# UHPLC-DAD-MS/MS phytochemical profiling of *Geranium robertianum L.* herba extract obtained by ultrasound-assisted extraction

**Emina M. Mrkalić[1], Miroslav M. Sovrlić[2,*], Elma M. Šaćirović[3], Anica M. Petrović[2,4], Miloš N. Milosavljević[5], Jelena B. Zvezdanović[6], Aleksandar Petrušić[7], Sandra S. Konstantinović[6]**

[1]University of Kragujevac, Institute for Information Technologies, Department of Science, Kragujevac, Serbia; emina.mrkalic@pmf.kg.ac.rs
[2]University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Kragujevac, Serbia; sofke-ph@hotmail.com
[3]University of Novi Pazar, Department of Biomedical Sciences, Novi Pazar, Serbia; elma.sacirovic96@gmail.com
[4]University of Kragujevac, Faculty of Medical Sciences, Center of Excellence for Redox Balance Research in Cardiovascular and Metabolic Disorders, Kragujevac, Serbia; petkovicanica0@gmail.com, salekkg91@gmail.com
[5]University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacology and Toxicology, Kragujevac, Serbia; milosavljevicmilos91@gmail.com
[6]University of Niš, Faculty of Technology, Leskovac, Serbia; sakisandra12@yahoo.com, jzvezdanovic@tf.ni.ac.rs
[7]University of Belgrade, Faculty of Social Pharmacy and Pharmaceutical Ligislation, Belgrade, Serbia; sakisandra12@yahoo.com, jzvezdanovic@tf.ni.ac.rs

* *Corresponding author: sofke-ph@hotmail.com*

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**Abstract**: This study aimed to investigate the polyphenolic profile of *Geranium robertianum* **L.** herba using ultrasound-assisted extraction (UAE) followed by UHPLC-DAD-MS/MS analysis. The extraction was performed with 38% polyethylene glycol (PEG) in water as solvent, under optimized conditions (1:10 w/v ratio, 60 W, 50 °C, 45 min). The qualitative analysis revealed a chemically diverse extract, rich in phenolic compounds, particularly ellagitannins and flavonoids. Identified ellagitannins included brevifolincarboxylic acid, geraniin, HDDP-galloyl-glucose isomer, di-galloyl-HHDP-glucoside isomer, and a castalagin derivative. In the flavonoid fraction, compounds such as quercetin and its glycosides (quercetin-glucuronide, quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, rutin), kaempferol derivatives, myricetin, myricitrin, luteolin-8-C-glucoside (orientin), and methylellagic acid rhamnoside were detected. Additionally, structurally complex flavonoids such as dihydroxy tetramethoxy flavones and sugar-conjugated quercetin forms further emphasized the phytochemical richness of the extract. The presence of these polyphenolic compounds suggests potential antioxidant, anti-inflammatory, antimicrobial, and photoprotective activities. These findings support the traditional use of *G. robertianum* and highlight its relevance as a source of multifunctional bioactive compounds. The results provide a basis for further pharmacological evaluation and indicate potential for future application in the development of phytopharmaceutical and cosmetic formulations.

**Keywords**: *Geranium robertianum*, Phytochemical profiling, Ultrasound-assisted extraction, UHPLC-DAD-MS/MS

## 1. Introduction

*Geranium robertianum L.* (commonly known as Herb Robert or Red Robin) is an annual or biennial herbaceous plant from the family *Geraniaceae*, widely distributed across the temperate regions of the Northern Hemisphere, including Europe, Asia, North Africa, and North America [1,2]. Traditionally, this species has been used in folk medicine for the treatment, wound healing, gastrointestinal ailments and inflammatory conditions, which has stimulated contemporary interest in the investigation of its chemical profile and pharmacological potential [3]. Phytochemical studies have demonstrated that *G. robertianum* is rich in a variety of polyphenolic compounds, notably ellagitannins, flavonoids (such as quercetin and kaempferol), and several organic acids, including gallic, ellagic, chlorogenic, caffeic, and ferulic acids [4]. These constituents have been associated with significant antioxidant, anti-inflammatory, and antimicrobial activities, highlighting the potential of this plant for further applications in pharmaceutical and cosmetic formulations [5]. The extraction of bioactive compounds is a fundamental step in the isolation of phytochemicals from plant matrices, as it ensures the recovery of pharmacologically active constituents for subsequent characterization and formulation. The selection of the optimal extraction approach is determined by the physicochemical properties of target compounds, solvent polarity, and the required yield, with non-conventional methods increasingly favored for their rapidity and enhanced extraction performance [6]. Ultrasound-assisted extraction (UAE) is a modern, efficient, and environmentally friendly technique increasingly used for isolating bioactive compounds from plant materials. This method significantly reduces extraction time and solvent consumption compared to conventional techniques such as maceration, while simultaneously providing higher yields. Due to its green, cost-effective, and highly productive characteristics, UAE is considered one of the leading techniques for the extraction of phytochemicals [7].

