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Comparative chemical analysis of volatile compounds of *Warionia saharea* leaves using hydrodistillation and headspace solid-phase microextraction (HS-SPME)

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Abstract

The objective of this study is to report the comparative chemical analysis of essential oil (EO) obtained by hydrodistillation (HD) and volatile fraction (VF) detected by headspace solid-phase micro-extraction (HS–SPME) isolated from *Warionia saharea* leaves using Gas Chromatography–Retention Indices (GC-RI) and GC–Mass Spectrometry (GC-MS). 39 volatile compounds identified in hydrodistilled essential oil (HD), representing 92.7% of the total oil, while HS-SPME revealed 25 components constituting 94.1% of the volatile material. The chemical composition of the HS-SPME and HD extract comprised mainly of phenylpropanoids (36.9%) and oxygenated sesquiterpenes (52.8%), respectively. The comparative analysis of two chemoprofiles obtained by two methods shows both qualitative as well as quantitative differences. The current study is the first report involving rapid analysis of volatile components of W. saharea by HS-SPME.

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Keywords: *Warionia saharea*, Essential oil, Volatile fraction, Hydrodistillation, Headspace solid-phase micro-extraction.

1.Introduction

Chemically, volatile constituents produced by aromatic plants are very complex natural mixtures. They can contain up to more than 100 components at quite different concentrations [1]. They are involved in communication and interaction like plant–plant interaction and plant-animal interactions. Indeed, they may attract pollinating insects to promote the dispersal of pollen and seeds, as well can intervene in the protection of plants against pathogens and pests [2,3]. Several analytical methods combined with GC or GC-MS have been employed for the extraction the volatile constituents from plant matrix, including hydrodistillation (HD), soxhlet extraction, simultaneous distillation extraction (SDE), supercritical fluid extraction (SFE) and headspace techniques such solid-phase microextraction (SPME) [4]. Among these methods, Hydrodistillation (HD) is a conventional method used to extract EOs, because it can be easily implemented in industry and has no chemical pollution. However, it has certain disadvantages, particularly, the consumption of energy and time, the deterioration of heat-sensitive compounds and the alteration of essential oil quality [5]. In order to overcome these problems, developing an alternative rapid, sensitive, safe, and energy-conserving extraction technique is highly desirable. Thereby, headspace solid-phase microextraction (HS-SPME) is an easy, fast and modern sample preparation technique, to characterize the volatile fraction (VF) of aromatic and medicinal plants [6]. The analytes are adsorbed from a solid sample by headspace extraction, using a polymer-coated fused silica fiber. The compounds are then desorbed by exposing the fiber in the injection port of a gas chromatographic apparatus (Figure1).

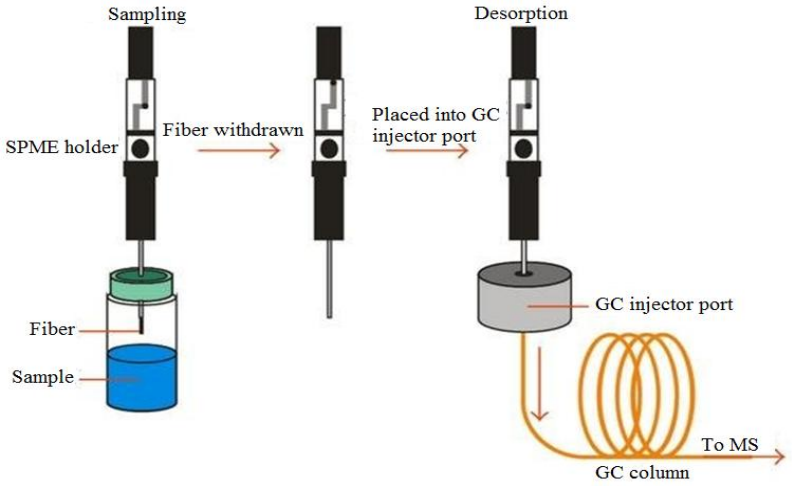


Figure 1. Diagram of analysis with HS-SPME-GC-MS.

