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ESSENTIAL OIL COMPONENTS AND ANTIMICROBIAL ACTIVITY OF PEPPERMINT (MENTHA PIPERITA) FROM MONTENEGRO

SUMMARY

Chemical composition of the essential oil of Mentha piperita L. (peppermint) from Montenegro was analyzed by gas chromatography-mass spectrometry (GC-MS) and its antimicrobial activity was evaluated. GC-MS analysis showed that major components of peppermint oil were menthol (33.15%), menthon (19.61%), 1,8 cineole (6.37%) and methyl acetate (5.63%).

Clinically isolated bacteria strains, Gram-positive (Staphylococcus aureus and Bacillus subtilis) and Gram-negative (Escherichia coli), were used for the antimicrobial activity tests, and results were compared with peppermint essential oil originated from Spain. The obtained results revealed that the essential oil of M. piperita from Montenegro has rather strong antibacterial activity, especially against Bacillus subtilis. These results confirm the potential use of M. piperita from Montenegro and its essential oil in the medical field as well as in the food industry.

Keywords: *Mentha piperita L.*, essential oil, chemical composition, antimicrobial activity

INTRODUCTION

The genus *Mentha (Lamiaceae*) is composed of 19 geographically widespread species and 13 named hybrids (Chambers and Hummer, 1994). Peppermint (*Mentha piperita L.*) originated from Mediterranean region, but nowadays it is cultivated throughout all regions of the world. It is a hybrid mint, a cross between water mint *Mentha aquatic* and spearmint *M. spicata* L. (Frampton, 2009). *M. piperita* is a perennial herb, 50–90 cm high, and a prototypical member of the mint family (Singh et al. 2015). Peppermint generally grows best in moist, shaded locations and prefers acid, neutral and basic, light, medium soils. Flowering is from mid to late summer, while flowers are purple or pinkish having false spikes with numerous inconspicuous bracts and rarely bear seeds (Clark and Menory, 1980). The medicinal parts are the dried leaves, the fresh flowering plant, the whole plant and the essential oil extracted from the

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aerial parts of the flowering plant. In traditional medicine peppermint and its oil have been used as an aromatic, antispasmodic, antiseptic and also in the treatment of cancers, colds, cramps, indigestion, nausea, sore throat and toothaches (Briggs, 1993). The fresh or dried leaves are the culinary source of mint and are used in breath fresheners, drinks, antiseptic mouth rinses, toothpaste, chewing gums, mint teas, beverages, jellies, syrups, candies, ice creams etc. (Hoffmann and Lunder, 1984). Peppermint essential oil and its constituents are commercially used in food, pharmaceutical and cosmetics industries. Thus, menthol is widely used in toothpaste, toothpowder, mouth fresheners, chewing gums, chewing tobacco, candies, analgesic balms, cough drops etc (Soković et al. 2009).

The chemistry of peppermint oil is very complex and highly variable. The relative concentrations vary depending on climate, cultivar, and geographic location (Lis-Balchin et al. 1997). Peppermint volatile oil composed primarily of menthol, menthone, menthofuran and menthyl acetate. Other pharmacologically active ingredients include bitter substances, caffeic acid, flavonoids, polymerized polyphenols, carotenes, tocopherols, betaine, choline and tannins (Soković et al. 2009). Measured low to moderate levels of phenolics with antioxidant activity were reported from peppermint (Zheng and Wang, 2001). Peppermint oil possesses antibacterial activity in vitro, against both Gram-positive and Gram-negative bacteria as well as antiviral and fungicidal activities (Hussain et al. 2010, Iscan et al. 2002, Farshbaf et al. 2004; Soković et al. 2009).

It was previously acknowledged that M. piperita essential oil and its major oil components are generally regarded as safe and full toxicology has been obtained (Soković et al. 2009).

Today, due to consumer awareness and negative perception of artificial preservatives, use of herbal extracts as "natural" products are fast developing segment of the industry. The huge production of essential oils (>70,000 tonnes per annum) with estimated market value of more than 700 million US $, indicate that production and its consumption is increasing (Djilani and Dicko, 2012). Thus there are numerous initiatives all over the world related to the cultivation of aromatic and medicinal plants, including Montenegro.

