ORIGNAL ARTICLE

ANTIMICROBIAL ACTIVITY OF TOPICAL FORMULATION CONTAINING *EUGENIA CARYOPHYLLATA L.* (KRUNFUD) AND *MYRITUS COMMUNIS L.* (ADES) ESSENTIAL OILS ON SELECTED SKIN DISEASE CAUSING MICROORGANISMS

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ABSTRACT

BACKGROUND: ***Aromatic plants are usually used in traditional medicine as antimicrobial agents. Their essential oils, isolated by hydro-steam distillation, have been known since antiquity to posses' antimicrobial property. The objective of this study was to formulate topical antimicrobial agents for the treatment of human skin diseases from myrtle and clove essential oils.***

METHODS: ***The plant were collected from Bale Zone, North Shewa Zone and Botanical Garden of Department of Drug Research, Ethiopian Health and Nutrition Research Institute on Augest to October, 2007. Eessential oils of both plants were collected by hydro-steam distillation in the Formulation Research Laboratory of Department of Drug Research. After the preparation of 1% Eugenia caryophyllus and 4% Myritus communis essential oil formulation in five different formulation bases; the antimicrobial activity were detected in the Microbiology Laboratory by using diffusion assay methods.***

RESULTS*: In the assayed antimicrobial activity topical formulation of 1% Eugenia caryophyllus and 4% Myritus communis essential oils have shown significantly high antimicrobial activity against: Staphylococcus aureus, Streptococcus pneumonia, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus flavus, Trachophyton mentagraphyte, Trachophyton vericusom, Microsporum cannis, Candida albicans, Cryptococcus neoformans than commonly used drugs. Both topical formulations were more effective in the hydrophilic bases than the lipophilic bases used in the study.*

CONCLUSION*: Our results of topical formulation in hydrophiic ointment or macrogol blend base support the concept that essential oil may be useful for the treatment of microbial infection. Therefore, we suggest that the topical formulation of these oils can be used as alternative drugs for the treatment of skin disease. However, skin sensitivity study of the topical formulations must be performed to ensure their safety.*

KEY WORDS: ***Antimicrobial, Eugenia caryophyllus oils, Myritus communis oils, Skin disease, bases in formulation***

INTRODUCTION

Skin disease that are caused by bacteria and fungal strains have become prevalent in the world, due to the emergence of disease such as HIV/AIDS, compounded with increased use of common recreational areas like swimming (1,2). Due to different factors, these diseases are more common in developing countries where Ethiopia is not exception (3). As a result of failure to treat skin diseases by the available antimicrobial agents due emergence of emergence of resistant microorganisms; now a days many researchers focused on the search for new drug from natural products (4,5).

Because of their long history of use in the treatment of different human diseases, most of the herbal medicines are believed to be safer and effective than synthetic drugs. According to WHO, herbal medicines serve the health needs of about 80% of the world population (6). Aromatic plants especially their essential oil and mixture of natural compound isolated by distillation, were among these herbs that have been used as traditional medicine to treat bacterial and fungal diseases (7). Essential oils are volatile products of aromatic plants secondary metabolism, normally formed in special cell or group of cells found in many leaves and stems. It is concentrated or stored either in particular region of the plant or in various organs in the same plants (8).

Previous studies showed that essential oils of aromatic plants have antimicrobial activity against bacterial and fungal pathogens (9-12). Their antimicrobial activity is attributed to the presence of small terpenoid and phenolic compounds (phenolic acids, polyphenols and flavonoids) which are related to their lipophilic character that leads to accumulation on membrane and to the subsequent membrane associated event such as energy depletion (8).

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In Ethiopia various aromatic plants having different essential oils, are being used for aroma-treatment (fumigation) of diseases (13). *Myritus communis* and *Eugenia caryophillata* were from these aromatic plants that have been used for the treatment of microbial diseases in Ethiopia (13). Essential oils of these aromatic plants contain reported major compounds that were active against different bacterial and fungal strains. *Myritus communis* contain α-pinene, limonene and 1,8-cineole and *Eugenia caryophillata* contain euginol and eugenyl acetate (8,14,15).

Even though, various studies showed the potentials of these essential oils to be an important source of biologically active compounds useful for the treatment of microbial diseases; no study have been conducted yet on its topical formulation of these plants (14, 15).

The objective of the present study was to fomulate effective antimicrobial agents from myrtle and clove essential oils, which can serve as topical antimicrobial agents for the treatment of human skin diseases caused by bacterial and fungal strains.

