



# A Comparative Study on the Phytochemicals and Antimicrobial Activities of the Ethanol and Petroleum Ether Extracts of the Leaves of *Albizia Lebbeck* and Its Mistletoe

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**Abstract:** A Comparative study on the phytochemical constituents and antimicrobial activities of the ethanolic and petroleum ether extracts of the leaves of *Albizia lebbeck* and its mistletoe was carried out. The phytochemical screening was carried out by adopting standard methods. Agar well diffusion method was employed for the antibacterial and antifungal screenings of the extracts of the leaves of *Albizia lebbeck* and its mistletoe. The phytochemical screening results revealed the presence of flavonoids, quinone, saponins, terpenoids, anthraquinone and steroids in the ethanol extract of *Albizia lebbeck* leaves while the ethanol extract of the mistletoe leaves revealed the presence of flavonoids, phenols, quinone, saponins, terpenoids, anthraquinones and steroids. The petroleum ether extract of the leaves of *Albizia lebbeck* revealed the presence of terpenoids, anthraquinone and steroids, while the petroleum ether extract of the mistletoe leaves revealed the presence of saponins, terpenoids, anthraquinone and steroids. For the antibacterial activities screening the results revealed the ethanol extract of the mistletoe leaves to have greater zone of inhibition in *Salmonella typhi* (16 mm), *Staphylococcus aureus* (20 mm) and *Escherichia coli* (15 mm) than the ethanol extract of the *Albizia lebbeck* leaves in *Salmonella typhi* (14 mm), *Staphylococcus aureus* (16 mm) and *Escherichia coli* (13 mm). For the petroleum ether extracts, the extract of the mistletoe leaves have greater zone of inhibition also in *Salmonella typhi* (16 mm), *Staphylococcus aureus* (20 mm) and *Escherichia coli* (14 mm) than the extract of the *Albizia lebbeck* leaves in *Salmonella typhi* (13 mm), *Staphylococcus aureus* (18 mm) and *Escherichia coli* (13 mm). For the antifungal activities screening, the results revealed the ethanol extract of the mistletoe leaves to have greater zone of inhibition in *Aspergillus fumigatus* (16 mm), *Aspergillus niger* (14 mm), *Fusarium oxysporum* (9 mm) than the ethanol extract of *Albizia lebbeck* leaves in *Aspergillus fumigatus* (14 mm), *Aspergillus niger* (13 mm) and *Fusarium oxysporum* (8 mm). For the petroleum ether extracts, the extracts of the mistletoe leaves have greater zone of inhibition in *Aspergillus niger*, while the extract of the *Albizia lebbeck* leaves have greater zone of inhibition in *Aspergillus fumigatus* but they both have the same zone of inhibition in *Fusarium oxysporum* (7 mm). Generally, the results of the antimicrobial screening showed the extracts of the leaves of the mistletoe to be more active than the leaves of the *Albizia lebbeck*. The leaf extracts of *Albizia lebbeck* and mistletoe are useful phytochemicals which possess both antibacterial and antifungal activities.

**Keywords:** Phytochemical Screening, Antifungal Activity, Antibacterial Activity, Mistletoe, *Albizia Lebbeck*, Agar Well Diffusion

## 1. Introduction

Since ancient times, nature has been an important source of medicinal agents. This fact is illustrated by the large

number of natural products currently in use in medical practice. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. This interest in drugs of plant origin is due to several reasons, namely, the

frequent inefficiency of conventional medicine, possible development of side effects of synthetic drugs, and that a large percentage of the world's poor population doesn't have access to conventional pharmacological treatment. In addition, the long history of use of folk medicine suggests that "natural" products are usually harmless.

*Albizia lebbeck* is a species of *Albizia*. It is a leguminous plant which belongs to the family of *Fabaceae*. It is widely cultivated and naturalised in India, New Guinea, Northern Australia and other tropical and subtropical regions. Being one of the most widespread and common species of *Albizia* worldwide, it is often simply called "sirir" though this name may refer to any common member of the genus [1]. It is a tree which grows to a height of 18–30 m tall with a trunk 50 cm to 1 m in diameter. The leaves are bipinnate, 7.5–15 cm long, with one to four pairs of pinnae, each pinnae with 6–18 leaflets. The flowers are white, with numerous 2.5–3.8 cm long stamens, and very fragrant. The fruit is a pod 15–30 cm long and 2.5–5.0 cm broad, containing six to twelve seeds [2].

