SHORT REPORT

A C G publications

Rec. Nat. Prod. 8:1 (2014) 61-65

records of natural products

Antimicrobial and Antioxidant Activity of the Essential Oils Obtained from *Mentha longifolia* L. Hudson, Dried by Three Different Techniques

Dragana Stanisavljević[1], Sofija Đorđević[*2], Marina Milenković[3], Miodrag Lazić[4], Dragan Veličković[1], Novica Ranđelović[5] and Branislav Zlatković[6]

[1]*College of Agriculture and Food Technology, Ćirila i Metodija 1, 18400 Prokuplje, Serbia*
[2]*Institute for Medicinal Plant Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia*
[3]*Department of Microbiology and Immunology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia*
[4] *Faculty of Technology, University of Niš, Bulevar oslobodjenja 124, 16000 Leskovac, Serbia*
[5] *Faculty of Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia*
[6] *Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia*

(Received October 05, 2012; Revised April 22, 2013, Accepted September 19, 2013)

Abstract: The way of drying the fresh herbal material influences the chemical content and the biological activities of their essential oils. The influence of the different drying methods of the herb *Mentha longifolia* (L.) Hudson on the antioxidant and antimicrobial activity of the extracted essential oils has been analyzed in this study. Drying has been carried out in three ways: in the natural way, in the laboratory oven (45°C) and in the absorptional low-temperature condensational drier (35°C). The antioxidant activity of the essential oil has been estimated by FRAP and DPPH assays, while the antimicrobial activity has been estimated by the diffusible and micro-delusional method, testing on the nine types of bacteria and two types of fungi. The essential oil obtained from the herb dried in the natural way has shown the highest antioxidant activity and the lowest from the herb dried in the laboratory oven. *Bacillus subtilis*, *Micrococcus luteus* and *Enterococcus faecalis* have shown the highest sensitivity on the three samples. The oil obtained from the herb dried in the absorptional low-temperature drier has shown the strongest antimicrobial effect.

Keywords: *Mentha longifolia* (L.) Hudson; drying; antioxidant; antimicrobial activity.

1. Plant Source

Species of the genus of *Mentha* L. (Lamiaceae) are widespread from the polar circle to the trope and from the depressions to the sub-alps zone on all continents. The number of the species of *Mentha* L. is huge and still disputed. Serbian Flora has 11 species [1, 2]. As a medicinal part we use the leaf or the plant, and the drug is officinal in many national pharmacopoeia. Owing to their complex chemical

[*] Corresponding author: E- Mail: sdjordjevic@mocbilja.rs (S. Đorđević), Phone +381-11-3031658.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 11/18/2013 EISSN: 1307-6167

composition, the preparations made of *Mentha* species, show anti-inflammatory, antimicrobial, spasmodic, carminative, antioxidant properties. In the traditional and conventional medicine these preparations are used for better digestion, they improve the secretion of the gall. In addition, the essential oils isolated from *Mentha* species, are used externally, in the preparations which alleviate pains in the muscles and in the treatments against neuralgia, but the largest quantities are used in cosmetic and food industry for production of different sweets and beverages [3, 4].

English horsemint *Mentha longifolia* (L.) Hudson is the perennial herbaceous aromatic and melliferous herb with aerial part which has the nice and fresh scent of menthol or lemon [5]. The aerial part of the herb in bloom contains the essential oil with the dominant components of piperitone, piperitone oxide, carvone, menthone, limonene, 1,8-cineole (depending on chemo type), flavonoids and tannins. The preparations show the carminative and stimulating effect towards gastrointestinal tract, alleviate colds, inflammation of respiratory organs, headaches, pains in muscles and joints [3].

There is the great number of data concerning the analyses of the essential oils of *M. longifolia*. Jančić and the collaborators have summed up the data of the chemical content of essential oil and have given the range of presence of certain components and the division into chemo types [6].

