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Phenolic Constituents and Antioxidant Properties of *Xanthosoma violaceum* Leaves

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An extract of *Xanthosoma violaceum* leaves was subjected to a polyphenol profile determination, including total polyphenols, and antioxidant activity evaluation. Analysis of the extract resulted in the isolation of a new flavone *C*-glycoside, apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-apiofuranoside (**1**), as well as known flavone *C*-glycosides, including vitexin (**2**), isovitexin (**3**), isovitexin 4′-*O*-rhamnopyranoside (**4**), apigenin 6-*C*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside] (**5**), and apigenin 6,8-di*C*-β-D-glucopyranoside (**6**). The antioxidant activity of the extract was assessed by means of two different in vitro tests: bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH test) and peroxidation induced by the water-soluble radical initiator 2,2′-azobis(2-amidinopropane) hydrochloride, on mixed dipalmitoylphosphatidylcholine/linoleic acid unilamellar vesicles (LP-LUV test). In both tests used, the extract and a fraction II showed a significant antioxidant/free-radical scavenging effect (fraction II, EC_{50} = 11.6 μg/mL) in comparison to α-tocopherol (EC_{50} = 10.1 μg/mL).

KEYWORDS: ***Xanthosoma violaceum*; new cocoyams; *C*-glycosyl flavones; free-radical scavenging/antioxidant activity**

INTRODUCTION

X. violaceum Schott (Araceae) is a herbaceous perennial plant of tropical American origin, widely distributed in Dominican Republic, Puerto Rico, Guatemala, and Ecuador; its roots and cormels are typical tropical crops (*1, 2*). Because many developing countries in the tropics depend on *Xanthosoma* species, collectively known as "new" cocoyams, as a source of carbohydrates (*2*), these edible aroids are an unexploited source of food and industrial starches. Among the *Xanthosoma* species, *X. sagittifolium* (L) Schott is generally considered as the main cultivated species and therefore the composition of its cormels and leaves has been studied with regard to minerals (*3*), carbohydrates (*3*), phenols (*4*), and carotenoids (*5*), whereas the phenolic composition of *X. violaceum* leaves is unknown. In our continuing search for antioxidative plant extracts from Central and South American species (*6*−*8*), *Xanthosoma violaceum* was investigated phytochemically and biologically. An analytical preliminary evaluation of a polar leaf extract (EXV) of *X. violaceum* showed high levels of phenolic compounds, especially flavones, whose effectiveness as antioxidant agents is reported in the literature (*9, 10*). In this study we investigated the in vitro antioxidant and free-radical scavenging activities of EXV extract determined both in homogeneous solution, employing the bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH test), and in a membrane system employing, as an experimental model, the peroxidation induced by the water-soluble radical initiator 2,2′-azobis(2-amidinopropane) hydrochloride, on mixed dipalmitoylphosphatidylcholine/linoleic acid unilamellar vesicles (LP-LUV test). Since the interaction with the microenvironment of the lipid cell bilayers plays a key role in the membrane-dependent antioxidant activity, the employment of both in vitro tests may give useful information on the actual effectiveness and suitability of potential antioxidants (*7*). Also, the total phenolic content of the extract was determined, the major constituents were isolated and their chemical structures established, and the flavone quantitative analysis was obtained by an HPLC analytical method.

MATERIAL AND METHODS

General Experimental Procedures. Melting points are uncorrected. UV spectra were obtained with a Perkin-Elmer 550 SE spectrophotometer. For NMR experiments, a Bruker DRX-600 spectrometer was used, operating at 599.2 MHz for ^{1}H and 150.9 MHz for ^{13}C and using the UXNMR software package; DEPT, ^{1}H−^{1}H DFQ-COSY (double-quantum filtered COSY), and ^{1}H−^{13}C HSQC and HMBC experiments were obtained using conventional pulse sequences. 1D TOCSY (*11*) (selective excitation spectra) were acquired as previously reported (*6*). Chemical shifts are expressed in δ (ppm) referring to the following solvent center peaks: δ_H 3.34 and δ_C 49.0 for CD_3OD. The FABMS

