



## Anti-fungal Activity of Neem Oil

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### ABSTRACT

The aim of the study is to determine the antifungal activity of neem oil. The objective of the study is to determine the antifungal activity of neem oil. Neem oil is a vegetable oil pressed from the fruits and seeds of neem (*Azadirachta indica*), an evergreen tree which is endemic to the Indian subcontinent. It is composed of triglycerides and contains many triterpenoid compounds, which are responsible for the bitter taste. Ayurvedic uses of neem include the treatment for acne, fever, leprosy, malaria, ophtalmia and tuberculosis. Various parts of neem tree show antifungal activities which can show effects on various fungal micro-organisms. The study may help in formulation of economical and new antifungal agents derived from neem oil.

**Keywords:** antifungal activity, neem oil, *Azadirachta indica*.

### INTRODUCTION

Infectious diseases are the major cause for health problems in developing countries like India. The resistance of micro organisms to various antibiotics has led to immense clinical problems for the treatment of various infectious diseases<sup>1</sup>.

This is due to the use of commercial antimicrobial drugs to treat infectious diseases<sup>2</sup>. There was an alarming increase of fungal infections which include different types of fungal pathogens<sup>3</sup>. People are aware of the importance of the fungal infection.

It was necessary for the preparation of oil from medicinal plants<sup>4</sup>. Therefore neem tree was selected.

Neem oil is a vegetable oil pressed from the fruits and seeds of the neem (*Azadirachta indica*), an evergreen tree which is endemic to the Indian subcontinent<sup>5</sup>.

It is composed of many triglycerides and contains many tetrapenoid compounds, which are responsible for the bitter taste<sup>6</sup>.

Ayurvedic uses of neem include the treatment of acne, fever, leprosy, malaria, ophtalmia and tuberculosis<sup>7</sup>. Various parts of neem tree show antifungal activity which can show effects on various fungal micro organisms, so this oil is extracted in a concentrated form<sup>8</sup>.

Therefore the aim of the study is to determine the effect of neem oil on different types of fungi.

### MATERIALS AND METHODS

**Materials:** fungal strains used were *Aspergillus niger*, *Aspergillus flavus* & *Trichoderma viride* were obtained from biozone research technologies Pvt. Ltd. Reagents are procured from himedia. The Samples were screened for antifungal activity against *Aspergillus niger*,

*Aspergillus flavus* & *Trichoderma viride* using agar well diffusion method<sup>9</sup>.

#### Preparation of Fungal Spore

Fungi are removed from the substrate surface using fine forceps and were broken and opened in sterilized water in order to provide a spore suspension<sup>10</sup>. The filamentous fungi were grown on Sabouraud dextrose agar (SDA) slants at 28°C for 10 days<sup>11</sup>.

The spores were collected using sterile double distilled water and stored in refrigerator<sup>12</sup>. A glass container is sterilized with ethanol and was sprayed on its surface. A sterilized pipette is used to transfer few drops of sterilized water into the glass slide. Alternatively it is pipetted on to the centre of the agar plate and is carefully shaken to spread the suspension. The prepared spores are checked every 24 hours to establish its germination. Once the spores have germinated, a small piece of spore containing that agar is isolated and examined via compound microscope for its quality.

#### Agar Well Diffusion Method

It is a method which shows the movement of the molecules through the matrix that is formed by the gelling of agar.

When this method is performed under controlled conditions, the degree of movement of the molecules can be related to the concentration of the molecule.

This phenomenon forms the basis of agar diffusion assay that is used to determine the susceptibility of the resistance of the fungal strain to an antifungal agent.

Antifungal activity was carried out using disc diffusion method. Petri plates were then prepared with 20 ml of sterile SDA (Hi- media, Mumbai). The test culture was



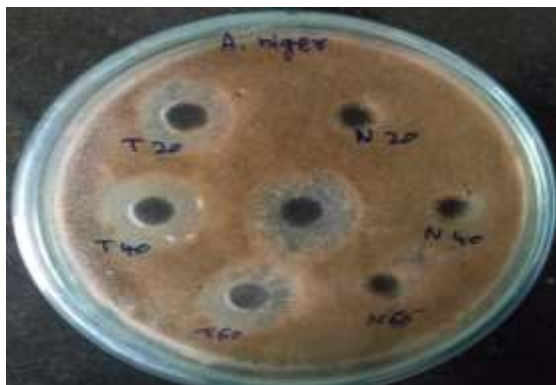
swabbed on the top of the solidified media and allowed to dry for 10 min<sup>13</sup>.

