

Original Research Article

Evaluation of anti-dermatophytic activity of medicinal plants derived products

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ABSTRACT

Background: 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*, *Cocos nucifera* were used as antifungal agent against different dermatophyte species.

Methods: A hospital-based study consisting of 320 patients clinically diagnosed having dermatophytic infection who reported to the Dermatology outpatient department (OPD), Adesh medical college, Bathinda was conducted for the period of 2 years. Socio-demographic and clinical information was collected and sample was taken from the edge of infected area which was then collected in 2 ml of Eppendorf. Extracts of medicinal plants were then explored against dermatophyte. The data was evaluated using appropriate statistical method.

Results: 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* and *Cocos nucifera* did not show any zone of inhibition around the well.

Conclusions: This 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.

Keywords: Medicinal plants, Dermaophyte, Keratinized tissue

INTRODUCTION

Dermatophytosis is most common superficial skin infection in India. Dermatophytes are fungi that invade within keratinized tissues; skin, hair and nails and causes infection. 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 anthrophilic which spread 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 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*.¹

In last 10 years the prevalence of dermatophyte has increased. 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.²

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.³

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.⁴

Many studies reported that some medicinal plants are very useful to treat various skin diseases including dermatophyte infections. As these medicinal plants are natural so have low cost, high availability, fewer side effects and valuable resources.

Development of antifungal compounds with diverse chemical structures and novel mechanisms of action is necessary because there has been an alarming increase in the incidence of new and re-emerging infectious diseases as well as resistance to currently used drugs. The investigations on new antifungal substances should be continued and all possible strategies and techniques need to be explored further.

The study aims at finding the anti-dermatophyte activity of medicinal plants like *Melaleuca alternifolia*, *Zingiber officinale*, *Allium sativum*, *Azadirachta indica*, *Citrus limonum*, *Curcuma longa* and *Cocos nucifera*.

METHODS

The present research was conducted in the centre for Interdisciplinary biomedical research, Adesh university, Bathinda in the period of June 2017-June 2019. The sample was collected from the out-patient department of dermatology, Adesh hospital, Bathinda. Total 320 patients were included in this study in which 272 patients were having skin fungal infection and 48 patients were having nail fungal infection. After taking an informed consent/assent from all the participants and recording their socio-demographic information a detailed history was taken including age, sex, occupation, any allergy, duration of infection and other demographic profile. Patient unwilling to give consent were excluded from the study. The specimens were taken from the edge of the infected area and collected in a sufficient amount in 2 ml Eppendorf by using blunt edge of a sterile surgical blade. The

collected specimens were labelled with name, gender, age and OPD number

Extraction of medicinal plant- In present study different medicinal plant extract was evaluated through agar well method. Medicinal plants *Melaleuca alternifolia* (tea-tree oil), *Azadirachta indica* (neem oil), *Curcuma longa* (turmeric), *Allium sativum* (garlic extract), *Zingiber officinale* (ginger extract), *Cocos nucifera* (coconut oil) and *Citrus limonum* (lemon oil) were used.

Fresh rhizomes of turmeric, ginger and garlic were purchased from local market. All the papery skin from rhizomes was removed and washed properly to remove all the soil. The fresh rhizomes were then peeled and sliced. The aqueous extract of turmeric, garlic and ginger was prepared freshly.

Allium sativum aqueous extract was prepared from garlic cloves. All the papery skin was removed and it was washed in sterilized MilliQ water. Then cloves were 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 were also prepared from ginger. The rhizomes were washed with sterilized MilliQ water and peeled properly, and then crushed into mortar pestle to make paste. The paste was dissolved in 10 ml MilliQ water and then filtered. Thus, the aqueous extracts obtained were explored against the dermatophytes.

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

Every time fresh prepared garlic, turmeric and ginger extract was used for antifungal susceptibility test

Melaleuca alternifolia (tea-tree oil) 10 ml was used from 'The body shop'. It is an essential oil that comes from steaming the leaves of the Australian tea tree.

Cocos nucifera (coconut oil), bio-organic extra virgin Coconut oil was used.

Citrus limonum (lemon oil), PRZ essential lemon oil was used.

Azadirachta indica (neem oil) 30 ml was used from 'juicy chemistry'. It is a vegetable oil pressed from the fruits and seeds of the neem.

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 µl 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 µl 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.

The statistical analysis was performed using the statistical

package for social sciences (SPSS) software version 20.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as measures of central tendency such as mean and standard deviation and categorical variables were presented as absolute numbers and percentage. Observations were analyzed using the Chi square test, Z test and proportion test, wherever applicable. For all statistical tests, a $p < 0.05$ was considered significant.

RESULTS

In present study total 320 cases of dermatophytes were reported in which *Trichophyton mentagrophytes* was the most common isolated fungi followed by *Trichophyton rubrum*, *Epidermophyton floccosum*, *Trichophyton tonsurans*, *Microsporum gypseum*, *Trichophyton verrucosum* and *Microsporum canis*.

Table 1: In-vitro activities of medicinal plants antifungal agents against 7 dermatophyte species.

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

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*, *Curcuma longa* and *Cocos nucifera* 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* and *Cocos nucifera* did not show any zone of inhibition around the well (Table 1).

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.⁵

Sagar and Vidyasagar, also reported the use of suitable medicinal plants for dermatophyte infections in their study.⁶ The improved and better responses of these home remedies, minimum side effects, and cost effectiveness make these therapies favourable 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*, *Curcuma longa* and *Cocos nucifera* 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 mentagophytes*, *Trichophyton rubrum*, *Mycosporum gypsum* and *Mycosporum canis*.⁷ They used oils of these medicinal plants while in current study their extract were used as antifungal agents and zone of inhibition was also higher in this study.

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 *Mycosporum canis*, *Mycosporum gypsum*, and *Trichophyton mentagophytes*.⁸

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 *Mycosporum canis* and *Trichophyton rubrum*.⁹

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.¹⁰

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.¹¹

Shaikh et al reported that *Allium sativum*, *Zingiber officinalis*, *Glycyrrhiza glabra*, *Curcuma longa* and *Azadirachta indica* have potent antifungal properties.¹²

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).¹³

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.¹⁴ Similarly current study reported that garlic and ginger show good antifungal activity as compare to other medicinal plants against seven different dermatophyte species.

CONCLUSION

The antimicrobial properties of medicinal plant have been observed and documented all over the world. In present work the medicinal plants *Melaleuca alternifolia*, *Zingiber officinale*, *Allium sativum*, *Azadirachta indica* has shown good antifungal activity against different dermatophyte species. This study showed that the plant extract and oils from plants possesses active ingredients that could be

employed in their purified form to cure dermatophytes. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity. These preliminary results, obtained from in vitro experiments, may be supplemented by other more comprehensive studies *in vivo*, both in controlled greenhouse conditions and in open field to practically evaluate the use of these extract in the frame of an integrated pest management system. Subsequently, this can help researchers to look for new bio stimulatory agents.

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