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Article

Volatile Compounds of *Baccharis punctulata*, *Baccharis dracunculifolia* and *Eupatorium laevigatum* obtained using Solid Phase Microextraction and Hydrodistillation

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Neste trabalho foram comparados perfis cromatográficos dos óleos provenientes de hidrodestilação de folhas de *Baccharis punctulata*, *Baccharis dracunculifolia* e *Eupatorium laevigatum* e os perfis cromatográficos obtidos utilizando-se HS-SPME. Várias plantas nativas do Brasil ainda não foram estudadas quanto à sua composição química. Técnicas convencionais de extração como a hidrodestilção, podem resultar em alterações da composição química original destes óleos. O uso da HS-SPME provê uma alternativa mais branda de extração, prevenindo transformações químicas e resultando em informações complementares sobre a composição de voláteis das plantas. Pela primeira vez, cumarina e cumaran foram identificados no "headspace" de folhas de *E. laevigatum*, após dano mecânico, por HS-SPME. As semelhanças e diferenças na composição dos voláteis, verificadas através de ambas as técnicas de extração, são discutidas, mostrando a complementaridade destas técnicas, as possíveis implicações destes resultados no que diz respeito a compostos infoquímicos e as possíveis transformações químicas durante o processo de hidrodestilação.

In this work the qualitative chromatographic profiles of the volatile oil obtained with fresh chopped leaves of *Baccharis punctulata*, *Baccharis dracunculifolia* and *Eupatorium laevigatum*, using HS-SPME were compared with their hydrodistilled oils. Several Brazilian native plant species have not yet been studied regarding their volatile compounds composition. Conventional techniques employed for the investigation of volatile compounds, such as hydrodistillation, may impart chemical changes to the original oil composition. The use of HS-SPME provides alternative milder extraction conditions, preventing chemical transformations and supplying complementary information about volatiles composition. Coumarin and coumaran were detected by the first time among volatile components of *E. laevigatum* leaves after mechanical damage, only when using HS-SPME. Differences and similarities perceived between volatile compounds profiles using both extraction techniques are discussed, showing that they are complementary and may bring insight about fresh leaf volatiles playing infochemical roles and about chemical transformations caused by hydrodistillation.

Keywords: *Baccharis punctulata, Baccharis dracunculifolia, Eupatorium laevigatum*, infochemicals, solid phase micro extraction

Introduction

Plant biogenic volatile organic compounds (BVOC) play several roles related to intra and inter species interactions, and are also important in various branches of industry, such as flavour and fragrances, pesticides, and perfumery industry.[1,2] Several factors may influence plant volatiles composition, such as freshness, grinding and drying processes, environmental conditions, extraction techniques etc.[1,3] Hydrodistillation (HD) as well as steam distillation (SD) are conventional techniques of isolation of volatile compounds, where temperature and pH may promote artifact formation, being also time consuming. On the other side, headspace solid phase micro extraction (HS-SPME) provides solventless extractions under mild temperatures and shorter extraction times.[1,4,5]

Several recent research works take advantage of the mild temperatures of HS-SPME to obtain information on

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BVOC of fresh plants *ex situ*, in order to achieve different purposes. Some examples are: comparison of BVOC released by different parts of various plants[6,7] analysis of volatile compounds of *Eruca sativa*,[8] *Hypericum triquetrifolium*,[9] and *Myrtus communis*[10] responsible for different aroma notes, and analysis of volatiles of fresh-cut pineaple during storage or UV-induced stress.[11] Some reports also compare information gathered with HS-SPME with those obtained using other techniques, such as HD[12,13] or SD[14-16] or several other extraction techniques.[3]

Former work of this group has already shown the potential of HS-SPME technique to extract compounds that were not found in the hydrodistilled oil of exotic plants (*Eucalyptus*) and that may play a role as infochemicals.[4,5]

Three Brazilian native species *Baccharis punctulata*, *Baccharis dracunculifolia*, and *Eupatorium laevigatum* (Asteraceae) are widely distributed in the South part of Brazil and were the object of this study. These species represent a social-economic potential because of their therapeutic use related to several diseases. The scientific literature presents some information about the volatile oils of *B. dracunculifolia* and *E. laevigatum*, although there is no report about *B. punctulata* essential oil.[17-19] However, neither one of these species has had their volatile compounds investigated by HS-SPME. The aim of this work is to compare the volatile oil composition of the three just mentioned native species of Rio Grande do Sul state using two different techniques, the conventional hydrodistillation and HS-SPME. Differences and similarities among results obtained with both techniques are discussed.

Experimental

Plant material

Adult fresh leaves of *B. punctulata* were collected in January 26th, 2005 and in September, 21th, 2005 in the km 307.5 of the BR 116, nearby Guaiba city in the state of Rio Grande do Sul, Brazil. In both samplings leaves of shrubs were sampled in the right and left side of the BR 116. Adult leaves of three shrubs of *B. dracunculifolia* were collected in the same days in the fields of São Maximiamo Farm (km 308 of BR 116 nearby Guaiba city in state of Rio Grande do Sul). Sampling of *E. laevigatum* young plants was performed in October 19th, 2005 in the same São Maximiamo Farm and its surroundings. Topographic coordinates of São Maximiamo Farm are 30° 10' 47" S and 51° 23' 33" W. Leaves of ten shrubs were sampled in the farm and on the edge of BR 116 road. *E. laevigatum* adult leaves were sampled in September 7th, 2004 by the sides of BR 386 road, approximately 12 km before Soledade city.

