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Original Article

Essential oil compositions, antibacterial and antioxidant activities of various populations of *Artemisia chamaemelifolia* at two phenological stages

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ABSTRACT

Artemisia chamaemelifolia Vill., Asteraceae, has been used as an antimicrobial, antifungal, antiparasitic and antitumor. This study determined the variation in chemical composition, and antibacterial and antioxidant activity of *A. chamaemelifolia* collected at two phenological stages within five natural habitats in northern Iran. The highest oil yield was obtained from the Shahkoh population with 1.10 ml/100g dry matter harvested at the 50% flowering stage. The highest values of 1,8-cineole (31.82%) was obtained from the Pelor population at the 50% flowering stage. The highest percentages of artemisia ketone (12.27%), camphor (17.21%), and borneol (13.50%) were obtained from the Kandovan population, harvested before flowering. The highest content of chrysanthenone (18.14%) was obtained from the Gadok population before flowering. The essential oil of the Kandovan population harvested at the 50% flowering stage had the highest percentages of Davanone D (28.44%) and Davanone (28.88%). The *A. chamaemelifolia* oils inhibited the growth of four bacterial pathogens, while these same oils exhibit weak antioxidant (DPPH) activity. The results indicated *A. chamaemelifolia* contained three chemotypes: 1,8-cineole, davanone and/or Davanone D, and chrysanthenone. The antibacterial properties of the essential oils obtained from various populations of *A. chamaemelifolia* at two phenological stages may be significant from a pharmaceutical stand point.

Introduction

The genus *Artemisia* L. (commonly known as wormwood), one of the largest and most widely distributed genus of the family Asteraceae, includes perennial, biennial, and annual herbs plus small shrubs (Watson et al., 2002; Iranshahi et al., 2007). The genus is of special interest because many *Artemisia* species have botanical and pharmaceutical properties, characterized scents and tastes due to the content of monoterpenes and sesquiterpenes. The plants have folk and conventional medicine applications (Mucciarelli et al., 1995; Kordali et al., 2005a,b).

The major classes of phytoconstituents of *Artemisia* species are terpenoids, flavonoids, coumarins, caffeoylquinic acids, and sterols (Bora and Sharma, 2011); making the genus an important source of biological compounds used in insecticides,

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antimalarials, cytotoxins, antihepatotoxic, fungicides, antibacterials, and allelochemicals (Chauhan et al., 2010; Bora and Sharma, 2011). A notably important drug found in this genus is artemisinin, the antimalarial drug isolated from *A. annua* (Bora and Sharma, 2011). Other species of *Artemisia* have also been noted for their potential use at in-depth investigations on biological activities, especially those species that affect the central nervous and cardiovascular systems (Bora and Sharma, 2011).

In Iran, the genus *Artemisia* consists of 34 species of herbaceous annual and perennial plants, two of which are endemic: *A. melanolepis* and *A. kermanensis* (Rechinger, 1963-1998). Species of *Artemisia* have been used in Iranian traditional medicine as astringents, antidiarrheals, antimicrobials, antihelminthics, anti-toxins, for their antiparasitic activity and for the treatment of infectious wounds, indigestion, digestive problems, and for children suffering from constipation, colitis, and abdominal cramps (Schlimmer, 1970; Zargari, 1989; Ghorbani, 2005; Ghasemi Pirbalouti, 2010; Ghasemi Pirbalouti et al., 2013a). Generally, chemical investigations on Iranian species of *Artemisia* have indicated the presence of monoterpenes, sesquiterpenes, and especially sesquiterpene lactones (Rustaiyan and Masoudi, 2011).

Artemisia chamaemelifolia Vill., Asteraceae, known as "Dermane-e-Babonehie" in Persian, is a shrubby aromatic plant, 30 to 50 cm tall, with small, yellow flowers that grows in north and northeast Iran (Rechinger, 1963-1998; Mozaffarian, 2008). The plant is unique, it can exhibit chromosome numbers 8 and 9, compared with most plants in the genus *Artemisia* that have either 8 or 9 chromosomes but not both. Marco et al. (1996) has reported some new tricyclic sesquiterpenes from *A. chamaemelifolia* subsp. *chamaemelifolia*. Trendafilova-Savkova et al. (2003) has identified silphiperfolane sesquiterpene acids in *A. chamaemelifolia*. Generally, *A. chamaemelifolia* has been used as an antimicrobial, antifungal, antitumor, and, as an antihelminthic for children. The species produces alkaloids, saponins, flavonoids, and polyphenols.

Several studies (Letchamo et al., 1995; Tanko et al., 2005; Nejhad Ebrahimi et al., 2008; Ghasemi Pirbalouti et al., 2013b; Ghasemi Pirbalouti et al., 2014) have shown that some medicinal and aromatic plants' genetic characteristics can be affected by ecological factors, including precipitation, temperature, plant competition, harvesting and post-harvest schedules, and nitrogen concentration in the soil. Thus, the essential oil composition of *Artemisia* may be expected to vary with genetic, chemotype, environmental conditions, harvesting time, and geographic origin; including climate, topography, elevation, and edaphic factors.

Knowledge of population diversity is the main prerequisite and the first step in plant breeding and domestication programs (Allard, 1999). Native plant populations are frequently suitable as germplasm for improving plant breeding and domestication programs, as bioclimatic preferences and geographic distances play a major role in plant differentiation (Rahimmalek et al., 2009; Ghasemi Pirbalouti and Mohammadi, 2013; Ghasemi Pirbalouti et al., 2013c). In Iran, *A. chamaemelifolia* grows in the wild in several bioclimatic zones extending from the semi-humid in northern Iran to the semi-arid in northeastern Iran (Rechinger, 1963-1998). To our knowledge, every document on diversity of oil chemical compositions, oil yield, antibacterial and antioxidant activities of various populations and harvest time of *A. chamaemelifolia* are available.