Džamić and the collaborators have analyzed the essential oil of *M. longifolia* gathered on the mountain Zlatar and they have discovered that *trans*-dihydrocarvone (23.64%) and piperitone (17.33%) as the main components have shown significant antifungal and antioxidant activities [7]. Analyzing the content of the essential oils of *M. longifolia* from Tajikistan, it has been concluded that different regions have a great influence not only on the morphological differences but also on the degree of the variation of the chemical content of the oil [8].

Drying is one of the oldest ways of preserving food products, based on the principle of kseroanabioza. The samples of medicinal herbs have been mostly dried naturally in the shade, then using an oven or condensation low temperature drying. The significant influence of different ways of drying herbal material on the chemical content has been proved, as well as the biological activities of the extracted essential oils *Salvia officinalis* and *Juniperus phoenicea* [9, 10].

The herb in bloom was gathered in July 2009 in the south of Serbia (the mountain Pasjača). The voucher specimen was deposited at the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac" of the Faculty of Biology, University of Belgrade (number 16469). Concerning the fact that there have been no records of the antioxidant and antimicrobial potential of the essential oils from the herb *M. longifolia* dried in three different ways, the aim of this study has been to analyze whether the way of drying the herbal material causes the significant differences in the biological activities of isolate.

2. Previous Studies

In our previous study, the influence of drying on the yield and content of the essential oils extracted from the herbs of English horsemint, which have been dried in the three mentioned ways, has been examined. The results showed that there were differences in the yield and content of the analyzed oils. The yield of essential oils was 0.6% (laboratory oven), 0.9% (natural drying) and 1.1% (low-temperature drier). The content of the main component of piperitone, was different [11]. We analyzed the non-volatile fraction of the ethanol extract of these samples, in terms of chemical composition and antioxidant activities. The highest content of the total phenols and flavonoids had the extract obtained from the raw material dried naturally, and in that way the antioxidant activity was highest [12].

3. Present Study

The samples of the essential oils were obtained by hydro-distillation from the herb *M. longifolia* which have been dried in three ways: sample 1 - natural drying in the shade on a draughty placefor 15 days, sample 2, - in the laboratory oven "Stockli" Switzerland at the temperature of 45°C for 2 days, sample 3 - in the absorptional low-temperature drier ("NT-KS/60S" FREON EKO Serbia) at the temperature of 35°C for 2 days. All chemical substances used in the experimental work were of

analytical purity. The culture media for specific pathogens, used for the research of the antimicrobial activity, were obtained from the Institute of Immunology and Virology, Torlak, Serbia.

The total antioxidant activity (TAA) was examined using the Ferric Reducing Antioxidant Power (FRAP) assay, which was based upon reduction of Fe^{3+}-2,4,6-tris-(2-pyridyl)-*s*-triazine complex (Fe^{3+}-TPTZ) in acidic conditions. The calibration curve of ferrous sulfate (100-1000 μM) was used, and results were expressed in μmol Fe^{2+}/mg of dry weight extract (FRAP value). The relative activity of the samples was compared to L-ascorbic acid [13, 14]. The antioxidant activity was determined by DPPH assay [14]. The principle of the assay is the reduction of the violet 2,2-diphenyl-1-picrylhydrazil (DPPH) radical in the reaction with "scavengers" of free radicals to the yellow-colored diphenylpicrylhydrazil. This change of color represents the measure of highness of "scavengers" and is determined by the spectrophotometry. Lowering the absorption of solution of DPPH is in relation to the hydrogen donor activity of the examined compound and is measured at 517 nm on the spectrophotometer. The activity of the sample is shown as IC_{50} (the concentration of the sample necessary for the neutralization of 50% of DPPH radicals).

The agar diffusion and broth microdilution method were used for the research of the antimicrobial activity *in vitro* [15], as well as the following microorganisms: *Micrococcus luteus* ATCC 10240, *Micrococcus flavus* ATCC 10240, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* NCIMB-9111, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10259 and *Candida albicans* ATCC 24433.

