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Variations of Essential Oil Compositions of *Achillea millefolium* L. subsp. *millefolium* Taxa Growing in Bingol (Turkey)

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Abstract

In this study, the aerial parts of *A. millefolium* subsp. *millefolium* taxa collected from six different localities of Bingol were hydro-distilled and the chemical composition of essential oils were analyzed by means of GC and GC/MS. Oil components present in all samples were α-pinene (4-17%), sabinene (3-20%), 1,8-cineole (23-29%) and β-cimen (1-4%). The oil yield for the two taxa collected from Ilıcalar and Genç was determined as 0.2% (v/w) with main components 1,8-cineole (31.18%), β-bisabolene epoxide (8.51%), 1,3,6-octatriene (7.44%) and α-terpieneol (7.19%) and 1,8-cineole (25.31%), sabinene (20.25%), β-pinene (5.39%) and α-pinene (4.21%) respectively. The samples collected from Ekinyolu and Sancak yielded 0.3% oil with 1,8-cineole (33.28%), β-pinene (8.83%), α-pinene (5.83%) and sabinene (5.56%) and 1,8-cineole (23.86%), β-thujone (23.46%), sabinene (8.92%) and α-pinene (4.73%) respectively. The oil of sample collected from Karlıova contained 1,8-cineole (28.88%), trans-chrizantenone (13.31%), α-pinene (7.16%), β-pinene (6.43%) and borneol (5.44%) and the oil yield was 0.4% (v/w). The only sample collected from Yedisu yielded 0.5% (v/w) oil, with 1,8-cineole (24.33%), cyclohexenol (15.36%), α-pinene (11.24%) and sabinene (10.18%).

Key words: *Achille millefolium* subsp. *millefolium,* essential oil, GC/MS, 1,8-cineol

Bingöl'de Yetişen *Achillea millefolium* L. subsp. *millefolium* Taksonlarının Uçucu Yağ Kompozisyonlarının Varyasyonları

Özet

Bu çalışmada Bingöl ilinin altı farklı lokalitesinden toplanan *Achillea millefolium* subsp. *millefolium*'un toprak üstü kısımlarından elde edilen uçucu yağların kompozisyonu GC ve GC/MS yöntemleriyle analiz edilmiştir. Tüm taksonlarda bulunan ortak yağ bileşenleri α-pinen (%4-17), sabinen (%3-20), 1,8-sineol (%23-29) ve β-simen (%1-4) olarak tespit edilmiştir. Ilıcalar ve Genç'ten toplanan taksonlardan %0.2 verimle yağ elde edilmiş ve ana bileşenleri sırasıyla 1,8-sineol (%31,18), β-bisabolen epoksit (%8.51), 1,3,6-oktatrien (%7.44), α-terpieneol (%7.19) ve 1,8-sineol (%25.31), sabinen (%20.25), β-pinen (%5.39), α-pinen (%4.21) olarak bulunmuştur. Ekinyolu ve Sancak'tan toplanan taksonlar %0.3 verimle, sırasıyla 1,8-sineol (%33.28), β-pinen (%8,83), α-pinen (%5.83), sabinen (%5.56) ve 1,8-sineol (%23.86), β-thuyon (%23.46), sabinen (%8.92), α-pinen (%4.73) ana bileşenlerini vermiştir. Kalıova'dan toplanan *A. millefolium* subsp. *millefolium* örneğinin ana bileşenleri %0.4 verimle 1,8-sineol (%28.88), trans-krizantenon (%13.31), α-pinen (%7.16), β-pinen (%6.43) ve borneol (%5.44) olarak bulunmuştur. Sadece Yedisu'dan toplanan taksondan %0.5 yağ verimi elde edilmiş ve ana bileşenleri 1,8-sineol (%24.33), siklohekzanol (%15.36), α-pinen (%11.24) ve sabinen (%10.18) olarak belirlenmiştir.

