Characterization of *Lavandula multifida* volatile composition from Morocco by headspace solid-phase Microextraction (HS-SPME) and Hydrodistillation coupled to GC–MS

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The essential oil and volatiles compounds of *Lavandula multifida* L., aerial parts collected in South-East Morocco, obtained by hydrodistillation (HD) and headspace solid phase Microextraction (HS-SPME), were analyzed using Gas Chromatography-Flame Ionization Detector (GC-FID) and GC-Mass Spectrometry (GC-MS). 31 components, accounting 94.3% in the total essential oil, were identified by HD and 21 compounds, representing 90.2% of the headspace, were characterized by HS-SPME. The major components identified are carvacrol (57.9% ; 65.6%), carvacrol methyl ether (7.6% ; 4.6%), p-cymen-8-ol (3.9% ; 4.8%) and spathulenol (3.8% ; 8.6%) of essential oil and volatile compounds detected in HS-SPME, respectively. By comparison of HD and HS-SPME extraction, only quantitative differences of some components can be observed in both aromatic profiles, while qualitatively are rather similar. This study demonstrates that HD and HS-SPME modes could be complimentary extraction techniques in order to obtain the complete characterization of plant volatiles.

Keywords: Headspace sampling; Essential oil; Gas chromatography; Hydrodistillation; *Lavandula multifida*; Solid phase Microextraction

1. Introduction:

Lavandula multifida, species of the genus *Lavandula*, is a small semi-evergreen perennial shrub native of the South-Western Europe and North Africa (Politi *et al.*, 2002). It is mainly distributed in pre-Saharan zones, growing on the rocky outcrops and more or less drained calcareous soils, the borders of rivers of temporary drainage, between 800 in 2000 altitude (Zuzarte *et al.*, 2012). *L. multifida* (Figure 1) occupies an important place in traditional Moroccan medicine. It is effectively used to treat up to 20 diseases, such as stomach problems, bleeding, wound healing, arthritis (Zuzarte *et al.*, 2012). These leaves and the stems are also used to prepare decoctions against rheumatism, to ease cough and treat colds and as a beneficial digestive system (El Hilaly *et al.*, 2003). It is endowed with antimicrobial activity (Ameziane *et al.*, 2007; Zuzarte *et al.*, 2012), antioxidant effects (Ramchoun *et al.*, 2009) and anti-inflammatory properties (Sosa *et al.*, 2005). Many studies of the GC and GC-MS analysis of *L. multifida* essential oils produced from plan growing in the different parts of the Mediterranean contour has been also reported and indicate that carvacrol is the main constituent of this specie (Bellakhdar *et al.*, 1985; Chograni *et al.*, 2007; Chograni *et al.*, 2010; Denier *et al.*, 1985; García-Vallejo *et al.*, 1989; Zuzarte *et al.*, 2012). This phenolic compound is mostly responsible for the aroma and biological activities and is the characteristic constituent of *L. multifida* since this compound does not usually appear as dominant in oils of other *Lavandula* species except the essential oil of *Lavandula canariensis* L. Mill., growing in Australia (Paúl *et al.*, 2004).

Generally, the composition of the essential oils varies with the species and extraction method. Indeed, various methods for the extractions of volatile components have been proposed. Hydrodistillation (HD) is a conventional method used to extract essential oils from aromatic plants; it can be used in industry and has no chemical pollution (Benyelles *et al.*, 2014). However, one of the disadvantages of this method is that essential oils undergo chemical alterations and the heat-sensitive compounds can easily be destroyed. Therefore, the quality of the essential oil extracts is extremely impaired (Illes *et al.*, 2000). Recently, the solid-phase microextraction (SPME) technique has been introduced as an alternative to the conventional technique as a sample preconcentration method prior to chromatographic analysis (Benyelles *et al.*, 2014). There are two typical SPME applications, sampling gases head space (HS) and sampling solutions or direct immersion (DI) (Deng *et al.*, 2005). In the DI-SPME mode, the fibre is inserted into the sample medium and the analytes are transported directly to the extraction phase, while in the HS-SPME mode, the analyte is transported through a layer of gas before reaching the coating. Combined with GC/MS, HS-SPME can be used for the analysis of volatile components of natural products and foods (Delgado *et al.*, 2010). Indeed, the analytes are adsorbed from a solid sample by headspace extraction, using a polymer-coated fused silica fibre. The compounds are then desorbed by exposing the fibre in the injection port of a gas chromatographic apparatus. SPME have diminished decomposition of plant compounds and cells, minimized activity of

enzyme, and decreasing loss of those constituents (Nam-Sun and Dong-Sun, 2002). However, some parameters involved in optimizing SPME as the choice of the appropriate fibre, time and temperature of extraction and desorption and equilibrium time.

