Seasonal variation in the chemical composition, antioxidant activity, and total phenolic content of *Artemisia absinthium* essential oils

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ABSTRACT

Background: The genus *Artemisia* belonging to the Compositae (*Asteraceae*) family and many traditional uses from the *Artemisia* species were reported. *Artemisia absinthium* is one of the species in this genus and commonly used in the food industry in the preparation of aperitifs, bitters, and spirits. **Objective:** Evaluation of the effect of different harvesting stages on the composition of essential oil and antioxidant capacity of *A. absinthium*. **Materials and Methods:** Essential oils from the aerial parts of *A. absinthium*, collected in three stages (preflowering, flowering, and after-flowering) from plants grown in the North Khorasan province of Iran were obtained by steam distillation and the chemical composition of the oils was analyzed by gas chromatography-mass spectrometry and antioxidant activity and total phenolic content were determined by 2-diphenyl-1-picrylhydrazyl assay and Folin-Ciocalteu method. **Results:** Analysis of the isolated oils revealed the presence of 44 compounds, mainly alpha-pinene, sabinene, beta-pinene, alpha-phellandrene, p-cymene and chamazulene. Alpha-phellandrene, and chamazulene were major compounds in preflowering stage, but beta-pinene and alpha-phellandrene were major in flowering and past-flowering stages. Flowering stage had highest yield and after flowering stage had lowest yield. The essential oil of preflowering stage had the highest amount of antioxidant compound (chamazulene). Preflowering stage with highest amount of phenolic compounds had the strongest antioxidant activity with the lowest amount of EC_{50}. **Conclusion:** This study showed that the harvesting stage had significant effects on chemical composition and antioxidant properties of essential oils, and chamazulene is main compound for antioxidant activity in *A. absinthium*.

Key words: Antioxidant activity, *Artemisia absinthium,* Harvesting stage, Total phenolic content

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INTRODUCTION

The genus *Artemisia* is among of the largest and widely family in the *Asteracea* family.[1] Many traditional uses from the *Artemisia* species were reported as antipyretic, antiseptic antimalarial, antiviral, antitumor, antihemorrhagic, anticoagulant, antianginal, antioxidant, antihepatitis, antiulcerogenic, antispasmodic, anticomplementary.[2] There are some studies on the essential oils of different species of *Artemisia*, especially on those used for antispasmodic, antipyretic, anti-inflammatory activities.[3,4] And these essential oils possess an antiparasitic efficiency.[5] *Artemisia absinthium* is an aromatic plant of the family *Asteraceae*, subfamily *Asteroideae* and distributed in Europe and Asia.[2] *A. absinthium* is commonly used in food industry in the preparation of aperitifs, bitters, and spirits.[6] The essential oil of this plant has been the subject of previous investigations.[7] *A. absinthium* commonly is used for its diuretic and antiseptic properties and the treatment of nematode infection.[8] Aerial parts of *A. absinthium* are an easily accessible source of natural antioxidants and antidepressant.[9] The *A. absinthium* oil has insecticidal properties.[10] Many studies have been reported antioxidant, cytotoxic, antimalarial, anti-protozoal, antipyretic, antimicrobial, anthelmintic, and antidepressant activities

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of *A. absinthium*.[9,11-13] *Artemisia* species were divided into two sub-groups with regard to oil composition; one group was characterized by the presence of camphor and 1.8-cineole and the second group contained mostly a-thujone.[8] Thujone is a natural constituent of *Artemisia* species. Thujone was said to be harmful to public health.[14]

The composition of *A. absinthium* varies from country to country with respect to the soil composition on which it is grown, and the content of beta-thujone often exceeds that of alpha-thujone depending on the plant source.[15-17] Due to various usages of *Artemisia* species or their oils, we were interested in studying essential oil contents and compositions of *A. absinthium* in Iran and so far, several articles were published dealing with the essential oil of *A. absinthium* but there is no report on the effect of harvesting stages on the antioxidant activity and total phenolic contents of essential oil of *A. absinthium*, so in the present work, we have investigated the essential oil composition of and antioxidant activities of the isolated essential oils of *A. absinthium* collected from Iran in different seasons.