All the samples were identified by Prof. Dr. Nelson Ivo Matzenbacher and a voucher specimen of *B. punctulata* (ICN 157537), *B. dracunculifolia* (ICN 143383) and *E. laevigatum* (ICN 029328) has been deposited at the herbarium of Universidade Federal do Rio Grande do Sul, Brazil.

Hydrodistillation

Fresh leaves of *B. punctulata, B. dracunculifolia and E. laevigatum* were subjected to hydrodistillation in a modified Clevenger apparatus for 5 hours. A cooling system using ethylene glycol mixed to water kept condenser temperature between −2 °C and 4 °C. Anhydrous sodium sulphate, previously heated to 400 °C, was employed to eliminate essential oil humidity.

HS-SPME

HS-SPME was performed using fibers coated with 7 μm poly (dimethylsiloxane) (PDMS), 0.5 g of fresh chopped leaves and 4 mL of phosphate buffer (pH 7) inside 10 mL clear flask vials. Samples were kept at 30 °C in a temperature-controlled block for a minimum of 24 h, and headspace extractions were performed during 30 min. Fresh leaves used for HS-SPME were from the same batch of leaves used for hydrodistillation. More details on HS-SPME method development are reported in a former research work.[4,5] Fibers were supplied by Supelco (Oakville, ON, Canada), and were conditioned according to supplier's instructions before use. For each plant species, at least 5 replicates of extraction were performed. For *E. laevigatum*, chopped leaves headspace from adult and young plants were extracted separately.

Chromatographic analysis

Chromatographic analyses were performed with a Shimadzu gas chromatograph G17A coupled to a mass spectrometer detector QP 5050A. Two capillary columns were used under the following conditions: (*i*) OV-5 (Ohio Valley, Marietta, USA, dimensions 30 m × 0.25 mm × 0.25 μm); oven temperature programme starting from 60 °C raising at 3 °C min^{-1} to 250 °C; injector and detector were kept at 250 °C; helium flow at 1 mL min^{-1}. (*ii*) Supelcowax 10 (Supelco, Bellefonte, USA, dimensions 30 m × 0.25 mm × 0.25 μm); oven temperature programme starting from 40 °C raising at 3 °C min^{-1} to 220 °C; injector and detector were kept at 220 °C; helium flow at 1 mL min^{-1}. Injection of a 1% hexanic solutions of essential oils were made in the split mode (1:10), while HS-SPME analyses were made in the splitless mode and only in the OV5 column.

The oils were also analyzed in a Shimadzu GC-FID 17A, under similar chromatographic conditions on both columns. Linear temperature programmed retention indices (LTPRI) were calculated using the retention data of a 1% hexanic solution of linear alkanes (C9 to C24), along with retention data of the compounds of the three essential oils. Identification of volatile components was done comparing injections of pure compounds with unknown ones, keeping the same chromatographic conditions. When pure compounds were not available, comparison with retention data and LTPRI reported in the literature was used for tentative identification of the compounds. Comparison of mass spectra of the 6th edition of the Wiley library and the unknown compounds spectra was also employed for tentative identification.[20]

Results and Discussion

Differences found in volatile compounds of plants isolated with HS-SPME and conventional methods such as HD and SD are reported in the literature. Sometimes HS-SPME provides a larger scope of compounds,[4,21] in other cases, HS-SPME detects a lesser amount of compounds[22] and in some other cases differences found are only quantitative.[13] Quantitative differences are normally expected when dealing with techniques that are based in different principles of extraction, such as HD (exhaustive) and SPME (equilibrium). However, area percent of volatile components are normally reported to provide the order of magnitude of each compound in the whole mixture, as presented in the Table 1. Besides that, the various fiber coatings present different chemical affinity for the analytes and several extraction parameters (temperature, time, etc) may influence extraction results. Also, distinct plant tissues present different matrix effects on the volatile compounds.[1,13,22] For all these reasons, HS-SPME sampling conditions should be carefully planned in order to obtain meaningful data. The Table 1 shows a total of 122 compounds detected in the three species under study using hydrodistillation and HS-SPME as extraction techniques. Compounds detected by both techniques are 60 for *B. punctulata*, 42 for *B. dracunculifolia* and 33 for *E. laevigatum*. Besides those compounds, 15 more components were found only by HS-SPME in the headspace of *E. laevigatum* and of *B. dracunculifolia*.

Other twenty compounds were detected only in the hydrodistilled oil of the species under investigation. In *B. punctulata* essential oil the main compounds are a non identified sesquiterpene followed by bicyclogermacrene, a sesquiterpene, *cis*-cadin-4-en-ol, and (*Z*)-ocimene. The major components of *B. dracunculifolia* and *E. laevigatum* are nerolidol, an important component for the perfumery industry,[17] and the oxygenated sesquiterpene laevigatin, respectively.[19] Figure 1 presents the chromatographic profile of the hydrodistilled oils of *B. punctulata*, *B. dracunculifolia*, and *E. laevigatum* and also point to some small peaks that were detected only by HD, whose names are written in underlined font.

