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Antimicrobial activity of *Artemisia sieberi* essential oil from central Iran

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ABSTRACT

Background and objectives: The *Artemisia* genus of Asteraceae family is represented by 34 species in Iran. *Artemisia sieberi* grows wild in different regions of Iran and grows in desert and semi- desert climate and has forage value for animals and also medicinal properties for humans. In this study we examined the antimicrobial effects of *A. sieberi.*

Materials and Methods: The antimicrobial activity of *A. sieberi* essential oil was evaluated against different microorganisms including Gram positive bacteria, Gram negative bacteria, yeast and fungi by disc diffusion method and micro broth dilution assay.

Results: The oil with main components of α- thujone, β- thujone and camphor showed antimicrobial activity against different microorganisms with varying types of pathogens. Gram positive bacteria and fungi were more sensitive than Gram negative ones. Among Gram positive bacilli, *Listeria monocytogenes* and *Bacillus cereus* and among Gram positive cocci, *Streptococcus mutans* were more sensitive than others.

Conclusion: The antimicrobial properties of this oil showed that the *A. sieberi* essential oil has good potential use in the food and cosmetic industry.

Keywords: Antimicrobial activity, *Artemisia herba alba, Artemisia sieberi,* Essential oil, Iran.

INTRODUCTION

The genus *Artemisia* is one of the largest and widely distributed genuses of the Asteraceae. *Artemisia siberi* besser (*Artemisia herba alba* Asso Var. *laxifolia* Boiss) is named locally "Dermaneh" and is widely distributed in the desert area of Iran (1). This plant has forage value for animals and also medicinal properties for humans. Some pharmacological effects of *A. sieberi* plant such as spasmolytic, vermicidal (2), insecticidal (3), anticandidal (4) and asexual reproduction inhibition of some filamentous fungi (5) were confirmed.

There are some investigations on chemical composition and antimicrobial activity of *A. sieberi* essential oil. Analysis of *A. sieberi* essential oil ingredients have shown differences in its constituents depending on the region of cultivation even within one country. It has been found that *A. sieberi* oil from north of Iran (Tehran and Semnan Province) contain camphor, 1, 8- cineole (6, 7), from northeast of Iran (Khorasan Province) contain; α-thujone, β-thujone and camphor with antifungal activity against soil born bacteria (8). In animal dermatophytosis, clinical improvement has been observed with 3% Artemisia solution (*A. sieberi* oil) in comparison with clotrimazole (9). Literature survey indicates antimicrobial activity of *A. sieberi* oil against limited microorganisms. The aim of this study was to evaluate the antimicrobial activity of *A. sieberi* essential oil that extracted from aerial part of plant against a large number of microorganisms including Gram positive and Gram negative bacteria, in addition to yeast and filamentous fungi.

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MATERIAL AND METHODS

***A. sieberi* essential oil.** *A. sieberi* essential oil and its major components [α- thujone (32.9%), β-thujone

(13.3%), camphor (22.9%)] was obtained from Barij Essence pharmaceutical Company, Kashan, Iran. *A. sieberi* was gathered from the desert of Kashan (central of Iran). The oil ingredients were analyzed by gas chromatography (GC) at Barij Essence Pharmaceutical Industries (Kashan, Iran). The GC apparatus was Agilent technology (HP) 6890 system with capillary column of HP-5MS (60m×0.25mm, film thickness 0.25_µm). The oven temperature program was initiated at 40 °C, held for 1 min then raised up to 230 °C at a rate of 3°C/min held for 10 min. Helium was used as the carrier gas at a flow rate 1.0 ml/min. The detector and injector temperatures were 250 and 230 °C, respectively.

Microbial strains. All experiments were tested against a panel of microorganisms, including Gram-positive cocci; *Staphylococcus aureus* ATCC 25923, *Staphylococcus saprophyticus* ATCC 13518, *Staphylococcus epidermidis* ATCC 12228, clinical isolate of *Streptococcus agalacteae*, *Streptococcus pneumoniae* ATCC 49615, *Enterococcus faecalis* ATCC 29212, *Streptococcus sanguis* PTCC 1449, *Streptococcus salivarius* PTCC 1448, *Streptococcus mutans* PTCC 1601, *Enterococcus faecium* ATCC 25778; Gram positive spore bacilli *Bacillus subtilis* ATCC 6051, *Bacillus cereus* ATCC 1247 *and* non-spore bacilli *Listeria monocytogenes* ATCC 7644; Gram negative bacilli; *Escherichia coli* ATCC 8739, *Salmonella typhimorium* ATCC 14028, *Shigella dysentery* RI 366, *Shigella flexneri* (Clinical isolate), *Klebsiella pneumoniae* ATCC 10031, *Proteus vulgaris* RI 231, *Enterobacter aerugenes* NCTC 10009, *Pseudomonas aeruginosa* ATCC 9027; Fungi; *Aspergillus niger* ATCC 16404, *Aspergillus flavus* (field isolate); and *Candida albicans* ATCC 10231. Bacterial strains were cultured overnight at 37 ºC on nutrient agar and blood Agar. Yeast and fungi were cultured at 30 ºC on sabouraud dextrose agar.

