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RESEARCH ARTICLE

Chemical composition and antimicrobial activity of essential oils from *Centaurea appendicigera* and *Centaurea helenioides*

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Abstract
The chemical components and antimicrobial activity of the essential oils from *Centaurea appendicigera* C. Koch and *Centaurea helenioides* Boiss, two different endemic members of the genus *Centaurea* L. (Asteraceae), were studied. The essential oils of air-dried *C. appendicigera* and *C. helenioides* were obtained by hydrodistillation in a Clevenger-type apparatus and analyzed by GC-MS. Forty-five and fifty-one components were identified in the essential oils of *C. appendicigera* and *C. helenioides*, respectively, and the main components of these taxa were found to be *β*-caryophyllene (17.5%) from *C. appendicigera* and caryophyllene oxide (18.2%) from *C. helenioides*. The antimicrobial activity of the isolated essential oil of the plants was also investigated, and demonstrated moderate antibacterial activity against Gram-positive, Gram-negative bacteria, and yeast-like fungi.

Keywords: *Centaurea appendicigera, Centaurea helenioides, Asteraceae, essential oil, antimicrobial activity, GC-MS*

Introduction

Centaurea L. is one of the most important genera of the family Asteraceae. The genus *Centaurea* is represented by 179 native species and 109 of them are endemic in Turkey (Davis, 1988; Güner et al., 2000). Many members of this genus, such as *C. behen* L., *C. cyanus* L., *C. calcitropa* L., were used in Anatolian folk treatment (Baytop, 1995; Yeşilada et al., 1999). *C. appendicigera* C. Koch and *C. helenioides* Boiss both are endemic taxa for Turkey and distributed mainly in East Anatolia. They are herbaceous perennial herbs growing in screes and dry lands (Wagenitz, 1975). According to the Red Data Book of Turkish Plants (Ekim et. al, 2000), their IUCN threatened categories are LR (Lower Risk).

Essential oils are a very complex mixture of natural compounds. The constituents of the oils are mainly terpenes or oxygenated compounds derived from these hydrocarbons. The chemical composition of essential oils differs in each species or subspecies. The compositional studies of essential oils have been carried out extensively by using gas chromatography-mass spectrometry (GC-MS), which is based on the comparison of the relative retention times/indices and mass spectra of the specific natural compounds found in an essential oil (Adams, 2004; Flamini et al., 2002; Dural et al., 2003; Skaltsa et al., 2003; Simiç et al., 2004; Javidnia et al., 2005; Yaylı et al., 2005; Fokialakis et al., 2002; Jovanovic et al., 2004; Küçük et al., 2006).

Volatile constituent studies on *Centaurea* species were described in our previous work (Yaylı et al., 2005) and in the literature (Ertuğrul et al., 2003; Dural et al., 2003; Senatore et al., 2005). As a result of our literature search, no published record has been found for

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the volatile chemical composition and antimicrobial activity of the essential oils of *C. appendicigera* and *C. helenioides*. However, in our previous chemical investigation of the chloroform extract of air-dried flowers of *C. helenioides*, grosheimin and cynaropicrin sesquiterpenes were isolated and characterized by spectral techniques (Yaylı et al., 2006). Hence, the systematic research was carried out by the extraction of the essential oil constituents of the plants by hydrodistillation in a Clevenger-type apparatus. The obtained crude essential oils were then investigated by GC-MS. Identification of the compounds was made by a typical library search (NIST, WILEY) and literature comparison (Adams, 2004; Flamini et al., 2002; Dural et al., 2003; Ertuğrul et al., 2003; Skaltsa et al., 2000, 2003; Simiç et al., 2004; Javidnia et al., 2004, 2005; Couladis et al., 2002; Fokialakis el al., 2002, 2003; Yaylı et al., 2005; Jovanovic et al., 2004; Küçük et al., 2006).

Materals and methods

Plant material

C. appendicigera C. Koch was collected in Rize, İkizdere Ovit Mountain (A8), scree in alpine meadows and *C. helenioides* was collected in Trabzon-Çaykara Demirkapı (Haldizen) plateau, alpine meadows (at heights of ~2285 m and 2920 m) in the northeastern part of Turkey on 24 and 30 July 2005, respectively. Voucher specimens (no. Coşkunçelebi 553-2005 and 559-2005, KTUB) were deposited in the Herbarium of the Department of Biology, Karadeniz Technical University, Turkey. The plant was identified immediately after collection (Davis, 1988) and air-dried at room temperature for later analysis.

