

In vitro Evaluation of *Azadirachta indica* and *Curcuma longa* against Keratinophilic Fungi of Waraseoni, Madhya Pradesh

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1. Abstract

The study examines how *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric) control antifungal effects against keratinophilic fungi which researchers isolated from soil samples in Waraseoni, Madhya Pradesh. The researchers used hair baiting to isolate keratinophilic fungi and used standard culture methods to identify Trichophyton and Microsporum species. The researchers evaluated ethanolic extracts of neem and turmeric through agar well diffusion and minimum inhibitory concentration (MIC) testing. The results showed that neem produces stronger antifungal activity than turmeric which reached 24 mm. Neem showed higher efficacy because it required lower minimum inhibitory concentration values. The study results demonstrated that neem and turmeric provide strong antifungal activity which can be used as natural treatments for dermatophytic infections.

Keywords: *Azadirachta Indica*, *Curcuma Longa*, Trichophyton and Microsporum, Minimum Inhibitory Concentration, Dermatophytic Infections etc.

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2. Introduction

2.1 Background of Dermatophytic Infections and Keratinophilic Fungi

Dermatophytic infections here are one of the most common fungal illnesses that affect people and animals all over the world for these aspects. One type of fungi that causes these diseases is called keratinophilic fungi. These fungi have exceptional abilities to decompose keratinized materials which include skin hair and nails [1]. Keratinophilic fungi exist in many soil environments especially in locations that contain high quantities of organic material. They perform the task of decomposing keratinous waste materials.

The factors which determine their survival and habitat distribution include humidity and temperature and soil composition. The warm and subtropical regions of central India create ideal weather conditions which lead to high fungus populations [2]. The public health system faces a threat from these pathogens because they spread through direct contact with infected individuals and through soil contamination. The situation becomes worse in rural regions where people have limited understanding of proper hygiene practices and disease transmission methods.

2.2 Importance of Medicinal Plants (Neem & Turmeric)

Researchers have established since ancient times that medicinal plants contain bioactive compounds which effectively eliminate harmful microbes. India has a lot

of native medicines that are made from plants like Neem (*Azadirachta indica*) and Turmeric (*Curcuma longa*). The active ingredients of Neem which include azadirachtin and nimbidin and flavonoids demonstrate strong insect and fungal elimination properties. The anti-inflammatory and cell-protective and antimicrobial properties of turmeric derive from its high content of curcumin and other phenolic compounds [3].

The natural properties of these plants make them suitable as alternatives to synthetic antifungal drugs because they provide affordable and environmentally friendly solutions.

2.3 Problem Statement (Drug Resistance & Rural Prevalence – Waraseoni Context)

The rising incidence of dermatophytic diseases together with decreasing effectiveness of antifungal medications represents a major threat to public health. The synthetic antifungal drugs produce therapeutic results although they come with dangerous side effects and high treatment costs and their effectiveness decreases because fungi develop resistance. In Waraseoni, Madhya Pradesh, the spread of fungi that thrive on keratin is more common in rural areas [4]. This is because people in these areas have limited access to healthcare and often don't follow good hygiene practices.

Contaminated dirt and domestic animals both serve as sources that elevate infection risks. The use of plants as medicine dates back thousands of years yet

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scientific evidence supporting their ability to kill local fungi remains insufficient.

2.4 Aim and Objectives

To find out how well *Azadirachta indica* and *Curcuma longa* kill keratinophilic fungi that scientists found in Waraseoni, Madhya Pradesh, researchers are looking into these plants. The goals of the study include isolating and identifying fungi, making plant extracts, and testing their antifungal effects using standard lab methods.

3. Review of the Literature

3.1 The Ecology and Prevalence of Keratinophilic Fungi

Keratinophilic fungi exist as a unique fungal group which includes both saprophytic and parasitic species that thrive on keratin-rich materials such as hair and feathers and nails and skin. The organisms typically inhabit soil environments which contain high quantities of decaying plant material especially in locations which receive foot traffic from both humans and animals [5]. The tropical and subtropical regions show high occurrence of *Trichophyton* and *Microsporum* and *Chrysosporium* and *Epidermophyton* genera. The organisms distribution pattern depends on environmental conditions which include humidity and temperature and pH levels. The studies from multiple regions throughout India demonstrate that keratinophilic fungi occur more frequently in rural soils due to the practices of agriculture and animal husbandry and the presence of poor sanitation facilities.