Traditionally the plant is considered a very potent tool for various therapies. The plant extract is evaluated in allergic rhinitis [3] and memory and learning of mice [4]. The bark is anthelmintic; relieves toothache, strengthens the gums and the teeth. It is also for leprosy, deafness, boils, scabies, syphilis, paralysis and weakness. The leaves are good for ophthalmia. The flowers are anti asthmatic and aphrodisiac emollient in action, maturant; their smell is useful in hemicranias. The root is astringent and prescribed for ophthalmia. The seeds are used for, aphrodisiac, brain tonic, used for gonorrhoea and tuberculosis glands; the oil is applied topically in leucoderma [3]. Pharmacologically, it is used as anti histaminic, antiasthmatic, antifertility, antimicrobial, spermicidal and ophthalmic agent. Its uses also include environmental management, forage, medicine and wood. It is cultivated as a shade tree in North and South America [5]. It serves as protein, energy and mineral supplement for animals. Its bark is used as ointment in skin diseases. All parts of the plants are recommended for the treatment of snake bite. It is also prescribed as antihelminthic. The leaves are good for ophthalmic diseases and night blindness. Traditionally, the bark and the seeds are used for diarrhea, dysentery and piles [6].

Among the plant species, *Albizia lebbeck* seem to possess numerous pharmacological properties. They are widely used as antiasthma, antiseptic, antidyseric, antitubercular, antioxidant activity, anti-microbial agents. *Albizia lebbeck* is used in Indian traditional system and folk medicine as well to treat several inflammatory pathologies such as asthma, arthritis and burns [7]. Recently, it was found that the alcoholic extract of *Albizia lebbeck* has antihistaminic property, by neutralizing the histamine directly or due to corticotrophin action as evidenced by raising cortisol levels in plasma [8]. It is also reported in Indian folk medicine that *Albizia lebbeck* has antiseptic, antidyseric and antitubercular activities [7]. *Albizia lebbeck* has been useful in the treatment of Alzheimer's and Parkinson's diseases [9]. The bark has acrid taste and its extract showed antimicrobial

activity [10]. It has also immunomodulatory effect [11]. In addition, it is used for bronchitis, leprosy, paralysis and helminth infections [7]. *Albizia lebbeck* is also psychoactive. Its sweet-smelling gum or resin is used in cosmetics in some African countries. They also prepare an infusion (hot or cold) from the bark and roots to treat skin diseases such as scabies, inflamed eyes and bronchitis.

Mistletoe is the common name for most obligate hemiparasitic plants in the order Santalales. They attach to and penetrate the branches of a tree or shrub by a structure called the haustorium, through which they absorb water and nutrients from the host plant. The name mistletoe originally referred to the species *Viscum album*. A family of *Loranthaceae*. The stem is yellowish and smooth, freely forked, separating when dead into bone-like joints. The leaves are tongue-shaped, broader towards the end, 1 to 3 inches long, very thick and leathery, of a dull yellow-green colour, arranged in pairs, with very short footstalks.

Mistletoe an ever green parasitic plant is an excellent medicinal herb one should not do without. People in our fast moving times with the tensions of modern living and working under stress surely need an aid like Mistletoe. This may have been an effective treatment, as mistletoe is a nervine and a narcotic, and has a profound effect on the nervous system. It has also been used as a natural remedy for hypertension, headaches, menopausal symptoms, infertility, arthritis, and rheumatism. Mistletoe extract is also frequently used to help treat convulsive coughing, bronchic asthma and asthmatic attacks. Its calming properties help relieve the psychological tension that occurs when an asthmatic has difficulties. The leaves of the herb when taken as a tea are known to have a significant standing as a home remedy. The objective of this study was to compare the phytochemical constituents and antimicrobial activities of the extracts of the leaves of *Albizia lebbeck* and its mistletoe.

## 2. Materials and Methods

### 2.1. Plants Source and Identification

Fresh leaves of *Albizia lebbeck* and its epiphyte (mistletoe) were collected from Sheda Science and Technology Complex (SHETSCO), Sheda, Abuja, Nigeria. The leaves were identified and authenticated in the herbarium unit of the Biological Science Department of Ahmadu Bello University, Zaria, Nigeria. The leaves were rinsed with distilled water to remove sand and any other dirt. They were dried for two weeks under shed and ground using exel mixer grinding machine.

### 2.2. Preparation of Crude Extracts

The powdered leaves of *Albizia lebbeck* and its mistletoe were extracted using Soxhlet extraction method [12], with ethanol and petroleum ether as extraction solvents. The powdered leaves (50g) of *Albizia lebbeck* was transferred into a Soxhlet flask in a 300 ml of absolute ethanol and extracted for eight hours. The extraction solvent was

recovered using rotary evaporator and the extract was further concentrated by evaporation using water bath. The extraction was repeated using petroleum ether as the extraction solvent.

