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Analysis of *Juniperus communis* subsp. *alpina* needle, berry, wood and root oils by combination of GC, GC/MS and ^{13}C-NMR

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ABSTRACT: The essential oils from needles, berries, wood and roots of *Juniperus communis* L. subsp. *alpina* were obtained by hydrodistillation, fractionated on column chromatography and analysed by GC, GC-MS and ^{13}C-NMR. Chemical compositions of wood and root oils are reported here for the first time. Eighty-two, 65, 76 and 54 components were identified, respectively, in needle, berry, wood and root oils. The chemical compositions of the essential oils from needles, berries and wood were characterized by a high proportion of monoterpene hydrocarbons. Root oil exhibited a quite different composition. Sesquiterpenes, especially those bearing a tricyclic skeleton (cedrane and longifolane), constituted the main fraction while monoterpenes were present at very low contents. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: *Juniperus communis* L. subsp. *alpina*; mountain juniper; Cupressaceae; essential oils; ^{13}C-NMR; GC-MS; chemical composition

Introduction

The genus *Juniperus* (Cupressaceae) consists of 68 species and 36 varieties growing principally in the northern hemisphere. The genus is divided into three sections: *Caryocedrus* (one species), *Juniperus* (= *Oxycedrus*, nine or 10 species) and *Sabina* (the remaining species).[1] *Juniperus communis* L. is generally recognized to have four varieties (or subspecies): *communis*, *depressa*, *megistocarpa* and *saxatilis*.[2] The last one, *Juniperus communis* L. var *saxatilis* Pall., has several synonyms: *J. sibirica* Burgsd., *J. communis* L. var *montana* Aiton, *J. communis* ssp *nana* Willd., *J. nana* Willd., *J. alpina* S.F. Gray, *J. communis* L. var *alpina* Suter and *J. communis* L. subsp. *alpina* (Suter) Celak. In Corsica, *J. communis* L. is represented by two subspecies: *J. communis* L. subsp. *communis* and *J. communis* L. subsp. *alpina* (Suter) Celak.[3]

J. communis L. subsp. *alpina*, commonly named 'mountain juniper', is a small shrub, 0.5–1.5 m high, with creeping stems and twigs. In Europe, it grows generally from 1700 to 2500 m altitude but in Corsica it is widespread from 1000 m altitude.[4] The essential oil, obtained from aerial parts, is locally produced and commercialized.

The essential oils and/or extracts from needles, berries or wood of juniper have been the subject of several studies.[5] However, most studies have concerned *J. communis* subsp. *communis*. Only a few reports have dealt with the oils of *J. communis* subsp. *alpina*. They are summarized below (we conserved the synonym employed by the authors).

The first study on the chemical composition of *J. communis* subsp. *alpina* needle oil dates back to 1973 and reported an α-pinene rich oil from the UK.[6] The needle oil of *J. sibirica* from Mongolia also contained α-pinene as the major component (32–55%) and sabinene was present at appreciable contents (up to 14%).[7] A sample of needle oil of *J. communis* ssp *nana* from Turkey contained essentially sabinene (30.5%) and α-pinene (29.6%).[8] Six samples of *J. sibirica* from Italy exhibited a chemical variability: three samples contained a main constituent, α-pinene (44.8–46.2%), two samples were characterized by α-pinene and sabinene (respectively, 18.7/37.3% and 13.4/18.8%), while the last sample exhibited a quite different composition with sabinene and terpinen-4-ol as the major constituents (respectively, 25.3 and 23.4%).[9] Four compositions have been distinguished for samples of needle oil of *J. communis* var *saxatilis* Pall. from Norway: α-pinene (82%), α-pinene/sabinene (47/22%), sabinene/α-pinene (41/22%) and α-pinene/limonene (51/24%).[10,11] The chemical composition of *J. communis* var *nana* Willd. needle oil from Bulgaria was also dominated by α-pinene (28.3–42.0%) while limonene was the second component (8.1–10.0%).[12] Recently Adams,[13] as part of a study on juniper belonging to the section *Juniperus*, reported the GC/MS analysis of

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two samples of needle oil of *J. communis* var. *saxatilis* from Switzerland and Mongolia. Sabinene (32.8%) and α-pinene (14.1%) were the main constituents of the first sample while α-pinene (58.2%) was the major component of the second.[1,13] Cavaleiro analysed by GC-MS the needle oil of *J. communis* subsp. *alpina* from Portugal.[14] Almost all out of the 25 analysed samples exhibited α-pinene as major component, accompanied by sabinene, terpinen-4-ol or limonene. However, in a few samples, α-pinene and limonene had similar contents.

