

GC/MS Analysis of Volatile Compounds of the Essential Oil of the Leaves of *Mentha pulegium* growing in Morocco

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Abstract: The essential oils of leaves of *Mentha pulegium*, a traditional herbal medicine in Morocco, were extracted by hydrodistillation and analysed by gas chromatography equipped with flame ionisation detector (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS), to determine the chemical composition of the volatile fraction and identify their chemotypes. Twenty eight constituents were identified. The essential oil yield and the percentage of identified compounds were 1.66% and 97.34% respectively. The major component was piperitone (35.56%), other predominant constituents were: piperitenone (21.18%), α -terpineol (10.89%), pulegone (6.452%), piperitone oxide (4.02%), menthol (3.28%), menthone (3.09%), neomenthol (2.80%), menthofuran (2.15%), isomenthone (1.56%), carvone (1.13%), geranyl acetate (1.06%), germacrene D (1.03%) and limonene (1.02%).

Keywords: *Mentha pulegium*, essential oil, GC/MS, piperitone.

1. Introduction

Aromatic and medicinal plants were used for centuries as remedies for human diseases because they contain chemical components of therapeutic value [1]. In the last few years, plant products and their modified derivatives have been rich sources for clinically useful drugs. According to the World Health Organization (WHO) [2], more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [3]. The family of Lamiaceae contains an extremely wide variety of aromatic plants mainly in temperate countries. Among this rich array of plants yielding essential oils, the genus *Mentha* includes 20 species that spread all over the world. *Mentha pulegium* is one of the *Mentha* species commonly known as pennyroyal. Its native species of Europe, North Africa, Asia Minor, and near East [4] have been traditionally used as antiseptic for treatment of cold, sinusitis, cholera, food poisoning, bronchitis and tuberculosis [5] and also as antifatulent, carminative, expectorant, diuretic, antitussive, menstruate [6]. Plant essential oils and their components have been known to have biological activities, especially antimicrobial [7], antifungal [8] insecticidal [9], antiparasitic, spasmolytic and antioxidant activities [10]. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition [11]. Essential oils were used in ancient Rome, Greece and Egypt and throughout the Middle and Far East as perfumes, food flavours, deodorants and pharmaceuticals [12]. The flowering aerial parts of *Mentha pulegium* has been traditionally used for its

antiseptic properties for treatment of infectious diseases [13].

Morocco is blessed with a rich source of medicinal and aromatic plants, many of which have not been previously investigated for their chemical constituents and biological potentials. *Mentha pulegium* is a plant belonging to the Lamiaceae family, which grows in Morocco and is a potential source of essential oils. In this work we determined the chemical composition and the essential oil yield of *Mentha pulegium* leaves from Morocco.

2. Experimental

Plant material

The leaves of *Mentha pulegium* were collected in Mai 2009 at Skoura near Boulmane (90 km in the south east of Fez. The coordinates: latitude: 35 ° 42 '21 " longitude: 4 ° 32' 31"; altitude: 3200 m). The climate was semi-humid with strong continental influence with an annual average temperature of 20°C. The plants were then isolated from the other specimen and conserved for extraction.

Essential oil extraction

The leaves of *Mentha pulegium* were shade dried (30 days) at room temperature, minced and immediately hydro-distilled (100g) for 2.5 h using a modified Clevenger-type apparatus. The oil was extracted from the distillate with hexane and then dried over anhydrous sodium sulfate. After filtration, the solvent was removed by

distillation under reduced pressure in a rotary evaporator at 35°C and the pure oil kept at 4°C in the dark, until the moment of analysis.

Gas chromatography analysis (GC-FID)

The isolated oil was diluted with hexane, and 1.5 µL was sampled for the gas chromatographic analysis. Trace GC gas chromatograph (ULTRA S/N 20062969, Thermo Fischer) equipped with HP-5MS non polar fused silica capillary column (50 m x 0.32 mm, film thickness 1.25 µm) was used. The operating conditions were as follows: oven temperature program from 40°C (2 min) to 260°C at 4°C/min heating rate, kept for 10 min at the final temperature, split injection ratio 1:20, carrier gas nitrogen, flow rate 1 mL/min, temperature of injector and detector (FID) fixed at 260°C and 280°C, respectively.