Warionia saharae is a somewhat thistle-like aromatic plant of 1 to 3 m of height, with white latex and fleshy, pinnately-partite leaves [7]. The thick trunk is covered of a gray peel, structural of very wavy terminal leaf bouquets and of capitulate of yellow flowers. The flowering season has been recorded from April to June, while it may extend to July or August if the spring rains are abundant and well-spaced [8]. In Morocco, *W. saharae* grows on slopes of the High Atlas, the Anti-Atlas and Saharian Atlas on the coast of western Morocco and desert areas on basic and siliceous rocks from 0 to 1300 m. This plant, known locally by the vernacular name of "afessas", is considered to have medicinal properties [9]. A decoction of dried leaves is used as antirheumatic, for gastrointestinal disorders and against epileptic crisis [10]. Crude extracts of the plants showed cytotoxic activities against a cancer cell line (KB cells) [11] and its essential oils possessed antibacterial properties [12], antioxidant effects [13] and antifungal activities [14]. Many studies on the essential oil of *W. saharae* obtained by hydrodistillation of different organs of the plant have been conducted using GC and GC-MS analysis [12-16]. However, there is no previous report on the chemical composition of *W. saharae* leaves volatile fraction (VF) extracted by HS-SPME. Therefore, this study aimed to

characterize and to compare the *W. saharae* leaves volatile constituents obtained by HD and HS-SPME extraction techniques by using a combination of GC and GC-MS.

2. EXPERIMENTAL

2.1. Plant material and EO isolation

The leaves of *W. saharae* were harvested in March 2013 from Errachidia (south-eastern Morocco). Identification of the species was confirmed by biology unity and voucher specimens were deposited in the herbarium of Faculty of Sciences and Technology of Errachidia. The dried vegetal material (100 g) was water-distillated (3 h) using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [17]. The essential oil obtained was dried under anhydrous sodium sulfate and stored at 4 °C in the dark before analysis. The average yield of essential oil was about 1.1%

2.2. Volatile compounds by HS-SPME

The dried and pulverized leaves of *W. saharae* were subjected directly to HS-SPME. The SPME fiber (Supelco) coated with divinylbenzene/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 (3.5 g 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 fiber 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 fiber 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 based on the 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-RI and GC-MS analysis

The analysis and identification of volatiles compounds of *W. saharae* leaves were carried out using the methodology reported previously in our works [5,18].

3. RESULTS AND DISCUSSION

3.1. Essential oil analysis

The essential oil of *W. saharae* leaves was extracted by hydrodistillation appearing as blue-green color viscous liquid with a percentage yield of 1.1% (v/w). The analysis of this oil was carried out by GC-RI and GC-MS, and a total of thirty-nine components, representing 92.7% 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 authentic compound library (Table 1 and Figure 2).

Table 1. Chemical composition of EO and VF from *W. saharae* leaves.

