Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (4):146-150
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Effect of Geographic Locations on Chemical Composition of *M. Spicata* L. Essential oils from Moroccan Middle-Atlas

Nadia Zekri[1, 2], Hanane Elazzouzi[1,2], Aziz Drioche[2], Ahmed Satrallah[3], Mohamed Alaoui El Belghiti[1] and Touria Zair[2]

[1]Laboratory of Chemistry - General Physics – University Med V, Faculty of Sciences, 4- Avenue IbnBattouta,B.P.1014 RP, Rabat, Morocco
[2]Laboratory of Chemistry of Bioactive Molecules and Environment, University Moulay Ismail, Faculty of Sciences, BP 11201.Zitoune, Meknes, Morocco
[3]Laboratory of Chemistry, Institute of Agronomy and veterinary –Hassan II, Avenue AllalEl Fassi, Madinat , Al Irfane, B.P. 6203, Rabat, Morocco

---

ABSTRACT

Mentha is one of the most common herbs which have been known for its medicinal and aromatherapeutic properties since ancient time. Mentha spicata L. is among the most used species in Morocco to flavor tea and for industrial and pharmaceutical purposes. The objective of this work was to evaluate the effect of origin region on yields and chemical composition of M. spicata L. essential oils (EOs). Leaves of spearmint were collected from Meknes and Azrou (Middle-Atlas). The extraction of essential oils was performed by hydro-distillation using Clevenger apparatus and then their chemical composition was identified by gas chromatography coupled with mass spectrometry (GC-MS). The results of chromatographic analyses have shown diverse chemical profiles of studied species. The spearmint EO from Azrou (2.4%) presented the higher yield than that from Meknes (1.54%). However, both EOs were dominated by carvone and limonene with higher rates of EO from Meknes (74.94–13.17%) than that from Azrou (71.56-10.50%) respectively. These monoterpenes are considered as interesting molecules that are needed in industrial and pharmaceutical goals.

Keywords: Lamiaceae, *Mentha spicata* (L), essential oil, carvone.

INTRODUCTION

Mentha spicata L. (*Mentha Viridis* L.), also known as spearmint or Moroccan mint (El Hassani et al., 2010), belongs to the Lamiaceae family. It is called in Arabic "Naânaa" or "Liqama" and "mint" in English. The market for mints exceeded trillions of dollars [1]. The production of mint essential oils (EOs) is the second largest behind those of the *Citrus* about 4,000 t / year of peppermint EOs and 2000 t / year of spearmint EOs. The use of mint goes back to the Egyptians who used it as perfume and culinary herb. Later, the Romans were introducing it in the dental hygiene. The antiseptic properties and antispasmodic of mint EO are widely known recently. Moreover, experiments have shown that menthol is present in mint EO exhibits an antifungal effect against various plant pathogens.

The area occupied by the mint in Morocco reached 3300ha in several regions, the Settat region (57 km from Casablanca) shelters the most concentrated area for mint parcels. The production is estimated around 8400 hectares [2]. The plant is also known for its ability to improve memory [3]. The extract of boiled leaves is used to relieve flatulence, dizziness and remedy for inflammation, bronchitis, and to control vomiting during pregnancy [4].

M. spicata L. contains many phenolic compounds, flavonoids, coumarins, alkaloids, steroids and terpenoids [5;6;7]. It acts as analgesic, stimulant, expectorant and carminative and also exhibits antiseptic, antispasmodic and diuretic properties. The spearmint oil is also of economic importance and widely used in the pharmaceutical, cosmetic, food, confectionery and beverage industry [8;9].

Spearmint contains monoterpenoids as carvone, limonene, menthone, menthol, pulegone and dihydrocarveol. The major constituent is carvone along with some other compounds as limonene, phellandrene, dihydrocarveol, cineol and linalool [10]. The concentration of carvone in the spearmint oils varies according to the genetic structure, climatic, agricultural practices and the region of origin [1].

