

Provided for non-commercial research and education use.

Not for reproduction, distribution or commercial use.



This article was published in an CASRP journal. The attached copy is furnished to the author for non-commercial research and education use, including for instruction at the authors institution, sharing with colleagues and providing to institution administration.

Other uses, including reproduction and distribution, or selling or licensing copied, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding CASRP's archiving and manuscript policies encouraged to visit:

<http://www.casrp.co.uk/journals>

© 2016 CASRP Publishing Company Ltd. UK.



Available online at [www.casrp.co.uk/journals](http://www.casrp.co.uk/journals)

# CASRP PUBLISHER

International Journal of Advanced Biological  
and Biomedical Research 4(3) (2016) 253–256



Original Article

Open Access

## Antimicrobial activity of essential oils of cultivated oregano (*Origanum vulgare*) against clinical isolates of *Escherichia coli*

Morad Soleimani Sarghashk<sup>1,\*</sup>, Musa Khammari<sup>1</sup>, Davood Khammari<sup>1</sup>, Saphora Bazi<sup>2</sup>

<sup>1</sup>Department of Plant Breeding and Biotechnology, Collage of Agriculture, University of Zabol, Zabol, Iran.

<sup>2</sup>Department of Biology, Faculty of Science, Payame Noor University, Zabol, Iran.



### Abstract

The present study was carried out to determine the potential antibacterial effect of essential oil of *Origanum vulgare* against antibiotic resistant *E. coli*. In this study, the essential oil of *Origanum vulgare* obtained by hydrodistillation was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) in order to determine their chemical composition. The minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this essential oil. The results in tables 1 showed that essential oil of *Origanum vulgare* had inhibitory effect against most isolated plates. The least MIC value of essential oil of *Origanum vulgare* was 0.62 mg/ml and the highest MBC value of essential oil of *Origanum vulgare* were 5 mg/ml and 10mg/ml. The present studies confirm the use of this essential oil as antibacterial agent. Further research is required to evaluate the practical values of therapeutic applications.

© 2016 Published by CASRP publishing company Ltd. UK. Selection and/or peer-review under responsibility of Center of Advanced Scientific Research and Publications Ltd. UK.

**Keywords:** Essential oil, *Origanum vulgare*, *E. coli*, Antibacterial activity.

### 1. Introduction

Microorganisms occur nearly everywhere in nature and affect the wellbeing of people in a great many ways. Many different microbial species normally inhabit various parts of our bodies, such as the oral cavity, skin and intestinal tract. Essential oils (EO) are products of plant secondary metabolites. They are a mixture of compounds, mainly mono and sesquiterpenes, carbohydrates, alcohols, aldehydes and ketones. The biological activity is in

\*Corresponding author: Department of Plant Breeding and Biotechnology, Collage of Agriculture, University of Zabol, Zabol, Iran.

© 2016 The Authors. This is an open access article under the terms of the Creative Commons Attribution-Non Commercial- No Derives License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Received 19 November 2016

