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

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<th>Abstract</th>
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<td>Article History</td>
<td>Background: Dermatophytosis 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.</td>
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<td>Received on: 01-08-2023</td>
<td>Objective: To evaluate the antifungal activity of <em>Melaleuca Alternifolia</em>, <em>Zingiber officinale</em>, <em>Allium sativum</em>, <em>Azadirachta indica</em>, <em>Citrus limonum</em>, <em>Curcuma longa</em>, and <em>Cocos nucifera</em> against the different dermatophytes causing skin infections.</td>
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<td>Revised on: 18-08-2023</td>
<td>Methods: The <em>Melaleuca Alternifolia</em> (tea tree oil), <em>Azadirachta indica</em> (neem oil), <em>Cocos nucifera</em> (coconut oil) and fresh juice of <em>Citrus limonum</em> (Lemon) and aqueous extracts of <em>Zingiber officinale</em>, <em>Allium sativum</em> and <em>Curcuma longa</em> were explored for antifungal susceptibility testing as per CLSI guidelines by agar well diffusion method.</td>
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<td>Accepted on: 11-09-2023</td>
<td>Results: Anti-fungal potentials of the oils and aqueous extracts of different medicinal plants were tested against <em>Trichophyton mentagrophytes</em>, <em>T. rubrum</em>, <em>T. tonsurans</em>, <em>T. verrucosum</em>, <em>Epidermophyton floccosum</em>, <em>Microsporum gypseum</em>, <em>M. canis</em>. All the seven dermatophytes were susceptible to <em>Melaleuca Alternifolia</em>, <em>Zingiber officinale</em> and <em>Allium sativum</em>, showed inhibitory zone ranges from 40±2.0mm to 45±2.0mm. Whereas, <em>Azadirachta indica</em> showed inhibitory zone ranges from 18±2.0mm to 35±2.0mm for all the seven dermatophytes followed by <em>Citrus limonum</em>. On the contrary <em>Curcuma longa</em> and <em>Cocos nucifera</em> not effective against any tested dermatophytes.</td>
</tr>
</tbody>
</table>

| 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 infections. |

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

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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* associated 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*,
Tineafaciei, Tinea barbareae, Tinea corporis, Tinea mannum, Tineacrupis, Tineapedis and Tinea unguium. Other clinical variants are Tinea imbricata, 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 asse some 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 Azadirachta indica has wide range of medicinal properties and it has been found to be effective against some dermatophyte like Trichophyton rubrum, Epidermophyton floccosum, Microsporum nanum and Trichophyton violaceum [8]. Lawsonia inermis 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 were also used 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, Zingiber officinale, Allium sativum, Azadirachta indica, 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 eppendorf 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/PM/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 CSLI guidelines-2017 [9]. In current investigation the medicinal plants Melaleuca Alternifolia (tea-tree oil), Azadirachta indica (neem oil), Curcuma longa (turmeric), Allium sativum (garlic extract), Zingiber officinale (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. The aqueous extract of turmeric, 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.

Zingiber officinale extracts was also prepared 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.
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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Scientific name of plant</th>
<th>Common name of plant</th>
<th>Part use</th>
<th>Volum e used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Melaleuca alternifolia</td>
<td>Tea tree</td>
<td>oil</td>
<td>100µl</td>
</tr>
<tr>
<td>2.</td>
<td>Azadirachtaindica</td>
<td>Neem</td>
<td>oil</td>
<td>100µl</td>
</tr>
<tr>
<td>3.</td>
<td>Cocosnucifera</td>
<td>Coconut</td>
<td>oil</td>
<td>100µl</td>
</tr>
<tr>
<td>4.</td>
<td>Citrus limonum</td>
<td>Lemon</td>
<td>Fresh juice</td>
<td>100µl</td>
</tr>
<tr>
<td>5.</td>
<td>Curcuma longa</td>
<td>Turmeric</td>
<td>Aqueou s extract</td>
<td>100µl</td>
</tr>
<tr>
<td>6.</td>
<td>Allium sativum</td>
<td>Garlic</td>
<td>Aqueou s extract</td>
<td>100µl</td>
</tr>
<tr>
<td>7.</td>
<td>Zingiber officinalae</td>
<td>Ginger</td>
<td>Aqueou s extract</td>
<td>100µl</td>
</tr>
</tbody>
</table>

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 wels of 5 mm in diameter, 4 mm deep and 2 cm apart were punched in with a sterile cork-borer. Then 100µl of medicinal plant extracts were loaded into each well in sterile 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 Zingiber officinalae, Allium sativum, Citrus limonum, Curcuma longa and oil from Cocosnucifera, Melaleuca Alternifolia and Azadirachtaindica were investigated as an antidermatophytic 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 officinalae 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 officinalae show inhibitory zone 45±2.0mm for Trichophyton Mentagrophytes, Trichophytonrubrum, Trichophyton Tonsurans, Microsporumgypseum and 45±2.0mm for Microsporumcanis, 40±2.0mm for Trichophyton Verrucosum.

