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Flavonol glycosides from *Chenopodium foliosum* Asch

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ABSTRACT

Three new flavonol glycosides, namely 6-methoxykaempferol-3-*O*-β-gentiobioside, gomphrenol-3-*O*-β-gentiobioside and gomphrenol-3-O-α-L-rhamnopyranosyl-(1 → 2)[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside as well as the known patuletin-3-*O*-β-gentiobioside and spinacetin-3-*O*-β-gentiobioside were isolated from the aerial parts of *Chenopodium foliosum* Asch. The structures of the compounds were determined by means of spectroscopic methods (1D and 2D NMR, UV, IR, and HRMS). DPPH free radical scavenging activity of the new compounds was low or lacking.

1. Introduction

The genus *Chenopodium* L. (Amaranthaceae) comprises about 150 species, most of these are cosmopolites and are distributed mainly in subtropical and temperate regions (Uotila and Tan, 1997). *Chenopodium* is represented by nearly 18 species in the Bulgarian flora, mostly ruderal plants and widely distributed in the country (Grozeva, 2007). Phytochemical investigations on *Chenopodium* species revealed the presence of triterpene saponins, ecdysteroids, flavonoids and essential oils (Kokanova-Nedialkova et al., 2009). Many species of this genus have been used traditionally in indigenous systems of medicine for treatment of numerous ailments. Biological properties of chenopods include antimicrobial, cytogenetic, anthelmintic, cytotoxic, immunomodulatory, hypotensive, haemagglutinative, trypanocidal and spasmolytic activity (Yadav et al., 2007). *Chenopodium foliosum* Asch has also been known in Bulgarian folk medicine as "garliche" or "svinski yagodi" (swine's berries). The decoction of its aerial parts has been used for treatment of cancer and as an immunostimulant. Although some monoterpenes have been detected in the essential oil from *C. foliosum* (Dembitsky et al., 2008) no detailed phytochemical investigation of this plant has been undertaken so far. The present study reports the isolation and chemical characterization of two new flavonol gentiobiosides and a trioside together with two known flavonol glycosides. The evaluation of DPPH free radical scavenging capabilities of the new compounds has also been done.

2. Results and discussion

A phytochemical investigation of *C. foliosum* led to the isolation and structural identification of five flavonol glycosides **1–5**. The known compounds **1** and **5** were identified as patuletin-3-*O*-*β*-gentiobioside and spinacetin-3-*O*-*β*-gentiobioside (Fig. 1), respectively by comparing their spectroscopic data with those reported in the literature (Aritomi et al., 1985). The signals in the ^{1}H and ^{13}C spectra of the isolated compounds **2–4** were unambiguously assigned using 2D NMR techniques, i.e., COSY, HSQC and HMBC. Multiplicities were determined using ^{1}H and HSQC spectra. Determination of the sugars as D-glucose and L-rhamnose was done according the method of Noe and Freissmuth (1995) using capillary electrophoresis.

Compound **2** was isolated as optically active pale-yellow crystalline powder. Its molecular formula was established as $C_{28}H_{32}O_{17}$ by means of HRLSI–MS showing a $[M+H]^+$ at *m*/*z* 641.1738. The IR spectrum showed absorption bands for hydroxyl groups (3458–3210 cm^{-1}), unsaturated carbonyl (1615 cm^{-1}) and conjugated double bonds (1563, 1471 cm^{-1}). The UV spectrum (MeOH) of **2** was typical for 3-OH substituted flavonols. The bathochromic shift of the maximum at 342 nm after addition of $AlCl_3$/HCl (Δ = 22 nm) and NaOAc (Δ = 40 nm) indicated the presence of free hydroxyl groups on 5 and 7, respectively (Markham, 1982). A TLC analysis of sugar portion of compound