As the contribution of these peaks are minor and can hardly be seen in Figure 1, they will be highlighted in the following Figures. As it is possible to see from Table 1, the majority of the components of these three species were detected in the headspace of chopped leaves and also in the essential oil. Although differences found between results of both techniques were minor, they may convey meaningful information. It is well known that very small amounts of volatile compounds can act as important signals in the recognition of food source by insects. Some interactions between plants and phytophagous insects involve specific volatile chemical cues in the range of parts per million or even parts per billion of plant weight.[23,24] In most insect species food location is heavily dependent on olfactory cues. Some investigations have demonstrated highly specific plant odor neurons responding selectively to single compound at very low concentrations.[25-31] As an example to this the *Melanophila acuminate* antennae can detect guaiacol derivatives at concentrations as low as a few parts per billion.[24,32] Buttery and Ling also detected several hydrocarbons as the major components of a low concentration mixture (*ca.* 10 parts *per* billion) in corn roots, which acted as attractants of insects, such as corn root worm (*Diabrotica* spp.).[23]

Figure 2A presents a clearer picture of some compounds detected only by HD in *B. dracunculifolia* in the region where monoterpenes elute. Chromatographic peaks representing α-terpinene, γ-terpinene, terpinolene, and terpinen-4-ol were found only in the hydrodistilled oil and were not detected in the headspace of chopped leaves of the same plant (Figure 2B). A similar pattern is observed for *B. punctulata*, where α-terpinene and terpinen-4-ol were also detected only in the essential oil (Table 1). Several researchers have already reported the tendency of sabinene and α-thujene to undergo acid catalysed hydration, resulting in α-terpinene, terpinolene, γ-terpinene and terpinen-4-ol.[33,34] As hydrodistillation is carried out at higher temperatures than SPME and may provide lower pHs in aqueous medium, the presence of those monoterpenes may be regarded as artifacts of hydrodistillation.

Figure 3 shows some other *B. dracunculifolia* and *B. punctulata* volatile compounds that were detected by only one of the extraction techniques employed and not by the other.

Table 1. Compounds detected in the headspace of chopped leaves and in the hydrodistilled oil of *Baccharis punctulata*, *Baccharis dracunculifolia*, and *Eupatorium leavigatum*

No.	Compound	LTPRI OV5	LTPRI Lit.[20]	LTPRI Wax	*B. punctulata*		*B. dracunculifolia*		*E. laevigatum*	
					HD %area	SPME %area	HD %area	SPME %area	HD %area	SPME %area
monoterpene hydrocarbons					20.13	16.02	31.09	46.17	1.72	0.36
1	α-thujene	927	930	1023	0.26[b]	0.32[b]	0.08[b]	0.09[b]	0.41[b]	0.07[b]
2	α-pinene	934	939	1029	0.31[a,b]	0.39[a,b]	8.00[b]	8.70[b]	0.28[b]	0.07[b]
3	camphene	949	954	1067			0.06[b]	0.11[b]		
4	sabinene	973	975	1120	0.59[b]	0.94[b]	0.44[b]	1.38[b]	0.09[b]	tr[b]
5	β-pinene	978	979	1110	0.32[a,b]	0.50[a,b]	12.17[b]	14.95[b]	0.12[b]	tr[b]
6	myrcene	990	991	1170	0.41[a,b]	0.19[a,b]	1.99[b]	4.73[b]	0.11[b]	tr[b]
7	monoterpene	-	-	-			tr[c]	0.15[c]		
8	α-phellandrene	1004	1003	1167	0.14[a,b]	tr[a,b]				
9	α-terpinene	1017	1017	1183	**0.06**[a,b]		**0.10**[a,b]			
10	limonene	1028	1029	1203	6.00[a,b]	4.21[a,b]	7.65[b]	14.66[b]	0.23[b]	tr[b]
11	1,8-cineole	1028	1031	-			0.05[b]	0.90[b]		
12	(*Z*)-ocimene	1035	1037	1242	6.33[a,b]	7.25[a,b]	0.40[b]	0.50[b]		
13	(*E*)-ocimene	1046	1050	1259	4.96[a,b]	2.09[a,b]			0.39[b]	0.11[b]
14	γ-terpinene	1058	1060	1249	0.09[a,b]	tr[a,b]	**0.15**[b]		0.05[b]	tr[b]
15	terpinolene	1088	1089	1288	tr[a,b]	tr[a,b]	**0.11**[b]		0.05[b]	tr[b]
16	linalool	1097	1091	1563	0.25[b]	tr[b]	0.10[b]	0.09[b]		
17	monoterpene	1114	-	1312	**tr**[c]					
18	allo ocimene	1126	1132	-	0.59[b]	0.07[b]				
oxigenated monoterpenes					0.61	0.05	0.72	0.26		0.06
19	terpin-4-ol	1178	1177	1605	**0.14**[a,b]		**0.24**[b]			
20	α-terpineol	1189	1189	-	0.17[a,b]	tr[a,b]	0.38[b]	0.17[b]		
21	coumaran	-	-	-						**tr**[c]
22	hexenyl ester	-	-	-						**tr**[c]
23	hexenyl ester	-	-	-						**tr**[c]
24	hexenyl ester	-	-	-						**tr**[c]
25	*trans*-geraniol	1251	1253	-	**tr**[b]					
26	nq - 69(100), 95(85), 55(65), 110(45), 152(33)	1259	-	1315			0.08[b]	0.14[b]		
sesquiterpene hydrocarbons				19.38	62.67	22.09		25.11	82.90	
27	nq – 150(100), 135(93), 107(32), 77(26), 151(10)	1310	-	1412	**0.10**					
28	sesquiterpene	-	-	-						**tr** [c]
29	δ-elemene	1338	1338	1483	0.96[b]	0.68[b]	0.09[b]	0.15[b]	0.11[b]	0.23[b]
30	sesquiterpene	-	-	-						**tr**[c]
31	sesquiterpene	-	-	-						**tr**[c]
32	α-ylangene	1374	1375	-				**0.20**[c]		
33	β-bourbonene	1387	1388	1520			0.10[b]	0.23 [b]		**tr**[c]