Anahtar kelimeler: *Achille millefolium* subsp. *millefolium*, uçucu yağ, GC/MS, 1,8-sineol

Introduction

Achillea L. is the largest and the most important genus of the family Asteraceae. Members of the genus *Achillea* are usually perennial herbaceous plants spread in the northern hemisphere (Davis, 1975). This genus is represented in Turkish flora by 43 species, 13 subspecies and 2 varieties altogether 58 taxa, 30 of which are endemic in Turkey (Guner et al., 2012).

A. millefolium subsp. *millefolium* (yarrow) milfoil has medicinal value. Some *Achillea* species have ethnopharmacologic importance as known to be used in folk remedies for various purposes (Baytop, 1995). *Achillea* species are used as medicinal plants against fever, common cold, and digestive complaints, and are topically used for slow-healing wounds and skin inflammations and *A. millefolium* has been used due to its anti-inflammatory, spasmolytic, haemostatic, and cholagogue effects (Si et al., 2006). The herbal tea of *A. millefolium* is used especially against diseases of the gastrointestinal tract in folk medicine (Skocibusic et al., 2004).

Essential oils of *Achillea* species were the subject of many studies. For example, *A. millefolium* has been studied extensively because of its economic and therapeutic importance. *Achillea* species have been used medicinal, agricultural, cosmetic and fragrance properties (Kocak et al., 2010). In particular, *A. millefolium* has been used as medicine by many cultures for hundreds years and is now listed in several pharmacopoeias (Newall et al., 1996; Blumenthal et al., 2000).

In this study, variations of essential oils compositions of *A. millefolium* subsp. *millefolium* taxa collected from six different localities of Bingol province were determined and evaluated.

Materials and Methods

Plant Source

A. millefolium subsp. *millefolium* specimens were collected from natural habitats in Bingol, Karlıova (Kayapinar village, 2000m, 10.07.2012 Herb. No: BIN-238), Yedisu (Yedisu district, 1520 m, 11.07.2012, Herb. No: BIN-239), Ekinyolu (Ekinyolu village, 1150 m, 11.07.2012, Herb. No: BIN-240), Ilicalar (Alatpe village, 1200 m, 13.06.2012, Herb. No: BIN-241), Sancak (Sudugunu village, 1650 m, 11.07.2012, Herb. No: BIN-242) and Genc (Çayirtepe village, 1200 m, 13.06.2012, Herb. No: BIN-243). Voucher specimens are kept at the Bingol University Herbarium (BIN).

Isolation of the Essential Oils

Air-dried aerial parts of the plant materials (100 g) were subjected to hydro-distillation using a Clevenger-type apparatus for 3 h.

GC and GC/MS Analysis

The essential oils were analyzed using GC-FID-MS (Agilent Technologies 5975C insert MSD with Triple-Axis Detector system, Agilent Technologies 7890A GC system) in central research laboratory, Bingol University. HP88 column (60m x 0,25 mm i.d., film thickness 0,25 μm) was used with helium as the carrier gas. Injector temperature was 250°C, split flow was 1.3 ml/min. The GC oven temperature was kept at 50°C for 2 min. and programmed to 150°C at a rate of 5°C /min and then kept constant at 150°C for 15 min than raised to 240°C at a rate of 5°C/min. n-Alkanes were used as reference points in the calculation of relative retention indices (RRI). MS were taken at 70 eV and a mass range of 35-425. Component identification was carried out using spectrometric electronic libraries (Wiley and Nist).The identification constituents of the essential oils are listed in Table 1.

Results and Discussion

The essential oils yields of *A. millefolium* subsp. *millefolium* collected from six different localities in Bingol were found as 0.4% (Karliova), 0.5% (Yedisu), 0.2% (Ilicalar), 0.3% (Ekinyolu), 0.3% (Sancak), and 0.2% (Genc) v/w, respectively. The analyses results of *A. millefolium* subsp. *millefolium* essential oils are listed in Table 1.

Twenty-nine components were identified representing 92.61% of the oil isolated from Karliova patterns. 1,8-cineole was determined to be present at the high percentage (28.88%). The presence of trans-chrizantenone (13.31%), α-pinene (7.16%), β-pinene (6.43%) and borneole (5.44%) were also important for the oil profile.