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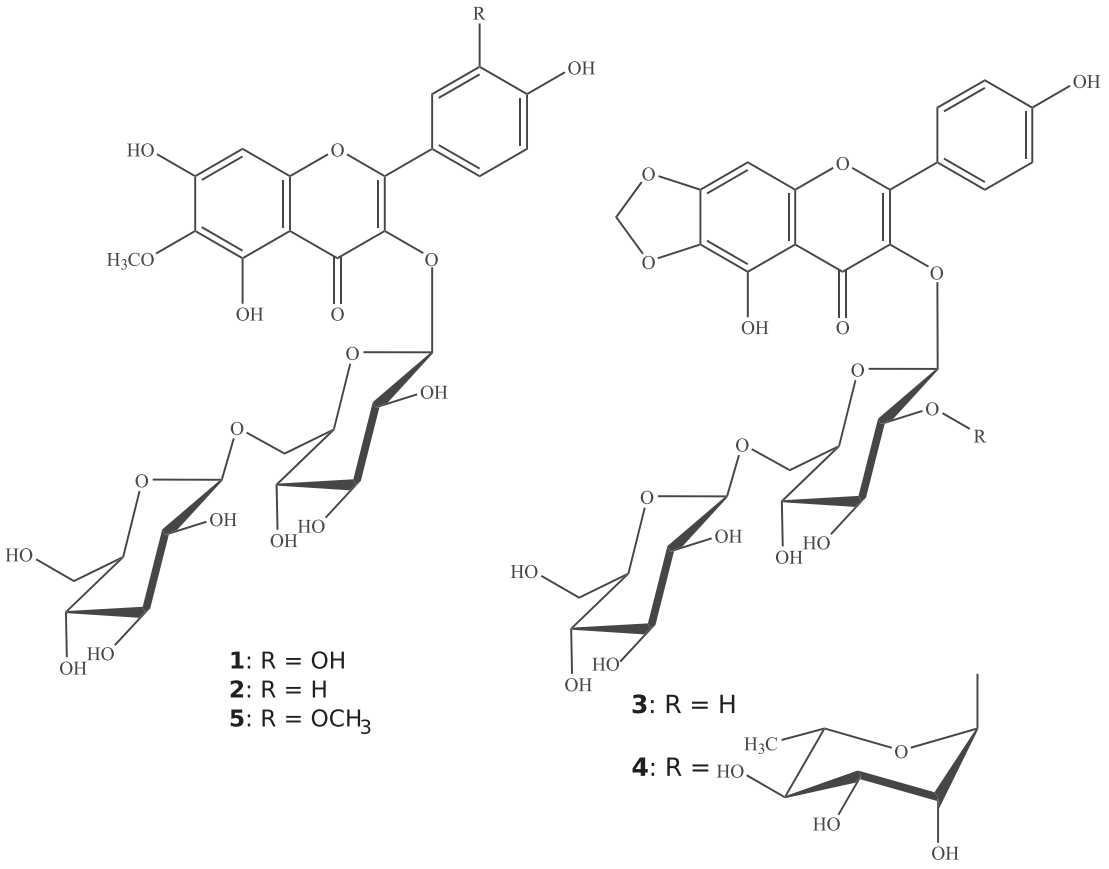

Fig. 1. Structures of the isolated compounds.

2 hydrolysate established the presence of glucose. The ^{1}H NMR spectrum (Table 1) was typical for a trifold-substituted A-ring showing only a singlet at δ_H 6.46. A broad singlet at δ_H 12.64 was due to OH-5 proton involved into a hydrogen bond. Two AB-type coupling proton signals at δ_H 6.87 and 8.00, showed that position 4′ of the B-ring was oxygenated. The three-proton singlet at δ_H 3.73 gave cross-peak with C-6 (δ_C 131.4) in the HMBC experiment and was attributed to the methoxyl group at position 6. In addition, two doublets at δ_H 5.35 (J = 7.6 Hz) and 4.02 (J = 7.7 Hz) belonging to the anomeric glucosyl protons of β configuration appeared in the ^{1}H NMR spectrum, as well. The former signal gave cross-peak in the HMBC experiment with C-3 (δ_C 132.8). This evidence confirmed that sugar moiety was attached at position 3. The signals of remaining sugar protons appeared mostly as double doublets or triplets in the range of δ_H 3.85–2.81. The coupling constants (J) ranging from 8.8 to 9.6 Hz were typical for axial/axial coupling of glucose protons. The ^{13}C NMR data of **2** (Table 1) showed signals of a methoxy group, five hydrogen bearing aromatic carbons as well as ten quaternary carbons that were typical for 3-*O*-glycosidated 6-methoxykaempferols (Agrawal, 1989). The gentiobiose-type linkage of the sugar moiety was confirmed by HMBC showing cross peaks between methylene protons (H-6″) of inner glucose (δ_H 3.85 and 3.44) and anomeric carbon (C-1‴) of terminal sugar (δ_C 103.1). In addition, the anomeric proton (H-1‴) of terminal glucose (δ_H 4.02) correlated with methylene carbon (C-6″) of inner sugar (δ_C 68.0). Thus, compound **2** was identified as 6-methoxy-3,5,7,4′-tetrahydroxy-flavon 3-*O*-β-D-glucopyranosyl(1 → 6)-*O*-β-D-glucopyranoside or 6-methoxykaempferol-3-*O*-β-D-gentiobioside (Fig. 1).