The chemical composition of the berry oil of *J. communis* subsp. *alpina* has been the subject of a few studies. In a sample from Spain, α-pinene (50.6%) was the major component accompanied by lower contents of limonene and myrcene (respectively 13.4 and 13.1%).[15] Six out of seven samples of *J. communis* subsp. *alpina* berry oil from Portugal contained α-pinene in large proportion (67.1–76.6%), while the last one was characterized by α-pinene (29.3%), γ-cadinene (10.3%), limonene (10.1%) and myrcene (8.6%).[14] A similar composition (α-pinene/sesquiterpenes) was reported for another sample from Portugal.[16]

The present work deals with the composition of the essential oils hydrodistilled from needles, berries, wood and roots of *Juniperus communis* L. subsp. *alpina* from Corsica. The analysis was carried out by combination of CC, GC/RI, GC/MS and ^{13}C-NMR spectroscopy. The chemical compositions of wood and root oils are reported here for the first time.

Experimental

Plant material and extraction of essential oils

Plant material of *J. communis* L. subsp. *alpina* was collected from different bushes growing in the centre of Corsica (Vizzavona, altitude 1200 m). Essential oils were isolated by water distillation for 4 h (needles and berries), 5 h (wood) or 6 h (roots) from fresh material using a Clevenger-type apparatus. Berries were crashed and wood and roots cut into small pieces.

Chromatographic fractionation of essential oils

Needle oil

The oil (12 g) was first chromatographed on a silica gel column (200–500 μm) and two fractions (F1, F2) were eluted, respectively, with pentane and diethyl oxide. The fraction F2 was further chromatographed on silica gel (35–70 μm) and four sub-fractions (F2.1–F2.4) were eluted with mixtures of pentane/diethyl oxide of increasing polarity. Finally, fraction F2.4 was resubmitted to chromatography on silica gel (35–70 μm).

Berry oil

The total oil (6 g) was submitted to flash chromatography using pentane with increasing amounts of diethyl oxide (up to 100%) as eluent and four fractions (F1–F4) were obtained.

Wood oil

The oil (2 g) was first chromatographed on a silica gel column (200–500 μm) and two fractions (F1, F2) were eluted, respectively, with pentane and diethyl oxide. The fraction F1 was further chromatographed on $AgNO_3$ (15%) impregnated silica gel column (35–70 μm), leading to six sub-fractions (F1.1–F1.6) by elution with pentane. Fraction F2 was chromatographed on silica gel (35–70 μm) and four sub-fractions (F2.1–F2.4) were eluted with mixtures of pentane/diethyl oxide of increasing polarity.

Root oil

The crude oil (2 g) was first chromatographed on a silica gel column (200–500 μm). Two fractions (F1, F2) were eluted, respectively, with pentane and diethyl oxide. Fraction F1 was rechromatographed on $AgNO_3$ (15%) impregnated silica gel column (35–70 μm) and eight sub-fractions (F1.1–F1.8) were eluted with pentane. Fraction F2 was rechromatographed on silica gel (35–70 μm), leading to seven sub-fractions (F2.1–F2.7) by elution with mixtures of pentane/diethyl oxide of increasing polarity.

Analytical GC

GC analysis was carried out with a Perkin-Elmer Autosystem apparatus equipped with two flame ionization detectors, and fused-silica capillary columns (50 m × 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polydimethylsiloxane) and BP-20 (polyethyleneglycol). The oven temperature was programmed from 60 to 220 °C at 2 °C/min and then held isothermal (20 min). Injector temperature was 250 °C (injection mode, split). The carrier gas was helium.

GC-MS analysis

GC-MS analyses were performed with a Hewlett-Packard 6890 gas chromatograph, equipped with a HP1 fused-silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., film thickness, 0.25 μm) and interfaced with a Hewlett-Packard Mass Selective Detector 5973 (HP Enhanced ChemStation software, version A.03.00). The oven temperature programme was 70–220 °C (at 3 °C/min), then 220 °C (for 15 min): injector temperature, 250 °C; carrier

gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; interface temperature, 250 °C; MS source temperature, 230 °C; MS quadrupole temperature, 150 °C; ionization energy, 70 eV; ionization current, 60 μA; scan range, 35–350 u.

^{13}C-NMR analysis

All NMR spectra were recorded on a Bruker AC 200 Fourier transform spectrometer operating at 50.323 MHz for ^{13}C, equipped with a 10 mm (or 5 mm) probe, in $CDCl_3$, with all shifts referred to internal TMS. ^{13}C-NMR spectra were recorded with the following parameters: pulse width (PW), 5 μs (or 3 μs) (flip angle 45°); acquisition time, 1.3 s for 32 K data table with a spectral width (SW) of 12 500 Hz (250 ppm); CPD mode decoupling; digital resolution, 0.763 Hz/point. In a typical procedure, 200 mg (or 70 mg) of the mixture (fraction of chromatography) were diluted in 2 ml (or 0.5 ml) of $CDCl_3$. The number of accumulated scans ranged between 2000 and 10 000 for each sample, depending on the available amount of each fraction. Exponential line broadening multiplication (LB = 1 Hz) of the free induction decay (FID) was applied before Fourier transformation.

