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ORIGINAL ARTICLE

Aromatic Plants of Morocco: GC/MS Analysis of the Essential Oils of Leaves of *Mentha piperita*

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ABSTRACT

In this work the chemical composition of essential oils obtained from *Mentha piperita* were determined. *Mentha* species from the Lamiaceae family are widely distributed in Morocco. In this study, the essential oils of *Mentha piperita* collected from Atlas median in the region of Meknes (Morocco) were obtained by hydro-distillation of the aerial parts and analysed by gas chromatography equipped with flame ionisation detector (GC-FID) and gas chromatography coupled to a mass spectrometry system (GC/MS) for their chemical composition**.** Twenty nine compounds were identified in leaves oil representing 58.61% of the total oil composition. The yield of essential oil of *Mentha piperita* was 1.02% and the major compound in aerial parts was: Menthone (29.01%), followed by menthol (5.58%), menthyl acetate (3.34%), menthofuran (3.01%), 1,8-cineole (2.40%), isomenthone (2.12%), limonene (2.10%), α-pinene (1.56%), germacrene-D (1.50%), B-pinene (1.25%), sabinene (1.13%) and pulegone (1.12%).

Key words: *Mentha piperita*, chemical composition, GC/MS, GC-FID, Menthone

Introduction

Mentha piperita. (Fam. Lamiaceae) is the species found in Morocco. The leaf essential oil of *Mentha piperita* has been reported in varying details from Russian [1], India [2], Greece [3] and from Yugoslavia [4]. Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value [5]. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [6]**.** Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use [7]. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition [8]. Also the Essential oils are used in traditional medicine for their antiseptic action, are constituted 1% of plant secondary metabolites and are mainly represented by terpenoids, phenypropanoids or benzenoids, fatty acid derivatives and amino-acid derivatives [9]. Plant essential oils and their components have been known to exhibit biological activities, especially antimicrobial [10], antifungal [11,12], Antibacterial [13,14,15], Antimycotic [16] and antioxidant activities [17]. Essential oils were used in ancient Rome, Greece and Egypt and throughout the Middle and Far East as

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perfumes, food flavours, deodorants and pharmaceuticals [18]. Essential oils have many therapeutic effects, which include vasodilatation, irritation, hypersecretion (in saliva and sweat secretions), hyperperistaltism, the stimulation of heart muscle, and they aid the distribution of drugs and antiseptics [19]. The essential oils which were utilised centuries ago in cosmetics usually show interesting biological features. The oils also help increase the flow of digestive fluids, improve digestion and eliminate gas and stomach cramping [20]. 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 of *Mentha*, includes 20 species that spread all over the world. *Mentha piperita,* commonly known as pennyroyal, is traditionally used in the treatment of flatulent dyspepsia and intestinal colic due to its carminative and antispasmodic properties [21].

In the light of this work we have determined, the chemical composition of essential oils of leaves of *Mentha piperita* collected in the region of Meknes from Morocco.

Materials and methods

Vegetal material and Essential oil extraction:

The visible parts of *Mentha piperita* have been collected during March 2009 in the region of Meknes, the climate is semi-humid with strong continental influence having an annual average temperature of 20°C. The plants were then isolated from the other specimen and conserved for extraction.

The essential oils were extracted by hydro-distillation using an apparatus of Clevenger type. The extraction took 3 hours for mixing 250g of plants in 1600 ml of distilled water. After filtration the solvent is eliminated by pressure distillation reduced in rotary evaporator and pure oil was stored at 4°C in obscurity till the beginning of analysis. The essential oils yield is demonstrated by the oil quality (in ml) obtained for 100g of dried leaves.

Gas chromatography analysis (GC-FID and GC/MS)

The chemical composition of leaf oil from *Mentha piperita* in Morocco was determined by GC-FID and GC-MS using a CP-SIL 5HP fused silica column.