The essential oils were dissolved in the absolute ethanol. Antimicrobial activity of the essential oils was tested using two concentrations 2% and 4 % (m/v), while the absolute ethanol was used as a control. Agar diffusion method was performed on Müller-Hinton and Sabouraud agar to test sensitivity of bacteria and *C. albicans*, respectively. The overnight cultures of tested microbial strains were diluted in saline in order to adjust the turbidity of the suspension to 0.5 McFarland standard (approximately 10^8 CFU/mL). One drop of the sample (20 μL) was poured on the agar prepared as required. After incubation (18 h at 37 °C for bacteria and 48 h at 26 °C for *C. albicans*) diameters of zones of inhibition (in mm) were measured. Ampicillin and nystatin were used for the control of the sensitivity of tested microorganisms. The solution of the essential oils in DMSO in successive dilutions in the range from 1.56 to 200 μL/mL (v/v) was used for the determination of MIC.

All the analyses were conducted in triplicate, while the results were shown as the arithmetic mean ± standard deviation.

The results of the antioxidant activity of the essential oils of *M. longifolia* dried in different ways are shown in Table 1.

Table 1. Antioxidant activity of the essential oils obtained from *M. longifolia*

The way of drying of herbal material	Antioxidant activity	
	FRAP (μmol Fe^{2+}/mL±SD)	DPPH (EC_{50}, μL /mL± SD)
1. natural drying	1423.6±8.8	5.8±0.1
2. laboratory oven	730.6±8.9	9.4±3.9
3. low-temperature drier	1101.5±34.3	7.4±1.1

The essential oil obtained from the naturally dried herbal raw material shows the highest antioxidant activity (determined by FRAP and DPPH assays). The value of the antioxidant capacity (determined by FRAP assay) of the essential oil obtained from the mint dried naturally is two times higher compared to the obtained value from the herb dried in the laboratory oven. The value EC_{50} of the oils obtained from the herb dried in the oven is 50% higher than the one of the naturally dried herb.

The results of the antimicrobial activity of the essential oils extracted from *M. longifolia*, dried in three different ways are shown in Table 2. All tested samples showed significant antimicrobial activity in comparison with ampicillin and nystatin.

Table 2. Antimicrobial activity of the essential oils obtained from *M. longifolia*

Microorganism	Zone of inhibition in mm[a]								MIC (μL/mL)			MIC (μg/mL)	
	Essential oil[b]												
	Sample 1		Sample 2		Sample 3								
	2%	4%	2%	4%	2%	4%	Amp[c]	Nys[d]	S 1	S 2	S 3	Amp	Nys
M. luteus ATCC 9341	15.0	16.0	20.0	25.0	24.5	27.5	33.0	n.t.	12.5	6.2	12.5	2.5	n.t.
M. flavus ATCC 10240	19.0	23.5	20.0	21.5	16.0	20.0	36.0	n.t.	25.0	25.0	50.0	3.0	n.t.
S. aureus ATCC 25923	17.0	17.5	20.0	23.0	17.5	28.5	26.0	n.t.	50.0	50.0	50.0	0.5	n.t.
S. epidermidis ATCC 12228	25.0	26.0	24.0	28.5	20.0	26.0	12.0	n.t.	25.0	50.0	25.0	0.2	n.t.
E. faecalis ATCC 29212	17.5	19.5	17.0	19.5	18.0	25.0	16.0	n.t.	12.5	6.2	12.5	5.0	n.t.
B. subtilis ATCC 6633	16.5	19.0	18.5	17.0	21.5	27.5	15.0	n.t.	1.6	1.6	3.1	n.t.	n.t.
E. coli ATCC 25922	21.0	21.0	19.5	21.5	20.5	29.0	18.0	n.t.	25.0	25.0	25.0	2.0	n.t.
K. pneumoniae NCIMB-9111	18.5	24.5	21.5	21.0	28.5	32.0	23.0	n.t.	12.5	25.0	6.2	4.0	n.t.
P. aeruginosa ATCC 27853	20.5	22.0	13.5	17.0	16.5	18.5	n.t.	n.t.	25.0	50.0	25.0	12.8	n.t.
C. albicans ATCC 10259	19.0	21.5	17.5	23.0	20.0	24.5	n.t.	21.0	50.0	50.0	50.0	n.t.	3.0
C. albicans ATCC 24433	22.5	23.5	19.5	25.5	18.0	26.0	n.t.	22.5	25.0	25.0	50.0	n.t.	5.0

