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Antimicrobial Effect of Essential Oil of *Thymus capitatus* from Northern Cyprus and Its Gargle Preformulation

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DÖY, YÖ, NA and DŞ designed the study, performed the statistical analysis and wrote the protocol. Author DÖY wrote the first draft of the manuscript. Authors YÖ, BT and DŞ managed the analyses of the study. DÖY, YÖ and DŞ managed the literature searches. Authors DÖY, YÖ, NA and DŞ revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study is to prepare herbal gargle preformulations making use of essential oil of aerial parts of *Thymus capitatus* growing wild in Northern Cyprus and comparing antimicrobial efficacy between these formulations with pure essential oil.
Place and Duration of Study: Department of Pharmaceutical Technology, Faculty of Pharmacy, Near East University, Nicosia, TRNC and Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Ankara University, Ankara, Turkey, between January 2017 and June 2017.
Methodology: Preformulations with three different concentrations of *Thymus capitatus* essential oil were prepared by using simple preparation method. Preformulation studies were done in lab with

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less than 500 ml. There is not specific process parameters. Manufacturing process and process parameters will be clarified with further studies. These compositions and the essential oil were tested *in-vitro* for antimicrobial activity studies by using broth microdilution method. *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* RSKK 574, *Salmonella paratyphi* C, *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas aeruginosa* ATCC 27853 as bacteria and *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019 as yeasts were used for antimicrobial activity tests.
Results: All trials were found to be more effective than EO, and a significant effect was observed when compared to the values of standard antimicrobial agents.
Conclusion: The*Thymus capitatus* growing in Northern Cyprus could be used as a herbal raw material, essential oil source in developing herbal gargle preformulations to reduce fungal and bacterial infections in mouth.

Keywords: Thymus capitatus; essential oil; herbal gargle; antimicrobial activity; preformulation.

1. INTRODUCTION

*Mentha spp,Origanum spp. and Thymus spp.*had been used in various forms of anti-inflammatory and antiseptic oral solutions, since ancient times [1,2,3,4].

Thymus spp. is one of the most common among these species worldwide due to the fact that its constituents have been used as antihelmintic, antimicrobial, antiseptic, antispasmodic, antitussive, astringent, bactericidal, carminative, disinfectant, expectorant, insecticide, secreto-motor and tonic [5,6,7]. Thyme is useful for treating laryngitis and relieving inflammation when it is used as a gargle form. Also thymol, is one of the active compound of thyme essential oil, has bactericidal effect especially on enterobacteria and cocci bacteria [6]. Thyme oil, yielded from *Thymus vulgaris* L. and *Thymus zygis* Loefl. ex L., takes place in the EMA's list of "Herbal Medicines for human use" [8].

Genus *Thymus* of the Lamiaceae family is rich in essential oil. *T. capitatus,* locally known as "*tülümbe*" in Turkish Republic of Northern Cyprus (T.R.N.C.) is one of the two species growing wild in Cyprus [9,10]. This species was reported as one of the most known and used plant for various pharmacological effects by Cypriot people [11,12]. In addition, mild upper respiratory infections have been treated by consuming medicinal teas of *T. capitatus* and also its leaves were chewed for treating mouth ulcers, traditionally in T.R.N.C. [13]. *Thymus capitatus* is a potential thymol source with regard to our previous results [14]. Major components were thymol (62.3%) followed by *p*-cymene (10.9%), carvacrol (6.7%) and γ-terpinene (5.1%) [14]. The possible regional variations of essential oil constituents of wild growing *T. capitatus* species in T.R.N.C. investigated in the previous study of some of our researchers. It has been shown to be the thymol chemotype [14].

Even though antimicrobial effects of *Thymus spp.* essential oil against various microorganisms have been researched well before [15,16], there is neither any data of a gargle preformulation development with *Thymus spp.* essential oil nor a comparison with antimicrobial effectiveness of both essential oil and any preformulation.

The aim of this study is to compare the antimicrobial efficacy of pure essential oil of *Thymus capitatus* growing wild in Northern Cyprus and a potentiality of herbal gargle preformulation development by using this essential oil. To the best of our knowledge, this is the first investigation of this type.

2. MATERIALS AND METHODS

The plant materials, isolation of the essential oil and GC/MS analysis are in parallel with those in our previous studies to obtain the essential oil for this study [14].

