Antioxidant Constituents of the Aerial Parts of *Globularia alypum* Growing in Morocco

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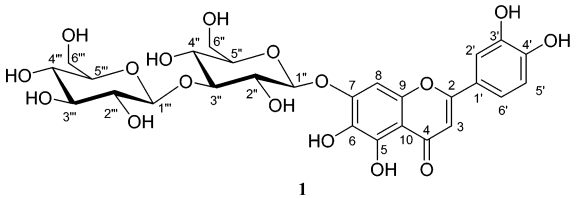
Three new phenolic compounds were isolated from the aerial parts of *Globularia alypum*. Their structures were determined as 6-hydroxyluteolin 7-*O*-laminaribioside (**1**), eriodictyol 7-*O*-sophoroside (**2**), and 6′-*O*-coumaroyl-1′-*O*-[2-(3,4-dihydroxyphenyl)ethyl]-*β*-D-glucopyranoside (**3**). In addition, three phenylethanoid glycosides (acteoside, isoacteoside, and forsythiaside) and two flavonoid glycosides (6-hydroxyluteolin 7-*O*-*β*-D-glucopyranoside and luteolin 7-*O*-sophoroside) were also isolated and are reported here for the first time in this plant. The structures of compounds **1**−**3** were established on the basis of their spectroscopic data analysis. Evaluation of the antioxidative activity, conducted in vitro, showed that the isolated phenylethanoids and flavonoid glycosides possess strong effects of this type.

Globularia alypum L. is a wild plant belonging to the family Globulariaceae. It is a perennial shrub found throughout the Mediterranean area. The plant is known for its uses in indigenous systems of medicine for a variety of purposes.[1] In the Moroccan traditional pharmacopoeia, *G. alypum*, named locally "Ain Larneb", is one of the most prominent plant remedies.[2] It is used as a hypoglycemic agent, laxative, cholagogue, stomachic, purgative, and sudorific, and also in the treatment of cardiovascular and renal diseases.[2,3] Several studies have shown the genus *Globularia* to be a rich source of phenolic compounds. Specifically, in a flavonoid survey of the Tubiflorae, Harborne and Williams[4] found 6-hydroxyluteolin to be the most abundant phenolic aglycon in all of the *Globularia* species they examined, including *G. alypum*. Furthermore, other flavonoid derivatives such as apigenin, luteolin, and quercetin have also been reported from *Golubularia* species.[5−7] More recent studies have revealed the presence of a number of phenylethanoid derivatives in *G. trichosantha*,[8,9] *G. dumulosa*,[10] *G. cordifolia*,[11] *G. orientalis*,[12] *G. davisiana*,[13] and *G. sintenisii*.[14] However, comparatively little is known about the phenolic profile of *G. alypum*. So far, the only chemical investigations of *G. alypum* are those of Bernard et al.,[5,15] Chaudhuri and Sticher,[16−18] and Ben Hassine et al.,[6] where the presence of several glycosidic iridoids, flavonoids, a lignan diglucoside, and syringin was indicated, but no phenylethanoid glycosides were reported.

In this study, three new compounds (**1**−**3**), in addition to two flavonoid glycosides (6-hydroxyluteolin 7-*O*-*β*-D-glucopyranoside and luteolin 7-*O*-sophoroside), three phenylethanoid glycosides (acteoside, isoacteoside, and forsythiaside), and syringin (the only glycoside already described in *G. alypum*), were isolated from an aqueous MeOH extract of the aerial parts of *G. alypum*. The structures of **1**−**3** were elucidated through MS, CID MS, tandem MS-MS, and 1D and 2D homonuclear and heteronuclear NMR spectroscopic data analysis.