The GC (Trace GC ULTRA, Thermo Fischer) analysis equipped with flame ionisation detector (GC-FID), Varian capillary column (CP-SIL 5HP, 60m length, 0.32mm of diameter and Film thickness 0.25 µm). The column temperature was programmed from 40 to 280°C for 5°C/min. The temperature of the injector was fixed to 250°C and the one of the detector (FID) to 260°C. The debit of gas vector (nitrogen) was fixed to 1ml/min.The volume of injected specimen was 0.5µl of diluted oil in hexane solution (10%). The percentage of each constituent in the oil was determined by area peaks.

The identification of different chemical constituents was done by gas phase chromatography (Ultra GC Trace) coupled with mass spectrometry (PolarisQ, Thermo Fischer) (GC/MS). The utilised column was; Varian capillary column (CP-Sil 5 HP; 60m length, 0.32mm of diameter and Film thickness 0.25 µm). The column temperature was programmed from 50 to 280°C for 3°C/min. The temperature of the injector was fixed to 240°C and the one of the detector to 200°C. Electrons impact: 70ev. The debit of gas vector (Helium) was fixed to 1.5ml/min.The volume of injected specimen was of 1µl of diluted oil in hexane solution (10%). The constituents of essential oils were identified in comparison with their Kovats Index, calculated in relation to the retention time of a series of lineary alkanes (C_4- C_{28}) with those of reference products and in comparison with their Kovats index with those of the chemical components gathered by Adam [22] and in comparison with their spectres of mass with those gathered in a library of (NIST-MS) type.

Results and discussion

The retention time and chemical composition of essential oils of *Mentha piperita* are presented in Figure 1 and Table 1.

The compounds of *Mentha piperita* from Morocco are listed in order of their elution on the CP-Sil 5 HP column (Figure 1). In total, twenty nine volatile constituents, representing 58.61 % of the total composition, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were Menthone (29.01%), followed by menthol (5.58%), menthyl acetate (3.34%), menthofuran (3.01%), 1,8-cineole (2.40%), isomenthone (2.12%), limonene (2.10%), α-pinene (1.56%), germacrene-D (1.50%), B-pinene (1.25%), sabinene (1.13%) and pulegone (1.12%). The essential oils yield of *Mentha piperita* collected from region of Meknes (Morocco) was 1.02%. It is relatively higher than other plants industrially exploited as a source of essential oils: *Artemisia herba-alba* (0.59%), *Artemisia absinthium* (0.57%) and *Artemisia pontica* (0.31%) [23], Thymus (1%) [24], lavender (0.8-2.8%), menthe (0.5-1%), néroli (0.5-1%) and Laurel (0.1-0.35%) [25], Artemisia (0.65%) [26] and Tetraclinis (0.22%) [27]. In this study the yield is low to those of *Laurus Nobilis* essential oils analyzed in Morocco by [28], which the yield was 1.86% and the yield was (1.62%) of *Juniperus phoenicea* essential oil [29].

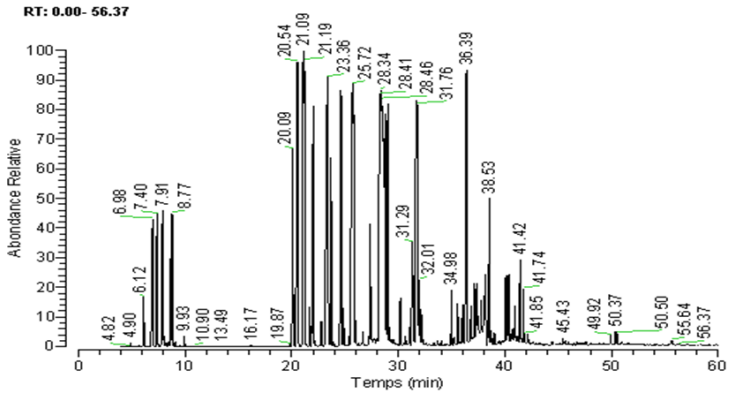

Fig. 1: Chromatogram of *Mentha piperita*

Table 1: Chemical composition of leaves of essential oils of *Mentha piperita* from Morocco

