Supporting Information

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Phytochemical Characterization of *Filipendula ulmaria* by UPLC/Q-TOF-MS and Evaluation of Antioxidant Activity

Milda Pukalskienė[*],Petras Rimantas Venskutonis[2] and Audrius Pukalskas[3]

Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas LT-50254, Lithuania

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Experimental details

On-line HPLC/UV/DPPH• scavenging assay, identification and quantification of bioactive compounds

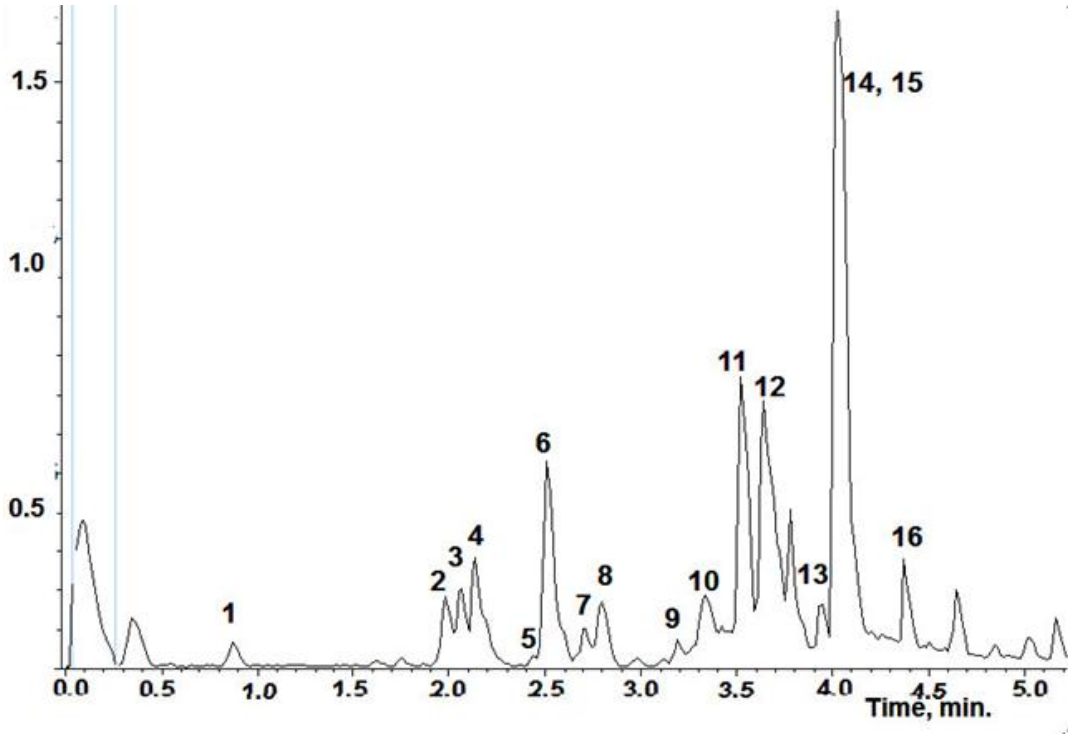
The system consisted of a Waters Reagent Manager pump (Milford, USA), Rheodyne 7125 manual injector (Rheodyne, RohnertPark, CA) with 20 µL injection loop and Discovery HS C_{18} 250 × 0.46 cm (5 µm) analytical column (Supelco Analytical, Bellefonte, USA). The linear binary gradient was formed at a constant flow rate of 1 mL min^{-1} using solvent A (2.5% acetic acid in water) and solvent B (100% acetonitrile). Analysis was started with 95% A and 5% B, afterwards B increased to 30% in 40 min, to 60% in 10 min and finally to 100% in 5 min. The column was washed for 5 min with 100% B, then in 3 min the gradient was returned to initial conditions and the column was equilibrated for 5 min before the next analysis. The applied gradient enabled to achieve good resolution and good peak shape. Compounds eluted from the column were detected with Waters 996 photodiode array detector (Milford, USA) in the range from 210 to 450 nm. After UV detection the 5 × 10^{-6} M DPPH• solution in methanol was added to the main eluent at a ratio 1:0.7 using Agilent 1100 series quaternary pump (Agilent Technologies, Inc. Santa Clara, CA). DPPH• solution were freshly prepared in methanol every day and kept protected from light. The mixture was introduced into a 15 m (0.25 mm ID) reaction coil made of PEEK (polyetheretherketone) tubing (Interchim, Frankfurt, Germany). Decrease of absorbance after the reaction of radical scavengers with DPPH• was detected photometricaly as a negative peak at 515 nm with variable-wavelength Shimadzu SPD-20A UV detector (Shimadzu Corporation, Kyoto, Japan). HPLC-MS system was equipped with Waters 996 photodiode array detector set for scanning from 210 to 450 nm and Waters Micromass ZQ mass detector operating in ESI negative ionization mode and starting scanning from 100 to 800 *m/z* till 16 min, then switched to *m/z* interval 200 – 1500 till 28 min, and after that the scanning interval was set back to 100 – 800 *m/z* till the end of chromatogram (Waters, Milford, USA). Capillary voltage was set to 3 kV, source temperature 120 °C, cone voltage 30 V, cone gas flow 80 L/h, desolvation temperature 300 °C, desolvation gas flow 310 L/h. The flow rate was 1 mL/min, the injection volume of 1% extract solutions was 20 µL the gradient was as described in (on line HPLC-UV-DPPH• method). The eluting constituents were identified by comparing their retention times, UV and MS spectra with reference compounds. Quantitative analysis was performed by using external available standards. Calibration curves were drawn using concentrations (1.5 – 250 µg/mL) of standard solutions: gallic acid (y = 0.0039x + 0.9566; R^2 = 0.999); catechin (y = 0.0073x + 5.2735; R^2 = 0.995); rutin (y = 0.0023x – 4.3059; R^2 = 0.996); hyperoside (y = 0.0025x – 2.8756; R^2 = 0.996); luteolin-7-glucoside (y = 0.0046x + 1.7192; R^2=0.990); astragalin (y = 0.0012x – 1.7406; R^2 = 0.995). Obtained curves represented the dependence between integrated chromatographic peak areas and corresponding amounts of injected standards. The concentration of identified compounds were calculated by linear regression of plots and expressed in mg/100 g on a dry weight (DW) of the herbs.