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Table 1. ^{13}C NMR and ^{1}H NMR Data of Compounds 1 in CD_3OD[a]

aglycone			sugar			
position[b]	δ_C	δ_H (J_{HH} in Hz)[c]	position[b]	δ_C	δ_H (J_{HH} in Hz)[c]	HMBC δ_C
2	164.2		Glc-1″	75.6	4.96 d (7.5)	72.0 (Glc-2″), 109.2 (C-6), 162.1 (C-5), 165.1 (C-7),
3	103.1	6.58 s	Glc-2″	72.0	4.27 dd (9.0, 7.5)	
4	182.4		Glc-3″	80.1	3.49 t (9.0)	
5	162.1		Glc-4″	71.3	3.56 t (9.0)	
6	109.2		Glc-5″	82.4	3.42 m	
7	165.1		Glc-6″	62.3	3.78 dd (12.0, 4.5), 3.87 dd (12.0, 3.5)	
8	105.1		Ap-1‴	77.1	5.01 d (2.0)	70.8 (Ap-2‴), 105.1 (C-8), 157.8 (C-9), 165.1 (C-7)
9	157.8		Ap-2‴	70.8	4.00 d (2.0)	
10	105.5		Ap-3‴	80.1	-	
1′	122.9		Ap-4‴	72.3	4.08 d (10.0), 3.82 d (10.0)	
2′ and 6′	129.6	7.98 d (8.5)	Ap-5‴	65.1	3.60 br s	
3′ and 5′	116.7	6.95 d (8.5)				
4′	161.9					

[a] Assignments confirmed by 1D TOCSY and 2D COSY, HSQC, HMBC experiments. [b] Glc = β-D-glucopyranosyl, Ap = β-D-apiofuranosyl. [c] ^{1}H–^{1}H coupling constants in the sugar unit were measured from TOCSY and COSY spectra in Hz.

were recorded in a glycerol matrix in the negative-ion mode on a VG ZAB instrument (XE atoms of energy 2−6 kV). Semipreparative HPLC separations were carried out with a Waters Model 6000A pump equipped with a U6K injector and a Model 401 refractive index detector. Quantitative HPLC analysis was performed with a Shimadzu LC-10AD system equipped with a Model SPD-10AV UV−vis detector and a Rheodyne Model 7725 injector (Millipore, Boston, MA), loop 20 μL. Peak areas were calculated with a Shimadzu Chromatopac C-R6A integrator. TLC analysis was performed on Si gel SiF_{254} (Merck) and visualized with the spray reagents cerium sulfate (saturated solution in dilute H_2SO_4) or vanillin (3 g of vanillin, 4 mL of HCl, 100 mL of MeOH). 1,1-Diphenyl-2-picrylhydrazyl radical, dipalmitoylphosphatidylcholine, 2,2′-azobis(2-amidinopropane) hydrochloride, linoleic acid, quercetin, and α-tocopherol were purchased from Sigma-Aldrich (Milan, Italy).

Plant Material. The leaves of *X. violaceum* Schott (Araceae) were collected near Riobamba, Ecuador, in Feb 1996 and identified by Dr. M. Tapia, ESPOCH. A specimen of the plant (X.V. 1, 1996) used in this study has been deposited at the Herbarium of ESPOCH, Riobamba, Ecuador.

