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ACTIVITY OF GINGER OIL AGAINST LIPOPHILIC YEAST LIKE FUNGUS *MALASSEZIA*

MEENAKSHI SHARMA, RICHA SHARMA* and SUNITA MAHARIYA
Laboratory of Mycology and Microbiology, Department of Botany, University of Rajasthan, Jaipur-302004, India.
*E-mail : richa.phd.15@gmail.com

Activity of ginger oil *(Zingiber officinale)* on yeast like fungus *Malassezia furfur* which causes Pityriasis versicolor, chronic superficial fungal disease of the skin have been studied by using two different methods: Disc diffusion and Microdilution methods. Results indicated that, in screening of ginger oil by disc diffusion method, the diameter of inhibition zone was found to be 37.5 mm which was greater than the diameter of inhibition zone of reference antibiotic i.e 16.5mm. In Microdilution method, the MIC of ginger oil was found to be 0.03µl/ml against *Malassezia furfur*. These findings support the use of *Zingiber officinale* (ginger) oil as a traditional medicine for the treatment of Pityriasis versicolor disease.

Keywords: Ginger Oil; *Malassezia furfur*; Pityriasis versicolor; Skin; *Zingiber officinale.*

Introduction

Pityriasis versicolor (Tinea versicolor) is a chronic, superficial fungal disease of the skin caused by the lipophilic, yeast like fungus *Malassezia.* This organism is a 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. Synonyms of this disease are Pityriasis versicolor, Tinea flava, Dermatomycosis furfuracea and Tinea versicolor. *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. Versicolor refers to the variety and changing shades of colors present in this disease. Affected areas include the back, chest, abdomen, neck, and upper limbs. The most common symptom of Pityriasis versicolor are light brown or white patches on the skin. These patches are most noticeable during summer season. Infectious diseases accounts for high proportion of health problems in the developing countries including India. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created[1]. The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease. This situation forced the researchers to search for new antimicrobial substance from various sources including medicinal plants[2]. There are alarming reports of opportunistic fungal infections[3]. There is an increasing awareness amongst clinicians and microbiologists pertaining to importance of infection caused by opportunistic fungi[4]. In folk medicine, medicinal herbs and plant products were used in treating a wide spectrum of infections and other diseases. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries, because plant-derived drugs have been reported to be safe and without side-effects. A survey of literature reveals that there are many essential oils which possesses antifungal activity. Treatments contains high doses of antibiotic due to resistance and side effects of this antibiotic, patients take more time for cure. Therefore, we need to search plant derived antifungal drugs which are safe and without side-effects. Hence, it is of interest to determine the scientific basis for the traditional use of medicinal plants. The herbal medicines may be in form of powders, liquids, or mixtures, which may be raw or boiled, ointments, liniments, and incisions[5]. In diseases of microbial origin, the plants function as a result of antimicrobial activity against the causative agents[6]. Zingiberaceae is among the plant families that are widely distributed throughout the tropics, particularly in Southeast Asia. It is an important natural resource that provides man with many useful products for food, spices, medicines, dyes, perfume and aesthetics[7]. The term '*Zingiber*' is derived from the Sanskrit word '*shringavera*', owing to their 'horn-shaped' rhizomes.

volatile oils and are used in traditional medicine and as spices. The ethnomedical and pharmacological activities of *Z. officinale* have been reviewed by various authors[8-10]. Ginger essential oil is utilized for bactericidal, fungicidal, respiratory tract infections, simulative and medicine effects. In recent years, several reports have been published concerning the composition and/or the biological properties (antimicrobial, antioxidant, anticancer and a stimulated effect on the immune system) of Zingiberaceae extracts. These studies have emphasized the existence of marked chemical differences among oils extracted from different species or varieties.

These variations are likely to influence the antimicrobial activity of the oil and are generally a function of three factors i.e genetically determined properties, the age of the plant and the environment. In the present study, antifungal activities of ginger oil were investigated with the aim to discover the medicinal potential of this oil for future application as a anti-*Malassezia furfur* agent in Pityriasis versicolor disease.

Material and Methods

Oil of *Zingiber officinale* (ginger) were tested for their antifungal activity against test organism *Malassezia furfur*, the causal organism of Pityriasis versicolor infection.

Extraction of oil- In winter season, extraction of oil from the fresh rhizome of *Zingiber officinale* (ginger) were carried out by standard hydrodistillation method and all operations were carried out at room temperature. The fresh rhizomes of ginger were washed to remove soil, peeled and sliced. Sliced rhizomes of fresh ginger (250gm) were placed in a flask together with distilled water (1L). After hydrodistillation, 100% pure essential oil were collected, dispensed into dark bottles and stored at 4°C until used. Essential Oil were ready to use for disc diffusion test and determination of minimum inhibitory concentration (MIC).

Culture preparation- Fresh culture of yeast, *Malassezia furfur* were maintained on a Sabouraud's Dextrose Agar for inoculum preparation. A loopful of 24 hr surface growth of yeast was transferred to 0.9% NaCl solution, vortex 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 were screened for their antifungal activity against *Malassezia furfur* by disc diffusion method[11]. Fresh culture of yeast was used for inoculum preparation. Using a sterile cotton swab, yeast culture were swabbed on the surface of sterile Sabourauds Dextrose agar plates. Filter paper discs of 6 mm diameter were prepared and sterilised. Using an ethanol dipped and flamed forceps, oil saturated discs of 100% concentration were aseptically placed over Sabouraud's Dextrose agar plates seeded with the respective test microorganism. The antibiotic discs of gentamycin (30mcg/disc), clotrimazole (10mcg/disc) and ketoconazole (10mcg/disc) were also aseptically placed over the seeded Sabourauds Dextrose agar plates as a standard drugs for comparison to antifungal activity of ginger oil. The plates were incubated at 37°C for 24 hours. The diameter of the inhibition zones was measured in mm and the activity rated on the basis of the size of the inhibition zone.

