Journal of Research in Ecology

An International Scientific Research Journal

ISSN No: Print: 2319 –1546; Online: 2319– 1554

Original Research

The evaluation of chemical composition, antimicrobial activity and drug interaction in the essential oil of *Artemisia sieberi*

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ABSTRACT:

Problems in the treatment of infections caused by antibiotic-resistant strains led to vast studies on new antimicrobial drugs, including medicinal plants. *Artemisia* has been used in the traditional medicine for a variety of clinical disease including treatment of malaria; suppress inflammation and infectious diseases. The aim of this study was to investigate the antimicrobial effects of essential oil from *Artemisia sieberi* and to compare its drug interaction with other antibiotics. In this study, essential oil was extracted from the aerial parts of *Artemisia sieberi* and its components were analyzed, then *in vitro* antibacterial properties were evaluated. Antibacterial effect of essential oil derived from the plant was analysed against six strains of bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis* and *Bacillus cereus*) by disc diffusion method and the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined. The synergistic and antagonistic effects of this plant with seven standard antibiotics (cephalothin, oxacillin, gentamyicin, ampicillin, vancomycin, erythromycin and piperacillin) were analyzed. In this study, camphor (25.17%) had the highest combination of essential oil and plant oil in the concentration of 0.00015 mg/ml and showed inhibitory effect against *Escherichia coli*. According to the results of this study, essential oil has inhibitory effect on the growth of pathogenic bacteria. Therefore, for the clinical application of essential oil, further researches are necessary.

Keywords:
Antibacterial, Antibiotic, *Artemisia sieberi*, Drug interactions, Essential oil.

Article Citation:
Maryam Moazen Zade and Mohammad Dakhili
The evaluation of chemical composition, antimicrobial activity and drug interaction in the essential oil of *Artemisia sieberi*
Journal of Research in Ecology (2017) 5(1): 771-779

Dates:
Received: 13 Jan 2017 **Accepted:** 06 May 2017 **Published:** 28 June 2017

Web Address:
http://ecologyresearch.info/documents/EC0249.pdf

Journal of Research in Ecology

INTRODUCTION

Since ancient time, medicinal herbs have been used in the treatment of various diseases. One of the active principal responsible for the medicinal activity is essential oils which have abundant biological effects. Recently, secondary metabolites of medicinal plants such as essential oils and plant extracts have been studied for their antimicrobial effects and it has become clear that most of the essential oils extracted from the medicinal plants have antifungal, anti-parasitic, antibacterial and antiviral properties. So plant essential oils have been used in herbal pharmacology, medical microbiology, phytopathology and other allied fields (Kordali *et al*., 2005). Nowadays, investigating the antimicrobial effects of plant extracts especially the plants that traditionally are used for medical purposes are one of favorite topics of researchers. Two main reasons have been stated for this interest. First, the more or less frequent compounds found in plants over the years and have been tested on humans are massive potential storage of various drugs, including compounds that are the inhibitors of various microorganisms and now they can be used as sources for new drugs, including antimicrobial compounds. The second reason is the antimicrobial resistance due to the non-normative usage of current antimicrobial drugs by the general folk as well as the high rate of drug allergies to the chemical compounds that intensifies the necessity of new drugs (Behmanesh *et al*., 2007).

Artemisia sieberi Besser is a widely distributed plant in Iran. It is distributed in the vast steppes of Iran. There are different opinions about the correct name of Iranian steppic *Artemisia*. Some authors believed that the Iranian specimens are *A. sieberi* and *A. herba-alba*. *Artemisia herba-alba* is thought to be the plant translated as "wormwood" in English versions of the Bible. It is usually 20-35 cm in height, with vertical stem, woody, thick, branching, straight brittle branches, many-flowered, sometimes oblique and almost stellar branches, covered with gray fuzz, or in parts without fuzz, yellowish, dense branching can be observed almost from the base (Podlech,1986). *A. sieberi* is a highly aromatic bush which is the main and dominant element of plant communities in arid and semi-arid steppes of Iran. *A. sieberi* belongs to the family of Asteraceae and has all aerial parts such as stems, leaves, flowers, fruits and seeds. It also have a sharp and penetrating fragrance (Mozaffarian, 2007). The essential oil of *A. sieberi* is 2.1% in dry weight that contains 25.17 camphor, 6.28, 1,-8 cineole and 6.20 α-terpineol and its antibacterial properties are investigated.

