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Chemical composition and stability of the hydrosol obtained during the production of essential oils. III. The case of *Myrica gale* L., *Comptonia peregrina* (L.) Coulter and *Ledum groenlandicum* Retzius.

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Abstract
The chemical composition of the oils and hydrosols of *Myrica gale*, *Comptonia peregrina*, and *Ledum groenlandicum* collected from wild plantations in the Grondines region of the province of Quebec, Canada, were determined by GC/FID and GC/MS analyses. The composition of the oil of *M. gale* shows great variations from one country to another, both qualitatively and quantitatively. Same variations seem to occur also in the *C. peregrina* oil. The components obtained from the hydrosols were mainly monoterpene alcohols, aldehydes and ketones and as such the compositions of the hydrosols are very different of the corresponding oils: the oil with the most important percentage of hydrocarbon compounds, the compositions of the two phases are different. It appears that in some cases the composition of hydrosols at room temperature may change over a two year period of storage. The *L. groenlandicum* hydrosol is relatively stable except that the main compound, *p*-mentha-1(7),8-dien-1-ol undergoes a *trans*- to *cis*- isomerization process meanwhile both *cis*- and *trans*-*p*-mentha-2,8-dien-1-ol disappear during the shelf-life study. Possible hydration processes may occur in the hydrosol during the shelf-life.

Keywords: *Myrica gale*, *Comptonia peregrina*, *Ledum groenlandicum* essential oil, hydrosol, shelf-life, 3-hexen-1-ol, *p*-mentha-1(7), 8-dien-2-ol.

1. Introduction
In previous papers of this series, we compared the composition of the essential oil and the hydrosol obtained from various plants growing wild or cultivated in the Grondines region, Province of Quebec [1, 2]. The purpose of this series was to identify the possibility of commercialization of a quantitative important sub-product of the essential oil production. After a short review of the corresponding essential oil compositions, we present some results about the chemical stability of hydrosols obtained from *Myrica gale*, *Comptonia peregrina*, and *Ledum groenlandicum*.

2. Experimental
2.1 Plant material
Aerial parts of *M. gale* L. (Bog myrtle, Sweet gale) and *C. peregrina* (L.) Coulter (Sweet-fern), two members of the *Myricaceae* family, and of *L. groenlandicum,* Ericaceae, (Labrador tea), were collected from spontaneous plants growing wild in the Grondines region during summer time. These plants are common in the Quebec province and are described in literature [3].

2.2 Oil and hydrosol extraction
All the samples were produced in a small plant located in Grondines, on the North side of the St-Lawrence River, between Quebec City and Three-Rivers. Typical batch involves 300 kg of fresh material and produces 50 kg of hydrosol. A 4-liter bottle of each hydrosol was kept at room temperature for shelf life studies. 100 mL hydrosols are submitted three times to extraction using 24 mL of chloroform HPLC grade solvent. These three fractions are gathered and are concentrated by partial evaporation until 2 mL and kept over dry $MgSO_4$. Liquid samples are stored in a room at -5 °C before the first GC or GC/MS analysis.

2.3 Oil analysis

Essential oils are analyzed by gas chromatography on a HP 5890, equipped with a flame ionisation detector (GC-FID), and two capillary columns: a Supelcowax 10 and a DB-5 column (30 m × 0.25 mm × 0.25 µm). Samples are also analyzed by gas chromatography, HP 5890, coupled with an HP 5972 mass spectrometer at 70 eV (GC/MS) and equipped either with a DB-5 or Supelcowax column (same as above). Injection port and detector temperature are 220 and 260 °C, respectively. The temperature program for both GC-FID and GC/MS is 40 ºC for 2 min, then 2 ºC/min until 210 °C and held constant for 33 min. Identification of the components is done by comparison of their linear retention indices (RI) with standards, by comparison of their mass spectra with literature data [4–6] and with our own data bases. An internal standard, 400 µl (tetradecane-chloroform solution – 0.4:100) was added to the extract before each GC analysis. Quantitative data are obtained electronically from GC-FID area percentages. The FID response factors for compounds relative to tetradecane are taken as one.

2.4 Hydrosol analysis

Hydrosols are submitted to GC-FID and GC/MS analyses using the same procedure as that used for the essential oils. All the samples were tested for aerobic and facultative anaerobic heterotrophic bacteria to estimate the density of the bacterial population. The measured values are essentially 0, far below the admitted value for drinking water: <5 CFU/100 mL. Total coliforms or atypical bacteria = 0 in each case.

3. Results and discussion

3.1 *Myrica gale*

The essential oil of this plant obtained from various sources was described elsewhere (Table 1). α-Pinene is the main compound of the oil produced in several European countries [7–11]. However, the second largest compound is different from one country to another. 1, 8-Cineole is the second important compound observed in the Netherland and Spain and cadinene is also the second important compound observed in Switzerland and Finland. The sample from France is apart with high percentage of germacrone [12], a compound also observed in Scotland. Oil from Japan is characterized by β-elemenone [13]. In North America, myrcene is the main component observed in various locations followed by limonene [14–17] although in one case selin-11-en-4α-ol is the second major compound [15]. Thus, the composition of the oil of *M. gale* is far from uniformity.

The four main compounds measured in the present oil are germacrone (13.5%), germacrene B (7.5%) - two compounds unobserved in this oil until now in North America - myrcene (7.8$_5$%), and β-caryophyllene (5.6$_5$%) (Table 2). A long list of hydrocarbon mono- and sesquiterpenes are also present as well as several oxygenated and unidentified compounds. Again, the detailed composition of the oils is different from one site to another in the Province of Quebec.

A special note must be added relatively to the germacrone yield. This compound is known to be thermally unstable. Through a Cope rearrangement, it isomerizes to β-elemenone in the injection port of the chromatograph and on the column itself above 200 °C [18]. Thus the formation of a peak of β-elemenone probably is an artefact due to some isomerization in the injection port. It corresponds to a small fraction of germacrone collected in the oil. Moreover, on the chromatogram between the peak of β-elemenone and that of germacrone, one can observed a positive deviation of the base line. This deviation corresponds to the Cope rearrangement on the analytical column. From an evaluation of the involved surface of the deviation, the germacrone percentage appearing in Table 2 is probably underestimated by *ca*. 10%. This deviation is more important on the polar column since the germacrone elution time is *ca.* 3 min higher and the temperature of the oven is higher by *ca.* 6 °C at the moment of elution. Noteworthy is the presence of an isomer of tentatively identified as (*E*, *E*)-germacrone. From the RI value published in literature, this isomer could be the (*Z*, *Z*) one [19].

The fresh hydrosol contains 110 mg/L of volatile organic compounds (VOCs) of which *ca*. 90% appear in Table 3. pH values decrease from 4.1 for the fresh hydrosol to 3.6 after 3 months of shelf-life at room temperature: it is similar to a literature value: 3.7 to 3.8 [20]. The hydrosol composition appears in Table 3. Only four oxygenated monoterpenes are common to both oil and hydrosol. They are: 1,8-cineole (0.8% in oil), linalool (0.2%), terpinen-4-ol (0.2%), and α-terpineol (0.15%). Five oxygenated sesquiterpenes are also common to oil and hydrosol: germacrone (13.5%), selin-11-en-4-α-ol, α-bisabol and *trans*-nerolidol (~2.2%, each), and eremoligenol (0.2%).

