Gas Chromatography-Mass Spectrometry Analysis and Comparison of Volatile Components Obtained by Hydrodistillation and Headspace Solid Phase Microextraction (HS-SPME) from *Teucrium luteum subsp. Flavovirens*

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Abstract:

The main purpose of this work is to investigate the comparative chemical analysis of essential oil (EO) isolated by hydrodistillation (HD) and volatile fraction (VF) detected by headspace solid-phase micro-extraction (HS–SPME) obtained from Teucrium luteum subsp. flavovirens using Gas Chromatography–Retention Indices (GC-RI) and GC–Mass Spectrometry (GC-MS). 63 volatile compounds identified in hydrodistilled essential oil (HD), representing 89.9% of the total oil, while HS-SPME revealed 50 components constituting 99.3% of the volatile material. The chemical composition of EO comprised mainly of of oxygenated sesquiterpenes (48.6%) while, hydrocarbon monoterpenes were detected in higher concentrations in VF (54.1%). The comparative analysis of two chemoprofiles obtained by two methods shows quantitative and semi-qualitative differences. The current study is the first report involving rapid analysis of volatile components of T. luteum subsp. flavovirens by HS-SPME.

Keywords: Essential oil, Hydrodistillation, Headspace solid-phase micro-extraction, *Teucrium luteum subsp. flavovirens,* Volatile fraction.

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Introduction

Natural products are chemically very complex mixtures of several components at very different concentrations produced by aromatic and medicinal plants (Frauendorfer and Schieberle 2005). In nature, these products, also called secondary metabolites, often play an important role through their involvement in communication and interaction such as plant-plant interaction and plant-animal interactions (Isah 2019). For instance, the volatile aroma constituents 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 (Herrera and Pellmyr 2002; Das et al. 2013). Moreover, several analytical methods combined with GC or GC-MS have been employed for the extraction of the volatile constituents from plant matrix, such as hydrodistillation (HD), which is a conventional method widely used to extract EOs, because of its easy and simple implementation in industry and has no chemical pollution (Rehman et al. 2017; Shaimerdenova et al. 2018). Amongst these methods, Hydrodistillation (HD). However, it has certain disadvantages, particularly, the consumption of energy and time and the deterioration of heat-sensitive compounds as well as the alteration of essential oil quality (Znini et al. 2014). Thus, it is very crucial to develop alternative rapid, sensitive, safe, and energy-conserving extraction techniques. For instance, headspace solid-phase microextraction (HS-SPME) is a modern sample preparation technique in gas phase making to characterize especially the volatile fraction (VF) of aromatic and medicinal plants (Delgado et al. 2010; Al-Massarani et al. 2018). Indeed, the mechanism takes place in two ways, the first consists of the adsorption of the volatile molecules from the solid matrix by extraction of the head-space using silica fibers coated with polymer. The second is based on the absorption of molecules adsorbed on the fiber in the injector of a gas chromatograph apparatus (Figure 1).