Isolation of the essential oils

The air-dried whole plants (~52 g, each) of *C. appendicigera* and *C. helenioides* were hydrodistilled in a Clevenger-type apparatus using an ice bath for cooling (4 h). The resulting oils were dissolved in HPLC grade *n*-hexane (0.5 mL), dried over anhydrous sodium sulphate, and stored at 4-6°C in a sealed brown vial. The extracts (1 μL) were directly injected into the GC-MS instrument. The percentage yields of the oils from *C. appendicigera* and *C. helenioides* calculated on a moisture-free basis were 0.18 and 0.15 ± 0.1 (v/w), respectively.

GC-MS analysis

GC-MS analyses were as described previously (Yayli et al., 2005).

Identification of components

The components of the oil were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Willey) and confirmed by comparison of their retention indices with data published in the literature (Adams, 2004; Flamini et al., 2002; Skaltsa et al., 2000, 2003; Jovanovic et al., 2004; Javidnia et al., 2005; Ertuğrul et al., 2003; Yayli et al., 2005).

Antimicrobial activity

All test microorganisms were obtained from the Refik Saydam Hifzissihha Institute (Ankara, Turkey) and are as follows: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas auroginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 709 ROMA, *Candida albicans* ATCC 10231.

Agar well diffusion method

The agar well diffusion method was adopted (Ahmad et al., 1998; Perez et al., 1990). Overnight cultures of microorganisms were adjusted to approximately 10^6 cfu/mL according to McFarland turbidity standards and spread over the appropriate media (Mueller-Hinton agar (Difco, Detroit, MI) for bacteria, Sabouraud Dextrose agar (Difco, Detroit, MI) for yeast) in Petri dishes. Wells of 5 mm diameter were punched into the agar medium and filled with 100 μL of essential oil solutions. The plates were incubated at 37°C for 18-48 h, and the inhibition zones around the wells were measured (data not shown). The antimicrobial effects of solutions that produce 6 mm zones of inhibition were tested quantitatively in respective broth media by using double dilution, and the minimal inhibition concentration (MIC) values (μg/mL) were determined.

The antibacterial and antifungal assays were performed in Mueller-Hinton broth at pH 7.3 and buffered yeast nitrogen base (Difco, Detroit, MI) at pH 7.0, respectively, in 96-well plates according to the National Committee for Clinical Laboratory method. The MIC was defined as the lowest concentration that showed no growth. Ampicillin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. The samples were dissolved in chloroform to prepare sample stock solution. Chloroform with dilution of 1:10 was used as solvent control. The results are shown in Table 4.

Results and Discussion

For the study of essential oil composition, dried whole plants of *C. appendicigera* and *C. helenioides* [with the yields of 0.18 and 0.15 ± 0.1 (v/w) on dry weight basis,

respectively] were subjected to hydrodistillation in a Clevenger-type apparatus (Yu et al., 2004; Baser et al., 2002; Tunalıer et al., 2002). The general chemical profile of the essential oils, the percentage content, and retention indices of the constituents, are summarized in Table 1. The essential oils of *C. appendicigera* and *C. helenioides* were analyzed by GC-MS with HP-5 column. A total of 45 and 51 compounds were identified in the hydrodistillates from both plants on the basis of a typical library search and literature data with selecting only the components showing matches exceeding 80%, which represented about 85.5 and 83.7% of the essential oils

Table 1. Identified components in the essential oils of *C. appendicigera* and *C. helenioides*[a].

