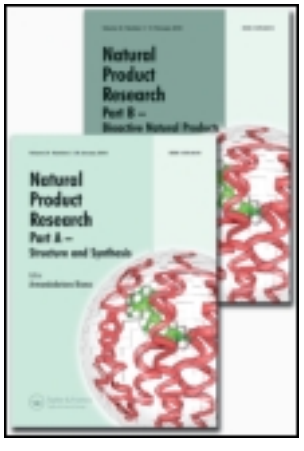

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Volatile constituents of aerial parts of three endemic Centaurea species from Turkey: Centaurea amanicola Hub.-Mor., Centaurea consanguinea DC. and Centaurea ptosimopappa Hayek and their antibacterial activities

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Volatile constituents of aerial parts of three endemic *Centaurea* species from Turkey: *Centaurea amanicola* Hub.-Mor., *Centaurea consanguinea* DC. and *Centaurea ptosimopappa* Hayek and their antibacterial activities

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The volatile constituents of the aerial parts of *Centaurea amanicola* Hub.-Mor., *Centaurea consanguinea* DC. and *Centaurea ptosimopappa* Hayek were extracted by hydrodistillation and analysed by GC and GC–MS. Altogether 94 components were identified. Sesquiterpenoids, fatty acids and carbonylic compounds were the most abundant components in the oils. Hexadecanoic acid and (*Z*,*Z*)-9,12-octadecadienoic acid were the main fatty acids in all the examined samples, that showed different patterns of composition. The study on the biological activity of the oils showed an action mainly against the Gram-positive pathogens.

Keywords: *Centaurea amanicola*; *Centaurea consanguinea*; *Centaurea ptosimopappa*; Essential oil; GC/MS; Antimicrobial activity

1. Introduction

The genus *Centaurea* L. belongs to the Cynareae tribe of the Asteraceae family and comprises approximately 500–600 species of annual, biennal and perennial grassy plants distributed all around the world, particularly in Asia, North Africa and America [1,2]. The taxonomy of the genus is still complex; in Turkey *Centaurea* is the third largest genus after *Astragalus* and *Verbascum* and is represented by 190 taxa, of which 117 are endemic (endemism ratio: 61.6%) [3]. The high endemism ratio shows that Turkey is one of the gene centers of the genus. The research in herbal medicine has increased in developing countries as a way to rescue ancient traditions and as an alternative solution to health problems in cities. Therefore, with the increasing acceptance

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of traditional medicine as an alternative form of health care, the screening of plants for active compounds has become very important. Many species of the genus *Centaurea* L. have traditionally been used for the treatment of various ailments [4–7] and the genus has also been the subject of many antimicrobial activity studies [8–15]. As a continuation of our research on the volatile components of some *Centaurea* species [15–19] in the present study, we report the volatile components of the aerial parts of *Centaurea amanicola* Hub.-Mor. (section Cynaroides), *Centaurea consanguinea* DC. (section Acrolophus) and *Centaurea ptosimopappa* Hayek (section Ptosimopappus), endemic plant species distributed in the Mediterranean and southeastern Anatolian regions of Turkey [1]. *Centaurea amanicola* is biennial or perennial, stem 65–80 cm, erect, with purple flowers. *Centaurea consanguinea* is perennial, stem 35–70 cm, profusely branched from near base with spreading branches and purple flowers. *Centaurea ptosimopappa* is an endemic species widespread and frequent locally in the Amanos and Casus mountains. Previous investigations on various extracts allowed the identification of apigenin, scopoletin, α-amyrin, β-sitosterol and six aromatic compounds in *Centaurea amanicola* [20] and of nine guaiane-type sesquiterpene lactones and two butyrolactone lignans in *C. ptosimopappa* [21]. To the best of our knowledge, no analyses have been previously reported on *C. consanguinea*.

