SHORT REPORT

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

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Abstract: Antioxidant properties of *Filipendula ulmaria* (meadowsweet) extracts obtained with increasing polarity solvents, acetone, methanol and water were evaluated by the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging, oxygen radical absorbance capacity (ORAC) assays and by the content of total phenols. Methanol extract was screened by the on-line HPLC-UV-DPPH$^{\bullet}$ scavenging assay, while phytochemical composition of extracts isolated at different plant vegetation phases was determined by HPLC-PDA-ESI-MS and UPLC-Q-TOF-MS^2. Eight compounds were identified and quantified, some other compounds were identified tentatively based on the obtained fragments and comparison with literature data. As a conclusion, the results demonstrate that *F. ulmaria* is a promising species for the development of novel high added value preparations.

Keywords: *Filipendula ulmaria*; antioxidant activity; total phenolic compounds; flavonoids; plant vegetation stage.

1. Plant Source

Filipendula ulmaria was harvested from Kaunas Botanical Garden at Vytautas Magnus University (Lithuania). Plant material was collected during plant butonization (June 1–22), flowering (July 10), and seed ripening (August 10–22) stages. The plants were air dried at room temperature in a ventilated room (20–25 °C), protected from direct sun light.

2. Previous Studies

In previous studies flavonoids, tannins, phenolic glycosides (salicylates), volatile oils, minerals and vitamin C were reported in the aerial parts of *F. ulmaria* [1]. Quercetin-4'-*O*-glucoside, quercetin-3'-glucuronide, quercetin-3-*O*-arabinoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamno-glucoside, kaemferol-4'-glucoside, quercetin, quercetin-3-*O*-glucofuranoside, quercetin-4'-*O*-galactopyranoside, and quercetin-3-*O*-glucopyranoside were the main flavonoids found in the leaves and flowers [2-6], wheras gallic, ferulic, vanillic, *p*-coumaric, caffeic and vanillic acids were the most abundant phenolic acids in the leaves and roots [4,7]. Other important polyphenolics are ellagitannins, rugosins A, B, B_1, D, E_1, E_2, and tellimagrandins I_1, I_2 and II [3,6,8].

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3. Present Study

Crude extracts were obtained from 20 g of ground material with 400 mL of acetone and methanol by constant shaking during 24 h and then filtered and concentrated in a rotary evaporator at 40 ºC temperature. Aqueous extracts were prepared from 10 g of plant material by three extraction steps, 30 min each, under constant shaking; first with 100 mL of water, second and third with 50 mL of water at 70–80 °C. After each step the extract was filtered and combined extracts were freeze-dried. Antioxidant capacities of extracts were tested by $DPPH^{\bullet}$ scavenging, $ABTS^{\bullet+}$ decolorization and oxygen radical absorbance capacity (ORAC) assays as described elsewhere [9-11]. The values obtained by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$assays were expressed as effective concentration (EC_{50}) and trolox equivalent antioxidant coefficient (TEAC) values, respectively (Table 1).The total content of phenols (TPC) was determined with Folin-Ciocalteu reagent [12]. The EC_{50} of *F. ulmaria* extracts decreased in the following order: aqueous > acetone > methanol indicating that the latter extract demonstrating the lowest EC_{50} (0.25 mg/mL) was the strongest $DPPH^{\bullet}$ scavenger. The comparative TEAC values in $ABTS^{\bullet+}$ assay ranged from 1.15 to 0.73. The highest TEAC value was determined for acetone extract, which may contain more lipophilic antioxidants compared to other extracts. The extracts of *F. ulmaria* were also strong antioxidants in ORAC assay (Table 1). The highest values expressed in trolox equivalents (g TE/g extract) were determined for aqueous extract, followed by the acetone and methanol extracts. To the best of our knowledge, this method has not been applied previously for *F. ulmaria* extracts. The variations in EC_{50}, TEAC and ORAC values obtained for *F. ulmaria* extracts suggest that the differences in the composition of extracts obtained by different polarity solvents might be remarkable. The TPC values in the analyzed extracts were from 22.5 to 106.8 mg/GAE g, i.e. in a remarkably wider range as compared with other antioxidant activity indicators. The highest TPC was determined for methanol extract, followed by the acetone and water extracts.

Table 1. Antioxidant properties of *F. ulmaria* extracts.

Extracts	Yield, % (g/g DW)	*EC_{50} mg/mL ($DPPH^{\bullet}$)	**TEAC ($ABTS^{\bullet+}$)	ORAC g TE/ g extract	TPC mg /GAE g
Acetone	8.53 ± 0.26	0.28 ± 0.02	1.15	1.82 ± 0.46	40.84 ± 1.61
Methanol	28.61 ± 1.12	0.25 ± 0.03	0.97	1.26 ± 0.03	106.81 ± 4.01
Aqueous	8.08 ± 0.32	0.41 ± 0.02	0.73	1.99 ± 0.15	22.50 ± 2.02

*EC_{50} = mg/mL extract/1 mg/mL $DPPH^{\bullet}$ decreasing the initial 1 mg/mL $DPPH^{\bullet}$ concentration by 50%; **TEAC = the concentration of a trolox solution (g/mL) having the antioxidant capacity equivalent to 1 g/mL extract solution.

