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## ANTIPSEUDOMONAL ACTIVITY OF *ARTEMISIA QUETTENSIS* ESSENTIAL OIL AND ITS SYNERGY WITH IMIPENEM

Elham Saffari<sup>1</sup>, Mohammad Ali Nasiri Khalili<sup>\*2</sup> and Jalil Fallah Mehrabadi<sup>3</sup>

Department of Chemistry and Essential Oil Technology<sup>1</sup>, School of Chemistry, Islamic Azad University of Pharmaceutical Science, Tehran, Iran.

Department of Biochemistry and Biophysics<sup>2</sup>, Education and Research Center of Science and Biotechnology, Malek Ashtar University of Technology, Tehran, Iran.

The Lister Laboratory of Microbiology<sup>3</sup>, Tehran, Iran.

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*Artemisia quettensis*,  
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### Correspondence to Author: Mohammad Ali Nasiri Khalili

Department of Biochemistry  
and Biophysics, Education and  
Research Center of Science and  
Biotechnology, Malek Ashtar  
University of Technology,  
Tehran, Iran.

**E-mail:** manasiri@alumni.ut.ac.ir

**ABSTRACT:** Over the past two decades, to increase the antimicrobial spectrum of antibiotics, effective compounds of plants have been used synergistically with antibiotics. The plants of genus *Artemisia* (Asteraceae) have been conventionally used for prevention and medication of several ailments. Due to the significance of antimicrobial activity of *Artemisia* species, this study aimed to evaluate the effect of essential oil of *Artemisia quettensis* individually, and in combination with imipenem, to inhibit the growth of *Pseudomonas aeruginosa*. The singular activity of essential oil and activity, when combined with an antibiotic, was hence elucidated. The essential oil was obtained through hydrodistillation from aerial parts of the plant and analysis using GC and GC-MS. The most dominant components were homoadamantane (9.38%), camphor (7.91%) and eugenol (10.46%). The oil and antibiotic showed high antibacterial activity against *Pseudomonas aeruginosa* with minimal inhibitory concentration (MIC) 0.5  $\mu\text{L/mL}$  and 16  $\mu\text{g/mL}$ , respectively. The main purpose of this research is the synergistic effect, the oil and antibiotic showed MIC 0.2  $\mu\text{L/mL}$  and antibiotics 4  $\mu\text{g/mL}$ , respectively. This study showed that *Artemisia quettensis* oil has significant antibacterial activity against *Pseudomonas aeruginosa* infections.

**INTRODUCTION:** Antibiotic resistance is the potential of a microbe to resist the effects of antibiotic drugs previously used to treat them. The spread of resistance to currently available antibiotics is a global concern<sup>1</sup>. With the spread of bacterial resistance to antibiotics, medicinal plants are important elements of traditional medicine in virtually all cultures.

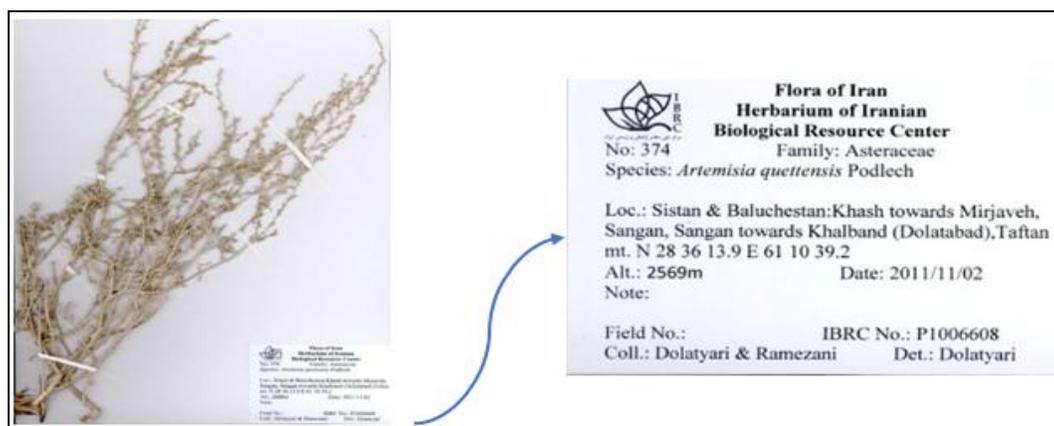
Essential oils (EOs) are a very interesting group of secondary metabolites that are useful sources of antibacterial, antioxidants, anti-inflammatory, anti-cancer compounds for human diseases. Many studies have been published on the antibacterial activity of (EOs) proving a reduction in the bacterial resistance<sup>2</sup>.

