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Essential Oil Composition of Aerial Parts of Two *Anthriscus* Pers. Species From Turkey

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Abstract: In this study, the essential oil of dried flowering aerial parts of *Anthriscus cerefolium* (L.) Hoffm. and *Anthriscus nemorosa* (M.Bieb.) Spreng. were analyzed by means of GC / GC-MS. As a result 35 and 34 components were identified representing 88.1 % and 90.5 % of the oil *A. cerefolium* and *A. nemorosa,* respectively. The main constituents of *A. cerefolium* were caryophyllene (16.9 %), δ-cadinene (16.4 %), *trans*-pinocarveol (12.5 %), spathulenol (7.5 %) and caryophyllene oxide (6.8 %); whereas caryophyllene (15.8 %), caryophyllene oxide (14.5 %), δ-cadinene (13.4 %), germacrene D (8.9 %) and *trans*-pinocarveol (6.2 %) were detected the major constituents of *A. nemorosa*. Studied plant samples were found to be rich in respect to essential oils. The results were discussed in consideration of natural products, renewable resources, chemotaxonomy and potential medical uses of these plants.

Key words: *Anthriscus*, essential oil, GC / GC-MS.

Introductin

The Apiaceae family has a cosmopolitan spreading, but most of Apiaceae taxa are confined to northern temperate areas, and high altitudes in the tropic regions [1]. Apiaceae is a large family, composed of more than 3.700 species belonging to 434 genera all around the world [2,3]. In Turkey, Apiaceae is represented more than 465 taxa, with a 30 % endemism rate [4-6]. Apiaceae taxa are mostly aromatic and economically important plants are well known with regard to their economic significance and varied profile of essential oils [7]. The genus *Anthriscus* Pers. is belonging to Apiaceae family, and represented by eight species in Flora of Turkey [8]. The Apiaceae taxa are generally rich in secondary metabolites and embodies numerous genera of high economic and medicinal value yielding flavonoids, alkaloids, coumarins, acetylenes, terpenes and essential oils [9]. Recent studies have shown that natural products and especially essential oils display potential as antimicrobial agents for various uses in medical applications [10]. Plant materials widespread use has raised the interest of scientists in basic research of extracts and essential oils; especially, the anti-microbial, anti-cancer and anti-oxidant activities [11,12]. Essential oils are compounds made of several organic volatile substances and they are produced and stored in the secretion canals of some plants, especially aromatic and medicinal herbs. At room temperature essential oils are usually liquid and they are responsible for the aromas of plants and widely distributed in nature and were found especially in conifers, Myrtaceae, Rutaceae, and Apiaceae families [13]. Essential oils have antiseptic, antispasmodic, expectorant, carminative, eupeptic, anticancer, antimicrobial, antioxidant, usage as free radical scavenging agents [14,15]. Because of antioxidant and antimicrobial activities, essential oils are serving as natural addi-tives in foods and food products [16]. Plant essential oils have been serve as the alternative additives or

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processing aid as green technology, phytotheraphy, aromatheraphy, pharmaceutical, cosmetic, veterinary products, textile, tobacco, biocides and insecticides industries [17]. A wide variety of essential oils are known to possess antifungal and antibacterial features, serving as chemical defense agents against plant pathogens and they exhibit cytotoxic effects being able to act as anti herbivorous agents [18,19]. According to literature records, the diversity of the oils and chemical differences among *Anthriscus* can be the consequence of the diverse geographical conditions, genetic and other factors [20]. Though the chemical composition of different essential oils from *Anthriscus* species has been reported, this study is a part of our ongoing research on phytochemical investigation of medicinal and aromatic plants. In some populations of *Anthriscus*, where above ground foliage was removed by mowing and/or herbicide application, showed large bare soil patches around the tap roots long after the control action. This suggests some residual allelopathic effect may be preventing the re-establishment of surrounding vegetation.

