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Antibacterial Effects of *Origanum vulgare* Essence Against Multidrug-Resistant *Acinetobacter baumannii* Isolated From Selected Hospitals of Tehran, Iran

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Background: Infection due to *Acinetobacter baumannii* has become a significant challenge to modern healthcare systems. The rapid emergence and global dissemination of *A. baumannii* as a major nosocomial pathogen is remarkable and it demonstrates its successful adaptation to the 21st century hospital environment. Recent studies have discussed about essential oil of *Origanum vulgare* against a range of bacteria, including various species of *Staphylococcus*, *Pseudomonas*, *Bacillus* and *Escherichia coli*.

Objectives: The present study aimed to investigate the inhibitory effects *O. vulgare* essence against multidrug-resistant (MDR) strains of *A. baumannii* from selected hospitals in Tehran, Iran.

Materials and Methods: This oil was obtained using the hydrodistillation method and analyzed by gas chromatography mass spectrography (GC/MS). The antimicrobial activity against MDR isolates was achieved using disc diffusion method and macro-broth dilution assay.

Results: Analysis of the essential oil revealed the presence of pulegone (68.59%) piperitone (7.8%), piperitenone (7.8%), 1, 8-cineole (1.3%), and carvacrol (1.6%) as the major components. The results showed a significant activity against MDR *A. baumannii* with inhibition zones and minimal inhibitory concentration values in the ranges of 7-15 mm and 20-35 µL/mL respectively.

Conclusions: This investigation showed that the essence oil of *O. vulgare* had a potent antimicrobial activity against MDR *A. baumannii*. Further research is required to evaluate the practical values of therapeutic applications.

Keywords:Acinetobacter baumannii; Drug Resistance; Origanum; Anti-Infective Agents; Essential Oil

1. Background

Acinetobacter baumannii is a Gram-negative bacteria and important opportunistic pathogen, causing a variety of nosocomial infections, especially in intensive care units (ICUs). These infections include bacteremia, surgical-site infections, secondary meningitis, urinary tract infections, and ventilator associated pneumonia (1, 2). These organisms have been implicated in a diverse range of infections such as respiratory tract, blood stream, skin and soft tissue. The rapid emergence and global dissemination of *A. baumannii* as a major nosocomial pathogen is remarkable and demonstrates its successful adaptation to the 21st century hospital environments (3). Over the last 10 years, *A. baumannii* emerged as one of the most problematic pathogens and its treatment has been limited to only a few antibiotics. The ability of *A. baumannii* to survive for extended periods on environmental surfaces is notorious and likely important for transmission within the health care settings. Multidrug resistance (MDR) is common in health care-associated *A. baumannii* infections (4). With the increase in population and urban growth and reduced use of synthetic drugs, many medicinal herbs have been replaced. However, due to the expansion of MDR bacteria in the world, attention to medicinal herbs application is unavoidable (5).

The genus *Origanum* belongs to the family of *Labiatae* and includes many species that are commonly found as wild plants in the Mediterranean areas as well as Euro-Siberian and Irano-Siberian regions (6). *O. vulgare* is species of the *Origanum* genus wildly growing in Iran. This plant is widely spread all over the country, particularly in Gilan, Mazandaran and West Azerbaijan provinces (7). The major components of the *Origanum* essential oil are carvacrol and pulegone. Results of various studies indicated that the antioxidant effects of oregano might be related to the dominant components including carvacrol and pulegone, presentin its essential oil (8). The essential oil of various *Origanum* species can contain a number of additional chemical constituents (9) including linalool, gamma-terpinene, p-cymene and terpinene-4-ol (9, 10). *Origanum* has long been valued for its culinary, fragrant and

medicinal properties. These qualities are the results of natural chemicals in the plant. One source for these qualities is the volatile or "essential" oil. The composition of the essential oil depends on both its growth location and the genetics of the plant. High-potential antimicrobial effects of the essential oil of *Origanum* against a range of bacteria, including various species of *Staphylococcus*, *Pseudomonas, Bacillus* and *Escherichia coli* have been reported. The most combined studies on the pulegone *Origanum* oil has been focused on its antibacterial effects (9, 10).

