Antimicrobial Activity of Topical Formulations Containing *Thymus vulgaris* Essential Oil on Major Pathogens Causing Skin Diseases

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The *in vitro* antimicrobial performance of topical semisolid formulations of the essential oil from *Thymus vulgaris* L. was assessed on major pathogenic microorganisms causing skin diseases. The essential oil was obtained by hydrodistillation and formulated into five different semisolid vehicles. The inhibition zones of the active constituents released from their respective bases were determined by using agar well diffusion method. The formulations showed inhibition against the growth of microorganisms used in this study at a concentration of 1% (v/w). Bioactive compounds were released better from the hydrophilic preparations than from the hydrophobic ones. The release from macrogol blend was particularly better. The inhibition zone from macrogol blend against both the standard and clinical isolate of *Pseudomonas aeruginosa* was also found to be better than those of Fuciderm® cream (2% sodium fucidate) and tetracycline hydrochloride ointment (3% tetracycline).

Keywords: *Thymus vulgaris*, agar well diffusion, antimicrobial activity, topical formulation, zone of inhibition

INTRODUCTION

It has been repeatedly stressed that traditional herbal remedies still cater for the health care needs of developing countries and, indeed, the majority of Ethiopian population. The main practice of the Ethiopian traditional medicine is based on ethnobotany where plants play the most critical role in the sustainable use of the practice. Modern medicines are either too expensive, or their supply is irregular and uncertain. Furthermore, they are not effective against all diseases and some may even no longer be effective due to microbial resistance (Urga *et al*., 2004). The use of medicinal plants is not limited to the traditional system of health care only. Medicinal plants are also important raw materials for the manufacture of useful therapeutic agents, complex semi-synthetic compounds and taxonomic markers in the search for new bioactive compounds (Addis *et al*., 2001).

Essential oils are rich sources of biologically active compounds and constitute a major source of natural organic compounds possessing antibacterial, antifungal, antiviral, insecticidal and antioxidant properties, and are used in food preservation, aromatherapy and fragrance industries (Burt, 2004). The antimicrobial properties of essential oils, which can be isolated from diverse parts of plants, are well recognized for many years. Preparations of essential oils have been widely used as naturally occurring antimicrobial agents in pharmacology, physiopathology, medicinal and clinical microbiology, and food preservatives (Dorman and Deans, 2000).

Thymus vulgaris L. (Lamiaceae), the vernacular name of which is 'tosign', widely grows in Ethiopia. The essential oil of *T. vulgaris* is used as antiseptic, antifungal and vermifuge as well as for the treatment of

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dermatitis. The decoction of the whole plant parts is used against tinea nigra, tinea capitis, acne, stomach trouble and chloasma (Duke, 1985; Abebe and Ayehu, 1993; Abebe, *et al*., 2003). *In vitro* studies have shown that the oil has antifungal and antibacterial activity against a number of fungi and bacterial species (WHO, 1999). *T. vulgaris* contains 1 to 2% essential oil known as thyme oil. Studies have showen that the major aromatic constituents of thyme oil are thymol, carvacrol, *p*-cymene, terpinene, *trans*-caryophyllene and borneol (Dagne *et al*., 1998; Asfaw *et al*., 2000). The antimicrobial property is mainly associated with the high phenolic (thymol and carvacol) content of the oil (Knobloch *et al*., 1989; Hammer *et al*., 1999; Rasooli *et al*., 2002; Seung *et al*., 2005). The essential oil has showen an antibacterial activity comparable to those of the pure isolated components of the oil such as thymol, carvacol and terpinene (Marino *et al*., 1999; Dorman and Deans, 2000), and standard commercial antimicrobial agents (Hammer *et al*., 1999; Shin *et al*., 2004). Although *T. vulgaris* is traditionally used for many skin diseases in Ethiopia, to the best knowledge of the authors, little or no scientific work has been reported on the topical formulations prepared from *T. vulgaris* growing in Ethiopia. Therefore, the objective of the present study is to develop an effective topical formulation from the *T. vulgaris* essential oil against bacterial and fungal pathogenic microorganisms causing skin disorders.

MATERIALS AND METHODS

Materials:

Chemicals. Cetostearyl alcohol, liquid paraffin, hard paraffin, propylene glycol, stearyl alcohol, and wool fat (Sigma-Aldrich, Steinheim, Germany), polyethylene glycol (PEG) 4000 and PEG 600 (BDH, Poole, England), sodium lauryl sulfate (Labort Fine Chem., India), white petrolatum USP (EPHARM, Addis Ababa, Ethiopia), and anhydrous sodium sulphate (Sigma-Aldrich, GMBH, Germany) were used as received.