MATERIALS AND METHODS

Materials

N-hexanol (99.6%, Merck) was used as the internal standard for the gas chromatography-flame ionization detector (GC-FID) calibration analysis. Pure alpha-pinene (147524, 98% Aldrich), sabinene (275166, 99% Aldrich), beta-pinene (80609, analytical standard [Fluka]), p-cymene (30039, analytical standard [Fluka]), and chamazulene (91595, analytical standard [Fluka]) were used as the standards as the five important components of the *A. absinthium* essential oil. Two, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu (FC) reagent, sodium carbonates anhydrous, gallic acid, and ascorbic acid were purchased from Sigma-Aldrich GmbH (Sternheim, Germany). High-performance liquid chromatography-grade methanol was supplied by Merck (Darmstadt, Germany).

Plant materials

The aerial parts of *A. absinthium* were collected in May 2013 (preflowering stage), July 2013 (flowering stage) and November 2013 (after flowering stage) from North Khorasan in Iran. The plant was identified by the Research Center of Natural Products Health (NPH), North Khorasan University of Medical Sciences (Iran). The voucher specimen has been deposited at the herbarium of the NPH (No: Maximum parsimony-27). The aerial parts were air-dried at room temperature in the shade and following the extraction procedures the aerial parts were finely grinded using laboratory equipment and the dried samples were kept within sealed bag in the cold and dry place until they were used.[18]

Hydrodistillation

The plant (80 g of dried material) was submitted to hydrodistillation for 3 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia (1975). The volatile distillate was collected over anhydrous sodium sulfate and refrigerated prior to analysis.[19]

Gas chromatography-flame ionization detector analysis

Gas chromatography analysis was carried out using a Shimadzu Technologies, GC-2010 plus FID chromatograph with a data base-5 column (30 m × 0.25 mm; 0.25 μm film thickness). Helium was used as the carrier gas with flow rate 0.9 mL/min and a split ratio of 1:20. A sample injection volume of 2 μL in each analysis and the internal standard method was used to obtain the highest possible precision for quantitative GC measurements. The amounts of alpha-pinene, beta-pinene, alpha-phellandrene, p-cymene, sabinene, and chamazulene quantified by calculating the area under the chromatographic peaks divided by the area of n-hexanol as an internal standard (A_s/A_{is}). In order to obtain the calibration curves, several solutions with different concentrations of these compounds in ethanol were injected into the GC-FID and the area under each peak was calculated, and the results were precisely obtained.[20] The five linear calibration curves were fitted using a linear regression line with $R^2 \geq 0.98$. Finally, using the calibration curves, the extraction yield (Y) was determined using Eq. (1).

$$Y = \frac{\text{Total mass of four components in } \textit{Artemisia absinthium} \text{ by Clevenger method}}{\text{Total mass of dried aerial parts of } \textit{Artemisia absinthium}} \quad (1)$$

Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry (MS) analysis was carried out using a Shimadzu-QP2010SE 15A operating at 70 eV ionization energy, equipped with a Rtx-5MS (phenyl methyl siloxane 30 m × 0.25 mm, 0.25 μm film thicknesses) with He as the carrier gas, flow rate 0.9 mL/min and a split ratio of 1:20. Acquisition mass range was 35–300 and scan time was 0.5 s/scan. Retention indices were determined using retention times of n-alkanes that had been injected after the oil under the same chromatographic conditions. The retention indices for all components were determined according to the Van Den Dool method using n-alkanes as standard.[21] The compounds were identified by comparison of retention indices (retention indices, repeats-in-toxin-5 MS) with those reported in the literature and by comparison of their mass spectra with the Wiley and Nist libraries or with the published mass spectra.[22,23]

2-diphenyl-1-picrylhydrazyl: Free radical scavenging assay

The antioxidant activity of the essential oils was measured on the basis of the scavenging activity of the stable radical DPPH according to the method of Wang.[24] The essential oils at different concentration range (20–100 μg) were mixed in the freshly prepared 0.5 mM DPPH in ethanol and 0.1 M acetate buffer (pH 5). Absorbance at 517 nm was determined after 30 min. The scavenging activity was calculated using Eq. 2.

$$\% \text{ DPPH scavenging activity} = \frac{\left(\left[\begin{array}{l}\text{A 517 of control}\\ -\text{A 517 of sample}\end{array}\right]\right)}{\left(\text{A 517 of control}\right)} \quad (2)$$

The percentage of scavenging activity was plotted against the sample concentration to obtain effective concentration (EC_{50}) defined as the concentration of sample necessary to scavenge 50% of the DPPH radicals and it was calculated using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego CA, USA). Butylated hydroxytoluene and ascorbic acid were used as reference antioxidants.

