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Volatile constituents of the flowerheads of three *Echinacea* species cultivated in Iran

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ABSTRACT: Three medicinal species of the genus *Echinacea* (Asteraceae), i.e. *E. purpurea*, *E. pallida* and *E. angustifolia*, were cultivated in the experimental field of the Medicinal Plants and Drugs Research Institute of Shahid Beheshti University (Tehran, Iran). The essential oil of flowerheads of the studied species was isolated by hydrodistillation. The essential oils were analyzed by GC and GC-MS. In total, 36, 30 and 36 constituents were identified and quantified in *E. purpurea*, *E. pallida* and *E. angustifolia*, respectively. Sesquiterpene hydrocarbons were the main group of compounds in *E. purpurea* (70.9%), *E. angustifolia* (70%) and *E. pallida* (62.6%). The content of germacrene-D in *E. purpurea* (57%) was higher than that in *E. pallida* (51.4%) and *E. angustifolia* (49.6%) as the principal component in all samples. Also, the monoterpene hydrocarbons were observed in the oil of *E. purpurea* (6.4%) and *E. angustifolia* (1.2%), while these compounds were completely absent in *E. pallida* oil. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: Asteraceae; *Echinacea* spp.; flowerheads; essential oil; germacrene-D; Iran

Introduction

The genus of *Echinacea* (Asteraceae) consists of nine species.[1] Most are native to North America and distributed on barren and dry prairies from Texas to Saskatchewan and from west of the Rocky Mountains to Minnesota.[2–4] Three species of *Echinacea*, i.e. *E. purpurea* (L.) Moench., *E. angustifolia* DC. and *E. pallida* (Nutt.) Nutt. have been found to show medicinal properties.[5] Today, preparations of *Echinacea* species are used as herbal drugs nearly worldwide.[6] These preparations contain different mixtures of various forms of *Echinacea*, and are used as self-medication and prescription drugs for immunostimulation and wound healing.[7]

In recent years, the importance of identifying and characterizing the biologically active constituents of *Echinacea* has been increasingly recognized, and considerable research has been carried out.[8]

Most of the studies reported so far have emphasized the analysis and quantification of the lipophilic (e.g. polyacetylenes and alkamides) and hydrophilic (e.g. polysaccharides and polyphenols) fractions of the hydroalcohlic and/or organic extracts of these plants.[9–18]

A report indicated that the aerial parts oil from *E. purpurea* contained germacrene-D, β-caryophyllene, valencene and 7-epi-α-eudesmol as the major components.[19] For the aerial parts oil of *E. pallida* and *E. angustifolia*, the occurrence of borneol, bornyl acetate, germacrene-D, caryophyllene and caryophyllene oxide as the principal constituents has been reported.[20] Becker determined that the root oil of *E. purpurea* contained mainly germacrene-D, caryophyllene, α-humulene and caryophyllene oxide.[21] In the study by Heinzer *et al*., the root oil of *E. purpurea* was reported to contain 8(*Z*)-pentadecen-2-one, (*E*,*E*)-dodeca-2,4-dienylisovalerate, palmitic and linoleic acids in addition to some alkene derivatives of isovaleric acid.[22] For the achene oils of these plants, the occurrence of α-pinene, β-pinene, myrcene, carvomenthone, β-caryophyllene and germacrene-D as the major components has been reported.[5] The headspace volatile components of roots, leaves and flowers of these species from Canada have also been studied.[23] Recently, the significance of climate change on the essential oil composition of *E. purpurea* under subtropical conditions of India has been reported.[24]

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In recent years, these plants have been imported to Iran and *E. purpurea* is cultivated as a valuable medicinal plant. Also, various preparations made from *E. purpurea* are used in Iranian herbal medicine as an immunostimulating agent against viral and bacterial infections. Here, the composition of the oil from flowerheads of three *Echinacea* species, cultivated in Iran, is reported.