The *Artemisia* genus (Asteraceae) comprises about 500 species from South Asia, North America, and European countries (100) and 34 species that are found wild all over Iran with the common Persian name of 'dermane' that provide valuable (EOs) notably for the pharmaceutical industry<sup>3</sup>. *Artemisia quettensis* Podlech belongs to the Asteraceae

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family and is narrowly distributed in the Southern heights of Iran **Fig. 1**. *Pseudomonas aeruginosa* (PA) is recognized as one of the primary reason for infections in hospitals. The ability of this opportunistic human pathogen to acquire resistance to a broad range of antibiotics has made effective therapy more difficult<sup>4</sup>. The high level of antibiotic resistance in (PA) involves several mechanisms, including the overexpression of active efflux systems, the production of modifying enzymes, a decrease in outer membrane permeability and structural alterations of topoisomerases II and IV, involved in quinolone resistance<sup>5</sup>. Carbapenems

such as meropenem and imipenem (IPM) are potent broad-spectrum antibacterial agents used to treat *Pseudomonas* infections. These antibiotics bind to critical penicillin-binding proteins, and thereby disrupt the growth and structural integrity of the bacterial cell wall. However, the resistance of non-fermenting gram-negative bacteria, including (PA), to (IPM) and meropenem is increasing<sup>6</sup>. Among many antibiotics against (PA), (IPM) is considered the last option of treatment against serious infections caused by (PA) but IPM resistance is prevalent in many areas of the world, and this problem has increased<sup>7,8</sup>.



**FIG. 1: THE PHOTO OF HERBARIUM SAMPLE OF ARTEMISIA QUETTENSIS**

The use of natural products with therapeutic properties, for a long time, was the main source of important therapeutic agents<sup>9</sup>. Medicinal plants are considered a major source of new chemical compounds with curative effects<sup>10</sup>. They have a broad range of substances that can be used to treat infectious diseases because they are beneficial sources of antibacterial compounds such as alkaloids, flavonoids, terpenoids<sup>11</sup>.

## **MATERIAL AND METHODS:**

**Plant Materials:** Aerial parts of *Artemisia quettensis* Podlech were collected from Sistan & Baluchestan in February 2014. The voucher specimen was prepared and deposited at the Herbarium and Botanical Lab, Research Center of Iranian Biological (National ID 14001906001) Tehran, Iran, IBRC No P1006608.

**Extraction and Identification of the Oil:** The extraction of the (EO) was carried out by hydrodistillation for 6 h using a Clevenger type apparatus<sup>12</sup>. The oil was obtained and stored in at 4 °C in the dark vial and the presence of anhydrous

sodium sulfate. The analysis of the (EO) was performed with Gas Chromatography-Mass Spectroscopy (GC/MS). The GC apparatus was an Agilent technology HP 6980 system, with HP-5MS capillary column (60 m length; 0.25 mm i.d; 0.25 mm film thickness). Helium was used as the carrier gas at a flow rate of 1 ml/min. The oven temperature program was as follows: 1 min at 100°C, held for 1 min, then heightened to 280 °C at a rate of 5 °C/min and held for 25 min. The chromatograph was equipped with a split/splitless injector used in the splitless mode. The relative proportion of each compound was expressed as a percentage obtained by peak area normalization. Identification of components was assigned by comparison of their retention indices (RI) and mass spectra fragmentation with NIST (National Institute of Standards and Technology)<sup>13</sup>.

**Pseudomonal Isolates:** The isolates of (PA) were obtained from pseudomonal infections, and *Pseudomonas aeruginosa* ATCC 27853 were provided from the microbiology laboratory of Imam Khomeini Hospital.

**Antibiotics:** The antibiotics-standard gentamicin (10 µg/mL), imipenem (10 µg/mL), ceftazidime (30 µg/mL), ciprofloxacin (5 µg/mL)

#### Determination of MIC Value by Disk Diffusion

**Method:** Disk diffusion method was used to determine the MIC value of oil. The Mueller-Hinton agar was poured in Petri dishes and the paper discs were impregnated with 2 ml of (EO), and antibiotic were placed on the inoculated agar surface. The diameter of the inhibition zone was measured. The larger the diameter of the area, the more sensitive the strain<sup>14</sup>. The synergistic effect of the combination of the (EO) and antibiotics was assessed so that, 2 ml of (EO) was saturated to the antibiotic disc to determine the zones of inhibition. The obtained results were compared with those of the antibiotics tested on the same strains alone and by the same method<sup>15</sup>.