The extraction procedure was repeated for the powdered leaves (50g) of the mistletoe.

### 2.3. Phytochemical Screening

The phytochemical screening was carried out for all the extracts by adapting to standard methods [13, 14].

### 2.4. Test for Steroids

Acetic anhydride (2ml) was added to the extract (0.5g), each with 2 ml of H<sub>2</sub>SO<sub>4</sub> (tetraoxosulphate (VI) acid). A colour change from violet to blue or green in some samples indicated the presence of steroids.

### 2.5. Test for Terpenoids

The extract (0.2g) of the whole plant sample was mixed with chloroform (2ml) and concentrated tetraoxosulphate (VI) acid (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face indicated the presence of terpenoids.

### 2.6. Test for Flavonoids

The extracts (0.2g) were treated with of tetraoxosulphate(VI) acid (5 ml). The formation of an orange colour indicated the presence of flavonoids.

### 2.7. Test for Phenols

Ferric chloride test: Extracts (0.2g) were treated with ferric chloride solution (5ml). Formation of bluish black colour indicated the presence of phenol.

### 2.8. Test for Saponins

The extract (0.2g) was shaken with distilled water (5ml). Formation of frothing, appearance of creamy mist of small bubbles, indicated the presence of saponins.

### 2.9. Test for Quinone

The Extracts (0.2g) were treated with tetraoxosulphate (VI) acid (5ml). The Formation of orange colour indicated the presence of quinone.

### 2.10. Test for Tannins

Each portion of plant extracts (5g) was stirred with distilled water (10ml), Filtered and ferric chloride reagent added to the filtrate. A blue- green precipitate was taken as evidence for the presence of tannin.

### 2.11. Test for Anthraquinones Derivatives

The powdered leaves (3g) was taken in a dry test tube and chloroform (10ml) was added. The mixture was shaken for 5 minutes. The extract was filtered and an equal volume of ammonia solution (10ml) was added to the filtrate and

shaken. A non bright pink colouration in the upper aqueous layer indicated the presence of free anthraquinones.

## 2.12. Antimicrobial Screening

### 2.12.1. Source of Test Organisms

The pure culture of clinical isolates of fungi include: *Aspergillus fumigatus*, *Aspergillus niger*, and *Fusarium oxysporum*; and bacteria includes *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*. They were obtained from University of Abuja Teaching Hospital, Gwagwalada, Abuja, Nigeria.

### 2.12.2. Culture Media

Mueller Hinton agar (MHA) and Potatoe Dextrose Agar (PDA) were used for the antimicrobial sensitivity test. They were prepared according to the manufacturer's instruction and the experiment was carried out in Biotechnology Advanced Research Centre, Sheda Science and Technology Complex (SHESTCO), Sheda, Abuja, Nigeria.

### 2.12.3. Antibacterial Activities of the Leaves Extracts

The activities of ethanolic and petroleum ether extracts of *Albizia lebbek* and its mistletoe leaves were evaluated on bacterial isolates which includes: *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli* using agar well diffusion method [15, 16]. The bacteria isolates were allowed to grow on Mueller Hinton Broth (MHB) for 24 hours at 37°C. The bacterial spores were harvested after sporulation by pouring a mixture sterile glycerol and distilled water to the surface of the plate and later scraped the spores with sterile glass rod. The bacterial isolates suspension were standardized to an Optical Density (OD) 600 nm of 0.5 using spectrophotometer before use. 0.2 ml of the standardized bacterial suspension was evenly spread on Mueller Hinton Agar (MHA) using a glass spreader. Wells were then bored into the agar media using a sterile 6 mm cork borer, the wells were filled up with 0.2 ml of the various concentration of the extract (950, 850 and 750 mg/ml), certain precaution was taken to avoid spillage of the suspension to the surface of the agar medium. The plates were allowed to stand in the lamina flow hood (safety cabinet) for 30 seconds to allow proper diffusion of the extract. The Bacteria plates were incubated at 37°C for 24 hrs. Streptomycin was prepared (10µg/ml) which served as a positive control and DMSO (Dimethyl sulfoxide) served as a negative control.