Determination of total phenolic content

The total phenolic content in the essential oils was determined using FC phenol reagent method.[25] Briefly, 100 μL extract (1000 mg/L) was added with diluted FC reagent (1/10, 500 μL). After 1-min of reaction, sodium carbonate (Na_2CO_3) (20%, 1.5 mL) was added to each tube, then tubes were vortexed and incubated for 120 min at room temperature. The absorbance was read at 760 nm using an ultraviolet-visible spectrophotometer. The analysis was performed in triplicates. The standard curve was prepared using 50–500 mg/L solutions of gallic acid in methanol. Total phenol values were expressed as gallic acid equivalents (mg GA/g of dry material), which is a common reference compound for phenolic compounds.

RESULTS

The highest yield of the oil was obtained from the flowering stage (E2) (0.685%) and in preflowering stage (E1), the essential oil yield decreased, a lower yield was obtained from after-flowering stage (E3) as shown in Table 1. The data obtained from the essential oils are shown in Table 2 and the main compounds in essential oils were alpha-pinene, beta-pinene, alpha-phellandrene, p-cymene, sabinene, and chamazulene, their chemical structures are shown in Figure 1. Standard curve for determination of phenolic compounds was: $Y = 0.003X + 0.068$ ($R^2 = 0.98$). Table 1 shows total phenolic content in terms of gallic acid equivalents. The radical scavenging effects of the essential oils were concentration dependent, and all samples with the highest concentration had strong scavenging effect, as shown in Figure 2. Results were reported as EC_{50}, which is defined as the amount of antioxidant required to inhibit 50% of DPPH free radicals. EC_{50} values of essential oils are shown in Table 1.

Table 1: Yield and DPPH EC_{50} radical scavenging activity and total phenolic contents of essential oils of aerial parts of *Artemisia absinthium*

Stages	Observed yield (w/w %)	Total phenoli content (mg GAE/g DW) (%)	EC_{50} (mg/mL)
Preflowering	0.603	48.49	3.307
Flowering stage	0.685	47.89	4.110
After-flowering	0.565	31.73	4.269
BHT			0.02
Ascorbic acid			0.018

DPPH: 2-diphenyl-1-picrylhydrazyl, EC_{50}: Effective concentration, GAE: Gallic acid equivalent, BHT: Butylated hydroxytoluene

DISCUSSION

Effects of seasonal variation on extraction yield and chemical compositions

Extraction yield

The highest yield of the oil was obtained from the flowering stage (E2) (0.685%) as shown in Table 1. Our results are in agreement with other researches, for example, *Artemisia annua* L. and *Mentha spicata* exhibited maximum essential oils yield during flowering stage.[26,27]

Chemical composition of essential oils

In E1, hydrocarbon monoterpenes formed the most abundant portion of the oil (54.43%). In E2, the main components were hydrocarbon monoterpenes (53.22% of the total oil). Hydrocarbon monoterpenes (58.23%) were the major part of E3. Sesquiterpene compounds were major in E1. As shown in Table 2, the main compounds identified from different seasonal phases were almost the same but the amounts of the corresponding components were different and in all samples, the highest contents were hydrocarbon monoterpenes while the lower contents were oxygenated sesquiterpenes and 32 compositions were same in three stages. Main compounds in essential oils from three stages were alpha-pinene (5.88%, 5.35%, and 3.23%), sabinene (8.74%, 7.85%, and 0%), beta-pinene (12.29%, 16.81%, and 31.87%), alpha-phellandrene (16.4%, 12.87%, and 9.76%), p-cymene (7.05%, 6.05%, and 8.18%), and sesquiterpene chamazulene (13.88%, 5.4%, and 4.18%).

