ORIGINAL ARTICLE

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Rec. Nat. Prod. 10:1 (2016) 58-67

records of natural products

Comparison of the Chemical Composition of "*Cystoseira sedoides* (Desfontaines) C. Agardh" Volatile Compounds Obtained by Different Extraction Techniques

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(Received March 24, 2013; Revised April 03, 2015; Accepted April 05, 2015)

Abstract: The volatile fraction of the brown alga *Cystoseira sedoides* (Desfontaines) C.Agardh is prepared from the crude extract through the following three extraction methods: Hydrodistillation (HD), focused microwave assisted hydrodistillation (FMAHD) and supercritical fluid extraction (SFE). The volatile fractions are analyzed by gas chromatography-flame ionization detector-mass spectrometry (GC-FID-MS), the chemical components are identified on the basis of the comparison of their retention indices with literature and their mass spectra with those reported in commercial databases. The chemical composition of the volatile fractions obtained by different extraction techniques fall into three major chemical classes: fatty acids and derivatives, sesquiterpenes, and hydrocarbons and derivatives. Others Compounds belonging to different chemical classes are found in that chemical composition.

Keywords: *Cystoseira sedoides*; volatile fraction; hydrodistillation; focused microwave; supercritical fluid extraction.

1. Introduction

The volatile compounds of marine origin have been rarely studied; although they could be used as a source of original flavouring agents in food and perfume industries. The odors connected to marine plants are much less familiar than those connected to terrestrial plants, may be due to the difficulty of harvesting the marine flora and its environment is, so far, poorly known. Currently, there is a rapid development of the chemical study of marine origin products (algae, sponges) and most research works are performed on the isolation of new secondary metabolites [1]. We can also point out the development of the research on the volatile compounds extracted from marine algae [2]. The various researches carried on the volatile compounds of algae showed that the green and red algae are not of a big importance from the smell point of view in particular. The volatile fraction contains

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 08/01/2015 EISSN:1307-6167

mainly sulphurated and halogenated organic compounds [3] but also the monoterpenoids and halogenated monoterpenoids [4]. In contrast to green and red algae, the volatile compounds of brown algae in particular the species of the genus *Dictyopteri*s and *Ectocarpus* present a very pleasant marine note. This "beach odor" is related to the presence of C_{11}-hydrocarbons which represents a class of pheromones responsible for the chemical defense of the species and of the chemoattraction of male gametes [5]. The odor of brown algae is also due to the presence of a large variety of monoterpenoids and sesquiterpenoids in their volatile fraction and essential oils [6]. The essential oils, volatile fractions and lipidic extracts are prepared from raw natural materials via several techniques. Currently, the supercritical fluid extraction [7], the subcritical fluid extraction [8], the focused microwave extraction [9] and the ultrasound extraction [10] have experienced a very rapid development in the field of natural products. There are many benefits from using these recent processes instead of conventional organic solvents. These include, among others: achieving higher purity extracts, no residual solvent, single step processing, reduced operating costs, selective fractionation, faster separation, being eco-friendly (green extraction) [11] and physiologically compatible. Furthermore, the oxygen-free operating system prevents oxidation. The low temperatures minimize thermal degradation of sensitive materials and the resulting extract is microbially sterile [12]. The green extraction methods mentioned above have been widely used in preparing volatile oils and extracts of algae [13-14].

The brown alga *Cystoseira sedoides* is endemic to the Algerian coast [15]. This species was also reported in low abundance off the Tunisian coast and off the Italian coast in the extreme south (Pantelleria) [16].

The aim of this study is to compare the chemical composition of volatile fraction of the endemic brown alga *Cystoseira sedoides* obtained by three methods: hydrodistillation, focused microwave hydrodistillation and supercritical fluid extraction. The volatile fractions were analyzed by GC/MS using electron impact ionization. The identification of the compounds was done on the basis of Kovat's indices and of mass spectral data base.

2. Materials and Methods

2.1. Plant material and chemicals

The plant material was collected off the Mediterranean coast of Algeria at the site called "the Carroubiers", on the west of Tipaza (36°37' 12 " NR, 2°39' 00 " E), in June 2006. This sample belongs to: Phaeophyceae class, Fucales order, Sargassaceae family, *Cystoseira* genus and *sedoides* (Desfontaines) C.Agardh for the species [17-18]. The alga was identified by Dr. H. Seridi and a voucher specimen (HS 01) of this species was deposited in the herbarium of Laboratory of Biological Oceanography and Marine Environment - USTHB - Algeria.

