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Benefits and opportunities for using of *Micromeria dalmatica*

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Abstract. *Micromeria dalmatica* Benth. is a Balkan endemic medicinal and aromatic plant widely used as herb or spice. Methanolic and aqueous extracts of three populations of *M. dalmatica* were evaluated for free radical scavenging activity against DPPH radicals. All studied extracts showed significant activity. No difference was found in the antiradical properties between methanolic and aqueous extracts. Comparative analysis of metabolites of plant material of the studied populations was done by GC–MS. The results showed that *M. dalmatica* is rich in essential nutrients as phenolic and organic acids, triterpenes (amyrin), sterols, fatty acids (saturated and unsaturated). The content of these important biologically active substances determines *M. dalmatica* as a valuable spice and medicinal plant.

Key words: DPPH, GC/MS, phenolic acids, triterpenes.

Introduction

According to Chater & Guinea (1982), there are 21 species of *Micromeria* in Europe. At least eighteen of them could be considered in Central and Eastern Europe (CE Europe). Even though CE Europe covers a much larger area, the occurrence of *Micromeria* species here could be restricted to the Balkans and Eastern Mediterranean. *Micromeria* species are traditionally used as medicinal plants and spices. Numerous studies have proved antimicrobial, antioxidant, gastroprotective, hepatoprotective, cytotoxic, anti-inflammatory, and analgesic activity of different *Micromeria* species (Said et al. 2002, Abu-Gharbieh et al. 2013, Bukvicki et al. 2015). Of the four species occurring naturally in Bulgaria (Assyov et al. 2012), *M. dalmatica* Benth. (Fig. 1) is most extensively collected and widely used as a spice. The composition of essential oil of *M. dalmatica* has been well studied (Kostadinova et al. 2007, Radulović & Blagojević, 2012, Bukvicki et al. 2015). Kostadinova et al. (2007) reported that high quantities of essential oil characterize *M. dalmatica* from Bulgaria with the domination of monoterpenic ketones: pulegone (35,8%), piperitenone (18,6%), menthone (15,8%) and piperitone (8,0%). It has been found that the essential oil of *M. dalmatica* is suitable for use in food preservation due to its good antimicrobial properties (Bukvicki et al. 2015). Although many *Micromeria* species such as *M. barbara, M. croatica, M. nervosa, M. juliana, M. graeca* have been studied for phenolic compounds and antioxidant properties (Vladimir-Knežević et al. 2011, Bakkour et al. 2014, Ghanem et al. 2014, Brahmi et al. 2017, Sarikurkcu et al. 2020), data concerning *M. dalmatica* are insufficient (Nikolova et al. 2016). The flavonoid composition of the species has been previously studied. Methylated derivatives of flavones have been identified as the most common flavonoid structures among *Micromeria* species (Tomas-Barberan et al. 1991, Marin et al. 2001, Nikolova et al. 2017).

The present study aimed to assay the free radical scavenging activity of methanolic and aqueous extracts of different populations of *M. dalmatica* and determine their metabolic composition by GC/MS because of the use in culinary and herbal medicine.

Figure 1. *Micromeria dalmatica* in its natural locality – Slavyanka Mts. (photo: Ina Aneva).

Material and Methods

Plant material

The material for analysis was collected from three natural populations of *M. dalmatica* in Bulgaria:

- Slavyanka Mts, Shabran locality, GPS: 41° 24' N, 23° 36' E, 1800 m a.s.l.
- Pirin Mts., 41° 25' N, 23°43' E, 800 m a.s.l.
- Mesta River Valley, GPS: 41° 28' N, 23°03' E, 650 m a.s.l.

DPPH radical scavenging activity

One part of the air-dried plant material (1 g) of each population was extracted with methanol by classical maceration for 24 h, another (1 g) with hot water for 6 h. The obtained extracts were evaporated to dryness and then analyzed for DPPH radical scavenging activity, according to Stanojević et al. (2009). Results are presented as IC50 values (μg mL^{-1}) - extract concentration providing 50% inhibition of the DPPH solution. The IC50 values were calculated by Software Prizm 3.00. All experiments were carried out in triplicate.

GC–MS analysis.

Methanolic extracts and their lipid and phenolic fractions were obtained as described by Nikolova et al. (2016). The fractions and methanolic extract of each sample were silylated with 50 μL of N,O-bis-

(trimethylsilyl)trifluoro-acetamide (BSTFA) in 50 µL of pyridine for 2 h at 50°C. The spectra were recorded on a Thermo Scientific Focus GC combined with a Termo Scientific DSQ mass detector, as described previously (Nikolova et al. 2016).

