

**IN VITRO CYTOTOXIC AND ANTI-INFLAMMATORY ACTIVITY OF
BRUCKENTHALIA SPICULIFOLIA EXTRACTS AND FRACTIONS**

**Dragana Pavlović^{1*}, Nikola Stojanović², Bojan Zlatković³, Niko Radulović³,
Nada Kovačević⁴**

¹University of Niš – Faculty of Medicine, Department of Pharmacy, Niš, Serbia

²University of Niš – Faculty of Medicine, Department of Medicine, Niš, Serbia

³University of Niš – Faculty of Sciences and Mathematics, Niš, Serbia

⁴University of Belgrade – Faculty of Pharmacy, Department of Pharmacognosy,
Belgrade, Serbia

*dragana.pavlovic@medfak.ni.ac.rs

According to our previous studies, *Bruckenthalia spiculifolia* Rchb. (Ericaceae) possess strong antioxidant activity and hydrogel loaded with 2% ethanolic extract from its flowers and leaves is a potential southing and wound healing agent (1). Ethanolic extracts of leaves, flowers, and herba, alongside fractions obtained by systematic solvent extraction method with solvents of the increasing polarity of liquid-liquid fractionation of areal parts of *B. spiculifolia*, were prepared. To estimate *in vitro* anti-inflammatory activities of eight different extracts and fractions, isolated rat peritoneal macrophages were employed. MTT test was used for the cytotoxicity determination, while the anti-inflammatory potential lipopolysaccharide-induced NO production model was utilized (2). Experimental procedures with animals complied with the rules of the European Union Normative. Resident rat peritoneal macrophages were harvested by peritoneal lavage and used for both procedures. Phytochemical analysis was done by RP-HPLC coupled with DAD detection. Tested samples statistically significantly decreased macrophage ability to metabolize MTT, mainly in a concentration-dependent manner. All of them also significantly inhibited NO production from macrophages stimulated with lipopolysaccharide, which can be related to either their cytotoxic or anti-inflammatory activity. Quantified polar compounds (especially quercetin-3-O-glucoside) could be associated with better antioxidant and anti-inflammatory effects, as well as with a lower cytotoxic potential. Indeed, fractions obtained with ethyl acetate and water, but also ethanol extract obtained after lipids removal with petroleum ether, showed the most promising effects. These results point out that *B. spiculifolia* is worth further investigation in order to detect new naturally occurring anti-inflammatory agents.

References

1. Pavlović D.R., Tasić-Kostov M., Marčetić M., Lakušić B., Kitić D., Savić S., Kovačević N. Evaluation of *in vivo* effects on surfactant-irritated human skin, antioxidant properties and phenolic composition of five Ericaceae species extracts. RIV ITAL SOSTANZE GR 2013; 90(4): 255-264.
2. Radulović N.S., Todorovska M.M., Zlatković D.B., Stojanović N.M., Randjelović P.J. Two goitrogenic 1,3-oxazolidine-2-thione derivatives from Brassicales taxa: Challenging identification, occurrence and immunomodulatory effects. FOOD CHEM TOXICOL 2017; 110:94-108.

Acknowledgments

This research was supported by the Ministry of Education and Science of the Republic of Serbia (Grant No. 451-03-68/2022-14/200113) and the Faculty of Medicine University of Niš Internal Scientific Project No. 15.

***IN VITRO CITOTOKSIČNA I ANTIINFLAMATORNA AKTIVNOST EKSTRAKATA I
FRAKCIJA BRUCKENTHALIA SPICULIFOLIA***

**Dragana Pavlović^{1*}, Nikola Stojanović², Bojan Zlatković³, Niko Radulović³,
Nada Kovačević⁴**

¹Univerzitet u Nišu – Medicinski fakultet, Katedra Farmacije, Niš, Srbija

²Univerzitet u Nišu – Medicinski fakultet, Odsek Medicina, Niš, Srbija

³Univerzitet u Nišu – Prirodno matematički fakultet, Niš, Srbija

⁴Univerzitet u Beogradu – Farmaceutski fakultet, Katedra za farmakognosiju,
Beograd, Srbija

*dragana.pavlovic@medfak.ni.ac.rs

Prema našim prethodnim istraživanjima, *Bruckenthalia spiculifolia* Rchb. (Ericaceae) poseduje jaku antioksidativnu aktivnost, a hidrogel sa 2% etanolnog ekstrakta cveta i lista ove biljne vrste pospešuje zarastanje rana (1). Pripremljeni su etanolni ekstrakti listova, cvetova i nadzemnog dela biljne vrste *B. spiculifolia*, kao i frakcije dobijene sistematskom ekstrakcijom rastvaračima rastućeg polariteta tečno-tečno frakcionisanjem nadzemnih delova biljke. Za procenu lokalne antiinflamatorne aktivnosti ukupno osam različitih ekstrakata i frakcija korišćeni su izolovani peritonealni makrofagi pacova. MTT test je služio za određivanje citotoksičnosti, a model produkcije NO izazvane lipopolisaharidom za *in vitro* kvantifikaciju antiinflamatornog potencijala (2). Eksperimentalne procedure sa životinjama su usklađeni sa zahtevima Evropske unije. Peritonealni makrofagi pacova su sakupljeni peritonealnim ispiranjem i korišćeni za obe procedure. Fitohemijska analiza je izvršna pomoću RP-HPLC sa DAD detekcijom. Testirani uzorci su statistički značajno smanjili sposobnost makrofaga da metabolišu MTT i to uglavnom na dozno-zavisan način. Svi oni su takođe značajno inhibirali proizvodnju NO iz makrofaga stimulisanih lipopolisaharidom, što se može dovesti u vezu sa njihovom citotoksičnom ili antiinflamatornom aktivnošću. Kvantifikovana polarna jedinjenja (naročito kvercetin-3-O-glukozid) mogu biti povezana sa boljim antioksidativnim i antiinflamatornim efektima, kao i sa nižim citotoksičnim potencijalom. Zaista, frakcije dobijene sa etil acetatom i vodom, ali i etanolni ekstrakt dobijen nakon uklanjanja lipida petroletom, su pokazale najbolje efekte. Izloženi rezultati ukazuju na to da vrstu *B. spiculifolia* treba dalje ispitati u cilju pronalaženja novih prirodnih antiinflamatornih agensa.

Literatura

1. Pavlović D.R., Tasić-Kostov M., Marčetić M., Lakušić B., Kitić D., Savić S., Kovačević N. Evaluation of *in vivo* effects on surfactant-irritated human skin, antioxidant properties and phenolic composition of five Ericaceae species extracts. RIV ITAL SOSTANZE GR 2013; 90(4): 255-264.
2. Radulović N.S., Todorovska M.M., Zlatković D.B., Stojanović N.M., Randjelović P.J. Two goitrogenic 1,3-oxazolidine-2-thione derivatives from Brassicales taxa: Challenging identification, occurrence and immunomodulatory effects. FOOD CHEM TOXICOL 2017; 110:94-108.

Zahvalnica

Ovo istraživanje podržali su Ministarstvo prosvete i nauke Republike Srbije (br. 451-03-68/2022-14/200113) i Interni naučni projekat Medicinskog fakulteta Univerziteta u Nišu br. 15.