This hydrosol is characterized by a small decrease of the pH values and an increase of the quantities of VOCs over a one year shelf-life. Thus, there is an apparent acidification of the hydrosol and a parallel increase in the percentages of the 6 carbon atom compounds and a decrease for several other compounds (Table 3). The percentage of *cis*-3-hexen-1-ol increases from *ca*. 9% to 36% (Table 2). From the observed results, there is no obvious process to explain this behaviour. Unsaturated fatty acids, in the presence of oxygen, are known to decompose in leaf alcohol [21]. Formation of *cis*-3-hexenol is followed by its oxidation to leaf aldehyde. In olive oil, these processes seem very fast: in the presence of enzymes it takes a few hours [22]. A twelve month period in hydrolate media cannot be excluded to support this process. Since oleic, linoleic or linolenic acids are not observed in the present study, one cannot conclude to the origin of the C_6 compounds. One can speculate on the presence of higher members of unsaturated fatty acids. Relatively to the unidentified compounds B and C, from the differences in RI values observed on the polar and a-polar columns, Δ [$RI_{(Swax-10)}$ − $RI_{(DB-5)}$] = 840 (Table 3), we may suggest that these compounds are diols. Their molecular mass is probably 170 as suggested on the MS of compound C. Such compounds, having a base peak at *m/z* = 126, were observed in the essential oil of *Chenopodium multifidum* as *p*-menthen-5-ene-1,2-diol isomers[23]. Moreover, the similarity of these RI values and of their MS supports their identity as members of the pinanediol family [24] (Fig. 1).

Finally, the sum of the products associated with linalool decreases by *ca*. 10%. For a complete discussion on linalool degradation, see ref. [1].

Table 1: Composition (%) of the main compounds observed in the oil of *M. gale* from various regions

S. No.	Compound	Switzerland	Netherland	Scotland[1]	Spain	Finland	France	Japan	USA	Canada[2]	Canada[3]		
1	α-Pinene	17.8	25.0	12.3	41.4	17.8	12.2	1.9	4.7	3.0-17.8	2.2	2.0	3.9
2	α-Phellandrene	6.4 ?	2.5	3.8	3.6	-	-	1.3	-	n.d.[4]-5.1	0.7	4.3	9.9
3	Myrcene		1.3	1.9	2.8	6.4	-	-	29.0	16.2	20.3	18.9	23.2
4	*p*-Cymene	4.7	6.0	1.1	-	-	2.0	10.6	5.5	4.4-5.5	-	-	4.5
5	Limonene	10.0	5.0	1.5	8.4	10.0	8.1	-	14.6	10.0-14.6	15.3	8.9	11.2
6	1,8-Cineole	7.1	20.0	10.6	13.6	7.1	5.3	7.7	0.1	0.1-7.1	-	-	-
7	β-Caryophyllene	2.6	0.5	0. 2	0.3	2.6	-	0.4	5.5	< 0.1-5.5	11.2	9.1	9.3
8	β-Elemenone	-	14.3	-	-	-	-	13.3	-	-	-	-	-
8	δ/γ-Cadinene	12.9	-	-	5.5	12.9	-	-	0.3	0.3-12.9	2.0	5.3	1.6
10	Selin-11-en-4α-ol	-	-	-	-		-	-	-	n.d.-14.6	2.4	8.1	-
11	Germacrene b	-	-	-	-	11.6	-	-	-	-	-	-	-
12	Germacrone	-	p	11.6	-	-	25.0	1.1	-	-	-	-	-
13	Ref.	7	8	11	10	11	12	13	14	15	16	16	17

[1]: Mean values observed on 10 individual plants; [2]: Barry's Bay, Ontario; [3]: province of Quebec; [4]: not determined; p: present.

Table 2: Composition (%) of the oil of *M. gale* observed in the Province of Quebec

S. No.	Compounds	This study			Other studies: ref.		
		RI [1]	RI [2]	%	16		17
1	α-Thujene	935	1026	0.2	0.4	0.4	0.5
2	α-Pinene	940	1019	1.1_5	2.2	2.2	3.9
3	Camphene	953	1057	-	t[3]	0.3	
4	β-Pinene	977	1107	0.1	0.3	0.2	0.3
5	Myrcene	992	1169	7.9	20.3	7.7	23.2
6	α-Phellandrene	1001	1164	3.8_5	0.7	2.1	9.9
7	α-Terpinene	1017	1177	0.1			0.1
8	*p*-Cymene	1026	1276	1.5	10.3	6.7	4.5
9	Limonene	1031	1194	4.1	15.3	10.4	11.2
10	β-Phellandrene	1031	1201	0.7_5	2.5	1.8	1.5
11	1,8-Cineole	1034	1199	0.8	-	-	
12	*cis*-β-Ocimene	1046	1242	1.0	1.8	0.8	4.3
13	*trans*-β-Ocimene	1058	1261	1.1	0.9	0.7	4.5
14	γ-Terpinene	1067	1248	0.3	0.2	t	0.3
15	Terpinolene	1098	1288	0.1_5			0.1_5
16	Linalool	1112	1557	0.2	0.2	0.8	
17	Isopentyl isovalerate	1119	1306	0.1			0.3
18	Terpinen-4-ol	1179	1593	0.2			
19	α-Terpineol	1190	1698	0.1_5			
20	δ-Elemene	1352		-			0.4_5
21	α-Terpinyl acetate	1354	1688	0.7			
22	Citronellyl acetate	1360	1679	0.2			
23	α-Copaene	1376	1489	0.2_5	1.1	1.7	0.4
24	Geranyl acetate	1389	1768	0.2_5			0.8
25	β-Elemene	1390	1582	0.3	1.0	0.5	
26	Benzyl isovalerate	1392	1888	0.3			
27	*cis*-α-Bergamotene	1404	1557	0.3			0.8_5
28	α-Gurjunene			-	2.4	3.5	0.6
29	β-Caryophyllene	1415	1582	5.6	11.2	18.6	9.3
30	α-Gurjunene			-	2.1	3.5	
31	γ-Elemene	1433	1631	1.0			
32	*trans*-α-Bergamotene	1436		-			0.3
33	α-Humulene	1453	1656	1.9	3.7	7.8	1.5
34	Drima-7,9(11)-diene	1471	1668	0.5			
35	γ-Gurjunene	1474		-			2.6
36	*trans*-Cadina-1(6),4-diene	1476	1652	0.5			0.2_5
37	β-Chamigrene	1479	1666	0.8			

