Journal of Chromatography B 1125 (2019) 121713

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Effect of pressure variation on the efficiency of supercritical fluid extraction of wild carrot (*Daucus carota* subsp. *maritimus*) extracts

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ARTICLE INFO

Keywords:
Daucus carota subsp. *maritimus*
Supercritical carbon dioxide extraction
Hydrodistillation
Geranyl acetate

ABSTRACT

The present study was conducted to check the comparative qualities of essential oils prepared by hydrodistillation (HD) and supercritical fluid techniques. It constitutes the first attempt to investigate the chemical composition of *Daucus carota* subsp. *maritimus* extracts using supercritical fluid technology (SFE) as an environmentally clean innovative method of extraction. The effect of pressure on the nature of extractable substances from wild carrot has been performed at a constant temperature of 50 °C and two different pressures (100 and 300 bar). The experimental results showed that pressure had a significant enhancing effect on the fluid transport properties and therefore on yield values. The extraction yield increases from 1.167 to 2.986% while increasing pressure. The chemical compositions of the essential oils prepared by HD and SFE were analyzed on the basis of gas chromatography–mass spectroscopy (GC–MS). Thus, we noticed that all analyzed samples were enriched in geranyl acetate and β-bisabolene, and that the quantities of different identified substances were extremely sensitive to the extraction method and to the pressure variation in case of SFE.

1. Introduction

The use of essential oils of aromatic plants and spices dates back thousands of years, since they are made up of mixtures of volatile secondary metabolites rapidly absorbed by the body inducing a wide variety of benefits such as relieving the symptoms of insomnia, headaches and asthma. Many essences such those of lemon and rosemary are able to improve the immune system and relieve stress [1]. Several scientific investigators indicated that the chemicals makeup, the chemotypes and therefore, the biological properties depends, among other various conditions, on the variable extraction methods [2]. It is important to mention that the traditional techniques, as hydrodistillation, suffer from many drawbacks including low yield, losses of volatile compounds, long extraction times, degradation of unsaturated constituents and are known to be energy intensive methods [3]. In this sense, the extraction of unadulterated essential oils is required for clinical use [4]. Supercritical fluid extraction (SFE) technology, suggested as an innovative and clean extraction technique with several advantages, has progressed an increasing attention [5]. Supercritical carbon dioxide is the most commonly used because it is a safe, non-flammable, inexpensive, inert, odorless, colorless, tasteless, nontoxic, readily available solvent and of low critical pressure (74 bar) and temperature (32 °C) [6]. The supercritical fluid properties such as temperature and pressure are considered relatively low to facilitate the high selectivity of extracting various types of compounds, even those thermally unstable [7]. Supercritical fluid was first carried by Zosel in 1970 out at the Max Plank, Institute, to extract caffeine from coffee beans. Since then, numerous studies have sought the applicability of this new technology for the extraction of essential oils from different kinds of aromatic plants [2].

Nowadays, the interests for plants from the Apiaceae family are diversified more and more, where they are utilized commercially as

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https://doi.org/10.1016/j.jchromb.2019.121713
Received 7 May 2019; Received in revised form 26 June 2019; Accepted 8 July 2019
Available online 10 July 2019

