Pharmacognostic characters of *Albizia lebbeck* (L.) Benth. leaves: macroscopy, microscopy, and phytochemical analysis

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**Author contributions**
Both Ogochukwu Immaculate Obika and Ifeanyi E. Obika made significant contributions to the design, writing, editing, and intellectual content revision of the manuscript. Ogochukwu Immaculate Obika was responsible for collecting plants and interpreting the results. Both authors have thoroughly read, reviewed, and approved the final version of the manuscript.

**Competing interests**
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**Abstract**

**Background:** *Albizia lebbeck* (L.) Benth, a medium to large tree belonging to the family Fabaceae, is commonly used as a medicinal plant for various disease conditions. **Aim:** The aim of this study was to establish a pharmacognostical profile for the leaves of *Albizia lebbeck* (L.) Benth in order to aid its identification and authentication. **Method:** Standard macroscopic methods were employed to analyze the physical parameters of the *Albizia lebbeck* (L.) Benth leaves. Fresh leaf samples and dried leaf powder were studied under a microscope to reveal the plant's microscopic features. Preliminary phytochemical investigation of the plant material was also conducted using standard methods. **Result:** The results showed the presence of fibers, epidermal cells, stomata, sclereids, prismatic crystals, vascular bundles, and parenchyma cells. Preliminary phytochemical analysis revealed the presence of phenols, flavonoids, glycosides, terpenes, tannins, saponins, carbohydrates, and quinones. **Conclusion:** These pharmacognostical and preliminary phytochemical observations can be considered as standard for future studies.

**Keywords:** microscopy; *Albizia lebbeck*; pharmacognostic; macroscopy; phytochemical
**Introduction**

*Albizia lebbeck* (L.) Benth belongs to the order Fabales, family Fabaceae, and subfamily Mimosoideae. It is commonly known as the Siris tree, woman's tongue, and East Indian walnut. Initially identified as *Mimosia lebbeck* by Carl Linnaeus, the species was later assigned its current genus by George Bentham. The genus Albizia is named after Filippo del Albizzi, a Florentine aristocrat who introduced the cultivation of *Albizia julibrissin* in 1749. The species name “lebbeck” is derived from the Arabic word for this plant, “laebach”. The name “woman’s tongue” refers to the rattling sound made by the wind-agitated pods and their enclosed seeds, which is likened to women’s chatter [1].

*Albizia lebbeck* (L.) Bent is native to deciduous and semi-deciduous forests in Asia, including eastern Pakistan, India, Sri Lanka, and Burma. It has become a popular ornamental and plantation tree in tropical and northern sub-tropical regions, including the Greater and Lesser Antilles, Central America, Colombia, Venezuela, and Brazil [2]. This deciduous tree has an open, large, spreading crown and typically reaches a height of 15–20 meters, with some exceptional specimens growing up to 30 meters [3].

*Albizia lebbeck* (L.) Bent is commonly used in African traditional medicine to treat various ailments, including diarrhea, bronchial asthma, flu, cough, eczema, insect bites, allergies, piles, hernia, malaria, gonorrhea, scrofulous swellings, boils, earache, toothache, yaws, scabies, inflamed eyes, piles, hemorrhoids, bronchitis, asthma, fever, pain, epilepsy, wounds, inflammation, and leprosy [4, 5]. Research has been conducted on *Albizia lebbeck* (L.) Bent based on its ethnomedical uses dating back nearly thousands of years, and it has been found to have numerous pharmacological potentials, including anti-asthmatic, anti-anaphylactic, anti-diarrheal, anti-ulcer, anti-malarial, anti-allergic, anti-diabetic, anti-inflammatory, anti-oxidant, anti-arthritic, nootropic and anxiolytic, hepatoprotective, and diuretic effects, as well as effects on the reproductive system, pulmonary eosinophilia, and allergic conjunctivitis [2, 4, 6–8].

