Research Article

International Journal of Indigenous Herbs and Drugs
Content Available at www.saap.org.in ISSN: 2456-7345

IN-VITRO ANTI-DERMATOPHYTIC ACTIVITY OF DIFFERENT MEDICINAL PLANT EXTRACTS

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Article Info

Article History
Received on: 01-08-2023
Revised on: 18-08-2023
Accepted on: 11-09-2023

Abstract

Background: Dermato phytosis is most common superficial skin infection and dermatophytes are fungi that invade within keratinized tissues; skin, hair and nails.In many studies reported that some medicinal plant are very useful to treat various skin disease included dermatophyte, because these medicinal plants are natural so have low cost, high availability, few side effects and valuable resources.

Objective: To evaluate the antifungal activity of *Melaleuca Alternifolia, Zingiber officinale, Allium sativum, Azadirachtaindica, Citrus limonum, Curcuma longa*, and *Cocosnucifera* against the different dermatophytes causing skin infections.

Methods: The *Melaleuca Alternifolia* (tea tree oil), *Azadirachtaindica* (neem oil), *Cocosnucifera* (coconut oil) and fresh juice of *Citruslimonum* (Lemon) and aqueous extracts of *Zingiberofficinale*, *Alliumsativum* and *Curcumalonga* were explored for antifungal susceptibility testing as per CLSI guidelines by agar well diffusion method.

Results: Anti-fungal potentials of the oils and aqueous extracts of different medicinal plants were tested against *Trichophyton Mentagrophytes, T. rubrum, T. tonsurans, T. verrucosum, Epidermophyton Floccosum, Microsporum gypseum, M. canis.* All the seven dermatophytes were susceptible to *Melaleuca Alternifolia, Zingiber officinale* and *Allium sativum*, showed inhibitory zone ranges from 40±2.0mm to 45±2.0mm. Whereas, *Azadirachta indica* showed inhibitory zone ranges from 18±2.0mm to 35±2.0mm for all the seven dermatophytes followed by *Citrus limonum*. On the contrary *Curcuma longa* and *Cocosnucifera* not effective against any tested dermatophytes.

Conclusion: The current research 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.

Key words: medicinal plants, dermatophytes, keratinized tissue, antifungal activity, skin infection.

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DOI: https://doi.org/10.46956/ijihd.v8i5.493

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Introduction

Dermatophytosis is most common superficial skin infection and dermatophytes are fungi that invade within keratinized tissues; skin, hair and nails. On the basis of their genera, dermatophytes can be classified into three groups: *Trichophyton, Epidermophyton* and *Microsporum.* Dermatophyte can be classified on the basis of their natural habitat like *anthropophilic* which spread in human beings and easily get transfer from one person to others, *geophilic*a ssociated with soil which transfer to human from soil and *zoophilic* which infect animals and easily get transfer from animals to human beings. Clinically dermatophyte have been classified into *Tineacapitis,*

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Tineafaciei, Tineabarbae, Tineacorporis, Tineamannum, Tineacruris, Tineapedis and *Tineaunguium*. Other clinical variants are *Tineaimbricata, Tinea pseudoimbricata* and *Majocchi granuloma* [1].

In last 10 years the prevalence of dermatophytes drastically enhanced. Hot and humid weather, working conditions and other predisposing factor play important role to increase the infection in human beings but development of resistant strains to a range of antifungal drugs also important factor to increase the prevalence of infection [2].

Over the time, a vast range of antifungal agents have been used to treat dermatophytosis Griseofulvin, oral Imidazole, Ketoconazole, oral azoles Fluconazole, Itraconazole and topical allylamines like Terbinafine, Butenafine and Naftifine [3]. The drugs Fluconazole, Itraconazole and Terbinafine have effective on dermatophytosis when used for systemic treatment [4]. Despite the availability of a wide range of antifungal agents, the failure in treatment has been extensively reported. This may be multifactorial and the reasons include the severity of the dermatophytosis, causative agents, immune suppressed patients and some antifungal drugs may modify blood levels, insufficient drug administration, and discontinuation of therapy [4-6]. These antifungal agents are effective for treatment of dermatophytosis but these antifungal agents have also many side effects for patients as these drugs require long treatment for cure as well assome dermatophyte species have acquired of resistance to antifungal drugs after long use. Therefore, there is need to search new cost-effective alternatives having good anti- dermatophytic activity and not harmful for human health. Traditional medicinal practice has been known for centuries in many parts of the world for the treatment of various human ailments without harmful effects. In many studies reported that some medicinal plant are very useful to treat various skin disease included dermatophyte, because these medicinal plants are natural so have low cost, high availability, few side effects and valuable resources [7].