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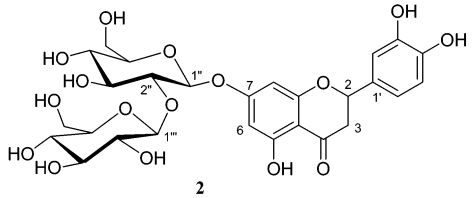

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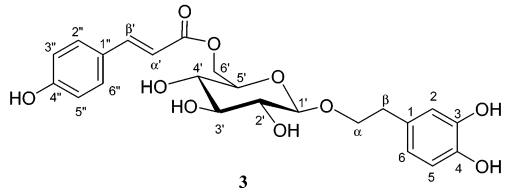

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The ESIMS of compound **1**, conducted in both negative- and positive-ion mode, showed quasimolecular ions at *m/z* 627 $[M + H]^+$ and *m/z* 625 $[M - H]^-$, respectively, indicating a molecular weight of 626, in agreement with a $C_{27}H_{30}O_{17}$ formula, as confirmed by HRMS analysis. The positive ESIMS spectrum of compound **1** showed ions at *m/z* 627, 465, and 303. The intense aglycon ion at *m/z* 303 was obtained by loss of two neutral fragments of mass 324, indicating the presence of two hexose residues. Although the *m/z* 303 ion was suggestive of compound **1** being a quercetin-based derivative, the daughter collision-induced decomposition (CID) MS-MS spectrum of the ion at *m/z* 303 was different from that of quercetin. In particular, the fragment observed at *m/z* 169, corresponding to $^{1,3}A^+$ fragmentation, showed substitution of the A ring by three OH groups. Subsequent analysis of the ^1H, ^{13}C, COSY NMR spectra of **1** determined the two carbohydrates to be *β*-D-glucopyranose, as confirmed by acid hydrolysis followed by TLC and HPLC. The remaining resonances in the NMR spectra were consistent with those of luteolin[19] except that

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the ^{13}C NMR chemical shifts associated with C-5, C-6, and C-7 were shifted significantly, indicating substitution in this region of the molecule. The protonated carbon resonance of luteolin, usually located around 99 ppm, assigned to C-6, was absent in compound **1**, being replaced by a quaternary carbon resonance at 131.4 ppm. This was accompanied by an upfield shifts of the C-5 (147.2 ppm) and C-7 (151.9 ppm) resonances, which were consistent with an oxygen substituent attached to C-6.[20] This was also confirmed in the ^1H NMR spectrum, where two singlets at 6.71 and 6.98 ppm were observed for H-3 and H-8, respectively. This was supported by the observation of carbon signals at 103.2 and 95.1 ppm, corresponding, respectively to C-3 and C-8 in the ^{13}C NMR spectrum. The position of attachment of sugars to the aglycon was determined by UV analysis, which showed that the 5, 3′, and 4′ hydroxyl groups were free.[4,21] Thus, the position of glucosylation must be at the 7-position. This was confirmed by the observation of a NOE effect between H-1″ of glucose and H-8 of luteolin, consistent only with a connection between O-1″ of glucose and C-7 of luteolin. An 1→3 interglycosidic linkage was established on the basis of ^1H and ^{13}C NMR spectra, where two anomeric glucose protons at 4.64 and 5.27 ppm were observed. Each glucosyl proton was determined through 2D COSY experiment, and their corresponding carbon resonances were assigned through HETCOR analysis. In the ^{13}C NMR spectrum, the C-3″ signal (inner glucose) was shifted downfield to 82.8 ppm compared to that of the terminal glucose.[19] This led to the assignment of the structure of 6-hydroxyluteolin-7-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranoside for **1**. 6-Hydroxyluteolin 7-*O*-diglucoside has been previously reported in *G. cordifolia*, but the interglycosidic linkage was not defined.[4] The 6-hydroxyluteolin 7-*O*-gentiobioside (1→6 linkage) from *Lomatogonium corinthiacum*[22] and the 7-*O*-sophoroside (1→2 linkage) from *G. elongata*[23] have been reported, while the 7-*O*-laminaribioside (1→3) analogue has not been described previously.