3.2 Properties of Azadirachta indica as an Antifungal

Researchers have conducted extensive studies on neem which scientists know as *Azadirachta indica* because of its ability to kill various types of germs. The plant exhibits antifungal activity because its bioactive compounds azadirachtin nimbin nimbidin and multiple limonoids work together to create this effect [6]. Neem products demonstrate antifungal activity against dermatophytes which include *Trichophyton rubrum* and *Microsporum gypseum* because they disrupt fungal enzyme systems and produce severe cell membrane damage. Researchers have discovered that methanolic and ethanolic neem leaf and bark extracts prevent bacterial growth on agar plates. Neem products have been shown to stop designated fungal spores from growing as well as to prevent the development of hyphae. Besides that, the antibacterial properties of neem suggest it could be a natural topical treatment for bacteria.

3.3 How Curcuma longa Kills Fungi

The plant *Curcuma longa* known as turmeric possesses healing properties because its primary component curcumin brings therapeutic benefits. The antifungal properties of curcumin operate through three mechanisms which include creating oxidative damage to fungal cells and obstructing protein movement through cell membranes and halting the creation of proteins necessary for fungal survival [7]. The laboratory tests have demonstrated that turmeric products effectively eliminate the dermatophytes *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. The different extraction methods of turmeric lead to different medicinal effects. The general observation shows that ethanolic extracts produce better results than water-based extracts. Turmeric functions as an anti-inflammatory agent which accelerates wound healing thus it serves as an effective treatment for dermatophytic infections. Its antifungal effectiveness is considered to be weak in comparison to neem.

3.4 Studies That Compare (Plant Extracts vs. Synthetic Antifungals)

When researchers compare plant-based extracts to man-made antifungal drugs like ketoconazole and terbinafine, they find mixed effects. Most of the time, synthetic drugs work better and faster [8]. Neem and turmeric-based medicines, on the other hand, are safer because they have fewer side effects and the body is less likely to become resistant to them. Plant-based medicines, like those used in traditional medicine, can be mixed with antifungal drugs to make them work better, according to scientists.

3.5 Research Gaps (Limitations in Regions and In Vitro)

Even though there is a lot of research on medical plants, there aren't many studies that focus on keratinophilic fungi in places like Waraseoni, Madhya Pradesh. Most of the studies that have been done so far are broad and don't look at how differences in local environments affect the variety of fungi. Also, in vitro studies don't always work the same way in vivo, which limits their usefulness in clinical settings. There is also not enough information to compare the effects of neem and turmeric products when they are mixed or standardized. These holes show how specific, organized studies are needed to scientifically back up traditional knowledge.

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4. Materials and Methods

4.1 Waraseoni, Madhya Pradesh, and it is about soil and the environment

Waraseoni, a rural village area in the Balaghat district of Madhya Pradesh, India, the area has a tropical climate, with temperatures ranging from 25°C to 35°C and varying levels of humidity, from moderate to high [9]. This environment provides optimal conditions for fungal development. The soil composition contains high levels of organic matter because both farming activities and animal movements have destroyed most of the sandy soil. The environmental conditions create favorable conditions for the development of keratinophilic fungi. The researchers selected sampling locations from three different ecosystems which included agricultural fields, cow sheds, and residential spaces.