Antimicrobial screening. Antimicrobial activity of the essential oil was determined, using disc diffusion method and micro broth dilution assays. The bacterium inoculate was prepared by suspending overnight colonies from nutrient or blood agar media in sterile normal saline. The *Candida albicans* and fungi inoculate were prepared by suspending colonies from 48 and 72 h sabouraud dextrose agar cultures in RPMI 1640 medium (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) buffered with 0.165 M morpholine propane sulfonilic acid (MOPS) (Merck KGaA, Darmstadt, Germany). This inoculate was adjusted to 0.5 McFarland (1×10^6 - 1×10^8 CFU ml^{-1}). Using a sterile cotton swab, the suspensions were cultured on Muller Hinton Agar (non-fastidious) or Todd Hewitt Agar supplemented with 0.5% yeast extract and 0.001% tween 20 (enriched THA) for fastidious bacteria and sabouraud dextrose Agar (fungi). Subsequently, sterile discs (6 mm in diameter) (Padtan Teb Co, Tehran, Iran) was saturated with 2.5, 5, 7.5, 15 µl of oil that dissolved in 10 µl of dimethyl sulfoxide (DMSO). Antibiotic disc and disc containing DMSO were used as controls. The culture plates were incubated at 37 ºC for 24 and 48 hours for bacteria and fungi respectively. The inhibition zone (IZ) was diameter in millimeters and recorded (10). The minimal inhibitory concentration (MIC) values of essential oil against different microorganism were determined by micro broth dilution assay. The oil was serially diluted two fold with 10% DMSO which contains 8-0.0125 µl ml^{-1} of oil. RPMI 1640 with L-glutamine but lacking bicarbonate buffered with 0.165 M MOPS was used as broth media for fungi and yeast (11). Cation adjusted Muller Hinton broth (12) and Enriched Todd Hewitt broth was used as a broth media for non-fastidious and fastidious bacteria, respectively (13).

After shaking, 100 µl of the essential oil was added to each well. The suspension of each organism was adjusted to 1×10^5 -1×10^6 CFU ml^{-1} and then 100 µl was added to each well and cultivated at 35 ºC. MIC values were defined as the lowest concentrations of oil that inhibit bacteria and fungi after 24 and 48 h, respectively. Minimal bactericidal concentration (MBC) values were recorded from the first tube that showed no growth on solid media. All experiments were done in triplicates.

RESULTS

The antimicrobial activity of *A. sieberi* essential oil and their potency were quantitatively and qualitatively assessed by determining the MIC and IZ diameter as given in Tables 1-3. The analysis of oil showed that this oil had some antimicrobial activity against different microorganisms and varied according to the type of pathogen. The inhibitory zones significantly increased in a dose dependent manner. Gram positive bacteria and fungi were more sensitive than Gram negative bacteria. The antimicrobial activity of oil was comparable with antibiotics. *B. cereus*

and *L. monocytogenes* were sensitive among Gram positive bacilli. *S. mutans* were more sensitive than other Gram positive cocci (Table 1). No clear correlation between MIC values and inhibition diameter was found. Inhibition diameter of some Gram negative bacteria is larger than Gram positive ones but the MIC values of Gram positive ones were smaller or MIC values of *S. mutans* did not correlate with inhibition diameter. These results show that these two methods are not necessarily comparable. MBC values of *B. subtilis*, *A. niger* and *A. flavus* were several fold to MIC values. This oil showed inhibitory effect against these microorganisms.

Table1. The antimicrobial activity of A. *sieberi* oil against Gram positive bacteria

	Inhibition Diameter (mm)					**minimal inhibitory growth (µl/ml)**	
	Essential oil (µl)				**Vancomycin**		
	2.5	**5**	**7.5**	**15**	**30 µg**	**MIC**	**MBC**
S. aureus	NE	10±0.5	14±0.8	18±0.6	17±0.6	2	4
S. epidermidis	NE	NE	10±0.0	16±0.5	19±0.0	4	8
S. saprophyticus	7.5±0.0	11±0.4	19±0.1	28±0.6	18±0.3	2	4
B. cereus	12±0.1	21±0.2	36±0.0	42±0.5	20±0.6	1	2
B. subtilis	NE	10±0.0	11.5±0.1	18±0.3	19±0.4	4	>16
L. monocytogenes	13±0.0	16±0.0	17.5±0.5	19.5±0.4	25±0.3	1	2
S. pneumoniae	NE	10±0.1	12±0.3	18±0.5	13±0.2	4	8
E. agalactiue	NE	NE	10±0.1	15±0.9	19±1.1	4	8
E. faecalis	NE	NE	9±0.6	12±0.5	15±0.4	4	8
S. faecium	NE	9±0.1	11±0.3	15±0.0	16±0.3	4	8
S. mutans	NE	NE	8±0.1	13±0.0	30±0.2	0.5	1
S. sanguis	NE	NE	11±0.1	17±0.1	22±0.0	4	8
S. salivarius	8±0.2	10±0.0	11±0.0	12±0.3	20±0.8	2	4