The present study determined the variations in chemical composition, oil yield, antibacterial, and antioxidant activities of populations of *A. chamaemelifolia* collected during vegetative and 50% flowering stages of growth (50% flowers open on a plant or 50% plants in bloom) from various geographical regions of Iran.

Materials and methods

Plants material

Samples of plant tissues collected from wild populations of *A. chamaemelifolia* growing in various regions of northern Iran (Mazandaran and Golestan provinces) were used in this study. A total of three replicate samples from ten individual plants were gathered during the vegetative and 50% flowering stages of growth. The samples, 30 to 50 g of the plant aerial parts (5-10 cm above ground level), were harvested from June 25 to August 15 2012. Selected geographic sites and characteristics of accessions differed (Table 1).

Table 1
Geographical and climate of natural habitats of *Artemisia chamaemelifolia* populations.

Region	Province	Altitude (m a.s.l)	Latitude	Longitude	†P[a]	T[b]	‡pH	E.C.[c]	O.C.[d]	S.T.[e]
Pelor	Mazandaran	2560	35° 52′ N	58° 41′ E	463	7.0	6.36	0.89	1.31	Loam
Kandovan	Mazandaran	3032	36° 14′ N	58° 11′ E	470	7.0	7.66	0.68	2.71	Loam
Gadok	Mazandaran	2340	35° 08′ N	57° 43′ E	286	8.9	7.45	0.78	0.33	Sandy loam
Shahkoh	Golestan	2450	36° 33′ N	54° 33′ E	250	9.2	7.95	2.94	0.87	Sandy loam
Shahvar	Golestan	2240	36° 13′ N	54° 13′ E	250	9.2	7.95	1.79	0.86	Sandy loam

[a]P: Annual precipitation (mm), [b]T: Average temperature (°C), [c]E.C.: electrical conductivity (dS.m^{-1}), [d]O.C.: organic carbon (%), and S.T.: Soil texture.
†Meteorological information was obtained from weather stations located within the study area and the surrounding zone; each value is the mean of 10 to 15 year data.
‡Soil characteristics are based on average of samples taken from three farms in each region.

Each sample was labeled and the location was recorded using a Global Positioning System (GPS, Vista Garmin) receiver. Soil physical and chemical characteristics, including pH, electrical conductivity (EC), percentage of organic carbon (% OC), and soil texture were determined. Climatic conditions of the locations were determined by using collected data from the nearest meteorology station. Plants were identified and authenticated by Prof. V. Mozaffarian, and a representative voucher specimen (No. 1021) was deposited in the Herbarium of Research Center of Natural Resources of Mazandaran, Iran.

Essential oil extraction

The fresh aerial tissue from the *A. chamaemelifolia* samples were air-dried for one week at room temperature (30 ± 5°C), and then ground to a fine powder using a Moulinex food processor and passed through a 20 mesh sieve to remove large pieces of debris. The essential oil was extracted from 30 g of ground tissue in 500 ml of water contained in a 1 liter flask and heated using a heating jacket at 100°C for 3 h in a Clevenger-type apparatus; according to procedures outlined in the British Pharmacopoeia. The collected essential oil was dried over anhydrous sodium sulfate and stored at 4°C until analyzed.

Identification of the oil components

The essential oil composition was determined by GC and GC-MS analysis. The analysis was performed using a gas chromatograph (Agilent Technologies 7890 GC) equipped with a FID detector, using HP-5ms 5% capillary column (30 m x 0.25 mm, 0.25 µm film thicknesses). The carrier gas was helium at a flow of 0.8 ml/min. Initial column temperature was 60°C and was programmed to increase at 4°C/min to 280°C. The split ratio was 40:1 and the injector temperature was set at 300°C. The purity of helium gas was 99.999%. Oil samples (0.1 µl) were injected manually.

The GC-MS analysis was done on the Agilent Technologies 5975 Mass system. Mass spectra were recorded at 70 eV with a mass range from *m/z* 50-550. Retention indices were calculated for all components using a homologous series of *n*-alkanes (C_5-C_{24}) injected under conditions used with the oil samples. Identification of oil components was accomplished based on the comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (Willey/ChemStation data system) (Weyerstahl et al., 1997; Tellez et al., 1999; Weyerstahl et al., 1999; Adams 2007; McLafferty 2009). The percentage composition was computed from the GC peak areas.

Antibacterial test

Clinical isolates of bacteria strains, Gram-positive (Bacillus cereus and Listeria monocytogenes), and Gram-negative (Pseudomonas aeruginosa and Salmonella typhimurium), were obtained from Food Microbiology Laboratory, Veterinary Medicine Faculty (I.A.U.) Iran. The identification of the bacterial strains was confirmed using PCR-RFLP and conventional morphological and biochemical tests. For the antibacterial tests, the density of bacterial cultures required for the test was adjusted to 0.5 McFarland standards (1.0 x 10^7 CFU/ml) and measured using a spectrophotometer (Eppendorf, AG, Germany). The MIC values were evaluated using the broth serial dilution method according to standard methods (Cockerill, 2012). Bacterial strains were cultured overnight at 37°C in MHB.