Extraction and Isolation. The powdered, air-dried leaves (200 g) were defatted at room temperature with *n*-hexane and $CHCl_3$ and then extracted with MeOH to give 13.2 g of residue, which was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH-soluble portion (5.5 g). An aliquot (2.0 g) of this was chromatographed over a 100 × 5 cm Sephadex LH-20 column using MeOH as eluent. Fractions (9 mL) were collected and checked by TLC (Si gel, *n*-BuOH−HOAc−H_2O (60:15:25)) giving fractions 9−25 (I) (180.1 mg), fractions 26−60 (II) (300.4 mg) used for biological assays and total phenol determination, and fractions 60−150 (III) (1.5 g). Part of fraction II (150 mg) was separated by RP-HPLC on a 300 × 7.8 mm i.d. C-18 μ-Bondapack column, at a flow rate of 2.5 mL/min using MeOH−H_2O (4:6) as the eluent to give vitexin (**2**) (*6*) (25.1 mg, t_R = 12.1 min), apigenin 6-*C*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside] (**5**) (*12, 13*) (7.8 mg, t_R = 13.8 min), apigenin 6,8-di-*C*-β-D-glucopyranoside (**6**) (*14*) (8.5 mg, t_R = 14.6 min), apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-apiofuranoside (**1**) (4.1 mg, t_R = 16.7 min), isovitexin 4′-*O*-rhamnopyranoside (**4**) (*15*) (10.6 mg, t_R = 19.8 min), and isovitexin (**3**) (*16, 17*) (13.1 mg, t_R = 22.3 min).

Compound 1: amorphous powder; mp 198−202 °C; $[\alpha]^{20}_D$ −60.5° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 335 (4.42), 270 (4.30); ^{1}H and ^{13}C NMR see **Table 1**; FABMS *m*/*z* 563 $[M - H]^-$. Anal. Found: C, 55.38; H, 4.98. Calcd for $C_{26}H_{28}O_{14}$: C, 55.33; H, 5.00.

Isovitexin 4′-*O*-rhamnopyranoside (4): amorphous powder; mp 202−206 °C; UV (MeOH) λ_{max} 333 (4.38), 268 (4.28) nm; FABMS *m*/*z* 577 $[M - H]^-$, 431 $[(M - H) - 146]^-$; aglycone ^{1}H NMR δ 6.39 (1H, s, H-8), 6.56 (1H, s, H-3), 6.94 (2H, d, *J* = 8.0 Hz, H-3′ and H-5′), 7.84 (2H, d, *J* = 8.0 Hz, H-2′ and H-6′); aglycone ^{13}C NMR δ 184.0 (C-4), 167.4 (C-7), 165.6 (C-2), 162.3 (C-4′), 160.9 (C-5), 158.7 (C-9), 129.4 (C-6′ and C-2′), 122.0 (C-1′), 116.7 (C-3′ and C-5′), 108.7 (C-6), 106.0 (C-10), 103.1 (C-3), 98.0 (C-8), 5; sugar chain ^{1}H NMR β-D-glucopyranosyl δ 3.42 (m, Glc-5), 3.47 (br t, Glc-3), 3.53 (br t, Glc-4), 3.71 (dd, *J* = 12.0, 4.5 Hz, Glc-6a), 3.86 (dd, *J* = 12.0, 2.5 Hz, Glc-6b), 4.35 (dd, *J* = 9.0, 7.5 Hz, Glc-2), 4.92 (d, *J* = 7.5 Hz, Glc-1), α-L-rhamnopyranosyl 1.26 (d, *J* = 6.5 Hz, Rha-6), 3.50 (br t, Rha-4), 3.62 (m, Rha-5), 3.87 (dd, *J* = 9.0, 2.5 Hz, Rha-3), 4.04 (dd, *J* = 2.5, 1.5 Hz, Rha-2), 5.57 (d, *J* = 1.5 Hz, Rha-1); sugar chain ^{13}C NMR β-D-glucopyranosyl δ 61.8 (Glc-6), 70.7 (Glc-4), 71.8 (Glc-2), 74.5 (Glc-1), 79.8 (Glc-3), 81.6 (Glc-5), α-L-rhamnopyranosyl 18.0 (Rha-6), 70.0 (Rha-5), 70.9 (Rha-2), 71.1 (Rha-3), 72.9 (Rha-4), 98.1 (Rha-1).

