



Essential oil components, phenolic content and antioxidant activity of *Anthriscus cerefolium* and *Anthriscus sylvestris* from Iran

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ABSTRACT

Purpose: The Apiaceae family (Umbelliferae) is one of the largest families of flowering plants. The genus *Anthriscus* of this family is considered of high importance because of its use in folk medicines and flavoring throughout the world. Three species of this genus are represented in the Flora of Iran. The main objective of this study was to evaluate the essential oil composition, phenolic content and antioxidant activity of *Anthriscus cerefolium* and *Anthriscus sylvestris*. **Research Method:** The essential oil samples were isolated by hydrodistillation in a Clevenger type apparatus and analyzed using GC and GC-MS methods. The antioxidant activity and total phenolic content were determined by DPPH scavenging assay and Folin-Ciocalteu method, respectively. **Findings:** Oxygenated monoterpenes constituted the principal fraction of essential oils obtained from *A. cerefolium* (rich in estragole), while aliphatic esters were detected to be the main class of compounds isolated from *A. sylvestris* (rich in chrysanthenyl acetate). Among the essential oils and methanolic extracts from two *Anthriscus* species at vegetative stage the highest antioxidant activity was observed for essential oil of *A. sylvestris* (IC₅₀=71.3 µg.ml⁻¹) followed by essential oil of *A. cerefolium* (IC₅₀=115 µg.ml⁻¹). In addition, the amounts of total phenolic contents of *A. cerefolium* and *A. sylvestris* methanolic extracts at full flowering stage (76.7 and 74.6 mg GAE.L⁻¹) were determined. Other important group of compounds and their biological properties needs to be studied in *Anthriscus* species due to their potential pharmacological and food industry value. **Research limitations:** No limitations were founded. **Originality/Value:** Since the essential oil of *A. sylvestris* at vegetative stage demonstrated the noticeable antioxidant ability which makes it well qualified to be used as natural ingredients to synthetic antioxidants in food industry.

INTRODUCTION

The family of the *Apiaceae* (Umbelliferae) is one of the most important families of flowering plants comprises over 3700 species scattered throughout the world, mainly in the northern temperate regions and high altitudes in the tropics. Several species of the *Apiaceae* are well known as a source of essential oils which are used for different purposes including nutrition, medicine, beverages, spices, repellents, staining, cosmetics, fragrances and industrial uses (Sayed-Ahmad et al., 2017; Zengin et al., 2019). The genus *Anthriscus Pers.* (commonly known as beaked parsley, rough chervil, beaked chervil) with 12 species growing in Europe, eastern North America, Africa, New Zealand and temperate parts of Asia, belongs to the family *Apiaceae*, subfamily *Apioideae*, tribe *Scandicineae* (Pavlović et al., 2011; Hendrawati et al., 2012). Three species of this genus [*A. cerefolium* (L.) Hoffm., *A. sylvestris* (L.) Hoffm., and *A. nemorosa* (M. B.) Speng.] are represented in the Flora of Iran (Mozaffarian, 2007). The chervil plants are generally well known for their strong and distinctive flavors and in some cases providing important nutrients which can fortify the consumer's diet to enhance its nutritional value. Some of the *Anthriscus* species are used as flavoring agent and spice for culinary purposes. The young aerial parts of the chervil are nearly always used fresh as vegetable, but can be preserved by drying, deep freezing or by making a pesto-like preparation (Pavlović et al., 2011; El Gendy et al., 2015). Aerial parts, roots and fruits of several *Anthriscus Pers.* species have been traditionally used in folk medicine of many countries for the treatment of asthma, bronchitis (Kim et al., 2019), hypertension (Fejes et al., 2000), rheumatism, gastrointestinal ailments, inflammation, and stomach-ache (Bagci et al., 2016). The species are also used as hematinic, tonic (Lim et al., 1999), antipyretic, antitussive, diuretic, analgesic, cough remedy (Bagci et al., 2016) detoxifying agent (Baser et al., 1998). In recent years, reports concerning the cytotoxic (Lai et al., 2018), antimicrobial (Pavlović et al., 2011; Lai et al., 2018), antioxidant (Lim et al., 1999; Lai et al., 2018), memory-enhancer, anxiolytic and antidepressant (Bagci et al., 2016), antilipoperoxidant (Fejes et al., 2000), anticancer (Ikeda et al., 1998; Lim et al., 1999), anti- allergic (Kim et al., 2019), apoptotic (Jeong et al., 2007), insecticidal (Kozawa et al., 1982), allelopathic (Lyytinen & Lindström, 2019) properties of various *Anthriscus* species are also available in the literatures.

