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Full Length Research Paper

Additive and inhibitory effect of antifungal activity of *Curcuma longa* (Turmeric) and *Zingiber officinale* (Ginger) essential oils against *Pityriasis versicolor* infections

Richa Sharma[1]*, Gajanand Sharma[2] and Meenakshi Sharma[3]

[1]Lab No-1, Laboratory of Mycology and Microbiology, Department of Botany, University of Rajasthan, Jaipur, India.
[2]Department of Chemical Sciences, Suresh Gyan Vihar University, Jagatpura, Jaipur, India.
[3]Lab No-1, Laboratory of Mycology and Microbiology, Department of Botany, University of Rajasthan, Jaipur, India.

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The antifungal potential of *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) essential oils alone and in combinations, against common causes of *Pityriasis versicolor* infections in humans was investigated via *in vitro* investigations, in order to determine a suitable dosage for use in clinical trials. The antifungal activity of oils was screened against *Malassezia furfur* by using disc diffusion method and microdilution method. Turmeric oil showed strong antifungal activity (55 mm, MIC 0.1 µl/ml) while ginger oil had good antifungal activity (37.5 mm, 0.03 µl/ml) and their mixture showed excellent antifungal activity (65 mm, MIC 0.02 µl/ml) against *M. furfur*. The inhibition zone of mixture of oils (turmeric and ginger) is higher than single oils and reference antibiotics. Gentamycin and Streptomycin showed inhibition zone of 16.5 and 17 mm against *M. furfur*. From our findings, the results provide a scientific validation for the use of these essential oils in the treatment of dermatophytic infections and could be used in future for the development of anti-skin disease agents.

Key words: *Pityriasis versicolor*, *Curcuma longa* and *Zingiber officinale* oils.

INTRODUCTION

Pityriasis versicolor (PV) is a superficial mycosis, affecting the superficial layer of stratum corneum (Marcon and Powell, 1992). The causative organism is Malassezia furfur, a yeast like lipophilic fungus. PV is common in the postpubertal age where sebaceous glands are active and in individuals who sweat more (Schmidt, 1997). There is often a positive family history of the disease. An increase in humidity, temperature and hyperhidrosis are important predisposing factors (Silva-Lizama, 1995; Boussida et al., 1998). 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 (Petmy et al., 2004). Because of the antimicrobial properties of essential oils, the aromatherapy has been used for treatment of serious skin diseases, in special, superficial mycoses (Burt, 2004). In the present study, antifungal activities of turmeric and ginger oils were investigated with the aim to discover the medicinal potential of these oils for future application as an antifungal agent for *P. versicolor* disease.

*Corresponding author. E-mail: richa.phd.15@gmail.com.

MATERIALS AND METHODS

Malassezia furfur was isolated from infected skin scrapings of tinea patients at the E.S.I.C. hospital, Jaipur and maintained on a Sabouraud's Dextrose Agar media and identified by microscopy and various biochemical tests.

Extraction of oil

Fresh rhizomes of turmeric and ginger were purchased from local market of Sodala, Jaipur in the month of October to December, 2009. In winter season, extraction of oil from the fresh rhizome of

Curcuma longa (turmeric) and *Zingiber officinale* (ginger) were carried out by standard hydrodistillation method, Clevenger's apparatus and all operations were carried out at room temperature (Clevenger, 1928). The fresh rhizomes of turmeric and ginger were washed to remove soil, peeled and sliced. Sliced rhizomes of fresh turmeric and ginger (250 g) were placed in a separate flask together with distilled water (1 L). After 5 to 6 h, 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 4℃ until used. Essential oil was ready to use for disc diffusion test and determination of minimum inhibitory concentration (MIC).