Compound **2** was obtained as an amorphous powder. Its molecular weight was concluded to be 612 Da on the basis of ESIMS, which showed parent ions at *m/z* 611 $[M - H]^-$and 613 $[M + H]^+$ respectively in the negative- and positive-ion mode. In addition, a loss of neutral fragments, corresponding to a mass of 324, was observed in both the negative- and positive-ion mode, suggesting the presence of two hexose residues. This loss gave an ion located at *m/z* 287 (negative) and 289 (positive), corresponding to an aglycon of 288 Da. The CID MS-MS of the ion at *m/z* 289 exhibited five main diagnostic fragmentations at *m/z* 271, 179, 163, 153, and 135. Taking into account the reported data concerning the fragmentation of the flavonoid skeleton, the obtained data indicated a 5,7,3′,4′-tetrahydroxyflavanone.[24] The negative ESIMS-MS showed an ion signal at *m/z* 475 $[M - H - 135]^-$, corresponding to an $^{1,3}A^-$ fragmentation. This indicated that the two glycoside moieties are located in the A ring. In addition, the ions observed at *m/z* 355 and 235 and corresponding to successive losses of 120 units from the *m/z* 475 ion suggested an 1→2 interglycosidic linkage (Figure S1, Supporting Information). Compound **2** was identified as eriodictyol 7-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, as confirmed by acid hydrolysis followed by TLC and HPLC. The presence of the flavanone skeleton was confirmed through ^1H and COSY NMR analysis, which showed two double doublets located, respectively, at 3.17 ppm (H-3a) and 2.78 ppm (H-3b) and a doublet at 5.34 ppm (H-2), in agreement with previous data.[25] While flavonols and flavones were already observed, no flavanone was previously reported from the family as either an aglycon or a glycoside. Until now, only 7-*O*-rutinoside and 7-*O*-neohesperidoside have been isolated as disaccharide derivatives of eriodictyol.

Compound **3** was isolated as an amorphous powder, for which the UV spectrum showed λ_{max} at 344 (sh), 325 and 304 (sh) nm, indicating its phenolic nature. The ESIMS of **3** revealed a molecular weight of 462, consistent with the molecular formula $C_{23}H_{26}O_{10}$, which was confirmed by HRMS analysis. This was in good agreement with the observation of the three methylene, 14 methine, and six quaternary carbon resonances in its ^{13}C NMR and DEPT spectra. The ^1H NMR spectrum of **3** showed the presence of seven aromatic proton signals located between *δ* 6.53 and 7.39. These protons were observed as a set of resonances corresponding to a *p*-substituted phenyl group (A_2B_2 spin system [*δ* 7.39 ppm (2H, d, *J* = 8.6 Hz, H-2″, H-6″); 6.79 ppm (2H, d, *J* = 8.6 Hz, H-3″, H-5″)]) and a set of resonances corresponding to the ABX spin system of a 1,3,4-trisubstituted phenyl group [*δ* 6.67 ppm (1H, d, *J* = 1.8 Hz, H-2); 6.53 (1H, dd, *J* = 8.2, 1.8 Hz, H-6); 6.63 (1H, d, *J* = 8.2 Hz, H-5)]. Additionally, a set of a *trans*-coupled olefinic protons [*δ* 7.61 ppm (1H, d, *J* = 16.1 Hz, H-*β*′); 6.74 (1H, d, *J* = 16.1 Hz, H-α′)], two geminated benzylic protons at *δ* 2.79 ppm (2H, *J* = 7.4 Hz, H-*β*) and two nonequivalent protons at *δ* 3.71 and 3.95 ppm (each 1H, *m*, H-α), and resonances corresponding to a sugar moiety were observed. Through NOESY NMR experiments, correlations between the *β*′ *trans*-coupled olefinic proton and the protons located at 7.39 ppm (H-2″, H6″) were observed. In addition, correlations between the benzylic protons and those of the ABX system were also observed, which is in agreement with the protons of *trans*-*p*-coumaric acid and 3,4-dihydroxy phenylethanol moieties. This was also confirmed by ES-IMS-MS analysis, where fragments corresponding to the *trans*-*p*-coumaroyl moiety were observed at *m/z* 145 (negative) and 147 (positive) (Figure S2, Supporting Information). All protons of the sugar unit were assigned unambiguously from COSY and HETCOR experiments, and the structure of the sugar moiety was identified as *β*-D-glucose, as confirmed by acid hydrolysis followed by TLC and HPLC. The coumaroyl moiety was concluded to be present at the C-6′ position of the glucose, on the basis of the significant deshielding of the H-6′ signals of the glucose unit (4.49 ppm, dd, *J* = 12.0, 2.0 Hz and 4.32 ppm, dd, *J* = 12.0, 6.0 Hz). This assumption was confirmed through ESIMS-MS analysis, where the observed signal ions where all in agreement with the fragmentations allowed from the proposed structure (Figure S2). From the above results, compound **3** was determined as 6′-*O*-coumaroyl-1′-*O*-[2-(3,4-dihydroxyphenyl)ethyl]-*β*-D-glucopyranoside.