In this context, the aim of this work was to compare the chemical composition of *L. multifida* essential oil and the volatile compounds extracted by HD and using HS-SPME, respectively. In both cases, the analysis was carried out using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The parameters of HS-SPME technique were optimised to improve analysis efficiency.

2. Materials and methods:

2.1. Plant material and essential oil isolation

The aerial parts (leaves and flowers) of *L. multifida* was harvested in May 2007 in the wild in south-east of Morocco (Errachidia). Voucher specimens were deposited in the herbarium of Faculty of Sciences and Technology of Errachidia (Morocco). The essential oil used in this study was the same we used in our previous study (Laghchimi *et al.*, 2014). It was prepared by hydrodistillation for 3h using a Clevenger type apparatus, according to the method recommended in the European Pharmacopoeia (European Pharmacopeia, 1997), and analyzed by gas chromatography (GC) and gas chromatography/mass spectroscopy (GC-MS). The essential oil yield was approx 1.20% (Laghchimi *et al.*, 2014).

2.2. Volatile compounds by HS-SPME

The dried and pulverized aerial parts of *L. multifida* were subjected directly to HS-SPME. The SPME device (Supelco) coated with divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 2cm-50/30 µm) was used for extraction of the plant volatiles. Optimization of conditions was carried out using fresh aerial parts of the plant (700 mg in a 20 ml vial) and based on the sum of total peak areas measured on GC-FID. The temperature and the equilibration time were selected, respectively, after three different experiments at 50, 70 and 90°C, and after three different experiments at 60, 90 and 120 min. The extraction time was selected after three different experiments at 15, 30 and 60 min. After sampling, SPME fibre was inserted into the GC and GC-MS injection ports for desorption of volatile components (5 min), both using the splitless injection mode. Before sampling, each fibre was reconditioned for 5 min in the GC injection port at 260°C. HS-SPME and subsequent analyses were performed in triplicate. The coefficient of variation (9.6% < CV < 13.4%) calculated on the basis of total area obtained from the FID-signal for the samples indicated that the HS-SPME method produced reliable results. In the same way, the CV of the major compounds was always less than 15%.

2.3. GC analysis

GC analyses were carried out using a Perkin-Elmer Autosystem (Waltham, MA, USA) XL GC apparatus equipped with dual flame ionization detectors (FID) and fused-silica capillary columns (60 m x 0.22 mm i.d.; film thickness 0.25 µm) coated with Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethyleneglycol). The oven temperature was programmed from 60 to 230°C at 2 °C/min and the held at 230°C for 35 min. Injector and detector temperatures were maintained at 280°C. Samples were injected in the split mode (1/50) using helium as carrier gas (1 ml/min); the injection volume of pure oil was 0.1 µl. For HS-SPME-GC analysis, only Rtx-1 (polydimethylsiloxane) column was used and volatile components were desorbed in a GC injector with a SPME inlet liner (0.75 mm. I.D., Supelco).

2.4. GC-MS analysis

Samples were analysed with a Perkin-Elmer Turbo mass detector (quadrupole), coupled to Perkin-Elmer Autosystem XL chromatograph equipped with Rtx-1 and Rtx-wax fused-silica capillary columns. The carrier gas was helium (1 ml/min), the ion source temperature was 150°C, the oven temperature was programmed from 60 to 230°C at 2°C/min and the held at 230°C for 35 min, the injector was operated in the split (1/80) mode at a temperature of 280°C, the injection volume was 0.2 µl of pure oil, the ionization energy was 70 eV, EI (Electron Impact)-MS were acquired over the mass range 35-350 Da. The volatile fractions sampling by HS-SPME were analyzed only on a Rtx-1 capillary column and volatile components were desorbed in a GC injector with a SPME inlet liner (0.75 mm. I.D., Supelco).