Quantitative HPLC Analysis. To prepare a standard solution containing vitexin and isovitexin, accurately weighed amounts of each compound were dissolved in MeOH. Serial dilutions with a concentration range of 12.5−50.0 μg/mL for both vitexin and isovitexin were prepared. Quantitative HPLC was conducted using a 150 × 3.9 mm i.d. C-18 μ-Bondapack column. The mobile phase was a gradient starting from 20% to 100% of MeOH (solvent B) in 0.01 M phosphate buffer, pH 5.0 (solvent A), over 46 min. The elution gradient was 20−30% of B from 0 to 6 min, 30−40% from 6 to 26 min, 40−50% from 26 to 36 min, and 50−100% from 36 to 46 min. The analyses were carried out in triplicate, at a flow rate of 1.0 mL/min with the UV detector set at λ 270 nm and the injection volume at 20 μL. The peak assignment was based on the retention time of each compound. Calibration graphs were plotted showing a linear relationship between concentration versus peak areas for both reference compounds. The regression equations were $y = 795760x - 59808$ ($R = 0.9996$) for isovitexin (**3**) and $y = 426701x - 31676$ ($R = 0.9977$) for vitexin (**2**), where *y* is the peak area and *x* the concentration used. EXV was redissolved in MeOH and analyzed under the same chromatographic conditions as used for vitexin and isovitexin. The attribution of the chromatographic peaks was based on the single retention times and confirmed by analysis in comparison with the isolated compounds. The total concentration of the isovitexin derivatives (compounds **1** and **3**−**6**) expressed as an isovitexin (**3**) equivalent was 6.7%; vitexin (**2**) represented 5.5% of the extract.

Quantitative Determination of Total Phenols. The *X. violaceum* dried *n*-BuOH extract and fractions I−II, dissolved in MeOH, were analyzed for their total phenolic content according to the Folin-Ciocalteu colorimetric method (*6*). Total phenols were expressed as apigenin equivalents (μg/mg extract). Results are reported in **Table 2**.

Oxidation of Linoleic Acid in LA/DPPC LUVs (LP-LUV Test). The method involves the spectrophotometric determination of the accumulation of products (conjugated dienes) of peroxidation, induced by the water-soluble peroxyl radical generator 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH), of linoleic acid in mixed dipalmi-

Table 2. Total Phenol Content and Free-Radical Scavenging Activity of the *n*-BuOH Extract, Fractions I and II, from *X. violaceum*

extract and fractions	phenol content[a] (μg/mg of extract)[b]	DPPH test EC_{50}[c]	LP-LUV test IC_{50}[c]
n-BuOH extract (EXV)	254 ± 1.0	103.6 (91.2–116.0)[d]	35.4 (31.3–39.5)[d]
fraction I	26 ± 2.1	172.7 (146.5–198.9)[d]	
fraction II	335 ± 3.5	11.6 (9.8–13.4)[d]	27.5 (23.6–31.4)[d]
quercetin[e]		2.3 (1.86–2.84)[d]	0.9 (0.2–1.6)[d]
α-tocopherol[e]		10.1 (8.8–11.4)[d]	

[a] Mean ±SD of three determinations by the Folin–Ciocolteu method. [b] Apigenin equivalents. [c] In units of μg of extract or compound/mL. [d] 95% confidence limits. [e] Positive control.