MATERIALS AND METHODS

Study area and Period: Plants were collected from from Bale Zone, North Shewa Zone and from botanical garden of Department of Drug Research, Ethiopian Health and Nutrition Research Institute, Ethiopia in Augest-October,2007. The identities of the plants were taxonomically identified and the samples were deposited in the Department of Drug Research Herbarium. The essential oils of these plants were extracted in November, 2007 in the Formulation Research Laboratory of Department of Drug Research. After preparation of Topical formulation, the in vitro experimental study or antimicrobial assay was conducted in the Department of Drug Research, Ethiopian Health and Nutrition Research Inistitute from December, 2007 to March, 2008.

Preparation of Topical Formulations: Essential oils of the identified plants were extracted by hydro-distillation of the plant material. The oils were separated from the aqueous layer by using a separatory funnel and dehydrated with anhydrous sodium sulphate and stored in clean, dark brown bottles. Cetostearyl alchol, Liquid paraffin, Hard paraffin, Propylene Glycol, Stearyl alcohol, and Wool fat (Sigma-Aldrich Chemie GmbH, Steinheim, Germeny), PEG 4000 and PEG (BDH, Poole, England), Sodium lauryl sulfate (Labort Fine Chem. Pvt. Ltd., India) and White soft paraffin USP (Ethiopian Pharmaceutical Manufacturing, Addis Ababa, Ethiopia) were used in the preparation.

Topical formulation containing 1%v/w *Eugenia caryophillata* essential oil and 4%v/w *Myritus communis* essential oil were prepared separately in five different formulation bases. Topical formulations containing 2%v/w *Eugenia caryophillata* and 8% v/w *Myritus communis* oils were prepared in the three lipophilic ointment bases (Table 1).

Control Antimicrobial Formulations: Topical formulations of Fuciderm 2% Cream (Delta Ltd, Lot: 28, Syria) and Mycoril 1% Cream (Remedica Ltd, Lot: IMAPT 33989) were used in the study as a positive control for bacterial and fungal topical treatment, respectively.

Test organism: The following bacterial and fungal strains were used in the study: *Staphylococcus aureus* (ATCC or American Type Culture Collection 25923 and clinical isolate), *Pseudomonas aeruginosa* (ATCC 27853 and clinical isolate) and *Streptococcus pyogen* (ATCC 19615 and clinical isolate), *Aspergilus niger* (ATCC 10535 and clinical isolate), *Aspergilus flavus* (ATCC 13697 and clinical isolate), *Trichophyton mentagraphyte* (ATCC 18748 and clinical isolate), *Trichophyton verrucosum* (clinical isolate), *Microsporum cannis* (clinical isolates), *Candida albican* (clinical isolate), and *Cryptococcus neoformans* (clinical isolate). All the clinical isolates were taken from sample of patients with skin disorder are stored at the Bacteriology and Mycology Laboratory, Ethiopian Health and Nutrition Research Institute. All the organisms were periodically characterized and maintained in the Microbiology Laboratory, Department of Drug Research during the study period.

Test for Antimicrobial Activity: An agar cup diffusion, long period methods were used as antimicrobial activity tests (16,17). Fiffteen millileter of molten and cooled (45^0C) agar were added to sterile petri dish (90mm) and allowed to solidify. Three equidistant holes 10mm apart from the edge of the plate were formed in the agar by removing the plug made with sterile cork borer (10mm). Using of 5 ml syringe with a gauge needle, weighed amounts (0.1 ml ≈0.2 g) of a designated topical agent were added to each well. A volume of 0.2 ml suspension of overnight Nutrient broth (Oxoid Ltd, UK) culture having standard turbidity of the respective bacteria and overnight Sabouraud Dextrose broth (Oxoid Ltd, UK) culture and young culture of respective fungus were used as a test organisms inoculated into 7 ml of molten and cooled (45 °C) Muller-Hinton agar (Oxoid Ltd, UK) for that of bacteria and Sabouraud Dextrose Agar (Oxoid Ltd, UK) for fungi. The agar suspension of organisms was thoroughly mixed by WHIRLI MIXER™ (Nickel ELECTRO, Ltd., AVON) and poured onto the previously prepared test plate containing antimicrobial agents. After the plate solidified for 2-3 minutes, they were inverted and allowed to stand for 1 hour at room temperature and incubated at 37^0C (bacteria) and 25^0C (fungi) for 24 hours in case of bacteria and yeasts; for 48 hours for molds and up to five days for dermatophytes. After

incubation the diameter of zones of inhibition (excluding cup size) was measured and compared with negative (formulation bases with out drug) controls and positive (standard topical formulation or drugs) controls. Results were recorded as presence or absence of zone of inhibition (16). Both inhibitory zone diameter around the cups or well containing the formulations and percentage of relative inhibition zones were recorded as antimicrobial activity of the formulation. The absences of inhibition zones were recorded as zero. The test was performed in triplicate to insure reliability of the results.