The alga was manually sorted out to remove any trace of epiphytes. It was then air-dried under shade without any other treatment.

Non-stabilized diethyl ether, dichloromethane and ethyl acetate of analytical grade were purchased from Biochim-Sial (Algiers - Algeria). The pure alkane standards (C_7 - C_{30}) were purchased from Prochima - Sigma (Tlemcen - Algeria).

2.2. Extraction

2.2.1. Preparation of crude extract

The extraction was performed in a 2.5 L - round flask with 314 g of plant material and 2 L of diethyl ether (batch extraction). The solvent was removed by vacuum distillation leading to 1.55 g of a crude extract (concrete), that is to say a yield of 0.495 %. The yield was calculated according to the weight of the plant material before distillation (expressed in percentage, w/w of the dry alga).

2.2.2. Preparation of the volatile fractions

The volatile fractions were obtained from the crude extract by the three techniques: Hydrodistillation (HD), Supercritical fluid extraction (SFE) and Focused microwave-assisted hydrodistillation (FMAHD).

2.2.2.1. Hydrodistillation (HD)

The volatile fraction was prepared on a modified Dean-Stark system. A mass of 405 mg of crude extract was crossed by a stream of steam over 45 min with 300 mL of water. The essential oil is recovered by liquid – liquid extraction with non-stabilized diethyl ether and dried on sodium sulfate. The hydrodistillation afforded yellow viscous oil with a marine odor, the mass obtained was 48.67 mg corresponding to a yield of oil on moisture free basis of 12.0 % (w/w).

2.2.2.2. Supercritical fluid extraction (SFE)

The experiment was carried out on an HP 7680 supercritical fluid extraction unit (Hewlett Packard, Les Ulis, France) equipped with an extractor vessel of 7 mL volume. A mass of 300 mg of crude extract (concrete) was heated to 40°C, mixed with about 5 g of 2 mm diameter glass beads and then filled into the extractor. The aim of the operation was to obtain a thin layer of concrete around the glass beads, and also to offer the maximum of contact surface between the concrete and the supercritical fluid (solvent). The mixture leaving the extractor (essential oils + solvent) passes through a trap collector. The latter is a cylindrical tube filled up with Tenax GC (60/80 Mesh, Interchim, Montluc¸ France) as a stationary phase. The essential oil is extracted by washing out the trap collector with 50 mL of dichloromethane and 50 mL of diethyl ether. The carbon dioxide used as solvent had 99.999 % purity. The optimized experimental parameters, previously determined, are set as follows: (i) CO_2 density being 0.6 g/mL, (ii) CO_2 flow being 1 mL/min, (iii) equilibrium time being 5 min; (iv) experiment temperature being 40 °C, (v) trap temperature being 0 °C, (vi) extraction time being 30 min, and (vii) extraction pressure being 91 bar. We have noticed that the increase in pressure causes the extraction of heavy products, which causes the increase in color intensity of the essential oil. Under the above conditions, a mass of 301 mg of crude extract afforded 113 mg of a yellow oil with an intense marine odor. The yield of oil on moisture free basis was 37.5 % (w/w)

2.2.2.3. Focused microwave-assisted hydrodistillation (FMAHD)

The focused microwave oven is a Discover, manufactured by CEM (Matthews, NC, USA), equipped with a modulator of power and an infrared temperature capture. The crude algal extract (50 mg) was mixed with water (50 mL) and poured in a pyrex tube. The latter is raised above by a Dean-Stark system used to condensate the heteroazeotrope mixture essential oil-water. The optimal conditions related to the ratio mass of extract/volume of water, the irradiation power of the magnetron and the irradiation time were previously determined by a kinetic study based on the variation of the yield of oil obtained in function of each of these parameters. These are 56 mg/50 mL, 180W and 10 min, respectively. Under these conditions, the yield of oil on moisture free basis was 12.7 % (w/w).

2.3. Chromatographic analysis

2.3.1. Gas chromatography (GC)

GC analyses were performed on an HP 4890A equipped with a flame ionization detector (FID) and a Chromatography Data Station (CDS) with optional software modules for data acquisition, data processing and instrument control.

