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Chemical Composition and Antibacterial Activity from Essential Oil of *Artemisia sieberi* Besser subsp. *Sieberi* in North of Iran

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Abstract: The chemical composition and antibacterial effect of *Artemisia siberi* essential oil were studied in this research. The composition of essential oil from aerial parts was analyzed by GC/MS and its antibacterial effect were determined by disc diffusion method. Artemisia ketone (48.5%), 1, 8-cineole (19.7%), selin-11-en-4-a-ol (4.6%) and lavandulon (2.8%) were the major constituents of this herbal medicine. Inhibitory zone against *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Escherichia coli* around discs contained 100 mg mL^{-1} of *Artemisia siberi* essential oil were 18, 13 and 12 mm, respectively. Further studies for the determination of anti *Pseudomonas* infection in animal model are suggested.

Key words: *Artemisia siberi*, essential oil composition, antibacterial, *Pseudomonas aeroginosa*

INTRODUCTION

It has long been recognized that naturally occurring substances in higher plants have anti bacterial activity (Cha *et al*., 2005). 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 and Buzelyte, 2006).

Artemisia species has been known as a folk medicine resource, which used for its anti-inflammatory, diuretic agent and treatment of epidemic hepatitis (Qiu Guo *et al*., 2004). Essential oils make a major contribution in to the plants biological activity as well (Judzentiene and Buzelyte, 2006). Sesquiterpene lactones and acetylenes have been reported from the some species of *Artemisia* such as *A. assoana*, *A. lantana* and *A. pedemontana* (Preze-Alonso *et al*., 2003) and artemisia ketone, 1, 8-cineole, davanone, camphor, β-thujone, myrcene and germacrene-D have been also reported in essential oil of *A. absinthium, A. scoparis* and *A. vulgaris* (Chericoni *et al*., 2004; Preze-Alonso *et al*., 2003; Morteza Semnani *et al*., 2004; Rana *et al*., 2003; Perazzo *et al*., 2003). Numerous studies in the literature have reported the antibacterial and antifungal activity of oils isolated from various species of *Artemisia* (Qiu Gua *et al*., 2004). Essential oil of aerial parts of *Artemisia annua* with camphor (44%) and germacrene-D (16%) inhibited the growth of *Entrococcus faecalis* (Juteau *et al*., 2002).

Artemisia siberi Besser subsp. *Siberi* has been known as endemic medicinal herbs with wide dispersal that used by the rural healers in traditional medicine in Chaharbagh region located in north of Iran.

In this research project study, the chemical constituents and antibacterial activity of volatile oil obtained from *A. siberi* in this region were assessed.

MATERIALS AND METHODS

Plant material: Aerial parts in blooming of *Artemisia sieberi* was collected in late August of 2005 from Chaharbagh region, 70 km east of Gorgan in Golestan province in the north of Iran. Subsequently, it was dried in the shade for one week and was powdered. Their botanical name identified in the Plant Systematic Laboratory, College of Science, Islamic Azad University of Gorgan Branch, Iran where voucher specimens were deposited.

Two hundred grams of the dried powders were separately subjected to hydro distillation for 2 h, in full glass apparatus. The oil was isolated using a Clevenger type apparatus and stored frozen in dark glass bottles until they were used (Orav *et al*., 2006).

Oil analysis: The oils were analyzed by GC (9-A-shimadzu) and GC-MS (Varian-3400) column (DB-1, 60 mm 0.25 mm fused silica capillary column film thickness 0.25 μm using a temperature program of 50-250°C at a rate of 4°C min^{-1}, injector temperature 260°C, carrier gas: Helium, the constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by comparison of their retention indices with those of authentic compounds or with literature data. The components of the oils were

identified by matching their mass spectra and retention indices with those of the Wiley 275 library in the computer library and literature. The yield of each component was calculated per kg of the plant material, while its percentage composition was determined from the peak areas of the total oil composition.

Tested organisms: The test organisms used in the study were obtained from Persian Type Culture Collection, Tehran, Iran (PTCC), namely: *E. coli* (PTCC No. 1330), *P. aeroginosa* , *Staphylococcus aureus* (PTCC No. 1112). Suspension equal to 0.5 McFarland were prepared for each tested organism in Muller Hinton Broth.

Essential oil dilution and disc preparation: The essential oil was serial diluted for antibacterial study by Dimethylsulphoxide (DMSO) with final concentration: 100, 50, 25, 12.5 and mg mL^{-1}. Blank disc were saturated by each dilution of essential oil and after drying used for study.

Disc that saturated by DMSO were prepared as negative control and disc contain Gentamycin (Pad Tan Teb, Tehran, Iran) were used as positive control.

Antimicrobial activity: The antibacterial effects were tested by the disc diffusion method, briefly, Muller Hinton Agar plates were cultured with a standardized inoculums (1.5×10^{8} cfu mL^{-1} equal to 0.5 McFarland) of each bacterial strains, then the saturated discs with different concentration of crude essential oils were carefully placed on the plates, then were incubated aerobically at 37°C and inhibition zones were measured after 24 h. The inhibition zones were compared with the control disc containing Gentamycin as positive control. Each test was repeated 3 times and means inhibition zone were recorded. Inhibitory zone≥12 mm used as good inhibitory effect of extract.