2.5. Components identification

Identification of the components was based: (i) on the comparison of their GC retention indices (RI) on non polar and polar columns, determined relative to the retention time of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data (Joulain and König, 1998) and (ii) on computer matching with commercial mass spectral libraries (Hochmuth *et al.*, 2001) and comparison of spectra with those of our personal library. Relative amounts of individual components were calculated on the basis of their GC peak areas on the two capillary Rtx-1 and Rtx-Wax columns, without FID response factor correction.

3. Results and discussion

The chemical analysis results obtained by both HD and HS-SPME methods are given in Table 1.

3.1. Essential oil analysis

GC carried out analysis of the aerial parts essential oil from *L. multifida* and GC–MS using the methodologies described in the section 2. A total of twenty nine components, representing 93.34% of the total oil content, were identified by comparison of their electron ionization-mass spectra (EI-MS) and their retention indices (RI) with those of our own authentic compound library (Table 1 and Figure 2). Among them, eleven monoterpene hydrocarbons (**4**, **7**, **9-12**, **14**, **16-19**), nine oxygenated monoterpenes (**13**, **15**, **20-22**, **24-28**), two sesquiterpene hydrocarbons (**29**, **30**), three oxygenated sesquiterpenes (**35-37**). Furthermore, three compounds absent from reference libraries (**23**, **38**, **39**) were also detected.

The essential oil was characterized by a large amount of monoterpenic fraction with 81.4% of the total oil, which the oxygenated monoterpenes account 72.9% and hydrocarbon monoterpenes represent only 8.2%. Phenolic compounds had the highest contribution to this fraction comprising 69.6% of the total oil composition with carvacrol **27** being the main component detected (57.9%), followed by carvacrol methyl ether (2-methoxy-p-cymene) **26** (7.6%), p-cymen-8-ol **24** (3.9%) and eugenol **28** (0.2%). However, the content of sesquiterpenic fraction did not exceed 7.6% of the total oil content mostly attributed to oxygenated sesquiterpenes with a percentage of 6.6%. Spathulenol (tricyclic sesquiterpenoid) **35** was the main constituent of this fraction accounting for 3.8% of the total oil content (Figure 3). It should be noted that this essential oil was characterized by the presence of three oxygenated derivatives of octane: **5**, **6** and **8** (1.9%) and three compounds, absent in reference libraries (**23**, **38**, **39**), with a percentage 3.7% of the total oil. These results were in accordance with those previously reported in the literature referring carvacrol as the major component of essential oil of *L. multifida* growing wild in the Mediterranean region (Portugal, Spain, Morocco, and Tunisia) (Chograni *et al.*, 2007; Chograni *et al.*, 2010 ; Lopez-Reyes *et al.*, 2010 ; Tataoui-Elaraki *et al.*, 1993 ; Zuzarte *et al.*, 2012). However, it appears that our sample differs significantly by the presence of spathulenol and carvacrol methyl ether with appreciable amounts and the total absence of β-bisabolene.

3.2. Volatile compounds by HS-SPME

The optimization of the HS-SPME sampling parameters was carried out using the aerial parts of *L multifida* and was based on the sum of the total peak areas obtained by GC-FID. The maximum sum of total peak area was obtained at a temperature of 70 °C, an equilibrium time of 60 min, and an extraction time of 30 min. The sum of total peak area increased according to the increase in the temperature until 70°C. These results were in accordance with those recently reported for the volatile components of *Salvia aucheri mesatlantica* (Znini *et al.*, 2014).

The volatile compounds of the aerial parts of the same plant were analyzed using HS-SPME with optimized parameters (Table 2 and Figure 4). The GC and GC-MS analysis allowed the identification of 21 components, representing more than 90% of the volatile composition. They are grouped into three hydrocarbon monoterpenes (**4**, **12**, **18**), five oxygenated monoterpenes (**24-28**), five hydrocarbon sesquiterpenes (**29**, **31-34**), two oxygenated sesquiterpenes (**35**, **36**), five non-terpenic compounds (**1-3**, **5**, **8**) and a phenol derivative compound (**13**). The volatile fraction obtained was characterized by large amount of oxygenated compounds which amounted for 85.5% and the amount of hydrocarbons compounds was drastically reduced (4.8%).

Phenolic compounds are the main family of oxygenated compounds with a percentage of 75.6%, which carvacrol (**27**) is the major constituent (65.6%), followed by carvacrol methyl ether **26** (4.6%), p-cymen-8-ol **24** (4.8%) and eugenol **28** (0.6) while the spathulénol **35** was present with a content of 8.6%. Among the main hydrocarbon compounds, we noted, in particular, p-cymene (**12**) (precursor of carvacrol) and p-cymenene (**18**) which fully represents 2.6% (Figure 4). To our knowledge, this study is the first study on the analysis of the volatile fraction of the species *L. multifida* by HS- SPME.