Medicinal plants are good source of low-cost phytochemicals, which have high availability and few side effects [2]. A commonly grown tree *Azadirachtaindica*has wide range of medicinal properties and it has been found to be effective against some dermatophyte like *Trichophyton rubrum*, *Epidermophyton floccosum*, *Microsporumnanum*and *Trichophytonsviolaceaum*[8]. *Lawsoniainermis*is another small shrub which is very common in India and shows good results against dermatophytes [4]. Essential oils from *Curcuma longa, Citrus lemon* and *Zingiber officinale*alone or in combination with extracts have also been used to treat infections caused by dermatophytes.

Therefore, the current study based on the investigation of antidermatophytic activity of some medicinal plants like*Melaleuca Alternifolia, Zingiberofficinale, Allium sativum, Azadirachtaindica, Citrus limonum, Curcuma longa, Cocosnucifera*. These are natural products which are cost-effective, ecofriendly without any side effects and alternative herbal treatment for dermatophytes.

Materials and Methods

The present research was conducted in the Centre for Interdisciplinary Biomedical Research, Adesh University, Bathinda. The samples were collected from the Out Patient Department of Dermatology, Adesh Hospital, Bathinda. After taking written informed consent total 320 patients were included in this study in which 272 patients were skin and 48 patients were nail. The full medical history, including age, sex, occupation, any allergy, duration of infection and other demographic profile was recorded. The specimens were collected in a sufficient amount in 2 ml eppendrof by using blunt edge of a sterile surgical blade and it taken from the edge of the infected area. The collected specimens were labeled with date, code name, gender, age, and infection site.

Ethical Approval

The research project was approved by the Institutional Ethics Committee (AU/EC/FM/38/2018) and Institutional Research Committee of Adesh University, Bathinda, Punjab, India.

Collection and Preparation of medicinal plant extracts

In present study different medicinal plant extract was evaluated through agar well method as per the antifungal CLSI guidelines-2017 [9]. In current investigation the medicinal plants *Melaleuca Alternifolia* (tea-tree oil), *Azadirachtaindica*(neem oil), *Curcuma longa* (turmeric), *Allium sativum* (garlic extract), *Zingiberofficinale*(ginger extract), *Cocosnucifera* (coconut oil), *Citrus limonum* (lemon oil) were used. Fresh rhizomes of turmeric, ginger and garlic were purchased from local market. Remove the all-papery skin from the rhizomes and wash it properly to remove soil. The fresh rhizomes then peeled and sliced. Theaqueous extractofturmeric, garlic and ginger was prepared freshly.

Allium sativum aqueous extract was prepared from garlic cloves (1.0g). Remove the all-papery skin from each clove and washed in sterilized MilliQ water. Then cloves grinded into paste in sterile ceramic mortar pestle followed by the addition of 10 ml MilliQ water. The mixture was filtered and final concentration was stored in sterile container and explored for the sensitivity test.

*Zingiberofficinale*extractswas alsoprepared from ginger (1.0g). The rhizomes were washed with sterilized MilliQ water and peeled properly, followed by crushed in to mortar pestle to make paste. The paste was dissolved in 10 ml MilliQ water and filtered. Thus, the aqueous extracts were explored against the dermatophytes.

Curcuma longa extracts was prepared from the turmeric rhizomes (1.0g), washed, peeled and crushed in to mortar pestle to make paste. The paste was dissolved in 10 ml MilliQ water and filtered. Thus, the aqueous extracts were explored against the dermatophytes.