vegetables, spices or drugs because of their richness on useful secondary metabolites [8]. Carrot, the main species belonging to this family, is like many others ((e. g., onions, fennel, tomatoes and potatoes), the most commonly consumed vegetable worldwide. Carrot is nutritionally important since it is considered as an excellent source of provitamin A, carotenoids, vitamins C, D, E, K, B_1, B_6, biotin and minerals [9]. Up to now, it occupies a privileged status thanks to their nutritional aspects as a very powerful root vegetable utilized in soup, salad, cakes and juice [9]. In addition to their nutritional values, carrots are used in traditional herbal medicine due to its antilipemic, stomachic, hypotensive, carminative and diuretic properties [10]. Interestingly, carrot seed essential oil is extensively used as a flavoring agent in food products and as a fragrance in perfumes, soaps and cosmetics [11] because of its anthelmintic, antiseptic, diuretic, hepatic, stimulant and tonic properties [12]. Moreover, it has been reported that the wild carrot root is edible while young, but quickly becomes too woody to consume [13]. Those pleasant nutritional and pharmacological properties motivated us to investigate a comparison of the effect of both conventional hydrodistillation and innovative supercritical fluid extraction on the yield and chemical composition of the essential oils from wild carrot, *Daucus carota* subsp. *maritimus*. According to the current literature, the essential oil from wild *D. carota* has been found to contain many compounds. Carotol, geranyl acetate, sabinene, *α*-pinene, are the most frequently encountered [13,14]. *D. carota* (subspecies not specified) has been also used in folk Tunisian medicine in the case of nephritic colic because of its carminative, aperitive, emmenagogue and diuretic properties [15]. Furthermore, this plant has been used as remedy for the treatment of cutaneous infections and has reputation for being antidiarrheal [15]. According to Alapetite [16], *D. carota* can be divided into three subspecies, viz., *carota*, *maximus*, and *maritimus*. Interestingly, the flowers and roots essential oils from Tunisian subspecies *maritimus* possesses interesting antibacterial activity against Gram (+) and Gram (−) bacteria. Based on the literature, the supercritical carbon dioxide extraction of carrot fruits essential oil showed that Serbian *D. carota* L. (subspecies not specified), is chiefly composed of carotol (30.3%) [17]. Moreover, despite only one study which reported the extraction of essential oil from *D. carota* subsp. *maritimus* by hydrodistillation [15], no report have been published on identifying the essential oil chemical composition from aerial parts of this medicinal subspecies prepared by supercritical carbon dioxide methodology neither in Tunisia nor in other countries. In this context, and continuing our research on the chemical characterization of Tunisian *Daucus* genus [18,19], the present work is the first study on the chemical composition of *D. carota* subsp. *maritimus* volatile oils extracted by supercritical fluid technique with an emphasis on the effects of various sample preparation and extraction parameters on the yield and characteristics of the oils.

2. Materials and methods

2.1. Plant material

The aerial parts of *D. carota* subsp. *maritimus* were gathered during the flowering/fruiting stage from CE of Tunisia (Monastir) in July 2018. Taxonomical identity was assigned by Ridha El Mokni, a teacher-researcher in Botany, Cryptogamy and Plant Biology in the Faculty of Pharmacy of Monastir, Tunisia, where some voucher specimens have been deposited. Herbal parts were air-dried at ambient temperature in the shade and mixed well. Immediately prior to SFE, the sample was ground in a blender to produce powder.

2.2. Raw material

The supercritical extraction was carried out using CO_2 of 99.9% in purity. Immediately before load the raw material into an extractor that was grinded to the sizes of 0.3–0.5 mm and the humidity level is defined to be 9.7 (w/w).

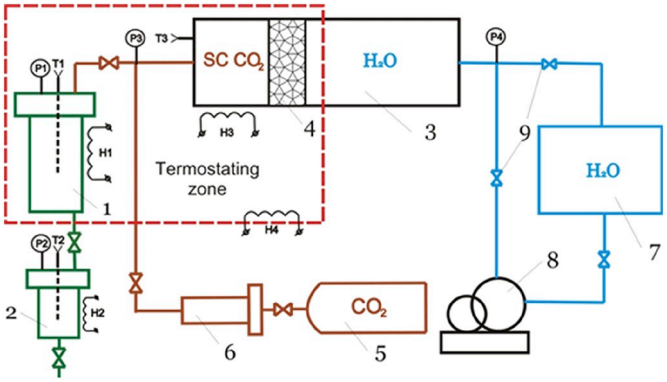

Fig. 1. Circuit diagram of the experimental plant for extraction with supercritical CO_2: (1) extractor, (2) separator, (3) power cylinder, (4) separating plunger, (5) cylinder with CO_2, (6) filter for CO_2 clearing, (7) reservoir with distilled water, (8) dosing high pressure pump, and (9) valves, P1, P2, P3, and P4 are the pressure transducers; T1, T2, and T3 are the temperature transducers; H1, H2, H3, and H4 are heaters.