Previous studies have reported that *Albizia lebbeck* (L.) Bent contains carbohydrates, potassium, copper, amino acids (arginine, lysine, glutamic acid, and aspartic acid), fatty acids (linoleic acid and α-tocopherol), keto acids (phosphonoendyruvate, glyoxalate, oxaloacetate, α-oxoglutarate, vicenin-2, reynoutrin, rutin, myricitrin, and robinin), alkaloids, flavonoids (gerodale, luteolin, and isookannin), tannins, saponins (d-catechin, d leucocyanidin, friedellan-3-one-and γ-stioseterol, triterpiondiosaponinlabbekanin D, lebbckanins D, F, G & H, echinocystic acid, flavon, vicenin II, and β-stiositerol), oleanen-type saponins (lebbecosides A and B), glycoside (albizinin), hemolysin (lebbecalyxain), oleanene triterpene (albizziasaponins A–E), and triterpeno saponin (lebbecoside C) [4, 6, 7, 9].

Medicinal plants have been the foundation of healthcare worldwide since ancient times and are still widely used. They are also the primary source of pharmaceuticals and new drug development, as well as healthcare products [10]. In many African countries, such as Nigeria, traditional medicine is the mainstay of primary healthcare, and medicinal plants are the major raw materials for these medicines [5].

People living in rural areas know from personal experience that traditional remedies are a valuable source of natural products for maintaining human health. However, they may not understand the science behind these medicines, but they do know that some medicinal plants are highly effective only when used at therapeutic doses [11]. The problems that often hinder the widespread use of traditional medicine, especially in Africa, are poor documentation or scarcity of records, as well as the lack of evidence or a complete absence of stringent quality control measures [12].

The screening of medicinal plant materials continues to represent potential sources of compounds for the discovery of new drugs. The World Health Organization not only encourages the use of traditional medicines but also recommends scientific evaluation of the medicinal properties of plant extracts [13]. Pharmacognostic study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostical evaluation provides valuable information regarding the morphology, microscopical, and physical characteristics of the crude drugs. Pharmacognostic studies have been conducted on many important drugs, and the resulting observations have been incorporated into various pharmacopoeias. Pharmacognostic study provides scientific information regarding the purity and quality of plant drugs when the plant source of the crude drugs has not yet been scientifically identified [14].

Pharmacognostic parameters are carried out on plant samples to establish appropriate data that may be utilized not only for identification but also to establish the purity and standard of crude drugs. Therefore, the first step towards the authentication of herbal drugs is the authenticated raw material for which pharmacognostic parameters are very important [3]. *Albizia lebbeck* is a plant that has been confused with other species due to their relative similarities and can easily be adulterated [15]. Adulteration is the conscious or erroneous substitution of crude drugs wholly or partly with another plant material or low-quality substances [16]. This adulteration can be prevented by standardization using various evaluation parameters [17]. Lack of documentation leading to adulteration, which may hinder further acceptance of the use of herbal medicine, has highlighted the importance of standardization and characterization of plant materials to be used as medicine. Hence, this study aims to scrutinize the pharmacognostic characters of *Albizia lebbeck* (L.) Bent via macroscopic, microscopic, and phytochemical analysis.

**Method**

**Materials**

Slides, cover slips, microscope, 40-mesh sieve, grinder, plant material, blade, petri dish, forceps, sodium hypochlorite 3% W/V, HCl, safranin, lactophenol blue, chloral hydrate, phone camera 8 mp.

**Collection of plant**

The leaves of the *Albizia lebbeck* (L.) Bent were collected from flowering and fruiting tree in the botanical and zoological garden University of Lagos, Akoka, Nigeria. The site is located on latitude 6°31’06.5”N and longitude 3°24’02.6”E. A voucher specimen was made and deposited at the department of Pharmacognosy herbarium, CMUL Ibi-araba.