- Percentage of Relative inhibiton zone diameter

$$\%RIZD = \frac{(IZD \text{ of sample} - IZD \text{ of negative control})}{IZD \text{ of positive control (commonly used drugs)}} \quad X\,100$$

%RIZD is percentage of rilative inhibition zone diameter, IZD is inhibition zone diameter. These value would indivate the antimicrobial potency of the topical preparations by companceting the activity due to the bases in the formulation and at the same time will compare the activity of this topical preparation with that of commenly used drugs.

RESULTS

Essential oils were extracted from *Eugenia caryophillata and Myritus communis* with the yield of 10.8 % and 0.5% based on the weight of the plant used for extraction, respectively. Topical preparation containing 1% *Eugenia caryophillata* and 4% *Myritus communis* essential oils exhibited antimicrobial activity. Topical formulation of both essential oils in hydrophilic ointment bases exhibited the highest antimicrobial activity, followed by macrogol-blend ointment bases. One percent *E. caryophillata and 4% M. communis* essential oils preparation in macrogol cream base, simple ointment bases and white soft paraffin showed no inhibition of microbial growths. Even at 2% *E. caryophillata* and 8% *M. communis* essential oils preparation in macrogol cream base, simple ointment bases and white soft paraffin were performed still no inhibition of microbial growth. However, 1% *E. caryophillata* and 4% *M. communis* preparation in hydrophilic and macrogol-blend ointments inhibited the growth of bacteria and fungi (Table 2 and 3).

Table 1. Formulation bases used in the study for the topical preparation of the essential oils, Ethiopian Health and Nutrition Research Inistitute, 2008.

Codes	Bases	Compositions	Proportions (%)
BASE 1	Macrogol Cream	Cetomacrogol emulsifying wax	9
		Liquid paraffin	6
		White soft paraffin	15
		Water	70
BASE 2	Hydrophilic	Sodium lauryl sulfate	1
	ointment	Propylene Glycol	12
		Stearyl alcohol	25
		White soft paraffin	25
		Water	37
BASE 3	Macrogol blend	PEG 4000	20
	ointment	PEG 600	80
BASE 4	Simple ointment	Stearyl alcohol	5
		Hard paraffin	5
		Wool fat	5
		White soft paraffin	85
BASE 5	White soft paraffin	White soft paraffin	100

Table 2. Antibacterial inhibition zone diameter of topical formulations by excluding the size of the borer, Ethiopian Health and Nutrition Research Inistitute, 2008.

Test sample	Code	Inhibition Zone Diameter (mm)					
		Standard bacterial strain			Clinical Isolate strains		
		Sa	Stp.	Pa	Sa	Stp	Pa
1% Eugenia caryophyllatas in							
Macrogol cream base	Base 1	0	0	0	0	0	0
Hydrophilic ointment	Base 2	20	15	6	18	14	6
Macrogol-blend ointment	Base 3	16	14	7	14.5	13	5
Simple ointment	Base 4	0	0	0	0	0	0
White soft paraffin	Base 5	0	0	0	0	0	0
4% Myritus communis in							
Macrogol cream base	Base 1	0	0	0	0	0	0
Hydrophilic ointment	Base 2	22	16	9	20	20	6.3
Macrogol-blend ointment	Base 3	10	5	3	9	5	3
Simple ointment	Base 4	0	0	0	0	0	0
White soft paraffin	Base 5	0	0	0	0	0	0
Negative controlsa							
Macrogol cream base	N1	0	0	0	0	0	0
Hydrophilic ointment	N2	2	5	0	2	4	0
Macrogol-blend ointment	N3	0	0	0	0	0	0
Simple ointment	N4	0	0	0	0	0	0
White soft paraffin	N5	0	0	0	0	0	0
Positive control							
Fuciderm 2% cream	P1	23.3	15	6	20.6	13	4

Sa, *Staphylococcus aureus*, Stp, *Streptococcus pyogen*, pa, *Pseudomonas aeruginosa*

Table 4. Antimicrobial activity profile indicated in Percentage of Rilative Inhibition Zone diameter, Ethiopian Health and Nutrition Research Inistitute, 2008.