Keeping in view the medicinal importance of this plant and wide use by population asa tea flavoring agent in Morocco, the valorization of this species remains not enough compared to its large uses. In this respect, the evaluation of the variation in the chemical composition of *M. spicata* L. essential oils from some regions in Middle-Atlas, has been the main objective of the present work.

MATERIALS AND METHODS

Plant material

Spearmint leaves were collected on July from two regions in Middle-Atlas: Meknes (Latitude: 33° 53′ 42″; Longitude: 5° 33′ 17″; Altitude: 522m)and Azrou(Latitude: 33° 25′ 59″; Longitude: 5° 13′ 01″; Altitude: 1278m). The species from Meknes is harvested whereas that from Azrou is spontaneous. The climate is semi-humid with strong continental influence with an annual average temperature of 20°C.

Dried leaves of spearmint (100g) were subjected to steam distillation for 3 h using a Clevenger-type apparatus. The essential oils were dried with anhydrous sodium sulphate and collected and stored in sterile dark containers at 4°C until use. For calculation of essential oil yields, three replicates were performed for each plant material.

Chromatographic analysis of spearmint essential oils

The chromatographic analyses were performed using a gas chromatograph Hewlett Packard (HP 6890 series) type equipped with a HP-5 capillary column (30m x 0.25 mm x 0.25 microns film thickness) , a FID detector set at 250 ° C and fed with a gas mixture H2/air. The mode of injection is split; the carrier gas used is nitrogen with a flow rate of 1.7 ml / min. The column temperature is programmed at a rate of 4 mounted ° C / min from 50 to 200 °C for 5 min. The unit is controlled by a computer system type "HP ChemStation" managing the operation of the device and to monitor chromatographic analyzes. GC-MS was carried out on chromatograph Hewlett Packard (HP 6890) coupled to a mass spectrometer (HP 5973 series). Fragmentation is performed by electron impact at 70eV. The used column was a capillary-type HP 5SM (30 mx 0.25 mm x 0.25 mm). The column temperature is programmed at a rate of 4 mounted °C/ min from 50 to 200 °C for 5 min. The carrier gas is helium with a flow rate set at 1.7 ml / min. The injection mode is split type.

The constituents of the EOs were identified by comparison of their Kovàts Index (KI)[11], calculated in relation to the retention time of a series of linear alkanes (C_7 - C_{40}). The calculated indices were compared with those of the chemical constituents gathered by Adams [12]. Their mass spectra were then matched with those stored in the NIST library / EPA / NIH MASS SPECTRAL LIBRARY; Version 2.0, 2002.

RESULTS AND DISCUSSION

-Yields of spearmint essential oils

The yields have been calculated from dry plant material. This is the essential oil of wild mint from Azrou which has the highest yield (2.4%) compared to that grown in Meknes (1.54%). Similarly, the yields of EO from 4 Turkish regions are between 2.41 and 2.74% [13]. In addition, [1] have found that spearmint EOs from Siwa (Egypt) have produced very attractive yields that range between 1.59 and 3.90%.These rates are considered as higher compared to those reported in the literature (< 2%)[14;15]; that is consistent with those obtained by EOs extracted from mint originated of Cairo (Egypt), they do not exceed 1.30% [1], and that calculated from the essence of Tunisian mint (1.1%) [16], and Indian one (0.65%) [17]. Recently, Zhao et al. [18], on their work on variation in chemical composition of spearmint EOs originated from seven Chinese provinces, have found that the yields varied from 0.5 to 0.8%. Therefore, the change in the region of origin leads to an increase or decrease in yields, this is due to the geographical location and climatic conditions.