The essential oils and antimicrobial standard (ampicillin) used in the antimicrobial tests were dissolved in 5% DMSO and diluted to the highest concentration (500 µg/ml) to be tested. Serial two-fold dilutions of the high concentration were made to develop a concentration range from 16 to 500 µg/ml in 10 ml sterile test tubes that contained BPW. After incubation at 37°C for 24 h, bacterial growth was determined by measuring the absorbance at 630 nm using a spectrophotometer (Zampini et al., 2005). To determine the MBC of the essential oils, 5 µl from the MIC tubes were transferred to agar plates and incubated at 37°C for 24 h. The MBC was the minimum concentration of essential oil in which no viable bacteria could be observed. All experiments were replicated three times.

Antioxidant test

The DPPH radical scavenging activity of essential oils was determined using the method proposed by Hung et al. (2005). The essential oils at concentrations of 16 to 500 µg/ml were mixed with an equal volume of 0.2 mM methanol solution of DPPH. The disappearance of the DPPH after 30 min of incubation at room temperature was determined by spectrophotometry at 515 nm in a spectrophotometer zeroed with methanol. The absorbance of the DPPH radical without antioxidant served as the control and was measured daily. The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically and the percentage inhibition was calculated according to the equation:

$$\% \text{ inhibition} = \left[\frac{A_{C(a)} - A_{A(t)}}{A_{A(t)}} \right] * 100$$

where AC(0) is the absorbance of the control at $t = 0$ min and AA(t) is the absorbance of the antioxidant at $t = 30$ min. The food preservative butylhydroxyanisole (BHA) was used as positive control.

Statistical analysis

Data was analyzed as a 5x2 factorial with a completely randomized design and three replicates. Means of the main essential oil constituents, oil yield, and antioxidant activity (IC_{50}) were compared using Duncan's Multiple Range Test at $p \leq 0.05$ level. Analytical data for Hierarchical cluster analysis were treated by means of the SPSS statistical software.

Results and discussion

Essential oil yield

All essential oils extracted from the aerial parts of *A. chamaemelifolia* harvested in pre and 50% flowering growth stage produced a clear, green liquid. The population of *A.*

chamaemelifolia and the harvesting time had significant impacts ($p \leq 0.05$) on the essential oil yield (Fig. 1a). The highest essential oil yields were obtained from the plants collected in the Shahkoh and Shahvar populations (Golestan province) with 0.86 and 0.85 ml/100 g dry matter, respectively (Fig. 1b). The harvesting time also influenced oil content of *A. chamaemelifolia* ($p \leq 0.01$) (Fig. 1c).

The highest oil yield was obtained from the Shahkoh population (1.10 ml/100 g dry matter) harvested at the 50% flowering stage, while the lowest essential oil yields were obtained from the three populations of Mazandran province (0.32 to 0.42 ml/100 g dry matter) harvested during the vegetative growth stage (Fig. 1a) and showed only a relatively small increase (0.63 to 0.73 ml/100 g) at 50% flowering stage. These results are in agreement with a previous study by Morteza-Semnani et al. (2008) in which the oil yield of aerial parts of *A. chamaemelifolia* collected from Nour (Mazandaran), Iran, at flowering stage was 0.70% (v/w). Our results indicated that the Golestan populations had oil yields higher than the Mazandaran populations at both harvesting times.

Differences in oil yield in our study can be partly attributed to the differences in environmental conditions in the two provinces. The climate at Golestan is dryer and warmer than the climate at Mazandaran; conditions that probably provide a better growth, leading to a higher accumulation of oil in the aerial parts of *A. chamaemelifolia*. Simon et al. (1992) reported that water stress increased essential oil accumulation via higher density of oil glands due to the reduction in leaf area.

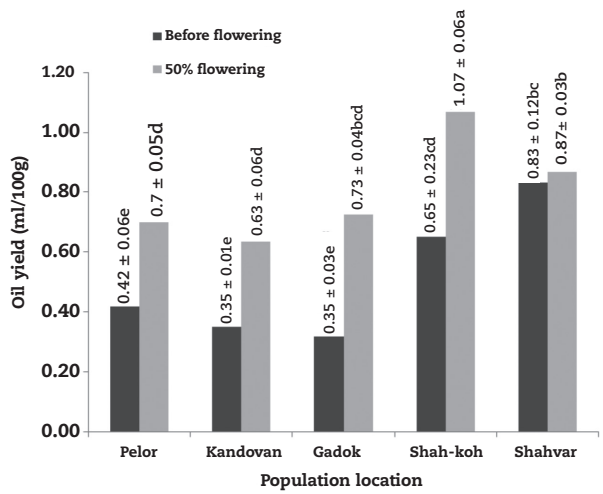

Figure 1 - Interaction effect of population and harvesting time (A), simple effect of population (B) and simple effect of harvesting time (C) on the oil yield of *Artemisia chamaemelifolia* (In each graph, similar letter indicates a non significant effect at $p \leq 0.05$).

Chemical composition of essential oil

Within various populations of *A. chamaemelifolia*, 59 volatile components were identified, representing 82 and 91% of total volatiles for both harvesting times (Table 2). Analysis of the essential oils indicated the some major constituents were 1,8-cineole, artemisia ketone, filifolone, chrysanthenone, camphor, borneol, terpin-4-ol, bornyl acetate, davanone, Davanone D, viridiflorol, and α-bisabolol. In general, the percentages of artemisia ketone, chrysanthenone, camphor, and bornyl acetate in the essential oil extracted from the aerial parts of *A. chamaemelifolia* collected before flowering, were higher than those from plants collected at the 50% flowering stage. While, the highest values of 1,8-cineole, davanone, and (*E*)-davanone in the essential oil of *A. chamaemelifolia* were obtained from plants harvested at the 50% flowering stage.

