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Antifungal activity of *Ocimum sanctum* Linn. (Lamiaceae) on clinically isolated dermatophytic fungi

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ABSTRACT

Objective: To assess antifungal activity of *Ocimum sanctum* leaves against dermatophytic fungi.**Methods:** Antifungal activity of *Ocimum sanctum* leaves was measured by 38 A NCCLS method. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of various extracts and fractions of *Ocimum sanctum* leaves were also determined. **Results:** *Ocimum sanctum* leaves possessed antifungal activity against clinically isolated dermatophytes at the concentration of 200 μ g/mL. MIC and MFC were high with water fraction (200 μ g/mL) against dermatophytic fungi used. **Conclusions:** *Ocimum sanctum* has antifungal activity, and the leaf extracts may be a useful source for dermatophytic infections.

1. Introduction

Fungal infections are one of the major health problems in tropical countries, and tinea is one of the most common fungal infection. Climate of the region, living conditions like nutrition, hygiene and socio-economic status, genetic susceptibilities and other such conditions play an important role in the prevalence of dermatophytic infection in a particular region. Dermatophytes invade in to the keratinophilic region of the body and cause dermatophytosis. The drugs used against dermatophytosis exhibit several side effects and have limited efficacy. Hence there is a distinct need for the discovery of new, safer and effective antifungal drugs^[1–4]. Herbal medicines are popular as remedies for diseases by vast majority of population in the world^[5–8]. The world health organization

(WHO) estimates that about three quarters of the world population currently use herbs and other forms of traditional system of medicines for treating their diseases.

Herbal formulations are popular among rural and urban community in India^[9–12]. Part of the reason is their popularity, acceptability and the belief that all natural products are safe^[13–17]. Recent studies proved that the plants used in traditional medicine contain a wide range of bioactive compounds that can be used to treat infectious diseases^[18–22]. *Ocimum sanctum* is a medicinal shrub and is commonly called as Tulsi. Traditionally the leaves of this plant were used as a medicine for asthma and other respiratory problems^[9–12]. No report is available on the antifungal activity of the *Ocimum sanctum*. Biologically active compounds and extracts isolated from such plant species were used in herbal medicine and they have been the centre of interest as on date.

This work was carried out to investigate the antifungal activity of the leaves of *Ocimum sanctum* against the clinical isolates of dermatophytic fungi from the patients attending the dermatology section of Bharath Heavy Electrical Limited (BHEL) Hospital, Trichirappalli, India

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and Annal Gandhi Memorial Government Hospital, Tiruchirappalli, India.

2. Materials and methods

The plant material used in this study was collected from Tiruchirappalli, Tamil Nadu, India. It was identified and authenticated by the Botanist of Department of Plant Biotechnology, Bharathidasan University, Tiruchirappalli. Fresh leaves were collected and shade dried. The dried leaves were ground to powder and stored in an air tight container until further use. Known quantity of *Ocimum sanctum* leaf powder was subjected for cold extraction with water and 100% ethyl alcohol and the aqueous extracts were collected. The extracts were dried in vacuum desiccator and were stored in a sterile container for further use.

Known quantity of *Ocimum sanctum* coarse powder was also successively extracted with various solvents like hexane, benzene, chloroform, ethyl acetate, methanol and water. Different fractions collected were filtered and evaporated in vacuum. Coding was given to various extracts and fractions and was stored till use. The dried extracts and fractions were weighed and dissolved in 5% dimethyl sulfoxide (DMSO) and was used for *in-vitro* testing of its activity against dermatophytes.

Five different clinical isolates of dermatophytic fungi like *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis*, *Microsporum gypseum* and *Epidermophyton floccosum* were taken for this study.

The selected isolates were grown on sabouraud dextrose agar (SDA). Twenty one day old culture of dermatophytic fungi was scraped with a sterile scalpel and macerated with sterile distilled water. The suspension was adjusted spectrophotometrically to an absorbance of 0.600 at 450 nm. By this way the fungal inoculum was prepared. For further study known quantity of this inoculum was used.

Susceptibility testing was performed by the reference broth micro dilution method^[23,24]. Minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) were determined after 21 days of incubation at 35 °C by 38 A NCCLS method. Medicinal plants are the good source of macromolecules and secondary metabolites. To know the phytoconstituents of *Ocimum sanctum*, the extracts were subjected to the analysis of macromolecules and secondary metabolites by using thin layer chromatography and high performance thin layer chromatography methods.

