The Essential Oil Constituents and Antimicrobial Activity of *Anthemis aciphylla* Boiss. var. *discoidea* Boiss.

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The essential oil of aerial parts, leaves and flowers of the endemic *Anthemis aciphylla* Boiss. var. *discoidea* Boiss. (Asteraceae) were obtained by hydrodistillation. The oils were analyzed both by GC and GC-MS on a polar column. The monoterpenes α -pinene (9–49%) and terpinen-4-ol (22–32%) were characterized as the main constituents. An unknown component isolated from the essential oil was characterized by means of MS, HR-MS, FT-IR, 1D- and 2D-NMR techniques as isofaurinone (1). Furthermore, the biological activity of the essential oils was evaluated in various human pathogenic microorganisms using the broth microdilution method. Weak to moderate inhibitions (0.06–1.0 mg/ml) was observed.

Key words *Anthemis aciphylla* var. *discoidea*; Asteraceae; essential oil; isofaurinone; antimicrobial activity

The genus *Anthemis* L. (Asteraceae) is represented in the flora of Turkey by 81 taxa belonging to 51 species, 29 of which are endemic to Turkey. *Anthemis aciphylla* Boiss. var. *discoidea* Boiss. is also one of the endemic species.^{1,2} *Anthemis* species are known to possess various biological activities and are commonly used in folk medicine. *A. nobilis* L., commonly known as Roman chamomile, is used like German chamomile (*Matricaria chamomilla* L.) and in Turkey, it is considered superior to German chamomile. Dried flowers of *A. altissima* L., *A. hyalina* DC., *A. arvensis* L., *A. auriculata* Boiss., *A. chia* L., *A. cotula* L. and *A. tinctoria* L. are used as a replacement for German chamomile and as stimulant, emmenagogue, and carminative infusions.³

A literature survey showed the presence of acetylenes, dehydromatricaria ester, thioenolether, and several sesquiterpenes which were isolated from the aerial parts and the roots of *A. aciphylla* var. *discoidea*.⁴ To the best of our knowledge, this is the first report on the essential oil composition and biological activity of the different parts of this species.

Results and Discussion

Dried aerial parts, leaves and flowers were subjected to hydrodistillation and the obtained essential oils were analysed by GC and GC/MS. Overall 66, 77 and 62 compounds were characterized in these oils of *A. aciphylla* var. *discoidea*, respectively. The main components were identified as α -pinene (49.4, 9.4, 39.0%) and terpinen-4-ol (21.8, 24.3, 32.1%), respectively. Non-oxygenated monoterpenes were the predominant group of constituents in the essential oil of aerial parts and flowers while oxygenated monoterpenes were the predominant group of constituents in the leaves. The cumulative results are given in Table 1.

Interestingly, an unknown constituent with 222 [M⁺] was detected in the GC-MS analysis. The amount of this compound was 5.2% in the leaf oil which enabled its isolation by silica gel column chromatography to afford a new sesquiterpene, isofaurinone (1) related to faurinone (2) from *Valeriana officinalis* first reported by Hikino *et al.*,⁵ which is known to be a rearranged derivative of eudesmane. Some stereochemical variations have been reported.^{5,6} Compound