The highest levels of 1,8-cineole, camphor, terpin-4-ol, bornyl acetate, and borneol were obtained from the Pelor population, while the highest levels of artemisia ketone, davanone, and (*E*)-davanone were obtained from the Kandovan population; also the highest levels of chrysanthenone, viridiflorol, and filifolone were obtained from the Gadok population. The highest level of α-bisabolol was obtained from the Shahvar population. Our results indicated three chemotypes: the 1,8-cineole (Pelor population); the davanone and (*E*)-davanone (Kandovan population); and the chrysanthenone (Gadok population).

Significant differences were observed among the various populations and the two harvesting times, in terms of 1,8-cineole, artemisia ketone, filifolone, chrysanthenone, camphor, borneol, davanone, and (*E*)-davanone ($p \leq 0.01$) and viridiflorol ($p \leq 0.05$) concentrations in *A. chamaemelifolia* oils (Table 2). The highest percentage of 1,8-cineole (31.82%) was observed in the essential oil extracted from the Pelor population at 50% flowering. The highest levels of artemisia ketone (12.27%), camphor (17.21%), and borneol (13.50%) were obtained from the Kandovan population before flowering; while the highest levels of chrysanthenone (18.14%) was obtained from the Gadok population before flowering. Davanone (28.44%), and (*E*)-davanone (28.88%) were the highest in the Kandovan population at 50% flowering; viridiflorol (9.74%) was obtained from the Gadok population at 50% flowering, and α-bisabolol (8.33%) was obtained from the Shahvar population at 50% flowering.

A study by Morteza-Semnani et al. (2008) on chemical composition of the essential oil of the flowering aerial parts of *A. chamaemelifolia* from Nour (North Iran), indicated the major constituents were vulgarone B (38.8%), santolinyl acetate (10.5%), and 14-hydroxy-9-*epi*-pcaryophyllene (8.4%). A comparison of our results with the report by Morteza-Semnani et al. (2008), suggests differences in the volatile composition of the plant material that could be attributed to the geographic origin of the plant and chemotype. A study by Sefidkon et al. (2002) indicated the main constituents of the oils of three *Artemisa* species from north Iran (Semnan) as follows: *A. aucheri*, verbenone (21.5%), camphor (21.0%) 1,8-cineole (8.3%), and *trans*-verbenol (8.1%); *A. santolina*, neryl acetate (13.4%), bornyl acetate (10.9%), (*Z*)-verbenol (9.9%), lavendulol (8.8%), linalool (6.9%), and 1,8-cineole (6.5%); *A. sieberi*, camphor (49.3%), 1,8-cineole (11.1%), and bornyl acetate (5.8%). Results of a study by Morteza-Semnani and Akbarzadeh (2005) demonstrated the main components of *A. scoptaria* oil from northern Iran (Behshar, Mazabdaran) were camphor (37.9%), 1,8-cineole (27.8%), and borneol (21.1%)

Table 2
Effect of different populations and harvesting times on chemical compositions (%) of essential oil of *Artemisia chamaemelifolia*.