3. Result

MIC value is used to evaluate antimicrobial nature of plant extracts and minimum quantity of antimicrobial compound required to kill or arrest multiplication of all microorganisms present in the medium or body fluid. Extracts and fractions of *Ocimum sanctum* leaves showed effective antifungal activity against all dermatophytic fungus tested and the MIC ranged from (125.00±25.00) μ g/mL to (425.00±66.14) μ g/mL. Methanol fraction yielded effective MIC against *Trichophyton mentagrophytes* with MIC value of about (125.00±25.00) μ g/mL concentrations. All the extracts and fractions effectively inhibited the growth of *Trichophyton mentagrophytes* at lower concentration from (125.00±25.00) μ g/mL to (266.66±101.03) μ g/mL, whereas other fungal

growth inhibition needed at least (291.66±28.86) μ g/mL of extracts and fractions. Among extracts alcoholic extract showed best activity. MIC required to inhibit the growth of *Trichophyton mentagrophytes* was (166.66±14.43) μ g/mL and (383.33±28.86) μ g/mL against *Trichophyton rubrum*, (366.66±38.11) μ g/mL against *Microsporum gypseum*, (383.66±14.43) μ g/mL against *Microsporum nanum*, and (366.66±57.73) μ g/mL against *Epidermophyton floccosum* (Table 1). Among fractions methanol fraction exhibited best activity against all dermatophytic fungus: (125.00±25.00) μ g/mL against *Trichophyton mentagrophytes*, (350.00±66.14) μ g/mL against *Trichophyton rubrum*, (375.00±25.00) μ g/mL against *Microsporum gypseum*, and (383.33±14.43) μ g/mL against *Epidermophyton floccosum*.

The results of preliminary quantitative phytochemical screening of aqueous and alcoholic extracts of *Ocimum sanctum* revealed the presence of multiple polar and non-polar chemical constituents (Table 2). Steroids, terpenoids, flavonoids, phenolic compounds, lignin, proteins, carbohydrates were present in both extracts. Hexane fraction showed the availability of steroid, terpenoids, benzene extract also indicated the availability of steroid. Alkaloid was also available in benzene fraction along with steroid. Chloroform and ethylacetate fractions yielded alkaloid, terpenoids and flavonoids. Methanol and water fractions showed the presence of terpenoids, flavonoids, phenolic compounds. Though carbohydrates, reducing sugars, proteins and aminoacids were available in all the extracts and fractions, they were not effectively involved in any of the inhibitory activities of fungus as they are the primary metabolites.

Ocimum sanctum showed the presence of proteins in all the fractions and extracts. *Ocimum sanctum* showed seven to eleven peaks in various fractions in HPTLC. The Rf was between 0.03 and 0.90. Tannins and steroids were the two major secondary metabolites seen in *Ocimum sanctum*.

4. Discussion

The incidence of dermatophytosis has been high since 1980s. It is still a serious problem in today. This is because of the antifungal drug resistance of the pathogen and the side effect exhibited by the drugs used to cure fungal diseases. Hence, there is a great demand for an efficient and safer treatment. Medicinal herbs could be an alternate source for the treatment of dermatophytosis. Herbal medicines are generally safer and free from side effects. Another important reason for this revival is that the effectiveness of many traditional medicines is now an accepted fact^[1].

This study showed that the leaf extracts of *Ocimum sanctum* has fungicidal activity. Few previous studies have comprehensively investigated the activity of medicinal plant leaves, bark extracts and oil against dermatophytes and other filamentous fungi^[19].

The MICs reported previously are based on broth micro dilution assay and NCCLS methods^[24]. Very few works were done on the fungicidal action of the plants *Ocimum sanctum*. The antifungal activity of other plant parts were also been reported earlier^[25].

Ethanol extract of *Azadiracta indica* leaves showed MIC and MFC at 250 μ g/mL concentration against *Trichophyton rubrum* and *Microsporum nanum*^[25]. Crude methanol extract of *Piper solmsianum* exhibited anti fungal activity

Table 1Minimum inhibitory concentration of *Ocimum sanctum* extracts and fractions(μ g/mL)(Mean \pm SD)(n=3).

