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Analysis of the essential oil from aerial parts of *Eupatorium cannabinum* subsp. *corsicum* (L.) by gas chromatography with electron impact and chemical ionization mass spectrometry

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Abstract

Eupatorium cannabinum subsp. *corsicum* (L.), an aromatic plant, is an endemic subspecies from Corsica. The essential oil from aerial parts of *E. cannabinum* subsp. *corsicum* was studied by GC, GC/MS and ^{13}C NMR. One hundred and forty-seven components were identified representing 93.6% of the total amount. The main constituents are germacrene D (28.5%), α-phellandrene (19.0%) and *para*-cymene (5.2%). A particularity of this essential oil is the presence of monoterpene esters derived from nerol, lavandulol, borneol, thymol and 8,9-dehydrothymol. These compounds have been investigated using GC/MS in different ionization modes like electron impact (EI), positive chemical ionization (PCI) and negative chemical ionization (NCI).
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Keywords: *Eupatorium cannabinum*; Asteraceae; Essential oils; Volatile organic compounds; Terpene esters; Chemical ionization (CI)

1. Introduction

The genus *Eupatorium* (Asteraceae) from the tribe Eupatorieae, comprises 45 species present in North America and in Asia [1]. Species of the genus *Eupatorium* are perennial plants with opposed, divided or simple leaves with white, pink or crimson flowers [2]. The type of species *Eupatorium cannabinum* has extended its range into Europe and India [1].

The extract of aerial parts of *E. cannabinum* contains sesquiterpene lactones, pyrrolizidine alkaloids, flavonoides, diterpenes and triterpenes [1,3–5].

The essential oil of aerial parts of *E. cannabinum* has been the subject of some investigations [3,6–10]. The first study on the essential oil of aerial parts of *E. cannabinum* (origin and subspecies not specified) was undertaken by Hendriks et al. [3]. Thirty-four constituents were identified by GC/MS(EI) and the essential oil was dominated by germacrene D, α-terpinene, neryl acetate, *para*-cymene and thymyl methyl oxide (relative percentages not given). Thereafter, using GC/MS(EI) and GC/MS(NCI), Hendriks et al. [6] identified nearly 70 compounds in aerial parts of *E. cannabinum*. Perineau et al. [7] have identified 14 components in essential oil of *E. cannabinum* (origin and subspecies not specified) representing 52% of the total amount: cyperone (16.9%), (*E*)-(*E*)-α-farnesene (12.3%) and spathulenol (6.1%) as major components. In essential oil of leaves of *E. cannabinum* (subspecies not specified) from Northern India [8], 14 constituents were identified representing approximately 99% of the total oil: caryophyllene oxide (28.5%), calarene (14.2%), β-selinene (10.2%), β-myrcene (6.2%), limonene (5.8%) and (*E*)-β-caryophyllene (5.0%) as major components. The essential oil of aerial part of *E. cannabinum* subsp. *cannabinum* from South Italy [9] was dominated by germacrene D (33.5%), α-farnesene (12.9%), δ-2-carene (6.5%), *para*-cymene (4.5%), (*E*)-β-caryophyllene (4.5%), α-phellandrene (3.6%), bicyclogermacrene (3.0%), elemol (2.8%) and α-cadinol (2.7%). Fifty-five compounds were identified by GC and GC/MS(EI) representing 94.1% of the essential oil. Recently, Flamini et al. [10] reported the essential oil of leaves

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and flowers–fruits of *E. cannabinum* (subspecies not specified) from South Tuscany (Central Italy), 66 compounds were identified in the leaves oil and 70 in the flowers–fruits oil, accounting for 78.9% and 86.1% of the total oils, respectively. The main constituents were germacrene D (leaves: 29.2%, flowers–fruits: 20.3%), bicyclogermacrene (leaves: 4.6%, flowers–fruits: 12.3%), thymyl methyl oxide (leaves: 7.8%, flowers–fruits: 12.2%) and spathulenol (leaves: 7.3%, flowers–fruits: 10.8%).

E. cannabinum subsp. *corsicum* (L.) is an endemic subspecies from Corsica, Southerner Italy, Sardinia and the Cevennes with pink flowers which appears from July to September. The genus *Eupatorium* is represented in Corsica by the only species *cannabinum* and by the subspecies *corsicum* alone. This one is well represented in the river banks of relative low altitude (0–1100 m) and is widespread on the whole island. The Corsican populations were different from the continental ones by a smaller size (50–80 cm) and with the presence of many individuals with indivisible leaves [2].

According to our knowledge, the essential oil of aerial parts of *E. cannabinum* subsp. *corsicum* is reported for the first time. In the present work, the essential oil from aerial parts of *E. cannabinum* subsp. *corsicum* was studied by GC, GC/MS(EI), GC/MS(CI) and ^{13}C NMR, after fractionation over silica gel. The aim of the present work was to obtain knowledge about the essential oil of *E. cannabinum* from Corsica island for a possible valorization. The objective of this study was also to demonstrate the complementarity of different spectroscopic techniques for the identification of components in a complex mixture.

2. Materials and methods

2.1. Plant material

The aerial parts of *E. cannabinum* subsp. *corsicum* were collected in July 2003 from Corsica (France) from four central localities (Barchetta, Francardo, Caporalino, Corte). Voucher specimens were deposited in the herbarium of University of Corsica, Corte, France.