2.3. Supercritical fluid extraction

The measurements were made through the use of an experimental CO_2 apparatus (Fig. 1) [19,20]. The apparatus provides an opportunity to make complex researches of extracting processes at pressures up to 400 bar and temperature intervals from 25 to 100 °C with the maximum flow of supercritical fluid of 1.7 (± 0.05) kg/h. Raw material grinded (170 g) is loaded into a cylindrical extractor of 1 L (70 mm in inside diameter and 260 mm internal height) in volume and substances are extracted at a pressure of 100 bar and a temperature of 50 °C. Obtained extract is taken out. Next, we obtain an extract once again from the same portion of raw material at 300 bar and 50 °C. The obtained extracts are weighted to determine the yield percentage (wt). In separator, a temperature and a pressure are supported within 20 °C and 5 bar being optimum for separation of an extract from gaseous CO_2. The extraction time (2 h), a rate of SC CO_2 flow (1.5 ± 0.05 kg/h), and temperature (50 °C) are constant for all processes. Set parameters provide a total extraction for every process.

2.4. Compositional analysis of (SFE) extracts

The compositional analysis of extracts obtained was carried out using Shimadzu GCMS-QP2010 plus chromatograph-mass spectrometer in Supelco SLB TM-5 ms column (30 m × 0.25 mm × 0.25 µm) in "split" mode. High-purity helium (99.9999%) with a flow rate of 1 mL min^{-1} was used as a carrier gas. The column temperature was raised from 60 °C (the hold time was 4 min) to 150 °C at a rate of 10 °C/min after that up to 280 °C at a rate of 5 °C/min. The temperature of an injector was 280 °C, an interface, and a detector was 250 °C. The ionization was performed by an electron impact with electron energy of 70 eV. The cathode emission current was 150 µA, a range of recorded ions was 45–500 *m/z*. The identification of components was performed by means of NIST08 and FFNSC mass-spectra libraries. Before analysis, a test portion was diluted in n-hexane by a factor of 500. 1 mL of diluted test portion was injected with split of 1:40 [21].

2.5. Hydrodistillation

100 g of air-dried *D. carota* subsp. *maritimus* aerial parts were subjected to a hydrodistillation process during 2 h, using a Clevenger-type apparatus. The essential oil was collected, dehydrated using anhydrous sodium sulphate and stored in hermitically sealed glass container at low temperature (−5 °C) until analysis.

2.6. GC-FID and GC–MS analyses of essential oil extracted by hydrodistillation

GC analyses of the extracts were performed using a gas chromatograph (Agilent 7890A, Palo Alto, CA, USA), equipped with a 30 m × 0.25 mm i.d. with 0.25 µm stationary film thickness HP-5 capillary column (Agilent J&W) and a flame ionization detector (FID). The following temperature program was used: from 60 °C to 246 °C, at a rate of 3 °C/min and then held at 246 °C for 20 min (total analysis time 82 min). Other operating conditions are the following: carrier gas helium (purity ≥99.9999% – Air Liquide Italy); flow rate, 1.0 mL min^{-1}; injector temperature, 250 °C; detector temperature, 300 °C. Injection of 1 µL of diluted sample (1:100 in hexane, w/w) was performed with 1:10 split ratio, using an autosampler (Agilent, Model 7683B). GC–MS analyses were carried out using a gas chromatograph (Agilent 6890N) equipped with a 30 m × 0.25 mm i.d. with 0.25 µm stationary film thickness HP-5 ms capillary column (Agilent J&W) coupled with a mass selective detector having an electron ionization device, EI, and a quadrupole analyzer (Agilent 5973). The temperature program was the same used for GC. Other chromatographic operating conditions are the following: carrier gas helium (purity ≥99.9999%); flow rate 1.0 mL min^{-1}; injector temperature, 250 °C. Injection of 1 µL of diluted sample (1:100 in hexane, w/w) was performed with 1:20 split ratio, using an auto sampler (Agilent, Model 7683B). The MS conditions were as follows: MS transfer line temperature, 240 °C; EI ion source temperature, 200 °C with ionization energy of 70 eV; quadrupole temperature, 150 °C; scan rate, 3.2 scan s^{-1} at *m/z* scan range: (30 to 480). To handle and process chromatograms and mass spectra was used the software MSD ChemStation (Agilent, rev. E.01.00.237).