Compound **3** was isolated as optically active pale-yellow crystalline powder. Its molecular formula was established as $C_{28}H_{30}O_{17}$ by means of HRLSI–MS exhibiting a $[M+H]^+$ at m/z

Table 1
NMR data of compound **2** (^{1}H 600 MHz; ^{13}C 150 MHz; DMSO-d_6, 298 K).[a]

Position	δ_c, Mult.	δ_H (J in Hz)	HMBC
2	156.4, qC		
3	132.8, qC		
4	177.4, qC		
5	152.2, qC	12.64, br. s, OH	C6, C10
6	131.4, qC		
7	158.3[b], qC		
8	94.1, CH	6.46, s	C4, C6, C7, C9, C10
9	151.8, qC		
10	104.0, qC		
1′	121.0, qC		
2′ and 6′	130.9, CH	8.00, d (8.9)	C2, C4′
3′ and 5′	115.1, CH	6.87, d (8.9)	C1′, C4′
4′	159.9, qC		
6-OCH_3	59.9, CH_3	3.73, s	C6
1″	101.1, CH	5.35, d (7.6)	C-3, C-3″
2″	74.1, CH	3.17, dd (9.0, 7.6)	C1″, C3″
3″	76.2, CH	3.22, t (9.0)	C2″, C4″
4″	69.7, CH	3.13, dd (9.6, 9.0)	C3″, C5″, C6″
5″	76.3, CH	3.29, ddd (9.6, 5.8, 1.5)	C4″
6″	68.0, CH_2	3.85, d (11.8), 3.44, dd (11.8, 5.8)	C4″, C5″, C1‴
1‴	103.1, CH	4.02, d (7.7)	C6″, C2‴, C3‴
2‴	73.4, CH	2.82,m	C1‴, C3‴
3‴	76.6[c], CH	2.93, t (8.8)	C2‴, C4‴
4‴	69.7, CH	2.96, dd (9.2, 8.8)	C3‴, C5‴, C6‴
5‴	76.5[c], CH	2.80, m	–
6‴	60.8, CH_2	3.51, dd (11.7, 1.8), 3.34, dd (11.7, 5.7)	C5‴

[a] Chemical shifts were referenced to the solvent signals (residual DMSO-d_6 at δ_H 2.50 for protons and δ_C 39.51 for carbons).
[b] The signal was missing in ^{13}C NMR spectrum, but it gave cross peak in HMBC experiment.
[c] The signals could not be assigned unambiguously to the certain carbon and may be exchanged.