2.2. Essential oil isolation

The fresh aerial parts of the plants were water-distillated (5 h) by using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [11] and the essential oils yields were 0.05–0.07%.

2.3. GC analysis

GC analyses were carried out using a Perkin-Elmer Autosystem XL GC apparatus equipped with dual flame ionization detection (FID) system and fused-silica capillary columns (60 m × 0.22 mm I.D., film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethyleneglycol). The oven temperature was programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. Injector and detector temperatures were maintained at 280 °C. Samples were injected in the split mode (1/50), using helium as carrier gas (1 mL/min); injection volume, 0.2 μl of pure oil.

Retention indices (*I*) of compounds were determined relative to the retention times of series of *n*-alkanes with linear interpolation, using software from Perkin-Elmer.

2.4. GC/MS(EI and CI) analysis

GC/MS(EI) conditions: Samples were analysed with a Perkin-Elmer Turbo mass detector (quadrupole), coupled to a Perkin-Elmer Autosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. Carrier gas: helium (1 mL/min), ion source temperature: 150 °C, oven temperature programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally at 230 °C (35 min), injector temperature: 280 °C, energy ionization: 70 eV, electron ionization mass spectra were acquired over the mass range 35–350 Da, split: 1/80, injection volume: 0.2 μl of pure oil.

GC/MS(CI) conditions: The PCI and NCI mass spectra were recorded on the same apparatus equipped with a Rtx-Wax column and specific ionization chemical source. Ionizing gas: methane (CH_4) or ammonia (NH_3). Carrier gas: helium (1 mL/min), ion source temperature: 150 °C, source pressure: 0.2 mbar, oven temperature programmed from 60 °C to 230 °C at 2 °C/min and then held isothermal 230 °C (35 min), injector temperature: 280 °C, energy ionisation: 70 eV, chemical ionisation mass spectra were acquired over the mass range 60–350 Da, split: 1/80, injection volume: 0.2 μl of pure oil.

2.5. NMR analysis

All NMR spectra were recorded on a Bruker Avance 400 Fourier Transform spectrometer operating at 100.13 MHz for ^{13}C NMR, equipped with a 5 mm probe, in deuterated chloroform, with all shifts referred to internal tetramethylsilane (TMS).

^{13}C NMR spectra were recorded with the following parameters: pulse width = 4 μs (flip angle 45°); acquisition time = 2.7 s for 128 000 data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 5000 for each sample (around 40 mg of the oil in 0.5 mL of C^2HCl_3). Exponential line broadening multiplication (1 Hz) of the free induction decay was applied before Fourier transformation. ^{1}H NMR were recorded with the following parameters: flip angle 30°; acquisition time = 2.56 s for 32 000 data table with a spectral width of 7000 Hz (17.5 ppm).

2.6. Oil fractionation

The essential oil of aerial parts of *E. cannabinum* subsp. *corsicum* (5 g) was separated by flash chromatography (silica

Table 1
Constituents of essential oil of aerial parts of *Eupatorium cannabinum* subsp. *corsicum*