Table 1. Continuation

No.	Compound	LTPRI OV5	LTPRI Lit.[20]	LTPRI Wax	*B. punctulata*		*B. dracunculifolia*		*E. laevigatum*	
					HD %area	SPME %area	HD %area	SPME %area	HD %area	SPME %area
34	β-elemene	1392	1391	1596	0.39[b]	1.13[b]	0.41[b]	0.35[b]	0.23[b]	0.21[b]
35	α-gurjunene	1411	1410	1662	0.05[b]	0.51 [b]				
36	methyl eugenol	1413	1404	2023			0.20[b]	0.11[b]		
37	sesquiterpene	1415	-	-				0.12		
38	(*E*)-caryophyllene	1421	1419	1675	0.63[b]	6.80[b]	2.79[b]	5.68[b]	1.62[b]	1.28[b]
39	coumarin	-	1224[d]	-						0.12[a]
40	β-copaene	1430	1432	1673	0.06[b]	0.27[b]				
41	sesquiterpene	-	-	-						**0.30**[c]
42	sesquiterpene	1436	-	1697	**0.05**[c]					
43	aromadendrene	1441	1441	1609	**0.13**[a,b]		0.47[b]	0.36[b]	**0.08**[b]	
44	sesquiterpene	1442	-	1648	0.09[c]	0.40[c]				
45	sesquiterpene	1446	-	1689			**0.20**[c]			
46	sesquiterpene	1447	-	1645					0.09[c]	0.23[c]
47	sesquiterpene	1452	-	-			0.28[c]	0.33[c]		
48	α-humulene	1456	1455	1675	0.16[b]	0.20[b]	0.72[b]	0.84[b]		
49	(*E*)-β-farnesene	1457	1457	1676	0.27[b]	3.89[b]			0.60[b]	1.33[b]
50	sesquiterpene	1460	-	-				**1.10**[c]		
51	β-santalene	1462	1460	1759			**1.82**[b]			
52	allo-aromadendrene	1463	1460	-				**0.70**[c]		
53	trans-cadina-1(6).4-diene	1474	1477	-	0.10[b]	0.25[b]				
54	γ-muurolene	1477	1480	1691	0.17 [b]	0.28[b]				
55	sesquiterpene	1480	-	-			1.08[c]			
56	germacrene D	1485	1485	1714	2.66[b]	16.05[b]	2.87[b]	9.12[b]	11.66[b]	44.88[b]
57	β-selinene	1488	1490	-				0.29[c]		
58	trans-muurola-4(14),5-diene	1493	1494	1729	**0.15**[b]					
59	bicyclogermacrene	1500	1500	1739	9.73[b]	22.93[b]	5.44[b]	7.63[b]	9.33[b]	30.44[b]
60	α-muurolene	1506	1500	1798			0.20[b]	0.10[b]		
61	β-bisabolene	1508	1506	1775	0.43[b]	4.45[b]			0.58[b]	1.71[b]
62	sesquiterpene	1510	-	1779			0.91[c]	1.33[c]		
63	γ-cadinene	1515	1514	1786	0.39[b]	0.81[b]	0.20[b]	0.47[b]		
64	sesquiterpene	1517	-	-					0.16[c]	0.43[c]
65	sesquiterpene	1520	-	-	0.33[c]	0.07[c]				
66	δ-cadinene	1524	1523	1762	2.06[b]	2.79[b]	0.91[b]	1.29[b]	0.38[b]	1.03[b]
67	nq – 145(100), 131(65), 187(59), 105(56), 202(45)	1532	-	1846	0.73	0.13				
68	sesquiterpene	1535	-	-			2.55[c]	0.09[c]		
69	α-cadinene	1538	1539	1796	0.11[b]	tr[b]				
70	sesquiterpene	1544	-	1791	0.12[c]	0.80[c]				
71	sesquiterpene	1542	-	1779					0.09[c]	0.21[c]
72	sesquiterpene	1549	-	1879	0.36[c]	0.30[c]				
73	sesquiterpene	1551	-	1880					0.18[c]	0.44[c]
74	sesquiterpene	1554	-	2015			0.83[c]	0.20[c]		

