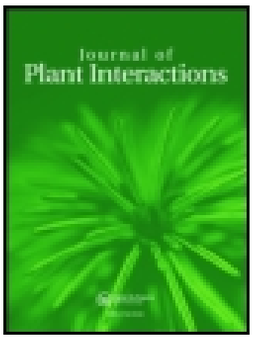

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Composition and allelopathic effect of essential oils of two thistles: *Cirsium creticum* (Lam.) D.'Urv. ssp. *triumfetti* (Lacaita) Werner and *Carduus nutans* L.

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Abstract
Cirsium and *Carduus* are two of the main genera of true thistles. Different species in these genera share a quantity of secondary metabolites and have interesting ecological properties. The essential oils of two species, *Cirsium creticum* and *Carduus nutans*, were analysed, showing the presence as main compounds of 4-ethyl guaiacol (15%), hexadecanoic acid (10.6%), (*E*)-β-damascenone (7.8%), dihydroactinidiolide (6.0%) and 4-vinyl guaiacol (4.5%) for *C. creticum* and hexadecanoic acid (18.6%), hexahydrofarnesylacetone (7.8%), heptacosane (5.9%), 4-vinyl guaiacol (5.8%), pentacosane (3.8%) and eugenol (3.6%) for *C. nutans*. The oils were evaluated at different doses for their effect on germination and initial radical elongation of three species, *Raphanus sativus* L. (radish), *Lactuca sativa* L. (lettuce), and *Lepidium sativum* L. (garden cress), usually utilized in allelopathic assays: they showed a weak inhibitory activity against seed germination and radical elongation of the three test species.

Keywords: *Cirsium creticum,* Carduus nutans*, Asteraceae, thistle, essential oil, antigerminative activity*

Introduction

Allelopathy is one expression of the general phenomenon of chemical interaction and is probably of widespread significance in the functioning of natural communities. In fact, a number of plants have inhibitory effects on the growth of neighboring or successional plants by releasing allelopathic chemicals into the soil, either as exudates from living tissues or by decomposition of plant residues. The study of compounds produced by plants which inhibit or stimulate the germination and the development of other plants is important for understanding the mechanisms of the ecological interaction (Rice 1984, Harborne 1988). *Cirsium* and *Carduus* are two of the main genera of true thistles (Family Asteraceae, Tribe Carduinae, Subtribe Carduineae) that are respectively native and non-native in North America; *Carduus is* a predominantly Eurasian genus, while *Cirsium* is a Palearctic genus that contains North American thistles. The genus *Cirsium* (from the Greek *kirsos*, meaning 'swollen vein'), comprises biennial or perennial herbs, rarely annuals. Most species are considered weeds, but some are cultivated in gardens for their aesthetic value, as they are known for their effusive flowered heads (usually purple, rose, yellow, or white). Thistles were used as a remedy against swollen veins and blessed thistle is believed to be a galactagogue. *Cirsium* sp. extracts have shown antimicrobial, cytotoxic and antioxidant activity (Loizzo et al. 2004, Orhan et al. 2007) antihemorrhagic effects, antidepressant action (Park et al. 2006) and antihepatotoxic activity (Park et al. 2004). The genus is well known also for its ecological properties, as it possesses allelopathic activity (Chon et al. 2003). *Cirsium* thistles are also used as food plants by the larvae of some Lepidoptera species. (Miyazawa et al. 2003a). *Carduus* is a genus of about 90 species well known in traditional medicine as many species exert different activities, such as antioxidant (Nichita 2005), antitumor (Xie et al. 2005), antimicrobial (El-Lakany et al. 1997), anti-inflammatory and protective activity on liver cells (Hoh et al. 2007). *Carduus* species are used as food plants by the larvae of some Lepidoptera

