

**SECONDARY METABOLITES AND ANTI-CHOLINESTERASE ACTIVITY OF
CHAMAECYTISUS HEUFFELII SUBSP. JANKAE AERIAL FLOWERING PARTS****Violeta Milutinović^{1*}, Marjan Niketić^{2,3}, Silvana Petrović¹**¹University of Belgrade – Faculty of Pharmacy, Department of Pharmacognosy,
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Chamaecytisus heuffelii subsp. *jankae* (Velen.) Niketić (Fabaceae) is a dwarfish shrub, which range includes eastern part of the Balkan Peninsula (Albania, Bulgaria, Serbia) (1). The aim of the investigation was to chemically characterize aerial flowering parts of this plant: the dried methanol extract (TM), its alkaloid (AF) and non-alkaloid (NAF) fractions, and to assess their ability and the ability of detected flavonoids to inhibit enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), *in vitro* in the Ellman spectrophotometric test. Plant material was collected in eastern Serbia (Jelašnička Klisura gorge), dried, powdered and extracted with methanol, after dichloromethane pre-extraction. Using solvents of different polarities and pH value, AF (yield 14.35%) and NAF (yield 57.78%) were obtained from TM. By LC-MS analysis, flavonoids luteolin-8-C-glucosylpentoside (159.81 mg/g), genistin (22.38 mg/g), vitexin (6.32 mg/g), rutin (4.25 mg/g), and genistein (0.86 mg/g) were detected and quantified in TM, while luteolin-8-C-glucosylpentoside (212.70 mg/g), rutin (1.38 mg/g), and genistin (1.03 mg/g) in NAF. Using GC-FID-MS analysis, quinolizidine alkaloids sparteine, 17-oksosparteine, and lupanine were identified in AF. TM and NAF exhibited significant and comparable anti-cholinesterase activity (IC₅₀AChE 0.54 and 0.72 mg/mL; IC₅₀BuChE 0.45 and 0.68 mg/mL). All detected flavonoids evinced the ability to inhibit enzymes; the most active were genistein and vitexin (IC₅₀AChE 54.75 and 88.35 µg/mL; IC₅₀BuChE 27.83 and 49.77 µg/mL). Considering the quinolizidine alkaloids toxicity and AF poor anti-cholinesterase activity, it might be concluded, regarding safety profile and medical potential, that the fraction of the herb methanol extract, flavonoid-rich and alkaloid-free (NAF), is good candidate for further research.

References

1. Niketić M, Tomović G, Bokić B, Buzurović U, Duraki Š, Djordjević V, Djurović S, Krivošej Z, Lazarević P, Perić R, Prodanović D, Radak B, Rat M, Ranimirović M, Stevanović V. Material on the Annotated Checklist of Vascular Flora of Serbia. Nomenclatural, taxonomic and floristic Notes III. Bull. Nat. Hist. Mus. Belgr. 2021; 14: 77-132.

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SEKUNDARNI METABOLITI I ANTIHOLINESTERAZNA AKTIVNOST HERBE *CHAMAECYTISUS HEUFFELII* SUBSP. *JANKAE*

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Chamaecytisus heuffelii subsp. *jankae* (Velen.) Niketić (Fabaceae) je polužbun, koji raste na području istočnog dela Balkanskog poluostrva (Albanija, Bugarska, Srbija) (1). Cilj istraživanja bio je da se izvrši hemijska karakterizacija nadzemnih delova u cvetu ove biljke: suvog metanolnog ekstrakta (TM), njegove alkaloidne (AF) i frakcije bez alkaloida (NAF), i da se *in vitro*, u Ellmanov-om spektrofotometrijskom testu, ispita njihova sposobnost, kao i sposobnost detektovanih flavonoida da inhibiraju enzime acetilholinesterazu (AChE) i butirilholinesterazu (BChE). Biljni materijal je sakupljen u istočnoj Srbiji (Jelašnička klisura), osušen, samleven i ekstrahovan metanolom, nakon pre-ekstrakcije dihlormetanom. Primenom rastvarača različite polarosti uz promenu pH vrednosti, iz TM su dobijene AF (prinos 14,35%) i NAF (prinos 57,78%). LC-MS analizom, u TM detektovani su i kvantifikovani flavonoidi luteolin-8-C-glukozilpentoza (159,81 mg/g), genistin (22,38 mg/g), viteksin (6,32 mg/g) i rutin (4,25 mg/g) i genistein (0,86 mg/g), a u NAF luteolin-8-C-glukozilpentoza (212,70 mg/g), rutin (1,38 mg/g) i genistin (1,03 mg/g). GC-FID-MS analizom, u AF identifikovani su hinolizidinski alkaloidi spartein, 17-oksosparteina i lupanin. TM i NAF ispoljili su značajnu i uporedivu antiholinesteraznu aktivnost (IC_{50AChE} 0,54 i 0,72 mg/mL; IC_{50BChE} 0,45 i 0,68 mg/mL). Svi detektovani flavonoidi su pokazali sposobnost da inhibiraju enzime; najaktivniji su bili genistein i viteksin (IC_{50AChE} 54,75 i 88,35 µg/mL; IC_{50BuChE} 27,83 i 49,77 µg/mL). Uzimajući u obzir toksičnost hinolizidinskih alkaloida i slabu antiholinesteraznu aktivnost AF, može se zaključiti da je frakcija metanolnog ekstrakta herbe bogata flavonoidima i oslobođena alkaloida (NAF), u pogledu bezbednosnog profila i lekovitog potencijala dobar kandidat za dalja ispitivanja.

Literatura

1. Niketić M, Tomović G, Bokić B, Buzurović U, Duraki Š, Djordjević V, Djurović S, Krivošej Z, Lazarević P, Perić R, Prodanović D, Radak B, Rat M, Ranimirović M, Stevanović V. Materijal za Kritičku listu vrsta vaskularne flore Srbije. Nomenklatura, taksonomski i floristički prilozi III. Bull. Nat. Hist. Mus. Belgr. 2021; 14: 77-132.

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