Supporting Information to:

Phytochemical Characterization of *Rhododendron ferrugineum* and 
in vitro Assessment of an Aqueous Extract on Cell Toxicity

Andrea Louis, Frank Petereit, Matthias Lechtenberg, Alexandra Deters, Andreas Hensel

**Affiliation**
Institute of Pharmaceutical Biology and Phytochemistry, University of Münster, Münster, Germany

**Correspondence**

_Prof. Dr. Andreas Hensel_

University of Münster
Institute of Pharmaceutical Biology and Phytochemistry
Hittorfstraße 56
48149 Münster
Germany
Phone: +49/251/83 33380
Fax: +49/251/83 38341
ahensel@uni-muenster.de
Analytical Data of Isolated Compounds Already Known from Published Literature

**Gossypetin 3-O-β-D-galactopyranoside (10):** ESI-MS: [M+Na]⁺ m/z 503; ¹H-NMR (CD₃OD, 400 MHz): δ 7.96 (1H, d, J = 1.9 Hz, H-2’), 7.72 (1H, dd, J = 1.9 and 8.5 Hz, H-6’), 6.87 (1H, d, J = 8.5 Hz, H-5’), 6.27 (1H, s, H-6), 5.13 (1H, d, J = 7.8 Hz, H-1’), 3.84 (1H, H-4’), 3.82 (1H, H-2’), 3.55 (1H, H-3’), 3.63 (1H, H-6’a), 3.54 (1H, H-6’b), 3.46 (1H, H-5’). ¹³C-NMR (CD₃OD, 100 MHz): δ 150.1 (C-2), 135.7 (C-3), 179.9 (C-4), 155.0 (C-5 and C-7), 99.7 (C-6), 126.3 (C-8), 146.6 (C-9), 105.4 (C-10), 123.2 (C-1’), 118.2 (C-2’), 145.8 (C-3’), 158.9 (C-4’), 116.1 (C-5’), 123.4 (C-6’), 105.8 (C-1’), 70.1 (C-2’), 75.3 (C-3’), 73.4 (C-4’), 77.3 (C-5’), 62.0 (C-6’).

**Gossypetin 3-O-β-D-glucopyranoside (11):** ESI-MS: [M+Na]⁺ m/z 503; ¹H-NMR (CD₃OD, 400 MHz): δ 7.83 (1H, d, J = 1.9 Hz, H-2’), 7.72 (1H, dd, J = 1.9 and 8.5 Hz, H-6’), 6.87 (1H, d, J = 8.5 Hz, H-5’), 6.27 (1H, s, H-6), 5.22 (1H, d, J = 7.6 Hz, H-1’), 3.70 (1H, H-6’a), 3.57 (1H, H-6’b), 3.48 (1H, H-2’), 3.42 (1H, H-3’), 3.34 (1H, H-4’), 3.21 (1H, H-5’). ¹³C-NMR (CD₃OD, 100 MHz): δ 150.0 (C-2), 135.7 (C-3), 179.9 (C-4), 155.0 (C-5 and C-7), 99.7 (C-6), 126.3 (C-8), 146.6 (C-9), 105.4 (C-10), 123.2 (C-1’), 118.0 (C-2’), 146.0 (C-3’), 159.2 (C-4’), 116.1 (C-5’), 123.6 (C-6’), 104.7 (C-1’), 78.3 (C-2’), 75.9 (C-3’), 71.3 (C-4’), 78.5 (C-5’), 62.7 (C-6’).

**6,8-Dimethylnaringenin (farrerol) (1):** ESI-MS: [M+H]⁺ m/z 301; ¹H-NMR (CD₃OD, 400 MHz): δ 7.32 (2H, d, J = 8.5 Hz, H-2’ and H-6’), 6.82 (2H, d, J = 8.5 Hz, H-3’ and H-5’), 5.28 (1H, d, J = 12.7 Hz, H-2), 3.05 (1H, m, H-3a), 2.69 (1H, m, H-3b), 1.99 (3H, s, CH₃ at C6), 1.98 (3H, s, CH₃ at C8). ¹³C-NMR (CD₃OD, 100 MHz): δ 198.5 (C-2), 164.3 (C-7), 160.4 (C-5), 159.4 (C-4’), 158.9 (C-9), 131.7 (C-1’), 129.0 (C-2’ and C-6’), 116.5 (C-3’ and C-5’), 104.9 (C-10), 104.2 (C-6), 103.4 (C-8), 80.2 (C-2), 44.2 (C-3), 8.3 (CH₃ at C6), 7.6 (CH₃ at C-8).