**Phytochemical screening**

**Alkaloids.** (1) Mayer’s test: filtrates were treated with Mayer’s reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids. (2) Wagner’s test: filtrates were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids. (3) Dragendorff’s test: filtrates were treated with Dragendorff’s reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

**Carbohydrate.** Molish’s test: 1 mL of concentrated sulphuric acid was added slowly from the sides of the test tube. A deep violet colour at the junction of two layers indicates the presence of carbohydrates. **Flavonoids.** Treat the filtrate with dilute NaOH, followed by addition of dilute HCl. A yellow coloration is observed with NaOH which turns colourless with the addition of dilute HCl. **Glycosides.** 2 mL chloroform and 2 mL acetic acid were added to 2 mL of filtrate and observed for a blue to green coloration. **Phenols.** 2 drops of lead acetate were added to 2 mL of filtrate. The formation of white substance at the bottom of the test tube indicates the presence of phenolic compounds. **Quinones.** 1 mL of concentrated nitric acid was added to 2 mL of filtrate. The colour change to red indicates the presence of quinones. **Saponins.** Frothing test: 2 mL of the filtrate was shaken in a test tube for 20 minutes and let to rest for 5 minutes. Formation of foam that remains stable after 5 minutes of rest indicates the presence of
saponins. **Steroids.** In 1 mL filtrate, 2 mL of acetic anhydride and 2 mL of H₂SO₄ were added and was observed for a blue or green coloration. **Tannins.** Ferric chloride test: 2 drops of ferric chloride was added to 2 mL filtrate and observed for brownish green-black or a blue-black coloration. **Terpenoids.** 5 mL filtrated was treated with 2 mL of chloroform and 3 mL of concentrated sulphuric acid, it was then observed for a reddish-brown colour of interface to indicate terpenoid.

**Macroscopy**  
The macroscopic study is the morphological description of the plant parts which was carried out by a naked eye. Macroscopic examination was done by visual and physical examination of 10 randomly selected leaflets. Shape and surface characteristics including texture, type, margin, and venation of the leaves were determined by touching and examining the leaf samples under daylight with both the naked eye and a hand lens, while size was determined by measurement with a graduated ruler.

**Microscopy**  
The microscopic study is the anatomical study which is done by taking appropriate sections of the plant parts to be further studied.  
**Preparation of Specimens.** (1) For fresh sample: the fresh leaf was scraped gently with a blade to obtain both the adaxial and abaxial epidermal thin layer and thin slices of the midrib and petiole was cut carefully. It was cleared by soaking in 3% W/V sodium hypochlorite for about 10 seconds to bleach, it was then rinsed in clean water at room temperature and mounted onto a slide. The specimen was allowed to air dry and then stained with Safranin and or Lactophenol blue. Using a drop of glycerol as the mountant, the specimen was covered with a cover slip. (2) For powdered sample: the fresh leaves were allowed to air dry for about 2 weeks until crispy. The dried leaves were then ground with a coffee grinder and sieved with a 40-mesh sieve. It was cleared by adding chloral hydrate to the sample and warming with low heat from a gas burner. It was stained with a drop of Lactophenol blue after which a drop of Hydrochloric acid was added to the sample and using a drop of glycerol as the mountant, the specimen was covered with a cover slip.

**Microscopic Analysis of Specimens.** The mount was viewed under the light microscope with magnification of 10× and 40×. Micrographs were taken by means of phone camera.

**Result**  
**Phytochemical Analysis**  
To conduct phytochemical studies, the leaf material was first powdered and then extracted by boiling in water over a water bath. The resulting mixture was then filtered, and the filtrate was tested for the presence or absence of different phytochemicals, such as alkaloids, steroids, quinones, glycosides, tannins, phenols, and flavonoids. The results of these tests are recorded in Table 1 and were detected using the prescribed methods.