Wells were made on the media using a well borer. Different concentrations of the sample (20, 40 and 60 µL per well) were loaded on the wells. Ketakonazole (10 µg /well) was used as a positive control.

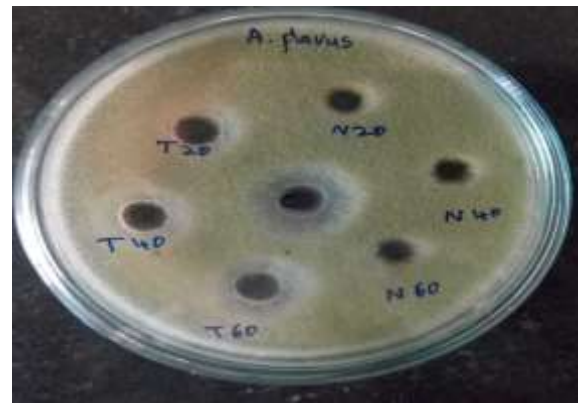
These plates were incubated for 48 hrs at 28 °C. Zone of inhibition was recorded in millimeters.

**RESULTS**

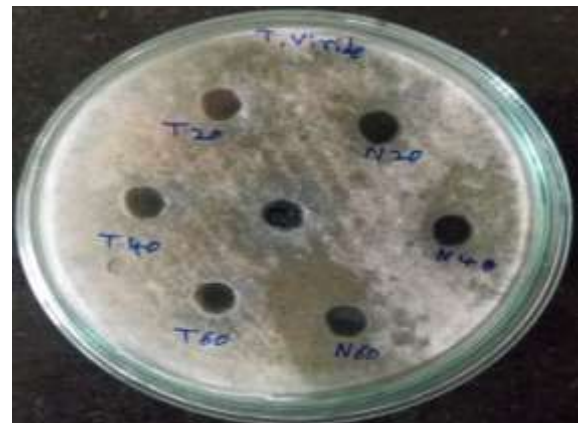
Neem oil showed significant antifungal activity against *Aspergillus niger* (Fig.1) and *Trichoderma viride* (Fig.3) at different concentrations such as 20muL and 40muL but it didn't showed any reaction against *Aspergillus flavus* (Fig.2). 60muL concentration of neem oil showed reactions against all the three fungi. The zone of inhibition is found to be more against *Aspergillus flavus*, when ketakonazole of 10muG was used (Table 1).



**Figure 1**



**Figure 2**



**Figure 3**

Figures show antifungal activity of samples by using well diffusion method

N- Neem Oil; C- Clove Oil; K – Ketakonazole

**Table 1: Antifungal Activity using Agar Well Diffusion Method**

Sample	Concentration (µL)	Zone of inhibition (in mm)		
		<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Trichoderma viride</i>
Neem oil	20	8	-	11
	40	11	-	14
	60	10	11	16
Ketakonazole	10 µg	17	19	15

**DISCUSSION**

The traditional use of plants as medicine provides the basis for indicating specific medical conditions. It is important to scientifically analyse these plants which have been used in traditional medicines.

The method that is used in extracting oil are Agar well Diffusion method. This study determines the antifungal activity of neem oil against various species of fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viride*. The essential oil caused a significant decrease in the activity of the above mentioned fungi and it causes

suppression in their growth at concentrations 20muL, 40muL, and 60muL. Ketakonazole was taken as a positive control in well diffusion method.

Petri plates were prepared with 20ml of sterile SDA.

Then the culture was layered down on the top of the solidified media and allowed to dry for 10min. These plates were then incubated for 48hrs at 28 degree Celsius and the zone of inhibition was identified and recorded. The neem oil shows effective reaction against the fungi at 60muL concentration. Whereas at 20muL and 40muL concentrations, it becomes less effective.

**CONCLUSION**

Today, most of the people are unaware of the uses of the medicinal plants like neem and in case of any fungal infections, they use allopathic medicines to treat it which may cause some side effects. Neem oil showed effective antifungal activity against different types of fungi. It has many medicinal properties such as healing abilities, antioxidant activity etc. Therefore People instead of using allopathy medicines can move in a natural way to heal fungal infections.

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