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Pharmacognostic and Biochemical Validation of Albizia lebbeck (L.) Benth. Bark: A Multifaceted Investigation into its Phenolic Content and Antidiabetic Enzyme Inhibitory Potential

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ABSTRACT

The present study aimed to evaluate the phytochemical composition, physicochemical properties, and antidiabetic enzyme inhibitory potential of *Albizia lebbeck* (L.) Benth. bark extract. Physicochemical analysis revealed acceptable levels of total ash (16.60%), sulphated ash (12.66%), water- and alcohol-soluble extractive values (20.21% and 12.49%, respectively), and loss on drying (17.96%), confirming the quality and stability of the crude drug. Organoleptic and fluorescence analyses further aided in authentication and characterization. Phytochemical screening confirmed the presence of key bioactive constituents, including flavonoids, tannins, phenols, alkaloids, saponins, and glycosides—compounds widely known for their antioxidant and antidiabetic properties. The total phenolic content was determined to be 329.53 mg GAE/g of extract, indicating potent antioxidant potential. Enzymatic assays revealed significant α-glucosidase inhibition with an IC_{50} value of 305.52 µg/mL, suggesting its potential to delay carbohydrate absorption and regulate postprandial blood glucose. Additionally, the extract exhibited moderate DPP-4 inhibitory activity (42.87% at 100 µg/mL), further supporting its antidiabetic relevance. While less potent than standard inhibitors (Acarbose and Diprotin A), the findings provide a strong basis for developing *Albizia lebbeck* as a complementary natural therapeutic for managing type 2 diabetes. Further in vivo studies and compound isolation are recommended.

Keywords: *Albizia lebbeck, Pharmacognostic evaluation, Phytochemical screening, α-Glucosidase inhibition, DPP-4 inhibition, Total phenolic content, Antidiabetic activity, Herbal standardization.*

1. INTRODUCTION

Diabetes mellitus, particularly type 2 diabetes (T2DM), is a chronic metabolic disorder characterized by elevated blood glucose levels due to insulin resistance and/or impaired insulin secretion. The global prevalence of diabetes continues to rise at an alarming rate, placing an enormous burden on healthcare systems, especially in developing countries. According to the International Diabetes Federation (IDF), over 537 million people were affected by diabetes in 2021, and this number is expected to rise significantly in the coming decades. Persistent hyperglycemia in diabetic patients leads to long-term damage and dysfunction of various organs, including the heart, kidneys, eyes, and nerves (Bellary et al., 2021; Bjornstad et al., 2023; Bukhsh et al., 2020).

One of the key strategies in managing T2DM is to control postprandial hyperglycemia. This can be achieved by inhibiting carbohydrate-hydrolyzing enzymes such as α-glucosidase, which breaks down disaccharides into absorbable monosaccharides. Another important target is the dipeptidyl peptidase-4 (DPP-4) enzyme, which degrades incretin hormones like GLP-1 that stimulate insulin secretion. Synthetic inhibitors such as Acarbose (for α-glucosidase) and Sitagliptin or Diprotin A (for DPP-4) are clinically used, but they are often associated with side effects such as gastrointestinal discomfort, hepatotoxicity, and cost-related challenges. This has led to a renewed interest in exploring plant-based alternatives that are effective, safer, and more affordable (Ma et al., 2022; Magliano et al., 2020; Vounzoulaki et al., 2020; Zhang et al., 2021).

Medicinal plants have been extensively used in traditional systems of medicine, including Ayurveda, for the treatment of diabetes. One such plant is *Albizia lebbeck* (L.) Benth., commonly known as Siris or Indian Walnut. It belongs to the family Fabaceae and is widely distributed across India and tropical Asia. Various parts of the plant, particularly the bark, have been used traditionally for their astringent, anti-inflammatory, antimicrobial, and antidiabetic properties. The bark is rich in secondary metabolites such as flavonoids, tannins, saponins, and phenolic compounds, which are known to exert therapeutic effects including antioxidant, anti-inflammatory, and enzyme inhibitory activities.

Despite its widespread traditional use, scientific validation of the pharmacological potential of *Albizia lebbeck* bark, particularly its mechanism-based antidiabetic activities, remains limited. Therefore, the present study was designed to evaluate the phytochemical composition, physicochemical characteristics, and antidiabetic enzyme inhibitory activities—specifically targeting α-glucosidase and DPP-4 enzymes—of *Albizia lebbeck* bark extract. The physicochemical analysis serves as a critical quality control measure to establish the identity, purity, and stability of the crude drug material. Organoleptic and fluorescence analysis further help in authentication and detection of adulteration. Phytochemical screening allows the identification of major bioactive classes, while total phenolic content estimation provides insight into the antioxidant capacity of the extract (Al-Masri, 2023; Ardeshiri et al., 2022; Avoseh et al., 2021; Tasneem et al., 2022). By combining these approaches with functional enzyme inhibition assays, this study aims to provide a comprehensive understanding of the therapeutic potential of *Albizia lebbeck* bark in diabetes management. The findings may offer scientific evidence supporting its traditional use and lay the groundwork for future research focused on isolating active constituents, evaluating in vivo efficacy, and developing standardized herbal formulations for the treatment or supportive management of type 2 diabetes.