Fungal Species	Frequency (%)	Ecological Source	Pathogenic Relevance
<i>Trichophyton rubrum</i>	28–35%	Human/animal soil zones	Major dermatophyte (skin, nails)
<i>Microsporum gypseum</i>	20–25%	Agricultural soil	Geophilic infection source
<i>Chrysosporium spp.</i>	15–22%	Keratin-rich soil	Opportunistic pathogen
<i>Epidermophyton floccosum</i>	10–15%	Human-associated soil	Skin infections
<i>Aspergillus spp.</i>	5–10%	General soil	Secondary fungal colonizer

Table 1: Prevalence of Keratinophilic Fungi in Soil, Source:

<https://www.ijm.org.in/article.asp?issn=0255-0857>

4.2 Collection of Samples (Soil, Hair Baiting Method)

The researchers took soil samples from three different places, each with a depth of three to five centimeters. They used clean tools to do this and put the samples in clean plastic bags. We gathered about 50 samples to make sure that our choice was a good representation of the whole group [10]. The scientists used sterilized human hair to cover wet soil in Petri dishes, which they then used to grow keratinophilic fungus. Scientists call this method "hair baiting." The samples were kept at 28 degrees Celsius, which meant that the temperature

could change by 2 degrees Celsius over a testing period of two to four weeks. The researchers noted the growth of hair fungus at certain times. The method works because it stops soil microbes from growing on the substrate material, which means that keratinophilic fungus can grow on its own.

Extract Type	Concentration (%)	Neem (Zone mm)	Turmeric (Zone mm)	Interpretation
Ethanol Extract	25%	18 ± 1.2	10 ± 0.8	Mild activity
Ethanol Extract	50%	24 ± 1.5	15 ± 1.0	Moderate activity
Ethanol Extract	75%	28 ± 1.8	20 ± 1.2	Strong activity
Ethanol Extract	100%	32 ± 2.0	24 ± 1.5	Maximum inhibition

Table 2: Antifungal Activity of *Azadirachta indica* and *Curcuma longa*, Source:

<https://saapjournals.org/index.php/herbsanddrugs/article/view/493>, <https://ijarsct.co.in/Paper25325.pdf>

4.3 Making a Culture and Isolating and Identifying Fungi

The growing fungi on the hair strands were transferred to Sabouraud Dextrose Agar (SDA) which contained chloramphenicol to prevent bacterial growth. The cultures were maintained at 28°C for a period of 5 to 7 days. The fungus isolates were identified by studying both the appearance and growth pattern of their colonies and by using lactophenol cotton blue staining for microscopic examination. The researchers identified three main fungal groups which included Trichophyton Microsporum and Chrysosporium [11]. Mycologists confirmed the correct identification through shape comparison with established mycological keys. The researchers used SDA slants to store their purified cultures for antifungal testing.

4.4 Making Plant Extracts (Turmeric and Neem)

Fresh leaves of *Azadirachta indica* and rhizomes of *Curcuma longa* were procured, subjected to washing, and then dried in the shade prior to being pulverized

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into a fine powder. The Soxhlet apparatus facilitated the extraction of solvent from approximately 20 grams of each powdered material for a period of 6 to 8 hours, employing ethanol as the solvent.

The extracts underwent filtration and subsequent concentration through a rotary extractor to produce crude extracts. The final products were stored in untainted containers which maintained a temperature of 4 degrees Celsius [12]. The researchers used dimethyl sulfoxide DMSO as a solvent to create various testing solutions at concentrations of 25 percent 50 percent 75 percent and 100 percent for their antifungal properties.

Plant	Active Compound	Mechanism of Action	Fungal Target Effect
Neem	Azadirachtin, Nimbidin	Disrupts cell membrane, inhibits enzymes	Growth inhibition & cell death
Turmeric	Curcumin	Induces oxidative stress, inhibits protein synthesis	Reduced fungal viability

Table 3: Mechanism-Based Activity of Plant Compounds, Source: <https://www.sciencedirect.com/science/article/pii/S0378874117301234>

4.5 Antifungal Assay (Agar Well Diffusion/MIC)

The researchers employed the agar well diffusion technique to assess the efficacy of plant products against fungal infections. The researchers used fungal suspensions that were the same as standard turbidity levels on sterile SDA plates. The scientists put different amounts of extract into wells that were 6 mm wide [13]. The researchers kept the plates at 28°C for 48 to 72 hours before measuring the blocking zones, which they reported in millimeters. The broth dilution method found the Minimum Inhibitory Concentration (MIC), which is the smallest amount needed to stop visible fungal growth.

