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ORIGINAL RESEARCH ARTICLE

Comparative Antifungal Study of Essential Oil with Allopathic Drugs against *Malassezia furfur*

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ABSTRACT

The present study aim to evaluate the potential inhibitory effect of *Curcuma longa* (turmeric) oil on lipophilic, yeast like fungus *Malassezia furfur* which causes Pityriasis versicolor disease, a common superficial fungal skin disease. The antifungal activity of turmeric oil was screened by using disc diffusion method and microdilution method. Results indicated that, in screening of turmeric oil by disc diffusion method, the diameter of inhibition zone was found to be 55 mm which was greater than the inhibiton zone of reference antibiotics i.e streptomycin and gentamycin of 17 mm and 16.5 mm respectively. By using microdilution method, the Minimum inhibitory concentration (MIC) of turmeric oil was found to be 0.1µl/ml against *Malassezia furfur*. These findings provide promising information for the potential use of *Curcuma longa* (turmeric) oil as a traditional herbal medicine for the treatment of Pityriasis versicolor disease.

Keywords: Skin disease, Superficial, *Malassezia furfur*, *Curcuma longa*

INTRODUCTION

Pityriasis versicolor disease is a chronic, superficial fungal infection of the skin caused by the lipophilic, yeast like fungus *Malassezia.* This organism is saprophytic yeast that is part of the normal skin flora. *Malassezia* (formerly known as *Pityrosporum*) is a genus of related fungi, classified as yeasts naturally found on the skin surfaces of many animals including human beings. Common names of Pityriasis versicolor disease are Tinea flava, Dermatomycosis furfuracea and Tinea versicolor[7]. *Malassezia furfur* is the causative agent of Pityriasis versicolor disease. High temperature, humidity, use of oils and hyperhidrosis are the main factors responsible for the occurrence of this disease. Tinea versicolor is world wide in its distribution, but is said to be more common in tropical and temperate climates. Versicolor refers to the variety in changing shades of colors present in this disease. Pityriasis versicolor occurs most commonly in adolescents and young adults, but can occur in children and infants as young as two weeks of age. Tinea versicolor also occurs commonly in patients who are immune-suppressed[1]. It is most evident in the summer because the organism produces azelaic acid, which inhibits pigment transfer to keratinocytes, thereby making infected skin more demarcated from uninfected, evenly pigmented skin. The colour varies according to the normal pigmentation of the patient, exposure of the area to sunlight, and the severity of the disease. Affected areas include the back, chest, abdomen, neck, and upper limbs. The most common symptoms of Pityriasis versicolor disease are light brown or white patches on the skin. These patches are most noticeable during summer season. Lesions occur on the trunk, shoulders and arms, rarely on the neck and face, and fluorescence a pale greenish colour under Wood's ultra-violet light. Superficial mycoses of the skin are among the most common dermatological infections, and causative organisms include dermatophytic, yeasts, and non-dermatophytic filamentous fungi. Numerous essential oils have been tested for *in-vitro* as well as *in-vivo* antifungal activity and some pose much potential as antifungal agents. Herbs and spices are generally considered safe and proved to be effective against certain

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ailments. Essential oils and their consitituents have a long history of applications as antimicrobial agents. Production of essential oils by plants is believed to be predominately a defense mechanism against microorganisms and indeed essential oils have been shown to possess antimicrobial and antifungicidal properties. Essential oils and their components are gaining importance because of their relatively safe status and their wide acceptance by the consumers. The rhizome of turmeric ***(Curcuma longa)*** has a rich history in India as spice, food preservative, and coloring agent and has been used for centuries. The continuing research indicates that turmeric and its active principle curcumin have unique antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory and antimicrobial properties[11]. Turmeric composed with atleast 7% of a yellow volatile oil containing tumerone and zingiberene as major constituents and sesquiterpenes and monoterpenes types of compounds, yellow colouring material including curcumin (1.8-5.4%), bisdemethoxycurcumin and turmeric oil exhibit antifungal activity against various dermatophytes and other fungi[2]. Turmeric oil inhibits dermatophytes and pathogenic molds *in vitro* but its main component curcumin has no antifungal activity[3]. Investigations concerning the evaluation of the biological activities of essential oils of some medicinal plants have revealed that some of them exhibit antibacterial, antifungal and insecticidal properties[4]. Because of the antimicrobial properties of essential oils, the aromatherapy has been used for treatment of serious skin diseases, in special, superficial mycoses[8]. Our present study analyze the inhibitory effect of turmeric oil against *Malassezia furfur* for economic, natural and safe antifungal drug without any side effects and as an alternative for expensive allopathic medicines.