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species including *Coleophora therinella*. A review on *Cirsium* and *Carduus* (Jordon-Thaden & Louda 2003) shows that multiple secondary metabolites are shared by different species in these genera. The best-known group of secondary metabolites in *Cirsium* and *Carduus* are flavonoids; Jordon-Thaden and Louda (2003) report the presence of 78 flavonoids from *Cirsium* spp. and 31 from *Carduus* spp. With ten flavonoid groups shared between them. The compounds most often reported for both *Carduus* and *Cirsium* were: apigenin, cirsimaritin, kaempferol, linarin, luteolin, pectolinarin, and quercetin (with various glycosides attached). Among these, flavones and flavonols are the two main classes of flavonoids reported to exhibit biological activity towards insects. Other common secondary metabolites in *Cirsium* and *Carduus* species are sterols, triterpenes, polyacetylenes and acetylenes which generally are typical of Asteraceae (Harborne, 1999). Polyacetylenes from the Asteraceae have been reported to have major antifeedant properties, including activity against root-feeding insects of thistles. Other metabolites isolated from *Cirsium* and *Carduus* spp. are alkaloids, carbohydrates, norisoprenoids and essential oils (Block et al. 1969, Nazaruk et al. 2002, Miyazawa et al. 2003b, Esmaeili et al. 2005, Wilson et al. 2006).

Cirsium creticum (Lam.) D'Urv., slough thistle, locally named *cardo di Creta*, is a biennal herbaceous plant 1–3 m tall, known for its effusive purple flowered heads that are particularly spiny; the radially symmetrical disk flowers are situated at the end of the branches. The leaves are alternate and entire. The seed has tiny tufts of hair, which allows them to be carried by the wind. *Carduus nutans* L. (musk thistle), together with *C. acanthoides*, is considered the major weed worldwide (USDA Plant Database 2002). It is a biennial herb with showy red-purple flowers and sharply spiny stems and leaves. Mature plants range in height from 1–1.5 m tall and have multi-branched stems. The leaves are dark green, coarsely bipinnately lobed, with a smooth, waxy surface and sharp yellow-brown to whitish spines at the tips of the lobes. The large globose flower heads contain hundreds of tiny individual flowers. Each plant may produce thousands of straw-colored seeds adorned with plume-like bristles. Flowering occurs from late spring to late summer, and seed dissemination occurs approximately one month after the flowers form. A single flower head may produce 1200 seeds and a single plant up to 120,000 seeds, which are wind dispersed. The seeds may remain viable in the soil for over ten years, making it a difficult plant to control.

Our research group is carrying out a series of studies on the possible allelopathic properties of medicinal plants that, being rich in active principles, are considered a primary source of potential allelochemicals (De Feo et al. 2002, Arminante et al. 2006, Rigano et al. 2006, Senatore et al. 2007). It has been demonstrated that terpenoids are involved in multiple ecological functions in plants, such as protection against herbivores and microbial diseases, attraction of pollinators and biochemical interactions among plants. Evidence for allelopathic interactions by plants containing volatile compounds has been frequently described (De Feo et al. 2002, Armirante 2006). To confirm the hypothesis that essential oils can play an important role in regulating interactions among plants, we carried out *in vitro* experiments in order to verify the possible effects of the essential oils from *C. creticum* and *C. nutans* on germination and initial radical elongation of three species, *Raphanus sativus* L. (radish), *Lactuca sativa* L. (lettuce), and *Lepidium sativum* L. (garden cress), usually utilized in allelopathic assays.

Materials and methods

Essential oil isolation

The oils from air-dried and ground aerial parts of plants were isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus according to the method recommended in the *European Pharmacopoeia* (2004). The oils were dried over anhydrous sodium sulphate and stored under N_2 at 4°C in the dark until tested and analysed. The samples yielded 0.13% of yellow oil (w/w) for *C. creticum* and 0.11% of yellow oil (w/w) for *C. nutans*.