Separation was achieved by using a fused-silica capillary column HP-5 MS (30 m x 0.25 mm i.d. and 0.25 μm film thickness). The operating conditions were set as follows: the injector and detector temperatures were 250°C and 280°C respectively, the oven temperature program was 5 min isothermal at 80°C, subsequently at 3 °C/min up to 250°C and finally held at that temperature for 10 min. The oil was dissolved in ethyl acetate (10 % w/w) and a volume of 0.2 μL was injected using the split mode of the split/splitless inlet with a split ratio of 1:10, under a column head pressure of 12 psi and helium as carrier gas at a flow rate of 1 mL/min.

2.3.2. Gas chromatography–mass spectrometry (GC/MS)

The GC–MS apparatus was a gas chromatograph HP-6890 with a split/splitless injector, and an HP-5972 mass-selective detector, equipped with a Varian (Walnut Creek, CA, USA), CP Sil 8 CB capillary column (30m length, 250 μm i.d., 0.25μm film thickness) equivalent column to DB5 and/or

HP5. Analyses were performed in the electron ionization (EI) mode at 70 eV, mass spectra were obtained by automatic scanning of the mass range *m*/*z* 40–550, transfer line and ion source temperatures of 280 and 250 °C were used respectively. Samples were injected (1 µL) with a splitting ratio of 1:90, and the injector temperature was set at 250°C. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. Gas chromatographic conditions were set as seen above.

Most components were identified from their GC retention indices with either of those reported in literature [19-21]. The retention indices were determined in relation to a homologous series of *n*-alkanes (C_7 - C_{30}) under the same operating conditions. The modified Van den Dool and Kratz formula was used for the determination of retention indices [22].

Further identification was made by comparison of their MS spectra with either mass spectra stored in the following mass spectra libraries: Wiley 7N (Data Base DB1 (Wiley, NY, USA)), NBS 75K (DB2 (HP database)) and Mass Finder 3 (DB3 (D.H. Hochmuth, www.massfinder.com)). Additional identification of components is performed by comparing their mass spectra with those reported in literature of the same compounds obtained by organic synthesis or isolated from a natural source. Component relative concentrations were calculated on the basis of GC peaks without using correction factors.

3. Results and discussion

3.1. Comparison of the extraction yield of volatile fractions

Examination of the values of extraction efficiencies of the three extraction methods shows that supercritical fluid extraction (SFE) provides a very high yield (37.5 %) in comparison to the values of yields obtained by hydrodistillation (HD) (12.0 %) and focused microwave-assisted hydrodistillation (FMAHD) (12.7 %). It must be pointed out that the yields for the three techniques were calculated on moisture free basis.

It should be noted that the mechanism of SFE is based on the solubilization of the components in the solvent (supercritical fluid), under the extraction conditions described above. At the used extraction pressure (91 bar), the density of carbon dioxide is relatively high, which leads to a great solubility of the compounds of the volatile fraction: this explains the high value of yield. In the case of HD and FMAHD, the extraction process is the same, but the heating mechanism is different. The process for both methods is based on steam distillation of volatile compounds. It must be pointed out that FMAHD is performed in 10 min, so that HD requires 45 min.

3.2. Chemical composition of the three volatile fractions

The chemical composition and the relative content of the volatile fractions obtained by HD, FMAHD and SFE analyzed by GC-FID-MS are reported in Table 1, as well as the calculated linear Kovats indices, those given by the literature [19-21] and the additional identification method based on the use of databases of mass spectra (DB1, DB2 and DB3).

Table 1. Chemical composition of volatile fractions of *Cystoseira sedoides* obtained by three extraction methods.

PN	**Compounds**	**$KI^a_{(cal)}$**	**$KI^b_{(Lit)}$**	**Relative Content (%)**			**MS Identif,[c]**
				HD	**FMAHD**	**SFE**	
1	3-Hexene-1-ol	850	844	-	1,9	0,8	DB1, DB2
2	Benzaldehyde	962	964	0,1	-	-	DB1, DB2
3	Hexanoic acid	968	967	0,3	1,9	0,7	DB1, DB2
4	(*E*)-3-Hexenoic acid	988	983	0,4	2,6	1,9	DB1, DB2
5	(*E*)-2-Hexeneoic acid	1000	1005	-	-	0,6	DB1, DB2
6	5-Ethyl-2(5H)-Furanone	1005	983	0,2	-	-	DB1, DB2
7	Benzenacetaldehyde	1050	1045	0,4	-	-	DB1, DB2