S. No.	Components	RI†	Pelor-Veg‡	Kandovan-Veg	Gadok-Veg	Shahkoh-Veg	Shahvar-Veg	Shahkoh-Fl	Shahvar-Fl	Pelor-Fl	Kandovan-Fl	Gadok-Fl
Monoterpenes												
Hydrocarbons												
1	santolinatriene	911	0.00 ± 0.00¶	0.08 ± 0.11	2.07 ± 1.58	1.06 ± 0.23	0.97 ± 0.14	1.59 ± 0.05	1.27 ± 0.20	0.01 ± 0.02	1.28 ± 0.20	0.64 ± 0.18
2	α-thujane	930	0.08 ± 0.07	0.08 ± 0.11	0.04 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.06	0.05 ± 0.05	0.12 ± 0.00	0.00 ± 0.00	0.50 ± 0.08
3	α-pinene	936	1.07 ± 1.13	0.33 ± 0.21	0.24 ± 0.41	0.27 ± 0.06	0.21 ± 0.04	0.16 ± 0.02	0.12 ± 0.02	0.21 ± 0.01	0.45 ± 0.16	0.74 ± 0.21
4	camphene	950	2.13 ± 1.04	2.70 ± 0.80	0.27 ± 0.14	0.73 ± 0.05	0.64 ± 0.17	0.59 ± 0.04	0.38 ± 0.05	1.85 ± 0.07	0.00 ± 0.00	0.15 ± 0.05
5	verbenene	955	0.06 ± 0.10	0.08 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
6	sabinene	975	0.65 ± 0.43	0.10 ± 0.13	3.56 ± 4.50	0.24 ± 0.05	0.26 ± 0.07	2.30 ± 0.14	2.58 ± 0.71	0.96 ± 0.07	1.32 ± 0.12	2.43 ± 0.01
7	β-pinene	979	0.15 ± 0.13	0.10 ± 0.13	0.02 ± 0.04	0.06 ± 0.05	0.06 ± 0.05	0.00 ± 0.00	0.03 ± 0.05	0.13 ± 0.01	0.00 ± 0.00	0.52 ± 0.04
8	α-terpinene	1017	1.18 ± 0.33	0.35 ± 0.04	0.87 ± 0.70	0.25 ± 0.06	0.16 ± 0.01	0.49 ± 0.04	0.35 ± 0.06	1.29 ± 0.04	1.04 ± 0.06	1.84 ± 0.17
9	pseudocumene	1021	0.00 ± 0.00	0.00 ± 0.00	1.30 ± 0.65	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.65 ± 0.04
10	p-cymene	1026	1.45 ± 0.52	1.06 ± 0.86	1.64 ± 1.44	0.16 ± 0.02	0.11 ± 0.11	0.52 ± 0.06	0.38 ± 0.06	1.52 ± 0.10	0.00 ± 0.00	0.85 ± 0.14
11	limonene	1030	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12	γ-terpinene	1057	1.84 ± 0.53	0.45 ± 0.16	1.31 ± 1.14	0.34 ± 0.06	0.24 ± 0.02	0.75 ± 0.06	0.58 ± 0.08	2.05 ± 0.15	0.00 ± 0.00	1.28 ± 0.21
Alcohols												
13	yomogi alcohol	1001	0.31 ± 0.32	1.18 ± 0.23	1.78 ± 0.82	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
14	1-octanol	1068	0.05 ± 0.09	0.00 ± 0.00	0.12 ± 0.20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
15	artemisia alcohol	1081	0.31 ± 0.13	0.95 ± 0.16	0.00 ± 0.00	0.08 ± 0.07	0.05 ± 0.08	0.11 ± 0.10	0.09 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16	α-terpinolene	1087	0.52 ± 0.16	0.17 ± 0.06	2.03 ± 0.55	0.19 ± 0.02	0.10 ± 0.09	0.37 ± 0.02	0.26 ± 0.03	0.50 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
17	*cis*-sabinene hydrate	1097	0.41 ± 0.11	0.00 ± 0.00	0.60 ± 0.34	0.40 ± 0.04	0.45 ± 0.12	0.68 ± 0.02	0.74 ± 0.11	0.86 ± 0.19	0.00 ± 0.00	0.41 ± 0.04
18	linalool	1097	0.60 ± 0.13	0.31 ± 0.01	1.10 ± 1.61	1.38 ± 0.40	1.04 ± 0.25	1.97 ± 0.06	1.91 ± 0.27	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
19	cis-β-terpineol	1137	0.55 ± 0.24	0.00 ± 0.00	0.40 ± 0.52	0.03 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.36 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
20	borneol	1162	11.32 ± 7.95ab	13.50 ± 1.93a*	1.05 ± 0.67c	10.44 ± 1.27ab	3.76 ± 0.50ab	10.78 ± 0.17ab	9.73 ± 1.45ab	6.85 ± 0.40ab	0.40 ± 0.27c	0.58 ± 0.37c
21	terpinen-4-ol	1174	3.97 ± 1.05a	1.36 ± 0.04bc	3.24 ± 2.80ab	0.73 ± 0.14c	0.54 ± 0.03c	1.64 ± 0.06bc	1.26 ± 0.13bc	4.05 ± 0.12a	0.33 ± 0.11c	2.50 ± 0.39bc
22	α-terpineol	1186	0.40 ± 0.11	0.07 ± 0.10	0.26 ± 0.23	0.49 ± 0.27	1.16 ± 0.01	1.49 ± 0.04	0.67 ± 0.09	0.35 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
23	myrtenol	1192	0.53 ± 0.13	0.12 ± 0.17	0.10 ± 0.09	0.33 ± 0.04	0.25 ± 0.03	0.00 ± 0.00	0.02 ± 0.04	0.45 ± 0.01	0.00 ± 0.00	0.21 ± 0.03
24	trans-piperitol	1203	0.04 ± 0.06	0.00 ± 0.00	0.08 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.09	0.04 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
25	trans-carveol	1214	0.03 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
26	unknown alcohol-1	1285	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.04 ± 1.37	18.88 ± 2.81	21.07 ± 0.43	17.90 ± 2.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Oxides												
26	1,8-cineole	1033	21.23 ± 5.49c	8.05 ± 0.29de	1.27 ± 0.31g	5.36 ± 1.22ef	4.67 ± 1.07efg	13.81 ± 0.60c	10.00 ± 1.29d	31.83 ± 2.03a	0.64 ± 0.25g	3.34 ± 27fg
Ketones												
27	*filifolone*	1103	0.12 ± 0.12c	0.00 ± 0.00c	3.71 ± 1.29a	0.00 ± 0.00c	0.00 ± 0.00c	0.34 ± 0.05c	0.26 ± 0.02c	0.00 ± 0.00c	0.00 ± 0.00c	2.08 ± 0.92b
28	isophorone	1119	0.16 ± 0.28	0.00 ± 0.00	1.98 ± 0.32	0.00 ± 0.00	0.00 ± 0.00	0.44 ± 0.01	0.30 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	1.15 ± 0.20

