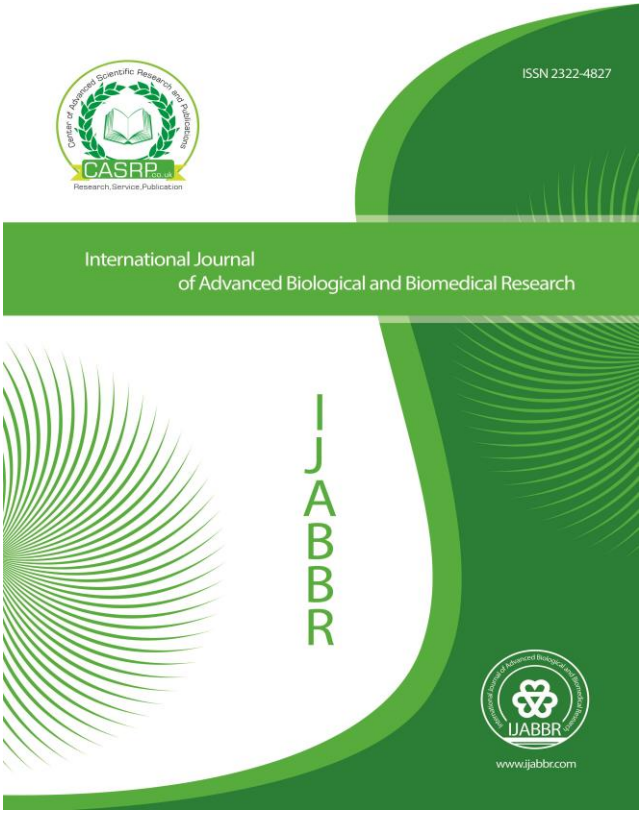

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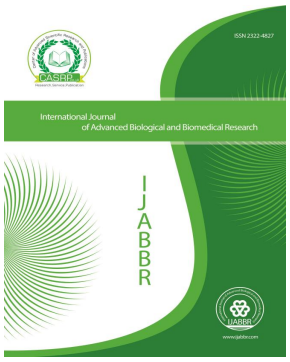

Original Article Open Access

Antimicrobial activity of essential oils of cultivated oregano (*Origanumvulgare*) against clinical isolates of *Escherichia coli*

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Abstract

The present study was carried out to determine the potential antibacterial effect of essential oil of *Origanumvulgare* against antibiotic resistant *E.coli.* In this study, the essential oil of *Origanumvulgare* 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 *Origanumvulgare* had inhibitory effect against most isolated plates. The least MIC value of essential oil of *Origanumvulgare* was 0.62 mg/ml and the highest MBC value of essential oil of *Origanumvulgare* 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.

Keywords: Essential oil, Origanumvulgare, 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

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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 *Origanumvulgare* 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 (*Origanumvulgare*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 γ-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 *Origanumvulgare* against antibiotic resistant *E.coli.*

2. Materials and methods

2.1. Plant materials

The seed *Origanumvulgare* 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 *Origanumvulgarevulgare* 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 4ºC.

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 µL of indicator solution (prepared by dissolving a 10 mg extract in 2 ml of DMSO) and 10 µL of Mueller Hinton Brothwere added. Finally, 10 µL of bacterial suspension (10^6CFU/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 37ºC 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 *Origanumvulgare* had inhibitory effect against most isolated plates. The least MIC value of essential oil of *Origanumvulgare* was 0.62 mg/ml and the highest MBC value of essential oil of *Origanumvulgare* were 5 mg/ml and 10mg/ml.

Table 1
Minimum inhibitory concentration of *Origanumvulgare* 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. lichiniformis. The infusion exhibited maximum activity against B. laterosporus (17.5 mm mean zone of inhibition+/-1.5 Standard deviation) followed by B. polymyxa (17.0 mm+/-2.0 SD) and essential oil of oregano exhibited maximum activity against S. saprophyticus (16.8 mm+/-1.8 SD) followed by B. circulans (14.5 mm+/-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 (*Origanumvulgare),* of the family Limiaceae*,* were asessed 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 *Citrobacter*species with mean zone of inhibition of 24.0 mm ± 0.5.

The aqueous infusion also showed significant inhibitory activity against *Klebsiellapneumoniae* (20.1 mm ± 6.1 SD), *Klebsiellaozaenae* (19.5 mm ± 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 ± 0.5 SD), *Salmonella typhi* (22.4 mm ± 1.5 SD) and *Escherichia coli* (19.0 mm ± 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 *Aspergillusflavus*, *Aspergillusnigar and Aspergilluspterus.* 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 Moradishowed that main component of leaf and flower oils origanumvulgarecarvacol (46/5% and 60/6%), γterpinene (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).

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