GC analysis

Analytical gas chromatography was carried out on a Perkin-Elmer Sigma 115 gas chromatograph fitted with a HP-5 MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness). Helium was the carrier gas (1 ml min^{-1}). Column temperature was initially kept at 40°C for 5 min, then gradually increased to 250°C at 2°C min^{-1}, held for 15 min and finally raised to 270°C at 10°C min^{-1}. Diluted samples (1/100 v/v, in *n*-hexane) of 1 μl were injected manually at 250°C, and in the splitless mode. Flame ionization detection (FID) was performed at 280°C. Analysis was also run by using a fused silica HP Innowax polyethylenglycol capillary column (50 m × 0.20 mm i.d.; 0.20 μm film thickness).

GC-MS analysis

GC-MS analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica HP-1 capillary column (30 m × 0.25 mm i.d.; 0.33 μm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V. Gas chromatographic conditions were as reported above; transfer line temperature, 295°C. Analysis was also run by using a fused silica HP Innowax polyethylenglycol

capillary column (60 m $\times$ 0.25 mm i.d.; 0.33 μm film thickness).

Qualitative and quantitative analyses

Most constituents were identified by gas chromatography by comparison of their retention indices (*I*) with either those of the literature (Jennings & Shibamoto 1980, Davies 1990) or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_8–C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with either those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature (Jennings & Shibamoto 1980, Adams 2001) and our home made library. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Allelopathic bioassay

A bioassay based on germination and subsequent radical elongation was used to study the possible allelopathic effects of the two essential oils on seeds of *Raphanus sativus* L. cv '*Saxa*', *Lactuca sativa* L., and *Lepidium sativum* L. The seeds were surface-sterilized in 95% ethanol for 15 s and sown in Petri dishes (90 mm diameter), containing five layers of Whatman filter paper, impregnated with 7 ml of distilled water (control) or 7 ml different doses of the two essential oils (0.06, 0.125, 0.25, 0.625, 1.25 and 2.5 mg/ml). The oils were dissolved in a water-acetone mixture (98:2). Controls performed with this mixture alone showed no appreciable differences with control. The germination conditions were as follows: For radish and cress seeds $20 \pm 1.1^{\circ}$C and for lettuce seeds $24 \pm 1.1^{\circ}$C, with natural photoperiod. Seed germination process was observed directly in Petri dishes, each 20 hours; seed was considered germinated when the radical protrusion became evident. After 120 hours, the effects of radical elongation were measured. Each determination was repeated six times, using Petri dishes containing 10 seeds each.

Results and discussion

The volatile components detected from the aerial parts of the plants and their percentage contribution are shown in Table I, according to their elution order on a HP-5 MS column. The essential oil from *Cirsium creticum* was a mixture of 56 substances accounting for the 94.4% of the total oil. Its mass fraction was 0.13% on a dry weight basis. 4-Ethyl guaiacol (15.0%), hexadecanoic acid (10.6%), (*E*)-β-damascenone (7.8%), dihydroactinidiolide (6.0%) and 4-vinyl guaiacol (4.5%) were recognized as the main constituents. Generally, the most abundant components of the oil were carbonylic compounds (33.8%), phenols (23.1%), acids (14.6%) and hydrocarbons (12.7%). In the first fraction predominated (*E*)-β-damascenone (7.8%) and dihydroactinidiolide (6.0%), while in the second the most abundant compounds were 4-ethyl guaiacol (15%) and 4-vinyl guaiacol (4.5%). Among the acids, hexadecanoic acid (10.6%) was the predominant while the most abundant hydrocarbons were nonacosane (3.1%) and heptacosane (2.4%). Other components in the oil were sesquiterpenes (5.6%) and monoterpenes (4.1%). In the first group sesquiterpene hydrocarbons (4.1%), with a prevalence of italicene (1.7%) and widdrene (1.4%), predominated over oxygen containing sesquiterpenes (1.5%) represented solely by (*E,Z*)-farnesol. Among monoterpenes, oxygen containing monoterpenes were the most abundant (2.6%) with α-terpineol (1.9%) being the main compound.