2. MATERIAL AND METHODS

Collection and Botanical Authentication of Plant Material

The bark of *Albizia lebbeck* (L.) Benth., a deciduous tree known for its therapeutic properties in traditional medicine, was collected during the month of March and April 2022 from the Six Mile region in Kamrup district, Assam, India. The collection site was selected based on the natural occurrence and ethnomedicinal use of the species by local communities. The collected bark specimens were identified based on macroscopic characteristics and authenticated by a botanist. A voucher specimen (MK/AXS/3492) was retained and deposited in the institutional herbarium for future reference.

Sample Cleaning, Drying, and Powder Preparation

The freshly collected bark was washed thoroughly under running tap water to remove dirt and debris, followed by rinsing with distilled water to ensure purity. The cleaned samples were dried under shade at room temperature for 10 to 12 days to avoid degradation of heat-sensitive phytoconstituents. Once completely dried, the bark was mechanically ground into coarse powder using an electric grinder. The resulting powder was sieved through a #40 mesh to achieve uniform particle size and stored in clean, dry, airtight amber-colored glass containers to protect it from light and moisture until further analysis.

Evaluation of Physicochemical Parameters

Standard physicochemical analyses were conducted in accordance with Ayurvedic Pharmacopoeia and WHO guidelines to determine the identity, purity, and quality of the powdered bark. The parameters evaluated included total ash, sulphated ash, water-soluble ash, acid-insoluble ash, alcohol- and water-soluble extractive values, moisture content (loss on drying), organoleptic features, and fluorescence characteristics under UV and visible light (Majid et al., 2021; Upadhyay et al., 2019).

Total Ash Value

Five grams of the powdered bark were accurately weighed and transferred to a pre-weighed silica crucible. The sample was incinerated in a muffle furnace at 450°C until a uniform white ash was obtained, indicating complete combustion. The crucible was cooled in a desiccator and weighed. The total ash value was calculated as a percentage of the air-dried sample, representing the total amount of inorganic residue present (Majid et al., 2021; Upadhyay et al., 2019).

Sulphated Ash Value

For sulphated ash estimation, 5 g of powdered bark were ignited in a crucible until carbon-free ash was obtained. After cooling, 1 ml of concentrated sulphuric acid was added to the residue and carefully heated to drive off fumes. The sample was then further incinerated at 800°C until no black particles remained. The crucible was again cooled, and the residue was weighed. The procedure was repeated until two consecutive weighing showed a difference of less than 0.5 mg. The sulphated ash value was expressed as a percentage of the air-dried material (Majid et al., 2021; Upadhyay et al., 2019).

Water-Soluble Ash

The total ash from the previous test was boiled with 20 ml of distilled water for 5 minutes. The solution was filtered through an ashless filter paper, and the insoluble portion was collected and incinerated. The final residue was weighed, and the difference between the total ash and the residue gave the water-soluble ash. This was calculated as a percentage of the air-dried bark sample (Majid et al., 2021; Upadhyay et al., 2019).

Acid-Insoluble Ash

The ash obtained was treated with 20 ml of dilute hydrochloric acid and boiled for 5 minutes. The mixture was filtered, and the insoluble matter was collected, washed, dried, incinerated, and weighed. The acid-insoluble ash was calculated as a percentage of the air-dried sample and used to assess the presence of siliceous materials like sand or grit (Majid et al., 2021; Upadhyay et al., 2019).

Water-Soluble Extractive Value

To determine the water-soluble extractives, 5 grams of the powdered sample were macerated with 100 ml of distilled water in a conical flask. The mixture was shaken intermittently for five hours and left undisturbed for 24 hours at room temperature. The filtrate was evaporated to dryness in a tarred dish using a water bath. The residue was dried at 100°C, cooled in a desiccator, and weighed. The extractive value was calculated as a percentage of the air-dried material (Majid et al., 2021; Upadhyay et al., 2019).

Alcohol-Soluble Extractive Value

Similarly, 5 grams of bark powder were macerated with 100 ml of 80% ethanol. The mixture was shaken intermittently for six hours and allowed to stand for 18–24 hours. After filtration, 25 ml of the extract was evaporated to dryness on a water bath, dried at 100°C, cooled, and weighed. The alcohol-soluble extractive value was expressed as a percentage with reference to the air-dried bark (Majid et al., 2021; Upadhyay et al., 2019).

Loss on Drying (Moisture Content)

To estimate the moisture content, 5 g of powdered bark were weighed and placed on a tarred evaporating dish. The sample was dried in a hot air oven at 105°C for five hours and then at hourly intervals until a constant weight was obtained. The final weight loss was recorded and expressed as a percentage of the air-dried material, indicating the amount of volatile moisture present in the sample (Majid et al., 2021; Upadhyay et al., 2019).

Organoleptic Evaluation

The organoleptic characteristics, such as colour, taste, texture, and odour, were assessed manually. The bark of *Albizia lebbeck* was found to be coarse in texture, brownish in colour, with a slightly bitter and astringent taste and a characteristic earthy odour. These features serve as important qualitative markers for identification and quality control of the plant material (Majid et al., 2021; Upadhyay et al., 2019).

Fluorescence Analysis

Fluorescence analysis was performed by treating the powdered bark with different reagents, including hydrochloric acid, sulphuric acid, nitric acid, sodium hydroxide, and ammonia. The treated samples were observed under visible light and UV light at wavelengths of 254 nm and 366 nm. The appearance of specific fluorescence or color changes provided insight into the presence of certain phytochemicals and served as a supportive tool for crude drug authentication (Majid et al., 2021; Upadhyay et al., 2019).

