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Research Paper

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Antifungal potential of blends of specific essential oils against *Tinea capitis* and *Tinea corporis*

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Abstract- Since ancient times, folk medicines have advantageous from the use of natural products and plant derivatives, such as essential oils, to inhibited different types of diseases. In Nature, essential oils play an important role in protecting the different types of infections on human beings. Combinations of different essential oils are often an option for therapeutic use for human health. Several researchers have examined in detail the modes of action of essential oils and most of their components and combinations. Approximately 90 types of essential oils with more than thousand combinations can be identified as being suggested for dermatological use. This research explores the antifungal properties of essential oils as natural treatment against pathogens responsible for dermatological infections. The purpose of this research is to describe the properties of essential oils, principally as antifungal agents, and their role in the form of combinations.

***Keywords*:** Essential oils, antifungal, human health, natural treatment, dermatological infections.

I. INRODUCTION

Skin is the prime and largest mechanical barrier against the exterior environment and microorganisms' attack. It is responsible for various functions like heat regulation, protecting the primary organs and tissues [1, 2]. The uppermost epidermal layer is roofed by a defensive keratinous surface which permits for the elimination of microorganisms. In the references of body protection, the skin also have natural microflora which allows additional defense by competitive inhibiting pathogenic microbial growth. The various natural microflora of skin consists of the species of *Corynebacterium*, *Staphylococci*, *Streptococci*, *Brevibacterium*, and *Candida* as well as *Propionibacterium* [3–8].

The common infections of skin caused by microorganisms include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Microsporum* spp., *Epidermophyton* spp., and *Trichophyton* spp., *P. acnes*, and *Brevibacterium* spp. [3, 8–11]. Skin infections create some most specific common reasons for persons to search medical involvement and are measured the most frequently collision of all infections. More than six million people are affected by chronic wounds and approximately 17% of clinical visits for medicines. Skin diseases are a major reason of perilous illness and death [8, 13, 14]. The therapeutic rate of chronic wounds is affected by bacterial infections, inflammation, pain and blood flow [15–17]. In some regions of the world, various infections are unresponsive to all known antibiotics [20]. Unresponsive activities are producing more severe conditions like simple ulcers now requiring treatment with systemic antibiotics [21]. The World Health Organization warned that, in future there will be a huge problem without antibiotics [22]. Therefore, the oldest forms of treatment are effective resolution to treat skin infections and wounds i.e., the medicine of natural products [18, 23]. Most of the developing countries are used complementary and alternative medicines [24– 27]. Essential oils are one of the most popular and effective natural products among several natural alternative medicines as in dermatology [28–30]. Infect, of all complementary and alternative medicines, essential oils are the most prevalent choice for treating fungal skin infections [13, 31]. The use of essential oils in the treatment of skin infections, in the nursing and hospitals is growing worldwide, especially in the United States and the United Kingdom [1, 27, 32–35].

Furthermore, many researchers have reported the major components of plant, the aroma therapeutic research literatures [1, 2, 26, 32, 36–43] recognize the treatment of dermatological infections with essential oils which are apart from the many other compounds of plants. The present study is focused on the antidermatotic effects of specific essential oils against two very common dermatophytic infections: *Tinea capitis* and *Tinea corporis.*

II. MATERIALS AND METHODS

Organisms and media

In this study, the strains of *Tinea capitis* and *Tinea corporis* were collected from the Microbial Type Culture Collection and Gene Bank (MTCC) CSIR-Institute of Microbial Technology, Chandigarh, India. The selected fungal strains were *Trichophyton tonsurans* (8475) and *Microsporum canis* (3270). The test strains were maintained and cultured on Sabouraud dextrose agar (SDA) and Emmons modification of Sabourauds Agar.

Natural essential oils and drugs

Three different essential oils of were used as herbal antifungal agent at different concentrations and blends. Ketoconozole used as positive control which was synthetic chemical antifungal agent. The essential oils of lemongrass, citronella and sweet basil were obtained from the *Cymbopogon flexuosus, Cymbopogon winterianus and Ocimum basilicum* plants respectively. These plants were self-cultivated in the region of Kannauj, Uttar Pradesh.

Assessment of selected oils components

All the different components of lemongrass, citronella and basil essential oils were identified through gas chromatography mass spectrometry (GC-MS). There were 64, 50 and 21 chemical components of lemongrass, citronella and basil essential oils were recorded respectively.