The aim of this study is to determine the chemical composition together with the antimicrobial properties of the essential oil from leaves of cultivated M. piperita from Montenegro, as natural sources of antiseptics with potential applications in the pharmaceutical and food industry.

MATERIAL AND METHODS

Preparation of herb material

Fresh leaves of cultivated *M. piperita* were collected manually from Zeta (central part of Montenegro) at the beginning of June. Herb material was milled in a domestic coffee mill and, after sieving in ERWEKA set of sieves, sample with a mean particle diameter size of approximately 0.8 mm was obtained. A prepared batch was kept in an airtight resalable polypropylene bag and stored at

+6 ºC for maximum 3 days before use, in order to avoid losses of volatile compounds.

Essential oil preparation

Previously prepared *M. piperita* leaves (80 g) was submitted to hydrodistillation in a Clevenger-type apparatus for 2 hours according to Yugoslav Pharmacopoeia IV (1984). The obtained oil was dried over anhydrous sodium sulphate, measured, poured in hermetically sealed dark-glass containers and stored at 4 °C until analyzed by GC-MS.

Hydrodestillated peppermint essential oil for pharmacetucal purposes, originated from Spain, was purchased in pharmacy (produced by D.B.C.H, Spain) and used for results comparation.

Gas chromatography - mass spectrometry (GC-MS)

The GC-MS analyses were carried out using a Shimadzu 2010+ gas chromatograph-mass spectrometer equipped with a ZB-5ms (30 m x 0,25 mm x 0,25 μm) capillary column. The column temperature was programmed from 35 °C (5 min) to 300 °C at 5 °C/min. The injection port temperature was 260 °C while the interface temperature was 305 °C. The samples of essential oil were injected by splitting and the split ratio was adjusted to 1:100. Helium was used as the carrier gas at a flow rate of 1.2 ml/min and 61.8 kPa inlet pressure. The MS conditions were: the ionisation voltage 70 eV, scanning interval 1.5 s, detector voltage 1.0 kV and *m/z* range 40 - 500. The components were identified by comparing their mass spectral data with those in the WILEY229 and the NIST107 mass spectra libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature and whenever possible, by co-injection with authentic standards (Fluka, Great Britain).

Antimicrobial screening

The antimicrobial activity of the oil was evaluated by the disc diffusion method, using Mueller–Hinton agar for bacteria, by determination of inhibition zones. Two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria were used. Test microorganisms were obtained from Department for medical microbiology of Institute of Public Health in Podgorica. Cefalexin, and erythromycin were used as positive control in order to control the sensitivity of the *S. aureus* and *B. subtilis* and cefalexin and nalidixic for *E. coli*

A 100 μl suspension of any tested bacteria, containing about 10^8 cells/ml, was spreaded on Mueller Hinton Agar (MHA) using sterile swabs. Sterile blank disk 6.0 mm in diameter were impregnated with 5, 10 and 20 μl essential oil/disk and finally placed on the agar surface. Plates were incubated at 37 °C for 24 hours and then the inhibition zones were measured in diameters. Disks soaked in the solvent (5% DMSO) were used as a negative control.

All tests of inhibitory activity were carried out in duplicate and the developing inhibition zones were compared with those of reference disks.

RESULTS AND DISCUSSION

The chemical composition of the hydrodistilled M. piperita essential oil from Montenegro as well as commercial sample of M. piperita essential oil from Spain is shown in Table 1. GC-MS analyses of peppermint essential oil from Montenegro revealed the presence of 36 compounds representing 100% of the total oil. The major components were menthol (33.15%), menthone (19.61%), 1,8 cineole (6.37%) and methyl-acetate (5.63%). Other major compounds in obtained essential oil were isomenthone (4.68%), neomenthol (3.90%), piperitone (3.23%), germacrene D (3.77%) and □-caryophyllene (1.05%).

