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Antifungal and antioxidant activity of *Mentha longifolia* (L.) Hudson (Lamiaceae) essential oil

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ABSTRACT: The present study describes the antifungal and antioxidant activity of *Mentha longifolia* (L.) Hudson essential oil. This plant is native to Europe, Central Asia and Australia. It is used as carminative, stomachic and stimulant and also in aromatherapy. The essential oil profile was determined by GC and GC-MS. The main compounds in the oil were *trans*-dihydrocarvone (23.64%), piperitone (17.33%) and *cis*-dihydrocarvone (15.68%). Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were recorded using the microdilution method. Commercial antimicotic bifonazol was used as a control. The concentration of 10 μl/ml showed fungicidal activity against *Aspergillus* and *Fusarium* species, *Penicillium funiculosum* and *Trichoderma viride*. Concentration of 5 μl/ml was efficient against *Trichophyton menthagrophytes* and yeast *Candida albicans*. The most sensitive micromycetes were *Cladosporium fulvum*, *C. cladosporium cladosporioides* and *Penicillium ochrochloron* where concentration of 2.5 μl/ml was lethal. The antioxidant activity of essential oil was evaluated by means of the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method. The essential oil of *M. longifolia* was able to reduce DPPH radicals into the DPPH-H form, and this activity was dose-dependent. The oil exhibited significant potential for antioxidant activity, and reduced DPPH to 50% (IC_{50}=0,659 ml/ml of solution).

Key words: *Mentha longifolia*, essential oil, antifungal, antioxidant activity, DPPH.

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INTRODUCTION

Mentha longifolia (L.) Hudson is perennial herb 40-120 cm high with musty scent. Stem white or grey-villous, sometimes sparsely hairy. Leaves are sessile or shortly petiolate usually oblong elliptical, hairs simple. Extremely variable in height, leaf size and shape, indumentum and inflorescence and complicated by the occurrence of hybrids (Harley, 1972). Species of *Mentha* genus are widespread except South America and Antarctic. *Mentha* spp. have been used as a folk remedy for treatment bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints due to their antiinflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue and anticatharral activities (Gulluce *et al*. 2007).

The objectives of this study were to analyze essential oil composition and to determine its potential antifungal and antioxidant activities of this species.

MATERIAL AND METHODS

Essential oil used in experiment was obtained from Institute for Medicinal Plant Research "Dr Josif Pancic". The oil was distillated from plant material collected on locality Gradina, Zlatar. Herbal material is deposited in Herbarium of Institute for Medicinal Plant Research "Dr

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Josif Pancic" S/N. Chemical composition of the essential oils of *M. longifolia* was analyzed using gas chromatography (GC) and gas chromatography - mass spectroscopy (GC-MS).

Gas chromatography (GC) and gas chromatography - mass spectrometry (GC-MS): Qualitative and quantitative analyses of the oils were performed using GC and GC-MS. The GC analysis of the oil was carried out on a GC HP-5890 II apparatus, equipped with split-splitless injector, attached to HP-5 column (25 m x 0.32 mm, 0.52 μm film thickness) and fitted to FID. Carrier gas flow rate (H_2) was 1 ml/min, split ratio 1:30, injector temperature was 250°C, detector temperature 300°C, while column temperature was linearly programmed from 40-240°C (at rate of 4°/min). The same analytical conditions were employed for GC-MS analysis, where HP G 1800C Series II GCD system equipped with HP-5MS column (30 m x 0.25 mm, 0.25 μm film thickness) was used. Transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in m/z range 40-400. Identification of the individual oil components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with those held in Wiley275 library of mass spectra. Confirmation was performed using AMDIS software and literature (Adams 2007). For the purpose of quantitative analysis area percents obtained by FID were used as a base.

Tests for antifungal activity. The fungi used in this study were: *Alternaria alternata* (ATCC 13963), *A. flavus* (ATCC 9170), *Aspergillus niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), *A. versicolor* (ATCC 11730), *Cladosporium cladosporioides* (ATCC 13276), *C. fulvum* (TK 5318), *Fusarium tricinctum* (CBS 514478), *F. sporotrichoides* (ITM 496), *Penicillium funiculosum* (ATCC 10509), *P. ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061), *Trichophyton menthagrophytes* and *Candida albicans* (clinical isolates). The molds were from Mycotheca of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade. The fungi were maintained on malt agar (MA) and Sabouraud dextrose agar (SDA) (Booth 1971). The cultures were stored at 4°C and subcultured once a month.

