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# Composition and antimicrobial activity of essential oils from *Centaurea sessilis* and *Centaurea armena*

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**Abstract**

The essential oils of air-dried *Centaurea sessilis* and *Centaurea armena* obtained by hydrodistillation were analyzed by gas chromatography–mass spectrometry (GC–MS). Forty and twenty components were identified in the essential oils and the main component of these taxons was β-eudesmol in the ratios of 12.4% and 19.3% from *C. sessilis* and *C. armena*, respectively. The antimicrobial activity of the isolated essential oil of the plants was also investigated. They showed moderate antibacterial activity against Gram-positive and Gram-negative bacteria, but no antifungal activity was observed against two yeastlike fungi.


*Keywords:* *Centaurea sessilis*; *Centaurea armena*; Asteraceae; Essential oil; Antimicrobial activity; GC–MS

## 1. Introduction

The genus *Centaurea* L. (Asteraceae) is represented in Turkey with 179 native species, 109 of which are endemic (Davis, 1988; Güner et al., 2000). Many member of this genus, such as *Centaurea cyanus* L., *Centaurea behen* L., *Centaurea calcitrapa* L., are used in Anatolian folk medicine (Baytop, 1995; Yeşilada et al., 1999). This genus with the rate of 61 is among the richest genera in terms of endemic species (Davis, 1988). *Centaurea sessilis* Wild. and *Centaurea armena* Boiss. both are endemic taxa for Turkey and distributed mainly in Eastern Anatolia. They are herbaceous perennial herbs grown in mountain slopes and dry lands (Wagenitz, 1975).

Essential oils are mostly natural mixtures of terpenes/terpenoids, most of which are obtained from aromatic and pharmaceutical plants. The chemical composition of essential oil differs in each species or subspecies and is characteristic for the species in question. Identification of individual components of complex mixtures such as terpenes/terpenoids in essential oils requires the use of several techniques. One of the most popular methods of studying essential oil composition is gas chromatography–mass spectrometry (GC–MS), which allows the identification of the specific natural compounds found in an essential oil by comparing their relative retention times/indices and their mass spectra (Adams, 1995; Flamini et al., 2002; Skaltsa et al., 2003, 2000; Jovanovic et al., 2004; Warthen et al., 1997; Javidnia et al., 2005; Ertugrul et al., 2003).

Volatile constituents studies are available in the literature on *Centaurea* species: *Centaurea thessala* subsp. *drakiensis*, *Centaurea zuccariniana*, *Centaurea spruneri*,

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*Centaurea raphanina* subsp. *mixta*, and *Centaurea pelia* (Lazari et al., 1999, 2000), *C. calcitrapa* and *Centaurea solstitialis* (Buttery et al., 1986; Binder et al., 1990a,b), *C. calcitrapa*, *Centaurea gloriosa*, and *Centaurea moschata* (Karawya et al., 1975; Saleh et al., 1981; Kustrak and Radic, 1985), *Centaurea pseudoscabiosa* subsp. *pseudoscabiosa*, and *Centaurea hadimensis* (Flamini et al., 2002), and *Centaurea kotschyi* var. *kotschyi* and *C. kotschyi* var. *decumbens* (Ertugrul et al., 2003). To our knowledge, there are no published reports on the chemical composition and antimicrobial activity of the essential oils of *C. sessilis* and *C. armena*. As part of this systematic research, the essential oil constituents of the plants were extracted by hydrodistillation in a Clevenger-type apparatus. The obtained crude essential oils were then investigated by GC–MS technique. Identification of the compounds was made by a typical library search (NIST, WILLEY) and literature comparison (Adams, 1995; Flamini et al., 2002; Skaltsa et al., 2003, 2000; Jovanovic et al., 2004; Warthen et al., 1997; Yu et al., 2004; Javidnia et al., 2005; Ertugrul et al., 2003). Therefore, we focused our study on the chemical composition and antimicrobial property of the essential oils of *C. sessilis* and *C. armena*.

## 2. Results and discussion

The essential oils, with pale yellow colors, were obtained by hydrodistillation in a Clevenger-type apparatus from whole plants of *C. sessilis* and *C. armena* with the yields of 0.25% and 0.18% (v/w) on dry weight basis, respectively. The essential oils of *C. sessilis* and *C. armena* were analyzed by GC–MS with HP-5 column. The general chemical profile of the essential oils, the percentage content, and retention indices of the constituents are summarized in Table 1. A total of 40 and 20 components were characterized on the basis of a typical library search and literature data with selecting only the components showing matches exceeding 80%, which represented about 66.7% and 82.7% of the essential oils from *C. sessilis* and *C. armena*, respectively (Adams, 1995; Flamini et al., 2002; Skaltsa et al., 2003, 2000; Jovanovic et al., 2004; Warthen et al., 1997; Javidnia et al., 2005; Ertugrul et al., 2003).