Qualitative analysis of phytochemical composition by UPLC/Q-TOF/MS^2

Analytical system consisted of ultra-performance liquid chromatograph Waters Acquity UPLC with binary solvent manager, sample manager, column manager with Acquity BEH, C_{18} column, 2.1 × 100 mm, particle size 1.7 µm (Waters, Milford, USA) and PDA detector. The flow rate was 0.4 mL/min, injection volume 2 µL (1 mg of extract was dissolved in 1 mL of methanol before injection). The mobile phase was composed of solvent A (0.4% formic acid in ultrapure water) solvent B (100% acetonitrile) and the following gradient was applied: analysis was started with 95% A and 5% B; than concentration of solvent B was increased to 20% in 3.0 min., and in following 5 min to 50%. The column was washed with 100% B in following 8 min, the gradient was returned to initial conditions. The eluents from the UPLC were directed to MaXis 4G Q-TOF MS^2 (Bruker Daltonic, Bremen, Germany) operating in ESI negative mode. Capillary voltage of + 4500 V, with the end plate offset of - 500 V, and collision cell energy of 30 eV were used. Nitrogen was used as the nebulizing and drying gas at flow rate of 10.0 L/ min and pressure of 2.5 bar, respectively. Nitrogen was introduced into the collisional cell as the collision gas. Fullscan and auto MS^2 was acquired at 2.5 Hz acquisition rate,

scanned mass range was from 100 to 2000 *m/z*. The compounds were identified based on their spectral properties comparing with standarts and on literature survey.

S2: Chromatogram of *F. vulgaris* obtained by UPLC - Q-TOF - MS/MS obtained in negative mode



Quantitative analysis of flavonoids and phenolic acids

Total amount of identified compounds in methanol extract was higher than in extracts obtained with acetone or water. In this case, quantitative analysis was performed using methanol extracts of plant material harvested at different plant vegetation stages. It may be observed that the concentrations of the quantified compounds during vegetation were changing in a wide range. For instance, the concentrations of gallic acid during blooming and seed ripening were 10.11 and 26.50 mg/100 g DW, respectively. The highest concentration of spiraeoside (1104.62 mg/100 g DW) and hyperoside (686.83 mg/100 g DW) were determined during plant blooming. It seems that these compounds are intensively biosynthesized during blooming, because their concentrations during butonization period were several times lower, only 99.6 and 77.5 mg/100 g DW, respectively. Rutin and catechin were also intensively biosynthesized during blooming; their concentrations at this phase were 421.61 and 446.50 mg/100 g DW, respectively. However, the concentration of rutin was found even higher at seed ripening period (458.22 mg/100g DW); while the concentration of catechin during seed ripening period remarkably decreased and constituted 156.94 mg/100 g DW. It is obvious that spiraeoside, rutin, catechin and hyperoside were the most abundant flavonoids found in *F. ulmaria* plant. The concentration of astragalin was quite similar during blooming and seed ripening; 27.61, and 32.60 mg/100 g DW, respectively, while during butonization period its concentration was below LOQ. Chlorogenic acid and luteolin-7-*O*-glucoside were present at the lowest concentrations. For instance, the concentration of chlorogenic acid was below its LOQ during all vegetation periods, while luteolin-7-*O*-glucoside was quantified only during seed ripening period when it constituted 17.14 mg/100 g DW, (this compound was not detected during plant butonization and blooming). These findings clearly show the importance of the selection of a proper harvesting time for obtaining the most valuable plant raw material.

S1: Concentrations of bioactive compounds (mg/ 100 g DW) in *F. ulmaria* collected during different vegetation periods