

Antibacterial Properties and Chemical Composition of the Essential Oil of *Artemisia sieberi* Grown in Kerman, Iran

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ABSTRACT

Bacterial resistance to multiple antibiotics is a major health problem. There is an increasing concern about the occurrence of antibiotic resistant bacteria and consequently interests for the development of new antimicrobial agents are increasing worldwide. *Artemisia sieberi* belongs to the Asteraceae family. The genus *Artemisia* is nearly 400 species. Several species and varieties of the plant grow wild or are cultivated in numerous parts of Asia and Europe. Essential oil of *Artemisia sieberi* was obtained by hydro-distillation of dried aerial parts and characterized by gas chromatography and gas chromatography-mass spectrometry. 8 constituents was characterized and Camphor (31.5%), 1,8-cineole (11.2%) and p-cymene (8.2%) were identified as major components of the essential oil. The oil was evaluated for its antibacterial activity against four pathogenic bacteria such as *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1015), *Salmonella enterica* (PTCC 1709) and *Listeria monocytogenes* (ATCC 7644) using broth dilution method. Minimum bactericidal concentration of the oil on the growth of *S. aureus*, *B. cereus*, *S. enterica* and *L. monocytogenes* were 0.7, 1.5, 6.25 and 1.5% respectively. The essential oil of *Artemisia sieberi* might be replaced by synthetic antimicrobial agents.

Keywords: Antibacterial properties, Chemical composition, *Artemisia sieberi*, Essential oil

INTRODUCTION

Based on investigators estimation, there are 250,000 to 500,000 species of plants on Earth [1]. 1 to 10 percent of these are used as foods by both humans and animal species. It is possible that even more are used for medicinal purposes [2]. Conventional medicine is progressively receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become unsuccessful. Plants are rich in an extensive variety of secondary metabolites, such as tannins [3], terpenoids [4], alkaloids [5], and flavonoids [6], which have been found *in vitro* to have antimicrobial effects. *Artemisia* genus is a large, diverse genus of plants with between 200 to over 400 species belonging to the family of Asteraceae. 29 of them are reported in Iran some are endemic [7]. *Artemisia sieberi* is an aromatic plant which has been widely distributed in the semi-desert regions of Iran [8]. *Artemisia* sp. comprises hardy herbaceous plants and shrubs, which are known for the powerful chemical components in their essential oils. Some constituents from the genus *Artemisia* have been shown anti-malarial [9], anti-viral [10], anti-oxidant [11], anti-spasmodic [12] anti-tumor, anti-

hemorrhagic, anti-coagulant, anti-hepatitis, anti-complementary, anti-ulcerogenic and interferon-inducing activity [13,14]. The aim of this study was to evaluate the antibacterial properties of *Artemisia* essential oil as well as its chemical composition.

MATERIALS AND METHODS

Plant collection and isolation of essential oil

Aerial parts of *Artemisia sieberi* were collected in May 2014 from Hezar Mountain, Kerman, Iran. The collected plant was powdered and hydro-distilled for 4 hours by Clevenger apparatus. The oil was collected and dried over anhydrous sodium sulfate and stored in sealed vial at -4°C until chemical analysis and antibacterial assay [15].

GC-MS analysis

The oil was analyzed using a Hewlett Packard 6890 Gas Chromatograph linked with Hewlett Packard 5973 mass spectrometer system equipped with a HP5-MS capillary column (30 m \times 0.25 mm, film thickness 0.25 μm , Agilent Technologies Wilmington, DE, USA). The oven temperature was programmed from 40°C – 250°C at a rate of $3^{\circ}\text{C}/\text{min}$ and pressure at 16.0 kPa. The ion source was set at 200°C with ionization voltage of 70 eV and interface temperature of 250°C . Helium at a flow rate 1ml/min was used as the carrier gas. Identification of components of oil was based on comparing their spectra and retention index (RI) (determined with reference to the homologous series of n-alkanes $\text{C}_6\text{-C}_{23}$, under identical experimental condition) with those of the Wiley 275 library (Wiley, New York) in the computer library and literature. Percentage composition was calculated using the summation of the peak areas of the total oil composition. Identification of constituents was done on the basis of retention index (RI) (determined with reference to the homologous series of n-alkanes $\text{C}_6\text{-C}_{23}$, under identical experimental condition), MS library search (NIST and WILEY), and by comparison with MS literature data. The relative amounts of individual components were calculated based on GC peak area (FID response) without using the correction factor [16].