Allium sativum show inhibitory zone 40±1.5mm for Trichophyton mentagrophytes and Microsporum gypsum, 45±2.0mm for Trichophytonrubrum, 35±2.0mm for Epidermophyton Floccosum and Trichophyton Tonsurans, 40±1.5mm for Trichophyton Verrucosum and 40±1.0mm for Microsporumcanis. Azadirachta indica showed inhibitory zone35±2.0mm for Trichophyton Mentagrophytes, 35±1.5mm for Trichophytonrubrum, 18±2.0mm for Epidermophyton Floccosum, 20±2.0mm for Trichophyton Tonsurans, Microsporumcanis and Trichophyton Verrucosum and 25±1.5mm for Microsporum gypsum (Table 2). In which Melaleuca Alternifolia, Zingiber officinalae, 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.
Table 2: *In-vitro* antifungal zone of inhibition (mm, in diameter) of medicinal plants against clinical isolates dermatophyte species

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>T. mentagrophytes</th>
<th>T. rubrum</th>
<th>E. floccosum</th>
<th>T. tonsurans</th>
<th>M. gypseum</th>
<th>T. verrucosum</th>
<th>M. canis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melaleuca alternifolia</td>
<td>Tea tree oil</td>
<td>45± 2.0mm</td>
<td>45± 1.5mm</td>
<td>40± 2.0mm</td>
<td>45± 2.0mm</td>
<td>45± 2.0mm</td>
<td>45± 2.0mm</td>
<td>40± 1.5mm</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Ginger extract</td>
<td>45± 2.0mm</td>
<td>45± 2.0mm</td>
<td>45± 1.5mm</td>
<td>40± 2.0mm</td>
<td>45± 2.0mm</td>
<td>40± 2.0mm</td>
<td>45± 2.0mm</td>
</tr>
<tr>
<td>Allium sativum</td>
<td>Garlic extract</td>
<td>40± 1.5mm</td>
<td>35± 1.0mm</td>
<td>45± 1.0mm</td>
<td>40± 1.5mm</td>
<td>45± 1.5mm</td>
<td>45± 1.5mm</td>
<td>40± 1.0mm</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>Neem oil</td>
<td>35± 2.0mm</td>
<td>35± 1.5mm</td>
<td>20± 2.0mm</td>
<td>25± 1.5mm</td>
<td>20± 2.0mm</td>
<td>20± 2.0mm</td>
<td>20± 1.0mm</td>
</tr>
<tr>
<td>Citrus limonum</td>
<td>Lemon</td>
<td>18± 2.0mm</td>
<td>18± 2.0mm</td>
<td>15± 2.0mm</td>
<td>15± 1.5mm</td>
<td>15± 1.5mm</td>
<td>20± 2.0mm</td>
<td>15± 2.0mm</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>Turmeric</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Cocos nucifera</td>
<td>Coconut oil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
</tbody>
</table>

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*, *Cocos nucifera* against *Trichophyton Mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum*, *Trichophyton Tonsurans*, *Micosporum gypseum*, *Trichophyton verrucosum* and *Micosporum canis*.

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* terpinene-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 (diallyltiosulfinate) 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 *Azadirachta indica* 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...
Similarly, in the present study the aqueous extracts of Zinger officinalis and Citrus limonum fresh juice showed zone of inhibition ranges from 40±2.0mm to 45±2.0mm and 15±2.0mm 20±2.0mm respectively. Bokhart, [19] 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. Dilget 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 Microsporum canis and Trichophytonrubrum. Adiguzel et al[21] reported that the methanol extract essential oil of Satureja hortensis plant has a strong antimicrobial activity against eight bacteria and three fungi. According to Abinuat 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, Glycyrrhiza Glabra, Curcuma longa and Azadirachta indica has potent antifungal properties. Shahithaet al. [24] reported that the medicinal plant Lawsonia inermis (henna) showed good results against dermatophyte and reported maximum zone of inhibition against Microsporum canis (41mm), Trichophytonequinum (30mm), Trichophytonrubrum (27mm) and Micosporum gypseum (24mm).

Imo and Za’aku, [25] reported in the review article that Garlic (Allium sativum) and Ginger (Zingiber officinalis) 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, Zingiberofficinalis, 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.

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**Reference**