3.3. Comparison of two methods

For comparison purposes, differences found in volatile compounds of plants isolated with HS-SPME and conventional methods such as HD are reported in the literature. Sometimes HS-SPME provides a larger scope of compounds (Rohloff, 2002; Liang *et al.*, 2005). In other cases, HS-SPME detects a lesser amount of compounds (Paúl *et al.*, 2004) and in some other cases differences found are quantitative but not qualitative (Koedam, 1987). In fact, quantitative but not qualitative differences have been found in the chemical composition of both analysed samples. The current investigation revealed that higher amounts of oxygenated compounds are found in the HS-SPME (85.5%) as compared to the oil obtained by HD (81.4%) while, hydrocarbons compounds were detected in lower concentrations in the HS-SPME as compared to the hydro-distilled oil (4.8%, 9.2%, respectively). Carvacrol was the principal component of this species in the HD and HS-SPME extracts with 57.9 and 65.6% respectively. It should be noted that among the 31 compounds previously detected in the essential oil, only 13 of them were identified in the HS-SPME. Conversely, 8 components (**1-3,13, 31-34)** detected in HS-SPME were absent in essential oil (Figure 5).

To simplify the analysis, the comparison was limited to major components (the content is greater than 1%) identified for the two different methods and five compounds that are presented in Figure 6. For instance, the maximum relative amounts of some oxygenated compound were isolated by HS-SPME such as carvacrol (65.6% vs 57.9% for HD), *p*-cymen-8-ol (4.8% vs 3.9% for HD) and spathulenol (8.6% vs 3.8% for HD). However, the carvacrol methyl ether was isolated by HD with a large amount

7.6% (4.6% for HS-SPME). This may result from the thermal decomposition of carvacrol, which justifies the reduction of its content in the essential oil.

In general, it was difficult to establish a direct correlation between the chemical compositions of HD and HS-SPME techniques since the first technique is based on the liquid quasi-total extraction of plant volatiles and the latter technique is controlled by a solid/gas equilibrium step and a competition between interfering molecules at binding sites on the fibre. However, the presence and / or absence of certain compounds in the two samples can be explained also by the influence of the temperature and the extraction time (Table 2). Indeed, during HD (180 min) at 100°C, the most volatile compounds and water-soluble compounds are lost in the gaseous phase and in the hydrolate under the effect of heat and acid pH, respectively, whereas compounds with more high-molecular-mass and low volatility compounds could be extracted by use of HD. In addition, the presence of three unknown compounds (**23**, **38**, **39**) only in essential oil, as artifact products, can be explained by the chemical transformations that may undergo some compounds such as oxidation, hydrolysis or thermal decomposition (Schossler *et al.*, 2009). This type of transformation has been observed in other plant species containing sensitive compounds at elevated temperatures (Liang *et al.*, 2005). However, with HS-SPME extraction at 70°C for 30 min, it is the fiber affinity of each compound that monitors the sampling of the volatiles limiting or favoring their extraction. It is apparent that amounts of the low-boiling and high volatility compounds could be extracted by HS-SPME.

In the same way, it is probable that the amount of plant material used for sample preparation might be one of the major reasons which explain the difference of chemical HS-SPME and HD data. Indeed, the amount of plant material used for the HS-SPME analysis was smaller (0.7 g), while the production of hydrodistilled essential oil needed the use of 100 g of plant material. HS-SPME analysis allowed a qualitative estimate of volatile compounds using a small quantity of material (Paolini *et al.*, 2008).

4. Conclusions:

The present study is the first report which describes the comparison of volatile compounds of the aerial partof *Lavandula multifida* from Morocco obtained by HS-SPME method. Only quantitative differences of some components can be observed in both aromatic profiles, while qualitatively both aromatic mixtures are rather similar. Concerning the plant chemistry, we conclude that this species was mainly dominated by carvacrol with a content of 57.9 and 65.6% obtained by Hydrodistillation and HS-SPME, respectively. Finally, this study has demonstrated that HS-SPME extraction can be considered as an alternative or a complimentary technique for isolating volatiles from aromatic plants.

