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Phytochemical composition and antioxidant properties of *Filipendula vulgaris* as a source of healthy functional ingredients

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ABSTRACT

Polyphenols and antioxidant potential of *Filipendula vulgaris* were evaluated. The total phenolic content (TPC) in methanol, acetone and water extracts was 346.6, 389.9 and 131.9 mg gallic acid equivalents/g; however, methanol gave higher total yield of phenolics from dry herb weight. Supercritical fluid extraction resulted in low yields and extract activities. Based on DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging assays methanol and acetone extracts with higher TPC values were also stronger antioxidants. Analysis of extracts by UPLC with quadrupole-time of flight mass spectrometer (Q-TOF-MS) resulted in full identification of 18 phenolic compounds. An on-line HPLC – diphenylpicrylhydrazyl radical scavenging (HPLC-UV-DPPH$^{\bullet}$) assay revealed that dimers and trimers of trigalloyl-hexahydroxydiphenoyl-hexoses were the major antioxidants in the extracts. Some minor compounds such as gallic acid, catechin, rutin, luteolin-7-O-glucoside, hyperoside, spiraeoside, and astragalin were also quantified by HPLC-electro spray ionization mass spectrometry (HPLC-ESI-MS). Quercetin-3-O-(2″-O-galloyl)-β-galactopyranoside was isolated from methanol extract of *F. vulgaris* and its structure was identified in this species by proton, carbon and heteronuclear multiple-bond correlation NMR spectral data for the first time. In general the study demonstrated that *F. vulgaris* biosynthesizes a wide range of polyphenolics, which were previously demonstrated as possessing health benefits. Consequently, the plant may be considered as a promising source of ingredients for functional food and nutraceuticals.

1. Introduction

The plant kingdom is an important source of bioactive compounds for functional foods, nutraceuticals, pharmaceuticals, cosmetics and other applications. Although hundreds of different medicinal and aromatic plants have been used in folk medicine and foods since ancient times, the activities of many species still rely on empirical knowledge, which has not been supported by the scientific data obtained by modern analytical techniques, or such data are rather scarce and fragmented. Underutilized medicinal plants may contain valuable bioactive

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Abbreviations: TPC, total phenolic content; DPPH$^{\bullet}$, 2.2-diphenyl-1-picrylhydrazyl free radical; ABTS$^{\bullet+}$, 2.2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); UPLC, ultra performance liquid chromatography; HPLC, high performance liquid chromatography; Q-TOF, quadrupole-time of flight; ESI, electro-spray ionization; MS, mass spectrometry; CID, collision induced dissociation; ISCID, in source collision induced dissociation; NMR, nuclear magnetic resonance; HMBC, heteronuclear multiple-bond correlation; HHDP, hexahydroxydiphenoyl

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constituents such as polyphenols, carotenoids, vitamins and other bioactives (Andarwulan et al., 2012); comprehensive characterization of such plant species remains an interesting task (Shahidi & Zhong, 2010), both from the scientific and practical application points of view.

Filipendula is a genus consisting of 12 species of perennial herbaceous flowering plants in the family *Rosaceae*, native to the temperate regions of the Northern Hemisphere. Well-known species include *Filipendula ulmaria* and *Filipendula vulgaris*, both native to Europe, and *Filipendula occidentalis* and *Filipendula rubra*, native to North America. *F. vulgaris* (dropwort) traditionally has been used in medicine as a febrifuge for the treatment of several inflammatory diseases, rheumatoid arthritis, gout, as well as astringent and stomachic remedies. The leaves are usually used in decoction to treat stomach ache and diarrhoea, kidney problems, breathlessness, wheezing, sore throats and congestion, and for easing influenza symptoms (Radulovič et al., 2007). Data on the application of *F. vulgaris* in foods have not been reported until now, however other species of the same genus, *F. ulmaria* were suggested as a source of potential ingredients for functional beverages; hot water extract of *F. ulmaria* contained high amounts of phenols, particularly quercetin and salicylic acid (Harbourne, Jacquier, & O'riordan, 2009).