38	γ-Muurolene	1478	1686	0.7	2.2	1.7	
39	γ-Curcumene	1484	1688	0.8			0.6_5
40	β-Selinene	1488	1706	1.1			-
41	ar-Curcumene	1488	1768	1.1			0.7_5
42	δ-Selinene	1497	1690	-			0.4
43	α-Selinene	1497	1712	1.2			0.25
44	α-Zingiberene	1502	1720	0.8			
45	β-Bisabolene	1513	1732	-			0.4
46	(*E*,*E*)-α-Farnesene	1515	1751	1.2			1.8
47	β-Curcumene	1517	1738	0.9			1.0
48	δ-Cadinene	1527	1749	2.0	2.0	5.3	1.6
49	*trans*-Calamenene	1532	1816	0.15			
50	(*E*)-Cadina-1,4-diene	1534	1775	-			0.4
51	Double peak?	1535	1757	1.4			
52	Selina-4(15),7(11)-diene	1540	1762	1.2_5			
53	Selina-3,7(11)-diene	1542	1762	2.6_5			
54	Unidentified **A**	1545	1833	1.0			
55	Unidentified **B**	1548	1897	1.3			
56	Germacrene **B**	1556	1805	7.5			
57	Cadina-1(10),6,8-triene	1563	1876	0.3			
58	*trans*-Nerolidol	1566	2041	2.0	0.7	1.1	0.2_5
59	β-Vetivenene	1566	1865	0.5			
60	Caryophyllene oxide	1579	1957	0.6	2.5	3.5	3.5
61	Unidentified **C**	1582	1874	1.2			
62	β-Elemenone	1600	2060	0.3			
63	Humulene epoxide II	1601	2021	0.3			0.4
64	Eremoligenol	1625	2166	0.2			1.15
65	1-epi-Cubenol	1625	2011	0.3			
66	Unidentified **D**	1625		1.3			
67	Selin-11-en-4α-ol	1652	2229	2.3	2.4	8.1	
68	(*Z*,*Z*)-Germacrone [4]	1667					
69	α-Bisabolol	1686	2202	2.2	0.5	1.0	
70	(*E*,*E*)-Germacrone	1694	2197	13.5			
71	Juniper camphor	1711	2271	0.1_5			
72	Guaiol acetate	1760	2275	0.4			
73	Pentadecanal		2040	t			0.2
74	α-Eudesmol acetate	1795	2307	0.4			0.2 [5]
75	Total (%)			91.4	88.5	91.3	95.2

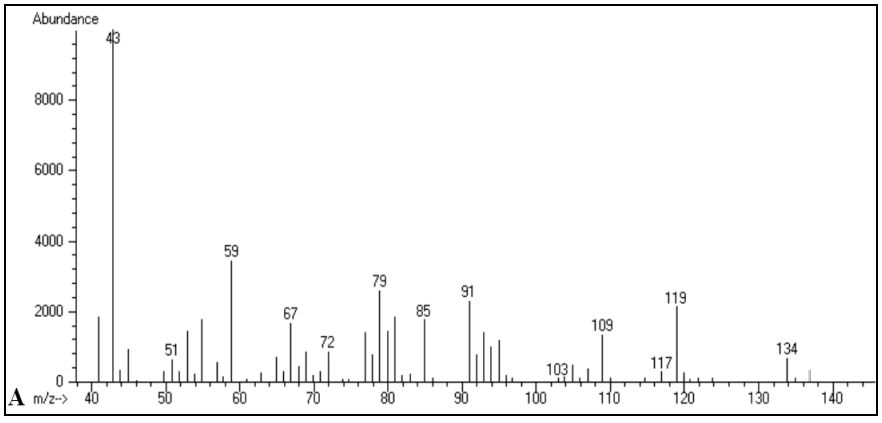
[1]: retention index on DB-5; [2]: retention index on S-wax 10; [3]: traces, < 0.05%; [4]: tentative identification, see text; [5]: this compound was observed only between 30 and 60 min of the extraction time. Mass spectrum of unidentified compounds: *m/z*(intensity):
unidentified **A**:202(100), 91(72), 93(69), 131(66), 77(52), 105(52), 145(52), 187(52), 79(40) …
unidentified **B**: 131(100), 202(72), 145(66), 187(60), 91(50), 41(58), 77(32), 105(32), 79(28) …
unidentified **C**: 126(100), 41(92), 123(76), 220(64), 55(52), 81(48), 43(42), 107(42), 67(40), 91(40) … This compound was also observed at the trace level in a not reported sample of the essential oil of *L. groenlandicum*[31].
unidentified **D**: 105(100), 161(46), 59(44), 43(32), 91(32), 41(28), 147(24), 93(20) …

Table 3: Composition (%) of the hydrosol of *M. gale*

S. No.	Identification	RI		Shelf-life (month)				
		DB-5 col.	S-wax	0	3	6	12	24
1	3-Hydroxy-2-butanone	698	1293	0.1	1.0	1.1	1.4	1.5
2	*trans*-3-Hexen-1-ol	859	1352	0.2	0.6	0.7	0.8_5	0.7
3	*cis*-3-Hexen-1-ol	864	1386	9.0	29.0	34.6	37.6	34.1
4	*trans*-2-Hexen-1-ol	876	1407	1.1	2.1	2.0	2.3	1.9
5	Hexanol	878	1360	1.6	3.5	3.2	3.7	3.3
6	Hexanoic acid	984-1013 [1]	1847	2.0	2.9	3.1	3.3	2.2
7	*cis*-3-Hexenoic acid		1956	t	t	t	t	t
8	*trans*-3-Hexenoic acid	1013-1033 [1]	1967	3.4	4.0	3.9	3.7	2.5
9	1,8-Cineole (0.8%)*	1035	1200	2.0	6.4	7.9	9.4	10.0
10	Benzyl alcohol	1040	1871	3.1	3.4	3.0	3.3	4.4
11	*trans*-2-Hexenoic acid	1036-1062[1]	1970	2.2	3.0	3.7	3.3	0.8
12	γ-Hexalactone	1058	1683	0.7	0.8	0.9	0.8_5	0.9
13	*trans*-Linalool oxide (fur.)	1084	1442	0.2	0.2	0.38	0.3_5	0.5