*RI	Constituents	**Area (%)	*** Mass range (m/z)	Method of identification
1147	Menthone	29.01	(154),112,41,69,55,43,56,70,27,39,139	RI, GC/MS
1163	Menthol	5.58	(155),71,81,95,55,41,82,69,123,96,67	RI, GC/MS
486	Menthyl acetate	3.34	(74),43,74,15,42,59,31,29,44,14,28	RI, GC/MS
1141	Menthofuran	3.01	(150),108,150,79,109,39,77,41,91,51,53	RI, GC/MS
1058	1,8-Cineole	2.40	(154),43,93,81,71,69,84,68,108,41,55	RI, GC/MS
2545	Isomenthone	2.12	(354),55,43,41,57,69,56,83,81,113,97	RI, GC/MS
1017	Limonene	2.10	(136),68,93,39,67,41,27,53,79,94,92	RI, GC/MS
947	α -Pinene	1.56	(136),93,91,39,121,77,92,79,43,41,105	RI, GC/MS
1514	Germacrene D	1.50	(204),161,105,91,41,119,79,81,93,77,27	RI, GC/MS
933	β-Pinene	1.25	(136),93,91,69,39,77,92,79,53,41,27	RI, GC/MS
896	Sabinene	1.13	(136),93,41,91,77,79,39,27,69,94,43	RI, GC/MS
1220	pulegone	1.12	(152),152,81,67,109,82,41,137,69,95,55	RI, GC/MS
942	Camphene	1.09	(136),93,79,91,77,41,121,67,27,107,39	RI, GC/MS
1147	Menthone	1.08	(154),112,41,69,55,43,56,70,27,39,139	RI, GC/MS
957	Myrcene	1.07	(136),41,93,69,39,27,53,79,77,67,91	RI, GC/MS
1163	Neomenthol	0.50	(156),71,95,81,41,55,43,69,82,138,57	RI, GC/MS
1441	Cadinene	0.40	(204),161,189,204,105,91,133,119,95,41,55	RI, GC/MS
1170	Piperitone oxide	0.07	(168),69,55,41,97,72,43,71,139,126,39	RI, GC/MS
1493	β-caryophyllene	0.05	(204),93,133,91,41;79,69,105,107,120,77	RI, GC/MS
975	Cis-ocimene	0.04	(136),93,41,79,39,91,77,92,27,80,53	RI, GC/MS
968	1-Octen-3-ol	0.04	(128),57,72,29,41,55,27,85,58,39,43	RI, GC/MS
997	α-Terpinene	0.03	(136),93,91,136,121,77,92,79,43,41,105	RI, GC/MS
1271	Linalyl acetate	0.03	(196),93,43,41,69,80,121,68,55,71,79	RI, GC/MS
1051	β-Terpinolene	0.02	(136),93,121,91,136,79,77,105,39,41,107	RI, GC/MS
1157	Piperitone	0.02	(152),82,110,39,41,27,95,137,109,54,152	RI, GC/MS
1137	Borneol	0.01	(154),95,41,110,93,55,67,139,121,96,69	RI, GC/MS
1136	Terpinen-4-ol	0.01	(154),71,111,93,43,86,41,69,55,68,154	RI, GC/MS
1081	Linalool	0.01	(136),71,41,43,93,55,69,80,39,121,27	RI, GC/MS
1175	α-terpineol	0.01	(154),59,93,121,136,81,43,68,95,67,41	RI, GC/MS
1223	piperitenone	0.01	(150),150,107,135,82,109,108,122,91,121,79	RI, GC/MS
Total Identified Constituents (%)		58.61%		

Yields (%) 1.02* RI: Retention Index was determined by GC-FID on a CP-Sil 5 HP column.
** Air (%) was determined by mass spectrometry (PlarisQ)
*** Mass range (m/z) was determined by mass spectrometry (PlarisQ).