[a]Average values; [b]% (v/v) in absolute ethanol; n.t.=not tested; [c]Amp=Ampicillin 10 μg /disc; [d]Nys=Nystatin 100 units/disc; Sample 1 (S 1) - natural drying in the shade on a draughty place lasting for 15 days; Sample 2 (S 2) - in the laboratory oven at 45°C two days; Sample 3 (S 3) - in the absorptional low-temperature drier at 35°C two days.

In the smples of essential oils dominate components are respectively: piperitone (50.8, 43.1, 71.1%), carvone (20.0, 2.9, 5.0), menthone (0.6, 17.5, 0%), limonene (6.3, 1.6, 2.4%), *trans*-caryophyllene (4.3, 4.1, 5.4%), γ-muurolene (3.1, 4.3, 3.6%), 1,8-cineole (1.3, 0.8, 1.2%), *cis*-dihydrocarvone (3.5, 0.6, 0.3%). Essential oil, of natural dried herb is richer in components: α-and β-pinene, sabinene, cis-β-ocimene and α-terpineol. The content of myrcene, iso-menthone, *trans*-dihydrocarvone, pulegone, piperitone oxide is higher in the essential oil from the herb dried in a laboratory oven. Finally, the essential oil from the herb dried in the low-temperature drier contains not only piperitone but also a higher percentage of *trans*-β-ocimene and ocimenol [11]. Mint belongs to the Lamiaceae family of plants, which are known to store their essential oils on or near the leaf surfaces [16]. This might account for the loss of volatile compounds in herbs *M. longifolia*, dried in different temperatures.

In the sample one, piperitone, carvone and *cis*-dihydrocarvone as the dominant component (50.8 20.0, 3.5 respectively) in synergy with other components show significant antioxidant activity, which is similar with the results of other authors [7, 17].

The tested samples of the essential oils exhibited significant inhibitory effect against Gram positive bacteria *B. subtilis*, *M. luteus* and *E. faecalis*. Sample 3 demonstrated the best antimicrobial activity. Piperiton as a major component (71.1%) in this sample in synergy with other components showed excellent effects, which is in accordance with the results of other authors [17-19].

The results obtained in the process of measuring of the antioxidant and antimicrobial activity of the essential oils of *M. longifolia* provide a foothold to the traditional use of the essential oils of the tested specimen, but also to the possibility of using them as natural preservatives in food products (7). The results of our tests confirmed that the way of drying raw herbal material influences not only the chemical content but also the biological activity of isolate.

Acknowledgments

This research was supported by the Ministry of Education and Science of Republic of Serbia (Project N° III 46001).

References

[1] R.M. Harley (1972). *Mentha*, In: T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Valentine, S.M. Walters, (eds.): Flora Europaea, Cambridge University Press, Cambridge, Vol 3, pp.183-186.