The major components of essential oils in *Artemisia* species in Turkey were reported as camphor and 1.8-cineole.[15] In the leaf oil of *A. absinthium* collected from Ardabil, 1,8-cineole, borneol, and camphor were the major

Table 2: The effect of seasonal variation in chemical composition of the essential oils of *Artemisia absinthium*

Compound	RI	Chemical constituents(%) of the essential oil in preflowering stage (E1)	Chemical constituents(%) of the essential oil in preflowering stage (E1)	Chemical constituents(%) of the essential oil in after-flowering stage (E3)
Alpha-thujene	927	0.21	0.21	0.58
Alpha-pinene	933	5.88	5.88	3.23
Camphene	950	-	-	0.15
Sabinene	974	8.74	8.74	-
Beta-pinene	976	12.29	12.29	31.87
Beta-myrcene	993	0.71	0.71	0.85
Alpha-phellandrene	1005	16.4	16.4	9.76
Alpha-terpinene	1018	0.23	0.23	0.29
P-cymene	1026	7.05	7.05	8.18
Limonene	1030	-	-	1.49
Beta-phellandrene	1033	0.75	0.75	-
1, 8-cineole	1036	0.16	0.16	0.31
Trans-beta-ocimene	1051	0.17	0.17	0.35
Gamma-terpinene	1062	0.38	0.38	0.1
Trans-sabinene hydrate	1073	0.26	0.26	0.46
Alpha-terpinolene	1089	0.14	0.14	0.3
Linalool	1105	0.61	0.61	1.93
Beta-thujone	1119	-	-	2.33
Trans-pinocarveol	1139	0.3	0.3	0.98
Terpinene-4-ol	1186	0.42	0.42	1.42
Alpha-terpineol	1189	0.11	0.11	0.11
Nerol	1226	-	-	0.18
Myrtenal	1233	-	-	0.7
Cuminal	1239	0.26	0.26	0.16
Citral	1241	0.35	0.35	0.49
Thujone-3-ol	1289	0.27	0.27	0.4
Thymol	1294	0.42	0.42	0.2
Carvacrol	1298	0.48	0.48	0.44
Eugenol	1365	-	-	0.12
Alpha-copaene	1378	0.19	0.19	0.37
Beta-bourbonene	1384	0.12	0.12	1.26
Alpha-cedrene	1410	0.15	0.15	0.32
Beta-caryophyllene	1418	0.25	0.25	0.15
Humulene	1458	0.87	0.87	0.15
Alpha-curcumene	1470	-	-	0.14
Germacrene-D	1480	2.14	2.14	2.07
Beta-selinene	1487	0.78	0.78	1.24
Delta-cadinene	1529	0.64	0.64	1.81
Germacrene-B	1562	0.41	0.41	0.13
Spathulenol	1577	-	-	0.6
Viridiflorol	1596	0.34	0.34	0.46
Chamazulene	1716	13.88	13.88	4.18
Phytane	1795	0.87	0.87	-
Trans-verbenol	1148	-	-	0.1
Total		77.12	77.12	80.36
Monoterpene hydrocarbons		54.43	54.43	58.23
Oxygenated monoterpenes		2.92	2.92	8.3
Sesquiterpene hydrocarbons		19.43	19.43	11.82
Oxygenated sesquiterpenes		0.34	0.34	1.06

RI=Retention indices

components in this oil.[28] In another research on essential oils of *A. absinthium* L., major constituents were thujone and trans-sabinyl acetate.[29] In another investigation on the chemical composition of essential oil from the leaves of *A. absinthium* collected from the region of Guigou and Errachidia, α-Thujone, sabinyl acetate, and β-thujone were the major components in this oil.[30] Thujone is probably the most important biologically active compound of absinthe.[31] But in this study, this compound wasn't main and major compound. There was alpha-thujene, and there wasn't beta-thujene in E1 and beta-thujene was higher in E2.

Seasonal variation effects on antioxidant activity and total phenolic content

Total phenolic contents

The quantitative determination of phenolic compounds was done with FC method.[32] The essential oil that displayed the highest concentration of total phenols was the E1 (48.49 mg

