

In Vitro Antimicrobial Activity of Albizia Lebbeck

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ABSTRACT

Albizia Lebbeck belonging to the Leguminosae family has been investigated for antimicrobial activities. The pods of Albizia lebbeck extracts of ethanol. The crude ethanolic extract of this plant were subjected to microbiological investigation by the disc diffusion method. The results compared with a standard Antibiotic, Taxim. The activity of Albizia lebbeck k was studied on microbes namely- E. coli, B. subtilis. The antimicrobial activity was assessed at the doses of 25 And 50 µg /ml and the results of zone of inhibition were compared with the activity of positive control. Maximum antimicrobial activity was reported with 50 mg/ml concentration of Ethanol against B. subtilis (22±1 mm), followed by. The aqueous extract showed the Maximum zone of inhibition against E. coli (17±1 mm). It was observed that with ethanolic extract ZOI was higher as compared to control group and similar to standard.

Key words: Albizia lebbeck; antimicrobial activity

I. INTRODUCTION

Since ancient time Plant kingdom is a source of various bioactive constituents that has been used by people all over the world to cure various diseases from flu to cancer.[1] Since earliest, man has identified medicinal plants with classic pharmacological capacity in the light of their remedial actions. It is generally accepted that plant-based medicines are better than synthetic drugs as these are much safer for humans and environment.[2] Medicinal plants occupy an important position in the socio-cultural, spiritual and medicinal arena of rural people of India. India is rich with indigenous plants which are used in herbal medicine to cure diseases and heal injuries.[3] These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-

diabetic, and antimicrobial functions. Medicinal plants are cheaper and more easily available most of the population in the world, thus, there is need to increase the use of medicinal plants as a potential source of new drugs [4].

Albizia lebbeck (A. lebbeck) Benth is widely distributed in India and is also found in South Africa and Australia, Bangladesh. And it is also called as Indian siris, flea tree, frywood, or Laback in Arabic. Albizia lebbeck is commonly used in ayurvedic and unani system of medicines [5].

The plant also contains saponins, macrocyclic alkaloids, tannins, and flavanols. The plant extract is evaluated in allergic rhinitis and memory and learning of mice [6]. 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 [7]. 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 [8]. Phytochemical investigations showed that the pod of the A. lebbeck contains 3', 5-dihydroxy-4', 7 dimethoxy flavone, and N-Benzoyl-L-phenyl alaninol. The pods show antispermatic effect [9].

An antimicrobial is an agent that kills microorganism (microbicide) or stops their growth of bacteriostatic antimicrobial medicines can be grouped according to the microorganisms they act primarily against [10]. For example, antibiotics are used against bacteria, and antifungals are used against fungal they can also be classified according to their function [11]. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of

antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis [12]. The main classes of antimicrobial agents are disinfectants (non-selective agents, such as bleach, which kill a wide range of microbes on non-living surfaces to prevent the spread of illness [13].

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology, and prediction of therapeutic outcome. In this research, we focused on the use of antimicrobial testing methods for the *in vitro* investigation of extracts and pure drugs as potential antimicrobial agents.

For this reason, discovery of new antibiotics is an exclusively important objective. Natural products are still one of the major sources of new drug molecules today. They are derived from prokaryotic bacteria, eukaryotic microorganisms, plants, and various animal organisms. Microbial and plant products occupy the major part of the antimicrobial compounds discovered until now. Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites, and new synthesized molecules as potential antimicrobial agents.

To evaluate the anti-microbial activity of *Albizia lebbek* ethanol pods extract tested on both Gram-negative and Gram-positive bacterial strains viz., *Escherichia coli*, and *Bacillus subtilis*. The pods extract of *Albizia lebbek* was tested against gram-positive and gram-negative bacteria by standard cup plate method. The anti-microbial screening of ethanol extract of *Albizia lebbek* showed that 25,50 and 100 mg/ml doses are effective against, *E. coli*, and *Bacillus*. Fresh pods of *Albizia lebbek* washed with water to get rid of contaminants like dirt and other.

Albizia lebbek Benth. (Shirish, Family: Leguminosae) is a deciduous tree with compound leaves, flat oblong fruits, round cream-colored seeds, grows wild. The plant is found throughout India, Bangladesh, tropical and subtropical Asia, and Africa. Barks are used in toothache and diseases of the gum [14]. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhoea. Ethanolic extract of pods possesses antiprotozoal, hypoglycaemics indehiscent, 12-35cm long and 3-6 cm wide, rolling along the structure, containing 3-12 seed [15].