8	Heptanoic acid	1080	1076	0,2	0,5	0,1	DB1, DB2
9	Undecane	1103	1100	-	0,2	-	DB1, DB2
10	Nonanal	1105	1100	0,1	-	-	DB1, DB2
11	2,6-Dimethylcyclohexanol	1118	1112	0,6	-	-	DB1, DB2
12	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	1155	1145	0,4	-	-	DB2
13	Octanoic acid	1170	1167	0,2	t	t	DB1, DB2
14	3-Ethyl-4-methyl-(1H)-pyrrole-2,5-dione	1245	1238	1,0	-	0,2	DB2
15	Nonanoic acid	1272	1267	t	0,5	t	DB1, DB2
16	Phenylacetic acid	1276	1274	-	-	0,8	DB1, DB2
17	3,3-dimethyl-2,7- Octanedione	1294	1290	0,7	-	-	DB2
18	Tridecane	1300	1300	t	0,4	0,2	DB1, DB2
19	(3E) - Hexenyl tiglate	1315	1315	0,5	-	-	DB2
20	Methylgeranate	1325	1323	0,1	-	-	DB2
21	Unknown A: (M^+ 152) ($C_{10}H_{16}O$)	1330	n,a	0,2	t	t	
22	5-Pentyl-2(5H)furanone	1337	1339	0,1	-	-	DB1, DB2
23	α-Cububene	1346	1345	0,2	0,4	0,1	DB2, DB3
24	Unknown B: (M^+ 204) ($C_{15}H_{24}$)	1355	n,a	0,3	t	t	
25	Decanoic acid	1368	1364	0,2	t	t	DB1, DB2
26	β– Bourbounene	1387	1387	0,2	0,9	0,2	DB2, DB3
27	β– Cubebene	1390	1390	0,8	0,7	t	DB2, DB3
28	Tetradecane	1400	1400	t	0,2	t	DB1, DB2
29	Unknown C: (M^+ 222) ($C_{15}H_{26}O$)	1420	n,a	4,2	6,0	1,5	
30	Peculiaroxide	1425	1416	5,2	11,5	2,8	DB3
31	α –Ionone	1430	1428	0,3	-		DB3
32	Aromadendrene	1440	1439	0,1	0,5	t	DB2, DB3
33	6,10-Dimethyl-5,9-undecadien-2-one	1460	1455	0,1	-	-	DB1, DB2
34	β- Ionone epoxide	1465	1460	1,3	-	-	DB3
35	γ - Gurjunene	1478	1475	-	0,9	0,2	DB2, DB3
36	2,6-Di(t-butyl)-4-hydroxy-4-methyl-2,5-Cyclohexadien-1-one	1480	1478	-	0,5	0,1	DB2
37	epi-Bicyclosesquiphellandrene	1482	1482	0,2	2,5	0,2	DB2, DB3
38	Germacrene D	1485	1484	0,2	t	t	DB2, DB3
39	β -Ionone	1490	1487	3,7	-	-	DB2, DB3
40	1-Pentadecene	1492	1492	1,6	-	-	DB1, DB2
41	Pentadecane	1500	1500	0,3	2,4	0,4	DB1, DB2
42	Epi-Zonarene	1510	1501	-	2,0	-	DB3
43	4,7-Dimethyl-1-tetralone	1515	1509	-	0,4	-	DB2
44	δ –Αμορπηενε	1520	1513	t	t	0,3	DB2, DB3
45	δ – Χαδινενε	1524	1522	0,8	1,2	0,3	DB2, DB3

