

**INVESTIGATION OF POLYPHENOLS, FURANOCOUMARINS AND  
ANTIOXIDANT ACTIVITY OF LEAF AND FLOWER EXTRACTS OF  
*HERACLEUM SIBIRICUM* (APIACEAE)**

**Ljuboš Ušjak<sup>1</sup>, Milica Drobac<sup>1</sup>, Marjan Niketić<sup>2</sup>, Silvana Petrović<sup>1</sup>**

<sup>1</sup>University of Belgrade - Faculty of Pharmacy, Department of Pharmacognosy,  
Belgrade, Serbia

<sup>2</sup> Natural History Museum, Belgrade, Serbia

Siberian cow parsnip, *Heracleum sibiricum* L. (Apiaceae), is autochthonous to northern, eastern and central Europe, Balkan Peninsula and Siberia (1). Infusion prepared from aerial parts of this plant is used in folk medicine on Balkan Peninsula as aperitif and to treat diarrhea and other gastrointestinal complaints (2). Previous studies of *H. sibiricum* have not included polyphenol analysis, while furanocoumarins were analyzed in roots, fruits and leaves (1, 3).

In this work, chemical composition and antioxidant activity of methanol and dichloromethane extracts obtained from leaves and flowers of this plant were investigated.

Dried plant material, collected on Mts Stara Planina (Serbia) in July 2014, was extracted successively with dichloromethane and methanol (in both cases by bimaceration 72+48 h; drug/solvent=1:10 w/V). Solvents were removed under reduced pressure. The composition of obtained dry extracts was analyzed by LC-MS methods. Additionally, for methanol extracts, total phenolics contents were determined spectrophotometrically using Folin-Ciocalteu reagent. Antioxidant activity of methanol extracts was tested by spectrophotometric FRAP and DPPH assays, and of dichloromethane extracts by DPPH assay.

LC-MS analysis showed that investigated leaf and flower dry methanol extracts were very similar. In both extracts, significant amounts of quercetin and kaempferol glycosides (154.30 and 144.20 mg/g of dry extract) were determined. Dominant were vincetoxicoside A (quercetin 7-*O*-rhamnosyl 3-*O*-glucoside; 26.83 and 29.61 mg/g), kaempferitrin (kaempferol 3,7-di-*O*-rhamnoside; 33.62 and 34.68 mg/g) and one quercetin deoxyhexosyl deoxyhexoside (69.06 and 79.91 mg/g). Six more glycosides of these flavonols were detected in the leaf extract and five more in the flower extract. Also, chlorogenic acid (19.31 and 4.12 mg/g) was identified in both extracts. Spectrophotometrically determined total phenolics contents were 126.08 and 125.33 mg gallic acid equivalents/g.

In the leaf dry dichloromethane extract, by LC-MS method, small amounts of furanocoumarins isopimpinellin and bergapten were detected (totally 0.22 mg/g of dry extract). Their presence was also previously shown (3). In the flower extract, besides these two, six more furanocoumarins were identified; all present in small amounts (totally 20.07 mg/g). The most abundant was bergapten (4.84 mg/g), followed by byakangelicol (3.29 mg/g).

Total antioxidant (FRAP values 1.08 and 0.94 mmol Fe<sup>2+</sup>/g) and antiradical activities (DPPH SC<sub>50</sub> values 64.10 and 64.77 µg/mL) of the leaf and flower dry methanol extracts were correlated with their total phenolics contents. Anti-DPPH activities of the dry dichloromethane extracts were expectedly lower (SC<sub>50</sub> values 636.19 and 790.20 µg/mL).

*Heracleum sibiricum* leaves and flowers represent new sources of compounds with known antioxidant activity.

## References

1. Ušjak L, Drobac M, Niketić M, Petrović S. Chemosystematic significance of essential oil constituents and furanocoumarins of underground parts and fruits of nine *Heracleum* L. taxa from Southeastern Europe. *Chem Biodivers.* 2018; 15: e1800412.
2. Ivancheva S, Stantcheva B. Ethnobotanical inventory of medicinal plants in Bulgaria. *J Ethnopharmacol.* 2000; 69: 165-172.
3. Ozek G, Yur S, Goger F, Ozek T, Andjelković B, Godjevac D, Sofrenić I, Aneva I, Todorova M, Trendafilova A. Furanocoumarin content, antioxidant activity, and inhibitory potential of *Heracleum verticillatum*, *Heracleum sibiricum*, *Heracleum angustisectum*, and *Heracleum ternatum* extracts against enzymes involved in Alzheimer's disease and type II diabetes. *Chem Biodivers.* 2019; 16: e1800672.

