Vegetos (2024) 37:82–93
https://doi.org/10.1007/s42535-023-00572-x

RESEARCH ARTICLES

Chemical composition of the essential oil and isolation of two main constituents of *Mentha pulegium* L.

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Received: 5 August 2022 / Revised: 30 November 2022 / Accepted: 6 January 2023 / Published online: 21 January 2023

Abstract

Aromatic and medicinal plants are the reservoirs of a vast quantity of molecules with different activities. Pennyroyal or *Mentha pulegium* L. is one of the miraculous plants that is very rich in aromatic essence and mostly used for their medicinal virtues. The purpose of the present work is to determine the chemical composition of the essential oil of the flowering tops of pennyroyal, which were harvested during July 2017 in M'Rirt (Middle-Atlas, Morocco), and to separate its constituents. The analysis of the essential oil, extracted by hydrodistillation, was made using gas chromatography coupled with mass spectrometry (GC/MS). The results of this analysis showed that the essential oil of pennyroyal contains two main compounds: pulegone and piperitenone with a rate of 71.97 and 26.04%, respectively. These two molecules represent 98.01% of the identified products. Subsequently, this essence was split on the open column of silica using an elected official of growing polarity in order to isolate certain compounds. Two main constituents were isolated and identified by the analysis of their spectroscopic data.

Keywords *Mentha pulegium* · Essential oil · Isolation · Pulegone · Piperitenone

Introduction

Throughout the world, the life expectancy of the human species continues to increase; the desire to satisfy its needs and those of future generations allows it to surely strive to discover the extraordinary beneficial virtues of aromatic and medicinal plants. Nowadays, the majority of the world population is turning to traditional medicine and pharmacopoeia and thus to the use of plants and plant extracts to deal with health problems. Indeed, aromatic and medicinal plants are very valuable source for the production of new, precious and miraculous chemical molecules. These molecules are often likened to active ingredients with a specific properties that give them a unique character. Today, no one ignores the importance of the plants and the discovery of natural products.

Mentha pulegium L. is a species of the family Lamiaceae and of the genus *Mentha* (Quezel et Santa 1962; Guignard et Dupont 2004). Pennyroyal (*Mentha pulegium* L.) is one of the most important aromatic and medicinal plants most used in traditional medicine and is marketed in Morocco as an essential oil, and its production fluctuates very dramatically from year to year (Direction de la protection de végétaux-Rabat 1999). According to several researches conducted, the flowering aerial parts of Pennyroyal are frequently used for their antimicrobial properties to treat colds, sore throats, coughs, hoarseness, bronchitis, lung infections and chills of all kinds; cholera, food poisoning, and tuberculosis. It also plays as an excellent digestive material (Zargari 1990; Bellakhdar 1997; Chraibi et al. 2018; Lahsissene et Kahouadji 2010). It is recognized as stimulating and exciting chemical for the nervous system (Aid et al. 2003). The dried leaves of this species are rolled into cigarettes and smoked to relieve asthma (Salhi et al. 2010). Furthermore, it is also used as an anti-flatulent, carminative, expectorant, diuretic, antitussive and menstruation agent (Newall 1996). Several publications describe the chemical composition of

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the essential oil of the aerial part of pennyroyal in which the authors report that the essential oil of this plant is characterized by the majority presence of ketones, although it is rich in oxygenated monoterpenes. Indeed, the described compositions are dominated either by the pulegone; menthone; piperitone; piperitenone; or sometimes by neo-menthol or menthol (El Arch et al. 2003; Silva et al. 2015; Ouakouaket al. 2015; Zantar et al. 2015; Mohammadhosseini et al. 2021; Mollaeia et al. 2021; Teixeira et al. 2012; Sarikurkcu et al. 2012). Within the framework of the valorization of aromatic and medicinal plants of Morocco, the present study aims to identify the chemical composition of the essential oil of pennyroyal and to isolate its majority compounds.

Material and methods

Plant material

The plant material consists of the flowering tops of *Mentha pulegium* L. harvested from M'Rirt (Middle-Atlas, Morocco) in the flowering month of July 2017. The aerial part of the plant has been dried away from the light and humidity at room temperature. This plant under study was identified at the Botany and Plant Ecology Laboratory of the Scientific Institute of Rabat (Morocco).

Extraction of the essential oil

The extraction of the essential oil was done by hydrodistillation in a Clevenger type device. This extraction was repeated three times in order to confirm the yield obtained by the used mode. The essential oil collected at the end of the distillation, measured in mL per 100 g of the dry plant, was introduced into a hermetically sealed dark glass bottle to preserve it from heat and light (Afnor 1996), then kept in the refrigerator at a temperature of 4 °C.

Analysis and identification of the chemical composition of the EO by GC and GC/MS

The chromatographic analysis of the EO from the aerial part of *Mentha pulegium* L. was performed on a gas phase chromatography of the Thermo Electron type (Trace GC Ultra) coupled to a mass spectrometer of the Thermo Electron Trace MS system type (Thermo Electron: Trace GC Ultra; Polaris Q MS), the fragmentation is performed by an electron impact with an intensity of 70 eV. The chromatography is equipped with a DB-5 type column (5% phenyl-methyl-siloxane) (30 m × 0.25 mm × 0.25 µm film thickness), a flame ionization detector (FID) powered by an H_2/Air gas mixture. The temperature of the column is programmed at the rate of a rise of 4 °C/min from 50 to 200 °C during 5 min. The injection mode is split (leakage ratio: 1/70, flow rate ml/min) and the used vector gas is the nitrogen with a flow rate of 1 ml/min. The identification of the chemical composition of the EO of *Mentha pulegium* L. was performed based on the comparison of their kovats indices (KI) and Adams with those of the reference products known in the literature (Kovats 1965; Adams 2007). It was supplemented by a comparison of indices and mass spectra with different references (Adams 2007). Kovats indices compare the retention time of any product with that of a linear alkane of the same carbon number. They are determined by injecting a mixture of alkanes (standard C7-C40) under the same operating conditions.