14	Octanol	1085	1563	0.9	0.3	0.2	0.2	
15	Fenchone	1098	1375	t	t	0.3	0.3	0.2
16	Heptanoic acid	1099-1103 [1]	1961	t	t	t	t	t
17	*cis*-Linalool oxide (fur.)	1100	1473	0.2	0.2	0.3_5	0.2	0.2_5
18	Linalool (0.2%)*	1114	1557	5.9	3.3	2.0	1.1	0.3
19	Unidentified **A**	1116	1630	3.7	2.4	1.3	0.9	0.7
20	α-Thujone	1117	1398	0.2	0.2	0.3	0.3	0.4
21	Maltol	1121	1963	0.2	0.1	t	0.1	0.2
22	2-Phenylethyl alcohol	1122	1904	0.6	0.5	0.4	0.5	0.3_5
23	Ipsdienol	1155	1684	0.7	0.5	0.3	0.2	t
24	2,6-Dimethyl-1,5,7-octatrien-3-ol	1161	1745	2.2	1.5	1.1	0.9	1.0
25	Isoborneol	1165	1655	1.0	0.7	0.6	0.6	0.7
26	*p*-Menth-5-en-2-one I	1168	1535	0.2	0.1	0.1	-	-
27	Borneol	1169	1701	1.1	0.4	0.3	0.3	0.3
28	*p*-Menth-5-en-2-one II	1172	1537	0.4	0.1_5	0.1	-	-
29	Benzoic acid	1165-1173 [1]	2439	-	2.1	1.5	2.3	1.0
30	Terpinen-4-ol (0.2%)*	1179	1594	10.5	6.9	5.1	5.1	3.9
31	Octanoic acid	1180-1183 [1]	2061	0.7	0.3	0.2	0.1	0.8
32	Cryptone	1182	1584	0.3	t	0.5	0.5	0.3
33	*p*-Cymen-8-ol	1186	1846	1.7	1.5	0.5	0.8_5	1.0
34	Methyl salicylate	1189	1760	0.7	0.4	-	-	-
35	α-Terpineol (0.1_5%)*	1190	1681	10.2	6.7	5.4	5.3	3.8
36	*trans*-Sabinol (OH vs IPP)	1197	1793	4.1	2.5	1.9	1.8	1.6
37	*trans*-Piperitol?	1205		0.6	0.2	0.1	-	0.1
38	*trans*-Carveol	1219	1825	0.6	0.3	0.2	0.2	0.1
39	Nerol	1229	1802	0.6	0.3	0.2	0.2	0.1_5
40	Citronellol	1234	1769	1.7	0.6	0.4	0.2	-
41	2,6-Dimethyl-7-octene-2,6-diol	1235	1983	-	0.3	0.4	0.6	1.3
42	*cis*-Sabinol (OH *vs* IPP)	1238	1881	1.9	0.9	0.6	0.4	0.2
43	Carvotanacetone	1245	1667	0.8	0.5	0.3_5	0.3_5	0.3
44	Piperitone	1250	1707	0.8	0.5	0.3_5	0.4	0.3
45	Geraniol	1257	1849	0.7	0.5	0.4	0.4	0.2
46	Thymol	1305	2170	0.2	t	0.1	t	t
47	Carvacrol	1313	2205	0.4	t	0.4	0.1	0.2
48	*p*-Vinylguaiacol	1318	2167	0.4	0.1_5	0.2	0.2	0.2
49	*p*-Menthan-1,8-diol	1321	2091	0.6	0.3	0.2	0.2	0.4
50	Unidentified **B**	1330	2169	0.4	0.1	0.2	0.3	0.4
51	Unidentified **C**	1341	2185	0.1	0.1	0.2	0.2	0.4
52	Eugenol	1359	2157	0.2	0.1	0.2	0.1	0.1
53	Benzylacetone	1360	2091	0.2	0.1_5	0.0	0.0	0.1
54	Hydroxycitronellol	1361	2184	0.3	0.1	0.2	0.3	0.3
55	Benzyl isovalerate	1392	1886	0.2	0.1	0.0	0.2	0.3
56	*trans*-Nerolidol (2.0%)*	1566	2040	0.2	0.0	0.0	0.0	-
57	Eremoligenol (0.2%)*	1626	2160	1.3	0.5	0.2	0.1	0.2
58	γ-Eudesmol	1637	2153	0.2	t	t	0.1	t
59	Selin-11-en-4α-ol (2.3%)*	1653	2231	2.3	0.5	0.3	0.1	-
60	(*E*,*E*)-Germacrone (13.5%)*	1694	2198	3.5	0.5	0.5	0.3	0.2
61	α-Bisabolol (2.2%)*	1694	2201	0.8	0.1	0.2	0.0	-
62	Rosifoliol	1804		-	0.5	0.4	0.5	0.6
63	Total (%) analyzed amount of VOCs			90.0	94.8	93.2	96.8	85.2

[1]:acids have an important tailing on the DB-5 column; indicated values correspond to the beginning and the top of the peak, respectively; integrations are obtained on the polar column. *: common compounds in oil and hydrosol (% in oil); unidentified **A**, **B**, and **C**: see Fig. 1.



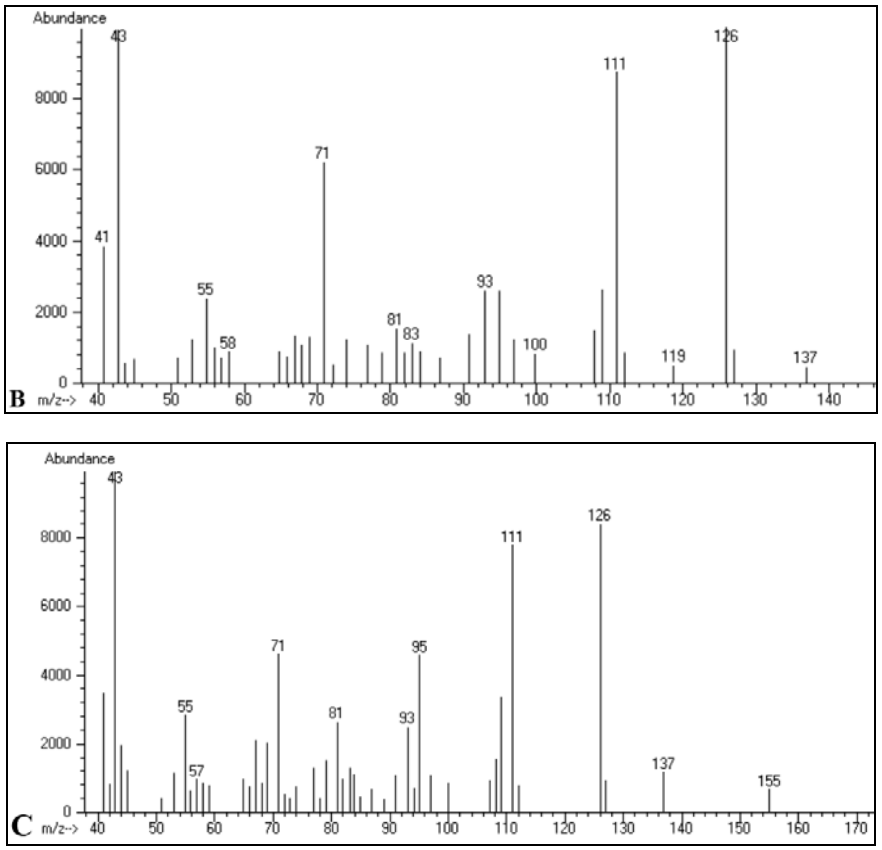


Fig 1: Unidentified compounds **A**, **B** and **C** observed in the *Myrica gale* hydrosol.

3.2 Comptonia peregrina

The essential oil was described elsewhere [14, 15, 25]. It contains many hydrocarbon mono- and sesqui-terpenes as well as several oxygenated compounds (Table 4). α-Pinene (0.8-7.0%), myrcene (7.3-12.6%), 1,8-cineole (3.4-17.8%), and γ-terpinene (<0.1-14.7 %) are the main monoterpenes. The main sesquiterpenes are β-caryophyllene (8.9-36.2%) and α-humulene (0.9-9.7%). β- and α-selinene, γ- and δ-cadinene (each ca. 2±1%) as well as *trans*-nerolidol (2.4%) are among sesquiterpenes. Although the identified compounds are common there are great variations in percentages from one study to another. However, there are several minor new reported compounds, particularly three C_6 alcohols, several esters, sesquiterpenes and oxygenated sesquiterpenes. The difference in the preparation of the samples is probably responsible for these differences as well as locations of collection and chemotypes. The samples reported in previous studies were prepared at the laboratory level. In the present study, the oil and the hydrosol were prepared concurrently at the semi-industrial level (see section 2.2).

Some rather rare compounds must be mentioned. 2,6-dimethyl-1,5,7-octatrien-3-ol was identified in a few essential oils. For example, its concentration is 0.3% in the essential oil of the leaves of *Eucalyptus oleosa* [26]. *p*-Menth-5-en-2-one I and II were characterized as minor constituents of the essential oil of *Eucalyptus dives* Schau [27]. Noteworthy is the presence of vitispirane A and B in this sample. In both cases, the reported RI values either on the a-polar column or a polar column (Table 4) are in agreement with the published one [28, 29].