Identification of components

Identification of the individual components was based on: (i) comparison of their GC retention indices (RI) on apolar and polar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation ('Target Compounds' software from Perkin-Elmer), with those of authentic compounds or literature data; (ii) computer matching with a laboratory-made mass spectral library and commercial libraries,[17,18] and comparison of spectra with literature data;[19,20] (iii) comparison of the signals in the ^{13}C-NMR spectra of all the fractions of chromatography with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software.[21–23]

A few constituents were identified by comparison of their spectral data (MS and/or ^{13}C-NMR) with those reported in the literature: (i) MS and ^{13}C-NMR, *p*-mentha-1,8-dien-4-ol (**37**),[24] amorpha-4,11-diene (**89**),[19] γ-eudesmol (**129**),[25] manool (**144**)[26] and ferruginol (**146**);[27] (ii) MS, selina-4(15), 7(11)-diene (**112**);[19] iii) ^{13}C-NMR: α-campholenyl acetate (**63**),[28] longiborneol (**121**),[29] sesquithuriferol (**120**)[30] and juniper cedrol (**119**).[31]

Results

The distillation of *J. communis* L. subsp. *alpina* needles and berries yielded clear and lightly coloured oils while wood and root oils were more viscous and coloured. The essential oil yields, calculated from fresh material, were 0.9, 1.3, 0.2 and 0.1%, respectively, for needles, berries, wood and roots.

In order to obtain a detailed composition, each oil was fractionated on column chromatography (SiO_2) and all the fractions were analysed by GC, GC/MS and ^{13}C-NMR. Indeed, fractionation of the oil followed by combined analysis of the fractions by GC/MS and NMR has proved to be efficient for the analysis of particularly complex essential oils.[32–35]

The components identified in the needle, berry, wood and root oils of *J. communis* L. subsp. *alpina* are reported in Table 1, together with their percentage, their

Table 1. Chemical composition of essential oils from needles, berries, wood and roots of *J. communis* l subsp. *alpina*

No.	Compounds	RI BP-1	RI BP-20	Percentage[a]				Identification
				Needles	Berries	Wood	Roots	
1	α-Thujene	924	1028	tr	tr	—	—	GC-RI, GC-MS, ^{13}C-NMR
2	α-Pinene	934	1028	24.4	22.1	7.5	1.4	GC-RI, GC-MS, ^{13}C-NMR
3	α-Fenchene	944	1060	tr	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
4	Camphene	946	1069	0.1	0.2	0.1	—	GC-RI, GC-MS, ^{13}C-NMR
5	Verbenene	948	1127	tr	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
6	Sabinene	967	1122	0.2	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
7	β-Pinene	973	1112	0.8	1.5	0.7	—	GC-RI, GC-MS, ^{13}C-NMR
8	Myrcene	982	1162	3.6	6.3	1.4	—	GC-RI, GC-MS, ^{13}C-NMR
9	δ-2-Carene	999	1131	0.5*	—	0.3*	—	GC-RI, GC-MS, ^{13}C-NMR
10	α-Phellandrene	999	1167	3.6*	—	1.2*	—	GC-RI, GC-MS, ^{13}C-NMR
11	δ-3-Carene	1007	1147	0.1	—	0.7	1.3	GC-RI, GC-MS, ^{13}C-NMR
12	α-Terpinene	1011	1181	0.2	0.1	—	—	GC-RI, GC-MS, ^{13}C-NMR
13	*p*-Cymene	1013	1271	0.8	0.2	2.4	—	GC-RI, GC-MS, ^{13}C-NMR
14	Limonene	1025	1205	30.9*	49.3*	19.0*	0.2*	GC-RI, GC-MS, ^{13}C-NMR
15	β-Phellandrene	1025	1214	12.6*	1.2*	8.9*	0.1*	GC-RI, GC-MS, ^{13}C-NMR
16	*(Z)*-β-Ocimene	1025	1232	—	tr*	—	—	GC-RI, GC-MS, ^{13}C-NMR
17	*(E)*-β-Ocimene	1036	1251	—	tr	—	—	GC-RI, GC-MS, ^{13}C-NMR
18	γ-Terpinene	1050	1245	0.1	0.1	—	—	GC-RI, GC-MS, ^{13}C-NMR

Table 1. (*Continued*)