Preparation of formulations of essential oils against selected fungus

Herbal formulation was prepared by the accords of only selected essential oils. The formulations were prepared with the accords of two and three essential oils at different concentrations. In each formulation the base amount of one essential oil placed more than 40%. In this study the lemongrass oil, basil oil and citronella oil denoted by L, B and C respectively (Table 1.1). Apart from the lemongrass, basil, citronella oil and ketoconazole, there were 31 formulations formed.

Sr. No.	Name of the Formulation	Percentage of essential oils	
1.	LC-1	10	90
2.	LC-2	20	80
3.	LC-3	30	70
4.	LC-4	40	60
5.	LC-5	50	50
6.	CL-1	10	90
7.	CL-2	20	80
8.	CL-3	30	70
9.	CL-4	40	60
10.	BL-1	10	90
11.	BL-2	20	80
12.	BL-3	30	70
13.	BL-4	40	60
14.	BL-5	50	50
15.	LB-1	10	90
16.	LB-2	20	80
17.	LB-3	30	70
18.	LB-4	40	60
19.	BC-1	10	90
20.	BC-2	20	80
21.	BC-3	30	70
22.	BC-4	40	60
23.	BC-5	50	50
24.	CB-1	10	90
25.	CB-2	20	80
26.	CB-3	30	70
27.	CB-4	40	60
28.	LBC-1	30	30 40
29.	LBC-2	20	40 40
30.	LBC-3	10	40 50
31.	LBC-4	10	50 40

Table 1.1. The formulation blends of lemongrass (L), citronella(C) and basil (B).

Disc diffusion assays

The disc diffusion assay was performed in sterilized petri plates of 10 cm diameter as per the method described in Indian Phamiacopoeia. After solidification of Sabouraud Dextrose Agar and Emmons modification of Sabourauds agar in petri plates, the suspension culture of fungus (*Trichophyton tonsurans* 8475 and *Microsporum canis* 3270) was spread on respective plates. Autoclaved and 5 mm diameter dried discs of Whatman filter paper no. 1 were used as discs. The different amounts of concentration of essential oils and formulations were loaded on discs and dimethyl sulfoxide (DMSO) solvent was used for dilution of the essential oil formulations and antifungal drug. Anti-fungal drug Ketocanzole was used as positive control. After that the plates were incubated for the period of 18 to 24 hours on 29℃ for checking minimum inhibitory concentration. Petri plates were incubated at 29℃ for 24 to 48 hours to check zone of inhibition. The values of minimum inhibitory concentration and zone of inhibition mentioned in result section as mean of tests was performed in triplicates.

Zone of inhibition test

Determination of antifungal activity of the selected essential oils and formulations against *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270 was done with the zone of inhibition test. Five microliters of essential oils and formulations of essential oils were pipetted onto sterile paper disks. The petri plates were incubated at 29℃ for 24 to 48

hours to check zone of inhibition. Areas of clearing around the disks after incubation were measured and indicate that the oils have some antifungal activity. Diameters of zones of inhibition were measured in millimeters and recorded (Table 1.2).

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of essential oils and formulations as antifungal agent was determined after 24 hours of incubation period. The process of inhibition of fungal growth was started around the disks as an initial clear line that sowed the minimum inhibitory concentration of respective oil and formulation. MIC of different formulations against *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270 displayed in the table 1.2.

III. RESULTS

Susceptibility of Trichophyton tonsurans 8475 and *Microsporum canis* 3270 *to formulations*

Table 1.2 shows the susceptibility of *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270 to three essential oils and their all formulations and ketoconazole as antifungal drugs using the disc diffusion method. Zones of inhibition with all the formulations varied from 0.73 to 7.63 mm, whereas, zones of inhibition to lemongrass, basil, citronella oil and ketoconazole ranged from 0.93 to 7.63 mm against *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270.