Screening of essential oil using disc diffusion method

Oil was screened for their antifungal activity against *M. furfur* by disc diffusion method (Rios et al., 1988). Standard size Whatman No. 1 filter paper discs, 6.0 mm in diameter, sterilized by dry heat at 140℃ in an oven for one hour were used to determine antifungal activity. SDA medium for disc diffusion test was prepared. After sterilization, it was poured into sterilized petri plates and allowed to solidify. Then, one day old fresh culture of yeast will be used for inoculum preparation. A suspension that was just turbid (~0.5 McFarland standard) by visual inspection was prepared by suspending yeast in 0.9% NaCl solution and the homogeneous suspension was used for inoculation. Using a sterile cotton swab, yeast culture were swabbed on the surface of sterile Sabourauds dextrose agar plates. Sterilized filter paper discs were soaked in neat, undiluted (100%) concentration of oils. Using an ethanol dipped and flamed forceps, oil saturated discs of 100% concentration were aseptically placed over Sabourauds dextrose agar plates seeded with the respective test microorganism. The antibiotic discs of gentamycin (30 mcg), streptomycin, clotrimazole and ketoconazole (10 mcg disc-1) were also aseptically placed over the seeded Sabourauds dextrose agar plates as a standard drugs for comparison of antifungal activity of lemon and orange oils. The plates were incubated at 37℃ for 24 h. The diameter of the inhibition zones was measured in millimetre. Three replicates were kept in each case and average values were calculated. The activity of oils was measured by the following formula:

AI (Activity index) = Inhibition zone of sample / Inhibition zone of standard

Determination of minimum inhibitory concentration using microdilution method

The modified microdilution method Provine and Hadley (2000) was followed to determine MIC. 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. A suspension that was just turbid (~0.5 McFarland standard) by visual inspection was prepared by suspending the selected fungi in 0.9% NaCl solution, vortexing, and homogeneous suspension was used for inoculation. Different concentrations of lemon and orange oils 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 oils, as well as a oilfree control, by a centered down-up motion to form a two dimensional inoculum. The tubes were then incubated at 30℃ for 48 h 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 respective fungi in oil-free medium as a control was detected (48 h for yeasts). Afterwards, the growth in all tubes at different concentrations of lemon and orange oil were compared with that of the oil free control in order to determine inhibition.

RESULTS

In our present work, *M. furfur* was found main etiological agent of *P. versicolor* disease hence the antimycotic studies was carried out on *M. furfur*. The conventional treatment of fungal disease is limited, and part of the reason is due to the limited spectrum of the currently antifungal drugs, and the expensive treatment, particularly due to the need of prolonged therapy. Thus, new drugs and alternative therapies are necessary, including natural products. We report here the antimycotic study of lemon and orange oil against *M. furfur in vitro*. The results of the present work on the antifungal activity of turmeric and ginger oil against *M. furfur* studied by two methods, that is, disc diffusion and microdilution method are presented in Tables 1, 2 and 3.

In our study, turmeric and ginger oils presented higher diameter of inhibition zones than gentamycin, streptomycin, clotrimazole and ketoconazole. The diameter of the inhibition zone obtained against turmeric, ginger and mixture of oils (turmeric+ginger) at 100% concentration of pure oil was 65 mm, respectively by disc diffusion method. The inhibition zone of mixture of oils (turmeric and ginger) is higher than single oils and reference antibiotics, that is, *M. furfur* was found to be resistant against clotrimazole and ketoconazole.

Other reference antibiotics, that is, gentamycin and streptomycin showed inhibition zone of 16.5 and 17 mm, respectively. According to our study by comparing with the reference drugs, turmeric and orange oil was found to be more effective in inhibiting the growth of *M. furfur*. Results of MIC of turmeric and ginger oils against *M. furfur* are summarized in Tables 2 and 3. The results show that turmeric, ginger and mixtures of oil exhibited inhibitory action at 0.1, 0.03 and 0.02 µl/ml concentrations against *M. furfur*. Both oils can be used for the development of natural antifungal agent against *P. versicolor* infections.

DISCUSSION

The traditional use of plants as medicines provide the basis for indicating which essential oils may be useful for specific medical conditions. It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Mitscher et al., 1987). Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required (Harris, 2002). Various publications have documented the antimicrobial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil,

Table 1. Antifungal activity of *C. longa* (turmeric), *Z. officinale* (ginger) and mixture of oils against *M. furfur*.