No.	Compounds	RI BP-1	RI BP-20	Percentage[a]				Identification
				Needles	Berries	Wood	Roots	
19	*p*-Cymenene	1072	1438	—	0.1	0.2	—	GC-RI, GC-MS, ^{13}C-NMR
20	Terpinolene	1080	1282	1.2	0.9	—	0.2	GC-RI, GC-MS, ^{13}C-NMR
21	Linalool	1083	1542	0.3	0.2	0.2	—	GC-RI, GC-MS, ^{13}C-NMR
22	Isonopinone	1088	1496	—	—	tr	—	GC-RI, GC-MS, ^{13}C-NMR
23	α-Fenchol	1100	1577	tr	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
24	α-Campholenal	1102	1491	—	tr	—	—	GC-RI, GC-MS, ^{13}C-NMR
25	*cis*-*p*-Menth-2-en-1-ol	1108	1558	0.2*	tr	0.3	—	GC-RI, GC-MS
26	Nopinone	1108	1577	tr*	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
27	*trans*-*p*-Menth-2-en-1-ol	1124	1622	0.1*	—	0.3	—	GC-RI, GC-MS
28	*trans*-Pinocarveol	1124	1646	tr*	0.1	—	—	GC-RI, GC-MS, ^{13}C-NMR
29	*trans*-Verbenol	1127	1677	—	0.1	—	—	GC-RI, GC-MS, ^{13}C-NMR
30	Isopulegol	1134	1583	tr	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
31	*trans*-Pinocamphone	1140	1515	tr	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
32	Isopinocamphone	1151	1551	tr*	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
33	Borneol	1151	1701	tr*	0.1	0.1	—	GC-RI, GC-MS, ^{13}C-NMR
34	Cryptone	1157	1665	0.1	—	0.5	—	GC-RI, GC-MS, ^{13}C-NMR
35	*p*-Cymen-8-ol	1160	1848	0.1	0.2	0.4	—	GC-RI, GC-MS, ^{13}C-NMR
36	Terpinen-4-ol	1163	1598	0.4	{0.4	2.4	0.2	GC-RI, GC-MS, ^{13}C-NMR
37	*p*-Mentha-1,8-dien-4-ol	1163	1681	0.1		—	—	GC-RI, GC-MS, ^{13}C-NMR
38	Myrtenal	1170	1634	tr	0.2*	—	—	GC-RI, GC-MS, ^{13}C-NMR
39	α-Terpineol	1174	1693	2.4	0.4*	8.4	0.3	GC-RI, GC-MS, ^{13}C-NMR
40	*cis*-Piperitol	1180	1674	0.1*	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
41	Verbenone	1180	1707	—	0.2*	—	—	GC-RI, GC-MS, ^{13}C-NMR
42	Myrtenol	1180	1790	0.2*	0.2*	1.1*	0.3	GC-RI, GC-MS, ^{13}C-NMR
43	*p*-Mentha-1(7),2-dien-6-ol	1180	1801	tr*	—	0.2*	—	GC-RI, ^{13}C-NMR
44	α-Campholenol	1185	1790	0.1	—	—	—	GC-RI, ^{13}C-NMR
45	*trans*-Piperitol	1191	1741	0.1	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
46	*trans*-Carveol	1198	1828	0.1	0.2	0.2	—	GC-RI, GC-MS, ^{13}C-NMR
47	Fenchyl acetate	1208	1466	tr*	0.1*	—	—	GC-RI, GC-MS, ^{13}C-NMR
48	Citronellol	1208	1761	0.6*	0.1*	1.0	—	GC-RI, GC-MS, ^{13}C-NMR
49	*cis*-Carveol	1208	1858	tr*	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
50	Thymyl methyl oxide	1215	1591	0.1*	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
51	Cuminaldehyde	1215	1779	tr*	—	0.1	—	GC-RI, GC-MS, ^{13}C-NMR
52	Carvone	1221	1741	tr*	0.2	0.3	—	GC-RI, GC-MS, ^{13}C-NMR
53	Carvacryl methyl oxide	1224	1601	—	—	—	0.2	GC-RI, GC-MS, ^{13}C-NMR
54	Piperitone	1228	1722	0.1	—	0.2	—	GC-RI, GC-MS, ^{13}C-NMR
55	Geraniol	1232	1852	—	—	0.3	—	GC-RI, GC-MS, ^{13}C-NMR
56	*trans*-Myrtanol	1232	1868	—	0.1	—	—	GC-RI, GC-MS, ^{13}C-NMR
57	*cis*-Myrtanol	1242	1869	0.1	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
58	Phellandral	1252	1715	tr	—	0.4	—	GC-RI, GC-MS, ^{13}C-NMR
59	Thymol	1266	2186	tr	—	tr	—	GC-RI, GC-MS, ^{13}C-NMR
60	Bornyl acetate	1271	1577	0.2	0.2	0.1	—	GC-RI, GC-MS, ^{13}C-NMR
61	Carvacrol	1276	2215	0.1	—	tr	—	GC-RI, GC-MS, ^{13}C-NMR
62	Myrtenyl acetate	1306	1684	0.8	0.4	0.6	—	GC-RI, GC-MS, ^{13}C-NMR
63	α-Campholenyl acetate	1316	1681	0.4*	tr*	tr*	—	GC-RI, ^{13}C-NMR
64	*trans*-Carvyl acetate	1316	1732	0.2*	0.1*	0.1*	—	GC-RI, GC-MS, ^{13}C-NMR
65	Citronellyl acetate	1334	1658	0.5*	0.1*	0.4*	—	GC-RI, GC-MS, ^{13}C-NMR
66	α-Terpinyl acetate	1334	1693	6.0*	1.6*	9.1*	—	GC-RI, GC-MS, ^{13}C-NMR
67	Neryl acetate	1340	1726	—	tr	—	—	GC-RI, GC-MS, ^{13}C-NMR
68	α-Longipinene	1353	1466	—	—	—	0.3	GC-RI, GC-MS, ^{13}C-NMR
69	Geranyl acetate	1358	1746	0.1	tr	0.1	—	GC-RI, GC-MS, ^{13}C-NMR
70	*trans*-Myrtanyl acetate	1360	1746	tr	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
71	*cis*-Myrtanyl acetate	1365	1746	0.3	0.1	0.1	—	GC-RI, GC-MS, ^{13}C-NMR
72	α-Copaene	1376	1489	—	—	0.1	—	GC-RI, GC-MS, ^{13}C-NMR
73	α-Funebrene	1383	1503	—	—	—	0.2	GC-RI, GC-MS, ^{13}C-NMR
74	β-Elemene	1389	1586	0.2	1.1	1.1	—	GC-RI, GC-MS, ^{13}C-NMR
75	Sativene	1392	1521	—	—	—	0.4	GC-RI, GC-MS, ^{13}C-NMR
76	Isolongifolene	1397	1524	—	—	—	0.1	GC-RI, GC-MS, ^{13}C-NMR
77	Cinnamyl acetate	1408	2151	tr	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
78	Longifolene	1409	1569	—	—	—	11.5	GC-RI, GC-MS, ^{13}C-NMR
79	β-Funebrene	1415	1569	—	—	—	0.7*	GC-RI, GC-MS, ^{13}C-NMR
80	α-Cedrene	1415	1570	—	—	—	6.7*	GC-RI, GC-MS, ^{13}C-NMR
81	*(E)*-Caryophyllene	1419	1595	—	—	0.4	—	GC-RI, GC-MS, ^{13}C-NMR
82	β-Copaene	1421	1580	—	—	—	tr*	GC-RI, GC-MS, ^{13}C-NMR
83	β-Cedrene	1421	1596	—	—	—	1.4*	GC-RI, GC-MS, ^{13}C-NMR
84	Geranyl acetone	1428	1854	tr	—	0.3	—	GC-RI, GC-MS, ^{13}C-NMR
85	Thujopsene	1431	1620	—	—	—	1.1	GC-RI, GC-MS, ^{13}C-NMR