No.	Components	I_a	I_p	Percent	Identification	Ref.	Fra.
1	(*Z*)-Hex-3-ene-1-ol	818	1377	0.2	RI, EI		**II.9**
2	(*Z*)-Hex-2-ene-1-ol	827	1405	0.1	RI, EI		**II.9**
3	Hexanol	834	1357	0.1	RI, EI		**II.9**
4	α-Pinene	930	1024	0.2	RI, EI		**I.1**
5	Camphene	943	1066	1.6	RI, EI		**I.1**
6	β-Pinene	970	1111	0.3	RI, EI		**I.1**
7	β-Myrcene	981	1166	0.8	RI, EI		**I.1**
8	δ-2-Carene	996	1140	3.9	RI, EI		**I.1**
9	α-Phellandrene	996	1169	19.0	RI, EI		**I.1**
10	Phenylacetaldehyde	1011	1624	tr	RI, EI		**II.4**
11	*para*-Cymene	1012	1271	5.2	RI, EI		**I.6**
12	β-Phellandrene	1020	1215	0.1	RI, EI		**I.3**
13	Limonene	1022	1205	0.7	RI, EI		**I.4**
14	(*Z*)-β-Ocimene	1026	1238	0.2	RI, EI		**I.5**
15	(*E*)-β-Ocimene	1037	1254	1.5	RI, EI		**I.5**
16	Nonanal	1081	1395	tr	RI, EI		**II.2**
17	Linalol	1082	1547	0.2	RI, EI		**II.6**
18	Undecane	1100	1100	tr	RI, EI		**I.1b**
19	(E)-4,8-Dimethyl-1,3,7-nonatriene	1105	1309	tr	RI, EI	[13]	**I.9**
20	*cis*-*para*-Menth-2-ene-1-ol	1106	1561	0.1	RI, EI	[13]	**II.8**
21	*trans*-*para*-Menth-2-ene-1-ol	1123	1625	0.1	RI, EI	[13]	**II.9**
22	Neryl oxide	1137	1469	tr	RI, EI		**II.4**
23	Lavandulol	1148	1671	tr	RI, EI		**II.7**
24	Borneol	1149	1692	tr	RI, EI		**II.8**
25	Terpinene-4-ol	1161	1672	tr	RI, EI		**II.6**
26	α-Terpinéol	1172	1692	0.2	RI, EI		**II.8**
27	*cis*-Piperitol	1179	1742	tr	RI, EI		**II.6**
28	Decanal	1182	1498	tr	RI, EI		**II.2**
29	*trans*-Piperitol	1189	1738	0.1	RI, EI		**II.7**
30	8,9-Dehydrothymol	1193	1961	0.1	RI, EI	[13]	**II.4**
31	β-Cyclocitral	1197	1611	tr	RI, EI		**II.4**
32	Nerol	1208	1793	0.1	RI, EI		**II.8**
33	Pulegone	1214	1637	tr	RI, EI		**II.5**
34	Thymyl methyl oxide	1215	1586	2.1	RI, EI		**II.1**
35	Carvotanacetone	1221	1669	tr	RI, EI	[13]	**II.5**
36	Carvacryl methyl oxide	1224	1594	tr	RI, EI		**II.1**
37	Linalyl acetate	1237	1555	tr	RI, EI		**II.2**
38	Thymol	1267	2157	tr	RI, EI		**II.5**
39	Bornyl acetate	1269	1579	0.6	RI, EI		**II.2**
40	Lavandulyl acetate	1271	1606	0.4	RI, EI		**II.3**
41	Carvacrol	1275	2183	tr	RI, EI		**II.6**
42	(*E*)-(*E*)-2,4-Decadienal	1288	1798	tr	RI, EI		**II.5**
43	Tridecane	1300	1300	tr	RI, EI		**I.1′**
44	7-α-[*H*]-Silphiperfol-5-ene	1326	1429	0.3	RI, EI, ^{13}C	[12,13]	**I.1′**
45	α-Terpinyl acetate	1330	1688	tr	RI, EI		**II.2**
46	Bicycloelemene	1334	1481	0.1	RI, EI	[13]	**I.10**
47	Presilphiperfol-7-ene	1335	1414	0.2	RI, EI	[13]	**I.1′**
48	Neryl acetate	1342	1725	2.3	RI, EI		**II.3**
49	7-β-[*H*]-Silphiperfol-5-ene	1348	1454	0.6	RI, EI, ^{13}C	[12,13]	**I.1′**
50	Silphin-1-ene	1348	1474	1.6	RI, EI, ^{13}C	[12,13]	**I.1′**
51	α-Cubebene	1350	1460	0.1	RI, EI		**I.3**
52	Geranyl acetate	1358	1752	tr	RI, EI		**II.4**
53	(*E*)-β-Damascenone	1362	1810	tr	RI, EI		**II.4**
54	Cyclosativene	1377	1483	tr	RI, EI		**I.1′**
55	α-Copaene	1377	1496	0.2	RI, EI		**I.1′**
56	Silphiperfol-6-ene	1377	1496	0.6	RI, EI, ^{13}C	[12,13]	**I.1′**
57	Pethybrene	1377	1512	tr	RI, EI	[13]	**I.1′**
58	Modhephene	1382	1522	0.8	RI, EI, ^{13}C	[12,13]	**I.1′**
59	β-Cubebene	1387	1537	0.4	RI, EI		**I.3**
60	β-Elemene	1387	1589	0.7	RI, EI		**I.6**
61	β-Bourbonene	1388	1519	tr	RI, EI		**I.2**
62	α-Isocomene	1388	1533	0.2	RI, EI	[13]	**I.1′**

Table 1 (*Continued*)