**Macroscopy observation**  
As shown in Figure 1, the leaf of *Albizia lebbeck* is compound and opposite bipinnate, consisting of 8–10 paired leaflets. The leaf color is lime-green, with the abaxial side being darker than the adaxial side. This color is maintained even after drying, and the leaf withers quickly when detached from the tree. To further examine the leaflets, single leaflets were analyzed using the described methods, and the resulting parameters are recorded in Table 2.

**Microscopy observation**  
**Epidermal thin layer.** The outlines of *Albizia lebbeck* (L.) Benth leaf epidermis are depicted in Figure 2 and Figure 3, as viewed under a light microscope at ×10 and ×40 magnification. Adaxial epidermal cells exhibit a bi-layer structure, with a jigsaw shape and deeply undulated cell walls at the top, and a rectangular shape with straight cell walls below (Figure 2). At ×40 magnification, the nuclei of adaxial epidermal cells appear as boysenberry-purple spots (Figure 2). Abaxial epidermal cells have a wavy shape, with diacytic stomata and nuclei located within the guard cells and towards the cell walls (Figure 3). Stomata and guard cells are exclusively present on the abaxial surface of the leaf.

**Table 1 preliminary phytochemical screening of Albizia lebbeck (L.) Benth**

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Present</th>
<th>Flavonoids</th>
<th>Present</th>
<th>Glycosides</th>
<th>Present</th>
<th>Phenols</th>
<th>Present</th>
<th>Quinone</th>
<th>Present</th>
<th>Saponins</th>
<th>Present</th>
<th>Steroids</th>
<th>Absent</th>
<th>Tannins</th>
<th>Present</th>
<th>Terpenoid</th>
<th>Present</th>
</tr>
</thead>
</table>

**Table 2 The morphological features of Albizia lebbeck (L.) Benth leaves**

<table>
<thead>
<tr>
<th>Features</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>2–4cm</td>
</tr>
<tr>
<td>Width</td>
<td>1–3cm</td>
</tr>
<tr>
<td>Shape</td>
<td>Oblong</td>
</tr>
<tr>
<td>Venation</td>
<td>Cross-venulate</td>
</tr>
<tr>
<td>Margin</td>
<td>Entire</td>
</tr>
<tr>
<td>Leaf type</td>
<td>Simple</td>
</tr>
<tr>
<td>Leaf surface (texture)</td>
<td>Smooth</td>
</tr>
</tbody>
</table>

**Figure 1** The pictures of *Albizia lebbeck* (L.) Benth taken in the botanical and zoological garden of the university of Lagos with GPS location 6°31'06.5"N 3°24'02.6"E (6.518460, 3.400730). A, The close-up view of Albizia leaves and flowers on the tree; B, The collected plant material in the Lab few hours after picking; C, The front view of the tree; D, The back view of the tree.
Transverse section of leaf lamina. Figure 4 depicts a transverse section of the lamina through the midrib, revealing the dorsoventral structure of the leaflet. The leaflet is thin and slightly concave, with an abaxial midrib. The epidermal cells are covered with a very thin, smooth cuticle, and the adaxial epidermis is double-layered while the abaxial epidermis is single-layered. Stomata are visible on the abaxial epidermis. The palisade cells are parenchymatous and 1-2 layered, followed by spongy mesophyll. Some cells contain prismatic crystals of calcium oxalate. Oil glands are present and scattered throughout the lamina, but larger glands are observed around the midrib. The lower epidermis of the midrib is convex, while the upper epidermis is somewhat convex. Both midrib epidermis are followed by collenchyma cells, then parenchyma cells, and finally, the vascular bundle sandwiched within. The vascular bundle is large, circular, and collateral, consisting of xylem towards the upper epidermis and phloem towards the lower epidermis, and is bounded by sclerenchyma cells with thick-walled, lignified fibers and a parenchyma sheath. The xylem is surrounded by some fibers, and the metaxylem is sandwiched by the protoxylem. The veinlets are scattered around the mesophyll cells, mostly at the palisade layer, and project above the surface level of the lamina. The vein has a small collateral vascular bundle, and vascular strands are scattered between the palisade and spongy parenchyma.