Constituents of the samples were identified by comparing: mass spectra fragmentation patterns with those of a computer library [22,23] and linear retention indices (RI) based on a homologous series of C_8-C_{26} n-alkanes with those reported in literature (Stein et al., 2008). The Table 2 shows the chromatographic results, expressed as GC peak area percentages.

3. Results and discussion

3.1. Effect of pressure variation on the extraction yield

The technology of the supercritical carbon dioxide is considered as one of the most potentially useful new methods suitable for essential oils extraction of better quality [24]. In this study, we intend to study the behavioral changes in the yields and the quality of wild *D. carota* subsp. *maritimus* essential oils obtained by hydrodistillation and supercritical fluid carbon dioxide under appropriate conditions of temperature and pressure. The variation of the yields of extractive substances derived from successive supercritical fluid extraction from one and same raw material at 100 and 300 bar and a constant temperature of 50 °C are presented in Fig. 2. Similar trends were described previously and reported that extraction yield enhanced significantly with increase of pressure at a constant temperature, due to an increase of the solubility [3]. In fact, the reduction of the surface between CO_2 molecules, increases its density and the contact surface between the extractable compounds and the extraction solvent [3]. Moreover, we noticed a significant difference in the quantity of the essential oil recovered by hydrodistillation compared to the innovative SFE procedure (Fig. 2). The yield of extractable organic volatiles from *D. carota* subsp. *maritimus* obtained using conventional hydrodistillation was more than seven times lower than that of the extraction achieved via supercritical fluid presenting a better selectivity for components of interest.

3.2. Effect of pressure variation on the supercritical fluid extractive substances

Data on the compositional analysis of both essential oils obtained by sequential SFE processes are presented in Table 1. The experiment

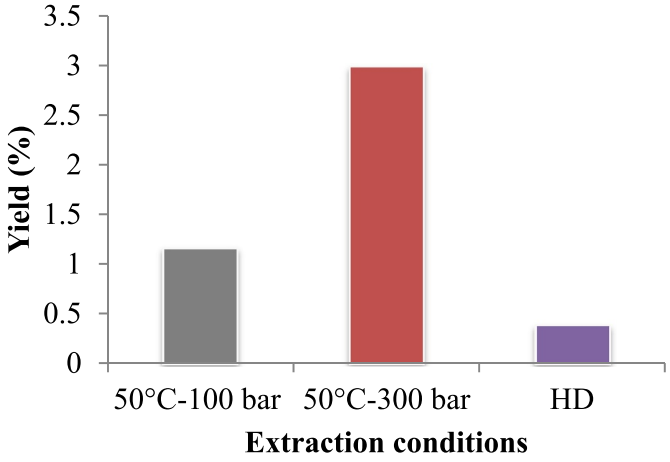

Fig. 2. Comparison of SFE and hydrodistillation yields of *D. carota* subsp. *maritimus* essential oil.

consisted on a first 120 min extraction at 100 bar as described in section 2.3, to remove mainly the essential oil preferentially extracted at low pressure followed by a second extraction at 300 bar applied on same raw material. Thus, a total of 35 organic volatiles, representing 92.7% of the whole constituents, were identified in the essential oil derived by SC CO_2 at 100 bar, whereas the SC CO_2 extract obtained at 300 bar revealed the presence of 60 compounds, 32 of which were identified and 45.31% of constituents were unidentified. Out of the 35 compounds detected at 100 bar, 10 were quantified in low percentages (0.06–1.71%), and were completely absent at 300 bar. Out of the 32 compounds, 8 were detected only in the oil extracted at 300 bar, among them; α-bisabolol reached 1.53%.

