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Short Communication

Antibacterial Activity of Anti-Aphthous Spray and Oral Drop: Two Thymus Commercial Products

Mohammad Reza Nahaei[1], Parisa Rahbarfam[1], Mahsa Kalajahi[1], Solmaz Maleki Dizaj[2], Farzaneh Lotfipour[2*]

[1]*Department of Biological Sciences, Tabriz Higher Education Institute of Rab-Rashid, Tabriz, Iran.*
[2]*Faculty of Pharmacy and Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

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ABSTRACT

Background: Today, traditional medicine is developed globally as an important source for health care of the world's population. The current study describes the antibacterial activity of *thymus* commercial products against both Gram-positive including *Staphylococcus aureus*, *Streptococcus pyogenes* and Gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa.*

Methods: Two commercial products of *thymus* with standard expiration date (and in three different batch numbers) including anti-aphthous *spray* and oral drop were purchased from the pharmacies of Tabriz city. *Minimum Inhibitory Concentration* (MIC) and disk diffusion are used to investigate the antibacterial efficiency of the mentioned products.

Results: The results of disk diffusion method showed zones of growth inhibition against *S. aureus and S. pyogenes* for the investigated products. Based on MICs, *thymus* oral drop had inhibitory effects against *S. aureus*, *S. pyogenes* while anti-aphthous *spray* showed inhibitory effects against *S. aureus*, *S. pyogenes* and *P. aeruginosa*. The findings also indicated that the *thymus* anti-aphthous *spray* had more inhibitory effects than *thymus* oral drop.

Conclusion: This study showed that *thymus* can be used as an optimistic antibacterial agent against the selected microorganisms.

Introduction

Medicinal plants have been known during human history. These plants make many chemical compounds and according to reports, 12,000 compounds have been isolated from them so far. These compounds may present biological functions, including antifungal and antibacterial activities. Herbal drugs may possess helpful pharmacology effects and the same potential as conventional drugs. Therefore, the growth in utilization of medicinal plants and also the development of attentiveness has improved the skill of pharmacists and chemists in order to reduce current problems such as side effects of conventional drugs.[1-4]

Like other medicinal plants, aromatic plants have been applied for their medicinal properties for centuries. The genus *thymus* contains about 350 species of herbaceous plants and is native to temperate regions in Europe, North Africa and Asia.[1] *Thymus* species have medicinal and cosmetics applications.[3] The antifungal and antibacterial activity of *thymus* is reported by several investigators.[2-4] Oussalah et al reported antimicrobial activity of *thymus vulgaris (with* a MIC $\leqslant$ 0.1% (v/v)) for some pathogenic bacteria (including *Escherichia coli*, *Listeria monocytogenes*, *Salmonella paratyphi* and *Staphylococcus aureus*).[5] Maksimović et al confirmed that essential oil of *thymus* possesses notable in vitro antimicrobial activity against *S. aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *E. coli*.[1] Marino et al assessed the antimicrobial activity of *thyme* essential oils against nine strains of Gram-negative bacteria and six strains of Gram-positive bacteria. Their results revealed that all the *thyme* essential oils had a remarkable bacteriostatic effect against the tested microorganisms. Their results also showed that the antimicrobial activity was more evident against the Gram-positive bacteria and *E. coli* was the most sensitive species even in the lowest concentration of oil.[6] The earlier investigates have exposed that most features of *thymus* medicinal uses are associated to the numerous levels of thymol,

***Corresponding Author:** Farzaneh Lotfipour , E-mail: lotfipoor@tbzmed.ac.ir

carvacrol, and phenolic derivatives with strong and wide-spectrum antimicrobial activity.[1,7]
The aim of this study was to investigate the *in vitro* efficiency of some *thymus* commercial products against selected pathogens including both Gram-positive (*S. aureus*, *S. pyogenes*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa).*

Material and Methods

Media and chemicals

All media including *Mueller-Hinton agar* (MHA) and *Mueller-Hinton broth* (MHB) were obtained from *Merck* Company (Darmstadt, *Germany). Thymus* commercial products (anti-aphthous *spray* and oral drop) were purchased from Tabriz, Iran.