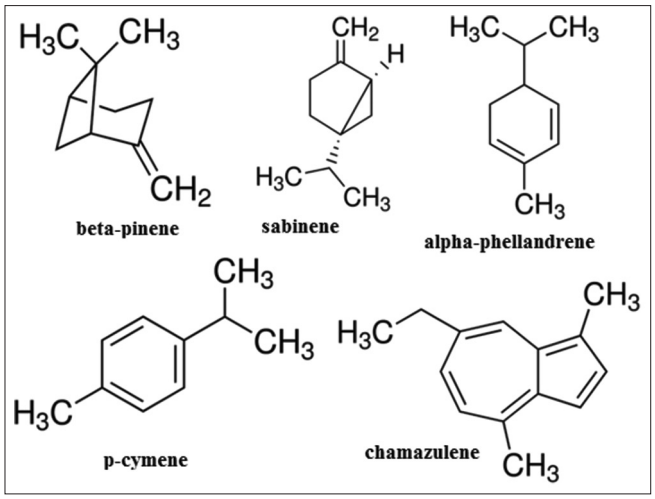

Figure 1: Chemical structures of the main compounds of *Artemisia absinthium*

GAE/g dry weight [DW]) and the essential oil that showed the lowest concentration of total phenols was the E3 (31.73 mg GAE/g DW). The difference was due to growing times and changes of seasons. The phenolic content of some *Artemisia* has already been determined, such as the total phenol contents of the leaf oil of *A. absinthium* was determined to be 168.67 ± 9.50 μg gallic acid equivalent/mg sample.[28]

2-diphenyl-1-picrylhydrazyl radical scavenging activity

E1 exhibited the highest radical scavenging potential with lowest amount of EC_{50} (EC_{50} = 3.307 mg/mL) followed by E2 (EC_{50} = 4.11 mg/mL) and E3 (EC_{50} = 4.26 mg/mL). Because E1 had highest amount of phenolic content as shown in Table 1, and this essential oil had highest radical scavenging potential. Our results are agreement with other studies, for example, the high contents of total phenolic compounds and total flavonoids in *Artemisia* extracts indicated that these compounds contribute to the antiradical and antioxidative activity.[11] In another research, the leaf essential oil of *A. absinthium* reduced the concentration of DPPH free radical (61.4 ± 1.4%, 10 mg/ml of essential oil).[28]

Effect of chemical composition on radical scavenging activity

Five compounds sabinene, beta-pinene, alpha-phellandrene, p-cymene, and chamazulene were major compounds in essential oil of *A. absinthium* that constituted 58.36% in E1, 48.98% in E2, 53.99% in E3. Among identified compounds, chamazulene and alpha-phellandrene were dominate compounds in E1, alpha-phellandrene is a hydrocarbon monoterpene and chamazulene is a sesquiterpene hydrocarbon and this compound in chamomile known to have anti-inflammatory and antioxidant properties because this compound has conjugated structure,[33,34] and good antioxidant activity in E1 can attribute to this compound.

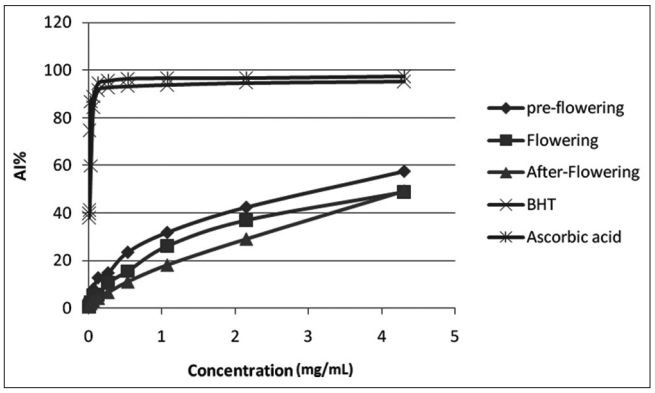

Figure 2: Radical scavenging activity of essential oils of *Artemisia absinthium* with seasonal variation

Sabinene was higher in E1, and there wasn't in E3, in some studies determined that sabinene is a good antioxidant compound.[35] p-cymene that was higher in E3 was able to reduce nociceptive behavior and p-cymene had a stronger antinociceptive effect but exhibited a poor antioxidant potential.[36] The antioxidant activity of the essential oil could be attributed to chamazulene, alpha-phellandrene, and sabinene that they were major in the essential oil of preflowering stage.

CONCLUSION

The results of antioxidant activities and chemical composition of the oils are compatible with phenolic compounds in essential oil and the relations between antioxidant activities and total phenolic obtained from various essential oils suggest close correlations and this study has shown that some compounds such as chamazulene, sabinene, and alpha-phellandrene were responsible for antioxidant activity in essential oil of preflowering stage.

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Conflict of interest

There are no conflict of interest.

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