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RESEARCH ARTICLE

Essential oil variation in the populations of *Artemisia spicigera* from northwest of Iran: Chemical composition and antibacterial activity

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Abstract

Context: *Artemisia spicigera* C. Koch (Asteraceae) is a perennial shrubby herb and is generally distributed in Armenia, Iran, and Middle Anatolia. This species traditionally has been used in medicines.

Objective: The aim of this research is to study the chemical composition and antibacterial activity of essential oils from *Artemisia spicigera* populations in northwest of Iran.

Materials and methods: The essential oil of *A. spicigera* was obtained by hydrodistillation from eight populations collected from different regions of East Azerbaijan and West Azerbaijan provinces (Iran) and analyzed by gas chromatography-mass spectrometry (GC-MS). The antibacterial activity of the oils was investigated against four Gram-positive and four Gram-negative bacteria using MIC determinations and the agar-gel diffusion method.

Results: Fourteen compounds were identified as the main components of the essential oils and the most abundant constituents are 1,8-cineole, camphor, α-thujone, camphene, β-thujone and *p*-cymene. Essential oil of population No. 1 showed the highest activity against *Escherichia coli*, *Enterobacter aerogenes*, *Serratia marcescens* and *Staphylococcus aureus* but the highest activity against *St. saprophyticus*, *Bacillus megaterium*, and *B. cereus* was found with population No. 6 and for *Citrobacter amalonaficus* with population No. 5. MIC values of essential oils ranged from 6 μg/mL against *Bacillus megaterium* to 12 μg/mL against *Citrobacter amalonaficus*.

Discussion: This study demonstrates the occurrence of 1,8-cineole/camphor/camphene chemotype of *A. spicigera* but there is also significant chemical variation between the studied populations. The findings showed the studied oils have good antibacterial activity, and thus potential to be used as natural health products.

Keywords: Biological activity, chemical variation, volatile compounds

Introduction

Plant essential oils and extracts have been used for many thousands of years in food preservation, pharmaceuticals, alternative medicines and natural therapies (Cimanga et al., 2002; Sylvestre et al., 2006). Essential oils are potential sources of novel antimicrobial compounds, especially against bacterial pathogens (Simic et al., 2004). Essential (volatile) oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, notably antibacterial, antifungal, and antioxidant properties (Baratta et al., 1998; Cosentino et al., 1999; Bounatirou et al., 2007). Biological activity of essential oils depends on their chemical composition, which is determined by the plant genotype and is greatly influenced by several factors such as geographical origin, environmental, and agronomical conditions (Rota et al., 2004; Yesil Celiktas et al., 2007).

The genus *Artemisia* is one of the largest in the Asteraceae family, consisting of more than 800 species, which are wide spread all over the world (Judzentiene &

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Buzelyte, 2006). *Artemisia* species have been known as a folk medicine resource, which used for its anti-inflammatory activity, and as a diuretic agent and treatment of epidemic hepatitis (Qiu Guo et al., 2004).

Artemisia spicigera C. Koch is a perennial shrubby herb. It is generally distributed in Armenia and also in the Middle Anatolia and Iran. It is an Iran–Turanian element that grows on dry slopes, steppes and rarely in salty areas with altitude about 300–2000 m (Guvenalp et al., 1998). Some researchers have reported the chemical composition of essential oils of *A. spicigera* (Aleskerova et al., 1986; Guevenalp, 1998; Guvenalp et al., 1998; Sefidkon et al., 2003; Kordali et al., 2005, 2007; Demirci et al., 2005; Rabie et al., 2006; Anon., 2008). The above mentioned studies display the different oil chemotypes, which strongly correlate with a different geographical origin, the plant material, the vegetative period, and method used for isolating the essential oils. With the increasing tendency for the use of volatile oils in both the food and the pharmaceutical industries, a systematic examination of plant extracts for antimicrobial activity is very important (Verdian-Rizi et al., 2008).

Although there are some reports about antispasmodic and antiseptic (Grainger & Wichtl, 2004), antifungal, antiulcer (Minaiyan et al., 2006) and antioxidant activity (Guvenalp et al., 1998) of essential oils of *A. spicigera*, our bibliographical studies indicated that there are few reports about its antibacterial activity. Kordali et al. (2005) and Kotan et al. (2007) reported that *A. spicigera* oils have antibacterial activities over a very wide spectrum.