Culture media. Muller-Hinton agar (Oxoid, UK), sabouraud dextrose agar (Oxoid, UK), yeast extract (LABORT, India), tryptone soya broth (Oxoid, UK), Sabouraud dextrose broth (Oxoid, UK), Muller-Hinton broth (IDG, UK), nutrient broth (Oxoid, UK) were used in the present study.

Test organisms:

Bacterial strains. *Pseudomonas aeruginosa* (ATCC 27853 and clinical isolate) *Staphylococcus aureus* (ATCC 13709 and clinical isolate), and *Streptococcus pyogen* (ATCC 19615 and clinical isolate) were used for antibacterial activity testing.

Fungal strains. *Aspergilus flavus* (ATCC 13697), *A. niger* (ATCC 10535 and clinical isolate), *Candida albicans* (clinical isolate), *Trichophyton mentagraphyte* (ATCC 18748), *T. verrucosum* (clinical isolate), *Cryptococcus neoformans* (clinical isolate) and all the bacterial strains used were obtained from Bacteriology and Mycology Laboratory, Infectious and Non-Infectious diseases Research Department, Ethiopian Health and Nutrition Research Institute (EHNRI). All bacterial and fungal cultures were periodically characterized and maintained at 4 °C on nutrient and Sabouraud dextrose agar, respectively.

Commercial topical antimicrobial products:

Antibacterial agents. Tetracycline hydrochloride ointment, (USP 3% tetra-cycline) Galentic Pharma, Batch No: 529,

India), Fuciderm® cream (2% sodium fucidate Lot No: 88, Syria), Gentamycine cream (0.1% gentamycine HOE Pharmaceuticals Sdn. Bhd, Batch No: 55964015, Malaysia) were purchased from local drug retail outlets.

Antifungal agents. Mycoril® cream (1% clotrimazole, Remedica Limassol Industrial State, Lot No: 33989, Cyprus), Fungigen® cream (2% miconazole BP cream, Batch No: CJ07003, Galentic Pharma India) also obtained from local drug retail outlets in Addis Ababa were used as positive controls.

Methods:

Extraction of essential oil. The dried leaves of *T. vulgaris* were collected from North Shewa around 100 km north of Addis Ababa. The plant was taxonomically identified and sample specimen was deposited in the Department of Drug Research Herbarium, EHNRI. The essential oil was extracted from the dried leaves by hydrodistillation using Clevenger type apparatus. The oil was collected and dehydrated with anhydrous sodium sulphate and stored in a clean, dark brown bottle.

Preparation of topical formulations. Five different formulation bases were prepared by the fusion method except for the white soft paraffin which was prepared in accordance with the formula given in Table 1. A 1% v/w of the essential oil of *T. vulgaris* topical formulations was prepared by incorporating the oil into the soft mass of the different dermatological bases. The bases, without the essential oil, were used as negative controls while the commercial topical products, namely, gentamycine and Fuciderm® creams, tetracycline hydrochloride ointment, and the antifungal creams, Mycoril® and Fungigen®, were employed as positive controls.

Antimicrobial testing:

Inoculum preparation. The bacterial strains were sub-cultured at 37 ºC for 24 h and fungal cultures were sub-cultured at 25 °C

Table 1. Dermatological bases used and their compositions.

Ingredients	Compositions (%)				
	Base 1	Base 2	Base 3	Base 4	Base 5
Cetomacrogol emulsifying wax	9	-	-	-	-
Hard paraffin	-	-	-	5	-
Liquid paraffin	6	-	-	5	-
Macrogol 4000	-	-	20	-	-
Macrogol 600	-	-	80	-	-
Propylene glycol	-	12	-	-	-
Sodium lauryl sulfate	-	1	-	-	-
Stearyl alcohol	-	25	-	-	-
White soft paraffin	15	25	-	85	100
Wool fat	-	-	-	5	-
Water to make	100	100	-	-	-

Base 1 = Macrogol cream base, Base 2 = Hydrophilic ointment, Base 3 = Macrogol blend ointment base, Base 4 = Simple ointment, Base 5 = White petrolatum alone

for 48 h in nutrient broth and liquid Sabouraud dextrose broth, respectively. The growth was inoculated into 4 ml of broth and the turbidities of both cultures were equilibrated with that of 0.5 McFarland standards (Barry and Thornsberry, 1991).