In the oil from *Carduus nutans* 54 compounds were identified, accounting for the 92.2% of the total oil. The mass fraction was 0.11% on a dry weight basis. The most abundant components were hexadecanoic acid (18.6%), hexahydrofarnesylacetone (7.8%), heptacosane (5.9%), 4-vinyl guaiacol (5.8%), pentacosane (3.8%) and eugenol (3.6%). On the whole, fatty acids and esters (26.0%) and carbonylic compounds (25.2%) constituted the main fractions. Among fatty acids hexadecanoic acid (18.6%) was the predominant, while hexahydrofarnesylacetone (7.8%) predominated over the 17 carbonylic compounds. Also hydrocarbons (18.2%) were quite abundant with good amounts of heptacosane (5.9%) and pentacosane (3.8%). Phenols accounted for the 11.2% of the oil and 4-vinyl guaiacol (5.8%) and eugenol (3.6%) were the most representative compounds of this fraction. As in *C. creticum*, sesquiterpenes (4.5%) and monoterpenes (2.9%) were scarce.

Tables II and III report the effects of different doses of the essential oils of *C. nutans* and *C. creticum* on germination and radical elongation of radish, lettuce and cress, respectively. The oils showed different allelopathic activity, with the essential oil of *C. creticum* the more potent. Both oils, however, acted in a dose-dependent way.

Radish seed appeared to be more sensitive to *C. nutans* essential oil, both for germination and radical elongation, whereas this species together with lettuce was affected severely by the oil of *C. creticum*. In fact, the major doses tested of this oil totally inhibited germination of these two test seeds. The lower doses of *C. nutans* essential oil showed a stimulatory activity. It is well known that low doses of inhibitory compounds can stimulate the plant growth (Stebbing 1982). The different allelopathic effects can be due to diverse chemical composition of the essential oil, and in particular to the content of volatile ketones, reported in the specific literature as

Table I. Constituents of the essential oil from aerial parts of *Cirsium creticum* (Cc) and *Carduus nutans* (Cn).

