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SHORT COMMUNICATION

Antifungal activity of *Nepeta rtanjensis* essential oil

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Abstract: The chemical composition and antifungal activity of the essential oil of an endemic Serbian plant *Nepeta rtanjensis* Diklić & Milojević was studied. The essential oil was isolated from cultivated plants. Inhibition of mycelia growth of five micromycetes, two *Alternaria* species originally isolated from *N. rtanjensis*, *Cladosporium cladosporoides*, *Trichoderma viride* and *Bipolaris spicifera*, were tested using the agar dilution method. The essential oil of *N. rtanjensis*, the main component of which was 4$a\alpha$,7α,7$\alpha\beta$-nepetalactone, showed strong antifungal activity against all the tested micromycetes. The minimum inhibitory concentration of *N. rtanjensis* essential oil ranged from 0.6 to 1.4 μg mL^{-1}. The fungi most sensitive to the tested oil were *Alternaria* species, while *Trichoderma viride* was the most resistant.

Keywords: *Nepeta rtanjensis*; essential oil; antifungal activity.

INTRODUCTION

Nepeta is a genus of perennial or annual herbs from the Lamiaceae family which is found in Asia, central and southern parts of Europe, the Middle East and North Africa.[1] *Nepeta rtanjensis* is an endemic and critically endangered (CR B_{2c}), aromatic plant which grows only on a few localities on the Rtanj Mountain in South-East Serbia.[2]

Nepeta species are widely used in folk medicine because of their medical properties. The essential oil of *N. rtanjensis* possesses strong antibacterial effect against different strains of *Staphylococcus aureus*, even stronger than most synthetic antibiotics.[3]

Nepeta species can be divided into two groups: nepetalactone-containing and nepetalactone-free species. The most frequent component in *N. govaniana*, *N.*

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cadmea, *N. cephalotes*, *N. racemosa, N. binaludensis* and *N. sulforiflora* is 4*aα*,7*α*,7*αα*-nepetalactone. From the oils of *N. nuda* spp. *albiflora* and *N. rtanjensis,* 4*aα*,7*α*,7*αβ*-nepetalactone was isolated, while *N. asterotrichus* and *N. sintenisii* oils are rich in 4*aβ*,7*α*,7*aβ*-nepetalactone. The main component of the nepetalactone-free species is 1,8-cineol, found in *N. heliotropifolia*. Caryophyllene oxide was the main component in the oil of *N. cilicia, N. betonicifolia* and *N. nuda*. ssp. *nuda*, while *α*-pinene was found in the oil of *N. glomerulosa* and *β*-caryophyllene in *N. fissa* oil.[4]

The main component of essential oil of *N. rtanjensis* is 4*aα*,7*α*,7*aβ*-nepetalactone. In wild populations of *N. rtanjensis*, the amount of 4*aα*,7*α*,7*aβ*–nepetalactone in the is oil 86.4 %, while in the oil of cultivated plants, this component is presented with 77.9 %.[3,4]

It is well known that fungal infection can be a great threat to plant, animal and human health. Medicinal plants are a good source of natural products with strong antimicrobial activities without harmful effects. The use of natural antimicrobial compounds is important in the control of human, animal and plant diseases of microbial origin.

The aim of this investigation was to evaluate the antifungal activity of *N. rtanjensis* essential oil against selected fungi.

EXPERIMENTAL

Plant material and isolation of essential oil

Nepeta rtanjensis was collected on experimental fields of the Institute for Biological Research "Siniša Stanković", Belgrade, Serbia. The plants were rapidly micropropagated *in vitro*, transferred to the greenhouse for acclimatization, and subsequently planted in an experimental field.[5] Herbal material is deposited at the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, Belgrade (16064 BEOU).

The essential oil was isolated from air-dried aerial parts of *N. rtanjensis*, collected during the pre-flowering stage, by hydrodistillation for 2 h in a Clevenger type apparatus.

Gas chromatography–mass spectrometry (GC/MS)

Analyses of isolated oil were performed by GC/FID and GC/MS on a fused silica capillary column PONA (crosslinked methyl silicone gum, 50 m×0.2 mm, 0.5 μm film thickness). A Hewlett-Packard, model 5890, series II gas chromatograph equipped with split–splitless injector was used for the GC/FID analysis. Sample solution in ethanol (0.2 %) was injected in split mode (1:100) at 250 °C. The detector temperature was 300 °C (FID), while the column temperature was linearly programmed from 40–280 °C, at a rate of 2 °C min^{-1}. In the case of the GC/MS analysis, a Hewlett-Packard, model 5971A MSD was used. The transfer line was kept at 280 °C. Carrier gas (H_2) flow rate was 1 mL min^{-1}.

Identification of the constituents of the essential oil

Identification of each individual compound was made by comparison of their retention times with those of pure components, matching mass spectral data with those from the Wiley library of 138000 MS spectra. For the library search, a PBM-based software package was used.

Fungal strains used

Two groups of micromycetes were tested: the autochthonous species (*Alternaria* sp. 1 from leaves and *Alternaria* sp. 2 from seeds of *N. rtanjensis*) and selected fungal species (*Cladosporium cladosporioides*, *Trichoderma viride* and *Bipolaris spicifera*) from the Mycotheca of the Department of Algology, Mycology and Lichenology, Faculty of Biology, University of Belgrade.