β-Eudesmol (12.4%), caryophyllene oxide (10.0%), phytol (6.4%), spathulenol (4.9%), and 6,10,14-trimethyl-2-pentadecanone (3.1%) were the main constituents of the essential oil of *C. sessilis*. The main components of the essential oil of *C. armena* were β-eudesmol (19.3%), calarene (10.3%), 6,10,14-trimethyl-2-pentadecanone (5.7%), and β-caryophyllene (5.4%).

The chemical class distribution of the essential oil components of the plants are reported in Table 2. The compounds were separated into six classes, which were monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenes, and others (Table 2). As it can be seen in Tables 1 and 2, 14 compounds were common in the essential oils of *C. sessilis* and *C. armena* with the total ratios of 45.8% and 64.8%, respectively. Sesquiterpenoid components were the main constituents of both oils in the ratios of 35.1% and 34.8%.

The major compounds for the chemical class distributions in the essential oils of *C. sessilis* and *C. armena* are reported in Table 3 and β-eudesmol was the most abundant constituent among all in the ratios of 12.4% and 19.3% from *C. sessilis* and *C. armena*, respectively.

The antimicrobial activities of the essential oils of *C. sessilis* and *C. armena* were tested in vitro by 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, but no antifungal activity was observed against the two yeastlike fungi.

The test extracts showed better antimicrobial activity against the Gram-positive bacteria when compared to the Gram-negative bacteria. The essential oils of *C. sessilis* and *C. armena* showed antibacterial activity against *Yersinia pseudotuberculosis* ATCC 911, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Bacillus subtilis* ATCC 6633. However, no antimicrobial activity was observed against the other five microorganisms tested, three bacteria and two fungi (Table 4). This is the first chemical composition analysis, performed by GC–MS analytical method, and antimicrobial activity report for the essential oils of *C. sessilis* and *C. armena*.

## 3. Experimental

### 3.1. Plant material

*C. armena* Boiss. and *C. sessilis* Wild. were collected in Gümüşhane (A8)-Çerçi village and Bayburt new industrial park, meadows (at heights of ~1745 and 1150 m) in the northeastern part of Turkey, in July 2004, respectively. Voucher specimens (No. Coşkunçelebi 446-2004 and 444-2004, 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.

### 3.2. Isolation of the essential oils

The air-dried whole plants (~60 g, each) of *C. sessilis* and *C. armena* were powdered by a blender and hydrodistilled in a Clevenger-type apparatus using ice bath for cooling system (3 h). The oils were taken by dissolving in HPLC grade *n*-hexane (0.5 mL) and kept at 4 °C in a sealed brown vial. One microliter of the sample was directly injected into the GC–MS instrument. The percentage yields of the oils from *C. sessilis* and *C. armena*

Table 1
Identified components in the essential oils of *C. sessilis* and *C. armena*[a,b]