F. vulgaris accumulates high concentrations of phenolics, which are responsible for antibacterial activity, salicylates and pharmacologically active plant heparin (Imbrea, Butnariu, Nicolin, & Imbrea, 2010). The roots of dropwort were reported to contain high content of tannins. Oszmianski, Wojdylo, Lamer-Zarawska, and Swiader (2007) reported that trolox equivalent antioxidant capacity (TEAC) of *F. vulgaris* root extracts in the reaction with DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ were 0.72 and 1.86 mM/kg dry roots, respectively. They also found that catechin (7.69 g/kg) and catechin-4-benzyl thioether (7.46 g/kg) were major phenolics in the extracts, whereas epicatechin (0.35 g/kg) and epicatechin-4-benzyl thioether (0.2 g/kg) were present in smaller amounts. Dropwort flowers accumulate some essential oil consisting mainly of 2-phenylethanol and *cis*-3-hexenyl acetate (Dobson, Groth, & Bergstrom, 1996) and inhibiting the growth of several tested microorganisms (Radulovič et al., 2007). However, information about phytochemical composition and antioxidative properties of *F. vulgaris* is very scarce. Therefore, the main purpose of this study was to evaluate the properties of *F. vulgaris* using a more systematic approach, focused on comprehensive assessment of phytochemical composition and antioxidant potential of extracts isolated by different extraction techniques and solvents.

2. Materials and methods

2.1. Plant material, chemicals and reagents

F. vulgaris (dropwort) was grown in Kaunas Botanical Garden at Vytautas Magnus University (Lithuania). The plants were collected during blooming period (10–20 July 2010), air dried at room temperature in a ventilated room protected from direct sunlight. The leaves and green steams were used for further analysis.

Organic solvents and reagents were of analytical and HPLC grade. 2,2-Diphenyl-1-picryhydrazyl (DPPH$^{\bullet}$, 98%), 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS, 98%), acetic and formic acids, Na_2HPO_4, KCl, $Na_2HPO_4 \times 12H_2O$, $K_2S_2O_8$, NaCl and HPLC grade and LC–MS grade acetonitrile were purchased from Sigma Aldrich (Steinheim, Germany). Analytical grade acetone and methanol were obtained from StanLab (Lublin, Poland). Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), Folin–Ciocalteu reagent and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA), hyperoside from CarlRoth, GmbH (Karlsruhe, Germany), ellagic acid from Fluka Biochemica (Buchs, Switzerland), luteolin-7-glucoside, (+) catechin and astragalin from Chromadex (Irvine, CA, USA).

2.2. Extraction procedure

The composition of plant phytochemicals is usually very complex consisting of compounds with different chemical and physical properties; therefore, different methods and solvents were applied to *F. vulgaris* plant material. Dried leaves and blossoms of plants were ground in a laboratory mill Vitek (An-Der, Austria). Ground sample was additionally sieved by using 1.0 mm sieve. Crude extracts were obtained from 20 g of sieved material with 400 mL of acetone and methanol by constant shaking during 24 h (Sklo Union LT, Teplice, Czech Republic). The extracts were filtered using a 0.3 μm filter (Filtrak, Niederschlag, Germany) and concentrated using a rotary evaporator Rotavapor R–114 (Büchi, Flavil, Switzerland) in vacuum (0.06 MPa) at 40 °C. It should be noted that acetone and methanol, which were used in this study for analytical purposes, although being toxic solvents, are allowed for all uses by the Directive 2009/32/EC (2009); however, their maximum residue limits in the extracted food ingredient should not exceed 10 mg/kg.

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; the combined extracts were freeze dried in a Maxi Dry Lyo apparatus (Hetto-Holton AIS, Allerod, Denmark).

Supercritical fluid extraction (SFE) was carried out with CO_2 (99.9%, AGA, Malmo, Sweden) according to previously described extraction procedure (Venskutonis, Škėmaite, & Sivik, 2008) in a 200 mL extraction vessel, which was filled with ground plant particles placed in between glass wool. Dynamic extraction followed after 15–20 min of static extraction. Extraction was performed at 42 and 20 °C temperatures and 15, 25 and 35 MPa pressures. In addition 1% of a modifier, ethanol was applied at 25 MPa, which was dosed by an extra pump. The organic solvent in this case was evaporated from the extract using Rotavac Heidolph apparatus (Schwabach, Germany). CO_2 flow rate was 16–17 g/min at atmospheric pressure, the extraction time varied between 2 and 4 h. Three replicates were extracted for each parameter set and the results are presented as mean values.

2.3. Fractionation of crude extract

Methanol extract (10 g) was diluted with 500 mL of distilled water and 100 mL of hexane and the mixture was shaken in