### 2.12.4. Antifungal Activities of the Leaves Extracts

The antifungal activities of ethanolic and petroleum ether extracts of *Albizialebbek* and its mistletoe leaves were evaluated on fungal isolates which includes; *Aspergillus fumigates*, *Aspergillus niger*, and *Fusarium oxysporum*, while the fungal isolates were allowed to grow on a Potatoe Dextrose Agar (PDA) (Oxoid) at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with sterile glass rod. The harvested fungal spores were standardized to an Optical Density (OD) 600 nm of 0.5 using

spectrophotometer before use. 0.2 ml of fungal spore suspension was evenly spread on PDA (Oxoid). Wells were then bored into the agar media using a sterile 6 mm cork borer, the wells were filled up with 0.2 ml of the various concentration of the extract (950,850 and750mg/ml), certain precaution was taken to avoid spillage of the suspension to the surface of the agar medium. The plates were allowed to stand in the lamina flow hood (safety cabinet) for 30 seconds to allow proper diffusion of the extract. The fungal plates at 25°C for 96 hrs followed by observation of zones of inhibition and recorded in mm. Griseofulvin (30µg/ml) served as positive control for fungi. DMSO (Dimethyl sulfoxide) served as a negative control.

### 3. Results and Discussions

#### 3.1. Phytochemical Screening

The medicinal properties of the plants could be attributed to the presence of one or more detected plant natural products. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *Albizia lebbeck* and its mistletoe leaves. Petroleum ether extract of *Albizia lebbeck* leaves contains steroids, terpenoids and anthraquinones. These secondary metabolites exerts antimicrobial activity through different mechanisms, thus suggesting the potential use of plant for the treatment and prevention of these bacteria [17].

On the other hand, the ethanol extract of *Albizia lebbeck* and its mistletoe leaves revealed the presence of saponins, steroids, terpenoids, anthraquinones, flavonoids and quinones but phenols was present in its epiphyte but not found in the host plant. Flavonoids and steroids containing herbs have been reported in treatment of small pox and ulcers in the mouth [18, 19]. These observations therefore, support the use of *Albizia lebbeck*in herbal cure remedies. The presence of the secondary metabolites in *Albizia lebbeck* and its mistletoe leaves supports the traditional medicine use of this plant in the treatment of different ailment. Saponin was found to be present in *Albizia lebbeck* and its mistletoe leaves extracts and has supported the usefulness of this plant in managing inflammation. The non sugar part of saponin has a direct antioxidant activity which may result in benefit like reduced cancer and heart diseases [20, 21, 22]. Steroidal compounds present in *Albizia lebbeck* and its mistletoe leaves extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones [23].

Research work was carried out on steroidal extract from

some medicinal plants which exhibited antimicrobial activities on some bacterial isolates [24, 25, 26]. Flavonoids and other constituent of *Albizia lebbeck* and its mistletoe extract exhibited a wide range of biological activities like antimicrobial, antiinflammatory, antiangiogenic, analgesic, antiallergic, cytostatic and antioxidant properties [19].

**Table 1.** Phytochemical screening of ethanolic and petroleum ether extracts of *Albizialebbeck*and its mistletoe leaves.

parameter	<i>Albizialebbeck</i> leaves		Mistletoe leaves	
	Ethanol extract	Pet. ether extract	Ethanol extract	Pet. ether extract
Flavonoids	+	-	+	-
Phenols	-	-	+	-
Quinones	+	-	+	-
Saponins	+	-	+	+
Terpenoids	+	+	+	+
Anthraquinones	+	+	+	+
steroids	+	+	+	+

+ means present; - means absent

#### 3.2. Antibacterial Activities

The activity of the extracts was based on the zone of inhibition. The greater the zone of inhibition, the more active the extract was. For the petroleum ether extracts of the leaves of *Albizia lebbeck* and that of the mistletoe, the extract of the mistletoe has greater zone of inhibition against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* than the *Albizia lebbeck* leaves. For the ethanol extracts of the leaves of *Albizia lebbeck* and mistletoe, the extract of the mistletoe has greater zone of inhibition against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* than the *Albizia lebbeck*. This showed that the mistletoe leaves have more antibacterial action than the *Albizia lebbeck* leaves. All the two extracts of the plants tested showed varying degree of antibacterial activities against gram positive and gram negative bacteria of the petroleum ether and ethanol extracts compared favorably with that of the standard antibiotic (Streptomycin). the mistletoe plant tested has the highest zone of inhibition to be 20mm as compared with the extract of *Albizia lebbeck*.

The range of inhibition of the ethanol extract was between 8mm to 20mm, while that of the petroleum ether extract was 7mm to 20mm. The results indicated the potential use of the plants in the management of bacterial diseases caused by *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* since these bacteria are important pathogenic bacteria causing a large number of diseases in human being and animals.