Test for antifungal activity

The fungi were maintained on malt agar (MA). The cultures were stored at + 4 °C and subcultured once in a month. The mycelial growth test with malt agar was used to investigate the antifungal activity of the essential oil.[6] The minimum inhibitory concentration (*MIC*) of oil necessary for inhibition of mycelial growth of the fungal strain was determined. Different concentrations of essential oil (0.6–1.4 μg mL^{-1}) were diluted in Petri dishes with MA. All fungal species were tested in triplicate. Essential oil was added into MA and poured into Petri dishes. The tested fungi were inoculated at the centre of the plates. The plates were incubated for three weeks at room temperature, the *MIC* was determined after this period. Petri plates with the commercial fungicide, Quadris (0.6–6.0 μg mL^{-1}), were used as controls.

RESULTS AND DISCUSSION

The results of the chemical analysis of the essential oil of *Nepeta rtanjensis* are presented in Table I. The aerial parts of *N. rtanjensis* contained 1.0 % of oil. The main component in this oil was 4*aα*,7*α*,7*aβ*-nepetalactone (79.89 %).

TABLE I. Composition of *N. rtanjensis* essential oil

Component	Content, %
α-Pinene	3.3
β-Pinene	0.4
2-Methoxy-*p*-cresol	1.1
4*aβ*,7*α*,7*aβ*-Nepetalactone	6.3
α-Copaene	1.3
4*aα*,7*α*,7*aβ*-Nepetalactone	79.9
Germacrene D	1.8
δ-Cadinene	2.1
Total	96.2

It was found that the essential oil isolated from *N. rtanjensis* had a strong antifungal activity against all the examined micromycetes. The most efficient impact of *N. rtanjensis* essential oil on mycelia growth *in vitro* was found for the *Alternaria* species (Table II).

TABLE II. Minimal inhibitory concentrations (*MIC* / μg mL^{-1}) of *N. rtanjensis* essential oil and Quadris

Micromycetes	Essential oil	Quadris
Alternaria sp. 1	0.8	4.0
Alternaria sp. 2	0.6	4.0
Cladosporium cladosporioides	1.0	3.0
Trichoderma viride	1.4	> 6.0
Bipolaris spicifera	1.0	3.0

It can be seen that both *Alternaria* species, which were originally isolated from *N. rtanjensis*, showed the highest sensitivity to this oil. Oil concentrations of 0.6–0.8 μg mL^{-1} inhibited the growth of mycelia of *Alternaria* species. The minimal inhibitory concentration of oil for *Cladosporium cladosporioides* and *Bipolaris spicifera* was 1.0 μg mL^{-1}. The highest *MIC* (1.4 μg mL^{-1}) of oil was against *Trichoderma viride*. The commercial fungicide, Quadris, showed lower antifungal activity than *Nepeta* oil, with *MIC* of 3.0–4.0 μg mL^{-1}. Quadris inhibited mycelial growth of *C. cladosporioides*, *B. spicifera* and *Alternaria* species at 3.0–4.0 μg mL^{-1}. *T. viride* was also the most resistant fungus to Quadris with a *MIC* higher than 6.0 μg mL^{-1} (Table II).

In previous investigations of the antifungal activity of different oils, it was found that *Alternaria alternata* was more sensitive than *T. viride*.[7] A strong resistance of *T. viride* was also observed in a previous investigation of the antifungal activity of essential oils. Analyzes of antifungal activity of some essential oils, *Achillea atrata* and Lauraceae plants, showed that *T. viride* was the most resistant fungus.[8,9]

The present research proved that the essential oil from *N. rtanjensis* has a strong antifungal activity and that this oil can inhibit the growth of the mycelia of some fungi. The results of a previous investigation suggested that *N. rtanjensis* essential oil can inhibit the growth of *Aspergillus niger* colonies.[3] According to the literature, this is only data concerning the antifungal activity of *N. rtanjensis* oil. The present research enlarges the number of fungi species known to be sensitive to this oil.

The antifungal activities of essential oils isolated from other *Nepeta* species have been reported. Iridodial β-monoenol acetate isolated from essential oil of *N. leucophyla*, and actidine isolated from *N. clarkei*, showed strong antifungal activity. Iridodial β-monoenol acetate was most effective against *Sclerotium rolfsii*, while actidine was highly active against *Macrophomina phaseolina*. Both fungi are soybean pathogens. The essential oil from *Nepeta hindostana* has an inhibitory effect on *Pythium aphanillermatum*, *P. debaryanum* and *Rhyzoctonia solani*.[10]

Due to their low mammalian toxicity, susceptibility to biodegradation and strong antimicrobial activity, essential oils can be used as bioagents.[11]

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ИЗВОД

АНТИФУНГАЛНА АКТИВНОСТ ЕТАРСКОГ УЉА *Nepeta rtanjensis*

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У овом раду презентован је хемијски састав и антифунгална активност етарског уља ендемичне биљке *Nepeta rtanjensis* Diklić & Milojević. Етарско уље је изоловано из култивисаних биљака. Инхибиција мицелијалног раста пет микромицета, две врсте рода *Alternaria,* изоловане са *N. rtanjensis*, *Cladosporium cladosporioides*, *Trichoderma viride* и *Bipolaris spicifera*, тестирана је макродилуционом методом. Етарско уље *N. rtanjensis*, чија је главна компонента 4*aα*,7*α*,7*aβ*-непеталактон показује јаку антифунгалну активност у односу на све тестиране микромицете. Минимална инхибиторна концентрација (*MIC*) етаског уља била је у распону од 0,6 μg mL^{-1} до 1,4 μg mL^{-1}. Највећу осетљивост на тестирано уље показале су врсте рода *Alternaria* док је *Trichoderma viride* била најотпорнија.

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