



## *In vitro* anti-Fungal activity of benzene and hydro-alcoholic leaves extract of *Albizia lebbek*

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### ABSTRACT

**Objective:** The present study was aimed to evaluate the *in vitro* anti-Fungal activity of benzene and hydro-alcoholic leaves extract of *Albizia lebbek*. **Materials and methods:** Preliminary phytochemical investigations were carried out for qualitative detection of phytoconstituents by standard procedures, anti fungal activity of benzene and hydro-alcoholic leaves of *Albizia lebbek* plant extracts was compared with that of standard Fluconazole (10µg/ml) by measuring the diameter of the Zone of Inhibitions of test and standard samples. **Results:** Hydro-Alcoholic extract (1000µg/ml) obtained from leaves of *Albizia lebbek* showed excellent activity on comparing to that of standard drug Fluconazole (10µg/ml) and benzene extract. **Conclusion:** In conclusion the present study supports the traditional medicine use of *Albizia lebbek* plant extracts in treating different infections caused by pathogenic fungi either by using a single or combined extracts.

**Keywords:** anti-Fungal activity, benzene, hydro-alcoholic, leaves extract, Fluconazole, *Albizia lebbek*.

### INTRODUCTION:

In developing countries and particularly in India low income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infections [1]. Skin, hair, nail, and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytoses [2, 3]. Dermatophytoses are one of the most frequent skin diseases of human, pets and livestock [4]. The disease is widely distributed all over the world with various degrees and more common in men than in women. Patients have a reduced risk to get infectious diseases from resistant pathogens than people from urban areas treated with traditional antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. More than 1300 plant species are known to be potential sources of antimicrobial components but only some of them have been studied scientifically [5, 6]. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many

parts of the world [7]. This use has been supported by the isolation of active antifungal compounds from plant extracts [8]. These compounds represent secondary metabolites that serve as defense agents against invading micro-organisms. Traditional healers claim that their medicine is cheaper and more effective than modern medicine. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens.

The present study was designed to evaluate the *in vitro* anti-Fungal activity of *Albizia lebbek* plant extracts. From our literature survey carried out on the above said plant, there was no scientific claim on anti-Fungal activity carried out on the leaves and flowering tops of the plant. Hence an attempt had been made in our present dissertation to evaluate the activity on various crude extracts obtained from leaves and flowering tops of the *Albizia lebbek* plant extracts. The percentage of inhibition and MIC are also recorded.

### 2. MATERIALS AND METHODS:

#### 2.1. Collection of plant material

The leaves of tree *Albizia lebbek* Benth. was collected from areas in around Narayana Group of Medical Institutions, Chinthareddypalem, Nellore, A.P, India. The plant was authenticated by Dr. S. Md. Khasim, Head, Department of Botany, Acharya Nagarjuna University, Guntur.

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One of the specimen was deposited in the medicinal garden of Narayana Pharmacy college.

## 2.2. Preparation of extract

After the leaves were collected, they were washed with fresh water to remove the soil and adhered matters. Sufficient leaves were dried under shade at room temperature then they were powdered using a grinder mixer to obtain a coarse powder and then passed through a 40-mesh sieve. Extraction solvent extraction by continuous hot (sox let extractor) and by cold maceration method.

## 2.3. Preliminary phytochemical screening

The preliminary phytochemical investigations were carried out for qualitative detection of phytoconstituents. Qualitative tests were conducted for all the extracts to identify the various phytoconstituents. The various tests observations are recorded in Table no 1.

## 2.4. Preparation of cell or spore suspension

The stock cultures of *Candida albicans* (MTTC No-183) and *Aspergillus niger* (MTTC No- 281) and 3 loops full of the material is taken and mixed in 0.5ml of distilled water separately for both the species.

## 2.5. Dilution of preparation

Both the samples i.e. Benzene extract and Hydro-alcohol extract of the leaves of *Albizia lebbek* were weighed and dissolved in Dimethylsulphoxide (DMSO) with the aid of sonification process to prepare following dilutions, 10, 20, 40, 60, 80, 100, 200, 1000µg/ml and thus DMSO is used as control.

## 2.6. Standard

Fluconazole (10µg/ml) antifungal agent is used as standard.

## 2.7. In vitro Pharmacological evaluation

### 2.7.1. Anti-fungal activity:

Samples are dissolved in to required concentration and taken in to watch glasses. Then Watt man filter paper disc were prepared and placed in watch glasses containing different concentrations of test samples. Later Paper discs were removed and dried. A cell or spore suspension of *Aspergillus niger* and *Candida albicans* are prepared and 0.2ml of suspension was shifted to sterile petri-dish then sterilized Potato dextrose agar medium was poured in to medium and allowed to solidify. Discs with sample were pleased in to petri plates carefully then allowed it for incubation at 24°C for 5 days. The plates are removed and the diameter of the Zone of Inhibitions of test and standard samples are measured.

## 3. RESULTS

### 3.1. Preliminary phytochemical evaluation

Various extracts have been prepared by suitable methods. The dried extracts were subjected to various chemical tests to detect the presence of different phyto-constituents present in them and are tabulated in Table no 1.