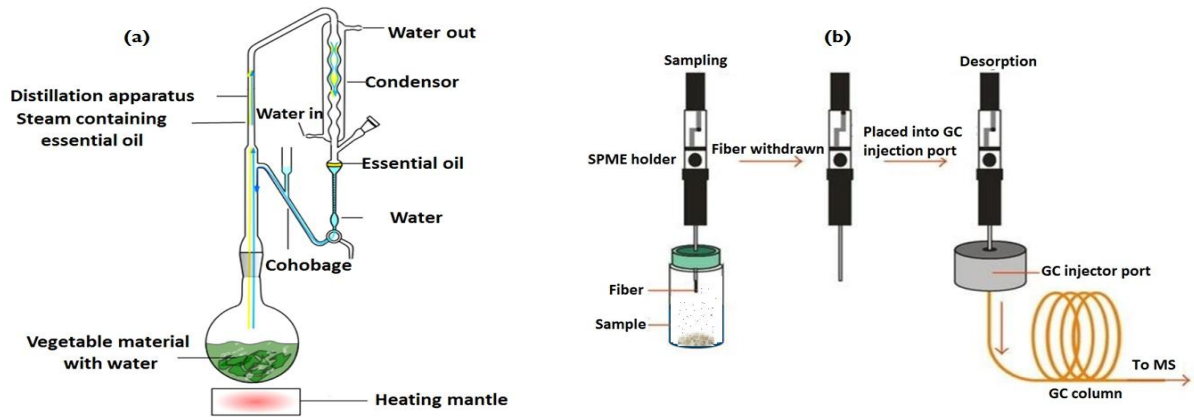

Figure 1. Hydrodistillation apparatus (a) and schematic diagrams of the HS-SPME-GC-MS analysis (b)

Teucrium luteum subsp. *flavovirens* (Batt.) Greuter & Burdet (synonym of *T. polium* subsp. *flavovirens*) is an aromatic and endemic plant growing in the wild in the Middle and High Atlas of Morocco (Navarro and El Oualidi 2000, Marzouk and El-Badan 2018). It generally prefers arid and perhumid bioclimatic conditions. In the Errachidia region, it grows in rocks and boulders, in low and medium mountains as well as on limestone and siliceous substrates (El Oualidi et al. 2002). Locally, this plant is used in traditional medicine and known by the vernacular name "Jaadah or Tayrart" (Figure 2). According to the literature, there are only two paper that described the composition of the EO of *T. luteum* subsp. *flavovirens* from Morocco (Ouknin et al. 2019. Znini et al 2021). However, no previous report on the chemical composition of a volatile fraction (VF) from *T. luteum* subsp. *flavovirens* extracted by HS-SPME. Therefore, this study aimed to characterize and compare the *T. luteum* subsp. *flavovirens* volatile constituents obtained by HD and HS-SPME extraction techniques by using a combination of GC and GC-MS.

Figure 2. *T. luteum* subsp. *flavovirens* in its native habitat in south-eastern of Morocco.

Materials and Methods

Plant material and EO isolation

The aerial parts of *T. luteum* subsp. *flavovirens* were collected wildly, at the full flowering stage, from Tizi n'talghoumt (32°37'48" N and 4°31'45" O) located in mountain zones with

altitudes up to 1907 m from the south-east of Errachidia (Morocco). A voucher specimen (CIM HERB # 41) was deposited in the Herbarium of Faculty of Sciences and Techniques, Errachidia, Morocco. The essential oil used in this study was the same we used in our previous study (Znini al. 2021). 100g of air-dried material was subjected to hydrodistillation for 180 min using a Clevenger-type apparatus. The EO obtained was dried under anhydrous sodium sulfate and stored at 4 °C in the dark before analysis.

Volatile compounds by HS-SPME

Approximately 1 g of homogenized dried leaves of *T. luteum* subsp. *flavovirens* were placed in a 20 mL headspace transparent glass vial (Supelco) to be subjected directly to HS-SPME. Subsequently, the headspace glass vial was heated at 70 ˚C for 60 min in order to reach thermal equilibrium. Later, the SPME fiber (DVB/CAR/PDMS, 2cm-50/30 µm) was inserted into the headspace of the glass vial to absorb volatile organic compounds for 30 minutes. After sampling, the SPME fiber was immediately removed from the sample and the analytes were thermally desorbed in the injector port of the GC/MS for 5 min at 250 °C using the splitless injection mode.

GC-RI and GC-MS analysis

The analysis processes of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) were performed with reference to Znini et al. (2019). The identification of the components was based on the comparison of their GC retention indices (RI) 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 on computer matching with commercial mass spectral libraries (Hochmuth et al. 2001) and comparison of spectra with those of our library.

