

Exploration of Antifungal Potential of *Azadirachta Indica* against *Microsporum Gypseum*

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Throughout ancient times, human civilizations have greatly exploited plants which have directly or indirectly served the humanity for diagnosis and treatment of various ailments. Herbal drugs have the provision to endanger multifarious range of phytochemical and biochemical compounds which can be acclimated for the performance of diverse biological functions. Many of these phytoconstituents and herbal products have salutary effects on long-term health when consumed by the human and can be efficaciously used to treat various human ailments. The present investigation was conducted to evaluate the antifungal potential of various extracts of dried powder of leaves of *Azadirachta indica* by means of paper disc diffusion method, with petroleum ether, ethyl acetate, ethanol, and aqueous solvents in 25 μ ml, 50 μ ml and 100 μ ml concentrations against *Microsporum gypseum*. Clotrimazole was used as a standard. The findings from the research revealed that *Azadirachta indica* is a potent antifungal agent against *Microsporum gypseum*. The ethanol extract of fenugreek using 100 μ ml concentrations depicted the highest zone of inhibition of 20.520 + 0.84 mm and 47.720% of mycelial inhibition against a tested pathogen. While drug extracts in other solvents also revealed reasonable to least antifungal potential. This finding tells us that *Azadirachta indica* extracts tested proved to be a potent antifungal agent against *Microsporum gypseum*. It was found that ethanol extract of *Azadirachta indica* is best effective against tested strain. This exploration of *Azadirachta indica* extracts has confirmed its importance, particularly in the area of influence on dermatophytic fungal strain.

Keywords: Antifungal, *Azadirachta Indica*, *Microsporum Gypseum*.

The previous investigations about *Azadirachta indica* have been not only restricted to its known antifeedant activity on insects, but also as potent antifungal drug¹. Fungi are a varied collection of saprophytic and parasitic eukaryotic microorganisms. Dermatophytoses is a skin disorder or skin infection which is caused by the fungi to the human skin. Superficial mycosis includes mild to chronic infections of

scalp, skin, nail, and hair. Cutaneous infective agents like *Trichophyton rubrum*, *Microsporum gypseum*, and *Epidermophyton floccosum* are vital reasons responsible for dermatophytoses. Dampness, unhygienic conditions and sweat are major causative factors for the emergence of dermatophytic infection. The resistance and side effects of the synthetic drugs are the major concern and motivated many researchers for



developing newer and also safer drugs of herbal origin^{2,3}. Roughly 25 % of the world population is infected from fungal infections of nails and skin, with a major prevalence in hot and humid areas⁴. There is progressive interest on the isolation of phytoconstituents with antifungal potential, from which terpenoids have revealed to produce either fungi static or fungicidal power on several harmful fungi.

Azadirachta indica was used as an air purifier and an ornamental tree in last century in arid zones of Africa and can be presently seen in many tropical Asian countries around the globe⁵. *Azadirachta indica* can be grown or cultivated in soil even with poor nutrition and also have capacity to survive in extreme temperatures of severe hot and even deep frost⁶. *Azadirachta indica* elaborates an immense array of phytoconstituents which are not only biologically active but are also chemically diverse and structurally variable with approximately 150 compounds isolated from different parts of the tree⁷. The leaves of *Azadirachta indica* constitutes 10-25% crude fibre, 45-50 % carbohydrates, 15-20 % crude protein, 2.5-7.0 % fat, 7.7-8.5% ash, 0.8-2.4% calcium, 0.13-0.24 % phosphorus⁸ with amino acids^{9, 10}. Different varieties of sugars, carbohydrates, and polysaccharides have also been isolated from the bark and gum of *Azadirachta indica*^{11, 12}. Carotenoids were also reported from a two-dimensional TLC profile of leaves extracts of neem¹³. Neem has multifarious pharmacological action on the human skin and soft tissue. It is clinically approved in many skin infections without any side effects.

In King George Medical College, Lucknow-India, *Azadirachta indica* lotion was formulated from extract of dried leaves and clinical trial was conducted, results were promising and beneficial in the treatment of ring worm and scabies. Extracts of Neem oil and some pure isolates (Nimbidin, Nimbidiol, and Nimbin) can also inhibit fungal growth on animals^{14, 15, 16} and humans¹⁷. Leaf extracts were shown good improvement within 15-20 days in chronic cases¹⁸. The present investigation aimed to compare the antifungal activity of various extracts of dried leaves of *Azadirachta indica* by means of paper disc diffusion method, with petroleum ether, ethyl acetate, ethanol, and aqueous solvents in 25

µml, 50 µml and 100 µml concentrations against *Microsporum gypseum*.