2. Objectives

We aimed to assess the antibacterial effects *O. vulgare* essence oil against MDR strains of *A. baumannii* according to the Clinical Laboratory Standards Institute (CLSI) 2013 guidelines and Kirby Bauer method.

3. Materials and Methods

Plant material and extraction procedure flowering aerial parts of *O. vulgare* was collected from Khoramabad, Lorestan, Iran, in June 2010. The essential oil was prepared by steam distillation of the aerial parts of the plant. After drying with sodium sulfate at 4°C, the oil was kept for the gas chromatography (GC) injection system. Analysis and identification of the oil composition was performed by Barij Essence company.

The gas chromatography-mass spectrography (GC/MS) results indicated that this oil has many antibacterial compounds. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-5MS (60 m × 0.25 mm, film thickness: 0.25 m). The oven temperature program was 40°C, held for one minute, then raised up to 230°C at a rate of 3°C/minute, held for 10 minutes. Helium was used as the carrier gas at a flow rate of 1.0 mL/min.

3.1. Bacterial Strain and Culture Conditions

A. baumannii strains were isolated from ICU units (Children's Medical Center and Imam Khomeini Hospital, Tehran, Iran) and identified with standard microbiological methods (culture, biochemical tests, catalase, oxidase, citrate, morphology and genetic techniques). *A. baumannii* was isolated from clinical specimens such as blood (n = 4), respiratory secretions (n = 29), pus and wound swabs (n = 10), cerebrospinal fluid (n = 7), and urine (n = 25). The testing was performed using the following antibiotics: amikacin (AMK), oxacillin (OX), kanamycin (K), gentamicin (GM), neomycin (N), imipenem (IMP), ciprofloxacin (CIP), ceftazidime (CAZ), ceftriaxone (CRO) and polymyxin (CST). The purpose of using these antibiotics with selected MDR strains of *A. baumannii* was to compare the effects of antibiotics with that of the *O. vulgare* essence.

3.2. Determination of Minimum Inhibitory Concentration

To determine the MIC of the *O. vulgare* essence, broth dilution method (macrodilution) was used according to the CLSI guidelines. Dimethyl sulfoxide (DMSO) was used to dissolve the essential oil; 10 μL of the essence was dissolved in 90 μL of DMSO. The final concentration was about 0.1 mg/mL. The oil was kept at 4°C away from direct light and heat. In sterile tubes containing 5 mL Mueller Hinton broth, *A. baumannii* was cultured and incubated at 37°C for 18-24 hours. After the incubation, for each sample, five tubes containing 1 mL Mueller Hinton broth were prepared and respectively 15, 20, 25, 30 and 35 μL of the essential oil were added to them. After mixing, add bacterial suspension reached to a final volume of 5×10^6 (CFU/mL) and was incubated at 37°C for 18-24 hours. The results were evaluated after the incubation. In the series of experiments, a positive control tube (without oil) and a negative control tube (without bacteria) were used. After the incubation time of opacification, the bacterial growth was assessed visually. Any opacification or clear slight was considered as resistance. *A. baumannii* ATCC# 19606 was employed in this study as a reference strain. To evaluate the antimicrobial effects of the *O. vulgare* essential oil, the diffusion method (disk diffusion) was used.

3.3. Antimicrobial Susceptibility Test

Susceptibility to various classes of antibiotics was determined by the disc diffusion method in accordance with the CLSI guidelines. The test was performed using the following antibiotics: AMK (30 μg), OX (30 μg), K (30 μg), GM (10 μg), N (30 μg), IMP (10 μg), CIP (5 μg), CAZ (30 μg), CRO (30 μg) and CST.