In order to compare the quality of *D. carota* subsp. *maritimus* essential oil extracted at 100 to that of the extract derived at 300 bar, the percentages of the main compounds are depicted in Fig. 3. The oxygenated monoterpene, geranyl acetate, identified as the chemotype and most beneficial ingredient from *D. carota* subsp. *maritimus*, was abundant at a higher percentage of 26.43% in the essential oil obtained at 100 bar comparing to that of the derived extract prepared at 300 bar (12.85%). Moreover, the others main compounds constituting the essential oil extracted at 100 bar are β-bisabolene (20.39%) and elemicin (8.26%), those constituents were less abundant and detected at only 11.53% and 5.35% in the extract obtained at 300 bar (Fig. 4).

The significant variability in the amounts of the main chemical classes in both extracts should be highlighted (Fig. 5). We noticed that the low molecular weight compounds (monoterpenes) were already obtained at 100 bar (43.36%), their percentage in the extract derived at 300 bar regularly decreased up to 18.26%.It is apparent from the obtained results that the increase in pressure from 100 to 300 bar led to a decrease in the percentage of sesquiterpenes hydrocarbons from 31.36% to 15.53%, in contrast the amount of oxygenated sesquiterpenes slightly increased (4% for 100 bar and 7.48% for 300 bar). Moreover, the higher concentration of phenyl derivatives found at low pressure (100 bar) with a percentage reaching up to 14.05% and they are less abundant at 300 bar (11.31%).

Previous researches revealed that pressure can significantly influence essential oil yield and their chemical constituents. This outcome was in agreement with the study of Donelian et al. [25] which noted that the pressure is a predominant factor in the variation in the concentration of the principal components of the patchouli essential oil.

Aliev et al. [21], also, reported that the increase of the oxygenated sesquiterpenes in the extract obtained at 300 bar was due to the solvent permeation into the deeper cellular structure of the raw material by virtue of pressure rise and the extraction of hard-to-get oils.

Consequently, it was verified that, depending on the pressure condition of $SCCO_2$, it is possible to obtain an essential oil of high quality.

Table 1
Compositional analysis of extracts from *D. carota* subsp. *maritimus* derived at 100 and 300 bar.

Compounds	Ret. Time	Area of peak (%)		*m/z*
		50 °C-100 bar	50 °C-300 bar	
α-Pinene	8.393	4.82	1.06	TIC
Camphene	8.869	0.11	-	TIC
Sabinene	9.469	4.20	1.12	TIC
β-Pinene	9.617	2.51	0.68	TIC
Myrcene	9.853	1.33	0.38	TIC
Limonene	10.859	0.90	0.28	TIC
(*E*)-*β*-ocimene	11.202	0.12	-	TIC
α-Campholenic aldehyde	12.901	0.14	-	TIC
trans-Pinocarveol	13.227	0.24	0.21	TIC
trans-Verbenol	13.297	0.53	0.47	TIC
Pinocarvone	13.601	0.20	0.13	TIC
Terpinen-4-ol	13.910	-	0.05	TIC
Myrtenal	14.191	0.30	0.26	TIC
Verbenone	14.406	0.26	0.18	TIC
trans-Carveol	14.545	0.06	-	TIC
Carvone	14.962	0.77	-	TIC
Geraniol	14.974	-	0.59	TIC
Bornyl acetate	15.582	0.18	-	TIC
α-Terpinyl acetate	16.553	0.11	-	TIC
Neryl acetate	16.636	0.15	-	TIC
α-Longipinene	16.769	2.38	0.94	TIC
Geranyl acetate	16.991	**26.43**	**12.85**	TIC
β-Elemene	17.336	0.28	0.11	TIC
(*E*)-Caryophyllene	17.954	2.56	1.12	TIC
Bicyclogermacrene	18.008	-	0.55	TIC
ϒ-Elemene	18.009	1.12	-	TIC
Sesquisabinene	18.153	0.29	0.12	TIC
(*E*)-*β*-Farnesene	18.258	0.31	0.14	TIC
α-Humulene	18.570	0.65	0.38	TIC
Vanillin methyl ether	18.769	-	0.32	TIC
Methyl isoeugenol	19.054	2.72	1.91	TIC
α-Bulnesene	19.172	1.34	0.64	TIC
***β*-Bisabolene**	19.348	**20.39**	**11.53**	TIC
Germacrene B	19.608	0.34	-	TIC
Elemicin	19.936	**8.26**	**5.35**	TIC
α-Elemol	20.149	-	0.12	TIC
Caryophyllene oxide	20.916	0.96	0.68	TIC
cis-Thujopsene	22.208	1.70	-	TIC
α-Bisabolol	22.210	-	1.53	TIC
Asarone	22.318	3.07	3.73	TIC
Juniper camphor	23.089	3.04	5.15	TIC
n-Hexadecanoic acid	27.746	-	1.41	TIC
n-triacontane	39.901	-	0.70	TIC
Monoterpenes hydrocarbons		13.99	3.52	
Oxygenated monoterpenes		**29.37**	**14.74**	
Sesquiterpenes hydrocarbons		**31.36**	**15.53**	
Oxygenated sesquiterpenes		4	7.48	
Phenyl derivatives		14.05	11.31	
Other		-	2.11	
Total identified (%)		92.77	54.69	
Total unidentified (%)		6.92	45.31	