Accepted 18 December 2016

Available online 25 December 2016

iThenticate screening 22 November 2016

English editing 15 December 2016

Quality control 21 December 2016

direct dependence of the genetically induced specific chemical composition of an EO. Considering this premise, the study of the antibacterial action of the essential oil of *Origanum vulgare* against strains of multiresistant bacteria, from nosocomial origin and isolated from clinical materials from diverse anatomical sites in the body was motivated. Since ancient ages essential oils (Eos) and other extracts of plants have evoked interest as sources of natural products, they are also called volatile are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, or extraction, but the method of steam distillation is most commonly used for commercial production of Eos (Van de Braak and Leijten, 1999; Tabrizi et al., 2016). Oregano (*Origanum vulgare* sub sp. *Hirtum*), a herb of the Labiatae family that has been used widely in cooking and folk healing, is the common oregano that thrives naturally in almost every region of Greece, especially on the edges of fields, dry and uncultivated or waste ground. Chemical analysis of the oregano EO revealed the presence of several ingredients, most of which possess important antioxidant and antimicrobial properties (Ozkan et al., 2003). Carvacrol and thymol, the two main phenols that constitute about 78–85% of oregano Eos, are principally responsible for the antimicrobial activity (Kokkini et al., 1997). In addition, other minor constituents such as the monoterpene hydrocarbons  $\gamma$ -terpinene and *p*-cymene also contribute to the antibacterial activity of the oil (Burt, 2004). In the literature, there are many reports relating the chemical composition and the antimicrobial properties of the Eos of various oregano species, and their application in various commercial preparations, as antimicrobials and antioxidants (Baydar et al., 2004). The present study was carried out to determine the potential antibacterial effect of essential oil of *Origanum vulgare* against antibiotic resistant *E. coli*.

## 2. Materials and methods

### 2.1. Plant materials

The seed *Origanum vulgare* was collected in the region of Iran (Zahedan and Kerman, Southeastern, Iran) and planted in Kerman Azaduniversity herbarium received approval and dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

### 2.2. Distillation of essential oil

The seed *Origanum vulgare vulgare* was ground prior to the operation and then 300 g of ground rosemary was submitted to water distillation for 4h using a Clevenger apparatus. The distilled essential oil was dried over anhydrous sodium sulfate, filtered and stored at 4°C.

### 2.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant essential oil

The broth microdilution method. All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 mg/ml to 10.00 mg/mL. To each well, 10  $\mu$ L of indicator solution (prepared by dissolving a 10 mg extract in 2 ml of DMSO) and 10  $\mu$ L of Mueller Hinton Broth were added. Finally, 10  $\mu$ L of bacterial suspension ( $10^6$  CFU/mL) was added to each well to achieve the concentration of  $10^4$  CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18-24 h. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated to provide the MIC and MBC values for the tested extracts. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the essential oil at which the incubated microorganism was completely killed.

## 3. Results and discussion

The results in tables 1 showed that essential oil of *Origanum vulgare* had inhibitory effect against most isolated plates. The least MIC value of essential oil of *Origanum vulgare* was 0.62 mg/ml and the highest MBC value of essential oil of *Origanum vulgare* were 5 mg/ml and 10mg/ml.

**Table 1**Minimum inhibitory concentration of *Origanum vulgare* essential oil against *E. coli*.

Bacterial cod	MIC/MBC	Bacterial cod	MIC/MBC
1	5/10	11	0.62/1/25
2	5/10	12	5/10
3	2/5/5	13	2/5/5
4	2/5/5	14	5/10
5	1/25/2/5	15	2/5/5
6	5/10	16	0.62/1/25
7	5/10	17	5/10
8	2/5/2/5	18	2/5/5
9	5/10	19	2/5/5
10	5/5	20	5/10