R_i[a]	R_i[b]	Component	Identification[c]	%[d] Cc	%[d] Cn
863		(*Z*)-3-Hexenol	R_i, MS		0.1
961		Benzaldehyde	R_i, MS, Co-GC	t	
979		1-Octen-3-ol	R_i, MS		t
1048	1663	Phenylacetaldehyde	R_i, MS, Co-GC	2.7	2.4
1087		Guaiacol	R_i, MS, Co-GC		0.1
1098	1553	Linalool	R_i, MS, Co-GC		1.4
1104	1398	Nonanal	R_i, MS	1.5	2.6
1113		2-Phenylethanol	R_i, MS, Co-GC		1.0
1134	1637	4-ketoisophorone	R_i, MS	0.3	
1158	1548	(*E*)-2-Nonenal	R_i, MS	0.5	0.7
1183	1856	*p*-Cymen-8-ol	R_i, MS		1.1
1189	1706	α-Terpineol	R_i, MS, Co-GC	1.9	
1201	1243	2 -Penthyl furan	R_i, MS, Co-GC	0.3	1.3
1200	1200	Dodecane	R_i, MS, Co-GC		0.7
1206	1510	Decanal	R_i, MS	1.6	0.7
1208		α-Ionene	R_i, MS	0.3	0.4
1235		Geraniol	R_i, MS, Co-GC		0.4
1264	2035	4-Ethyl guaiacol	R_i, MS	15.0	1.7
1276	1465	Eucarvone	R_i, MS	0.4	
1278	2190	Nonanoic acid	R_i, MS, Co-GC	1.9	
1290	2471	Indole	R_i, MS, Co-GC	0.2	1.2
1295		Undecan-2-one	R_i, MS		0.4
1296		Dihydroedulan I	R_i, MS	t	0.6
1298	2239	Carvacrol	R_i, MS, Co-GC	1.9	
1304	1797	*p*-Methoxyacetophenone	R_i, MS, Co-GC	0.5	0.5
1312	2180	4-Vinyl guaiacol	R_i, MS	4.5	5.8
1313	1827	(*E,E*)-2,4-Decadienal	R_i, MS	0.3	
1323		a $C_{13}H_{18}$	MS	1.1	
1343		Dehydroionene	R_i, MS	1.2	
1353	2186	Eugenol	R_i, MS, Co-GC	1.7	3.6
1356		α-Longipinene	R_i, MS		0.4
1382	1838	(*E*)-β-Damascenone	R_i, MS	7.8	1.2
1400		Tetradecane	R_i, MS, Co-GC		0.3
1410	1538	Italicene	R_i, MS	1.7	
1413		α-Cedrene	R_i, MS		0.1
1415	1612	Caryophyllene	R_i, MS, Co-GC		2.2
1437	1628	Aromadendrene	R_i, MS	0.5	
1449	1625	Widdrene	R_i, MS	1.4	
1451	1868	(*Z*)-Geranyl acetone	R_i, MS	1.8	0.6
1452	1673	(*E*)-β-Farnesene	R_i, MS	0.4	0.9
1455		α-Humulene	R_i, MS		0.1
1477		a $C_{14}H_{22}$	MS	0.2	
1482	1957	(*E*)-β-Ionone	R_i, MS, Co-GC	2.6	
1486	2354	Dihydroactinidiolide	R_i, MS	6.0	1.8
1492	1741	Valencene	R_i, MS	0.1	
1500	1500	Pentadecane	R_i, MS, Co-GC	0.6	0.3
1508	1812	Tridecanal	R_i, MS	1.2	0.8
1523		Megastigmatrienone[#]	R_i, MS	0.4	
1560		(*E*)-Nerolidol	R_i, MS		0.2
1566	2503	Dodecanoic acid	R_i, MS, Co-GC	0.3	2.4
1580	2150	Caryophyllene oxide	R_i, MS, Co-GC		0.6
1590	2512	Benzophenone	R_i, MS, Co-GC	2.7	
1592		2,6-Diisopropylnaphtalene	R_i, MS	0.9	
1600	1600	Hexadecane	R_i, MS, Co-GC	0.6	0.2
1618	1935	Tetradecanal	R_i, MS		0.8
1648		Campherenone	R_i, MS	0.3	
1743	2348	(*E,Z*)-Farnesol	R_i, MS	1.5	
1768	2672	Tetradecanoic acid	R_i, MS, Co-GC	0.4	0.2
1778		Phenanthrene	R_i, MS	0.3	
1815	2138	Hexadecanal	R_i, MS		0.7
1835	2131	Hexahydrofarnesyl acetone	R_i, MS	3.9	7.8
1873	2740	Pentadecanoic acid	R_i, MS, Co-GC	0.1	2.2
1925		Hexadecanoic acid methyl ester	R_i, MS, Co-GC		t
1957	2931	Hexadecanoic acid	R_i, MS, Co-GC	10.6	18.6
2035	2387	Octadecanal	R_i, MS		3.2
2073	2975	Heptadecanoic acid	R_i, MS, Co-GC	0.4	
2120		(*Z*)-9-Octadecenoic acid	R_i, MS, Co-GC	0.8	1.6
2122	3157	(*Z,Z*)-9,12-Octadecadienoic acid	R_i, MS, Co-GC		0.8

Table I (*Continued*)

R_i^a	R_i^b	Component	Identification[c]	%[d] Cc	%[d] Cn
2172	3402	Octadecanoic acid	R_i, MS, Co-GC	0.4	0.2
2300	2300	Tricosane	R_i, MS, Co-GC	t	2.1
2400	2400	Tetracosane	R_i, MS, Co-GC		0.6
2500	2500	Pentacosane	R_i, MS	1.1	3.8
2600	2600	Hexacosane	R_i, MS	0.3	0.7
2700	2700	Heptacosane	R_i, MS	2.4	5.9
2800	2800	Octacosane	R_i, MS	0.8	0.8
2900	2900	Nonacosane	R_i, MS	3.1	2.8
3000		Triacontane	R_i, MS	0.2	
3100	3100	Hentriacontane	R_i, MS	1.1	
		Total		94.4	92.2

R_i^a: Retention index on a HP-5MS column; R_i^b: Retention index on a Innowax column; [c]: Ri = retention index identical to bibliography; MS = identification based on comparison of mass spectra; Co-GC = retention time identical to authentic compounds; [d]: t = trace, less than 0.05%. #: correct isomer not identified.