Fractionation of the essential oil of pennyroyal by chromatography in liquid phase at low atmospheric pressure

In this present study, we were interested in fractionating the components of the essential oil extracted from *M. pulegium* consisting of its main compounds: pulegone (71.97%) and piperitenone (26.04%). After the glass column was filled and stacked with a silica-eluent mixture, 3 g of the essential oil was weighed, diluted in ether and then gently added to the silica column by agitating it against the walls. The essential oil was then gently incorporated into the silica column until it reaches the upper limit of the column. Then, the eluent slided over the walls of the latter in order to cover the silica grains. A reservoir filled with the eluting solvent was finally placed above the column in order to feed (alimenter) it gradually during the experiment. The polarity gradient of the solvent in the reservoir gradually increased from a hexane-ether solvent (95/5) to a final hexane-ether solvent (90/10). The eluted products were collected progressively in numbered glass tubes. The plates of the chromatography on thin layer (TLC) plates with a migration solvent formed from hexane-ether (90/10) were used at each step for monitoring and controlling the purifications. At the end of the experiment, the column was washed with an increasingly high polarity solvent until the hexane-ether composition is reached (50/50). Due to the discoveries revealed by TLC plates, the identical compounds were grouped together to form the preponderant fractions of the essential oil containing the eluent. After the elimination of the eluting solvent by rotary evaporator, the obtained various fractions were weighed and stored away from light at temperature below 4 °C. This step permitted to collect two oxygenated fractions F_1 and F_2. The step of the fractionation of the EO is shown in Fig. 1. The molecular structures of the two fractions will be identified and confirmed later using different chromatographic and spectroscopic methods.

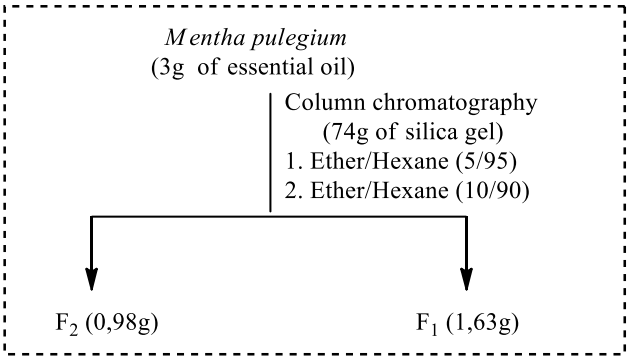

Fig. 1 Scheme of fractionation of the essential oil of *M. pulegium*

Spectroscopic analyzes by nuclear magnetic resonance (^{1}H and ^{13}C)

Due to the analysis of the fractions obtained by GC/MS, we have used the methods of proton nuclear magnetic resonance (^{1}H NMR) and carbon 13 (^{13}C NMR) to confirm the identities of the compounds and lead to their exact structural identifications. The fractions F_1 and F_2 of the EO of *M. pulegium* obtained by LPC, have checked their purity by GC and GC/MS, were then analyzed by proton and carbon 13 Nuclear Magnetic Resonance (^{1}H NMR and ^{13}C NMR) in order to confirm their structural identities. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a nuclear magnetic resonance spectrometer of the «AVANCE 300 (MHz) type from BRUKER». The chemical shifts δ are given relative to the reference (TMS) universally accepted by ^{1}H NMR and ^{13}C NMR. The used solvent is the deuterated chloroform ($CDCl_3$).

Results and discussion

Yield and chemical composition of the essential oil of *Mentha pulegium*

The average yield of the EO extracted from *M. pulegium* is around 5.2 ± 0.25. The identification of the volatile constituents of the EO of *M. pulegium* was performed by gas chromatography and gas chromatography coupled with mass spectrometry (GC/MS). The relative chromatogram for this analysis is shown in Fig. 2.

Twelve chemical substituents have been identified in the EO of *M. pulegium* (Table 1). The results presented in Table 1 indicate that the essential oil of *M. pulegium* is dominated by monoterpenes (99.66%), with a preponderance of oxygenated compounds (99.39%) and marked by high percentages of pulegone (71.97%) and piperitenone (26.04%). The hydrocarbon monoterpenes (0.27%) and sesquiterpenes are in the minority in this essence (0.12%).

Liquid chromatographic analysis of the fractionated essential oil of *M. pulegium*

The fractionation of 3 g of the essential oil of *M. pulegium* by the liquid phase chromatography method (LPC) on silica gel made possible to obtain two fractions F_1 and F_2, representing 54.33% (1.63 g) and 32.67% (0.98 g), respectively of the total essential oil. The TLC analysis made it possible to check the purity of the two fractions of the crude EO, and to compare the migration of the sample of the commercial pulegone to that fractionated by LPC. We have found that the separation of the two fractions is perfect and that the degree of the purity of our sample (pulegone) is better than that of the commercial pulegone.

The more precise study of the chemical composition of the two fractions required other spectroscopic and analytical

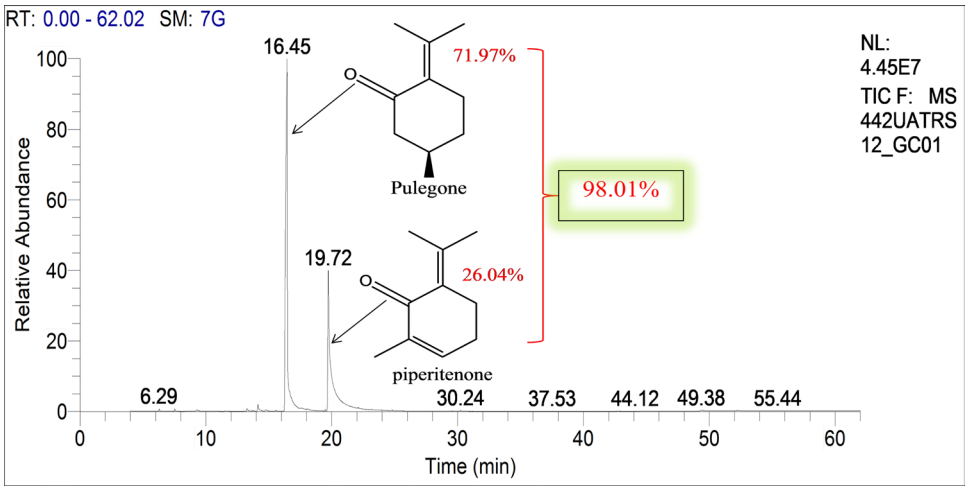

Fig. 2 Chromatogram of the essential oil of *M. pulegium* L.

Table 1 Chemical composition of the EO of *M. pulegium*

Monoterpenes		Sesquiterpenes	
Hydrocarbons (0.27%)	Oxygenated (99.39%)	Hydrocarbons (0.06%)	Oxygenated (0.06%)
α-pinene (0.14%) β-pinene (0.13%)	1,8- cineole (0.10%) Trans-p-menth-2-en-1-ol (0.28%) Cis-Chrysanthenol (0.80%) α-terpineol (0.10%) Trans-pulegol (0.06%) Pulegone (71.97%) Piperitenone (26.04%) Thymol (0.04%)	α-guaiene (0.06%)	11- α H-Himachal -4-en-1- β-ol (0.06%)

analyses in order to rigorously give the structure of each compound. These are the analyses: GC, GC/SM, RMN ^{1}H, RMN ^{13}C and DEPT.

Fraction analysis F_1 and F_2 of the EO by GC and GC/SM

According to the fractionation of the essential oil by chromatography on an open column of silica, both fractions were analyzed by GC and GC/MS. The chromatographic spectra (GC) of EO and its fractions are shown in Fig. 3.