Results and discussion

EO analysis

The EO of *T. luteum* subsp. *flavovirens* aerial parts was extracted by HD appearing as a pleasant-smelling yellow oil with a percentage yield of 0.9% (v/w). The analysis of this oil was carried out by GC-RI and GC-MS, and a total of sixty-three components, representing 89.9% 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). These included 24 alcohols (52.8%),18 hydrocarbons (27.0%), 10 ketones (5.0%), 4 ethers (3.1%), 5 esters (1.3%) and 2 aldehydes (0.7%) (Table 2).

Table 1. Chemical composition of EO and VF from *T. luteum* subsp. *flavovirens.*

N°	Components	RI *a*	% HD	% SPME
1	**α-Pinene**[e]	931	**4.5**	**19.8**
2	Camphene[e]	936	-	0.9
3	2,4-Thuja-10-diene[e]	939	-	1.4
4	1-Octen-3-ol[a]	959	0.2	0.3
5	Sabinene[e]	963	0.4	2.2
6	**β-Pinene**[e]	**969**	**6.9**	**15.4**
7	Myrcene[e]	978	0.6	**4.2**
8	Mentha-1,4,8-triene[e]	992	-	0.4
9	Lavander lactone[c]	995	-	0.8
10	2-Methylbutyl isobutyrate[d]	1000	-	0.5
11	P-Cymene[e]	1008	0.3	**3.9**
12	Cineole 1,8[f]	1017	0.1	-
13	Limonene[e]	1017	1.5	**5.9**
14	2-Methylpropyl ester[d]	1031	-	1.8
15	Linalol[a]	1077	1.4	1.5
16	α-Thujone[c]	1079	0.5	0.2
17	1-Octen-3-yl-acetate[d]	1085	0.2	0.3
18	β-Thujone[c]	1090	0.1	-
19	α-Campholenal[b]	1097	0.1	-
20	Nopinone[c]	1101	0.1	1.6
21	Camphre[c]	1113	0.8	0.5
22	trans-Pinocarveol[a]	1116	0.9	1.1
23	cis-Verbenol[a]	1121	0.8	0.3
24	Menthone[c]	1125	0.3	0.1
25	Pinocarvone[c]	1131	0.4	1.3
26	Borneol[a]	1142	0.2	0.3
27	Terpinen-4-ol[a]	1154	0.4	0.4
28	Myrtenal[b]	1162	0.6	1.1
29	α-Terpineol[a]	1165	0.4	0.3
30	Myrtenol[a]	1172	0.4	-

31	Verbenone[c]	1173	0.3	**2.5**
32	trans-Carveol[a]	1191	0.5	0.1
33	Carvone[c]	1209	0.8	0.3
34	Carvotanacetone[c]	1214	1.4	1.8
35	Geraniol[a]	1228	0.2	0.1
36	Chrysanthenyl acetate[d]	1236	0.4	0.1
37	Bornyl acetate[d]	1262	0.2	0.5
38	Carvacrol[a]	1275	2.1	0.2
39	Myrtenyl acetate[d]	1300	0.1	0.1
40	α-Terpinyl acetate[d]	1327	0.4	0.2
41	α-Copaene[e]	1370	0.4	1.0
42	β-Bourbonene[e]	1378	0.2	0.2
43	β-Elemene[e]	1383	0.5	0.2
44	**trans-Caryophyllene**[e]	**1413**	**2.7**	**3.8**
45	γ-Elemene[e]	1424	0.5	-
46	α-Humulene[e]	1446	1.9	1.8
47	Dehydrosesquicineol [f]	1455	0.5	-
48	Germacrene D[e]	1472	1.8	0.6
49	β-Selinene[e]	1478	0.8	-
50	cis-β-Guaiene[e]	1482	0.4	0.3
51	Epicubebol[a]	1484	0.9	-
52	Bicyclogermacrene[e]	1487	1.1	-
53	Cubebol[a]	1503	1.1	0.2
54	*7-epi a-Selinene*[e]	1509	1.6	-
55	δ-Cadinene[e]	1511	0.9	0.5
56	β-**Elemol**[a]	**1533**	**5.0**	**16.4**
57	**E-Nerolidol**[a]	**1552**	**31.0**	**0.8**
58	Caryophyllene oxyde[f]	1567	1.6	0.1
59	epoxyde Humulene II[f]	1592	0.9	-
60	Epicubenol[a]	1612	0.4	-
61	γ-Eudesmol[a]	1616	1.1	-
62	τ-Cadinol*[a]	1624	1.1	-
63	τ-Muurolol*[a]	1624	0.6	-
64	β-Eudesmol[a]	1633	1.4	0.8
65	Valerianol*[a]	1637	0.1	-