In general *Anthriscus* taxa are moderately disturbed habitats and edges, in moist or mesic sites. They can form dense colonies in ditches, dykes, road verges, meadows, hay fields, stream banks and hedge rows. They are found at lower frequencies in pastures, open woodlands or waste land. In Turkey *A. cerefolium* and *A. nemorosa* generally flowering in 4-8 months and usually spreading rocky slopes, water meadows, rocky limestone cliffs, an altitude of 450-1300 m and 500-3200 m respectively. In late April and early May, linear-lanceolate cotyledons of *Anthriscus* spacies appear followed quickly by the compound true leaves with lobed leaflets; by the end of June seedlings have about six leaves while older, mature plants form a dense canopy of leaves. The fruits mature in late June and July; by August the flowering stems senesce and the brown stalks overtop the canopy of green basal leaves. Seeds fall gradually from the mature plants through late July, August and September and some may persist on the dead stems through the winter. The pattern of vegetative reproduction, budding from the root crown, allows chervil to achieve dense populations with minimal seedling recruitment. Young plantlets are formed early in the growing season and usually remain attached to their parent root stalk throughout the year. By the end of the first year the tap root is well formed and can provide considerable resources for initiation of growth the following spring.

In this study essential oil properties of *A. cerefolium* and *A. nemorosa* are the subject of a detailed research. To the best of our knowledge, the essential oil of the aerial parts at flowering stage of these plants in Bingol area from east part of Turkey have not been investigated before. Therefore, the aim of this study is to provide essential oil composition of these species, that might be helpful in potential usefulness, biological activities and chemotaxonomy of *Anthriscus* taxa.

Materials and methods

Plant materials

A. cerefolium was collected from south of Yelesen village, steppe and stony areas, Bingöl/ Turkey, at an altidude of 1600-1700 m., by Omer Kilic, collect no: 4886. *A. nemorosa* was collected from north of Dikme village, Quercus foresty areas, Bingöl / Turkey, at an altidude of 1500-1600 m., by Omer Kilic, collect no: 5729. Plant materials were identified by taxonomist O. Kilic with volume 4 of Flora of Turkey and East Aegean Islands [2]. The aerial parts of plants were dried and 200 g each were cut into small pieces and was boiled with hydrodistillation using a Clevenger apparatus (in Bingol University, Department of Park and Garden Plants) to get essential oils. Voucher specimens were deposited in the Bingol University, Department of Park and Garden Plants.

Isolation of the essential oil

Dried flowering aerial parts of *A. cerefolium* (200 g) and *A. nemorosa* (200 g) were exposed to hydrodistillation using a Clevenger apparatus for 3 hour, in Bingol University, Department of Park and Garden Plants.

Gas chromatography-Mass spectrometry

The essential oil of plant samples were analyzed with 60-m long column packed with CP-Wax 52 CB 0.25 mm i.d. in Bingol University, with two repetitions. The column and analyzes circumstances were the same as in GC-MS. The percentage of the essential oil composition was calculated from GC-FID peak areas without correction factors. A Varian 3800 gas chromatograph, exactly interfaced with a Varian 2000 ion trap mass spectrometer, was used with a splitless injection mode and an injector temperature of 260°C. The oven temperature was 45°C held for 5 min, then increased to 80°C at a rate of 10°C min^{-1}, and to 240°C at a rate of 2°C min^{-1}. Helium was the carrier gas which used at a stable pressure of 10 psi; the transfer line temperature was 250°C; with an electron impact ionisation mode an acquisition range of 40 to 200 m z^{-1} and a scan rate of 1 us^{-1}. Alkanes were used as reference points in the calculation of relative retention indices (RRI).

Supplementary identification was determined using Wiley and Nist libraries, mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz [21, 22]. The relative amounts were calculated on the basis of peak-area ratios. The essential oil composition of studied samples are seen in Table 1.