Analysis of the fraction F_1

The analysis of the fraction F_1 made it possible to identify a total of 55 compounds (Table 2), by comparing the retention indices in GC and the mass spectra in GC/MS with those of the reference products known in the literature. These compounds are distributed as follows:

- Four compounds previously identified in the crude essential oil are: α-pinene, β-pinene, 1,8-cineole and the pulegone.

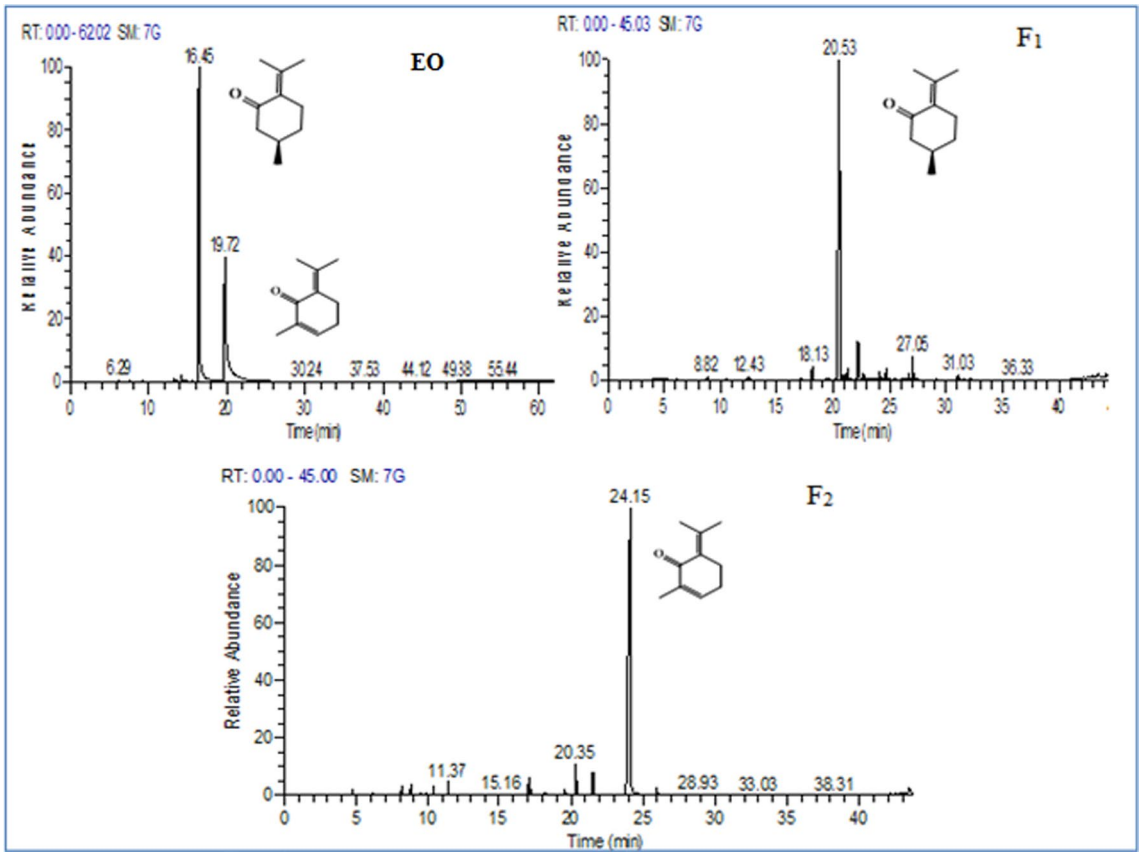

Fig. 3 Chromatograms of the EO of *M. pulegium* and its fractions (F_1, F_2)

Table 2 Chemical compounds identified in the fraction F_1 of the EO of *M. pulegium*

Compounds	KI	Brute formula	M	Area %
3-methyl-2-buten-1-ol	733	$C_5H_{10}O$	86	0.10
5-methylene-2-norbornene	810	C_8H_{10}	106	0.02
Santolinatriene	908	$C_{10}H_{16}$	136	0.02
2E, 4E hexadienol	916	$C_6H_{10}O$	916	0.04
α-thujene	930	$C_{10}H_{16}$	136	0.02
α-pinene	939	$C_{10}H_{16}$	136	0.30
Sabinene	969	$C_{10}H_{16}$	136	0.03
β-pinene	979	$C_{10}H_{16}$	136	0.15
p-cymene	1024	$C_{10}H_{14}$	134	0.07
Limonene	1029	$C_{10}H_{16}$	136	0.35
1,8-cineole	1031	$C_{10}H_{18}O$	154	0.15
Linalool	1096	$C_{10}H_{18}O$	154	0.03
Myrcenol	1122	$C_{10}H_{18}O$	154	0.02
α-campholenal	1126	$C_{10}H_{16}O$	152	0.12
Thymol, methyl ether	1235	$C_{11}H_{16}O$	164	0.07
Menthofuran	1164	$C_{10}H_{14}O$	150	0.07
Borneol	1169	$C_{10}H_{18}O$	154	0.02
Cyclopent-2-enone	1185	$C_{10}H_{14}O$	150	0.32
Myrtenal	1195	$C_{10}H_{14}O$	150	0.02
Trans-4-caranone	1196	$C_{10}H_{16}O$	252	0.29
p-cymen-9-ol	1205	$C_{10}H_{14}O$	150	0.02
2-methoxy thiophenol	1211	C_7H_8OS	140	0.26
Trans-cyclocitral	1219	$C_{10}H_{16}O$	152	2.15
Pulegone	1233	$C_{10}H_{16}O$	152	77.92
Carvotanacetone	1247	$C_{10}H_{16}O$	152	0.02
Piperitone	1252	$C_{10}H_{16}O$	152	0.56
Piperitone cis-epoxide	1254	$C_{10}H_{16}O$	168	1.24
Piperitone trans-epoxide	1256	$C_{10}H_{16}O_2$	168	0.15
Carvenone	1258	$C_{10}H_{16}O$	152	0.02
Perilla aldehyde	1271	$C_{10}H_{14}O$	150	0.07
p-menth-1-en-7-al	1275	$C_{10}H_{16}O$	152	5.28
2-ethyl-endo-fenchol	1287	$C_{12}H_{22}O$	182	0.78
3-oxo-p-menth-1-en-7-al	1333	$C_{10}H_{14}O_2$	166	0.03
Transdimethoxy citral	1341	$C_{12}H_{22}O_2$	198	0.03
Mentho thiophene	1342	$C_{10}H_{14}S$	166	0.05
Dihydroisojasmatone	1342	$C_{11}H_{20}O$	168	1.20
Piperitenone	1343	$C_{10}H_{14}O$	150	0.11
4aα,7α, 7aβ-nepetalactone	1360	$C_{10}H_{14}O_2$	166	0.19
Piperitenone oxide	1368	$C_{10}H_{14}O_2$	166	2.73
Carvacrol acetate	1372	$C_{12}H_{16}O_2$	192	0.06
Trans-mentholactone	1373	$C_{10}H_{18}O_2$	170	1.60
Trans-p-mentha-8-thiol-3-one	1373	$C_{10}H_{18}OS$	186	0.05
Dihydrojasmone	1380	$C_{11}H_{18}O$	166	0.04
8-epi-dictaminol	1380	$C_{12}H_{18}O$	146	0.06
2E,6Z nonadienaldiethylacetal	1381	$C_{13}H_{24}O_2$	212	0.04
4a-α,7-β, 7a-α-nepetalactone	1392	$C_{10}H_{14}O_2$	166	0.03
Cis-jasmone	1392	$C_{11}H_{16}O$	164	0.72
Methyl perillate	1393	$C_{11}H_{16}O_2$	180	0.08
(2E,3Z)-2-ethylidene-6-methyl-3,5-heptadienal	1395	$C_{10}H_{14}O$	150	0.22
(Z)-isoeugenol	1406	$C_{10}H_{12}O_2$	164	0.04
2(3H)-furanone, dihydro-5,5-dimethyl-4-(3-oxobutyl)	–	$C_{10}H_{16}O_3$	184	1.00