66	α-Eudesmol*[a]	1637	1.8	0.2
67	Bulnesol[a]	1640	0.3	-
68	α-Bisabolol[a]	1650	0.5	-
69	α-Cyperone[c]	1723	0.3	-
			89.9	**99.3**

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

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

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

[a-f] Superscripts represent the functional group of compounds (a = alcohol; b = aldehyde; c = ketone; d = ester; e = hydrocarbon; f = ether).

Regarding compounds of the terpene group, this EO was characterized by a large amount of sesquiterpenic fraction with 61.4% of the total oil, which the 17 oxygenated sesquiterpenes account 48.6% and 12 hydrocarbon sesquiterpenes represent 12.8%. However, the content of monoterpenic fraction was 28.3% of the total oil, equally attributed to 6 hydrocarbon and 27 oxygenated monoterpenes with a percentage of 14.2 and 14.1%, respectively. E-Nerolidol **57** was the main constituent accounting for 31.0% of the total oil content, followed by β-Pinene **6** (6.9%), β-Elemol **56** (5.0%) and α-Pinene **1** (4.5%). The chromatogram of hydrostilled oil is given as shown in Figures 3.

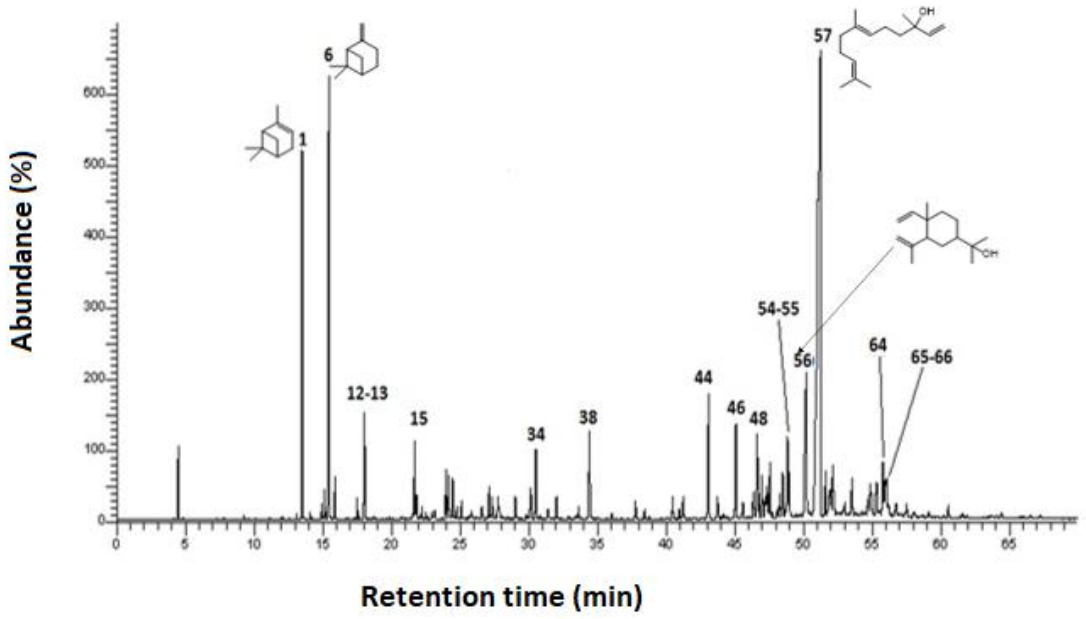

Figure 3. Chromatographic profile of the EO from *T. luteum* subsp. *flavovirens* obtained by HD. The separation was carried out on a polar column (Rtx-1)

