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Volatile components of *Centaurea calcitrapa* L. and *Centaurea sphaerocephala* L. ssp. *sphaerocephala*, two Asteraceae growing wild in Sicily

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ABSTRACT: The volatile components of the flowerheads of *Centaurea calcitrapa* L. (C.c.) and *Centaurea sphaerocephala* L. ssp. *sphaerocephala* (C.s.) were obtained by hydrodistillation and identified by GC and GC–MS. Altogether 96 components were identified, 66 in *C. calcitrapa* and 68 in *C. spaerocephala*, mostly fatty acids (32.8%, C.c. and 44.2%, C.s.) and hydrocarbons (32.3%, C.c. and 15.9%, C.s.). 9,12-Octadecadienoic acid (15.8%, C.c.) and hexadecanoic acid (30.7%, C.s.) were the most abundant fatty acids; tricosane (8.0%, C.c.) and heptacosane (4.9%, C.s.) were the main hydrocarbons. Sesquiterpenes were also present as hydrocarbons (10.1% for 14 components in C.c. and 9.2% for 13 components in C.s.) and as oxygen-containing sesquiterpenes (2.0% for four components in C.c. and 13.6% for nine components in C.s.). Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: *Centaurea calcitrapa* L.; *Centaurea sphaerocephala* L. ssp. *sphaerocephala*; Asteraceae; antimicrobial activity; essential oil; 9,12-octadecadienoic acid; hexadecanoic acid; β-eudesmol

Introduction

The botanical genus *Centaurea* (Asteraceae) is a large genus comprising about 1000 species and is widespread all around the world. The aerial parts of several species of *Centaurea* are used in the popular medicine of many countries and in some cases a scientific evidence of these activities has been pointed out, such as antibacterial,[1,2] hypoglycemic,[1,2] antimicrobial,[1] cytotoxic and phytotoxic[3]. The genus was an object of numerous chemical studies, leading to the isolation of sesquiterpene lactones[4–7] and flavones[8–10] as the main secondary metabolites of its species. Recently, we reported on the volatile components of *Centaurea cineraria* L. subsp. *umbrosa* (Lacaita) Pign. and *Centaurea napifolia* L. (Asteraceae), two species growing wild in Sicily.[11] As a continuation of our researches on this plants,[7–12] we have now investigated the chemical composition and the antimicrobial activity of the essential oils obtained from the flowerheads of *Centaurea calcitrapa* L. and *Centaurea sphaerocephala* L. ssp. *sphaerocephala*, both growing wild in Sicily. *C. calcitrapa*, locally named 'fiordaliso stellato' (common star thistle), is a grassy biennial plant, up to 60 cm tall, with hermaphroditic flowers. It blooms from June to August and its leaves are consumed in salads. *C. sphaerocephala*, locally named 'fiordaliso delle spiagge', is a perennial grassy plant with scattered leaves, 50 cm tall, that blooms from June to September. In previous investigations *C. sphaerocephala* has been shown to contain various polyacetylenic compounds,[13] lignans[14] and sesquiterpene lactones,[9] while for *C. calcitrapa* sesquiterpene lactones and lignans were described.[15–17] Volatile components obtained from acetone extracts of flowers, leaves, stems and flowerhead buds of *C. calcitrapa* have also been described previously.[18]

Experimental

Plant Material

Flowerheads of *Centaurea calcitrapa* L. and *Centaurea sphaerocephala* L. ssp. *sphaerocephala* were collected at the full flowering period from plants grown at Capo Zafferano and Cefalù (Palermo, Sicily), respectively, in July and August 2003. The plants were identified by Mr Emanuele Schimmenti. Herbarium samples (PAL 03-178 for *C. calcitrapa* and PAL 03-182a for *C. sphaerocephala* ssp. *sphaerocephala*) were deposited in the Palermo Botanical Garden, Italy.

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Isolation of the Essential Oil

Freshly collected flowerheads were subjected to hydrodistillation according to the standard procedure described in the *European Pharmacopoeia*[19] for 3 h using *n*-pentane 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.

