



Original Article

Antifungal Screening of *Curcuma longa* and *Zingiber officinale* against Dermatophytes Causing Superficial Mycosis

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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 dermatophytic 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 *Trichophyton mentagrophytes* and *Microsporum audouinii* by using disc diffusion method. *C. longa* (turmeric) oil showed good antifungal activity (55 mm and 57 mm), *Z. officinale* (ginger) oil had also good antifungal activity (69 mm and 59 mm) against *T. mentagrophyte* and *M. Audouinii* respectively as compared to reference antibiotics i.e. Clotrimazole (39 mm against *T. mentagrophyte* & 45 mm against *M. audouinii*) Ketoconazole (34 mm against *T. mentagrophyte*, 36 mm against *M. audouinii*). The present study provides a scientific validation for the use of these essential oils in the treatment of superficial fungal infections.

Keywords: Clotrimazole, Dermatophytes, Essential oils, Superficial mycosis.

1. INTRODUCTION

Dermatophytes are responsible for serious human pathogenic infections that have increased during the last decades, particularly among high risk patients (Pfaller et al, 2006) ¹. These infections are a major cause of morbidity-associated superficial mycoses, with frequent relapses and often refractory to therapy. Dermatophytosis constitutes a group of superficial fungal infections of the epidermis, hair and nails (Sharma and Jasuja, 2012) ². Most fungal infections are located on the skin's outermost layer (epidermis). Dermatophytic infections are one of the earliest known

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fungal infections of mankind and are very common throughout the world. Dermatophytosis constitutes a group of superficial fungal infections of the epidermis, hair and nails. Recently there has been an increase in the incidence of fungal infections. This increase may be a result of frequent usage of antibiotics, immunosuppressive drugs and various conditions like organ transplantations, lymphomas, leukemia and human immunodeficiency virus (HIV) infections (Petmy *et al.*, 2004) ³. In the last years, research in aromatic and medicinal plants, and particularly their essential oils (EO), has attracted many investigators. EO have traditionally been used during centuries for their antifungal properties (Rios and Recis, 2005) ⁴. More recently, several studies have shown evidence of the huge potential of these natural products as antifungal agents justifying their current use in a number of pharmaceutical, food, and cosmetic products. Therefore, it is not surprising that EO are one of the most promising groups of natural products, for the development of broad-spectrum, safer and cheaper antifungal agents. The oils extracted from the (peels) of *C. sinensis* and *C. lemon* hold good promise as an antifungal agent, which could be used in therapeutic remedy against human pathogenic fungi on account of its various antifungal properties (Sharma *et al.*, 2012) ⁵. The present study was designed to evaluate the *in vitro* anti dermatophytic activity and chemical composition of essential oils to find out the alternative herbal medicine for the treatment of superficial fungal infections of skin.

2. MATERIALS AND METHODS

Trichophytonmentagrophytes and *Micro sporumaudouinii* was isolated from infected skin scrapings and hair collected from Government and Private Hospitals of Badaun (U.P) and maintained on a Sabouraud's Dextrose Agar and identified by microscopic, macroscopic and various biochemical tests.

Extraction of Essential oils

In winter season, extraction of Oils from the fresh rhizomes of *C. longa* (turmeric) and *Z. 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 and peeled. Sliced rhizomes of fresh *C. longa* (turmeric) and *Z. officinale* (ginger) (250 gm) were placed in a separate flask together with distilled water (1L). After 5-6 hours, oil were 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°C 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 *T. mentagrophytes* and *M. audouinii* by disc diffusion method

(Gould and Bowie, 1952) ⁶. Standard size Whatman No.1 filter paper discs, 6.0 mm in diameter, sterilized by dry heat at 140°C 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 petriplates and allowed to solidify. A suspension that was just turbid by visual inspection was prepared by suspending in 0.9 % NaCl solution and the homogeneous suspension was used for inoculation and test inoculum was maintained at 1-5×10⁶CFU/ml. The spore suspension of each of the fungi was prepared from 8 to 10-day-old cultures separately. The suspension was vortexed and 0.1 aliquots were spread over the respective agar medium plates. Sterilized filter paper discs were soaked in neat, undiluted (100 %) concentration of single oils. An oil-saturated disc of 100 µl concentration per disc was placed on an agar plate containing fungal spore suspension. Similarly, solutions of standard antibiotics (Clotrimazole and Ketoconazole (Sigma) of 10 mcg/disc concentration) for antifungal activity were prepared and impregnated in the filter-paper discs. These discs were then placed over the plates preceded with respective microorganisms. The plates were incubated at 30°C for 48-72 hours. Three replicates were kept in each case and average values were calculated. 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 oils was measured by the following formula:-
Activity Index = Inhibition zone of sample / Inhibition zone of standard

