

**ANTIFUNGAL POTENTIAL OF DALBERGIA SISSOO LEAF**

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**ABSTRACT**

Infectious disorders are frequently treated with medicinal plants. Infectious diseases caused by various microorganisms continue to be the main cause of morbidity and mortality worldwide. There is a tonne of synthetic antifungal substances on the market. The treatment of associated illnesses can be done extremely well with these antimicrobial drugs. However, their usefulness is constrained because many microorganisms are able to develop resistance to different antimicrobial medicines. Most of these antibiotics also have negative effects. Therefore, the infectious diseases caused by the bacteria constitute a serious threat to public health around the world. Therefore,

the demand for developing natural anti-microbial drugs is considerable. With reference to various pieces of Ayurvedic literature, a strong desire to work on the plant. The most important public health achievement will be the introduction of natural antifungal medications to treat the fungal disease. This study's objective was to assess the antifungal potential of methanol and aqueous extracts of Dalbergia sissoo leaf material. Dalbergia sissoo leaves were processed into aqueous and methanolic extracts for this study. The agar well diffusion method was used to examine these extracts' antifungal properties. Dalbergia sissoo leaves were found to suppress the growth of *Trichophyton rubrum*, *Epidermophyton floccusum*, and *Microsporum canis* in both aqueous and methanolic extracts. The extract's antifungal activity was on par with that of fluconazole. In this investigation, it was found that Dalbergia sissoo leaves have strong antifungal capability.

**KEYWORDS:** Dalbergia sissoo; antifungal capability; aqueous and methanolic extract.

## INTRODUCTION

Ayurveda is a holistic medical system. Ayurveda is an ancient healing discipline from India that was used extensively around 5,000 years ago during the Vedic period. Ayurvedic formulations are often made from the various components of healing herbs. Herbal/Ayurvedic formulations are an efficient way to treat a variety of infectious disorders brought on by bacteria, fungus, viruses, and worms.

The need for finding new antimicrobial drugs from non-traditional sources is highlighted by the pathogenic microorganisms' growing resistance to synthetic antimicrobial agents. Numerous compounds of plant origins have been found to possess potent antibacterial activities. According to reports, the Leguminosae plant *Dalbergia sissoo* possesses antibacterial properties against a variety of microorganisms. Its effectiveness against certain worms has also been looked into.

*Dalbergia sissoo* Linn (Synonyms-Shisham or Sisam), a large deciduous tree found throughout India, has been reported in folk medicine and is used mainly as aphrodisiac, abortifacient, expectorant, anthelmintic and antipyretic. It is also used in conditions like emesis, ulcers, leucoderma, dysentery, stomach troubles and skin diseases<sup>1-3</sup>. Since no information is available on antifungal activity of *Dalbergia sissoo* leaves, the present study was undertaken to investigate the antifungal activity of *Dalbergia sissoo* leaves.

*Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, and *Rhizopus stolonifera*, four different fungal strains, were evaluated against *Dalbergia sissoo* leaves in a different study. The antifungal activity was effective from 20°C to 25°C. Methanol extracts are said to be the most potent of the many extracts.

Humans can get ringworm, a fungal skin ailment. Numerous distinct fungal organisms that are all members of the "Dermatophytes" category are what cause ringworm. Dermatophytes are the species of the genera *Microsporum*, *Trichophyton*, and *Epidermophyton*.

## MATERIAL AND METHOD

### Materials

#### Fungal Culture Maintenance

Fungal strains were provided by MTCC, IMTECH, Chandigarh, India at DSRRAU, Jodhpur. Following Three species of dermaophytes are selected for this study- *Trichophyton rubrum* (MTCC NO.296), *Epidermophyton floccosum* (MTCC NO.7880), and *Microsporum cannis* (MTCC NO.2820).