Twenty-nine components were identified representing 95.90% of the oil isolated from Yedisu patterns. 1,8-cineole was determined as a major component (24.33%). The presence of cyclohexenol (15.36%), α-pinene (11.24%) and sabinene (10.18%) were also important for the oil profile.

Thirty-one components were identified representing 90.61% of the oil isolated from Ekinyolu patterns producing high concentration of 1,8-cineole (33.28%). β-pinene (8.83%), α-pinene (5.83%) and sabinene (5.56%) were also identified.

Twenty-eight components were identified representing 92.11% of the oil isolated from Ilicalar patterns. 1,8-cineole was determined to be present at high percentage (31.18%). The presence

of β-bisabolene epoxide (8.51%), 1,3,6-octatriene (7.44%) and α-terpieneol (7.19%) were also found as an important components of oil profile.

Twenty-six components were identified representing 92.93% of the oil isolated from Sancak patterns. The dominant components was determined as 1,8-cineole (23.86%) while β-thujone (23.46%), sabinene (8.92%) and α-pinene (4.73%) were also identified.

Twenty-eight components were identified representing 95.26% of the oil isolated from Genc patterns. The main component of sample was found as 1,8-cineol (25.31%) and the other components were found as sabinene (20.25%), β-pinene (5.39%) and α-pinene (4.21%).

According to Kocak et al (2010) δ-cadinene (19.03%), limonene oxide (10.13%), alloaromadendrene (6.37%), caryophyllene oxide (5.71%), and trans-caryophyllene (4.89%) were among the main components in the oil isolated of *A. millefolium* subsp. *millefolium* from Elazig pattern.

The major component of all studied *A. millefolium* subsp. *millefolium* taxa was 1,8-cineole (eucalyptol), suggesting that the essential oils which were analyzed belongs to the 1,8-cineole chemotype. In addition to 1,8-cineole, α-pinene, β-pinene, sabinene, and α-terpineole were detected in all patterns. The essential oil composition of *Achillea* species revealed that 1,8-cineole was the most abundant compound, ranging from trace levels to 47.7% as in essential oils of Balkan *Achillea* (Radulovic et al., 2007).

In conclusion, this study demonstrates the occurrence of 1,8-chemotype of all samples (*A. millefolium* subsp. *millefolium*) grown in Bingol. Especially 1,8-cineole is known its anti-inflammatory effects and it is also considered useful for sinusitis and bronchitis (Santos and Rao, 2000). Therefore, this result gives some clues about usability of these taxa as natural products especially in medicinal field.

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Table 1. Constituents of the essential oils of *A. millefolium* subsp. *millefolium* taxa (% Rate)