Gas chromatography-mass spectrometry analysis (GC/MS)

GC/MS analyses were performed on a Thermo Fischer capillary gas chromatograph directly coupled to the mass spectrometer system (model GC ULTRA S/N 20062969; PolarisQ S/N 210729). HP-5MS non polar fused silica capillary column (50 m x 0.32 mm, 1.25 µm film thickness) was used under the following conditions: oven temperature program from 40°C (2 min) to 280°C at 5°C/min, and the final temperature kept for 10 min; injector temperature 250°C; carrier gas He, flow rate 1 mL/min; the volume of injected sample was 1.5 µL of diluted oil in hexane; splitless injection technique; ionization energy 70 eV, in the electronic ionization (EI) mode; ion source temperature 200°C; scan mass range of m/z 40-650 and interface line temperature 300°C. The constituents of essential oils were identified based on their Kovats Index, calculated in relation to the retention time of a series of alkanes (C₄-C₂₈) as reference products, in comparison with those of the chemical compounds gathered by Adams table [14], and the similarity of their mass spectra with those gathered in the NIST-MS library, or reported in the literature [15-16].

3. Results and Discussion

The essential oil average yield obtained in this study was 1.66%. It is higher than reported for other plants industrially exploited as source of essential oils: Lavender (0.8-1.8%), Mint (0.5-1%), Neroli (0.5-1%), Laurel (0.1-0.35%) [17] and *Lippia rotundifolia* (0.01%) [18], but lower as in case of *Mentha rotundifolia* and *Mentha pulegium* of Morocco, which contained very high level essential oil, (4.33%) and (2.33%), respectively [19]. The yields, related to dry material, varied from sample to sample between 3.0% and 5.1%. In the *Mentha pulegium* leaf essential oil (Table 1), 28 compounds were identified, which made up 97.34% of the total essential oil. This total oil yield was similar to the values reported for *Mentha pulegium* oil of Tunisia: 96.13% [20] and 92.26% [7], and

that of north-western Himalayas, India (between 87-98%) [21], but different from 90.40% found in *Mentha*

The results of chemical composition study of the essential oils are shown on Table (1). The major component was piperitone (35.56%), other components present in appreciable contents being: piperitenone (21.18%), alpha-terpineol (10.89%), pulegone (6.452%), piperitone oxide (4.02%), menthol (3.28%), menthone (3.09%), neomenthol (2.80%), menthofuran (2.15%), isomenthone (1.56%), carvone (1.13%), geranyl acetate (1.06%), germacrene D (1.03%) and limonene (1.02%).

The retention data and chemical composition of *Mentha pulegium* essential oil are presented in Fig. 1 and Table 1.

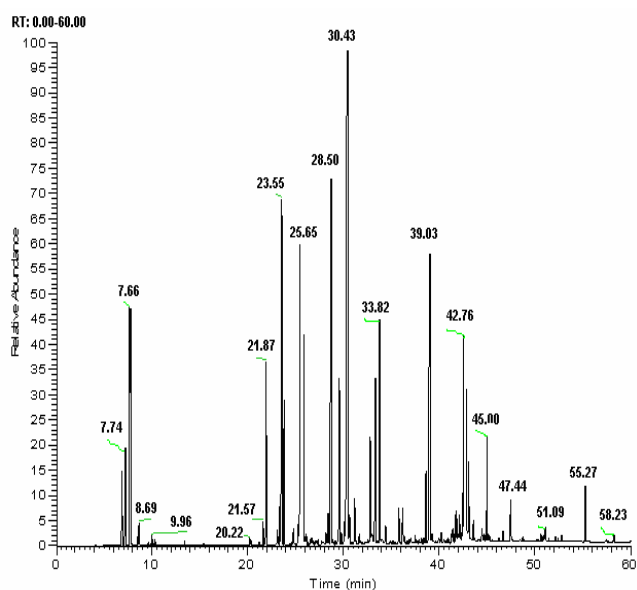


Figure 1. Chromatogram of *Mentha pulegium* essential oil

Intense studies on *Mentha pulegium* essential oil composition have been already published [20, 23, 25-27]. The essential oils composition determined in our study showed a relatively similar pattern to those published for other geographical regions: piperitone (38.00%), piperitenone (33.0%), α -terpineol (4.7%) and pulegone (2.3%), were reported as the major component in an essential oil from Iran [23], menthone, isomenthone, isomenthol, menthol and pulegone in Uruguay [21], pulegone, piperitenone and isomenthone in Bulgaria [24], pulegone (43,3-87,3%), menthone (6.7%), isomenthone (22.6%), piperitone (2.13%) and piperitenone (26.73%) in Algeria [25], and piperitone (1.3-3.2%), pulegone (65.9-83.1%), menthone (8.3-8.7%), isomenthone (3.8-4.0%), neo-menthol (0.7-1.3%), pulegol acetate (0.1-1.2%), γ -terpinene (0.9-1.2%), β -caryophyllene (0.1-0.9%) and β -caryophyllene oxide (0.3-1.9%) in India [21]. On the other part, this composition is different from that of the essential oil of *Mentha pulegium* leaves studied in Tunisia and Morocco, where the major components were menthol (48.56%) [7], and pulegone (73.33%) [19], respectively, and to the results reported for *Mentha rotundifolia* oil in Uruguay, where the major component was piperitenone oxide (80.8%) [25].