-Variation in chemical composition of EO spearmint according to the region origin

The chromatographic analyses of spearmint essential oils have identified thirty three compounds each other that represent approximately 97.65% of the total chemical composition for Meknes and 96.07% for Azrou (Table 1). The monoterpenes are more abundant in the two species that sesquiterpenes but the EO from Meknes which contains the highest percentage in monoterpenes (93.42%) compared to Azrou (88.75%). Conversely, it contains 4.81% against 4.05% of sesquiterpenes in the essence from Meknes.

Table. 1: Chemical composition of EO *M. spicata* L. from Azrou and Meknes (Middle-Atlas)

Compound	Calculated Kovàts Index	Aire%	
		Meknes	Azrou
α-pinene	933	0.51	0.37
β-pinene	975	0.79	0.58
p-mentha-1(7),8-diene	994	0.07	---
limonene	1028	**13.17**	**10.50**
Ocimene (E)-β	1059	0.08	-----
p-mentha-3,8-diene	1065	**1.01**	**0.79**
Terpinolene	1098	0.08	0.10
Hydrocarbon monoterpenes		**15.71**	**12.34**
Borneol	1165	0.41	0.78
terpinen-4-ol	1177	0.38	0.65
α-terpineol	1191	0.16	0.12
trans-4-caranone	1195	**1.01**	**2.74**
trans-carveol	1227	0.16	0.22
Carveol cis	1232	0.06	-----
Pulegone	1238	0.46	0.16
Carvone	1242	**74.91**	**71.56**
p-cymen-7-ol	1287	0.07	0.08
γ -terpinen-7-al	1292	0.09	0.10
Oxygenated monoterpenes		**77.71**	**76.41**
iso-dihydrocarveol acetate	1329	0.10	**2.07**
cis-carvyl acetate	1364	0.08	0.44
Acetates		**0.18**	**2.51**
α-Yalengene	1375	-----	0.08
β-Bourbonene	1384	**0.89**	**1.04**
β-Elemene	1391	0.06	0.09
β-Caryophyllene	1418	**0.76**	**0.76**
β-Coparene	1428	0.13	0.16
6,9-Guaiadiene	1444	0.13	0.15
Spirolepechinene	1452	0.10	0.12
Cis-Cadina-1(6),4-diene	1462	0.25	0.15
Cis-Muurola-4(14),5-diene	1466	---	0.11
Germacrene D	1479	**0.87**	**0.61**
Trans-calamenene	1522	0.24	0.33
Hydrocarbon sesquiterpenes		**3.43**	**3.6**
Spathulenol	1575	0.08	0.14
Trans-Sesquisabinene hydrate	1583	----	0.13
Globulol	1590	0.14	0.23
1,10-di-epi-Cubenol	1614	0.11	0.17
Hinesol	1640	0.17	0.30
α-Cadinol	1654	0.12	0.24
Oxygenated sesquiterpenes		**0.62**	**1.21**
Total		**97.65**	**96.07**

Bold: abundance

In general, there is a slight modification of the chemical composition of the *M. spicata* L. oils (Table. 1). This change is remarkable at major compounds (Carvone and Limonene). Its rates are in the range of 74.91 and 13.17% for EO from Meknes and 71.56% and 10.50% for that from Azrou. Other compounds are present in both species but EO from Meknes has shown higher levels than Azrou: Trans-4-caranone (2.74-1.04%), iso-dihydrocarveol acetate (2.07-0.10%) and α-bourbonene (1.04-0.89%) respectively. However, compounds such as α-yalengene (0.08%), cis-Muurola-4 (14), 5-diene (0.11%) and trans-sesquisabinene hydrate (0.13%) are present only in the EO from Azrou.