Table II. Effects of different doses of the essential oil of *Carduus nutans* on germination and radical elongation of radish, lettuce, and cress.

	Germination inhibition (%)			Root elongation inhibition (%)		
Doses	Radish	Lettuce	Cress	Radish	Lettuce	Cress
Control	0 (±1.5)	0 (±0.6)	0 (±1)a	0 (±0.6)a	0 (±0.6)a	0 (±0.59)a
Essential oil						
0.06 µg/ml	10.31 (±0.5)	−16.67 (±1)	−6.67 (±0.57)a	15.63 (±0.75)b	−12.50 (±1)a	−1.82 (±0.3)a
0.125 µg/ml	14.43 (±0.5)	−28.33 (±2.3)	−6.67 (±0.57)a	28.13 (±0.28)c	−6.25 (±2.3)a	−7.27 (±0.2)a
0.25 µg/ml	24.74 (±0.5)	−43.33 (±1.15)	−3.33 (±0.57)a	28.13 (±0.4)c	43.33 (±1.15)ab	12.73 (±0.41)b
0.625 µg/ml	27.84 (±0.5)	−38.33 (±1.5)	−3.33 (±0.57)a	28.13 (±0.35)c	38.33 (±1.5)ab	9.09 (±0.5)bc
1.25 µg/ml	41.24 (±1.1)	−21.67 (±2.6)	0 (±1.0)a	40.63 (±0.25)c	37.50 (±2.6)ab	40.00 (±0.3)c
2.50 µg/ml	48.45 (±2.3)	6.67 (±3.5)	52.22 (±2.3)b	62.50 (±0.35)c	56.25 (±3.5)c	94.55 (±0.17)d
Significance	Ns	Ns	**	**	**	**
			F = 8.038	F = 13.084	F = 4.970	F = 76.156

Data are expressed as the mean of six experiments ± SD. The negative symbol (−) indicate a stimulatory effect.

Table III. Effects of different doses of the essential oil of *Cirsium creticum* on germination and radical elongation of radish, lettuce, and cress.

	Germination inhibition (%)			Root elongation inhibition (%)		
Doses	Radish	Lettuce	Cress	Radish	Lettuce	Cress
Control	0 (±1.5)	0 (±0.6)	0 (±1)a	0 (±0.25)	0 (±0.3)	0 (±1.3)a
Essential oil						
0.06 µg/ml	20.62 (±2.1)	33.33 (±1)	−6.67 (±0.57)a	−15.74 (±0.3)	6.25 (±1.3)	12.73 (±0.8)a
0.125 µg/ml	20.62 (±0.57)	50.00 (±1)	0 (±0.1)ab	16.67 (±0.37)	50.00 (±0.3)	23.64 (±0.4)a
0.25 µg/ml	79.38 (±1)	88.33 (±0.6)	0 (±1.7)ab	87.96 (±0.3)	93.75 (±0.1)	81.82 (±0.6)b
0.625 µg/ml	100 (0)	100 (0)	41.11 (±1.1)b	100 (0)	100 (0)	94.55 (±0.15)bc
1.25 g/ml	100 (0)	100 (0)	96.67 (±0.5)bc	100 (0)	100 (0)	99.45 (±0.05)c
2.50 µg/ml	100 (0)	100 (0)	100 (0)c	100 (0)	100 (0)	100 (0)c
Significance	Ns	Ns	Ns	Ns	Ns	*

Data are expressed as the mean of six experiments ± SD. The negative symbol (−) indicate a stimulatory effect.

powerful germination inhibitors (Asplund 1968, Fischer 1986, Duke & Oliva 2004). It is worthy to note that in the Mediterranean area aromatic shrubs play important roles in determining the vegetational pattern (Vokou 1993).

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