TABLE 1. Chemical composition of essential oil of *Mentha pulegium* from Morocco

No.	Compound	^a RT (min)	^b KI	Area (%)	^c M
1	limonene	9.96	1033	1.02	136
2	sabinene	20.22	984	0.82	136
3	camphene	21.57	980	0.01	136
4	α -myrcene	23.01	981	0.01	136
5	α -terpineol	23.55	1175	10.89	154
6	menthyl acetate	25.00	515	0.02	74
7	pulegone	25.65	1220	6.42	152
8	cadinene	26.56	1529	0.01	204
9	piperitenone	28.50	1223	21.12	150
10	neomenthol	29.50	1159	2.82	156
11	piperitone	30.43	1237	35.56	152
12	β -caryophyllène	31.01	1415	0.42	204
13	trans-ocimène	31.91	1040	0.14	136
14	isomenthone	32.02	1192	1.56	354
15	menthofuran	32.50	1155	2.15	150
16	menthol	33.82	1171	3.28	155
17	1-Octen-3-ol	34.50	969	0.09	128
18	germacrene D	35.90	1474	1.03	204
19	geranyl acetate	36.50	1352	1.06	196
20	terpinolene	37.20	1080	0.54	136
21	piperitone oxide	39.03	1230	4.02	168
22	α -Terpinene	40.01	1057	0.05	136
23	Linalyl acetate	42.00	1252	0.02	196
24	menthone	42.76	1142	3.09	154
25	terpinen-4-ol	43.14	1159	0.02	154
26	carvone	45.00	1182	1.13	152
27	1,8-cineole	46.50	1028	0.01	154
28	linalool	51.09	1109	0.03	136
Total Identified Constituents				97.34	
Yield (%)				1.66	

^aRT: Retention time obtained from the chromatogram (Fig1).^bKI: Kovats Index determined by GC-FID on a HP-5MS column.^cMolecular mass

The essential oil content shows variations in plants of different geographical origin and also in different parts of the plant. The composition of *Mentha pulegium* oil collected from the Batak in the Rhodope Mountains (Bulgaria) showed oil yields obtained by water and steam distillation of 1.54% and 1.48%, respectively, and the composition was characterized by a high content of pulegone (42.9-45.4%), piperitenone (21.7-23.1%) and isomenthone (11.3-12.8%) [24]. Similar studies reported pulegone (73.4%) and isomenthone (12.9 %) in Uruguay [22]; pulegone (43.5%) and piperitone (12.2%) in Egypt

[28]; pulegone (41.8%) and isomenthone (11.3%) in Tunisia [29]. In a previous study on the chemical composition of essential oil from *Lippia rotundifolia*, Brazil [18], considerable differences were observed between leaves and flowers: α -pinene (8.7%, and 1.8%), myrcene (5.1%, and 3.6%), limonene (26.0%, and 7.9 %), cis-pinocampone (4.5% and 3.1%) and myrtenal (22.3%, and 16.7%), respectively. The essential oils obtained from leaves and stems from *Mentha pulegium* in Tunisia contained: menthol (46.60-49.86%, and 40.57-51.61%), 1,8-cineole (13.53-17.31%, and 11.10-18.46%), and menthone (11.13-12.34%, and 7.32-20.04%), respectively [7]. The main constituents of the essential oils of flowers, leaves and stem from basil (*Ocimum basilicum* L.) in Turkey, were estragole (58.26%, 52.60% and 15.91%), limonene (19.41%, 13.64% and 2.40%), and p-cymene (0.38%, 2.32% and 2.40%) respectively [30].

4. Conclusion

The aim of this study was to describe the chemical composition of essential oils of *Mentha pulegium* from atlas mean in Morocco. The essential oils, obtained from leaves by hydro-distillation, were analysed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC/MS). Twenty eight compounds were identified and the yield of essential oils was 1.66%. The total identified compounds accounted for about 97.34% of the oil, and were characterized as piperitone (35.56%), piperitenone (21.18%), α -terpineol (10.89%), pulegone (6.452%), piperitone oxide (4.02%), menthol (3.28%), menthone (3.09%), neomenthol (2.80%), menthofuran (2.15%), isomenthone (1.56%), carvone (1.13%), geranyl acetate (1.06%), germacrene D (1.03%) and limonene (1.02%).

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