Check for updates

Original Article

Natural Product Communications
June 2019: 1–3
© The Author(s) 2019
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1934578X19851687
journals.sagepub.com/home/npx

Micromeria myrtifolia: Essential Oil Composition and Biological Activity

Cengiz Sarikurkcu[1], Olcay Ceylan[2], and Sanja Ćavar Zeljković[3,4]

Abstract

Detailed chemical composition of the essential oil of *Micromeria myrtifolia* Boiss. & Hohen., as well as its antioxidant and enzymatic activity, was analyzed. The most abundant constituents of the oil were sesquiterpene hydrocarbons, β-caryophyllene (40.8%) and α-copaene (17.9%), followed by oxygenated sesquiterpene, caryophyllene oxide (13.5 %). Antioxidant activity of the oil was tested via 8 different methods based on reduction of both radicals and stable metal cations. The oil of *M. myrtifolia* showed weaker, but still comparable activity with the known antioxidants, butylated hydroxyanisole and disodium edetate, that are used as positive controls. In addition, the essential oil of this aromatic plant species revealed strong inhibitory effects against tyrosinase and α-amylase. Present results suggest the use of the essential oil of endemic *M. myrtifolia* in a wide variety of applications in food and pharmacological industries.

Keywords

Micromeria myrtifolia, essential oil, GC-FID, GC-MS, antioxidant activity, enzyme inhibition

Received: November 9th, 2018; Accepted: February 20th, 2019.

Micromeria species (family Lamiaceae) are used in folk medicine as herbal teas as a substitute for mint, mostly for treatment of intestine pain and inflammation, fever, asthma, and disorders of respiratory system.[1] *Micromeria myrtifolia* Boiss. & Hohen. is native to flora of Israel, Lebanon, and Turkey.[2,3] Its aerial parts are traditionally used as tea infusion in the treatment of cold and flu[4] and skin disorders.[1] The essential oil composition of this species from Lebanon and few localities from Turkey is described,[2,3,5] and there are very few studies on its biological activity, such as antioxidant,[3] antifungal,[6] and cytotoxic activities. Therefore, it seems reasonable to perform a detailed investigation of the chemical composition of the essential oil as well as the antioxidant and enzyme inhibitory activity of *M. myrtifolia*.

The yield of essential oil of *M. myrtifolia* was determined as 0.2% (v/w), and the chemical composition is presented in Table 1. A total of 16 compounds were identified in the oil. The major class of the compounds was sesquiterpene hydrocarbons (76.3%), with β-caryophyllene (40.8%) and α-copaene (17.2%) as the most abundant representatives. Next were oxygenated sesquiterpenes (17.3%) with caryophyllene oxide (13.5%) as the major one.

The chemical composition of the essential oil of *M. myrtifolia* has already been reported,[2,3,5] and the results are comparable, which suggests quite a high stability of the oil against different environmental factors. Ozek et al[5] reported very low yield of the oil (0.03%) from the plant material collected at Bilecik (Turkey), with the major compounds being β-caryophyllene (42.56%) and caryophyllene oxide (8.69%). Carikci analyzed the oil of *M. myrtifolia* from Antalya and Tekirgad (Turkey), where the major compounds were β-caryophyllene (40.5%) and caryophyllene oxide (33.9%), respectively. Finally, the majors of the essential oil of the same species from Lebanon were again β-caryophyllene (15.5%) and caryophyllene oxide (14.8%).[3]

[1] Department of Analytical Chemistry, Faculty of Pharmacy, Suleyman Demirel University, Isparta, Turkey
[2] Department of Biology, Faculty of Science, Mugla Sitki Kocman University, Turkey
[3] Centre of Region Haná for Biotechnological and Agricultural Research, Department of Phytochemistry, Faculty of Science, Palacky University, Olomouc, Czech Republic
[4] Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Genetic Resources for Vegetables, Medicinal and Special Plants, Crop Research Institute, Olomouc, Czech Republic

Corresponding Author:
Sanja Ćavar Zeljković, Centre of Region Haná for Biotechnological and Agricultural Research, Department of Phytochemistry, Faculty of Science, Palacky University, Šlechtitelů 27, 78371 Olomouc, Czech Republic.
Email: zeljkovic@vurv.cz

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Table 1. Chemical Composition of the Essential Oil of Aerial Parts of *Micromeria myrtifolia*.