Antimicrobial activity tests. The antimicrobial activity of the formulated products, the commercial products, and negative controls (base only) were tested by using agar well diffusion technique (Rodheaver *et al*., 1980). Sterilized and molten Muller-Hinton agar (15 ml) (for bacteria) and Sabouraued dextrose agar (for fungi) were poured into a sterile Petri dish and allowed to solidify at room temperature. Equidistant holes were made on the solidified media by a standard borer (size 10 mm). The wells were filled with the test formulations, positive controls (commercial products) or the negative controls (approximate weight of each was 0.2 g) with the help of 5 ml disposable syringe. The thoroughly mixed nutrient agar (7 ml each) inoculated with 0.5 ml of microbial suspensions equivalent to 0.5 McFarland standard were poured and spread uniformly over the surface of all plates. After 2 h incubation at room temperature to allow the diffusion of bioactive substances from the base to the seeded medium, the plates were incubated at 37 ºC for 24 h (for bacteria) and at 25 °C for 24 h in the case of *C. albicans* and *C. neoformans*, 48 h for *A. flavus* and *A. niger*, and five days for *T. mentagraphyte* and *T. verrucosum*. The diameters of the zones of inhibition were measured. Experiments were performed in triplicate.

RESULTS AND DISCUSSION

The activity of the essential oil of *T. vulgaris* which was formulated into five different bases was compared against scores of both standard and clinical isolates of bacteria and fungi. As shown in Tables 2 and 3, only formulations 2 and 3 showed

Table 2. Antibacterial activity of topical formulations of *Thymus vulgaris* essential oil.

	Formulation code	Zone of inhibition (mm) including the diameter of borer					
		Standard organisms			Clinical isolates		
		Pa	*Sa*	*Sp*	*Pa*	*Sa*	*Sp*
Test formulation	Form. 1	-	-	-	-	-	-
	Form. 2	16.0	24.0	23.0	15.0	28.0	21.0
	Form. 3	30.0	24.0	29.0	18.6	29.5	30.0
	Form. 4	-	-	-	-	-	-
	Form. 5	-	-	-	-	-	-
Negative control	Base 1	-	-	-	-	-	-
	Base 2	-		15.0	-	12.0	11.0
	Base 3	-	-	-	-	-	-
	Base 4	-	-	-	-	-	-
	Base 5	-	-	-	-	-	-
Positive control (drug)	Tetracycline HCl ointment	15.0	40.0	40.0	15.0	40.0	28.0
	Gentamycine cream	17.0	40.0	40.0	15.0	34.0	35.0
	Fuciderm cream	20.0	56.0	42.0	18.0	44.0	45.0

1% *Thymus vulgaris* essential oil in: macrogol cream base (Form. 1); hydrophilic ointment (Form. 2); macrogol blend ointment (Form. 3); simple ointment (Form. 4); white petrolatum (Form. 5).
Pa = Pseudomonas aeruginosa; Sa = Staphylococcus aureus; Sp = Streptococcus pyogen
- No inhibition zone

activities against fungal and bacterial strains employed in this study.

Table 2 shows the antibacterial profiles of *T. vulgaris (*1% v/w) essential oil formulations against standard and clinical isolates of *S. aureus, P. aeruginosa,* and *S. pyogen* strains. As shown in the table, Formulation 3 displayed better antibacterial effects on the standard and clinical isolate of *P. aeruginosa* than the commercial anti-bacterial agents; tetracycline hydrochloride ointment (3% w/w tetracycline), gentamycine cream (0.1% w/w gentamycine) and Fuciderm® cream (2% w/w sodium fucidate). Moreover, it has activities against the remaining bacterial strains which are comparable to those of the commercial formulations. Similar to its activity against bacteria, Formulation 3 showed better antifungal activity against the standard *A. niger* and the clinical isolate of *T. verrucosum* as compared to the positive control Fungigen® cream (2% w/w of miconazole) (Table 3). It also showed better antifungal activity against the standard *T. mentagraphyte*, *A. niger* and clinical isolates of *A. niger, C. albicans, T. verrucosum* than the commercially available Mycoril® cream (1% w/w of miconazole).

The better release of the oil from macrogoal blend preparation (Formulation 3) may be partly ascribed to the fact that polyethylene glycols have an excellent solubility in water, and hence, have a solvent power over numerous compounds that are sparingly soluble in water. Polyethylene glycols are used as skin penetration enhancers as they are compatible with many substances (Reynolds, 1982). Similar results were also reported that polyethylene glycol-

Table 3. Antifungal activity of topical formulations of *Thymus vulgaris* essential oil.