639.1558. The IR spectrum showed absorption bands for hydroxyl groups (3361–3211 cm^{-1}), unsaturated carbonyl (1680 cm^{-1}) and conjugated double bonds (1561, 1480 cm^{-1}). The UV spectrum of **3** in MeOH was typical for 3-OH substituted flavonols. The bathochromic shifts of the maximum at 342 nm after addition of $AlCl_3$/HCl (Δ = 30 nm) indicated the presence of free hydroxyl group at 5 position. On the other hand, the spectrum after addition of NaOAc was almost identical to the one in MeOH which was indication for blocked or missing 7-OH group (Markham, 1982). A TLC analysis of sugar portion of compound **3** hydrolysate established the presence of glucose. The ^{1}H NMR spectrum in DMSO-d_6 (Table 2) showed a typical flavonoid pattern with a para-substituted ring B characterised by two doublets (J = 8.9 Hz, J = 8.8 Hz), each integrating for two protons, at δ_H 8.03 and δ_H 6.89 ppm. A broad singlet centred at δ_H 12.58 ppm belongs to the 5-OH group, involved in a hydrogen bond with the C-4 keto group (δ_C 177.9, Table 2). A trisubstituted ring A carrying a methylenedioxy group was indicated by a singlet signal at δ_H 6.85 for the single aromatic proton and two doublets at δ_H 6.160 and 6.155 (J = 1.0 Hz) for the methylenedioxy protons. In the HSQC spectrum the latter protons showed a cross-peak with the carbon signal at δ_C 102.7 (Table 2). The HMBC experiment revealed a correlation between methylenedioxy protons and the carbons at 6 (δ_C 129.3) and 7 (δ_C 153.9) position. The ^{1}H and ^{13}C NMR data of **3** were in good agreement with literature data for 3-*O*-glycosidated 6,7-methylenedioxy-3,5,4′-trihydroxyflavones (Kohda et al., 1990; Mosquera et al., 2008). Additionally, in the ^{1}H NMR spectrum of **3** appeared two doublets at δ_H 5.36 (J = 7.2 Hz) and δ_H 3.99 (J = 7.7 Hz) indicating two sugar units. The downfield doublet exhibits a cross-peak with the signal of C-3 (δ_C 133.2) showing clearly the position of glycosylation. The ^{13}C NMR signal pattern of the sugar carbons (Table 2) was similar to that of compound **2**. Similarly, the HMBC spectrum confirmed gentiobiose-type linkage where the anomeric proton and carbon (δ_C 102.9) of the terminal glucose showed cross-correlation with methylene carbon (δ_C 67.7) and protons (δ_H 3.84 and 3.44) of the inner sugar, respectively. Thus, **3** was identified as 6,7-methylenedioxy-3,5,4′-trihydroxyflavone 3-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside or gomphrenol-3-*O*-β-D-gentiobioside (Fig. 1).

Compound **4** was isolated as optically active pale-yellow crystalline powder. Its molecular formula was established as $C_{34}H_{40}O_{21}$ by means of HRLSI–MS showing a $[M+H]^+$ at m/z 785.2140. The IR spectrum showed absorption bands for hydroxyl groups (3384 cm^{-1}), unsaturated carbonyl (1637 cm^{-1}) and conjugated double bonds (1561, 1519 cm^{-1}). The sugar portion of compound **4** hydrolyzate showed a major spot on TLC corresponding to glucose as well as one minor spot due to rhamnose. The UV spectra and aglycone signals in ^{1}H and ^{13}C NMR spectra (Table 2) of **4** were similar to those of compound **3** pointing to the same aglycone. The ^{1}H, ^{13}C and 2D NMR confirmed again the presence of a gentiobiosyl-substituent at OH-3 (a cross-peak between δ_H 5.50 and δ_C 132.8 in HMBC spectrum) substituted with a rhamnopyranosyl moiety. An additional anomeric proton signal at δ_H 5.09 (J = 1.3 Hz) in ^{1}H NMR spectrum gave a HMBC correlation with C-2″ (δ_C 77.0) of primary glucopyranosyl moiety. The cross-peak between proton H-2″ (δ_H 3.45) of primary glucose and anomeric carbon C-1‴′ (δ_C 100.6) of rhamnopyranose was also observed. Accordingly, the sugar signals in ^{13}C NMR were typical for triglycosides with primary β-D-glucopyranose glycosidated at C-2″ (downfield shifted to δ_C 77.0) and C-6″ (downfield shifted to δ_C 67.7) with α-rhamnopyranose and β-glucopyranose (Kikuchi and Matsuda, 1996; Veitch et al., 2010). Thus, the structure of **4** was established as 6,7-methylenedioxy-3,5,4′-trihydroxyflavone 3-*O*-α-L-rhamnopyranosyl-(1 → 2)[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside (Fig. 1).