[2] R. Jančić (1998). Diversion of the specimen *Mentha* L. in Serbia, In: M. Kojić, R. Jančić: *Mentha piperita* and other specimens of *Mentha* L., Institute for Medicinal Plant Research, Belgrade, 13-36.
[3] PDR for Herbal Medicines (2004). Third Edition, Thomson PDR at Montvale, 283-284.
[4] N. Mimica-Dukić and B. Božin (2008). *Mentha* L. Species (Lamiaceae) as promising sources of bioactive secondary metabolites, *Curr. Pharm. Design*. **14**, 3141-3150.
[5] M. Janković (1974). Genus *Mentha* L., In: Flore de la Republique Socialiste de Serbie, M. Josifović, ed: SANU, Department of Mathematics and Natural Sciences, Belgrade, vol VI, 524-525.
[6] R. Jančić, D. Stošić, N. Mimica-Dukić and B. Lakušić (1995). Aromatic Plants of Serbia, NIP Children newspapers, Belgrade, 247-254.
[7] A.M. Džamić, M.D. Soković, M.S. Ristić, M. Novaković, S. Grujić-Jovanović, V. Tešević and P.D. Marin (2010). Antifungal and antioxidant activity of *Mentha longifolia* (L.) Hudson (Lamiaceae) essential oil, *Bot. Serb.* **34**, 57-61.
[8] F.S. Sharopov, V.A. Sulaimonova and W.N. Setzer (2012). Essential oil composition of *Mentha longifolia* from wild populations growing in Tajikistan, *J. of Medicinally Active Plants*. **1**(2), 76-84.
[9] I.H. Sellami, I.B. Rebey, J. Sriti, F.Z. Rahali, F. Limam and B. Marzouk (2012). Drying sage (*Salvia officinalis* L.) plants and its effects on content, chemical composition, and radical scavenging activity of the essential oil, *Food Bioprocess Tech.* **5**, 2978-2989.
[10] M. Ennajar, J. Bouajila, A. Lebrihi, F. Mathieu, A. Savagnac, M. Abderraba, A. Raies and M. Romdhane (2010). The influence of organ, season and drying method on chemical composition and antioxidant and antimicrobial activities of *Juniperus phoenicea* L. essential oils, *J. Sci. Food Agric.* **90**, 462-470.
[11] D. Stanisavljević, S. Đorđević, M. Ristić, D. Veličković and N. Ranđelović (2010). Effects of different drying methods on the yield and the composition of essential oil from herb *Mentha longifolia* (L.) Hudson, *Biologica Nyssana*. **1**(1-2), 89-93.
[12] D. Stanisavljević, S. Stojičević, S. Đorđević, B. Zlatković, D. Veličković, I. Karabegović and M. Lazić **(**2012). Antioxidant activity, the content of total phenols and flavonoids in the ethanol extracts of *Mentha longifolia* (L.) Hudson dried by the use of different techniques,*CICEQ*. **18**(3), 411−420.
[13] R. Szöllösi and I. Szöllösi Varga (2002). Total antioxidant power in some species of Labiatae (Adaptation of FRAP method), *Acta Biol. Szeged.* **46,** 125–127.
[14] S. Đorđević, S. Dobrić, S. Petrović, M. Milenković, D. Vučićević, S. Žižić and J. Kukić (2007). Antimicrobial, anti-inflammatory, anti-ulcer and antioxidant activities of *Carlina acanthifolia* root essential oil, *J Ethnopharmacol*. **109**, 458-463.
[15] F. Candan, M. Unlu, B. Tepe, D. Daferera, M. Polissiou, A. Sökmen and A. Akpulat (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae), *J Ethnopharmacol*. **87**, 215-220.
[16] D.A. Moyler (1994). Spices – recent advances in Charalambous, *ed*: Spices, herbs and edible fungi, Elsevier Science, Amsterdam, Netherland, 1-70.
[17] H. Nikšić, E. Kovač-Bešović, E. Makarević and K. Durić (2012). Chemical composition, antimicrobial and antioxidant properties of *Mentha longifolia* essential oil, *J. Health Sci*. **2,** 192-200.
[18] M. Mahboubi and G. Haghi (2008). Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil, *J. Ethnopharmacol.* **119,** 325–327.
[19] N. Sarac and A. Ugur (2009). The in vitro antimicrobial activities of the essential oils of some lamiaceae speciecfrom Turkey, *J. Med. Food*. **12** (4), 902-907.