N°[a]	Components	RI *a*[b]	RI *p*[c]	%[d] HD	%[e] SPME
1	*α*-Thujene	921	1025	0.1	-
2	*α*-Pinene	928	1025	0.3	-
3	Camphene	941	1060	0.5	-
4	Sabinene	961	1114	0.2	-
5	*β*-Pinene	966	1104	0.1	-
6	Myrcene	975	1153	0.2	-
7	*α*-Terpinene	1005	1144	0.2	-
8	*para*-Cymene	1007	1250	0.6	0.5
9	1,8-Cineol*	1016	1176	2.7	0.3
10	Limonene*	1016	1186	0.3	-
11	*(E)-β*-Ocimene	1031	1195	0.1	-
12	γ-Terpinene	1044	1207	0.4	-
13	*(E)*-hydrate Sabinene	1048	1232	0.1	-
14	Linalol oxyde THF E	1052	1422	0.1	-
15	Fenchone	1071	1401	-	0.5
18	Terpinolene	1074	1262	0.2	
17	**Linalool**	**1083**	**1504**	**15**	**1.8**
18	*(Z)-para*-menth-2-en-1-ol	1104	1542	0.2	-
19	Camphor	1118	1463	5.3	-
20	Terpinen-4-ol	1159	1553	1.8	-
21	*α*-Terpineol	1171	1647	3.0	-
22	**Estragol**	**1176**	**1671**	**-**	**10.4**
23	Dihydrocarvone	1180	1626	-	0.8
24	**p-Propylanisol**	**1184**	**-**	**-**	**13.6**
25	Nerol	1208	1747	0.7	-
26	**Pulegone**	**1210**	**1676**	**0.7**	**13.2**
27	Carvotanacetone	1218	1660	0.5	-
28	*(Z)*-Anethol	1228	-	-	0.7
29	Geraniol	1234	1794	2.8	-
30	***(E)*-Anethol**	**1260**	**1828**	**0.3**	**12.2**
31	**Thymol**	**1266**	**2189**	**-**	**10.6**
32	**2-Undecanone**	**1273**	**1592**	**-**	**10**
33	Carvacrol	1279	2135	1.4	2.1
34	2-Undecanol	1289	1717	-	0.7
35	Peperitenone	1315	1909	-	3.4
36	*α*-Terpinyl acetate	1330	1686	0.3	-
37	*(E)-β*-Damascenone	1360	1755	0.3	-
38	α-Copaene	1379	1488	-	0.9
39	2-Dodecanone	1385	1711	-	0.3
40	*(E)*-Caryophyllene	1414	1570	0.1	1.2
41	Geranyl acetone	1428	1820	0.2	-
42	(E)-α-Bergamotene	1432	1580	-	0.6
43	α-Curcumene	1471	1769	-	2.0
44	α-Muurolene	1496	1719	-	0.4
45	α-Bisabolene	1500	1720	-	0.5
46	*δ*-Cadinene	1513	1704	0.1	-
47	β-Sesquiphellandrene	1516	1765	-	0.9
48	Elemol	1533	2019	0.4	-
49	***(E)*-Nerolidol**	**1552**	**1990**	**15.6**	**1.1**
50	Agarospirol	1614	2143	1.1	-

51	γ-Eudesmol	1617	2101	0.9	-
52	**β-Eudesmol**	**1642**	**2199**	**33.9**	**5.4**
53	*α*-Eudesmol	1646	2188	0.9	-
54	2-Pentadecanone	1679	1974	0.5	-
55	2-Heptadecanone	1881	2179	0.6	-
Total				**92.7**	**94.1**

[a] The numbering refers to elution order on apolar column (Rtx-1).

[b] RIa = retention indices measured on the apolar column (Rtx-1).

[c] RIp = retention indices measured on the polar column (Rtx-Wax).

[d] % = relative percentages of components are given on the apolar column except for components with an asterisk (*) (percentages are given on the polar column).

[e]% = relative percentages of components obtained by GC-FID (on RTX-1: apolar column) with peak-area normalization under optimized HS-SPME parameters: temperature: 70°C; equilibrium time: 90 min; extraction time: 30 min.

The EO was characterized by a large amount of sesquiterpenic fraction with 53.0% of the total oil, which the oxygenated sesquiterpenes (6 compounds) account 52.8% and hydrocarbon sesquiterpenes (2 compounds) were scarcely represented (0.2%). This fraction was highly dominated by β-eudesmol **52** and nerolidol E **49** accounting for 33.9 and 15.6%, respectively. However, the content of monoterpenic fraction represent 30.3% of the total oil content, mostly attributed to oxygenated monoterpenes (13 compounds) with a percentage of 27.8% and monoterpene hydrocarbons (12 compounds) represent only 2.5%. This fraction was characterized by a large amount of Linalool **17** with 15.0% of the total oil. Also, it should be noted that this essential oil was characterized by the presence of one phenylpropanoid **30** (0.3%) and five non-terpenic oxygenated compounds: **36**, **37**, **41**, **54** and **55** with a percentage 1.9% of the total oil. The chemical structures of these three major compounds are presented in Figure 3.