Antifungal assay. In order to investigate the antifungal activity of essential oils, the modified microdilution technique was used (Hanel & Raether 1988; Daouk *et al.* 1995). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (vol/vol). The spore suspension was adjusted with sterile saline to a concentration of approximately

Table 1. Chemical composition (expressed as %) of *M. longifolia* essential oil

Constituents	KIE	KIL	%
α-pinene	928.9	932	1.13
sabinene	969.1	969	0.27
β-pinene	971.5	974	0.37
3-octanol	995.3	988	0.45
p-cymene	1021.0	1020	0.76
limonene	1024.8	1024	1.81
1,8-cineole	1027.6	1026	8.18
linalool	1098.2	1095	2.72
isoamyl isovalerate	1104.7	1102	0.15
α-terpineol	1189.1	1186	1.36
cis-dihydrocarvone	1193.4	1191	**15.68**
trans-dihydrocarvone	1202.5	1200	**23.64**
isodihydrocarveol	1212.9	1212	0.85
neoisodihydrocarveol	1226.4	1226	7.87
cis-carveol	1229.1	1226	0.10
cis-3-hexenyl 3-methylbutanoate	1235.1	1232	0.49
carvone	1241.8	1239	0.27
piperitone	1252.2	1249	**17.33**
dihydroedulan I	1284.7	1289	0.60
thymol	1291.5	1289	0.25
diosphenol	1296.8	1309	0.34
p-vinylguiacol	1310.6	1309	0.16
isodihydrocarvylacetate	1326.5	1326	0.62
neoisodihydrocarvylacetate	1356.2	1356	4.17
piperitenone oxide	1364.2	1366	0.20
α-copaene	1372.2	1374	0.11
β-bourbonene	1381.1	1387	0.65
geosmin	1393.9	1399	0.12
β-caryophyllene	1415.3	1417	1.37
β-gurjunene	1425.2	1431	0.10
α-humulene	1449.6	1452	0.13
spathulenol	1572.0	1577	0.30
caryophyllene oxide	1577.9	1582	2.11
humulene epoxide II	1603.2	1608	0.13
caryophylla-4(12),8(13)-dien-5α-ol	1630.9	1639	0.17
Total			**95.00**

*__KIE__=Kovats (retention) index experimentally determined
**__KIL__=Kovats (retention) index –literature data (Adams, 2007)

Table. 2. Minimal inhibitory (MIC) and fungicidal concentrations (MFC) of *M. longifolia* essential oil and bifonazole (μl/ml)

	M. longifolia		bifonazol	
Fungi	**MIC**	**MFC**	**MIC**	**MFC**
Alternaria alternata	5	10	10	10
Aspergillus niger	2.5	10	10	10
Aspergillus ochraceus	10	10	10	15
Aspergillus flavus	10	10	10	15
Aspergillus versicolor	2.5	10	10	10
Cladosporium cladosporioides	1	2.5	10	10
Cladosporium fulvum	2.5	2.5	5	10
Fusarium tricinctum	2.5	10	15	20
Fusarium sporotrichioides	2.5	10	15	20
Penicillium funiculosum	2.5	10	15	20
Penicilium ochrochloron	2.5	2.5	15	20
Trichoderma viride	10	10	15	20
Trichphyton mentagrafites	5	5	10	15
Candida albicans	2.5	5	10	15

1.0×10^6 in a final volume of 100 μL per well. The inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum. The MICs determination was performed by a serial dilution technique using 96-well microtitre plates. Investigated essential oils were dissolved in MA or SDA broth with fungal inoculum. The microplates were incubated for 72 h at 28°C.

Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were determined. The lowest concentrations without visible growth were defined as MIC. MFC were determined as the lowest concentrations with no visible growth after reinoculation of the original inoculum. The commercial fungicide bifonazole was used as a positive control.