The fungal stains procured from MTCC; IMTECH Chandigarh were in freeze dried condition (in dormant form). So, the revival of the stains was done. After revival all fungal cultures were allowed to grow in incubator at 200 rpm and 25°C to 30°C temperature for 7 to 10 days by using potato dextrose broth. After incubation the turbidity in each flask confirms the growth of culture and placed them at 4°C till further use. Thereafter the fungal cultures were used for antimicrobial studies.

#### Parts of Dalbergia sissoo Plant

*Dalbergia sissoo* leaves of the drug were collected in month of August 2021 from the herbal garden of University Post Graduate Institute of Ayurveda Studies & Research, Jodhpur. The leaves were thoroughly washed with running tap water 2-3 times and finally washed with sterile distilled water followed by shade-drying on paper towel at room temperature for 15 days. Leaves were exposed to direct sunlight also for a few days. After drying, the plant materials were ground in a grinding machine.

## METHODS

### Aqueous Extracts

Fifty grams of grounded plant material was extracted with 150 ml sterile double distilled water for 24 h as in the case of methanol. The mixture was filtered with sterile five-layered muslin cloth and centrifuged at 5000 rpm. The supernatant obtained was concentrated to N/5 volume with rotary evaporator. The concentrated extract was then UV sterilized and stored at 4 °C for further use.

### Methanolic extracts

In a tightly sealed container at room temperature, fifty grams of grounded plant material was extracted with 150 ml methanol. The extract was protected from light and kept overnight on a rotary shaker at Seminal Applied Sciences Pvt. Ltd. Jaipur, Rajasthan in India. The extract

was filtered with a five layered sterile muslin cloth. The procedure was repeated three times to obtain clear and colorless filtrate. The methanol from the filtrate was removed by rotary evaporation. Extracts were stored at 16 °C overnight and were subsequently freeze-dried at 60 °C in a 20 mL vacuum for 24 h. The extract was then sterilized with UV and stored in an airtight container at 4 °C for further use.

### Antifungal Potentiality Test

Agar well diffusion technique (Adeniyi *et al*) was adopted for this study with some modification. Sabouraud dextrose agar (Hi-Media- M063) poured into pre-sterilized petri-plates. Fungal suspensions (1 ml of each) were spread over the solidified potato dextrose agar plates and allowed to dry for few minutes. Stains were incubated at 25°C or 30°C for 30 days according to MTCC protocol to enhance sporulation.

There after five wells were punched with a sterile corn borer. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 20 mg/ml. Four wells were filled with 20 µl, 40 µl, 60 µl and 80 µl of diluted extract. In fifth well fluconazole (40 µl) was filled as the standard for comparison of antifungal activity. Plates were then incubated at 37 °C for 72 h. Following an incubation period of 72 hrs, plates were removed from the incubator and antifungal activity was evaluated by measuring zones of inhibition of fungal growth.

### OBSERVATION AND RESULTS

The methanolic and aqueous extracts prepared from the leaves of *Dalbergia sissoo an* along with fluconazole were inoculated into wells punched in pre-seeded agar plates. After incubation at 37 °C for 72 h, the clear growth inhibition zone around the well was measured and recorded as a measure of antifungal activity. The results represented in Figure 1 show the antifungal potential of methanolic extract along with fluconazole against *Trichophyton rubrum* and *Epidermophyton floccosum*. and Figure B show that aqueous of *Dalbergia sissoo* leaves extract along with fluconazole exhibit significant antifungal activity.