MATERIAL AND METHODS

Herbal sampling

In early autumn, *A. sieberi* were collected from the villages around the city of Qom, Iran and dried in a completely dark place. It was then approved by the Research Institute for Forest and Rangelands, Iran. In order to separate the essential oil, 100 g of powdered dried herb (weighed by Clevenger apparatus) were extracted for 4 h with the distillation speed of 1ml per minute. Moisture in the resulting oil was removed with sodium sulfate and kept in -4^0 C, until use (Behmanesh *et al*., 2007).

Analysis of essential oils

In order to measure the quality of the prepared essential oil Gas Chromatography, Mass Spectrometry (GC-MS) was used. The analysis of test samples was done by experts in a chemical laboratory in Azad University of Kerman, Iran and is available in library (Ramezani *et al*., 2004).

GC-MS analysis

Mass Spectrometer connected to a Varian 3400 gas chromatograph, column DB-5 with a length of 30 m, inner diameter of 25 μ and the thickness of stationary phases layer was 25.0 μ. In ion trap detector, the flow rate of helium carrier gas was 50 ml/min and ionization energy in the mass spectrometer was 70 eV. Column

Table 1. Chemical composition of *Artemisia sieberi* essential oil estimated using GC-MS

S.No	Compounds	RI[a]	(%)[b]
1	α-Pinene	923	1.16
2	Camphene	938	2.90
3	Sabinene	959	5.15
4	ß-Pinene	964	1.21
5	Dehydro-cineole	978	3.17
6	α-Terpinene	1013	1.23
7	Cymene-p	1023	1.13
8	1,-8 Cineole	1038	6.28
9	Cis arbusculone	1040	1.21
10	g-Terpinene	1041	2.32
11	Cis sabinene hydrate	1057	2.28
12	Trans arbusclone	1063	1.28
13	Chrysanthenone	1125	2.91
14	Camphor	1133	25.17
15	Cis chrysanthenol	1152	1.45
16	Pinocarvone	1172	1.96
17	Terpinen	1174	1.30
18	α-Terpineol	1185	6.20
19	Promecarb	1200	1.13
20	Methyl cinnamate	1209	2.10

[a] Retention indices, as determined on a DB-5 HAV column.
[b] Percentages obtained by FID peak-area normalization

heat program was set from 40 to 220° C. The temperature of injection chamber was 230°C (Ramezani *et al*., 2004; Eloff, 1998).

Experimental strains

Six bacterial strains viz; *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 33196), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 21778), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 29997) were purchased from the center of the microbial collection of Pasteur Institute of Iran (http://www.pasteur.ac.ir). Then in order to culture the bacteria Mueller Hinton agar medium was used. To conduct the sensitivity test, 24 h cultures and single colonies were harvested. Colonies were regularly evaluated in terms of purity to reduce the possibility of error and pollution (Sardashti and Pourramazani, 2012).

Antibacterial activity of essential oils

Antibacterial activity of essential oil was determined using two models viz; disc diffusion method by measuring the inhibition zone and broth dilution method for measuring the Minimum Inhibitory Concentration (MIC) (Halawani, 2009). In disc diffusion method, the 100ml of the bacterial suspension (0.5 McFarland equal to 1.5×10^8 cfu/ ml), Mueller-Hinton agar (Merck). On culture media, blank disc (6 mm diameter) containing 30 ml of the diluted essential oils was used.

Blank disc containing 30 ml of dimethyl sulfoxide was used as negative control. To compare the antimicrobial effects of discs oxacillin and gentamicin were used as a positive control. After 24 h of incubation at 37°C, the diameter of inhibitory zone was measured. The test was repeated thrice (Halawani, 2009).