46	4,4,7α-Trimethyl-5,6,7,7α-tetrahydro2(4H)-benzofuranone	1528	1527	2,0	0,4	0,4	DB1, DB2
47	*cis*-Calamenene	1535	1528	1,2	-	0,3	DB2
48	α - Calacorene	1547	1544	1,3	1,8	0,3	DB2, DB3
49	*trans*- Cadina-1,4-diene	1534	1533	0,8	0,4	t	DB2, DB3
50	Dodecanoic acid	1565	1565	0,8	0,6	0,4	DB1, DB2
51	Gleenol	1580	1585	-	4,2	1,0	DB3
52	Axenol	1590	1586	4,9	-	-	DB3
53	1-Hexadecene	1595	1588	0,2	-	-	DB1, DB2
54	Hexadecane	1600	1600	0,2	t	t	DB1, DB2
55	Allo-aromadendrene epoxide	1630	1639	t	1,0	t	DB2, DB3
56	β-Oplopenone	1610	1607	t	t	0,3	DB3
57	Cubenol	1645	1645	3,6	2,4	0,4	DB3
58	α-Cadinol	1653	1652	1,3	t	t	DB2, DB3
59	α-Eudesmol	1660	1658	t	t	0,3	DB2, DB3
60	8-Heptadecene	1665	1661	1,4	t	t	DB1, DB2
61	Cadalene	1672	1675	0,4	0,4	-	DB2, DB3
62	Tridecanoic acid	1677	1677	0,6	t	t	DB1, DB2
63	1- Heptadecene	1680	1678	0,6	-	0,3	DB1, DB2
64	Germacra-4(15),5,10(14)-trien-1a-ol	1684	1685	0,7	t	t	DB3
65	14-Norcadin-5-en-4-one	1694	1691	0,3	1,5	0,5	DB2
66	Heptadecane	1700	1700	0,1	3,4	1,0	DB1, DB2
67	Pentadecanal	1715	1710	-	0,4	-	DB1, DB2
68	Tetradecanoic acid, methyl ester	1730	1722	-	1,7	0,6	DB1, DB2
69	Tetradecanoic acid	1750	1750	14,6	9,1	8,1	DB1, DB2
70	Tetradecanoic acid, ethyl ester	1790	1795	t	0,4	t	DB1, DB2
71	1,2-Epoxyhexadecane	1810	1798	-	0,2	0,2	DB1, DB2
72	Pentadecanoic acid, methyl ester	1830	1827	-	0,3	0,2	DB1, DB2
73	Hexadecanal	1835	1830	0,5	-	-	DB1, DB2
74	6, 10, 14-Trimethyl 2-Pentadecanone	1855	1850	0,2	1,0	0,5	DB1, DB2
75	Pentadecanoic acid	1860	1851	t	1,2	1,0	DB1, DB2
76	1-Hexadecanol	1878	1874	0,3	-	-	DB1, DB2
77	9-Hexadecenoic acid, methyl ester	1880	1877	-	3,0	1,8	DB1, DB2
78	Pentadecanoic acid, 14-methyl-, methyl ester	1888	1884	-	5,8	3,2	DB1, DB2
79	9-Hexadecenoic acid	1910	1898	9,8	2,9	10,0	DB1, DB2
80	Phytol	1945	1942	0,4	0,4	1,0	DB1, DB2
81	Hexadecanoic acid	1960	1959	19,6	11,0	27,5	DB1, DB2
82	Hexadecanoic acid, ethyl ester	1990	1989	-	1,0	0,7	DB1, DB2
83	Octadecanal	2015	2012	0,9	0,3	0,3	DB1, DB2
84	Heptadecanoic acid	2025	2022	0,2	-	0,4	DB1, DB2

85	9-Octadecenoic acid (Z)-, methyl ester	2080	2086	t	0,6	0,6	DB1, DB2
86	9, 12-Octadecadienoic acid (Z,Z)-, methyl ester	2090	2097	-	-	0,2	DB1, DB2
87	9,12,15-Octadecatrienoic acid, methyl ester	2105	2098	0,2	2,5	0,2	DB1, DB2
88	Octadecanoic acid, methyl ester	2130	2124	-	-	1,7	DB1, DB2
89	8-Heptadecenoic acid	2145	2140	-	-	0.2	DB1, DB2
90	9-Octadecenoic acid (Z)-	2165	2161	4,4	0,9	13,9	DB1, DB2
91	9-Octadecenoic acid, ethyl ester	2180	2175	-	0,6	0,6	DB1, DB2
92	Octadecanoic acid	2210	2200	1,0	-	2,7	DB1, DB2
93	5, 8, 11, 14-Eicosatetraenoic acid, methyl ester	2222	2217	0,3	1,3	0,5	DB1, DB2
94	5,8,11,14,17-Eicosapentaenoic acid, methyl ester	2290	2282	0,5	t	2,4	DB1, DB2
95	5,8,11,14-Eicosatetraenoic acid, ethyl ester	2310	n.a.	t	t	3,4	DB1, DB2