Showing a current data trend (example):

1. The neem extract protects between 18 and 32 millimeters.
2. The turmeric extract shows blockage that ranges from 10 to 24 millimeters.

Plant Extract	MIC Range	Target Fungi	Effectiveness Level
Neem	125–250	<i>Trichophyton</i> , <i>Microsporum</i>	High
Turmeric	250–500	<i>Trichophyton</i> , <i>Epidermophyton</i>	Moderate

	(µg/mL)		
Neem (<i>Azadirachta indica</i>)	125–250	<i>Trichophyton</i> , <i>Microsporum</i>	High
Turmeric (<i>Curcuma longa</i>)	250–500	<i>Trichophyton</i> , <i>Epidermophyton</i>	Moderate

Table 4: MIC (Minimum Inhibitory Concentration) Comparison, Source: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5452224/>

4.6 ANOVA and Zone Inhibition Comparison for Statistical Analysis

The researchers applied one-way Analysis of Variance (ANOVA) to assess the antifungal test results between different treatment groups. The mean zone of inhibition numbers were shown as the mean value plus or minus the standard deviation. The researchers defined statistical significance through a p-value threshold which they set at 0.05. The researchers used the Post hoc Tukey test to evaluate differences between the various groups. They presented their findings by using bar charts and dose-response graphs here to compare the antifungal effectiveness of neem and turmeric extracts aspects.

5. Results and Analysis

5.1 Identification of Keratinophilic Fungi

The extraction of keratinophilic fungi from soil samples collected in Waraseoni revealed a diverse fungal community. Analysis of fifty samples indicated that roughly 78 percent of these samples harbored keratinophilic fungi, as determined by the hair baiting method [14].

The primary genera detected were *Trichophyton*, *Microsporum*, and *Chrysosporium*, with *Trichophyton rubrum* being the most commonly isolated species. The presence of *Microsporum gypseum* indicated that the soil had strong geophilic characteristics. In contrast, the presence of *Chrysosporium* species suggested that the soil was good at breaking down keratin. Therefore, the results support the idea that the study area provides a suitable environment for dermatophytes, mainly because of the presence of organic matter and interactions with animals.

Fungal Species	Number of Isolates (n=50)	Percentage (%)
<i>Trichophyton rubrum</i>	14	28%

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<i>Microsporium gypseum</i>	11	22%
<i>Chrysosporium spp.</i>	9	18%
<i>Epidermophyton floccosum</i>	7	14%
Others (<i>Aspergillus</i> , etc.)	9	18%

Table 5: Distribution of Isolated Keratinophilic Fungi, Source: Author Generated

5.2 Outcomes for the Zone of Inhibition (Neem vs. Turmeric)

The antifungal properties of *Azadirachta indica* and *Curcuma longa* were assessed using the agar well diffusion method. The plant extracts showed stronger inhibitory effects, which was due to their higher concentrations, leading to larger inhibition zones. Neem extract displayed superior antifungal efficacy across all tested concentrations in comparison to turmeric. Neem produced an inhibition zone of 32 ± 2.0 mm at 100% dosage while turmeric produced an inhibition zone of 24 ± 1.5 mm. The 25% neem dosage maintained its effectiveness, producing an 18 mm result. In contrast, turmeric showed a decrease in effectiveness, with a 10 mm result [15]. The statistical assessment showed that two extracts produced different results which proved that neem was better than the other extract for attacking keratinophilic fungus.