In sterile conditions, the bacterial concentrations equivalent to 0.5 McFarland (1.5×10^8 CFU/mL) were cultured on Mueller Hinton agar medium using sterile cotton swabs. Blank discs with 6 mm diameters as well as the essence concentrations according to the determined MIC were placed on Muller Hinton agar media. Disks containing 30 μL of dimethyl sulfoxide were used as negative controls and colistin disc was used as positive control. After 18-24 hours of incubation at 37°C, the zones of growth inhibition were measured. Each concentration was repeated three times for each of the bacteria and the average was documented.

4. Results

A total of 75 *A.baumannii* strains were isolated from the ICU units. The frequency of the isolated *A. baumannii* according to clinical specimens were as follows: cerebrospinal fluid (CSF) (9%), respiratory tract (39%), wound (13.4%), blood (5.3%), and urine (33.3%). The antimicrobial susceptibilities of *A. baumannii* isolates are presented in Table 1 indicates that most of the *A. Baumannii* isolates have high resistancy to antibiotics, which is important for high-risk patients.

The minimum concentration of essence was determined by the absent of significant growth; also, determining MIC in tubes with no turbidity was carried out by culture. The minimum bactericidal concentration (MBC) value was the first tube showing no growth on solid medium. The results of this research stated that the MIC of

the essence illustrated antibacterial effects more than the selected antibiotics. The inhibition zone results of the *O. vulgare* essence against five MDR strains of *A. baumannii* (isolates that were resistant to all the antibiotics) were 7, 8, 10, 15, 12 and 15 mm, respectively. The MIC results for the *O. vulgare* essence were 20, 24, 28 and 35 (µL/mL), respectively (Table 2 and Figure 1). The results illustrated that although the isolated *A. baumannii* was resistant to many antibiotics with high MIC, it was highly sensitive to the *O. vulgare* essence with low MIC. The research demonstrated that the *O. vulgare* essence may have proper antimicrobial effects against major pathogens.

Analysis of the essential oil revealed the presence of pulegone (68.59%) piperitone (7.8%), piperitenone (7.8%), 1, 8-cineole (1.3%), and carvacrol (1.6%) as the major components (Table 3).

Table 1. Antibiotic Susceptibility Test Results by Disc Diffusion Method [a, b]

Antimicrobial Agent, Concentration, µg	R	I	S
Oxacillin, 30	100	0	0
Gentamicin, 10	60	36	4
Kanamycin, 30	68	32	0
Neomycin, 30	89	7	4
Ciprofloxacin, 5	21	30	49
Ceftazidime, 30	31	60	9
Ceftriaxone, 30	76	24	0
Polymyxin, 10	9	20	71
Amikacin, 30	75	5	20
Imipenem, 10	45	43	12

[a] Abbreviations: I, intermediate; R, resistance; S, sensitive.
[b] Data are presented as %.

Table 2. Zone of Growth Inhibition and Minimum Inhibitory Concentration Values of the *O. vulgare* Essence Against *Acinetobacter baumannii* [a]

Organisms	Inhibition Zone Diameter, mm		Minimum Concentration Values, µL/mL	
	Essential Oil	Antibiotics [b]	MIC	MBC
***A.baumannii* (3)**	7	0	2.4	2.6
***A.baumannii*(64)**	8	0	2.4	2.6
***A.baumannii* (69)**	10	0	3.5	3.7
***A.baumannii* (75)**	12	0	2.8	3
***A.baumannii* (77)**	15	0	2	2.2

[a] Abbreviations: MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration.
[b] Neomycin, Gentamicin, Amikacin, Kanamycin, Oxacillin, Imipenem, Ciprofloxacin, Ceftazidime, Ceftriaxone and Polymyxin.