2. Results and discussion

The composition of the volatile fractions from aerial parts of *C. amanicola* Hub.-Mor. (A), *C. consanguinea* DC. (C) and *C. ptosimopappa* Hayek (P), all endemic in Turkey, is listed in table 1 according to their retention indices on a DB-5 column. The yield of oils were: 0.16, 0.12 and 0.14%, for A, C, and P, respectively. The oils were pale yellow in colour and had a characteristic odour. Totally, we identified 94 components: 61 in A (93.3% of the oil), 40 in C (90.1% of the oil), 64 in P (91.6% of the oil). The compounds may be grouped in six main classes: sesquiterpenes (21.3–28.4%), acids and their derivatives (20.9–25.8%), carbonylic compounds (16.9–20.3%), phenols (4.1–18.1%) hydrocarbons (3.1–7.4%), monoterpenes (0.2–10.3%). In accordance with the results obtained in previous studies on oils from other *Centaurea* sp. endemic in Turkey [22–25], the oils were characterized by a low content of monoterpenes and the amount of these constituents was lower most in the *C. consanguinea* oil (0.2%) which also showed the minimum content of sesquiterpenes (21.3%). Among the sesquiterpenes, oxygen containing compounds were present in a concentration a little higher in comparison to those of sesquiterpene hydrocarbons and in all the analyzed oils only caryophyllene oxide was present in appreciable amounts (4.3–12.0%). The oil of *C. amanicola* was characterized by the highest contents of caryophyllene oxide (12.0%) and caryophyllene (5.4%) while in *C. consanguinea* germacrene B (8.3%) and caryophyllene oxide (7.3%) represented the most abundant sesquiterpenes. The oil of *C. ptosimopappa* showed a large number of sesquiterpenes, all present in low concentration, except β-eudesmol (8.2%). In this oil, β-selinene and caryophyllene oxide were present in quite similar amounts, 4.1 and 4.3%, respectively. Among fatty acids hexadecanoic and (*Z,Z*)-9,12-octadecadienoic acids were the most abundant and their total content in each oil was quite similar although they presented some quantitative differences. In fact, the former showed the higher concentration (15.0%) in *C. amanicola* while the latter showed the greater amount in *C. ptosimopappa* (6.1%).

Table 1. Volatile constituents of the aerial parts of *C. amanicola* Hub.-Mor. (A), *C. consanguinea* DC. (C) and *C. ptosimopappa* Hayek (P).

K_i	Component	Identification	A	C	P
961	Benzaldehyde	1, 2, 3	2.2	0.2	0.7
982	1-Octen-3-one	1, 2	0.2		0.7
986	1-Octen-3-ol	1, 2, 3			1.1
1001	2-Pentylfuran	1, 2	0.1	0.6	1.8
1015	(*E*,*E*)-2,4-heptadienal	1, 2	1.8		0.4
1045	Phenyl acetaldehyde	1, 2, 3	3.3	1.1	2.6
1089	Guaiacol	1, 2, 3	1.8	14.9	2.4
1101	Linalool	1, 2, 3			2.1
1102	Nonanal	1, 2		2.1	0.5
1180	Naphtalene	1, 2, 3	0.3	1.6	1.2
1185	*p*-Cymen-8-ol	1, 2	2.9		7.6
1189	Methyl salicylate	1, 2, 3	3.0		
1201	Safranal	1, 2	2.8	0.8	
1204	Decanal	1, 2, 3	0.6	1.2	
1224	Benzothiazole	1, 2	0.1	1.2	
1239	Thymol methyl ether	1, 2			1.6
1294	Dihydroedulan I	1, 2	0.9		
1304	*p*-Methoxyacetophenone	1, 2			6.8
1313	4-Vinylguaiacol	1, 2	9.4		
1353	α-Cubebene	1, 2	1.6		0.9
1355	Eugenol	1, 2, 3		3.2	
1356	α-Longipinene	1, 2			0.8
1362	Cyclosativene	1, 2	1.4		1.2
1371	α-Copaene	1, 2	1.7	1.3	0.5
1381	(*Z*)-β-Damascenone	1, 2	0.4	0.7	1.0
1406	α-Cadinene	1, 2			0.7
1414	Caryophyllene	1, 2	5.4	2.0	0.7
1461	*allo*-Aromadendrene	1, 2	0.9		
1484	β-Selinene	1, 2			4.1
1486	Dihydroactinidiolide	1, 2	0.7		0.4
1494	α-Selinene	1, 2	0.8		0.5
1500	β-Himachalene	1, 2			0.7
1508	α-Amorphene	1, 2			0.7
1564	Ledol	1, 2	0.9		0.7
1565	Germacrene B	1, 2		8.3	
1566	Dodecanoic acid	1, 2, 3	1.4	0.3	
1572	Spathulenol	1, 2	0.5		1.6
1577	Caryophyllene oxide	1, 2, 3	12.0	7.3	4.3
1588	α-Copaen-4-ol	1, 2		0.8	
1604	Cedrenol	1, 2		0.9	
1648	β-Eudesmol	1, 2	0.5		8.2
1672	(*Z*)-α-Bisabolene epoxide	1, 2	0.8		
1846	Hexahydrofarnesylacetone	1, 2	6.0	13.5	
1865	Pentadecanoic acid	1, 2, 3	0.3	0.3	1.6
1967	Hexadecanoic acid	1, 2, 3	15.0	14.2	13.6
2225	(*Z*,*Z*)-9,12-octadecadienoic acid	1, 2, 3	5.2	5.7	6.1
2500	Pentacosane	1, 2	0.4	0.8	0.8
2525	Tricosanal	1, 2			2.1
2700	Heptacosane	1, 2	1.1	2.1	1.4
2900	Nonacosane	1, 2	0.1	1.9	1.3

K_i: Retention index on a DB-5 column. 1: retention index identical to bibliography; 2: identification based on comparison of mass spectra; 3: retention time identical to authentic compounds.