No.	Components	I_a	I_p	Percent	Identification	Ref.	Fra.
63	Sativene	1390	1525	tr	RI, EI		**I.1′**
64	(*E*)-β-Damascone	1391	1805	tr	RI, EI	[13]	**II.5**
65	Bornyl isobutyrate	1402	1641	1.2	RI, EI, CI, ^{13}C	S, [13]	**II.1**
66	β-Isocomene	1406	1571	0.2	RI, EI	[13]	**I.1′**
67	Lavandulyl isobutyrate	1410	1677	tr	RI, EI, CI	S, [17]	**II.1**
68	β-Ylangene	1418	1568	tr	RI, EI		**I.2**
69	(E)-β-Caryophyllene	1419	1593	3.1	RI, EI		**I.4**
70	β-Copaene	1426	1585	0.1	RI, EI		**I.2**
71	Geranyl acetone	1426	1846	tr	RI, EI		**II.5**
72	Neryl propionate	1428	1784	tr	RI, EI		**II.2**
73	*trans*-α-Bergamotene	1430	1580	tr	RI, EI		**I.3**
74	Aromadendrene	1436	1599	0.1	RI, EI		**I.2**
75	Alloaromadendrene	1451	1631	0.2	RI, EI		**I.2**
76	α-Humulène	1451	1661	1	RI, EI		**I.10**
77	8,9-Dehydrothymyl isobutyrate	1454	1922	0.2	RI, EI, CI	[6,13]	**II.2**
78	Thymyl isobutyrate	1458	1889	0.7	RI, EI, CI, ^{13}C	S, [6,13]	**II.1**
79	2-Phenyl ethyl 2-methylbutyrate	1460	1950	tr	RI, EI		**II.3**
80	β-Ionone	1462	1923	tr	RI, EI		**II.5**
81	2-Phenyl ethyl isovalerate	1463	1964	tr	RI, EI		**II.3**
82	Neryl isobutyrate	1469	1780	0.3	RI, EI, CI, ^{13}C	S, [6,13]	**II.1**
83	γ-Muurolene	1471	1681	0.2	RI, EI		**I.3**
84	Germacrene D	1477	1701	28.5	RI, EI		**I.9**
85	trans-β-Bergamotene	1479	1678	tr	RI, EI		**I.4**
86	Cubebol	1485	1880	0.1	RI, EI		**II.7**
87	Lavandulyle 2-methylbutyrate	1490	1759	0.3	RI, EI, CI	S, [17]	**II.1**
88	Tridecan-2-one	1491	1806	tr	RI, EI		**I.4**
89	Ledene	1492	1686	0.2	RI, EI		**I.3**
90	α-Muurolene	1494	1716	tr	RI, EI		**I.3**
91	Bicyclogermacrene	1494	1724	1.7	RI, EI		**I.10**
92	Bornyl 2-methylbutyrate	1494	1729	1.2	RI, EI, CI, ^{13}C	S	**II.1**
93	Lavandulyl isovalerate	1494	1761	0.1	RI, EI, CI	S, [17]	**II.1**
94	(*E*)-(*E*)-α-Farnesene	1496	1744	0.9	RI, EI		**I.10**
95	β-Bisabolene	1500	1718	0.4	RI, EI		**I.10**
96	Bornyl isovalerate	1501	1753	0.2	RI, EI, CI	S, [6]	**II.1**
97	4-*epi*-Cubebol	1504	1928	0.2	RI, EI		**II.8**
98	γ-Cadinene	1507	1748	0.2	RI, EI		**I.4**
99	Calamenene	1511	1818	tr	RI, EI		**I.3**
100	Presilphiperfolane-9-α-ol	1515	1992	tr	RI, EI, ^{13}C	[13,19]	**II.9**
101	δ-Cadinene	1516	1748	0.6	RI, EI		**I.4**
102	α-Cadinene	1531	1779	tr	RI, EI		**I.4**
103	Elemol	1531	2052	tr	RI, EI		**II.8**
104	α-Calacorene	1539	1894	tr	RI, EI		**I.9**
105	Nerolidol E	1544	2013	0.1	RI, EI		**II.6**
106	β-Calacorene	1548	1932	tr	RI, EI		**I.9**
107	8,9-Dehydrothymyl 2-methylbutyrate	1548	1996	0.2	RI, EI, CI		**II.2**
108	8,9-Dehydrothymyl isovalerate	1548	2006	0.2	RI, EI, CI	[6]	**II.2**
109	Thymyl 2-methylbutyrate	1551	1964	0.2	RI, EI, CI	S	**II.I**
110	Thymyl isovalerate	1551	1975	0.3	RI, EI, CI	S, [6]	**II.1**
111	Germacrene B	1554	1816	0.1	RI, EI	[13]	**I.10**
112	Neryl 2-methylbutyrate	1554	1857	0.2	RI, EI, CI	S	**II.1**
113	1,5-Epoxysalvial-4-(14)-ene	1554	1959	tr	RI, EI		**II.4**
114	Maaliol	1559	1973	tr	RI, EI	[13]	**II.7**
115	Neryl isovalerate	1560	1867	0.1	RI, EI, CI	S, [6]	**II.1**
116	Palustrol	1562	1914	tr	RI, EI		**II.5**
117	Spathulenol	1563	2103	0.3	RI, EI		**II.7**
118	Gleenol	1572	2008	tr	RI, EI		**II.5**
119	β-Germacrenol	1573	2019	tr	RI, EI		**II.5**
120	Caryophyllene oxide	1575	1960	0.2	RI, EI		**II.5**
121	Globulol	1576	2051	0.1	RI, EI		**II.9**
122	Viridiflorol	1580	2054	0.1	RI, EI		**II.7**
123	Ledol	1588	1997	tr	RI, EI		**II.7**
124	Apiol dill	1589	2327	tr	RI, EI		**II.4**
125	Copaborneol	1592	2159	0.3	RI, EI	[13]	**II.7**

Table 1 (*Continued*)