Recently, an increasing interest in scientific researches concerning discovery of natural products including the plant extracts, essential oils of aromatic plants and their components that can be applied to the food, cosmetic, perfume and pharmaceutical industries has gained an increasing interest (Bakkali et al., 2008; Shahwar et al., 2012). Likewise, scientific research reveals that the antioxidant capacity of biologically active compounds which isolated from plant gives beneficial effect to human health. For that reason, antioxidant activity is widely used as a parameter to describe nutritional health food or plants and their natural active components (Lobo et al., 2010).

Nowadays, the use of natural substances such as medicinal plant extracts are considered as an alternative to the employ of synthetic preservatives to prolong the storage stability of food products owing to their notable antioxidant, since consumers are keeping away from consumption of products with harmful synthetic additives or chemicals because of their potentially health hazard and side effects such as carcinogenicity and toxicity (Riahi et al., 2013). Previous studies have demonstrated that antioxidant activity of plant extracts may be associated with their level of phenolic compounds (Tungmunnithum et al., 2018). Therefore, international attention has been directed toward discovery of phenolic compounds in crude extracts of plant materials as functional and beneficial ingredients that can be applied to the food industry, due to their ability to postpone oxidative degradation of lipids and thereby

improve the quality and nutritional value of food (Trumbeckaite et al., 2011; Riahi et al., 2013).

The *Anthriscus* species have been demonstrated to possess important groups of compounds, such as flavonoid glycoside (Dall'Acqua et al., 2006; Žemlička et al., 2014), flavolignans (Ikeda et al., 1998), lignans, coumarins (Jeong et al., 2007), Furanocoumarins, furanocoumarin ethers (Fujioka et al., 1999) and cyclopropane fatty acids (Kuiper & Stuiver, 1972). Moreover, chemical composition of essential oil obtained from different *Anthriscus* species were presented by various studies (Lemberkovics et al., 1994; Bos et al., 2002; Nickavar et al., 2009; Pavlović et al., 2011; Kiliç, 2017; Lai et al., 2018).

Previous studies suggest that chemical composition of plants can be influenced by a number of intrinsic and external factors such as genetic background, physiological condition of plant, climatic and agronomic conditions, type of plant part, growing stage of the plant, method of extraction and postharvest processing and storage conditions (Ramezani et al., 2009; Norouzi & Norouzi, 2018). In compliance with this variation, the biological activities of plant can be expected to vary, based on chemical composition variability. Thus, the findings of investigation of plant chemical composition at different phenological stages and its coherence with the biological activities can be considered to select the optimal harvesting time of this plant for relevant industries to be used in foods, cosmetics and pharmaceuticals (Djouahri et al., 2017). So the aim of this study was to assess the volatile components, phenolic content and antioxidant activity of two *Anthriscus* species: *A. cerefolium* (called isti-ot) and *A. sylvestris* (called jajigh), the former is one of the most popular vegetation in the region used as vegetable in the spring and fall seasons.

MATERIALS AND METHODS

Plant materials

The fresh aerial parts of two *Anthriscus* Pers. Species (*A. cerefolium* & *A. sylvestris*) were collected during vegetative (March) and full flowering (May) stages in 2018 from Lajayer Rural District, Germe, Ardabil province, Iran, GPS coordinates: 38°58'02.3"N 48°15'07.3"E. The plant materials were air dried at room temperature in shadow (until reached final moisture content of 10% wet basis) and were ground to fine powder, used for further extractions.

Isolation of the essential oils

The essential oils of all air-dried samples (100 g) were isolated by hydrodistillation employing a Clevenger-type apparatus for 3 h. The oil was dried over anhydrous sodium sulfate, and then, was kept in a sealed dark vial at 4°C until further analysis. Experiments were carried out in the University of Mohaghegh Ardabili.