The presence of (*Z*,*Z*)-9,12-octadecadienoic acid is noteworthy because this acid, considered an essential fatty acid, is the precursor of prostaglandins PG_1 and PG_2. A quite similar amount of hexadecanoic acid (11.8%) was previously detected in *Centaurea dichroa* Boiss et Heldr endemic in Turkey [25]. Among the esters, the most

representative was methyl salicylate, determined only in *C. amanicola* in a concentration of 3.0%, while only methyl benzoate was detected in all oils, even if in very low amounts (trace-0.2%). The fraction of aldehydes (5.6–11.3%) and the ketones (7.5–14.7%) contained more components. The most representative aldehydes were phenyl acetaldehyde and benzaldehyde (3.3 and 2.2%, respectively, both in *C. amanicola*). The most abundant ketone in *C. amanicola* and *C. consanguinea* was hexahydrofarnesyl acetone (6.0 and 13.5%, respectively), whereas in *C. ptosimopappa* was *p*-methoxyacetophenone (6.8%). The oil of *C. consanguinea* showed the highest content of phenols (18.1%), constituted by guaiacol (14.9%) and eugenol (3.2%). Also in the oil of *C. ptosimopappa* guaiacol was the most abundant phenol (2.4%) while 4-vinyl guaiacol (9.4%) was the main phenol in the oil of *C. amanicola*. This is the first time that this kind of phenols is found in an essential oil from Turkish Centaureae [22,25].

The essential oils were tested against 10 selected micro-organisms both Gram-positive and Gram-negative. The oils were active mainly against the Gram-positive pathogens. *Bacillus subtilis* and *Staphylococcus aureus* were the most affected by the oil of *C. amanicola* with a MIC of 25 $\mu g\,mL^{-1}$ and a MBC of 50 $\mu g\,mL^{-1}$. The same values of MIC and MBC were found for *Bacillus cereus* and *B. subtilis*, the most affected by the oil of *C. consanguinea*. The MIC and MBC values against the other Gram-positive bacteria were 50 $\mu g\,mL^{-1}$ for all the oils. Among Gram-negative bacteria only *Escherichia coli* was affected by the oils of *C. amanicola* and *C. consanguinea* with a MIC and a MBC of 50 $\mu g\,mL^{-1}$ for both. The oil of *C. ptosimopappa* was the one with lesser activity against the tested bacteria. These results may be attributed to the different oils composition; in fact, the activity of the oil of *C. amanicola* may be ascribed to its content of 4-vinyl guaiacol, caryophyllene and caryophyllene oxide, while the activity of *C. consanguinea* can be attributed to the presence of guaiacol and eugenol. These compounds may act in synergy with other oil components, for instance aldehydes such as (*Z*)-2-hexenal and (*E,E*)-2,4-heptadienal, alcohols and terpenes (1-octen-3-ol, linalool, limonene, *p*-cymene, caryophyllene); as a matter of fact these compounds, though present in traces in the studied oils, were found very active against the growth of different bacteria [26,27]. The present results, when compared with the data previously obtained [14] by testing the antimicrobial activities of the acetone, chloroform and ethanol extracts from *C. amanicola* and *C. ptosimopappa*, confirm that *S. aureus* is the bacterium most affected by the metabolites of *C. amanicola*. Further studies on this plant may supply useful informations for its use in popular medicine.

3. Experimental

3.1. *Plant material*

Aerial parts of *C. amanicola* were collected at Osmaniye, Yarpuz, Yağlipinar Mountain, 1900 m on 10 July 2005 (Voucher specimen no: 625). Aerial parts of *C. consanguinea* were collected at Bingol, 110 km from Muş to Bingol on 10 June 2005 (Voucher specimen no. 665). Aerial parts of *C. ptosimopappa* were collected in Hatay, the Amanos Mountain above Dörtyol (Turkey), 850–950 m above sea level in June 2005 (36°51′ N, 36°13′ E, voucher specimen: Çelik 2148–2153). All voucher specimens were deposited in the Herbarium of the Department of Biology, Çanakkale Onsekiz Mart University (COMU).

