



In Vitro Evaluation of the Anti-Dermatophytic Activity of Medicinal Plant-Derived Products

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Abstract

Dermatophytes are a group of closely related fungi which are able to invade the keratinized tissue skin, hair and nail. In this study different medicinal plants like *Melaleuca alternifolia*, *Zingiber officinale*, *Allium sativum*, *Azadirachta indica*, *Citrus limonum*, *Curcuma longa*, were used as antifungal agent against different dermatophyte species. All dermatophyte species were found sensitive for *Melaleuca alternifolia*, *Zingiber officinale* and *Allium sativum*. These medicinal plants showed very good results as antifungal against dermatophytes while *Azadirachta indica* and *Citrus limonum* were moderate sensitive and *Curcuma longa* did not show any zone of inhibition around the well. This study provides a scientific validation for the use of these medicinal plants in the treatment of dermatophytic infection and could be used in future for dermatophytic infection and other skin infection.

Keywords

Medicinal plants, Dermophytes, Keratinized tissue, *Azadirachta*, *Citrus*

1. Introduction

Dermatophytosis is one of the most common superficial fungal infections in India. It is caused by dermatophytes, a group of keratinophilic fungi that invade keratinized tissues such as the skin, hair, and nails, resulting in infections commonly known as ringworm or tinea. Based on their taxonomy, dermatophytes are classified into three genera: *Trichophyton*, *Microsporum*, and *Epidermophyton*. They can also be categorized according to their natural habitat into anthropophilic, zoophilic, and geophilic species. Anthropophilic dermatophytes primarily infect humans and are transmitted through person-to-person contact. Zoophilic dermatophytes mainly infect animals but can be transmitted to humans through direct or indirect contact with infected animals. Geophilic dermatophytes are naturally found in soil and occasionally infect humans following exposure to contaminated soil.

Clinically dermatophyte infections have been classified into *Tinea capitis*, *Tinea faciei*, *Tinea barbae*, *Tinea corporis*, *Tinea manuum*, *Tinea cruris*, *Tinea pedis*, and *Tinea unguium*. Other clinical variants are *Tinea imbricata*, *Tinea pseudoimbricata*, and *Majocchi granuloma* (Narain and Kumar, 2016). Hot and

humid weather, working conditions of people and other predisposing factor play important role to increase the infection but development of resistant strains to a range of antifungal drugs is also an important factor to increase the prevalence of infection (Mousavi and Kazemi, 2015). From last many decades many antifungal drugs were used to treat the dermatophytosis like griseofulvin used from last six decades and imidazole and ketoconazole were used from last four decades. Many other antifungal drugs like terbinafine, fluconazole, itraconazole etc. are also popular in clinical practice (Dabas et al., 2017). These antifungal agents are effective for treatment of dermatophytosis but also have many side effects for the patients. These drugs need longer periods of treatment for cure and with time some dermatophyte species acquire resistance from these drugs, therefore there is need to search new alternatives which have anti-dermatophyte activity and not harmful for human health and are also cost effective. Different traditional medicinal plants are used from centuries in many parts of the world for the treatment of dermatophytes (Sharma and Malik, 2015). Numerous studies have demonstrated that medicinal plants possess significant therapeutic potential in the treatment of various skin diseases, including dermatophytic infections. Plant-derived antifungal agents are considered promising alternatives to conventional antifungal drugs because they are widely available, cost-effective, environmentally friendly, and generally associated with fewer adverse effects. However, the increasing incidence of fungal infections and the emergence of resistance to currently available antifungal drugs have created an urgent need for the development of novel antifungal compounds with diverse chemical structures and unique mechanisms of action. Therefore, continued investigation of medicinal plants and their bioactive constituents is essential to identify safe and effective antifungal agents. The present study aims to evaluate the anti-dermatophytic activity of selected medicinal plants, including *Melaleuca alternifolia*, *Zingiber officinale*, *Allium sativum*, *Azadirachta indica*, *Citrus limon*, and *Curcuma longa*, against dermatophyte species.