Extract / Fraction	MIC				
	<i>Trichophyton mentagrophytes</i>	<i>Trichophyton rubrum</i>	<i>Microsporium gypseum</i>	<i>Microsporium nanum</i>	<i>Epidermophyton floccosum</i>
Aqueous extract	266.66 \pm 101.03	375.00 \pm 86.60	291.66 \pm 28.86	200.46 \pm 24.86	416.66 \pm 14.43
Alcoholic extract	166.66 \pm 014.43	383.33 \pm 28.86	366.66 \pm 38.11	383.33 \pm 14.43	366.66 \pm 57.73
Hexane fraction	150.00 \pm 025.00	400.00 \pm 00.00	400.00 \pm 25.00	400.00 \pm 00.00	383.33 \pm 52.04
Benzene fraction	200.00 \pm 000.00	383.33 \pm 14.43	408.33 \pm 38.18	416.66 \pm 28.86	425.00 \pm 25.00
Chloroform fraction	141.66 \pm 014.43	400.00 \pm 50.00	350.00 \pm 50.00	425.00 \pm 25.00	441.66 \pm 38.18
Ethylacetate fraction	191.00 \pm 014.43	391.63 \pm 38.18	383.33 \pm 28.86	383.33 \pm 28.86	416.66 \pm 28.86
Methanol fraction	125.00 \pm 025.00	350.00 \pm 66.14	375.00 \pm 25.00	441.66 \pm 38.18	383.33 \pm 14.43
Water fraction	208.33 \pm 014.43	333.33 \pm 38.18	400.00 \pm 25.00	366.66 \pm 38.11	425.00 \pm 66.14

Table 2Qualitative phytochemical analysis of *Ocimum sanctum* extracts and fractions.

Test	Extracts				Fractions			
	Aqueous	Ethanol	Hexane	Benzene	Chloroform	Ethyl acetate	Methanol	Water
Alkaloids	-	+	+	+	-	-	-	-
Steroids	+	+	-	+	+	+	+	-
Terpenoids	+	+	+	+	+	+	-	-
Flavonoids	+	+	-	+	+	+	+	+
Saponins	+	+	-	-	-	-	-	+
Phenolic compounds	+	+	-	-	-	-	+	+
Tannins	+	+	-	-	-	-	+	+
Lignin	+	+	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-
Fat and Oil	-	+	-	-	-	-	-	-
Inulin	+	+	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-
Proteins	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+
Aminoacids	+	+	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+	+	+

+: presence; -: absence.

against *Microsporium canis*, *Microsporium gypseum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Trichophyton rubrum*[26]. The studies of Dahanukar *et al*[27] showed that the ethanolic extract of *Azadiracuta indica* leaves demonstrated much more significant anti dermatophytic activity as compared with the aqueous extract and maximum antimycotic activity of *Eucalyptus globus* (88%), *Catharanthus roseus* (88%) followed by *Ocimum sanctum* (85.50%). The essential oil of *Melaleuca alternifolia* also called tea tree oil has increasing attention overseas as a natural topical antiseptic. Tea tree oil is known as an excellent treatment for fungal infections in general and vaginal candidosis and dermatophytosis in particular[28].

Some Indian plants have gained reputation in the treatment of fungal infections[1, 29], but no systematic study has been made so far to investigate their efficacy. Monica Bedi *et al*[30] reported that garlic (*Allium sativum*) and *Scontains ajoene* had antifungal activity. They have also reported that the tea tree oil has been widely used typically for the treatment of bacterial and fungal infections. They showed *in-vitro* activity against *Propionibacterium acnes*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

The present study revealed that *Ocimum sanctum* leaf extracts showed both inhibitory and fungicidal activity against the dermatophytes studied. Secondary metabolites

may be the reason for inhibition and fungicidal activity of this plant leaf extracts and fractions. TLC and HPTLC also confirmed the presence of these compounds. It is evident that the antidermatophytic activity of this plant may be due to secondary metabolites present in it. *Ocimum sanctum* plant leaf contains alkaloids, glycosides, saponins, tannins, volatile oil and ascorbic acid. Leaf wax contains *n*-alkenes. Essential oil consists of methyl chavicol, camphor, β -caryophyllene, eugenol, caryophyllene camphene, α -pinene *etc*. Antidermatophytic activity of *Ocimum sanctum* may be due to these metabolites.

However, the mechanism of *in-vitro* activity is unclear. But these plant leaves are still used as medicine in India. Till now there is no report about side effects of *Ocimum sanctum*. This plant may be a useful source for treating dermatophytic infections.

Conflict of interest statement

We declare that we have no conflict of interest.

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