Table 1. (*Continued*)

No.	Compounds	RI BP-1	RI BP-20	Percentage[a]				Identification
				Needles	Berries	Wood	Roots	
86	*(E)*-β-Farnesene	1446	1667	—	0.1	—	—	GC-RI, GC-MS, ^{13}C-NMR
87	β-Humulene	1454	1668	—	{0.3	—	—	GC-RI, GC-MS, ^{13}C-NMR
88	α-Humulene	1454	1668	0.1		0.4	—	GC-RI, GC-MS, ^{13}C-NMR
89	Amorpha-4,11-diene	1467	ND	—	—	—	tr	GC-RI, GC-MS, ^{13}C-NMR
90	Selina-4,11-diene	1470	1675	—	—	—	0.3	GC-RI, GC-MS, ^{13}C-NMR
91	γ-Muurolene	1471	1678	—	tr	0.4	—	GC-RI, GC-MS, ^{13}C-NMR
92	γ-Gurjunene	1472	1664	—	—	—	tr*	GC-RI, GC-MS, ^{13}C-NMR
93	α-Curcumene	1472	1768	—	—	—	tr*	GC-RI, GC-MS, ^{13}C-NMR
94	α-Neocallitropsene	1477	1695	—	—	—	0.1	GC-RI, GC-MS, ^{13}C-NMR
95	Germacrene D	1478	1707	—	2.0	0.5	—	GC-RI, GC-MS, ^{13}C-NMR
96	γ-Curcumene	1480	1682	—	—	—	tr	GC-RI, GC-MS, ^{13}C-NMR
97	β-Selinene	1486	1719	tr	0.2	0.4	0.1	GC-RI, GC-MS, ^{13}C-NMR
98	Valencene	1490	1717	—	—	—	1.7	GC-RI, GC-MS, ^{13}C-NMR
99	α-Selinene	1495	1719	0.1	0.6	{0.8	tr	GC-RI, GC-MS, ^{13}C-NMR
100	α-Muurolene	1495	1719	—	—		—	GC-RI, GC-MS, ^{13}C-NMR
101	β-Himachalene	1501	1717	—	—	—	tr*	GC-RI, GC-MS, ^{13}C-NMR
102	β-Bisabolene	1501	1722	—	—	—	tr*	GC-RI, GC-MS, ^{13}C-NMR
103	β-Curcumene	1501	1731	—	—	—	0.1*	GC-RI, GC-MS, ^{13}C-NMR
104	Cuparene	1508	1825	—	—	—	0.4*	GC-RI, GC-MS, ^{13}C-NMR
105	γ-Cadinene	1509	1756	0.3	0.4	0.8	tr	GC-RI, GC-MS, ^{13}C-NMR
106	*trans*-Calamenene	1511	1837	—	—	{0.1	0.2*	GC-RI, GC-MS, ^{13}C-NMR
107	*cis*-Calamenene	1511	1837	—	—		—	GC-RI, GC-MS, ^{13}C-NMR
108	β-Sesquiphellandrene	1511	1768	—	—	—	0.1*	GC-RI, GC-MS, ^{13}C-NMR
109	Nootkatene	1511	1815	—	—	—	0.1*	GC-RI, GC-MS, ^{13}C-NMR
110	δ-Cadinene	1517	1754	0.4	1.2	1.8	0.2	GC-RI, GC-MS, ^{13}C-NMR
111	α-Calacorene	1526	1919	—	—	0.1	—	GC-RI, GC-MS
112	Selina-4(15),7(11)-diene	1531	1778	—	tr*	0.1*	0.6	GC-RI, GC-MS
113	α-Cadinene	1531	1791	—	tr*	0.1*	—	GC-RI, GC-MS, ^{13}C-NMR
114	β-Elemol	1536	2080	0.8	0.5	1.3	—	GC-RI, GC-MS, ^{13}C-NMR
115	Selina-3,7(11)-diene	1540	1778	—	tr	0.1	0.3	GC-RI, GC-MS, ^{13}C-NMR