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Table 1. Chemical composition of aerial parts essential oil from *L. multifida* from Morocco.

N° [a]	Components	RI l [b]	RI a [c]	RI p [d]	% HD[e]	% SPME[e]
1	Hexanal	770	770	-	-	0.1
2	E-2-Hexenal	830	830	-	-	0.1
3	Heptanal	876	876	-	-	0.1
4	α-Pinene	936	930	1007	0.7	0.5
5	Oct-1-en-3-ol	962	958	1398	0.8	0.3
6	Octan-3-one	969	961	1219	0.6	-
7	Myrcene	987	977	1132	0.8	-
8	3-Octanol	981	977	1344	0.5	0.2
9	α-Phellandrene	1002	992	1137	0.1	-
10	2-Carene	1000	1001	1123	0.6	-
11	3-Carene	1010	1004	1150	0.1	-
12	*para*-Cymene	1015	1007	1230	1	1
13	*para*-Crésol	1062	1064	-	-	0.2
14	Limonene	1025	1015	1169	0.7	-
15	1,8-Cineol	1024	1015	1183	0.3	-
16	(*Z*)-b-Ocimene	1029	1019	1201	1.1	-
17	(*E*)-b-Ocimene	1041	1029	1214	0.2	-
18	*para*-Cymenene	1075	1065	1380	0.3	1.6
19	Terpinolene	1082	1071	1242	2.6	-
20	Linalool	1086	1074	1496	0.3	-
21	α-Thujone	1089	1078	1372	0.5	-
22	Camphor	1123	1113	1460	1.7	-
23	NI 1	-	1119	1415	0.5	-
24	***para*-Cymen-8-ol**	1169	1153	1790	**3.9**	**4.8**
25	α-Terpineol	1176	1165	1645	0.5	0.2
26	**Carvacrol methyl ether**	1226	1221	1549	**7.6**	**4.6**
27	**Carvacrol**	1278	1283	2136	**57.9**	**65.6**
28	Eugenol	1331	1327	2105	0.2	0.6
29	(*E*)-b-Caryophyllene	1421	1414	1551	0.9	0.5
30	α-Sesquisabinene	1435	1434	1600	0.1	-
31	Aromadedrène	1443	1447	-	-	0.6
32	γ-murolène	1474	1471	-	-	0.3
33	Calamenène	1517	1512		-	0.2
34	d-Cadinène	1520	1516		-	0.1
35	**Spathulenol**	1572	1557	2059	**3.8**	**8.6**
36	Caryophyllene oxyde	1578	1571	1920	2.5	0.1
37	β-Eudesmol	1641	1655	2156	0.3	-
38	NI 2	-	2043	2594	2.4	-
39	NI 3	-	2346	2981	0.8	-

	Total	**94.3**	**90.3**
	Monoterpene Hydrocarbons	8.2	3.1
	Oxygenated monoterpenes	72.9	75.8
	Sesquiterpene Hydrocarbons	1.0	1.7
	Oxygenated sesquiterpenes	6.6	8.7
	Oxygenated non-terpenic compounds	1.9	0.8
	Phenol derivative (**13**)	0.0	0.2
	Unidentified compounds (**23**, **38**, **39**)	3.7	0.0

[a]Order of elution are given on a polar column (Rtx-1);
[b] **RI *l*** = Retention indices on the apolar column (Rtx-1) in literature;
[c] **RI *a*** = Retention indices on the apolar column (Rtx-1) ;
[d] **RI *p*** = Rtention indices on the polar column (Rtx-Wax);
[e] Relative percentages of components (%) are calculated on GC peak areas on the apolar column (Rtx-1) except for components with identical RI a (concentration are given on the polar column).

Table 2. Comparison of HS-SPME and HD for separation of the volatile components of *L. multifida.*

Characteristic	**HS-SPME**	**HD**
Amount of sample required (g)	0.7	100
Extraction time (min)	30	180
Extraction temperature (°C)	70	~ 100
Separation time by GC-MS (min)	5	35
Major compound identified	Carvacrol (65.60%)	Carvacrol (57.90%)
Total number of components identified	**21** (90.30%)	**29** (94.30%)

Figure 1. *Lavandula multifida* in its native habitat in south-eastern of Morocco.