In this research work, we studied the chemical composition of the hydrodistilled oils of *A. spicigera* from Iranian origin that were collected from different populations in the northwest Iran, and then comparing the results with previous reports. The behavior of antibacterial activities of essential oils prepared from the species was studied against some Gram-positive and Gram-negative bacteria. According to our bibliographical studies, there are few reports on the antibacterial effects of essential oils of *A. spicigera* (Kordali et al., 2005; Kotan et al., 2007).

Materials and methods

Plant materials

Artemisia spicigera is perennial and constitutes the vegetation cover of dry and semi-arid regions. *A. spicigera* is a ligneous plant that is 25–50 centimeters tall, the bottom of plant is woody and divided, its different flowering branches are completely erected, covered with papill as white and highly dense at the start while decreasing towards the end. They are green and occasionally yellow, angular and have branches at the top.

The aerial parts of *A. spicigera* were collected from eight different populations from East Azarbaijan (Azarbayjane-Sharghi) and West Azerbaijan (Azarbayjane-Gharbi) provinces (northwest Iran) in August 2008 (Figure 1). Voucher specimens of the species are deposited in the local herbarium of the Department of Biology, Faculty of Science, Bu-Ali Sina University, Hamedan, Iran. The localities and collection information are recorded in Table 1. The plant materials were authenticated by Prof. Shahin Zarre who is a botanist in Tehran University.

Isolation of essential oils

Aerial parts of the plants were air-dried in the shade at room temperature. The dried plant samples (500 g) were subjected to direct hydrodistillation (plant material in boiling water) using a Clevenger type apparatus (Faraz Teb Tajhiz, Tehran, Iran) for 4 h. The oils were dried over anhydrous Na_2SO_4 for 4 h and stored at 5°C, for further analysis (Chehregani et al., 2010; Mohsenzadeh et al., 2011).

Identification of components

Essential oil components were identified by GC-MS via peak matching and by utilizing their retention indices on an Innowax FSC column (Shimadzu, Kyotone, Japan). The oven temperature was programmed from 50°C to 250°C at 3°C/min. Carrier gas was helium with a flow rate of 1.5 mL/min. Temperatures of the injector and FID detector were 250 and 300°C respectively. Oil samples were injected without solvent (0.1 μL) using the split mode injection (1:60 ratio). Temperature-programmed retention indices (RI) were calculated using a homologous series of *n*-alkanes (C9-C20) (Vandendool & Kratz, 1963; Curves et al., 1985). Computer matching against commercial libraries (Wiley and Mass Finder Version 2.1) (Adams, 1995; Mc Lafferty & Stauffer, 1989; Joulain et al., 2001), Baser Library of Essential Oil Constituents, which was built from genuine compounds and components of known oils, and the reported MS literature library data (Jennings & Shibamoto, 1980; Joulain & Koenig, 1998) were utilized in the final characterization of oil components.

Test organisms

Standard strains of the following microorganism were used as test organisms: *Enterobacter aerogenes* (PTCC 1221), *Serratia marcescens* (PTCC 1111), *Escherichia coli (*Lio*), Citrobacter amalonaticus* (PTCC 1499), *Bacillus cereus* (PTCC 1247), *Bacillus megaterium* (PTCC 1017), *Staphylococcus saprophyticus* (Lio), and *Staphylococcus aureus* (ATCC 6633). Some microorganisms were obtained from the Persian Type Culture Collection, Tehran, Iran and others locally isolated (Lio). For use in the experiments, the organisms were sub-cultured in nutrient broth and nutrient agar (Oxoid, Cambridge, UK). Diagnostic sensitivity test agar (DST) (Oxoid) was used for antibiotic sensitivity testing.