116	*(E)*-Nerolidol	1547	2038	tr	tr	0.7	—	GC-RI, GC-MS, ^{13}C-NMR
117	Spathulenol	1568	2124	0.1	0.2	0.5	—	GC-RI, GC-MS, ^{13}C-NMR
118	Caryophyllene epoxide	1573	1987	—	—	0.2	—	GC-RI, GC-MS, ^{13}C-NMR
119	Juniper cedrol[b]	1582	2100	—	—	—	0.3	GC-RI, ^{13}C-NMR
120	Sesquithuriferol	1592	2110	—	—	—	0.4*	GC-RI, ^{13}C-NMR
121	Longiborneol	1592	2157	—	—	—	8.2*	GC-RI, ^{13}C-NMR
122	Cedrol	1598	2127	—	—	—	37.7	GC-RI, GC-MS, ^{13}C-NMR
123	Humulene epoxide II	1600	2038	tr	—	0.2	—	GC-RI, GC-MS, ^{13}C-NMR
124	10-*epi*-γ-Eudesmol	1607	2105	tr	—	—	—	GC-RI, GC-MS, ^{13}C-NMR
125	8-*epi*-Cedrol	1607	2162	—	—	—	0.9	GC-RI, GC-MS, ^{13}C-NMR
126	α-Acorenol	1614	2127	—	—	—	0.4	GC-RI, ^{13}C-NMR
127	1-*epi*-Cubenol	1619	2059	tr	0.1	0.3	0.2	GC-RI, GC-MS, ^{13}C-NMR
128	Cubenol	1620	2062	—	—	0.2*	—	GC-RI, GC-MS, ^{13}C-NMR
129	γ-Eudesmol	1622	2170	0.1	0.1	0.8*	—	GC-RI, GC-MS, ^{13}C-NMR
130	τ-Cadinol	1629	2170	0.4*	0.2*	0.5*	—	GC-RI, GC-MS, ^{13}C-NMR
131	τ-Muurolol	1629	2186	0.2*	0.2*	0.7*	—	GC-RI, GC-MS, ^{13}C-NMR
132	β-Eudesmol	1642	2231	0.5	0.6	1.1	—	GC-RI, GC-MS, ^{13}C-NMR
133	α-Cadinol	1642	2231	0.5	0.6	1.9	—	GC-RI, GC-MS, ^{13}C-NMR
134	α-Eudesmol	1645	2221	0.2	0.1	1.0	0.2	GC-RI, GC-MS, ^{13}C-NMR
135	β-Bisabolol	1651	2140	—	—	—	0.2*	GC-RI, ^{13}C-NMR
136	Cadalene	1651	2224	—	—	—	tr*	GC-RI, GC-MS, ^{13}C-NMR
137	Juniper camphor[c]	1681	2302	—	—	—	0.7	GC-RI, GC-MS, ^{13}C-NMR
138	*(2Z,6E)*-Farnesal	1688	2219	—	—	0.2	—	GC-RI, GC-MS, ^{13}C-NMR
139	*(2E,6E)*-Farnesol	1699	2353	0.4	—	2.2	—	GC-RI, GC-MS, ^{13}C-NMR
140	*(2E,6E)*-Farnesal	1715	2264	tr	—	0.3	—	GC-RI, GC-MS, ^{13}C-NMR
141	Nootkatone	1781	2527	—	—	—	3.3	GC-RI, GC-MS, ^{13}C-NMR
142	*(2E,6E)*-Farnesyl acetate	1816	2265	—	—	0.2	—	GC-RI, GC-MS, ^{13}C-NMR
143	Dehydroabietane	2041	2489	—	—	tr	0.1	GC-RI, GC-MS, ^{13}C-NMR
144	Manool	2048	2628	0.1	0.1	0.2	—	GC-RI, GC-MS, ^{13}C-NMR
145	Totarol	2268	ND	—	—	—	0.9	GC-RI, GC-MS, ^{13}C-NMR
146	Ferruginol	2283	ND	—	—	—	0.1	GC-RI, GC-MS, ^{13}C-NMR
	Total identified			96.8	95.3	89.3	84.4	