1 was prepared from (–)-carvomenthone by a multi-step synthetic procedure.⁷ However, a new structure (3) for faurinone was proposed with careful analysis of ¹H- and ¹³C-NMR spectra by Bos *et al.*⁸ However, to the best of our knowledge, the stereochemistry indicates it is a new essential oil derivative from a natural source. Influenced by the biogenetical evidence that the C-7 isopropyl group is in the β -orientation, circular dichroism studies along low resolution NMR was employed formerly to deduct the absolute chemistry of the eudesmane sesquiterpenes.⁵ In the present work, structure elucidation of 1 was carried out using spectral techniques. A new sesquiterpene (1) was obtained as a colorless oil, whose molecular formula, C₁₅H₂₆O was established by high resolution electron impact mass spectroscopy (HR-EIMS) ([M]⁺ *m/z* 222.1977). The FT-IR spectrum of 1 indicated the presence of a carbonyl (1716 cm^{–1}) group, while the ¹³C-NMR spectrum of 1 showed 15 carbon signals. As indicated in Table 3, the ¹H- and ¹³C-NMR spectra of 1 showed the presence of two secondary methyl groups [δ _H 0.83 (6H, d, *J*=6.8 Hz, H-12, 13); δ _C 19.5 (q), 19.7 (q)], one tertiary methyl [δ _H 0.92 (3H, s); δ _C 25.2 (q)] and one acetyl [δ _H 2.15 (3H, s); δ _C 28.2 (q), 212.4 (s)]. The ¹H-NMR spectral data of isofaurinone (1) were similar to those of faurinone (2) except for the chemical shifts of one secondary methyl [δ _H 0.74 (3H, d, *J*=5.0 Hz, H-12 or H-13)] and one tertiary methyl group [δ _H 1.03 (3H, s, H-14)]. Compound 1 showed the correlations between (i) H-3/C-1 and C-4; (ii) H-6/C-4 and C-8 (iii) H-12/C-7, C-11 and C-13; (iv) H-13/C-7, C-11 and C-12; (iv) H-15/C-4 and C-5 in the HMBC spectrum (Fig. 1), and the NOEs between (i) H-7 and H-9; (ii) H-12/H-8 β and H-11; (iii) H-13/H-6 β and H-11; (iv) H-14/H-3 β and H-9 β ; and (v) H-15/H-3 α and H-6 α in the NOESY spectrum (as seen in Fig. 1). From the above described spectral evidence, the relative structure of the new compound (1) was named isofaurinone, which was deduced to be a regioisomer of the acetyl group of faurinone (2) at C-5. The sesquiterpene-skeleton of 1 was very rare. Only 3 α ,11-dihydroxy-isoiphonnan-4-one-[α -xylopyranoside] from *Iphiona scabra* is known in the natural kingdom.⁹

Furthermore, the essential oils were subjected to an an-

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Table 1. The Composition of the Essential Oils of *Anthemis aciphylla* var. *discoidea*