Leaflet petiole. Figure 5 displays a transverse section of the petiolule, which has a somewhat frog-like outline. The uniseriate peripheral parenchymatous epidermis is covered with a smooth cuticle, and simple and unicellular trichomes are occasionally observed on the epidermis. The cortical region contains two small collateral vascular bundles, each surrounded by a sclerenchyma sheath. The phloem encircles the xylem, and the cortical region presents 3-4 layers of collenchyma and 2-3 layers of parenchyma, with the endodermis containing crystals. The medullary region is well-developed and made up of cells of varying sizes, with the vascular system surrounded by a sclerenchyma sheath. The vascular bundles are open and collateral, consisting of upper sclerenchyma cells and thick-walled pericyclic fibers, followed by an outer phloem and inner xylem with xylem fibers. The vascular bundle covers the pith region, which is wide and parenchymatous, containing oil glands and crystals.

Dry powdered sample. During the powder microscopy of Albizia lebbeck (L.) Bent leaves, we observed a mass of mesophyll cells, as depicted in Figure 6A, as well as parenchymatous cells and helix vessels, as shown in Figure 6B. Figure 6C displays epidermal cells and mesophyll cells containing calcium oxalate crystals. Figure 6D shows fragments of sclerenchyma with vessels and the upper epidermis, while Figure 6E depicts fibers and fragments of stomata, prismatic crystals, and leaf venation.
Figure 4 TS of leaf lamina through midrib viewed at 10× and 40×. (A, B) Leaf lamina through midrib at 10×; (C, D, E, F) Midrib at 40×. E, Epidermis; PC, Palisade Cell; VB, Vascular Bundles; MC, Mesophyll Cell; C, Collenchyma; X, Xylem; PH, Phloem; S, Sclerenchyma; OG, Oil Glands; ST, Stoma; V, Veinlets; VS, Vascular Strand; Cr, Crystals; SM, Spongy Mesophyll.
Figure 5 Leaf petiole viewed at 10×. (A, B, C) Cross-sectional of petiole with focus on the medullary region; (D, E) Cross-sectional of petiole with focus on the cortical region. PP, Pith Parenchyma; P, Parenchyma; PF, Pericyclic Fibre; VB, Vascular Bundles; C, Collenchyma; S, Sclerenchyma; X, xylem; MR, Medullary rays; E, Epidermis; En, Endodermis; Ph, Pith; T, Trichome.
Discussion

In this study, a phytochemical analysis and a detailed examination of both fresh and powdered leaves of *Albizia lebbeck* (L.) Benth were carried out with appropriate staining. The World Health Organization refers to the use of poor quality, adulterated, and counterfeit products as a significant risk affecting the use of herbal medicines [18]. Despite the availability of sophisticated modern research tools to evaluate crude drugs, microscopy is still recommended by the World Health Organization and the American Herbal Pharmacopoeia as the simplest, quickest, and most cost-effective way of establishing the purity and authenticity of herbal materials [19, 20]. The pharmacognostic characteristics of herbal drugs are essential in their authentication, given that every plant has distinctive features that...
make it unique. Macroscopic and microscopic studies of the herbs should be the first and foundational step to authenticate herbs, followed by preliminary phytochemical analysis, which should precede chemical characterization [21, 22]. In the macroscopic and microscopic analysis of Albizia lebbeck (L.) Benth leaves, features of diagnostic value can be confirmed for the plant drug. In the phytochemical analysis of Albizia lebbeck (L.) Benth leaves, secondary plant metabolites that may be responsible for various pharmacological actions are established for the genuineness and viability of crude drug material. Therefore, the results of these investigations could serve as a basis for proper identification, collection, and investigation of the plant [14].