Results and Discussion

Free radical scavenging activity

Methanolic and aqueous extracts of three populations of *M. dalmatica* were evaluated for free radical scavenging activity against DPPH radicals. All extracts showed significant activity with IC50 values less than 50 µg mL^{-1} which is a prerequisite for more detailed research. No significant difference in antiradical properties was found between methanol and aqueous extracts. This result emphasizes the importance of using the plant as a spice for culinary purposes, such as an aqueous extract. The differences between the antioxidant potential of the individual populations are also insignificant. The received data confirms our previous report (Nikolova et al. 2016) and support considerable free radical activity established for other *Micromeria* species (Vladimir-Knežević et al. 2011, Ghanem et al. 2014).

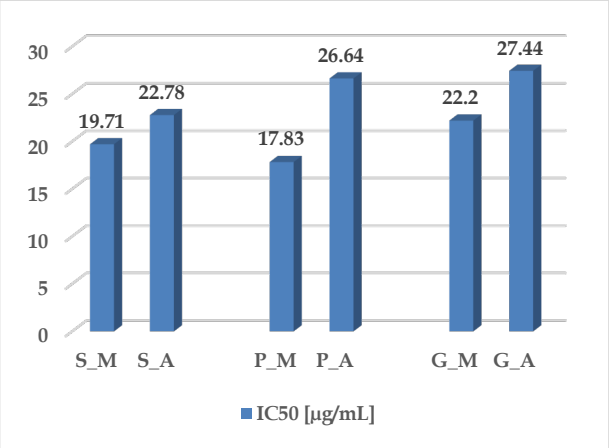

Figure 2. Free radical scavenging activity of methanolic and aqueous extracts of three populations of *M. dalmatica* S-M - methanolic extract of *M. dalmatica* Slavyanka population, S-A - aqueous extract of *M. dalmatica* Slavyanka population, P-M - methanolic extract of *M. dalmatica* Pirin population, P-A - aqueous extract of *M. dalmatica* Pirin population, G-M - methanolic extract of *M. dalmatica* Mesta River Valley population, G-A - aqueous extract of *M. dalmatica* Mesta River Valley population.

GC/MS-based analysis of *M. dalmatica* extracts

Bioactive compounds of methanolic extracts of three *M. dalmatica* populations were analyzed by GC/MS technique (Table 1). Fatty, phenolic and organic acids, sterols, triterpenes were established. Nine phenolic acids were identified. Caffeic and rosmarinic acids were determined in the most considerable amount. Hydroxycinnamic, chlorogenic and protocatechuic were determined as predominant too. Rosmarimic acid has been reported as the most abundant of *M. thymifolia* and *M. graeca* phenolic profile (Vladimir-Knežević et al. 2011, Brahmi et al. 2017, Sarikurkcu et a., 2019). At the same time, chlorogenic acid has been determined as the main phenolic acid of *M. barbata* (Ghanem et., 2014). Phenolic acids have been determined as the main contributors to antioxidant properties (Sato et al. 2011).

In the lipid fraction, fatty acids and triterpenes were detected as main components. A variety of fatty acids was found, especially of the fraction from the Slavyanka population. Unsaturated fatty acids: octadecatrienoic acid (α-linolenic acid C18:3) present in a significant amount. Other identified fatty acids were palmitic acid (C16:0), linoleic acid (18:2), oleic acid (C18:1), stearic acid (C18:0), myristic acid (C14:0). Unsaturated fatty acids prevent a wide range of health problems, including heart disease, depression, asthma and rheumatoid arthritis (Tur et al. 2012, Swanson et al. 2012). High content of linolenic acid has been reported for other *Micromeria* species (Ristić et al. 1997). ß-Amyryn, ß-sitosterol and unidentified triterpene derivative were found in the lipid fraction. Some terpeneoids, including sitosterol and triterpene acids - oleanolic and ursolic acids, have been reported for *M. persica* (Kordkolaei et al. 2020). Triterpenoids are compounds with antimicrobial, anti-inflammatory and antitumor activities (Pinto et al. 2008, Vázquez et al. 2012). Also, β-sitosterol is being studied for its potential to reduce benign prostatic hyperplasia (BPH) and blood cholesterol levels (Field et al. 1997, Wilt et al. 2000). These beneficial for human health effects, and the high content of mentioned above compounds in *M. dalmatica* determines the species as valuable for human use.

Table 1. Identified metabolites of three populations of *M. dalmatica*.