Gas Chromatography

GC analyses were carried out using a Perkin-Elmer Sigma-115 gas chromatograph fitted with FID and a data-handling processor. A DB-5 (J&W Scientific, Folsom, CA, USA) fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was employed. The operating conditions were as follows: temperature column, 40 °C, with 5 min initial hold, then rising to 260 °C at 2 °C/min, then held at 260 °C (20 min); carrier gas, He at 1.0 ml/min; injection mode, splitless (1 μl 1:1000 *n*-pentane solution); injector and detector temperatures, 260 °C and 290 °C, respectively. Retention indices (R_i) for all compounds were determined using *n*-alkanes (C_8–C_{24}) as standards. Components relative concentrations were obtained by peak area normalization. No response factors were calculated.

Gas Chromatography–Mass Spectrometry

GC–MS analyses were performed on a Hewlett-Packard 5890 A gas chromatograph linked on-line with a HP Mass Selective Detector MSD 5970 HP. The column was a HP-1 (Hewlett-Packard, Palo Alto, CA, USA) fused-silica capillary column (30 m × 0.25 mm i.d.; 0.33 μm film thickness). The temperature programme was the same as for GC analysis; interface temperature, 295 °C; mass range, 29–350 *m*/*z*; ionization energy, 70 eV; multiplier energy, 2000 V; scan time, 1 s; carrier gas, He at 1.0 ml/min. Peak identification was based on comparison of their mass spectral data with those of the NIST 98 and Wiley 5 Libraries and those described in literature[20,21] and by comparison of their retention indices with literature data.[22] In many cases, the essential oils were subjected to chromatography with authentic compounds available in our laboratories.

Antimicrobial Activity

The antimicrobial activity of the entire oils was evaluated by the *in vitro* paper-disk diffusion method[23] against 10 selected Gram-positive and Gram-negative bacteria (*Bacillus cereus* PCI 213, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATTC 25923, *Streptococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella paratyphi A* ATCC 12176), as previously described.[24]

Results and Discussion

The hydrodistillation of flowerheads of *C. calcitrapa* and *C. sphaerocephala* ssp. *sphaerocephala* gives essential oils of yellow colour without a particular smell and in a yield of 0.20% and 0.12% (fresh weight), respectively. The identified components and their percentages are given in Table 1, where the components are listed in order of their elution from DB-5 column. The fatty acids (32.8–44.2%) and hydrocarbons (16.5–34.3%) fraction was dominant in both of the oils analysed, although with some differences concerning the main components. 9,12-Octadecadienoic acid (15.8%) was the major component of the oil from *C. calcitrapa*, whereas hexadecanoic acid (30.7%) was the main component of the oil from *C. sphaerocephala*. Among hydrocarbons (15.9–32.3%), tricosane (8.0%), heptacosane (7.8%) and nonacosane (6.5%) showed high concentrations in *C. calcitrapa*, whereas the major component of this fraction in *C. sphaerocephala* was heptacosane (4.9%). The aldehydes represented 4.2% (C.s.) and 6.3% (C.c.) of the total oils and in both phenyl acetaldehyde was the dominant component. Among ketones hexahydrofarnesyl acetone predominated in *C. sphaerocephala* (1.9%), while (*Z*)-β-damascone is the main compound in *C. calcitrapa* (1.2%). The sesquiterpene fraction was constituted by several components. In *C. calcitrapa* sesquiterpene hydrocarbons predominated, with β-bisabolene (2.0%), germacrene D (1.8%) and caryophyllene (1.6%) being the only components that attained relative percentages higher than 1.5%. In *C. sphaerocephala* the oxygen containing sesquiterpenes predominated with β-eudesmol (5.4%), humulene epoxide II (1.8%) and aromadendrene oxide II (1.7%) the main components of this fraction. A previous paper[18] reported benzene (4.26 μg/g), caryophyllene (3.09 μg/g), germacrene D (1.80 μg/g) and 2-pentanone (1.39 μg/g) as compounds present in the volatiles obtained from acetone extracts of flower tissues in >1 μg/g concentration. In this paper, among the volatiles from acetone extracts of buds, stems and leaves of *C. calcitrapa*, it was described for the first time the presence of *cis*- and *trans*-theaspirane (0.11–0.17 μg/g), already reported in nine other plant families, hypothesizing that these compounds may be artefacts. In our work we have not found these components, but it is not possible to draw any final conclusion because the plants come from different localities and the volatiles were obtained by different procedures.