Several years ago, this laboratory produced a hydrodistilled extract from the aerial part of *C. peregrina* [30]. The oil and the hydrosol were extracted together with diethyl ether. Thus, the extracted VOC sample contained both water-insoluble and water-soluble compounds. These compounds appear in Table 4 and 5 with an asterisk.

The fresh hydrosol contains 296 mg/L of VOCs of which ca. 99% are reported in Table 5. The total measured quantities do not change significantly all along the experimental period. pH values are 4.3 for the fresh hydrosol and 4.1 after a 6 month period storage at room temperature. The compositions of the fresh hydrosol after various months of shelf-life appear in Table 5 and fig. 2. Alcohols, ketones, a few aldehydes and organic acids are included in these compositions. *cis*-3-Hexenol and *trans*-2-hexenol, hexanol, 1,8-cineole, linalool, terpinen-4-ol, and α-terpineol are the main components of the hydrolate. They are also found in the essential oil. Four sesquiterpenols are observed in both oil and hydrosol of *C. peregrina*.

The concentration of the main C_6 compounds just above-identified shows a small increase, ~20%, after a two-year shelf-life. The percentages of α-terpineol and terpinen-4-ol decrease by about 15%, the decrease of linalool is more important: it is divided by a factor of 3.1. Finally, the decrease of the percentage of benzaldehyde by a factor of 2.5 is also noticeable. The decrease of the concentration of nerol and geraniol and their isomerization to linalool as well as the isomerization/oxidation of the latter towards the formation of linalool oxides, 2,6-dimethyl-7-octene-2,6-diol, and hodiendiol (2,6-dimethyl-3,7-octadiene-2,6-diol) was discussed in the first paper of this series[1]. In the same paper, the rapid decrease of the citral concentration was discussed. In the hydrosol of *Melissa officinale*, the decrease of citronellal seems very fast [1]. Benzaldehyde, a compound that seems to disappear in less

than three months in the *Asarum canadense* hydrosol decreases by 60% over a two year period at room temperature (Table 5). If the oxidation of benzaldehyde was observed in various conditions, the presence of benzoic acid is very limited and does not offer an explanation for the loss of benzaldehyde in acidic aqueous media.

Table 4: Composition (%) of the essential oil of *C. peregrina*

S. No.	compound	This study			Other studies, ref.:		
		RI [1]	RI [2]	%	15	15	25
1	*trans*-2-Hexenal *	857	1220	t[3]			
2	*cis*-3-Hexenol *	863	1376	0.1			
3	*trans*-2-Hexenol *	876	1395	0.2			
4	Hexanol *	879	1359	0.3			
5	Styrene	895	1263	0.1			
6	α-Thujene *	935	1026	0.3			0.1
7	α-Pinene *	940	1019	3.1	7.0	5.5	0.8
8	Camphene *	953	1057	0.1	0.1	<0.1	
9	Benzaldehyde *	961	1526	0.1			
10	Sabinene *	976	1123	0.1		0.1	0.5
11	β-Pinene *	977	1107	0.8	1.2	0.9	0.3
12	Myrcene	992	1169	7.3	9.9	11.1	12.6
13	α-Phellandrene *	1003	1161	0.1			
14	*cis*-3-Hexenyl acetate *	1009	1326	0.2			
15	α-Terpinene *	1017	1177	0.7	0.9	0.6	0.2
16	*trans*-2-Hexenyl acetate	1020	1342	0.1			
17	*p*-Cymene *	1026	1276	1.2	3.4	1.0	0.3
18	Limonene *	1032	1194	1.1	2.3	1.3	0.3
19	1,8-Cineole *	1035	1199	9.7	17.8	11.0	3.4
20	*cis*-β-Ocimene *	1046	1243	2.0	3.5	1.8	2.4
21	*trans*-β-Ocimene *	1058	1261	2.5	3.9	7.7	2.2
22	γ-Terpinene *	1067	1248	4.2	14.7	<0.1	0.8
23	Terpinolene *	1098	1288	0.6	1.0	0.4	0.2
24	Linalool *	1112	1556	3.4			5.4
25	Nonanal	1115	1382	0.1			
26	4,8-Dimethyl-1,3,7-nonatriene	1127	1316	0.1			
27	Terpinen-4-ol *	1179	1594	0.9	1.3	7.2	0.5
28	α-Terpineol *	1190	1697	0.7	1.7	1.6	0.5
29	4-Phenyl-2-butanone	1244	1844	0.1			
30	Vitispirane A [4]	1282	1519	0.2			
31	Vitispirane B [4]	1282	1526	0.1			
32	Safrole	1290	1879	-			0.6
33	Bornyl acetate	1291	1565	0.1			
34	*trans*-Pinocarvyl acetate	1306	1647	0.1			
35	2-Undecanone	1306	1594	t			
36	Myrtenyl acetate	1332	1695	0.1			
37	δ-Elemene	1342	1458	0.1			
38	α-Terpinyl acetate	1353	1692	0.1			
39	α-Copaene	1376	1481	0.2		0.2	
40	β-Elemene *	1390	1583	1.0			
41	β-Caryophyllene *	1416	1583	27.0	8.9	36.2	23.7
42	*trans*-α-Bergamotene	1436	1583	t			0.5
43	α-Humulene *	1453	1656	1.3	4.2	0.9	9.7
44	(*E*)-β-Farnesene + ?	1462	1661	0.3			
45	4,5-di-epi-Aristolochene	1471	1685	0.3			
46	γ-Muurolene *	1479	1684	0.4			0.7
47	β-Chamigrene	1479	1667	0.6			
48	Unidentified sesquiterpene A	1479	1653	1.0			
49	γ-Curcumene	1484	1688	0.8			0.7
50	ar-Curcumene	1488	1768	0.5			-
51	β-Selinene *	1488	1706	2.5	0.8	0.9	1.5
52	α-Selinene *	1498	1712	2.2			1.2
53	γ-Gurjunene	1499	1716	1.5			
54	α-Muurolene	1503	1718	0.5		1.0	1.0
55	2-Tridecanone *	1503	1808	0.5			

56	(*E*,*E*)-α-Farnesene	1515	1752	1.0			
57	γ-Cadinene *	1517	1748	1.7	2.8	2.7	3.0
58	δ-Cadinene *	1527	1778	2.5			4.3
59	Zonarene [5]	1527					
60	Calamenene *	1527	1831	t	0.2	0.1	
61	(*E*)-γ-Bisabolene	1534	1771	0.5			1.0
62	α-Cadinene	1536	1816	0.2			0.7
63	Selina-3,7(11)-diene	1543	1763	0.5			0.6
64	Germacrene B	1556	1806	1.5			3.1
65	*trans*-Nerolidol	1566	2042	2.3	1.5	3.7	2.1
66	*cis*-3-Hexenyl benzoate	1570	2110	0.3			
67	Caryophyllene oxide *	1579	1958	0.9			4.1
68	Ethyl dodecanoate	1594	1843	t			
69	β-Oplopenone	1595	1958	t			
70	Humulene epoxide II	1601	2021	0.1			1.5
71	1-epi-Cubenol	1624		0.3			
72	Eremoligenol	1625	2184				0.5
73	Caryophylla-4(12),8(13)-dien-5-ol	1632	2258	0.1			
74	γ-Eudesmol	1637	2153		t		
75	τ-Cadinol *	1639	2156	1.2			1.2
76	τ-Muurolol	1639	2172	0.3			0.3
77	β-Eudesmol	1645	2176		0.4		
78	α-Eudesmol	1656	2199				
79	α-Cadinol*	1650	2212	0.2			0.5
80	Selin-11-en-4α-ol (isomer?)	1652	2257	0.3			
81	α-Eudesmol	1656	2199				
82	(*E*,*E*)-Germacrone	1694	2197	0.4			0.9
83	Juniper camphor	1694	2257	0.1			
84	Farnesol-(*E*,*E* ?)	1719	2350		0.2		
85	Hexahydrofarnesylacetone	1844	2125	0.1			
86	Tricosane	2295	2300	t			
87	Tetracosane *	2400	2400	t			
88	Pentacosane	2490	2501	1.0			1.5
89	Phytol		2606	0.3			
90	Total (%)			97.1	96.9	95.4	95.4