Total Phenolic Compounds assay

The total phenolic content (TPC) of the RG-PV-HF extract was quantified using the Folin–Ciocalteu (FC) colorimetric method, with modifications based on the procedure described by Olayinka and Anthony (2009) (Olayinka & Anthony, 2009). In this assay, a known volume of the extract was mixed with the Folin–Ciocalteu reagent, which reacts with phenolic

compounds under alkaline conditions to form a blue-coloured complex. After the initial reaction with the FC reagent, sodium carbonate solution was added to the mixture to neutralize the medium and facilitate colour development. The reaction mixture was incubated in the dark at room temperature for 30 minutes to allow complete development of the blue colour. The absorbance of the resulting solution was measured at 760 nm using a UV-Visible spectrophotometer. A standard calibration curve was prepared using various concentrations of gallic acid, and the total phenolic content of the extract was expressed in terms of milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g). All experiments were performed in triplicate, and the results were reported as mean ± standard deviation.

Inhibition of alpha glucosidases enzyme

The ability of the RG-PV-HF extract to inhibit α-glucosidase activity was determined using a modified spectrophotometric method as described by Nair et al. (2013), with slight alterations to suit laboratory conditions (Nair et al., 2013). In this assay, α-glucosidase enzyme was incubated with different concentrations of the plant extract in phosphate buffer (pH 6.8) at 37°C for 10 minutes. Subsequently, p-nitrophenyl-α-D-glucopyranoside (pNPG), the chromogenic substrate, was added to initiate the reaction. After an additional incubation period of 20 minutes, the reaction was terminated by adding a stop solution (commonly sodium carbonate or a similar alkaline reagent). The amount of p-nitrophenol released, which reflects enzymatic activity, was measured spectrophotometrically at 405 nm. Acarbose was used as a standard inhibitor for comparison. The percentage inhibition of α-glucosidase was calculated, and IC_{50} values were derived from dose-response curves.

DPP-4 Inhibition Assay

The dipeptidyl peptidase-4 (DPP-4) inhibitory activity of the RG-PV-HF extract was assessed using a modified protocol based on the method originally reported by Al-Masri et al. (2009) (Al-masri et al., 2009). The assay involved the incubation of recombinant human DPP-4 enzyme with varying concentrations of the extract in Tris-HCl buffer (pH 8.0) at 37°C for 15 minutes. The fluorogenic substrate Gly-Pro-AMC (7-amino-4-methylcoumarin) was then added to initiate the enzymatic reaction. After a specific incubation time, the release of fluorescent AMC, which indicates DPP-4 activity, was measured using a fluorescence microplate reader at excitation and emission wavelengths of 355 nm and 460 nm, respectively. Diprotin A served as the reference standard. The inhibitory potential was expressed as a percentage of enzyme activity relative to the control, and IC_{50} values were calculated to quantify potency.

Statistical analysis

All experimental results were expressed as mean ± standard deviation (SD) from at least three independent replicates (n = 3). The data were analyzed using one-way analysis of variance (ANOVA) to determine the level of significance among different groups or concentrations. When ANOVA showed significant differences ($p < 0.05$), Tukey's post hoc test was applied for multiple comparisons to identify statistically significant pairs. IC_{50} values for enzyme inhibition assays were calculated using non-linear regression analysis (dose-response curve fitting) with the help of GraphPad Prism software version 8 (GraphPad Software Inc., USA). A significance level of $p < 0.05$ was considered statistically meaningful throughout the analysis.

3. RESULTS

Physicochemical Analysis

The physicochemical parameters of the bark of *Albizia lebbeck* (L.) Benth. provide essential insights into the quality, purity, and stability of the crude drug material. These parameters are crucial for standardization and serve as fundamental tools in identifying adulteration, improper storage, and overall acceptability in herbal formulations. The total ash content was found to be 16.60% w/w, indicating the total amount of inorganic residue remaining after incineration. A high ash value may suggest the presence of inherent mineral content or contamination with soil or other extraneous material. However, the acid-insoluble ash, which was 4.37%, specifically represents the siliceous matter, such as sand or silicates, and was within a permissible range, suggesting minimal contamination. The sulphated ash value was 12.66%, reflecting the content of oxidizable organic matter that is converted to sulphates upon treatment with sulphuric acid. This helps determine the presence of non-volatile inorganic salts and is often higher than the total ash due to the sulphation process. The water-soluble ash was 6.04%, which represents the portion of ash soluble in water and gives an estimate of the water-soluble inorganic components. This parameter supports the suitability of the sample for aqueous formulations. The water-soluble extractive value was noted to be 20.21%, indicating a significant quantity of water-soluble phytoconstituents such as tannins, sugars, and glycosides. This suggests that a substantial portion of the active constituents can be extracted using aqueous solvents, which is advantageous for developing traditional decoctions or herbal teas.