RESULTS

Artemisia siberi is a perennial herb, 50-150 cm in height, known as locally name Dermaneh. Flowering time in August and setting seeds in September. This species grows in open fields, road sides and wast ground. Often forming dense colonies in arid and semi-arid stepic grasslands in mountainous region in Golestan province over the 2500 m above sea level.

Chemical composition: Water distillation of dried aerial parts in blooming of *A. siberi* yielded 94% (v/w) and about 22 constituents were identified by means of GC /MS analysis (Table 1). Artemisia ketone (48.5%) and 1,8-cineole (19.6%) were the major constituents of this essential oil.

Table 1: Chemical composition of *Artemisia siberi* L.

Chemical composition	RI	RT	Percentage
Santoline teriene	259	5:59	0.297
α-pinene	408	6:48	0.239
β-pinene	489	8:09	0.312
Yomogi alkohol	529	8:49	3.769
ρ-cymene	588	9:48	0.864
1, 8-cineol	606	10:06	19.620
Artemisia ketone	678	11:18	48.417
Artemisia alcohol	727	12:07	1.174
Trans verbenol	885	14:45	0.758
Lavandulol	942	15:43	2.707
Terpinen-4-ol	972	16:12	1.557
α-terpineol	1007	16:47	0.866
Lavandulyl acetate	1276	21:16	1.267
Geranyl acetat	1523	25:23	0.984
Caryophyllene (g-epi-E)	1626	17:06	0.847
Lavandulyl isovalerate	1847	30:47	1.542
Lavandulyl 2-methyl butyvate	1850	30:50	1.483
Caryophyllene oxide	2024	33:54	0.088
Cadin-4-en-7-ol(cis)	2152	35:52	2.466
Caryophyllen-4(14),8(15) -diexe-5-a-ol	2159	35:59	1.250
β-eudesmol	2191	36:31	1.380
Selin-11-en-4-a-ol	2202	36:42	4.589
Others not identified (3 unknown)			3.502

Table 2: Diameter of inhibitory zone (mm) of crude essential oil, *Artemisia sieberi* against three tested bacteria

Essential oil concentration	100	50	25	12.5
	---------- mg mL^{-1} ----------			
S. aureus	13	10	8	R
E. coli	12	8	8	R
P. aeroginosa	18	15	10	R

R = Resistant

Anti bacterial activity: The best antibacterial effect were found against *P. aeroginosa* with Inhibitory zone 18 mm (Table 2).

DISCUSSION

Literature studies showed that there are not enough research about ethno pharmacology, the essential oil components and *in vitro* antibacterial activity of *A. siberi*.

Appearance of phonological state of plants is affected by the genetic and environmental factors, especially in medicinal plants, these factors affected on the rates of not only the yield but also the secondary metabolites.

Therefore for obtaining the best yield of plants the surveying on and determining the phonological stage of the plant is necessary (Fieldsend and Morison, 2000). The rural healers believed that the proper therapeutically effect of *A. siberi* is in flowering stage in the late of August.

In present study Artemisia ketone and 1, 8-cineole were the major constituent of this species, similar to some other species of *Artemisia* such as *A. pedemontana* (Perez-Alonso *et al*., 2003), *A. annua* (Jutteau *et al*., 2002), *A. absanthium* and *A. parviflora* (Orav *et al*., 2006).

Cha *et al.* (2005) and Zhang *et al.* (2005) in two separated studies were reported that the *A. lavandulaefolia*, *A. scoparia* and *A. capillaris* have a good anti microbial activity against different genera of bacteria with the presence of β-caryophyllene, 1,8-cineole, α-terpineole and β-pinene in their essential oil. All of these constituents were present in high or low concentration in present studied *Artemisia* species, it means that this species can show good antibacterial activity. We found that the Gram-negative bacteria especially *P. aeroginosa* is the most sensitive bacteria to essential oil of *A. siberi* and *S. aureus* was a Gram positive sensitive tested bacteria.

P. aeruginosa is a leading cause of nosocomial infections, ranking second among the Gram-negative pathogens reported to the NNIS and is important for immunosuppressed patients and in patients hospitalized with cancer, cystic fibrosis and burns (NNIS, 1998). The increasing frequency of multi-drug-resistant *Pseudomonas aeroginosa* stimulated the medical researcher to look for efficacious antimicrobial options which are severely limited (Orav *et al.*, 2006). The conclusion drown from this study indicated that this herb, demonstrated a suitable anti *Pseudomonas* activity with good *in vitro* inhibitory effect. Further study are suggested to determine which of the chemical constituent of this essential oil carry the best anti *Pseudomonal* activity and also we recommend *in vivo* study of the same substance in an animal model.

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