PN: Peak Number, [t] = Trace (percentage value less than 0.05%), n.a.: not available
$KI^{a}_{(cal)}$: Linear retention index relative to C_7-C_{28} n-alkanes on the CP Sil 8 CB column (eq. DB-5 or HP-5).
$KI^{b}_{(Lit)}$: literature retention index reported by Adams [21]. [c] MS-tentatively identified on the basis of computer matching of the mass spectra of peaks with the Wiley 7N (**DB1),** NBS 75K (**DB2)** and Mass Finder 3 (**DB3)** libraries and those reported by Adams [21] and others references [19,20].

The chromatogram profiles given as a supporting information (S1) reveal the presence of 64, 51 and 54 compounds (constituents in trace amounts are not considered) corresponding to 98.5%, 98.8% and 98.5% of total volatile fraction composition obtained by HD, FMAHD and SFE, respectively. Three components have not been identified: **A** (PN 21), **B** (PN 24) and **C** (PN 29). Two of them (A and B) exist in traces in the oils extracted by SFE and FMAHD.

The volatile fraction of *Cystoseira sedoides* is characterized by the presence of six chemical classes (Figure 1). fatty acids and derivatives (esters) (**FA-D**), hydrocarbons and derivatives (alcohol, aldehydes) (**HC-D**), monoterpenes (**Mon**), sesquiterpenes (**Sesq**), diterpenes (**Dit**) and a mixture of other chemical classes (**OCC**). The relative content of each chemical class is given on Figure 1. The most important class is that of fatty acids and derivatives with a content of 53.1 %, 48.5 % and 84.3 % for HD, FMAHD and SFE, respectively. In each case, the major fatty acid was hexadecanoic acid (PN 81) with a percentage of 19.6 %, 11.02 %, 27.5 % for HD, FMAHD and SFE, respectively. Fatty acids, especially unsaturated, are known to be precursors of the biosynthesis of various metabolites in the living organisms [23]. Moreover, it must be pointed out that the vast majority of essential oils of terrestrial species [24], marine macroalgae [25] and microalga [26] contain fatty acids. The elevated content of fatty acids (84.3%) in the oil extracted by SFE can be interpreted by their high solubility in the supercritical fluid at the extraction pressure (91 bar) [27].

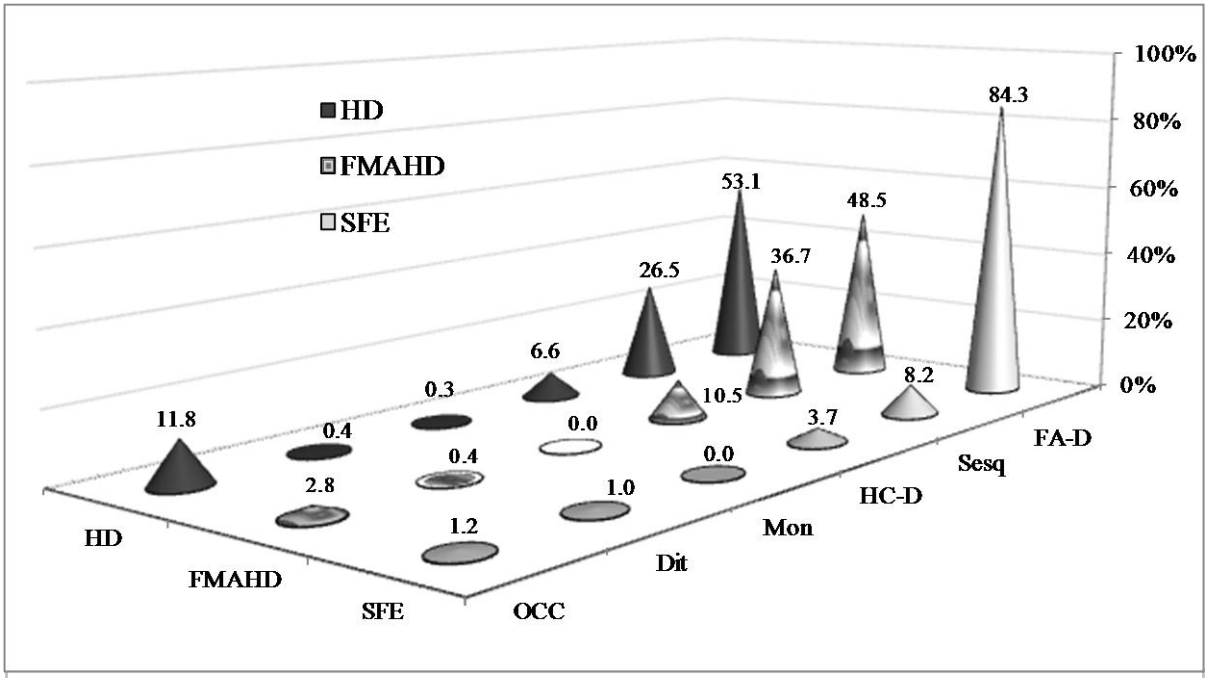
The fatty acids class is followed by that of sesquiterpenes, their content in oils extracted by the three methods are 26.5 %, 36.7 % and 8.2 % for HD, FMAHD and SFE, respectively. With 36.7%, the oil extracted by FMAHD is the one which gave the highest content in sesquiterpenes. This fact can be explained by the high relative volatility of sesquiterpenes compared to the other chemical classes as well as their ability to absorb the microwave energy more easily [25]. The main sesquiterpene in the different oils is peculiaroxide (PN 30) and its highest relative content is obtained in the FMAHD oils with 11.5%. Gleenol (PN51) is also present, but only in the oils extracted by SFE and FMAHD, even though axenol (PN52) is only present in that extracted by HD. Gleenol and axenol are two biologically important axane sesquiterpenes and epimers [28]. The presence of axenol only in the oil obtained by HD is probably the result of a rearrangement reaction (epimerization) of gleenol, due to the high temperatures encountered during the hydrodistillation. Calorific energy provided by hydrodistillation