The alcohol-soluble extractive value was 12.49%, showing the presence of moderate amounts of alcohol-soluble compounds like alkaloids, flavonoids, and sterols. This suggests that hydroalcoholic extraction methods may effectively isolate both polar and moderately non-polar constituents from the bark. The loss on drying, recorded at 17.96%, indicates the moisture content and volatile matter present in the sample. This relatively high moisture value suggests that the crude drug must be properly dried and stored in airtight conditions to prevent microbial growth and degradation. Overall, the physicochemical

parameters of *Albizia lebbeck* bark are within acceptable ranges and support its suitability as a quality raw material for pharmacognostical and phytopharmaceutical applications. The results also provide a baseline for quality control in future formulations using this plant part.

Table 1. Physicochemical Analysis of Bark of *Albizia lebbeck* (L.) Benth.

S. No.	Parameter	Values (% w/w)
1	Total ash	16.60
2	Sulphated ash	12.66
3	Water-soluble ash	6.04
4	Acid-insoluble ash	4.37
5	Water-soluble extractive value	20.21
6	Alcohol-soluble extractive value	12.49
7	Loss on drying	17.96

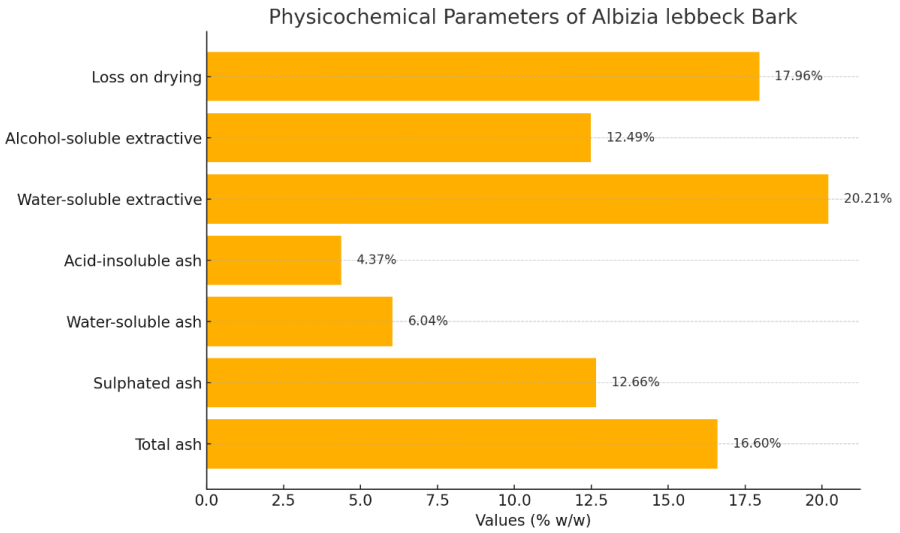

Figure 1. Physicochemical Analysis of Bark of *Albizia lebbeck* (L.) Benth.

Organoleptic Properties

Organoleptic evaluation is a primary and essential step in the identification and quality assessment of crude plant drugs. It includes the examination of physical characteristics such as colour, odour, taste, shape, and surface texture, which help in establishing the sensory profile of the plant material. The colour of the *Albizia lebbeck* bark in its natural form was observed to be dark brown, which is typical for mature bark rich in tannins and polyphenolic compounds. Upon pulverization, the colour changed to pinkish brown, indicating the exposure of internal tissues and possible oxidation of certain constituents during grinding. The odor of both the bark and its powdered form was reported as none, meaning it lacked any distinctive aromatic or pungent smell. This neutral odor is beneficial in formulations where strong scents may be undesirable or interfere with other active ingredients. The taste of both forms was noted as astringent, which aligns well with the traditional use of *Albizia lebbeck* bark in treating conditions like diarrhea, wounds, and inflammations. Astringency is often due to the presence of tannins, which are well-known for their tissue-constricting and antimicrobial properties. In terms of shape, the bark was described as curved, a feature common to tree barks when stripped and dried, and helps in macroscopic identification. The powdered form was naturally coarse, which is consistent with initial pulverization steps prior to extraction or further processing. The surface texture of the bark was wrinkled, which is typical for dried, fibrous barks, and helps in distinguishing it from smooth or hard barks of other species. After grinding, the texture became non-wrinkled, as expected, due to loss of structural integrity. In summary, the organoleptic properties of *Albizia lebbeck* bark are characteristic and match well with standard monographic descriptions. These features support its proper identification and can be used as a reference point for

detecting adulteration, substitution, or degradation in raw herbal materials.

Table 2. Organoleptic Properties of Bark of *Albizia lebbeck* (L.) Benth.

Description	Bark Form	Powder Form
Colour	Dark Brown	Pinkish Brown
Odour	None	None
Taste	Astringent	Astringent
Shape	Curved	Coarse Powder
Surface	Wrinkled	Non-wrinkled

Fluorescence Characteristics

Fluorescence analysis is a rapid and effective qualitative method used to assess the identity and purity of crude drugs. Many natural compounds emit characteristic fluorescence when exposed to ultraviolet (UV) light, especially after treatment with specific reagents. These changes in color under visible and UV light offer valuable clues for preliminary phytochemical screening and serve as an important diagnostic feature in pharmacognosy.

