegalensis* showing coax= calcium oxalate crystal (prism-shaped) (x400)

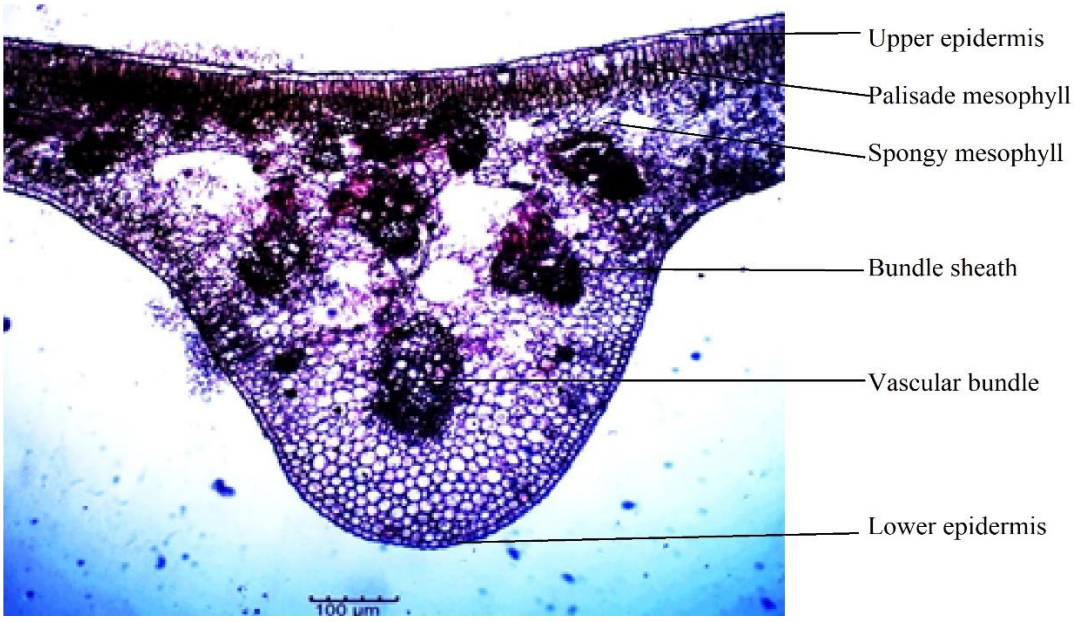

Figure 8: A cross-sectional view of *L. senegalensis* leaves

Table 5: Qualitative phytochemical screening of *L. senegalensis* leaf

S/N	Phytochemical	Crude extract	n-hexane fraction	Ethylacete fraction	n-butanol fraction
1	Tannins	+	-	-	+
2	Saponins	+	+	+	+
3	Steroids	+	-	+	+
4	Alkaloids	+	-	+	+
5	Flavonoids	+	-	+	+
6	Phenols	+	-	+	+
7	Glycosides	+	-	-	+
8	Hydrogen cyanides	+	-	+	+
9	Terpenoids	+	+	+	-

Key: + = present, - = absent

Table 6: Analytical standards of the powdered leaf of *Lasimorpha senegalensis* Schott**.**

Parameters	% Composition
Moisture content	9.2±0.07
Total ash	8.4±0.01
Acid-insoluble ash	0.9±0.00
Water soluble ash	2.6±0.02
Alcohol soluble extractive	41.5±0.01
Water soluble extractive	16.5 ±0.01

physicochemical metric that shows whether or not a crude drug sample is exhausted is its extractive yield [19].

The leaves of *L. senegalensis* contained phytoconstituents that were very soluble in alcohol (41.5%). This suggests that a sizable percentage of the phytochemical substances found in the plant are alcohol soluble. As a result, alcohol might work well as a solvent to extract the bioactive substances from the plant. Consequently, our work can serve as a benchmark to help future researchers differentiate *L. senegalensis* leaves from those of other closely related plant species.

CONCLUSION

This study provided parameters for the identification, standardization, and preparation of a monograph on the leaf of *Lasimorpha senegalensis* Schott.

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AUTHORS' CONTRIBUTION

Onugwu O. S: project design and execution, Okoh G. M: writing and analyzing the result, Ezugwu O. C: Editing and corrections.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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