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Antimicrobial Effects of *Trachyspermum ammi* and *Cymbopogon citratus* Essential Oil Topical Formulations on Pathogenic Fungi

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Evaluation of the antimicrobial activity of semisolid formulations of *Trachyspermum ammi* and *Cymbopogon citratus* essential oils against five common strains of pathogenic fungi was carried out using the agar well diffusion method. The results indicated that 1 % v/w of *T. ammi* and *C. citratus* oils prepared in some bases exhibited remarkable antifungal activity with zone inhibition diameters greater than those for standard antifungal agents. The growth of all five fungal strains was inhibited when *T. ammi* oil and *C. citratus* oil were formulated separately in macrogol blend ointment or hydrophilic ointment base. The properties of base into which the oil was incorporated affected its activity. The hydrophilic formulations exhibited higher antifungal activities compared to their lipophilic counterparts and all the formulations were intended for topical use.

Key words: Agar well diffusion, antifungal activity, essential oils, semisolid formulation, zone of inhibition.

INTRODUCTION

Essential oils belong to one of the most promising groups of natural compounds for the development of safer antimicrobial agents [1]. In a recent study, essential oils of *Trachyspermum ammi* and *Cymbopogon citratus* were shown to possess strong antimicrobial activity against pathogenic human bacteria and fungi [2]. *T. ammi* (Netch azmud) belongs to the family Apiacae. Its essential oil is rich in thymol (45-55 %) [3]. An ointment containing thymol was formerly used in western medicine as a topical preparationtion in eczema, psoriasis, tinea, and other cutaneous affections [4]. In Ethiopia, the fruits and roots of *T. ammi* are used to treat stomach complaints, as a vermifuge and as an abortifacient [5-6]. The essential oils are thought to be strongly antiseptic and parasiticidal [7].

C. citratus (DC.) Stapf. (Family Poaceae), a spice producing plant known by the commercial name Lemongrass, is an aromatic plant believed to have a wide range of therapeutic effects. Lemongrass is considered by herbalists to have antibacterial, antifungal, and fever-reducing effects. It has been demonstrated in various studies to be effective against 22 strains of bacteria and 12 types of fungi [8]. Its essential oil has a high content of citral (>70 %). Citral is an oxygenated aldehydic terpenoid which has been recognized as having antifungal properties [8].

The purpose of this study was to assess the inhibitory effect of the semisolid formulations of *T. ammi* and *C. citratus* essential oils separately in different bases against five common and clinically significant pathogenic fungi.

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MATERIALS AND METHODS

Chemicals

Cetostearyl alcohol, liquid paraffin, hard paraffin, propylene glycol, stearyl alcohol and wool fat were from Sigma-Aldrich Chemie GmbH (Riedstr, Steinheim, Germany), sodium lauryl sulphate from Labort Fine Chem. Pvt. Ltd (India), white petrolatum from Ethiopian Pharmaceutical Manufacturing (Addis Ababa, Ethiopia), while Macrogol 4000 and Macrogol 600 were from BDH (Poole, England).

Test organisms and culture media

Two standard test microorganisms and three clinical isolates were included in the study. The standard organisms *Trichophyton mentagrophytes* (ATCC 18748) and *Aspergilus niger* (ATCC 10535) together with the clinical isolates *Candida albicans, Microsporum canis* and *Trichophyton verrucosum* were obtained from the Bacteriology Laboratory, Ethiopian Health and Nutrition Research Institute. All the microorganisms were maintained on Sabouraud's dextrose agar (Oxoid) plates at 4º C prior to testing.

Standard antifungal preparation

The topical antifungal agent used in this study was clotrimazole cream (1 % w/w) (HOE Pharma, Malaysia) obtained from the Quality Control Laboratory, DACA.

Plant material and preparation of the essential oils

Ripe and dried fruits of *T. ammi* were bought from farmers at markets within Addis Ababa while the leaves of *C. citratus* were collected from the botanical garden of the Department of Drug Research, Ethiopian Health and Nutrition Research Institute. The identity of the plants was confirmed by comparison with authentic herbarium specimens deposited in the herbarium of the Department of Drug Research, Ethiopian Health and Nutrition Research Institute. The essential oils were obtained by steam distillation of the plant materials. The oil was separated from the aqueous layer using a separatory funnel and stored in clean, dark brown bottles after dehydration with anhydrous sodium sulphate.