Medicinal plants deeply connected with our lives, especially those who not only hold culinary importance, but also possess combative ability against number of microbes. They become cynosure in our homes, commercial and research sector. Their natural origin paves expedient outcomes, some in form of natural preservatives along with aiding our immune system to fight against pathogenic organisms. World Health Organization (WHO) supported the use of herbal medicines as safe therapy for the treatment of different diseases. Medicinal plants would be the paramount basis to find a range of drugs (Santos et al., 1985). The study of Saeed and Tarig, infusion and essential oil exhibited antibacterial activity against *Staphylococcus saprophyticus*, *S. aureus*, *Micrococcus roseus*, *M. kristinae*, *M. nishinomiyaensis*, *M. lylae*, *M. luteus*, *M. sedentarius*, *M. varians*, *Bacillus megaterium*, *B. thuringiensis*, *B. alvei*, *B. circulans*, *B. brevis*, *B. coagulans*, *B. pumilus*, *B. laterosporus*, *B. polymyxa*, *B. macerans*, *B. subtilis*, *B. firmus*, *B. cereus* and *B. lichiniiformis*. The infusion exhibited maximum activity against *B. laterosporus* (17.5 mm mean zone of inhibition $\pm$ 1.5 Standard deviation) followed by *B. polymyxa* (17.0 mm $\pm$ 2.0 SD) and essential oil of oregano exhibited maximum activity against *S. saprophyticus* (16.8 mm $\pm$ 1.8 SD) followed by *B. circulans* (14.5 mm $\pm$ 0.5 SD). While all these tested isolates were found resistant to decoction of oregano (Saeed and Tarig, 2009). The study of Chaudhay, the oil, aqueous infusion and decoction of oregano (*Origanum vulgare*), of the family Lamiaceae, were assessed for antibacterial activity against 11 different genera of Gram-ve bacilli viz., *Aeromonashydrophila*, *Citrobacter* sp., *Enterobacteraerogenese*, *Escherichia coli*, *Flavobacterium* sp., *Klebsiellaozaenae*, *K. pneumoniae*, *Proteus mirabilis*, *Pseudomonasaeruginosa*, *Salmonella typhi*, *S. paratyphi B*, *Serratiamarcescens* and *Shigelladysenteriae*, by disc diffusion method. Oregano oil exhibited the highest activity against *Citrobacterspecies* with mean zone of inhibition of 24.0 mm  $\pm$  0.5.

The aqueous infusion also showed significant inhibitory activity against *Klebsiellapneumoniae* (20.1 mm  $\pm$  6.1 SD), *Klebsiellaozaenae* (19.5 mm  $\pm$  0.5 SD) and *Enterobacteraerogenes* (18.0 mm). Besides, all isolates were found resistant to the aqueous decoction of oregano seeds (Chaudhay et al., 2007). The oregano oil exhibited significant inhibitory activity against *Citrobacter* spp., (24.0 mm  $\pm$  0.5 SD), *Salmonella typhi* (22.4 mm  $\pm$  1.5 SD) and *Escherichia coli* (19.0 mm  $\pm$  2.2 SD) (Table 1). It has long been acknowledged that oregano oil is among the most active against strains of *E. coli* and also presents antimicrobial activity against pathogenic microorganisms like *Salmonella choleraesuis*, *S. typhi*, *S. typhimurium* and many others related GNB strains of *Enterobacteriaceae* family (Penalver et al., 2005) and *H. pylori* (Stamatis et al., 2003). The study of Coelho de Costa the result show that of the four strains of *A. baumannii*, three (01, 02 and 03) corresponding to 75% were inhibited by the essential oil of *O. vulgare* at the 0.125% concentration. The strain 04 (25%) was inhibited by the 0.5% concentration. The four strains (100%) of *E. coli*, *S. faecalis* and *S. aureus* were inhibited with the 0.125% concentration. From the four strains of *K. pneumoniae*, three (75%), strains 01, 02 and 04 were inhibited at the 0.125% concentration, on the other hand, the strain 03 was inhibited in the 0.25% concentration. Considering the strains of *P. aeruginosa*, three of them (01, 02 and 03) corresponding to 75% were inhibited at the 0.5% concentration, however, the strain 04 (25%) was inhibited in a lesser concentration (0.25%) (Coelho de Costa et al., 2009). The study of Ashraf, the antimicrobial activity of methanol, chloroform and aqueous extracts were determined against nine different gram negative and gram positive bacterial strains and three fungal stains.

The bacterial strains were *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), *Micrococcus luteus* (ATCC 9341), *Pseudo-monas aeruginosa* (ATCC 33347), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 19430), *Shig-ellaflexneri* (ATCC 25929), *Salmonella paratyphi A* (ATCC 9150) and *Proteus mirabilis* (ATCC 49565)

and fungal strains were *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus terreus*. Agar well diffusion method was followed in this study. The comparative analysis of antibacterial activity reflects that among these three extracts, chloroform and methanol extracts shows promising result by exhibiting maximum anti-bacterial activity, whereas aqueous extract is not active against most of these strains (Ashraf et al., 2011). The study of Morad showed that main component of leaf and flower oils of *Origanum vulgare* are carvacol (46/5% and 60/6%),  $\gamma$ -terpinene (13/91) and 16/64% and p-cymene (13.54% and 7/21%). *E. coli* and *S. aureus* had similar sensitivity to essential oils, but *S. typhimurium* showed more sensitivity (Moradi et al., 2014).