Microorganisms

The microorganisms used in this study (*Staphylococcus aureus* PTCC 1112, *Streptococcus pyogenes* PTCC 1447, *Escherichia coli* PTCC 1338, *Pseudomonas aeruginosa* PTCC 1074) were obtained from Iran's Biotechnology Institute of Scientific and Technical Research (Tehran, Iran).

Inoculum preparation

The bacteria were activated according to supplier protocols to prepare the bacteria's stock cultures. Then, a single colony from the stock cultures was transferred into Mueller Hinton Broth. After overnight incubation (at 37 ˚C), cells were collected by centrifugation at 3000 rpm. Cells were washed and re-suspended with a sterile physiologic saline solution to prepare the bacteria inoculum equal to 10^8 CFU.mL^{-1}.

Sample preparation of commercial products and Determination of MICs

MIC determination was performed by broth macro dilution method according CLSI protocol. Ten tubes contain 1 ml sterile buffer were used as serial dilution tubes. Then, 1 ml of samples (anti-aphthous *spray* and oral drop each from three different batches) was added in the first tube and twofold serial dilutions were prepared using sterile buffer. Then, 100 µl of bacterial inocula and 900 µl of Mueller-hinton broth were transferred into the tubes and all tubes were incubated at 35˚C for 24 h. After 24 h incubation of dilution tubes, MICs of the products was determined for most sensitive bacterial species. To this end, the first tube of the series with no sign of visible growth after streak culture to the agar plates and incubation was considered as the MICs.[7] This process has been done three times.

Disc diffusion method

The filter paper discs with identical diameter were autoclaved and impregnated by 30 µl of solution of the antimicrobial products. The disks were placed on the nutrient agar plates and incubated at 37 °C for 24 h. The inhibition zone diameters were read for samples and recorded.[7]

Results

MIC results

Based on the obtained results for MIC determination, both *thymus* commercial products exhibited inhibitory effects on selected microorganisms. *Thymus* oral drop exhibited inhibitory effects against *S. aureus*, *S. pyogenes* whereas anti-aphthous *spray* showed inhibitory effects against *S. aureus*, *S. pyogenes* and *P. aeruginosa* as well. The results also showed that the *thymus* anti-aphthous *spray* had more inhibitory effects compared to *thymus* oral drop. The results of MIC tests against four selected microorganisms are shown in Table 1 and the pertaining plates pictures are shown in Figures 1 to 4.

Figure 1. Streak cultures with *thymus* Anti-aphthous *Spray sample* on *S. aureus* PTCC 1112 (Second batch).

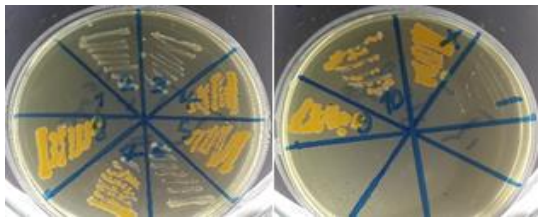

Figure 2. Streak cultures with *thymus* Anti-aphthous *Spray sample on S. pyogenes* PTCC 1447 (Second batch).

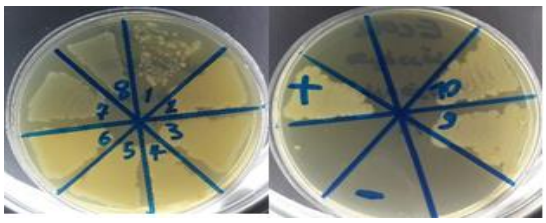

Figure 3. Streak cultures with *thymus* Anti-aphthous *Spray sample on E. Coli* PTCC 1338 (Second batch).