3.2. *Isolation of the essential oils*

The air-dried samples were ground in a Waring blender and then subjected to hydrodistillation according to the standard procedure described in the *European Pharmacopoeia* [28] for 3 h using *n*-hexane as a solvent. The extracts were dried over anhydrous sodium sulphate and then stored in sealed vials, at −20°C, ready for the GC and GC–MS analyses.

3.3. *GC and GC/MS*

GC analyses were performed on a Perkin-Elmer Sigma-115 gas chromatograph equipped with a FID and a data handling processor. The separation was achieved using a DB-5 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Column temperature: 40°C, with 5 min initial hold, and then to 260°C at 2°C min^{-1}, 260°C (20 min) using He as the carrier gas (1.0 mL min^{-1}); injection mode splitless (1 μL of a 1 : 1000 *n*-pentane solution). Injector and detector temperatures were 250 and 290°C, respectively. Retention indices (R_i) were determined in relation to a homologous series of *n*-alkanes (C_8–C_{24}) under the same conditions. Components relative concentrations were obtained by peak area normalization. No response factors were calculated.

GC–MS analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica HP-5 capillary column (30 m × 0.25 mm), 0.33 μm film thickness, coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V. Helium was used as carrier gas (1 mL min^{-1}). Gas chromatographic conditions were the same used for GC analysis. Interface temperature 295°C; mass range was 29–350 m/z, ionization energy 70 eV, multiplier energy 2000 V, scan time 1 s. Peak identification was accomplished by comparison of their mass spectra with those stored on the GC–MS data bases (NIST 02 and Wiley 275) and reported in literature [29,30]. The identification of the oil components was also possible by comparison of their linear retention indices with those from literature [29,31]. Whenever possible, co-injection with standard was also performed. Results are reported in table 1. In this table, we reported the compounds present in concentrations higher than 0.5%. Therefore among monoterpenes which were not reported are *p*-cymene (0.5%, P), pinocarvone (0.5%, A), α-terpineol (0.4%, A), limonene (0.3%, A), α-pinene (0.1%, P), 1,8-dehydrocineole and terpineol-4 (0.1%, C). Among sesquiterpenes which were not reported in the table are: β-elemene (t, A and 0.3%, C), α-cedrene, eremophilene and widdrol (0.5%, P), γ-gurjunene, 1S-*cis*-calamenene and viridiflorol (0.5%, A), β-oplopenone (0.3%, A) and aromadendrene oxide II (0.4%, C). Acids and esters present in low amounts were tetradecanoic acid (0.5%, A, t in C and 0.2%, P), heptadecanoic acid (0.4%, C and 0.3%, P), hexyl valerate and methyl benzoate (0.2%, A), ethyl benzoate (0.1%, P). The carbonilic compounds not reported in table were: 6-methyl-5-hepten-2-one (0.3%, A; 0.5%, C and 0.3%, P), octanal (0.2%, A and 0.5%, P), eicosanal (0.5%, P), (*E*)-2-octenal (t, in A and 0.5%, P), *p*-methyl benzaldehyde (0.4%, P), (*E*)-2-nonenal (0.3%, P), *p*-methylacetophenone (0.5%, A and 0.1%, P), (*E*)-2-dodecenal (0.2%, C and 0.5%, P), heptanal (0.3%, A and P), heptan-2-one (t in A and 0.3%, P), (*Z*)-2-hexenal (0.1% A and P). The hydrocarbons detected in low concentration were: octane (0.1%, A), nonane (0.1%, P), tricosane (0.3%, A, 0.4%, C and 0.2%, P), tetracosane (0.1%, A and C),

hexacosane (0.1%, A, C and P), octacosane (0.3%, A and C and 0.1%, P), tetracontane (0.1%, A and C), entriacontane (0.2%, A and 0.1%, P). Other compounds present in low amounts were hexanol (t, in P), *p*-methyl anisole (0.1%, P) and pentadecanol (0.1%, A).

3.4. *Antimicrobial assay*

The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using the broth dilution method [32] as previously described [33]. Ten bacterial species, selected as representative of the class of Gram-positive and Gram-negative, were tested: *Bacillus cereus* (ATCC 11778), *B. subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATTC 29212), *E. coli* (ATCC 25922), *Proteus mirabilis* (ATCC 25933), *P. vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi Ty2* (ATCC 19430). The values of MIC and MBC for the Gram-negative bacteria *P. mirabilis*, *P. vulgaris*, *P. aeruginosa* and *S. typhi Ty2* were 100 or $>100\,\mu g\,mL^{-1}$ for the oils tested.

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