S. No.	Components	RRI	Pelor-Veg	Kandovan-Veg	Gadok-Veg	Shahkoh-Veg	Shahvar-Veg	Shahkoh-Fl	Shahvar-Fl	Pelor-Fl	Kandovan-Fl	Gadok-Fl
Ketones												
29	artemisia ketone	1059	2.27 ± 1.75c	12.27 ± 1.94a	0.36 ± 0.40c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.38 ± 0.15c	0.14 ± 0.19c
30	α-thujone	1115	1.80 ± 1.74	0.15 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.39±0.03	0.00 ± 0.00	0.00 ± 0.00
31	chrysanthenone	1123	0.70 ± 0.45c	0.30 ± 0.10c	18.14 ± 4.25a	0.00 ± 0.00c	0.00 ± 0.00c	2.25 ± 0.16c	1.63 ± 0.31c	0.00 ± 0.00c	0.21 ± 0.09c	6.48 ± 0.96b
32	camphor	1141	8.92 ± 4.84b	17.21 ± 2.75a	1.04 ± 0.47de	5.17 ± 3.53bcd	2.82 ± 0.51de	4.28 ± 0.20cde	2.96 ± 0.37de	7.90 ± 0.37bc	0.40 ± 0.27e	0.78 ± 0.25e
33	pinocarvone	1159	0.14 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.52 ± 0.04
Esters												
34	santolinyl acetate	1168	1.06 ± 0.50	3.52 ± 0.69	0.00 ± 0.00	0.00 ± 0.00	10.67 ± 0.76	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
35	chrysanthenyl acetate	1256	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.91 ± 0.26	2.71 ± 0.14	0.69 ± 0.04	0.70 ± 0.10	0.00 ± 0.00	1.69 ± 0.53	0.13 ± 0.07
36	bornyl acetate	1280	4.70 ± 2.89a	3.02 ± 0.40ab	0.29 ± 0.21cd	3.92 ± 0.28a	3.51 ± 0.74a	0.93 ± 1.62bcd	0.27 ± 0.47cd	2.46 ± 0.12abc	0.46 ± 0.09bcd	0.09 ± 0.13c
Aldehydes												
37	cuminic aldehyde	1234	0.10 ± 0.09	0.00 ± 0.00	1.18 ± 0.54	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00±0.00	0.12 ± 0.00	0.00 ± 0.00	0.54 ± 0.33
Phenol												
39	thymol	1286	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.54 ± 0.18
40	carvacrol	1295	0.82 ± 0.17	0.32 ± 0.01	0.32 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sesquiterpenes												
Hydrocarbons												
41	δ-elemene	1331	0.50 ± 0.01	1.04 ± 0.03	0.38 ± 0.21	0.55 ± 0.09	0.38 ± 0.17	0.00 ± 0.00	0.16 ± 0.28	0.60 ± 0.08	0.78 ± 0.21	2.21 ± 0.22
42	α-copaen	1369	0.29 ± 0.08	0.26 ± 0.01	0.10 ± 0.11	0.29 ± 0.03	0.10 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.14	0.00 ± 0.00	0.51 ± 0.37
43	β-elemene	1385	0.31 ± 0.11	0.43 ± 0.03	1.55 ± 0.23	0.17 ± 0.03	0.11 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.02	0.52 ± 0.11	0.92 ± 0.01
44	safranal	1392	0.00 ± 0.00	0.00 ± 0.00	6.95 ± 2.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
45	*trans*-caryophyllene	1412	0.34 ± 0.37	0.14 ± 0.19	0.08 ± 0.14	0.08 ± 0.07	0.05 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.06	0.00 ± 0.00	1.80 ± 0.02
46	germacrene-D	1474	1.11 ± 0.35	1.57 ± 0.31	0.28 ± 0.18	0.78 ± 0.08	0.78 ± 0.20	0.50 ± 0.01	0.54 ± 0.08	0.33 ± 0.05	0.89 ± 0.19	0.52 ± 0.04
47	bicyclogermacrene	1489	0.39 ± 0.09	0.69 ± 0.97	0.75 ± 0.34	0.34 ± 0.02	0.33 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.05	0.55 ± 0.12	1.79 ± 0.08
Alcohols												
48	eugenol	1350	0.29 ± 0.09	0.18 ± 0.01	0.24 ± 0.07	0.14 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.46 ± 0.26	0.93 ± 0.01
49	unknown alcohol-2	1517	11.60 ± 1.28	8.10 ± 1.23	6.02 ± 6.87	11.67 ± 0.66	11.31 ± 0.89	6.42 ± 0.54	6.44 ± 0.71	11.85 ± 0.69	3.88 ± 2.13	13.50 ± 1.37
50	nerolidol	1557	0.00 ± 0.00	0.00 ± 0.00	0.96 ± 0.52	0.76 ± 0.69	0.44 ± 0.04	1.42 ± 0.12	3.57 ± 0.43	0.00 ± 0.00	0.00 ± 0.00	1.80 ± 0.06
51	spathulenol	1569	1.33 ± 1.20	0.92 ± 1.30	1.96 ± 1.93	1.37 ± 0.28	1.75 ± 0.37	0.49 ± 0.07	1.40 ± 0.32	1.12 ± 0.34	1.39 ± 0.26	3.63 ± 0.14
52	viridiflorol	1590	1.58 ± 1.52b	1.86 ± 2.63b	1.93 ± 3.34b	1.55 ± 0.35b	2.32 ± 0.28b	1.53 ± 0.20b	4.83 ± 0.18b	0.00 ± 0.00b	0.00 ± 0.00b	9.74 ± 0.23a
53	β-eudesmol	1640	2.72 ± 0.88	3.11 ± 0.87	0.56 ± 0.64	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.54 ± 0.15	0.88 ± 0.11	1.57 ± 0.20
54	α-bisabolol	1668	0.46 ± 0.45b	0.00 ± 0.00b	0.72 ± 1.04b	6.64 ± 1.12a	7.98 ± 6.99a	4.48 ± 0.25ab	8.33 ± 0.09a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
55	β-bisabolol	1671	0.10 ± 0.17	0.00 ± 0.00	0.10 ± 0.17	0.28 ± 0.07	0.07 ± 0.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Oxides												
56	caryophyllene oxide	1574	0.33 ± 0.11	0.00 ± 0.00	0.25 ± 0.24	0.22 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.11	0.25 ± 0.05	0.00 ± 0.00	2.71 ± 0.11
Ketones												
57	davana ether	1507	0.00 ± 0.00	1.38 ± 0.85	1.66 ± 0.89	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.64 ± 0.56	5.35 ± 0.88	2.01 ± 0.04
58	davanone D	1564	0.00 ± 0.00c	1.19 ± 1.68c	7.50 ± 4.98b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	28.44 ± 1.04a	4.59 ± 0.65b
59	davanone	1583	0.00 ± 0.00c	3.45 ± 4.87c	8.17 ± 3.95b	1.44 ± 0.45c	2.13 ± 0.63c	0.00 ± 0.00c	0.32 ± 0.56c	0.50 ± 0.87c	28.88 ± 0.56a	3.64 ± 1.20c