The chemical composition of the studied oils agrees with that reported by some researches previously conducted. The spearmint oil obtained from Saîs valley (Morocco) was rich of carvone (73.01%), limonene (8.54%) and 1,8-cineole (6.70%) [19] but that from Greece was characterized by the predominance of carvone (71.8%) followed by 1,8-cineole (9%) but devoid of limonene. carvone (29.00%) was the major component of spearmint oil from Errachidia (South-East of Morocco) followed by trans carveol (14.00%)[20]. The oil, extracted from Tunisian M. *spicata* L., is also dominated by carvone and limonene with less important rates of carvone (40.8%) and higher amount of limonene (20.8%) than our plants followed by 1,8-cineole (17%)[17]. Moreover, Chauhan *et al.* [21] reported that the main constituents were carvone (76.65%), limonene (9.57%), cis-dihydrocarvone (2.04%), and 1,8-cineole (1.93%). Lately, Padalia *et al.* [22] studied the chemical composition of 16 cultivars of *Mentha* from the western Himalayan region (China) and reported that carvone (51.3%–65.1%), limonene (15.1%–25.2%), β-pinene (1.3%–3.2%), and 1,8-cineole (≤0.1%–3.6%) are the major components in five cultivars.

Furthermore, the essential oil from leaves of Algerian spearmint, was characterized by predominance of carvone (59.40 %), while limonene was present in appreciate percentage (6.12%) [23]. Likewise, in studies of Soković et al. [24], carvone (49.5 %) is also the main constituent of spearmint EO, followed by menthone (21.9 %) and limonene (5.8 %). In contrast, in studied *M. spicata* essential oils wild as well harvested, menthone was absent. In the north Indian plains, carvone content varies between 45.9% and 77.1% [25].

The amounts of carvone also varied in the essential oils of spearmint growing in different countries, such asCanada (59%–74%) [26], Egypt (46.4%–68.55%) [27; 28], Turkey (78.35%–82.2%) [29; 30], China (55.45%–74.6% [31], Bangladesh (73.2%) [8], and recently Zhao *et al.* [19] (46.7%–65.41%).

Although, lower rates of carvone were reported in the spearmint essential oil from Iran (22.4%) [29], while Rasooli et al. [30] have identified a different chemical composition of spearmint oil, in other region from Iran, which α-terpinene (19.7%), piperitone oxide (19.3%), isomenthone (10.3%), and β-caryophyllene (7.6%) were the main constituents. A species from Turkey was rich in linalool (82.8%)[31] (Baser et al., 1999), whereas the Serbian *M. spicata* essential oil were dominated by menthone (21.9%), carvone (49.5%), limonene (5.7%), 1,8-cineole (3%), and β-mycrene (2.3%)[24] (Sokovic et al., 2009). In the Zagazig Region (Egypt), Omar *et al.* [32] in their studies have also reported that menthone (32.43%), 1,8-cineole (18.79%), *cis-iso*pulegone (16.65%), pulegone (10.01%), β-pinene (7.12%), α-cadinol (5.30%), and α-pinene (5.03%) were the main components of the *M. spicata* essential oil harvested from *M. spicata* essential oil harvested from Sfax (South of Tunisia) showed a different chemical profile which the main components were identified as L-menthone (32.74%), pulegone (26.67%), 1,8-cineole (11.16%), and menthol (11.42%)[33].

Furthermore, essential oils, richer in carvone, are widely used as spices in the flavor and fragrance industries in Europe; carvone may also be used as a pesticide, food flavoring, feed flavoring, in feed additive, in personal care products and as veterinary medicine [34]. Indeed, limonene is also used to make medicine, to promote weight loss, treat cancer, and treat bronchitis. In foods, beverages, and chewing gum, limonene is used as a flavoring. In pharmaceuticals, it is added to help medicinal ointments and creams penetrate the skin. In manufacturing, it is used as a fragrance, cleaner (solvent), and as an ingredient in water-free hand cleanser.

The differences in oil content and composition may be attributed to several factors: the method used, the used plant parts, the products and reagents used in the extraction, the environment, the plant genotype, geographical origin, the harvest period of the plant, the degree of drying, the drying conditions, temperature and drying time and the presence of parasites, viruses and weeds [28].