		Studied populations		
Metabolites	RI	MD_S	MD_G	MD_P
Fatty acids and triterpenoids				
Tetradecanoic acid (C14:0)	1721	1,4±0,2	5,6±1	1,0±0,03
Pentagecanoic acid (C15:0)	1822	0,3±0,1	1,5±0,5	0,3±0,07
9-*cis*- Hexadecanoic acid (C16:1)	1915	5,6±0,8	3,6±0,9	3,0±0,4
Hexadecanoid acid (C16:0)	1920	127±17	163,5±10	102,6±14
Octadecadienoic acid (C18:2)	2088	30,8±5	34±8	18,9±5
Octadecatrienoic acid (C18:3)	2098	114,5±8	57±4	29,3±6
Octadecanoic acid (C18:0)	2124	1,2±0,3		
Tetracosanoic acid (C24:0)	2728	42,4±8	7,4±5	36,1±4
ß-Sitosterol	3335	78,85±12	82±8	76,4±6
ß-Amyrin	3400	17,4±3	12,9±6	16,7±4
Triterpene derivative	3421	28,4±4	30,2±5	31,7±4
Phenolic and organic acids				
Succinic acid	1302	35,4±2	34,1±3	26,5±5
Glyceric acid	1313	16,6±4	22,9±4	10,3±2
Mallic acid	1469	128±13	57,1±8	54±5
Pyroglytamic acid	1516	19,3±3	3,7±0,9	9,7±2
4(p)-Hydroxybenzoic acid	1637	7,6±3	5,7±2	5,2±1
Vanilic acid	1754	5,5±2	4,9±1	5,9±0,1
Protocatechuic acid	1809	12,0±3	5,5±2	4,4±2
Quinic acid	1843	12,7±4	15±7	20±9
Syringic acid	1887	1,0±0,1	3,7±2	1,9±0,9
p-Hydroxycinnamic acid *trans c*	1934	20,3±5	38±2	40,9±4
Ferulic acid *trans*	2103	1,2±0,9	2,5±0,6	1,6±0,1
Caffeic acid *trans*	2142	140,2±8	124±9	151±7
Chlorogenic acid	3108	8,3±3	9,8±2	5,7±0,8
Rosmarinic acid	3510	182±7	83±5	103,5±5

Legend: MDS *M. dalmatica* Slaviyanka; MDG *M. dalmatica* Godeshevo; MDP *M. dalmatica* Pirin. Relative metabolites quantification was based on an internal standard added at the beginning of the extraction using the calculated areas for both components.

The present study revealed that extracts of *M. dalmatica* are rich in compounds that positively affect humans.

Conclusion

The results showed that *M. dalmatica* is rich in essential nutrients such as fatty acids (saturated and unsaturated), triterpenes (amyrin), phenolic and organic acids that make it a valuable spice and medicinal plant.

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References

Abu-Gharbieh, E., Shehab, N.G., Khan, S.A. (2013): Anti-inflammatory and gastroprotective activities of the aqueous extract of *Micromeria fruticosa* (L.) Druce ssp. *serpyllifolia* in mice. Pakistan Journal of Pharmaceutical Sciences 26(4): 799-803.

Assyov, B., Petrova, A., Dimitrov, D., Vassilev, R. (2012): [*Conspectus of the Bulgarian Vascular flora. Distribution Maps and Floristic Elements*]. 4th ed., Sofia, Bulgaria, BBF. [in Bulgarian]

Brahmi, F., Guendouze, N., Hauchard, D., Okusa, P., Kamagaju, L., Madani K., Duez, P. (2017): Phenolic profile and biological activities of *Micromeria graeca* (L.) Benth. ex Rchb. International Journal of Food Properties supl.2: 2070-2083.

Bukvicki, D., Stojkovic, D., Sokovic, M., Nikolic, M., Vannini, L., Montanari, C., Marin, P.D. (2015): Potential application of *Micromeria dalmatica* essential oil as a protective agent in a food system. LWT - Food Science and Technology 63: 262-267.

Chater, OA, Guinea, E. (1972). Micromeria Bentham. pp.167-170. In: Tutin, G.T., Heywood, H.V., Burges, A.N., Moore, M.D., Valentine, H.D., Walters, M.S., Webb A.D. (eds), Flora Europaea, 3. London, Cambridge University Press.

Field, F.J., Born, E., Mathur, S.N. (1997): Effect of micellar beta-sitosterol on cholesterol metabolism in CaCo-2 cells. Journal of Lipid Research 38(2): 348-60.

Ghanem, N., Hamaoui, B.E, El-Achi, N., Bakkour, Y., Alwan, S., Houmaisi, F., El-Nakat, J.H., El-Omara, F. (2014): Identification of nonvolatile phenolic acids and flavonoids with antioxidant activity in *Micromeria barbata* extract by RF-HPLC. International Journal of Pharmacy and Chemistry 4(4): 142-145.

Kordkolaei, S.K., Kanani, M.R., Tabefam, M., Sarvestani, N.N., Hamburger, M., Farimani, M.M. (2020): Terpenoids and phenolics of *Micromeria persica*. Natural Product Research 34: 2913-2918.