Test sample	Code	Inhibition Zone Diameter (mm)									
		Standard strain			Clinical Isolate strains						
		Sa	Stp	Pa	Sa			Stp		Pa	
1% Eugenia caryophyllatas in											
Hydrophilic ointment	Base 2	80.7	66.7	100	77.7			76.9		150	
Macrogol-blend ointment	Base 3	71.7	60	116	72.5			100		125	
4% Myritus communis in											
Hydrophilic ointment	Base 2	89.7	73.3	150	90			123		156	
Macrogol-blend ointment	Base 3	44.8	33.3	50	87.1			38.5		75	
1% Eugenia caryophyllatas in											
		An	Af.	Tm	An	Af	Tm	TV	Mc	Ca	Cn
Hydrophilic ointment	Base 2	246	159	145	163	106	105	125	112	81	194
Hydrophilic ointment	Base 2	246	159	145	163	106	105	125	112	81	194
Macrogol-blend ointment	Base 3	173	132	136	173	97	125	125	112	13 5	171
4% Myritus communis											
Macrogol-blend ointment	Base 3	100	64.7	90.9	100	67	75	85	62	75	88
Hydrophilic ointment	Base 2	164	137	81.8	155	100	80	85	76	75	119
Macrogol-blend ointment	Base 2	100	64.7	90.9	100	67	75	85	62	75	88

Sa, *Staphylococcus aureus*, Stp, *Streptococcus pyogen*, pa, *Pseudomonas aeruginosa* An, *Aspergilus niger*, Af, *Aspergilus flavus*, Tm, *Trichophyton mentagraphyte*, Tv, *Trichophyton verrucosum*, Mc, *Mycrosporum cannis*, Ca, *Candida albican*, Cn, *Cryptococcus neoformans*

Table 3. Antifungal inhibition zone diameter of topical formulation by excluding the size of the borer, Ethiopian Health and Nutrition Research Inistitute, 2008.

Test sample	Codes	Inhibition Zone Diameter (mm)									
		Standard fungi strains			Clinical Isolate of fungi strains						
		An	Af.	Tm	An	Af	Tm	Tv	Mc	Ca	Cn
1% Eugenia caryophyllatas in											
Macrogol cream	Base 1	0	0	0	0	0	0	0	0	0	0
Hydrophilic ointment	Base 2	33	30	36	22	23	30	35	25	17	34
Macrogol-blend ointment	Base 3	19	22.5	30	19	18	25	25	19	21.6	27.3
Simple ointment	Base 4	0	0	0	0	0	0	0	0	0	0
White soft paraffin	Base 5	0	0	0	0	0	0	0	0	0	0
4% Myritus communis in											
Macrogol cream	Base 1	0	0	0	0	0	0	0	0	0	0
Hydrophilic ointment	Base 2	24	26.3	32	21	22	25	27	19	16	21
Macrogol-blend ointment	Base 3	11	11	20	11	12	15	17	10.6	12	14
Simple ointment	Base 4	0	0	0	0	0	0	0	0	0	0
White soft paraffin	Base 5	0	0	0	0	0	0	0	0	0	0
Negative controls											
Macrogol cream	N1	0	0	0	0	0	0	0	0	0	0
Hydrophilic ointment	N2	6	3	14	4	4	9	10	6	4	3
Macrogol-blend ointment	N3	0	0	0	0	0	0	0	0	0	0
Simple ointment	N4	0	0	0	0	0	0	0	0	0	0
White soft paraffin	N5	0	0	0	0	0	0	0	0	0	0
Positive control											
Mycoril 1% cream	P1	11	17	22	11	18	20	20	17	16	16

An, *Aspergilus niger*, Af, *Aspergilus flavus*, Tm, *Trichophyton mentagraphyte*, Tv, *Trichophyton verrucosum*, Mc, *Mycrosporum cannis,* Ca, *Candida albican*, Cn, *Cryptococcus neoformans*

