IN VITRO STUDY OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF *ORIGANUM VULGARE* AGAINST *STAPHYLOCOCCUS AUREUS* ATCC 29213 AND *ESCHERICHIA COLI* ATCC 25922

Sylvia Atanasova-Stamova[1], Silvia Mihaylova[2], Neli Ermenlieva[3], Emilia Georgieva[4]

[1]Department of Pharmaceutical chemistry, Faculty of Pharmacy, Medical University of Varna
[2]TS Assistant Pharmacist, Medical College, Medical University of Varna
[3]Department of Microbiology and Virusology, Faculty of Medicine, Medical University of Varna
[4]TS Medical Laboratory Assistant, Medical College, Medical University of Varna

ABSTRACT

INTRODUCTION: Essential oils are aromatic and volatile liquids, extracted from different plant parts with antibacterial, antifungal, antiviral, antioxidant, and biological properties. For example, the essential oil of oregano presents antioxidant and antimicrobial activities.

AIM: The aim of this study is to evaluate the potential antimicrobial activity of two (for external and internal use) commercial essential oils of *Origanum vulgare* against *Staphylococcus aureus* ATCC 29213 and *Escherichia col*i ATCC 25922.

MATERIALS AND METHODS: The study was conducted in Medical College, Varna, Bulgaria. The antimicrobial activity of oregano essential oils was determined by the use of the Kirby-Bauer disk diffusion susceptibility test.

RESULTS: Antimicrobial activity of oregano essential oils (for external and internal use) was efficient in the control of *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. This inhibitory effect was observed by the inhibitory diameters above 21 mm.

CONCLUSION: The future evaluation of natural essential oils with a relatively high in vitro activity could be researched in the treatment of various infectious diseases.

Keywords: *essential oils, Lamiaceae family,* monoterpenes, *antibacterial activity, natural antimicrobials, bacterial strains*

Address for correspondence:
Sylvia Atanasova-Stamova
Faculty of Pharmacy
Medical University of Varna
84 Tzar Osvoboditel Blvd
9002 Varna, Bulgaria
e-mail: sylviastamova@yahoo.com

Received: November 20, 2021
Accepted: December 4, 2021

INTRODUCTION

The antibacterial activity of essential oils has been widely discussed for a long time. Essential oils are aromatic and volatile liquids, extracted from specific plant parts with antibacterial, antifungal, antiviral, antioxidant, and biological properties (1). The use of various natural compounds to combat microorganisms is increasingly present (2). The phenolic compounds included in essential oils, for exam-

ple, carvacrol, eugenol, and thymol are active against many microorganisms (3). This is why the essential oil of oregano presents antioxidant and antimicrobial activities, probably due to the presence of carvacrol and thymol (4,5). According to the Ph. Eur. 8th Edition, sum of the contents of carvacrol and thymol (both $C_{10}H_{14}O$) must be minimum 60 per cent in the essential oil (6). Carvacrol and thymol are cyclic organic compounds containing a methyl group, hydroxyl group, and an isopropyl group. The two structures differ from each other in terms of the position of the hydroxyl group. The key difference between carvacrol and thymol is that carvacrol contains a hydroxyl group at the ortho position of the benzene ring whereas thymol contains a hydroxyl group at the meta position of the benzene ring (7). The chemical structures of carvacrol and thymol are presented in Fig. 1.

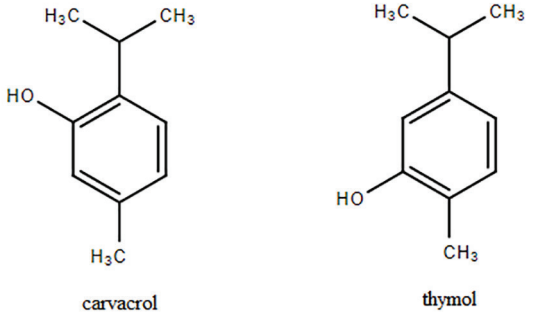


Fig. 1. Chemical structures of carvacrol and thymol.

The main focus of the study is to compare the potential antibacterial activity of oregano essential oils with different uses—for external and internal use.

AIM

The aim of this study is to evaluate the potential antimicrobial activity of two (for external and internal use) commercial essential oils of *Origanum vulgare* against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922.

MATERIALS AND METHODS

The study was conducted in Medical College, Varna, Bulgaria. In this experiment, the used essential oils are 100% pure with certified organic ingredients and are commercially available (Alteya Organics LLC; doTERRA (Europe) Ltd.). The antimicrobial activity of oregano essential oils was determined by the use of Kirby-Bauer disk diffusion susceptibility test against *Escherichia coli* and *Staphylococcus aureus*. We made dense seed of 0.5 MF standardized bacterial culture on the surfaces of Mueller–Hinton agar media (HiMedia®, provided by Ridacom, Bulgaria). A stock solution of 10% (v/v) oregano oil in dimethyl sulfoxide (DMSO) (1%) was prepared. Sterile filter discs (HiMedia®, provided by Ridacom, Bulgaria) were soaked with the appropriate volume (3, 5, 10 µL) of the test compounds (oregano essential oil—topical form, and oregano essential oil—internal form) and after the surfaces of the culture media have dried, the discs were placed on it. Controls were set for the solvent, which was used for the preparation of suspensions of the active substances—DMSO. All samples were performed in triplicate.