Preparation of Topical Formulations

Formulations containing 1 % v/w *T. ammi* oil and 1 % v/w *C. citratus* oil were prepared separately in five different bases as shown in Table 1.

Antifungal activity of the topical formulations

Culture preparations

Cultures of the test organisms were prepared following the method adopted from Leven *et al.* [9]. The cultures were then sub-cultured in liquid Sabouraud's Dextrose Broth (SDB) medium (Oxoid) for 48 h at 25 °C. The turbidity of each cell suspension was measured at 530 nm and adjusted with sterile distilled water to match that of a 0.5 ml solution of McFarland standard to obtain approximately 1 x 10^6 to 5 x 10^6 cfu/ml. The mixture was diluted 1:100 in sterile distilled water to give the starting inoculums (1 x 10^4 cfu/ml) used in the test.

Antifungal activity assay

Antifungal activity of the formulated products against the test organisms was determined by the agar well diffusion method [10]. A 0.2 ml aliquot of overnight Sabouraud's dextrose broth culture of the respective microorganisms was seeded into 20 ml of molten and cooled Sabouraud's dextrose agar. A single well was bored into the seeded plates using a sterile 9 mm diameter cork borer. After removal of the agar plugs, 0.1 ml or approximately 0.2 g of the semisolid test preparations was transferred into the well. The plates were incubated at 25 °C for 3 to 7 days, except for the dermatophytes, which were incubated for up to 3 weeks at the same temperature. The zone diameters of the resulting zones of inhibition were measured and recorded.

The standard drug was included as a positive control and a separate agar plate with the formulation bases (vehicles) excluding the essential oil or standard drug was prepared for negative control. The inhibitory effect of the different preparations was judged by direct visual

comparison of the test cultures with the control cultures and all the tests were carried out in triplicate.

RESULTS

Steam distillation of *T. ammi* seeds produced a clear, colorless to pale yellow oil with a yield of 8.9 %, while *C. citratus* yielded 0.6 % of essential oil. The various topical preparations of both essential oils (1 % v/w) exhibited antifungal activity. The formulation bases used as vehicles for topical preparation and their compositions are given in Table 1. Bases 2 and 3 are hydrophilic compared to other bases. Antifungal activity profiles of topical formulations of the essential oils and the formulation bases are also shown in Table 2.

T. ammi essential oil in macrogol blend ointment base (1 % v/w) was most effective against *T. mentagrophytes* and *M. canis* as compared to the same oil in hydrophilic base. *C. citratus* in macrogol blend and hydrophilic ointment base (1 % v/w) was equally effective against *T. verrucosum* and *T. mentagrophytes* while *C. albicans* was the least responsive fungus to the *C. citratus* essential oil formulations.

The essential oil preparations of *T. ammi* and *C. citratus* in macrogol cream and simple ointment base as well as in white petrolatum ointment base showed little or no inhibition on the growth of test fungi (Table 2).

Table 3 shows the comparative performance of the commercial antifungal agent and the essential oil preparations. *T. ammi* in macrogol blend ointment base and clotrimazole cream were equally effective against *T. verrucosum*. *C. albicans* was less susceptible towards *C. citratus* essential oil preparation compared to the commercial antifungal agent. *A. niger* was more susceptible to *T. ammi* in bases 2 and 3 compared to clotrimazole cream. The activity of *T. ammi* in macrogol blend ointment base against *M. canis* and *T. mentagrophytes* was significantly higher than that of clotrimazole cream. The susceptibility of *M. canis* and *T. mentagrophytes* to *T. ammi* and *C. citratus* essential oil preparations in both bases 2 and 3 was significantly higher compared to the standard antifungal agent. *C. citratus* oil in bases 2 and 3 demonstrated activity comparable to that of clotrimazole cream against *T. verrucosum*.

Table 1: Formulation bases used as vehicles for the preparation of topical formulation of *C. citratus* and *T. ammi* essential oils.