Table 2 (continued)

Compounds	KI	Brute formula	M	Area %
α- thujaplicin	1411	$C_{10}H_{12}O_2$	164	0.13
(Z)-caryophellene	1408	$C_{15}H_{24}$	204	0.19
(E)-caryophyllene	1419	$C_{15}H_{24}$	204	0.23
4,8-β-epoxy caryophellene	1424	$C_{15}H_{26}O$	222	0.03
Total				99.59

- 51 newly identified compounds are: 3-methyl-2-buten-1-ol, 5-methylene-2-norbornene, santolinatriene, 2E, 4E hexadienol, α-thujene, sabinene, p-cymene, limonene, linalool, myrcenol, α-campholenal, thymol, methyl ether, menthofuran, borneol, cyclopent-2-enone, myrtenal, trans-4-caranone, p-cymen-9-ol, 2-methoxy thiophenol, trans-cyclocitral, carvotanacetone, piperitone, cis-piperitone epoxide, trans-piperitone epoxide, carvenone, Perilla aldehyde, p-menth-1-en-7-al, 2-ethyl-endofenchol, 3-oxo-p-menth-1-en-7-al, transdimethoxy citral, mentho thiophene, dihydroisojasmatone, piperitenone, 4aα,7α, 7aβ-nepetalactone, piperitenone oxide, transmentholactone, carvacrol acetate, trans-p-mentha-8-thiol-3-one, dihydrojasmone, 8-epi-dictaminol, 2E, 6Z nonadienal diethylacetal, 4a-α,7-β, 7a-α-nepetalactone, Cis-jasmone, methyl perillate, (2E,3Z)-2-ethylidene-6-methyl-3,5-heptadienal, (Z)-isoeugenol, 2 (3H)-furanone, dihydro-5,5-dimethyl-4-(3-oxobutyl), α-thujaplicin, cis-caryophellene, tans-caryophyllene, and 4,8-β-epoxy caryophellene.
- Seven compounds of the parent essential oil are absent from this fraction: trans-p-menth-2-en-1-ol, cis-chrysanthenol, α–terpineol, trans-pulegol, thymol, α-guaiene and 11- α H-himachal -4-en-1- β-ol.

Thus, the main constituent of the fraction F_1 is the pulegone which presented the highest percentage (77.92%). This compound was also predominant in the essential oil of *M. pulegium* (71.97%). The other six compounds with a percentage greater than 1% are: Trans-cyclocitral (2.15%); Cis-epoxide of piperitenone (1.24%); p-menth-1-en-7-al (5.28%); dihydro-isojasmatone (1.20%), piperitenone oxide (2.73%) and menthalactone (1.60%). For other 48 compounds (remaining) of the fraction F_1, they are given with a percentages lower than 1%.

Analysis of the fraction F_2

The chromatographic analysis of fraction F_2 revealed 51 chemical compounds (Table 3). In this fraction, three chemical compounds are initially identified in the essential oil «mother»: pulegone, trans-p-menth-2-en-ol and piperitenone. This latter constitutes a majority compound of this fraction with a very high percentage of 84.72%, and it represents a percentage of 26.04% in the parent EO. Other 48 constituents are newly identified compounds. All of these 48 compounds have an abundance less than 1%, except for three compounds which have a percentage greater than 1%. It is artemisia ketone (1.09%); p-menth-3-en-8-ol (1.58%) and perilla aldehyde (2.31%).

Discussion of the results of the two fractions F_1 and F_2

The seven chemical compounds: 2E, 4E-hexadienol, linalool, borneol, pulegone, piperitenone, piperitenone oxide and carvacrol acetate were present in both the fractions F_1 and in F_2 with different proportions. These results show that fractionation of the essential oil by liquid chromatography on silica gel is an effective method that effeciently separates the majority of compounds and reveals new compounds of the essential oil. However, it is necessary to optimize the parameters to obtain even better results. These parameters are, among others, the length and width of the column, the elution flow rate, the progressive variations of the level of polarity of the eluent, the size of the silica beads and the elution time. According to the chromatographic results obtained above, we noted that the majority compound (pulegone) of the fraction F_1 contains 77.92% of its total chemical composition; while it represents only 71.97% of all constituents of the EO of *M. pulegium.* Concerning the fraction F_2, of which the piperitenone contains 84.72% of the totality of its chemical composition, it only represents 26.04% of the totality of the constituents of the same EO. Then, we can conclude that the fractionation of the EO by chromatography on a silica column made it possible to highlight a number of compounds greater than the number highlighted in the starting essential oil. This number is explained by their presence in very low percentages. The fractionation also permits separating and concentrating the major compounds of the essential oil. The EO of *M. pulegium* initially studied contained 12 identified compounds, while the fraction F_1 contains 55 compounds and the fraction F_2 contains 51 identified compounds. In addition, the separation on a silica column allowed us to achieve the objective which was to separate, identify and confirm the structure of pulegone and piperitenone which are the main compounds of the essential

Table 3 Chemical compounds identified in the fraction F_2 of the EO of *M. pulegium*