*: see text; [1, 2, 3]: see footnotes, Table 2; [4]: RI are in agreement with [24-25] and MS is in agreement with [4]; [5] : From the MS trace, the ratio δ-cadinene/zonarene = ~8; Mass spectrum of unidentified compound **A,** *m/z*(intensity): 91(100), 161(88), 105(82), 41(72), 119(60), 133(52), 79(48), 93(48), 81(48), 77(40), 67(36), 204(36), 55(33), 107(30) …

Table 5: Composition (%) of the hydrosol of *C. peregrina*

S. No.		RI		Shelf-life (month)				
	Identification (% in oil)	**DB-5**	**S-wax**	**0**	**3**	**6**	**12**	**24**
1	1-Penten-3-ol *	675	1167	t [1]	0.8	1.0	1.1	1.2
2	3-Hydroxy-2-butanone	698	1293	t	0.8	1.1	1.2	1.3
3	Isopentanol	728	1208	0.1	1.8	2.1	2.0	1.6
4	*cis*-2-Pentenol	768	1331	0.1	1.1	1.2	1.2	1.4
5	*trans*-2-Hexen-1-al *	858	1218	0.8	0.9	1.0	1.0	0.6
6	*trans*-3-Hexen-1-ol *	860	1369	1.1	1.0	0.9	0.7	1.1
7	*cis*-3-Hexen-1-ol (0.1%)[2]*	864	1386	11.0	11.9	12.3	12.4	14.0
8	*trans*-2-Hexen-1-ol (0.2%)*	876	1407	12.9	13.8	14.1	14.3	16.0
9	Hexanol (0.3%) *	878	1360	7.6	8.0	8.3	8.3	8.8
10	Benzaldehyde (0.1%) *	963	1521	5.0	4.6	4.2	3.4	2.1
11	Hexanoic acid	984-1013[3]	1847	1.2	1.0	1.0	1.1	1.0
12	*trans*-3-Hexenoic acid	1013-1033[3]	1967	0.6	0.6	0.7	0.7	0.7
13	1,8-Cineole (9.7%)*	1035	1200	16.8	15.9	15.3	15.1	15.8
14	Benzyl alcohol *	1040	1871	1.4	1.4	1.4	1.5	1.7
15	*trans*-2-Hexenoic acid	1036-1062[3]	1970	1.3	1.2	1.0	1.4	1.3
16	Acetophenone *	1058	1683	2.3	2.1	2.0	1.7	1.2
17	*trans*-Linalool oxide (fur.) *	1084	1442	0.6	0.6	0.6	0.7	1.0
18	*cis*-Linalool oxide (fur.) *	1100	1473	0.7	0.8	1.0	1.0	1.2
19	Linalool (3.4%) *	1114	1557	9.7	7.5	6.4	5.0	3.1
20	α-Thujone	1116	1398	0.2	0.1	0.1	t	0.1

21	Maltol	1121	1963	0.3	0.3	0.3	0.3	0.3
22	2-Phenylethyl alcohol	1123	1905	0.3	0.3	0.3	0.3	0.2
23	*trans*-Pinocarveol	1145	1649	0.3	0.2	0.2	0.2	0.3
24	Borneol	1169	1691	0.3	0.2	0.3	0.3	0.3
25	δ-Terpineol	1172	1672	0.6	0.5	0.5	0.4	0.3
26	Benzoic acid	1173	2439	t	0.1	t	0.1	0.3
27	Terpinen-4-ol (0.9%) *	1179	1594	7.1	6.3	6.2	5.9	5.6
28	Octanoic acid	1180-1183[3]	2061	t	t	t	t	t
29	Methyl salicylate	1188	1764	0.3	0.3	0.3	0.3	0.3
30	α-Terpineol (0.7%) *	1190	1681	10.7	9.6	9.6	9.6	9.4
31	Myrtenol	1195	1783	1.0	0.8	0.8	0.7	0.8
32	2-Hydroxycineole	1204	1830	0.3	0.2	0.2	0.2	0.2
33	2-Hydroxycineole isomer	1223	1852	0.3	0.3	0.3	0.3	0.5
34	2,3-Dihydrobenzofuran	1224	2383	t	t	t	t	t
35	Nerol	1229	1802	0.3	0.2	0.1	0.1	0.1
36	Citronellol	1234	1769	t	0.2	0.1	0.1	0.1
37	2,6-Dimethyl-7-octene-2,6-diol	1243	2022	t	0.4	0.6	1.0	1.9
38	4-Phenyl-2-butanone	1245	1847	3.1	2.7	2.7	2.4	2.0
39	Geraniol	1257	1849	0.5	0.6	0.6	0.7	0.4
40	*p*-Menthane-1,8-diol isomer[4]	1309		t	0.2	0.3	0.7	1.6
41	*p*-Vinylguaiacol	1318	2167	~0.2	t	t	0.1	0.2
42	4-Phenyl-3-buten-2-one *	1339	2095	0.3	0.2	0.2	2.1	1.7
43	Benzenepropanoic acid	1342	2630	0.3	0.3	0.3	0.4	0.3
44	Eugenol *	1360	2156	1.0	0.8	0.7	0.6	0.3
□□	*trans*-Nerolidol (2.3%) *	1567	2022	t	n.m.	n.m.	t	t
46	τ-Cadinol (1.2%) *	1633	2134	t	n.m.	n.m.	t	t
47	Selin-11-en-4α-ol (isomer[4])	1650	2202	t	n.m.	n.m.	0.4	0.1
48	Juniper camphor (0.1%) *	1692	2256	t	n.m.	n.m.	t	t
49	Total of identified compounds (%)			99	96	95	98	89

[1]: traces, < 0.1%; [2]: percentage in oil; [3]: See footnote 3, Table 2; [4]: needs to be ascertained; *: see text.

3.3 Ledum groenlandicum

The oil of this wild growing plant is a complex mixture of mono- and sesquiterpenes, hydrocarbons, and oxygenated compounds. It was recently described in details in this journal [31]. During the present study, the oil corresponds to that of the A sample described in this paper.

The VOC content of the fresh hydrosol is 630 mg/L of which *ca*. 85% are reported in Table 6. The comparison with the data for the previous two hydrosols as well as with those obtainedelse where [1, 2], shows that the higher percentage of oxygenated compounds in oils, the richer in VOCs is the hydrosols. PH values of the hydrosol stay constant over the period of observation: 3.6±0.1. This is a rather acidic value.

