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Original Article

A Comparative Study on Antidermatophytic Activity of Essenial Oils with Standard Drugs

Richa Sharma [1,*], Ved Prakash Mamoria [2]

[1]Assistant Professor, Department of Microbiology, Mahatma Gandhi University of Medical Science & Technology, Jaipur, India

[2] Professor & Head, Department of Microbiology, Mahatma Gandhi University of Medical Science & Technology, Jaipur, India.

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ABSTRACT

This study aimed to evaluate the in vitro antifungal activity (AA) of the Essential oils (EO) of *C. longa* (turmeric) and *Z. officinale* (ginger) oil against dermatophytes causing superficial fungal infections.The antifungal activity of Essential oils was screened against *Trichophyton tonsurans* and *Microsporum canis* by using disc diffusion and microdilution method. *C. longa* (turmeric) oil showed strong antifungal activity (65 mm, MIC 0.1 µl/ml and 50mm, MIC0.3 µl/ml), *Z. officinale* (ginger) oil had good antifungal activity (62 mm, 0.01 µl/ml and 52mm, 0.2 µl/ml) against *T. tonsurans* and *M. canis* while their mixture showed excellent antifungal activity (82 mm, MIC 0.02 µl/ml and 79mm, MIC 0.04 µl/ml) against *T. rubrum* and *M.gypseum* respectively. The oil of *C. longa* and *Z. officinale* were dominated by α-zingiberene (30-70%) and aromatic turmerone (31.1%) respectively by gas chromatography analysis. The mixture of oils (*C. longa* + *Z. officinale*) were found to have highly significant inhibition activity against *T. tonsurans* and *M. canis* as compare to both single oils and reference antibioticsi.e. Clotrimazole (36mm against *T.tonsurans*, 41 mm against *M.canis*) and Ketoconazole (34mm against *T.tonsurans*, 36 mm against *M.canis*). Present study provides a scientific validation for the use of these essential oils in the treatment of dermatophytic infections and could be used in future for development of anti- skin diseases agents. A synergistic action of essential oils displayed an excellent antifungal activity against dermatophytes.

Keywords: Clotrimazole, dermatophytes, essential oils, synergistic.

1. INTRODUCTION

Fungal infections are quite widespread and have affected a growing number of people in recent years. Most fungal infections are located on the skin's outermost layer (epidermis). Fungal infections in the lower layers of skin, internal organs and blood are rarely seen. Dermatophytic infections are one of the earliest known fungal infections of mankind and are very common throughout the world. Dermatophytosis constitutes a group of superficial fungal

Corresponding author *
Dr. Richa Sharma,
Department of Microbiology, Mahatma Gandhi University of Medical Science & Technology, Jaipur
E-mail: richa.phd.15@gmail.com.

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) [1]. Dermatophytosis constitutes a group of superficial fungal infections of the epidermis, hair and nails (Sharma and Jasuja, 2012) [2]. 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 (Burt, 2004) [4]. 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* antidermatophytic 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

T.tonsurans and *M.canis* was isolated from infected skin scrapings, Department of Dermatology, OPD at the S.M.S hospital and E.S.I.C. hospital Jaipur and maintained on a Sabouraud's Dextrose Agar and identified by microscopic, macroscopic and various biochemical tests

Plant Materials

Fresh rhizomes of *C. longa* (turmeric) and *Z. officinale* (ginger) were purchased from local market of Sodala, Jaipur in the month of October to December, 2011. These plant materials were identified by Department of Botany, University of Rajasthan, Jaipur.

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) [6]. 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).

Analysis of Essential oils

Gas chromatography analysis of the essential oils was performed using Finnigan Focus gas chromotograph, Thermoelectron Corporation, with capillary column of suplecowax (30 mm x 0.25 mm x 0.25 mm) thickness. GC ramp temperature was programmed as follows: initial temperature 80°C hold four minutes with rise of 4°C/min at 240°C. The carrier gas used was He (Helium) at the rate of 10 ml/min at constant volume. The column pressure corresponded to 100 Kpa. Injected temperature 200°C, detector temperature 240 °C. Oven conditions programmed as follows: Maximum temperature 240°C. Prep run time 10 min, equilibration time 0.50 min, oven run time 49 min. Intel SSL temperature was set at 200°C and split flow was 10 ml /min.