toylphosphatidylcholine/linoleic acid (DPPC/LA) unilamellar vesicles (LUVs) (*18*). Multilamellar liposomes (MLVs) were obtained by freshly prepared chloroform−methanol (1:1, v:v) concentrated solutions of DPPC and linoleic acid (molar ratio 1:0.125). The solvents were removed under nitrogen in a rotary evaporator, and the resulting film was kept overnight under vacuum to remove the residual solvents. Liposomes were prepared by adding 0.9% NaCl aqueous solution to the film and then heating at a temperature above that of the gel−liquid crystalline phase transition (60 °C) and vortexing three times for 1 min. Then, the samples were shaken for 1 h in a water bath at 60 °C to homogenize the liposomes. LUVs were prepared by submitting the previously prepared MLV dispersion to extrusion through 100 nm polycarbonate membranes (Avestin Inc., Ottawa, Canada) in a Liposo-Fast Basic extruder system (Avestin Inc., Ottawa, Canada). An aliquot (5 μL) of the EtOH/H_2O (7:3) solution containing different amounts of *X. violaceum* extract (EXV) and of fractions I and II was added to 1.2 mL of the LUV suspension (21 mg of DPPC/mL), after which the mixture was incubated for 20 min at 37 °C in a shaking water bath. Then the peroxyl radical generator AAPH was added to the suspension to obtain a final concentration of 10 μM. The oxidation was carried out at 37 °C (below the transition temperature of DPPC/LA LUVs) under air. At given times (5−90 min) 120 μL aliquots of the reaction mixtures were withdrawn and added to 1 mL of methanol. The accumulation of hydroperoxides (LOOH) formed from linoleic acid was determined spectrophotometrically by measuring the absorbance of the samples at 233 nm. The ratio of oxidation-induced change in absorbance with and without antioxidant addition was used to calculate a percent inhibition of oxidation (100% corresponding to complete protection and 0% corresponding to no difference from control) by the equations

$$[\mathrm{LOOH}]_{\mathrm{RI}} = A_{\mathrm{T90}} - A_{\mathrm{T5}}/eT_{\mathrm{SEC}}$$

$$[\mathrm{LOOH}]_{\mathrm{InH}} = A'_{\mathrm{T90}} - A'_{\mathrm{T5}}/eT_{\mathrm{SEC}}$$

$$\%\ \mathrm{inhibition} = ([\mathrm{LOOH}]_{\mathrm{RI}} - [\mathrm{LOOH}]_{\mathrm{InH}}/[\mathrm{LOOH}]_{\mathrm{RI}}) \times 100$$

where RI = radical initiator, A_{T90} and A_{T5} = absorbances at the end and at the beginning of the experiment; A'_{T90} and A'_{T5} = absorbances at the end and at the beginning of the experiment in the presence of the antioxidant, e (molar extinction coefficient of conjugated dienes) = 26 100 ± 400 M^{-1} cm^{-1}, T_{SEC} (time, sec) = 5100, $[LOOH]_{IR}$ = hydroperoxide concentration after addition of the radical initiator alone, and $[LOOH]_{InH}$ = hydroperoxide concentration after addition of the antioxidant. All experiments were carried out in triplicate and repeated at least three times. Results were calculated as a percentage decrease with respect to control values, and mean inhibitory concentrations (IC_{50}) were evaluated by using the Litchfield and Wilcoxon (*19*) test.

Bleaching of the Free Radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH Test). The antiradical activities of the *X. violaceum* extract and fractions and positive controls (rutin and α-tocopherol) were determined using the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and the procedures described by Rapisarda et al. (*18*). DPPH has an absorption band at 515 nm, which disappears upon reduction by an antiradical compound. An aliquot (37.5 μL) of the MeOH solution containing different amounts of the *n*-BuOH extract, or of fractions I and II, from *X. violaceum* and controls was added to 1.5 mL of freshly prepared DPPH solution (0.025 g/L in methanol); the maximum concentration employed was 200 μg/mL. An equal volume (37.5 μL) of MeOH was added to control tubes. Absorbance at 515 nm was measured on a Shimadzu UV-1601 UV−visible spectrophotometer 20 min after starting the reaction. The DPPH concentration in the reaction medium was calculated from a calibration curve analyzed by linear regression. The percentage of remaining DPPH (% $DPPH_{REM}$) was calculated as

$$\%\ \mathrm{DPPH}_{\mathrm{REM}} = [\mathrm{DPPH}]_T/[\mathrm{DPPH}]_0 \times 100$$

where T is the experimental duration time (20 min). All experiments were carried out in triplicate, and the mean effective scavenging concentrations (EC_{50}) were calculated by using the Litchfield and Wilcoxon test (*19*). Results are reported in Table 2.