[a] Order of elution and percentages are given on an apolar column (BP-1), except for compounds marked with an asterisk, where the percentage is on BP-20. RI (BP-1), RI (BP-20): retention indices measured, respectively, on apolar and polar columns. ND, retention indices not determined. tr < 0.05%.
[b] 2,2,6,9-tetramethyl-tricyclo[5.2.2.0^{3,7}]undecan-9-ol.
[c] eudesm-7(11)-en-4α-ol.

retention indices on both BP-1 (apolar) and BP-20 (polar) columns and the mode of identification. Obviously, fractionation of the oils by column chromatography and analysis of the fractions by ^{13}C-NMR contributed greatly to the accurate identification of the individual components and particularly sesquiterpenes.

Essential oil from needles

Analysis of the needle oil, by combination of chromatographic (CC, GC/RI) and spectroscopic techniques (GC/MS and ^{13}C-NMR) led to the identification of 82 components (60 monoterpenes, 21 sesquiterpenes, one diterpene), which represented 96.8% of the total amount (Table 1). The essential oil consisted chiefly of monoterpene hydrocarbons (79.1%) with limonene (30.9%), α-pinene (24.4%) and β-phellandrene (12.6%) as major components. Oxygenated monoterpenes (13.7%) were mostly represented by α-terpinyl acetate (6.0%) and α-terpineol (2.4%). The sesquiterpene fraction, which constituted 3.8% of the total amount, consisted mainly of bicyclic molecules bearing the bicyclo[4.4.0]decane skeleton such as δ- and γ-cadinene and τ-cadinol for the most important.

Essential oil from berries

The analysis of the essential oil from berries of *J. communis* L. subsp. *alpina* by CC, GC-RI, GC-MS and ^{13}C-NMR allowed the identification of 65 compounds which represented 95.3% of the whole oil (Table 1). Monoterpene hydrocarbons constituted the main group of compounds (82.0%), especially limonene (49.3%) and α-pinene (22.1%). Among the sesquiterpenes (7.9%), germacrene-D (2.0%), δ-cadinene (1.2%) and β-elemene (1.1%) were the major constituents. Oxygenated monoterpenes (5.3%) were largely represented by α-terpinyl acetate while the other compounds were present at very low content, less than 0.4%.

Essential oil from wood

A number of 76 compounds were identified in the essential oil from wood of *J. communis* subsp. *alpina*, meaning 89.3% of the total oil (Table 1). Monoterpene hydrocarbons (42.4%) constituted the main fraction of the investigated sample, followed by oxygenated monoterpenes (26.9%). Limonene (19.0%), α-terpinyl acetate (9.1%), β-phellandrene (8.9%), α-terpineol (8.4%) and α-pinene (7.5%) were the major constituents. In contrast with the needle and berry oils, the sesquiterpene fraction was present at an appreciable content (19.8%) and largely represented by oxygenated compounds such as (2*E*,6*E*)-farnesol (2.2%), α-cadinol (1.9%) and β-elemol (1.3%). The main sesquiterpene hydrocarbons were δ-cadinene (1.8%) and β-elemene (1.1%).

Essential oil from roots

In total, 54 constituents (nine monoterpenes, 42 sesquiterpenes and three diterpenes), meaning 84.4% of the total oil, were identified in the essential oil from roots of *J. communis* L. subsp. *alpina* (Table 1). The composition differed drastically from the former ones. Root oil was a sesquiterpene-rich oil (79.1%) while the identified monoterpene represented only 4.2% of the total oil. Among the oxygenated sesquiterpenes (52.5%), cedrol (37.7%) and longiborneol (8.2%) were the major constituents accompanied by lower contents of nootkatone (3.3%), 8-epi-cedrol (0.9%) and juniper camphor [eudesm-7(11)-en-4α-ol, 0.7%]. The sesquiterpene hydrocarbons were largely represented by longifolene (11.5%) and α-cedrene (6.7%) while β-cedrene, thujopsene and valencene accounted respectively for 1.4, 1.1 and 1.7%. The other compounds were present at contents lower than 0.5%.