In the present analysis, the bark powder of *Albizia lebbeck* exhibited distinct fluorescence characteristics under visible light, UV 254 nm, and UV 365 nm after treatment with various chemical reagents:

- The untreated bark powder appeared pinkish grey in visible light, brown under UV 254 nm, and blackish brown under UV 365 nm. This colour shift suggests the presence of natural polyphenolic compounds, such as tannins or flavonoids, known to fluoresce under UV light.
- On treatment with sodium hydroxide (NaOH), the powder developed a rusty brown colour in visible light and a reddish-brown hue under long-wave UV light (365 nm), indicating potential phenolic and flavonoid interactions with alkali.
- With ammonia (NH_3), a light brown to pale brown appearance was seen across all lighting conditions, reflecting mild reactivity, possibly with terpenoids or low-concentration alkaloids.
- The picric acid-treated sample showed a stable yellowish-brown colour in both visible and short-wave UV (254 nm), which turned brown under long-wave UV. This shift supports the presence of reactive phenolic structures.
- The petroleum ether treatment led to a greenish brown fluorescence at 254 nm, indicating the presence of non-polar constituents such as fixed oils or resins, although the visible and long-wave UV responses remained light brown.
- Treatment with hydrochloric acid (HCl) and 50% HCl resulted in reddish or light brown tones in visible light and greenish to brown shades under UV, likely due to acid-induced breakdown or activation of chromophoric groups.
- When exposed to sulphuric acid (H_2SO_4) and 50% H_2SO_4, the powder turned black or brownish black, particularly under UV light. This intense reaction suggests the presence of strong oxidizable compounds, such as lignins or steroidal components.
- The sample treated with 50% potassium hydroxide (KOH) showed reddish brown coloration consistently across all lighting conditions, aligning with strong alkali-reactive phytoconstituents such as anthraquinones or flavonoids.

The fluorescence responses of *Albizia lebbeck* bark powder under various reagent treatments provide strong preliminary evidence of the presence of diverse phytoconstituent classes including flavonoids, phenolics, tannins, and possibly terpenoids. These findings support the use of fluorescence analysis as a reliable quality control and authentication tool for crude herbal materials.

Table 3. Fluorescence Characteristics of *Albizia lebbeck* (L.) Benth. Bark Powder Under Visible and UV Light

Reagent / Treatment	Visible Light	UV Light (254 nm)	UV Light (365 nm)
Untreated Powder	Pinkish Grey	Brown	Blackish Brown
Powder + Sodium Hydroxide (NaOH)	Rusty Brown	Dark Brown	Reddish Brown
Powder + Ammonia (NH_3)	Light Brown	Pale Brown	Light Brown

Powder + Picric Acid	Yellowish Brown	Yellowish Brown	Brown
Powder + Petroleum Ether	Light Brown	Greenish Brown	Light Brown
Powder + Hydrochloric Acid (HCl)	Reddish Brown	Brown	Brown
Powder + Sulphuric Acid (H_2SO_4)	Black	Black	Brownish Black
Powder + 50% Hydrochloric Acid	Light Brown	Greenish Brown	Brown
Powder + 50% Sulphuric Acid	Yellowish Brown	Dark Brown	Yellowish Brown
Powder + 50% Potassium Hydroxide	Reddish Brown	Brown	Reddish Brown

Preliminary phytochemical screening

Phytochemical analysis is a foundational step in evaluating the medicinal potential of plant materials. The qualitative screening of *Albizia lebbeck* bark extract revealed the presence of a wide array of bioactive secondary metabolites, each associated with distinct pharmacological activities. The positive reaction for flavonoids, observed via the Shinoda test in ethanol, indicated the presence of polyphenolic compounds, confirmed by the appearance of a reddish pink/magenta coloration. Flavonoids are known for their strong antioxidant, anti-inflammatory, and anti-diabetic properties. Tannins and phenols, both detected using the Ferric chloride test in water and methanol respectively, gave characteristic blue-black to bluish-green colorations, supporting their strong presence. These compounds contribute significantly to astringent, antimicrobial, and wound-healing properties, which align with the traditional uses of *Albizia lebbeck*. Phytosterols were confirmed through the Salkowski test, producing a reddish-brown ring at the interface, indicating steroidal components that may contribute to anti-inflammatory and cholesterol-lowering effects. Glycosides, essential for cardiac and anti-inflammatory activity, showed a reddish-brown ring with a bluish layer in the Keller-Killiani test, confirming their presence. The presence of carbohydrates was confirmed by the Molisch's test, where a violet ring appeared at the junction in the aqueous extract, indicating basic nutritional content and a role in energy supply. Saponins, well-known for their immunostimulant and expectorant activity, were detected through the foam test, with persistent froth lasting more than 10 minutes, signifying a strong positive result. The Liebermann–Burchard test revealed deep green coloration, confirming the presence of terpenoids, a class of compounds with anti-inflammatory, anticancer, and antimicrobial potential. Alkaloids, commonly associated with analgesic and CNS-modulating activities, were detected using Wagner's reagent in dilute HCl, yielding a reddish-brown precipitate, affirming their presence in the extract. Proteins were identified through the Biuret test, with the appearance of a violet or purple coloration, indicating the presence of structural and enzymatic proteins in the extract. Conversely, fixed oils and fats showed a negative result in the spot test, as no permanent translucent spot was observed. Similarly, resins and amino acids were not detected through the acetone-water test and ninhydrin test respectively, suggesting their absence or presence in negligible amounts. The phytochemical profile of *Albizia lebbeck* bark extract demonstrates a rich presence of pharmacologically important compounds such as flavonoids, tannins, glycosides, saponins, and alkaloids. These findings validate the traditional medicinal use of the bark in managing inflammation, infections, and metabolic disorders. The absence of lipids, resins, and amino acids helps in further refining the extract's chemical characterization and guiding targeted isolation of active principles in future studies.

