

## RESEARCH ARTICLE

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## Antimicrobial activity of the essential oil of wild-growing *Micromeria thymifolia* (Scop.) Fritsch

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### Article info:

Received: 5 May 2014

Accepted: 13 November 2014

### ABSTRACT

The genus *Micromeria* Benth. (Lamiaceae, Nepetoideae) includes about 130 species, often aromatic. The essential oil and extracts of some *Micromeria* species have significant antioxidant, antibacterial and antifungal activities. *Micromeria thymifolia* is endemic species of the Balkan peninsula. It has been traditionally used in the Mediterranean area as condiment and medicinal plant. The aim of this study was to investigate antimicrobial properties of essential oil of wild *Micromeria thymifolia* against four Gram negative bacteria (*Escherichia coli* SY252, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076 and human pathogen *Burkholderia cepacia* ATCC25416), four Gram positive bacteria (*Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC6633, *Listeria innocua* ATCC33090) and two fungi strains (*Candida albicans* ATCC10231 and *Saccharomyces cerevisiae* ATCC9763). The MICs of *M. thymifolia* essential oil against tested bacteria and fungi was assessed using microtitre plate-based antimicrobial assay. MHB was used as growth media for bacteria, with exception of *L. innocua* when BHI was used, YPD was used for fungi. The results of our investigation showed that the essential oil of wild-growing *M. thymifolia* possess significant antimicrobial activity against all tested strains except the *P. aeruginosa*.

**Key words:** *Micromeria thymifolia*, essential oil, antimicrobial

## Introduction

The genus *Micromeria* Benth. (Lamiaceae, Nepetoideae) includes about 130 species (Šilić, 1984), with a distribution range extending from the Himalayan region to the Macronesian Archipelago and from the Mediterranean to South Africa and Madagascar (Brauchler et al., 2005). The essential oil and extracts of some *Micromeria* species have significant antioxidant, antibacterial and antifungal activities (Gulluce et al., 2004; Marinković et al., 2003). *Micromeria thymifolia* (Scop.) Fritsch is endemic species of the Balkan peninsula. It usually grows in clefts of fissured rocks and inside deep crevices mainly on karst, but also on serpentine, ranging from 30 m to 2000 m above sea level (Šilić, 1979). *Micromeria thymifolia* have been traditionally used in the Mediterranean area as condiment and medicinal plant.

The aim of this study was to investigate antimicrobial activity of essential oil of wild *Micromeria thymifolia* against four Gram negative bacteria, four Gram positive bacteria and two fungi strains.

## Materials and Methods

Air-dried aerial parts of plant material was cut up into small pieces and subjected to hydrodistillation for 2 h using Clevenger apparatus to obtain the essential oil in yield of 0.49%. The oil was yellow in color with density of 0.9256 g/cm<sup>3</sup>. Because of the high viscosity of the oil, the antimicrobial activity was tested with concentrated essential oil and with essential oil dissolved in ethanol in 1:9 ratio to increase solubility of essential oil and subsequently to increase its availability in media.

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*Micromeria thymifolia* was tested against four Gram negative bacteria (*Escherichia coli* SY252, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076 and human pathogen *Burkholderia cepacia* ATCC25416), four Gram positive bacteria (*Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC6633, *Listeria innocua* ATCC33090) and two fungi strains (*Candida albicans* ATCC10231 and *Saccharomyces cerevisiae* ATCC9763).

Selected bacterial strains were inoculated in Mueller-Hinton Broth (MHB, Biomedics, Madrid, Spain), with exception of *L. innocua* which was inoculated in Brain Heart Infusion Broth (BHI, LAB, Lanchashire, UK) and cultivated 24 h at 37°C. Selected fungi were inoculated in YPD Broth (1% Peptone, 0.5% Yeas extract, 2% Glucose, Difco & Co, Corpus Christi, TX, USA) and cultivated 24 h at 30°C.