| No. | Compounds | *C. sessilis* | | *C. armena* | | Exp. RI | Ident. (RI/MS) | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | *Q* (%) | % Area | *Q* (%) | % Area | | | |
| 1 | *n*-Heptanal | 83 | 0.2 | – | – | 901 | 900 | [c] |
| 2 | α-Pinene | 97 | 1.4 | – | – | 940 | 940 | [c] |
| 3 | Verbenene | 91 | 0.3 | – | – | 961 | 967 | [d] |
| 4 | Benzaldehyde | 90 | 0.3 | 85 | 1.1 | 966 | 963 | [c] |
| 5 | 4,4-Dimethyl-(*E*)-2-pentene | – | – | 95 | 4.0 | 972 | MS | [j] |
| 6 | β-Pinene | 95 | 0.6 | – | – | 984 | 982 | [c] |
| 7 | 2-Pentyl-furan | 91 | 1.1 | – | – | 998 | 990 | [d] |
| 8 | *n*-Octanal | 98 | 0.4 | – | – | 1008 | 1003 | [c] |
| 9 | *p*-Cymene | 91 | 0.2 | – | – | 1029 | 1029 | [c] |
| 10 | Limonene | 98 | 0.3 | – | – | 1032 | 1033 | [c] |
| 11 | *Trans*-pinane | – | – | 98 | 4.1 | 1041 | MS | [j] |
| 12 | Benzeneacetaldehyde | 91 | 0.5 | – | – | 1045 | 1045 | [c] |
| 13 | Linalool | 90 | 0.2 | – | – | 1091 | 1098 | [e] |
| 14 | *n*-Nonanal | 90 | 0.3 | – | – | 1098 | 1104 | [c] |
| 15 | 2,3,3-Trimethyl-3-cyclopentene acetaldehyde | 86 | 0.3 | – | – | 1117 | MS | [j] |
| 16 | *Cis*-Verbenol | 95 | 0.6 | – | – | 1135 | 1140 | [d] |
| 17 | α-Thujenal | 84 | 1.0 | – | – | 1195 | MS | [j] |
| 18 | Safranal | 86 | 0.5 | – | – | 1198 | 1201 | [f] |
| 19 | *n*-Decanal | 91 | 0.5 | – | – | 1205 | 1206 | [c] |
| 20 | Pulegone | 90 | 0.6 | – | – | 1221 | 1221 | [g] |
| 21 | 5-Pentyl-3H-furan-2-one | 86 | 0.5 | – | – | 1266 | MS | [j] |
| 22 | Dihydroedulan I | 92 | 1.3 | – | – | 1293 | 1289 | [e] |
| 23 | α-Copaene | 99 | 0.7 | 99 | 3.2 | 1378 | 1378 | [c] |
| 24 | β-Damascenone | 97 | 1.0 | 83 | 1.7 | 1385 | 1382 | [c] |
| 25 | Cyperene | 99 | 0.7 | – | – | 1400 | 1399 | [h] |
| 26 | β-Caryophyllene | 99 | 1.3 | 99 | 5.4 | 1421 | 1424 | [c] |
| 27 | *Cis*-Geranyl acetone | 87 | 1.1 | 96 | 2.2 | 1455 | 1453 | [d] |
| 28 | Germacrene D | – | – | 99 | 3.3 | 1481 | 1481 | [f] |
| 29 | Calarene | 94 | 2.4 | 98 | 10.3 | 1482 | MS | [j] |
| 30 | β-Ionone | 97 | 1.5 | – | – | 1486 | 1484 | [e] |
| 31 | Cetene | 94 | 0.5 | 97 | 1.4 | 1492 | MS | [j] |
| 32 | β-Bisabolene | 96 | 1.0 | 97 | 2.7 | 1510 | 1510 | [c] |
| 33 | α-Calacorene | 95 | 1.2 | – | – | 1546 | 1540 | [h] |
| 34 | Nor-copaanone | 82 | 1.5 | – | – | 1555 | MS | [j] |
| 35 | 1,5-Epoxysalvial-4(14)-ene | 95 | 1.2 | – | – | 1569 | MS | [j] |
| 36 | Spathulenol | 98 | 4.9 | 95 | 3.9 | 1579 | 1578 | [c] |
| 37 | Caryophyllene oxide | 94 | 10.0 | 98 | 4.7 | 1585 | 1584 | [c] |
| 38 | Salvial-4(14)-en-1-one | 95 | 1.6 | – | – | 1595 | 1598 | [i] |
| 39 | β-Eudesmol | 98 | 12.4 | 95 | 19.3 | 1653 | 1653 | [c] |
| 40 | Tetradecanol | – | – | 94 | 3.2 | 1676 | 1676 | [e] |
| 41 | Vulgarol B | 88 | 2.4 | – | – | 1688 | MS | [j] |
| 42 | (*E*)-2-Tetradecen-1-ol | – | – | 98 | 2.3 | 1713 | MS | [j] |
| 43 | 6,10,14-Trimethyl-2-pentadecanone | 99 | 3.1 | 91 | 5.7 | 1846 | 1845 | [d] |
| 44 | Farnesyl acetone | 86 | 0.7 | 90 | 1.2 | 1919 | 1918 | [h] |
| 45 | *Cis*-Phytol | 96 | 6.4 | 90 | 2.0 | 2113 | 2114 | [e] |
| 46 | Neophytadiene | – | – | 90 | 1.0 | 2218 | MS | [j] |
| | Total isolate identified | | 66.7 | | 82.7 | | | |

Q: Quality.
[a] Compounds are listed in order of elution.
[b] Retention index (RI) values are calculated from retention times relative to that of *n*-alkanes on the non-polar HP-5 column.
[c] Flamini et al. (2002).
[d] Jovanovic et al. (2004).
[e] Skaltsa et al. (2003).
[f] Ertugrul et al. (2003).
[g] Warthen et al. (1997).
[h] Skaltsa et al. (2000).
[i] Javidnia et al. (2005).
[j] NIST and Willey Libraries.