The determination of MIC

To determine the Minimum Inhibitory Concentration (MIC) of essential oils on the experimental strains, microbroth dilution method was used (Halawani, 2009). 100 µl of Mueller Hinton broth (Merck) was added to each microplate well. Essential oil was diluted in dimethyl sulfoxide (2%) as a solvent. In the first well, 100 µl of 1: 2 oil dilution was added and after mixing, 100 µl was taken from first plate and added to second plate and therefore serial dilutions was created in wells. Then 10 microliters of each bacterial suspension was added individually to the wells. To negative control well, the dimethyl sulfoxide (2%) without oil was added. Then, the microplate was stored for 18 h at 37°C. The lowest concentration of essential oils which showed the inhibited growth of bacteria was defined as MIC (Sandri *et al*., 2017).

MBC determination

To determine the Minimum Bactericidal Concentration (MBC), 10 µl of the contents of the wells at the end of 18 h of incubation was taken and cultured on the Mueller-Hinton agar (Merck). In order to study the growth of bacteria they were incubated for 24 h. The

Table 2. Antibacterial activity of *A. sieberi*

S. No	Pathogens	Inhibition zone (mm)								MIC	MBC
		Amp	Pip	Cef	Oxa	Gen	Ery	Van	Oil		
1	*E. coli* (ATCC 25922)	12±0.6	12±0.3	15±0.6	15±0.4	30±0.7	12±0.3	NI	15±0.3	0.0015	00015
2	*Enterococcus faecalis* (ATCC 33196)	20±0.5	26±0.4	26±0.7	NI	NI	19±0.4	Ni	17±0.7	0.0031	0.0031
3	*Pseudomonas aeruginosa* (ATCC 27853)	15±0.4	27±0.5	20±0.7	NI	21±0.5	20±0.3	NI	11±0.3	0.025	0.0125
4	*Bacillus cereus* (ATCC 21778)	30±0.5	24±0.5	36±0.5	11±0.4	32±0.7	33±0.5	21±0.3	12±0.4	0.05	0.05
5	*Staphylococcus aureus* (ATCC 25923)	37±0.6	31±0.7	40±0.8	27±0.3	30±0.7	36±0.6	20±0.5	28±0.6	0.0125	0.0125
6	*Klebsiella pneumoniae* (ATCC 29997)	NI	16±0.4	NI	NI	27±0.5	NI	NI	18±0.4	0.0062	0.0031

NI: No Inhibition; Amp: Ampicillin; Pip: Piperacillin; Cef: Cephalothin; Oxa: Oxacillin; Gen: Gentamycin; Ery: Erythromycin; Van: Vancomycin; Oil: *Artemisia sieberi* oil; MIC: Minimum Inhibitory Concentration (mg/ml); MBC: Minimum Bactericidal Concentration (mg/ml); Values are means of three replications ± SD.

lowest concentration of essential oil in which 99.9% of bacteria had no growth was considered as MBC (Duffy and Power, 2001). All the tests were repeated thrice.

Synergistic and antagonistic effects of *Artemisia sieberi* on antibiotics

To determine the effect of the combination of *A. sieberi* oil, antibiotic disc diffusion method was used. Synergistic and antagonistic effects of *A. sieberi* on antibiotics was done by the use of Sub-Minimum Inhibitory Concentration (Sub-MIC) that as per the results of MIC test, its dilution was 1 to 2 up to 1 to 4. Bacterial suspension at the concentration of half McFarland equal to 1.5×10^8cfu/ ml at a rate of 100 µl was added on the agar medium containing sub-MIC of essential oil for Meadow cultivation and antibiotic disc with determined concentrations was placed on agar surface. Then, the diameter of the inhibition zones of discs was recorded after 24 hours of incubation at 37°C. To investigate the interaction effect of essential oil on antibiotics, the discs of vancomycin (30 mg), cephalothin (30 mg), piperacillin –tazobaktam (110 mg), ampicillin (10 mg), gentamycin (10 mg), erythromycin (15 mg) and oxacillin (1 mg) were used (Soleimani *et al*., 2010).

