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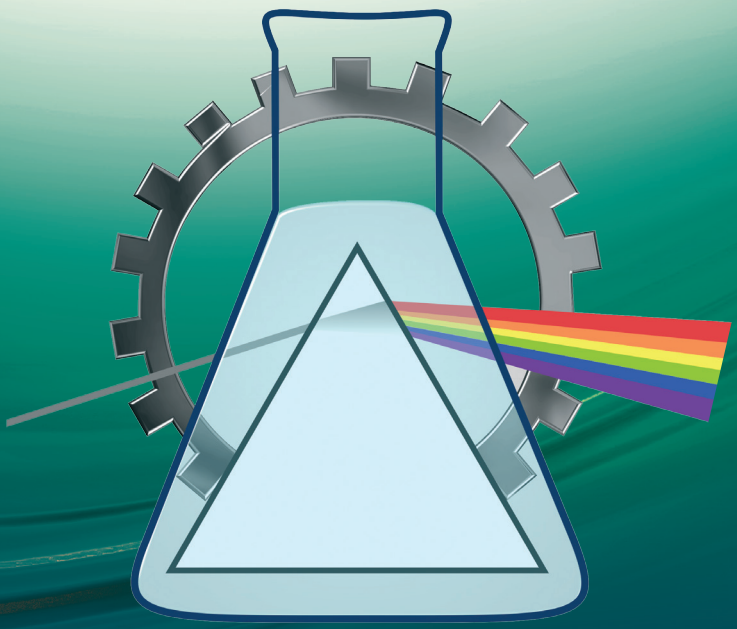
# SCIENCE AND INDUSTRY
## challenges and opportunities



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**Edited by:**
**prof. dr hab. Zbigniew Hubicki**

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20-031 Lublin, ul. Idziego Radziszewskiego 11
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# PERCOLATION VS ULTRASOUND ASSISTED EXTRACTION: IMPACT ON CHEMICAL COMPOSITION AND PHARMACOLOGICAL ACTIVITIES OF *Vaccinium myrtillus* FRUIT EXTRACTS

**N. KRGOVIĆ[1], J. MUDRIĆ[1], M. MARTINOVIĆ[2], A. ŽUGIĆ[1], V. M. TADIĆ[1],**
[1]Institute for Medicinal Plants Research "Dr. Josif Pančić", 11000 Belgrade, Serbia,
[2]University of Niš, Faculty of Medicine, Department of Pharmacy, 18000 Niš, Serbia.

**Introduction:** Fruits, particularly berries, are one of the most important sources of phenolic compounds. Apart from diet, fruits are used for the production of herbal preparations intended for use in phytotherapy. At the industrial scale, percolation is the most widespread conventional extraction method for the recovery of phenolics from plant material, while ultrasound-assisted extraction, an unconventional extraction method, is considered a highly efficient alternative [1]. According to numerous reports, anthocyanins, the main phenolic constituents of wild bilberry (*Vaccinium myrtillus* L., Ericaceae), are connected with anti-inflammatory, antioxidant, vasoprotective, as well as hypoglycemic and hypolipidemic effects [2]. In this context, our study aimed to compare the phenolics profile, *in vitro* antioxidant and hypoglycemic activities of dry hydroethanolic extracts prepared from fresh berries (*Myrtilli fructus*) by percolation (P) and ultrasound-assisted extraction (UAE).

**Experimental:** *Plant material and preparation of extracts:* The ripe fruits of wild bilberry were collected from a locality in Montenegro (Bijelo Polje) in August 2021. Before the analysis, the fruits were stored at –4 °C in the freezer. Percolation and ultrasound-assisted extraction were employed for the preparation of extracts from fresh berries (1:10, w/v) with 50% ethanol. The extraction was performed at room temperature, which lasted for 24 h in the case of percolation, and 2 h for the UAE.
*Chemical analysis:* To determine total phenolics, the Folin–Ciocalteu (FC) spectrophotometric method was applied [3], while total anthocyanins were estimated by following the procedure with 0.1% hydrochloric acid in methanol, described in 10$^{th}$ European Pharmacopoeia [4]. The HPLC method was used for the identification and quantification of active principles in the bilberry extracts. Analysis was performed on Agilent 1200 HPLC system equipped with a photodiode-array (PDA) detector and Lichrospher 100RP 18e column (250 × 4.6 mm; 5.0 µm particle size). The mobile phase consisted of 0.1 M phosphoric acid solution (phase A) and pure acetonitrile (phase B). Total run time was 60 min, flow rate 0.8 mL/min, injection volume 4 µL, and column temperature 25 $^{o}$C; the PDA detector was set at 520 nm. For flavonoids and phenolcarboxylic acids analysis, different chromatographic conditions were applied, and the total run time was 70 min, flow rate 1.0 mL/min, injection volume 10 µL and column temperature 25 $^{o}$C; PDA detector was set at 260, 280, and 325 nm.
*In vitro pharmacological activities:* The antioxidant capacity of extracts was assessed by the DPPH, FRAP and ABTS [5] assays. The hypoglycemic potential was evaluated by the ability of extracts to inhibit α-amylase [6] and α-glucosidase [7], using potato

starch/3,5-dinitrosalicylic acid and p-nitrophenyl-α-D-glucopyranoside methods, respectively.

**Results:** Colorimetrically assays revealed higher total phenolic (39.67 vs 45.94 mg GAE/g) and total anthocyanins content (26.46 vs 31.00 mg CGE/g) in UAE extract. Regarding HPLC analysis, the same chemical profile was observed in both extracts, with little differences in the determined active principles amount. The main identified and quantified secondary metabolites included anthocyanins (delphinidin-3-O-glucoside: 3.08 vs 4.53 and cyanidin-3-O-glucoside: 3.77 vs 4.48, being the most abundant), phenolic acids (gallic acid: 0.30 vs 0.27; protocatechuic acid: 0.81 vs 0.78; chlorogenic acid: 2.02 vs 2.02 mg/g) and flavonols (hyperoside: 0.91 vs 0.77; quercetin: 0.09 vs 0.10 mg/g). All applied antioxidant assays, DPPH ($IC_{50}$: 149.16 vs 141.06 μg/ml), ABTS ($IC_{50}$: 26.64 vs 23.85 μg/ml), and FRAP (0.81 vs 1.09 mmol $Fe^{2+}$/g), showed the similar antioxidant capacity of investigated extracts. By testing the ability of extracts to inhibit carbohydrate-digesting enzymes, it was noticed almost equal for α-amylase ($IC_{50}$: 900 vs 770 μg/ml) and stronger inhibitory potential of the UAE for α-glucosidase ($IC_{50}$: 74.86 vs 35.85 μg/ml).

**Conclusions:** Considering the chemical composition and pharmacological properties of bilberry extracts, a similar trend was remarked for the UAE and P extraction methods.

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