Essential oil analysis procedure

GC analysis of the essential oils was performed using a Shimadzu GC-9A gas chromatograph, equipped with flame ionization detector (FID) and DB-5 fused silica column (30 m×0.25 mm i.d., film thickness 0.25 µm). Oven temperature was programmed from 50 to 240°C at the rate of 3°C.min⁻¹; initial and final temperatures were held for 5 and 10 minutes, respectively. Detector (FID) temperature was 265°C and injector temperature was 250 °C. Helium was used as carrier gas with a linear velocity of 32 cm.s⁻¹. The percentages of compounds were calculated by the area normalization method. GC-MS analysis were carried out in an Varian 3400 GC/MS system equipped with a DB-5 fused silica column (30 m×0.25 mm i.d., film thickness 0.25 µm); oven temperature was 50–240°C at a rate of 4°C.min⁻¹, transfer line

temperature 290°C, carrier gas, helium, with a linear velocity of 31.5 cm.s⁻¹, split ratio 1:60, ionization energy 70 eV, scan time 1 s, and mass range 50–550 m.z⁻¹. The components of the essential oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds, and confirmed by comparison of their retention indices calculated relative to homologous series of n-alkanes (C5-C24), either with those of authentic compounds or with data reported in the literature (Adams, 2007).

Methanolic extract

25 g of the powdered samples were extracted in Soxhlet apparatus with methanol (MeOH) at 60°C for 4 h. The solvent evaporated at reduced pressure using a rotary evaporator and extracts stored in the dark at +4 °C until further tests.

Determination of antioxidant activity by DPPH scavenging assay

The scavenging effect of DPPH free radical was measured according to modified method of Kondo et al. (2002). The reaction system consisted of 0.1 ml extract or essential oil at different concentrations (5, 10, 20 and 30 µg.ml⁻¹) and 2 ml of DPPH methanolic solution (0.21 mM). The reaction mixture was shaken and left for 30 min at room temperature in the dark, and the absorbance was measured at 517 nm in a spectrophotometer. Ascorbic acid was used as was used as reference. Inhibition of free radical DPPH in percent (I%) was calculated in following way: $I\% = [(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control reaction (containing all reagents except the extract) and A_1 is the absorbance in the presence of the sample. The concentration of sample required to decrease the initial DPPH absorbance by 50% was calculated as IC₅₀.

Determination of total phenolic content

The total phenolic contents were determined by using Folin-Ciocalteu method. A volume of 1 mL of the plant extract (1 mg.ml⁻¹) was mixed with 1 ml of 10% Folin-Ciocalteu's reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 mL of sodium carbonate solution (7.5%, w.v⁻¹). After 30 min incubation at room temperature with intermittent shaking, the absorbance was measured at 760 nm. Gallic acid was used as a reference standard for plotting calibration curve ($Y = 0.0031x + 0.1$, $R^2 = 0.976$). Total phenols were expressed in terms of gallic acid equivalents (mg gallic acid.l⁻¹).

RESULTS AND DISCUSSION

Essential oil composition

The constituents of the hydrodistilled essential oil isolated from two *Anthriscus* species at different phenological stages were analyzed by GC-MS. The essential oil components along with retention indices and their relative percentages are presented in Table 1, where the compounds are given in order of their elution from the DB-5 column.

A hundred and four components were detected in the essential oils obtained from different samples representing 93.40% to 98.20% of the total oil composition. Both quantitative and qualitative differences were observed among all essential oils.

Oxygenated monoterpenes were the main class of compounds extracted from vegetative and flowering stages of *A. cerefolium* (93.83% and 88.13%, respectively), while aliphatic esters were detected to be the main fraction of essential oils obtained from *A. sylvestris* at vegetative (66.90%) and flowering (42.48%) stages.

Sesquiterpene hydrocarbons were the second fraction of the essential oils extracted from *A. cerefolium* at vegetative and flowering stages. Among groups of the chemical compounds,

the oxygenated monoterpenes with 7.5% of the total oil were the second predominant fraction of essential oil isolated from *A. sylvestris* collected before flowering phase, whereas monoterpene hydrocarbons were second fraction of the essential oil of this species at flowering stage.