No.	Components	I_a	I_p	Percent	Identification	Ref.	Fra.
126	Humulene 6,7-epoxide	1594	2011	tr	RI, EI		**II.5**
127	Eudesm-4(15)-ene-6-ol	1610	2025	0.3	RI, EI	[13]	**II.5**
128	1-10-*epi*-Cubenol	1610	2054	tr	RI, EI		**II.4**
129	*epi*-Cubenol	1615	2033	0.2	RI, EI		**II.6**
130	γ-Eudesmol	1616	2149	tr	RI, EI		**II.8**
131	*iso*-Spathulenol	1619	2206	tr	RI, EI	[13]	**II.8**
132	τ-Cadinol	1625	2152	0.3	RI, EI		**II.7**
133	τ-Muurolol	1625	2170	0.3	RI, EI		**II.7**
134	8,9-Dehydrothymyl tiglate	1627	2205	tr	RI, EI, CI	[6]	**II.2**
135	Cubenol	1631	2023	0.1	RI, EI		**II.4**
136	Thymyl tiglate	1634	2169	0.6	RI, EI, CI, ^{13}C	[6]	**II.2**
137	α-Cadinol	1643	2218	1.0	RI, EI		**II.9**
138	Neryl tiglate	1652	2054	tr	RI, EI, CI	[6]	**II.2**
139	Valeranone	1658	2087	0.1	RI, EI	[13]	**II.4**
140	α-Bisabolol	1665	2199	0.2	RI, EI		**II.6**
141	Pentadecan-2-one	1682	2010	tr	RI, EI		**II.4**
142	Mintsulfide	1724	2128	tr	RI, EI	[13]	**II.1**
143	(*E*)-Phytol	2099	2604	0.1	RI, EI		**II.8**
144	Tricosane	2300	2300	0.1	RI, EI		**I.1′**
145	Tetracosane	2400	2400	tr	RI, EI		**I.1′**
146	Pentacosane	2500	2500	tr	RI, EI		**I.1′**
147	Heptacosane	2700	2700	tr	RI, EI		**I.1′**
	Monoterpene hydrocarbons			33.5			
	Oxygen containing monoterpenes			12.6			
	Sesquiterpene hydrocarbons			43.3			
	Oxygen containing sesquiterpenes			3.6			
	Others			0.6			
	Total identified			93.6			

Order of elution are given on apolar column (Rtx-1). I_a = retention indices on the apolar column (Rtx-1), I_p = retention indices on the polar column (Rtx-Wax), % = percentages of components, tr = trace (<0.05%), EI = mass spectra in electron impact mode, CI = mass spectra in chemical ionization mode, ^{13}C = ^{13}Carbon-nuclear magnetic resonance, Ref. = references, S = synthesized compounds, Fra. = fraction.

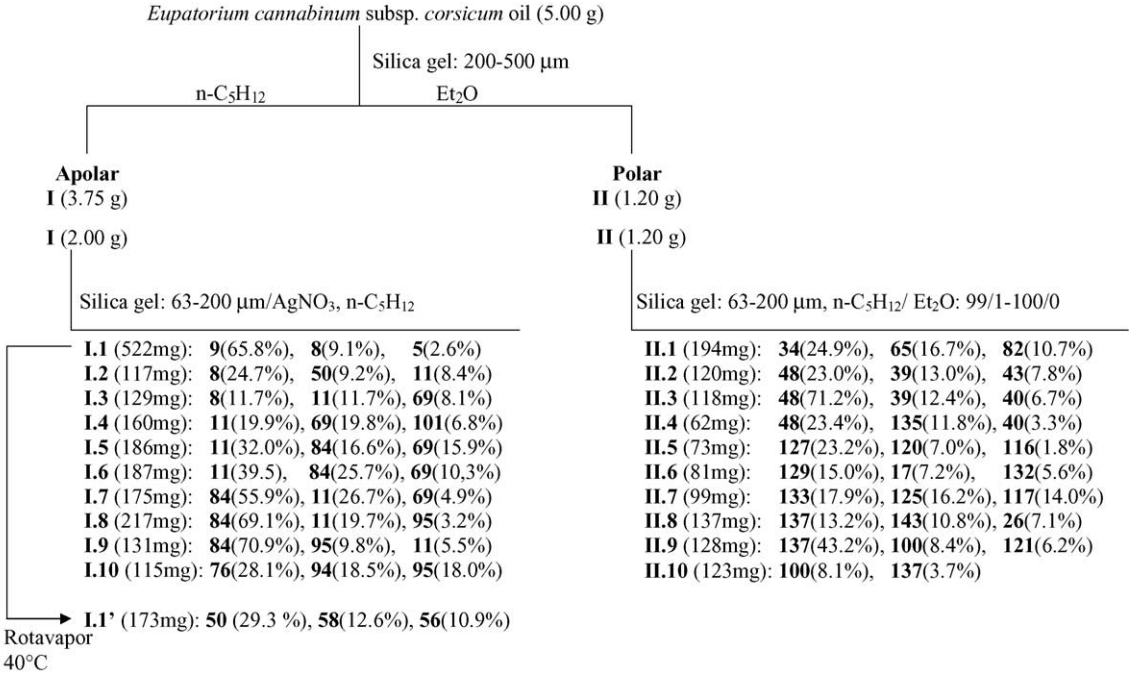

Fig. 1. Separation of *Eupatorium cannabinum* subsp. *corsicum* oil by flash chromatography.

gel, 200–500 μm) using pentane (n-C_5H_{12}) and diethyl oxide (Et_2O) to give two fractions: apolar (**I**: 3.75 g) and polar (**II**: 1.20 g) (Fig. 1). The apolar fraction **I** (hydrocarbons) was separated by flash chromatography (20% $AgNO_3$ impregnated silica gel, 63–200 μm) using pentane as eluent. The polar fraction **II** (oxygenated compounds) was separated by flash chromatography (silica gel, 63–200 μm) using pentane with increasing amount of diethyl oxide up to 100% as eluent. Hydrocarbon monoterpenes (camphene 5, δ-2-carene 8, α-phellandrene 9) of fraction **I.1** were eliminated with the rotavapor at 40 °C to obtain the fraction **I.1**′. All the fractions were analysed by GC and GC/MS(EI) (Table 1).