RESULTS AND DISCUSSION

The dried leaves of *X. violaceum*, defatted with *n*-hexane and chloroform, were extracted with MeOH, and this extract was partitioned between water and *n*-BuOH. The dried *n*-BuOH extract (EXV) had a total phenolic content, determined by the Folin-Ciocalteu method (*6*) and expressed as apigenin equivalents, equal to 254.5 μg/mg (**Table 2**). With regard to the DPPH test (*6*), the free-radical scavenging effect elicited by this extract was concentration-dependent, so that the EC_{50} value was calculated as 103.6 μg/mL with respect to α-tocopherol (EC_{50} 10.1 μg/mL), used as positive control. EXV extract showed a strong, concentration-dependent antioxidant activity also in the LP-LUV test (IC_{50} 35.4 μg/mL of extract). The similar antioxidant activity of EXV extract observed in homogeneous solution (DPPH test) and in a membrane system (LP-LUV test) suggest that the antioxidant compounds contained in EXV extract may act as scavengers both of radicals generated in the aqueous environment and/or within lipidic membranes. EXV extract gave two main phenolic fractions, I and II, by gel filtration on a Sephadex LH-20 column. In comparison to the parent extract, fractions II were more potent in both the DPPH (EC_{50} 11.6 μg/mL) and LP-LUV (EC_{50} 27.5 μg/mL) tests and showed a higher level of total phenols (335.1 μg/mg, **Table 2**), suggesting that it contained a higher concentration of the antioxidant principles. Fraction I was less potent than the parent extract in the DPPH test and showed a minor total phenolic content.

Thus, with the aim of isolating and characterizing the constituents of *X. violaceum* responsible for the observed activity, fraction II was chromatographed by HPLC, giving, as major constituents, the new flavone *C*-glycoside **1**, as well as vitexin (**2**) (*6*, *17*), isovitexin (**3**) (*16*, *17*), isovitexin 4′-*O*-rhamnopyranoside (**4**) (*15*), apigenin 6-*C*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (isovitexin 6″-O-*β*-D-glucopyranoside) (**5**) (*12*, *13*), and apigenin 6,8-di*C*-*β*-D-glucopyranoside (**6**, vicenin-2) (*14*) (**Figure 1**). Vitexin (**2**) and isovitexin (**3**), characteristic components of EXV extract, were chosen as markers to determine the flavone content of this extract, and the isolated compounds were used as standards for quantitative analytical HPLC analysis. The results showed that the content of the total isovitexin derivatives (compounds **1** and **3**−**6**) expressed as isovitexin equivalents was 6.7%, and the concentration of vitexin (**2**) was 5.5%. The structures of the known compounds were determined by NMR and MS (*12*−*17*). The ^{13}C NMR data for isovitexin 4′-*O*-rhamnopyranoside (**4**) are

