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Antibacterial activity of *Artemisia absinthium* essential oil from the Northeast of Iran

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Abstract

Background: *Artemisia absinthium* is a species from the family Asteraceae. Traditionally, *Artemisia* species have been used as antipyretic, antiseptic, antimalarial, antiviral, antitneoplastic, antihepatitis and antispasmodic plants. In the present study, we evaluated the antibacterial activity of *A. absinthium* essential oil collected from North khorasan, Iran.
Materials and Methods: The essential oil of *A. absinthium* was obtained by steam distillation and the chemical composition of the oil was analyzed using GC-MS method. Antibacterial activity was investigated by disc diffusion method against two bacteria namely, *Staphylococcus aureus* and *Escherichia coli*.
Results: Analysis of the isolated oil revealed the presence of 35 compounds. Major compounds of *A. absinthium* essential oil were α-pinene, α-phellandrene, p-cymene, sabinene and chamazulene. Zones of bacterial growth inhibition around the discs were 32 mm for *S. aureus* and 30 mm for *E. coli* in disc diffusion method and 33 mm for *S. aureus* and 32 mm for *E. coli* in well diffusion method. The results showed that the antibacterial activity of *A. absinthium* essential oil against *E. coli* and *S. aureus* was markedly more than that of gentamicin.
Conclusion: Based on our data, *A. absinthium* essential oil should be subjected to more evaluation for antibactical activity.

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Introduction

The *Artemisia* genus, belonging to the family Asteraceae, possesses various important medicinal properties. *Artemisia* species have been used as antipyretic, antiseptic antimalarial, antiviral, antispasmodic, antineoplastic, antihemorrhagic, anticoagulant, analgesic, antioxidant, antihepatitis, antiulcerogenic and anticomplementary herbs (Tan et al, 1998; Abu Zarga et al,1995). *A. absinthium* grows in temperate regions of Eurasia and Northern Africa and has been commonly used for its diuretic and antiseptic properties (Mozaffarian, 1996; Tan et al, 1998; Deans and Kennedy, 2002). Also, it shows antimicrobial, cytotoxic, antimalarial, anti-protozoal and antidepressant activities (Mahmoudi et al, 2009; Canadanovic-Brunet et al, 2005; Omer et al, 2007; Tariq et al, 2009). Essential oils of *Artemisia* species have been used for different medicinal purposes (Ahameethunisa and Hopper, 2010). The extracts and essential oil of the plants of this genus have shown to exhibit marked antibacterial activity (Fiamegos et al, 2011; Guangrong et al, 2008; Hedi et al, 2013; Karabegovic et al, 2011). The composition of *A. absinthium* extracts varies from country to according to the soil composition (Kordali et al, 2005; Semnani and Akbarzadeh, 2005) and the analysis of the essential oil from different geographical regions has shown noticeable variations in its chemical composition (Sharopov et al, 2012; Orav et al, 2006). Considering these variations in chemical contents of *Artemisia* species, in the present work, we studied the composition and antibacterial activity of *A. absinthium* essential oil collected from North Khorasan, Iran.

Materials and Methods

Plant materials

The aerial parts of *A. absinthium* were collected from North Khorasan, Iran, in May 2013. A voucher specimen (No. 13035) was deposited at the Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. The aerial parts were air-dried at room temperature in the shade.

Preparation of essential oil

To prepare the essential oil, 80 g of dried materials were submitted to hydrodistillation for 3 hr, using a Clevenger-type apparatus. The volatile distillate was collected over anhydrous sodium sulfate and refrigerated at -20 ºC for future analysis (Yamini et al, 2008).

Gas Chromatography-Mass Spectroscopy analysis

Analysis was performed using a GCMS-QP2010SE (Shimadzu, Japan). Helium was used as the carrier gas (0.9 ml/min) and RTX-5MS (30 m × 0.25 mm, 0.25 µm) was used as the capihhy column. The column temperature was kept at 50° C for 5 min, then heated to 260° C with a 5° C/min rate and kept at 260° C for 10 min. MS were taken at 70 ev ionization energy, mass scan range was 30-350 amu and scan time was 2 sec per scan. The retention indices were calculated in relation to a series of *n*-alkanes (C_5-C_{30}) as standards (under the similar operating conditions) and compounds were identified by comparison of their retention indices from Rtx-5MS column with those reported in the literature and their mass spectra in NIST and Wiley libraries (Massada, 1976; Adams, 2005).

