Professional paper
10.7251/AGSY15051127M

CHEMICAL COMPOSITION AND ANTIOXIDANT POTENTIAL OF ESSENTIAL OIL AND METHANOL EXTRACT FROM MINT (Mentha piperita L.) GROWING IN MACEDONIA

Ljupcho MIHAJLOV*, Sanja KOSTADINOVIĆ VELIČKOVSKA

Faculty of Agriculture, University "Goce Delčev", Krste Misirkov bb, 2000 Štip, Macedonia
*Corresponding author: ljupco.mihajlov@ugd.edu.mk

Abstract

The chemical composition and antioxidant potential of essential oil and methanol extract from Macedonian *Mentha Piperita L.* was object of this study. The plant was organically produced from for the first time at south-east region of Macedonia (41°49`N, 21° 59`E) on the overlapping area of two climate types: the Mediterranean and Continental climate.

The GC-FID and GC-MS analyses of essential oil indicated menthol and menthone as the most abundant compounds with 48.05 and 20.4% respectively. The other identified and quantified compounds such camphene, sabinene, *p*-cymene, *β*-pinene, limonene, *cis*-carvon, menthol acetate, piperitone and piperitone oxide were presented in the levels below 10%.

The total phenolic compounds and total flavonoids were presented at higher level in methanol extract with abundance of 22.42±1.14 mg of gallic acid equivalent/g DM and 0.79±0.34 mg luteolin/g DM respectively, in comparison to essential oil with the levels of 10.12±0.89 mg galiic acid equivalent/g DM and 0.54±0.11 mg luteolin/g DM.

The antioxidant potential measured by two radicals DPPH and ABTS indicated higher values for methanol extract in comparison to essential oil.

Keywords: *Mentha piperita L., essential oil, phenolic compounds, flavonoids, antioxidant potential*

Introduction

The aromatic plant *Mentha piperita L.* belonging to the family Lamiaceae which is grown in regions of Eurasia, South Africa, the north, west and east portions of Europe, as well as Turkey and Russia.

The chemical composition and antimicrobial activity of different varieties of Mentha plant were object of study of many researchers. In the work of Arman et al., the essential oil from *Mentha mozaffarianii* had significant antimicrobial activity against gram positive bacteria *Bacillus subtilis*, *B. pumulis*, *Staphylococcus aureus* and *S. Epidermidis* as well as moderate activity against gram negative bacteria such *Escherichia coli*, *Klebsiella pneumoniae*) and no activity against *Pseudomonas aeruginosa, Aspergillus niger*. The major components of the oil were piperitenone (59.5 %), *cis*-piperitenone epoxide (14.9 %), and pulegone (8.5 %), (Arman et al.2011). Chemical composition and antimicrobial activity of *Mentha pulegium* from Iran was studied by Morteza-Semnani et al. (2011). According to their findings, the major constituents of this essential oil were pulegone (54.6 %) and menthone (15.1 %). Carvone-Rich Essential Oils from *Mentha longifolia (L.) Huds. ssp. schimperi Briq*. and *Mentha spicata L*. grown in Sudan had oxygenated monoterpenes comprised 81.5% and 88.7%, while monoterpene hydrocarbons comprised 14.7% and 9.2% (M. H. Younis and S. M. Beshir 2011). Zeinali et al. (2005), reported fifteen principal components in the oils of 12 variety of Iranian mints accessions. The oils obtained from Mint variety Mzin 9 and Mzin 10 contained the highest value of *p*-cymene with the levels of 48.9 and 48.6%. In the mint oils from variety Mzin 5 and Mzin 11 was quantified cis-carveol over 70%. Carvon oxide was the most dominant compound in Mzin 4 with the level of 52.5%. Rissanen et al. (2002), stated that oil

yield and composition of *Mentha piperita L.* depends of plant density and growing season. According to their findings, the highest oil concentrations were present in highest plant densities in the first growing season.

The chemical composition and antioxidant potential of essential oil and methanol extract from Macedonian organically produced *Mentha Piperita L.* was object of this study.

The plant was organically produced, described in details in research of Mihajlov et al. (2015), from for the first time at south-east region of Macedonia (41°49`N, 21° 59`E), on the overlapping area of two climate types: the Mediterranean and Continental climate.

Materials and Methods

Oil isolation and analysis: A sample of 250 g of dried leaves and stems was mixed with 500 mL of tap water in flask and water distilled for 2 h using a Clevenger-type apparatus (Fig. 1). The oil content was measured based on mL oil per 100 g dry matter (mL/DM). The oil compositions were analyzed by GC (FID) and GC/MS. Gas chromatography was carried out with an Agilent HP 6890 gas chromatograph equipped with flame-ionization detector (FID) and quantitation was carried out by addition of pure standards as well as area normalization and neglecting response factors.

