Evaluation of antifungal activity of
Salvadora persica Linn. leaves

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Abstract
Invasive fungal infections are significant causes of morbidity and mortality, particularly
in immuno-compromised patients. In vitro antifungal activity of dried leaf extract of Salvadora
persica Linn. was assessed against Aspergillus niger, A. flavus, A. xylinium and Candida
albicans by zone of inhibition method using Clotrimazole as a positive control. The leaf extract
was found active against all three species of Aspergillus but the extract did not show significant
activity against C. albicans.

Keywords: Salvadora persica, Tooth Brush Tree, Barapilu, Antifungal activity, Aspergillus
niger, Aspergillus flavus, Aspergillus xylinium and Candida albicans.

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Introduction
Higher incidence of fungal infection caused by various species of fungi has been reported, hence, research on
bioactive substances that may lead to the discovery of new compounds is required. There has been a dramatic increase in the
use of antifungal agents for the treatment of both systemic and localized fungal infection but the expanded use of
antifungal agents has accelerated the development of resistance to antifungal drugs followed by frequent therapeutic
failures and increasing mortality rate. The most important and dreadful species of fungi includes Aspergillus niger, A.
flavus, A. xylinium and Candida albicans. Despite advances in antifungal therapies, many problems related to drug
resistance and toxicity remains to be solved, for example, the toxicity of Amphotericin B and development of clinically resistance strains of various fungal species on continuous use of Azoles.

This situation highlights the need for advent of safe, novel and effective antifungal agents. The main objective of
the present study is to explore the antifungal potential of Salvadora persica Linn., commonly known as Tooth Brush Tree (Hindi — Barapilu) belonging to family Salvadoraceae. It is a native of Arabia, grown upon the sea
cost. In India it is found in western and southern parts especially throughout the tropics in open places on saline land and
often on black cotton soil. The leaves are elliptic-ovate or ovate-lanceolate, fleshy, 3.8-6.8 cm in length and 2.0-3.2 cm in
width; flowers greenish-white or greenish-yellow; fruit globose or round, smooth, red when ripe.

In traditional medicine the fruit is considered to be hot and digestive and beneficial in enlarged spleen, rheumatism
and tumours. The leaves are also used for external application in rheumatism, their juice is given in scurvy and shoots are
pungent and used as an antidote to poison. Their poultice is used for piles and tumours. The leaves are bitter, corrective and astringent to the bowels, tonic to the liver, diuretic, analgesic, anthelmintic and used to strengthen the
teeth. The leaves are also used in asthma and cough. Since the crude extract of
**S. persica** twigs and roots have already been reported to possess *in vitro* antimicrobial effects and leaves contain alkaloid salvadoricine (indole-2-acetyl-3-methyl), flavonoids; quercetin and ruffin are already reported, present study aims at evaluation of leaf extract for its antifungal activity.

**Materials and Methods**

**Preparation of ethanolic extract**

The leaves were collected from Alagar hills which is 15 km away from Madurai, Tamil Nadu in the month of November. About 700 g of dried leaves were obtained from fresh leaves weighing about 3kg. They were powdered and extracted with 50% hot ethanol using the Soxhlet apparatus for about 48 hours. After extraction, the ethanolic extract was filtered through Whatmann filter paper No. 1. The filtrate was dried in the vacuum distillation and then in dessicator.

**Antifungal activity**

Cup-Plate method was used for screening the antifungal activity of pure and dried 50% hot ethanolic leaf extract. A commercial sample of Clotrimazole was used as a standard and Sabourad’s dextrose (SDS) agar was used as culture medium. The petri dishes were filled to depth of 4.5mm with SDS medium and were placed on a level surface so as to ensure that the level of the medium was of uniform thickness. The petri dishes were sterilized at 160-170°C for 1 hour before use.

Small sterile borer of uniform size having an internal diameter of 6-8 mm and made up of stainless steel was placed at 10cm height. Four holes were made in the medium with the sterile borer. Two holes for the extract, one hole for positive control (Clotrimazole) and one for solvent control dimethyl sulfoxide (DMSO). Solutions of the standard and the extract being examined were prepared in four different concentrations (1mg/ml, 2mg/ml, 3mg/ml and 4mg/ml) using sterile DMSO as a solvent. For experiment 0.1ml of the solution of Clotrimazole, DMSO and leaves extract was filled with the help of 1.0 ml syringe. The zones of the inhibition were measured in diameter (cm) produced around the hole after incubation at 37°C for 24 hours.

**Results and Discussion**

The results of present study are shown in Table 1. The zone of inhibition of ethanolic leaf extract with four different concentrations were found to be comparable with that of standard drug in case of *A. niger*, *A. flavus*, and *A. xylinium*, reflecting the potency of extract against these pathogens, whereas diameter of zone of inhibition of *C. albicans* were found to be far less then standard drug. In the case of *A. niger*, when the concentration of the 3 mg/ml and 4 mg/ml were used, the diameter of zone of inhibition, was less in comparison to 1mg/ml and 2 mg/ml concentration used, but in the cases of *A. xylinium* and *A. flavus*, it was observed that increase in concentration inevitably

**Table 1: The comparison of zone of inhibition values at different concentrations of 50% ethanolic leaf extract of Salvadora persica with Clotrimazole as standard drug**

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Concentration (mg/ml)</th>
<th>Zone of inhibition (cm) with leaf extract</th>
<th>Zone of inhibition (cm) with Clotrimazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>1</td>
<td>2.00</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.52</td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.60</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.70</td>
<td>2.56</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>1</td>
<td>1.46</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.70</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.10</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.31</td>
<td>2.56</td>
</tr>
<tr>
<td><em>Aspergillus xylinium</em></td>
<td>1</td>
<td>1.46</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.66</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.00</td>
<td>2.10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.80</td>
<td>2.30</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>1</td>
<td>0.05</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.05</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.08</td>
<td>2.15</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.07</td>
<td>2.35</td>
</tr>
</tbody>
</table>
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increases the diameter of zone of inhibition.

The use of ethanol as a solvent for extraction and subsequent good antifungal activity exhibited by the leaf extract are in agreement to the previously reported work performed on stem of *S. persica* for antifungal activity.\(^{13}\)

Conclusion

Almost all the antifungal agents, in-use shown toxic side effects\(^{14}\) and are relatively expensive. In search of efficacious, less toxic and economical antifungal agent this study was performed and it was found that 50% ethanolic leaf extract of *S. persica* possesses good antifungal activity when compared with Clotrimazole. The future prospective includes the isolation and characterization of active constituents responsible for antimycotic activity of the plant.

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