Sensitivity testing

For bioassays, a suspension of about 1.5×10^8 cells per mL in sterile normal saline was prepared as described by Forbes et al. (1990). Sensitivity testing was determined using the agar-gel diffusion method (Russell & Furr, 1977; Chehregani et al., 2010; Mohsenzadeh et al., 2011). In each disk, 30 μL of essential oil was loaded. The isolated

Figure 1. A map of Iran showing the localities (*) of the studied populations of *Artemisia spicigera* collected from East Azarbayjan (Azarbayjan-Sharghi) and West Azarbayjan (Azarbayjane-Gharbi) provinces in Northwest of Iran.

bacterial strains were first grown in nutrient broth for 18 h before use. The inoculum suspensions were standardized and then tested against the effect of the essential oils with 30 µL for each disk in DST medium. The plates were later incubated at 37 ± 0.5°C for 24 h after which they were observed for zones of inhibition. The effects were compared with standard antibiotics (Mohsenzadeh et al., 2011), chloramphenicol (30 µg per disc), nalidixic acid (30 µg per disc), penicillin (10 µg per disc) and tetracycline (30 µg per disc) (purchased from Padtanteb Co., Tehran, Iran).

The MICs of essential oils were also determined by tube dilution techniques in Mueller-Hinton broth (Merck) according to NCCLS (2008). The experiments were repeated at least three to five times for each organism and the data are presented as the mean ± SE of 3–5 samples.

Results

The essential oil yields (v/w, on dried mass basis) of *Artemisia spicigera* ranged from 0.86 to 1.14% in the different study populations. The results of the analysis of essential oils are present in Table 2. Results show that fourteen compounds were identified in the oils of *A. spicigera* but there are significant variations between the populations. Three compounds, including 1,8-cineole, camphor and camphene, were constantly observed in the essential oils of all populations. In population No. 5, collected from Tabriz in East Azerbaijan, 1,8-cineole (47.15%) was in a higher percentage than other populations. The highest amount of camphor was evaluated in population No. 7, collected from Salmas in West Azerbaijan. Population No. 1 (collected from Soufian, East Azerbaijan) showed the highest percentage of camphene (18.68%). The highest amounts of *p*-cymene, α-thujone and β-thujone were determined in populations 4, 7, and 4, respectively (Table 2).

Antibacterial activity of the oils was studied against eight bacterial strains (Table 3). The oils inhibited the growth of bacterial strains producing a zone diameter of inhibition from 9.0 to 48.0 mm, depending on susceptibility of the tested bacteria. For the oil of population No. 1, the inhibition zones against some bacterial strains (*Escherichia coli, Enterobacter aerogenes, Serratia marcescens,* and *Staphylococcus aureus*) were higher than the

Table 1. Different localities of *A. spicigera* population that were studied in this research.

Locality number	Herbarium vouchers	Locations	Geographical characters	Collectors	Altitude (m)	Slope (%)	Direction of slope	Soil	Date of collection
1	17819	East Azerbaijan, Tabriz to Marand road, before the Soufian Cement Factory	N: 37° 41′ 15″ E: 45° 52′ 29″	Yousefi, Jalali, Taleb-Pour	1370	0	Flat	Loam	16 August 2008
2	17820	East Azerbaijan, Tabriz, Marand to Soufian, three way of Qareh Aghaj Village	N: 38° 18′ 47″ E: 45° 57′ 15″	Yousefi, Jalali, Taleb-Pour	1476	20	Sothern	Loam	16 August 2008
3	17821	East Azerbaijan, Tabriz, Vanyar, Opposite the river	N: 33° 07′ 56.7″ E: 46° 30′ 1.6″	Yousefi, Jalali, Taleb-Pour	1453	0	Flat	Salty Loam	18 August 2008
4	17822	East Azerbaijan, Tabriz, Nahand river margins, Soulcheh to Nahand	N: 33° 10′ 22.8″ E: 46° 28′ 52.9″	Yousefi, Jalali, Taleb-Pour	1620	0	Flat	Loam	19 August 2008
5	17823	East Azerbaijan, Tabriz, Khajeh city, watershed management area	N: 33° 09′ 24.9″ E: 46° 39′ 10.2″	Yousefi, Jalali, Taleb-Pour	1524	0	Flat	Loam	20 August 2008
6	17824	West Azerbaijan, Urmia to Salmas road, Km 25, opposite the Nazlou river	N: 38° 01′ 01″ E: 45° 06′ 45″	Yousefi, Jalali, Taleb-Pour	1774	0	Flat	Loam	23 August 2008
7	17825	West Azerbaijan, Salmas, railway bridge	N: 38° 16′ 44.3″ E: 45° 00′ 29″	Yousefi, Jalali, Taleb-Pour	1759	60	Northern	Loam-Sand	24 August 2008
8	17826	West Azerbaijan, Urmia, between the villages of Khan Takhti and Qareh Ashlagh	N: 38° 01′ 7.4″ E: 45° 01′ 29″	Yousefi, Jalali, Taleb-Pour	1824	15	Western	Loam-Sand	25 August 2008