MATERIALS AND METHODS

Preparation of Plant Extract

Azadirachta indica leaves were collected from local areas of Chandigarh and Mohali. Leaves were dried in shade and kept safely in dry area. The dried *Azadirachta indica* leaves were grounded to form a powder with the help of a mechanical grinder. Herbarium sheet is submitted with the Pharmacognosy department Chandigarh college of Pharmacy- Landran Mohali with voucher no. CCP/AI/070. Extracts were prepared by extracting *Azadirachta indica* leaves powder successively with petroleum ether, ethyl acetate, ethanol, and aqueous solvents and tested against *Microsporum gypseum*. The prepared extract was weighed and stored in airtight sample bottles. The filtered extracts were tested against dermatophytes at three different concentrations viz. 25 µml, 50 µml and 100 µml.

Procedure and Procurement of Strain

The antifungal potential of an extract of dried *Azadirachta indica* leaves were evaluated by the Paper disc diffusion method. The test organisms used were the dermatophyte strains of *Microsporum gypseum*, which was procured from IMTECH, Chandigarh. MTCC No. was 2829. Sabouraud Dextrose agar was used as a culture media according to the manufacturer's direction. The dermatophyte cultures were aseptically inoculated on Sabouraud agar plate and subjected to incubation at 28°C for approximately 3 days.

Phytochemical-Screening

Test for alkaloids

The ethanolic extract of leaves of *Azadirachta indica* was evaporated to dryness and the residue was subjected to indirect heat on a boiling water-bath with 2% HCL. After cooling, the mixture was filtered and few drops of Meyer's reagent were added. Turbidity or yellow precipitation confirms the presence of alkaloids.

Test for glycosides

2-5 ml of extract of leaves of *Azadirachta indica*, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were taken in a test tube. 0.5 ml of concentrated H₂SO₄ was added towards the walls

Table 1. Mean Zone of Inhibition in different solvents (mm) of the *Azadirachta indica*

Crude Drug	Conc.	Pet Ether	Ethyl Acetate	Ethanol	Aqueous	Clotrimazole
<i>Azadirachta indica</i>	25 µml	7.699 ± 0.85	9.891 ± 0.86	13.211±0.65	4.565±1.20	43.00±0.20
<i>Azadirachta indica</i>	50 µml	12.210 ± 0.65	15.130 ± 0.86	17.100±0.75	10.560±0.50	43.00±0.20
<i>Azadirachta indica</i>	100 µml	14.510 ± 0.75	18.360 ± 0.72	20.520±0.84	12.210±0.47	43.00±0.20

Table 2. Percentage inhibition (%) of various extracts of the *Azadirachta indica*

Crude Drug	Conc.	Pet Ether	Ethyl Acetate	Ethanol	Aqueous	Clotrimazole
<i>Azadirachta indica</i>	25 µml	17.883	23.000	30.720	10.604	100
<i>Azadirachta indica</i>	50 µml	27.723	34.577	39.201	23.849	100
<i>Azadirachta indica</i>	100 µml	33.744	42.697	47.720	28.395	100

Table 3. Phytochemical components of different extracts
(+ low concentration, ++ moderate concentration, +++ high concentration)

Components	Test	Petroleum ether	Ethyl acetate	Ethanol	Aqueous
Alkaloids	Meyers Reagent	++	++	++	+
Saponins	Froth Formation	++	+	+	++
Tannins	Ferric chloride	++	++	++	++
Glycosides	Keller-killiani	+++	+++	+++	++
Flavonoids	Pews Reagent	++	++	++	+
Reducing Sugars	Fehling Reagent	++	++	++	+

of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of cardiac glycosides.

Test for flavonoids

4 mg of extract of leaves of *Azadirachta indica* was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was integrated and orange or red colour was observed for presence of flavonoids.

Test for reducing sugars

To 0.5 ml of extract solution, 1 ml of di hydrogen monoxide and 5-8 drops of Fehling's solution was integrated at boiling and observed for brick red precipitate.

