Chemical Bulletin of "Politehnica" University of Timisoara, ROMANIA
Series of Chemistry and Environmental Engineering
Chem. Bull. "POLITEHNICA" Univ. (Timisoara) ***Volume 55(69), 2, 2010***

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 antiflatulent, 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 1.5µL 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 1mL/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 1mL/min; the volume of injected sample was 1.5µl of diluted oil in hexane; splitless injection technique; ionization energy 70eV, 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 (C_4- C_{28}) 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.

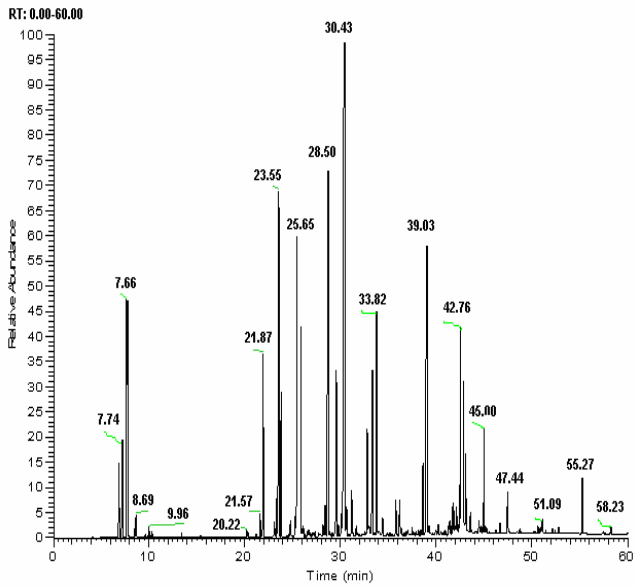

Figure 1. Chromatogram of *Mentha pulegium* essential oil

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%), ß-caryophyllene (0.1-0.9%) and ß-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***	

[a]*RT: Retention time obtained from the chromatogram (Fig1).*
[b]*KI: Kovats Index determined by GC-FID on a HP-5MS column.*
[c]*Molecular 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-pinocamphone (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%), pipéritone 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%).

ACKNOWLEDGEMENTS

We acknowledge the Regional Center of Interface, University Sidi Mohamed Ben Abdellah, Fez, Morocco for the gas chromatography coupled with mass spectrometry (GC/MS), and gas chromatography with flame ionization detection (GC-FID) analysis.

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Received: 10 April 2010
Accepted: 15 December 2010