No.	Compound	RRI	A (%)	B (%)	C (%)
1	α -Pinene	1032	49.4	9.4	39.0
2	Hexanal	1093	—	—	0.1
3	β -Pinene	1118	4.2	1.6	4.3
4	Sabinene	1132	0.5	0.1	0.5
5	Myrcene	1174	0.6	0.1	0.6
6	α -Terpinene	1188	0.3	tr	0.3
7	Limonene	1203	1.5	2.1	3.4
8	1,8-Cineole	1213	0.1	0.1	0.1
9	β -Phellandrene	1218	0.1	—	0.1
10	Amyl furan (=2-Pentyl furan)	1244	0.1	tr	0.1
11	(Z)- β -Ocimene	1246	0.1	—	0.1
12	γ -Terpinene	1255	0.6	0.1	0.4
13	(E)- β -Ocimene	1266	0.5	tr	0.5
14	<i>p</i> -Cymene	1280	0.5	0.5	0.4
15	Terpinolene	1290	0.3	0.1	0.2
16	Octanal	1296	0.1	0.1	—
17	Nonanal	1400	0.4	0.4	0.4
18	γ -Campholene aldehyde	1439	—	0.1	—
19	(E)-2-Octenal	1441	0.2	0.1	0.2
20	α -Copaene	1497	0.6	0.9	0.5
21	α -Campholene aldehyde	1499	—	0.8	—
22	Decanal	1506	—	0.4	—
23	Chrysanthenone	1522	—	0.1	—
24	β -Bourbonene	1535	0.2	0.8	0.3
25	(E)-2-Nonenal	1548	0.1	0.2	0.2
26	Linalool	1553	0.1	0.2	0.1
27	Octanol	1562	—	0.1	0.1
28	<i>trans-p</i> -Menth-2-en-1-ol	1571	0.1	0.2	0.1
29	Pinocarvone	1586	—	0.5	—
30	Terpinen-4-ol	1611	21.8	24.3	32.1
31	Octyl 2-methyl butyrate	1634	0.1	—	—
32	<i>cis-p</i> -Menth-2-en-1-ol	1638	—	0.2	0.1
33	Myrtenal	1648	—	0.5	—
34	Octyl 3-methyl butyrate (=Octyl isovalerate)	1654	0.2	—	—
35	(E)-2-Decenal	1655	tr	0.7	0.2
36	<i>cis</i> -Verbenol	1663	tr	0.7	—
37	Nonanol	1664	tr	—	—
38	(Z)- β -Farnesene	1668	0.1	—	—
39	<i>trans</i> -Pinocarveol	1670	0.2	1.1	0.2
40	<i>p</i> -Mentha-1,5-dien-8-ol	1674	—	0.2	—
41	<i>trans</i> -Verbenol	1683	0.3	2.7	0.3
42	Salicylaldehyde	1703	—	—	0.1
43	γ -Curcumene	1704	0.3	0.4	0.5
44	α -Terpineol	1706	0.2	0.5	0.4
45	Borneol	1719	—	0.3	—
46	α -Zingiberene	1726	0.2	—	0.4
47	Germacrene D	1726	1.0	0.9	1.2
48	(Z,E)- α -Farnesene	1737	—	0.1	—
49	β -Bisabolene	1741	0.3	0.3	0.4
50	Carvone	1751	—	0.4	—
51	Bicyclogermacrene	1755	0.1	—	—
52	<i>cis</i> -Piperitol	1758	—	0.1	—
53	(E)-2-undecenal	1764	0.1	0.4	—
54	Decanol	1766	—	—	0.1
55	δ -Cadinene	1773	0.3	0.3	0.4
56	γ -Cadinene	1776	0.1	0.3	0.1
57	(E,Z)-2,4-Decadienal	1779	0.1	0.1	0.1
58	<i>ar</i> -Curcumene	1786	tr	0.1	0.1
59	Myrtenol	1804	0.1	0.8	0.1
60	(E,E)-2,4-Decadienal	1827	0.2	0.5	0.2
61	<i>trans</i> -Carveol	1845	—	0.7	0.2
62	<i>p</i> -Cymen-8-ol	1864	—	0.4	—
63	(E)-Geranyl acetone	1868	—	tr	—
64	<i>cis</i> -Carveol	1882	—	0.1	—
65	Geranyl isovalerate	1893	—	0.1	—
66	Dodecyl acetate	1893	0.1	—	—

Table 1. continued

No	Compound	RRI	A (%)	B (%)	C (%)
67	Isofaurionone (=Compound 1)	1904	0.5	5.2	0.5
68	Caryophyllene oxide	2008	0.3	1.1	0.2
69	Salvial-4(14)-en-1-one	2037	0.1	0.6	—
70	Pentadecanal	2041	0.1	tr	0.1
71	Ledol	2057	—	0.4	—
72	Octanoic acid	2084	—	0.2	0.1
73	Hexahydrofarnesyl acetone	2131	0.3	1.0	0.3
74	Spathulenol	2144	0.3	0.8	0.3
75	(Z)-3-Hexen-1-yl benzoate	2148	0.1	—	—
76	Tetradecanol	2179	—	0.5	—
77	Nonanoic acid	2192	0.1	0.9	0.2
78	Thymol	2198	0.2	0.9	0.1
79	4-Vinyl guaiacol	2218	tr	—	tr
80	Carvacrol	2239	0.1	0.1	tr
81	α -Cadinol	2255	0.1	—	0.3
82	Selin-11-en-4 α -ol	2273	—	0.2	—
83	Decanoic acid	2298	0.4	1.5	0.6
84	Tricosane	2300	0.6	0.6	0.2
85	γ -Dodecalactone	2396	—	0.3	—
86	Pentacosane	2500	tr	0.2	tr
87	Dodecanoic acid	2503	0.5	1.7	0.5
88	1-Octadecanol	2607	0.1	0.5	—
89	Tetradecanoic acid	2670	0.2	0.9	0.1
90	Heptacosane	2700	0.2	0.1	0.1
91	Nonacosane	2900	0.2	0.5	0.1
92	Hexadecanoic acid	2931	0.3	1.3	0.2
Monoterpenes			58.6	14.0	49.8
Oxygenated monoterpenes			23.2	35.6	33.8
Sesquiterpenes			3.2	4.6	3.9
Oxygenated sesquiterpenes			1.3	8.3	1.3
Others			4.8	13.2	4.3
Identified compounds			66	77	62
Total			91.1	75.7	93.1