Compounds	KI	Brute formula	M	Area %
4-methyl-2-pentanol	755	$C_6H_{14}O$	102	0.03
Hexen-3-ol	776	$C_6H_{12}O$	100	0.02
2-hexanol	797	$C_6H_{14}O$	102	0.08
Octane	800	C_8H_{18}	114	0.04
4-methyl pentanol	838	$C_6H_{14}O$	102	0.01
Cis-2-hexenol	855	$C_6H_{12}O$	100	0.01
2E, 4E-hexadienol	912	$C_6H_{10}O$	98	0.02
1-methyl cyclopentanol	931	$C_6H_{12}O$	100	0.39
Allylisovaerate	938	$C_8H_{14}O$	142	0.84
4-methyl pentanoic acid	940	$C_6H_{12}O_2$	116	0.95
Octanal	998	$C_8H_{16}O$	128	0.13
1-methyl pentyl hydro peroxide	–	$C_7H_{16}O_2$	132	0.15
Trans-2-octen-1-al	1054	$C_8H_{14}O$	126	0.81
Artemisiaketone	1062	$C_{10}H_{16}O$	152	1.09
Linalool	1096	$C_{10}H_{18}O$	154	0.03
1,3,8-p-menthatriene	1110	$C_{10}H_{14}$	134	0.07
Cis-p-menth-2-en-ol	1121	$C_{10}H_{18}O$	154	0.08
Iso-3-thuyanol	1138	$C_{10}H_{18}O$	154	0.04
Trans-p-menth-2-en-ol	1140	$C_{10}H_{18}O$	154	0.02
Trans-sabinol	1142	$C_{10}H_{16}O$	152	0.06
Cis-pinene hydrates	1143	$C_{10}H_{18}O$	154	0.05
Allo-neo-ocimene	1144	$C_{10}H_{16}$	136	0.04
P-menth-3-en-8-ol	1150	$C_{10}H_{18}O$	154	1.58
Borneol	1169	$C_{10}H_{18}O$	154	0.08
Terpinen-4-ol	1177	$C_{10}H_{18}O$	154	0.22
Santalone	1180	$C_{11}H_{16}O$	164	0.01
Trans-p-mentha-1 (7), 8-dien-2-ol	1187	$C_{10}H_{16}O$	152	0.07
Dihydrocarveol	1193	$C_{10}H_{18}O$	154	0.04
Cis-carveol	1229	$C_{10}H_{16}O$	152	0.07
Cis pulegol	1229	$C_{10}H_{18}O$	154	0.02
Cis-p-mentha-1 (7), 8-dien-2-ol	1230	$C_{10}H_{16}O$	152	0.63
Thymol, methyl ether	1235	$C_{11}H_{16}O$	154	0.08
Pulegone	1237	$C_{10}\mathbf{H_{16}O}$	152	2.98
Carvacrol, methyl ether	1244	$C_{11}H_{16}O$	164	0.01
Perillaketone	1248	$C_{10}H_{14}O_2$	166	0.01
Perillaaldehyde	1271	$C_{10}H_{14}O$	150	2.31
Cogeijerene	1285	$C_{12}H_{18}$	162	0.02
2-ethyl-endo fenchol	1288	$C_{12}H_{22}O$	182	0.04
Methyl myrtenate	1294	$C_{11}H_{16}O_2$	180	0.05
Carvacrol	1299	$C_{10}H_{14}O$	150	0.03
p-vinyl-guacacol	1309	$C_9H_{10}O_2$	150	0.07
Piperitenone	1343	$C_{10}H_{14}O$	150	84.72
Piperitenone oxide	1368	$C_{10}H_{14}O_2$	166	0.06
Carvacrol acetate	1372	$C_{12}H_{16}O_2$	192	0.02
O-isopropenylanisole	–	$C_{10}H_{12}O$	148	0.81
Z-Isoeugenol	1407	$C_{11}H_{12}O$	164	0.04
α-thujaplicin	1411	$C_{10}H_{12}O_2$	164	0.12
Cis-cadina-1(6), 4-diene	1463	$C_{15}H_{24}$	204	0.05
Ethylvanillin	1360	$C_9H_{10}O_3$	166	0.03
δ-cadinene	1523	$C_{15}H_{24}$	204	0.01
γ-cadinene	1513	$C_{15}H_{24}$	204	0.02
Total				99.16

oil of *M. pulegium*, responsible for its important biological properties and its very appreciable therapeutic virtues. The fractionation of the EO permits highlighting 92 chemical compounds instead of 12 compounds identified in the crude EO. The disappearance of these compounds in the EO is probably linked to the presence of the two major compounds (pulegone and piperitenone) in the crude EO with very high percentages (98.01%) or their appearance is due to degradation of the chemical composition of the crude EO during the fractionation.

^{1}H and ^{13}C NMR analyzes of the two fractions F_1 and F_2

^{1}H NMR analyzes of the fraction F_1

The NMR spectrum of the proton in Fig. 4 revealed the presence of two singlets and a doublet, with little different chemical shifts, attributable to the protons of three methyl groups of the pulegone. For the other protons of the three CH_2 groups, they are distinguished by several unresolved massifs. The results are given in Table 4.

^{1}H NMR is an essential spectroscopic method for identifying and distinguishing the types of protons in an organic structure. Indeed, the comparison of the ^{1}H NMR spectrum of our sample (F_1) with that of the commercial sample (Pulegone) showed that they are practically identical.

Analysis by ^{13}C NMR and ^{13}C DEPT-NMR of the fraction F_1

To identify the carbons of the studied molecule, we have performed two analyses giving rise to two spectra: that one of the ^{13}C NMR and the other of the ^{13}C NMR-DEPT (Fig. 5).

The analysis of the first spectrum made it possible to compare the number and the position of the signals with the number and the nature of the carbons in the molecule. Concerning the counting of the second spectrum, it contributed to assigning the different signals to the types of primary, secondary, tertiary or quaternary carbons. We were able to confirm the presence of ten signals relating to the ten carbons present in the structure of the pulegone. We remember that the analysis of a product by NMR-DEPT spectroscopy of ^{13}C (135)