-: absent.
The percentages of major odorants constituting the essential oils are represented on bold.

3.3. Comparison of supercritical fluid extraction with the hydrodistilled essential oil

The present study constitutes also a good opportunity to compare the qualities of *D. carota* subsp. *maritimus* extracted by different techniques. Thus, the chemical composition of the aerial parts essential oil obtained by hydrodistillation is reported in Table 2. As a result, we noticed that carbon dioxide extracts were closer in chemical composition to the hydrodistilled essential oil characterised similarly by its specific richness in the oxygenated monoterpene odorant geranyl acetate (29%). All analyzed essential oils contained almost the same constituents. However the supercritical fluid extracts presented the highest numbers of organic volatiles than the hydrodistillate. Thus, It

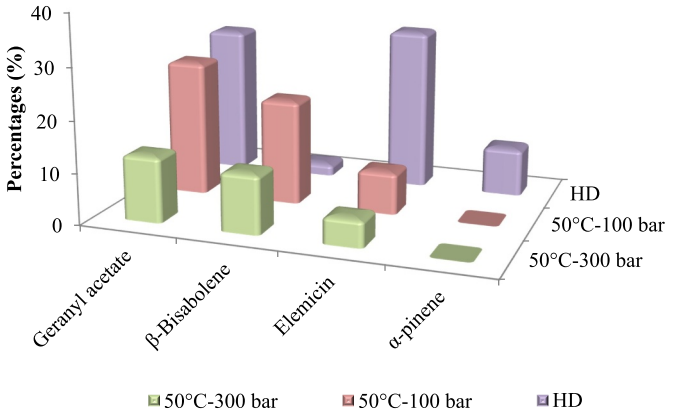

Fig. 3. Relative extraction efficiencies of representative Essential oil components from *D. carota* subsp. *maritimus* based on hydrodistillation and supercritical fluid extraction.

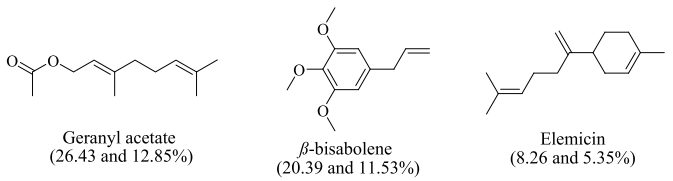

Fig. 4. Major constituents of *D. carota* subsp. *maritimus* SC CO_2 extracts.