A: aerial parts, B: leaves, C: flowers. RRI: Relative retention indices calculated against *n*-alkanes. %: calculated from FID data. tr: trace (<0.1%).

Fig. 1. Chemical Structures of Isofaurionone (1) and Faurionone (2, 3)

Fig. 2. HMBC and NOESY Spectra of Isofaurionone (1) in CDCl₃Table 2. ¹H- and ¹³C-NMR Data of Compound 1^{a)}

Position no.	¹ H (δ)	¹³ C (δ)
1 α	2.00 (m)	34.9 (t)
1 β	1.14 (m)	
2	1.72 (m)	18.0 (t)
3 α	1.66 (m)	34.8 (t)
3 β	2.18 (m)	
4		212.4 (s)
5		59.9 (s)
6 α	1.47 (br d, $J=11.8$)	33.4 (t)
6 β	1.11 (dd, $J=5.5, 11.8$)	
7	1.14 (m)	38.8 (d)
8 α	1.52 (m)	24.8 (t)
8 β	1.15 (m)	
9 α	1.42 (m)	35.2 (t)
9 β	1.88 (m)	
10		42.7 (s)
11	1.34 (m)	32.4 (d)
12	0.83 (d, $J=6.8$)	19.5 (q) ^{b)}
13	0.83 (d, $J=6.8$)	19.7 (q) ^{b)}
14	0.92 (s)	25.2 (q)
15	2.15 (s)	28.2 (q)

^{a)} Chemical shifts from TMS (multiplicity, J in Hz) in CDCl₃ (Assignments from ¹H-¹H COSY, NOESY, HMQC and HMBC spectra, see Fig. 1). ^{b)} May be interchanged in each vertical column.

timicrobial bioassay⁸⁾ for the determination of the minimal inhibitory concentrations (MIC). The results are given in Table 3. As an overall result, the essential oils showed weak to moderate inhibitory effects on the microorganisms used. Almost all of the essential oils tested were weaker in inhibition when compared to the standard antimicrobial agents chloramphenicol and ketoconazole at the same tested con-

centration. *Staphylococcus epidermidis* was most strongly inhibited by *Anthemis* oils with MIC values of 0.125, 0.06 and 0.25 mg/ml, respectively.

Table 3. Antimicrobial Activity (MIC, mg/ml) of *Anthemis aciphylla* var. *discoidea* Oils

Microorganisms	A	B	C	ST
<i>Escherichia coli</i>	1.0	0.5	1.0	0.06
<i>Staphylococcus aureus</i>	0.5	0.5	1.0	0.007
<i>Pseudomonas aeruginosa</i>	1.0	0.5	0.25	0.25
<i>Enterobacter aerogenes</i>	1.0	1.0	0.5	0.125
<i>Staphylococcus epidermidis</i>	0.125	0.06	0.25	0.007
<i>Salmonella typhimurium</i>	1.0	0.5	1.0	0.06
<i>Candida albicans</i>	0.5	0.25	0.5	0.06 ^{a)}

A: aerial parts, B: leaves, C: flowers, ST: chloramphenicol. a) Ketoconazole for the yeast.