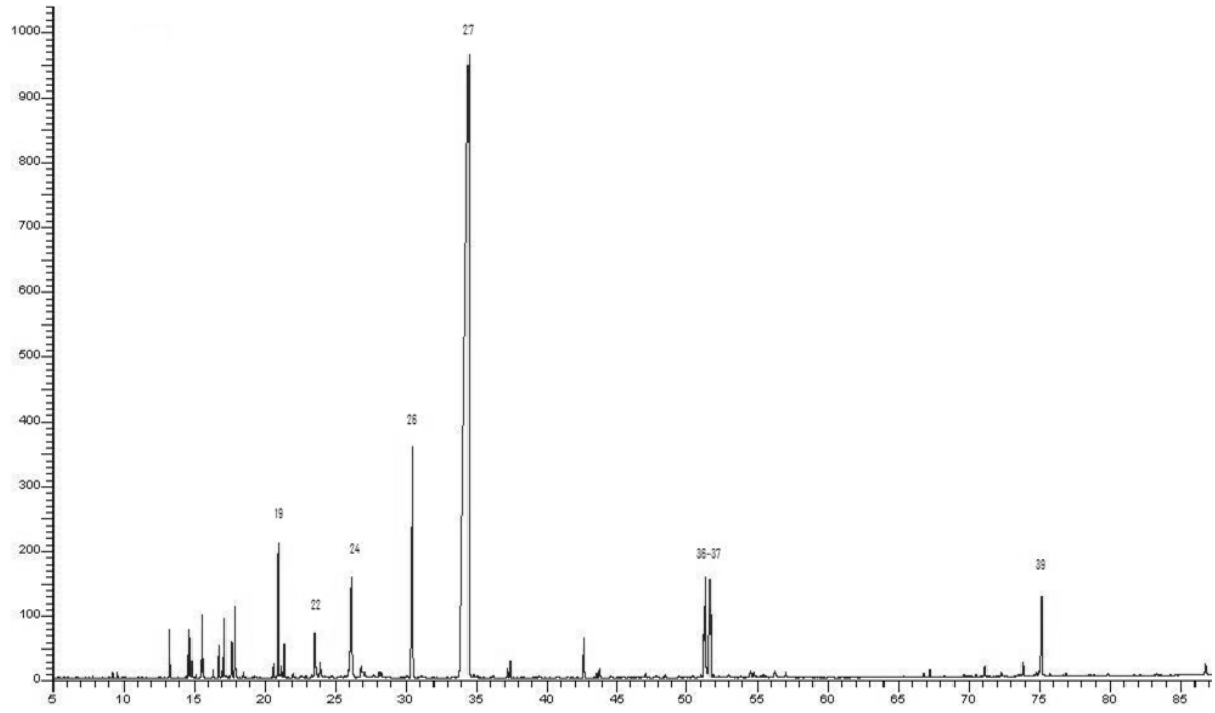

Figure 2. Chromatographic profile of the aerial parts essential oil from *L. multifida* obtained by HD. The separation was carried out on a polar column (Rtx-1).

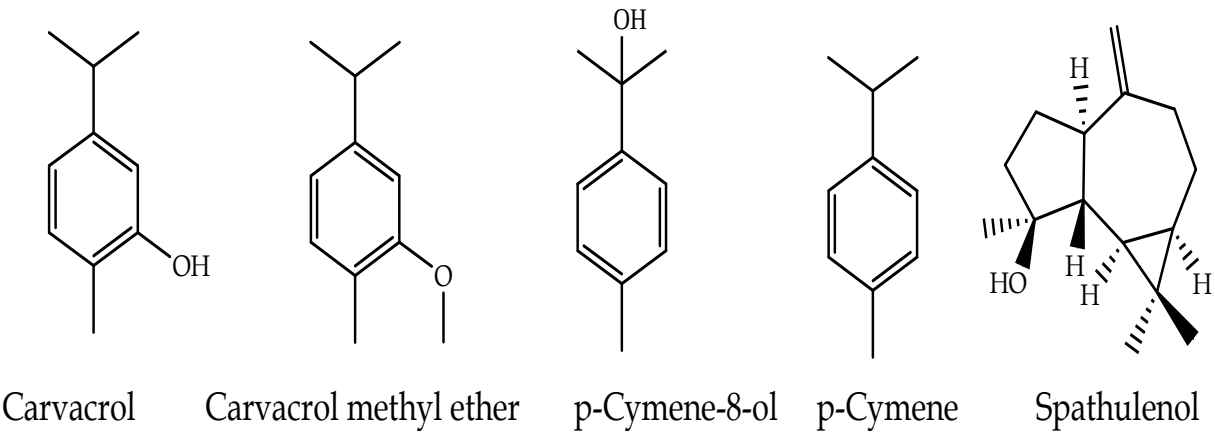

Figure 3. Major constituents detected in the hydro-distilled oil from aerial parts of *L. multifida.*