3. RESULTS AND DISCUSSION

In the present study, the antifungal activity of *C. longa* and *Z. officinale* essential oils against dermatophytes were evaluated. The selected test fungi were *T. mentagrophytes* and *M. audouinii*. In the current study, *T. mentagrophytes* and *M. audouinii* was found predominant dermatophytes from skin scrapings and hair. Disc diffusion method was employed for the screening of essential oils. The diameter of Inhibition zone of *C. longa* and *Z. officinale* was found 55 mm, 57mm and 69 mm, 59mm against *T. mentagrophytes* and *M. audouinii* respectively. The present results suggest that turmeric and ginger oil exhibits strong antifungal activity. This is in agreement with the findings of Valero and Frances (2006) ⁷ where the essential oils derived from many plants are known to exhibit antifungal activities. 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 (Harris, R, 2002) ⁸. Herbal drug preparation containing rhizome powder cured ringworm infection caused by *Trychophytonverrucosum* in 12 cattles and

Microsporumcanis in 21 dogs within 12-15 days of treatment (Sharma & Dwivedi, 1990)⁹. 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 (Apisariyakul et al, 1995)¹⁰. In the present study, essential oil of turmeric and ginger extracted by hydrodistillation method exhibited the strong antimycotic activity against *T. mentagrophytes* and *M. audouinii*. In screening of turmeric oil, the diameter of inhibition zone by disc diffusion method was found to be 55 mm and 57 mm against *T. mentagrophytes* and *M. audouinii* at 100% concentration of pure oil. Our work is in agreement with the observations of Wuthi-udomlert et al (2000)¹¹ 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. There are numerous scientific studies which prove the inhibitory effect of the essential oils against different fungi (Duarte et al, 2000 and Falahati et al, 2005)¹²⁻¹⁶. It is important to analyze that the plants which have been used in the medicines as a potential source of normal antimicrobial compounds (Mitscheretal, 1987)¹⁷. The present work coincides with the work of Sharma et al (2011)¹⁸ who also reported the additive and inhibitory effect of *C.longa* and *Z.officinale* essential oils against Pityriasisversicolor infections. The use of essential oils in treatment and prevention from infection has been in demand in the field of research from the past (Sherry et al, 2001)^{19, 20}. Both of these oils can be used for the development of the natural antifungal agents against *T. mentagrophytes* and *M. audouinii* to prevent dermatophytic infections.

Table 1: Antifungal Activity of C. longa and Z. officinale against T.mentagrophyte

Oil	Test strain	IZ of sample (mm)	AI (Ketoconazole)	AI (Clotrimazole)
<i>C. longa</i>	<i>T.mentagrophyte</i>	55mm	1.61	1.41
<i>Z. officinale</i>	<i>T.mentagrophyte</i>	69mm	2.02	1.76

Concentration of oil used 100%;
 IZ of standard Ketoconazole drug against *T.mentagrophyte* was 34 mm;
 IZ of standard Clotrimazole drug against *T.mentagrophyte* was 39 mm;
 Here IZ = Inhibition zone (in mm) including the diameter of disc (6mm);
 AI = Activity index

Table 1.1 Antifungal Activity of C. longa and Z. officinale against M.audouinii

Oil	Test strain	IZ of sample (mm)	AI (Ketoconazole)	AI (Clotrimazole)
<i>C. longa</i>	<i>M. audouinii</i>	57mm	1.58	1.26
<i>Z. officinale</i>	<i>M. audouinii</i>	59mm	1.63	131.1

Concentration of oil used 100%;
 IZ of standard Ketoconazole drug against *M.audouinii* was 36 mm;
 IZ of standard Clotrimazole drug against *M.audouinii* was 45 mm;

4. CONCLUSION

The present study clearly suggests that the extracted oils of *C. longa* and *Z. officinale* hold a good promise as an antifungal agent, which could be used in therapeutic remedy against human pathogenic fungi and can be used for the development of potential source of effective and economically viable herbal antifungal agent against fungal infections (Superficial mycosis).

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