Antioxidant assay. The antioxidant activity of essential oil was evaluated by means of the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method. This spectrophotometer assay uses stable radical DPPH as reagent (Blos 1958). Methanolic solution of investigated essential oil (200 μl) (with starting concentrations of 200, 300, 400, 500 μl/ml of solution) was added to 1800 μl methanolic solution of DPPH radical (concentration of 0,04 mg/ml) and after shaking reaction mixture was left to react in the dark for 30 minutes at room temperature. Absorbance of remaining DPPH radical was measured on 517 nm after that time (A_1) on Agilent GBC Cintra 40, UV-Visible spectrophotometer. Every concentration was done in triplicate and the same was done with Trolox and BHT, known antioxidants. Blank probes were done in the same way using methanol instead of investigated solution (A_0). The decrease of absorption of DPPH solution is calculated by equation:

Percentage of absorption decrease (on 517 nm) = $(A_0 - A_1)x100/ A_0$

Concentrations which decrease absorption of DPPH solution for 50% (IC_{50}) were obtained from the curve dependence of absorption of DPPH solution on 517 nm from concentration for each compound and standard antioxidant. For calculation of these values, Origin 6.0 software was used. Tests were cared out in triplicate.

RESULTS AND DISCUSSION

The results of chemical analysis of *Mentha longifolia* essential oil are presented in Table 1.

The main compounds in *M. longifolia* oil were *trans*- and *cis*-dihydrocarvone (23.64% and 15.68%) and piperitone (17.33%) followed by 1,8-cineole (8.18%) and neoisodihydrocarveol (7.87%).

According to recent investigations, dominant compounds in essential oil from *M. longifoila* flowers were

piperitone oxide, piperitenon oxide, *β*-caryophyllene, thymol, *cis*- and *trans*-dihydrocarvone and menthofuran (Mimica-Dukić 1992). These authors found that essential oil from aerial parts of flowering *M. longifolia* contains piperitone as the main compound followed by menthone, pulegone neo-menthol and isomenthone (Mimica-Dukić *et al.* 2003). Ghoulami *et al.* (2001) found high content of piperitone oxide and piperitenon oxide in sample *M. longifolia* oli from Maroko, while in Iranian samples *cis*-carveol (53-78%) was dominant (Zenali *et al.* 2005).

Minimum inhibitory and fungicidal concentrations (MIC and MFC) of *M. longifolia* essential oil investigated in this study are presented in Table 2.

The concentration of 10 μl/ml showed fungicidal activity against *Aspergillus* and *Fusarium* species, and *Alternaria alternata*, *Penicillium funiculosum* and *T. viride*. Concentration of 5 μl/ml was efficient against *Trichophyton menthagrophytes* and yeast *Candida albicans*. The most sensitive micromycetes were *Cladosporium fulvum*, *C. cladosporium cladosporioides* and *Penicillium ochrochloron* where concentration of 2.5 μl/ml was lethal. The essential oil exhibited fungicidal characteristics with MIC and MFC of 1-10 μl/ml. Fungistatic and fungicidal activity of bifonazole was 5-20 μl/ml (Fig.4.). Previous results indicate that essential oil of *M. longifolia* showed higher antimicrobial and antifungal activity than tested commercial substances (Mimica-Dukić *et al.* 2003).

Free radical scavenging capacities of the tested oil was measured by DPPH assay and results are shown in Figure 1. According to the results obtained, *M. longifolia* oil was found active with IC_{50} value of 0.659 ml/ml of solution. IC_{50} values of the synthetic antioxidants BHT was 0,328 mg/ml and Trolox 0.0637 mg/ml were determined in parallel experiments. The results for antioxidant activity obtained in this work are in correlation with recent results of other authors (Mimica-Dukić *et al.* 2003; Gulluce *et al.* 2007; Mkaddem *et al.* 2009).

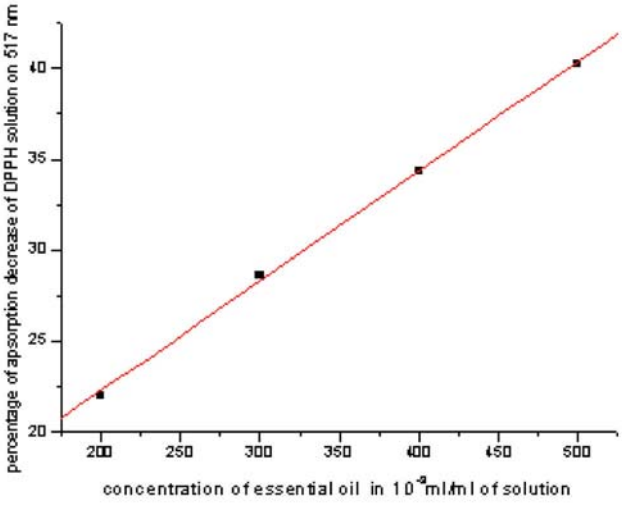

Fig. 1. Antioxidant activity of *M. longifolia* essential oil

Antioxidant and antifungal properties of the essential oils and various extracts from many plants are of great interest in both fundamental science and the food industry, since their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants by natural ones. The present study confirmed the antifungal activity of Serbian *M. longifolia* essential oil, as well.