Table 1. Continuation

No.	Compound	LTPRI OV5	LTPRI Lit.[20]	LTPRI Wax	*B. punctulata*		*B. dracunculifolia*		*E. laevigatum*	
					HD %area	SPME %area	HD %area	SPME %area	HD %area	SPME %area
sesquiterpenes and oxygenated sesquiterpenes					54.76	20.21	45.87	22.85	71.79	16.57
75	(*E*)-nerolidol	1562	1563	1028	0.28[b]	0.11[b]	22.16[b]	12.80[b]		
76	oxygenated sesquiterpene	1569	-	2084	0.32[c]	0.07[c]	1.07[c]	0.76[c]		
77	oxygenated sesquiterpene	1579	-	2068	0.87[c]	0.86[c]				
78	spathulenol	1580	1578	2099	0.88[b]	0.24[b]	8.81[b]	3.31[b]	0.30[b]	0.42[b]
79	globulol	1585	1585	2094	1.05[b]	0.23[b]				
80	sesquiterpene	1589	-	2092					0.26[c]	0.12[c]
81	β-copaen-4-α-ol	1589	1591	2159			3.39[b]	2.10[b]		
82	guaiol	1594	1601	2102	3.43[b]	2.06[b]				
83	viridiflorol	1597	1593	2162			3.54[b]	1.12[b]		
84	oxygenated sesquiterpene	1600	-		0.28[c]	0.10[c]				
85	oxygenated sesquiterpene	1605	-	2128	**0.32**[c]					
86	oxygenated sesquiterpene	1608	-	2198			1.71[c]	0.87[c]		
87	oxygenated sesquiterpene	1620	-	2136	0.24[c]	0.06[c]				
88	nq – 119(100), 161(66), 159(57), 105(54), 121(22)	1624	-	2219	1.56	0.57				
89	oxygenated sesquiterpene	1631	-	2187			0.56[c]	0.34[c]		
90	cis-cadin-4-en-7-ol	1636	1637	2116	6.77[b]	2.74[b]			0.31[b]	0.18[b]
91	oxygenated sesquiterpene	1643	-	-			0.63[c]	0.40[c]		
92	oxygenated sesquiterpene	1643	-	2185	1.99[c]	0.13[c]				
93	oxygenated sesquiterpene	1645	-	2203			1.01[c]			
94	torreyol	1645	1646	2147	0.53[b]	0.10[b]			0.47[b]	0.14[b]
95	α-cadinol	1656	1654	2211	3.17[b]	0.10[b]				
96	oxygenated sesquiterpene	1658	-	2217			1.45[c]	0.49[c]		
97	oxygenated sesquiterpene	1659	-	2149					0.61[c]	0.15[c]
98	sesquiterpene (201, 216)	1670	-	2166	14.67[c]	8.36[c]			2.31[c]	0.91[c]
99	(epi-α) bisabolol	1685	1685	2250	3.08[b]	0.70[b]				
100	(Z)-farnesol	1686	1686	2234					0.45[b]	0.16[b]
101	oxygenated sesquiterpene	1693	-	2391	2.20[c]	2.41[c]	0.65[c]	0.11[c]		
102	oxygenated sesquiterpene	1699	-	2255	2.83[c]	0.96[c]				
103	sesquiterpene	1700	-	2242					0.64[c]	0.12[c]
104	oxygenated sesquiterpene	1707	-		0.75[c]	0.19[c]				
105	oxygenated sesquiterpene	1718	-				0.23[c]	0.32[c]		
106	oxygenated sesquiterpene	1722	-	2298	**0.82**[c]					
107	nq – 143(100), 185(92), 129(64), 128(55), 157(54)	1727	-		0.71	0.11				
108	sesquiterpene (212,197)	1735	-		1.09	0.22				
109	sesquiterpene (214, 199)	1738	-	2333	**0.75**					
110	laevigatin	1738	-	2404[e]					59.63[c]	13.82[c]
111	oxygenated sesquiterpene	1739	-	-			0.38[c]	0.13[c]		
112	sesquiterpene (214,199)	1747	-	-			0.28[c]	0.09[c]		
113	sesquiterpene	1751	-	2357	7.14[c]	0.38[c]				

Table 1. Continuation

No.	Compound	LTPRI OV5	LTPRI Lit.[20]	LTPRI Wax	*B. punctulata*		*B. dracunculifolia*		*E. laevigatum*	
					HD %area	SPME %area	HD %area	SPME %area	HD %area	SPME %area
114	sesquiterpene (214,199)	1753	-	2252					3.11[c]	0.42[c]
115	sesquiterpene (214,199)	1775	-	2359	2.86[c]	0.33[c]			1.07[c]	0.08[c]
116	sesquiterpene	1785	-	-	0.19[c]	0.06[c]				
117	nq – 68(100), 57(89), 82(78), 95(69), 69(64)	1837	-	-	0.06	tr				
118	sesquiterpene (212,197)	1891	-	-	0.10[c]	tr[c]				
119	nq – 210(100), 209(35), 195(28), 165(27), 167(16)	1917	-	-	0.12	tr				
120	nq - 217(100), 189(35), 164(28), 157(27), 95(16)	1924							0.33	0.11
121	sesquiterpene (197, 212)	2069	-	-					2.64[c]	tr[c]
122	nq – 82(100), 57(98), 71(93), 95(92), 68(92)	2140	-	-					1.05	

nq: not quantified; empty cell means the compound was not detected or it was not possible to get its LTPRI due to co-elutions, similarity of mass spectra among several isomers, or lack of such information in the scientific literature; ( ):some of the major ions in the mass spectrum of nq compounds; -: whenever it was not possible to determine the LTPRI experimentally, as they were determined mainly for hydrodistilled oils; tr: compounds detected as traces level; compounds name written in bold and italics were detected either in the hydrodistilled oil or using SPME. Area % of these compounds is also underlined. Identification or tentative identification by: [a]co-injection with standard under the same analytical conditions in the OV-5 chromatographic column; [b]comparison of experimantal LTPRI with the ones found in the literature;[20] [c]comparison of experimental retention and mass spectra data with literature data;[20] [d]Pino *et al.*,[41]; [e]Maia *et al.*,[19].

