Supplementary data for the article:

Samardžić, S.; Arsenijević, J.; Božić, D.; Milenković, M.; Tešević, V.; Maksimović, Z. Antioxidant, Anti-Inflammatory and Gastroprotective Activity of Filipendula Ulmaria (L.) Maxim. and Filipendula Vulgaris Moench. *J. Ethnopharmacol.* **2018**, *213*, 132–137. https://doi.org/10.1016/j.jep.2017.11.013

Supplementary material

Antioxidant, anti-inflammatory and gastroprotective activity of *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench

Stevan Samardžić*, Jelena Arsenijević, Dragana Božić, Marina Milenković, Vele Tešević, Zoran Maksimović

* Corresponding author. Tel: +381 11 3951 321; E-mail: stevans@pharmacy.bg.ac.rs

A. Isolation and structure elucidation of constituents of *Filipendula vulgaris* Moench flowers.

1. General

Silica gel (0.063–0.200 mm, Merck, Germany), Sephadex LH-20 (GE Healthcare Bio-Science, Sweden) and octadecyl silica gel (Sigma-Aldrich, USA) were employed for column chromatography. Final purification was performed using TLC cellulose plates (Merck, Germany) or Agilent 1260 LC System equipped with an automated fraction collector 1364C and semi-preparative Zorbax SB-C18 column (9.4×250 mm, 5 μm).

NMR spectra (^{1}H, 400 MHz; ^{13}C, 100 MHz) were recorded on Bruker Ascend™ 400 instrument. Prior to analysis, all samples were dissolved in deuterated methanol (MeOH-d_4). TMS was used as the internal standard.

MS and UV spectral data of the isolated compounds were obtained by LC-PDA-MS analysis using Agilent 1260 liquid chromatograph. Mass spectral data were acquired with Agilent 6130 single quadrupole mass detector (negative ion mode, mass range 100–1000 m/z). Ion source (electrospray ionization) operating parameters were set as follows: drying gas flow 9 L/min (N_2), nebulizer pressure 40 psi, drying gas temperature 350 °C, capillary voltage 3500 V and fragmentor voltages 100 and 250 V. UV spectra were obtained with a G4212B PDA detector.

2. Isolation of constituents

2.1. Preparation of fractions F1 and F2

Dried and ground flowers of *F. vulgaris* (178 g) were successively bi-macerated with organic solvents of increasing polarity: *n*-hexane, dichloromethane and methanol (flowers : solvent ratio, 1:10, w/v). The resulting extracts were dried under reduced pressure. A portion (47 g) of the obtained methanol extract was suspended in water and partitioned with ethyl acetate. In the next step, the organic layer was separated and concentrated in vacuum to give 4.7 g of fraction (F1) further used in the isolation process.

The ethyl acetate insoluble fraction (8 g) of methanol extract of defatted *F. vulgaris* flowers, prepared according to procedure described by Ćebović and Maksimović (2012), was subjected to silica gel column chromatography and eluted with solvents of increasing polarity

(CH_2Cl_2/MeOH/H_2O/CH_3COOH, 90/10/0.4/0.1→70/30/1.2/0.3 v/v/v) to provide 40 subfractions (100 mL). Based on chemical composition similarity, subfractions 21–36 were combined to yield 2.6 g of the fraction here designated as F2.

2.2. Isolation procedure

Mixture of F1 (0.7 g) and F2 (0.65 g) was partitioned on Sephadex LH-20 column using ethanol as the mobile phase. Sixteen subfractions were collected (A1–A16, 50 mL each). Subfraction A10 was subsequently purified by semi-preparative RP-HPLC (30% acetonitrile, flow rate 3.3 mL/min) to give 5.3 mg of **1**. Compound **2** (150.1 mg) precipitated from combined subfractions A12–A14. Residue obtained after removal of **2** was submitted to preparative TLC (cellulose, 30% CH_3COOH) to yield 4.2 mg of **3**.

Mixture of F1 (1.6 g) and F2 (0.65 g) was partitioned on Sephadex LH-20. This time the elution was performed with mixtures of ethanol and methanol (80/20→0/100 v/v), mixture of methanol, acetone and water (80/14/6 v/v/v) and finally 70% acetone. Forty subfractions were obtained (B1–B40, 50 ml each). Subfractions B14–B15 were separated using preparative TLC (cellulose, 30% CH_3COOH) and 17.8 mg of mixture of **4** and **5** was isolated. Semi-preparative RP-HPLC of combined fractions B31–B32 (20% acetonitrile, flow rate 3.3 mL/min) yielded 6.1 mg of **6**.

For conduction of *in vivo* pharmacological experiments, additional amount of **2** was obtained by repeating the aforementioned procedure with minor adjustments, whereas new quantities of **6** were isolated by vacuum liquid chromatography separation of combined subfractions (B29–B30 and B33–B34, 262.9 mg) over octadecyl silica gel eluted with 20% acetonitrile.

3. Structure elucidation of the isolated constituents

UV and NMR (^1H, ^{13}C) spectra of the isolated compounds were in agreement with the previous reports. Their molecular masses corresponded to the determined structures. Based on literature data, compound **1** was identified as kaempferol 4′-*O*-*β*-D-glucoside (Lim et al., 2004; Scheer and Wichtl, 1987); **2** was determined to be spiraeoside (quercetin 4′-*O*-*β*-D-glucoside) (Fossen et al., 1998; Lim et al., 2004; Pavlović, 2008); **3** presented kaempferol 3-*O*-(2″-*O*-galloyl)-*β*-D-glucoside (astragalin 2″-*O*-gallate) (Isobe et al., 1980; Ochir et al., 2013); mixture of **4** and **5** was identified as mixture of quercetin 3-*O*-(2″-*O*-galloyl)-*β*-D-glucoside and quercetin 3-*O*-(2″-*O*-galloyl)-*β*-D-galactoside (2:1 w/w, based on HPLC-UV chromatograms and NMR spectra integration), respectively (Isobe et al., 1979; Kawakami et al., 2011; Kim et al., 1998; Ochir et al., 2013; Pakulski and Budzianowski, 1996; Yazaki et al., 1989); and finally, the structure of **6** was established as tellimagrandin II (Chen et al., 2014; Feldman and Sahasrabudhe, 1999; Wilkins and Bohm, 1976).