No noteworthy antimicrobial activity activity was detected against any of the bacteria tested. Only weak activity at a concentration of 0.1 mg/ml on *Bacillus cereus* and *B. subtilis* was detected for the oil of *C. sphaerocephala*, probably due to the presence of carvacrol (2.0%) and eugenol (1.1%) and some terpenoidic components.

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Table 1. Composition of the essential oils of *Centaurea calcitrapa* L. and *Centaurea sphaerocephala* L. spp. *sphaerocephala* (Asteraceae) growing wild in Sicily

K_i	Component	Identification[a]	C.c.[b]	C.s.[b]
800	Hexanal	R_i, MS	t	
855	(*Z*)-2-Hexenal	R_i, MS	t	
889	Heptan-2-one	R_i, MS	t	t
901	Heptanal	R_i, MS	t	
916	(*E*,*E*)-2,4-Hexadienal	R_i, MS		t
930	α-Thujene	R_i, MS		t
961	Benzaldehyde	R_i, MS, Co–GC	0.2	0.5
1001	2-Pentyl furan	R_i, MS	0.3	t
1001	Octanal	R_i, MS	0.1	
1015	(*E*,*E*)-2,4-Heptadienal	R_i, MS		t
1030	Limonene	R_i, MS, Co–GC		0.9
1045	Phenyl acetaldehyde	R_i, MS, Co–GC	1.6	1.2
1064	(*E*)-2-Octenal	R_i, MS	0.1	
1088	*trans*-Linalool oxide	R_i, MS		0.4
1101	Linalool	R_i, MS, Co–GC		0.9
1102	Nonanal	R_i, MS	0.8	0.3
1158	(*E*)-2-Nonenal	R_i, MS	t	0.2
1175	Terpineol-4	R_i, MS, Co–GC		0.2
1189	Methyl salicylate	R_i, MS	0.7	
1195	Hexyl butanoate	R_i, MS,		t
1201	Safranal	R_i, MS		0.5
1203	Decanal	R_i, MS	1.4	0.3
1242	*p*-Anisaldehyde	R_i, MS	0.1	
1260	(*E*)-2-Decenal	R_i, MS	1.4	0.3
1299	Carvacrol	R_i, MS		2.0
1300	Tridecane	R_i, MS, Co–GC	1.8	0.5
1301	Indole	R_i, MS, Co–GC	tr	
1304	*p*-Methoxyacetophenone	R_i, MS	1.4	
1452	Geranyl acetone	R_i, MS	0.4	
1307	Undecanal	R_i, MS	t	
1316	(*E*,*E*)-2,4-Decadienal	R_i, MS	0.6	0.2
1355	Eugenol	R_i, MS, Co–GC		1.1
1361	(*E*)-2-Undecenal	R_i, MS	t	
1362	Cyclosativene	R_i, MS		0.5
1365	Nonanoic acid	R_i, MS, Co–GC	t	
1370	α-Ylangene	R_i, MS	t	0.2
1372	α-Copaene	R_i, MS		0.6
1382	(*E*)-β-Damascenone	R_i, MS		0.5
1387	β-Elemene	R_i, MS	0.3	
1400	Tetradecane	R_i, MS, Co–GC		0.6
1405	α-Cedrene	R_i, MS		0.2
1412	γ-Gurjunene	R_i, MS	t	0.8
1412	(*Z*)-β-Damascone	R_i, MS	1.2	
1414	Caryophyllene	R_i, MS, Co–GC	1.6	0.9
1426	*cis*-Thujopsene	R_i, MS	0.4	
1431	α-*trans*-Bergamotene	R_i, MS	0.7	1.0
1440	Aromadendrene	R_i, MS	0.3	0.4
1448	(*E*)-β-Farnesene	R_i, MS	0.4	0.7
1448	α-Humulene	R_i, MS	0.4	0.7
1461	*allo*-Aromadendrene	R_i, MS	t	
1468	Decanoic acid	R_i, MS, Co–GC	t	
1478	Germacrene D	R_i, MS	1.8	1.0
1480	(*E*)-β-Ionone	R_i, MS	0.8	1.3
1484	β-Selinene	R_i, MS	0.7	
1486	Bicyclogermacrene	R_i, MS	0.3	
1492	1-Pentadecene	R_i, MS	t	
1500	Pentadecane	R_i, MS, Co–GC	0.5	0.4
1507	(*E*,*E*)-α-Farnesene	R_i, MS		0.8
1512	β-Bisabolene	R_i, MS	2.0	1.4
1529	Cadina-1,4-diene	R_i, MS	1.2	
1564	(*E*)-Nerolidol	R_i, MS	0.9	
1566	Dodecanoic acid	R_i, MS, Co–GC	0.4	1.8
1572	Spathulenol	R_i, MS	0.9	0.7
1577	Caryophyllene oxide	R_i, MS, Co–GC		1.8
1588	α-Copaen-4-ol	R_i, MS		0.6
1597	Cedrol	R_i, MS	0.2	
1610	Humulene epoxide II	R_i, MS		1.8
1631	Ledol	R_i, MS		0.5

