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Original Article

Volatile constituents of essential oils isolated from flowers and leaves of *Eupatorium cannabinum* L. from Iran

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Abstract

Hydro-distilled volatile oils from the flowers and leaves of *Eupatorium cannabinum* L. which gathered in Touchal on Mountain Alborz (Tehran province) in Iran were investigated mainly by a combination of GC and GC/MS. Thirty-nine components were identified in the oils. The main constituents of the essential oils in flowers and leaves were germacrene D (27.3%, 37.1%), gemacrene B (12.4%, 11.7%) and β-caryophyllene (8.6%, 10.1%). Although the main components of all the oils are similar, their percentages are different.

Keywords: *Eupatorium cannabinum*; Compositae; Essential oil composition; Germacrene D; Germacrene B.

Introduction

Eupatorium L. belongs to family Compositae and comprises about 1200 species, which occur largely in America, but a few species are also to be found in Europe, Asia, and Africa. It is classified in the tribe Eupatorieae. One species of this genus is found in Iran (1). We have studied the composition of essential oils flowers and leaves of *Eupatorium cannabinum* in Iran. This plant is a perennial herb, of common on the banks of rivers sides of ditches and in other damp places in Iran. As a medicinal plant, *Eupatorium cannabinum* has been traditionally used as febrifuge, cathartic, diuretic and scorbutic properties (2).

An infusion of the fresh herb act as a strong purgative and emetic. Due to its content of alkaloids, the plant should only be used under professional supervision (2). The essential oil of *E.cannabinum* was analysed in India by hydrodistillation of fresh leaves and fourteen compounds were identified (3). The volatile oil composition of this genus has been previously reported (8-22). The qualitative and quantitative compositions of the essential oils of 3 Eupatorium species have been investigated. (4). Six flavonoids have been isolated from leaf exudates of *E. cannabinum* from Netherlands (5). Recent research has shown that the plant might have anti tumor activity, though the plant also contains pyrrolizidine alkaloids that can cause damage or cancer to the liver (6, 7). Literature search did not reveal any reference to previous work on the essential oil of different parts of *E. cannabinum* from Iran.

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Experimental

Plant material

Flowers and leaves of *E. cannabinum* L. were collected from Touchal on Mountain Alborz (Tehran province), at an altitude of 1800m in May 2004. The voucher specimens have been deposited in the national herbarium of Iran (TARI).

Isolation procedure

Dried plant material (80-100g) was subjected to hydro-distillation for 3h using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature before analysis.

Gas chromatography

GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30m x 0.25 mm i.d., film thickness 0.25 µm). Oven temperature was held at 50ºC for 5 min and then programmed to 250ºC at a rate 3˚C/min. Injector and detector (FID) temperatures were 290ºC; helium was used as the carrier gas with a linear velocity of 32 cm/s.

Gas chromatography-Mass spectrometry

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d, film thickness 0.25 µm.). Oven temperature was 40-240ºC at a rate of 3ºC min, injector temperature 250ºC and transfer line temperature 260ºC , carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 eV, scan time 1 s, mass range 40-300 amu.

Identification of components

The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (8).

Results and discussion

Table 1 summarizes the identified compounds, their retention indices, their percentage composition and the method of identification. The constituents are arranged in order of their elution on the DB-5 column. The yields based on dry weight of samples were as follows: flowers, 0.1% and leaves, 0.2%. Thirty one compounds were identified, constituting 94.5% *Eupatorium cannabinum* flowers oil. The major components of this oil were found to be germacrene D (27.3%), germacrene B (12.4%), valencene (10.5%) and β-caryophyllene (8.7%). Thirty one compounds were identified constituting 97.5% leaves oil. The main constituents of pale yellow leaves oil were shown to be germacren D (37.1%), germacrene B (11.7%), β-caryophyllene (10.2%) and delta-2-carene (8.5%). Literature review showed that the chemical compositions of the essential oils from *E. odoratum* were examined in Cameroon and Congo, α-pinene (14.3%) and p-cymene (22.2%) being the most dominant (9). *Eupatorium coelestinum* collected from Vietnam showed that methyl chavicol were identified in stem and leaf (9.7% and 42.2% respectively). (10). Essential oil of *E. capillifolium* from Cuba was analyzed and p-cymene (23.7%) was the major constituents (11) and in Bingerville the main oil constituents of *E. odoratum* were identified as α-pinene (18.8%), beta pinene (10.5%) and pregeijerene (14.3%) (12).The essential oil of *E. cannabinum* in India had been investigated and beta caryophyllene oxide (28.5%) was the major component (3), but in our investigation this compound was found to be 8.7-10.2%. (8.7-10.2%). 6-methoxy flavones hispidulin and eupafolin have been identified for the first time from the aerial parts of eupatorium cannabinum. (13). *E. ballotaefolium* was characterized by limonene (15.3%) and (E)-beta ocimene (10.5%) as the major constituents (14). *E. triplinerve* and *E. paniculatum* (collected from Brazil) were analysed. *E. triplinerve* was dominated by 2, 5-dimethoxy p-cymene (69.7%) and in *E. triplinerve* the major constituent identified was beta-caryophyllene (46.8%) (15).The essential oils of six *Eupatorium* species were analysed and showed *E.macrophyllum* was rich in sabinene (46.7%), *E. laevigatum* was mainly constituted by a mixture of aristolone+laevigatin (23.6%). The main constituents of the oils of *E. squalidum*, *E. amygdalinum* and *E. conyzoides* were caryophyllene oxide (17.4-30.1%). The oils of *E. marginatum* were dominated by α-zingiberene (57.5%) (16 Maia, etal 2002). The major components of *Eupatorium triplinerve* were selina-4(15), 7(11)-dien-8-one (36.6%) and beta caryophyllene (14.7%) (17). Analysis of the essential oil of the leaves and flowers/ fruits of *Eupatorium cannabinum* in Tuscany