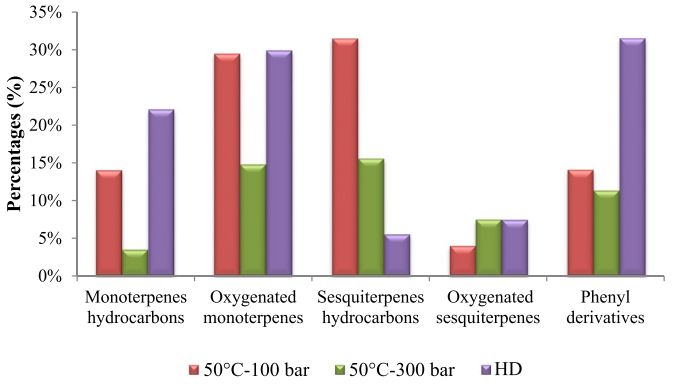

Fig. 5. Effect of extraction technique on *D. carota* subsp. *maritimus* chemical classes.

can be postulated that some compounds (as myrtenal, verbenone and some sesquiterpene hydrocarbons) were thermally degraded by hydrodistillation. Therefore, the investigations showed some differences in the quantities and nature of the detected components with respect to the type of extraction technique. Hydrodistillation process is more suitable for extracting Monoterpenes hydrocarbons (22%), oxygenated monoterpenes (29.8%) and phenyl derivatives (31.4%), while supercritical carbon dioxide can be useful as a selective procedure to extract sesquiterpenes hydrocarbons (Fig. 5). In fact, a biggest number of sesquiterpenes hydrocarbons, dominated by *β*-bisabolene, was efficiently extracted by means of SFE. Moreover, *β*-bisabolene abundant at 20.39% in the SFE essential oil prepared at (50 °C-100 bar) was extracted at a substantially lower percentage (2.4%) using hydrodistillation (Fig. 3). This important odorant, shows interesting anti-tumor properties against melanoma, hepatocellular carcinoma and leukemia cells [26]. Moreover, previous studies have reported the importance of Elemicin because of its antibacterial [27], antifungal, anti-cholinergic [1], antioxidant [28], anti-inflammatory, antiviral and analgesic [29] activities.

Table 2
Chemical compositions of *Daucus carota* subsp. *maritimus* hydrodistilled essential oil (%).

Number	Compound	RI[a]	%RA[b]	Identification[c]
1	α-Thujene	929	0.1	RI,MS
2	**α-Pinene**	937	**9.4**	RI,MS
3	CAMPHENE	952	0.2	RI,MS
4	SABINENE	976	1.7	RI,MS
5	β-Pinene	979	4.4	RI,MS
6	Myrcene	991	4.9	RI,MS
7	Ortho-cymene	1026	0.1	RI,MS
8	SYLVESTRENE	1031	1.2	RI,MS
9	LINALOOL	1099	0.4	RI,MS
10	Terpinen-4-ol	1178	0.2	RI,MS
11	Bornyl acetate	1285	tr	RI,MS
12	α-Longipinene	1350	0.4	RI,MS
13	**Geranyl acetate**	1386	**29.0**	RI,MS
14	β-Caryophyllene	1418	1.1	RI,MS
15	α-Humulene	1452	0.2	**RI,MS**
16	γ-Muurolene	1479	0.4	RI,MS
17	Ar-curcumene	1481	tr	RI,MS
18	γ-Curcumene	1484	0.4	RI,MS
19	*β*-Selinene	1492	0.4	RI,MS
20	(*E*)-methyl isoeugenol	1497	0.2	RI,MS
21	*β*-Bisabolene	1507	2.4	RI,MS
22	*δ*-Cadinene	1522	0.2	RI,MS
23	γ-Vetivene	1536	tr	RI,MS
24	**Elemicin**	1559	**31.4**	RI,MS
25	Eremoligenol	1630	0.2	RI,MS
26	**Cubenol**	1643	4.8	RI,MS
27	*β*-Eudesmol	1648	0.2	RI,MS
28	Eudesm-7(11)-en-4-ol	1691	2.2	RI,MS
Monoterpenes hydrocarbons		22		
Oxygenated monoterpenes		29.8		
Sesquiterpenes hydrocarbons		5.5		
Oxygenated sesquiterpenes		7.4		
Phenyl derivatives		**31.4**		
Total identified (%)		96.1		

The percentages of major odorants constituting the essential oils are represented on bold

[a] Retention index relative to n-alkanes on HP-5 capillary column.

[b] Relative area (peak area relative to the total peak area).

[c] Identification: MS, comparison of mass spectra with MS libraries.