Table 1. Chemical composition and characteristics of essential oils isolated *Anthriscus* species at various phenological stages

Compounds	RI	Percentage (%)			
		<i>A. cerefolium</i>		<i>A. sylvestris</i>	
		Vegetative stage	Flowering stage	Vegetative stage	Flowering stage
3-methyl-2-butenal (prenal)	746		0.01	0.18	
n-Hexanal	798		0.07		0.08
(Z)-2-hexenal	851		0.03		
(E)-2-Hexenal	854	0.02			
Nonane	860	0.05	0.09		0.17
Heptanal	903		0.01		
α -Pinene	940		0.03	1.33	2.5
2-Heptenal	954		0.04		
Camphene	955			0.14	0.34
Benzaldehyde	962		0.03		
β -Sabinene	978				0.23
β -Pinene	982	0.29	0.43		14.24
2,6-Dimethyl 2,6-octadiene	989			0.39	0.24
1-Decene	990			0.74	0.31
β -Myrcene	991		0.26	2.10	
2-Pentylfuran	993	0.03			
Dehydro-1,8-cineole	994	0.52			
2,3,6-Trimethylpyridine	1003			0.40	
α -Phellandrene	1005				0.27
cis-2-(2-Pentenyl)furan	-		0.03		
Hepta-2(E),4(E)-dienal	-		0.01		
Corylon	1024			0.51	
o-Cymene	1027			0.59	0.26
p-Cymene	1029			0.39	0.20
Limonene	1032	0.12	0.18	0.92	1.93
trans- β -Ocimene	1052		0.05		3.72
3,5-Octadien-2-ol	-		0.02		
Benzeneacetaldehyde	1044		0.01		
4-Methyl-1,5-Heptadiene	1048			0.12	
2-Octenal	-		0.01		
2,2-Dimethyl 3,4-pentadienal	-		0.03		
Hexylvinylcarbinol	1059	0.03	0.04		
3-Nonanone	-	0.12	0.03		
γ -Terpinene	1061				0.16
α -Terpinolene	1063	0.07	0.07		0.09
3-Decyne	1092				8.64
cis-4-Undecene	-	0.09	0.14		
Undecane	-	1.14	1.74	0.31	0.29
Nonanal	1101		0.07		
2,6-Dimethyleneoct-7-en-3-one	1107				0.23
5-Undecene	-		0.02		
2-Nonen-1-ol, (Z)	-		0.03		
cis-Verbenol	-			7.10	
3,4-Heptadiene	-		0.05		
1-(4-Methyl-3-cyclohexen-1-yl)ethanol	1149				1.08
2,4-Dimethylbenzaldehyde	1179				0.50
4-ethylbenzaldehyde	1181			0.20	
3-Nonanol	1091	0.06			
Estragole	1195	59.01	53.96	0.40	1.31
4-Ethyl-3-heptene	-	0.18	0.22		