2.1 Preformulation with Thyme Essential Oil

Preformulation studies were performed by using three different concentration of the thyme essential oil 8% (F1), 10% (F2) and 12.5% (F3) respectively. Propylene glycol is used as co-solvent, where as distilled water and sorbitol solutions are used as solvents with simple preparation method. Preformulation studies were done in lab with less than 500 ml. There is not specific process parameters. Manufacturing process and process parameters will be clarified with further studies.

2.2 Antimicrobial Activity Studies

Microorganisms used in the experiment were *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 (MRSA), *S. epidermidis* ATCC 12228, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *K. pneumoniae* RSKK 574, *P. aeruginosa* ATCC 9027, *P. aeruginosa* ATCC 27853 as bacteria and *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 as yeasts. The microorganisms used in this study were obtained from the culture collection of the Ankara University, Faculty of Pharmacy, Pharmaceutical Microbiology Department.

Antimicrobial activity studies were carried in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations by broth microdilution method with some modifications for antifungal assay [17,18]. Firstly, -80°C stocks were opened and colonies were isolated from fresh overnight culture of these strains from Mueller Hinton Agar (MHA, Merck) for bacteria and Sabouraud Dextrose Agar (SDA, Oxoid) medium for yeasts were transferred into Mueller-Hinton Broth (MHB, Merck) medium for bacteria and Sabouraud Dextrose Broth (SDB, Oxoid) medium for yeasts to obtain turbidity comparable to that of 0.5 McFarland standards. This ratio was then eluted at the appropriate concentration to give a final concentration of 5×10^5 CFU/mL for bacteria and $2{,}5\times10^3$ CFU/mL for yeasts.

Firstly, 100 µL MHB media for bacteria and SDB media for yeasts added to each well of the microplate. For each preparation to be tested, 100 µL of preparation added in the first well and two-fold 8 serial dilutions were made. Then, 100 µL bacterial/fungal suspensions were added to the wells and they were left for incubation. Final concentrations were ranging from 2.083 to 0.016, 2.5 to 0.019, 3.125 to 0.024 µg/mL respectively for F1, F2 and F3. After incubation for 20 h at 35±2° for the antibacterial assay and for 48 h at 35±2° for the antifungal assay, the last well with no growth of microorganism was recorded to represent the MIC (µg/mL). All experiments conducted in two parallel sets for controlling purposes.

3. RESULTS AND DISCUSSION

As it can be seen from Table 1, antimicrobial activity results indicate that all the formulations have significantly higher activity with lower activity concentration values than EO, on all microorganism studied. Except for *P. aeruginosa* and *S. parathyphi* C, all three formulations showed bactericidal/fungusidal activity at concentrations in the tested range. Visible growth of *P. aeruginosa* ATCC 9027 inhibited with 0.130, 0.156, 0.196 for F1, F2 and F3 respectively. For *P. aeruginosa* ATCC 27853 MIC value between approximately 0.2-0.3 µg/mL, remained above 0.1 µg/mL for all three formulas. F2 and F3 showed bactericidal activity at all concentrations in the tested range and F1 at the 0.033 µg/mL value for *S. paratyphi* C. When we compare the values for EO, *C. albicans* ATCC 10231(0.078 µg/mL), *C. parapsilosis* ATCC 22019 (0.039 µg/mL) and *E.faecalis* ATCC 29212 (0.039 µg/mL) seems to be most affected microorganisms. MIC values of EO vary between 0.312-0.039µg/mL for Gram positive bacteria and between 1.250-0,156 µg/mL for Gram negative bacteria.

Table 1. Antimicrobial activity results of *Thymus capitatus* essential oil (EO) and gargle formulas with three different concentrations (F1, F2, F3)

Minimum Inhibitory Concentration (MIC) results (µg/mL)						
Microorganism	**EO**	**F1**	**F2**	**F3**	**C**	**M**
Staphylococcus aureus ATCC 43300	0.312	≤ 0.016	≤ 0.019	≤ 0.024	0,312	-
Staphylococcus aureus ATCC 25923	0.156	≤ 0.016	≤ 0.019	≤ 0.024	0,312	-
Staphylococcus epidermidis ATCC 12228	0.312	≤ 0.016	≤ 0.019	≤ 0.024	0,078	-
Bacillus subtilis ATCC 6633	0.156	≤ 0.016	≤ 0.019	≤ 0.024	0,078	-
Enterococcus faecalis ATCC 29212	0.039	≤ 0.016	≤ 0.019	≤ 0.024	0,625	-
Escherichia coli ATCC 25922	0.312	≤ 0.016	≤ 0.019	≤ 0.024	0,0097	-
Pseudomonas aeruginosa ATCC 27853	1.250	0.260	0.312	0.390	0,625	-
Pseudomonas aeruginosa ATCC 9027	0.625	0.130	0.156	0.196	0,312	-
Salmonella paratyphi C	0.156	0.033	≤ 0.019	≤ 0.024	0,312	-
Klebsiella pneumoniae RSKK 574	0.156	≤ 0.016	≤ 0.019	≤ 0.024	0,039	-
Candida albicans ATCC 10231	0.078	≤ 0.016	≤ 0.019	≤ 0.024	-	1,56
Candida parapsilosis ATCC 22019	0.039	≤ 0.016	≤ 0.019	≤ 0.024	-	1,56