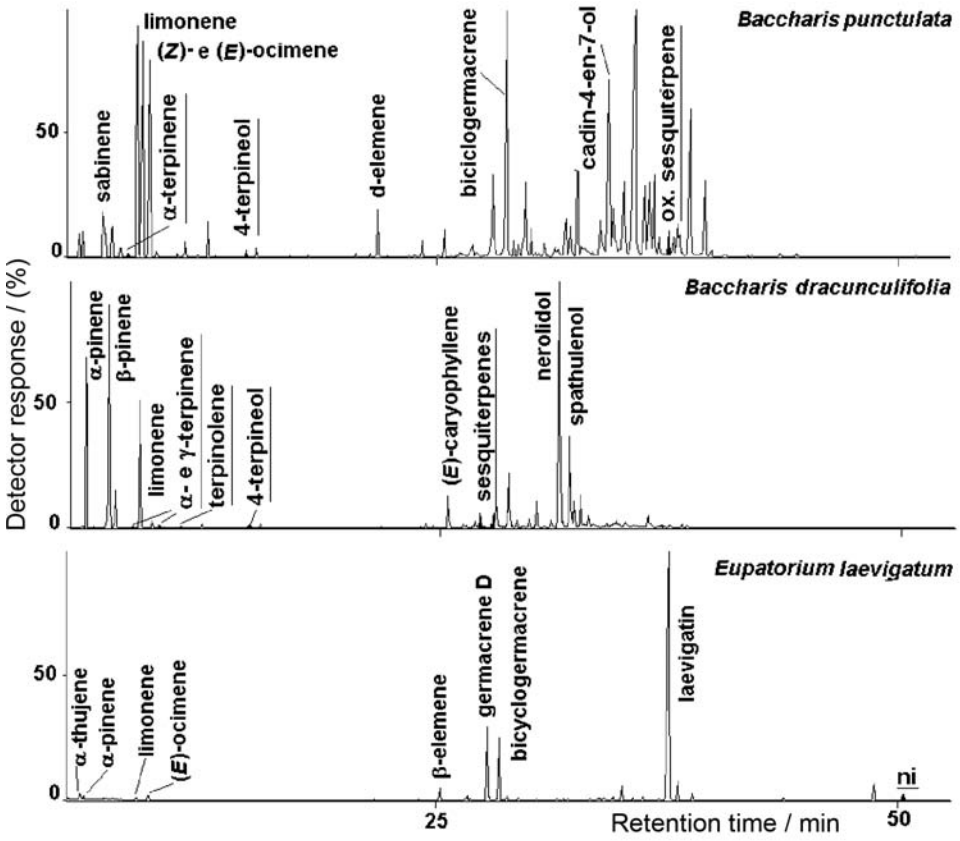

Figure 1. Chromatographic profile of the essential oils of *Baccharis punctulata*, *Baccharis dracunculifolia*, and *Eupatorium laevigatum*.

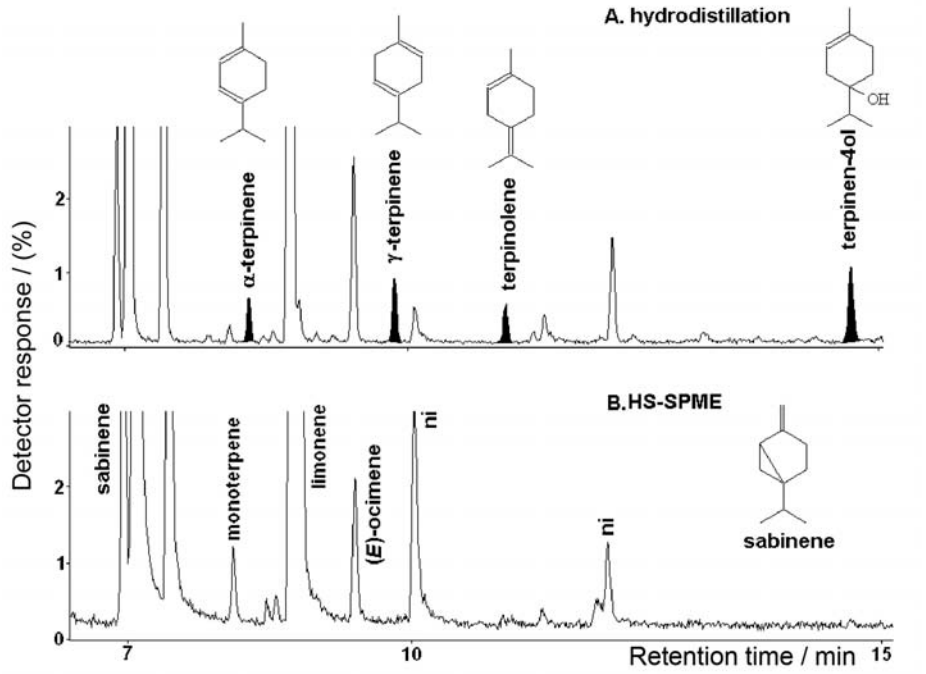

Figure 2. Part of the chromatographic profile of *B. dracunculifolia* hydrodistilled oil (A) and of the HS-SPME (B) of the chopped leaves of the same plant, showing marked peaks detected either by hydrodistillation or HS-SPME.