## 2. Results and Discussion

### *2.1 Qualitative profiling of phenolic compounds by UHPLC-DAD-MS*

Representative UHPLC-DAD-MS chromatogram of the extract, illustrating the retention times and peak intensities of the identified phenolic compounds, as determined by UV detection and mass spectral data (Figure 1.).

The chromatograms of the *G. robertianum* extract revealed a complex chemical profile, with numerous well-resolved peaks detected at different retention times, corresponding to various classes of polyphenolic compounds. he presence of multiple overlapping signals further underscores the chemical complexity of the extract and the diversity of phenolic constituents.

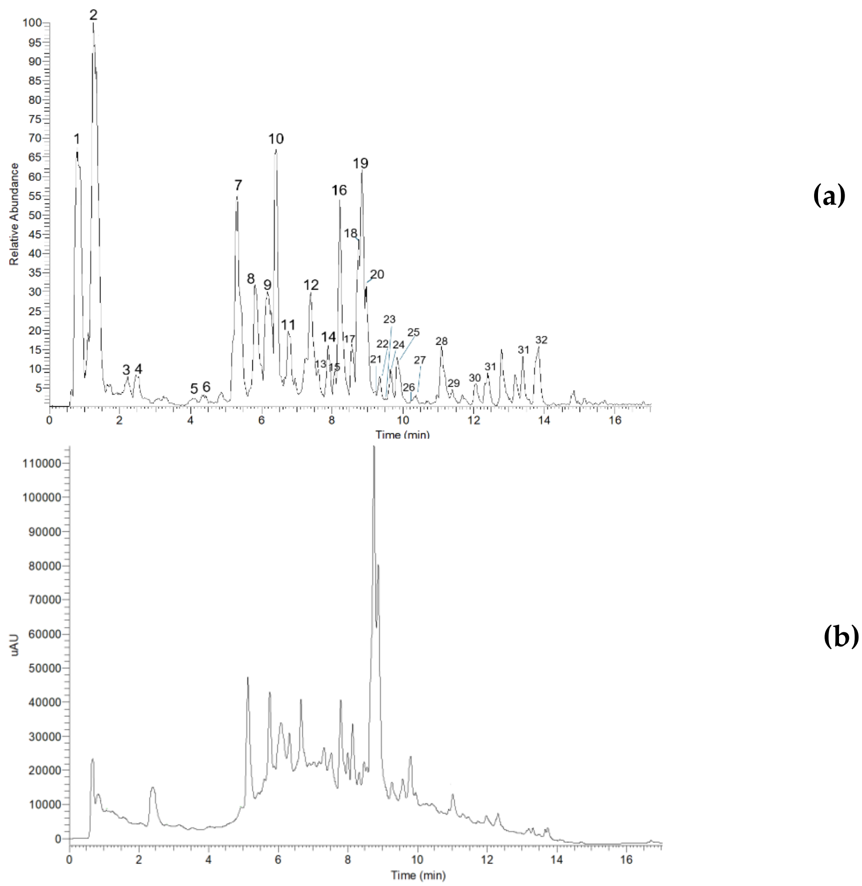


**Figure 1.** UHPLC-ESI-MS chromatogram of *Geranii robertiani herba* PEG extract ranked by base peak Intensity (a) and UHPLC-DAD chromatogram recorded at 330 nm (b)

A comprehensive qualitative analysis of the extract revealed the presence of a wide array of phenolic and polyphenolic compounds, especially phenolic acids, hydrolyzable tannins and flavonoids. including phenolic acids, ellagitannins and flavonoids (Table 1). Ellagitannins such as brevifolincarboxylic acid, geraniin, HDDP-galloyl-glucose isomer, di-galloyl-HHDP-glucoside isomer, and a castalagin derivative were detected, indicating a rich presence of hydrolyzable tannins in the extract.