MATERIALS AND METHODS

Collection of samples and identification

Skin scrapings from patients with superficial lesions were collected from Department of Dermatology, Venerology and Leprology, E.S.I.C. hospital, Jaipur. All the samples were collected in sterilized plastic bags. The preliminary microscopic examination of the material was done immediately. For this small portions of infected skin sample were examined under microscope for presence of yeast cells or hyphal fragments. Following to this, additional points were also recorded like sex (male or female), age of patient, nature of infection, occupation; symptoms, climatic influence, clothing and condition of personal hygiene were recorded for para-clinical data. Remaining infected skin samples were then transferred in triplicates on Sabouraud's dextrose agar slants. These cultures were maintained at 28±2^{O}C temperature in B.O.D incubator for further growth. After 7 days, the process of identification and biochemical test (catalse and urease) was performed for confirmation of *Malassezia species*.

Extraction of oil

In winter season, extraction of oil from the fresh rhizome of *Curcuma longa* (turmeric) was carried out by standard hydrodistillation method (Clevenger's apparatus) and all operations were carried out at room temperature. The fresh rhizomes of turmeric were washed to remove soil, peeled and sliced. Sliced rhizomes of fresh turmeric (250gm) were placed in a flask together with distilled water (1L). After 5-6 hours, oil was collected from the apparatus, anhydrous with sodium sulphate for removal of water traces, then this 100% pure essential oil were dispensed into dark bottles and stored at 4ºC until used. Essential oil was ready to use for disc diffusion test and determination of MIC.

Culture preparation

Culture of *Malassezia furfur* was maintained on a Sabouraud's Dextrose Agar at 28^{O}C for 48 hours. A loopful of 48 hours surface growth of yeast was transferred to 0.9% NaCl solution, vortex it and homogenous suspension was used for inoculation. Turbidity was adjusted to match that of a 5 McFarland standard.

Screening of essential oil using disc diffusion method

Oil was screened for their antifungal activity against *Malassezia furfur* by disc diffusion method[13]. Fresh culture of yeast used for inoculum preparation. Using a sterile cotton swab, yeast culture was swabbed on the surface of sterile Sabouraud's Dextrose Agar plates. Filter paper discs of 6 mm diameter were prepared and sterilized. Sterilized filter paper discs were soaked in neat, undiluted (100%) concentration of *Curcuma longa* (turmeric) oil. An oil-saturated disc was placed on an agar plate containing test organism. Similarly, standard antibiotic discs of gentamycin (30mcg/disc) and 10mcg/disc of streptomycin, clotrimazole and ketoconazole were also aseptically placed over the seeded Sabouraud's Dextrose Agar plates as standard drugs for comparison of antifungal activity of turmeric oil. The plates were incubated at 35^{O}C ±

2^{O}C for 24 hours. The diameter of the inhibition zones was measured in mm and the activity index was calculated on the basis of the size of the inhibition zone. The activity of turmeric oil was measured by the following formula:-