RESULTS

Components identified by GC-MS are summarized in Table 1. A total of 20 compounds were identified in *A. siberi*. The major identified compounds in the essential oil of *A. sieberi* were 1, 8– cineole (6.28), camphor (25.17), sabinene (5.15) and terpineol (6.20), respectively (Figure 1).

The results of antibacterial activity of essential oils by disc diffusion method are given in Table 2. The greatest inhibitory zone belonged to *Staphylococcus aureus* with a diameter of 28 mm and then *Klebsiella pneumoniae, Enterococcus faecalis, E. coli, Bacillus cereus* and *Pseudomonas aeruginosa*. The results of MIC and MBC indicate that *A. sieberi* has the highest effect on *Bacillus cereus* and *Klebsiella pneumoniae.*

Table 3. Synergistic and antagonistic effects of *Artemisia sieberi* on seven antibiotics

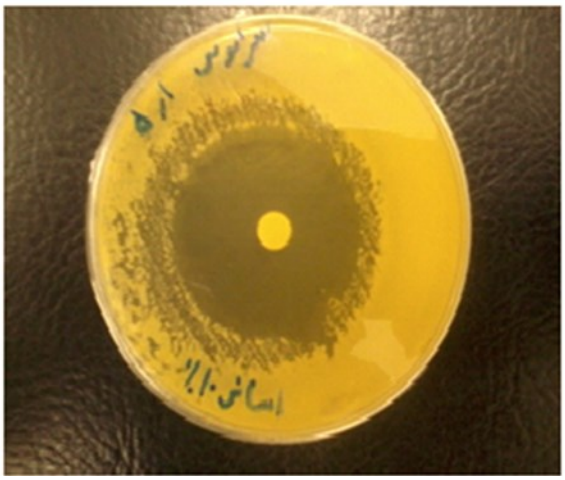
S. No	Pathogens	Inhibition zone (mm)						
		Amp A/AE	PIP A/AE	Cef A/AE	Oxa A/AE	Gen A/AE	Ery A/AE	Van A/AE
1	*Escherichia coli*	12.12±0	12.15±0.5	15.20±1.2	15.21±1.5	30.25±0.7	12.10±0.3	0
2	*Enterococcus faecalis*	20.25±0.6	26.28±0.3	26.30±0.4	0	0	19.12±0.8	0
3	*Pseudomonas aeruginosa*	15.18±0.4	27.31±0.6	20.25±0.9	0	21.29±1.2	20.26±1.5	0
4	*Bacillus cereus*	30.35±1.1	24.26±0.4	36.36±0	11.15±0.7	32.34±0.6	33.33±0	21.21±0
5	*Staphylococcus aureus*	37.50±1.6	31.35±0.5	40.45±0.8	27.30±0.5	30.320±0.5	36.34±0.3	20.25±0.7
6	*Klebsiella pneumoniae*	0	16.18±0.5	0	0	27.32±1.3	0	0

A: Inhibition zone for antibiotics
AE: Inhibition zone for antibiotics along with *Artemisia sieberi* essential oil

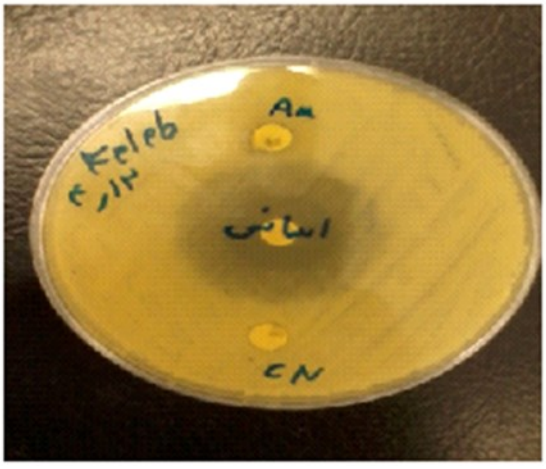
Synergistic and antagonistic effects of *Artemisia sieberi* on seven antibiotics and six strains of standard bacteria are given in Table 3. In the case of *Pseudomonas aeruginosa* essential oil enhances the effect of ampicillin, piperacillin, cephalothin, gentamicin and erythromycin. In the case of *Staphylococcus aureus* oil had a synergistic effect on the ampicillin, piperacillin, cephalothin, oxacillin, gentamicin and vancomycin. The results of drug interaction and essential oils on other bacterial strains varied.

