

**ANTIRADICAL ACTIVITY AND PHENOLIC CONSTITUENTS OF *STACHYS MILANII*  
AERIAL FLOWERING PARTS METHANOL EXTRACT****Jelena Kukić-Marković<sup>1\*</sup>, Violeta Milutinović<sup>1</sup>, Marjan Niketić<sup>2,3</sup>,  
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*Stachys milanii* Petrović (Lamiaceae) is an endemic of central and eastern part of Balkan Peninsula, growing mainly in river valleys on semi-saline soils, often along the edge of cereal fields and abandoned arable lands. It is a critically endangered and strictly protected species in Serbia, with just one proven habitat (1). So far it was investigated on herb essential oil composition and antimicrobial activity, and on fatty acid composition of seeds (2). Aim of this work was to investigate antiradical potential and phenolic constituents of dry methanol herb extract. Aerial flowering parts (herb) of *S. milanii* were collected in North Macedonia (Ovče Pole). Powdered, dried plant material was extracted by bimaceration with chloroform, and then with methanol. Obtained dry methanol extract was used for further analysis. Using appropriate spectrophotometric procedure strong, concentration-dependant anti-DPPH activity of extract was observed (SC<sub>50</sub>=34.15 µg/mL). Also spectrophotometrically, using Folin-Ciocalteu reagent, significant total phenolics content (0,47 mg gallic acid/mg dry extract) was determined. In TLC-DPPH test observed yellow zones indicated presence of few compounds which strongly neutralized purple DPPH radical. LC-MS analysis revealed presence of one phenolic acid, four phenylethanolic glycosides, eight flavonoid glycosides derivatives of 8-hydroxyflavones, and one apigenin glycoside. Using commercial standards in extract were identified and quantified: chlorogenic acid (1.63%) and acteoside (2.65%), as the main phenylethanolic. In 8-hydroxyflavones fraction six hypolaetine and two isoscutellarein derivatives were present; using compounds previously isolated from *S. alpina* subsp. *dinarica* Murb. herb, 4'-*O*-methylhypolaetine-7-*O*-[6'''-acetylallosyl(1→2)]-glucopyranoside and isoscutellarein-7-*O*-[6'''-acetylallosyl(1→2)]-glucopyranoside were identified, representing the most prominent flavonoids in investigated extract, respectively.

**References**

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**Acknowledgements**

This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade – Faculty of Pharmacy No: 451-03-68/2022-14/200161.

## ANTIRADIKALSKA AKTIVNOST I FENOLNI SASTOJCI METANOLNOG EKSTRAKTA HERBE *STACHYS MILANII*

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*Stachys milanii* Petrović (Lamiaceae) je endemit centralnog i istočnog dela Balkanskog poluostrva koji raste pretežno u dolinama reka na poluslanim zemljištima, često i po obodu žitnih polja i po napuštenim oranicama. U Republici Srbiji je kritično ugrožena i strogo zaštićena vrsta, sa samo jednim zabeleženim staništem (1). Biljka je do sada ispitivana u pogledu sastava i antimikrobne aktivnosti etarskog ulja herbe i kompozije masnih kiselina semena (2). Cilj ovog rada bio je da se ispituju antiradikalni potencijal i fenolni sastojci suvog metanolnog ekstrakta herbe. Nadzemni delovi *S. milanii* u cvetu prikupljeni su u Severnoj Makedoniji (Ovče Pole). Sprašen, osušen biljni materijal je ekstrahovan posupkom bimaceracije najpre hloroformom, a zatim metanolom. Dobijeni suvi metanolni ekstrakt korišćen je za dalju analizu. Odgovarajućim spektrofotometrijskim postupkom utvrđena je snažna, koncentraciono-zavisna anti-DPPH aktivnost ekstrakta ( $SC_{50}=34,15 \mu\text{g/mL}$ ). Spektrofotometrijski, korišćenjem Folin-Ciocalteu reagensa, utvrđen je i značajan sadržaj ukupnih polifenola (0,47 mg galne kiseline/mg suvog ekstrakta). U TLC-DPPH testu uočene žute zone ukazale su na prisustvo većeg broja jedinjenja koja snažno neutrališu ljubičasti DPPH-radikal. LC-MS analizom u ekstraktu su detektovani jedna fenolkarboksilna kiselina, četiri feniletanska heterozida, osam flavonoidnih heterozida derivata 8-hidroksiflavona i jedan heterozid apigenina. Korišćenjem komercijalnih standarda identifikovani su i kvantifikovani hlorogenska kiselina (1,63%) i akteozid (2,65%), kao glavni feniletanski heterozid. Frakciju derivata 8-hidroksiflavona činilo je šest heterozida hipolaetina i dva heterozida izoskutelareina; korišćenjem jedinjenja prethodno izolovanih iz herbe *S. alpina* subsp. *dinarica* Murb., identifikovani su 4'-*O*-metilhipolaetin-7-*O*-[6'''-acetilalozil(1→2)]-glukopiranozid i izoskutelarein 7-*O*-[6'''-acetilalozil(1→2)]-glukopiranozid, koji su ujedno predstavljali i najzastupljnije flavonoide u ekstraktu, redom.

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### Zahvalnica

Ovo istraživanje finansirano je od strane Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije kroz Ugovor sa Univerzitetom u Beogradu – Farmaceutskim fakultetom broj: 451-03-68/2022-14/200161.