**Table 1**. Identified phenolic compounds in the PEG extract of *Geranii robertiani herba*

| Peak No. | $t_R$, min MS | Compound | Peak No. | tR, min MS | Compound |
|---|---|---|---|---|---|
| 1. | 0.85 | Quinic acid | 17. | 8.56 | Myricitrin |
| 2. | 1.20 | Galloyl-quinic acid | 18. | 8.77 | Elagic acid |
| 3. | 2.16 | Galloylshikimic acid | 19. | 8.82 | Quercetin-glucuronide |
| 4. | 2.51 | Neochlorogenic acid (3-*O*-caffeoyl-quinic acid) | 20. | 8.90 | Quercetin-3-*O*-glucoside |
| 5. | 4.15 | *O*-p-coumaroyl-quinic acid | 21. | 8.94 | Rutin |
| 6. | 4.56 | Feruloyl hexaric acid isomer | 22. | 9.34 | Quercetin pentoside |
| 7. | 5.20 | Chlorogenic acid | 23. | 9.58 | Kaempferol-7-*O*-β-D-glucopyranoside |

| | | | | | |
|---|---|---|---|---|---|
| 8. | 5.84 | Brevifolincarboxylic acid | 24. | 9.66 | Quercetin arabinoside or xyloside |
| 9. | 6.13 | Geraniin | 25. | 9.84 | Quercetin-3-*O*-rhamnoside |
| 10. | 6.40 | HDDP-galloyl-glucose isomer 1- | 26. | 10.05 | Luteolin-8-C-glucoside (orientin) |
| 11. | 6.76 | Di*O*-galloyl-HHDPglucoside isomer 1 | 27. | 10.30 | Methylellagic acid rhamnoside |
| 12. | 7.40 | Castalagin derivative | 28. | 10.89 | Quercetin-3-*O*-rhamnoside |
| 13. | 7.61 | Kaempferol-8-*C*-*β*-D-glucopyranoside | 29. | 11.09 | Quercetin |
| 14. | 7.81 | Quercetin derivative | 30. | 12.39 | Kaempferide |
| 15. | 8.12 | Myricetin | 31. | 13.38 | Dihydroxy tetramethoxy flavones |
| 16. | 8.20 | Quercetin-3-*O*-*β*-D-2``-galloylgalactopyranoside) | 32. | 13.76 | Kemferol-dimetiletar |

Within the flavonoid fraction, a diverse range of compounds was identified, including quercetin and its derivatives (e.g., quercetin-glucuronide, quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, rutin, and quercetin pentoside), as well as kaempferol derivatives (kaempferol-8-C-β-D-glucopyranoside, kaempferol-7-O-β-D-glucopyranoside, and kaempferol dimethyl ether), myricetin and myricitrin, luteolin-8-C-glucoside (orientin), and methylellagic acid rhamnoside. Additionally, complex flavonoid structures such as dihydroxy tetramethoxy flavones and various sugar-conjugated forms of quercetin were also detected, further highlighting the remarkable chemical diversity of the extract.

## 3. Methodlogy

*3.1 Ultrasound-Assisted Extraction (UAE)*

Ultrasound-assisted extraction (UAE) of *Geranii robertiani herba* was carried out in order to obtain a polyphenol-rich extract under optimized conditions. Dried aerial parts of *G. robertianum* were obtained from the Institute for Medicinal Plant Research "Dr. Josif Pančić". Dried and powdered plant material was sieved to achieve a uniform particle size of 0.75 mm. The extraction solvent consisted of 38% (v/v) polyethylene glycol (PEG) dissolved in distilled water. A fixed plant material-to-solvent ratio of 1:10 (w/v) was used. The extraction was performed in an ultrasonic bath operating at a power of 60 W and a constant temperature of 50 °C. The duration of sonication was 45 minutes.

*3.2 Qualitative Analysis of Bioactive Compounds by UHPLC-DAD-MS/MS*

Qualitative analysis of the extract composition was performed using UHPLC on a Hypersil Gold C18 column (50 × 2.1 mm, 1.9 μm) at 25 °C with a Dionex Ultimate 3000 UHPLC+ system, coupled to a DAD and LCQ Fleet Ion Trap MS (Thermo Fisher Scientific, USA), as previously described [8,9]. The mobile phase consisted of 0.1% formic acid (A) and methanol (B), applied via a gradient at 0.25 mL/min. The injected volume was 1 μL. DAD spectra were recorded between 200–800 nm. MS analysis was done in negative ESI mode, with full scan (m/z 100–900) and data-dependent MS/MS (30 eV). Compound identification was based on retention time, UV–Vis and MS spectra, and comparison with standards and literature data.

## 4. Conclusions

*Geranium robertianum* extract was found to contain a diverse range of polyphenolic compounds, particularly ellagitannins and flavonoids, with known antioxidant, anti-inflammatory, and antimicrobial potential. These findings support its traditional use and indicate promising potential for application in phytopharmaceutical and cosmetic formulations.

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