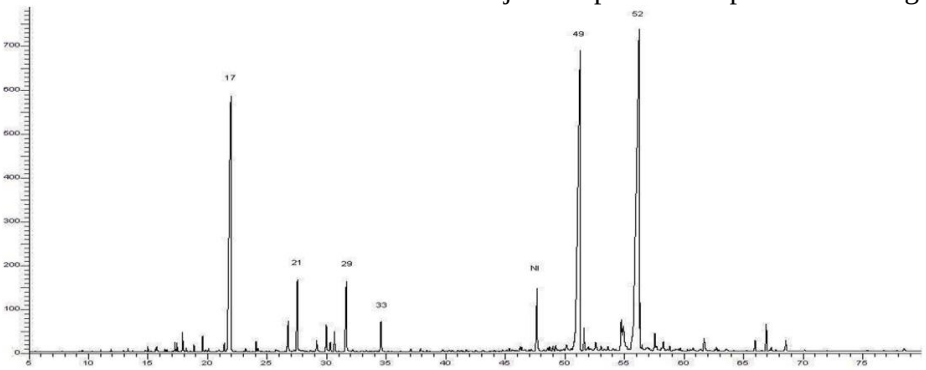
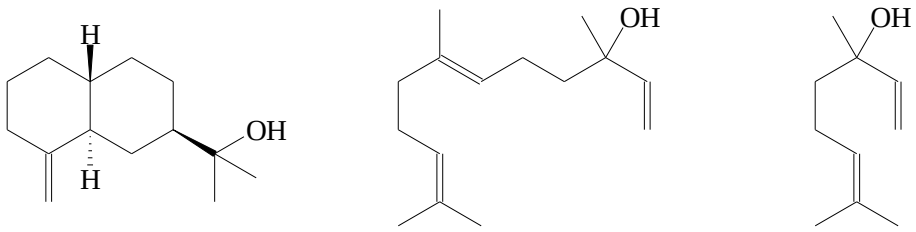


Figure 2. Chromatographic profile of the leaves essential oil from *W. saharea* obtained by HD. The separation was carried out on a polar column (Rtx-1).

These results agreed with the findings of Amezouar et al. who found that β-Eudesmol (38,12%) and Nerolidol (25,95%) were the major components of essential oil leaves of *W. saharae*, collected from Tata region, but not Linalool, which is absent in this oil [13]. Mezhoud et al. reported that the Linalool (27.7%), β-Eudesmol (25.7%) and (E)-Nerolidol were the main constituents of leaves oil of *W. saharae* collected from Bechar in the Southwest of Algeria [16]. Furthermore, previous studies reported that the essential oils of flowers and aerial parts from *W. saharae* were dominated by β-Eudesmol, (E)-Nerolidol and Linalool [12-16].



β-Eudesmol (E)-Nerolidol Linalol

Figure 3. Chemical molecular structure of three major constituents of *W. saharea* essential oil.

3.2. Volatile compounds by HS-SPME

The optimization of the HS-SPME sampling parameters was carried out using the leaves of *W. saharea* and was based on the sum of the total peak areas obtained by GC-FID. the maximum sum of the 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 the total peak area increased according to the increase in the temperature until 70 °C. The GC-RI and GC-MS analysis allowed the identification of twenty-five components, representing 94.1% of the total VF composition (Table 1 and Figure 4).