In addition to compounds **1**−**3**, syringin,[26] 6-hydroxyluteolin 7-*O*-*β*-D-glucopyranoside,[27] luteolin 7-*O*-sophoroside,[28] acteoside,[29] isoacteoside,[29] and forsythiaside[30] were also isolated in this study. Their structures were identified by comparison of their physical and spectroscopic data with those of published values. Among these compounds, only syringin was reported previously in *G. alypum*.[18] This work constitutes the first report of a luteolin diglucoside derivative in the Globulariaceae family, while the corresponding 7-*O*-glucoside was previously reported.[6] Harborne and Williams[4] indicated the absence of luteolin in *G. alypum*, while it was occasionally present in *G. incanescens*, *G. nudicaulis*, and *G. orientalis*. No specification was given about its glycosylation pattern. Acteoside and isoacteoside have been previously reported in several *Globularia* species including *G. trichosantha*,[8,9] *G. dumulosa*,[10] *G. davisiana*,[13]

G. cordifolia,[11] and *G. sintensii*,[14] while forsythiaside has never been obtained from any species in the Globulariaceae before. The occurrence of 6-hydroxyluteolin-based flavonoids in *G. alypum* supported the fact that this aglycon is characteristic of the Globulariaceae family.

The three newly described compounds and the known isolated phenolic compounds were found to have potent antioxidant properties, as indicated by their IC_{50} values, which showed their ability to efficiently scavenge free radicals.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. UV−visible spectra were recorded using a Kontron Uvikon 930 spectrophotometer fitted with a quartz cell. FT-IR spectra were recorded with a Nicolet Avatar 320 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD with a Varian Gemini-300 spectrometer at 300 and 75 MHz, respectively (proton decoupling mode for carbon). Positive- and negative-mode ESIMS were recorded on a Quattro LC MS/MS triple quadrupole mass spectrometer. HRMS were recorded on a Bruker Maldi-TOF instrument using 2,5-dihydroxybenzoic acid (DHB) as matrix. Analytical TLC was performed on Merck silica gel 60 F254 plates. Column chromatography was performed on a SPE column using a mixture of MeOH/H_2O (0/100 to 100/0) as eluent. Analytical HPLC was performed on a Kromasil reversed-phase C_{18} 5 μm (250 × 4.6 mm) column using a Varian apparatus including a 9012 solvent delivery system, a 9100 autosampler, and a 9065 polychrom diode array detector. Semipreparative HPLC was performed on a Kromasil C_{18} 10 μm column (250 × 20 mm) using an apparatus including a Millipore Waters 600 multisolvent delivery system, a Waters U6K manual injector, and a TSP-UV200 dual-wavelength UV/visible programmable detector. LC-MS analyses were performed with a chromatographic system (Alliance) consisting of a Waters 2695 separations module equipped with an autosampler and a Waters 2487 dual lambda absorbance detector. The column was a 150 × 2.1 mm Interchrom UP5ODB#15E (Uptisphere 5 μm ODB). The HPLC system was coupled on line to a Quattro LC MS/MS triple quadrupole mass spectrometer, and data acquisition and processing were performed using a MassLynx NT 3.5 data system.