The analysis was conducted using a HP-5 (5% Phenyl Methyl Siloxane) fused silica capillary column (30 m x 0.50 mm, film thickness 0.32 µm, J &W Scientific Inc., Rancho Cordova, CA). The operating conditions were as follow: injector and detector temperature: 250°C, carrier gas: helium; inlet pressure: 35.4 kPa. Oven temperature program was 50 - 220°C at the rate of 4°C/min. Quantitative data concerning the percentage contribution of each constituent were taken with this system. GC/MS analysis was carried out using an Agilent HP 6890 gas chromatograph fitted with the same column as described above, coupled to quadrupole 5973 MSD, which was operated at an ionization potential of 70 eV and electron multiplier energy 2000 V. The temperate program started at 50°C during the split injection and then programmed to 220°C with increment of 4°C/min. The oil components were identified by comparing their retention indices and mass spectra data (NIST 14 Standard Reference Database 1A) with those of authentic samples and published data: M. H. Younis and S. M. Beshir (2011), S. Kostadinović Veličkovska (2013), and S. Kostadinović et al. (2010).

Determination of total phenolic compounds and total flavonoids: 0.5 mL of the methanol extract and 0.5 mL of essential oil were dissolved in 5 mL distilled water and mixed with 0.5 mL of 10 times diluted Folin-Ciocalteu's reagent. 1 mL of saturated sodium carbonate (35 %) was added to the mixture and it was topped up to 10 mL distillated water. After three hours, the total phenolics were measured spectrophotometrically at 725 nm.

Determination of flavonoids: in the extract and essential oil was performed by method of Oomah et al, (1996). Methanolic extract and essential oil (10 µL) was three times dissolved in distillated water. Furthermore, 200 µL of diphenylboric acid 2-aminoethyl ester solution was added in the mixture and solution was measured at 404 nm. Luteolin as standard for calibration curve was used in the range from 0.1 to 10 mg/L.

Antioxidant assays: DPPH and ABTS: For DPPH assay, the antioxidant activities of the extract and essential oil were expressed as percentage of decolorization of a solution of the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical) at 517 nm.The Trolox equivalent antioxidant assay (TEAC) employed in this study gives a measure of the antioxidant activity of methanol extract and essential oil under study. For this purpose 10 mL of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) solution was prepared from 39.23 mg of ABTS and 7.17 mg of potassium thiosulphate disolved in Nanopure water to volume.

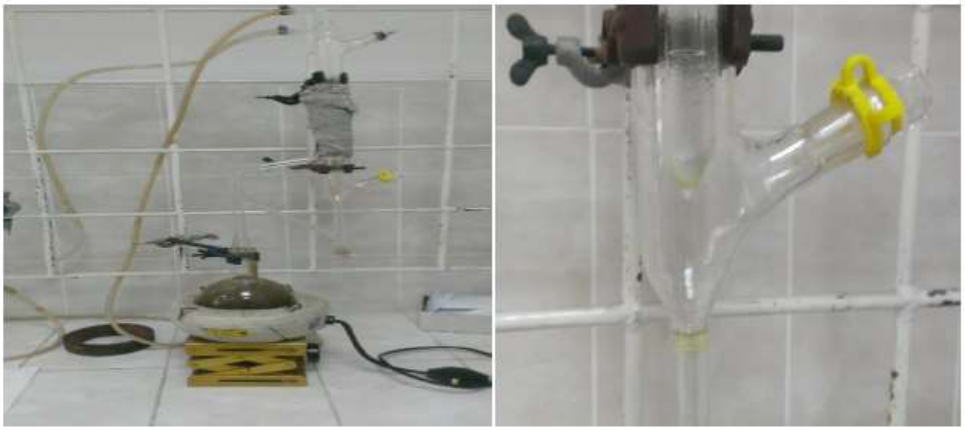

Fig. 1. Isolation of essential oil from *Mentha piperita L.* by hydrodestilation.

Results and Discussion

The qualitative and quantitative compositions of the essential oil obtained from Macedonian organically produced *Mentha piperita L.* was presented in Table 1. Twelve components were identified with total abundance of 85.96%. Four compounds had the highest abundance from all identified compounds, in particular: menthol (48.05%), menthone (20.4%), piperitone (8.3%) and piperitone oxide (4.9%). The relative percentages of all other compounds were in the level below 2.5%. The level of total phenolic compounds and total flavonoids for methanol extract and essential oil of organically produced *Mentha pipperita L.* as well as antioxidant activity were presented in Table 2. As we can see from the results, the total phenolic compounds expressed as gallic acid equivalent had higher abundance in comparison to total flavonoids expressed as mg of luteolin/g of dry matter.