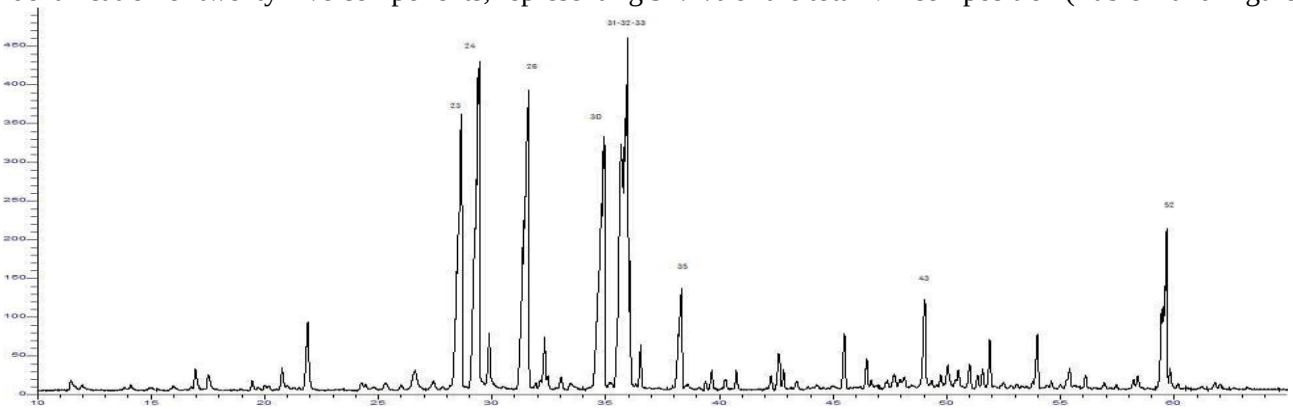


Figure 4. Chromatographic profile of volatile fraction from leaves of *W. saharea* detected by HS-SPME. The separation was carried out on apolar column (Rtx-1).

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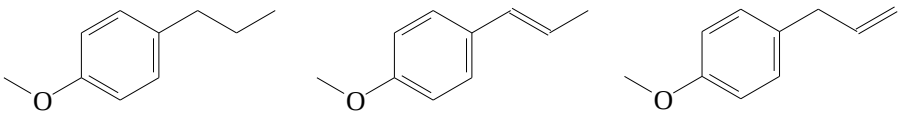
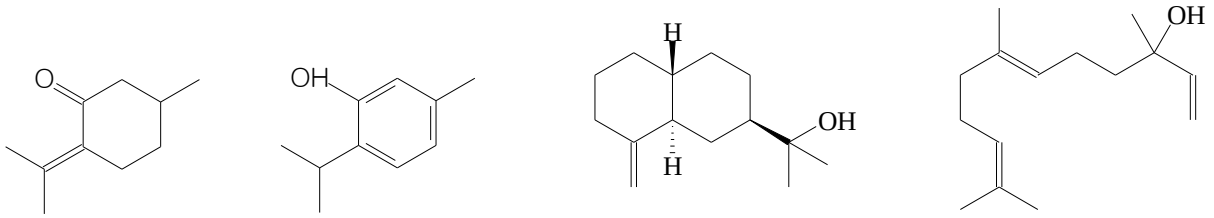


para-Propylanisol (E)-Anethol Estragol



Pulegone Thymol β-Eudesmol (E)-Nerolidol

Figure 5. Chemical molecular structure of the major constituents of *W. saharea* VF.

The chemical composition of the VF was strongly dominated by oxygenated compounds representing 80.6% of the total VF composition. They are grouped into four phenylpropanoids (22, 24, 28 and 30), eight oxygenated

monoterpenes (9, 15, 17, 23, 26, 31, 33 and 35), two oxygenated sesquiterpenes (49 and 52) and three non-terpenic oxygenated compounds (32, 34 and 39). However, the hydrocarbon fraction appeared in a small proportion (7%), comprising one monoterpene hydrocarbon (8) and six sesquiterpenes hydrocarbons (40, 42-45 and 47). Phenylpropanoid compounds are the main family of oxygenated compounds with a percentage of 36.9%, which para-Propylanisol (24) (13.6%), (E)-Anethol (30) with 12.2% and Estragol (22) with 10.4% were the major constituents. Among the oxygenated monoterpenes (32.7%), we noted, in particular, Pulegone (26) and Thymol (31) were the main components with 13.2 and 10.6%, respectively. However, the two oxygenated sesquiterpenes (6.5%) namely (E)-Nerolidol (49) and β-Eudesmol (52) represent 1.1 and 5.4%, respectively (Figure 5)

3.3. Comparison of two methods

In this study, dramatic qualitative and quantitative differences have been found in the composition of EO and VF obtained by HD and HS-SPME methods, respectively. Indeed, the total numbers of components in the case of HD were found to be more than that of HS-SPME. GC-FID and GC-MS analysis report of EO reveals 39 constituents, while as the HS-SPME extract reveals 25 constituents. The data in Table 2 revealed that higher amounts of oxygenated sesquiterpenes are found in EO obtained by HD (52.8%) as compared to the HS-SPME (6.5%) while, phenylpropanoids were detected in much higher concentrations in the HS-SPME as compared to the HD (36.9% *vs* 0.3%, respectively), Similarly, the non-terpenic oxygenated compounds contents of the EO (1.9%) were lower than those of the VF (11.0%). Besides, the concentrations of oxygenated monoterpenes are quasi-similar in both samples (34.3% (HD) *vs* 32.7% (HS-SPME).

