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### **Anti-Microbial Activity of Leaves Extract of *Albizia Lebbeck* Against Some Selected Pathogens**

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#### **ABSTRACT**

To evaluate the anti-microbial activity of *Albizia lebbeck* methanolic leaf extract tested on both Gram-positive and Gram-negative bacterial strains viz., *Staphylococcus aureus*, *Listeria monocytogens*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

The leaf extract of *Albizia lebbeck* was tested against gram-positive and gram-negative bacteria by standard disc diffusion method. The phytochemical analysis was carried out using chloroform, ammonium hydroxide, ferric chloride, sulphuric acid.

The anti-microbial screening of methanolic extract of *Albizia lebbeck* showed that 100, 200 and 500 mg/ml doses are effective against *S. aureus*, *E. coli*, *L. monocytogens*, *P. aeruginosa* and *Salmonella typhi*. The maximum inhibition zone is found against *Staphylococcus aureus* with 500 mg/ml dose. The phytochemical analysis was carried out which revealed the presence of saponins, tannins, terpenoids, steroids and flavonoids in the leaves of *A. lebbeck*.

The current study showed that methanolic extract of *A. lebbeck* have potent anti-microbial activity against *S. aureus* when compared with standard antibiotics like Ciprofloxacin and Streptomycin. These findings showed that *A. lebbeck* can be a significant replacement of antibiotics which were used against bacterial infections.

**KEYWORDS:** *Albizia lebbeck*, Anti-microbial activity, Methanolic extract

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## INTRODUCTION

The illness caused by the micro-organism is very old and common but human evolved many drugs against the micro-organism like antibiotics but because of irregular use, many pathogenic micro-organism registrants to the antibiotics. These medicines also have lot of side effects for humans. So currently the uses of herbal medicine increased because herbal medicine is good alternative for this type of illness because the presences of many compounds like secondary metabolites which are more effective, cheaper and safer. Now a day's not only developing, developed countries also depend on the wide range of herbal medicine. Medicinal plants have an important role in cultural, spiritual and curative aspects of rural and tribal peoples of India. India have wide range of native plants which are used to cure diseases and heal injuries, because of its vast and wide variations in soil and sub-continent climate is suitable for cultivation of medicinal properties. These plants exhibit a wide verity of pharmacological activities such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-spasmodic, anti-hypertensive, anti-diabetic and anticancer uses. Alternative remedies are being used by about 60% of the world's populations and 30% of the worldwide drugs sales are based on natural products. In previous few decades, there has been increasing demand and delivery of diverse herbal product for health benefits. In many developed countries, ethanomedicine still continue as dietary supplements such as called nutraceuticals. It is estimated that there are about 2500000 species of higher plants ubiquitously the world and most of them have not been investigated in detail for their pharmacological properties. However, in recent years by facing a big trouble of multidrug resistant micro-organisms, scientists are interested to development of plant based drugs as alternative. Plant derived antimicrobial agents are commercially proven drug used in modern medicines, were initially used in crude form in traditional or folk medicine or for the other purposes that suggested potentially useful biological activity.<sup>1-3</sup>

*Albizia lebbek*, locally known as siris or woman's-tongue. It belongs to family leguminosae and subfamily mimosoideae. It is broadly distributed in Asia from Eastern Pakistan through India and Sri Lanka to Burma and is also found in South Africa and Australia. It is used in Indian traditional system and folk medicine to treat several inflammatory pathologies such as asthma, arthritis and burns. The leaves, bark, seed and root are used in traditional medicine, where leaves are used in the treatment of night blindness and syphilis and barks for toothache, diseases of the gum treatment of inflammation, bronchitics, leprosy and also a quality of anthelminatic. Decoction of leaves and barks are protective against bronchial asthma and other allergic disorders. The root of the plant has astringent property and is useful in ophthalmia and skin diseases. Barks were mainly used in

dental infections and seeds are given in piles and diarrhoea. Flower of the plant are reputed for its aphrodisiac properties and are also being commonly used to treat anxiety, depression and insomnia in traditional Chinese medicine. Ethanolic and methanolic extracts of pods possesses antiprotozoal, antibacterial, antifertility activity, hypoglycaemic and anticancer properties. With this back ground the present work was aimed to screen the antimicrobial properties of *Albizia lebbeck* stem bark and root against the human pathogens.<sup>4-9</sup>