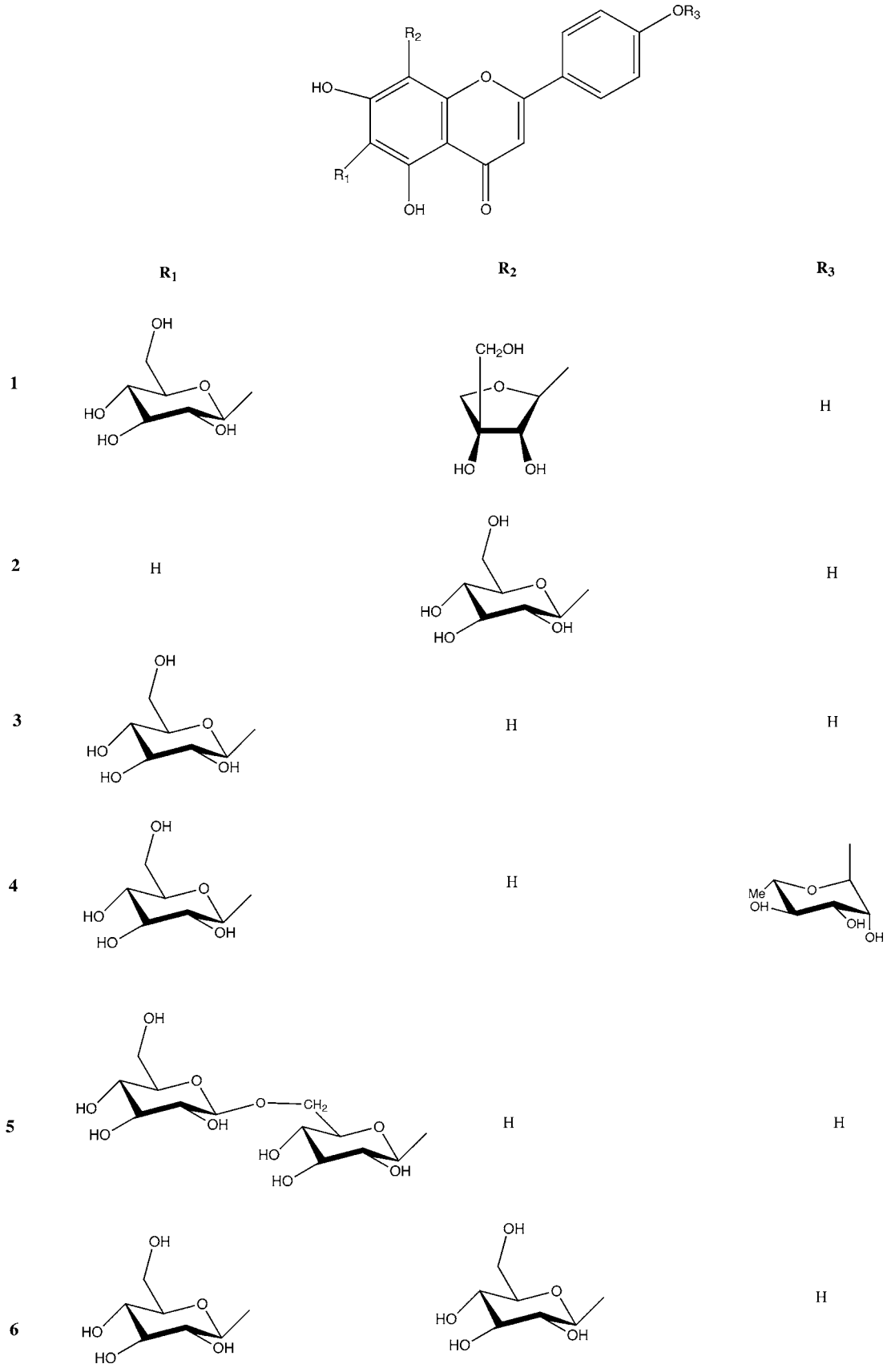

Figure 1. Compounds **1**–**6** isolated from *X. violaceum*.

reported for the first time. The structure identification of compound **1** was based on the following evidence.

The negative FABMS of compound **1** showed an $[M - H]^-$ ion at *m*/*z* 563, consistent with the molecular formula $C_{26}H_{28}O_{14}$, which was confirmed by combustion analysis and ^{13}C and DEPT NMR analysis. The ^1H and ^{13}C NMR spectra of **1** (**Table 1**) suggested that apigenin was the aglycone and β-D-glucopyranosyl (*6*) and β-D-apiofuranosyl (*6*) were the sugar residues. The C-bonds between sugars and the aglycone were revealed by the HSQC correlations between two anomeric proton and

carbon signals (δ_H 4.96, δ_C 75.6; δ_H 5.01, δ_C 77.1). A 6-C- and 8-C-substituted apigenin structure was indicated by the ^{1}H NMR spectrum (**Table 1**), showing in the aromatic region a one-proton singlet at δ 6.58 (1H, br s) typical of H-3 of a flavone, two signals at δ 6.95 and 7.98 (each 2H, d, $J = 8.5$ Hz), the multiplicity of which indicated the 4′-hydroxy-substituted ring B; the absence of any proton signal for ring A suggested that this ring was completely substituted. The ^{13}C NMR chemical shifts of the aryl carbons were superimposable on the corresponding carbons of both 6-*C*-glycosylated apigenin derivatives (isovitexin) and 8-*C*-glycosylated apigenin derivative (vitexin); particularly, the C-6 (δ_C 109.2) and C-8 (δ_C 105.1) resonances indicated a 6,8-di*C*-glycosylflavone (*14*).

