SUPPLEMENTARY MATERIAL

Chemical Composition and Antimicrobial Activities of *Perovskia artemisioides* Boiss. Essential Oil

S. Hafez Ghoran ^{a*}, B. Azadi ^b and H. Hussain ^c

^a *Department of Chemistry, Faculty of Sciences, Golestan University, Gorgan 4913815759, Iran.*

^b *Phytochemistry Department, Faculty of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.*

^c *UoN Chair of Oman's Medicinal plants and Marine Natural products, University of Nizwa, Nizwa, Sultanate of Oman.*

Abstract

The *Perovskia artemisioides* Boiss. essential oil obtained by hydrodistillation method of flowers wild growing in the north of Iran. The study led to the identification of Twenty-nine compositions by a combination HP-5 GC-FID and GC-MS analytical techniques. The constituents were identified in *P. artemisioides* essential oil with 1,8-Cineole (29.9%), Camphor (29.5%), and α -Pinene (7.8%) as main constituents as well as δ -3-Carene (5.1%), Camphene (3.3%), and β -Pinene (2.7%). The oil was identified by much larger amount of monoterpenes (87.7%) and sesquiterpenes (6.3%). The results of antimicrobial activity exhibit that the extracted essential oil has presented a high inhibiting activity against five microbial strains up to 18 mm. Also the MIC and MBC results displayed that *S. aureus*, *E. coli*, and *S. typhi* were inhibited by *P. artemisioides* essential oil. Therefore determination of essential oils in this research showed a relativity similar pattern to those published for the other species of *Perovskia*.

Keywords: Antimicrobial activity; Camphor; 1,8-Cineole; *E. coli*; Lamiaceae; *Perovskia artemisioides*.

*E-mail Address: S.Hafezghoran@gu.ac.ir and S.Hafezghoran@yahoo.com (Hafez ghoran S). Tel.: +98-9144425047

Experimental

Plant Material

The aerial parts of *P. artemisioides* were collected in full flowering stages in August 2013 from Golestan province (Bioclimatic zone: Humid; Attitude: 174 m), Iran. Voucher specimen (No. 6239) have been deposited in the Herbarium of the faculty of biology (Golestan University, Gorgan, Iran). Plant specimens were identified by Dr. Mohammad Fatemi from that institute. The dried flowering aerial parts for *P. artemisioides* were extracted by hydro-distillation method using Clevenger-type apparatus for 4-5 hours to give the following yield (w/w): 1.52%. Anhydrous sodium sulfate was used to remove water after extraction and the volatile oils stored in refrigerator at 4°C.

Isolation Procedure of Perovskia artemisioides Boiss

FID-GC analyses were measured by an Agilent 7890A gas chromatograph equipped with a split injector (250°C with ratio, 1:50) and 30 m × 0.32 mm film thickness with silica HP-5 capillary column. Column temperature was held at 50°C for 5 min and then temperature program was; ramp at 5°C/min to 150°C, then 10°C/min up to 280°C held for 3 min. The detector (FID) was held at 260°C and the carrier gas was helium (1.5 mL/min). GC-MS was carried out on an Agilent 5975C GC-MS system with a HP-5 fused silica column using the aforementioned chromatographic conditions.

Identification and Characterization of the Volatile Oil constituents

The retention indices (RI) for *P. artemisioides* Boiss. were individually determined by conjunction of the sample with containing the homologous series of C₈ to C₂₆ *n*-alkanes (Van del Dool & Kratz 1963). The constituents of *P. artemisioides* oil were identified by their retention indices compared with data published in the literature (Adams 2001), computer library (NIST/NIH libraries) and Wiley library (Massada 1976). Finally, the percentage of oil component was carried out by peak area normalization measurement.

Antimicrobial activity and determination of MIC and MBC

Antimicrobial activity was assayed against five bacteria by using disc diffusion method (NCCLS 2006). Gram-positive bacteria: *Staphylococcus aureus* (PTCC 1112) and *Bacillus cereus* (PTCC 1247); Gram-negative bacteria: *Salmonella typhi* (PTCC 1639); *Escherichia coli* (PTCC 1330) and *Pseudomonas aeruginosa* (PTCC 1214) were chosen for their pathogenicity and high frequency for which they are able to contaminate food products. Because volatile oils

are not miscible with water, we have used an emulsion-type culture medium using an agar solution (0.2%) explained by Remmal *et al* (1993), according to Mahboudi and kazempour (2009). The incubation is realized at 37°C for 20 - 24 hours. For the comparison of results, Gentamicin and Tetracycline were used as positive controls. Each experiment was carried out in triplicate wells and repeated at least three times. In addition, the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined using a broth dilution method as described Mezni *et al* (2014). Serial dilution values for MIC and MBC were used, ranging from 0.7-25 % (v/v) and 3-50 % (v/v) of the studied substances, respectively. Each test was performed in triplicate.

Table caption;

Table S1. Antimicrobial activity of *Perovskia artemisioides* Boiss. essential oil*.

(Table S1)

Microorganisms	Diameter of inhibition zone (IZ, mm)			MIC (v/v %)	MBC (v/v %)	p-value
	Essential oil	Gentamicin ^a	Tetracycline ^a			
<i>S. aureus</i>	26.14 ± 0.02	17.41 ± 0.04	11.91 ± 0.05	3	6	< 0.001
<i>B. cereus</i>	15.43 ± 0.05	31.12 ± 0.01	26.78 ± 0.03	25	> 50	> 0.05
<i>E. coli</i>	23.74 ± 0.03	19.34 ± 0.07	19.25 ± 0.06	6	12	< 0.001
<i>S. typhi</i>	21.29 ± 0.02	25.16 ± 0.03	20.48 ± 0.02	12	25	< 0.05
<i>P. aeruginosa</i>	18.86 ± 0.01	21.51 ± 0.03	10.67 ± 0.04	25	> 50	> 0.05

* Inhibition zone values are mean ± S.D of three separate experiments (p < 0.05).

^a Gentamicin and Tetracycline were used as positive controls.

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