†Retention indices (RI) relative to C5-C24 n-alkanes on HP-5MS capillary column.
*Means with different letter in a row are statistically significant at 5% level probability. Values of major compounds are given as means ± SD.
¶% GC peak.
‡ Veg: Vegetative or before flowering, Fl: 50% flowering stage.

The volatile compounds from *A. chamaemelifolia* could be grouped in the following chemical types: 40 monoterpenes (8.6-69.7%), including hydrocarbons (2.6-11.4%), alcohols (0.7-36.1%), oxides (0.6-31.8%), ketones (1.0-29.9%), esters (0.3-16.9%), aldehydes (0.0-1.2%), and phenols (0.0-0.8%); and 19 sesquiterpenes (14.9-72.0%), including hydrocarbons (0.5-10.1%), alcohols (6.6-31.2%), oxides (0.0-2.7%), and ketones (0.0-62.7%). A study by Morteza-Semnani et al. (2008) on the chemical composition of the flowering aerial parts of *A. chamaemelifolia* indicated the essential oil of the plant had sixteen monoterpenoids (26.3%), twenty sesquiterpenoids (65.1%), and thirteen non-terpenoids (3.7%).

Hierarchical cluster analysis

A hierarchical cluster analysis of the percentages of the main compounds in *A. chamaemelifolia* essential oils grouped populations into three distinctive clusters (Fig. 2). The first cluster was formed by oils from eight samples, including the three populations of *A. chamaemelifolia* collected at pre and 50% flowering from Mazandaran province, and two populations of the plant harvested at vegetative stage from Golestan province. The second cluster was formed by the essential oil from the Shahkoh population collected at 50% flowering. The third cluster was formed by the essential oil from the Shahvar population collected at 50% flowering. All clusters were clearly distinguished in quality and quantity of oil from the various populations of *A. chamaemelifolia*. Generally, the oil from the three chemotypes of *A. chamaemelifolia* included 1,8-cineole, davanone and/or (E)-davanone, and chrysanthenone. Similarly, results of a study by Perez-Alonso et al. (2003) indicated the presence of two chemotypes in *A. pedemontana*: camphor, 1,8-cineole and davanone. Earlier research showed that bornane derivatives (camphor, borneol and bornyl acetate), and 1,8-cineole are major characteristic components of many species of *Artemisia*, such as: *A. annua, A. vulgaris, A. diffusa, A. santonicum, A. spicigera, A. afra, A. asiatica, A. austriaca, A. pedemontana, A. cana, A. longifolia, A. frigida*, and *A. ludovician* (Perez-Alonso et al., 2003; Kordali et al., 2005a,b; Lopes-Lutz et al., 2008).

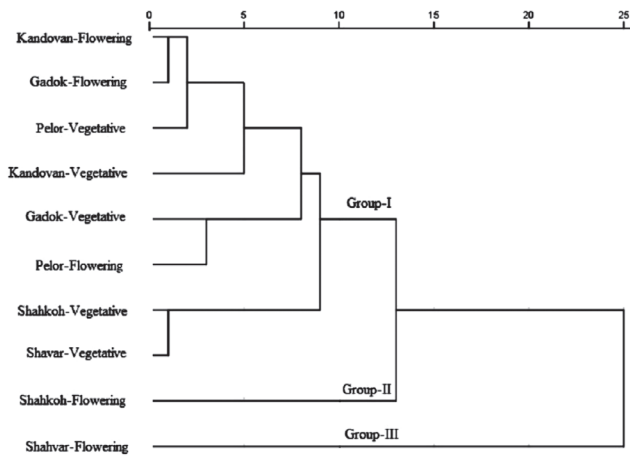

Figure 2 - Dendrogram obtained by hierarchical cluster analysis (HCA), based on the main compositions of the essential oils of *Artemisia chamaemelifolia*.

Antibacterial test

The antibacterial activity of the oils had varying degrees of inhibition against the tested microorganisms. Essential oils from the various populations of *A. chamaemelifolia* collected at the vegetative and 50% flowering growth stage demonstrated relatively high inhibitory activities against the four bacteria tested (Table 3). The MIC of the essential oils were within concentration ranges from 31 to 125 µg/ml, and the respective MBC were from 62 to 500 µg/ml. Generally, the antibacterial activity of the essential oils of the Shahkoh and Shahvar populations (Golestan province) harvested at 50% flowering was higher than samples collected before flowering. In addition, the antibacterial activity of the essential oils of the three populations from Mazandaran province collected at the vegetative growth stage was higher than that of plants harvested at 50% flowering. The essential oils of some populations with the highest percentage of monoterpenes had relatively higher inhibitory activity against bacteria, than the essential oils from the other populations.