2.7. Identification of essential oil constituents

Identification of the components was based:

(i) on comparison between calculated retention indices, on polar (I_p) and apolar (I_a) columns, and those of authentic compounds or literature data [12,13];
(ii) on computer matching with commercial mass spectral libraries [14,15] and comparison of mass spectra with those of our own library of pure standard authentic compounds or mass spectra of literature data [12,13,16,17];
(iii) on ^{13}C NMR, following the methodology first reported by Formácek and Kubeczka [18] and based on the comparison of ^{13}C NMR spectral data of components in the mixture with those of pure components or reported in the literature [12,19].

Component-relative concentrations were calculated based on GC peak areas without using correction factors.

Finally, we have checked that all compounds in the fractions were also present in the original oil by comparison of retention indices (I_a and I_p) and/or EI-mass spectra obtained in the fractions with those of the total oil.

3. Results and discussion

3.1. Essential oil composition

In the essential oil of aerial parts of *E. cannabinum* subsp. *corsicum*, 147 components were identified representing 93.6% of the total amount (Table 1). Among them, 57 were hydrocarbons (76.9%) and 90 were oxygenated compounds (16.7%). The essential oil of aerial parts of *E. cannabinum* subsp. *corsicum* was dominated by sesquiterpene and monoterpene hydrocarbons: 33 sesquiterpenes (43.3%) and 12 monoterpenes (33.5%). The main constituents in the essential oil are germacrene D (28.5%), α-phellandrene (19.0%) and *para*-cymene (5.2%).

The identification of 105 components were performed by comparing their EI-mass spectra and their retention indices with those of our own library.

Twelve components unknown to our own library (**65**, **67**, **78**, **82**, **87**, **92**, **93**, **96**, **109**, **110**, **112**, **115**) (Fig. 2) have been synthesised and studied by GC, GC/MS(EI and CI) (Tables 2 and 3), ^1H and ^{13}C NMR. The occurrence of these components in the essential oil was confirmed by comparison of retention indices and mass spectra obtained for synthesised compounds with those of the fraction **II.1**.

Thirty components remained unknown to our own library of authentic compounds:

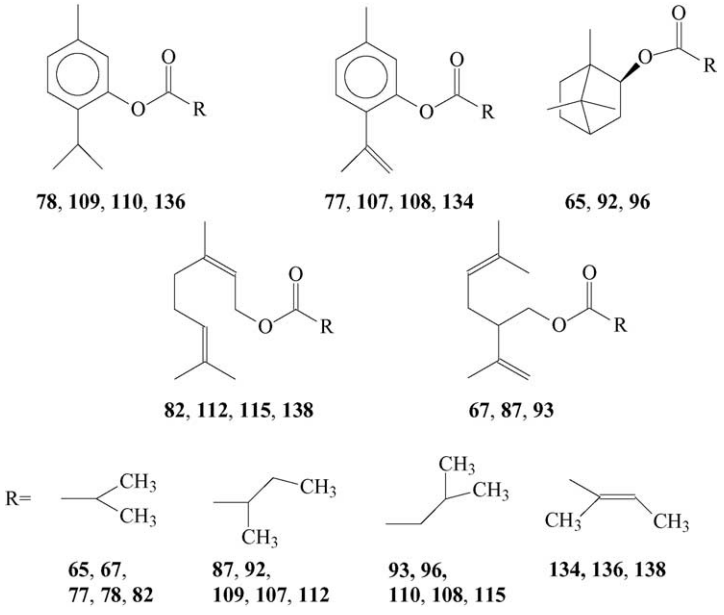

Fig. 2. Structure of esters identified in essential oil of *Eupatorium cannabinum* subsp. *corsicum*.

Table 2
EI-mass spectra of monoterpene esters

No.	Components	$M^{\bullet+}$ intensity (%)	Abundant ions (*m*/*z*)
78	Thymyl isobutyrate	9	150, 135[a], 43
109	Thymyl 2-methylbutyrate	5	150, 135, 57[a]
110	Thymyl isovalerate	6	150, 135[a], 57
136	Thymyl tiglate	4	83[a], 55
77	8,9-Dehydrothymyl isobutyrate	7	148[a], 133, 43
107	8,9-Dehydrothymyl 2-methylbutyrate	5	148, 133, 57[a]
108	8,9-Dehydrothymyl isovalerate	4	148[a], 133, 57
134	8,9-Dehydrothymyl tiglate	3	83[a], 55
65	Bornyl isobutyrate	4	95, 93, 71, 43[a], 41
92	Bornyl 2-methylbutyrate	5	95, 93, 71, 57[a], 41
96	Bornyl isovalerate	3	95, 93, 71, 57[a], 41
82	Neryl isobutyrate	–	93, 69, 43, 41[a]
112	Neryl 2-methylbutyrate	–	93, 69, 57, 41[a]
115	Neryl isovalerate	–	93, 69, 57, 41[a]
138	Neryl tiglate	–	93, 83, 69, 55, 41[a]
67	Lavandulyl isobutyrate	<1	93, 69, 43[a], 41
87	Lavandulyl 2-methylbutyrate	<1	93, 69, 57, 41[a]
93	Lavandulyl isovalerate	<1	93, 69, 57, 41[a]

$M^{\bullet+}$ = molecular ions.
[a] Base peak.