can be considered as a cause of the rearrangement of natural volatile compounds [29]. The three volatile fractions contain a class of hydrocarbons and derivatives (**HC-D**) as alkanes, alkenes and their alcohol and aldehyde derivatives. The oil extracted by FMAHD is the one containing the higher content in **HC-D** with 10.5%, followed by that of HD and SFE with 6.6 % and 3.7 %, respectively.

The class of diterpenes, only represented by the phytol (PN 80), is present in small amounts in the oils obtained by the three extraction methods. Phytol, known to be the biosynthetic precursor of chlorophyll [30], is present in essential oils [31] and solvent extracts [32]. For the class of monoterpenes, the methylgeranate (PN 20) is the only monoterpene ester identified in the oil extracted by HD. The oils obtained by FAMHD and SFE do not contain a monoterpene compounds.

It must be pointed out that our identification study of oil compounds based on the comparison of their retention indices and mass spectra with those present in commercial mass spectral libraries (DB1, DB2 and DB3) has not allowed the identification of three compounds: **A** (PN 21), **B** (PN 24) and **C** (PN 29). Their spectral data are given as a supporting information (S2). The unknown component A shows a molecular ion peak at *m/z* 152 corresponding to the molecular formula $C_{10}H_{16}O$. Our own investigation revealed a great similarity between the mass spectrum of A and that of β-cyclocitral ($C_{10}H_{16}O$) [21, 33]. The notable difference concerns the base peak which is pointed at *m/z* 55 and at *m/z* 41 for A and β-cyclocitral.

Under our chromatographic analysis conditions, the compounds B (PN24) and C (PN29) are eluted in the range of time corresponding to classical sesquiterpenes (cubebene). B presents a molecular ion peak and a base peak at *m/z* 204 and 131, respectively. According to our literature search, this compound is a sesquiterpene hydrocarbon belonging to nardosinane skeleton [34-35]. The mass spectrum of C shows a molecular ion peak at *m/z* 222 and a base peak at *m/z* 179. Our review of the literature has not allowed us to deduce information on the type of chemical skeleton; **C** seems to be a new oxygenated sesquiterpene.



FA-D: Fatty Acids and Derivatives, Sesq: Sesquiterpenes, HC-D: Hydrocarbons and Derivatives, Mon: Monoterpenes, Dit: Diterpenes, OCC: Other Chemical Classes.

HD: Hydrodistillation, FMAHD: Focused Microwave Assisted HydroDistillation, SFE: Supercritical Fluid Extraction

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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