1-Cyclohexene-1-carboxaldehyde	-		0.02		
β-Safranal	1202			1.90	3.00
p-Cumic aldehyde	1240				0.25
Chrysanthenyl acetate	1262		0.65	64.85	41.5
2-Octyne	-	0.07	0.36		
Anethole	1281		0.05		
Isobornyl acetate	1286			0.59	0.31
(E)-5-Tridecene	-	0.10	0.17		
Tridecane	-	0.08	0.14		
2,4-Nonadienal	-		0.03		
Bicycloelemene	1327		0.06		
Citronellyl acetate	1351				0.24
α-Cubebene	1352		0.07		0.13
β-Bourbonene	1385		0.04		
β-Elemene	1392		0.08		
o-tert-Butylphenol	-			1.66	
Methyleugenol	1401	34.3	34.12		
β-Caryophyllen	1418	0.51			2.31
Geranyl propionate	1445			0.40	0.18
β-Ionone	1470				0.26
Germacrene D	1483	0.33	1.25		0.75
α-Caryophyllene	1455	0.03	0.08		0.31
trans-β-Farnesene	1459		0.13	0.70	5.15
α-Amorphene	1482		0.04		
β-Ionone	-		0.07		
Geranyl ester	1475			0.40	0.18
Phenylethyl 2-methylbutyrate	1477		0.04		
alpha-Bergamotene	1434		0.34		
Bicyclogermacrene	1494		0.31		
α-selinene	1495			0.27	0.49
Zingiberene	1497	0.41			
α-Farnesene	1507				0.51
β-Bisabolene	1509	0.16	0.18	0.04	
delta-Cadinene	1512		0.16		
Tridecanal	-			0.66	
Myristicine	1520	0.03	0.03		0.18
β-sesquiphellandrene	1523	0.06			
Elemol	1551		0.02		
Elemicine	1554				0.14
α-Dendrolasin	1571			0.17	0.12
spathulenol	1578		0.21		
Caryophyllene oxide	1583		0.13	5.45	0.95
Neryl acetate	1381	0.05	0.05		0.07
Butanoic acid	1582	0.17	0.12		
calamenene	1537		0.04		
τ-Cadinol	1638		0.03		
Longiborneol	1592		0.02		
Iso-Caryophyllene	-		0.02		
Humulene oxide II	1609			0.39	
Myristic aldehyde	1611				0.46
Perhydrofarnesyl acetone	1830				0.51
Neophytadiene	1838	0.17	0.30	0.10	0.44
trans- α-Bergamotol	1698				0.30
n-Hexadecanoic acid	1972			1.10	0.05
Total (%)		98.20	97.07	93.40	95.53
Aldehyde		0.19	0.56	2.59	5.23
Monoterpene hydrocarbons		0.48	1.02	5.86	24.41
Oxygenated monoterpenes		93.83	88.13	7.50	1.31
Sesquiterpene hydrocarbons		1.50	2.80	1.01	9.65
Oxygenated Sesquiterpene		0.17	0.53	6.01	1.37
Aliphatic esters		0.05	0.70	66.90	42.48
Aromatic esters			0.04	0.20	
Alcohol		0.09	0.09		1.08
Other		1.89	3.20	3.33	10.00

Relative amounts of different classes of compounds of essential oils of different *Anthriscus* species were reported by previous researchers. Monoterpene hydrocarbons were the main group of constituents of essential oil produced by hydrodistillation of *A. cerefolium* fresh herb, while a phenol was the main component of oil isolated by supercritical fluid extraction from its fresh herb (Simandi et al., 1996). According the study of Hendawy et al. (2019) regarding the essential oil of *A. cerefolium*, cultivated in Egypt under different locations, oxygenated compounds were shown to be principal compound group of oils. In other study, oxygenated monoterpenes were the most noticeable fraction of all essential oil of *A. cerefolium* L. cultivated in Egypt and underwent at different treatments of NK fertilizers (El Gendy et al., 2015).

Monoterpene hydrocarbons fraction was identified as the main group of components in essential oil of fresh leaves, DCM extract of fresh leaves and DCM extract of air dried leaves of *A. sylvestris*, while sesquiterpene hydrocarbon fraction dominated DCM extract of freeze dried leaves (Kiliç, 2017). Sesquiterpene hydrocarbons constituted the principal fraction of essential oils obtained from the roots (Pavlović et al., 2011) and aerial parts (Bagci et al., 2016) of *A. nemorosa*.

In the present study, some compounds are only observed at one of the phenological stages. It is believed that synthesis of specific components in plants has relevance to the phenological stage in which the plants are (Norouzi & Norouzi, 2018). There were 21 similar components in essential oils of *A. cerefolium* at different phenological stages, while 19 components were in common to essential oil of *A. sylvestris* at vegetative and flowering stages. Furthermore, the essential oils of flowering stage in both species were more complex and therefore had more compounds in comparison to the essential oils of vegetative phase. This could be explained by the ability of older plant to produce alternative defensive mechanisms, since the constituents of essential oil play an important role in plants defense reactions (Ochoa-López et al., 2015). It can also be related to low rate of biosynthesis of essential oil components during the vegetative stage that may be due to partial inactivation of enzymes necessary to the biosynthesis of certain compounds (Ochoa-López et al., 2015).

In present study, twenty-eight compounds were identified in essential oil of *A. cerefolium* at vegetative stage, accounting for 98.20% of total oil. The main components of this essential oil were estragole (59.01%) and methyleugenol (34.3%). In a similar way, among 62 compounds existed in the essential oil of *A. cerefolium* during reproductive phase, estragole (53.96 %) and methyleugenol (34.12%) were as the main ones. The effect of different phenological stages on the composition of essential oil may be due to its effect on enzyme activity and metabolism of essential oil production (Sellami et al., 2009).