Bacterial strains

The bacterial strains were *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1015), *Salmonella enterica* (PTCC 1709) and *Listeria monocytogenes* (ATCC 7644). The inocula of the bacterial strains were prepared from 24 h cultures using Trypticase soy broth (Merck Company). The suspensions were adjusted to 0.5 of the McFarland standard turbidity 1.5×10^8 cells/mL in normal saline [17].

Antibacterial assay

The tube-dilution method was used to determine the minimum bactericidal concentration (MBC) of the essential oil of *Artemisia sieberi* against the bacteria under study. The essential oil was diluted with Trypticase soy broth medium (Merck Company) at the following concentration 50, 25, 12.5, 6.25, 3.1, 1.5, 0.75, 0.3, 0.15 % (v/v). Then 100 μL of bacterial suspension including 1.5×10^8 cells/ml (equal to turbidity of 0.5 Mc Farland solution concentration) was inoculated to each dilution separately and were incubated at 37°C for 24, 48, 72, 96 and 120 hours in agitation conditions by shaker incubator (Labnet, USA) with 150 rpm. After incubation period, from each

dilution was cultured on Muller-Hinton agar medium (Merck Company) and incubated at 37°C for 24 hours [18].

RESULTS AND DISCUSSION

Eight compounds were characterized and identified by GC-MS. The identified compounds were camphor, 1,8-cineole, p-cymene, camphene, thymol, Z-jasmone, davana ether, Z-davanone. The major constituents were Camphor (31.5%), 1, 8-Cineole (11.2%) and p-cymene (8.2%). The *Artemisia sieberi* essential oil showed antibacterial activity against four evaluated pathogenic bacteria including *Staphylococcus aureus* (PTCC 1431), *Bacillus cereus* (PTCC 1015), *Salmonella enterica* (PTCC 1709) and *Listeria monocytogenes* (ATCC 7644) using broth dilution method. Minimum bactericidal concentration of the oil on the growth of *S. aureus*, *B. cereus*, *S. enterica* and *L. monocytogenes* were 0.7, 1.5, 6.25 and 1.5% respectively.

The screening of medicinal plants for determination of antimicrobial properties is important for discovery potential new compounds. The most interesting area of application for essential oil is the inhibition of growth and reduction in numbers of the more serious food borne pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus* in foods [19]. There are several investigations on chemical composition and antimicrobial activity of *Artemisia sieberi* essential oil. The essential oil of *Artemisia sieberi* from our study was rich in Camphor, 1,8- Cineole and p-cymene and the oil compositions were different to the oil compositions in other regions. Some investigators identified chemical composition of *Artemisia sieberi* from south of khorasan province in Iran and reported β -thujone (19.7%), α -thujone (19.5%), Camphor (19.5%) and Verbenol (9.6%) as major components [20]. In similar study, chemical composition and antifungal properties of *Artemisia sieberi* has been investigated. Based on their findings, major components were β -thujone (23%), camphor (19.5%) and α -thujone (15%) and the oil had antifungal activity against *Candida glabrata* [21]. In another study, major components of *Artemisia sieberi* were β -thujone, α -thujone and camphor and *Listeria monocytogenes*, *Bacillus cereus* and *Streptococcus mutans* were more sensitive than other tested bacteria [22]. Analysis of *Artemisia sieberi* essential oil constituents has shown dissimilarities in its components. The presence of certain composition in essential oils determines the specific aroma of plants and the flavor of the condiment. Not only the type of cultivar but also the agronomical practices and environmental situations affect the composition of sensory important compounds [23]. This quantitative and qualitative difference may be due to the geographical, climatic, and soil, which in turn may affect the composition and other secondary metabolites of the plant.

CONCLUSION

The presence of bioactive substances in some medicinal plants can react with microbial pathogens and prevent microbial growth. Therefore, the plant extracts and essential oils are regarded as good candidates for replacing synthetic preservatives. The essential oil of *Artemisia sieberi* might be replaced by synthetic antimicrobials and preservatives in food industry.

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