No	Compounds	RRI	*Achillea millefolium* subsp. *millefolium* var. *millefolium*					
			Karliova	Yedisu	Ekinyolu	Ilicalar	Sancak	Genc
			%	%	%	%	%	%
1	α-pinene	948	7.16	11.24	5.83	3.91	4.73	4.21
2	Camphene	1028	3.30	-	0.53	-	-	0.64
3	β-pinene	1176	6.43	1.66	8.83	1.52	1.75	5.39
4	1,3,6-octatriene	1177	-	-	-	7.44	1.02	-
5	3-carene	1191	-	-	-	1.08	-	-
6	Sabinene	1208	3.27	10.18	5.56	4.25	8.92	20.25
7	α-terpinene	1217	0.57	-	1.05	-	-	0.93
8	Cis-ocimene	1266	-	-	2.20	-	-	-
9	1,6-octadiene	1267		-	-	-		3.65
10	γ-terpinene	1276	2.24	-	2.16	-	0.91	1.66
11	1,8-cineole	1323	28.88	24.33	33.28	31.18	23.86	25.31
12	β-cimen	1357	1.97	3.28	3.47	1.95	1.97	3.39
13	Artemisiaketon	1475	0.92	-	-	-	-	-
14	1,4-hexdiene	1509	-	-	-	0.40	-	-
15	1-octen-3-ol	1518	0.73	-	0.40	0.80	-	0.54
16	Nonanal	1559	0.19	-	0.13	-	-	-
17	Artemisaalcohol	1593	-	-	0.33	-	-	-
18	4-thujanol	1602	2.26	1.77	3.25	1.56	1.58	2.33
19	Trans-chrizanthenol	1630	0.63	-	-	-	3.60	-
20	Linalool	1646	-	0.73	-	-	1.09	-
21	Butanoic asid	1665	-	-	-	2.31	-	-
22	β-thujone	1680	-	-	-	-	23.46	-
23	Artemisiaalcohol	1693	-	-	-	0.75	-	-
24	Chrisanthenylacetate	1701	0.73	1.87	0.82	4.34	1.25	-
25	Caryophyllene	1709	-	1.45	2.33	0.85	-	1.53
26	Cis-sabinenhydrate	1714	2.93	1.63	-	1.35	-	1.74
27	Lavandulolacetate	1735	0.97	-	-	-	1.18	-
28	Benzaldehyde	1750	0.32	-	0.63	0.35	-	0.26
29	Endo-bornylacetate	1765	3.57	8.81	3.96	1.78	5.13	7.29
30	4-thujen-2	1780	-	-	-	-	1.33	-
31	Cis-verbanol	1789	-	1.51	-	-	-	-
32	Camphene	1799	-	0.95	-	-	0.57	-
33	Camphor	1812	-	1.48	3.92	1.90	1.47	2.80
34	Trans-chrysanthenon	1816	13.31	-	-	0.96	-	-
35	Bicyclo(2,2,1) heptan-3-one	1821		-	0.99	-	-	-
36	Germakren-D	1832	-	-	0.53	-	-	1.43
37	β-bisabolenepoxide	1840	-	-	-	8.51	-	-
38	Siklohekzanol	1843	-	15.36	-	-	-	-
39	2-butenal	1855	-	-	-	2.94	-	-
40	3-cyclohexen-1-methanol	1857	-	-	0.67	-	-	-
41	α-terpineol	1868	1.05	2.62	6.25	7.19	2.64	4.46
42	Endo-borneol	1891	-	-	0.74	-	-	-
43	Borneol	1992	5.44	0.63	-	-	1.87	2.97
44	α-phellandrene-8-ol	1909	-	0.74	-	-	-	-
45	Myrtenal	1914	0.87	-	0.51	-	-	-
46	Caren	2037		-	-	-	-	1.26
47	*E*-osimenol	2041		-	-	-	-	0.21
48	Artemisia triene	2048	-	0.57	0.46	1.67	-	-
49	Myrtenol	2064	0.78	0.51	-	0.36	-	-
50	Cis-carveol	2111	-	0.34	0.32	0.21	-	-
51	2-cyclohexen-1-ol	2139	-	0.16	-	-	-	-
52	Piperitone	2170	0.91	-	-	-	-	0.15
53	Verbenone	2206	-	0.23	-	-	-	-
54	β-bisabolene	2273	-	0.18	-	-	0.45	-
55	Cyclohexen	2343	-	0.27	-	-	-	0.24
56	γ-himashalen	2391	-	-	0.22	-	-	0.12
57	α-copaen	2397		0.25	-	-	1.03	-
58	α-cubebene	2399	1.23	-	-	-	-	-
59	Caryophyllene oxide	2423	0.92	1.51	0.20	0.95	0.64	0.98
60	Farnesol	2434	-	0.40	-	-	-	-
61	Phenol	2456	-	-	0.37	-	-	-
62	Spathulenol	2460	-	0.34	-	-	0.30	0.55
63	Chrysanthenone	2486	-	0.66	-	-	-	-
64	Bisabolene oxide	2550		-	-	-	0.35	-
65	Azulen	2559	0.24	-	-	-	-	-
66	2-naphtalen methanol	2585	-	-	-	-	1.72	-
67	Adamantene	2638	0.52	-	0.16	0.86	-	-
68	Dehydroaromadendrene	2744	0.27	0.24	0.51	0.74	-	0.26
69	2-cyclohexen-1-one	2763	-	-	-	-	0.11	0.71
		Total	92.61	95.90	90.61	92.11	92.93	95.26

*RRI. Relative Retention Indices