#	Compounds	RI[a]	RA (%)[b]	Identification[c]
1	Linalool	1106	4.7	GC, MS, RI
2	α-Cubebene	1340	0.7	GC, MS, RI
3	α-Copaene	1373	17.9	GC, MS, RI
4	β-Bourbonene	1383	0.2	GC, MS, RI
5	β-Cubebene	1385	5.3	GC, MS, RI
6	β-Caryophyllene	1419	40.8	GC, MS, RI
7	α-Humulene	1460	2.8	GC, MS, RI
8	Germacrene D	1486	1.9	GC, MS, RI
9	δ-Cadinene	1517	3.2	GC, MS, RI
10	α-Calacorene	1541	1.0	MS, RI
11	Germacrene B	1551	1.3	MS, RI
12	Caryophyllene oxide	1579	13.5	GC, MS, RI
13	10-*epi*-γ-Eudesmol	1615	1.2	MS, RI
14	δ-Cadinol	1636	0.9	GC, MS, RI
15	α-Cadinol	1659	1.8	GC, MS, RI
16	Cadalene	1676	1.3	MS, RI
	Total		98.5	

GC, gas chromatography; MS, mass spectrometry; RI, retention index.
[a]Retention index relative to *n*-alkanes on SGE-BPX5 capillary column.
[b]Percentage content.
[c]GC: co-injection with standards; MS: tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data; RI[7]: identification based on comparison of retention index with those of published data.[7]

Generally, essential oil composition within the *Micromeria* species is mostly comprised of oxygenated mono and sesquiterpenes. For instance, the oil of *M. fruticulosa* is rich in pinocarvone, borneol, and α-bisabolol,[8] and that of *M. pseudocroatica* in borneol and camphor.[9]

Table 3. Enzyme Inhibitory Activities (IC_{50}; mg/mL) of the Essential Oil From *Micromeria myrtifolia*.[x]

Assays	Essential oil	Kojic acid	Acarbose
Tyrosinase inhibition	1.65 ± 0.01	0.32 ± 0.02	
α-Amylase inhibition	1.89 ± 0.02	-	1.05 ± 0.01

[x]Different superscripts in the same row indicate significant differences ($P < 0.01$). "-", not determined.

The antioxidant activity of the essential oil of *M. myrtifolia* was assayed by 8 different assays, and results are presented as IC_{50} values (Table 2), meaning the concentration of the oil required to reduce 50% of antioxidant. The oil was very successful in β-carotene bleaching test, very close to butylated hydroxyanisole, which was used as positive control.

Furthermore, the oil was able to scavenge stable radicals, which was tested via α,α-diphenyl-β-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) spectrophotometric assays, reaching IC_{50} values of 25.36 ± 1.61 and 54.06 ± 2.4 mg/mL, respectively. Moreover, Formisano et al.[3] subjected the essential oil of *M. myrtifolia* to DPPH assays and their results are comparable with those presented here. These results are expected, because the oil of *M. myrtifolia* is not rich in potential terpenoid antioxidants, such as linalool and α-terpineol.[3] The essential oil from *M. myrtifolia* was also tested for its inhibitory activity against tyrosinase and α-amylase (Table 3), and in both cases the results for the oil are comparable with those for positive probes.

Experimental

Plant Material

The aerial parts (leaves, buds, and flowers) of *M. myrtifolia* Boiss. & Hohen. were collected from Nebiler village,

Table 2. Antioxidant Activity of the Essential Oil From *Micromeria myrtifolia*.

Assay	Essential oil	BHA	EDTA
β-Carotene bleaching[x]	80.4 ± 0.30	95.70 ± 0.13	-
Phosphomolybdenum[y]	4.69 ± 0.11	0.79 ± 0.01	-
Potassium ferricyanide reducing[y]	1.08 ± 0.06	0.04 ± 0.00	-
CUPRAC assay[y]	8.14 ± 0.47	0.14 ± 0.01	-
FRAP assay[y]	11.27 ± 0.90	0.06 ± 0.01	-
DPPH radical scavenging[z]	25.36 ± 1.61	0.14 ± 0.01	-
ABTS radical cation scavenging[z]	54.06 ± 2.47	0.19 ± 0.01	-
Ferrous ion chelating[w]	1.36 ± 0.07	-	0.07 ± 0.00

BHA, butylated hydroxyanisole; EDTA, disodium edetate; CUPRAC, cupric reducing antioxidant capacity; FRAP, ferric reducing antioxidant power; DPPH, α,α-diphenyl-β-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).
Different superscript letters in the same row indicate significant difference ($P < 0.01$). "-", not determined. [x]Inhibition (%) at 2 mg/mL concentration. [y]Effective concentration (EC_{50}; mg/mL) at which the absorbance was 0.5 for reducing power and phosphomolybdenum assays. [z]Inhibition concentration (IC_{50}; mg/mL) at which 50% of the DPPH radicals were scavenged. [w]Inhibition concentration (IC_{50}; mg/mL) at which 50% of the ferrous ion-ferrozine complex was inhibited.

Kavaklidere-Mugla on April 2016 (37°27′08″ N, 28°23′40″ E, 1055 m), authenticated by Dr Olcay Ceylan, and deposited at the Department of Biology, Mugla Sıtkı Koçman University (Mugla-Turkey), under the accession no. O.1486.