Table 2
NMR data of compounds **3** and **4** (^{1}H 600 MHz; ^{13}C 150 MHz; DMSO-d_6, 298 K).[a]

Position	**3**			**4**		
	δ_c, Mult.	δ_H (J in Hz)	HMBC	δ_c, Mult.	δ_H (J in Hz)	HMBC
2	156.9, qC			156.9, qC		
3	133.2, qC			132.8, qC		
4	177.9, qC			177.8, qC		
5	140.4, qC	12.58 br. s, OH		140.5, qC	12.61, br. s.	
6	129.3, qC			129.3, qC		
7	153.9, qC			153.8, qC		
8	89.6, CH	6.85, s	C6, C7, C9, C10	89.6, CH	6.83, s	C6, C7, C9, C10
9	151.8, qC			151.8, qC		
10	107.1, qC			107.1, qC		
1′	120.7, qC			120.7, qC		
2′ and 6′	130.9, CH	8.03, d (8.9)	C2, C3′, C4′, C5′	130.8, CH	8.02, d (8.9)	C2, C3′, C4′, C5′
3′ and 5′	115.1, CH	6.89, d (8.9)	C1′, C4′	115.1, CH	6.87, d (8.9)	C1′, C4′
4′	160.0, qC	10.19, br. s, OH		160.0, qC	10.20, br. s., OH	
O–CH_2–O	102.7, CH_2	6.160, d (1.0); 6.155, d (1.0)	C6, C7	102.7, CH_2	6.16, d (0.8); 6.15, d (0.8)	C6, C7
1″	100.8, CH	5.36, d (7.4)	C3,	98.3, CH	5.50, d (7.7)	C3, C3″
2″	74.1, CH	3.19, dd (9.0, 7.4)	C1″, C5″	77.2, CH	3.45, dd (9.2, 7.7)	C1″, C1‴′
3″	76.2, CH	3.21, dd (9.0, 8.5)	C2″, C4″	77.1, CH	3.37, m	C1″, C2″, C4″, C6″
4″	69.8, CH	3.09, dd (9.6, 8.5)	C3″	70.2, CH	3.07, dd (9.3, 9.0)	C3″
5″	76.6, CH	3.29, ddd (9.6, 6.3, 1.5)	C4″	76.6, CH	3.30, m	C4″
6″	67.7, CH_2	3.84, dd (12.0, 1.5); 3.44, dd (12.0, 6.3)	C4″, C5″, C1‴′	67.7, CH_2	3.8, m; 3.38, m	C1‴′, C4″, C5″
1‴′	102.9, CH	3.99, d (7.7)	C6″, C3‴′ or C5‴′	102.9, CH	3.95, d (7.7)	C-3‴′, C6″
2‴′	73.3, CH	2.78, dd (9.0, 7.7)	C1‴′, C3‴′	73.3, CH	2.74, td (8.8, 7.7)	C1‴′, C3‴′
3‴′	76.4, CH	2.84, dd (9.0, 8.8)	C2‴′, C4‴′	76.5, CH	2.83, dd (9.0, 8.8)	C2‴′, C4‴′
4‴′	69.6, CH	2.96, dd (9.5, 8.8)	C3‴′, C6‴′	69.7, CH	2.94, dd (9.3, 9.0)	C3‴′, C6‴′
5‴′	76.5, CH	2.71, ddd (9.5, 5.5, 2.1)	C4‴′	76.4, CH	2.67, ddd (9.3, 5.5, 2.3)	C4‴′
6‴′	60.7, CH_2	3.47, dd (12.0, 1.7,); 3.33, dd (12.0, 5.5)	C5‴′, C4‴′	60.7, CH_2	3.46, m; 3.32, m	
1‴′				100.6, CH	5.09, d (1.3)	C2″, C2‴′, C5‴′
2‴′				70.6, CH	3.74, dd (3.3, 1.3)	C3‴′, C4‴′
3‴′				70.5, CH	3.47, dd (9.5, 3.3)	
4‴′				71.8, CH	3.14, t (9.5)	
5‴′				68.3, CH	3.77, dd (9.5, 6.2)	C4‴′
6‴′				17.4, CH_3	0.82, d (6.2)	C5‴′, C4‴′

[a] Chemical shifts were referenced to the solvent signals (residual DMSO-d_6 at δ_H 2.50 for protons and δ_C 39.51 for carbons).