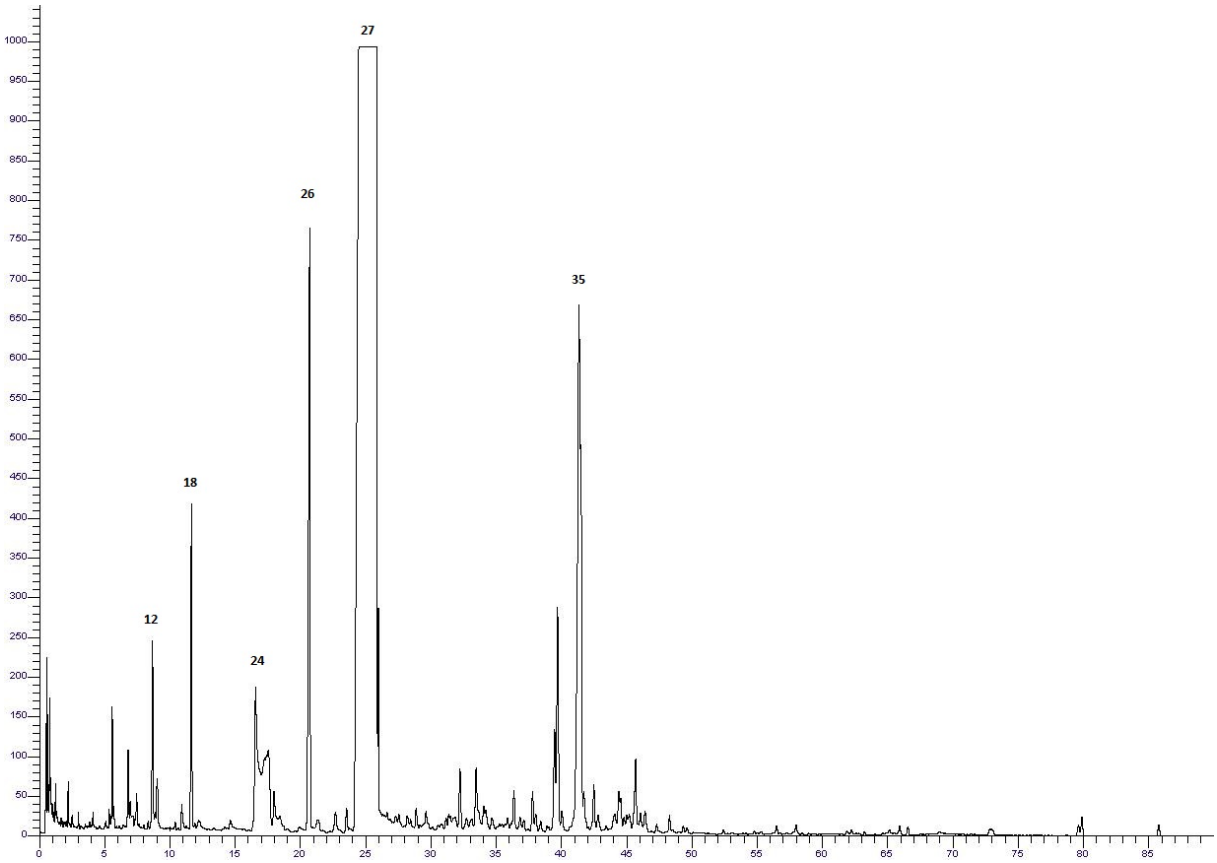

Figure 4. Chromatographic profile of volatile fraction from aerial parts of *L. multifida* detected by HS-SPME. The separation was carried out on apolar column (Rtx-1).