B. HPLC analysis of *F. vulgaris* and *F. ulmaria* lyophilized flower infusions

HPLC analysis of the lyophilized flower infusions of *F. ulmaria* and *F. vulgaris* was performed on an Agilent 1100 system coupled to a DAD detector and equipped with Zorbax Eclipse XDB-C18 analytical column (4.6×250 mm, 5 µm, Agilent). Quantification was done by the external standard method. Detailed description of the used procedures is given in the manuscript (section 2.3.). Mobile phase consisting of solvent A (H_3PO_4 in H_2O, pH 2.75) and solvent B (solvent A : acetonitrile, 10 : 90 v/v) was used for gradient elution as presented in the Table 1S. Program 1 was used for the quantification of astragalin, spiraeoside and salicylic acid, whereas the contents of gallic acid, ellagic acid, hyperoside and isoquercitrin were determined using program 2.

Table 1S. Elution programs.

Elution program 1			Elution program 2		
time (min)	solvent B (%v/v)	flow rate (mL/min)	time (min)	solvent B (%v/v)	flow rate (mL/min)
0	10	0.8	0	10	0.8
5	25	0.8	5	15	0.8
15	25	0.8	15	20	1.7
20	30	0.8	19	24	1.2
25	50	0.8	25	54	0.8
30	70	0.8	28	70	0.8
35	10	0.8	30	10	0.8
38	10	0.8	32	10	0.8

4. References

Ćebović, T., Maksimović, Z., 2012. Hepatoprotective Effect of *Filipendula hexapetala* Gilib. (Rosaceae) in Carbon Tetrachloride-induced Hepatotoxicity in Rats. Phytother. Res. 26, 1088–1091.

Chen, Y., Wang, J., Ou, Y., Chen, H., Xiao, S., Liu, G., Cao, Y., Huang, Q., 2014. Cellular antioxidant activities of polyphenols isolated from Eucalyptus leaves (*Eucalyptus grandis* × *Eucalyptus urophylla* GL9). J. Funct. Foods 7, 737–745.

Feldman, K.S., Sahasrabudhe, K., 1999. Ellagitannin Chemistry. Syntheses of Tellimagrandin II and a Dehydrodigalloyl Ether-Containing Dimeric Gallotannin Analogue of Coriariin A. J. Org. Chem. 64, 209–216.

Fossen, T., Pedersen, A.T., Andersen, Ø.M., 1998. Flavonoids from red onion (*Allium cepa*). Phytochemistry 47, 281–285.

Isobe, T., Fukushige, T., Noda, Y., 1979. A new flavonoid glycoside from *Polygonum nodosum*. Chem. Lett. 8, 27–30.

Isobe, T., Ito, N., Noda, Y., 1980. Minor flavonoids of *Polygonum nodosum*. Phytochemistry 19, 1877.

Kawakami, K., Shibukura, Y., Kanno, T., Furuki, T., Aketa, S., Hirayama, M., 2011. Identification of 2″-Galloylated Flavonol 3-*O*-Glycosides Accumulating in Developing Leaves of Persimmon. Phytochem. Anal. 22, 403–410.

Kim, H.J., Woo, E.-R., Shin, C.-G., Park, H., 1998. A New Flavonol Glycoside Gallate Ester from *Acer okamotoanum* and Its Inhibitory Activity

against Human Immunodeficiency Virus-1 (HIV-1) Integrase. J. Nat. Prod. 61, 145–148.

Lim, E.-K., Ashford, D.A., Hou, B., Jackson, R.G., Bowles, D.J., 2004. Arabidopsis glycosyltransferases as biocatalysts in fermentation for regioselective synthesis of diverse quercetin glucosides. Biotechnol. Bioeng. 87, 623–631.

Ochir, S., Yuki, T., Kanazawa, T., Nishizawa, M., Yamagishi, T., 2013. Two Galloylated Flavonoids as Antioxidants in *Rosa gallica* Petals. Chem. Nat. Compd. 49, 940–942.

Pakulski, G., Budzianowski, J., 1996. Quercetin and Kaempferol Glycosides of *Dionaea muscipula* from *in vitro* Cultures. Planta Med. 62, 95–96.

Pavlović, M., 2008. Doctoral dissertation: Proučavanje sastojaka *Anthemis triumfetti* (Asteraceae) i poređenje sa drugim vrstama podroda Cota. University of Belgrade – Faculty of Pharmacy, Belgrade.

Scheer, T., Wichtl, M., 1987. On the Occurrence of Kaempferol-4′-*O*-β-D-glucopyranoside in *Filipendula ulmaria* and *Allium cepa*. Planta Med. 53, 573–574.

Wilkins, C.K., Bohm, B.A., 1976. Ellagitannins from *Tellima grandiflora*. Phytochemistry 15, 211–214.

Yazaki, K., Shida, S., Okuda, T., 1989. Galloylhomoarbutin and related polyphenols from *Pyrola incarnata*. Phytochemistry 28, 607–609.