**Table No 1. Phytochemical screening of *Albizia lebbek* leaves Extracts**

Phytochemicals	Benzene extract	Hydro-alcoholic extract
Alkaloids	+	+
Glycosides	+	-
Tannins	+	+
Saponins	+	+
Steriods	-	-
Flavonoids	+	+
Carbohydrates	+	+
Protiens	+	-
Amino acids	+	-

### 3.2. In vitro pharmacological evaluation

#### 3.2.1. Anti-fungal activity

The Anti-Fungal activity was carried out for Benzene and Hydro-Alcoholic extracts of Leaves of *Albizia lebbek*, and the zone of inhibition of the above stated extracts at different concentrations are measured and are tabulated in Table no 2, 3, 4 & 5 respectively.

**Table No 2 - Zone of Inhibition of Benzene extract on *Aspergillus niger***

<i>Aspergillus niger</i>	
Concentration (µg/ml)	Zone of Inhibition (mm)
Control	-
10	-
20	-
40	-
60	2
80	2
100	3
200	5
1000	11
Standard	12

**Table No 3 - Zone of Inhibition of Benzene extract on *Candida albicans***

<i>Candida albicans</i>	
Concentration (µg/ml)	Zone of Inhibition (mm)
Control	-
10	-
20	-
40	-
60	1
80	2
100	2
200	4
1000	9
Standard	13

**Table No 4. Zone of Inhibition of Hydro-Alcoholic extract on *Aspergillus niger***

<i>Aspergillus niger</i>	
Concentration ( $\mu\text{g/ml}$ )	Zone of Inhibition (mm)
Control	-
10	-
20	-
40	-
60	1
80	2
100	3
200	5
1000	13
Standard	12

**Table No 5 - Zone of Inhibition of Hydro-Alcoholic extract on *Candida albicans***

<i>Candida albicans</i>	
Concentration ( $\mu\text{g/ml}$ )	Zone of Inhibition (mm)
Control	-
10	-
20	-
40	-
60	2
80	2
100	3
200	6
1000	14
Standard	12

#### 4. DISCUSSION

Many investigations were carried out to discover plant products that inhibit the fungi like *Aspergillus niger* and *Candida albicans*. These two species cause common infections in humans which are difficult to control effectively, and the pharmaceutical arsenal currently available against them is rather limited<sup>[9-12]</sup>. Hence, plant products that inhibit their growth without harming the host represent potential therapeutic agent.

As stated earlier, Benzene and Hydro-Alcoholic extracts of Leaves of *Albizia lebbbeck*, were detected for their antifungal activities against *Aspergillus niger* and *Candida albicans* which are usually considered to cause disease in keratinized epithelial structures such as hairs and nails and can invade the dermis, particularly in immunocompromised patients<sup>[13]</sup>. The antifungal activities of the plant extracts obtained using different organic solvents were compared with that of Fluconazole (10 $\mu\text{g/ml}$ ) and the % of inhibition was calculated. Extracts were obtained through the extracting action of the appropriate solvent on a dry plant and the active compounds are thus contained in the solvent used. Each type of extract is defined by the way it is prepared and the nature of the solvent. The extraction process is always studied to respect the integrity of the active molecules. In this experiment, Hydro-Alcoholic extract (1000 $\mu\text{g/ml}$ ) ob-

tained from leaves of *Albizia lebbbeck* showed excellent activity on comparing to that of standard drug [Fluconazole (10 $\mu\text{g/ml}$ )] and benzene extract.

Further studies are needed to determine the antifungal compound(s) in such plant extract (isolation, separation and identification) as well as its formulation to be applicable as alternative methods to be used in treatment of skin and skin structures diseases in human and animal. Therefore, such results are of a significant value that confirms the therapeutic potency of some plants used in traditional medicine. It should form a good basis for further phytochemical and pharmacological investigation<sup>[14]</sup>. Useful antimicrobial phytochemicals are: phenolics and polyphenols (such as simple phenols and phenolic acids, quinones, flavones, flavonoids, and flavonols, tannins, coumarins); terpenoids and essential oils; alkaloids; lectins and polypeptides; plus other compounds. The mechanisms thought to be responsible for these phytochemicals against microorganisms vary and depend on these compounds<sup>[15]</sup>. Their mechanism of actions may include enzyme inhibition by the oxidized compounds, and act as a source of stable free radical and often leading to inactivation of the protein and loss of function. They have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls and disrupt microbial membranes<sup>[16]</sup>, some have ability to intercalate with DNA, formation of ion channels in the microbial membrane, competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors<sup>[17]</sup>.

#### CONCLUSION

The ultimate conclusion of this study supports the traditional medicine use of *Albizia lebbbeck* plant extracts in treating different infections caused by pathogenic fungi either by using a single or combined extracts. It also suggests that a great attention should be paid to medicinal plants which are found to have plenty of pharmacological properties that could be sufficiently better when considering a natural food and feed additives to improve human and animal health.

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#### Conflict of Interest

We declare that we have no conflict of interest.

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