Screening of Essential oil using Disc Diffusion method

Oil was screened for their antifungal activity against *T. tonsurans* and *M. canis* by disc diffusion method (Gould and Bowie, 1952) [7]. 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 vortexes 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 and their mixtures (*C. longa* + *Z. officinale*). 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 28°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

Determination of Minimum Inhibitory Concentration using Modified Microdilution method

The modified Microdilution method of Provine and Hadley was adopted 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) equivalent to 1-5×106CFU/ml prepared by suspending the selected fungi in 0.9 % NaCl solution, vortexing, and homogeneous suspension was used for inoculation. Different concentration of single and

mixture of 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 oil-free control, by a centered down-up motion to form a two dimension inoculum. The tubes were then incubated at 30°C for 48-72 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 respective fungi in oil-free medium as a control was detected (after 48 hrs for filamentous fungi) afterwards, the growth in all tubes at different concentrations of oils was compared with that of the oil-free control in order to determine inhibition after 48 hours of incubation.

3. RESULTS

In the present study the antifungal activity of *C. longa* and *Z. officinale* essential oils (alone and in combination) against dermatophytes were evaluated (Tables 1& 1.1 and figures 2.0 & 2.9).In the present study *T. tonsurans* and *M. canis* was isolated from clinical samples of Department of Dermatology, OPD at S.M.S and E.S.I.C Hospital, Jaipur.. The results of the present work on the antifungal activity of mixture of oils (*C. longa* + *Z. officinale*) against *T. tonsurans* and *M. canis* with their activity index has been shown in (Tables 1& 1.1 and figures 2.0 & 2.9).Disc diffusion method was employed for the screening the essential oils. The diameter of inhibition zone of C. longa and Z.officinale was found 65 mm, 50mm and 62 mm, 52mm against *T.tonsurans & M.canis* espectively. Inhibition zone of mixture of oils was found to be highest than single oils and reference drugs. In screening of mixture of oils (turmeric + ginger) against dermatophytes, namely *T. tonsurans* and *M. canis*, we reported excellent antifungal activity by disc diffusion method. The diameter of the inhibion zone (IZ) obtained against mixture of oils at a concentration of 100% pure oils were 82 mm and 79 mm against *T. tonsurans* and *M. canis* respectively. Inhibition zone of mixture of oils was double than that of standard drugs and single oil used. These studies indicate that mixture of oils were very effective at a very low concentration of oils. MIC of mixture of *C. longa* and *Z. officinale* oils against *T.tonsurans & M.canis* was found 0.02 and 0.04 µl/ml respectively. These low concentrations were found very effective in inhibiting the growth of *T. tonsurans* and *M. canis* and no growth was observed from these concentrations to 2 µl/ml and showed fungicidal properties. Mixture of oils of *C. longa* and *Z. officinale* showed effective inhibition on the growth of *T. tonsurans* and *M. canis* than a single oil alone used.

4. DISCUSSION

In the present study additive and synergistic effects of essential oils studied. Mixture of *C. longa* and *Z. officinale* oils were found excellent antifungal activity against *T. tonsurans* and *M. canis.* In the present studies, inhibition zone of mixture of oils were found to be highly significant than single oils and reference antibiotics. MIC determined by microdilution method for mixture of oils was found at a very low concentration of oils as compared to MIC of single oils. In screening of mixture of *C. longa* and *Z. officinale* oils, diameter of IZ was found to be 82 mm and 79 mm against *T. tonsurans* and *M. canis* respectively. MIC of mixture of oil (*C. longa* + *Z. officinale*) was found 0.02 and 0.04 µl/ml against *T. tonsurans* and *M. canis* respectively. Our findings coincide (Prasad *et al.,* 2008 and Sharma *et. al*., 2012) [5, 8] reported the synergistic antifungal efficacy of essential oils of *Cymbopogonmartinii*, *Chenopodiumambrosiodes* and their combinations against dermatophytes and some filamentous fungi *in vitro* and *C. sinensis*, *C.lemon* oils were effective in inhibiting the growth of *A. niger, A. flavus, P.chrysogenum* and *P. verrucosum* respectively. Our work also coincides with the findings (Casella *et al*., 2002) [9] who studied the antifungal potential of tea tree and lavender essential oils alone and in combinations against *T. rubrum* and *T. mentagrophytes* and also effective inhibition by mixture of oils than single oil alone. Our results are also in agreement with (Gutirrez*et al.,* 2008) [10] who reported that essential oils in combinations showed synergistic activity against food borne pathogenic and spoilage bacteria and found that combination of basil, lemon balm, majoram, oregano, rosermary, sage and thyme in different combinations showed additive efficacy against *B.cereus, E.coli, L. monocytogenes* and *P. aeruginosa.* Bukovska *et al.,*2007 [3] studied the effect of a combination of thyme and oregano essential oils on mice and results indicated that combined treatment with appropriate concentrations of thyme and oregano essential oils decreased the mortality rate, accelerated gain in body weight, reduce the production of pre-inflammatory cytokines and reduced the macroscopic damage of the colonic tissue. Yujie*et al.*, 2007 [11] studied the antimicrobial activity of the essential oils from clove (*Syzygiumaromaticum*) and rosemary (*Rosmarinus officinalis*) in combinations and alone against *Staphylococcus epidermidis.* These results highlight the potential usefulness of essential oils to enhance the activities of conventional biocides. Our findings are in agreement with (Srivastava *et al*., 2008) [12] who investigated a novel combination of the essential oils of *Cinnamomum camphora* and *Alpiniagalanga* in checking alfatoxin B_1. The results showed that, the oil of *C. camphora* completely checked alfatoxin B_1 at 750 ppm (mg/l) while that of *A. galangal* showed complete inhibition at 500 ppm only and oil combination of *C. camphora* and *A. galangal* showed more efficacy than the individuals oils showing complete inhibition of alfatoxinB1 production even at 250 ppm. Results are in agreement with above workers. In our studies, mixture of oils was found to be more effective in inhibiting the growth of *T. tonsurans* and *M. canis* than single oils and standard drugs used. Hence the mixture of oils owing to its