Experimental

Plant material

This study was conducted during 2001–2003 at the experimental field of the Medicinal Plants and Drugs Research Institute of Shahid Beheshti University located in Evin (35°48′285″ N, 51°23′494″ E and altitude 1785 m), north of Tehran. The seeds of three evaluated *Echinacea* species, i.e. *E. purpurea*, *E. pallida* and *E. angustifolia* were obtained from the Forest and Range Management Research Institute of Italy. The seeds were sowed in the greenhouse in February 2001 and then the seedlings transplanted into the experimental field in May 2001. Samples of flowerheads (fully opened) were harvested in June–July 2003. Voucher specimens of *E. purpurea*, *E. pallida* and *E. angustifolia* with herbarium numbers of 200361, 200362 and 200363, respectively, were deposited at the Medicinal Plants and Drugs Research Institute Herbarium, Shahid Beheshti University of Tehran.

Isolation of the oils

Air-dried flowerheads (100 g) of each species were subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 °C until analysed. All of the oils were light yellow and had distinct sharp odor.

Gas chromatography

GC analysis was conducted using a Varian CP-3800 instrument equipped with a capillary DB-1 fused silica column (25 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of 1.1 ml/min. The oven temperature was held at 60 °C for 1 min, then programmed to 250 °C at a rate of 4 °C/min, and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280 °C, respectively.

Gas chromatography–mass spectrometry

GC-MS analysis was carried out on a Thermoquest-Finnigan Trace GC-MS instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from 60 to 250 °C at a rate of 5 °C/min, then held at 250 °C for 10 min; transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 ml/min; split ratio, 1/50. The quadrupole mass spectrometer was scanned over the range 45–46 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA.

Identification of the compounds

The constituents of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for *n-alkanes* (C_6–C_{24}) and the oil on a DB-1 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature.[25,26] For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Results and discussion

The essential oil contents of *E. purpurea*, *E. angustifolia* and *E. pallida* were 0.2, 0.08 and 0.1% (w/w), respectively. The analyses of essential oils from the flowerheads of the studied species are reported in Table 1, where all compounds are arranged in order of their elution on the DB-1 column. In total 36 constituents were identified and quantified in *E. purpurea* oil, 30 in *E. pallida* and 36 in *E. angustifolia*, representing 92.7, 88.4 and 92.3% of the total oil, respectively. A comparison among the compositions of the essential oils revealed both quantitative and qualitative differences. The GC and GC-MS analysis showed that the distribution of monoterpene hydrocarbons of the oil from *E. pallida* and *E. angustifolia* was drastically different from that of the oil of *E. purpurea*. Table 1 shows that the monoterpene hydrocarbons identified in the oil of *E. purpurea* (6.4%); in particular, α-phellandrene (3.2%), α-pinene (1.3%) and myrcene (0.9%) were present in higher amounts than in *E. angustifolia* oil (1.2%), while these compounds were completely absent in the oil of *E. pallida*. Sabinene and *p*-cymene were found only in the oil of *E. purpurea*. Bornyl acetate as an oxygenated monoterpene was not found in the oil of *E. purpurea*.

Sesquiterpene hydrocarbons were the main group of compounds in *E. purpurea* (70.9%), *E. angustifolia* (70%) and *E. pallida* (62.2%). Germacrene-D was the principal component corresponding to half of the total oil content in all samples. Germacrene-D has frequently been reported as a typical, mostly principal, constituent of *E. purpurea* oil.[5,6] In an earlier investigation on the essential oil composition of *E. purpurea* harvested in June from India,[24] myrcene (26.1%), β-pinene (13%) and germacrene-D (7.2%) were found to be the main constituents, while, in our study germacrene-D (57%),

Table 1. Percentage of chemical constituents of the oil of three *Echinacea* spp.