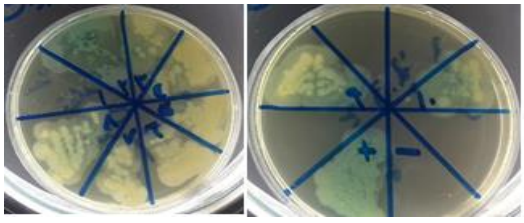

Figure 4. Streak cultures with *thymus* Anti-aphthous *Spray sample on P. Aeruginosa* PTCC 1074 (Second batch).

Table 1. The results of MICs against four selected microorganisms.

	MICs against:											
	S. aureus			*S. pyogenes*			*E. coli*			*P. aeruginosa*		
	Batch No.											
	1	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
Anti-aphthous *Spray*	1/4	1/2	1/2	1/4	1/2	1/2	ND*	ND	ND	1/2	1/2	ND
Oral Drop	1/4	1/4	1/4	1/4	1/2	1/2	ND	ND	ND	ND	ND	ND

*ND: Not Detected

Table 2. The results of disk diffusion method against four selected microorganisms.

	Mean zones of growth inhibition (mm) ± SD			
	S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*
Anti-aphthous *spray*	15±3	14±4	ND	10±2
Oral drop	11±3	10±2	ND	ND
Standard Amikacin disk	19±3	18±2	12 ±2	19±3

*ND: Not Detected

Disk diffusion method

The results of disk diffusion method showed "zones of growth inhibition" only for *S. pyogenes* and no "zones of growth inhibition" for other bacteria. The findings also indicated that "zones of growth inhibition" for *thymus* oral drop was greater than *thymus* anti-aphthous *spray*. The results of disk diffusion method against four selected microorganisms are shown in Table 2.

Discussion

The previous researches have shown that most aspects of *thymus* medicinal applications are related to the various levels of thymol and/or carvacrol, phenolic derivatives with strong and wide-spectrum antimicrobial activity.[1,8]

Based on MIC and MBC results, *thymus* oral drop exhibited inhibitory effects against Gram negative bacteria (*P. aeruginosa* and *E. coli*) whereas anti-aphthous *spray* showed inhibitory effects against both Gram positive and Gram negative bacteria (*S. aureus*, *S. pyogenes*, *P. aeruginosa).*

The results also showed that the *thymus* anti-aphthous *spray* had more inhibitory effects compared to *thymus* oral drop. The producer company did not provide information about the excipients incorporated into the formulations and it can be probably said that in the case of using the same concentration of thymus extract in both products, the solvents and excipients in the anti-aphthous spray may potentiate the antimicrobial activity of the thymus extract compared to that of oral drop. On the other hand, MIC values in the case of first batch are higher than the remaining two batches for anti-aphthous *spray and* oral drop. Martin et al tested and compared the antioxidant and antibacterial properties for decoction, infusion and hydroalcoholic extract of *thymus*. Their results showed that all samples had efficiency against Gram-positive (*S. aureus* and *S. epidermidis*) and Gram-negative (*E. coli*, *P. aeruginosa* and *E. aerogenes*) bacteria. They concluded that the use of *thyme* infusion and decoction (by both internal and external use) is safe without adverse reactions.[9]

The observed batch to batch variations in MIC and MBC results can be attributed to the differences between manufacturing processes and the variations during construction or formulation of these products or *lack of reproducibility of them.* If batch effects go undetected, the technologies developed for clinical outcomes (using data) may produce results that are more variable than expected. Then, development of precise and repeatable manufacturing processes with reproducible pharmaceutical products is necessary that can decrease batch to batch variations.[10] Also the assay method is of great importance in evaluation of antimicrobial activity.[11-13]