Experimental

General Experimental Procedures IR was measured using a JASCO-FT-IR-5300 apparatus. ¹H- and ¹³C-NMR spectra were recorded on Varian UNITY 600 system at 600 and 150 MHz, respectively. Chemical shifts are given relative to tetramethylsilane (TMS) at 0.0 ppm as internal standard in CDCl₃. 1D- and 2D-NMR data were also obtained using the same system, and the spectra were measured and reported in ppm. HR-MS analysis was conducted using a JEOL JMS-AX-500 system. All chemicals were obtained from Sigma/Aldrich.

Plant Material The plant material was collected in Eskişehir: Bozdağ, Turkey, in July 2001. Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskişehir, Turkey (ESSE 13180).

Hydrodistillation Air dried aerial parts, leaves and flowers of plant material was hydrodistilled for 3 h using a Clevenger-type apparatus to yield essential oil (0.09%, 0.11%, 0.35%, respectively).

Gas Chromatography (GC) The oils were analyzed by GC using a Hewlett Packard 6890 system. An HP-Innowax FSC column (60 m×0.25 mm inner diameter, with 0.25 μm film thickness) was used with nitrogen as carrier gas (1 ml/min). The oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, then kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. The injector temperature was set at 250 °C. The percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID, 250 °C). *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentages of the characterized components were as cited in Table 1.

Gas Chromatography-Mass Spectrometry (GC-MS) The essential oils were analysed by gas chromatography/mass spectrometry using a Hewlett-Packard GCD system. An Innovax FSC column (60 m×0.25 mm inner diameter, 0.25 μm film thickness) was used with helium as carrier gas. The GC oven temperature was as above. Split flow was adjusted at 50 ml/min. The injector temperature was 250 °C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 425. A library search was carried out using both the "Wiley GC/MS Library" and the in-house "Baser Library of Essential Oil Constituents".

Isolation of Isofaurione (1) The compound was isolated by micro-column chromatography. Silica Gel 60 G (ca. 1 g, Merck 7734) was used as the packing material and was added to a Pasteur pipette with wet *n*-hexane. *n*-Hexane in diethylether (100%→0%) was used as the eluent in the gradient system. Essential oil (15 mg) was applied and eluted with *n*-hexane:diethylether (99:1) to yield **1** (2.1 mg) as a colorless oily material.

FT-IR $\nu_{\max}$ (cm⁻¹): 2956, 2870, 1716 (C=O), 1463, 1385, 879; The ¹H- and ¹³C-NMR data are given in Table 2; HR-EI-MS 222.1977 [M]⁺ *m/z*: (Calcd for C₁₅H₂₆O 222.1984), EI-MS *m/z* (rel. int.)=222 (3) [M⁺], 207 (1), 179 (6), 161 (1), 149 (1), 137 (2), 125 (100), 109 (9), 95 (11), 81 (10), 69 (7), 55 (7), 43 (33).

Antimicrobial Assay *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus epidermidis* (ATCC 12228), *Enterobacter aerogenes* (NRRL 3567), *Salmonella typhimurium* (NRRL 4420), and *Candida albicans* (clinical isolate, Osmangazi University, Faculty of Medicine, Eskişehir, Turkey) were used as test microorganisms. The bacteria and yeast were refreshed in Mueller Hinton Broth (Merck, Germany) at 35–37 °C prior to use.

A micro-dilution broth susceptibility assay was performed according to our previously described method.¹⁰⁾ Stock solutions of the essential oils were prepared in diluted dimethylsulfoxide (Carlo-Erba, France). Dilution series were prepared from 2 mg/ml to 0.001 mg/ml in sterile distilled water in micro-test tubes from where they were transferred to 96-well microtiter plates. All microorganisms grown overnight in double strength Mueller Hinton Broth were standardized to approx. 10⁸ CFU/ml (using McFarland No: 0.5). The last row containing only the serial dilutions of sample without microorganism was used as a negative control. Sterile distilled water and medium served as a positive growth control. After incubation at 37 °C for 18–24 h, the minimal inhibitory concentrations (MIC) were determined (see Table 3). Chloramphenicol and ketoconazole (Sigma, Germany) were used as standard antimicrobial agents.

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