MIC= Minimal Inhibitory Concentrations, MBC= Minimal Bactericidal Concentrations, NE= No Effect

Table 2. Antibacterial activity of *A. sieberi* essential oil against Gram negative bacteria

	Inhibition Diameter (mm)					minimal inhibitory growth(µl/ml)	
	Essential oil (µl)				Gentamicin		
	2.5	5	7.5	15	10µg	MIC	MBC
E. aerugenes	NE	NE	NE	11.5±0.0	22±0.1	16	>16
E. coli	NE	8±0.0	9±0.0	10±0.0	20±0.3	>16	>16
S. typhi	NE	8.5±0.0	10±0.0	11±0.0	20±0.2	>16	>16
P. aeruginosa	NE	NE	NE	NE	22±0.0	>16	>16
P. vulgaris	NE	12.5±0.2	14.5±0.5	16.5±0.2	22±0.0	>16	>16
Sh. dysentery	7±0.0	12±0.0	13±0.0	18±0.2	23±0.0	8	8
Sh. flexneri	12±0.1	14±0.0	15±0.2	23±0.3	19±0.0	16	>16
K. pneumoniae	13±0.1	14±0.0	16±0.5	19±0.2	22±0.1	16	16

MIC= Minimal Inhibitory Concentrations, MBC= Minimal Bactericidal Concentrations, NE= No Effect

Table 3. The antifungal activity of *A. sieberi* essential oil against fungi and yeast

	Inhibition Diameter (mm)					minimal inhibitory growth (µl/ml)	
	Essential oil (µl)				Amphotricin B		
	2.5	5	7.5	15	100U	MIC	MFC
A. niger	NE	NE	NE	10±0.0	10±0.0	1	8
A. flavus	11±0.0	13±0.1	15±0.0	18±0.0	9±0.0	1	4
C. albicans	16±0.5	18±0.2	21±0.0	27±0.2	17±0.0	2	2

MIC= Minimal Inhibitory Concentrations, MFC= Minimal Fungicidal Concentrations, NE= No Effect

DISCUSSION

In general, screening of medicinal plants for antimicrobial activities is important for finding potential new compounds for medicinal and industrial purposes. In many cultures, some infectious diseases are known to have been treated with herbal remedies and also some pathogenic bacteria are commonly resistant to many antibiotics. So, interests at antimicrobial properties of extracts from aromatic plants particularly essential oils are very important. The genus of *Artemisia* belongs to the family Asteraceae is presented by 34 species in Iran that are found wild all over Iran (1). Our survey exhibited the main components of *A. sieberi* oil were very variable from different place of Iran (3, 6- 8). Bagheri et al (14) showed that grazing affects on essential oil compositions of *A. sieberi*. The essential oil of *A. sieberi* from our study was rich in β-thujone, α-thujone, camphor and its oil compositions were similar to the oil compositions of the Khorasan type (8).

Negahban et al. (3) suggested that *A. sieberi* oil containing camphor (54.7%), camphone (11.7%), 1,8-cineole (9.9%) may have potential as a fumigant agent against *Callosobruchus maculates*, *Sitophylus oryzae* and *Tribolium castaneum*. Farzaneh et al. (8) demonstrated that *A. sieberi* oil with β-thujone (19.8%), α-thujone (19.5%), and camphor (19.5%) being its major components was slightly effective against *Tiarosporella phaseolina*, *Fusarium moniliforme* and *Fusarium solani* but had high antifungal activity against *Rhizoctonia solani*. Also antifungal and antibacterial activity of β-thujone, α-thujone and camphor were reported in different studies (15,16).

Our results demonstrate that the antimicrobial activity of this oil is dependent on the type of pathogen and the antimicrobial activity of this oil against *B. cereus* and *L. monocytogenes* demonstrated the application of this oil in food industry as preservative and flavoring agents. Also, it can be used in cosmetic and toiletry products such as toothpastes because of antibacterial effect against *S. mutans*. The disc diffusion method and micro broth dilution assays were not completely comparable. Dissolution of *A. sieberi* in aqueous media was different from the agar layer. This oil exhibited a comparably good antimicrobial potential in the present study implying feasibility of its application as an antimicrobial agent but needing further investigation to evaluate the suitability of these antimicrobial properties in practical applications.

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