The results of disk diffusion method showed "zones of growth inhibition" only for *S. aureus and S. pyogenes* and no "zones of growth inhibition" for other bacteria in the case of both products. Maksimović et al reported antimicrobial efficiency for essential oil of *thymus* against *S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli*. They reported maximum activity for *S. aureus* and *E. coli*, moderate activity for *P. aeruginosa* and one of the tested strains of *K. pneumoniae* (MIC = 200 µl/ml), and *E. faecalis* expressed a higher degree of resistance.[1]

Conclusion

The present study is focused on the antimicrobial activity of two commercial products of *thymus*. The efficacy of these products can develop a new approach for using of the medical herbs in the incorporation into the drug formulations in order to treatment of infectious diseases. This strategy may consider more important in the case of the infectious diseases that have developed resistance to antibiotics. So therefore, herbal drugs may hold helpful pharmacology and the same potential as

conventional drugs. However, plant material comes with a variety of compounds that may have undesired effects that should be efficiently processed.

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Maksimović Z, Milenković M, Vučićević D, Ristić M. Chemical composition and antimicrobial activity of Thymus pannonicus All.(Lamiaceae) essential oil. Open Life Sci. 2008;3(2):149-54. doi:10.2478/s11535-008-0013-x
2. Didry N, Dubreuil L, Pinkas M. Antibacterial activity of thymol, carvacrol and cinnamaldehyde alone or in combination. Pharmazie. 1993;48(4):301-4.
3. Didry N, Dubreuil L, Pinkas M. Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. Pharm Acta Helv. 1994;69(1):25-8. doi:10.1016/0031-6865(94)90027-2
4. Horvath G, Kocsis B, Botz L, Németh J, Szabo L. Antibacterial activity of Thymus phenols by direct bioautography. Acta Biol Szeged 2002;46(3-4):145-6.
5. Oussalah M, Caillet S, Saucier L, Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157: H7, Salmonella typhimurium, Staphylococcus aureus and Listeria monocytogenes. Food control. 2007;18(5):414-20. doi:10.1016/j.foodcont.2005.11.009
6. Marino M, Bersani C, Comi G. Antimicrobial activity of the essential oils of Thymus vulgaris L. measured using a bioimpedometric method. J Food Prot. 1999;62(9):1017-23. doi:10.4315/0362-028x-62.9.1017
7. M.A. Wikler. Performance standards for antimicrobial susceptibility testing: eighteenth informational supplement. NewYork: Clinical and Laboratory Standards Institute (CLSI); 2008.
8. Nabavi SM, Marchese A, Izadi M ,Curti V, Daglia M, Nabavi SF. Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. Food Chem. 2015;173:339-47. doi:10.1016/j.foodchem.2014.10.042
9. Martins N, Barros L, Santos-Buelga C, Silva S, Henriques M, Ferreira IC. Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterisation. Food Chem. 2015;167:131-7. doi:10.1016/j.foodchem.2014.06.094
10. Leek JT, Scharpf RB, Bravo HC, Simcha D, Langmead B, Johnson WE, et al. Tackling the widespread and critical impact of batch effects in high-throughput data. Nat Rev Genet. 2010;11(10):733-9. doi:10.1038/nrg2825
11. Farajnia S, Hassan M, Hallaj-Nezhadi S, Mohammadnejad L, Milani M, Lotfipour F. Determination of indicator bacteria in pharmaceutical samples by multiplex PCR. J Rapid Methods Autom Microbiol. 2009;17(3):328–38. doi:10.1111/j.1745-4581.2009.00154.x
12. Azhdarzadeh M, Lotfipour F, Zakeri-Milani P, Mohammadi G, Valizadeh H. Anti-bacterial performance of azithromycin nanoparticles as colloidal drug delivery system against different gram-negative and gram-positive bacteria. Adv Pharm Bull. 2012;2(1):17-24. doi:10.5681/apb.2012.003
13. Azhar SL, Lotfipour F. Magnetic nanoparticles for antimicrobial drug delivery. Pharmazie. 2012;67(10):817-21.