In our research, commercially available peppermint oil from Spain had higher content of menthol (40.42%), of menthone (14.41%), isomenthone (8.01%), isopulegol acetate (5.32%) and had lower content 1,8 cineole (7,15%) and methyl acetate (1,38%), in comparison to peppermint oil from Montenegro (Table 1). It is noteworthy that oxygenate sesquiterpenes were not detected in oil sample from Spain, while spatulenol (0.52%), veridiflorol (1.54%) and α-cadinol (0.32%) were present in peppermint oil from Montenegro.

High menthol content is the main criterion in peppermint oil quality. According to European Scientific Organization for Phytotherapy (ESCOP), the menthol content should be 44% (ESCOP 1997).

The quality of aromatic herbs is greatly dependent on the growing area, the cultivation method and the production technology. We assume that the content of menthol would be much higher if herb leaves were collected during full flowering season (late June-July). Previous research confirms that plants subjected to shorter days (sunlight exposure) contained very small quantities of menthone and menthol. Also, it was found that, in order to get better results from leaf oil composition, leaf samples should be taken from the lower part, which contains more menthol and represents the oil content of most parts of mint (Aflatuni, 2005).

It is acknowledged that the specific chemical composition of herbal extracts produced in a particular geographical location is the result of a combination of factors such as genotype, ontogeny, light, temperature, water and nutrients (Pirbaoiliti et al. 2013). It was found that three follow-up years are needed in order to understand better the optimum harvesting time (Aflatuni et al. 2000). Previous investigations on M. piperita essential oil composition are consistent with our results in which menthol and menthone were found to be the major compounds (Iscan et al. 2002; Soković et al. 2009). However, research of *M. piperita* essential oil from Brazil revealed that menthyl acetate was the component obtained at the highest percentage (Scavroni et al. 2005).

Table 1. Chemical composition (%) of *Mentha piperita* essential oil

	Retention time	**Compound**	**from Montenegro %**	**from Spain %**
1.	11.228	α-pinene	0.70	1.06
2.	12.747	Sabinene	0.57	0.50
3.	12.882	β-pinene	0.97	1.50
4.	13.432	β-myrcene	0.76	0.30
5.	13.747	3-octanol	0.22	0.45
6.	14.619	p-cymene	0.11	0.15
7.	14.777	Limonene	1.77	2.85
8.	14.887	1,8 cineole	6.37	7.15
9.	15.073	cis-β-ocimene	0.34	0.03
10	15.420	trans-β-ocimene	0.07	-
11	15.778	γ- terpinen	0.30	0.06
12	16.200	cis-4-thujanol	0.32	0.20
13	16.671	α-terpinolene	0.11	0.02
14	17.211	Linalool	0.23	0.19
15	18.735	Isopulegol	-	1.88
16	19.025	Menthone	19.61	14.41
17	19.187	Menthofurane	1.64	2.47
18	19.258	Isomenthone	4.68	8.01
19	19.415	Neomenthol	3.90	4.97
20	19.756	Menthol	33.15	40.42
21	19.999	Neoisomenthol	0.49	
22	21.440	Pulegone	2.50	1.00
23	21.897	Piperitone	3.23	1.72
24	22.925	Methyl acetate	5.63	1.38
25	23.429	Isopulegol acetate	0.09	5.32
26	24.065	Bicycloelemene	0.06	0.08
27	25.253	Copaene	0.04	0.11
28	25.472	α-burbonene	0.42	0.05
29	25.617	β-elemene	0.23	0.60
30	25.697	Jasmine	0.46	-
31	26.418	β-caryophylene	3.57	2.36
32	27.985	Germacrene D	3.77	0.23
33	28.341	Bicyclogermacrene	1.01	0.09
34	28.885	δ-cadinene	0.30	0.09
35	30.311	Spatulenol	0.52	-
36	30.739	Veridiflorol	1.54	-
37	32.133	α-cadinol	0.32	-

Yield of *M. piperita* essential oils with respect to grouped components, is shown in Figure 1.

