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Antifungal effect of the essential oil of two *Micromeria* (*Lamiaceae*) species

Abstract

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The essential oils from two Balkanian endemic plants: *Micromeria thymifolia* (Scop.) Fritsch and *M. albanica* (Grisebach ex K. Malý) Šilić were investigated by GC and GC - mass spectrometry and their antifungal activities evaluated on the basis of their minimum inhibitory concentration (MIC). Both oils showed antifungal effect, the more active being those from *M. albanica*.

Introduction

Micromeria thymifolia (Scop.) Fritsch and *M. albanica* (Grisebach ex K. Malý) Šilić are endemic species of the Balkan peninsula (Šilic 1979). During the last ten years numerous studies have been performed concerning the antimicrobial activity of essential oils, especially of plants belonging to the *Lamiaceae* family. There is little data about the oil of *M. thymifolia* (Ševarda & al. 1979; Stanić & al. 1988) and *M. albanica* (Stojanović & al. 1999). The antifungal activity of the essential oil of *Micromeria thymifolia* was investigated by Kalodjera & al. (1994), while there is no report of antifungal activity of the essential oil of *M. albanica*.

Material and methods

Plant material was collected during the flowering period of *M. thymifolia* from Srbovac and samples of *M. albanica* from Prizrenska Bistrica. The aerial parts were dried at room temperature. Voucher specimens have been deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade.

The essential oils were isolated by a Clevenger type apparatus for 2 h. Their composition was determined using analytical GC/FID and GC/MS techniques as reported by Marinković & al. (2001).

In order to test the antifungal activity seven fungal species were used: *Aspergillus niger*

(ATCC6275), *A. ochraceus* (ATCC12066), *Penicillium ochrochloron* (ATCC9112), *Cladosporium cladosporioides* (ATCC13276), *Fusarium tricinctum* (CBS514478), *Phomopsis helianthi* (ATCC201540) and *Trichoderma viride* (IAM5061).

The antifungal activity was recorded according to the mycelial growth test (Ischii 1995). Different concentrations of essential oil (0,2–2 µl /ml) were diluted in Petri dishes containing Malt Agar (MA) (Booth 1971). After the cooling of the medium the tested fungi were inoculated in the center of the Petri dishes. The minimum inhibitory concentration (MIC) was determined by comparing it to the control after the 21-days incubation period at 24 ± 2 °C.

Results and discussion

The hydrodestillation of the aerial parts of both the *Micromeria* species gave yellowish pleasant-smelling peppermint-like essential oils. The oil yields were 0,99% for *M. thymifolia* and 0,88% for *M. albanica*. The results of the GC/MS analysis are given in Table 1.

Table 1. Quantitative composition (%) of the essential oil of *Micromeria* species.

Constituent (%)	*Micromeria thymifolia*	*Micromeria albanica*
α-pinene	0,50	0,39
β-pinene	0,86	1,10
myrcene	-	0,29
limonene	2,40	3,20
menthone	0,71	1,39
isomenthone	4,98	0,15
trans-isopulegone	1,00	0,81
isomenthol	0,47	-
cis-carveol	-	0,37
pulegone	32,81	13,43
piperitone	11,71	5,62
isopiperitone	-	0,92
carvacrol	-	1,77
piperitenone	25,70	9,72
piperitenone oksid	-	38,73
α-copaene	-	2,12
β-bourbonene	0,91	1,10
β-caryophyllene	2,39	-
caryophyllene	-	1,15

The oil of the *M. thymifolia* showed a similar chemical composition to the oils analyzed by other authors (Ševarda & al. 1979; Stanić & al. 1988), containing mainly monoterpene ketones: pulegone, piperitenone, piperitone and isomenthone. Characteristic odour of this oil is determined by the high content of ketone.

The oil of *M. albanica* was rich in monoterpene oxide - piperitenone oxide (38,73%) and also a high level of menthon and presence of carvacrole was found. As reported by Stojanović & al. (1999) the oil of *M. albanica* was characterized by piperitenon oxide (44%), but this component was only found in some *Calamintha* and *Mentha* species (Kokkalou & Stefanou 1990; Mimica-Dukić 1993; Mastelić & al. 1998).

The essential oil of *M. thymifolia* showed the antifungal effect, with MIC of 2 µl/ml for *A. niger*, *A. ochraceus*, *C. cladosporioides*, *F. tricinctum*, *T. viride* and *P. ochrocloron*, except for *P. helianthi* where MIC was 0,4 µl/ml. The obtained results showed high antifungal activity of the *M. albanica* essential oil on to selected fungal species. MIC was 0,2 µl/ml for *A. niger*, *A. ochraceus*, *P. helianthi, C. cladosporioides* and *P. ochrocloron*, but for more resistant fungal species *F. tricinctum* and *T. viride* MIC was 0,4 µl/ml (Table 2).

Table 2. Antifungal activity of the essential oil of *Micromeria* species.

Essential oil / Fungi	*M.thymifolia MIC* (µl/ml)	*M.albanica MIC* (µl/ml)
Aspergillus niger	2	0,2
A. ochraceus	2	0,2
Cladosporium cladosporoides	2	0,2
Penicillium ochrocloron	2	0,2
Phomopsis helianthi	0,4	0,2
Trichoderma viride	2	0,4
Fusarium tricinctum	2	0,4

In the Kalodjera & al.'s work (1993) pulegone has been shown to have a high activity against *A. niger*, *A. ochraceus* and *F. tricinctum*. Since the oil of *M. thymifolia* consists of mainly monoterpene ketones (pulegone, piperitenone, piperitone and isomenthone), it could be concluded that pulegone and some other components in this essential oil are responsible for the antifungal activity. The oil of *M. albanica* was characterized by high presence of piperitenone oxide which was not identified in the oil of *M. thymofolia.* It can be assumed that this component is responsible for the higher antifungal activity of *M. albanica* oil.

These results showed that the essential oils of selected *Micromeria* species have a fungistatic activity on all the tested fungal species. The testing and evaluation of the antifungal activity of the essential oil is difficult because of the volatility and the complexity of each oil. Because of the long period of fungal incubation, evaporation or decomposition of some components may occur during the tested period (Jansen & al. 1987). The techniques for testing antifungal activity have still not been developed, as they are for bacteria.

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