These results were in accordance with those previously reported by Ouknin et el. (2019) which repotted tat fifty-five compounds were identified *T. luteum* subsp. *flavovirens*, and β-Elemol (16.4%), α-Pinene (12.0%), trans-Caryophyllene (7.0%), α-Humulene (6.4%) and β-Pinene (5.7%) are the major components. However, it appears that our sample differs with other various *Teucrium polium* subspecies' essential oils. For instance, Sabzeghabaie and Asgarpanah (2015) reported that α-pinene (18.2%), elemol (14.5%) and β-pinene (10.1%) were the main constituents of the essential oil from *T. polium* fruits (subspecies not specified), growing from Iran. In addition, essential oil from Turkey (subspecies not reported) was characterized by β-pinene (10.2%), (E)-Nerolidol (9.5%) and α-pinene (7.7%) as the major components (Sarer and Konuklugil 1987).

Volatile compounds by HS-SPME

The volatiles emitted *T. luteum* subsp. *flavovirens* were extracted with HS-SPME apparatus using the fiber (Supelco) coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 2cm-50/30 µm). The GC-RI and GC-MS analysis allowed the identification of fifty components, representing 99.3% of the total VF composition (Table 1 and Figure 4). As shown in Table 2, these compounds are divided into 17 hydrocarbons (62.5%), 15 alcohols (23.0%), 9 ketones (9.1%), 7 ester (3.5%), 1 aldehyde (1.1%) and 1 ether (0.1%) (Table 2).

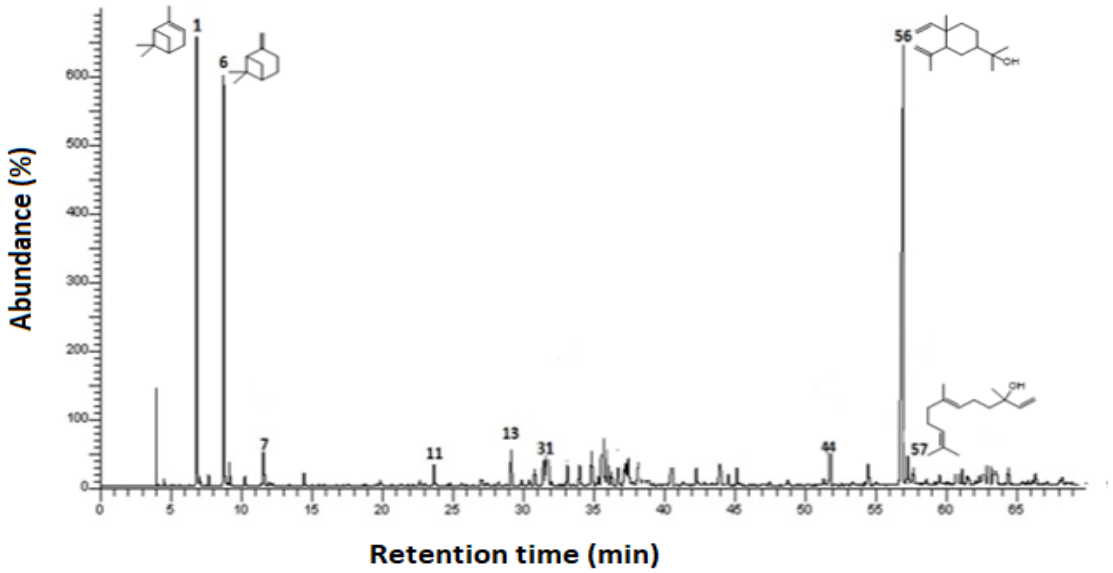

Figure 4. Chromatographic profile of volatile fraction from leaves of *T. luteum* subsp. *flavovirens* detected by HS-SPME. The separation was carried out on apolar column (Rtx-1)