On the other hand, menthanol extract had higher level of both classes of polyphenolics in comparison to the level of the same classes of polyphenolics in distillated essential oil. Furthermore, the level of total phenolic compounds for mentanol extract was more than double in comparison to total phenolics found in essential oil. However the difference for flavonoids was around 30% in favor to methanol extract. The higher antioxidant potential by two radicals (ABTS and DPPH) had methanol extract. More precisely, methanol extract had higher antioxidant potential than essential oil which was in good agreement with the results from total phenolics and total flavonoids. On the other hand, TEAC assay showed higher antioxidant potential for both samples in comparison to DPPH assay. If we compare the results from DPPH and ABTS assay for methanol extract and essential oil presented, we can concluded that results are more than double in favor to ABTS assay.

Table 1. Chemical composition of essential oil from *Mentha piperita L.* from Macedonia by GC-FID and GC-MS

	Compound	RT	Area (%)
1.	camphene	947	0.05
2.	sabinene	974	0.10
3	*p*-cimene	1025	0.05
4.	β-pinene	1027	0.01
5.	limonene	1034	1.2
7.	**menthone**	1110	**20.4**
8.	**menthol**	1175	**48.05**
9.	*cis*-carveol	1180	2.2
10.	menthol acetate	1183	0.7
11.	piperitone	1185	**8.3**
12.	piperitone oxide	1199	**4.9**
	Total		**85.96**

Table 2. Total phenolic compounds, total flavonoids and anthioxidant activity of methanol extract and essential oil from Mentha piperita L.

	Total phenolic compounds (mg GAE/g DM)	Total flavonoids (mg Luteolin/g DM)
Methanol extract of *Mentha piperita L.*	**22.42±1.14**	**0.79±0.34**
Essential oil from *Mentha piperita L.*	10.12±0.89	0.54±0.11
	DPPH assay (mg Trolox/g DM)	TEAC assay (mg Trolox/ g DM)
Methanol extract of *Mentha piperita L.*	12.98±1.05	**27.14±2.24**
Essential oil from *Mentha piperita L.*	7.14±0.87	**13.31±0.04**

Conclusion

We concluded that the region of South-east Macedonia had good potential for production of high-quality organically produced *Mint (Mentha piperita L.)* described in details in published surveys of Mihajlov et al. (2015), with appreciable amount of menthol and menthone.
Further investigations will include antibacterial, antifungal and antimicrobial activity of essential oil from different varieties of Mentha plants.

References

Arman M., Yousefzadi M., Zahra Khademi S., (2011). Antimicrobial Activity and Composition of the Essential Oil from Mentha mozaffarianii, J. Essent. Oil Res., 14, p.131-135.

Kostadinović Veličkovska S. (2013). Volatile profile and flavour of cold –pressed Citrus essential oils, LAMBERT Academic Publishing GmbH & Co. Saarbrücken, Germany.

Kostadinović S., Stefova M., Nikolova D., D. Nedelcheva, N. Martinez, D. Lorenzo, E. Dellacassa, H. Mirhosseini, (2010). Multivariate analysis discrimination of various cold-pressed lemon oils from different geographical regions, J Food, Agric. Environ. , 8(2), 132-136.

Mihajlov Lj., Zlatkovski V., and Ruzdik N.M., (2015). Obtained Yield and Financial Parameters of Organically Grown Mint in the Republic of Macedonia, British Journal of Applied Science & Technology, http://sciencedomain.org/issue/1213, Vol.(10) p.1-6.

Morteza-Semnani K., Saeedi M. and Akbarzadeh M., (2011). Chemical Composition and Antimicrobial Activity of the Essential Oil of Mentha pulegium L, Journal of Essential Oil Bearing Plants, 14, p.208-213.

Rissanen K. Aflatuni S. A., Tomperi P. H., Jalonen J. E. and Laine K. M., (2002). Herbage and Essential Oil Yield and Composition of Mentha piperita L. in Different Plant Densities in Northern Latitudes, J. Essent. Oil Res., 14, p.243-246.

Younis M. H. and Beshir S. M. (2004). Carvone-Rich Essential Oils from Mentha longifolia (L.) Huds. ssp. schimperi Briq. and Mentha spicata L. Grown in Sudan, J. Essent. Oil Res., 16, p.539-541.

Zeinali H., Arzani A., Razmjoo K. and Rezaee M. B. (2005). Evaluation of Oil Compositions of Iranian Mints (Mentha ssp.), J. Essent. Oil Res., 17, p.156-159.