Determination of the Minimum inhibitory concentration using microdilution method - The modified microdilution method Weseler *et al*[12] 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 16x125 mm glass tubes, autoclaved ,different concentrations of ginger 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°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 ginger oil was compared with that of the oil-free control in order to determine inhibition.

Results and Discussion

The results of the present work on the antifungal activity of ginger oil against *Malassezia furfur* studied by two methods are presented in Table 1 and 2. It is to be noted that the antifungal activity of ginger oil obtained by disc diffusion method (Table 1) is double than that of standard reference drugs. The diameter of the inhibition zone obtained against ginger oil at concentration of 100% pure oil was 37.5mm. In our study, ginger oil presented higher diameter of inhibition zones than standard drugs i.e gentamycin, clotrimazole and ketoconazole. Gentamycin showed the diameter of inhibition zone i.e 16.5mm. Clotrimazole and ketoconazole was found to be resistant against *Malassezia furfur*. Thus, ginger oil can be considered to be more effective than reference drugs tested. Hence, ginger oil can also be used as an efficient

and useful herbal drug against *Malassezia furfur*. In Table 2 the MIC of ginger oil against *Malassezia furfur* is presented. The results show that the ginger oil exhibited inhibitory action at 0.03 to1µl/ml concentrations against *Malassezia furfur* even after 4 days, no growth observed at that low concentrations. Therefore ginger oil can also be used as a natural antifungal agent against *Malassezia furfur*, the causal organism of Pityriais versicolor infections.

Table 1. Antifungal activity of ginger oil against *Malassezia furfur.*

Test Strain	Concentration of ginger oil in %	IZ of sample (ginger oil)	IZ of standard (Genta-mycin)	Activity index (AI)
Malassezia furfur	100%	37.5mm	16.5mm	2.27

Here ,Activity index (AI)=IZ of sample / IZ of standard
Where IZ=Inhibition zone (in mm) including the diameter of disc (6mm)

Table 2. MIC of ginger oil against *Malassezia furfur.*

Test Organism	Different concentrations of Ginger Oil used in µl/ml	Growth visually inspected in different concentrations of oil
Malassezia furfur	0.01	+2
	0.02	+1
	0.03	0
	0.04	0
	0.05	0
	0.06	0
	0.07	0
	0.08	0
	0.09	0
	0 .2	0
	0 .4	0
	0 .6	0
	0.8	0
	0.9	0
	1.00	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 ginger oil exhibits strong antifungal activity. This is in agreement to findings where the essential oils have been found to exhibit narrow as well as wide ranges of activity. The oil of ginger grass oil were toxic to *Pacielomyces sp, Fusarium solani, Fusarium moniliformis, Cornespora casicola* and various human pathogens[13]. Sabulal *et al.*[14] reported the antimicrobial activity of oil extracted from the rhizome of *Zingiber nimmonii* and oil showed significant inhibitory activity against human pathogenic fungi *Candida glabrata, C. albicans* and *Aspergillus niger* and the bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa*. In present study, essential oil of ginger extracted by hydrodistillation exhibited the strong antimycotic activity against *Malassezia furfur*. In screening of ginger oil, the diameter of inhibition zone by disc diffusion method was found to be 37.5 mm at 100% concentration of pure oil. This is in agreement with the observations of Bansod and Rai[15], who reported the antifungal activity of ginger oil against human pathogenic *Aspergillus niger* and *Aspergillus fumigatus* and in screening of ginger oil, diameter of inhibition zone was found to be 14mm and 15mm against *A.niger and A.fumigatus* (100µg/disc). In our findings, MIC of ginger oil obtained by microdilution method was 0.03µl/ml. The results of MIC of ginger oil are comparable to that of Barbosa *et al.*[16] who found that the MIC of ginger oil against gram negative strains (*Escherichia coli* and *Salmonella Enteritidis*) was 0.56% v/v. The 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 findings are similar to work of Nanasombat and Lohasupthawae[17] who found that the inhibitory acivity of ginger oil was greater than the ethanolic extracts and ginger oil seemed to be a more potent inhibitor to most bacterial strains than garlic oil. Ginger oil showed great inhibitory effect (4.2µl/ml) to *S. choleraesuis, S. senftenberg* and *E. coli* while garlic oil exhibited less inhibitory activity (16.4µl/ml) against *S. typhimurium*. Norajit *et al.*[18] also found that essential oil of ginger extracted by hydrodistillation had the highest efficiency against three positive strains of bacteria (*S.aureus, B.cereus and L. monocytogenes*), with a minimum concentration to inhibit B.*cereus* and *L.monocytogenes* of 6.25mg/ml. Present work is also in

agreement with the findings of Bansod and Rai[15] who reported the MIC of ginger oil by broth microdilution method to be >4%v/v against *Aspergillus niger* and *Aspergillus fumigatus*. Present work also coincides with the previous findings of Singh *et al.*[19] 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.

The present study thus confirms that the ginger oil possesses *in vitro* antifungal activity. Further the ginger oil in present study was found to be more effective against *Malassezia furfur* than the reference standard drugs. Ginger oil was found to be most effective in both the disc diffusion and microdilution methods. These results support that the ginger oil can be used to cure mycotic infections and may potentiate the efficacy of chemotherapeutics and may have role as a herbal, traditional medicine in the treatment of fungal infections.

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