Moreover, the chemical composition of the VF was characterized by a high content of monoterpenes (69.0%) while sesquiterpene accounted only for 26.9%. It was dominated highly by the 9 hydrocarbon monoterpenes (54.1%) followed by 6 oxygenated sesquiterpenes (18.1%), 23 oxygenated monoterpenes (14.9%) and 8 hydrocarbon sesquiterpenes (8.4%). The most abundant compounds were α-Pinene **1** (19.8%), β-Elemol **56** (16.4%), β-Pinene **6** (15.4%) Limonene **13** (5.9%), Myrcene **7** (4.2%) and Trans-Caryophyllene **44** (3.8%).

Table 2. Relative contents of the functional groups in the volatile organic compounds detected among *T. luteum* subsp. *flavovirens.*

Functional group	HD		HS-SPME	
	No	%	No	%
Alcohol	24	52.8	15	23.0
Aldehyde	02	0.7	01	1.1
Ketone	10	5.0	09	9.1
Ester	05	1.3	07	3.5
Hydrocarbon	18	27.0	17	62.5
Ether	04	3.1	01	0.1
Total	63	89.9	50	99.3

Comparison of two methods

For comparison purposes, quantitative and semi qualitative differences have been found in the chemical composition of EO and VF obtained by HD and HS-SPME methods, respectively. Indeed, quantitatively, 63 compounds were characterized in EO extracted by HD, representing 89.9% of the total oil, while 50 volatile constituents have been detected by HS-SPME, accounting for 99.3% of the total VF (Table1). Moreover, alcoholic and hydrocarbon identified compounds were the major by both extraction methods compared to other classes of compound. Besides, Figure 5 revealed that higher amounts of oxygenated sesquiterpenes (OS) are found in EO obtained by HD (48.6%) as compared to the HS-SPME (18.5%) while, hydrocarbon monoterpenes (HM) were detected in higher concentrations in the HS-SPME as compared to the HD (54.1% *vs* 14.2%, respectively), Also, the concentrations of oxygenated

monoterpenes (OM) are quasi similar in both samples (14.1% (HD) *vs* 14.9% (HS-SPME) while, the hydrocarbon sesquiterpenes (HS) contents of the EO (12.8%) were slightly higher than those of the VF (8.4%).

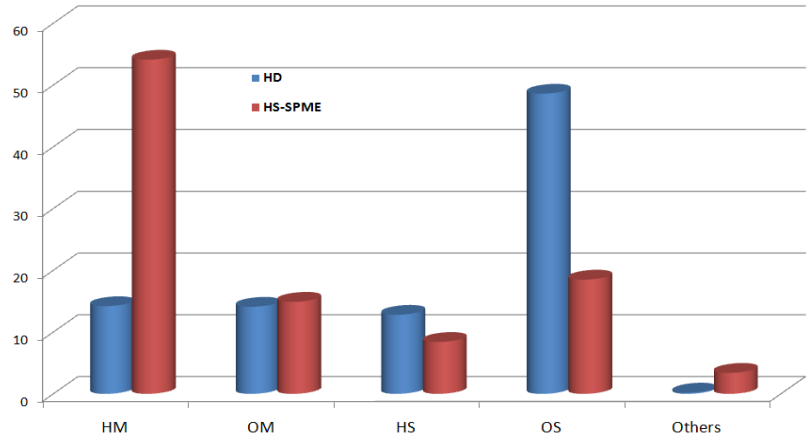

Figure 5. Terpene Compound class detected in the HS-SPME and HD of *T. luteum* subsp. *flavovirens.*

In addition, as shown in Table 1, the amounts of the main compounds differed between these two extraction methods. Indeed, Nerolidol **57** (31.0%) was identified as the major compound in HD, but was found in lower amounts in comparison to the HS-SPME extract (0.8%). Conversely, β-Elemol **56** (16.4%) could be identified as the predominant volatile compound but represented only 5.0% in hydrodistilled oil. Also, the α-Pinene **1** and β-Pinene **6** are isolated by HS-SPME with a large amount (19.8 and 15.4%, respectively) than HD extract (12.0 and 5.4%, respectively).