Table 4. Phytochemical Screening of *Albizia lebbeck* (L.) Benth. Bark Extract

Phytochemical Group	Type of Compound	Test Performed	Solvent Used	Reaction Inference	Observation Time	Result
Flavonoids	Polyphenolic compounds	Shinoda Test	Ethanol	Reddish pink/magenta coloration	Immediate	+
Tannins	Polyphenols	Ferric Chloride Test	Water	Blue-black or greenish-black color	Within 2 minutes	+
Phenols	Phenolic acids	Ferric Chloride Test	Methanol	Deep bluish-green coloration	Within 2 minutes	+
Phytosterols	Steroidal	Salkowski	Chloroform	Reddish-	After 5 min	+

	compounds	Test		brown ring at interface		
Glycosides	Secondary metabolites	Keller-Killiani Test	Ethanol	Reddish-brown ring with bluish layer	2–3 minutes	+
Carbohydrates	Primary metabolites	Molisch's Test	Water	Violet ring at junction	Immediate	+
Saponins	Glycosides (Triterpenoid)	Foam Test	Water	Persistent foam lasting >10 min	After 10 min	+
Terpenoids	Isoprenoids	Liebermann–Burchard Test	Chloroform + H_2SO_4	Deep green coloration	5 minutes	+
Alkaloids	Nitrogenous compounds	Wagner's Test	Dil. HCl	Reddish-brown precipitate	Immediate	+
Proteins	Structural/enzymatic	Biuret Test	Water	Violet or purple coloration	Within 2 minutes	+
Fixed Oils & Fats	Lipid-based compounds	Spot Test	Filter paper (dry)	Permanent translucent spot	After drying	–
Resins	Resinous exudates	Acetone-Water Test	Acetone	Precipitation or turbidity on water addition	Immediate	–
Amino Acids	Building blocks of proteins	Ninhydrin Test	Water	Purple/blue coloration	After heating	–

Total Phenolic Compounds determination

The total phenolic content (TPC) of the bark extract of *Albizia lebbeck* was quantitatively determined using the Folin–Ciocalteu method, and the results were expressed in terms of gallic acid equivalents (GAE) per gram of dry extract. The standard calibration curve for gallic acid produced a strong linear relationship, represented by the regression equation: $y = 0.8461x – 0.0875$, with a correlation coefficient $R^2 = 0.9898$. The high R^2 value (0.9898) indicates an excellent fit of the data points to the linear regression model, suggesting high reliability and accuracy in the quantification of phenolic compounds using this method. This confirms that the absorbance values measured during the assay were directly and proportionally related to the concentration of gallic acid used as standard. The total phenolic content of the bark extract was found to be 329.53 mg GAE/g, which is notably high. This significant concentration of phenolics suggests that *Albizia lebbeck* bark is a potent source of natural antioxidants. Phenolic compounds are known for their free radical scavenging, metal chelating, and reducing capabilities, which contribute to their protective effects against oxidative stress-related conditions such as inflammation, aging, cancer, and diabetes. The high total phenolic content in *Albizia lebbeck* bark extract underscores its therapeutic potential, particularly in formulations aimed at oxidative stress-mediated disorders. The strong linear regression model further validates the precision of the analytical method used. These findings support the ethnomedicinal use of the bark and encourage its further exploration as a functional phytopharmaceutical ingredient.

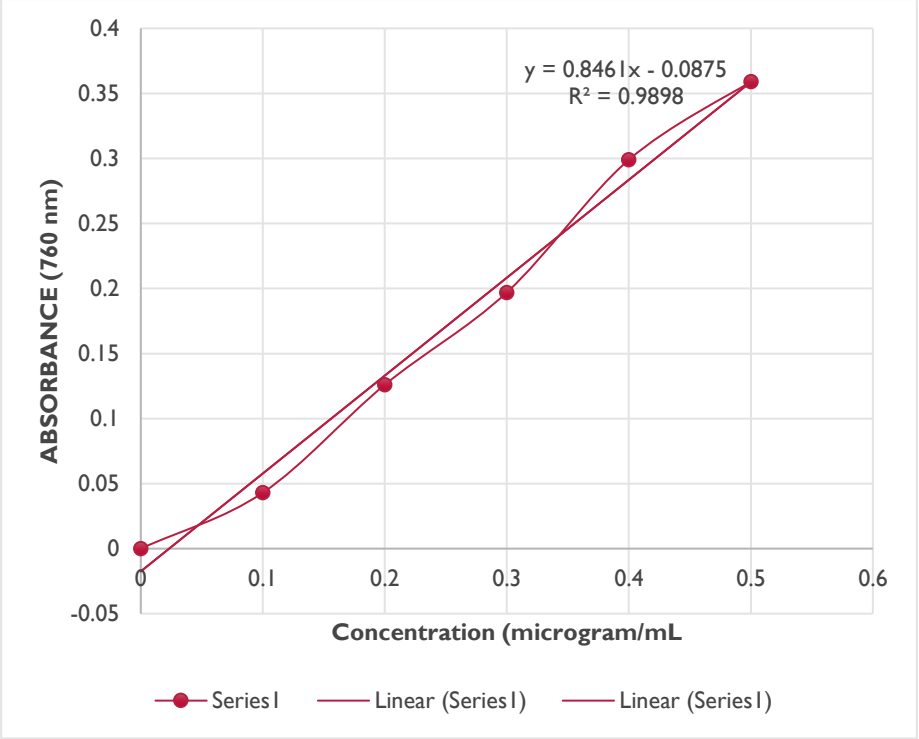

Figure 2. Standard curve of Gallic acid and linear regression analysis

Table 5. The linear regression analysis equations and the total phenolic content of *Albizia lebbeck* (L.) Benth. Bark extract