Table 3. The Main Constituents of the Essential Oil of *Origanum vulgare*

Relative Percentage Obtained From the Peak Area, %	Retention Index	Compound
1.3	36150	1,8 cineole
1.6	62289	Menthone
1.3	62256	Borneol
1.6	81229	4-Terpinol
1.4	70690	α-Terpineol
0.9	4603	m-ethyl cumene
68.59	3284245	Pulegone
1.9	62200	Karvol
7.8	37625	Piperitone
1.41	70679	Thymol
7.8	377046	Piperitenone oxide
1.6	62262	Carvacrol
0.9	36162	Isophorone
0.9	46388	Bornylacetate
0.5	36162	Trans-caryophyllene
0.5	1893	1-phenel-1,4-butanediol

Figure 1. Zone of Growth Inhibition of the *Origanum vulgare* Essence Against Multidrug-Resistant *Acinetobacter baumannii*

5. Discussion

Nosocomial infections caused by MDR strains of *A. baumannii* (MDR-AB) are currently among the most difficult ones to treat and they continue to present serious challenges to clinicians' empirical and therapeutic decisions in burned patient (11). Outbreaks of extensively, and pan multidrug-resistant (MDR) and pandrug-resistant (PDR) *A. baumannii*, have been reported worldwide. The results indicated that the number of *A. baumannii* isolated from respiratory tracts were more than other specimens. This recommends that *A. baumannii* is an important factor in respiratory tracts infections, especially in ICUs.

Health problems have been caused by *Acinetobacter* spp. and the possibility of transition between living and nonliving things as well as long-term survival in hospital environments enhance the appearance of this bacterium in hospital environments and its consequent infections (12).

Use of medicinal herbaceous drugs has been recommended for treatment of diseases since ancient periods. Humans have realized and used their beneficial effects. With the increase in population and the urban growth and reduced use of synthetic drugs, many of these of medicinal herbs have been replaced (13). The increase of antibiotic resistance is a serious problem worldwide. Some medications and antibiotics have completely lost their medicinal effects; this problem causes the development and creation of multiple disease resistances. Medicinal plants in the last decade were used as natural reservoirs of drug. One of their advantages is that they are natural reservoirs and laboratory experiments have shown no negative effects (14, 15). In this research, the antibacterial activity of the *O. vulgare* essence was assessed against important pathogens by making some minor changes to the CLSI recommended agar dilution method which was originally developed for analyzing the conventional antimicrobial agents activities, so it could be used to analyze plant extracts and essential oils for their antimicrobial activities (16, 17).

Ciprofloxacin and ceftriaxone are used to treat infections caused by *A. baumannii*. According to all the above results, ceftriaxone and ciprofloxacin-resistant strains were sensitive to the *O. vulgare* essential oil with low MIC. This shows the power of *O. vulgare* essential oil for the drug-resistant strains of *Acinetobacter baumannii*.

This research showed the high antimicrobial activity of *O. vulgare* essence against drug-resistant strains of *A. baumannii*. Due to the high resistance to drugs by *A. baumannii*, high prevalence of nosocomial infections, enormous economic costs, and restrictions on the use of broad-spectrum drugs by immune compromised people, applications of the *O. vulgare* essential oil against these pathogens is necessary. The *O. vulgare* essence can be effective enough to reduce the rate of infection transmission. According to the results of the current research, we hope that in future, *O. vulgare*be used with a wider range as a complementary therapy. Additional clinical researches and trials are necessary to completely confirm the above results for medical purposes.

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Authors' Contributions

Davoud Esmaeili: study concept and design, critical revision of the manuscript for important intellectual content, statistical analysis, administrative, technical and material support, and study supervision; Hossein Saghi: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. Abbas Bahador, Azad Khaledi, Ramazanali Ataee Kachoei, Ferdoes Amiri Dastjerdi: administrative, technical and material support, and study supervision.

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Molecular Biology Research Center and Department of Microbiology, Baqiyatallah University of Medical Sciences was responsible for the study design and procedure, collection, management, preparation, review, and approval of the manuscript.

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