Isolation and Analysis of the Essential Oils

The air-dried and grounded plant material (2.5 kg) was submitted for 5 hours to hydrodistillation by using a British-type Clevenger apparatus. The constituents of the oil were analyzed by means of gas chromatography-flame ionization detector (GC-FID) and GC-mass spectrometry (MS). Briefly, the GC-FID analysis of the essential oil was performed using a Thermo-Finnigan Trace GC/A1300 (E.I.) equipped with an SGE/BPX5 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm). Helium was the carrier gas, at a flow rate of 1 mL/min. Injector temperature was set at 220°C. The program used was 50°C to 150°C at a rate of 3°C/min, held isothermal for 10 min, and finally raised to 250°C at 10°C/min. Diluted sample (1/100, v/v, in methylene chloride), 1.0 µL, was injected manually and in the splitless mode. Quantitative data of the oil was obtained from FID area percentage data. The GC-MS analysis of the oil was performed with a Thermo-Finnigan Trace GC/Trace DSQ/A1300 (E.I. Quadrapole) with the same column and an electron ionization system with I.E. of 70 eV. Carrier gas was He at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220°C and 290°C, respectively. The temperature program was same as described above. The identification of compounds was based on comparison of their relative retention times with those of authentic samples on the SGE/BPX5 capillary column and by matching their mass spectra with those obtained from authentic samples and/or the Wiley 7N and TRLIB libraries spectra and published data.[7]

Biological Activity

Antioxidant activities of the samples were investigated by using β-carotene-linoleic acid, phosphomolybdenum, free radical scavenging DPPH radical and ABTS radical cation, ferrous ion chelating, and reducing power assays (potassium ferricyanide, cupric ion reducing, and ferric reducing antioxidant power). For enzyme inhibitory activities, the essential oils were analyzed toward α-amylase and tyrosinase. All assays are already described in the literature.[10] Results of biological activities are presented as IC_{50} values, which represent the concentration of the oil (mg/mL) required to inhibit 50% of the initial concentration of oxidant/enzyme.

Statistical Analysis

All the assays were carried out in triplicate. The results were expressed as mean and standard deviation values. Statistical differences between the samples were analyzed using Student’s *t*-test ($\alpha = 0.01$). All the analyses were carried out by using SPSS v22.0 software.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by project No. RO0418 (sustainable systems and technologies, improving crop production for higher quality of production of food, feed, and raw materials, under conditions of changing climate) funded by the Ministry of Agriculture, Czech Republic.

References

1. Azab A. *Micromeria*: Chemistry and medicinal activities. *Eur Chem Bull*. 2016;5(7):300-307.
2. Carikci S. The essential oil components of five *Micromeria* species grown in Anatolia. *BAÜ Fen Bil. Enst. Dergisi Cilt*. 2013;15(2):73-79.
3. Formisano C, Oliviero F, Rigano D, Saab AM, Senatore F. Chemical composition of essential oils and in vitro antioxidant properties of extracts and essential oils of *Calamintha origanifolia* and *Micromeria myrtifolia*, two Lamiaceae from the Lebanon flora. *Ind Crops Prod*. 2014;62:405-411.
4. Polat R, Satıl F. An ethnobotanical survey of medicinal plants in Edremit Gulf (Balıkesir-Turkey). *J Ethnopharmacol*. 2012;139(2):626-641.
5. Özek T, Kirimer N, Baser KHC. Composition of the Essential Oil of *Micromeria myrtifolia* Boiss. et Hohen. *J Essent Oil Res*. 1992;4(1):79-80.
6. Özcan M. Antifungal effects of *Micromeria myrtifolia* Boiss. & Hohen. in Boiss. and Prangos uechtritzii Boiss. Hawsskn decoctions. *Acta Aliment*. 1999;28(4):355-360.
7. Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. fourth ed. Illinois, USA: Allured Publishing Corporation, Carol Stream; 2007.
8. Ruščić M, Vuko E, Bezić N, Kremerb D, Dunkić V. Croatian *Micromeria fruticulosa* – Essential Oil Composition and Antiphytoviral Activity. *Nat Prod Commun*. 2017;12(7):1934578X 1701200-1135.
9. Kremer D, Müller ID, Stabentheiner E, et al. Phytochemical and Micromorphological Traits of Endemic *Micromeria pseudocroatica* (Lamiaceae. *Nat Prod Commun*. 2012;7(12):19345 78X1200701-19345781201670.
10. Zengin G, Sarikurkcu C, Gunes E, et al. Two *Ganoderma* species: profiling of phenolic compounds by HPLC-DAD, antioxidant, antimicrobial and inhibitory activities on key enzymes linked to diabetes mellitus, Alzheimer's disease and skin disorders. *Food Funct*. 2015;6(8):2794-2802.