## MATERIALS AND METHODS

### *Collection of plant material:*

The collection of plant material was done by pre survey of the Bhopal region and the site of location was recorded. Fresh leaves of *Albizia lebbeck* were collected from in and around Bhopal region during post monsoon of 2013 and 2014 (October to December) and placed in sealed plastic ziplock bags after removing excess moisture using 95% ethanol and further stored. Identification and preparation of voucher specimen of the selected plant had been done in the herbarium record of Department of Botany, Govt. M.V.M. Bhopal, and M.P.

### *Processing of the plant materials:*

After collection of leaves samples thorough washing was done with tap water in order to remove the soil and dust particles adhering to the plant parts. Finally, the leaves were rinsed with distilled water separately. Furthermore, plant samples were spread over the blotting paper in a room temperature for 7 days, until the samples were completely dried and free of moisture. During the course of drying, the plant samples were regularly turned and the blotting paper used for drying of plant samples was changed daily to avoid microbial contamination due to excessive moisture. The dried plant material was processed using mixer grinder to crush into powder form. The samples were wrapped in a paper and packed in polythene bags to avoid moisture absorption and contamination. The dry weight of the powdered samples (in Grams) was recorded using electronic balance.

### *Preparation of plant extract:*

Air shade dried and powdered plants part were extracted by sequential soxhlet extraction with different solvents of increasing polarity and separately by hot percolation method through soxhlet apparatus. The dried Powdered (200g.) of *Albizia lebbeck* leaves was packed with thimble and then extraction with (1000 ml) methanol. The extraction was done for 48 hours duration at a temperature not exceeding the boiling point of the solvent. There after resulting extract was filtered through

Whatman filter paper (No.1) and then concentrated under pressure at 45°C using the Eppendorf rotary vacuum concentrator to obtain a crude residue.

### ***Pytochemical Analysis:***

After the collection, identification and extraction of the plant leaves, the aqueous extract of plant leaves was used for the screening to determine the presence of phytochemicals.

**Detection of Steroid:** 200 mg plant leaves material was taken and mixed with 10 ml chloroform and then filtered. 2 ml filtered solution added with 2 ml acetic anhydride and few drops of concentrated sulphuric acid. Blue green ring indicates the presence of steroids.

**Detection of Terpenoids:** Crude extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated sulphuric acid was added carefully and shaken gently. A reddish brown colour indicated the presence of terpenoids.

**Detection of Saponins:** 2ml of distilled water was mixed with plant leaves extracts and shaken in a graduated cylinder for 15 min. Length wise. Formation of foam indicates the presence of saponin.

**Detection of flavonoids:** 50mg plant leaves extracts were treated with ammonium hydroxide solution. The yellow fluorescence indicates the presence of flavonoids.

**Detection of Tannins:** 50mg plant leaves material was dissolved in 5 ml of distilled water and few drops of neutral 5% ferric chloride solution were added. The formation of blue green colour indicates the presence of tannins.

**Detection of Quinones:** Concentrated sulphuric acid (1ml) was added to 1ml of the plant leaves extracts. Formation of red colour indicates the presence of quinones.