### The Relationship between Zone of Inhibition and Drug Sensitivity

Table No. 1

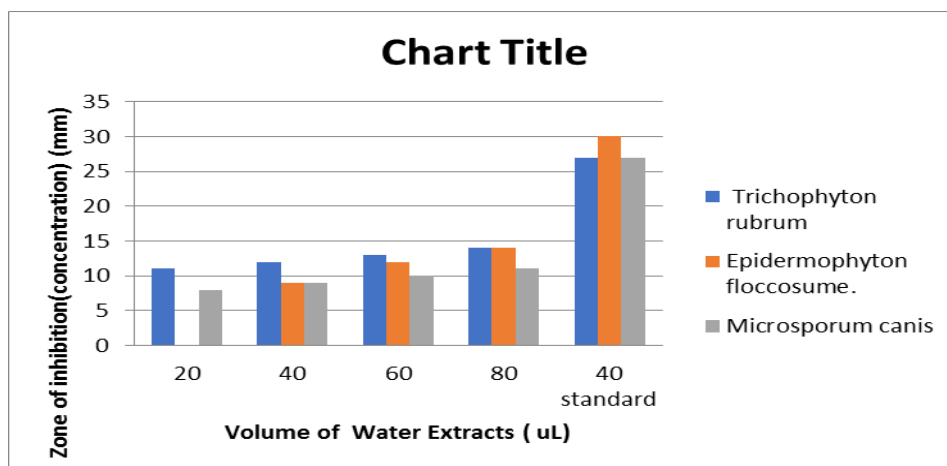
SL.NO.	Zone of Inhibition (mm)	Drug Sensitivity
1	No Inhibition/below 6	In Sensitive
2	6-9	Less Sensitive
3	9-12	Moderate Sensitive
4	Above 12	Highly Sensitive

### Zone of Inhibition of each Sample Extracts against Dermatophyton

#### ❖ Aqueous Extract - Observation

Table No: 2. Zone of inhibition for Aqueous extract of *Shinshapa*.

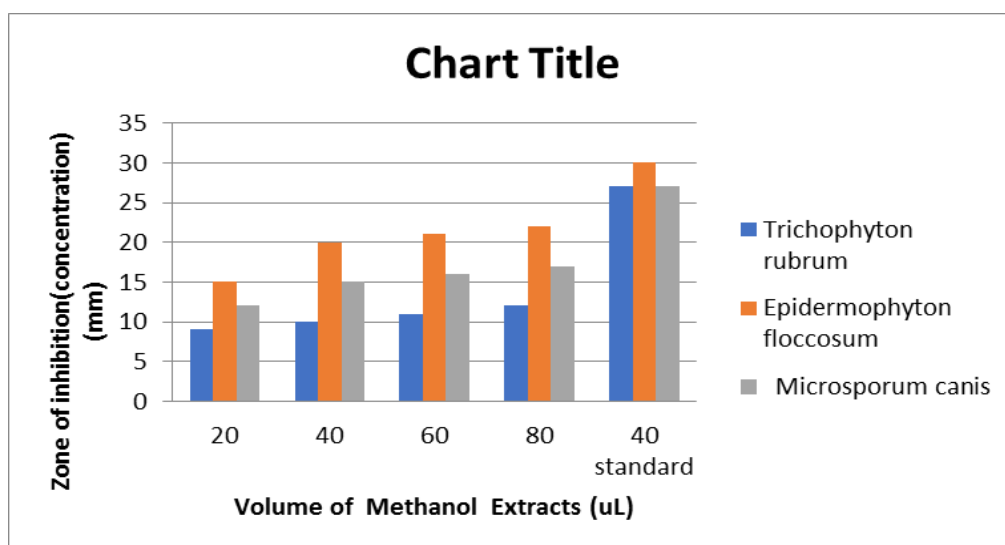
Sr.no.	Microbial Strains	Zone of inhibition (Increasing Volume)				
		20µl	40µl	60µl	80µl	40µl Standard
1	<i>Trichophyton rubrum</i>	11mm	12mm	13mm	14mm	27mm
2	<i>Epidermophyton floccosum</i>	-	9mm	12mm	14mm	30mm
3	<i>Microsporum canis</i>	8mm	9mm	10mm	11mm	27mm



#### ❖ Methanol Extract - Observations

Table No. 3 Showing of zone inhibition of methanol extract of *Dalbergia sissoo*.