-: Not applicable, C: Ciprofloxacin, M: Miconazole

Table 2. Stability results of *Thymus capitatus* essential oil gargle formulas (F1, F2, F3)

Tests	F1					F2					F3				
	t_o	5°C ±3°C		25°C ± 2°C		t_o	5°C ± 3°C		25°C ± 2°C		t_o	5°C ± 3°C		25°C ± 2°C	
		3M	6M	3M	6M		3M	6M	3M	6M		3M	6M	3M	6M
Appearance	Clear	Clear, light yellow		Clear, light brown		Clear	Clear, light yellow		Clear, light brown		Clear	Clear, light yellow		Clear, light brown	
pH	5.85	5.85	5.87	5.92	5.95	6.03	6.03	6.03	6.05	6.06	6.25	6.22	6.25	6.20	6.16

Preformulation of *Thymus* essential oil with 8% (F1), 10% (F2) and 12.5% (F3) dilutions showed significant antimicrobial activity against all microorganisms tested, when the values of standard agents' (ciprofloxacin and miconazole) are considered. Without using any preservatives, they were well closed and stored at room temperature and refrigerator. In two different storage conditions, the appearances and pHs of three formulas were not changed (Table 2).

The major contents of genus *Thymus* essential oils were thymol, carvacrol, γ-terpinene and *p*-cymene, respectively [19]. Northern Cyprus thyme essential oil was found to be rich in thymol percentage (62.3%) [14] which was rarely encountered in the nature and also were found to be relatively higher than other Mediterranean countries' samples [20,21]. The possible reasons of variations in essential oil compositions were mentioned as climatic, geographical and/or environmental factors in the literature for other *Thymus* species [22,23].

Some other studies have been carried on regional *T. capitatus* essential oils' antimicrobial activity. Dzamic et. al., 2015 found that wild growing Libyan *T. capitatus* essential oil showed high antibacterial and antifungal activity against many human pathogens, between the range of MIC values 1-2 µg/mL for bacteria and 0.2-1 µg/mL for fungae [15]. In the study of Tabti et. al., 2014 it has reported that *T. capitatus* essential oil have a significant antifungal activity with the MIC range of 0.1-0.5 µg/mL against some *Aspergillus spp.* [16]. Mkaddem et. al., 2010 reported that Tunusian *T. capitatus* essential oil had an antibacterial activity against *K. pneumoniae* and *E. coli* by disc diffusion method. In our study, essential oil MIC values ranged between 0.1-1.25 µg/mL for bacteria and 0.039-0.078 µg/mL for yeasts.

Our EO result values are comparable to other studies with *Thymus spp*. mentioned above. We think that the reason why we found activity at slightly lower values was due to the difference in the active substance rates, especially the high rate of thymol active substance in the oil. As a result, the preformulations prepared have been shown to reduce the antimicrobial activity concentration of the essential oil and show an efficacy comparable to antimicrobial agents against broad range of microorganisms and stability results show that these compositions have good physical and chemical stability in wo storage conditions at the predicted time interval.

4. CONCLUSIONS

We aimed to develop a mouthwash/gargle preformulation with thyme oil which has been used for a very long time in traditional therapy, as well as the main components of thyme oil, such as thymol and carvacrol, are used in various formulations in modern medicine for their antimicrobial activities. For this purpose, these three preformulation studies showed a higher efficacy than essential oil and we observed a remarkable efficacy compared to standard antimicrobial agents. Wildly growing *Thymus capitatus* in Northern Cyprus could be used as not only for an essential oil source but also this essential oil would be used for development of herbal gargle optimum formulations in order to reduce fungal and bacterial infections of mouth. Further studies will compare pure Thymol in order to understand the real efficacy of *Thymus capitatus*.

Regarding these studies, it is also planned to research the effectiveness of these formulations with future antiviral studies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was performed in accordance with the Helsinki declaration.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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