The percentage of relative inhibition of bacterial and fungal growth by compensating the effect of vehicle was tested. Accordingly, *Myritus communis* preparation in hydrophilic ointment showed higher activity against the standard organism of *P. auroginosa*, *A. niger*, *A. flavus* and clinical isolates of *P. auroginosa*, *S. pneumonia*, *A. niger* and *C. neoformans* when compared to commonly used drugs. One percent *Eugenia caryophillata* in hydrophilic and macrogol-blend ointments also showed higher activity against *P. auroginosa* clinical isolate and standard organism respectively. When compared to the tested drugs, higher activities were observed in 1% *Eugenia caryophillata* in hydrophilic and macrogol-blend ointments preparations against all standard fungal strain and all clinical isolates except *C. albicans* and *A. flavus* isolates, respectively. Comparable results with the antibiotics used were observed in 1% *Eugenia caryophillata* formulation in hydrophilic and macrogol-blend ointments against standard *P. auroginosa* and clinical isolates of *P. auroginosa*, *S. pneumonia*, respectively. Similarly, 4% *Myritus communis* preparations in macrogol-blend and hydrophilic ointments inhibited growth of standard *A. niger*, isolates of *A. niger* and *A. flavus*. The over all highest percentage of inhibition was recorded against standard *A. niger* followed by isolates of *C. neoformans* in hydrophilic prepation and then against both standard and isolates of A. niger in macrogol-blend ointment preparation of 1% *Eugenia caryophillata* (Table 4).

DISCUSSION

Eugenia caryophillata essential oil contain 78% Eugenol and 13% Eugenyl acetate and *Myrtus communis* contain 56% α-pinene and 33% 1,8-cineole as a major component, which were mainly responsible for their antimicrobial activity (14,15). Besides these major components, the variation in antimicrobial activity of the essential oils of the same plant in different formulation as indicated in table 2, 3, 4 were due to the nature of the base it is formulated in which has considerable effect on its efficacy (18,19). Factors that affect the release of a bioactive component from the base include its affinity to the base (18,19). Hydrophilic bases have less affinity for the oils while the lipophilic bases have higher affinity for oils than the agar medium used.

The result showed, both macrogol-blend and hydrophilic ointment base topical formulations of both essential oils have markedly higher activity when compared to the essential oil formulations in lipophilic bases (macrogol cream, simple ointment, and white soft paraffin) which exhibited no activity. These variation could be attributed to the lipophilic affinity of the oils for paraffin found in macrogol cream base, simple ointment base and white soft paraffin; which impairs the release of bioactive constituents of the essential oils into the more hydrophilic agar media. In addition to their hydrophilic affinity, the intrinsic antimicrobial property of sodium luaryl sulphate may have contributed for the higher antimicrobial activity of essential oil topical formulations in hydrophilic ointment bases. Whereas, the bland inherent activity and higher hydrophilic affinity of macrogol-blend ointment base compared to the agar media might have contributed for its higher antimicrobial activity. The effect of sodium luaryl sulphate were compensated in the expresion of percentage of relative inhibition (see the Mathimatical expresion for the calculation of percentage of relative inhibition).

This study showed above 100% relative inhibition of microbial growths indicating that the topical preparation of the essential oils had greater inhibition zone diameter (IZD) than the commonly used antibiotics. The percentage of relative inhibition diameter is inversily proportional to the inhibition zone diameter of the commertial antibiotic while directly proportional to that of the test sample.

This study indicated, in some cases percentage of relative inhibition zone diameters of both essential oils formulation on the bacterial clinical isolates exceed that of the standard organism. This may be due to the higher sensitivity patterns of the bacterial clinical isolates to that of the topical formulations than to commonly used drugs, while the standard organism seems less resistant to the commonly used drugs. Unlike bacterial clinical isolates, all standard organisms of fungal strains were more susceptible to both formulations.

The present study confirmed that the hydrophilic topical formulation of 1%v/w *Eugenia caryophillata* essential oil and 4%v/w *Myritus communis* posses strong in-vitro antimicrobial activity against all tested skin-disease causing organisms. The study also showed that the topical formulations had better antimicrobial activity compared to the tested drugs

In conclusion, our study showed that the essential oils of *E. caryophillata* and *M. communis* had better antimicrobial activity than control drugs in vitro. Because of their natural origin in which peoples can find it comforting, low risk of the microbial resistance to the mixture of the components that make up the oil having apparent diversity of antimicrobial mechanisms and their remarkable antimicrobial activity, we recommend the use of topical formulations of *E. caryophillata* and *M. communis* essential oils as an alternative treatment of skin diseases disorders after the toxicological and skin sensitivity study of the topical formulations.

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