In the fresh hydrosol about 50% of the VOCs are *p*-menthadienols including isopiperitenol, carveol, and perilla alcohol. From Table 6 one can observed an apparent isomerization of the *trans*-*p*-mentha-1(7), 8-dien-2-ol into the *cis*-isomer, the sum being constant over a one year period of shelf-life (Fig. 3). On the other hand, the *cis*- and *trans*-*p*-mentha-2,8-dien-1-ols practically disappear during a 12 month period of time from 3.6 to 0.6% with a parallel increase of the diol *trans*-sobrerol from 0 to 1,4%. There is also a major increase in the percentage of the unidentified **B** and **C** compounds from 0.7 to 7%. From the similarities observed in the RI values and in their mass spectrum, one may assumed they are isomers. Moreover, the difference Δ in the RI values is higher than 900 units and the similar Δ values for *trans*-sobrerol, may infer a diol structure for these **B** and **C** compounds (Fig 4), a situation very similar to what is observed above in the *M. gale* hydrosol, although these two couples **B** and **C** are different. Possible hydration processes may occur in the hydrosol during the shelf-life. It was shown that various products formed in the degradation of citral, and among them *cis* and *trans*-*p*-mentha-2,8-dien-1-ol, were subsequently converted mainly to *p*-cymen-8-ol and the more stable *p*-cymene [32, 33]. There is a small increase in the percentage of *p*-cymen-8-ol, not enough to compensate the decrease of the percentage of *cis*- and *trans*-*p*-mentha-2,8-dien-1-ol. *p*-Cymene is not water soluble and as such is not observed in the hydrosol.

The percentages of ketones, carvone, *p*-methylacetophenone, épi-cyclocolorenone, and eudesma-3,11-dien-2-one, decrease during the same although the decreases are of limited importance (sometimes inside the estimated error limits). The variation in the percentages of some alcohols increases (benzyl alcohol, phenylethyl alcohol, *p*-cymen-8- and -7-ols) and decreases (rosifoliol) again with small absolute values.

Finally, the presence of rare compounds such as isopiperitenols and isopiperitenone must be mentioned. They were observed in the essential oil of *Cymbopogon giganteus* [34] and of *Calamintha nepeta* subsp. *Nepeta* [35]. They were identified from their RI values [34, 36] and their mass spectra [37, 38].

Table 6: The composition (%) of the *L. groenlandicum* hydrosol during shelf-life and oil

S. No.		RI		Shelf-life (month)				Oil
	Identification	**DB-5**	**S-wax**	**0**	**3**	**6**	**12**	
1	*cis*-3-Hexen-1-ol	863	1391	0.1	0.2	0.2	t	-
2	6-Methyl-5-hepten-2-one	988	1341	-	t [1]	t	t	-
3	*trans*-3-Hexenoic acid	~1020	1955	0.2	0.2	0.2	0.4	-
4	Benzyl alcohol	1039	1872	t	0.3	0.3	0.4	-
5	*cis*-Linalool oxide (fur.)	1083	1442	t	0.1	0.2	0.1	-
6	*trans*-Linalool oxide (fur.)	1100	1468	0.1	0.1	0.3	0.1	-
7	Linalool	1113	1558	0.2	0.2	0.2	0.1	-
8	Phenylethyl alcohol	1122	1904	0.2	0.4	0.6	0.5	-
9	Dehydrosabina ketone	1128	1604	1.9	2.2	2.2	1.5	-
10	*trans*-*p*-Mentha-2,8-dien-1-ol	1130	1624	1.5	0.7	0.3	0.1	1.9
11	Nopinone	1135	1558	-	t	t	t	-
12	*cis*-*p*-Mentha-2,8-dien-1-ol	1145	1670	2.1	0.7	0.7	0.5	-
13	*trans*-Pinocarveol	1145	1648	1.0	1.5	1.4	1.0	0.8
14	Sabina ketone	1162	1614	1.5	1.8	1.9	1.6	-
15	Pinocarvone	1165	1554	0.4	0.4	0.4	0.2	-
16	Borneol	1171	1701	0.1	0.2	0.5	0.3	-
17	Terpinen-4-ol	1178	1596	2.8	2.8	2.7	1.9	2.3
18	*p*-Methylacetophenone	1182	1764	0.6	0.5	0.4	0.2	-
19	*p*-Cymen-8-ol	1186	1846	1.2	1.8	2.0	2.3	-
20	*trans*-*p*-Mentha-1(7),8-dien-2-ol	1189	1791	17.4	16.3	14.8	12.0	3.5
21	α-Terpineol	1191	1695	1.0	0.7	0.7	0.8	-
22	Myrtenal	1193	1611	0.55	0.55	0.5	0.3	1.3
23	Myrtenol	1195	1783	0.6	0.9	0.9	0.7	-
24	Isopiperitenol A [2]	1198	1746	3.7	1.7	0.9	0.2	-
25	Isopiperitenol B [2]	1217	1750	0.8	0.4	0.6	0.4	-
26	*trans*-Carveol	1220	1830	6.9	6.1	5.3	4.5	0.6
27	*cis*-*p*-Mentha-1(7),8-dien-2-ol	1234	1887	28.0	32.6	31.6	33.1	3.2
28	*cis*-Carveol	1236	1863	3.1	1.9	1.8	1.8	-
29	Carvone	1247	1723	3.8	3.3	2.8	1.5	-
30	Geranial	1277	1822	1.1	1.5	1.5	1.4	-
31	Geraniol	1266	1851	0.3	0.3	0.3	t	-
32	Isopiperitenone [2]	1281	1822	1.7	1.4	1.4	0.5	-
33	Perillaldehyde	1285	1761	0.5	0.3	0.2	0.2	-
34	Cuminol = *p*-cymen-7-ol	1298	2094	1.0	1.4	1.5	1. 8	-
35	Perilla alcohol	1299	1999	0.2	0.2	0.2	0.2	-
36	Unidentified **A**	1313	1920	0.6	0.4	0.4	0.5	-
37	*p*-Mentha-1,4-dien-7-ol	1326	2040	0.8	0.5	0.4	0.5	-
38	Unidentified **B**	1352	2275	0.15	1.1	2.1	6.5	-
39	*trans*-Sobrerol = (*E*)-*p*-menth-6-ene-2,8-diol	1378	2308	t	0.27	0.48	1.4	-
40	Unidentified **C**	1393	2384	0.22	1.0	2.0	6.7	-
41	Rosifoliol	1600	2096	0.3	0.1	0.1	0.1	-
42	epi-Cyclocolorenone	1754	2329	0.85	0.7	0.6	0.6	1.0
43	Eudesma-3,11-dien-2-one	1780	2470	1.1	0.7	0.6	0.5	1.0
43	**Total (%)**			88.9	87.8	88.8	85.4	15.6

[1]: traces (<0.05%); [2]: see text; Mass spectra of unidentified compounds, *m/z*(intensity):

A- 93(100), 136(92), 91(43), 77(40), 79(39), 121(24) … Could be a double peak. **B** and **C**: see figure 4.

Note: the *trans*-sobrerol MS is identical to that proposed for both the *cis*- and *trans*-sobrerol in the SciFinder base [38] and different from the one appearing in Fig 4. All these MS are different from that of *p-menth-6-ene-2,8-diol published* elsewhere [39].