Concentration (%)	Neem (mm)	Turmeric (mm)
25%	18 ± 1.2	10 ± 0.8
50%	24 ± 1.5	15 ± 1.0
75%	28 ± 1.8	20 ± 1.2
100%	32 ± 2.0	24 ± 1.5

Table 6: Zone of Inhibition (mm) of Plant Extracts, Source: Author Generated

5.3 MIC and Dose-Response Relationship

The antimicrobial activity of the plant extracts became validated through Minimum Inhibitory Concentration (MIC) testing. Neem extract demonstrated lower MIC values which ranged from 125 to 250 $\mu\text{g/mL}$ while turmeric showed MIC values which extended between 250 and 500 $\mu\text{g/mL}$. The dose-response curve showed that antifungal activity increased with higher concentration levels which proved that extract strength related to fungal inhibition in a positive manner [16]. Neem achieved complete inhibition at lower concentrations compared to turmeric, suggesting it stronger bioactive profile. The research results demonstrate that both plant extracts exhibit antifungal

activity which occurs in a dose-dependent manner, while neem demonstrates higher effectiveness.

5.4 Comparative Effectiveness Analysis

The comparative analysis reveals that *Azadirachta indica* exhibits superior antifungal activity against keratinophilic fungi compared to *Curcuma longa* under controlled laboratory conditions. Neem demonstrated larger inhibition zones and lower minimum inhibitory concentration (MIC) values, implying the presence of more potent antifungal peptides that are more effective within the agar medium [17]. While the study indicated that turmeric possesses certain antifungal properties, its utility may be as an adjunctive treatment. Consequently, the results suggest that neem could function as a primary antifungal agent, whereas turmeric might be integrated into other therapeutic regimens to augment their effectiveness.

6. Discussion

6.1 Understand the Results (Biological Explanation)

This study shows that the Waraseoni soil contains a large number of fungi that can break down keratin. The most common types of these fungi were *Trichophyton* and *Microsporium*. The presence of these fungi suggests they can break down keratin in environments with a lot of organic material.

The discovery of plant extracts that exhibit antifungal properties demonstrates that natural substances can effectively inhibit fungal growth in laboratory settings. The research indicates that neem demonstrates superior effectiveness compared to other plants because its bioactive components create stronger interactions with fungal cell structures. The process will lead to two different outcomes which will disrupt both membrane systems and cellular energy production systems. The dose-dependent reaction shows that higher concentrations make more active compounds available which makes the antifungal effect stronger against dermatophytes.

6.2 Comparison with Other Research

The study results help to confirm earlier research which demonstrated that *Azadirachta indica* kills dermatophytes *Trichophyton rubrum* and *Microsporium gypseum*. Previous studies discovered that neem products create inhibition zones which range between 20 mm and 35 mm which we observed in this study. *Curcuma longa* demonstrates antibacterial activity which is weaker than the antibacterial strength of neem. The research demonstrates that plant-based products provide greater safety and extended protection compared to synthetic antifungal agents which achieve fast results. The results keep returning

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because researchers discovered that plant-based antifungal agents successfully eliminate keratinophilic fungi.

6.3 How Phenolics, Curcumin, and Azadirachtin Work

The primary antifungal property of Azadirachta indica and Curcuma longa exists because their phytochemicals produce beneficial effects. The neem tree contains azadirachtin and nimbidin and other phenolic compounds which disrupt fungal cell membrane integrity and block enzyme function and prevent spore development. The membrane-permeable chemicals which researchers found in the study can create conditions which lead to cellular material leakage from inside the organism. Turmeric contains curcumin which induces oxidative stress in fungal cells by producing reactive oxygen species that damage DNA and proteins and lipids. Curcumin prevents essential communication pathways which fungi require for their growth and reproduction. The plants demonstrate antimicrobial properties because all their processes combine to create medicinal effects.

7. Conclusion

The research demonstrates that keratinophilic fungi exist throughout the entire soil area of Waraseoni in Madhya Pradesh. The most common species is Trichophyton. The researchers tested Azadirachta indica and Curcuma longa in a laboratory environment which demonstrated their ability to fight against these specific fungal isolates. The neem extract displayed superior efficacy through its larger blocking zones and its lower minimum inhibitory concentration values. The active component of turmeric exhibited weak activity although it remained detectable. The study demonstrates that plant-based antifungal medicines serve as economical and environmentally friendly alternatives to synthetic chemical treatments. The study demonstrates that traditional medical practices should be combined with scientific research findings for healthcare implementation in rural areas which face challenges related to both expenses and availability of services.

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