Test for saponins

5 ml of extract was shaken vigorously with 5 ml of distilled water in a test tube and heated. The formation of stable foam was accepted as an indication of the presence of saponins.

Antifungal Activity

In this research, Paper disc diffusion

method was employed, and some amount of Sabouraud Dextrose agar was dispersed in Petri dishes, which were allowed to solidify. A micropipette was employed to introduce 0.1 ml. Spores on agar medium and was spread with the help of glass rod spreader under aseptic conditions. Sterilized discs (5 mm, Whatman No. 1 filter paper) were prepared by soaking in different concentrations of the extract 25 µml, 50 µml and 100 µml for approximately 5- 6 hour. After this duration, discs were removed and then allowed to dry. To evaluate the antifungal potential of dried leaf extracts of neem, various discs impregnated with different concentrations of the dried neem leaves extracts were positioned on the fungal spore or mycelium with the help of sterilized forceps. The Petri dishes incubated at 28 °C for 72 hours. The antifungal potential was determined by measuring the zone of inhibition (ZOI) around the discs and percentage inhibition after the period of incubation¹⁹.

Data Analysis

Data from antifungal screening was

analyzed with the help of simple statistics from Microsoft Excel and recorded in appropriate tables as a mean standard deviation of the mean.

RESULTS AND DISCUSSIONS

Antifungal potential of extracts of leaves of *Azadirachta indica* against the tested fungal strain *Microsporum gypseum* can be seen in Table 1. The Pet ether extract of *Azadirachta indica* showed 7.69 mm ZOI at 25 µml concentration. 50 µml concentrations were moderately effective with 12.21 mm zone of inhibition. At 100 µml, the zone of inhibition was observed to be as 14.51 mm. The ethyl acetate extract showed a 9.89 mm inhibition zone at 25 µml concentration. 50 µml concentrations were effective with 15.13 mm inhibition zone. 18.36 mm inhibition zone was observed at 100 µml. The ethanol extract showed a 13.21 mm inhibition zone at 25 µml concentration. 50 µml concentrations were moderately effective with 17.10 mm inhibition zone. At 100 µml, the inhibition zone was observed to be as 20.52 mm. While it's aqueous extract showed a 4.56 mm inhibition zone at 25 µml concentration. 50 µml concentrations were effective with a 10.56 mm inhibition zone. 12.10 mm inhibition zone was observed at 100 µml concentration. The antifungal potential was determined by comparing the activity of extracts with the Clotrimazole, in which the zone of inhibition was 43mm. Percentage inhibition was also calculated, which was 47.72 % with 100 µml ethanol extract depicted in Table 2. The results of this study showed that of all the extracts screened, *Azadirachta indica* ethanol extract had higher inhibitory activity against the test organism in comparison to the standard drug. This could be as a result of better extraction with ethanol solvent. Other solvents also showed promising effects against the tested pathogen. As per results from previous studies, it was reported that topical application of a lotion prepared from 70% alcoholic extract of neem leaves was found to be effectual in chronic skin infections like ringworm infection, scabies and also eczema¹⁸. Antidermatophytic potential of neem leaf extract has also been reported against different species of dermatophytes including *Trichophyton rubrum*, *Microsporum gypseum* and *Epidermophyton floccosum*²⁰. Different extracts of neem leaf are

effective against variety of fungal strains, like *Trichophyton rubrum*, *Microsporum gypseum*, and *Epidermophyton floccosum*¹⁷. Aqueous extract of leaves of *Azadirachta indica* showed minimal percentage of inhibition against *Candida albicans* and *Microsporum gypseum*²¹. On other hand, as per results of my research the ethanol extract is showing a high zone of inhibition. Standardization is a significant consideration to make sure of the relevance between the phytoconstituents and its multifarious pharmacological activities. The phytochemical tests performed, and results were recorded in Table 3.

CONCLUSION

The present research confirmed the antifungal potential of crude extracts of *Azadirachta indica* leaves against *Microsporum gypseum*. The extent of antifungal potential varied depending on the polarity of the solvent utilized in the extraction process. From the study, it can be depicted that ethanol extract of neem leaves are promising in comparisons to other solvents used. Furthermore, quantitative phytochemical analysis can be conducted in the future to isolate and identify the phytoconstituents liable for the antifungal potential.

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