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Chemical composition and antimicrobial activity of essential oil of *Artemisia fragrans* Willd. in north-west of Iran

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Abstract
The essential oil of *Artemisia fragrans* Willd. (Asteraceae) growing wild in north-west of Iran was examined by GC and GC-MS methods. The yield of total volatiles was 0.9 % (v/w). In all, 36 compounds representing 91.4 % of the oil were identified. The main components of the oil were 1,8-cineole (19.7%), n-octane (12.5%), p-cymene (5.5%) and chrysanthenone (4.9%). Antibacterial activity was tested against Gram-positive and Gram-negative bacteria using the agar diffusion method. Activity was observed against two Gram-positive and one Gram-negative bacteria.

Keywords: *Artemisia fragrans*, essential oil, antibacterial activity, Gram-positive, Gram-negative bacteria.

1. Introduction
The Asteraceae (syn: Compositae) is one of the largest plant families, and more than 28,000 compounds have been identified in chemical studies of this family. The genus *Artemisia*, usually represented by small herbs and shrubs, is one of the important and most widely distributed genera of the Asteraceae. Thirty-four species of this genus are found in Iran, among which two are endemic: *A. melanolepis* and *A. kermanensis* [1]. Members of this genus have botanical and pharmaceutical interest due to their characteristic scent or taste and are used in the liqueur-making industry [2]. Several *Artemisia* species have medicinal importance and are used in traditional medicine for the treatment of a variety of diseases and complaints [3]. *Artemisia* essential oils have been used for various purposes such as flavorings, fragrances, rodent and mite repellents and as folk medicine for antispasmodic, anti-pyretic, anti-inflammatory and abortifacient activities [4]. The essential oils of some *Artemisia* species are also used in soaps, detergents, cosmetics and perfumes, and in aromatherapy [5]. The present paper reports a detailed analysis of the aerial parts oil of *A. fragrans* by GC and GC-MS, and their antibacterial activities.

2. Materials and methods
2.1 Plant material
The aerial parts of *Artemisia fragrans* Willd were collected in July 29, 2014 in Ardabil area at an altitude of 1450 m from North-west Iran. A voucher specimen has been deposited at the Herbarium of the Agriculture Research Centre Ardabil, Iran. The air-dried aerial parts of plant material (150 g) were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus, separately. The oil yield (v/w) on moisture free basis of aerial part was 0.9% (v/w). The oil was dried over anhydrous sodium sulfate and recovered with *n*-hexane, then stored in sealed vials and at low temperature before analysis.

2.2 Gas Chromatographic / Mass Spectral analysis
GC analysis was performed on a Shimadzu 15A Gas Chromatograph equipped with asplit/splitless injector (250 °C) and a flame ionization detector (250 °C). N_2 was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60 °C for 3 min and then heated to 220 °C with a 5 °C/min rate and kept constant at 220 °C for 5 min. Alkanes (C8- C18) were used as reference points in the calculation of relative retention indices (RRI). The relative percentages of the characterized components are given in Table 1. GC/MS analysis was

performed using a Hewlett Packard 5973 with an HP-5 ms column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60 °C for 3 min and programmed to 220 °C at a rate of 5 °C/min and kept constant at 220 °C for 5 min. The flow rate of helium as carrier gas was 1 mL/min. MS were taken at 70 eV. Identification of the constituents of the oils was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and with those of authentic samples [6]. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac, without the use of correction factors.

2.3 Antimicrobial activity

The *in vitro* antibacterial activities of the oils were evaluated by the disc diffusion method using Mueller-Hinton agar for bacteria [7]. Discs containing 15 µL of the oils were used and growth inhibition zones were measured after 24 h of incubation at 37 °C. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the oil that resulted in a complete inhibition of visible growth in the broth which was measured by microdilution broth susceptibility assay recommended by NCCLS [8]. The microorganisms used were: *Staphylococcus aureus*, *Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*.

3. Results & Discussion

The composition of the oil of the aerial parts of *A. fragrans* is given in Table 1. Thirty-six components, which represented about 91.4% of the total composition, were identified. The identified components of the oil consisted of monoterpenes (63.5%), sesquiterpenes (15.7%) and non-terpenoid compound (12.2%). As can be seen in Table 1, 1, 8-cineole (19.7%), *n*-octane (12.5%), *p*-cymene (5.5%) and chrysanthenone (4.9%) are the major components in the oil. The oil of this plant was rich in monoterpenoids constituents. Results obtained in the antibacterial study of the essential oil are shown in Table 2. With the agar disc diffusion assay, growth inhibition was observed with two Gram-positive and one Gram-negative bacteria; the oil was found to be active against Staphylococcus aureus, Enterococcus faecalis and Escherichia coli. Against *E. faecalis*, the oil was found to be more active. Antimicrobial activity of the oil measured by disc diffusion and minimal inhibitory concentration (MIC) methods showed that the oil of A. fragrans was active against most of the tested microorganisms except for Pseudomonas aeruginosa that was resistant to the oil (Table 2).

In the essential oil of A. fragrans was dominated by α-thujone (39.80%), followed by camphor (27.44%), *β*-thujone (13.18%), and eucalyptol (10.25%) [9]. the oils of *A. fragrans and A. austriaca*, growing in Tabriz, camphor (54.92% and 40.59%, respectively) and 1, 8-cineole (11.48% and 27.97%, respectively) [10]. Essential oil obtained from the dried flowering aerial parts of *Artemisia fragrans* Willd. Camphor (46.0%), 1, 8-cineole (23.7%) and camphene (7.9%) were reported as main constituents [11]. The main constituting compounds of the *A. fragrans* essential were camphor, 1, 8-cineole, α-terpinolene, γ-terpinene and carvacrol [12]. Furthermore, 1, 8-cineole (52.1%) and α-thujone (34.8%) were also detected in considerable amounts in the oil of *A. fragrans* collected from Tabriz region of Iran [13].