It is worth noting that three analyzed essential oils are predominantly composed of geranyl acetate (29%, 26.43% and 12.85%, in HDEO, SFE 100 bar and 300 bar, respectively). This main oxygenated monoterpene detected, is commonly used as a constituent in perfumes, soap industry and cosmetic due to its pleasant rose odor [30]. The published data indicated that geranyl acetate exhibits powerful pharmacological effects related to inflammation and pain-related processes, and antioxidant properties [31]. Moreover, many studies have demonstrated its excellent anti-*Candida*, its anti-fungal, anti-inflammatory properties and bactericidal effects against *Campylobacter jejuni* and *Escherichia coli* [32]. It can be seen from Table 3 that, the geranyl acetate representing the chemotype of Tunisian *D. carota* subsp. *maritimus* essential oils, was previously extracted from other *Daucus carota* subspecies from different origins including *D. carota* subsp. *carota* [33], *D. carota* subsp. *gummifer* [34,35] and *D. carota* subsp. *major* [14].

It is worth noting that screening of the literature on *D. carota* subsp. *maritimus* showed the lack of prior research studies on the chemical composition of its aerial parts essential oil. However, the comparison of the results of our study with the former research on essential oils extracted from different organs and using conventional hydrodistillation [15], demonstrated a considerable dissimilarity of the chemicals depending on the organ and on the region of collect. Therefore, sabinene (51.6%) identified in previous studies, as the major odorant in fresh flowers essential oil extracted via hydrodistillation of *D. carota* subsp. *maritimus* collected in Kroussia region (Sousse, Tunisia) [15], is rarely detected in the aerial parts volatiles oils of same species collected in Monastir region (present study). Moreover, dillapiole (46.6%), the predominant constituent of roots essential oil [15], is completely absent in three samples subject of this work.

4. Conclusion

The present study establishes, for the first time, the effect of pressure on the characteristics of supercritical carbon dioxide essential oils from Tunisian *D. carota* subsp. *maritimus*. The enhancement of extraction yield is observed as pressure is increased from 100 to 300 bar for which the maximum yield of 2.986% is reached. The extraction capacity of CO_2 is a function of pressure at a constant temperature and it influences clearly the nature and the class of odorants. Our investigation showed that *D. carota* subsp. *maritimus* essential oils are predominated by bioactive geranyl acetate, *β*-bisabolene and elemicin quantified at a greater concentration at a pressure of 100 bar and a temperature of 50 °C. Comparative analysis showed that the essential oils prepared by SFE and hydrodistillation techniques, are rather similar, containing quite the same dominant odorants. However, the supercritical fluid gave the best extraction yield and it was more selective towards sesquiterpenes hydrocarbons than hydrodistillation. Thus, CO_2 supercritical fluid methodology is presented as a fast, innovative, cleaner, economical, green and efficient method suitable to extract natural bioactive compounds from aromatic and medicinal plants.

Acknowledgements

The authors thank the Ministry of Higher Education and Scientific Research of Tunisia for financial support.

Funding sources

None.

Table 3
Percentages of geranyl acetate of the essential oils from *Daucus carota* species, current and previous studies.

Daucus carota species	Country or district	Method extraction	Plant organs	Geranyl acetate (%)
D. carota subsp. *maritimus* [present work]	Tunisia	Supercritical extraction	Aerial parts	26.43 (100 bar) 12.85 (300 bar) 29.0 (2 h HD)
D. carota [33]	Tunisia	Hydrodistillation	Mature seeds	48.75
D. carota [13]	Serbia	Hydrodistillation	Seeds	53.2
D. carota [34]	Vienna	Hydrodistillation	Fruits	28.1
D. carota subsp. *carota* [26]	Portugal	Hydrodistillation	Umbels with ripe seeds	65.0
D. carota subsp. *gummifer* [27]	Spain	Hydrodistillation	Fruits	51.74–76.95
D. carota subsp. *gummifer* [28]	Portugal	Hydrodistillation	Ripe umbels	37.0
D. carota ssp. *major* [14]	–	Hydrodistillation	Fruits	34.2

Declaration of Competing Interest

No conflict of interest to declare.

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