	Formulation code	Zone of inhibition (mm) including the diameter of borer						
		Standard organism			Clinical isolate			
		Af	*An*	*Tm*	*An*	*Ca*	*Cn*	*Tv*
Test formulations	Form. 1	-	-	-	-	-	-	-
	Form. 2	33.0	30.0	32.0	28.0	25.0	40.0	40.0
	Form. 3	26.0	25.5	30.0	22.5	25.0	25.5	32.0
	Form. 4	-	-	-	-	-	-	-
	Form. 5	-	-	-	-	-	-	-
Negative control	Base 1	-	-	-	-	-	-	-
	Base 2	13.0	16.0	-	14.0	14.0	13.0	20.0
	Base 3	-	-	-	-	-	-	-
	Base 4	-	-	-	-	-	-	-
	Base 5	-	-	-	-	-	-	-
Positive control (drug)	Fungigen® cream	30.0	24.0	40.0	27.0	27.0	40.0	27.0
	Mycoril® cream	27.0	21.0	28.0	20.0	18.0	26.0	28.0

1% *Thymus vulgaris* essential oil in: macrogol cream base (Form. 1); hydrophilic ointment (Form. 2); macrogel blend ointment (Form. 3); simple ointment (Form. 4); white petrolatum (Form. 5).
Af = Aspergilus flavus; *An = Aspergilus niger*; *Ca = Candida albicans*; *Cn = Cryptococcus neoformans*; *Tm = Trichophyton mentagraphyte*; *Tv = Trichophyton verrucosum*
- No inhibition zone

containing preparations show better release properties from dermatological preparations (Lara *et al*., 2001; Taddese *et al*., 2003; Messele *et al*., 2004; Tadeg *et al*., 2004). Moreover, the bioactive component(s) of the essential oil may be released more easily from hydrophilic bases than from hydrophobic preparations.

Formulation 2 has shown antibacterial activity against both standard and clinical isolates and its activity especially against the standard and clinical isolates of *P. aeruginosa* was comparable to those of the positive controls employed in the present study (Table 2). Moreover, consistent antifungal activity was observed for Formulation 2 against both standard and clinical fungal isolates but the antifungal activity against *T. verrucosum* was visibly better than the positive controls (Fungigen® and Mycoril® creams) (Table 3). Hydrophillic ointment (base 2) has shown activity against all microbes with the exception of *P. aeruginosa* (both standard and clinical isolate), *S. aureus* (standard) and *T. mentagraphyte* (standard) (Tables 2 and 3). The increased activity of this formulation is expected and could be attributed to the additional activity exerted by the base itself. Sodium lauryl sulphate, which is used as a surfactant, has bacteriostatic action against Gram-positive bacteria and is also used pharmaceutically as a protective skin cleaner as well as in medicated shampoos (Florence and Attwood, 1998). Therefore, the antimicrobial property of sodium lauryl sulphate might have contributed to the remarkable antimicrobial activities displayed by Formulation 2.

Thymol and carvacrol are hydrophobic in nature (Nostro *et al*., 2007). These two compounds are the major constituents oils obtained from several *Thymus* species (Cosentino *et al*., 1999), and are known to have antimicrobial activities (Didry, *et al*., 1993). Their mechanism of action is by disruption of the functions of bacterial cell wall and membrane which leads to a leakage of the cytoplasm thereby causing death of the microorganisms (Janssen *et al*., 1987). The active components easily diffuse into the surrounding area from the hydrophilic bases (bases 2 and 3) and inhibit the growth of the microorganisms. Owing to the hydrophobic nature of bases 1, 4, and 5, the active components will have better affinity for these bases than diffusing to the surrounding to produce the activity.

The results of the present study is in agreement with those of other studies conducted using the same essential oil. Manou and coworkers (1998) have shown that topical formulations containing the essential oil of *T. vulgaris* are active against standard strains of *P. aeruginosa*, *S. aureus*, *A. niger* and *C. albicans* at a concentration of 3% (v/v). In the present study, however, activity was demonstrated at a concentration of 1% v/w. An increased activity obtained in this work as compared to previous reports on the same essential oil, may be due to the nature of the bases in which the drug was formulated (Omotosho *et al*., 1986), the viscosity of the preparation (Florence and Attwood, 1998; Oyedele *et al*., 2000); and the different growth conditions of the plants from which the oil was obtained.

CONCLUSION

The *in vitro* antimicrobial activity of 1% topical formulation of *T. vulgaris* essential oil, in hydrophilic bases, was found to be comparable with those of the commercially available antimicrobial products. PEG-based bases were shown to be more efficient in releasing the bioactive compounds and can therefore be considered as possible vehicles for further dermatological formulation development.

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