The minimum inhibitory concentration (MIC) of *M. thymifolia* essential oil against tested bacteria and fungi was assessed using microtitre plate-based antimicrobial assay. MHB was used as growth media for bacteria, with exception of *L. innocua* when BHI was used, YPD was used for fungi.

For bacteria, antimicrobial activity of essential oil was detected by reading the OD<sub>600</sub> and by observing the changes in resazurin color. For fungi, antimicrobial activity of essential oil was detected only by observing the changes in resazurin color.

The minimum bactericide/fungicide concentration (MBC/MFC) is defined as the lowest concentration of antimicrobial substance that will kill bacteria/fungi.

## Results and Discussion

Essential oils of *Micromeria* species are well known because of their biological and pharmacological properties. Antimicrobial activity of *M. thymifolia* essential oil was tested on bacteria and fungi which could be found in food and some of them are agents of food poisoning (Nostro et al., 2009; Djenane et al., 2011). *L. innocua* is a non-pathogenic species and may be used as biological indicator for food-borne pathogen *L. monocytogenes* because of its similar response to physical, chemical or thermal treatments (Staszewski, 2011). The laboratory strains of *E. coli* SY252 was chosen to investigate whether the mechanism of action of essential oil is associated with structure of the bacterial cell wall. In our research essential oil of wild-growing *M. thymifolia* showed antimicrobial activity against all tested strains except the *P. aeruginosa* (Table 1).

In previous study carried with essential oils from three *Micromeria* species against micromycetes and bacteria, pulegone was suspected to be the main reason for strong antibacterial/antifungal activity detected (Marinković et al., 2002). Similarly, strong antimicrobial activity of pulegon was reported in numerous studies (Kalodera et al., 1994; Flamini et al., 1999).

**Table 1.** Values of MICs and MBCs/MFCs of *M. thymifolia* EO detected in microtitre plate-based antimicrobial assay

	MIC [%]	MBC(MFC) [%]	Tet <sup>1</sup> /Str <sup>2</sup> /Nys <sup>3</sup> [µg/well]	MBC (MFC):MIC
<b>G<sup>-</sup> bacteria</b>				
<i>Escherichia coli</i> SY252	5.00	/	3.05 <sup>2</sup>	/
<i>Salmonella enterica</i> ATCC13076	0.50*	5.00	12.50 <sup>2</sup>	10
<i>Pseudomonas aeruginosa</i> ATCC27853	/	/	50.00 <sup>2</sup>	/
<i>Burkholderia cepacia</i> ATCC 25416	2.50	/	12.50 <sup>2</sup>	/
<b>G<sup>+</sup> bacteria</b>				
<i>Bacillus subtilis</i> ATCC 6633	0.50*	5.00	3.13 <sup>2</sup>	10
<i>Enterococcus faecalis</i> ATCC 29212	0.61	5.00	12.50 <sup>1</sup>	8
<i>Staphylococcus aureus</i> ATCC 25923	0.50*	/	6.13 <sup>2</sup>	/
<i>Listeria innocua</i> ATCC 33090	0.50*	/	12.50 <sup>1</sup>	/
<b>Fungi</b>				
<i>Candida albicans</i> ATCC 10231	0.25*	1.00*	1.58 <sup>3</sup>	4
<i>Saccharomyces cerevisiae</i> ATCC9763	0.13*	0.25*	12.50 <sup>3</sup>	2

\*values obtained when EO was dissolved in ethanol in 1:9 ratio.

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Activity of essential oil could be associated with the structure of the outer membrane, goes absence of the effect in *P. aeruginosa*, which is known to have a high level of resistance to different antibiotics due to restrictive outer membrane (Aeschbach *et al.*, 1994). The results of our investigation showed that the essential oil of wild-growing *Micromeria thymifolia* possess high antimicrobial activity, and may be useful in food industry.

### Acknowledgement

This work was supported by Ministry of Science and Technologies of Republic of Serbia, Research projects 172053 and 172058. We are grateful to prof. Petar Marin for providing a plant material.

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