**Table 2.** Antibacterial activities of theethanolic and petroleum ether extracts of *Albizialebbeck* and its mistletoe leaves.

Bacteria	EXTRACTS	Concentration in mg/ml (zone of inhibition in mm)			Positive control streptomycin 10 µg/ml (zone of inhibition in mm)	Negative control DMSO
		950	850	750		
<i>Salmonella typhi</i>	ETA ALAB	14.00	11.00	10.00	26	NA
	ETA MLD	16.00	13.00	10.00	26	NA
	PET. ETHER ALAB	13.00	11.00	8.00	26	NA
	PET. ETHER MLD	16.00	14.00	10.00	26	NA
<i>Staphylococcus aureus</i>	ETA ALAB	16.00	14.00	11.00	28	NA

Bacteria	EXTRACTS	Concentration in mg/ml (zone of inhibition in mm)			Positive control streptomycin 10 µg/ml (zone of inhibition in mm)	Negative control DMSO
		950	850	750		
<i>Escherichia coli</i>	ETA MLD	20.00	16.00	13.00	28	NA
	PET. ETHER ALAB	18.00	15.00	11.00	28	NA
	PET. ETHER MLD	20.00	17.00	15.00	28	NA
	ETA ALAB	13.00	9.00	8.00	26	NA
	ETA MLD	15.00	11.00	8.00	26	NA
	PET. ETHER ALAB	13.00	9.00	7.00	26	NA
	PET. ETHER MLD	14.00	12.00	10.00	26	NA

NA means; not active, DMSO means; Dimethyl Sulfoxide, ETA means; Ethanol, ALAB means; *Albizia lebbbeck*, PET. ETHER means; petroleum ether, MLD means; mistletoe and NA – Not active.

## Antifungal Activities

For the ethanol extracts of the leaves of *Albizia lebbbeck* and the mistletoe, the mistletoe leaves extract has greater zone of inhibition against *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium oxysporum* than the leaves extract of the *Albizia lebbbeck*. For the petroleum ether extracts of the leaves of *Albizia lebbbeck* and the mistletoe, the leaves extract of

*Albizia lebbbeck* has greater zone of inhibition against *Aspergillus fumigatus* than the leaves extract of the mistletoe., but the leaves extract of mistletoe has greater zone of inhibition against *Aspergillus niger* than the leaves extract of *Albizia lebbbeck*. Both the leaves extracts of *Albizia lebbbeck* and the mistletoe were less active against *Fusarium oxysporum*.

The two extracts of the plants showed different ranges of inhibition, the ethanol extract ranges from 8 mm to 14 mm, while that of petroleum ether ranges from 7 mm to 13 mm. The minimum inhibition was *Fusarium oxysporum* at 750 mm/ml. The ethanol extract showed the highest inhibition at 14 mm.

**Table 3.** The antifungal activities of the ethanolic and petroleum ether extracts of *Albizia lebbbeck* and its mistletoe leaves.

FUNGI	EXTRACTS	Concentration in mg/ml (zone of inhibition in mm)			Positive control Griseofulvin 30µg/ml (Zone of inhibition in mm)	Negative control DMSO
		950	850	750		
<i>Aspergillus fumigatus</i>	ETA ALAB	14.00	10.00	9.00	22	NA
	ETA MLD	16.00	11.00	9.00	22	NA
	PET. ETHER ALAB	13.00	13.00	0.00	22	NA
	PET. ETHER MLD	11.00	9.00	8.00	22	NA
<i>Aspergillus niger</i>	ETA ALAB	13.00	11.00	0.00	23	NA
	ETA MLD	14.00	11.00	10.00	23	NA
	PET. ETHER ALAB	11.00	13.00	10.00	23	NA
	PET. ETHER MLD	12.00	10.00	0.00	23	NA
<i>Fusarium oxysporum</i>	ETA ALAB	8.00	7.00	0.00	20	NA
	ETA MLD	9.00	8.00	0.00	20	NA
	PET. ETHER ALAB	7.00	0.00	0.00	20	NA
	PET. ETHER MLD	7.00	0.00	0.00	20	NA

## 4. Conclusion

From the study, the mistletoe leaves extract appear to possess more antibacterial activity than the leaves extract of *Albizia lebbbeck*.

*Albizia lebbbeck* and its mistletoe leaves extracts have good potency for antimicrobial activity. These may represent new sources of antimicrobial agents that can be used for modern phytomedicine for the treatment of diseases.

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