

Antimicrobial activity of ethyl acetate extract of an endemic *Centaurea glaberrima* Tausch (Asteraceae)

Milica Miletic^{*1}, Marija Ivanov², Jelica Novaković¹, Pedja Janačković¹

¹University of Belgrade - Faculty of Biology, Department of Morphology and Systematics of Plants,
Studentski trg 16, 11000 Belgrade, Serbia

²Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", National Institute of Republic of
Serbia, University of Belgrade, Bulevar despota Stefana 142, 11000 Belgrade, Serbia

Introduction

Global health and modern medicine are facing a worldwide issue nowadays – antimicrobial resistance of microorganisms. A great interest in the pursuit of bioactive compounds from plants that can be antimicrobial drugs has been triggered due to multidrug resistance in pathogenic microorganisms and undesirable side effects of certain antibiotics (Alviano & Alviano, 2009).

Ethnobotanical data suggest that many *Centaurea* species are traditionally used for treatment of various ailments in people and animals, as well as for nourishment (Khammar & Djeddi, 2012). Extracts obtained from *Centaurea* exhibit wide range of biological activity (Khammar & Djeddi, 2012) with a numerous studies concerning antimicrobial activity (Güven et al., 2005; Karamenderes et al., 2006; Özcan et al., 2019; Dimkić et al., 2020).

Centaurea glaberrima Tausch (Asteraceae), Circum-Mediterranean Clade *sensu* Hilpold et al. (2014), is an endemic plant species that inhabits fields as well as a rocky cliffs and waste places in the West Balkans (Dostál, 1976).

There is no information on biological activity of *C. glaberrima* therefore the aim of this study was to investigate antimicrobial potential of ethyl acetate extract of this endemic species.

Materials and methods

Plant material

Plant material of *C. glaberrima* was collected in August 2021 at the Orjen Mountain (Montenegro). Voucher specimen (BEOU 38660) was deposited at the Herbarium of the University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac" (Thiers, 2016).

Extraction

Plant material, 10 g of air-dried *C. glaberrima* aerial parts, was milled into powder using a laboratory mill and submerged in 150 mL of ethyl acetate for 24 hours. After that the sample was ultrasonicated for 15 min and filtered afterwards. In order to re-extract the plant residue, procedure was repeated twice for 48 hours each time using additional 150 mL of solvent. After last repeat, sample was evaporated to dryness using rotary vacuum evaporator.

Antimicrobial activity

Antimicrobial potential of extract was investigated on four bacterial strains (*Escherichia coli* ATCC 35210, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* PAO1, and *Staphylococcus aureus* ATCC 6538) and three *Candida* strains (*Candida auris* ATCC 11903, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750) using microdilution method. Minimum inhibitory and minimum bactericidal

*milica.miletic@bio.bg.ac.rs

concentrations (MIC/MBC) were determined by a serial microdilution of *C. glaberrima* extract in 96-well microtiter plates following the protocol described by Kostić et al., 2017. Minimal inhibitory and minimal fungicidal concentrations (MIC/MFC) were determined according to the modified EUCAST, 2002 procedure.

Results and discussion

Ethyl acetate extract of *C. glaberrima* showed moderate antibacterial and anticandidal activity. *Escherichia coli* ATCC 35210 and *Pseudomonas aeruginosa* PAO1 were more susceptible to the extract (MICs 0.5 mg/ml) than *Klebsiella pneumoniae* ATCC 13883 and *Staphylococcus aureus* ATCC 6538 (MICs 1 mg/mL). Extract exhibited the strongest anticandidal activity against *Candida parapsilosis* ATCC 22019 (MIC 0.25 mg/mL) while *C. auris* ATCC 11903 and *C. tropicalis* ATCC 750 were equally susceptible (MICs 1 mg/mL).

Previous study of antimicrobial activity of several *Centaurea* species showed that the ethyl acetate extracts were the most active against bacteria and *Candida* species (Güven et al. 2005). Species from Circum-Mediterranean Clade are scarcely investigated from the aspect of biological activity, including antimicrobial activity. It was shown that extracts of capitula and aerial parts of *Centaurea kilaea* inhibited the growth of *P. aeruginosa* at concentrations 312 µg/mL which is lower than MIC from current study (0.5 mg/mL). Extracts also exhibited activity against *C. albicans* (MICs 312 µg/mL), and it was more susceptible than *Candida* strains investigated herein, with an exception of *C. parapsilosis* (0.25 mg/mL). Extract of aerial parts of *C. cuneifolia* inhibited the growth of *S. aureus* (MIC 625 µg/mL) (Şen et al., 2014) more than *C. glaberrima* extract (MIC 1mg/mL). Extracts of *C. cariensis* ssp. *maculiceps* and *C. cariensis* subsp. *microlepis* were active against *E. coli* (MICs 2 and 4 mg/mL, respectively), and *S. aureus* (MICs 2 mg/mL) (Tekeli et al., 2011) in concentrations higher than obtained in this study. *C. virgata* exhibited activity against *E. coli* as well (MIC 1 mg/mL) (Tekeli et al., 2011).

Conclusion

Centaurea glaberrima ethyl acetate extract showed promising results in microdilution assay against tested bacteria and yeasts. To the best of our knowledge, this is the first study concerning biological activity of this species and outcome encourages further investigation.

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