The highest TPC value was determined for methanol extract; therefore it was selected for a more detailed qualitative and quantitative analysis. Firstly, the on-line HPLC-UV-$DPPH^{\bullet}$ post column scavenging technique has been applied as a fast and efficient method for detecting individual antioxidants. UV and $DPPH^{\bullet}$ quenching chromatograms revealed the presence of a great number of individual radical scavengers represented by the negative peaks in the $DPPH^{\bullet}$ chromatogram (Fig. 1). Among 16 antioxidants identified in *F. ulmaria* methanol extract ellagitannins were the major constituents exhibiting a strong radical scavenging capacity, which comprised aprox. 69.4% of the total negative peak area (TNPA), while the peaks of flavonoids and phenolic acids constituted 14.1% and 6.2% of the TNPA, respectively. The results revealed that two compounds, **9** (17.4% of TNPA) and **10** (21.5% of TNPA), are stronger antioxidants than the others. Gallic acid (**1**), (R_t on UPLC - 0.88 min); catechin (**4**), (R_t on UPLC – 2.39 min); chlorogenic acid (**5**), (2.56 min.); rutin (**11**), (3.87 min.); hyperoside (**12**), (3.92 min.); luteolin-7-*O*-glucoside (**13**), (4.04 min.) spiraeoside (**14**), (4.16 min.) and astragalin (**15**), (R_t on UPLC – 4.21 min.) were identified in methanol extracts by comparing their elution time R_t in UPLC analysis (indicated in brackets) with those of available standards.

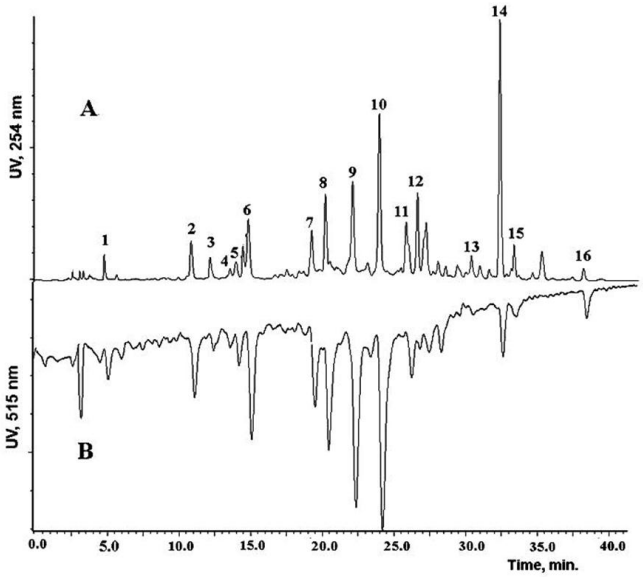

Figure 1. HPLC-UV-DPPH$^{\bullet}$ profiles of *F. ulmaria* methanol extract: A - UV 254 nm; B – DPPH$^{\bullet}$ scavenging at 515 nm (negative peaks indicate activity).

High resolution UPLC Q-TOF-MS was applied for the identification of other compounds, which was based on UPLC UV MS data and MS^2 fragmentation patterns and on the previously reported data [3, 13-16]. UPLC-MS chromatogram is given as Figure S1 in supporting information. The compound **9** gave a molecular ion $[M-H]^-$, *m/z* 937.0908 ($C_{41}H_{29}O_{26}$), and the fragments of 785.0824 and 767.0713 fitting molecular formulas $C_{34}H_{25}O_{22}$ and $C_{34}H_{23}O_{21}$, respectively. The loss of 152 and 170 amu were attributed to the loss of galloyl unit and gallic acid, respectively. The fragments of *m/z* 635.0925 ($C_{45}H_{15}O_5$) and 465.0699 ($C_{20}H_{17}O_{13}$) indicate the loss of hexahydroxydiphenoyl moiety from the molecular ion, and [M-H-gallic acid]$^-$ ion, respectively. The fragment of *m/z* 300.9985 ($C_{14}H_5O_8$) demonstrates the presence of ellagic group in the molecule, while the fragment of *m/z* 169.9134 ($C_7H_5O_5$) indicates on the presence of free galloyl residue. In addition, the fragment of *m/z* 419.0633 ($C_{19}H_{15}O_{11}$), according Sanz et al. [17], indicates one or several gallic acid groups, linked through a hydroxyl group to the gallic acid and not esterified to the glucose [17]. Taking into account all this data, this compound was tentatively identified as trigalloyl-hexahydroxydiphenoyl-glucose. Some of the detected compounds can be hydrolyzed to yield gallic and ellagic acids; UV spectra characteristics of this kind of compounds can be classified into two groups; first, with a characteristic UV spectrum of ellagic acid possessing two absorption maximum at 254 nm and 358 nm, and second with UV spectra characteristic to gallic acid and showing a single maximum at 272–277 nm [15]. Interpretation of MS^2 spectra of such compounds suggests different isomers of di- and trigalloyl glucoses. The loss of one or more fragments of 152 and 170 amu indicates on galloyl and gallic acid groups, while the loss of 302 amu indicates on hexahydroxydiphenoyl moiety; consequently, these compounds are considered to be ellagitannins [14-18]. For some of the detected compounds, particularly **7**, **8** and **10**, it was not possible to determine molecular formulas unambiguously from their exact monoisotopic masses.