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(Every time freshly prepared garlic, turmeric and ginger extract was used for antifungal susceptibility test)

Melaleuca alternifolia (tea-tree oil) was used from 'The body shop'. It is an essential oil that comes from steaming the leaves of the Australian tea tree.*Citrus limonum* fresh juice and *Cocosnucifera* (coconut oil), bio-organic extra virgin was used. *Azadirachtaindica* (neem oil) was used from 'juicy chemistry'. It is a vegetable oil pressed from the fruits and seeds of the neem.

The extracts of tea-tree oil, coconut oil, neem oil and lemon juice were prepared by 1:1 dilution method i.e. 500 µl oil/juice and 500 µl MilliQ water, from this 100 µl diluted solution was used for susceptibility testing.

Table 1: Details of Medicinal plants part and volume used for antifungal susceptibility testing

Sr. No.	Scientific name of plant	Common name of plant	Part use	Volume used
1.	*Melaleuaalternifolia*	Tea tree	oil	100µl
2.	*Azadirachtaindica*	Neem	oil	100µl
3.	*Cocosnucifera*	Coconut	oil	100µl
4.	*Citrus limonum*	Lemon	Fresh juice	100µl
5.	*Curcuma longa*	Turmeric	Aqueous extract	100µl
6.	*Allium sativum*	Garlic	Aqueous extract	100µl
7.	*Zingiberofficinale*	Ginger	Aqueous extract	100µl

Procedure of antifungal susceptibility test

The isolates from Sabroud dextrose agar (SDA) plates were sub cultured in 100 ml Sabroud dextrose broth to enhance sporulation up to 5 days at room temperature (37°C). After fungal growth the suspension mixed on spinner and then suspension was allowed to sediment for 30 minutes. With a sterilized Pasteur pipette 100µl clear culture suspensions from the side of the tube were streaked evenly over the surface of Sabouraud dextrose agar plates. 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 were 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 100µl of medicinal plants extracts were loaded into each well in sterilize conditions and kept the plates at 4°C for 30 min for diffusion of supernatant into agar. Incubated the plates at 25°C for 5-10 days and the zone of inhibition around the wells were measured in mm.

Results

In present study total 320 cases of dermatophytes were reported in which *Trichophyton Mentagrophytes* was the most common isolated fungi followed by *Trichophytonrubrum, Epidermophyton Floccosum*, *Trichophyton Tonsurans, Microsporumgypseum*, *Trichophyton Verrucosum* and *Microsporumcanis*. The antidermatophytic activity was carried out on these etiological agents. The effect of extracts of *Zingiberofficinale, Allium sativum, Citrus limonum, Curcuma longa* and oil from *Cocosnucifera, Melaleuca Alternifolia* and *Azadirachtaindica* were investigated as an anti-dermatophytic activity. In the present investigation, the *Melaleuca Alternifolia* diluted oil exhibited high degree of inhibitory activity against most of the seven tested organisms followed by *Zingiber officinale* and *Allium sativum* extracts. *Melaleuca Alternifolia* show inhibitory zone 45±2.0mm for *Trichophyton Mentagrophytes,* 45±1.5mm for *Trichophytonrubrum,* 45±2.0mm for *Trichophyton Tonsurans, Trichophyton Verrucosum, Epidermophyton Floccosum* and *Microsporumgypseum*, 40±1.5mm for *Microsporumcanis*(Table 2). *Zingiber officinale* show inhibitory zone 45±2.0mm for *Trichophyton Mentagrophytes, Trichophytonrubrum, Trichophyton Tonsurans,*45±1.5mm for*Epidermophyton Floccosum, Microsporumgypseum* and 45±2.0mm for *Microsporumcanis*, 40±2.0mm for *Trichophyton Verrucosum*.