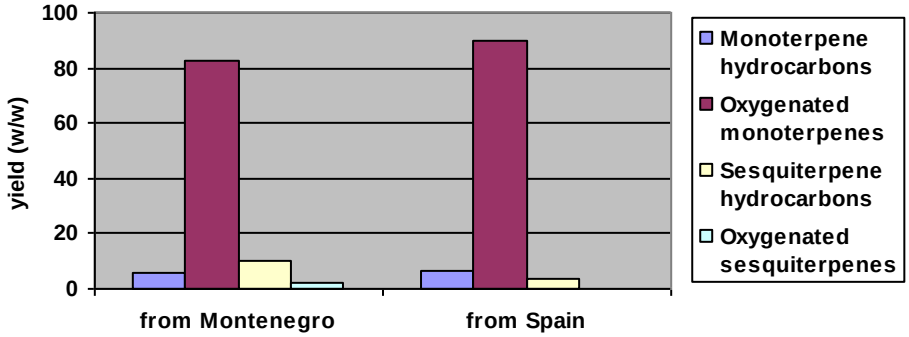

Figure 1. Yield (%, w/w) of *M. piperita* essential oils with respect to grouped components

The *M. piperita* essential oil from Montenegro (Figure 1) consisted mostly of oxygenated monoterpenes (82.6%) and sesquiterpenes (9.86%), while oil from Spain had 89.66% oxygenated monoterpenes (89.66%) and monoterpene hydrocarbons (6.47%).

As previously mentioned, the yield and composition of aromatic plants essential oil are, among other, influenced by harvest time (Araus et al. 2009). Thus, the researchers assume that the content of oxygenated monoterpenes might have been higher if herb was collected at flowering stage, due to the influence of phenological status.

Most of the antimicrobial activity of the essential oils has been attributed to the oxygenated monoterpenes, especially pronounced on whole cells, while hydrocarbon derivatives possess lower antimicrobial properties, as their low water solubility limits their diffusion through the medium (Bakkali et al. 2008). In the literature, it was reported that various chemical compounds have direct activity against many species of bacteria, such as terpenes and a variety of aliphatic hydrocarbons (alcohols, aldehydes and ketones) (Rios and Recio, 2005). The lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups, which is related to the present functional group and hydrogen- bounding parameters in all cases, are of main importance in the antimicrobial action of essential oils components (Couladis et al. 2004; Mancini et al. 2015).

It was found that most active antimicrobial compounds of essential oils are terpenes and phenolics and, thus, their mode of action might be similar to that of other phenolic compounds (Shunying et al. 2005). Individual essential oil contains complex mixtures of such compounds however, a little is known about the effect of interaction between individual constituents on antimicrobial activity.

Interactions between constituents may lead to additive, synergistic or antagonistic effects (Delaquis et al. 2002).

The antimicrobial plate diffusion assay for M. piperita essential oil, as summarized in the Table 2, showed that different microorganisms tested had different susceptibility to the same essential oil. The essential oil activities against tested microorganisms were increased with increased amount of investigated essential oil.

Table 2. Antimicrobial activity of the *Mentha piperita* essential oil and some standard antibiotics

	M. piperita essential oil (μl) from Montenegro				from Spain			Standard antibiotics (μg)		
								NK	CFK	ERM
	5	10	20		5	10	20	30	30	15
Inhibition zone (mm)										
Staphylococcus aureus	21	33	42		23	35	45		25	32
Escherichia coli	22	36	41		26	38	46	29	19	
Bacillus subtilis	21	43	50		27	46	52		27	34

Peppermint essential oil from Montenegro shows very high antibacterial activity against *Bacillus subtilis* which were significantly susceptible to the essential oil at concentration of 10 and 20μl, with significant diameters of growth inhibition zones (43 and 50 mm), respectively. Essential oil from Montenegro showed the strongest antimicrobial activity against medically important pathogens *Staphylococcus aureus* and *Escherchia coli*, ranged from 21 to 42 mm and from 22 to 41 mm, respectively. For comparison, used standard antibiotics diameters of growth inhibition zones ranged from 14 to 32 mm (for *S. aureus*) and 22 to 34 mm (for *E. coli*).