All isolated compounds share one common feature: an oxygenation at position 6, which was either methylated or involved in methylenedioxy group. Although 6-methoxy flavonols were common for some genera of Chenopodioideae (Sanderson et al., 1988) these compounds were rarely found in *Chenopodium* species (Kokanova-Nedialkova et al., 2009). Within Amaranthaceae gomphrenol has been found only in species belonging to subfamily Gomphrenoideae, so far. The occurrence of 6-methoxykaempferol, spinacetin, patuletin and gomphrenol derivatives in *Chenopodium* genus is reported here for the first time. More detailed survey on distribution of 6-methoxy and 6,7-methylenedioxy flavonols within Amaranthaceae is required in order to establish the significance of these compounds as chemotaxonomic markers.

The new compounds **2**, **3** and **4** were tested for DPPH free radical scavenging activity at 100 μM (Blois, 1958). Compounds **2** and **4** were not active while compound **3** showed weak DPPH free radical scavenging activity (18.0%) compared to vitamin C (97.4%) and BHT (48.8%) at the same concentration.

3. Experimental

3.1. General

Melting points (m.p.) were measured on a Kofler hot-stage microscope and were uncorrected. Optical rotations (OR) were measured on a Schmidt + Haensch UniPol L1000. Infrared (IR) spectra were recorded on a Bruker Tensor 27 spectrophotometer equipped with ATR accessory. UV spectra were run in MeOH or with the standard shift reagents (Markham, 1982) on a Varian Cary 50 spectrophotometer (Palo Alto, USA). ESI–MS spectra were measured on a ThermoQuest Finnigan TSQ 7000 (4 kV). HRLSI–MS spectra were recorded on a Finnigan MAT 95 (glycerin, Cs^+, 20 kV). NMR spectra were recorded on a Bruker BioSpin (Rheinstetten, Germany) Avance III 600 spectrometer at 600 MHz (^1H) and 150 MHz (^{13}C) in DMSO-d_6. Column chromatography (CC) was carried out with Diaion HP-20, MCI-gel (Supelco, USA) and LiChroprep C-18 (40–63 μm, using an over-pressure of 0.8–1.0 bar, Merck, Darmstadt, Germany) as stationary phase. Semi-preparative high performance liquid chromatography (HPLC) was performed on a Waters (Milford MA, USA) Breeze 2 high pressure binary gradient system consisting of a pump model 1525EF, manual injector 7725i and an UV detector model 2489. Separations were achieved on a semi-preparative HPLC column Kromasil C18 (250 mm × 21.6 mm, 10 μm) purchased from Eka Chemicals AB (Bohus, Sweden). Determination of the absolute stereochemistry of the sugars was done on a Biofocus 3000 apparatus (Biorad). Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck) using following mobile phases: H_2O–AcOH–MeOH–BuOH–$CHCl_3$ (2:1:6:2:10), EtOAc–AcOH–HCOOH–H_2O (100:11:11:27) and EtOAc–AcOH–HCOOH–H_2O (25:3:3:7). The chromatograms were observed under an UV light (254 and 366 nm) before and after spraying with 1% Natural Product Reagent A (Carl Roth, Germany) in MeOH. All solvents were of HPLC grade and were purchased from Merck or Sigma–Aldrich (Taufkirchen, Germany). All reagents were of analytical grades.

3.2. Plant material

Aerial parts of *C. foliosum* Asch were collected from Beglika, Western Rhodopes, Bulgaria from June to September 2007, at an altitude of 1600 m. The plant was identified and a voucher specimen (No. SOM-Co-1207) was deposited at the National Herbarium, Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences, Sofia, Bulgaria.