Antibacterial activity

The microorganisms used in this study were *S. aureus* (ATCC 6538p) and *E. coli* (ATCC 10536). The essential oil of the plant was tested for antibacterial activity using the disc and well diffusion methods on solid media Mueller-Hinton agar (MHA) plates. The sterile paper discs and wells of 6 mm diameter were placed on the agar plates with the appropriate media, and the bacteria density was adjusted to approximately 10^7 CFU/ml. Then, 50 µl of the essential oil was applied to test paper disc and well in plates and the agar plates were further incubated for 24 hr at 37°C. Finally, the zones of growth inhibition around the discs were measured. Gentamicin and DMSO were used as positive and negative controls, respectively (Firdaus et al, 2011).

Results

GC-MS analysis

Table 1 shows the components of the essential oil and their properties (the retention index and percentage composition).

Table 1. Chemical composition of the essential oil of *A. absinthium*.

No.	Compound	RI*	Chemical constituents (%)
1	α-thujene	927	0.21
2	α-pinene	933	5.88
3	sabinene	974	8.74
4	β- pinene	976	12.29

5	β-myrcene	993	0.71
6	α-phellandrene	1005	16.4
7	α-terpinene	1018	0.23
8	ρ-cymene	1026	7.05
9	β-phellandrene	1033	0.75
10	1,8-cineole	1036	0.16
11	Trans-β-ocimene	1051	0.17
12	γ-terpinene	1062	0.38
13	Trans-sabinene hydrate	1073	0.26
14	α-terpinolene	1089	0.14
15	linalool	1105	0.61
16	Trans-pinocarveol	1139	0.3
17	Terpinene-4-ol	1186	0.42
18	α-terpineol	1189	0.11
19	Cuminal	1239	0.26
20	Citral	1241	0.35
21	Thujone-3-ol	1289	0.27
22	Thymol	1294	0.42
23	Carvacrol	1298	0.48
24	α-copaene	1378	0.19
25	β-bourbonene	1384	0.12
26	α-cedrene	1410	0.15
27	β-caryophyllene	1418	0.25
28	Humulene	1458	0.87
29	Germacrene-D	1480	2.14
30	β-selinene	1487	0.78
31	Delta-cadinene	1529	0.64
32	Germacrene- B	1562	0.41
33	Viridiflorol	1596	0.34
34	Chamazulene	1716	13.88
35	Phytane	1795	0.87
	Total		77.12
	Monoterpene hydrocarbons		54.43
	Oxygenated monoterpenes		2.92
	Sesquiterpene hydrocarbons		19.43
	Oxygenated sesquiterpenes		0.34

RI, retention index obtained from RTX-5MS column by comparing each compound's retention time with that of *n*-alkenes (C_5–C_{30}).

In total, 35 compounds were identified in the essential oil, representing 77.12 % of total oil. The main compounds in the essential oil were α-pinene, β-pinene, α-phellandrene, p-cymene, sabinene and chamazulene (Figure 1).

Antibacterial activity

The results of evaluation of the antibacterial activity of the essential oil of *A. absinthium* (diameter of growth inhibition zones (mm)) against 2 bacterial strains (*S. aureus* and *E. coli*) assessed by disc-diffusion and well-diffusion methods, are shown in Table 2.