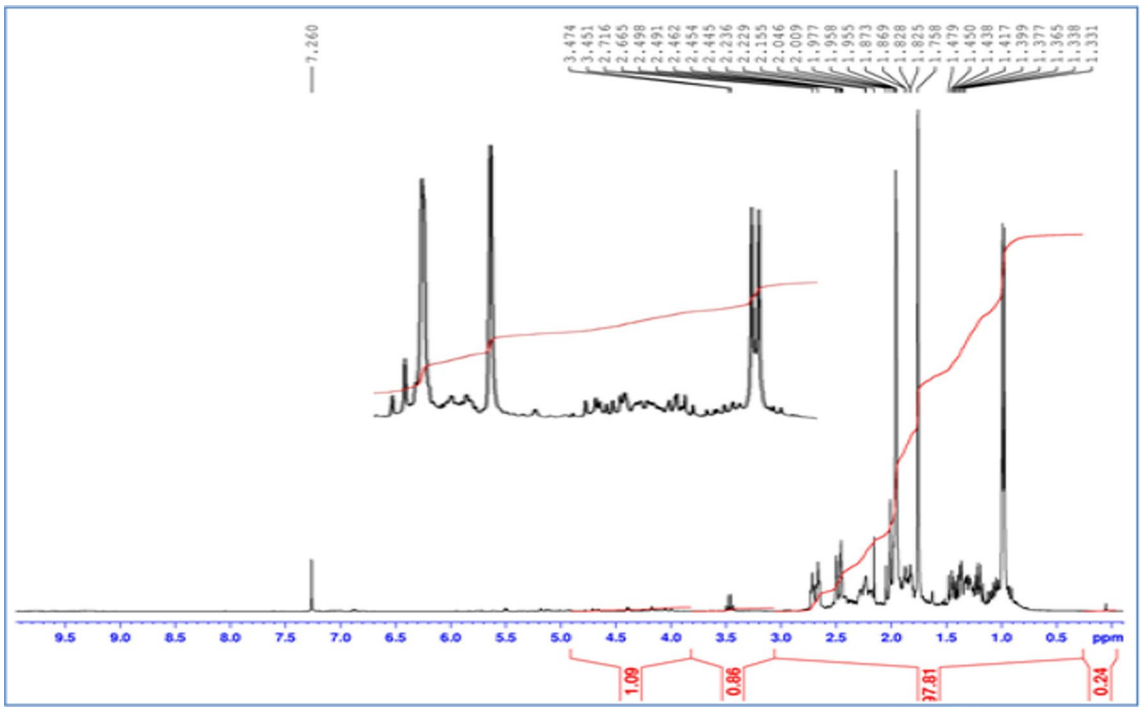

Fig. 4 ^{1}H NMR spectrum of pulegone

Table 4 Characteristics of ^{1}H NMR spectrum of pulegone

Identification of protons	Chemical shifts (δ) in ppm	Number of protons	Multiplicity	Coupling (MHz)
H_a	1.958	3H	Singulet	–
H_b	1.798	3H	Singulet	–
H_c	0.910 à 0.994	3H	Doublet	8.6

provides relevant information to facilitate the identification of organic structure. NMR-DEPT of ^{13}C indicates the appearance of the signals associated with the CH_3 and CH groups upwards. While the signals due to the CH_2 groups point downwards, and the atoms of quaternary ^{13}C do not appear. The simple method to approach the interpretation of ^{13}C-NMR-DEPT spectra is to start by looking for the signals that are present in the ^{13}C-NMR spectrum and

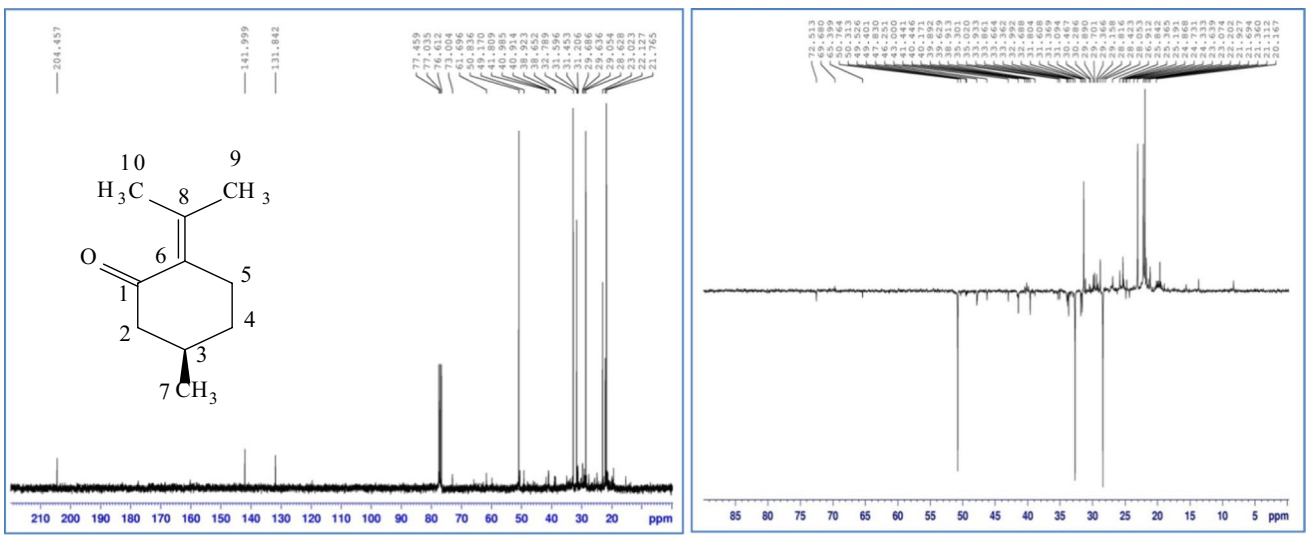

Fig. 5 ^{13}C NMR and ^{13}C NMR-DEPT spectrum of the pulegone

absent in the ^{13}C-NMR-DEPT. The signals that are absent in the latter are attributed to quaternary carbons. Indeed, three signals absent in the DEPT correspond to the three quaternary carbons of the molecule (C_1, C_6 and C_8). For the four signals which point upwards, they correspond to the three primary carbons of the CH_3 group (C_7, C_9 and C_{10}) and to the single tertiary carbon of the group CH (C_3). For the other three signals that point down, they correspond to the secondary carbons C_2, C_4 and C_5. The results of the analysis of the NMR spectrum of ^{13}C are shown in Fig. 6 and Table 5. In order to attribute to each carbon its adequate chemical shift, we relied on the data of electronic effects (electronic charges and densities, polarity of atoms) and on the bibliographic reference of David (David et al. 2016). Figure 7 summarizes the characteristics of the ^{13}C NMR spectrum of the pulegone from our sample and those found by David et al. in 2016.

Table 5 Characteristics of the ^{13}C NMR spectrum of the pulegone

Identification of carbons	Chemical shifts (δ) in ppm
C_1	204.457
C_8	141.999
C_6	131.842
C_2	50.836
C_4	32.789
C_3	31.453
C_5	28.628
C_9	23.02
C_{10}	22.127
C_7	21.765

^{1}H and ^{13}C NMR analysis of the fraction F_2

The analysis of the ^{1}H NMR and ^{13}C NMR spectra (Fig. 8) of the fraction F_2 made it possible to verify the presence of 14 protons and 10 carbons in the structure of the piperitenone. The results are shown in Tables 6 and 7.

The four signals absent in the DEPT correspond to the four quaternary carbons of the molecule (C_1, C_3, C_6 and C_8) (Fig. 7). Concerning the four signals which point upwards, they correspond to the three primary carbons of the CH_3 group (C_7, C_9 and C_{10}) and to the single tertiary carbon CH (C_2). For the two signals that point down, they correspond to the secondary carbons C_4 and C_5. The attribution of the chemical shifts to the different carbons of piperitenone (Fig. 9) is completed using the electronic effects (electronic charges and densities, polarity of the atoms) which distribute the charges inside the molecule. Figure 10 summarizes the characteristics of the ^{13}C NMR spectrum of piperitenone of our sample. The chemical shifts attributed to the various carbons are recorded in Table 7.