Table 2. Compound class distribution in the HS-SPME and HD of *W. saharea* leaves.

Compound class	**HD**	**HS-SPME**
Monoterpene hydrocarbons	3.2	0.5
Sesquiterpene hydrocarbons	0.2	6.5
Oxygenated monoterpenes	34.3	32.7
Oxygenated sesquiterpenes	52.8	6.5
Phenylpropanoids	0.3	36.9
Non-terpenic oxygenated compounds	1.9	11.0

Besides, as shown in Table 1, the amounts of the main compounds differed between these two extraction methods. Indeed, β-Eudesmol (33.9%), Nerolidol E (15.6%) and Linalool (15.0%) were identified as the major compounds in HD, but were found in lower amounts in comparison to the HS-SPME extract at 5.4, 1.1 and 1.8%, respectively. Conversely, phenylpropanoids (36.9%), such as *para*-Propylanisol (13.6%), (E)-Anethol (12.2%) and Estragol (10.4%) could be identified as the predominant volatile compounds, but not identified in hydrodistilled oil. So, it should be noted that among the 25 compounds previously detected in the HS-SPME, only 9 of them were present in the EO. Conversely, among 39 identified compounds in the EO, only 10 of them were detected in the HS-SPME. These chemical differences observed can be explained by the fact that the HD extraction technique is based on the liquid quasi-total extraction of plant volatiles and the HS-SPME technique is controlled by a solid/gas equilibrium step [18]. Thus, these differences were probably due to the solubilization and affinity of volatile compounds to the water and the fiber. Also, prolonged heating and high temperature may affect the quality and quantity of volatile constituents (Table 3). Indeed, 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. Normally, the quantities of low boiling and high volatility compounds could be extracted by HS-SPME [19]. However, during HD (180 min at 100 °C), the most volatile compounds and water-soluble compounds are lost in the gaseous phase and the hydrosol under the effect of heat and acid pH, respectively [5,18]. Essential oils with high solubility in water and susceptible to decomposition under temperature cannot be distilled. Also, some of the fragile and thermolabile constituents may get decomposed resulting in artifacts due to heating and long extraction time [4]. There is direct evidence of loss of some major and pharmacologically important minor chemical constituents from the hydrodistilled EO when compared to HS-SPME. In the same way, the amount of plant material used for sample preparation might probably be one of the major reasons which explain the difference between chemical HS-SPME and HD data. Indeed, the amount of plant material used for the HS-SPME analysis was smaller (3.5 g), while the production of hydrodistilled EO needed the use of 100 g of plant material (Table 2). HS-SPME analysis allowed a qualitative estimate of volatile compounds using a small quantity of material [20].

Table 3. Comparison of HS-SPME and HD for separation of the volatile components of *W. saharea* leaves.

Characteristic	HS-SPME	HD
Amount of sample required (g)	3.5	100
Extraction time (min)	30	180
Extraction temperature (°C)	70	~ 100
Separation time by GC-MS (min)	5	35
Total number of components identified	25 (94.1%)	39 (92.7%)

4. Conclusion

The chemical composition of HS-SPME extract obtained from the leaves of *W. sahare* was compared with the composition of EO obtained by HD of the same plant. GC-RI and GC-MS analysis of the EO revealed thirty-nine constituents, representing 92.7% of the oil, while HS-SPME analysis of the same plant material revealed twenty-five constituents, representing 94.1 % of the extract. The EO consisted predominantly of oxygenated sesquiterpene compounds, which the main components were β-eudesmol (33.9%) and nerolidol E (15.6%), while HS-SPME extract comprised mainly of phenylpropanoids (36.9%), which the main components were para-Propylanisol (13.6%), (E)-Anethol (12.2%) and Estragol (10.4%). According to our study, the chromatographic profiles obtained indicate that significant quantitative and qualitative differences between the chemical compositions of both analyzed samples were observed. In conclusion, this study demonstrates that HD and HS-SPME modes could be complimentary extraction techniques in order to obtain the complete characterization of plant volatiles.

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