3.3. Extraction and isolation

The aerial parts of *C. foliosum* were dried in the shade and powdered plant material (857 g) was extracted with CH_2Cl_2 (7 × 3 L). After filtration, the extracts were combined and the solvent was evaporated under reduced pressure to give 31.3 g of greenish waxy residue. Subsequently, the plant material was extracted with MeOH (7 × 3 L), 70% aq. MeOH (6 × 2 L) and 50% aq. MeOH (2 × 2 L). The resulting extracts were combined, concentrated under *vacuo* until most of the MeOH was removed and the aq. residue was successfully extracted with CH_2Cl_2 (8 × 300 mL). The aq. layer was conc. to 200 mL and then subjected to CC over Diaion HP-20 (7 cm × 75 cm) with eluent H_2O–MeOH (100:0 → 0:100) to obtain 86 fractions (500 mL each) that were combined into 23 pooled fractions (I–XXIII) on basis of the TLC profiles. The fractions XIII (2.46 g, 60% MeOH) and XIV (7.37 g, 70% MeOH) were separately subjected to CC over MCI gel (4 cm × 30 cm, 50 mL fraction volume) with eluent H_2O–MeOH (100:0 → 0:100). The combined fractions 27–31 (0.70 g, 50% MeOH) of XIV was chromatographed over RP-18 (4 cm × 25 cm, 50 mL fraction volume) with H_2O–MeOH (100:0 → 0:100). An isocratic semi-prep. HPLC purification of sub-fractions 20–25 (0.23 g, 40–50% MeOH) with MeOH–H_2O (38:62, 19.5 mL min^{-1}, 280 nm) as eluent gave pure 1 (8 mg) and 2 (11 mg). The fractions 32–35 (60% MeOH) of XIII and 32–34 (60% MeOH) of XIV were combined together on the basis of TLC analysis. This combined fraction (2.39 g) was further subjected to a CC over RP-18 and was eluted with H_2O–MeOH (100:0 → 0:100). The resulted sub-fraction 23–26 (0.75 g, 40–50% MeOH) was further separated by semi-prep. HPLC, isocratically eluted with MeOH–H_2O (40:60) and yielded compounds 3 (37 mg), 4 (59 mg), 5 (15 mg).

3.3.1. 6-Methoxy-3,5,7,4′-tetrahydroxyflavone 3-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (2)

Pale-yellow crystalline powder from MeOH–H_2O; m.p. 230–231 °C; $[\alpha]_D^{21.9}$ −138 (*c* = 0.1; DMSO); UV λ_{max} (MeOH) log (ε), 342 (4.30), 270 (4.25), (+$AlCl_3$) 369, 307sh, 277, (+$AlCl_3$/HCl) 364, 307sh, 278, (+NaOAc) 382, 307sh, 274, (+NaOAc/H_3BO_3) 356, 272; IR ν_{max} (ATR) cm^{-1} 3458–3210 (OH), 1615 (C=O), 1563, 1471 (C=C); ^1H NMR (600 MHz, DMSO-d_6) see Table 1; ^{13}C NMR (150 MHz, DMSO-d_6) see Table 1; ESI–MS *m*/*z* 641 $[M + H]^+$; HRLSI–MS found *m*/*z* 641.1738 $[M + H]^+$; calcd. for $C_{28}H_{33}O_{17}$ *m*/*z* 641.1718.

3.3.2. 6,7-Methylenedioxy-3,5,4′-trihydroxyflavone 3-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (3)

Pale-yellow crystalline powder from MeOH–H_2O; m.p. 235–236 °C; $[\alpha]_D^{22.1}$ −86 (*c* = 0.1; DMSO); UV λ_{max} (MeOH) log (ε), 342 (4.27), 278 (4.10), (+$AlCl_3$) 378, 297, (+$AlCl_3$/HCl) 372, 297, (+NaOAc) 339, 289, (+NaOAc/H_3BO_3) 346, 278; IR ν_{max} (ATR) cm^{-1} 3361–3211 (OH), 1680 (C=O), 1561, 1480 (C=C); ^1H NMR (600 MHz, DMSO-d_6) see Table 2; ^{13}C NMR (150 MHz, DMSO-d_6) see Table 2; ESI–MS *m*/*z* 639 $[M + H]^+$; HRLSI–MS found *m*/*z* 639.1558 $[M + H]^+$; calcd. for $C_{28}H_{31}O_{17}$ *m*/*z* 639.1561.