Configuration at C2 was not determined.

**Poriol 7-O-β-D-glucopyranoside (poriolin) (2):** ESI-MS: [M+Na]⁺ m/z 471; ¹H-NMR (CD₃OD, 400 MHz): δ 7.32 (2H, d, J = 8.5 Hz, H-2’ and H-6’), 6.81 (2H, d, J = 8.5 Hz, H-3’ and H-5’),
6.30 (1H, s, H-8), 5.35 (1H, dd, J = 2.8 and 13.1 Hz, H-2), 4.99 (1H, d, J = 7.2 Hz, H-1’’), 3.86 (1H, dd, J = 1.8 and 12.1 Hz, H-6’’a), 3.67 (1H, dd, J = 5.3 and 12.1 Hz, H-6’’b), 3.53 – 3.34 (4H, H-2’’, H-3’’, H-4’’ and H-5’’), 3.15 (1H, dd, J = 13.1 and 17.1 Hz, H-3a), 2.72 (1H, dd, J = 2.8 and 17.1 Hz, H-3b), 2.02 (3H, s, \text{CH}_3). 13C-NMR (CD$_3$OD, 100 MHz): δ 80.9 (C-2), 44.5 (C-3), 198.9 (C-4), 161.9 (C-5), 104.7 (C-6), 164.9 (C-7), 95.2 (C-8), 162.7 (C-9), 107.8 (C-10), 131.2 (C-1’), 129.2 (C-2’ and C-6’), 116.4 (C-3’ and C-5’), 159.2 (C-4’), 101.4 (C-1’’), 78.4, 78.2, 74.9 and 71.3 (C-2’’-C-5’’), 62.5 (C-6’’). Configuration at C2 not determined.

2R,3R-taxifolin 3-O-β-L-arabinopyranoside (5): ESI-MS: [M-H] m/z 435; [α]$_{20}^{20}$ = + 26.72 (c 0.1048, MeOH); CD (MeOH): [θ]$_{224}$ 30581, [θ]$_{252}$ 7715, [θ]$_{295} - 31557$, [θ]$_{328}$ 10360. 1H-NMR (CD$_3$OD, 400 MHz): δ 6.97 (1H, d, J = 1.9 Hz, H-2’), 6.85 (1H, dd, J = 1.9 and 8.1 Hz, H-6’), 6.79 (1H, d, J = 8.1 Hz, H-5’), 5.92 (1H, d, J = 2.1 Hz, H-6’), 5.90 (1H, d, J = 2.1 Hz, H-8), 5.13 (1H, d, J = 10.6 Hz, H-2), 4.80 (1H, d, J = 10.6 Hz, H-3), 3.83 (1H, d, J = 3.8 Hz, H-1’), 3.59 (1H, dd, J = 3.8 and 6.0 Hz, H-2’’), 3.55 (1H, dd, J = 3.4 and 6.0 Hz, H-3’’), 3.80 (1H, dt, J = 3.4, 3.4 and 7.2 Hz, H-4’), 3.92 (1H, dd, J = 7.2 and 11.6 Hz, H-5’’a), 3.38 (1H, dd, J = 3.6 and 11.6 Hz, H-5’’b). 13C-NMR (CD$_3$OD, 100 MHz): δ 83.9 (C-2), 76.4 (C-3), 196.2 (C-4), 165.6 (C-5), 97.6 (C-6), 169.3 (C-7), 96.6 (C-8), 164.4 (C-9), 102.5 (C-10), 129.1 (C-1’), 115.8 (C-2’), 146.7 (C-3’), 147.6 (C-4’), 116.4 (C-5’), 120.9 (C-6’), 101.5 (C-1’’), 71.2 (C-2’’), 73.2 (C-3’’), 66.9 (C-4’’), 63.5 (C-5’’).