Results and discussion

In this study, the main constituents of *A. cerefolium* were caryophyllene (16.9 %), δ-cadinene (16.4 %), *trans*-pinocarveol (12.5 %), spathulenol (7.5 %) and caryophyllene oxide (6.8 %); whereas caryophyllene (15.8 %), caryophyllene oxide (14.5 %), δ-cadinene (13.4 %), germacrene D (8.9 %) and *trans*-pinocarveol (6.2 %) were the major constituents of *A. nemorosa* (Table 1).

In a study the essential oil constituents of *A. nemorosa* from Turkey, β-caryophyllene (23.6 %), caryophyllene oxide (12.3 %), cadinene (12.1 %), and *trans*-pinocarveol (9.8 %) were found to be major compounds [23]; in this study, *A. cerefolium* and *A. nemorosa* have similar essential oil composition properties and are different from the cited study, producing high concentration of caryophyllene (16.9-15.8 %) and δ-cadinene (16.4-13.4 %) respectively; followed by low percentages of some constituents (Table 1). In another research, essential oil *A. nemorosa* were investigated and fourty one compounds were identified; however, the essential oil was poor in other classes of terpenoid compounds, especially monoterpenoids; germacrene-D (5.0 %), (*E*,*E*)-a-farnesene (3.9 %), and α-pinene (3.7 %) [24]. In generally, the essential oil of *A. nemorosa* was characterized by the presence of high levels of sesquiterpenoids. Literatures shows that considerable differences exist in the compositions of the essential oils between different species of the genus *Anthriscus*, especially in terms of the type of major components. According to the some studies in the literature β-phellandrene, β-myrcene, sabinene, (*Z*)-β-ocimene, α-pinene (in the oil of *A. sylvestris*), or methyl chavicol and 1-allyl-2,4-dimethoxybenzene (in the oil of *A. cerefolium*) were the main compounds [25,26]. *Anthriscus* species as to the variety of their components and their relative quantity; these essential oil composition differences can be most probably explained by the variability of the plant species and the existence of different chemotypes [27]. In another research, the essential oil obtained by hydrodistillation from the roots of *Anthriscus nemorosa* was analyzed by GC and GC-MS; among sixty-two compounds identified (representing 89.0 % of the total oil), the main components were *n*-nonane (12.1 %), *n*-hexadecanol (6.9 %), δ-cadinene (6.4 %), β-pinene (6.0 %) and germacrene D (5.4 %) [28]. It is noteworthy that *n*-nonane and *n*-haxadecanol were not found in this research; δ-cadinene, β-pinene and germacrene D were detected high percentages in the essential oil of two studied *Anthriscus* species; details of essential oil compositions of two studied *Anthriscus* species can be seen in Table 1. The water-distilled essential oil of *A. cerefolium* growing wild in Turkey was analyzed by GC-MS and four compounds were detected comprising the total oil were methyl chavicol (83.10 %), 1-allyl-2,4-dimethoxybenzene (15.15 %), undecane (1.75 %) and β-pinene (<0.01 %) [29]. It is noteworthy that our results were determined different in respect to main constituents. Comparison of the oil compositions from two species showed that the amounts of *trans*-pinocarveol, caryophyllene, δ-cadinene and spathulenol were higher in the oil of *A. cerefolium* than *A. nemorosa* (Table 1). The percentage of