Essential oil gained from *M. piperita* from Spain showed stronger antimicrobial activity (Table 2), probably due to the higher content of oxygenated monoterpenes, especially menthol. However, the difference in antibacterial activity between investigated peppermint oils was not high as expected, due to higher content of oxygenated compounds in Spanish oil. It was found that antimicrobial activity of peppermint oil is due to the presence of a mixture of

monoterpenes and oxygenated monoterpenes, particularly menthol (Iscan et al. 2002; Mimica-Dukić et al. 2003; Sivropoulou et al. 1995; Yadegarinia et al. 2006). The antimicrobial activity of the peppermint essential oil also could be associated with presence of 1,8 cineole and linalool, well-known chemical with their pronounced antimicrobial properties (Viljoen et al. 2003; Damjanović-Vratnica et al. 2011).

The components present in lower amount in oil, such as p-cymene, limonene, neoisomenthol and oxygenated sesquiterpenes might contribute to the antimicrobial activity, involved in some type of synergism with the other active compounds, which could be an explanation for rather similar antimicrobial activity of investigated peppermint essential oils. Identification of such compounds with wide biological activity is critical for mankind as it helps in the search for chemical structures (Damjanović-Vratnica et al. 2011) which should assist in designing new drugs as therapeautic against human pathogens.

CONCLUSIONS

Chemical composition of the hydrodistilled essential oil of *Mentha piperita L. (peppermint)* from Montenegro was analyzed by GC-MS and major components identified were menthol (33.15%), menton (19.61%), 1,8 cineole (6.37%) and methyl-acetate (5.63%).

This study has shown that *M. piperita* essential oil possesses significant activity against different microorganisms, especially against Bacillus subtilis, which suggest that investigated peppermint essential oil could be used as preservative materials on foods, since it is natural, and generally non-toxic to humans.

REFERENCES

Aflatuni, A. (2005): The yield and essential oil content of mint (Mentha ssp.) in Northern Ostrobothnia. Dissertation (Master), Faculty of Science, Department of Biology, University of Oulu, Finland, 50 pp.

Aflatuni, A., Heikkinen, K., Tomperry, P., Jalonen, J. & Laine, K. (2000): Variation in the extract composition of mints of different origin cultivated in Finland. Journal of Essential Oil Research, 12: 462-466.

Araus, K., Uquiche, E. & del Valle, J.M. (2009): Matrix effects in supercritical CO2 extraction of essential oils from plant material. Journal of Food Engineering, 92: 438–447.

Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. (2008): Biological effects of essential oil: a review. Food and Chemical Toxicology, 46: 446-475.

Briggs, C. (1993): Peppermint: medicinal herb and flavouring agent. Canadian Pharmaceutical Journal, 126: 89–92.

Chambers, H.L. & Hummer K.E. (1994): Chromosome counts in the Mentha collection at the USDAARS national germplasm repository. Taxon, 43: 423–432.

Clark R.K. & Menory R.C. (1980): Environmental effects or peppermint (Mentha piperita). Australian Journal of Plant Physiology, 7: 685–692.

Couladis, M., Tzakou, O., Kujundzić, S., Soković, M. & Mimica-Dukić, N. (2004): Chemical analysis and antifungal activity of Thymus striatus. Phytotherapy Research, 18: 40-42.

Damjanović-Vratnica, B., Đakov, T., Šuković, D. & Damjanović J. (2011): Antimicrobial effect of essential oil isolated from Eucalyptus globules Labill. from Montenegro. Czech Journal of Food Science, 29: 277–284.

Djilani, A. & Dicko, A. (2012): The therapeutic benefits of essential oils. In: Bouayed J, editor. Nutrition, Well Being and Health, pp. 155-178. InTech. 224 pp.

ESCOP (1997): Menthae Piperitae Aetheroleum (peppermint oil). In: Monographs on the Medicinal uses of plant drugs, European Scientific Cooperative on Phytotherapy. Exeter, UK, Fascicule 3.