Activity index = Inhibition zone of sample / Inhibition zone of standard

Determination of Minimum inhibitory concentration using microdilution method

The modified microdilution method was followed to determine MIC[12]. Media used for MIC was semisolid agar media (Brain Heart Infusion Agar) aliquots of semisolid agar media (Bacto Agar; Difco Laboratories) at a pH of approximately 7.4 were poured into a 16- by 125-mm glass tubes and autoclaved. Different concentrations of turmeric oil were added in media containing test-tubes, afterwards a standard platinum loopful (~0.001 ml, Himedia, Flexiloop) of the inoculum suspension was inserted deep into each tube of medium containing a different concentration of oil, as well as a oil-free control, by a centered down-up motion to form a two dimensional inoculum. The tubes were then incubated at 30^{O}C for 48 hours to determine the MIC. MIC was read to be the lowest concentration at which there was no visible growth of the organism. Then, by visual inspection, good growth of the yeast in oil-free medium as a control was detected (within 48 h for yeasts) afterwards, the growth in all tubes at different concentrations of turmeric oil was compared with that of the oil-free control in order to determine inhibition.

RESULTS AND DISCUSSION

Colonies raised on Sabouraud's Dextrose Agar slants showed cream to yellow colour. They were smooth initially and got dried and wrinkled with time. The yeast grew rapidly and matured in 5 days at 30-37^{O}C. Characteristic clusters of thick-walled, round, budding yeast like cells; short angular hyphal fragments were upto 8µm in diameter. These microscopic and macroscopic features and special lipid requirement in media are diagnostic characters of *Malassezia furfur*. Biochemical tests like catalse and urease were used to identify the presence of *Malassezia furfur*. Both the tests were positive for *Malassezia furfur*. In this work, the antifungal activity of turmeric oil against *Malassezia furfur* was studied and the results obtained showed the important antifungal activity of turmeric oil. *Malassezia furfur* is an important pathogen of human skin infections. Different yeasts were tested and the antifungal activity of turmeric oil was evaluated against *Malassezia furfur*. The results of the present work on the antifungal activity of turmeric oil against *Malassezia furfur* studied by two methods are presented in (**Table 1 & 2**). It is to be noted that the antifungal activity of turmeric oil obtained by disc diffusion method (Table 1) is double than that of standard reference drugs i.e gentamycin, streptomycin, clotrimazole and ketoconazole. The diameter of the inhibition zone obtained against turmeric oil at concentration of 100% pure oil was 55 mm. *Malassezia furfur* was found to be resistant against clotrimazole and ketoconazole. Other reference drugs i.e gentamycin and streptomycin showed inhibition zone of 16.5 mm and 17 mm respectively. Now we consider table-2 in which the MIC of turmeric oil against *Malassezia furfur* is presented. The results show that the turmeric oil exhibited inhibitory action at 0.1 to 2µl/ml concentrations against *Malassezia furfur*. Even after 4 days, no growth was observed at that low concentrations and control taken without oil showed 100% growth of *Malassezia furfur*. According to our study, by comparing with the reference drugs, turmeric oil was found to be more effective in inhibiting the growth of *Malassezia furfur*. Therefore turmeric oil can be used as a natural antifungal agent against *Malassezia furfur*, the causal organism of Pityriasis versicolor infection.

Table 1: Antifungal activity of turmeric oil against *Malassezia furfur*

Test Strain	Concentration of turmeric oil in %	IZ of sample (Turmeric oil)	IZ of standard (Gentamycin)	AI	IZ of standard (Streptomycin)	AI
Malassezia furfur	100%	55mm	16.5mm	3.33	17mm	3.23

***IZ=** Inhibition zone, including 6mm diameter of the filter paper disc; **AI=** Activity Index.*

Table 2: *MIC of turmeric oil against Malassezia furfur*

Test Strain	Different concentrations of turmeric Oil used in µl/ml	Growth visually inspected in different concentrations of oil
Malassezia furfur	0.02	+4
	0.04	+3
	0.06	+2
	0.08	+1
	0.1	0
	0.3	0
	0.5	0

0.7	0
0.9	0
1.1	0
1.3	0
1.5	0
1.7	0
1.9	0
Control without oil	100% growth

Growth was scored in the following manner:
4+, *growth comparable to that of the oil free control;* ***3+****, growth approximately 75% that of the control;* ***2+****, growth approximately 50% that of the control;* ***1+****, growth 25% or less that of the control; and* ***0****, no visible growth*