Code No.	Base	Composition	Proportions (%)
Base1	Macrogol cream base	Cetomacrogol emulsifying wax	9
		Liquid paraffin	6
		White petrolatum	15
		Water	70
Base 2	Hydrophilic ointment base	Sodium lauryl sulfate	1
		Propylene glycol	12
		Stearyl alcohol	25
		White petrolatum	25
		Water to	100
Base 3	Macrogol blend ointment base	PEG 4000	20
		PEG 600	80
Base 4	Simple ointment base	Stearyl alcohol	5
		Hard paraffin	5
		Wool fat	5
		White petrolatum	85
Base 5	White petrolatum ointment base	White petrolatum	100

Table 2: Antifungal activity profiles of topical formulations of the essential oils and the formulation bases

Test Sample (1 % v/w in base)	Code No.	Zones of Inhibition (mm)				
		CA	***MC***	***TV***	***AN***	***TM***
T. ammi						
Base 1	F. 1	–	–	–	–	–
Base 2	F. 2	15	25	16	14	50
Base 3	F. 3	19	80	20	18	80
Base 4	F. 4	–	–	–	–	–
Base 5	F. 5	1	5	–	1	20
C. citratus						
Base 1	F. 1	–	–	–	–	–
Base 2	F.2	8	13	17	13	80
Base 3	F. 3	10	18	17	12	80
Base 4	F. 4	–	–	–	–	–
Base 5	F. 5	–	1	–	1	13
Macrogol cream base	Base 1	–	–	–	–	–
Hydrophilic ointment base	Base 2	–	–	1	6	2
Macrogol-blend ointment base	Base 3	–	–	–	–	–
Simple ointment base	Base 4	–	–	–	–	–
White petrolatum	Base 5	–	–	–	–	–

F = Formulation, *CA = C. albicans, MC = M. canis, TV =T. verrucosum, AN =A. niger, TM = T. mentagrophytes,* – = no activity.

Table 3: Antifungal activity profile of the standard topical antifungal agent in comparison with some formulations of the essential oils

Code No.	Antifungal agents (1%)	Zones of Inhibition (mm)				
		CA	***MC***	***TV***	***AN***	***TM***
1	Clotrimazole cream	21	14	20	14	37
F. 2	*T. ammi* oil	15	25	16	14	50
F. 3	*T. ammi* oil	19	80	20	18	80
F. 5	*T. ammi* oil	1	5	0	1	20
F. 2	*C. citratus* oil	8	13	17	13	80
F. 3	*C. citratus* oil	10	18	17	12	80
F. 5	*C. citratus* oil	0	1	0	1	13

F = Formulation, *CA = C. albicans, MC = M. canis, TV =T. verrucosum, AN =A. niger, TM = T. mentagrophytes*

DISCUSSION

The results obtained indicate that the macrogol blend preparation of the essential oils is superior in performance to the other formulations used in this study. The higher antifungal activity observed in the macrogol blend ointment base may be attributed to the potentiation of the antifungal activity of the essential oils [10]. PEG additionally possesses properties such as water solubility and hence compatibility with many dermatological medicaments [11-12]. Formulations 1, 4 and 5 showed virtually little or no antifungal activity indicating that the active component(s) of the essential oils could not be released from this formulation to the media. This may be due to the affinity of the oil for petrolatum which impairs the release of its active constituents into the more hydrophilic agar medium [13]. The sensitivity pattern of the fungi to the various formulations showed that *C. albicans*, *T. verrucosum* and *A. niger* were equally responsive to *T. ammi* in hydrophilic ointment base with similar trends being observed for *T. ammi* in macrogol blend base. These findings are significant since candidiasis and aspergillosis are major public

health problems as opportunistic infections in immunocompromised patients and their treatment is complicated by the emergence of resistant strains [14-15].

The activity of *T. ammi* in macrogol blend ointment base against *M. canis* and *T. mentagrophytes* surpasses that of clotrimazole cream and the preparation can be a candidate drug for the treatment of infections caused by these fungi. The same can be said about the activity of formulation 3 of *C. citratus* oil against *T. mentagrophytes.*

The hydrophilic ointment base (negative control) showed limited activity against *A. niger* and *T. mentagrophytes*. This may be due to the presence of sodium lauryl sulfate as one of the components of the base. Sodium lauryl sulfate, usually employed pharmaceutically as a protective skin cleaner and in medicated shampoos, has been reported to possess antimicrobial properties [13]. Moreover, all salts of fatty acids are known to have variable fungicidal properties [16].

CONCLUSION

The results of this study clearly indicated that the rate and degree of release and hence the activity of the essential oil formulations may be modulated by the type and nature of the formulation. The findings of this study suggest that *T. ammi* and *C. citratus* essential oils if formulated in bases such as macrogol blend base or hydrophilic ointment base that deliver the drug at the target site to achieve the desired therapeutic concentration may be candidate preparations for alternative therapy in patients with topical infectious mycoses.

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