Table 2. The main chemical compositions of essential oils from aerial parts of *A. spicigera* collected from different populations.

		Populations							
RI*	Compounds	1	2	3	4	5	6	7	8
924	Tricyclene	0.37	0.36	**0.38**	–	–	0.13	0.33	0.27
930	α-Thujone	14.6	15.12	2.62	14.02	6.12	8.39	**21.2**	11.63
947	Camphene	**18.68**	3.75	4.98	2	2.09	3.53	2.41	1.26
972	*trans*-Sabinene hydrate	0.35	0.37	**0.38**	0.26	–	0.18	–	0.12
1017	*para*-Cymen-8-ol	0.38	0.42	–	–	–	–	–	**12.50**
1028	*p*-Cymene	3.81	3.75	3.78	**4.05**	1.46	1.78	1.19	2.38
1031	1,8-Cineole	27.24	26.98	35.61	21.32	**47.15**	9.1	20.09	10.8
1059	γ-Terpinene	0.3	0.2	0.43	0.4	**4.48**	–	0.17	–
1102	β-Thujone	5.02	4.9	1.14	**10.28**	2.27	2.94	9.79	–
1125	Chrysanthenone	3.42	3.39	1.33	2.34	3.25	6.56	1.69	**25.09**
1143	Camphor	30.68	31.02	37.97	30.05	28.81	15.25	**39.93**	27.97
1258	Carvone	0.21	0.19	0.18	0.2	**4.65**	–	0.1	0.22
1263	Chrysanthenyl acetate	0.2	0.27	–	0.33	**5.72**	4.28	–	0.86
1580	(+) Spathulenol	0.2	0.3	1.26	0.79	**8.34**	5.26	0.07	1.38

Amounts were expressed as percentage. Each datum represented the mean of three samples; 1-8, different studied populations. The bold data showed the highest amount of each compound through the populations.

*RI, Retention index of the compounds.

oils of other populations. The oil prepared from population No. 5 showed the highest inhibition zone (16.5 mm) against *Citrobacter amalonaficus*. The oil of population No. 6 showed the highest inhibition zones (36, 43, and 48 mm) against *Staphylococcus saprophyticus*, *Bacillus megaterium,* and *B. cereus*, respectively, and their effects are also greater than that of the studied standard antibiotics.

Since the comparison of the inhibition zone size merely is not trustworthy, the MIC of the plant oil provided from population No. 1 was also determined according to the method of NCCLS (2008). Results showed that the MIC of plant oil against the tested organisms varied between 6 μg/mL against *Bacillus megaterium* and 12 μg/mL against *Citrobacter amalonaficu*. The standard

Table 3. Antimicrobial activity of the essential oils provided from different populations of *A. spicigera* expressed as diameter of inhibition zone (mm) and minimum inhibitory concentration (MIC).