Kostadinova, E., Alipieva, K., Stefova, M., Stafilov, T., Antonova, D., Evstatieva, L., Matevski, V., Kulevanova, S., Stefkov, G., Bankova V. (2007): Chemical composition of the essential oils of three *Micromeria* species growing in Macedonia and Bulgaria. Macedonian Journal of Chemistry and Chemical Engineering 26 (1): 3-7.

Marin, P.D., Grayer, R.J., Veitch, N.C., Kite, G.C., Harborne, JB (2001): Acacetin glycosides as taxonomic markers in *Calamintha* and *Micromeria*. Phytochemistry 58: 943-947.

Nikolova, M., Aneva, I., Berkov, S. (2016): GC-MS metabolic profiling and free radical scavenging activity of Micromeria dalmatica. Biologica Nyssana 7(2): 159-165.

Nikolova, M., Aneva, I. Zhelev, P., Dimitrova, M. (2017): Flavonoid compounds and antioxidant activity of Bulgarian species of Micromeria. Annuaire de l'Université de Sofia "St. Kliment Ohridski", Faculte de Biologie 102(4): 7-13.

Pinto, S.A.H., Pinto L.M., Cunha, G.M., Chaves, M.H., Santos, F.A., Rao V.S. (2008): Anti-inflammatory effect of alpha, beta-amyrin, a pentacyclic triterpene from *Protium heptaphyllum* in rat model of acute periodontitis. Inflammopharmacology 16(1): 48-52.

Radulović, N.S., Blagojević, P.D. (2012): Volatile secondary metabolites of *Micromeria dalmatica* Benth. (Lamiaceae): biosynthetical and chemotaxonomical aspects. Chemistry & Biodiversity 9: 1303-1319.

Ristić, N., Palić R., Kitić D., Stojanović G. (1997): The fatty acids from some plants of *Micromeria* genus. Facta Universitatis, Series: Physics, Chemistry and Technology, University of Niš 1: 53-56.

Said, O., Khalil, K., Fulder, S., Azaizeh H. (2002): Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. Journal of Ethnopharmacology 83: 251–265.

Sarikurkcu, C., Hanine, H., Sarikurkcu, R.B., Sarikurkcu, R.T., Amarowicz, R. (2019): *Micromeria myrtifolia*: The influence of the extracting solvents on phenolic composition and biological activity. Industrial Crops and Products 145: 111923.

Sarikurkcu, C., Andrade, J.C., Ozer, M. S., de Lima Silva, J.M.F., Ceylan, O., de Sousa, E.O., Coutinho, H.D.M. (2020): LC-MS/MS profiles and interrelationships between the enzyme inhibition activity, total phenolic content and antioxidant potential of *Micromeria nervosa* extracts. Food Chemistry 328: art.126930.

Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., Sugawara, M., Iseki, K. (2011): In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. International Journal of Pharmaceutics 403: 136-138.

Stanojević, L., Stanković, M., Nikolić, L., Nikolić, V., Ristić, D., Čanadanovic-Brunet, J., Tumbas, V. (2009): Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L. extracts. Sensors 9: 5702–5714.

Swanson D., Block, R., Mousa, S.A. (2012): Omega-3 fatty acids EPA and DHA: health benefits throughout life. Advances in Nutrition 3 (1): 1-7.

Tomas-Barberan, F.A., Gil, M.I. Marin, P.D., Tomas-Lorente, F. (1991): Flavonoids from some Yugoslavian *Micromeria* species: Chemotaxonomical aspects. Biochemical Systematics and Ecology 19: 697-698.

Tur J.A., Bibiloni, MM, Sureda, A. (2012): Pons dietary sources of omega 3 fatty acids: public health risks and benefits. British Journal of Nutrition 107 (Suppl. 2): S23-S52.

Vázquez, L. H., Palazon, J., Navarro-Ocaña A. (2012): The pentacyclic triterpenes α,β-amyrins: a review of sources and biological activities. pp. 487-502. In: Rao V. (ed), Phytochemicals – a global perspective of their role in nutrition and health. Rijeka, Croatia, InTech Publisher.

Vladimir-Knežević, S., Blažeković, B., Štefan, M.B., Alegro, A., Kőszegi, T., Petrik J. (2011): Antioxidant activities and polyphenolic contents of three selected *Micromeria* species from Croatia. Molecules 16: 1454–1470.

Wilt, T., Ishani, A., MacDonald, R., Stark, G., Mulrow, C., Lau, J. (2000): Beta-sitosterols for benign prostatic hyperplasia. Cochrane Database of Systematic Reviews 2: CD001043.