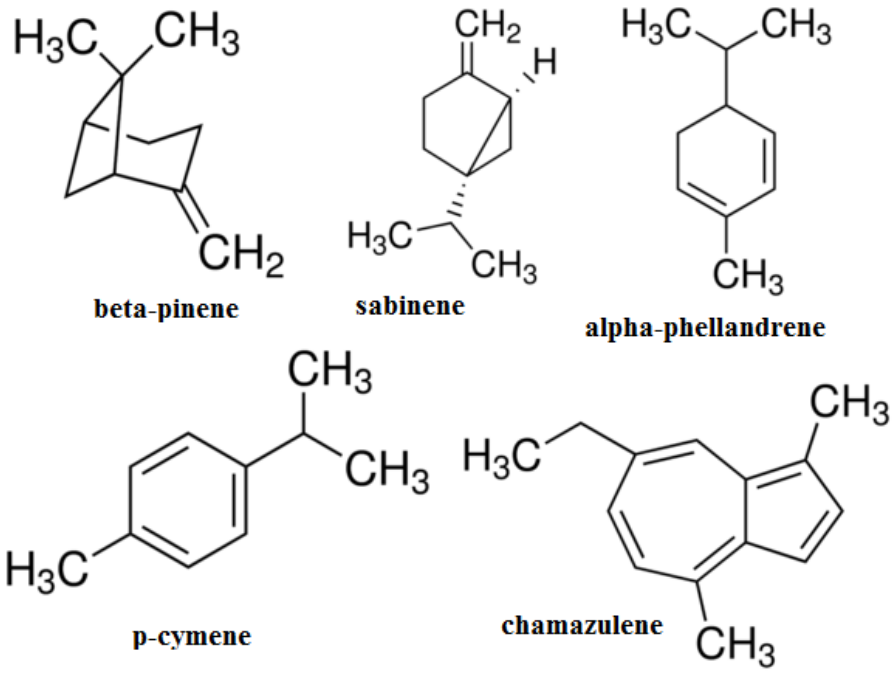

Figure 1. Chemical structures of the main components of *A. absinthium*.

Table 2. Antibacterial activity of *A. absinthium* was assessed by disc and well-diffusion methods. The effectiveness of essential oil is demonstrated by the size of the microorganism growth inhibition zone around the filter paper disc, which is typically expressed as the diameter of the zone in mm.

	Diameter of inhibition zones (mm) , Disc- diffusion method*		Diameter of inhibition zones (mm) , Well- diffusion method*	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
Essential oil	32 mm±0.016	30 mm±0.11	33 mm±0.08	32 mm±0.078
Gentamicin	28 mm±0.02	26 mm±0.05	31 mm±0.03	30 mm±0.065

*Expressed as the size of the growth inhibition zones (mm) as the average of triplicates.

As shown in Table 2, the essential oil of the plant inhibited Gram positive and negative microorganism strains and this effect was markedly higher than that of gentamicin.

Discussion

A study done in Turkey showed that the major components of the essential oils *of rtemisia* species were camphor and 1, 8-cineole (Kordali et al, 2005). In the oil obtained from the leaves of *A. absinthium* collected from Ardabil, Iran, 1, 8-cineole, borneol and camphor were the major components (Taherkhani et al, 2013). The major compounds in *A. absinthium* from India were borneol, methyl hinokiate, isobornyl acetate, β-gurjunene and caryophyllene oxide (Joshi, 2013). In this study, five compounds namely, sabinene, β-pinene, α-phellandrene, p-cymene and chamazulene were the major compounds in the essential oil of *A. absinthium* that constituted 58.36% of the essential oil. Among the identified compounds, α-phellandrene (a hydrocarbon monoterpene) and chamazulene (a sesquiterpene hydrocarbon) were the most abundant chemicals in the essential oil. Chamazulene is found in chamomile and is known to have anti-inflammatory and antioxidant properties due to its structure (Ornano et al, 2013). Antimicrobial activity of the plants of different areas of the world has been reported (Janovska et al, 2003). The disc and well-diffusion methods are dependent on the diffusion ability of the substances and in these methods, antibacterial property is expressed as diameter (mm) of the zone of inhibition (He et al, 2010). In other studies on antibacterial activity of the essential oil of *A. absinthium*, it was demonstrated that *A. absinthium* extract had significant antibacterial activity against *S. aureus* (Moslemi et al, 2012). Treatment with the methanolic extract of *A. absinthium* resulted in a 16 mm zone of inhibition against *S. aureus*, 10 mm against *Bacillus subtalis*, 9 mm against *Enterococcus faecalis*, and 8 mm against *Pseudomonas aeruginosa* while it had no activity against *Salmonella typhimurium* (Khalid et al, 2011). In another experiment, the main constituents of *A. absinthium* were sabinene, sabinyl acetate and and α-phellandrene. The antimicrobial activity of the oil was investigated against ten bacterial isolates and the minimal inhibitory/bactericidal concentration of the oil ranged between 0.08 and 2.43 mg/ml and 0.08 and 38.80 mg/ml (Mihajilov-Krstev et al, 2014). Also, in another work, the results showed that the inhibitory effects of the methanolic extract of *A. absinthium* against *B. subtilis, S typhmiruim, Saccharomyces cerevisiae, Bacillus cereus, Streptococcus thermophilus, Providencia alcalifaciens* and

Pseudomonas putida were higher than those of ofloxacin and novobiocin (Sengul et al, 2011). In this study, the antibacterial activity of the essential oil was attributed to the presence of chamazulene and α-phellandrene which were the major constituents of the essential oil.

Conclusion

In conclusion, the antibacterial activity of the essential oil of this plant was markedly higher than gentamicin against *E. coli* and *S. aureus*. Therefore, the essential oil of this plant can possibly be used as a natural preservative in food and pharmaceutical industries.

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Conflict of interest

The authors have no competing interests to declare.

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