The compound **10** gave the molecular ion peak of *m/z* 936.0867, which was assumed as a $[M-2H]^{2-}$ ion, fitting $C_{82}H_{58}O_{52}$ formula; the *m/z* value of a singly charged ion was calculated as 1873.1810. This peak gave the fragments of *m/z* 169.0139 (C_7H_5O) and 300.9989 ($C_{14}H_5O_8$), which could be assigned to galloyl and ellagic acid moieties, respectively. Summarising all this data, the compound **10** was tentatively identified as a dimer of trigalloyl-hexahydroxy-diphenoyl-glucose. Previuosly, Fecka [3] quantified the main compounds in *F. ulmaria* flower extracts and found that the major constituent was rugosin D (29.88 mg/g DW); it was later confirmed by Nitta et al. [19]. Consequently our results are in agreement with the previously reported findings.

The compound **7** had a characteristic to gallic acid UV absorption maximum at 274.5, and gave the molecular ion of *m/z* 860.0813. So far as it was not possible to determine the molecular formula of a singly charged ion, this peak was assumed as a $[M-2H]^{2-}$ ion, well-fitting $C_{75}H_{54}O_{48}$ formula. In this

case, the calculated mass for a singly charged ion species should be 1721.1700. In addition, this peak gave the fragments characteristic to ellagitannins; *m/z* 169.0136 ($C_7H_5O_5$) indicates the presence of free galloyl residue, while *m/z* 300.9989 ($C_{14}H_5O_8$) shows the presence of ellagic group. Consequently, this compound was considered to be hydrolysable tannin with a structure similar to rugosin E. The UV and MS spectral data of the compound **8** was identical to that of **7**; from this data it may be assumed that **7** and **8** are the isomers. Hydrolysable ellagitannins were reported in *F. ulmaria* extracts previously [5,8,17], however our data expands the existing knowledge on phytochemicals in this species; for instance, only monomers of di-, and trigaloyl hexahydroxydiphenoyl glucoses were found in *F. ulmaria* extract from Portugal [8]. Compound **14** was identified as spiraeoside (R_t = 4.16 min); a pseudomolecular ion $[M-H]^-$ at *m/z* 463.0872 suggested $C_{21}H_{19}O_{12}$ molecular formula and released unique MS^2 fragment at 301.0347 ($C_{15}H_9O_7$) corresponding to quercetin, while UV spectra showed max 253.4; 265.9 (sh), 365.7 nm. Spiraeoside was found as the major constituent in *F. ulmaria* by Krasnov et al., and Kahkonen et al. [5, 20] and our study confirmed their findings. The compounds **2** (R_t = 2.06 min) and **6** (R_t = 2.76 min) were tentatively identified as digalloyl-hexahydroxydiphenoyl glucoses: the precursor ion $[M-H]^-$, *m/z* 785 ($C_{34}H_{25}O_{22}$) gave *m/z* fragment of 633 ($C_{27}H_{21}O_{18}$) indicating on the loss of galloyl unit; an *m/z* fragment 615 ($C_{27}H_{19}O_{17}$) shows the loss of gallic acid unit, whereas *m/z* 483 ($C_{20}H_{19}O_{14}$) shows the hexahydroxydiphenoyl moiety. Finally, an *m/z* fragment 169 ($C_7H_5O_5$) shows the presence of a free galloyl residue. In addition, the compound **16** (R_t = 4.44 min) with ellagic acid-like UV spectrum was detected (284.9; 365.2 nm). This compound gave a molecular ion of *m/z* 585.0871, which could be assigned to molecular formula $C_{27}H_{21}O_{15}$. In MS^2 mode this compound gave three fragment ions, *m/z* 433.0762 with fragment loss of 152 amu indicating the residue of a galloyl unit. Another recorded fragment had *m/z* 281.0660; thus, the sum of these fragments *m/z* fits $C_{13}H_{13}O_7$ formula. An *m/z* of the third fragment was 169.0143 which fits molecular ion formula $C_9H_7O_3$ and clarly indicates that it is gallic acid. The compound **3** (R_t = 2.23 min.) gave a molecular ion of *m/z* 297.0618, which is well-fitting molecular formula $C_{13}H_{13}O_8$, while the fragment ion *m/z* 179.0344, corresponding to $C_9H_7O_4$ indicates the residue of caffeic acid. The loss of H_2O gave a fragment of *m/z* 161 matching $C_9H_5O_3$ formula; the last fragment of *m/z* 135.0250 was matching $C_4H_7O_5$.

Quantitative analysis of identified constituents showed that the concentrations of flavonoids and phenolic acids during vegetation were changing in a wide range; these results are presented in supporting information (Figure S2).

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