Table 1. Percentage composition of the oils of different parts of *Eupatorium cannabinum* L.

No	Compound	RI	Flowers	Leaves	Method of Identification
1	α-pinene	935	-	0.2	a,b
2	camphene	948	-	0.2	a,b
3	sabinene	978	-	0.1	a,b
4	myrcene	990	-	0.1	a,b
5	delta-2-carene	1000	0.4	8.5	a,b
6	α-phellandrene	1003	1.3	4.9	a,b
7	*p*-cymene	1024	1.6	0.8	a,b
8	limonene	1029	0.1	0.4	a,b
9	(Z)-β-ocimene	1036	-	0.2	a,b
10	benzenacetaldehyde	1042	0.4	-	a,b
11	(E)-β-ocimene	1048	0.2	1.5	a,b
12	terpinolene	1086	0.1	0.2	a,b
13	linalool	1096	0.1	0.1	a,b
14	nonanal	1102	0.4	0.2	a,b
15	phenyl ethylalcohol	1112	0.3	-	a,b
16	α-terpineol	1188	0.5	0.1	a,b
17	decanal	1202	0.1	t	a,b
18	nerol	1230	0.5	-	a,b
19	thymol<methyl ether>	1233	6	4.3	a,b
20	thymoquinone	1250	6.2	-	a,b
21	bornyl acetate	1284	0.1	0.1	a,b
22	thymol	1292	0.6	-	a,b
23	hexyl tiglate	1329	t	0.1	a,b
24	neryl acetate	1363	8.7	3.3	a,b
25	geranyl acetate	1385	0.2	-	a,b
26	β-cubebene	1389	0.3	0.3	a,b
27	β-elemene	1391	-	0.4	a,b
28	longifolene	1406	-	0.2	a,b
29	β-caryophyllene	1421	8.7	10.2	a,b
30	coumarine	1432	0.6	-	a,b
31	α-guaiene	1443	0.2	-	a,b
32	α-humulene	1457	1.3	1.7	a,b
33	α-patechoulene	1460	1.8	0.6	a,b
34	germacrene D	1485	27.3	37.1	a,b
35	valencene	1494	10.5	6.7	a,b
36	bicyclogermacrene	1498	0.9	1.6	a,b
37	β-himachalene	1502	-	0.5	a,b
38	δ- cadinene	1526	2.7	1.2	a,b
39	germacrene B	1560	12.4	11.7	a,b

RI: Retention indices relative to C_9-C_{24} n-alkanes on DB-5 column (standard compared with products of Fluka chemie company in Switzerland)

a: retention indices relative to n-alkanes on DB-5 column

b: compared with mass spectra

t: trace, less than 0.05

showed that cyperone (16.9%) was the main component (18). Pyrrolizidine alkaloids have been studied in *Eupatorium cannabinum* and *japonicum, viridiflorine, cynaustraline* (19). A study has been made on the essential oil from *E. fortunei, E. japonicum, E. chinense* and *E. cannabinum* and provides scientific methods for the identification of components of these species (20). The new diterpene glucoside has been isolated from *E. glutinosum* (21). Beta caryophyllene (12.4-41.7%), humulene (11.7-14.6%) and gamma-muurolene (10.4-19%) were the most prominent constituents of *E. betonicaeforme* (22). Comparing the oil compositon in *E. cannabinum* in Iran with other Eupatorium species showed that there is not any resemblance with them, but *E. cannabinum* in our study was rich of germacrene D and in other investigation beta caryophyllene has been predominant.

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