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# ISPITIVANJE POLIFENOLA, FURANOKUMARINA I ANTIOKSIDANTNE AKTIVNOSTI EKSTRAKATA LISTOVA I CVASTI *HERACLEUM SIBIRICUM* (APIACEAE)

Ljuboš Ušjak<sup>1</sup>, Milica Drobac<sup>1</sup>, Marjan Niketić<sup>2</sup>, Silvana Petrović<sup>1</sup>

<sup>1</sup>Univerzitet u Beogradu - Farmaceutski fakultet, Katedra za farmakognoziju,  
Beograd, Srbija

<sup>2</sup> Prirodnački muzej, Beograd, Srbija

Velika mečja šapa, *Heracleum sibiricum* L. (Apiaceae), autohtona je u severnoj, istočnoj i centralnoj Evropi, kao i na Balkanskom poluostrvu i u Sibiru (1). Infuz pripremljen od nadzemnih delova ove biljke se u narodnoj medicini na Balkanskom poluostrvu koristi kao aperitiv i za tretman dijareje i drugih gastrointestinalnih tegoba (2). Prethodna ispitivanja *H. sibiricum* nisu uključivala analizu polifenola, dok su furanokumarini do sada analizirani u korenu, plodovima i listovima (1, 3).

Cilj ovog rada bio je ispitivanje hemijskog sastava i antioksidantne aktivnosti metanolnih i dihlormetanskih ekstrakata listova i cvasti ove biljke.

Osušen biljni materijal (sakupljen na Staroj Planini u Srbiji u julu 2014. god.) ekstrahovan je sukcesivno dihlormetanom i metanolom (u oba slučaja bimaceracijom 72+48 h, sa odnosom droga rastvarač 1:10 *m/V*). Rastvarači su uklonjeni pod sniženim pritiskom. Hemijski sastav dobijenih suvih ekstrakata analiziran je LC-MS metodama. Dodatno, u slučaju metanolnih ekstrakata spektrofotometrijski je utvrđen sadržaj ukupnih polifenola pomoću *Folin-Ciocalteu* reagensa. Antioksidantna aktivnost metanolnih ekstrakata ispitana je spektrofotometrijskim FRAP i DPPH testovima, a dihlormetanskih ekstrakata DPPH testom.

LC-MS analiza pokazala je da su suvi metanolni ekstrakti listova i cvasti bili vrlo slični. U njima su određene značajne količine heterozida kvercetina i kemferola (154,30 i 144,20 mg/g suvog ekstrakta). Najzastupljeniji su bili vincetoksikozid A (kvercetin 7-*O*-ramnozil 3-*O*-glukozid; 26,83 i 29,61 mg/g), kemferitrin (kemferol 3,7-di-*O*-ramnozil; 33,62 i 34,68 mg/g) i jedan kvercetin dezoksiheksozil dezoksiheksozil (69,06 i 79,91 mg/g). U ekstraktu listova detektovano je još šest, a u ekstraktu cvasti još pet heterozida ovih flavonola. Takođe, u oba ekstrakta identifikovana je hlorogenska kiselina (19,31 i 4,12 mg/g). Spektrofotometrijski utvrđen sadržaj ukupnih polifenola iznosio je 126,08 i 125,33 mg ekvivalenata galne kiseline/g.

U suvom dihlormetanskom ekstraktu listova LC-MS metodom detektovane su male količine furanokumarina izopimpinolina i bergaptena (ukupno 0,22 mg/g suvog ekstrakta), čije je prisustvo i ranije utvrđeno (3). U ekstraktu cvasti, pored dva navedena, identifikovano je još šest furanokumarina i svi su takođe bili prisutni u malim količinama (ukupno 20,07 mg/g); najzastupljeniji je bio bergapten (4,84 mg/g), a po količini ga je sledio biakangelikol (3,29 mg/g).

Ukupna antioksidantna aktivnost suvih metanolnih ekstrakata listova i cvasti (FRAP vrednosti 1,08 i 0,94 mmol Fe<sup>2+</sup>/g), kao i antiradikalska aktivnost (DPPH SC<sub>50</sub> vrednosti 64,10 i 64,77 µg/mL) bile su u korelaciji sa sadržajem ukupnih polifenola. Anti-DPPH aktivnost suvih dihlormetanskih ekstrakata bila je očekivano niža (SC<sub>50</sub> vrednosti 636,19 i 790,20 µg/mL).

Može se zaključiti da listovi i cvasti *H. sibiricum* predstavljaju nove izvore jedinjenja sa poznatom antioksidantnom aktivnošću.

## Literatura

1. Ušjak L, Drobac M, Niketić M, Petrović S. Chemosystematic significance of essential oil constituents and furanocoumarins of underground parts and fruits of nine *Heracleum* L. taxa from Southeastern Europe. *Chem Biodivers.* 2018; 15: e1800412.
2. Ivancheva S, Stantcheva B. Ethnobotanical inventory of medicinal plants in Bulgaria. *J Ethnopharmacol.* 2000; 69: 165-172.
3. Ozek G, Yur S, Goger F, Ozek T, Andjelković B, Godjevac D, Sofrenić I, Aneva I, Todorova M, Trendafilova A. Furanocoumarin content, antioxidant activity, and inhibitory potential of *Heracleum verticillatum*, *Heracleum sibiricum*, *Heracleum angustisectum*, and *Heracleum ternatum* extracts against enzymes involved in Alzheimer's disease and type II diabetes. *Chem Biodivers.* 2019; 16: e1800672.

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