2. Methods

In the present study, the antifungal activity of selected medicinal plant extracts was evaluated against dermatophytes using the agar well diffusion method. The medicinal plants used included *Melaleuca alternifolia* (tea tree oil), *Azadirachta indica* (neem oil), *Curcuma longa* (turmeric), *Allium sativum* (garlic), *Zingiber officinale* (ginger), and *Citrus limon* (lemon oil). Fresh rhizomes of turmeric and ginger, along with garlic bulbs, were purchased from a local market. The plant materials were thoroughly washed with sterile Milli-Q water to remove adhering soil and debris. The outer skin was removed, and the rhizomes were peeled and sliced before extraction. Fresh aqueous extracts of garlic, ginger, and turmeric were prepared immediately before use. The aqueous extract of *Allium sativum* was prepared by removing the papery skin from fresh garlic cloves, washing them with sterile Milli-Q water, and crushing them into a fine paste using a sterile ceramic mortar and pestle. The paste was mixed with 10 mL of sterile Milli-Q water and filtered through sterile filter paper. The resulting filtrate was collected in a sterile container and used immediately for antifungal susceptibility testing. Similarly, the aqueous extract of *Zingiber officinale* was prepared by washing and peeling the fresh rhizomes, crushing them into a paste using a sterile mortar and pestle, mixing the paste with 10 mL of sterile Milli-Q water, and filtering the suspension. The filtrate obtained was used for antifungal evaluation against dermatophytes.

The aqueous extract of *Curcuma longa* was prepared following the same procedure. Fresh turmeric rhizomes were washed, peeled, crushed into a paste, mixed with 10 mL of sterile Milli-Q water, and filtered. The freshly prepared filtrate was used for antifungal susceptibility testing. Fresh aqueous extracts of garlic, ginger, and turmeric were prepared for each experiment to ensure maximum bioactivity.

Procedure of antifungal susceptibility test: The isolates from Sabouraud dextrose agar (SDA) plates were sub cultured in 100 ml Sabouraud dextrose broth to enhance sporulation for 5 days at room

temperature. After fungal growth, the suspensions were mixed on spinner and then allowed to sediment for 30 minutes. With a pipette 100 μ l clear culture suspensions were taken from the side of the tube and were streaked evenly over the surface of Petri dishes containing Sabouraud dextrose agar. Lids were left open for 2-3 minutes in a laminar air flow to allow the excess moisture absorbed into the agar before antifungal mixture was applied. After that wells of 5 mm in diameter, 4 mm deep and 2 cm apart were punched in with a sterile cork-borer. Then 100 μ l of medicinal plants extracts were loaded into each well in sterilize conditions and plates were kept at 4°C for 30 min for diffusion of supernatant into agar. The plates were incubated at 25°C for 5-10 days. The zone of inhibition around the wells were measured in mm and noted.

3. Results

Table 1: In-vitro activities of medicinal plants antifungal agents against 5 dermatophyte species.

S.No.	Common name	Scientific name	<i>T. rubrum</i>	<i>T. mentagrophytes</i>	<i>M.gypesum</i>	<i>M. canis</i>	<i>E. floccosum</i>
1.	Neem oil	<i>Azadirachta indica</i>	35 $\pm$ 1.5 mm	35 $\pm$ 2.0 mm	25 $\pm$ 1.5 mm	20 $\pm$ 2.0 mm	18 $\pm$ 2.0 mm
2.	Turmeric	<i>Curcuma longa</i>	R	R	R	R	R
3.	Garlic extract	<i>Allium sativum</i>	45 $\pm$ 2.0 mm	40 $\pm$ 1.5 mm	40 $\pm$ 1.5 mm	40 $\pm$ 1.0 mm	35 $\pm$ 1.0 mm
4.	Ginger extract	<i>Zingiber officinale</i>	45 $\pm$ 2.0 mm	45 $\pm$ 2.0 mm	45 $\pm$ 1.5 mm	45 $\pm$ 2.0 mm	45 $\pm$ 1.5 mm
5.	Lemon grass	<i>Cymbopogon citratus</i>	18 $\pm$ 2.0 mm	18 $\pm$ 2.0 mm	15 $\pm$ 1.5 mm	15 $\pm$ 2.0 mm	15 $\pm$ 2.0 mm

R= No zone of inhibition

In present study cases of dermatophytes were reported in which *Trichophyton mentagrophytes* was the most common isolated fungi followed by *Trichophyton rubrum*, *Epidermophyton floccosum*, *Microsporum gypesum*, and *Microsporum canis*. The anti-dermatophyte activity was carried out on these etiological agents. Medicinal plant such as *Melaleuca alternifolia*, *Zingiber officinale*, *Allium sativum*, *Azadirachta indica*, *Citrus limonum* and *Curcuma longa* were investigated in this study as anti-dermatophyte. All dermatophyte species were found sensitive for *Melaleuca alternifolia*, *Zingiber officinale* and *Allium sativum*. These medicinal plants showed very good results as antifungal against dermatophytes while *Azadirachta indica* and *Citrus limonum* were moderate sensitive and *Curcuma longa* did not show any zone of inhibition around the well (Table 1).