		A		B			
No.	Compounds	% Area	Q (%)	% Area	Q (%)	Exp. RI	Ident. LRI
1	2-Heptanone	0.4	91	-	-	892	892
2	Benzaldehyde	0.1	83	-	-	965	963
3	2-Pentylfuran	0.4	94	0.1	91	994	991
4	*n*-Octanal	0.2	91	0.1	91	1003	1001
5	Limonene	0.7	86	tr	93	1024	1027
6	Benzeneacetaldehyde	0.4	91	0.2	91	1046	1045
7	Linalool	0.4	81	0.2	91	1094	1091
8	*n*-Nonanal	0.4	90	0.6	91	1101	1102
9	1-Nonanol	0.2	86	-	-	1166	1169
10	Safranal	0.2	83	-	-	1198	1197
11	*n*-Decanal	0.6	91	0.1	91	1205	1204
12	Pulegone	-	-	0.2	83	1235	1237
13	Dihydroedulan I	-	-	2.6	92	1288	1289
14	Thymol	-	-	0.1	93	1296	1294
15	*n*-Undecanal	0.2	96	-	-	1306	1306
16	(*E*,*E*)-2,4-Decadienal	0.3	81	0.2	93	1317	1317
17	α-Copaene	1.6	99	0.6	98	1377	1377
18	(*E*)-β-Damascenone	1.8	87	0.6	96	1385	1385
19	Tetradecane	-	-	0.3	91	1400	1400
20	Dodecanal	1.0	91	-	-	1410	1409
21	β-Caryophyllene	17.5	99	3.5	99	1421	1420
22	β-Copaene	-	-	0.4	96	1431	1432
23	α-*trans*-Bergamotene	1.7	96	-	-	1438	1436
24	Aromadendrene	1.9	96	-	-	1442	1441
25	α-Humulene	2.1	99	1.0	98	1456	1455
26	(*E*)-β-Farnesene	-	-	0.7	97	1459	1458
27	δ-Gurjunene	7.6	85	-	-	1474	1473
28	γ-Muurolene	-	-	0.6	82	1478	1478
29	Germacrene-D	-	-	7.3	99	1483	1482
30	β-Ionone	-	-	1.5	98	1486	1485
31	Bicyclogermacrene	1.5	98	1.2	94	1497	1496
32	1-Pentadecene	0.4	99	-	-	1492	1491
33	(*E*,*E*)-α-Farnesene	-	-	0.9	98	1509	1508
34	β-Bisabolene	1.9	99	-	-	1510	1510
35	γ-Cadinene	4.5	93	-	-	1511	1513
36	α-Amorphene	1.4	95	0.6	88	1516	1516
37	δ-Cadinene	1.3	92	1.4	81	1526	1524
38	α-Cadinene	-	-	0.4	96	1540	1538
39	Nerolidol	-	-	0.6	95	1567	1564
40	1,5-Epoxysalvial-4(14)-ene			2.1	99	1569	1569
41	Spathulenol	2.7	98	5.1	98	1581	1578
42	Caryophyllene oxide	17.1	90	18.2	86	1586	1584
43	Salvial-4(14)-en-1-one	1.1	99	2.8	99	1596	1598
44	Humulene epoxide II	1.5	91	3.6	83	1611	1614

Table 1 Continued on next page

Table 1. Continued

45	*epi*-Cedrol	-	-	0.7	93	1620	1619
46	3-*iso*-Thujopsanone	-	-	3.7	83	1639	1643
47	β-Eudesmol	1.3	93	1.0	87	1653	1653
48	*ar*-Turmerone	2.2	90	1.3	80	1669	1669
49	Caryophyllenol-II	1.8	95	3.1	91	1675	1676
50	*epi*- α-Bisabolol	-	-	2.1	80	1688	1685
51	2-Pentadecanone	0.4	87	-	-	1699	1694
52	(*E*)-2-Tetradecen-1-ol	0.6	91	-	-	1714	1713
53	Pentadecanal	0.6	91	1.7	91	1715	1715
54	Benzyl benzoate	1.4	97	-	-	1767	1763
55	Hexahydrofarnesylacetone	0.4	91	1.0	99	1847	1846
56	Ethyl linoleolate	0.2	91	0.4	90	1894	1891
57	*n*-Nonacosane	-	-	0.3	89	1901	1900
58	Farnesyl acetone	-	-	0.3	91	1920	1919
59	*n*-Heneicosane	0.2	96	0.4	98	2101	2100
60	*cis*-Phytol	0.5	86	6.2	86	2115	2213
61	*n*-Docosane	-	-	0.1	91	2200	2200
62	Neophytadiene	-	-	0.1	96	2222	2218
63	*n*-Tricosane	0.6	99	1.0	99	2300	2300
64	*n*-Tetracosane	-	-	0.2	98	2400	2400
65	*n*-Pentacosane	0.6	96	0.8	99	2500	2500
66	*n*-Heptacosane	1.6	96	1.5	99	2700	2700