From a qualitative point of view, as shown in Table 1, 69 compounds were totally identified using HD and HS-SPME (Table 1) including 44 compounds Common to both extraction methods. However, among the 50 compounds previously detected in the HS-SPME, only 6 of them were absent in the EO. Conversely, among 63 identified compounds in the EO, only 19 of them were not 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 (Znini et al. 2019). Thus, these differences were probably due to the solubilization and affinity of volatile compounds to the water (Table 3). Indeed, during HD (180 min at 100 ° C), the most volatile compounds are lost in the gas phase under the effect of heat while the water-soluble compounds are lost in the aqueous phase under the effect of the

acidic pH (Znini et al. 2014; Znini et al. 2019). Therefore, EOs with high solubility in water and susceptible to decomposition under temperature cannot be distilled. Also, prolonged heating and high temperature can cause decomposition of some fragile and thermolabile constituents, resulting in artifacts due to heating and long extraction time (Rehman et al. 2017). However, the fiber affinity of each compound at and the extractive conditions (70 °C for 30 min) significantly influence the HS-SPME extraction; it therefore monitors the sampling of volatile constituents limiting or promoting their extraction. Normally, the quantities of low boiling and high volatility compounds could be extracted by HS-SPME (Mékaoui et al. 2019). Likewise, the quantity of plant material used in sample preparation is another determining factor in the difference between HS-SPME and HD chemical data. In fact, the amount of plant material used in the HS-SPME analysis was lower (1.0 g), while the production of hydrodistilled EO required the use of 100 g of plant material (Table 3). HS-SPME analysis allowed the qualitative identification of volatile compounds using a small amount of material (Paolini et al. 2008).

Table 3. Comparison of HS-SPME and HD for separation of the volatile components of *T. luteum* subsp. *flavovirens.*

Characteristic	**HS-SPME**	**HD**
Amount of sample required (g)	1.0	100
Extraction time (min)	30	180
Extraction temperature (°C)	70	~ 100
Total of components identified	**50** (**99.3**%)	**63** (**89.9**%)
Major compounds	**1** (19.8%), **56** (16.4%), and **6** (15.4%)	**57** (31.0%), **6** (6.9%), **56** (5.0%) and **1** (4.5%).

Conclusions

The chemical composition of HS-SPME extract obtained from *T. luteum subsp. flavovirens* was compared with composition of EO obtained by HD of the same plant. GC-RI and GC-MS analysis of the EO revealed sixty-three constituents, representing 89.9% of the oil, while HS-SPME analysis of same plant material revealed fifty constituents, representing 99.3% of the

extract. HD was able to detect more compounds, but HS-SPME analysis was reported as more efficient and convenient. The EO consisted predominantly of oxygenated sesquiterpene compounds, which the main components were-Nerolidol **57** (31.0%) followed by β-Pinene **6** (6.9%), β-Elemol **56** (5.0%) and α-Pinene **1** (4.5%), while HS-SPME extract comprised mainly of hydrocarbon monoterpenes (54.1%), which the main components were α-Pinene **1** (19.8%), β-Elemol **56** (16.4%) and β-Pinene **6** (15.4%). To sum up, the obtained chromatographic profiles revealed the presence of quantitative and semi-qualitative differences between the chemical compositions of both analyzed samples. So, this observation demonstrates that HD and HS-SPME modes could be complimentary extraction techniques to obtain the complete characterization of plant volatiles.

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