	Populations												MIC	
Bacterial strains	1	2	3	4	5	6	7	8	a	b	c	d	EO[a] (μL/mL)	STD (μg/mL)
Escherichia coli	**31 ± 4**	16 ± 2	15 ± 3.5	14 ± 3	11 ± 2	13.5 ± 4	20 ± 5	18 ± 3	24 ± 4.3	–	9 ± 1.4	–	7	8
Enterobacter aerogenes	**24 ± 4**	14 ± 3	13.5 ± 4	9 ± 1	15 ± 2	12.5 ± 4	16.5 ± 2	12.5 ± 2	19 ± 4	–	16 ± 3	20 ± 4	10	4
Serratia marcescens	**28 ± 6**	19 ± 5	12 ± 2	11 ± 2	19 ± 3	14 ± 4	22.5 ± 4	15 ± 2	20 ± 5	–	13 ± 1	21 ± 3	8	4
Citrobacter amalonaficus	15 ± 3	16 ± 4	14 ± 2	10 ± 1.5	**16.5 ± 2**	14 ± 4	15 ± 2	12 ± 1.4	17 ± 2	–	16 ± 1.6	15 ± 2	12	8
Staphylococcus aureus	**22 ± 2.5**	21.5 ± 4	16 ± 1.6	19 ± 2	19 ± 3	12 ± 1	14 ± 1.5	12 ± 2	21 ± 2	8.5 ± 1	8.2 ± 0.8	9 ± 1	8	2
Staphylococcus saprophyticus	19 ± 2	19 ± 3	17 ± 2	–	17 ± 2	**36 ± 5**	18 ± 2	11 ± 2	23 ± 5	18 ± 2	15.5 ± 3	–	8	4
Bacillus megaterium	38 ± 5	17.5 ± 2	21 ± 3	17.5 ± 3	18 ± 4	**43 ± 6**	27.5 ± 3	12 ± 3	22 ± 4	–	15 ± 4	11 ± 2	6	1
Bacillus cereus	21 ± 3	21 ± 4	15.5 ± 2	17 ± 2	14 ± 2	**48 ± 8**	35 ± 6	17 ± 4	25 ± 3	23 ± 3.6	22 ± 4	21 ± 3	8	1

1-8, Different studied populations. The bolded data indicates the most effective essential oils against the studied bacterial test organisms. a, Chloramphenicol; b, Penicillin; c, Tetracycline; d, Nalidixilic acid; EO, Essential oil; STD, Standard chloramphenicol antibiotic.
[a]MIC of essential oil was evaluated only for the most active populations.

chloramphenicol had MIC values varying between 1 and 8 μg/mL. The results indicated that standard antibiotic showed stranger activity than plant oil against most bacterial strains, but in a case (*Escherichia coli*), the effect of plant oil is greater than that of chloramphenicol. The lowest MIC (6 μg/mL) was detected for essential oil against *Bacillus megaterium* and for other bacterial strains were 7 (*Escherichia coli*), 8 (*Staphylococcus saprophyticus*, *St. aureus, Bacilus cereus,* and *Serratia marcescens*), 10 (*Enterobacter aerogenes*), and 12 μg/mL (*Citrobacter amalonaficus*) (Table 3).

Discussion

In this research, eight populations of *A. spicigera*, growing in the Northwestern areas of Iran, were studied regarding essential composition and the results of the analysis of the essential oils showed the presence of fourteen main compounds in the oils of *A. spicigera*. 1,8-Cineole, camphor, camphene, α-thujone, β-thujone and *p*-cymene were found to be the highest amounts, and between them, 1,8-cineole, camphor, and camphene were common in essential oils of the all populations. Our results are in accordance with those published by Demirci et al. (2005) and Guevenalp et al. (1998) that introduced 1,8-cineole, camphene and camphor as a major components of the essential oil in *A. spicigera* and very similar to reports of Aleskerova et al. (1986) and Anonymous (2008) that demonstrated the main compounds of essential oil are camphor and 1,8-cineole, but are different from some other reports.

In one study, the major compounds of the oils of *A. spicigera* were reported as camphor, limonene, 1,8-cineole and camphene (Sefidkon et al., 2003). 1,8-Cineole, limonene, camphor, camphene and β-thujone were also reported as the main compounds in the oil of *A. spicigera* (Rabie et al., 2003). Bicyclogermacrene, β-pinene and eogenol were reported by Guvenalp et al. (1998) as the main compounds. Camphor, acetate artemisil, β-thujone and artemisia alcohol were reported for a population of *A. spicigera* and, in another population, acetate artemisil, camphor, 1,8-cineole and β-ocymene were reported as the main compounds (Guvenalp et al., 1998). Guvenalp et al. (2003) also showed that α-pinene, 1,8-cineole and camphor are the main compounds in the oil of *A. spicigera* in some populations. Camphor, 1,8-cineole, caryophyllene oxide, borneol, α-terpineol, spathulenol, cubenol, β-eudesmol, and terpinen-4-ol were also reported by Kordali et al. (2005) as the main compounds.