**Check Board Titer Test:** The checkerboard method was used to evaluate the antimicrobial interactions between *Artemisia quettensis* essential oil (AQEO) and (IPM). Eight serial, twofold dilutions of AQEO and IPM was prepared and used in the MIC tests. 50 ml of each dilution of oil was added to the wells of 96-well plates in the vertical orientation, and 10 ml of (IPM) dilutions were added in a horizontal orientation. 100 ml of microbial suspension ( $10^6$  CFU/ml) was added to each well and incubated at  $35 \pm 1$  °C for 24 h.

Fractional inhibitory concentrations (FICs) were calculated as the MIC of the combination of *A. quettensis* oil and (IPM) divided by the MIC of oil or (IPM) alone. The results of this test were expressed as Fractional inhibitory concentration (FIC). The checkerboard test was used as the basis to calculate a fractional inhibitory concentration (FIC) Index according to the formulas:

FIC of *Artemisia quettensis* oil = (MIC in combination with imipenem) / (MIC of *A. quettensis* oil alone)

FIC of Imipenem = (MIC in combination *A. quettensis* oil) / (MIC of Imipenem alone)

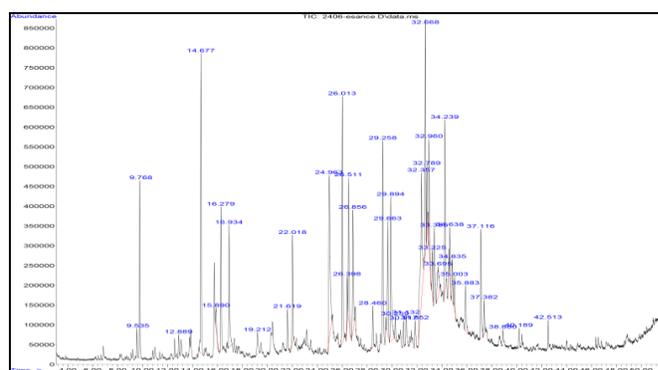
FICI = FIC of *Artemisia quettensis* oil + FIC of Imipenem

After calculating the FICI, the interpretation of the results was carried out using the European Committee's guidelines for the antimicrobial susceptibility test (EUCAST 2000).

Synergy (FIC Index  $\leq 0.5$ ), antagonism (FIC Index  $> 4.0$ ), and no interaction (FIC Index  $> 0.5-4.0$ )<sup>16,17</sup>.

## RESULTS:

**Phytochemical Composition:** The chemical profile of the tested EO **Fig. 2** was performed by GC-MS. As shown in **Table 1**, fifteen compounds were identified. The major components were homoadamantane (9.38%), camphor (7.91%), and eugenol (7.46%) followed by geranyl acetate (6.93%), spathulenol (5.27%) and 1,8-Cineole (4.14%). Although, homoadamantane was the main compound found in (AQEO), in addition to other constituents exhibiting relatively low proportion.



**FIG. 2: CHROMATOGRAPHIC PROFILE OF A. QUETTENSIS**

**Antibiotic Resistance Pattern:** The resistance pattern of the bacterial strains was studied by investigating their MIC using the disc diffusion test, and the results achieved are reported in **Table 2**. The activity of IPM antibiotic fluctuated among the strains, and most of them were not potent in therapeutic doses towards the tested bacteria. Among all tested MIC, only three strains numbered 5, 11, and 16 were susceptible to the antibiotic. The MIC values of IPM against (PA) strain were varied from 2 to 64 µg/mL **Table 2**.

#### Minimum Inhibitory Concentrations (MIC) of

**Essential Oil:** The results of the antibacterial activity of the (EO) are presented in **Table 2**. Twenty different strains of (PA) strains were used to evaluate the possible antipseudomonal activity of (AQEO). The oil exhibited the antipseudomonal activity against all strains of (PA) with MIC values in the range of 0.5 to 64 µg/mL.

**The Synergy Effect of Essential Oil and Antibiotic:** Considering the effect of synergism, which is the main purpose of our study, all

infectious specimens had high sensitivity, so that in the concentration of one-fourth of in singular activity of (EO) and one-half when combined with

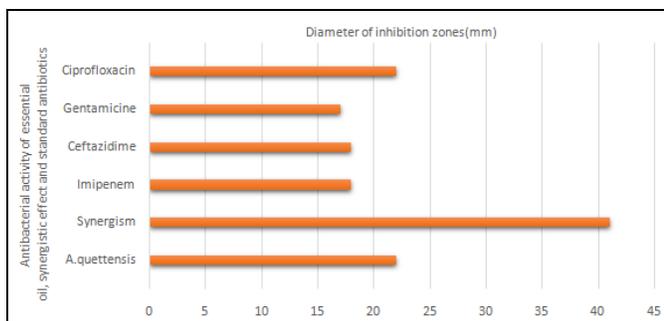
IPM inhibited the growth of (PA). In general, the effect of (EO) and the antibiotic combination could overcome resistant strains.