- 18 components were unambiguously identified by comparison of their EI-mass spectra and their retention indices with those of commercial libraries [13].
- six components (**77**, **107**, **108**, **134**, **136**, **138**) (Fig. 2) were studied by GC, GC/MS(EI) (Table 2) and GC/MS(CI) (Table 3) and the identification was based on the joint information from the EI- and CI-mass spectra. The stereochemistry of compound **136** (7.8%) (tiglate or angelate isomers) was determined using ^{13}C NMR by analogies of chemical shifts in the spectra of fraction **II.2** with those of thymyl isobutyrate **78** and ethyl tiglate.
- six components were confirmed by ^{13}C NMR, by comparison of chemical shifts in the spectra of fractions **I.1′** and **II.9** with those of literature [12,19]: 7-α-[*H*]-silphiperfol-5-ene **44** (5.9%), 7-β-[*H*]-silphiperfol-5-ene **49** (9.8%), silphin-1-ene **50** (29.3%), silphiperfol-6-ene **56** (10.9), modhephene **58** (12.6%) and presilphiperfolane-9-α-ol **100** (8.4%).

It is noticeable that the presence of tricyclic sesquiterpenes (silphinane, silphiperfolane, presilphiperfolane, modhephane, and isocomane skeletons) has never been reported in the essential oil of the genus *Eupatorium*.

The essential oil yields (0.05–0.07%) were very low and in accordance with those obtained from the leaves oil (0.05%) of *E. cannabinum* from South Tuscany [10].

The essential oil of *E. cannabinum* from Corsica was characterized by a greater number of monoterpene esters (9% of the total amount) primarily derived from nerol, lavandulol, borneol, thymol and 8,9-dehydrothymol (Fig. 1). These results were in accordance with those of Hendriks et al. [6] but not with those of other authors. For example, monoterpene esters were scarcely represented in essential oils of *E. cannabinum* from Northern India [8], Central and South Italy [9,10]. However, an important number of esters identified in our oil has not been reported in the other studies on essential oils of *E. cannabinum* [3,7–10]. The major differences of our study with those previously reported in the literature [3,6–10] were the great number of identified components and the use of different analytical techniques for their identification.

3.2. GC/MS(EI) of monoterpene esters

EI-mass spectra of thymyl and dehydrothymyl esters present molecular ions (**77**, **78**, **107**, **108**, **109**, **110**, **134**, **136**) and also fragments ions for the phenolic moiety and the loss of a methyl group (**77**, **78**, **107**, **108**, **109**, **110**). Conversely, the determination of molecular mass and the differentiation of linear esters (neryl and lavandulyl) in the electron impact mode were difficult. In effect, EI-mass spectra of neryl and lavandulyl esters show only small differences in the peak intensity and the molecular ions are absent (**82**, **112**, **115**, **138**) or present in a very low abundance (**67**, **87**, **93**). In all cases EI-mass spectra of monoterpene esters show alkyl ions (R^+) characteristic of the acid part at *m*/*z* 43 (**67**, **65**, **77**, **78**, **82**), *m*/*z* 57 (**87**, **93**, **92**, **96**, **107**, **108**, **109**, **110**, **112**, **115**), *m*/*z* 55 (**134**, **136**, **138**) and acyl ions ($RC{=}O^+$) at *m*/*z* 83 (**134**, **136**, **138**).

3.3. GC/MS(CI) of monoterpene esters

3.3.1. Using positive chemical ionization with ammonia as reagent gas

NH_3 PCI-mass spectra of all aromatic esters (thymyl and dehydrothymyl) show the higher abundance of the quasi-molecular $[M + NH_4]^+$ and $[M + H]^+$ ions. NH_3 PCI-mass spectra of bornyl, neryl and lavandulyl esters exhibit intense ions at *m*/*z* 154 $[M + NH_4 - RCOOH]^+$ and at *m*/*z* 137 $[M + H - RCOOH]^+$ resulting respectively from the loss of

Table 3
CI-mass spectra of monoterpene esters by different ionization modes: GC/MS(NH_3 PCI), GC/MS(NH_3 NCI) and GC/MS(CH_4 PCI)