Table 2
The chemical class distribution of the essential oil components of *C. sessilis* and *C. armena*

| Compound class | *C. sessilis* | | *C. armena* | |
|---|---|---|---|---|
| | %Area | Number of compounds | %Area | Number of compounds |
| Monoterpenes | 2.8 | 5 | 6.3 | 2 |
| Monoterpenoids | 5.3 | 7 | – | – |
| Sesquiterpenes | 9.8 | 8 | 26.6 | 6 |
| Sesquiterpenoids | 35.1 | 7 | 34.8 | 5 |
| Diterpenes | 6.4 | 1 | 3.0 | 2 |
| Others | 7.3 | 12 | 12.0 | 5 |
| The common compounds | 45.8 | 14 | 64.8 | 14 |

Table 3
The major components in the chemical class distribution of the essential oil constituents of *C. sessilis* and *C. armena*

| Compound class | *C. sessilis* | | | *C. armena* | | |
|---|---|---|---|---|---|---|
| | Major component | %Area | RI | Major component | %Area | RI |
| Monoterpenes | α-Pinene | 1.4 | 940 | *Trans*-pinane | 4.1 | 1041 |
| Monoterpenoids | α-Thujenal | 1.0 | 1195 | – | – | – |
| Sesquiterpenes | Calarene | 2.4 | 1482 | Calarene | 10.3 | 1482 |
| Sesquiterpenoids | β-Eudesmol | 12.4 | 1653 | β-Eudesmol | 19.3 | 1653 |
| Diterpenes | *cis*-Phytol | 6.4 | 2113 | *cis*-Phytol | 2.0 | 2113 |
| Others | Dihydroedulan I | 1.3 | 1293 | Tetradecanol | 3.2 | 1676 |

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

| Sample | Stock (μg/ml) | Microorganisms and inhibition zone (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Ec | Yp | Kp | Sm | Ef | Sa | Bs | Ca | Ct |
| *C. sessilis* | 1000 | – | + | – | – | + | + | + | – | – |
| *C. armena* | 700 | – | + | – | – | + | + | + | – | – |
| Ceftazidime | 10 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | | |
| Triflucan | 5 | | | | | | | | +++ | +++ |

Results were interpreted in terms of the diameter of the inhibition zones: (–), <5.5 mm; (+), 5.5–10 mm; (++), 11–15 mm; (+++), ⩾16 mm.
Ec, *Escherichia coli* ATCC 35218, Yp, *Yersinia pseudotuberculosis* ATCC 911; Kp, *Klebsiella pneumoniae* ATCC 13883; Sm, *Serratia marcescens* ATCC 13880; Ef, *Enterococcus faecalis* ATCC 29212; Sa, *Staphylococcus aureus* ATCC 25923; Bs, *Bacillus subtilis* ATCC 6633; Ca, *Candida albicans* ATCC 60193; Ct, *Candida tropicalis* ATCC 13803.

calculated on a moisture free basis were 0.25% and 0.18% (v/w), respectively.

### 3.3. Gas chromatography–mass spectrometry

GC–MS analyses were performed by using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was HP-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm). The carrier gas used was helium, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. One microliter essential oil solution in hexane (HPLC grade) was injected and analyzed with the column held initially at 60 °C for 2 min and then increased to 260 °C with a 5 °C/min heating ramp and subsequently kept at 260 °C for 13 min. The relative percentage amounts of the separated compounds were calculated from total ion chromatograms by a computerized integrator.

### 3.4. Antimicrobial activity assessment

All test microorganisms were obtained from the Refik Saydam Hifzissihha Institute (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Serratia marcescens* ATCC 13880, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 60193, *Candida tropicalis* ATCC 13803. Essential oils from *C. sessilis* and *C. armena* were dissolved in acetone to prepare sample stock solutions of 1000 and 700 μg/mL, respectively.

### 3.5. Agar well diffusion method

Simple susceptibility screening test using agar-well diffusion method as adapted earlier was used (Perez et al., 1999; Bagamboula et al., 2004; Erdemoğlu et al., 2003; Perez et al., 1990). Each microorganism was suspended in Brain Heart Infusion (BHI) (Difco, Detroit,

MI) broth and diluted to approximately $10^6$ colony forming unit (cfu) per mL. They were "flood-inoculated" onto the surface of BHI agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans* and *C. tropicalis*, SDA was used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 100 μL of the sample solutions were delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Ceftazidime (Fortum) (10 μg) and Triflucan (5 μg) were the standard drugs for antibacterial and antifungal activities, respectively. Acetone was used as solvent control. The tests were carried out in duplicates. Results were interpreted in terms of diameter of inhibition zone: (−): <5.5 mm; (+): 5.5–10 mm; (++): 11–15 mm; (+++): ⩾16 mm. The results are shown in Table 4.

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