No.	Compounds	*E. purpurea*	*E. pallida*	*E. angustifolia*	RI[a]
	Monoterpene hydrocarbons				
1	α-Pinene	1.3	—	tr	934
2	Sabinene	0.1	—	—	967
3	β-Pinene	0.6	—	tr	975
4	Myrcene	0.9	—	0.7	980
5	α-Phellandrene	3.2	—	tr	1000
6	*p*-Cymene	0.1	—	—	1013
7	Limonene	0.2	—	0.3	1023
8	δ-3-Carene	—	—	0.2	1035
	Oxygenated monoterpene				
9	Bornyl acetate	—	0.7	2.3	1271
	Sesquiterpene hydrocarbons				
10	δ-Elemene	0.1	tr	0.5	1337
11	α-Copaene	0.5	0.3	1.1	1380
12	β-Elemene	0.6	0.8	1.8	1390
13	β-Caryophyllene	4.6	3	7.4	1425
14	(*E*)-α-bergamotene	0.9	0.1	0.2	1433
15	β-Cedrene	0.1	tr	0.2	1446
16	α-Humulene	1.5	1.5	2.8	1457
17	*allo*-Aromadendrene	0.4	1	0.1	1465
18	γ-Curcumene	1.1	tr	tr	1476
19	Germacrene-D	57	51.4	49.6	1487
20	β-Bisabolene	1	0.7	2.4	1493
21	γ-Elemene	1.2	0.6	0.9	1498
22	(*E*,*E*)-α-farnesene	0.1	tr	0.3	1501
23	δ-Guaiene	—	—	0.1	1503
24	γ-Cadinene	0.4	0.9	—	1512
25	δ-Cadinene	1.4	2.3	2.4	1517
26	Cadina-1,4-diene	tr	—	0.2	1534
	Oxygenated sesquiterpenes				
27	(*Z*)-Nerolidol	0.2	0.2	0.1	1521
28	(*E*)-Nerolidol	0.8	1.3	1.9	1545
29	Spathulenol	2	4.3	2.7	1572
30	Caryophyllene oxide	1.8	1.2	1.7	1580
31	Guaiol	1.3	0.8	1.7	1601
32	Globulol	0.7	0.1	1.8	1614
33	Cubenol	0.8	1.4	—	1623
34	α-Cadinol isomer [1]	2.1	3.7	1.8	1631
35	6-Isocedrol	0.5	0.8	0.3	1638
36	α-Cadinol	2.4	4.3	2.2	1645
37	Unknown[b]	1.6	2.1	2.1	1652
38	*cis*-14-Muurol-5-en-4-one	0.7	1.3	1.3	1674
39	(*E*,*Z*)-farnesol	0.5	0.2	0.9	1686
40	(*Z*,*Z*)-farnesol	—	3.4	0.3	1689

[a] Retention indices.
[b] MS, *m/z* (relative intensity): 206[M]$^+$ (35), 163 (100), 145 (12), 136 (38), 105 (49), 93 (57), 79 (70), 67 (13), 55 (31).
tr, trace (<0.1%).

β-caryophyllene (4.6%) and α-phellandrene (3.2%) were characterized as the major components, which could be attributed to their chemotype variability.

In this study, the flowerheads oil from *E. purpurea* contained germacrene-D in higher amounts (57%) than *E. pallida* (51.4%) and *E. angustifolia* (49.6%). Other major sesquiterpene hydrocarbons were β-caryophyllene, δ-cadinene, α-humulene and β-bisabolene. δ-Guaiene as a sesquiterpene hydrocarbon was found only in the flowerhead oil of *E. angustifolia*. δ-Cadinene was detected in the oils of *E. angustifolia* (2.4%), *E. pallida* (2.3%) and *E. purpurea* (1.4%). The high content of germacrene-D and the presence of δ-cadinene in the flowerheads of *E. purpurea* are in agreement with earlier published results from Italy.[19]

The classification of the identified compounds, based on functional groups, is summarized in Table 2. *E. purpurea* is clearly distinct from the other species because of high amounts of monoterpene hydrocarbons; in particular, α-phellandrene (3.2%), and the characteristic lack of bornyl acetate as an oxygenated monoterpene. Oxygenated sesquiterpenes were the main group of compounds in *E. purpurea* (70.9%), *E. angustifolia* (69.9%) and *E. pallida* (62.2%). Finally, the greatest similarity of the oils was observed between *E. purpurea* and *E. angustifolia*, especially in sesquiterpene hydrocarbons.

Table 2. Compound-class composition of the flowerheads oil of three *Echinacea* spp.

Compound class	Content (%)*		
	E. purpurea	*E. pallida*	*E. angustifolia*
Monoterpene hydrocarbons	6.4	—	1.2
Oxygenated monoterpene	—	0.7	2.3
Sesquiterpene hydrocarbons	70.9	62.6	70
Oxygenated sesquiterpenes	15.4	25.1	18.8
Total	92.7	88.4	92.3

* Sum of mean percentage contents recorded in Table 1.

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