**Arbutin content**

HPLC quantification of arbutin (Roth; 99.2% purity, HPLC) using Ph. Eur., monograph “Uvae ursi folium” with external calibration against arbutin reference standard (Roth, content 99.3 %). Detection limit: 0.5 µg/mL, corresponding to 0.01 % in dried leave material with a signal-noise ratio of 3:1 (HPLC Shimadzu; SCL/CTO/SIL/SPD/LC-10A VP). For TLC investigations on arbutin a methanol/water extract (1:1 V/V) of leaf material was compared semi-quantitatively to serial dilutions of arbutin reference standard on silica gel 60 F$_{254}$ 0.2 mm (Merck), with
Pulverized leaf material (100 g, batch 2) was extracted three times at 8 °C with 1 L of water for 20 h. Extracts obtained after centrifugation (15,000 × g, 15 min) were combined and concentrated (< 40 °C). High molecular constituents were precipitated in ethanol (4 L). The precipitate was isolated by centrifugation (15,000 × g), suspended in 20 mL of water, dialysed (Cellulose membranes, MWCO 3500 Da; Roth) and lyophilized to yield 1.0 g raw polysaccharides (RPS). RPS was fractionated by AEC on DEAE-Sephacel® (30 x 2.5 cm; General Health Care) in the phosphate form and elution by a step gradient of deionized water, sodium phosphate buffers pH 6.0, ion strength 0.1, 0.25, 0.5, 1 mol/L, and 0.05 N NaOH, flow 100 mL/h, fraction size 2 mL. Carbohydrate-containing fractions were pooled, concentrated under vaccuum, dialyzed and lyophilized.

Carbohydrate analysis
Total carbohydrates in AEC- and FPLC-fractions were assayed using the resorcinol-sulphuric acid test [1]. Determination of total uronic acids was performed according to the method of Blumenkrantz [2] with o-hydroxydiphenyl modified for 96-well-microtiter plates using a mixture of glucuronic acid and galacturonic acid as reference. Quantification of monomeric carbohydrates was accomplished on ion-exchange HPLC with pulsed-amperometric detection (Dionex), Bio LC, with AS50 autosampler, G50 gradient pump, AS50 oven and ED50 electrochemical detector on a CarboPac™ PA1, analytical column, 2 x 250 mm, CarboPac™ PA1, guard column 2 x 50 mm and BorateTrap™ Trap, 4 x 50 mm. Elution with gradient program using water and NaOH 0.1 M for neutral sugars, and ternary gradient water, NaOH 0.1 M and NaOAc 0.5 mM for uronic acids. Polysaccharides were hydrolyzed with trifluoroacetic acid 2 mol/L at 121 °C for 1 h. Interglycosidic linkage of neutral sugars was analyzed using the partially methylated alditol acetates (PMAA) by GC-MS [3, 4]. GC was performed on an Agilent 6890N GC-MS system with mass selective detector on a HP5MS fused silica capillary column (i.d. 0.25 mm x 30 m, film thickness 0.25 µm) with helium as a carrier gas (1.5 bar). Reduction of acidic
polysaccharides to the carboxyl-reduced polymers was accomplished in the presence of carboximide and NaBH₄ following the method of [5]. The determination of molecular weight distribution of polysaccharides was performed by FPLC on a Superose® 6 column (GE Healthcare) using standard dextrans for calibration. Void volume was determined with DextranBlue®. Quantification of residual protein was performed using standard BSA (Applichem, content 98 %) as a reference [6].

**Fig. 1S** Anion exchange chromatography of RPS from *R. ferrugineum* on DEAE Sephacel® using a step gradient of water and 0.1 M, 0.25 M, 0.5 M and 1 M sodium phosphate buffer (SPB). Fraction size 2 mL. Numbers and lines indicated represent the pooled fractions.
References

5 Taylor RL, Conrad HE. Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. Biochemistry 1972; 11: 1383-1388.