Allium sativum show inhibitory zone 40±1.5mm for *Trichophyton mentgrophytes* and *Microsporum gypsum*, 45±2.0mm for *Trichophytonrubrum,* 35±1.0mm for *Epidermophyton Floccosum* and *Trichophyton Tonsurans,* 40±1.5mm for *Trichophyton Verrucosum*and40±1.0mm for *Microsporumcanis.*

Azadirachta indica showed inhibitory zone35±2.0mm for *Trichophyton Mentagrophytes,* 35±1.5mm for *Trichophyton rubrum.* 18±2.0mm for *Epidermophyton Floccosum*, 20±2.0mm for *Trichophyton Tonsurans*, *Microsporumcanis* and *Trichophyton Verrucosum and* 25±1.5mm for *Microsporum gypseum* (Table 2).In which *Melaleuca Alternifolia, Zingiber officinale, Allium sativum* effectively inhibit the human pathogenic seven dermatophyte species, these medicinal plants have potential as antifungal agents against dermatophytes. While *Azadirachta indica* and *Citruslimonum were* moderate sensitive and the diameter of zone of growth inhibition varied between 20± 2.0 mm to 35± 2.0 mm (in Neem) and 15± 2.0to 18± 2.0 mm (in lemon juice). Whereas *Curcuma longa* and *Cocosnucifera* were not show any zone of inhibition around the well. Therefore, the dermatophytes grow well in the presence of coconut oil and turmeric extract.

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Table 2: *In-vitro* antifungal zone of inhibition (mm, in diameter) ofmedicinal plants against clinical isolates dermatophyte species

Scientific name	Common name	*T.mentagrophytes*	*T.rubrum*	*E.floccosum*	*T.tonsurans*	*M.gypseum*	*T.verrucosum*	M.canis
Melaleucaalternifolia	Tea tree oil	45± 2.0mm	45± 1.5mm	40± 2.0mm	45 ± 2.0mm	45± 2.0mm	45 ± 2.0mm	40± 1.5mm
Zingiberofficinale	Ginger extract	45± 2.0mm	45± 2.0 mm	45± 1.5mm	40 ± 2.0mm	45± 1.5mm	40± 2.0 mm	45± 2.0mm
Allium sativum	Garlic extract	40± 1.5mm	45± 2.0mm	35± 1.0mm	45± 1.0mm	40± 1.5mm	45± 1.5mm	40± 1.0mm
Azadirachtaindica	Neem oil	35± 2.0mm	35± 1.5mm	18± 2.0mm	20± 2.0mm	25± 1.5mm	20± 2.0mm	20± 2.0mm
Citrus limonum	Lemon	18± 2.0mm	18± 2.0mm	15± 2.0mm	15± 1.5mm	15± 1.5mm	20± 2.0mm	15± 2.0mm
Curcuma longa	Turmeric	nil	Nil	nil	nil	nil	nil	nil
Cocosnucifera	Coconut oil	nil	Nil	nil	nil	nil	nil	nil

Discussion

In spite of the development of science and technology, but it fails to develop there is novel and efficient antifungal drugs, fungi are also eukaryotic and have similar mechanism like human beings. There is need of more efficient antifungal drugs which cure the specific fungi without any side effects on human beings. The duration of treatment depends upon the site and type of infection and symptom. The dosage depends on the clinical site and severity of infection and more important efficacy of the drugs [10].

As Sagar and Vidyasagar, [11] reported the use of suitable medicinal plants for dermatophyte infections. The improved and better responses of these home remedies, minimum side effects, cost effective make these therapies favorable choice to combat fungal infection including dermatophytes.

In present study the medicinal plants were explored as antifungal were *Melaleuca Alternifolia, Zingiber officinale, Allium sativum, Azadirachta indica, Citrus limonum, Curcuma longa, Cocosnucifera* against *Trichophyton Mentagrophytes, Trichophyton rubrum, Epidermophton floccosum, Trichophyton Tonsurans, Micosporum gypseum, Trichophytonverricosum* and *Micosporumcanis.*

According to Yu *et al*.[12] the antifungal activity of *Melaleuca Alternifolia* essential oil is occur due to the synergistic effects of their various components on fungus. The most abundant components of *Melaleuca Alternifolia*terpinen-4-ol which compromise the cytoplasmic membrane of both bacteria and fungi, had highest antifungal activity, followed by, α-terpineol, terpinolene, 1, 8-cineole which appear to be highly effective against fungus.

Further, Mao *et al*. [13] showed that the compounds the γ-terpinene and citral in ginger showed potent antifungal properties which inhibit the growth of fungi.