Extracts Name	Regression Equation	Total Phenolic content in GAE per gram of extract
Albizia lebbeck (L.) Benth. Bark	y = 0.8461x - 0.0875 R^2 = 0.9898	329.53

Inhibition of α-glucosidase enzyme

The α-glucosidase inhibitory activity of *Albizia lebbeck* (L.) Benth. bark extract was evaluated at various concentrations and compared with the standard drug Acarbose, a known α-glucosidase inhibitor used clinically for managing postprandial hyperglycemia in type 2 diabetes. The results demonstrate a concentration-dependent inhibition of the enzyme by both the extract and the standard. At a concentration of 50 µg/ml, the bark extract exhibited 39.88% inhibition, which gradually increased with higher concentrations, reaching a maximum of 94.92% inhibition at 800 µg/ml. In comparison, Acarbose demonstrated slightly higher inhibition at each corresponding concentration, with 49.86% inhibition at 50 µg/ml and 98.92% at 800 µg/ml. The calculated IC_{50} value (the concentration required to inhibit 50% of enzyme activity) for the *Albizia lebbeck* extract was 305.52 µg/ml, whereas Acarbose showed a significantly lower IC_{50} of 112.95 µg/ml, confirming its stronger inhibitory potential. However, the extract still demonstrated a promising inhibitory profile, especially considering its natural origin and potential for fewer side effects. The ability of *Albizia lebbeck* bark to inhibit α-glucosidase can be attributed to the presence of bioactive secondary metabolites such as flavonoids, tannins, phenols, and saponins, as confirmed by phytochemical screening. These compounds are known to interfere with carbohydrate-hydrolyzing enzymes, delaying glucose absorption in the intestine and thereby contributing to better glycemic control. The findings indicate that the bark extract of *Albizia lebbeck* possesses noteworthy α-glucosidase inhibitory activity in a dose-dependent manner, supporting its traditional use in the management of diabetes. Although not as potent as Acarbose, its high inhibition at elevated concentrations and natural origin make it a promising candidate for developing plant-based antidiabetic formulations or functional foods aimed at controlling postprandial blood sugar levels. Further studies involving compound isolation and in vivo evaluations are warranted to explore its therapeutic potential more fully.

Table 6. Results for α-glucosidase inhibition along with the IC_{50} values.

Sample	Concentration (µg/ml)	% Inhibition (Mean ± SD)	IC_{50} (µg/ml)
***Albizia lebbeck* (L.) Benth. Bark**	50	39.88 ± 0.79	**305.52**
	100	48.76 ± 0.99	
	200	65.84 ± 0.92	
	400	73.71 ± 0.99	
	800	94.92 ± 0.99	
Acarbose (Standard)	50	49.86 ± 1.01	**112.95**
	100	58.87 ± 0.98	
	200	72.74 ± 0.99	
	400	81.62 ± 1.02	
	800	98.92 ± 1.04	

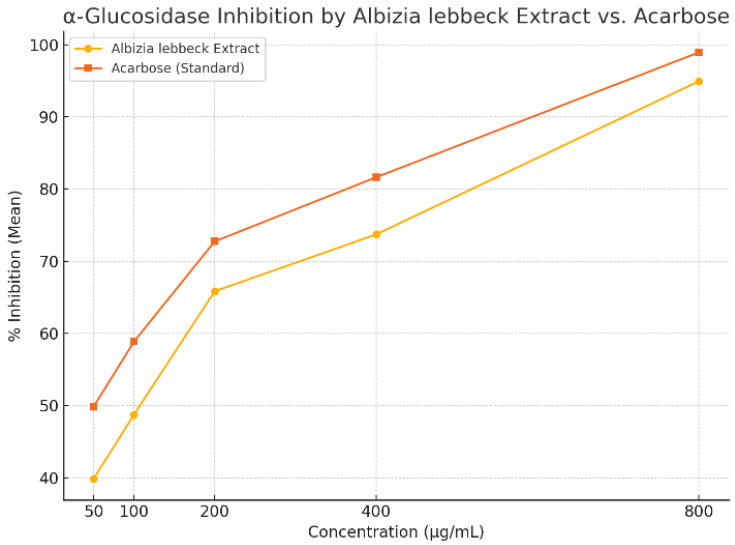

Figure 3. Results for α-glucosidase inhibition along with the IC_{50} values.