The phytochemical screening of the drug is a highly sensitive aspect in the process of standardisation and quality control, as the constituents not only vary from plant to plant but also in different samples of the same species depending on various atmospheric factors and storage conditions [5]. The therapeutic effect of medicinal plants is only exhibited due to the presence of a certain quantity of biologically active compounds [16]. The preliminary phytochemical screening of Albizia lebbeck (L.) Benth leaves shows the absence of steroids and the presence of alkaloids, phenols, flavonoids, glycosides, terpenes, tannins, saponins, carbohydrates, and quinones. These bioactive compounds have been widely reported for their pharmacological importance, thereby highlighting the medicinal value of the plant. In Shamugavadivelu and Subramanian's research on the phytochemical constitution of Albizia lebbeck (L.) Benth, steroids were present in the leaves [23]. Contrary to our findings, Jasiem et al. found neither flavonoids nor alkaloids in both ethanol and aqueous extracts of Albizia lebbeck (L.) Benth leaves [24].

Upon physical examination of 10 randomly selected leaf samples, the Albizia lebbeck (L.) Benth leaves were observed to be simple with smooth surfaces, lengths ranging from 2 cm to 4 cm, and widths ranging from 1 cm to 3 cm. The leaves were oblong in shape, had cross-venulelum venation, and entire margins. The macromorphology observed in this study is consistent with the description provided by Abdel-Ghani et al. [25].

Microscopic examination of the fresh leaf epidermal layer revealed irregularly shaped epidermal cells with wavy cell walls and nuclei present on both upper and lower surfaces of the leaves. Stomata with guard cells were observed on the abaxial surface and absent on the adaxial surface, indicating that the species is hypostomatic [26]. The transverse section of the leaf lamina through the midrib showed an abaxially prominent midrib and the presence of epidermis, palisade cells, vascular bundles, spongy mesophyll, collenchyma, xylem, phloem, fibers, sclerenchyma, oil glands, crystals, veinlets, vascular strands, and stomata. The leaflet petiole was found to be fagrofase-shaped and presented cortex parenchyma, pith parenchyma, pericyclic fibers, medullary rays, epidermal trichomes, two prominent cortex vascular bundles, and a medullary vascular bundle with pith. Powder microscopy of the leaves revealed the presence of fibers, mesophyll, crystals, fragments of vessels, stomata epidermal cells, and leaf venation. The microscopic features observed in this study are similar to the report by Abdel-Ghani et al. in their study of the morphology of Albizia lebbeck (L.) Benth cultivated in Egypt [13]. Jasiem et al. also observed cell walls, fibers, vessels, and stone cells in Albizia lebbeck (L.) Benth leaves, which agrees with our study [24]. The transverse section of the leaflet lamina observed by Vasanthi was similar to our study, and the leaf rachis he observed had similar features to the leaflet petiole observed in our study [11]. However, trichomes, which were reported to be present on the leaf blade in previous studies, were not observed in our study, challenging the findings of Vasanthi, Jasiem et al., and Abdel-Ghani et al. [11, 24, 25].

Conclusion

The leaf of Albizia lebbeck (L.) Benth possesses distinct macroscopic and microscopic features, as well as certain bioactive compounds. Some of the distinctive features of the plant include the lack of leaf blade trichomes, jigsaw epidermal cells with undulate cell walls, palisade cells, vascular bundles, spongy mesophyll, collenchyma, xylem, phloem, fibers, sclerenchyma, oil glands, crystals, veinlets, vascular strands, and hypostomatic diacytic stomata. The plant also contains therapeutic phytochemicals, including alkaloids, phenols, flavonoids, glycosides, terpenes, tannins, saponins, carbohydrates, and quinones. The parameters from this study can be used for future reference as a standard in scientific evaluation, identifying, and authenticating Albizia lebbeck (L.) Benth and its adulterants. The information from this work can also be incorporated into Herbal Pharmacopoeias to emphasize the future pharmacological and pharmaceutical benefits of the plant.

References

10. Immaculate OO, Bernard OE. Evaluation of Iron Concentration


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