Oil	Test strain	IZ of sample (mm)	AI (gentamycin)	AI (streptomycin)
Turmeric	*M. furfur*	55	3.33	3.23
Ginger	*M. furfur*	37.5	2.27	2.21
Mix of turmeric and ginger	*M. furfur*	65	3.94	3.82

Concentration of oil used 100%; IZ of standard gentamycin and streptomycin against *M. furfur* was 16.5 mm and 17 mm; Control showed growth of *M. furfur;* Here IZ = Inhibition zone (in mm) including the diameter of disc (6mm); AI = Activity index

Table 2. MIC of *C. longa* (turmeric) oil against *M. furfur.*

Test strain	Different concentrations of turmeric oil used in µl/ml	*M. furfur*
M. furfur	0.02	+4
	0.04	+3
	0.06	+2
	0.08	+1
	0.1	0
	0.2	0
	0.4	0
	0.6	0
	0.8	0
	1.0	0
	1.2	0
	1.4	0
	1.6	0
	1.8	0
	2.0	0
	Control without oil	100% growth

Table 3. MIC of *Z. officinale* (ginger) oil against *M. furfur.*

Test strain	Different concentrations of turmeric oil used in µl/ml	*M. furfur*
M. furfur	0.02	+4
	0.03	+3
	0.04	+2
	0.05	+1
	0.06	0
	0.07	0
	0.08	0
	0.09	0
	0.1	0
	Control without oil	100% growth

tea tree, celery seed and fennel (Lis-Balchin and Deans, 1997). Herbal drug preparation containing rhizome powder cured ringworm infection caused by *Trychophyton verrucosum* in 12 cattles and *Microsporum canis* in 21 dogs within 12 to 15 days of treatment (Sharma and Dwivedi, 1990).

Many Curcuma species are traditionally used for their medicinal properties. Antifungal, antibacterial and anti-inflammatory activity has been reported for species such as *C. longa, Curcuma zedoaria, Curcuma aromatic* and *Curcuma amada* (Apisariyakul, 1995). In our study, essential oils of turmeric and ginger and their mixtures displayed the strong antifungal activity against *M. furfur*. In screening of turmeric, ginger and their mixtures of oil, the diameter of inhibition zone by disc diffusion method (55, 37.5 and 65 mm) against *M. furfur* at 100%

concentration of pure oils were observed. 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. Our findings also coincides with the Bansod and Rai (2008) who reported the antifungal activity of ginger oil against human pathogenic *A. niger* and *A. fumigatus* and in screening of ginger oil, diameter of inhibition zone was found to be 14 and 15 mm against *A. niger* and *A. fumigatus* (100 µg/disc).

In the screening of mixture of oils, diameter of inhibition zone was found to be 65 mm against *M. furfur*. MIC of mixture of oils against *M. furfur* was 0.02 µl/ml. Our findings are similar to Prasad et al. (2008) who found the synergistic antifungal efficacy of essential oils of *Cymbopogon martini, Chenopodium ambrosiodies* and their combinations against dermatophytes and some filamentous fungi *in vitro*. Our work also coincides with the findings of Cassella et al. (2002) who studied the antifungal potential of tea tree and lavender essential oils alone and in combinations against *Trichophyton rubrum* and *Trichophyton mentagrophytes* and effective inhibition by mixture of oils than a single oils alone. In our findings, MIC of turmeric and ginger oil against *M. furfur* was obtained by microdilution method which are in very low concentrations.

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. That differences are possibly due to the medium used to assess antimicrobial activity and variation in the choice of test microorganism used in the present study. Our work concides with the findings of earlier workers who reported that the turmeric oil inhibits dermatophytes and pathogenic molds *in vitro* but its main component curcumin has no antifungal activity. Our work also coincides with the previous findings of Singh et al. (1980) who studied the antimicrobial activity of essential oil and oleoresins of *Zingiber officinale* against fungal and bacterial species, ginger oil was found to be more effective than its component oleoresins. Our findings are similar to work of Nanasombat and Lohasupthawae (2005) who found that the inhibitory activity of ginger oil was greater than the ethanolic extracts of ginger on bacterial strains. From the results, it is evident that *C. longa* (turmeric), *Z. officinale* (ginger) and mixture of oils possess potential inhibitory activity against *T. rubrum* and *M. gypseum in vitro*. The activity of mixture of oils were higher than those of single oils and standard antibiotics. The antifungal activity of combinations of two essential oils indicated their additive, synergistic or antagonistic effects against individual microorganism tested. Finally, this study confirms that mixture of oils possess higher antifungal activity and can be used to cure dermatophytic infections and may potentiate the efficacy of chemotherapeutics and may have role as a herbal, traditional medicine, pharmaceuticals in the treatment of superficial fungal infections of the skin.

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