No. (M)	GC/MS(NH_3 PCI) (%)				GC/MS(NH_3 NCI) (%)			GC/MS(CH_4 PCI) (%)							
	$[M+NH_4]^+$	$[M+H]^+$	$[M+NH_4-RCOOH]^+$	$[M+H-RCOOH]^+$	$[M-H]^-$	$[M-RCO]^-$	$[RCOO]^-$	$[M+H]^+$	$[M+H-RCOOH]^+$	Others (*m/z* 81)	$[M+C_2H_5]^+$	$[M+H-C_3H_6]^+$	$[M+C_2H_5-C_nH_{2n}CO]^+$	$[M+H-C_nH_{2n}CO]^+$	$[RCO]^+$
78 (222)	100	4	–	–	21	100	–	35	–	–	4	100	$100_{n=3}$	$96_{n=3}$	40
109 (234)	100	2	–	–	24	100	–	62	–	–	7	64	$12_{n=4}$	$100_{n=4}$	38
110 (234)	100	1	–	–	19	100	–	38	–	–	8	35	$12_{n=4}$	$100_{n=4}$	25
136 (232)	100	8	–	–	29	100	–	59	–	–	5	49	–	–	100
77 (220)	100	76	–	–	56	100	–	42	–	–	–	–	$14_{n=3}$	$100_{n=3}$	39
107 (222)	100	81	–	–	48	100	–	30	–	–	–	–	$9_{n=4}$	$100_{n=4}$	21
108 (222)	100	68	–	–	29	100	–	22	–	–	–	–	$11_{n=4}$	$100_{n=4}$	17
134 (230)	76	100	–	–	38	100	–	14	–	–	–	–	–	–	100
65 (224)	78	–	37	100	100	–	45	–	100	–					
92 (238)	66	–	35	100	100	–	47	–	100	–					
96 (238)	62	–	29	100	100	–	17	–	100	–					
82 (224)	9	–	27	100	33	–	100	–	67	100					
112 (238)	4	–	28	100	37	–	100	–	70	100					
115 (238)	5	–	25	100	57	–	100	–	51	100					
138 (236)	3	–	33	100	55	–	100	–	73	100					
67 (224)	47	100	–	29	100	–	73	10	100	33					
87 (238)	53	100	–	15	100	–	41	9	100	28					
93 (238)	52	100	–	13	100	–	40	8	100	32					

M: molecular weight, R: see Fig. 2. Relative abundances in percent of base peak are reported, (–): not detected.

carboxylic acid after association reactions and proton transfer. Adduct ions $[M+NH_4]^+$ characteristics of molecular mass are observed for bornyl, neryl and lavandulyl esters. NH_3 PCI-mass spectra of linear esters exhibit a significant difference: the protonated quasimolecular $[M+H]^+$ ions (absent in mass spectra of neryl esters) is a base peak of lavandulyl esters. In effect, the structure of lavandulyl esters stabilize the protonated $[M+H]^+$ ion by the interaction between the carbon-carbon double bond and the ester group.

3.3.2. Using negative chemical ionization with ammonia as reagent gas

NH_3 NCI-mass spectra of all monoterpene esters show the $[M-H]^-$ ion. NH_3 NIC-mass spectra of aromatic esters exhibit characteristic phenolate anions at *m/z* 149 for thymyl esters (**78**, **109**, **110**, **136**) and at *m/z* 147 for dehydrothymyl esters (**77**, **107**, **108**, **134**). On the contrary, NH_3 NCI-mass spectra of bornyl, neryl and lavandulyl esters show characteristic carboxylate anions $RCOO^-$. In contrast to EI-mode, neryl and lavandulyl esters are unambiguously differentiated by NCI; the $[M-H]^-$ ion is the base peak of lavandulyl esters whereas carboxylate anion $RCOO^-$ is a base peak of the neryl esters.

3.3.3. Using positive chemical ionization with methane as reagent gas

Thymyl and dehydrothymyl esters exhibit quasimolecular ions $[M+H]^+$ (**77**, **78**, **107**, **108**, **109**, **110**, **134**, **136**) and characteristic acyl cations $RC{=}O^+$ (**77**, **78**, **107**, **108**, **109**, **110**, **134**, **136**). The loss of isopropylen group (C_3H_6) is observed for thymyl esters (**78**, **109**, **110**, **136**) conversely for dehydrothymyl esters (**77**, **107**, **108**, **134**) this fragmentation is not observed probably due to the localization of the double bond conjugated with the aromatic ring. CH_4 PCI-mass spectra of six aromatic esters (**77**, **78**, **107**, **108**, **109**, **110**) show $[M+C_2H_5{-}C_nH_{2n}C{=}O]^+$ and $[M+H{-}C_nH_{2n}C{=}O]^+$ ions resulting respectively from the loss of $C_nH_{2n}C{=}O$ group after association reaction and proton transfer. The absence of these fragments on the mass spectra of two aromatic esters (**134**, **136**) could be explained by the localization of the carbon–carbon double bond on the acid part conjugated with the carbonyl group. CH_4 PCI-mass spectra of bornyl, neryl and lavandulyl esters show intense $[M+H{-}RCOOH]^+$ ions resulting from the decarboxylation of protonated ions. The protonated $[M+H]^+$ ions are observed only for lavandulyl esters (**67**, **87**, **93**).

These results on GC/MS(CI) of terpene components were in accordance with those previously reported in the literature [6,20,21]. The information obtained by GC/MS(CI) has been used on unknown terpene esters or identified with commercial libraries [13,17] for (i) the determination of molecular mass, (ii) the differentiation of compounds with similar EI-mass spectra, and (iii) the identification of acid and alcohol parts. The identification of compounds present in our own library (retention indices and EI-mass spectra) did not require the utilization of GC/MS(CI) data. It is in the case of lavandulyl

acetate (**40**) and neryl acetate (**48**) which can be differentiated by the utilization of retention indices and EI-mass spectra in our own library of standard compounds.

The present work demonstrates the complementarity of GC/MS using electron impact and chemical ionization for the analysis of complex mixture, particularly for differentiation of monoterpene esters with similar EI-mass spectra. We can provide the conclusion that chemical ionization (PCI and NCI) with different gases reagent is an effective method for the confirmation of the molecular mass and the determination of acid and alcohol parts of unknown compounds.

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