3.3.3. 6,7-Methylenedioxy-3,5,4′-trihydroxyflavone 3-O-α-L-rhamnopyranosyl-(1 → 2)[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside (4)

Pale-yellow crystalline powder from MeOH–H_2O; m.p. 193–194 °C; $[\alpha]_D^{22.3}$ −210 (*c* = 0.1; DMSO); UV λ_{max} (MeOH) log (ε), 340 (4.29), 278 (4.12), (+$AlCl_3$) 379, 298, (+$AlCl_3$/HCl) 373, 299, (+NaOAc) 337, 284, (+NaOAc/H_3BO_3) 342, 277; IR ν_{max} (ATR) cm^{-1} 3384 (OH), 1637 (C=O), 1560, 1519 (C=C); ^1H NMR (600 MHz, DMSO-d_6) see Table 2; ^{13}C NMR (150 MHz, DMSO-d_6) see Table 2; ESI–MS *m*/*z* 785 $[M + H]^+$; HRLSI–MS found *m*/*z* 785.2166 $[M + H]^+$; calcd. for $C_{34}H_{41}O_{21}$ *m*/*z* 785.2140.

3.4. Sugar analysis

3.4.1. TLC analysis

Compounds **2–4** (2.0 mg) were dissolved in 2 N HCl (H_2O–MeOH 1:1, 2 mL) and hydrolysed by heating at 100 °C for 2 h. After evaporation of the solvent under *vacuo*, the residue was sonificated in H_2O (3 mL) and the mixture extracted with EtOAc (3 × 5 mL). The water phase was evaporated under vacuum and redissolved in 50% MeOH. The sugar portions were co-chromatographed on a TLC with authentic samples (β-D-glucose and α-L-rhamnose). Mixtures of EtOAc–pyridine–H_2O (12:5:4) on cellulose (twofold development) and EtOAc–MeOH–H_2O–AcOH (13:3:3:4) on silica gel were used as mobile phase. The spots were visualized by spraying with anisidine-phthalate reagent (a solution of 1.23 g p-anisidine and 1.66 g phthalic acid in 100 mL 95% EtOH) and heating the plate for 10 min (110–120 °C).

3.4.2. CE determination of L-rhamnose and D-glucose

Derivatization procedure and CE method was performed according to Noe and Freissmuth (1995) with some modifications. Samples (1 mg) were dissolved in 0.5 mL 23% aq. trifluoroacetic acid (TFA) and sealed in a 1 mL glass tube. After 60 min reaction at 120 °C, the TFA solution was transferred into a pear-shaped flask using 2 mL of water. TFA was treated with additional water (3 times) and completely removed. Remaining hydrolysis product as well as D- and L- sugar references (1 mg per sample) were derivatized with 60 μL of 0.1 M S-(−)-1-phenylethyamin to afford the Schiff base, which was immediately reduced to the corresponding diastereoisomers using 22.5 μL of 0.46 M aq. sodium cyanoborohydride solution. 10 μL of every derivatized sugar reference solution were mixed to obtain a standard solution. To determine the signals of single sugars in the electropherogram, a standard diastereoisomer solution was spiked with single diastereoisomers. In order to determine the sugars in compounds isolated the standard diastereoisomer solution was spiked with single derivatized compounds.

3.5. DPPH free radical scavenging activity

Experiments were carried out according to the method of Blois (1958) with slight modifications. Briefly, a 2 mL of 0.1 mM solution of DPPH radical solution in methanol was mixed with 2 mL of 0.1 mM sample solutions and standards in methanol. Finally, after 30 min, the absorbance was measured at 517 nm. The results were expressed in terms of the percentage reduction of the initial radical adsorption by the test samples and compared with that exercised by vitamin C and BHT used as standards. All determinations were performed in triplicate (n = 3).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2011.08.002.

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