The nature of a sugar unit as β-D-apiofuranosyl (*6*) was deduced from the following evidence: in the 1D TOCSY experiment, selective excitation of the anomeric proton at δ 5.01 (H-1‴, d, $J = 2.0$ Hz) led to the enhancement only of H-2‴ (δ 4.00, d, $J = 2.0$ Hz), and the multiplicity of H-2‴ may be only derived from the presence of a quaternary carbon at C-3‴, characteristic of an apiofuranosyl structure. The ^{13}C NMR spectrum gave 11 carbon signals for the sugar moiety, of which three methylenes were ascribable to C-4‴ (δ 72.3) and C-5‴ (δ 65.1) of an apiofuranosyl unit and to C-6″ (δ 62.3) of a glucopyranosyl unit, respectively. Analysis of the correlated ^{13}C NMR signals in the HSQC spectrum and of the resonances of the quaternary carbon signal (δ 80.1, C-3‴) led to the identification of β-D-apiofuranosyl. For the glucopyranose unit, the 1D TOCSY (*6*) subspectrum obtained by irradiating at the anomeric proton signal at δ 4.96 ($J_{H-1,H-2} = 7.5$ Hz, β configuration) showed a set of coupled protons at δ 4.27, 3.49, 3.56, and 3.42 (all CH) and δ 3.78 and 3.87 (CH_2O; its sequence H-1″−H_2-6″ was established by the DQF-COSY spectrum. Analysis of the correlated ^{13}C NMR signals in the HSQC spectrum led to the identification of a β-D-glucopyranosyl unit (*6*). The relative positions of glucopyranose and apiofuranose were established unambiguously by the HMBC correlations observed between the anomeric proton signal at δ_H 4.96 (H-1″) and C-6 (δ_C 109.2), C-5 (δ_C 162.1), and C-7 (δ_C 165.1) and between the anomeric proton signal at δ_H 5.01 (H-1‴) and C-8 (δ_C 105.1), C-9 (δ_C 157.8), and C-7. Therefore, the structure of **1** was determined to be apigenin 6-*C*-β-D-glucopyranosyl-8-C-β-D-apiofuranoside (**1**).

The findings obtained in this study clearly demonstrate that EXV extracts of *X. violaceum* leaves possess antioxidant/free-radical scavenging properties, which are very likely due to the presence of the high phenolic content determined by the Folin−Ciocolteu method. The extract appears to contain, as major constituents, a series of characteristic *C*-glycosyl flavones, apigenin derivatives. Recently, a series of new *C*-glycosyl flavones, acacetin (4′-methoxy apigenin) derivatives, have been isolated from *Anthurium versicolor* (*6*), a species of the same family Araceae growing in the same tropical habitat as *X. violaceum.* Several *C*-glycosyl flavones proved to be effective in different tests as free-radical scavengers showing weak to moderate activities dependent on their structural features (*20*, *21*). Those with *o*-dihydroxy substitution on ring B (luteolin derivatives) were more active than those with a *p*-hydroxy group on ring B (apigenin derivatives) in both DPPH and superoxide anion radical scavenging tests (*20, 21*) and phosphomolybdenum assays (*20*), whereas the phloroglucinol A-ring gave a small contribution to the antioxidant activity. Glycosidation has been reported to decrease the radical scavenging activity of the host molecules. Thus, the free-radical scavenging properties of EXV extract seem to be correlated with the structures of the *C*-glycosyl flavones isolated, which are apigenin derivatives with different glycosidation patterns. The presence of polyphenols which contribute to cardioprotective and anticarcinogenic action of fruits and vegetables used in the human diet could increase the nutritional interest of *X. violaceum*. It is noteworthy that a species usually considered only for the carbohydrate content of the cormels revealed the presence of antioxidant phytonutrients in the leaves as well.

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