Conclusion

In this present work, we have studied the chemical composition of the essential oil of *Mentha pulegium* L. from M'Rirt (Middle Atlas, Morocco). Interesting results have been found; in particular, pulegone and piperitenone are the

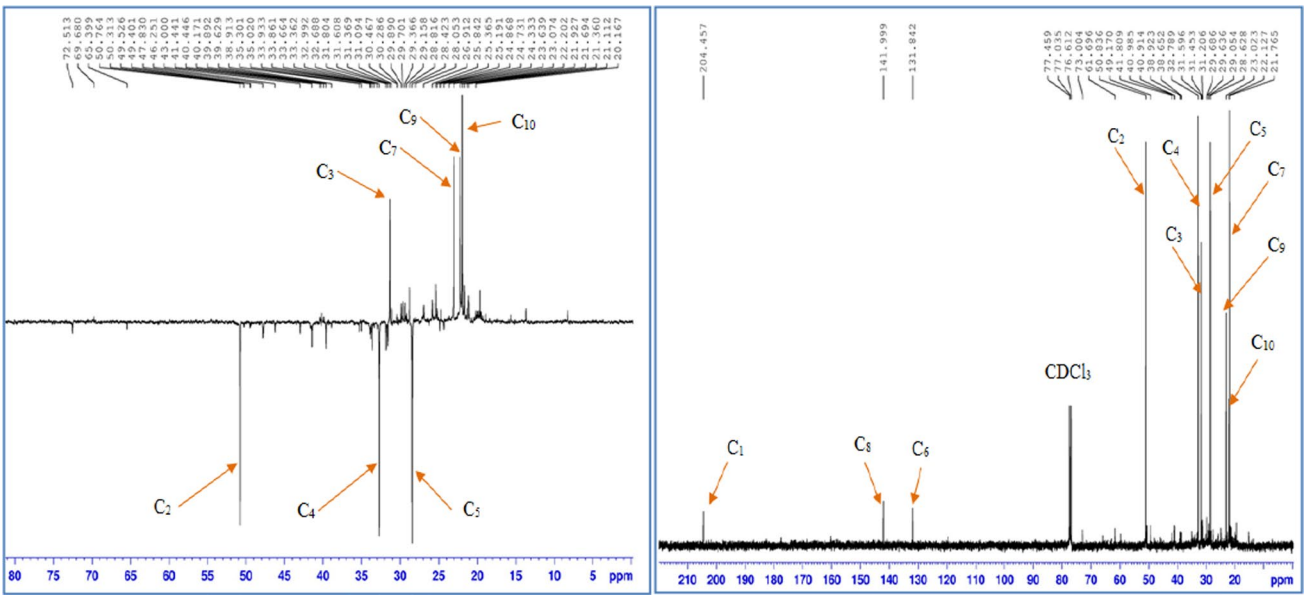

Fig. 6 NMR-DEPT spectrum of ^{13}C and ^{13}C NMR of pulegone

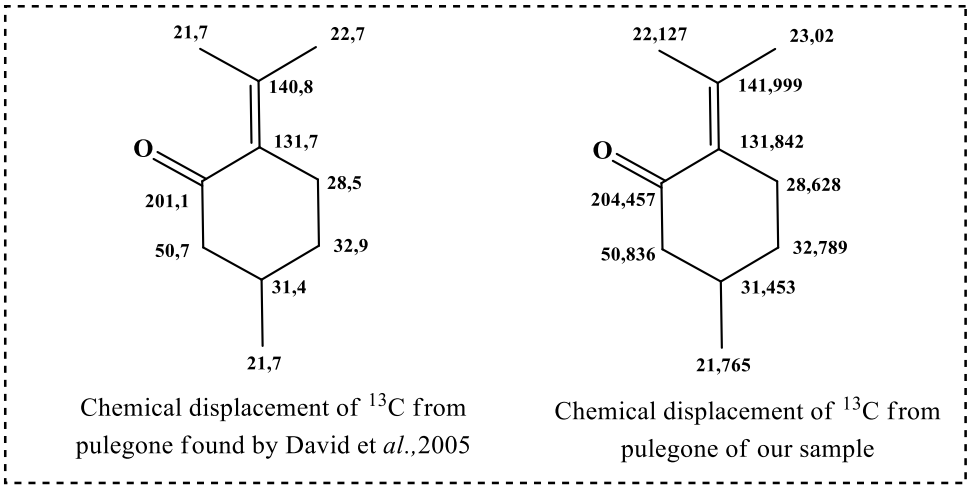

Fig. 7 Identification of the pulegone of the essential oil of *M. pulegium* by ^{13}C NMR