S.N.	Microbial Strains	Zone of inhibition (increasing volume)				
		20µl	40µl	60µl	80µl	40µl Standard
1	<i>Trichophyton rubrum</i>	9mm	10mm	11mm	12mm	27mm
2	<i>Epidermophyton floccosum</i>	15mm	20mm	21mm	22mm	30mm
3	<i>Microsporum canis</i>	12mm	15mm	16mm	17mm	27mm



## DISCUSSION

Sensitivity of drug sample can be explained on the basis the following scale which was developed by Arora D. S. et al (1997).

**Table: Relation between zone of inhibition and drug sensitivity.**

S. No.	Zone of inhibition (in mm)	Drug sensitivity
1.	Below 6	Insensitive
2.	6 to <9	Less sensitivity
3.	9 to <12	Moderate sensitivity
4.	$\geq 12$	High sensitivity

It is clear that the antifungal component within the extract could successfully inhibit fungal growth. On the basis of above scale, we can explain-

### Aqueous Extract

- ❖ *Trichophyton rubrum* shows high sensitivity in 80  $\mu$ l (greater than; 12 mm inhibition zone).
- ❖ *Epidermophyton floccosum* shows high sensitivity (greater than; 12 mm inhibition Zone) in 80 $\mu$ l volume and in 20  $\mu$ l volume its show. Moderate sensitivity (greater than;9 mm inhibition Zone)
- ❖ *Microsporum canis* shown moderate sensitivity in 80  $\mu$ l (9 – 12 mm inhibition zone) and in 20  $\mu$ l it shows mild sensitivity (less than; 9mm Inhibition Zone).
- ❖ All the three Fungi shows slightly greater than 25% of antifungal activity on comparing with standard.

### Methanol Extract

- *Trichophyton rubrum* shows moderate sensitivity in 80  $\mu$ l (9 - 12 mm) inhibition zone.
- *Epidermophyton floccosum* shows high sensitivity in 80  $\mu$ l (greater than; 12 mm) inhibition Zone.
- *Microsporum canis* shows high sensitivity in 80  $\mu$ l (greater than; 12 mm) inhibition zone.
- ❖ On comparing with the standard *Epidermophyton floccosum* shows almost 50% of Antifungal activity whereas *Epidermophyton floccosum* and *Microsporum canis* shows almost 25% of Antifungal activity.
- ❖ Methanol extract is more sensitive in comparison of same dose of Aqueous extract against all three organisms.

Both extracts showed moderate and high sensitivity against all organisms. Methanol extract showed slightly higher potential than water extract in the same volume. There was also slight augmentation in sensitivity with increasing concentration of both extracts. The extracts have more potential against *Epidermophyton floccosum* and *Microsporum canis* besides rest of two organisms.

Many other researchers have also confirmed great antimicrobial activity of *Dalbergia sissoo* seeds and leaves against gram-positive as well as gram-negative bacteria, fungi and other pathogenic micro-organisms.<sup>[1]</sup>

Phytochemical compounds are known to play important role in bioactivity of medicinal plants and these help to produce definite physiological action on the human body.<sup>[2]</sup>

Recent scientific studies suggest that antifungal potential of *Dalbergia sissoo* is also due the presence of some phytochemicals. According to Singh et al., 2009 the alkaloids, dehydrocorydalmine and oxyberberine isolated from *Dalbergia sissoo*, were found to exhibit antifungal activities against some fungal strains.<sup>[3]</sup> In the view of one another study its alkaloids are mostly beneficial when it is used superficially; it shows active inhibitor against bacteria and fungus. It inhibits the bacterial cell wall permeability and leak out its cytoplasm from the cell and bacterial cell unable to survive.<sup>[4]</sup>

## CONCLUSIONS

In conclusion, experiments described in this article demonstrate that the *Dalbergia sissoo* extracts exhibit the antifungal potential. More experiments are required to elucidate molecules that have antifungal potential from *Dalbergia sissoo* leaves.

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