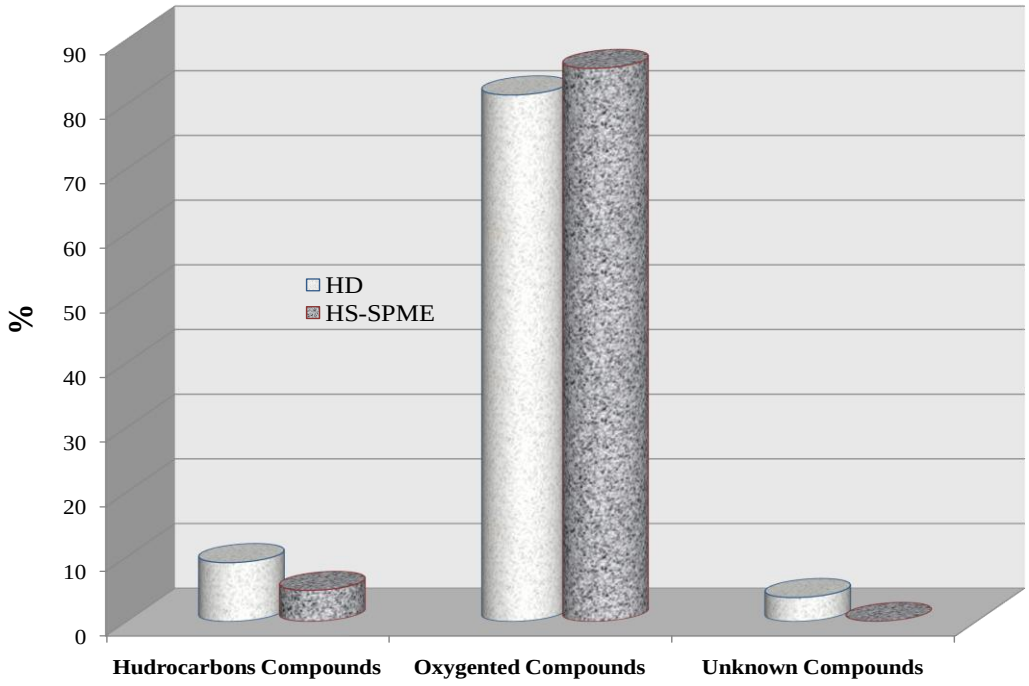

Figure 5. Class of extracted compounds by HD and HS-SPME methods.

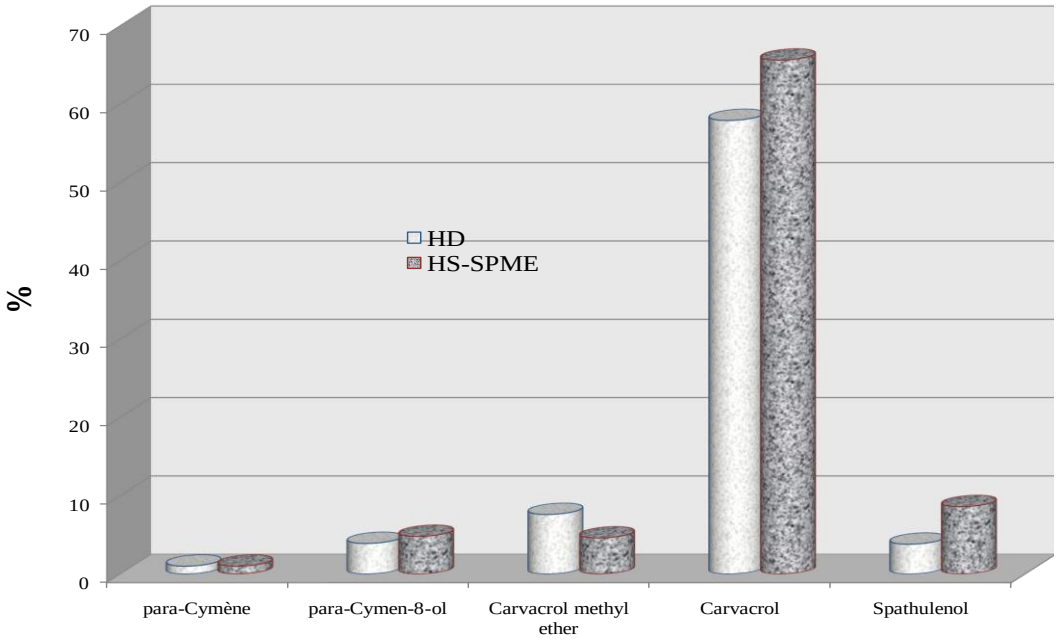

Figure 6. Comparison of the main volatile components (percentages above 1%) of the *L. multifida* obtained by HD and HS-SPME.

Conflict of interest

The authors declare that they have no conflict of interest.

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