Plant Material. Fresh aerial parts of *Globularia alypum* L. were collected from the Taza region, Morocco, in April 2003. Taxonomic identification was performed by Dr. R. Tellal, Department of Biology, University of El Jadida, Morocco. A voucher specimen (KS_2) has been deposited in the Herbarium of the Department of Biology, University of El Jadida, Morocco.

Extraction and Isolation. Fresh aerial parts were air-dried in shade at room temperature, and the dried aerial parts were powdered. A 100 g portion of the obtained powder was macerated for 48 h at room temperature with 500 mL of distilled water−methanol (3/2). This was filtered and concentrated under reduced pressure to provide a crude extract (10.75 g), which was stored at −20 °C until use. The aqueous phase obtained was extracted with hexane, and a further aqueous phase was subjected to passage over a SPE column. Elution was performed successively with H_2O, 10% MeOH, 40% MeOH, 50% MeOH, and 100% MeOH. The fractions obtained were concentrated under reduced pressure, lyophilized, tested for their scavenging activity, and analyzed by analytical HPLC. The 50% MeOH fraction, which was shown to be rich in natural antioxidant compounds, was subjected to semipreparative HPLC. Elution was performed with a mixture of solvents A (acetonitrile) and B (water with 0.5% acetic acid), eluting from 5 to 30% A in 120 min followed by a washing and a reequilibrating of the column. After several successive injections, samples corresponding to the same chromatographic peaks were controlled by analytical HPLC, concentrated under reduced pressure, and lyophilized. This operation gave the pure described compounds **1** (9.7 mg), **2** (7.0 mg), and **3** (10.5 mg).

6-Hydroxyluteolin 7-*O*-laminaribioside (1): amorphous solid; $[\alpha]^{20}_D$ −45.3° (*c* 0.1, MeOH/H_2O, 1:1); UV (MeOH) λ_{max} (log ϵ) 230 (3.8), 255 (3.5), 284 (4.13) and 346 (4.17) nm; IR (dried film) ν_{max} 3460 (OH), 1675 (C=O), 1620, 1575, 1570, 1520, 1510 cm^{-1} (aromatic ring); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.42 (2H, m, H-2′, H-6′), 6.98 (1H, s, H-8), 6.91 (1H, dd, J = 2.5, 8.0 Hz, H-5′), 6.71 (1H, s, H-3), 5.27 (1H, d, J = 8.0 Hz, H-1″), 4.64 (1H, d, J = 8.0 Hz, H-1‴), 3.69 (1H, m, H-4″), 3.57 (1H, m, H-2″), 3.54 (1H, m, H-3″), 3.30 (1H, m, H-5″), 3.29 (1H, dd, J = 12.0, 2.0 Hz, H-6″a), 3.24 (1H, dd, J = 12.0, 6.0 Hz, H-6″b), 3.20 (1H, m, H-2‴), 3.16 (3H, m, H-3‴, H-5‴, H-6‴a), 3.07 (1H, dd, J = 11.6, 2.0 Hz, H-6‴b), 3.04 (1H, m, H-4‴); ^{13}C NMR (300 MHz, DMSO-d_6) δ 183.0 (C, C-4), 165.3 (C, C-2), 151.9 (C, C-7), 151.7 (C, C-9), 149.9 (C, C-4′), 147.2 (C, C-5), 146.8 (C, C-3′), 131.4 (C, C-6), 122.0 (C, C-1′), 120.2 (CH, C-6′), 117.0 (CH, C-5′), 114.4 (CH, C-2′), 106.7 (C, C-10), 104.8 (CH, C-1‴), 103.2 (CH, C-3), 100.5 (CH, C-1″), 95.1 (CH, C-8), 82.8 (CH, C-3″), 78.0 (CH, C-5″), 77.7 (CH, C-5‴), 77.1 (CH, C-3‴), 76.5 (CH, C-2‴), 75.3 (CH, C-2″), 70.6 (CH, C-4‴), 70.2 (CH, C-4″), 61.5 (CH_2, C-6″), 61.3 (CH_2, C-6‴); negative LRESIMS *m/z* 625 (100) $[M - H]^-$, 463 (3), 445 (7), 301 (83); positive LRESIMS *m/z* 627 (36) $[M + H]^+$, 465 (16), 303 (100); HRMS *m/z* 627.1575 $[M + H]^+$ (calcd for $C_{27}H_{30}O_{18}$, 627.1561).