Leontiev*et al*. [14] reported that Allicin (diallylthiosulfinate) is a defense molecule from garlic (*Allium sativum* L.) with broad antifugal activities and many bacteria and fungi. Allicin inactivate the essential enzymes. The active ingredient allicin of garlic, partially inhibiting DNA and protein synthesis while RNA synthesis has completely inhibited in the presence of allicin [15]. Whereas, in another study Organosulfur compounds and phenolic compounds have been reported to be involved in the garlic antimicrobial activity [16].The garlic and ginger is popular as plants and food ingredient for flavouring and adding acidity, its juices have been reported to exhibit antibacterial activity against wide range of microbes. According to previous reports garlic has an anti-infective agent in traditional dietary and medicinal applications. *In-vitro*investigations of the antimicrobial activity of freeze dried and fresh garlic extracts against many bacteria [17], fungi and viruses [18] supports these applications.

Mahmoud *et al.* [8] reported that major compounds of *Azadirachtaindica* are 6-deacetylnimbin, azadiradione, nimbin, salannin and epoxy-azadiradione which active against different pathogenic fungi.

Sharma and Malik, [7]investigated that *Zingiber officinale, Citrus limonum* and *Curcuma longa* essentials oils alone and in combination showed good antifungal activity against *Trichophyton Mentagrophytes, Trichophytonrubrum, Micosporum gypseum* and

Micosporumcanis. Similarly, in the present study the aqueous extracts of *Zinger offcinale* and *Citruslimonum* fresh juice showed zone of inhibition ranges from 40±2.0mm to 45±2.0mm and 15±2.0mm 20±2.0mm respectively.

Bokhari, [19]eported that methanol extract and ethyl extract of lemon grass (*Cymbopogon citrates DC.*), lantana (*Lantana camara L.*), nerium (*Nerium oleander L)* basil (*Ocimumbasilicum L.*) and olive leaves (*Oleaeuropaea L.*) showed highest activities against *Trichophyton rubrum* followed by *Microsporumcanis, Micosporum gypseum*, and *Trichophyton Mentagrophytes*.

Dilig*et al*. [20] 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 *Microsporumcanis* and *Trichophytonrubrum*. Adiguzel*et al*.[21] reported that the methanol extract essential oil of *Saturejahortensis* plant has a strong antimicrobial activity against eight bacteria and three fungi. According to Abinu*et al.*[22] that scientists are more interested now days to use more plants extract for antimicrobial activities and identifying the compounds responsible for the antimicrobial properties. Shaikh *et al*.[23] reported that *Allium sativum, Zingiber Officinalis, GlycyrrhizaGlabra, Curcuma longa* and *AzadirachtaIndica* has potent antifungal properties.

Shahitha*et al*. [24] reported that the medicinal plant *Lawsoniainermis* (henna) showed good results against dermatophyte and reported maximum zone of inhibition against *Microsporumcanis* (41mm), *Trichophytonequinum* (30mm),*Trichophytonrubrum*(27mm) and *Micosporum gypsum* (24mm).

Imo and Za'aku, [25] reported in the review article that Garlic *(Allium sativum*) and Ginger (*Zingiber officinale*) has many medicinal properties and antifungal is one of them. Similarly current study reported that garlic and ginger showed good antifungal activity as compared to other medicinal plants against seven different dermatophyte species.

Conclusion

The antimicrobial properties of medicinal plant were observed and documented all over the world. In present work the medicinal plants *Melaleuca Alternifolia, Zingiberofficinale, Allium sativum, Azadirachta indica* was show good antifungal activity against different dermatophyte species. The compounds present in the plant extracts and oils indicated that they possess antifungal properties. This study also shows that the plant extract and oils possesses the active ingredients that could be employed in their purified form to cure dermatophytes.

Funding

None

Conflict Of Interest Statement

We declare that we have no conflict of interest.

Ethics Approval and Consent to Participate

Not applicable.

Availability of Data and Material

The authors confirm that the data supporting the findings of this study are available within the article.

Acknowledgements

The authors are grateful to the Management and Centre for Interdisciplinary Biomedical Research, Adesh University, Bathinda, Punjab, India, for providing laboratory and other necessary facilities for successful completion of work.

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