### ***Antimicrobial screening:***

Antimicrobial activity of different crude extracts of *Albizia lebbek* were tested on both Gram positive and Gram negative bacterial strains viz., *Staphylococcus aureus*, *Listeria monocytogens*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Stock cultures were maintained at 4°C on nutrient agar slants. Inoculums were prepared from the 24 hours old culture of standard bacterial isolates in nutrient broth. Overnight nutrient broth culture of the test organisms was seeded over the nutrient agar plates using sterile cotton swab so as to make lawn culture. Agar well diffusion method was followed to determine the antimicrobial activity. Wells of 10 mm diameter were punched over the agar plates using sterile gel puncher. The well were loaded with 50 mg/ml, 100 mg/ml, and 200 mg/ml 500 mg/ml of leaves extracts. Standard antibiotics solution as Control was used for comprising inoculums. The plates were incubated at 37°C for 18-24 hrs. Triplicates were maintained and the experiment was repeated trice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

## RESULTS AND DISCUSSION

### *Phytochemical analysis:*

The phytochemical analysis was carried out which revealed the presence of saponins, tannins, terpenoids, steroids and flavonoids in the leaves of *A. Lebbeck* which has been summarized in Table 1.

#### 1. Phytochemical analysis of *A. lebbeck*

Phytochemical	Occurrence
Steroids	++
Tannins	+
Quinones	+
Flavonoids	++
Saponins	+
Terpenoids	+

+ = present - = absent

Methanolic extracts were screened for the antibacterial activity against the selected pathogens. The antibacterial activity of stem barks extract of *Albizia lebbeck* were illustrated in Table -1. The present study demonstrated that methanolic extracts of *A. lebbeck* conferred the widest spectrum activities that inhibited the growth of all studied pathogens with the maximum zone of inhibition (Table 1). The methanolic extracts of *A.lebbeck* illustrated against the pathogens *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

#### 2. Antibacterial activity of leaves of *A. lebbeck* against pathogens (mm).

Test sample in different concentration	Microorganisms (Zone of inhibition in mm)				
	Gram's Positive		Gram' s Negative		
	<i>S.aureus</i>	<i>L. monocytogenes</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
<i>A.lebbeck</i> (100mg/ml)	7mm	-	-	3mm	-
<i>A.lebbeck</i> (200mg/ml)	11mm	-	6mm	9mm	-
<i>A.lebbeck</i> (500 mg/ml)	18mm		12mm	16mm	5mm
Ciprofloxacin(0.5mg/ml)	26mm	23mm	27mm	26mm	22mm
Streptomycin (1 mg/ml)	22mm	11mm	36mm	32mm	14mm
DMSO (20%)	-	-	-	-	-

Despite previous records, *A. lebbeck* extract didn't show any inhibitory effect. It could be because of ecological reasons. According to pharmacognosy science, it is clear that if plant is cultivated in different environmental conditions, it produces different types and concentration of

products. The other points that could be considered as the reason are plant growing location, height, climate, humidity, dryness and temperature, amount of sun light, sex, soil, rainfall and time of collection. Another problem due to medicinal effects of plants is geographical origin. The native place of plant is the best and the most effective plants are grown up named geographical origin. A plant that is grown in different places in or out of geographical origin doesn't have the same substances and effects; it is because the weather condition is very decisive in producing officinal substances.

The use of herbal medicines increased in recent years. In this experiment, antibacterial activity of different concentration of plant extract was monitored using disc-diffusion assay. The anti-bacterial screening of methanolic extract of *Albizia lebbek* showed that 100mg/ml, 200mg/ml and 500 mg/ml doses against *S. aureus*, *E. coli*, *L. monocytogens*, *Pseudomonas aeruginosa* and *S. typhi* are effective. The maximum inhibition zone is found against *Pseudomonas aeruginosa* with 500 mg/ml dose. The current study showed that methanolic extract of *A. lebbek* have potent anti-microbial activity against *S. aureus* when compared with standard antibiotics like Ciprofloxacin and Streptomycin. These findings showed that *A. lebbek* can be a significant replacement of antibiotics which were used against bacterial infections. This antibacterial activity of extract is because of phytochemical part of leaf extract. Phytochemical assay extract of *Albizia lebbek* leaves was done with methanol. Assay shows presence of steroid, tannins, flavanoids, saponins, quinones and terpenoids.

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