The Swedish botanist Carl Linnaeus published the first description of *Albizia lebbek* in 1753 in his landmark book "Species Plantarum." The species was previously Categorized by Linnaeus as *Mimosa lebbek*[15]. Botanists realized in the early 19th century that some *Mimosa* species required their own independent genus. Consequently, *Mimosa lebbek* was moved [16]. Based on morphological Variations seen in the flowers and fruits' structure, the transfer was made [17].

Albizia lebbek is a legume tree that can grow up to 30M tall in open areas, and 015M long in plantation areas. Leaves are bipinnate ranging from 1.5 to 6.5 cm long by 0.5 to 3.5 Cm wide [18].

Phytoconstituents of pod: For the determination of phytoconstituents in the pod of the plant, the ethanolic and petroleum ether extract of bark were made, and subjected to preliminary phytochemical screening, followed by the method given in WHO Guidelines and Ayurvedic Pharmacopoeia [19]. The results show the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids and proteins in the ethanolic extract whereas in the petroleum ether only fats and oils were present. Other constituents are 3', 5-dihydroxy-4', 7 dimethoxy flavones, N- Benzoyl-L- phenyl alaninol, abigenic acid, a triterpenoid sapogenin [20]. The present study was aimed to perform *In vitro* antimicrobial activity on *Albizia lebbek* pods by cup plate method.

II. MATERIALS AND METHODS

Plant collection and authentication

Pods of *Albezia lebbek* was obtained from the local places of Tirupati, AP. *Albezia lebbek* was authenticated by Dr. K. Madhava Chetty, M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

Extraction of plant material:

Fresh pods of *Albezia lebbek* washed with water to get rid of contaminants like dirt and other Impurities and were shade dried. These dried pods were ground and sieved to get a uniform, coarse Powder. Soxhlet apparatus was assembled which contains of a round bottom flask, a Soxhlet Extractor (made by glass) a condenser, and a collection flask. 500g Dried powder of pods were Placed inside the Soxhlet thimble (a small cylindrical container made of porous material). 500 ml of ethanol was poured inside the round bottom

flask and closed it with thimble. Upon setting up the apparatus round bottom flask was heated to vaporize the ethanol and enter the plant material, collecting the phytoconstituents, condensed and ethanol is dripped down into the sample thimble.

Number of cycles of process was repeated until the constituents are dissolved in the ethanol. Heating is then terminated and allowed it to cool. The solvent was transferred to clean container and kept for evaporation using rotatory evaporator [21].



Figure 2: soxhlet apparatus

Preliminary Phytochemical Analysis test

Extract Phytochemical analysis of the ethanol extract of pods was carried out using standard methods. The plant materials were checked for the presence of active constituents like alkaloids, glycosides, flavonoids, tannins, fixed oils and fats, resins and phytosterols [22].

The preliminary phytochemicals tests were carried out for all the extract as per standard methods prescribed by Brain and Turner 1975 and Evans 1996 [23].

Phytochemical screening methods are used for identification and authentication of raw and final herbal medicines in order to detect the species based specific compounds and characteristics [24].

1. Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

a) Mayer's test: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

b) Wagner's test: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

2. Flavonoids

a) Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of

yellow colour precipitate indicates the presence of flavonoids.

b) H₂SO₄ test: Extracts were treated with few drops of H₂SO₄. Formation of orange colour indicates the presence of flavonoids.

3. Tannins

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered, and ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

4. Resins

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

5. Anthraquinones

Add 10ml extract to isopropyl alcohol and add few drops of concentrated ammonium hydroxide solution. Formation of red colour will be seen after 2 min.

6. Glycosides

To the plant extract in the test tube add few ml of bromine water formation of yellow precipitate indicates the presence of glycosides

7. Phlobatanins

Add 0.2gm plant extract to test tube, now add 5ml distilled water and shake well, heated to Bolling. Presence of creamy miss of small bubbles

After phytochemical screening procedure we must start our experiment on antimicrobial activity on albezia lebbeck pods.

Preparation of micro-organism:

The organisms used in this study were Escherichia coli, bacillus subtilis. the strains were maintained on nutrient agar slants at 40C. A loopful of each bacterial strain was inoculated into 50 ml of

sterile nutrient broth in 100 ml conical flask. The flask was incubated on a rotary shaker for 24 hr to activate the strain.