Table 1: Chemical composition of the essential oil of *Artemisia fragrans*

Compound	RI[a]	%
cis-1-ethyl-3-methylcyclopentane	790	2.9
n-Octane	800	12.5
Tricyclene	927	0.5
α-Pinene	939	1.2
Camphene	954	1.3
3-Methylnonane	968	0.5
1, 2, 4-Trimethylbenzene	1026	3.8
n-Decane	1000	4.5
o-Cymene	1020	0.4
α-Terpinene	1017	0.8
p-Cymene	1025	5.5
1, 8-Cineole	1031	19.7
γ-Terpinene	1060	0.7
1, 5, 5-trimethyl cyclopentadiene	1026	1.1
β-Thujone	1114	1.3
Isophorone	1122	3.9
Chrysanthenone	1128	4.9
trans-Pinocarveol	1139	0.7
Camphor	1146	2.5
4-Terpineol	1177	1.3
ρ-Cymen-8-ol	1183	0.7
α-Terpineol	1189	1.0
n-Dodecane	1200	2.5
Verbenone	1205	1.1
Nordavanone	1231	1.2
Cuminal	1242	0.5
Piperitone	1253	0.7
ρ-Cymen-7-ol	1291	0.6
Piperitenone	1343	1.1
Germacrene-D	1485	1.1
Davana ether	1511	2.1
Spathulenol	1578	3.0
Caryophyllene oxide	1583	1.6
Hexadecane	1600	1.1
α-Cadinol	1654	0.8
Valeranone	1675	2.3

[a] Retention index determined with respect to a series of *n*-alkanes an HP-5 ms column.

Table 2: Antibacterial activity of the essential oil of *Artemisia fragrans*

Microorganisms	Essential oil[a]	
	IZ[b]	MIC[c]
Escherichia coli	9	11
Pseudomonas aeruginosa	NA	NA
Staphylococcus aureus	10	5.5
Enterococcus faecalis	12	15

[a] Essential oil tested at a concentration of 15 µl/disc.
[b] Diameter of inhibition zones including diameter of sterile disc (6 mm).
[c] Minimal inhibitory concentration, values are given as mg/ml.
NA = Not Active.

4. Conclusions

The oil was found to be active against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*. Against *E. faecalis*, the oil was found to be more active. Also the oil had no antibacterial activity on *P. aeruginosa*. The essential oil composition and the observed antimicrobial property showed that the essential oil of plant has a good potential for use in aromatherapy and pharmacy and supports its uses in traditional Medicine.

5. References

1. Mozaffarian V. A dictionary of Iranian Plant Names, Farhang Moaser Publishers, Tehran, Iran, 2007, 56-58.
2. Kordali S, Kotan R, Maui A, Coker A, Ala A, Yildirim A. Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish *Artemisia* absinthium, A. dracunculus, A. santonicum, and A. specigera essential oils. Journal of Agriculture & Food Chemistry. 2005; 53:9452-9458.
3. Burits M, Asres K, Bucar F. The antioxidant activity of the essential oils of Artemisia afra, Artemisia abyssinica and Juniperusprocera. Phytotherapy Research. 2001; 15:103-108.
4. Abu Zarga M, Qausasmeh R, Sabri S, Munsoor M, Abadalla S. Chemical constituents of *Artemisia arborescens* and the effect of aqueous extract on rat isolated smooth muscle. Planta Medica 1995; 61:242-245.
5. Kulkarni RN. Artemisia pallens. In: *Artemisia* Wright CW (Ed.) Taylor Francis, London 2002, 1-260.
6. Adams RP. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy, Allured Publishing Corp. Carol Stream, IL, USA, 2007, 1-804.
7. Baron EJ, Finegold SM. Methods for testing antimicrobial effectiveness. In: Diagnostic Microbiology. Stephanie M (Ed.). Baltimore, Mosby, 1990, 171-194.
8. National Committee for Clinical Laboratory Standards (NCCLS) Performance Standards for Antimicrobial Susceptibility Testing, 9th International Supplement. The Committee, Wayne, 1999, M100-S9.
9. Orhan E, Belhattab R, Şenol FS, Gülpinar AR, Hoşbaş S, Kartal M. Profiling of cholinesterase inhibitory and antioxidant activities of *Artemisia* absinthium, A. herba-alba, A. fragrans, Marrubium vulgare, M. astranicum, Origanum vulgare subsp. glandulossum and essential oil analysis of two Artemisia species. Industrial Crops and Products 2010; 32:566-571.
10. Morteza-Semnani K, Akbarzadeh M, Moshiri K. Essential oil composition of *Artemisia fragrans* Willd from Iran. Flavour and Fragrance Journal. 2005; 20:330-331.
11. Delazar A, Naseri M, Nahar L, Moghadam SB, Esnaashari S, Nazemiyeh H *et al.* GC-MS analysis and antioxidant activities of essential oils of two cultivated *Artemisia* species. Chemistry of Natural Compounds 2007; 43:112-114.
12. Movafeghi A, Djozan Dj, Torbati S. Solid-phase microextraction of volatile organic compounds released from leaves and flowers of *Artemisia fragrans*, followed by GC and GC/MS analysis. Natural Product Research. 2010; 24:1235-1242.
13. Barazandeh MM. Essential oil composition of Artemisia fragrans Willd. from Iran. Journal of Essential Oil Research. 2003; 15:414-415.