4. Discussion

In spite of the development of science and technology, it has failed to develop a novel and efficient antifungal drug and there is need of more efficient antifungal drugs which can cure the specific fungi without any side effects on human beings. The duration of treatment depends upon the site, type of infection and symptom. The dosage depends on the clinical site and severity of infection and more

important efficacy of the drugs (Lakshmipathy and Kannabiran, 2010) Sagar and Vidyasagar, also reported the use of suitable medicinal plants for dermatophyte infections in their study (Sagar and Vidyasagar, 2013). The improved and better responses of these home remedies, minimum side effects, and cost effectiveness make these therapies favorable choice to combat fungal infection including dermatophytes.

In present study the medicinal plants were used as antifungal substance were *Melaleuca alternifolia*, *Zingiber officinale*, *Allium sativum*, *Azadirachta indica*, *Citrus limonum* and *Curcuma longa* against different dermatophyte species like *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum*, *Trichophyton tonsurans*, *Mycosporum gypsum*, *Trichophyton verrucosum* and *Mycosporum*. Sharma and Malik, reported that *Zingiber officinale*, *Citrus limonum* and *Curcuma longa* essential oils alone and in combination showed good antifungal activity against *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Mycosporum gypsum* and *Mycosporum canis* (Sharma and Malik 2015).

Bokhari, reported that methanol extract and ethyl extract of lemon grass (*Cymbopogon citrates* DC.), lantana (*Lantana camara* L.), nerium (*Nerium oleander* L) basil (*Ocimum basilicum* L.) and olive leaves (*Olea europaea* L.) showed highest activities against *Trichophyton rubrum* followed by *Microsporum canis*, *Micosporum gypseum*, and *Trichophyton mentagrophytes*. (Bokhari, 2009) Dilig et al., conducted a study of antifungal activity with twenty different medicinal plants and result showed that garlic juice and methanol extract of calamansi were inhibitory to *Microsporum canis* and *Trichophyton rubrum* (Dilig et al., 2016). Adiguzel et al, reported that the methanol extract essential oil of *Satureja hortensis* plant has a strong antimicrobial activity against eight bacteria and three fungi (Adiguzel et al., 2007). According to Abinu et al, scientists are more interested now days to use more plants extract for antimicrobial activities and identifying the compounds responsible for the antimicrobial properties (Abinu et al, 2007). Shaikh et al (2018) reported that *Allium sativum*, *Zingiber officinalis*, *Glycyrrhiza glabra*, *Curcuma longa* and *Azadirachta indica* have potent antifungal properties. Shahitha et al, reported that the medicinal plant *Lawsoniainermis* (henna) show good results against dermatophyte. It showed maximum zone of inhibition against *Mycosporum canis* (41 mm), *Trichophyton equinum* (30 mm), *Trichophyton rubrum* (27 mm) and *Mycosporum gypsum* (24 mm) (Shahitha et al., 2013) Imo and Za'aku reported in their review article that Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) has many medicinal properties and antifungal activity is one of them (Imo and Za'aku 2019). Similarly, current study reported that garlic and ginger show good antifungal activity as compare to other medicinal plants against five different dermatophyte species.

5. Conclusion

The antimicrobial properties of medicinal plants have been widely recognized and documented across the world. In the present study, *Melaleuca alternifolia*, *Zingiber officinale*, *Allium sativum*, and *Azadirachta indica* exhibited significant antifungal activity against different dermatophyte species. These findings suggest that medicinal plant extracts and essential oils contain bioactive compounds with promising anti-dermatophytic potential, which could be further isolated and developed as effective natural antifungal agents. Plant-derived fungicides represent a valuable alternative to synthetic antifungal drugs due to their natural origin, lower toxicity, and reduced environmental impact.

Although the present findings are based on in vitro investigations, they provide encouraging evidence for the therapeutic potential of these medicinal plants. Further in vivo studies and clinical investigations are required to confirm their efficacy, safety, optimal dosage, and mechanisms of action. In addition, comprehensive studies under controlled and field conditions are needed to evaluate their practical application in integrated disease management strategies. The identification and characterization of the



active phytochemical constituents may facilitate the development of novel bioactive compounds and environmentally sustainable antifungal therapies for the management of dermatophytosis.

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