RESULTS AND DISCUSSION

The results of the reported antimicrobial activity of oregano essential oils (for external and internal use) against *Staphylococcus aureus* ATCC29213 and *Escherichia coli* ATCC25922 via disk-diffusion method are shown in Table 1, 2, and Fig. 1. The two essential oils showed high antimicrobial activity against these bacterial strains.

Regarding its antimicrobial activity, the result of the research confirms that the two used forms of oregano essential oils have antibacterial activity against Gram-positive bacteria such as *S. aureus*—zones of inhibition between 21 and 38 mm and against Gram-negative bacteria such as *E. coli*, with demonstrated zones of inhibition between 21 and 34 mm. These large zones of inhibition are comparable even to conventional antibiotics tested by this method. It is also possible that there is a synergistic effect

Table 1. Antimicrobial activity of oregano essential oils against Staphylococcus aureus ATCC29213 determined via disk-diffusion method (zones of inhibition in mm).

Essential Oil	10% Stock Solutions, µL		
	3	5	10
Oregano essential oil (internal use)	22 mm	30 mm	35 mm
Oregano essential oil (external use)	21 mm	34 mm	38 mm

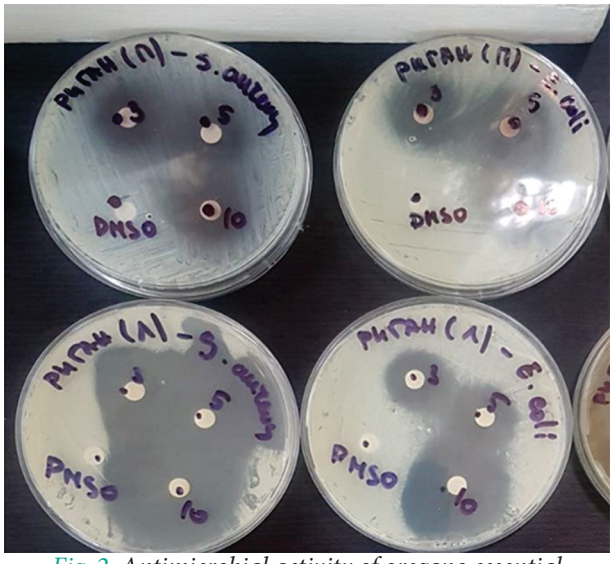


Fig. 2. Antimicrobial activity of oregano essential oils (internal form—above, and external form—below) against Staphylococcus aureus ATCC29213 and Escherichia coli ATCC25922 determined via disk-diffusion method.

Table 2. Antimicrobial activity of oregano essential oils against Escherichia coli ATCC25922 determined via disk-diffusion method (zones of inhibition in mm).

Essential Oil	10% Stock Solutions, μL		
	3	5	10
Oregano essential oil (internal use)	26 mm	30 mm	31 mm
Oregano essential oil (external use)	21 mm	25 mm	34 mm

between oregano oil and some antibiotics used in the treatment of infectious diseases. This would contribute mainly to the treatment of infections caused by multidrug-resistant strains of pathogens.

Other authors share similar results from in vitro sensitivity tests with Gram-negative and Gram-positive pathogenic strains with clinical significance in human medicine (8,9,10,11). De Aguiar et al. conclude that oregano is one of the EOs with the major potential antimicrobial against staphylococci. Tested via the disk diffusion method the authors report results with range inhibition zones 22–40 mm (10). Donaldson et al., 2005 also give encouraging results and discuss that from 14 EOs that they tested with diffusion test, oregano was the most active oil against *Staphylococcus aureus* and *Candida albicans* (8).

Essential oils have the potential to demonstrate synergic interaction with conventional drugs (12,13,14) and also between EOs and their active components (15). Studies have also been conducted that explored the potential for synergistic effects between oregano oil and antimicrobial agents such as biological silver nanoparticles (16). The results demonstrate a powerful antimicrobial activity that is extremely valuable in the fight against microbes of clinical importance. All presented options would contribute mainly to the treatment of infections caused by multidrug-resistant strains of pathogens—one of the main causes of high mortality worldwide. A number of studies discuss the possibility of using EOs as a natural antimicrobials with a strong effect against a number of polyresistant strains of bacteria (16,17,18).

CONCLUSION

The antimicrobial activity of oregano essential oils (for external and internal use) was efficient in the control of *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. This inhibitory effect was observed by the inhibitory diameters above 21 mm. The future evaluation of natural essential oils with a relatively high *in vitro* activity could be researched in the treatment of various infectious diseases.