CONCLUSION

Spearmint is a popular herb. It has been used to impart flavor, food preservations, and as a tradition medicine. The present work indicated that the geographic location influences the yields and chemical compositions of studied essential oils spearmint, either wild from Azrou or harvested from Meknes. The chemical profiles were slightly different. The yield of EO from Azrou was very important than that from Meknes. Both of them were rich of many components but EO from Meknes shown the highest rates than EO from Azrou: carvone (74.91-71.56%) and limonene (13.17- 10.50%) respectively, accompanied to other constituents which occurred in both plants at diverse percentages or absent in one of them. Therefore, the variation in the yields and the chemical composition depends to the region of origin with other factors as climatic conditions.

This species seems a source of interesting components like carvone and limonene that are widely employed for therapeutic, industrial and pharmaceutical purposes.

Due to the importance of this species for populations in the world, more researches are needed on isolation of carvone and limonene that exhibit biological activities mainly limonene that proved its efficiency to block cancer-forming chemicals and kill cancer cells in the laboratory but there are few informations if this occurs in humans.

REFERENCES

[1] Edris A.E, Shalaby A.S , Fadel H.M, Abd El-Wahab M.A. *Eur Food Res Technol*. **2003**, 218 :74-78

[2] El Fadl, A. and N. Chtaina, **2010**. Etude de base sur la culture de la menthe du Maroc. Programme Régional de lutte intégrée contre les organismes nuisibles (Integrated Pest Management) au Proche Orient. Office National de sécurité sanitaire des produits alimentaires (ONSSA).
[3] Adsersen A, Gauguin B, Gudiksen L, Jager AK. *J Ethnopharmacol*. **2006**, 104, 418–22.
[4] Kumar, A., and Chattopadhyay, S. *Food Chem*. **2007**, 100:1377–1384.
[5] Zheng, J., Wu, L. J., Zheng, L., Wu, B., Song, A. H. *J Asian Nat Prod Res*. **2003**, 5, 69–73.
[6] Bimakr, M., Russly, A.R., Taip F.S, Ganjloo, A., Saleena, F., Md Salleh, L., Selamat, J. Hamid, A and Zaidul I.SM. *Food and Bioproducts Processing*. **2011**, 89, 67-72.
[7] N. Zekri, S. Amalich, M. Alaoui El Belghiti, T. Zair, *Adv. Environ. Biol*. **2014**, 8(17),10–18.
[8] Chowdhury, J.U.; Nandi, N.C.; Uddin, M.; Rahman, M. *Bangl. J. Sci. Ind. Res.* **2007**, *42*, 79–82.
[9] Kanatt, S.R., R. Chander and A. Sharma, **2007**. *Food Chemistry*, 100: 451-458.
[10] Ullah N, Khurram M, Usman Amin M, Afridi H.F,Khan F.A, Khayam S.M.U, Ullah S, Najeeb U, Hussain J and Khan M.A. *Journal of Applied Pharmaceutical Science*. **2011**, 01 (07), 2011:72-76.
[11] Kovàts E. *Adv. Chromatogr*. **1965**, 1, 229–247.
[12] Adams R.P., Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectroscopy (Allured, Carol Stream, **2007**).