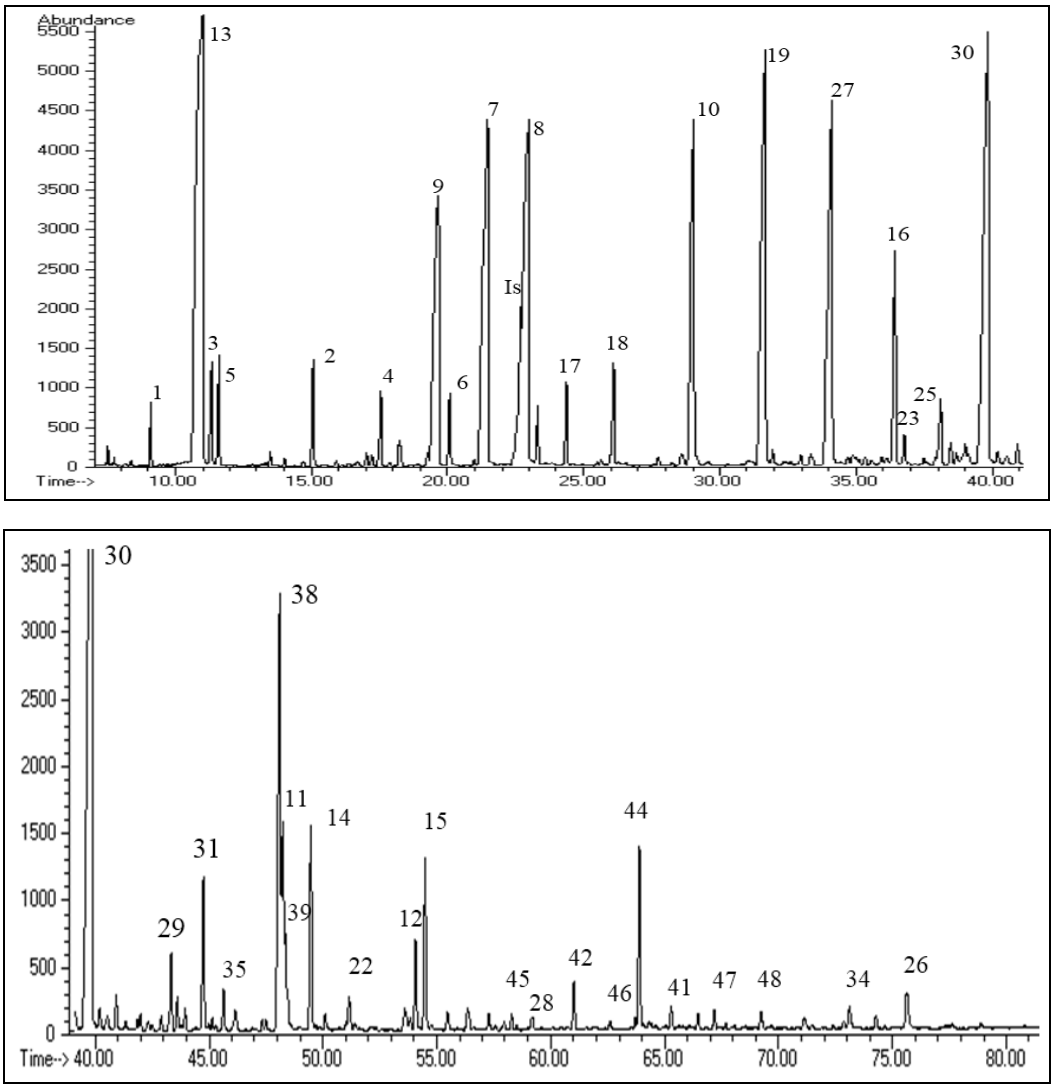


Fig 2: Chromatogram of the *C. peregrina* hydrosol on the polar column.
On the upper chromatogram, Is: internal standard, tetradecane. Numbers correspond to those appearing in Table 5.

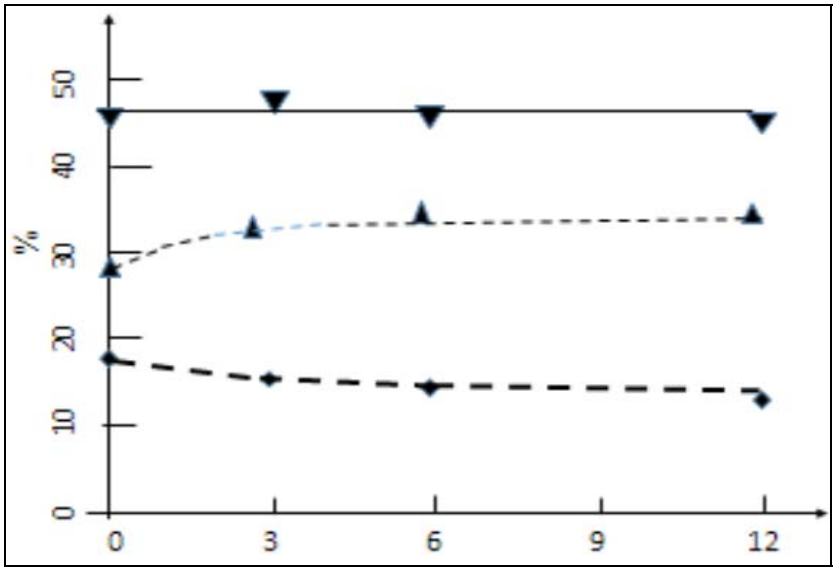


Fig 3: The variation in the % of *p*-mentha-1(7),8-dien-2-ol in the *L. groenlandicum* hydrosol:
◆: *trans*-; ▲: *cis*-; ▼: total *cis*- + *trans*-*p*-mentha-1(7),8-dien-2-ols.

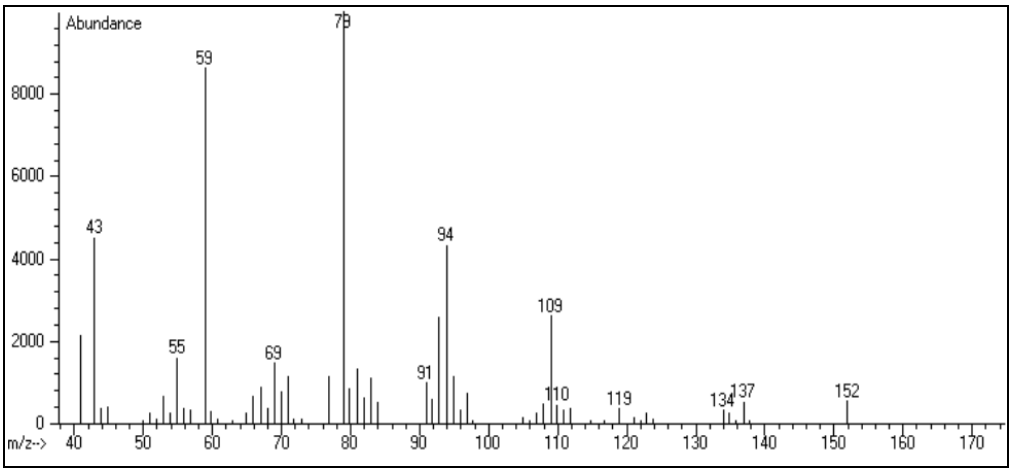


Fig 4: Mass spectrum of unidentified **B** and **C** in the *L. groenlandicum* hydrosol.

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