Eriodictyol 7-*O*-sophoroside (2): amorphous solid; $[\alpha]^{20}_D$ −35.6° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 289 (4.2) and 324sh (3.7) nm; IR (dried film) ν_{max} 3420 (OH), 1650 (C=O), 1610, 1540, 1520 cm^{-1} (aromatic ring); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.62 (1H, dd, J = 2.5, 8.5 Hz, H-6′), 6.92 (1H, d, J = 2.0 Hz, H-6), 6.39 (1H, d, J = 8.5 Hz, H-5′), 6.36 (1H, d, J = 2.0 Hz, H-8), 6.22 (1H, d, J = 2.5 Hz, H-2′), 5.34 (1H, dd, J = 5.0, 11.0 Hz, H-2), 5.20 (1H, d, J = 7.8 Hz, H-1″), 4.65 (1H, d, J = 7.8 Hz, H-1‴), 3.89 (1H, dd, J = 11.8, 2.0 Hz, H-6″a), 3.70 (2H, m, H-2″, H-6″b), 3.69 (1H, m, H-5″), 3.68 (1H, m, H-6‴a), 3.65 (2H, m, H-4″, H-6‴b), 3.53 (1H, m, H-4‴), 3.50 (1H, m, H-3″), 3.42 (1H, m, H-5‴), 3.40 (1H, m, H-3‴), 3.23 (1H, m, H-2‴), 3.17 (1H, dd, J = 5.0, 17.0 Hz, H-3a), 2.78 (1H, dd, J = 11.0, 17.0 Hz, H-3b); ^{13}C NMR (300 MHz, DMSO-d_6) δ 198.4 (C, C-4), 167.6 (C, C-5), 165.8 (C, C-9), 163.1 (C, C-7), 148.1 (C, C-4′), 147.9 (C, C-3′), 130.7 (C, C-1′), 120.4 (CH, C-6′), 116.0 (CH, C-5′), 112.9 (CH, C-2′), 103.2 (CH, C-1‴), 101.4 (C, C-10), 100.9 (CH, C-1″), 96.7 (CH, C-8), 92.3 (CH, C-6), 79.3 (CH, C-2), 79.0 (CH, C-2″), 76.5 (CH, C-5‴), 75.0 (CH, C-5″), 73.7 (CH, C-3‴), 72.9 (CH, C-2‴), 72.9 (CH, C-3″), 71.8 (CH, C-4‴), 69.6 (CH, C-4″), 62.8 (CH_2, C-6″), 61.1 (CH_2, C-6‴), 43.4 (CH_2, C-3); negative LRESIMS *m/z* 611 (100) $[M - H]^-$, 475 (53), 287 (28), 151 (99); positive LRESIMS *m/z* 613 (52) $[M + H]^+$, 451 (86), 433 (21), 331 (11), 289 (100); HRMS *m/z* 613.1786 $[M + H]^+$ (calcd for $C_{27}H_{32}O_{17}$, 613.1769).