The chemical compositions revealed that this leaves had compositions similar to those of other *Mentha piperita* essential oils analyzed in Serbia by [11], which the major component was menthol (37.4%), menthyl acetate (17.4%) and menthone (12.7%). Menthol and menthone were the main components of *Mentha piperita* [10]. Menthol (64.0%), menthyl acetate (9.2%) and menthofuran were dominant in *Mentha piperita* study in Italy by [30]. Also, menthanol (36.24%) and menthone (32.42%) were the major compounds of the *Mentha piperita* essential oil study iran [12]. Menthon (44.1%), menthol (29.5%), menthylacetate (3.8%) and menthofuron (0.9%) were tke major compounds of % *Mentha piperita* from Turkey [31]. Contrary it's different to the composition of essential oil of leaves of *Mentha piperita* study in Korea which the major component were linalyl acetate (28.2%), menthol (33.4%), 1,8-cineole (46.1%), limonene (64.5 and 94.2%), and p-menth-2-en-ol (34.5%) [17] and it's different to the composition of essential oil of *Mentha arvensis* study in India by [16] which de

major compounds were: menthol (71.40%), p-menthone (8.04%), iso-menthone (5.42%) and neo-menthol (3.18%). α-terpene (20.11%), pipertitinone oxide (17.10%) and trans-carveol (19.48%) were the main components of the oils of *Mentha piperita* [13]. The Chemical composition of *Mentha piperita* L essential oil study in Iran, contained α-terpinene (19.7%), isomenthone (10.3%), trans-carveol (14.5%), pipertitinone oxide (19.3%), and β-caryophyllene (7.6%) as the major compounds [32]. Intensive research on the chemical characteristics has been conducted on this species [33,34,35]. Previous reports [36,37] on the composition of its essential oil showed that pulegone was the main constituent, and its percentage ranged from 25 - 92%. The leaves essential oil of *Mentha piperita* has been reported in varying detail [38,32,39,34,40].

The essential oil content shows variations in plants of different geographical origin and also in different part of the tree: [41]; studied the composition of *Juniperus phoenicea* oil collected from the Portugal, Spain and Greece, they reported that the yields and the total oil obtained were (0.41% and 98.3%), (0.66% and 99%) and (0.58% and 88%) respectively and the composition is characterized by a high content of α-pinene (34.1%, 53.5% and 41.8%), β-phellandrene (19.2%, 5.9% and 3.5%) and β-caryophyllene (0.22%, 1.0% and 0.5%). In our studies on the chemical composition of fresh leaves *of Mentha spicata* from Italy [33], considerable differences were observed in the essential oil composition between Low and High-Friuli: 24 compounds have been identified in the essential oil of *Mentha spicata* from Low-Friuli. The monoterpenoids represent 80% of the total oil content. Carvone (3302 µg/g) and limonene (964 µg/g) are the most prominent components. 29 compounds have been identified in *Mentha spicata* from High-Friuli. The monoterpenoids represent 90.4%. The major components are carvone (7273 µg/g), and limonene +1.8 cineole (889 µg/g). [42], Studying of essential oil variations in leaves of *Mentha* species, the data indicated that was significant difference between essential oil yields in leaves of mint species. Furthermore, the essential oils, obtained from flower, leaves and stems from basil (*Ocimum basilicum L.)* from Mersin province (Bu¨yu¨keceli-Gu¨ lnar) in Turkey contained: 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%) [43]. In our studies on the chemistry of Uruguay [44], considerable differences were observed in the essential oil composition between *Mentha rotundifolia* and *Mentha pulegium*: Piperitenone (80.8%) and Pulegone (73.4%) and the total constituents identified is 93.5% and 99.3% respectively.

Conclusion:

In conclusion, the present study has been concerned with determining the chemical composition characteristics of essential oils extracted from the leaves of *Mentha piperita*, collected in the Meknes region of Morocco**.** The chemical analyses, by GC/MS, GC-FID, have allowed us to identify around 58.61% of the total volatile products for *Mentha piperita* and the yield of essential oils was 1.02%. Twenty nine volatile compounds were identified and the major constituent in leaves was Menthone (29.01%), followed by menthol (5.58%), menthyl acetate (3.34%), menthofuran (3.01%), 1,8-cineole (2.40%), isomenthone (2.12%), limonene (2.10%), α-pinene (1.56%), germacrene-D (1.50%), B-pinene (1.25%), sabinene (1.13%) and pulegone (1.12%). This yield of the plants essential oil that has been studied was important.

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