## References

- Baydar, H., Sagdic, O., Ozkan, G., Karadogan, T., 2004. Antibacterial activity and composition of essential oils from *Origanum*, *Thymra* and *Satureja* species with commercial importance in Turkey. *Food. Control.*, 15, 169-72.
- Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods – a review. *Int. J. Food. Microbiol.*, 94, 223-53.
- Chaudhry, N.M.A., Saeed, S., Tariq, P., 2007. Antibacterial effects of Oregano (*Origanum Vulgare*) against gram negative bacteria. *Pak. J. Bot.*, 39(2), 609-613.
- Da Costa, A.C., Cavalcanti dos Santos, B.H., Santos Filho, L., de Oliveira Lima, E., 2009. Antibacterial activity of the essential oil of *Origanum vulgare* L. (Lamiaceae) against bacterial multiresistant strains isolated from nosocomial patients. *Rev. Bras. Farmacogn.*, 19(1).
- Kokkini, S., Karousou, R., Dardioti, A., Krigas, N., Lanaras, T., 1997. Autumn essential oils of Greek oregano. *Phytochem.*, 44, 883-6.
- Moradi, M., Hassani, A., Ehsani, A., Hashemi, M., Raeisi, M., Naghibi, S.S., 2014. Phytochemical and Antibacterial properties of *Origanum Vulgare* ssp. *Gracile* growing wild in Kurdistan province of Iran. *J. Food. Q. Hazard. Contr.*, 120-124.
- Ozkan, G., Sagdic, O., Ozcan, M., 2003. Inhibition of pathogenic bacteria by essential oils at different concentrations. *Food. Sci. Technol Int.*, 9, 85-8.
- Saeed, S., Tariq, P., 2009. Antibacterial activity of oregano (*Origanum vulgare* Linn.) against gram positive bacteria. *Pak. J. Pharm. Sci.*, 22(4), 421-4.
- Santos, P.R.V., Oliveira, A.C.X., Tomassini, T.C.B., 1985. Controle Microbiológico de Produtos Fitoterápicos. *Rev. Farm. Bioquim.*, 31, 35-38.
- Stamatis, G., Kyriazopoulos, P., Goleyou, S., Basayiannis, V., Skaltsas, S., Skaltsa, H., 2003. In vitro anti-*Helicobacter pylori* activity of Greek herbal medicines. *J. Ethnopharmacol.*, 88(2-3), 175-179.
- Tabrizi, R., Zolala, F., Nasirian, M., Baneshi, M.R., Etmnian, A., Sekhavati, E., Khodadost, M., Haghdoost, A.A., 2016. Estimation of the prevalence of chronic kidney disease: The results of a model based estimation in Kerman, Iran. *Med. J. Islam. Repub. Iran.*, 30, 338.
- Van de Braak, S.A.A.J., Leijten, G.C.J.J., 1999. Essential oils and Oleoresins: A survey in the Netherlands and other major markets in the European union. CBI, centre for the promotion of imports from developing countries, Rotterdam, 116.

**How to cite this article:** Soleimani Sarghashk, M., Khammari, M., Khammari, D., Bazi, S., 2016. Antimicrobial activity of essential oils of cultivated oregano (*Origanum vulgare*) against clinical isolates of *Escherichia coli*. *International Journal of Advanced Biological and Biomedical Research*, 4(3), 253-256.

**Submit your next manuscript to CASRP Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.casrp.co.uk/journals](http://www.casrp.co.uk/journals)