The differences found between our results and those of other authors can be attributed to the fact that essential oils are a heterogeneous group of mixtures that are affected by environmental conditions. The quality and quantity of essential oils are related to growth stages, ecological conditions, and other factors based on the way in which the essential oil is extracted (Ozcan & Erkmen, 2001; Moreira et al., 2005; Chehregani et al., 2010). Since the essential oil compositions of the all populations were characterized by high percentage of 1, 8-cineole, camphor and camphene, we are able to introduce the chemotype of 1, 8-cineole/camphor/camphene in *A. spicigera* populations in the Northwestern regions of Iran. With the consideration of different geographical conditions for the studied populations (Table 1) we could not find a sharp relationship between the environmental conditions and plants essential oil constitutes.

Since *A. spicigera* is a medicinal plant (Grainger & Wichtl, 2004; Minaiyan et al., 2006), its antibacterial activity was investigated against some Gram-positive and Gram-negative bacteria. Results of antibacterial

tests showed good activities of the oils against the studied bacterial strains (Table 3). The MIC of essential oil against some bacterial strains was high and greater even than those of standard antibiotics, in some cases, and showed significant inhibition at low concentrations. On the other hand, it can be concluded from Table 3 that the oils of *A. spicigera* have significant inhibitory effect against all studied bacterial strains. Since there is a relationship between the chemical composition of the oils and their antibacterial activities (Imelouane et al., 2009), it can be said that the high antibacterial activity of the essential oils of *A. spicigera* is apparently related to presence of 1,8-cineole, camphor, camphene, α-thujone, β-thujone and *p*-cymene. Some previously reports were also showed that 1,8-cineole and camphor have antibacterial properties (Jalsenjak et al., 1987; Sur et al. 1991; Sivropoulou et al., 1997; Kim et al., 1997; Yu et al., 2003; Cha et al., 2005; Kordali et al., 2005; Cetin et al., 2009). Camphor and *p*-cymene (Royo et al., 1999), camphene and *p*-cymene (Imelouane et al., 2009), *p*-cymene and β-thujene (Pengelly, 2004) exhibit antimicrobial activity. Nevertheless, other minor components in essential oils of this species may influence on its antibacterial activity. Based on our results, population No.1 contained the highest amount of comphene and also a high percentage of camphor and 1,8-cineole in the whole, and antibacterial activity in this population is also high.

In addition, our results showed that Gram-positive bacteria are more sensitive to the oils than Gram-negative bacteria. Similar results have been reported previously by some other researchers (Zaika, 1988; Ouattara et al., 1997; Mangena & Muyima, 1999; Awouafack et al., 2008; Verdian-Rizi et al., 2008). The highest antibacterial effect was against to two Gram-positive bacteria (*Bacillus megaterium* and *B. cereus*) and the lowest antibacterial activity was against a Gram-negative bacterium (*Citrobacter amalonaficus*). Regarding the three compounds (1,8-cineole, camphene, and camphor) as the major constituents of essential oils in different populations of *A. spicigera*, it can be concluded that the high antibacterial activity against *Bacillus megaterium* and *B. cereus* is related to the presence of above-mentioned compounds. It is also known that antibacterial or biological activities of essential oils and extracts of medicinal plants may be subjected to a change, based on the variations in their chemical composition that may be related to the origin, the locality, the environmental conditions, and the developmental stages of the collected plant materials (Güllüce et al., 2003; Chehregani et al., 2010). However, major or trace compounds might give rise to the antimicrobial activity exhibited (Lopes-Lutz et al., 2008).

Conclusion

Results showed that 1,8-cineole, camphor and camphene are the main compounds of the studied populations, thus the occurrence of 1,8-cineole/camphor/camphene chemotype was introduced in the Northwestern regions of Iran, but there is high chemical variation between the populations. The findings also demonstrated that the essential oils of *A. spicigera* have excellent antibacterial activities and thus have great potential to be used as a resource for natural health products, but the quality of the essential oil and its biological activity is related to the its growing ecological conditions. This means that biological properties of medicinal plants, antibacterial activity for example, are affected not only genetically but also by their growing environmental and geographical conditions.

Declaration of interest

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