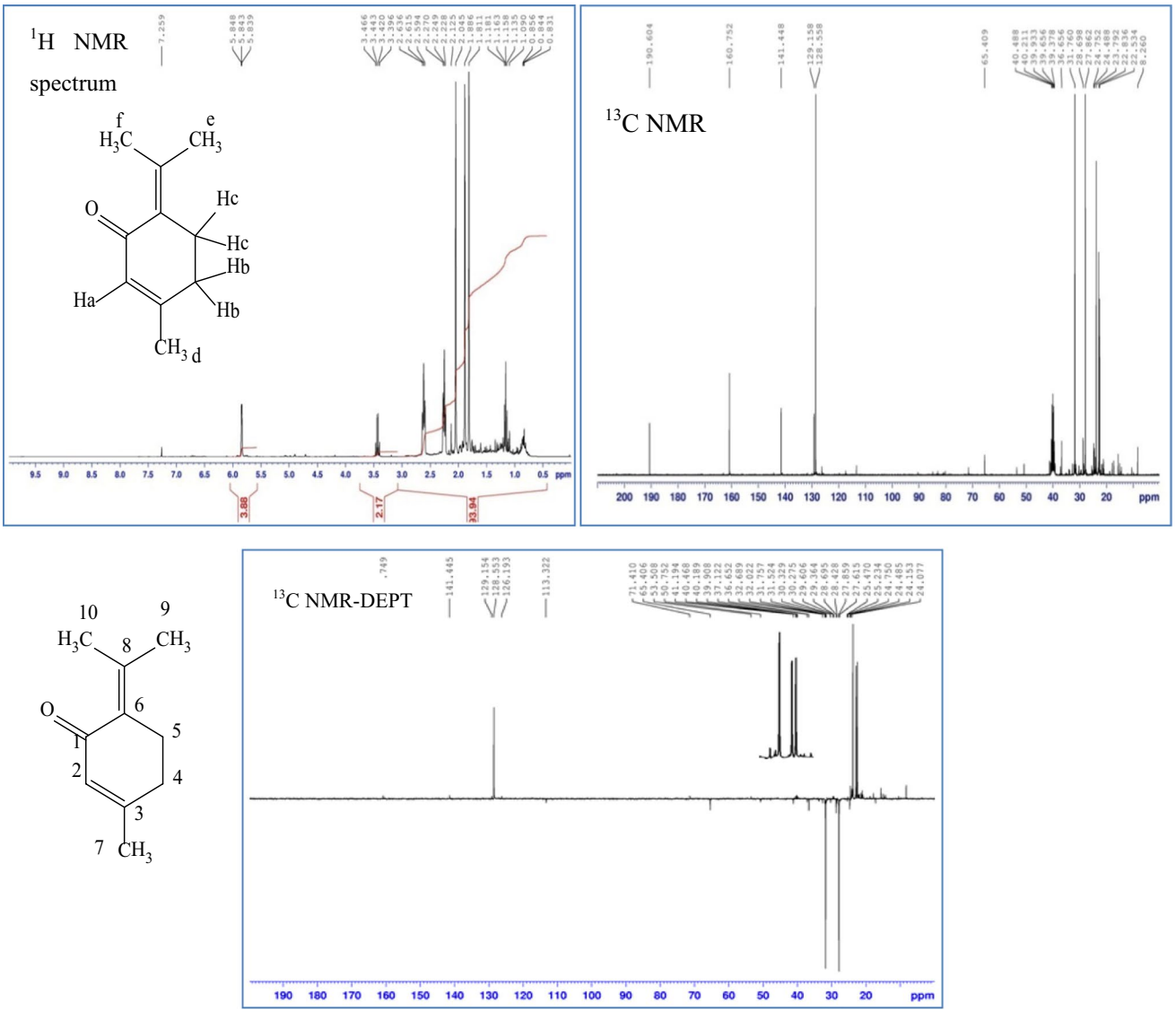

Fig. 8 ^{1}H NMR, ^{13}C NMR and ^{13}C NMR-DEPT spectrum of piperitenone

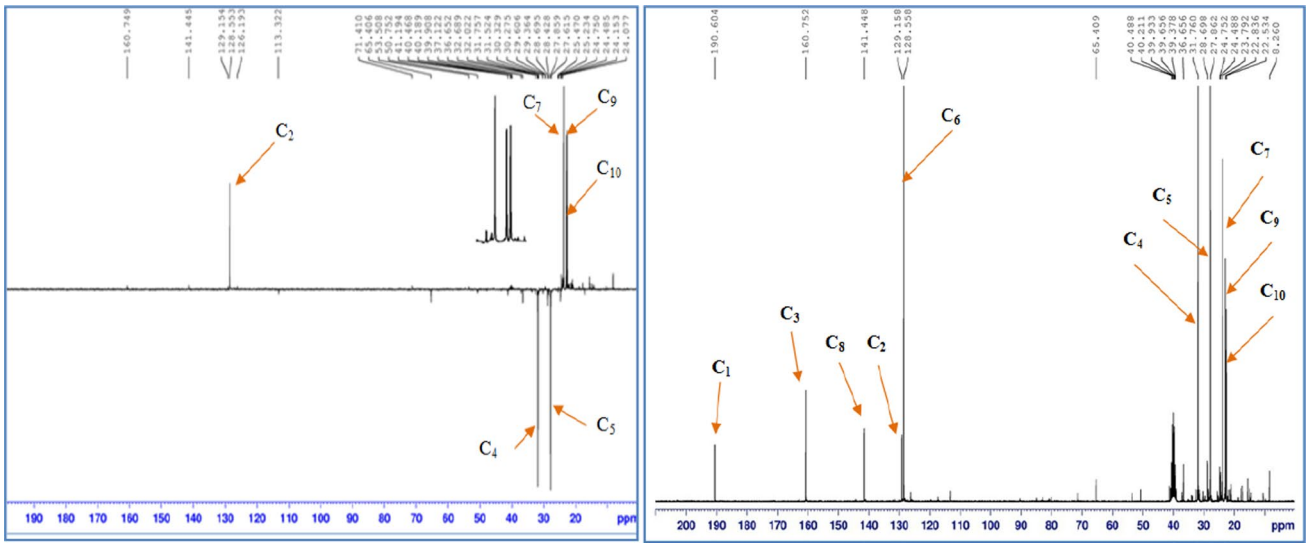

Fig. 9 NMR-DEPT spectrum of ^{13}C and ^{13}C NMR of piperitenone

main compounds of this oil sample with a rate of 71.97% and 26.04%, respectively. In addition, the isolation of these two main compounds was done for the purpose of using their therapeutic properties.

Table 6 Characteristics of the ^{1}H NMR spectrum of piperitenone

Identification of protons	Chemical shifts (δ) in ppm	Number of protons	Multiplicity	Coupling (MHz)
H_a	5.843	1H	Singulet	–
H_b	2.615	2H	Triplet	8.15
H_c	2.249	2H	Triplet	8.15
H_d	2.045	3H	Singulet	–
H_f	1.886	3H	Singulet	–
H_e	1.811	3H	Singulet	–

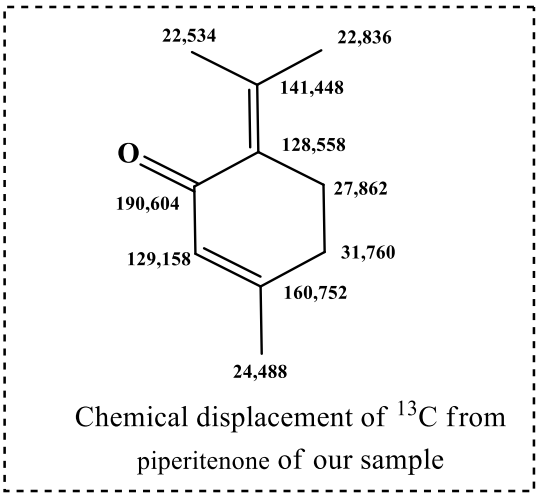

Fig. 10 Identification of piperitenone from the EO of *M. pulegium* by ^{13}C NMR

Table 7 Characteristics of the ^{13}C NMR spectrum of the pulegone

Identification of carbons	Chemical shifts (δ) in ppm
C_1	190.604
C_3	160.752
C_8	141.448
C_2	129.158
C_6	128.558
C_4	31.760
C_5	27.862
C_7	24.488
C_9	22.836
C_{10}	22.534

Acknowledgements We are grateful to Mr M. Ibn Tattou, Professor at the Scientific Institute of Rabat, for the species identification

Data availability The data used in this article to support the finding of this study are included in this article.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present paper.

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