Acknowledgement

This work was supported by the institutional Science Fund (Project No. 21012) of the Medical University of Varna.

REFERENCES

1. Dutra TV, Castro JC, Menezes JL, Ramos TR, do Prado IN, Machinski M, et al. Bioactivity of oregano (Origanum vulgare) essential oil against Alicyclobacillus spp. Ind Crops Prod. 2019;129:345-9. doi: 10.1016/j.indcrop.2018.12.025.
2. Castro JC, Endo EH, Souza MR, Zanqueta EB, Polonio JC, Pamphile JA, et al. Bioactivity of essential oils in the control of Alternaria alternata in dragon fruit (Hylocereus undatus Haw.). Ind Crops Prod. 2017; 97:101-9. doi: 10.1016/j.indcrop.2016.12.007.
3. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 1999;86:985–90. doi: 10.1046/j.1365-2672.1999.00780.x.

4. Li J, Ye F, Lei L, Zhao G. Combined effects of octenylsuccination and oregano essential oil on sweet potato starch films with an emphasis on water resistance. Int J Biol Macromol. 2018;115:547-53. doi: 10.1016/j.ijbiomac.2018.04.093.

5. Zhou Y, Sun S, Bei W, Zahi MR, Yuan Q, Liang H. Preparation and antimicrobial activity of oregano essential oil Pickering emulsion stabilized by cellulose nanocrystals. Int J Biol Macromol. 2018;112:7-13. doi: 10.1016/j.ijbiomac.2018.01.102.

6. European Pharmacopoeia. 8.0 Edition. pp. 1342-4.

7. Abdelhamid AG, Yousef AE. Carvacrol and Thymol Combat Desiccation Resistance Mechanisms in Salmonella enterica Serovar Tennessee. Microorganisms. 2021;10(1):44. doi: 10.3390/microorganisms10010044.

8. Donaldson JR, Warner SL, Cates RG, Young DG. Assessment of antimicrobial activity of fourteen essential oils when using dilution and diffusion methods. Pharm Biol. 2005;43 (8):687-95. doi: 10.1080/13880200500384932.

9. Melo AD, Amaral AF, Schaefer G, Luciano FB, de Andrade C, Costa LB, et al. Antimicrobial effect against different bacterial strains and bacterial adaptation to essential oils used as feed additives. Can J Vet Res. 2015;79(4):285-9.

10. de Aguiar FC, Solarte AL, Tarradas C, Luque I, Maldonado A, Galán-Relaño Á, et al. Antimicrobial activity of selected essential oils against Streptococcus suis isolated from pigs. Microbiologyopen. 2018;7(6):e00613. doi: 10.1002/mbo3.613.

11. da Silva Gündel S, de Souza ME, Quatrin PM, Klein B, Wagner R, Gündel A, et al. Nanoemulsions containing Cymbopogon flexuosus essential oil: Development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. Microb Pathog. 2018;118:268-76. doi: 10.1016/j.micpath.2018.03.043.

12. Langeveld WT, Veldhuizen EJ, Burt SA. Synergy between essential oil components and antibiotics: a review. Crit Rev Microbiol. 2014;40(1):76-94. doi: 10.3109/1040841X.2013.763219.

13. Thawabteh A, Juma S, Bader M, Karaman D, Scrano L, Bufo SA, Karaman R. The Biological Activity of Natural Alkaloids against Herbivores, Cancerous Cells and Pathogens. Toxins (Basel). 2019 Nov 11;11(11):656. doi: 10.3390/toxins11110656.

14. Zacchino SA, Butassi E, Cordisco E, Svetaz LA. Hybrid combinations containing natural products and antimicrobial drugs that interfere with bacterial and fungal biofilms. Phytomedicine. 2017;37:14-26. doi: 10.1016/j.phymed.2017.10.021.

15. Bassolé IH, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;17(4):3989-4006. doi: 10.3390/molecules17043989.

16. Scandorieiro S, de Camargo LC, Lancheros CA, Yamada-Ogatta SF, Nakamura CV, de Oliveira AG, et al. Synergistic and additive effect of oregano essential oil and biological silver nanoparticles against multidrug-resistant bacterial strains. Front Microbiol. 2016;7:760. doi: 10.3389/fmicb.2016.00760.

17. Uzair B, Niaz N, Bano A, Khan BA, Zafar N, Iqbal M, et al F. Essential oils showing in vitro anti MRSA and synergistic activity with penicillin group of antibiotics. Pak J Pharm Sci. 2017;30(5(Supplementary)):1997-2002.

18. Lu M, Dai T, Murray CK, Wu MX. Bactericidal property of oregano oil against multidrug-resistant clinical isolates. Front Microbiol. 2018;9:2329. doi: 10.3389/fmicb.2018.02329. Erratum in: Front Microbiol. 2021;12:713573.