Farshbaf, M.M., Omidbaigi, R., Pourbaig, M.V.M. & Ghaemi, A. (2004): Composition and antifungal activity of peppermint (Mentha piperita) essential oil. Iranian Journal of Pharmaceutical Research, 3: 68-69.

Frampton, A. (2009): The Complete Illustrated Book of Herbs. The Reader's Digest Association, 400 pp.

Hoffmann, B.G. & Lunder L.T. (1984): Flavonoids from Mentha piperita leaves. Planta Medica, 50: 361.

Hussain, A.I., Anwar, F., Nigam, P.S., Ashraf, M. & Gilani, A.H. (2010): Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species. Journal of the Science of Food and Agriculture, 90:1827-36.

Iscan, G., Kirimer, N., Kurkcuoglu, M., Baser, K.H. & Demirci, F. (2002): Antimicrobial screening of Mentha piperita essential oils. Journal of Agriculture and Food Chemistry, 50: 3943-3946.

Lis-Balchin, M., Deans S.G. & Hart S. (1997): A study of the variability of commercial peppermint oils using antimicrobial and pharmacological parameters. Medicinal Science Research, 25: 151–152.

Mancini, E., Senatore, F., Del Monte, D., De Martino, L., Grulova D., Scognamiglio M., Snoussi, M. & De Feo, V. (2015): Antimicrobial and antioxidant activities of five Thymus vulgaris L. essential oils. Molecules, 20: 12016-12028.

Mimica-Dukić, N., Božin, B., Soković, M., Mihajlovic. B. & Matavulj, M. (2003): Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Medica, 69: 413–419.

Pirbalouti A.G., Barani, M., Hamedi, B., Ataei Kachouei, M. & Karimi, A. (2013): Environment effect on diversity in quality and quantity of essential oil of different wild populations of kerman thym., Genetika, 45: 441-450.

Rios, J.L. & Recio, M.C. (2005): Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100: 80-84.

Shunying, Z., Yang, Y., Huaidong, Y., Yue, Y. & Guolin, Z. (2005): Chemical composition and antimicrobial activity of the essential oils of Chrysanthemum indicum. Journal of Ethnopharmacology, 96: 151-158.

Scavroni, J., Boaro, C.S.F., Marques, M.O.M. & Ferreira, LC. (2005): Yield and composition of the essential oil of Mentha piperita L. (Lamiaceae) grown with biosolid. Brazilian Journal of Plant Physiology, 17: 345-352.

Singh R., Muftah A.M., Belkheir, A. (2015): Antibacterial and antioxidant activities of Mentha piperita L. Arabian Journal of Chemistry, 8: 322–328.

Sivropoulou, A., Kokkini, S., Lanaras, T. & Arsenakis, M. (1995): Antimicrobial activity of mint essential oils. Journal of Agriculture and Food Chemistry, 43:2384–2388.

Sokovic, M.D., Vukojevic, J., Marin, P.D., Brkic, D.D., Vajs, V. & van Griensven L.J.L.D. (2009): Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities, Molecules, 14: 238–249.

Stojanova, A., Paraskevova, P. & Anastassov, C.H. 2000: A comparative investigation on the essential oil composition of two Bulgarian cultivars of Mentha piperita L. Journal of Essential Oil Research, 12: 438-440.

Yadegarinia, D., Gachkar, L., Rezaei, M.B., Taghizadeh, M., Astaneh, S.A. & Rasooli, I. 2006: Biochemical activities of Iranian Mentha piperita L. and Mentha communis L. essential oils. Phytochemistry, 67:1249–1255.

Viljoen, A., Vuuren, S.V., Ernst, E., Klepser, M., Demirci, B., Baser, H. & Wyk, B.E.V. 2003: Osmitopsis asteriscoides (Asteraceae) - the antimicrobial and essential oil composition of a Cape-Dutch remedy. Journal of Ethnopharmacology, 88: 137-143.

Zheng W. & Wang S.Y. 2001: Antioxidant activity and phenolic compounds in selected herbs. Journal of Agriculture and Food Chemistry, 49: 5165–5170.