Evaluation of DPP-4 Inhibition Assay

The DPP-4 (Dipeptidyl Peptidase-4) inhibition assay evaluates the ability of a compound to inhibit the enzymatic degradation of incretin hormones, particularly GLP-1, thereby enhancing insulin secretion and reducing postprandial glucose levels. This mechanism plays a key role in managing type 2 diabetes mellitus. In the present study, the DPP-4 inhibitory activity of *Albizia lebbeck* (L.) Benth. bark extract was compared to Diprotin A, a standard DPP-4 inhibitor. The control group exhibited 0.00% inhibition, confirming the reliability of the assay system. The standard Diprotin A at a concentration of 60 µg/mL showed a strong inhibitory effect of 88.94%, validating its high potency and serving as a reference for comparison. The bark extract of *Albizia lebbeck* exhibited 19.95% inhibition at 50 µg/mL and 42.87% inhibition at 100 µg/mL, demonstrating a clear dose-dependent response. Although the activity was significantly lower than that of Diprotin A, the extract still displayed notable DPP-4 inhibition, indicating the presence of bioactive phytochemicals capable of modulating this enzyme. The moderate inhibition observed may be attributed to phenolic compounds, flavonoids, and saponins present in the extract, as previously confirmed by phytochemical screening. These secondary metabolites are known to interfere with key metabolic enzymes and contribute to glucose regulation. *Albizia lebbeck* bark extract demonstrated a moderate yet promising DPP-4 inhibitory activity, increasing with concentration. While not as potent as Diprotin A, the natural extract offers potential as a complementary or supportive agent in diabetes management. Further fractionation, compound isolation, and in vivo validation are necessary to fully explore and optimize its DPP-4 inhibitory efficacy.

Table 7. DPP-4 Inhibition by *Albizia lebbeck* (L.) Benth. Bark and Diprotin A

Sample	Concentration (µg/mL)	% Inhibition (Mean ± SD)
Control	–	0.00%
Diprotin A	60	88.94%
Albizia lebbeck (L.) Benth. Bark	50	19.95%
Albizia lebbeck (L.) Benth. Bark	100	42.87%

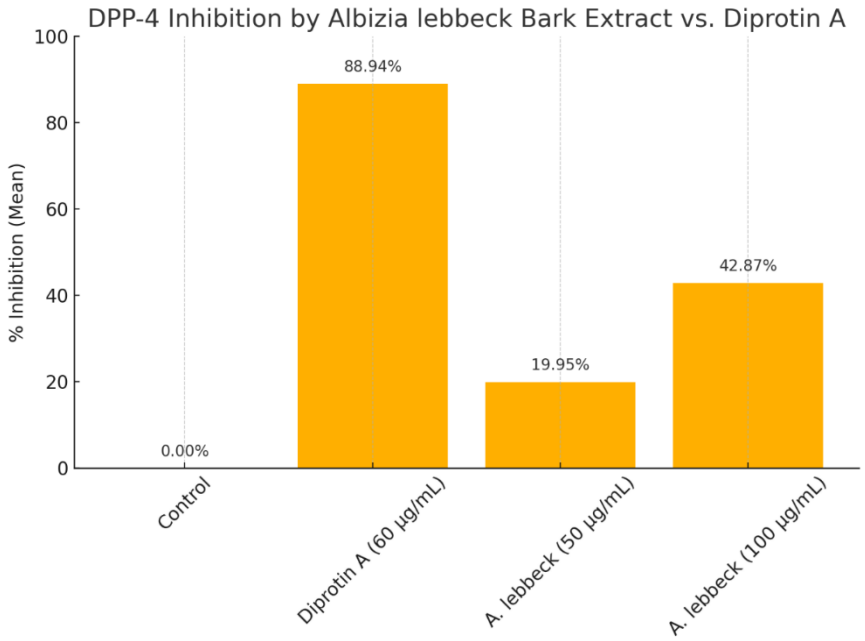

Figure 4. The effect of *Albizia lebbeck* (L.) Benth. Bark on the percentage of DPP-4 inhibition.

4. CONCLUSION

The study provides comprehensive insights into the pharmacognostical and pharmacological potential of *Albizia lebbeck* (L.) Benth. bark, reinforcing its traditional usage and unveiling its promise in modern phytotherapeutic applications. The physicochemical parameters such as total ash, water- and alcohol-soluble extractives, and moisture content confirm the purity, stability, and suitability of the crude bark as a medicinal resource. Organoleptic and fluorescence analyses further enhance its macroscopic and diagnostic identity, contributing to the standardization process. Phytochemical investigations revealed a diverse profile of bioactive compounds, including flavonoids, phenolic compounds, tannins, alkaloids, saponins, glycosides, and terpenoids. These phytoconstituents are known for their antioxidant, anti-inflammatory, and antidiabetic effects. Notably, the high total phenolic content (329.53 mg GAE/g extract) indicates strong antioxidant capacity, which may play a significant role in mitigating oxidative stress associated with diabetes. The α-glucosidase inhibition assay demonstrated a concentration-dependent inhibition, with an IC_{50} of 305.52 µg/mL, suggesting its potential to moderate postprandial hyperglycemia by delaying carbohydrate breakdown. Moreover, the extract showed moderate inhibition of DPP-4 enzyme (42.87% at 100 µg/mL), supporting its role in enhancing endogenous insulin secretion by preserving incretin hormones.

Although the extract was less potent than the standard drugs Acarbose and Diprotin A, its natural origin, presence of multiple bioactives, and safety profile make it a promising candidate for development as a supportive antidiabetic therapy. Future work should focus on isolating active compounds, conducting in vivo validations, and exploring formulation approaches for clinical application in managing type 2 diabetes.

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