6′-*O*-Coumaroyl-1′-*O*-[2-(3,4-dihydroxyphenyl)ethyl]-β-D-glucopyranoside (3): amorphous solid; $[\alpha]^{20}_D$ −25.4° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 344sh (3.1), 325 (3.4) and 304sh (3.0) nm; IR (dried film) ν_{max} 3480 (OH), 1695 (α,β-unsaturated ester), 1638 (olefinic C=C), 1610, 1540, 1520 cm^{-1} (aromatic ring); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (1H, d, J = 16.1 Hz, H-β′), 7.39 (2H, d, J = 8.6 Hz, H-2″, H-6″), 6.79 (2H, d, J = 8.6 Hz, H-3″, H-5″), 6.74 (1H, d, J = 16.1 Hz, H-α′), 6.67 (1H, d, J = 1.8 Hz, H-2), 6.63 (1H, d, J = 8.2 Hz, H-5), 6.53 (1H, dd, J = 8.2, 1.8 Hz, H-6), 4.49 (1H, dd, J = 12.0, 2.0 Hz, H-6′a), 4.33 (1H, d, J = 7.9 Hz, H-1′), 4.32 (1H, dd, J = 12.0, 6.0 Hz, H-6′b), 3.95 (1H, m, H-αa), 3.71 (1H, m, H-αb), 3.52 (1H, m, H-5′), 3.36 (1H, m, H-4′), 3.29 (1H, m, H-3′), 3.22 (1H, m, H-2′), 2.79 (2H, m, H-β); ^{13}C NMR (300 MHz, DMSO-d_6) δ 169.8 (C, C=O), 162.1 (C, C-4″), 147.7 (C, C-4), 147.4 (CH, C-β′), 145.5 (C, C-3), 132.0 (C, C-1), 131.7 (CH, C-3″, C-5″), 128.0 (C, C-1″), 121.8 (CH, C-6), 117.8 (CH, C-2″, C-6″), 117.4 (CH, C-2), 116.9 (CH, C-α′), 115.5 (CH, C-5), 105.1 (CH, C-1′), 78.5 (CH, C-3′), 76.0 (CH, C-5′), 75.6 (CH, C-2′), 72.9 (CH_2, C-α), 72.4 (CH, C-4′), 65.3 (CH_2, C-6′), 37.6 (CH_2, C-β); negative LRESIMS *m/z* 461 (72) $[M - H]^-$, 315 (8), 163 (4), 145 (100); positive LRESIMS *m/z* 463 (100) $[M + H]^+$, 381 (85), 309 (44), 147 (96); HRMS *m/z* 463.1630 $[M + H]^+$ (calcd for $C_{23}H_{26}O_{11}$, 463.1604).

Reduction of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical.[31] In a TLC autographic assay, methanolic solutions (0.1%) of the isolates were chromatographed on a silica gel plate using $CHCl_3$−MeOH−H_2O (61:32:7) for elution. After developing and drying, TLC plates were sprayed with a 0.2% 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in MeOH. Compounds showing yellow on purple spots were regarded as an antioxidant. In a spectrophotometric assay, 50 μL of a solution containing the compound to be tested was added to 5 mL of a 0.006% MeOH solution of DPPH. The studied compounds were tested in triplicate with MeOH as the negative control and BHT as the positive control. Absorbance at 517 nm was determined after 30 min, and the concentration required for a 50% reduction (IC_{50}) of DPPH radical was determined graphically. The obtained results were as follows: 6-hydroxyluteolin 7-*O*-laminaribioside (8.0 μM), eriodictyol 7-*O*-sophoroside (12.0 μM), 6′-*O*-coumaroyl-1′-*O*-[2-(3,4-dihydroxyphenyl)-ethyl]-β-D-glucopyranoside (18.0 μM), 6-hydroxyluteolin 7-*O*-β-D-glucopyranoside (11.0 μM), luteolin 7-*O*-sophoroside (12.0 μM), acteoside (20.0 μM), isoacteoside (20.0 μM), forsythiaside (21.0 μM), and BHT (40.0 μM).

Supporting Information Available: Figures showing CID MS-MS data for compounds **2** and **3**. This information is available free of charge via the Internet at http://pubs.acs.org/jnp.

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