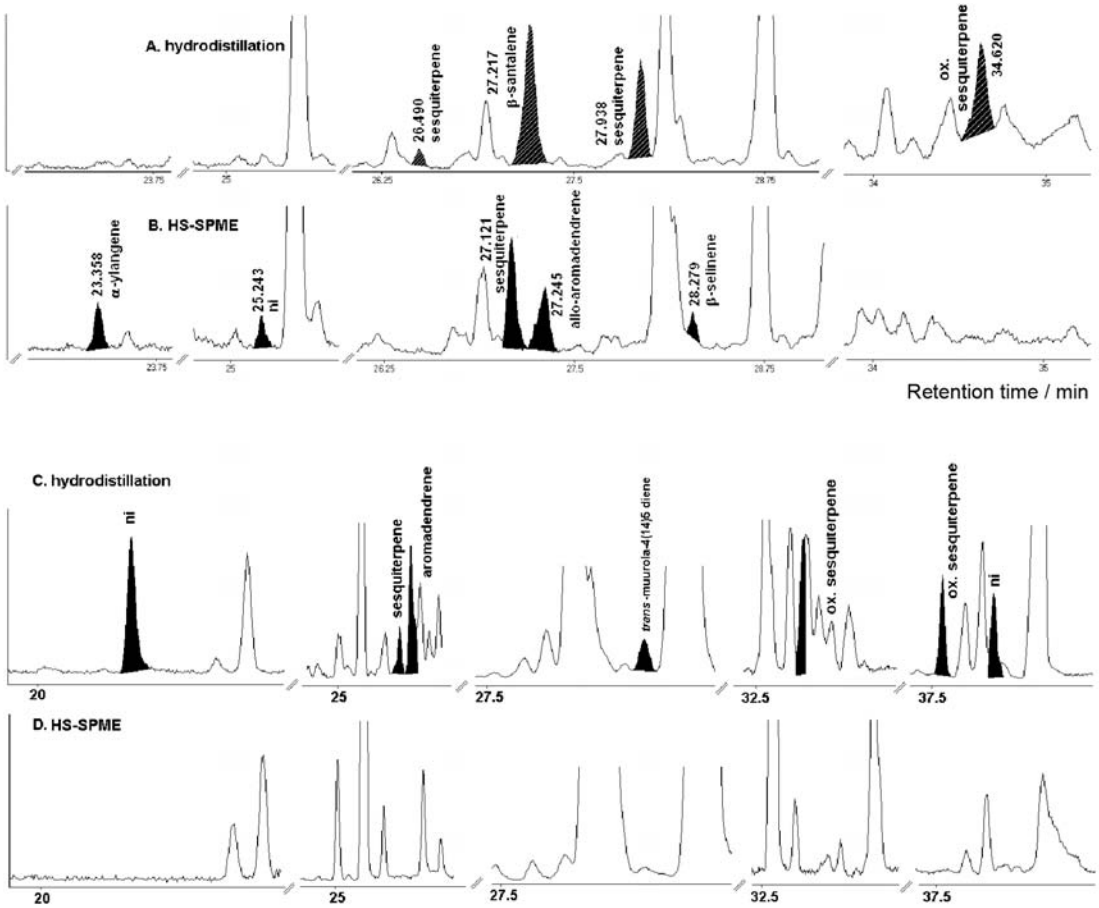

Figure 3. Part of the chromatographic profile of the hydrodistilled oils of *B. dracunculifolia* (A) and of *B. punctulata* (C) and of the HS-SPME of the chopped leaves of the same plants of *B. dracunculifolia* (B) and of *B. punctulata* (D), showing the marked peaks detected only by one of the techniques.

A reasonable explanation for the presence or absence of some of these compounds may not be straightforward, as sesquiterpene hydrocarbons undergo several rearrangements, which can be thermal, photochemical or acid-catalyzed. Chemical transformations of sesquiterpene compounds are complex processes, which may include oxidation, thermal decomposition or hydrolysis, being affected by many parameters, such as light, pH, and temperature.[35] Only HD detected some oxygenated compounds, and their presence may be understood as products of thermal oxidation occurring during the distillation process.[34] This type of transformation has already been observed in some other plant species containing thermally sensitive compounds, when conventional extraction processes using higher temperatures were employed.[4,16,21]

On the other side, some compounds were found only in the headspace of chopped leaves and were not detected in the hydrodistilled oil as is shown in Figure 4B (10 peaks: 5 sequiterpenes, 3 hexenyl esters, coumaran, and coumarin) where chromatographic peaks were marked in dark ink.

Figure 4A shows the corresponding chromatogram of the essential oil of the same plant. The presence of the so called green leaf volatiles (in this case hexenyl esters) are due to enzymatic cleavage of non volatile precursors, which was prompt by mechanical damage of fresh leaves, and has already been observed in former works when SPME was employed as extraction technique.[1,4,34] The presence of coumarin (1,2-benzopyrone or 2H-1-benzopyran-2-one) and coumaran (2,3-dihydrobenzofuran) is for the first time reported among volatile components of *E. laevigatum* leaves.[19] Unlike other simple coumarins, the biosynthesis of the coumarin itself is obscure. As far as it is known it is a derivative of *trans*-cinnamic acid (shikimate pathway), although it remains uncertain if 2-H-1-benzopyran-2-one is a true plant metabolite of the shikimic pathway or if it rises from chemical transformation of another precursor. This last hypothesis is considered plausible for *Melilotus* sp. (Fabaceae), since in this case the glucoside of 2'-hydroxycinnamic acid (coumarinic acid) rather than the coumarin is the true plant metabolite.[36]

The presence of coumarin in the headspace of *E. laevigatum* leaves is consistent with the chemotaxonomic profile of the Asteraceae family. First, species that belong to evolved families as Asteraceae have a general tendency to lack the shikimate derivatives in detriment of mevalonate derivatives and this tendency is also observed in the volatile chemistry.[37] Secondly, although the presence of 2-H-1-benzopyran-2-one is not common in other Asteraceae species, which mainly produce oxygenated coumarins, this plant group is one of the major coumarin producers among angiosperms and is highly specialized in the biosynthesis of simple coumarins.[38]

Coumarin may be a signaling compound among other plant species or even arthropods species. It is reported that this arylpropanoid completely inhibited the growth of alfafa (*Medicago sativa*) and bayard *grass (Echinochloa crus-galli*, var. oryzicola), what means it may be used for natural weed control.[39] Coumarin is also an attractant to herbivore insects, such as *Listroderes costirostris* (Coleoptera: Curculionidae) and *Sitona cylindricollis* (Coleoptera:Curculionidae).[40] On the other hand, coumarin is one of the feed deterrents produced by *Trifolium glanduliferum* (Fabaceae) and is probably responsible for the resistance of this plant against the red-legged earth mite *Halotydeus destructor* (Acari: Penthaleidae).