DISCUSSION

Nowadays, many studies are conducted regarding the inhibitory effects of natural antibiotics and plant essences which indicate the effort toward eliminating the use of chemical preservatives and using natural preservatives. It should be noted that components of essential oils can be affected by the different geographical areas of growth, age of the plant, the part of plant which is used, type of extraction method and the solvent used. Extractions that are taken through different methods and by the use of various solvents may have different antibacterial effects on a particular bacterium (Ulukanli, 2011). This study was conducted to investigate the antibacterial properties, synergistic and antagonistic effects of essential oil of *A. Sieberi*. The results of analysis of



1(a). Inhibition of *Bacillus cereus* (ATCC 21778) *Artemisia sieberi* essential oils



1(b). Inhibition of *Klebsiella pneumoniae* (ATCC 29997) by *Artemisia sieberi* essential oils

Figure 1. Antibacterial activity of *A. sieberi*

Artemisia sieberi compounds indicated that, 70% of compounds belong to oxygenated monoterpenes which was consistent with the findings of Nematellahi *et al*. (2006) on the analysis of *A. sieberi* essential oil who found that the oxygenated monoterpenes was 78%. Since these compounds are abundant, the antibacterial effect of essential oil can be attributed to these compounds (Nematollahi *et al*., 2006). In the study of Mahbobi *et al*. (2009) on *Artemisia sieberi*, they significant antimicrobial effect of essential oil was shown. The greatest antibacterial effect of essential oil of *Artemisia sieberi* was on Gram-positive bacteria and fungi (*Bacillus cereus* and *Streptococcus mutans*) and these findings are consistent with the results obtained from this study, but in our study the greatest antibacterial effect belonged to *Staphylococcus aureus*, and this was due to the bactericidal effect of the oil in the lowest concentration. In another study Behmanesh *et al*. (2007) studied the chemical composition and antimicrobial effects of *Artemisia sieberi* oil collected from four garden areas located in Esfahan, Iran. Analysis of oil by GC/MS and microbial studies against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* though cultivation and disc method indicated that the greatest impact of essential oil is on *Klebsiella pneumoniae*. According to MIC and MBC results obtained from *Artemisia sieberi*, *Escherichia coli* and *Klebsiella pneumoniae*, were sensitive to the lowest concentration