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Table 1. (*Continued*)

K_i	Component	Identification[a]	C.c.[b]	C.s.[b]
1648	β-Eudesmol	R_i, MS		5.4
1672	(*Z*)-α-Bisabolene epoxide	R_i, MS	t	0.4
1673	Cadalene	R_i, MS	0.5	
1678	Aromadendrene oxide II	R_i, MS		1.7
1720	(*E*,*E*)-Farnesol	R_i, MS		0.7
1769	Tetradecanoic acid	R_i, MS, Co–GC	4.2	2.0
1846	Hexahydrofarnesyl acetone	R_i, MS	0.9	1.9
1865	Pentadecanoic acid	R_i, MS	0.8	0.5
1950	Phytol	R_i, MS	2.3	1.6
1967	Hexadecanoic acid	R_i, MS, Co–GC	10.2	30.7
2033	Octadecanal	R_i, MS		0.7
2068	Heptadecanoic acid	R_i, MS		t
2115	(*Z*)-9-Octadecenoic acid	R_i, MS, Co		2.8
2125	9,12,15-Octadecatrienoic acid	R_i, MS, Co–GC	1.4	0.5
2169	Octadecanoic acid	R_i, MS, Co–GC		2.0
2200	Docosane	R_i, MS, Co–GC	0.4	
2225	9,12-Octadecadienoic acid	R_i, MS, Co–GC	15.8	3.9
2289	Tricosene-1	R_i, MS	0.5	
2300	Tricosane	R_i, MS, Co–GC	8.0	1.8
2400	Tetracosane	R_i, MS, Co–GC	1.6	0.5
2500	Pentacosane	R_i, MS	5.6	2.4
2600	Hexacosane	R_i, MS	0.4	0.2
2700	Heptacosane	R_i, MS	7.8	4.9
2800	Octacosane	R_i, MS	0.5	0.5
2900	Nonacosane	R_i, MS	6.5	2.9
3000	Triacontane	R_i, MS		0.2
3100	Hentriacontane	R_i, MS	0.7	1.6
	Total		94.0	98.5

K_i, retention index on a DB-5 column. [a] R_i, retention index identical to bibliography; MS, identification based on comparison of mass spectra; Co–GC, retention time identical to authentic compounds; C.c., *Centaurea calcitrapa*; C.s., *Centaurea sphaerocephala*; [b] t, trace, <0.05%.

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