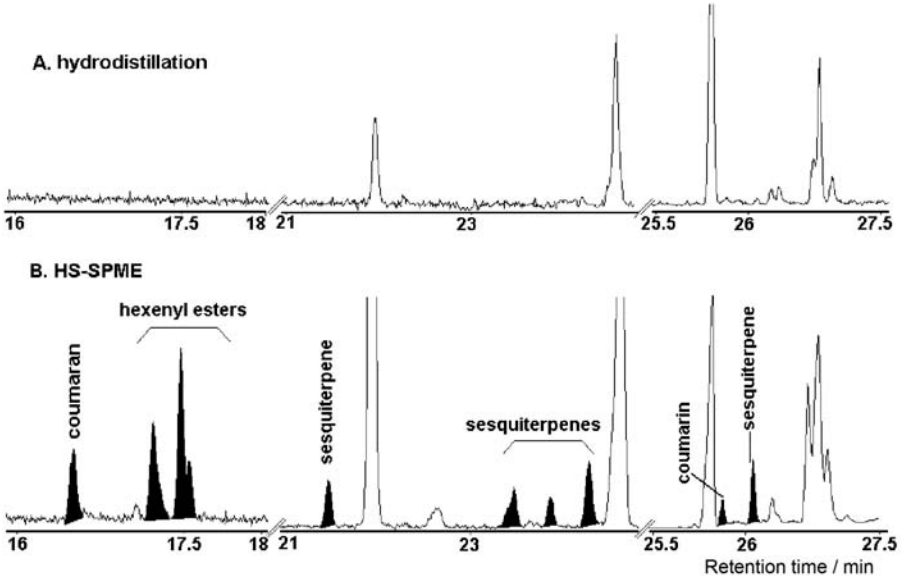

Figure 4. Chromatogram of volatile components of young leaves of *E. laevigatum.*

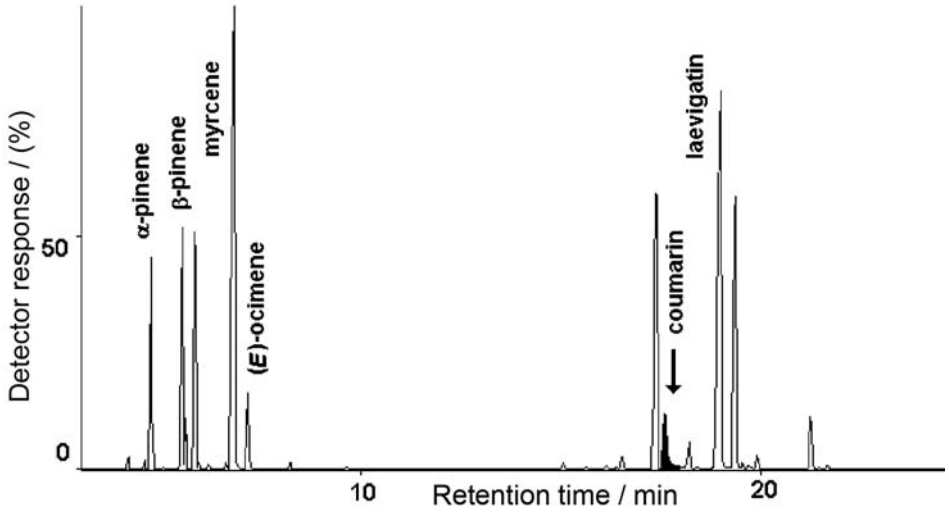

Figure 5. Chromatogram of volatile components of adult leaves of another individual of *E. laevigatum*.

Figure 5 presents a chromatogram of volatile components of adult leaves of another sample of *E. laevigatum*.

This additional analysis confirms the presence of coumarin in adult leaves of *E. laevigatum* and reinforces the results of experiments with young leaves, which were performed as five replicates.

The compound tentatively identified as coumaran was detected in the headspace of young leaves of *E. laevigatum* (Figure 4B), but was not in the headspace of adult leaves of the *E. laevigatum* (Figure 5). This fact can be explained by genetic, ontogenetic or ecological differences between samples and should be further investigated in order to elucidate the role of this substance as a signal. Its LTPRI on a SPB5 chromatographic column is reported to be 1224.[41] In this work it was not possible to experimentally determine the LTPRI of this compound, as the indices were only calculated for the essential oil components. However, coumaran eluted in the proper chromatographic region (between LTPRI 1189 (α-terminal) and 1251 (*trans*-geraniol)), and presented a 90% match with the mass spectrum of the 6th edition of Wiley mass spectra library. This compound may also play an ecological role as feed deterrant as it was found in *Cyperus nipponicus* (Cyperaceae) as responsible for the inhibition of polyphagous insects (*Spodoptera litura* (Lepidoptera: Noctuidae) feeding.[42]

Conclusions

A complete characterization of volatile components of plants may require the use of more than one extraction technique, as different principles of extraction and distinct extraction parameters (temperature, pH, solvent, etc) may contribute to various chromatographic profiles. Qualitative differences between hydrodistilled essential oils and the volatile compounds found in the headspace of *B. punctulata*, *B. dracunculifolia*, and *E. laevigatum* chopped leaves brought additional information about their composition and their possible chemical transformation during hydrodistillation process. The fact that some compounds were detected only by HS-SPME might possibly unveil their infochemical roles regarding plant defense of some species.

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