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Significance of changed climatic factors on essential oil composition of *Echinacea purpurea* under subtropical conditions†

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ABSTRACT: Variation pattern in the composition of essential oil, hydrodistilled from the over-matured flower heads of *Echinacea purpurea* (L.) Moench under subtropical climate was characterized by GC–MS and GC analysis. The plants flower from June till December and climatic factors such as temperature and humidity were found to affect both the content and the composition of the essential oil. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS: *Echinacea purpurea*; essential oil; subtropical conditions; GC–MS; NMR; germacrene D; β-pinene; myrcene; 1,8-pentadecadiene

Introduction

Essential oils from natural herbs form an integral component in the perfumery, flavour, fragrance and cosmetic industry. *Echinacea*, a herbaceous perennial of the family Asteraceae, although being a medicinal plant reputed to have immunomodulatory virtues, also has a strong aromatic nature. Herbal preparations of *Echinacea* spp. viz. *E. angustifolia*, *E. pallida* and *E. purpurea*, are used the world over for their immunostimulating effects.[1] The biological activity of *Echinacea* is not restricted to any single component, as it has been reported to contain a wide variety of bioactive molecules, e.g. alkamides, caffeic acid derivatives, glycoproteins, polyacetylenes and polysaccharides.[2,3] Essential oils present in the achenes of *Echinacea* spp. have an important role as phytochemical markers in differentiating the three species.[4,5] The present study was undertaken to unravel the effect of altered climatic conditions on the chemical composition of essential oil of *E. purpurea*, a temperate plant grown under subtropical conditions.

Experimental

Plant Material

E. purpurea, an introduced plant species maintained in the medicinal plant repository of our institute, formed the basic material for the present study. The plant material was collected during the year 2000–2001. A voucher specimen is deposited in the herbarium of our institute (RRL No. 59898).

Isolation Procedure

For quantitative analysis of the oil, flower heads harvested at the over-matured stage were dried at 45 ± 2 °C in an oven. The volatile oil was obtained by hydrodistillation of 100 g of this material with water (500 ml for each sample) in a Clevenger-type apparatus for 8 h. The oil samples so obtained were dried over anhydrous Na_2SO_4 (500 mg) and allowed to mature for about 20–30 days at a temperature of 5 °C.

Gas Chromatography

Analysis of the oil samples was done using a NUCON Model 5765 gas chromatography (GC) fitted with a 20 m × 0.25 mm i.d. fused silica capillary column coated with FFAP 0.25 μm film; carrier gas, He with a flow rate of 30 ml/min; injector temperature, 240 °C; temperature (FIDS), 260 °C; and injection volume, 0.2 μl. The programming was carried out from 90 °C for 2 min rsing at 7 °C/min to 180 °C, then at 15 °C/min to 220 °C.

Gas Chromatography–Mass Spectrometry

GC–MS spectra were recorded on a QP-2000 Shimadzu model fitted with a fused silica capillary column (20 m × 0.25 mm i.d.) coated with 0.20 μm CP-Sil 5. The GC was run from 100–270 °C at a programmed rate of 8 °C/min, with hold at 100 °C for 2 min; using He as the carrier gas

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Table 1. Metereological details of cultivation site (Jammu)

Month	Temperature (°C)		Relative humidity (%)	
	Max.	Min.	Highest	Lowest
June	37.0	24.7	78.0	39.2
July	33.8	25.3	92.2	60.9
August	34.0	24.9	92.8	62.4
September	34.4	25.5	91.8	52.7
October	32.5	19.5	80.5	37.5
November	28.8	17.4	86.6	43.8

at a pressure of 1.6 kg/cm^2 and injector temperature of 250 °C. The GC column was coupled directly to the quadrupole mass spectrometer operated in the electron impact (EI) mode at 70 eV. Mass spectra were recorded at a scan speed of 9 at *m*/*z* 700–10.

Identification of Components

Identifications were made by Library search programme on a mono- and sesquiterpenoids mass spectral database and by comparing RRT with those of reference samples.[6,7] The oil samples were directly subjected to analyses over ^{1}H-NMR (200 MHz) and ^{13}C-NMR (50.3 MHz) on a Bruker Model DPX-200 spectrometer, using $CDCl_3$ as solvent and TMS as internal standard. The spectra were analysed by comparing the chemical shift values of the oil components with those reported in the literature. Germacrene D, myrcene, α-pinene and limonene were identified by ^{1}H-NMR and ^{13}C-NMR spectra.

Results and Discussion

The composition of essential oil of *E. purpurea*, distilled from roots and aerial portions, has been reported previously.[8–10] In the present study, of the various parts of this plant analysed, the essential oil was found only in the over-matured floral heads (before the seed-bearing stage). In a subtropical climate (Table 1), the plants flowered from June to December and the samples were taken at the end of every month and subjected to chemical analysis. The analyses were carried out during two growing seasons (2000 and 2001). The content and composition of the essential oil exhibited a variable pattern during the growing season; the content varied from 0.12 to 0.3 ml/100 g during 7 months of the flowering period, with the highest content of 0.3% in October–December (Table 2 and 3). The essential oil was characterized by higher percentage of terpene hydrocarbons, especially the monoterpenoids, which constituted about 60–70% of the oil. The major terpene hydrocarbons found are α-pinene, β-pinene, myrcene, limonene, β-caryophyllene and germacrene D. The percentages of these terpene hydrocarbons exhibited a variable pattern during the growing season of 7 months.