The antimicrobial activity of the essential oils can be explained by the lipophilic nature of the monoterpenes contained in the oils (Cristani et al., 2007). Monoterpenes act by disrupting the microbial cytoplasmic membrane, which causes a loss in the membrane's impermeability to protons and larger ions. If the membrane integrity is disturbed, then the membrane properties of barrier, matrix for enzymes and energy transducer are compromised (Turina Adel et al., 2006; Gill and Holley, 2006). Cristani et al. (2007), supposing that the antimicrobial effect of monoterpenes is influenced by the net surface charge of microbial cell membrane. Attributing the activity of a complex mixture to a single or particular constituent, however, is difficult. Major or trace compounds in the oil might give rise to the antimicrobial activity exhibited. Possible synergistic and antagonistic effects of compounds in the oil are needed to be taken into consideration (Lopes-Lutz et al., 2008).

Antioxidant test

Free radicals cause auto-oxidation of unsaturated lipids in food (Kaur and Perkins, 1991), and the antioxidant activity of essential oils could be attributed to their hydrogen donating ability. Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods and biological systems. The potential antioxidant activity of the essential oils was determined by the scavenging activity of the stable free radical DPPH. In our study the antioxidant activity of essential oils of the various populations of *A. chamaemelifolia* collected at vegetative and 50% flowering stage was expressed as IC_{50} with a low IC_{50} value indicating the oil acts as a weak DPPH scavenger (Fig. 3). IC_{50} values were between 310.1 ± 11.9 to 809.8 ± 42.2 µg/ml for *A. chamaemelifolia* essential oils and 76.2 ± 11.3 µg/ml for BHA (positive control). Generally, the antioxidant activity of the essential oils of various population of *A. chamaemelifolia* harvested before flowering was higher than that of plants collected at 50% flowering (Fig. 3). A study by Lopes-Lutz et al. (2008) indicated that essential oils of seven *Artemisia* species exhibited weak antioxidant abilities for preventing the linoleic acid oxidation and reduction of DPPH radicals; while phenolic compounds, such as thymol and carvacrol present in oregano oil and BHA show potent antioxidant and DPPH radical scavenging activities.

Table 3
Effect of different populations and harvesting times on antibacterial activity of essential oil of *A. chamaemelifolia*.

Pathogens	Pelor-Veg		Kandovan-Veg		Gadok-Veg		Shahkoh-Veg		Shahvar-Veg		Shahkoh-Fl		Shahvar-Fl		Pelor-Fl		Kandovan-Fl		Gadok-Fl		Am
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
B. cereus	31	125	31	125	62	125	125	250	125	500	62	250	62	125	125	250	62	250	62	250	62
L. monocytogenes	62	125	31	62	62	250	62	125	31	62	31	62	31	62	31	125	125	250	31	125	62
P. aeruginosa	62	250	62	125	125	250	125	500	62	250	31	125	62	250	125	500	125	250	125	500	62
S. typhimurium	62	125	125	250	125	250	62	250	62	250	31	125	62	125	125	250	62	125	125	500	125

† Am: Ampicillin.
The essential oils and antimicrobial standard (ampicillin) were dissolved in 5% DMSO.
DMSO as negative control had not antibacterial activity.

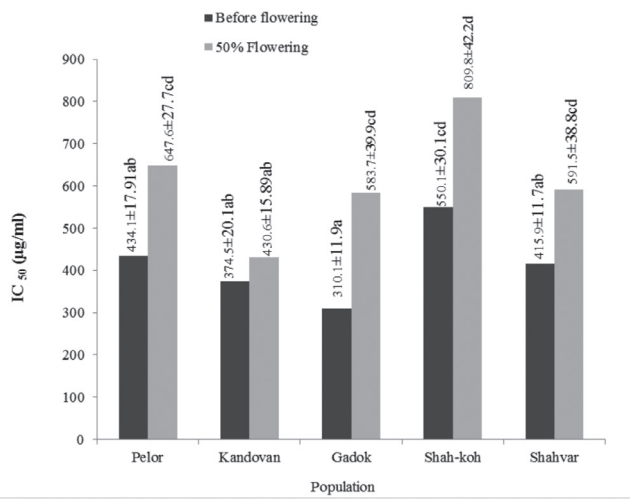

Figure 3 - Effect of harvesting time on various populations over antioxidant activity of the essential oil of *Artemisia chamaemelifolia* using DPPH assay
The essential oils at concentrations of 16-500 µg/ml were mixed with an equal volume of methanol solution of DPPH. The disappearance of the DPPH was determined by spectrophotometry at 515 nm. The absorbance of the DPPH radical without antioxidant served as control.

Conclusion

This study characterized and provided data on the antibacterial and antioxidant activity of the essential oils from populations of *A. chamaemelifolia* at two phenological stages. The main constituents of the essential oils of *A. chamaemelifolia* included 1,8-cineole, artemisia ketone, chrysanthenone, camphor, borneol, davanone, (*E*)-davanone, viridiflorol, and α–bisabolol. The essential oils showed antibacterial activity comparable with a standard antibiotic (ampicillin), but exhibited only weak antioxidant activity, failing to reduce DPPH radicals. The antibacterial activity of the essential oil is economically important considering the availability of herbal material for the production of natural antibacterial products and for its use in traditional medicine.

Authors contributions

AGP designed the study, supervised the laboratory work, contributed to chromatographic analysis, analysis of the data, and drafted the paper. MF (MSc student) contributed in collecting plant samples and running the laboratory work. LC contributed to critical reading of the manuscript. MA contributed in identification and confection of herbarium. All the authors have read the final manuscript and approved for submission.

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