According the study of Baser et al. (1998) the essential oil of *A. cerefolium*, grown wild in Turkey was characterized by methylchavicol, 1-allyl-2,4-dimethoxybenzene, undecane and β -pinene as the main constituents. It has been documented that essential oil isolated from *A. cerefolium* by hydrodistillation was found to contain methylchavicol (80%) and 1-allyl-2,4-dimethoxybenzene (16%), while the oil isolated by supercritical fluid extraction contained a much lower level of methyl chavicol (21.1%) and a higher amount of 1-allyl-2,4-dimethoxybenzene (57.4%) (Simandi et al., 1996). The main components of essential oil obtained from aerial parts of this species in Turkey were caryophyllene, γ -cadinene, trans-pinocarveol, spathulenol and caryophyllene oxide (Kiliç, 2017).

In another study, volatile constituents of chervil plant (*A. cerefolium*), cultivated in Egypt under different nutritional conditions were investigated. The oil compositions of all samples were distinguished by methyleugenol as the main component followed by estragole, 2-allyl-1,4-dimethoxybenzene, (-)-zingiberene and 1-nonene (El Gendy et al., 2015). Similar findings

were also observed by Hendawy et al. (2019) in plants cultivated in 4 different locations in Egypt.

The composition of essential oil of *A. sylvestris* was completely different from that of the *A. cerefolium*. Low percentages were observed for Estragole in the essential oil of *A. sylvestris* at vegetative (0.4 %) and flowering (1.31%) phases. Methyleugenol as the second main component of *A. cerefolium* samples was not present in *A. sylvestris* volatile oil. In the other hand, Chrysanthenyl acetate which was the major constituents of the essential oil of the *A. sylvestris* during ontogenesis, couldn't be detected in vegetative stage and was detected in low amount in the essential oil of flowering phase (0.65%) of *A. cerefolium*.

Essential oil obtained from vegetative stage of *A. sylvestris* contained 31 compounds representing 93.40% of the total oil composition and was dominated by Chrysanthenyl acetate (64.85%) and cis-Verbenol (7.1%). GC-MS analysis of *A. sylvestris* essential oil at flowering phase showed that among 46 components which represented 95.53% of the total oil, Chrysanthenyl acetate (64.85%) and β -Pinene (14.24%) were synthesized and accumulated as the major components.

The essential oil of *A. sylvestris* flower have been reported previously to contain Phenol, o-cresol, eugenol, β -myrcene, d-limonene, γ -terpinene, p-cymene, benzyl alcohol, phenethyl alcohol, l-linalool, β -farnesene and d-sabinyl acetate, while phenol, cresol (o-, m-, p-), guaiacol, eugenol, p-cymene, α -pinene, β -myrcene, d-limonene, γ -terpinene, terpinolene, β -farnesene, cis-3-hexen-1-ol, benzyl alcohol, phenethyl alcohol, sabinyl acetate, l- α -fenchyl acetate and chrysanthenyl acetate were identified from the leaves essential oil (Kurihara & Kikuchi, 1979). (-)-sabinen was shown to have highest percentage among volatile compounds from flowers, buds and leaves of *A. sylvestris* (Borg-Karlson et al., 1993). In another study Myrcene, α -pinene and β -pinene were the main components of the essential oil of this species (Valterová et al., 1997). Moreover, the chemical composition of essential oil and dichloromethane extracts of leaves and roots from *A. sylvestris* were analysed by GC and GC-MS. The results showed that β -Phellandrene, β -Myrcene in fresh leaves essential oil, β -Myrcene and trans-Sabinyl acetate in extract of fresh leaves, β -Phellandrene and Z- β -Ocimene in both essential oil and extract of fresh root were reported as major constituents (Bos et al., 2002).