With regard to monoterpenes, the content of α-pinene increased from 1.7% in June to 10.3% in August, but again exhibited a downward trend as the temperature and humidity decreased. However, β-pinene was more stable, as it showed little variation until November. The content of 3-carene was maximal at 4.4% in August and showed a pattern similar to that of α-pinene. By contrast, myrcene was reduced from 26.1% in June to 10.5% in December. Like α-pinene and 3-carene, the content of limonene was higher (6.1%) in August; *p*-cymene (1.0–3.5%) and β-farnesene (0.5–2.1%) also showed variability. The content of β-caryophyllene was maximal (9.0–9.3%) in October and November, when the weather changes and the temperature and humidity decrease. However, in December the β-caryophyllene was found to be low (4.1%), perhaps because dormancy set in due to the onset of winter.

The most abundant terpene found in the oil was germacrene D, which showed a steady rise from 7.2% in June (hot and humid weather) to 33.5%, a very steep rise, in December.

Variability was also observed in the concentration of 1,8-pentadecadiene, which was maximum in September (7.5%) and decreased as the achenes developed. The above results indicate that the various components of the essential oil of *E. purpurea* are specific to climatic factors, which influence their concentration. The variations in their concentrations under subtropical conditions may be due to expression of these characters under altered agroclimatic conditions, as *Echinacea* is primarily a plant grown commercially in countries having a temperate agroclimate.

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Table 2. Chemical composition of *E. purpurea* grown under subtropical conditions

No.	Compound name	RI	Percentage	Method of identification
1.	Hexenal	776	0.1–0.4	GC, RT
2.	Thujene	921	0.1–2.3	GC, RT
3.	α-Pinene	930	1.7–10.3	GC, MS, RT, ^{13}C-NMR
4.	Camphene	940	tr	GC, RT
5.	Sabinene	965	0.4–1.2	GC, RT
6.	β-Pinene	968	tr–13.0	GC, MS, RT, ^{13}C-NMR
7.	Myrcene	983	10.5–26.1	GC, RT, ^{13}C-NMR, ^{1}H-NMR
8.	α-Phellandrene	998	0.1–0.6	GC, RT
9.	3-Carene	1009	tr–4.4	GC, RT
10.	p-Cymene	1018	1.0–35	GC, RT, ^{13}C-NMR
11.	Limonene	1020	1.0–6.1	GC, RT, ^{13}C-NMR
12.	1,8-Cineole	1022	tr	GC, RT
13.	β-Phellandrene	1031	0.3–0.6	GC, RT
14.	γ-Terpinene	1047	0.2–0.7	GC, RT
15.	Terpinolene	1074	0.2–0.3	GC, RT
16.	α-Cubebene	1362	0.3–7.0	GC, MS, ^{13}C-NMR
17.	δ-Elemene	1365	0.5–1.0	GC, MS, ^{13}C-NMR
18.	α-Copaene	1370	0.1–0.7	GC, RT
19.	β-Cubebene	1390	0.5–1.5	GC, RT
20.	β-Elemene	1391	0.3–1.2	GC, RT
21.	β-Caryophyllene	1412	0.5–9.3	GC, RT, ^{13}C-NMR
22.	β-Farnesene	1422	0.5–2.1	GC, RT, ^{13}C-NMR
23.	Calarene	1430	tr	GC, RT
24.	α-Humulene	1441	0.1–0.5	GC, RT, MS
25.	Germacrene D	1468	7.2–33.5	GC, RT, MS, ^{13}C-NMR, ^{1}H-NMR
26.	Bicyclogermacrene	1476	0.3–0.6	GC, RT, MS
27.	α-Muurolene	1479	0.1–1.5	GC, RT
28.	1,8-Pentadecadiene	1488	1.0–7.5	GC, RT, MS
29.	δ-Cadinene	1512	0.2–0.5	GC, RT, MS
30.	α-Cadinene	1520	0.3–0.6	GC, RT, MS
31.	Calamenene	1548	tr	GC, RT
32.	Nerolidol	1561	0.5–0.6	GC, RT, MS
33.	β-Caryophyllene oxide	1570	0.7–2.2	GC, RT, MS
34.	Spathulenol	1575	0.1–1.5	GC, RT, MS
35.	Cubenol	1620	0.2–1.0	GC, RT, MS
36.	β-Eudesmol	1630	0.1–1.0	GC, RT, MS
37.	Epishiobunol	1662	0.5–1.5	GC, RT, MS
38.	β-Bisabolol	1674	0.5–0.6	GC, RT, MS

tr, trace (<0.1%).

Table 3. Variation pattern in the major components of *Echinacea purpurea*

Oil and components (%)	June	July	August	September	October	November	December
Oil	0.2	0.1	0.1	0.2	0.3	0.3	0.3
α-Pinene	1.7	5.5	10.3	3.0	2.7	4.5	6.6
β-Pinene	13.0	12.9	12.8	8.9	12.3	12.7	tr
3-Carene	0.6	2.0	4.4	1.0	0.7	tr	0.5
Myrcene	26.1	18.2	15.5	10.7	20.4	18.9	10.5
Limonene	2.0	1.0	6.1	2.1	2.7	4.8	1.5
β-Caryophyllene	2.5	1.5	0.5	4.1	9.0	9.3	1.5
Germacrene D	7.2	9.5	21.3	17.7	21.9	32.0	33.5
β-Caryophyllene oxide	1.8	1.0	0.7	0.7	2.2	1.0	0.50
1,8-Pentadecadiene	1.5	1.0	1.0	7.5	2.5	2.5	2.5

tr, trace (<0.1%).

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