**TABLE 1: MAIN CONSTITUENTS OF THE ESSENTIAL OIL ISOLATED FROM AERIAL PARTS OF *ARTEMISIA QUETTENSIS* PODLECH**

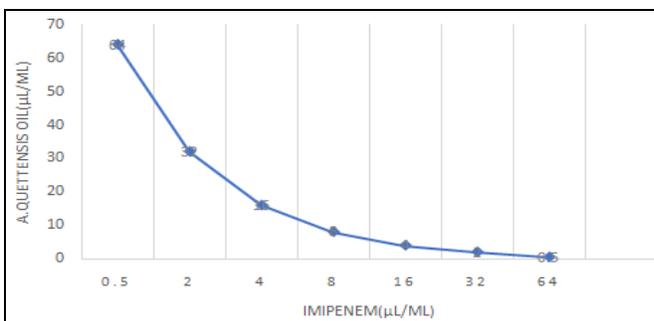
No.	Retention Time (min)	Area%	Name	Quality	Retention Index
1	9.537	0.68	P-cymene	95	1024
2	9.768	4.14	1,8-Cineole	99	1031
3	14.677	7.91	Camphor	98	1146
4	16.279	3.78	4-Terpineol	97	1177
5	16.931	3.89	alpha-Terpineol	91	1188
6	19.212	0.88	Pulegone	94	1237
7	21.62	1.09	l-Bornyl acetate	99	1288
8	22.021	2.54	Lavandulyl Acetate	91	1290
9	24.963	7.46	Eugenol	98	1359
10	26.011	6.93	Geranyl acetate	91	1381
11	26.509	4.03	cis-Jasmone	98	1394
12	26.858	3.92	Methyleugenol	98	1403
13	29.256	9.38	Homoadamantane	90	Not found
14	30.915	0.67	delta-Cadinene	97	1523
15	32.666	5.27	Spathulenol	98	1576

**TABLE 2: ANTIBACTERIAL ACTIVITY OF *ARTEMISIA QUETTENSIS*, IMPENEM AND SYNERGISTIC EFFECT AGAINST *PSEUDOMONAS AERUGINOSA***

Strain number	MIC ( $\mu\text{L/mL}$ )		MIC (synergism) ( $\mu\text{L/mL}$ )		FICI	FIC	FIC
	Essential oil	Imipenem	Essential oil	Imipenem	Combination	Imipenem	Essential oil
ATCC278531	0/5	16	0/2	4	0.4	0.25	0.65
1P	16	32	4	8	0.25	0.25	0.5
2P	16	64	4	16	0.25	0.25	0.5
3P	32	64	4	16	0.125	0.25	0.375
4P	32	64	4	16	0.125	0.25	0.375
5P	0/5	2	0/25	0/5	0.5	0.25	0.75
6P	64	128	16	32	0.25	0.25	0.5
7P	64	128	16	32	0.25	0.25	0.5
8P	16	64	4	1	0.25	0.25	0.5
9P	32	64	8	16	0.25	0.25	0.5
10P	64	64	16	16	0.25	0.25	0.5
11P	2	8	0/5	2	0.25	0.25	0.5
12P	8	32	2	8	0.25	0.25	0.5
13P	16	64	4	16	0.25	0.25	0.5
14P	32	64	8	16	0.25	0.25	0.5
15P	16	64	8	16	0.5	0.25	0.75
16P	2	8	0/5	2	0.25	0.25	0.5
17P	32	64	8	16	0.25	0.25	0.5
18P	32	64	8	16	0.25	0.25	0.5
19P	64	128	16	32	0.25	0.25	0.5
20 P	32	64	8	16	0.25	0.25	0.5



**FIG. 3: THE ANTIBACTERIAL ACTIVITIES (ZONES OF INHIBITION) OF ESSENTIAL OIL OF *A. QUETTENSIS* AND ITS SYNERGISTIC EFFECT WITH IMPENEM (mm)**



**FIG. 4: THE ISOBLE METHOD DESCRIBING SYNERGY *PSEUDOMONAS AERUGINOSA***

The FIC of *Artemisia quettensis* and FIC of IPM and also FIC index and their antimicrobial interaction on PA strains are shown in **Table 2**. 75 percent (75%) tested bacterial isolates were sensitive to this combination at MIC ranged from 0/25 to 64 µg/mL. Based on the results, the (AQEO) and (IPM) were studied synergistically on all pathogenic bacteria studied. The antibacterial activity of (AQEO) against (PA) was investigated. It showed high inhibition of bacterial growth, **Fig. 3**. Inhibition zones about 1 to 22 mm were recorded for PA (Isolates no. 5p, 11p, and 16p). Lower sensitivity was observed for isolates number 7p, 8p, 10p, 13p, and 18p.