Discussion

The compositions of the essential oils from needles, berries, wood and roots of *J. communis* L. subsp. *alpina* exhibited qualitative and/or quantitative differences. Monoterpene hydrocarbons constituted the main fraction of the needle, berry and wood oils. Although, limonene was the major constituent of the three oils, its content varied consequently from sample to sample: 30.9, 49.3 and 19.0%, respectively. Needle and berry oils differed by the content of limonene, which was by far the major component of the berry oil while the needle oil belonged to the limonene/α-pinene composition. The essential oil from wood is distinguishable from needle and berry oils by a more important sesquiterpene fraction, especially represented by alicyclic (2*E*,6*E*-farnesol), monocyclic (β-elemene, β-elemol) and bicyclic (δ-cadinene, α-cadinol, β-eudesmol) compounds. In contrast, the oil from roots exhibited a quite different composition. Sesquiterpenes, especially those bearing a bridged tricyclic skeleton (cedrane and longifolane), constituted the main fraction while monoterpenes were present at very low contents.

Compared with the chemical compositions of *J. communis* subsp. *alpina* needle oils reported in the literature, we observe that the needle oil from Corsica, dominated by limonene, is original. Indeed, several compositions have been reported for *J. communis* subsp. *alpina* needle oil: (i) α-pinene was by far the major component of oils from England (93%),[6] Norway (82%),[11] Mongolia (58.2%)[13] and Italy (44.8–46.2%);[9] (ii) α-

pinene and sabinene were the main components of samples from Norway (47 and 22%),[10] Mongolia (32–55 and up to 14%),[7] Turkey (30.5 and 29.6%)[8] and Italy (18.7–37.3 and 13.4–18.8%);[9] (iii) sabinene and α-pinene were also the major constituents of samples from Norway (41 and 22%)[10] and Switzerland (32.8 and 14.1%);[1,13] (iv) sabinene and terpinen-4-ol (25.3 and 23.4%) dominated the composition of one sample from Italy;[9] and finally (v) α-pinene and limonene were the main constituents of oils from Norway (51 and 24%)[10,11] and Bulgaria (28.3–42.0 and 8.1–10.0%).[12] Several samples of oil of Portuguese origin belonged to this fifth group, characterized by the pre-eminence of α-pinene over limonene.[14] However, a few samples exhibited approximately equal amounts of limonene and α-pinene. The Corsican limonene-rich oil differed from all the *J. communis* subsp. *alpina* needle oil reported in the literature. It is distinguishable from Portuguese oils by an important contribution of monoterpene acetates, such as α-terpinyl acetate.[14] Conversely, sabinene and terpinen-4-ol, largely represented in samples from Portugal, were negligible in the Corsican oil. Otherwise, several compounds, such as α-campholenyl acetate, *trans*-carvyl acetate, (2*E*,6*E*)-farnesal and manool, have been identified for the first time as constituents of *J. communis* L. subsp. *alpina* needle oil.

The composition of Corsican *J. communis* subsp. *alpina* berry oil, characterized by a high content of limonene (49.3%), which is by far the major component, differed from all other reported compositions from the Iberian peninsula. Indeed, α-pinene was the main constituent of the Spanish (50.6%) and Portuguese (67.1–76.6 and 29.3%) investigated oils, while limonene never exceeded 13.4%.[14,15]

To our knowledge, the chemical composition of the essential oils from wood and roots of the 'mountain juniper' is reported here for the first time. Nevertheless, it appears that the volatile constituents of wood oil of *J. communis* subsp. *alpina*, a monoterpene-rich oil, differed drastically from those of *J. communis* L. wood oil from southeast of France,[36] characterized by high contents of thujopsene (42.4%). They also differed from those of a wood extract of *J. chinensis*.[31]

The results presented here confirmed the variability of the needle and berry oils of *J. communis* subsp. *alpina*. The Corsican 'mountain juniper' needle and berry oils exhibited an unusual composition, dominated by limonene. The compositions of wood and root oils, reported for the first time, are characterized, respectively, by a high content of limonene and appreciable contents of sesquiterpenes for the former and by the pre-eminence of bridged tricyclic sesquiterpene alcohols for the later.

In this study, the combined analysis by GC/MS and ^{13}C-NMR appeared particularly preferable. For instance, the structure of some components such as myrtanyl acetates and piperitols was proposed by computer matching with mass spectra libraries. The stereochemistry of each stereoisomer was ensured by comparison of the ^{13}C-NMR spectra with those of authentic samples prepared by acetylation of myrtanols or reduction of piperitone. The information provided by ^{13}C-NMR was also useful for the identification of stereoisomers, such as cedrenes and funebrenes, α- and β-humulenes, *trans*- and *cis*-calamenenes, or sesquiterpene hydrocarbons, which co-elute on both columns, for instance α-selinene and α-muurolene or β-himachalene (co-elution with β-bisabolene and β-curcumene on apolar column and with valencene on polar column).

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