Sr. no.	formulations	Zone of inhibition(mm)		MIC(µl/ml)	
		T.T.	M.C.	T.T.	M.C.
1	Lemongrass	7.63	7.56	1	1
2	Citronella	0.93	1.40	400	600
3	Basil	2.66	2.36	500	200
4	Ketoconazole	7.63	7.56	0.1	0.1
5	LC-1	7.63	7.20	5	5
6	LC-2	7.00	7.10	10	20
7	LC-3	6.83	6.80	25	25
8	LC-4	6.20	7.00	95	35
9	LC-5	5.76	6.60	125	65
1	CL-1	3.26	4.33	380	140
1	CL-2	2.93	4.60	295	135
1	CL-3	3.53	6.26	240	95
1	CL-4	3.76	6.63	165	80
1	BL-1	2.76	3.26	305	170
1	BL-2	3.26	3.60	260	135
1	BL-3	3.73	3.66	195	105
1	BL-4	3.73	4.13	140	100
1	BL-5	6.26	6.86	200	95
1	LB-1	7.46	7.26	5	1
2	LB-2	7.53	7.13	10	5
2	LB-3	7.23	7.16	10	15
2	LB-4	6.46	6.83	65	30
2	BC-1	2.50	3.1	405	210
2	BC-2	2.53	2.8	410	235
2	BC-3	2.40	2.33	425	295
2	BC-4	2.16	2.03	435	315
2	BC-5	1.40	1.80	480	318
2	CB-1	1.26	0.73	410	550
2	CB-2	1.26	0.90	425	500
3	CB-3	1.06	1.83	455	395
3	CB-4	1.06	1.83	475	380
3	LBC-1	7.66	7.50	5	1
3	LBC-2	7.60	7.40	5	1
3	LBC-3	7.46	7.36	5	1
3	LBC-4	7.63	7.23	5	1

Table 1.2. The susceptibility of *Trichophyton tonsurans* 8475 (TT) and *Microsporum canis* 3270 (MC).

MICs of antifungal drugs

The limit of drug resistance was identified on the basis of susceptibility performance of the test strains. The selected strains were considered resistant at the MIC values ranging from 0.1 µl/ml to 600 µl/ml for all the essential oil and their formulations and ketoconazole. The MIC values of natural oils and formulations; lemongrass oil, LC-5, LB-1, LB-2, LB-3, LBC-1, LBC-2, LBC-3, LBC-4 were also effective as comparison to the chemical or synthetic antifungal drugs.

IV. DISCUSSION

In this study, susceptibility of test strains of *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270 were found to have some specific resistance with respective essential oil and their blends, across a wide range of concentrations (1 µl/ml to 600 µl/ml). Multi-drug resistance is a severe problem in the treatment of resourceful fungal infections of immunocompromised individuals such as transplant recipients and cancer patients undergoing cytotoxic chemotherapy [30]. Some specific essential oils and their blends identified as antifungal activity. Lemongrass oil, LC-5, LB-1, LB-2, LB-3, LBC-1, LBC-2, LBC-3 and LBC-4 formulations were highly active against both strains *Trichophyton tonsurans* 8475 and *Microsporum canis* 3270. The essential oils were tested for their interactive effects at different quantitative percentage level. Their synergistic effect seems to hold good potential in combination therapy. Essential oils in combination can exhibit enhanced efficacy because of the increased level of killing, a larger spectrum of action covering several infections by different pathogens and a short period of time of therapy resulting in the decreased likelihood of developing resistance [44]. In our study, lemongrass, citronella and basil oils being a potential

antifungal agent alone, also exhibited synergistic interaction with the blends of these essential oils.

V. CONCLUSION

When blends are formed, the purpose is to generate therapeutic synergy; the reasoning for the combinations is to initiate a forceful blend that has more than one mode of action. It is a complex section, because although a certain combination of essential oils may have a synergistic therapeutic effect. In our findings, essential oil especially Lemongrass oil and , LC-5, LB-1, LB-2, LB-3, LBC-1, LBC-2, LBC-3 and LBC-4 formulations were quite effective against *Trichophyton tonsurans* 8475 and *Microsporum canis*. Therefore, a preferred combination may be established for the natural treatment against selected fungal strains. These oils and their blends might be useful topically to deal with such infections. Our findings suggest that some specific essential oils in blends combination provide a natural, improved and safer clinical approach without any adverse effect towards the fungal infections caused by *Tinea capitis* and *Tinea corporis.* After all, this study concluded that the different formulations of essential oils, can initiate a synergistic antifungal effect. However, further investigations are needed to determine the synergistic effects of different oils and their compounds, as well as the best possible doses and methods of application in the field.

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