Free radical scavenging activities

The radical scavenging effect of the essential oils and methanolic extracts from two *Anthriscus* species at vegetative stage was investigated using DPPH as reagent (Fig. 1). The concentration of sample required to inhibit 50% of radicals (IC_{50}) is a parameter widely used to measure the antioxidant activity. The lower IC_{50} value demonstrates stronger antioxidant activity (Roby et al., 2013). In current study, the highest antioxidant activity was obtained for essential oil of *A. sylvestris* ($IC_{50}=71.3 \mu\text{g.ml}^{-1}$) followed by essential oil of *A. cerefolium* ($IC_{50}=115 \mu\text{g.ml}^{-1}$). IC_{50} values for extract of *A. cerefolium* and *A. sylvestris* were 982 and $1733 \mu\text{g.ml}^{-1}$, respectively.

It has been recommended that antioxidant activity of phytochemicals is related directly to the presence of active major components. Whereas, other compounds with lower amounts could play an important role in antioxidant capacity due to synergistic or antagonistic effects between the volatile components (Mastelic et al., 2008; Norouzi & Norouzi, 2018).

Fejes et al. (2000) assessed the In vitro antioxidant activity of aqueous extracts from different vegetative parts (root, herb) of *A. cerefolium* by various test methods. Based on their results, both root and herb extracts possessed DPPH radical scavenging capacity. However, the exact compounds which demonstrate radical scavenging activity are still unclear.

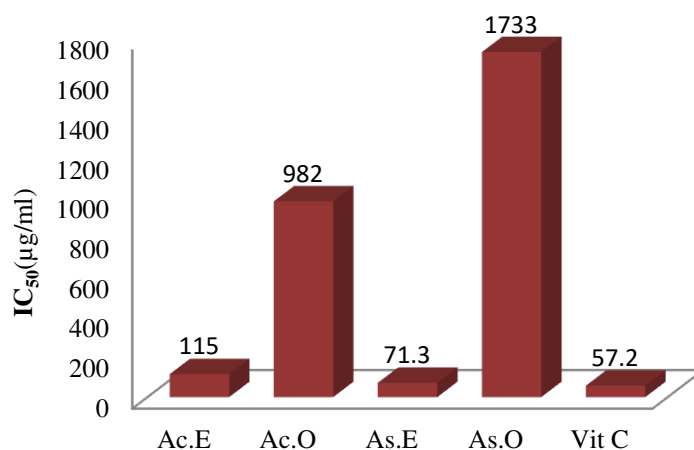


Fig. 1. IC₅₀ (µg.ml⁻¹) of the essential oils and methanolic extracts from two *Anthriscus* species at vegetative stage. Note: Ac.E, extract of *A. cerefolium*; Ac.O, essential oil of *A. cerefolium*; As.E, extract of *A. sylvestris*; As.O, essential oil of *A. sylvestris*

Findings by Milovanovic et al. (1996) highlighted that antioxidant activity of ethanolic extract of *A. sylvestris* was superior to apigenin, quercetin, or a tocopherol mixture. The antioxidant activity of the crude methanol extract of *A. sylvestris* was found to be related to luteolin-7-O-glucoside and chlorogenic acid (Dall'Acqua et al., 2006).

Total phenolic content

The total phenolic contents of *A. cerefolium* and *A. sylvestris* at full flowering stage were determined spectrophotometrically according to the Folin-Ciocalteu procedure which were 76.7 and 74.6 mg Galic acid equivalent per 1 liter of extract, respectively. Verma et al. (2007) and Ayan et al. (2007) suggested previously that flowering stage conduct the way in which phenolic compounds reach the highest levels. Accumulation of phenolics during the late vegetative phase can be attributed to the fact that during this stage, the plant protection is mainly secured by phenolic compound which are highly synthesized during this stage (Sellami et al., 2009).

CONCLUSION

Characterization of volatile compounds of two *Anthriscus* species growing wild in Iran revealed that changing in phenological stages can influence the pathway of the essential oils biosynthesis. Besides, climatic factors such as temperature, sunlight, relative humidity, rainfall and water etc. differ throughout various growth phases, and they can also cause some variation in essential oil composition. Moreover, essential oil of *A. sylvestris* at vegetative stage demonstrated the noticeable antioxidant ability (analogous to reference standard) which makes it well qualified to be used as functional ingredients and natural alternatives to synthetic antioxidants in food industry, since increasing attention has been directed toward finding naturally occurring antioxidant.

Conflict of interest

The authors declare no conflict of interest to report.

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