strong antifungal activity inhibiting heavy doses of inoculum having fungicidal properties. Therefore, both mixture of oils and single oils can be used as a natural antifungal agent against *T. tonsurans* and *M. canis*, the causal organism of tinea capitis and dermatophytic infections. From the results, it was evident that studies of mixture of oils possessed potential inhibitory activity against all the two selected human pathogenic fungi *in vitro.* The activity of mixture of oils was higher than those of single oils and standard antibiotics. The antifungal activity of combination of two essential oils indicated their additive, synergistic and antagonistic effects against individual microorganisms tested. Finally this study confirms that mixture of oils possessed higher antifungal activity and can be used to cure fungal infections and may potentiate the efficacy of chemotherapeutics and may have role as an herbal traditional medicine, pharmaceuticals in the treatment of superficial fungal infections of the skin.

Table 1: Antifungal Activity of C. longa, Z. officinale and mixture of oils against T. tonsurans

Oil	*Test strain*	*IZ of sample (mm)*	*AI (Ketoconazole)*	*AI (Clotrimazole)*
C. longa	*T. tonsurans*	65mm	1.91	1.80
Z. officinale	*T. tonsurans*	62mm	1.8	1.7
Mixture of oils	*T. tonsurans*	82mm	2.41	2.27

IZ of standard Ketoconazole drug against T. tonsurans was 34 mm;
IZ of standard Clotrimazole drug against T. tonsurans was 36 mm;
Here IZ = Inhibition zone (in mm) including the diameter of disc (6mm);
AI = Activity index

Table 1.1 Antifungal Activity of C. longa, Z. officinale and mixture of oils against M.canis

Oil	*Test strain*	*IZ of sample (mm)*	*AI (Ketoconazole)*	*AI (Clotrimazole)*
C. longa	*M.canis*	50mm	1.38	1.21
Z. officinale	*M.canis*	52mm	1.4	1.26
Mixture of oils	*M.canis*	79mm	2.19	1.92

IZ of standard Ketoconazole drug against M. canis was 36 mm;
IZ of standard Clotrimazole drug against M. canis was 41 mm;
Here IZ = Inhibition zone (in mm) including the diameter of disc (6mm); AI = Activity index

Figures

Fig 2: C. longa oil against T.tonsurans
Fig 2.1: C. longa oil against M. canis

Fig 2.2: Z.officinale oil against T.tonsurans
Fig 2.3: Z.officinale oil against M.canis

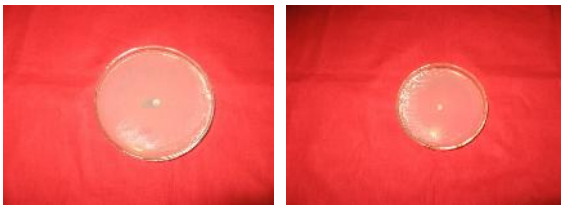

Fig 2.4: Mixture of oils against T.tonsurans
Fig 2.5: Mixture of oils against M.canis

Fig 2.6: Ketoconazole drug against T.tonsurans
Fig 2.7: Ketoconazole drug against M. canis

5. 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. The mixture of oils can be used for the development of potential source of effective and economically viable herbal antifungal against fungal infections (Superficial mycosis).

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