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REFERENCES

Adams R. 2007. Identification of essential oil components by gas chgromatography/mass spectrometry, 4th ed, Allured Publishing Corp., Carol Stream, IL, USA.

Blos MS. 1958. Antioxidant determination by the use of stabile free radical. *Nature* **181**: 1199-1200.

Booth C. 1971. Fungal Culture Media. In: Norris JR, Ribbons DW (eds.), Methods in Microbiology, Pp. 49-94, Academic Press, London & New York.

Daouk KD, Daghera MS, & Sattout JE. 1995. Antifungal activity of the essential oil of *Origanum syriacum* L. *J. Food Protect.* **58**: 1147-1149.

Dorman HJD & Deans SG. 2000. Antimicrobial agents from plants: antibacterial activity of plants volatile oils. *J. App. Microbiol.* **88**: 308-316.

Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A & Ozkan H. 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. *Food Chemistry* **103**: 1449-1456.

Hanel H & Raether W. 1988. A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. *Mycoses* **31**: 148-154.

Harley RM. 1972. *Mentha*. In: Tutin TG, Heywood VH, Burges NA, Moor DM, Valentine DH, Walters SM & Webb DA (eds.), Flora Europaea III, pp. 183-186, Cambridge University Press, Cambridge, UK.

Mimica-Dukić N. 1992. Ispitivanje sekundarnih biomolekula u nekim vrstama roda *Mentha*. Doktorska disertacija. Univerzitet u Novom Sadu.

Mimica-Dukić N, Božin B, Soković M, Mihajlović B, Matavulj M. 2003. Antimicrobial and antioxidant activities of three Mentha species essential oils. *Planta Med*. **69**: 413-419.

Mkaddem M, Bouajila J, Ennajar M, Lebrihi A, Mathieu F & Romdhane M. 2009. Chemical composition and antimicrobial and antioxidant activities of *Mentha* (*longifolia* L. and *viridis*) essential oils. *Journal of Food Science* (in press).

Zenali H, Arzani A, Razmjoo K & Rezaee MB. 2005. Evaluation of oil compositions of Iranian mints (*Mentha* ssp.). *J. Essent. Oil Res*. **17**: 56-159.

REZIME

Antifungalna i antioksidativna aktivnost etarskog ulja *Mentha longifolia* (L.) Huds. (Lamiaceae)

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U radu je ispitivano potencijalno delovanje etarskog ulja *Mentha longifolia* (L.) Huds. kao antifungalnog i antioksidativnog agensa. Vrsta *M. longifolia* je samonikla u Evropi, Centralnoj Aziji i Australiji. Koristi se kao karminativ, stomahik i stimulant u aromaterapiji. Kompozicija etarskog ulja je određivana korišćenjem gasne hromatografije (GH) i gasne hromatografije sa masenom spektroskopijom (GH-MS). Dominantne komponente u ispitivanom etarskom ulju su: *trans*-dihidrokarvon (23.64%), piperiton (17.33%) i *cis*-dihidrokarvon (15.68%). Antifungalna aktivnost je ispitivana metodom mikrodilucije i određivane su minimalne inhibitorne i fungicidne koncentracije ulja i komercijalnog fungicida bifonazola. Etarsko ulje je pokazalo fungicidno dejstvo pri koncentraciji 10 µl/ml za gljive iz rodova *Aspergillus* i *Fusarium* kao i za vrste *Penicillium funiculosum* i *Trichoderma viride*. Najosetljivije su mikromicete *Cladosporium fulvum*, *C. cladosporioides* i *Penicillium ochrochloron*, za koje je letalna koncentracija 2.5 µl/ml. Antioksidativna aktivnost je rađena korišćenjem DPPH kao hvatača slobodnih radikala. Etarsko ulje *M. longifolia* je pokazalo sposobnost redukcije DPPH radikala u DPPH-H formu. Antifungalna aktivnost je predstavljena redukcijom DPPH na 50% (IC_{50}=0,659 ml/ml rastvora).

Ključne reči: *Mentha longifolia*, etarsko ulje, antifungalna aktivnost, antioksidativna aktivnost, DPPH.