The present results suggest that turmeric oil exhibits strong antifungal activity. This is in agreement with the findings of Valero and Frances (2006) where the essential oils derived from many plants are known to exhibit antifungal activities[16]. Numerous essential oils have been tested for *in -vivo* and *in-vitro* antimycotic activity and some have demonstrated to be potential antifungal agents. Their mechanism of action appears to be predominately on the fungal cell membrane, disrupting its structure causing leakage and cell death; blocking the membrane synthesis; inhibition of the spore germination, fungal proliferation and cellular respiration[8]. Herbal drug preparation containing rhizome powder cured ringworm infection caused by *Trychophyton verrucosum* in 12 cattles and *Microsporum canis* in 21 dogs within 12-15 days of treatment[14]. Many *Curcuma* species are traditionally used for their medicinal properties. Antifungal, antibacterial and anti-inflammatory activity has been reported for species such as *Curcuma longa, Curcuma zedoaria, Curcuma aromatic* and *Curcuma amada*[2]. In our study, essential oil of turmeric extracted by hydrodistillation method exhibited the strong antimycotic activity against *Malassezia furfur*. In screening of turmeric oil, the diameter of inhibition zone by disc diffusion method was found to be 55 mm at 100% concentration of pure oil. Our work is in agreement with the observations of Wuthi-udomlert *et al* (2000) who reported the antifungal activity of turmeric oil against 29 clinical strains of dermatophytes and in screening of turmeric oil, diameter of inhibition zone was found to vary from 26.1 mm to 46 mm against 29 clinical strains of dermatophytes[17]. In our findings, MIC of turmeric oil obtained by microdilution method was 0.1µl/ml. Our results of MIC of turmeric oil are similar to those of Wuthi-udomlert *et al (*2000) who also observed antidermatophytic activity of turmeric oil against dermatophytes but with a difference in MIC values[17]. Those differences are possibly due to different composition of plant oils which varies according to local climatic and environmental conditions. Second, the medium used to assess antimicrobial activity and variation in the choice of test microorganism used in the present study. Our results of MIC of turmeric oil also coincide with the findings of Dhingra *et al* (2007) who reported that the MIC of turmeric oil against *Aspergillus flavus, Fusarium semitectum, Colletotrichum gloeosporioides and Colletotrichum musae* was 0.1%. Our work also agrees with the findings of Singh *et al* (2003) on the antifungal activity of *Curcuma longa* oil, which causes complete inhibition of mycelial growth of *Colletotrichum falcatum, Fusarium moniliforme, Fusarium oxysporum and Aspergillus niger.* Lai *et al* (2004) reported the antimicrobial activity and cytotoxicity of essential oil of *Curcuma zedoaria* against gram positive and gram negative pathogenic microorganisms[10,15].

It is interesting to note that the turmeric oil possessed strong free radical scavenging activity and reducing power as compared to standard antioxidants[18]. The results of our present study coincide with the findings of most of the earlier works[5,17] which also revealed that turmeric oil shows better inhibitory action against microorganisms than crude ethanol extract. These results suggest the use of turmeric oil in the formulation should improve skin infection and could be considered for scavenging free radicals from inflammatory conditions. Additionally, pre-clinical studies have been conducted with ointments containing essential oils, including *Trachyspermum ammi*[9] and *Curcuma longa*[2]. Natural products of plant origin have played significant role in the search for therapeutic drugs. Search for new antimcrobials is very important in recent times, due to tremendous increase of drug resistance in diverse pathogenic microorganisms.

In the present study *Curcuma longa* oil was observed as a very good inhibitor of *Malassezia furfur*. This study is a preliminary evaluation of

antifungal activity of turmeric oil for its possible use in preparation of herbal formulations. The results from this study support the antifungal activity of turmeric oil. Moreover it may support the use of *Curcuma longa (*turmeric) oil for treatment of fungal diseases or prevention of fungal growth. This herbal oil can be used in topical formulations and effective alternative for allopathic medicines in near future.

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