**DISCUSSION:** In addition to slowing the treatment process, the spread of antibiotic-resistant strains jeopardizes the lives of patients who are contaminated with these resistant strains. In this study, the antibacterial effects of (AQEO) and its synergistic effect with (IPM) were evaluated and the results indicated that 0/5 µg/mL of (AQEO) could inhibit (PA) and when combined with antibiotic it could decrease the MIC from 4 to 0/2 µg/mL, and it can be considered a significant potent. Briefly, the antibacterial effect in synergism was more potent than that of (IPM) on the same bacteria.

Despite some information on the antibacterial activity of (EO) in this species<sup>18,19</sup> and other plants on PA<sup>20,21,22,23</sup> to our knowledge, this is the first report on the antipseudomonal activity of (AQEO) against (PA) using disc diffusion assay. The results of<sup>24</sup> study were similar to those of this study, which *T. vulgaris* essential oil exerted a synergistic effect with piperacillin, cefepim, meropenem on (PA). The (EO) has been able to double the antibacterial activity of antibiotic, but the current study demonstrated that (EO) in synergistic effect increased the anti-bacterial effect four times.

In the study by<sup>25</sup>, the antibacterial activity of the *Artemisia annua* essential oil revealed that it had antibacterial properties against most isolates tested. Inhibition zone diameters varied from 6 (*Pseudomonas aeruginosa* and *Shigella flexneri*) to 45 mm (*Vibrio cholerae*). The (AQEO) used in this study has antibacterial activity against the tested strains with different diameters of inhibition zones from 1 to 22 mm. A study on the synergistic effect

of some (EOs) with antibiotic showed that 544 µl/mL of *Carum copticum* essential oil displayed effect on (PA) growth and when combined with gentamicin, exerted no effect<sup>26</sup>. In the present study (AQEO) at 0/5 µl/mL could inhibit the growth of (PA). This inconsistency in the findings can be due to the difference in the percentages of the chemical compounds among the (EOs). Homoadamantane and camphor, as the main components of (AQEO), seem to be responsible for the anti-pseudomonal effects of the oil, this oil is also equally or more effective when compared with standard antibiotics at a very low concentration.

Previously, many studies have indicated that (PA) resisted the action of the (EO) of *Thymus lanceolatus*, *C. coronarium*, *M. officinalis* Linn.<sup>27,28,29</sup> Our study revealed significant findings which prove the efficacy of (AQEO) against (PA). Since the World Health Organization has rated multidrug-resistant (MDR) *Pseudomonas aeruginosa* as a critical threat to human health, many studies have been carried out in recent years on the resistance of (PA) to different antibiotics<sup>30</sup>. Current results suggest that the potential use of this oil as pharmaceutical products can diminish harmful side effects and treatment costs of synthetic drugs.

Limited studies had been carried out on the (EO) of this species. While researchers have identified antibacterial activities of the extract from (AQEO), no synergistic study using (IPM) and (AQEO) on (PA) isolates has yet been published. Furthermore, it will be very important to investigate the synergistic behavior of natural products with (IPM) with the hope of enhancing their activity. The results of the synergistic action of oil with (IPM) demonstrate the potential use of (AQEO) to enhance (IPM) action. Additional research is required to assess the practical value of therapeutic applications.

**CONCLUSION:** Since, infectious diseases constitute a wide range of diseases, and the number of antibiotic-resistant microbial strains is increasing every day, the need for new and low-risk antibacterial agents is highly essential. Accordingly, the antibacterial activity of natural plants can create a way for obtaining new antibiotics. In general, the findings of the present study properly revealed the antibacterial activity of

(AQEO) and (IPM) on (PA). This study also showed that the recurrent utilization of this oil and antibiotic could lead to the inhibition of bacterial growth. Considering the inter-developmental effects of antibiotics and plant compounds, it seems that the recurrent application of these compounds can prove to be an appropriate solution for microbial resistance. The solution to this world health issue is only feasible through further and more comprehensible investigations.

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**CONFLICT OF INTEREST:** Nil

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