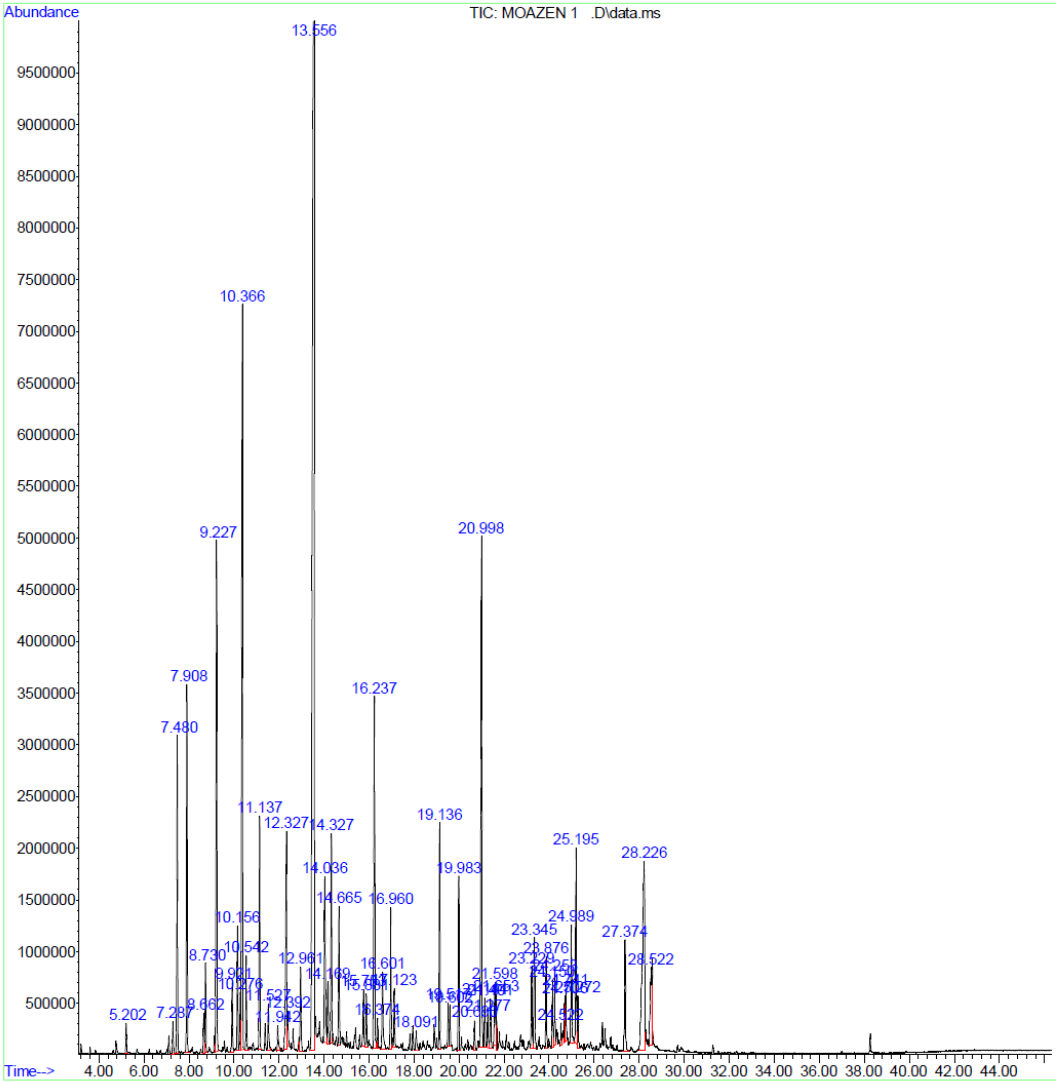


Figure 2. GC-MS chromatogram of *A. sieberi*

Table 4. Minimum inhibitory and bactericidal concentrations of essential oils against selected bacterial strain

S. No		Bacteria	*Escherichia coli*	*Enterococcus faecalis*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Staphylococcus aureus*	*Klebsiella pneumonia*
1	*Artemisia sieberi*	MIC (mg/ml)	250	150	250	125	250	125
2		MBC (mg/ml)	350	300	500	200	350	250

MIC was defined as the lowest concentration of the compounds which produced > 95% growth reduction compared with the growth in the control well.

of essential oil (0.0015 and 0.0031 mg) (Behmanesh *et al*., 2007).

Zadeh *et al*. (2012) investigated the antibacterial activity of aqueous extract of *Artemisia aucheri*, *Artemisia sieberi*, and *Hyssopus officinalis L* and indicated that the aqueous extract of *Artemisia aucheri, Artemisia sieberi* had inhibitory effect on *Escherichia coli* with MIC of 160 mg/ml and on *Staphylococcus aureus* and *Listeria monocytogenes* with MIC of 80 mg/ml. In this study, a minimum deterrence for *Artemisia sieberi* oil was on *Escherichia coli* is (0.0015 mg). This difference is due to the difference between the concentrations of the aqueous extract with essential oil (Dehghanzadeh *et al.,* 2012).