Unknown	RI	*m/z* (%)	A	B
Un-1	1395	204(5), 189(17), 137(100), 121(40), 95(24)	1.7	-
Un-2	1555	220(4), 205(20), 123(58), 106(78), 91(80), 79(100).	1.5	-
Un-3	1646	220(20), 204(41), 135(64), 105(83), 91(100), 79(76)	-	0.6
Un-4	1661	246(2), 206(10), 133(100), 119(30), 91(54), 79(38)	-	4.3
Un-5	1663	220(5), 205(15), 133(60), 119(75), 91(100), 79(77)	1.3	-
Un-6	1684	220(32), 177(90), 123(100), 107(48), 91(62), 81(55)	-	0.9
Un-7	1744	220(10), 218(52), 175(94), 105(80), 91(100), 79(82)	-	1.7
Un-8	1760	220(31), 177(80), 105(70), 91(100), 79(68), 67(46)	-	1.2
Un-9	1769	236(12), 218(22), 147(49), 105(57), 95(60), 81(100)	-	1.9
Un-10	1775	220(5), 218(53), 173(75), 105(77), 91(100), 79(64)	-	0.9
Un-11	1798	220(41), 189(100), 133(36), 105(40), 91(53), 79(32)	-	0.6
Un-12	1870	243(6), 223(18), 167(10), 149(100), 57(20)	-	1.0
Un-13	1881	302(2), 287(2), 97(80), 83(100), 69(80), 55(92)	1.4	-
Un-14	2146	287(7), 278(39), 121(60), 93(64), 79(84), 67(100)	-	0.9
Un-15	2150	302(15), 287(10), 178(27), 134(100), 119(60), 81(57)	1.5	-
Un-16	2619	429(7), 302(38), 207(30), 135(38), 71(77), 57(100)	1.4	-
Total			8.8	14.0
Total isolate		85.5	83.7	
Total unknown		8.8	14.0	
Total		94.3	97.7	

RI, retention index; LRI, literature retention index; tr, trace, less than 0.05%; Q, quality; A, *Centaurea appendicigera*; B, *Centaurea helenioides*.
[a]Compounds are listed in order of elution. RI (retention index) values are calculated from retention times relative to that of *n*-alkanes (C_6-C_{32}) on the nonpolar HP-5 column.

in *C. appendicigera* and *C. helenioides,* respectively (Adams, 2004; Dural et al., 2003; Ertuğrul et al., 2003; Skaltsa et al., 2000, 2003; Simiç et al., 2004; Javidnia et al., 2004, 2005; Couladis et al., 2002; Fokialakis et al., 2002, 2003; Yaylı et al., 2005; Jovanovic et al., 2004; Küçük et al., 2006).

The main components are completely different for both plants except for caryophyllene oxide with values of 17.1 and 18.2% in *C. appendicigera* and *C. helenioides,* respectively (Table 1). The other major compounds of the chemical class distribution were β-caryophyllene (17.5%), *n*-heptacosane (1.6%), *cis*-phytol (0.5 %), and (*E*)-β-damascenone (1.8%) in *C. appendicigera*, and germacrene-D (7.3%), dihydroedulan I (2.6%), *cis*-phytol (6.2%), pentadecanal, (1.7%), and neophytadiene (0.2%) in *C. helenioides*.

The chemical class distribution of the essential oil components are reported in Table 2. The compounds are classified into seven classes, which are monoterpene, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenes, diterpenoids, and others (Table 2). As shown in Tables 1 and 2, 30 compounds were common in the essential oils of *C. appendicigera* and *C. helenioides,* in which sesquiterpenes were the main constituents in *C. appendicigera* (43.0%) and sesquiterpenoids were the main constituents in *C. helenioides* (47.6%), respectively.

The major compounds for the chemical class distributions in the essential oils of *C. appendicigera* and *C. helenioides* are reported in Table 3, and β-caryophyllene and caryophyllene oxide were the main constituents in *C. appendicigera* and *C. helenioides,* with the ratios of 17.5 and 18.2%, respectively.