Culture media:

For bacterial cultures Mueller Hinton agar was dissolved in distilled water and sterilized by autoclaving at 15 psi for 20 minutes



Figure 3: Autoclave

Preparation of Inoculum

One day prior to the testing, inoculations of the above bacterial cultures were made in the nutrient broth and incubated at 37°C for 18 –24 hours. Preparation of test solutions Sample extracts was dissolved in solvent separately to give different solution [25].

Standard preparation:

Take 10mg of the antibiotic drug and add 10 ml water to the beaker. And dissolve solution properly without drug tresses. From the solution take 1ml solution add to volumetric flask and makeup to 10ml solvent which is 1000µg/ml, but we must convert them to 100µg/ml And next take 1ml from that solution add 10ml solvent which is 100 µg/ml From this solution take 25µg and 50µg solution through 1000µl micro pipette add to the agar medium

Test preparation:

Take 10mg of ethanol plant extraction and add 10 ml solvent to the beaker. Dissolve the solution properly without plant extraction tresses.

From solution take 1ml and add 10ml solvent in volumetric flask which is 1000µg/ml but we have to convert them to 100µg/ml Next take 1ml from that solution and add to volumetric flask and makeup to 10ml with the help of solvent which is 100µg/ml from this solution take 25µg/ml and 50µg/ml with the help of 1000µg micro pipette and add it the agar medium which is having bacteria

Procedure:

Antimicrobial activity of extracts Agar plates were prepared by pouring the medium in to each sterilized petri dishes and was allowed to set at room temperature. The bacterial culture was inoculated over the surface of agar medium using sterile cotton swab in inoculating chamber. The method adopted was well diffusion method. With the help of sterile borer of 5mm diameter, required holes (wells) were made in the agar petri plates. Different solutions of the extracts were poured in the holes using micropipette, while antibiotics discs placed firmly onto it. At the end all the plates were incubated at 37°C for 48 hours. The zone of inhibition was measured in mm for each organism.

III. RESULTS AND DISCUSSION

Table 1: Results of phytochemical screening

Phytoconstituents	Detection
Alkaloids	+
Carbohydrates	+
Phlobatanins	-
Glycosides	+
Tannins	+
Flavonoids	+
Antraquinones	+
Resins	+

+' represent Positive and '- 'represent Negative
 The preliminary phytochemical screening showed the presence of various phytoconstituents like Flavonoids, tannins, carbohydrates, Glycosides, Alkaloids, Antraquinones and Resins in Ethanolic extract of Albizia lebeck. Results were shown in the table 1.

Antimicrobial Activity

The inhibitory activity of the test compound on microbes was evaluated at various concentrations (25µg/ml, 50µg/ml). The results indicated a concentration dependent.

Table: Inhibition of antimicrobial activity

Name of microorganism	Ethanol Extraction of albezia lebeck		Antibiotic Taxim	
	25µg/ml	50µg/ml	25µg/ml	0µg/ml
E. coli	12	17	18	20
B. subtilis	17	22	19	20

DISCUSSION

Antimicrobial activity is concerned with the investigation of antimicrobial potential of Albizia lebeck plant Extract. The aim is to determine the zone of inhibition on few bacterial strains. The activity of Albizia Lebeck was studied on microbes namely- E. coli, B. subtilis. The antimicrobial activity was assessed at the doses of 25 and 50µg /ml and the results of zone of inhibition were compared with the activity of positive control. Maximum antibacterial Activity was reported with 50µg /ml concentration of Ethanol against B. subtilis (22±1mm), followed by. The aqueous extract showed the Maximum zone of inhibition against E. coli (17±1mm). It was observed that with ethanolic extract ZOI was higher as compared to control group and similar to standard.

The plants are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, insecticides, steroids; related active metabolites are of great medicinal use and have been extensively used in the new formulated drugs,

also combined with pervious drug to get more positive results and pharmaceutical industry. Recently, more plants have been reported for antimicrobial properties across the world

The above results show that the activity of ethanolic extracts of Albizia lebeck pods shows significant antimicrobial activities. This study also shows the presence of different phytochemicals with biological activity. The result of phytochemicals in the present investigation showed that the plant contains more or less same components like glycosides, anthraquinones, flavonoids, proteins, alkaloids, carbohydrates, tannins and resins. Results show that plant rich in tannin and phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms.

IV. CONCLUSION

The phytochemical analysis of Ethanolic extract of Albizia lebeck pods showed the presence of Alkaloids, carbohydrates, Glycosides, Tannins, Anthraquinones, Flavonoids, Resins. The

study revealed that the pods extract of *Albizia lebbek* has potent antimicrobial activity against the tested organism.

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