Table 1. Essential oil composition of aerial part of *Anthriscus* species

No.	Compounds	RT	RRI*	*A. cerefolium*	*A. nemorosa*
1	Heptanal	7.35	871	0.1	-
2	Hexanal	7.45	925	-	0.2
3	α-Thujene	7.55	938	0.2	-
4	α-Pinene	7.80	975	0.1	0.6
5	Camphene	8.35	985	-	0.2
6	Verbenene	8.95	996	-	-
7	Sabinene	9.20	965	0.2	1.9
8	β-Pinene	9.45	975	4.3	7.4
9	3-Octanone	9.65	981	-	0.1
10	β-Myrcene	9.78	988	4.1	-
11	α-Phellandrene	10.35	995	-	0.2
12	δ-3-Carene	10.45	1002	-	0.3
13	3-Octanol	10.90	1006	0.2	-
14	α-Terpinene	11.05	1015	3.1	4.6
15	p-Cymene	11.10	1023	-	0.3
16	β-Phellandrene	11.25	1028	0.2	0.1
17	β-Ocimene	11.30	1030	0.5	1.2
18	Limonene	11.40	1033	0.2	-
19	cis-Ocimene	11.50	1050	-	0.8
20	γ-Terpinene	12.25	1062	0.1	2.3
21	Sabinene hydrate	12.60	1070	0.2	-
22	α-Terpinolene	12.95	1082	-	0.4
23	Linalool	13.40	1095	-	0.1
24	Terpinen-4-ol	13.65	1125	0.8	-
25	*trans*-Pinocarveol	14.05	1138	12.5	6.2
26	*trans*-Verbenol	14.90	1142	0.1	-
27	Borneol	15.55	1175	-	0.3
28	3-Cyclohexen-1-ol	15.95	1205	0.2	-
29	*cis*-Chrysanthenyl acetate	16.50	1255	0.1	-
30	*trans*-Anethol	17.60	1280	-	0.3
31	Bornyl acetate	18.42	1305	0.2	-
32	α-Copaene	20.23	1352	0.1	-
33	β-Bourbonene	23.20	1382	-	0.3
34	β-Elemene	23.54	1395	5.1	4.3
35	β-Cubebene	24.05	1402	-	0.2
36	Caryophyllene	25.07	1412	16.9	15.8
37	γ-Elemene	25.30	1428	0.1	-
38	α-Humulene	25.55	1450	-	0.3
39	β-Farnesene	25.90	1460	0.4	0.7
40	Germacrene D	26.15	1476	5.8	8.9
41	Bicyclogermacrene	26.65	1495	0.3	-
42	α-Selinene	27.15	1500	-	0.2
43	Muuorelene	27.34	1505	0.2	-
44	δ-Cadinene	27.45	1508	16.4	13.4

table 1 (continued).

No.	Compounds	RT	RRI*	*A. cerefolium*	*A. nemorosa*
45	β-Bisabolene	27.80	1513	0.4	0.6
46	Spathulenol	28.35	1530	7.5	2.8
47	Germacrene B	28.70	1545	-	0.8
48	Caryophyllene oxide	29.30	1552	6.8	14.5
49	α-Cadinol	29.55	1598	0.2	-
50	Benzoic acid	30.35	1605	-	0.1
51	Hexadecanoic acid	35.42	1674	0.4	-
52	Tricosane	38.46	1795	0.1	-
53	Heptacosane	47.90	1853	-	0.1
	Total			88.1	90.5

some minor components in both oils were similar, but there are some differences between percentages of some other components; for example *trans*-pinocarveol, spathulenol, caryophyllene oxide and so on (Table 1). These differences can be the result of different ecological properties and might have been derived from local, climatic factors of two plant localities.

In conclusion, this study demonstrates the occurrence of δ-cadinene, caryophyllene oxide, caryophyllene, *trans*-pinocarveol chemotypes of *A. cerefolium* and *A. nemorosa* in Eastern Anatolian region of Turkey. The essential oil results have given some clues on the chemotaxonomy of the genus patterns and usability of *A. cerefolium* and *A. nemorosa* as natural product. So these plants can be used different purposes in industry and ethnobotany. In addition, many plant species are threatened due to overharvesting for medicinal or other use, so there is great need to protect plant diversity.

There is also a need to develop more sustainable ways of obtaining industrial products from renewable resources. The cultivation of medicinal and aromatic plants for industrial products can address these issues. The results showed that the analysis of essential oil composition will add some contributions on the usability of this plant as a crop and the chemotaxonomy of the genus patterns.

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