In our previous study, the chemical components and antimicrobial activity of essential oils from *Centaurea sessilis* and *Centaurea armena* were investigated (Yayli et al., 2005). The main components of these two taxons were β-eudesmol in the ratio of 12.4 and 19.3% in *C. sessilis* and *C. armena,* respectively. However, β-eudesmol has a much lower percentage, with values of 1.3 and 1.0% in C. *appendicigera* and *C. helenioides*, respectively. Caryophyllene oxide was the significant common component for all four members showing a minimum percentage of 4.7% in *C. armena* and a maximum percentage of 17.5% in *C. helenioides*. Benzaldehyde, β-damascenone, β-caryophyllene, spathulenol, caryophyllene oxide, β-eudesmol, 6,10,14-trimethyl-2-pentadecanone, and *cis*-phytol were the common compounds for the four different members (*C. sessilis*, *C. armena*, *C. appendicigera*, and *C. helenioides*) of the same genus.

The antimicrobial activities for the essential oils of *C. appendicigera* and *C. helenioides* were tested *in vitro* using the agar-well diffusion method with the microorganisms as seen in Table 4. The essential oils showed antibacterial activity against Gram-positive and Gram-negative bacteria and against the yeast-like fungus.

The test extracts showed better antimicrobial activity against Gram-positive bacteria in comparison to the

Table 2. The chemical class distribution of the essential oil components of *C. appendicigera* and *C. helenioides.*

Compound class	*C. appendicigera*		*C. helenioides*	
	% Area	Number of compounds	% Area	Number of compounds
Monoterpene	0.7	1	tr	1
Monoterpenoids	2.4	3	1.1	4
Sesquiterpenes	43.0	11	20.7	14
Sesquiterpenoids	28.1	8	47.6	14
Diterpene	-	-	0.1	1
Diterpenoid	0.5	1	6.2	1
Others	10.8	21	8.0	16
The common compounds	63.0	30	58.5	30

Table 3. Major compounds of the chemical class distribution in the essential oil components of *C. appendicigera* and *C. helenioides*.

Compound class	*C. appendicigera*			*C. helenioides*		
	Major component	% Area	RI	Major component	% Area	RI
Monoterpene	Limonene	0.7	1624	Limonene	tr	1624
Monoterpenoid	(*E*)-β-Damascenone	1.8	1385	Dihydroedulan I	2.6	1288
Sesquiterpene	β-Caryophyllene	17.5	1421	Germacrene-D	7.3	1483
Sesquiterpenoid	Caryophyllene oxide	17.1	1586	Caryophyllene oxide	18.2	1586
Diterpene	-	-	-	Neophytadiene	0.1	2222
Diterpenoid	*cis*-Phytol	0.5	2115	*cis*-Phytol	6.2	2115
Others	*n*-Heptacosane	1.6	2700	Pentadecanal	1.7	1715

Table 4. Screening results for antimicrobial activity of the essential oils from *C. appendicigera* and *C. helenioides*.

Sample	Stock solution	Microorganisms and MIC value (μg/mL)						
	μg/mL	Ec	Kp	Pa	Ef	Sa	Bc	Ca
C. appendicigera	1900	-	-	-	80	80	-	190
C. helenioides	3300	-	-	-	165	165	-	330
Ampicillin		8	32	>128	2	2	2	
Fluconazole								8

MIC, the lowest concentration causing total inhibition of microbial growth in μg/mL; Ec, *Escherichia coli* ATCC 25922; Kp, *Klebsiella pneumoniae* ATCC 13883; Pa, *Pseudomonas aeruginosa* ATCC 10145; Ef, *Enterococcus faecalis* ATCC 29212; Sa, *Staphylococcus aureus* ATCC 25923; Bc, *Bacillus cereus 702 Roma, Candida albicans* ATCC 10231; Amp., Ampicillin; Flu., Fluconazole; -, no activity at stock solution concentration.

Gram-negative bacteria. The essential oil extracts of *C. appendicigera* and *C. helenioides* showed antimicrobial activity against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 10231, but no antimicrobial activity was observed against the bacteria *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 10145, and *Bacillus cereus* 702 Roma. This is the first chemical composition analysis performed by GC-MS analytical method and antimicrobial activity report for the essential oils of *C. appendicigera* and *C. helenioides*.

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