Lopez *et al.* (2008) examined the chemical composition of essential oil from *A. sieberi*. *A. biennis, A. absinthium, A. frigida, A. dracunculus, A.ludoviciana, A. cana pursh* and *A. longifolia*. A total of 110 compounds in these oils were identified. The highest concentrations belonged to 1,-8 cineole which allocated 5.21 to 6.27 percent of essential oils. As well as camphor in *A. cana A. frigida, A.ludoviciana* and *A. longifolia* varied from 9.15 to 3.37%. The essential oil of mentioned species of *A. sieberi* had antimicrobial effects against bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis* and also against yeasts such as *Cryptococcus neoformans* and *Candida albicans* and dermatophytes such as *Microsporum canis, Microsporum gypseum, Fonsecaea pedrosoi, Trichophyton rubrum* and *Aspergillus niger*. Essential oil of *A. biennis* had highest activity against *Dermatophytes*, *Fonsecaea pedrosoi, Cryptococcus neoforman*, *Aspergillus niger* and *A. absinthium* essential oil had highest activity against *Styaphylococcus* (Lopez- Lutz *et al.*, 2008). In the present study also, the highest concentration of to 1,-8 cineole and camphor was observed and its anti-bacterial effects were identified. The results of inhibitory zone on *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa* confirms the antibacterial effect of this essential oil. The MIC of *Artemisia sieberi* essential oil is related to *Escherichia coli* and then *Enterococcus faecalis, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *B. cereus* strains respectively.

While studying the synergistic and antagonistic effects of *Artemisia sieberi* essential oil on seven standard antibiotics, the results achieved in the study showed that the diameter of inhibitory zone on *E. coli* strains indicated that the essential oil had antagonistic effects gentamiycin and Erythromycin. In case of *Enterococcus faecalis,* essential oil had synergistic effect on ampicillin, piperacillin, erythromycin cephalotin antagonistic effect against *erythromycin*. The essential oil had synergistic effect on the strains of *Pseudomonas aeruginosa* and also the same happened in case of piperacillin, gentamicin, cephalothin, ampicillin and erythromycin. Most essential synergistic effect is related to the antibiotic ampicillin in the inhabitation of *Staphylococcus aureus*

strain. The synergistic effect of oil is evident on the antibiotics of ampicillin, piperacillin, cephalothin, oxacillin and gentamicin against the growth inhabitation of *Bacillus cereus*. Regarding the strain of *Klebsiella Pneumoniae* a synergistic effect on the antibiotics piperacillin, gentamicin was found.

The greatest diameter of inhibitory zone in *Artemisia sieberi* essential oil is allocated to *Staphylococcus aureus*. 18 mm diameter of *Artemisia sieberi's* essential oil against *Klebsiella pneumoniae* strains indicated that this oil has good antibacterial activity compared to the ampicillin, cephalothin, oxacillin, erythromycin and vancomycin. *Artemisia sieberi* essential oil against the strains of *Pseudomonas aeruginosa* had 11 mm zone compared to the vancomycin and oxacillin antibiotics.

CONCLUSION

In summary, these results suggested that the essential oil of *Artemisia sieberi* with a high percentage have good antibacterial activity against gram-positive and gram negative bacteria. It seems that antibacterial activity of this essential oil is because of the interaction of its ingredients, but the impact of various factors such as ecological, geographical and climatic conditions cannot be ignored. In this study it was found that essential oils of *Artemisia sieberi* inhibits the growth of bacteria at various concentrations. The minimum inhibitory concentration of essential oil of *Artemisia sieberi* is varying in the range of 1.5 -50 micrograms per ml.

Artemisia sieberi oil in combination with antibiotics has shown synergistic effects on bacterial strains and can be used in making appropriate drugs with herbal origin and also as effective treatment for bacterial infections can be achieved. Also in the food industry, it can be used as a food preservative.

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