[13] Telci, I.; Demirtas, I.; Bayram, E.; Arabaci, O.; Kacar, O. *Ind. Crops Prod.* **2010**, *32*, 588–592.
[14] Kokkini S, Vokou D. *Econ Bot*. **1989**, 43, 192–202.
[15] Kokkini, S.; Karousou, R.; Lanaras, T. *Biochem. Syst. Ecol.* **1995**, *23*, 425–430.
[16] Snoussi M, Noumi E, Trabelsi N, Flamini G, Papetti A and De Feo V . *Molecules* **2015**, *20*, 14402-14424.
[17] Vermaa R.S, Pandeyab V, Padaliaa R.C, Saikiac D & Krishna B. *Journal of Herbs, Spices & Medicinal Plants.* **2011**, 17, Issue 3.
[18] Zhao, D.; Xu, Y.W.; Yang, G.L.; Husaini, A.M.; Wu, W. *Ind. Crops Prod.* **2013**, *42*, 251
[19] El Hassani, F.Z., A. Zinedine, S. Mdaghri Alaoui, M. Merzouki and M. Benmlih. *Industrial Crops Products*, **2010**, 32: 343-348.
[20] Znini, M.; Bouklah, M.; Majidi, L.; Kharchouf, S.; Aouniti, A.; Bouyanzer, A., Hammouti, B.; Costa, J., Al-Dyab, S.S. *Int. J. Electrochem. Sci.* **2011**, *6*, 691–704.
[21] Chauhan, R.S.; Nautiyal, M.C.; Tava, A. *J. Essent. Oil Bear. Pl.* **2010**, *13*, 353–356.
[22] Padalia, R.C.; Verma, R.S.; Chauhan, A.; Sundaresan, V.; Chandan, S.C. *Maejo Int. J. Sci., Technol.* **2013**, *7*, 83–93.
[23] Boukhebti, H.; Chaker, A.N.; Belhadj, H.; Sahli, F.; Ramdhani, M.; Laouer, H.; Harzallah, D. *Der. Pharm. Lett.* **2011**, *3*, 267–275.
[24] Sokovic, M.D.; Vukojevic, J.; Marin, P.D.; Brkic, D.D.; Vajs, V.; van Griensven, L.J.L.D. *Molecules* **2009**, *14*, 238–249.
[25] Bahl, J.R.; Bansal, R.P.; Garg, S.N.; Naqvi, A.A.; Luthra, R.; Kukreja, A.; Kumar, S. *J. Med. Arom. Plant Sci.* **2000**, *22*, 787–797. *Molecules* **2015**, 20,**14421.**
[26]Zheljazkov, V.D.; Cantrell, C.L.; Astatkies, T. *Agron. J.* **2010**, *102*, 1652–1656.
[27] Abd El-Waheb Mohamed, A. *Res. J. Agric. Biol. Sci.* **2009**, *5*, 250–254.
[28]Foda, M.I.; El-Sayed, M.A.; Hassan, A.A.; Rasmy, N.M.; El-Moghazy, M.M. *J. Am. Sci.* **2010**, *6*, 272–279.
[29]Telci, I.; Sahbaz, N.; Yilmaz, G.; Tugay, M.E. *Econ. Bot.* **2004**, *58*, 721–728.
[30]Telci, I.; Sahbaz, N. *J. Agron.* **2005**, *4*, 96–102.
[31]Hua, C.X.; Wang, G.R.; Lei, Y. *Afr. J. Biotechnol.* **2011**, *10*, 16740–16745.
[32]Hadjiakhoondi, A.; Aghel, N.; Zamanizadech-Nadgar, N.; Vatandoost, H. *DARU J. Pharm. Sci.* **2000**, *8*, 19–21.
[33]Baser, K.H.C.; Kürkçüoglu, M.; Tarimcilar, G.; Kaynak, G. *J. Essent. Oil Res.* **1999**, *11*, 579–588.
[34]Rasooli, I.; Gachkar, L.; Yadegarinia, D.; Bagher, M.R.; Astaneh, S.D.A. *Acta Aliment.* **2008**, *37*, 41–52.
[35]Omar, N.N.; El-Sayed, Z.I.A.; Romeh, A.A. *Res. J. Agric. Biol. Sci.* **2009**, *5*,1089–1097.
[36]Dhifi, W.; Jelali, N.; Mnif, W.; Litaiem, M.; Hamdi, N. *J. Food Biochem.* **2013**, *37*, 362–368.
[37]European Food Authority: Scientific Opinion on the safety assessment of carvone, considering all sources of exposure. *EFSA Journal* **2014**,12(7), 3806.