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Short communication

Antimicrobial activity of essential oil isolated from nepetalactone-low-productive chemotype of *Nepeta nuda* L. from the Central Balkans

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Introduction

Nepeta nuda L. represents a widespread *Nepeta* species that can be found in ruderal habitats across Balkan peninsula, characterized by the presence of various bioactive compounds from the group of terpenes and polyphenolics. Previous studies have indicated that *N. nuda* essential oils (EOs) can be rich in oxygenated monoterpenes such as 1,8-cineole (Aćimović et al., 2022), monoterpene iridoids such as nepetalactone (Gkinis et al., 2010), as well as sesquiterpenes including *β*-caryophyllene, *β*-farnesene (Mancini et al., 2009) and germacrene D (Aćimović et al., 2022). However, the composition of EOs can vary in response to environmental factors, genetic structure of populations, and developmental stage of plants (Petrović et al., 2024). *N. nuda* has been used in the traditional medicine of many nations, for treating wounds and urinary infections, due to its antimicrobial properties. The present study aimed to provide novel insights into the antimicrobial activity of EOs isolated from aerial parts of specific *N. nuda* chemotype from the Central Balkans, which is characterized by the low content of nepetalactone. This complemented the existing knowledge on bioactive properties of this remarkable plant, and pointed to chemotype-specific modes of action against selected bacterial and fungal pathogens.

Materials and methods

Isolation of N. nuda essential oil

N. nuda EO was obtained from aerial parts of flowering plants collected in 2022, in several locations in Eastern Serbia. Hydrodistillation of EOs from 100 g dry plant material was carried out for 2 h, using a Clevenger-type apparatus connected to a 2 L glass flask (Isolab, Germany), filled with 1.5 L of deionized water.

GC/MS analysis

Identification of volatile compounds in *N. nuda* EO extracts was performed using an Agilent 8890 gas chromatograph coupled to a 5977B mass selective detector (Agilent Technologies, USA) and an automated sample enrichment system (Centri®, Markes International Ltd., UK). Separation was achieved on an HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness) using helium as the carrier gas at a constant flow rate. The oven temperature was programmed from low to high temperature with a final isothermal hold, as described in Petrović et al., (2024). Compounds were identified by

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comparison of mass spectra with the NIST library (NIST05) and available reference standards.

Antimicrobial assay

The antimicrobial activity was evaluated using a modified microdilution method according to (Smiljković et al., 2018). The lowest concentration showing a visible reduction in color intensity was defined as the minimum inhibitory concentration (MIC). For determination of minimum bactericidal/fungicidal concentrations (MBC and MFC, respectively), aliquots from non-turbid wells were subcultured into fresh medium and incubated for an additional 24 h. The lowest concentration showing no visible growth was considered bactericidal. The assay included Gram-positive and Gram-negative bacteria, as well as the yeast *Candida auris*. Streptomycin and ketoconazole were used as positive controls for bacteria and yeast, respectively, as well as preservative sodium metabisulfite (E221). All microbial strains were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade.

Results and discussion

In the present study, GC/MS data revealed that *N. nuda* EO is particularly rich in oxygenated monoterpenoid 1,8-cineole. Interestingly, nepetalactones were present in relatively low amounts. Monoterpenes (α-pinene, β--pinene, sabinene), sesquiterpenes (β-bourbonene, germacrene D), and oxygenated sesquiterpenes (caryophyllene oxide) were also recorded. The antimicrobial activity of *N. nuda* EO was examined against selected bacterial and fungal strains using the microdilution method. The activity was assessed based on MIC, MBC, and MFC. *N. nuda* EO exhibited notable antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, including a methicillin-resistant (MRSA) strain. Although its antibacterial efficacy was lower than that of streptomycin standard, *N. nuda* EO generally demonstrated stronger inhibitory and bactericidal effects compared to the preservative sodium metabisulfite (E221). *N. nuda* EO also exhibited significant antifungal activity towards yeasts, *Candida parapsilosis* and *C. albicans*. Although its antifungal potential was lower compared to the reference antifungal agent (ketoconazole) the observed activity indicates that *N. nuda* EO possesses a broad-spectrum antimicrobial potential against both bacterial and fungal pathogens.

Conclusion

Observed results indicate that *N. nuda* EO exhibits strong antimicrobial potential, particularly towards bacterial pathogen *P. aeruginosa*. These activities might be attributed to the high content of terpenes belonging to the groups of monoterpenes and sesquiterpenes. Although not comparable with commercial antibiotics, *N. nuda* EO could find applications as a natural antimicrobial agent in the food, cosmetic, and pharmaceutical industries.

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