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New *C*-Deoxyhexosyl Flavones and Antioxidant Properties of *Passiflora edulis* Leaf Extract

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The flavonoids present in passion fruit (*Passiflora edulis*) leaves were identified by a high-performance liquid chromatography–diode array detection–tandem mass spectrometry (HPLC–DAD–MS/MS) method. Sixteen apigenin or luteolin derivatives were characterized, which included four mono-*C*-glycosyl, eight *O*-glycosyl-*C*-glycosyl, and four *O*-glycosyl derivatives. With the exceptions of *C*-hexosyl luteolin and *C*-hexosyl apigenin, all the compounds exhibited a deoxyhexose moiety. Moreover, the uncommon *C*-deoxyhexosyl derivatives of luteolin and apigenin have been identified for first time in *P. edulis* by HPLC–DAD–MS/MS. The antioxidative capacity of passion fruit leaves was checked against DPPH radical and several reactive oxygen species (superoxide radical, hydroxyl radical, and hypochlorous acid), revealing it to be concentration-dependent, although a pro-oxidant effect was noticed for hydroxyl radical.

KEYWORDS: *Passiflora edulis*; *C*-deoxyhexosyl flavones; mass spectrometry; flavonoids; antioxidant

INTRODUCTION

The genus *Passiflora* comprises approximately 450 species, but only a few are commercially exploited. *Passiflora edulis* Sims, usually called passion fruit, is the best known among them. It originated in Brazil and is now being cultivated in many other countries for its edible fruits and pharmacologic properties (*1*). In fact, *P. edulis* is very popular, not only because of its pleasant fruits but also because the infusion of their leaves has been largely used in American and European countries as sedative or tranquilizer, being much appreciated due to its agreeable taste (*2*). Leaf extracts are also used in many pharmaceutical preparations and are widely employed as a flavor and as a juice by the food industries (*3*). Besides the anxiolytic effects (*4, 5*), *P. edulis* leaves are recognized for their anti-inflammatory activity (*2, 6, 7*).

A wide number of *Passiflora* species have been studied with respect to their flavonoid composition. These studies concluded that they are a rich source of *C*-glycosyl flavones. In a great number of them, vitexin and/or isovitexin and their 2″-xylosyl derivatives predominate: *Passiflora serratifolia* (*8*), *Passiflora cyanea*, *Passiflora oerstedii*, and *Passiflora menispermifolia* (*9*), *Passiflora fetida* (*10*), *Passiflora pittieri*, *Passiflora alata*, and *Passiflora ambigua* (*11*), and *Passiflora serratodigitata* (*12*). In other species, mono-*C*-glycosyl flavonoid derivatives with an *O*-rhamnosyl group at the 7, 4′, and 2″ positions have been reported: *Passiflora platiloba* (*13*), *Passiflora coactilis* (*14*), *Passiflora biflora* (*15*), and *Passiflora palmeri* (*16*). In *P. edulis*, orientin 2″-rhamnoside and luteolin 7-*O*-(2-rhamnosylglucoside) have been identified (*5*). Mareck and co-workers (*17*) also described two *C*-deoxyhexosyl derivatives, 6-*C*-chinovoside and 6-*C*-fucoside of luteolin.

Previous studies have demonstrated the utility of the high-performance liquid chromatography–diode array detection–electrospray ionization tandem mass spectrometry (HPLC–DAD–MS/MS-ESI) method in the preliminary characterization of *C*-glycosyl flavones (*18*) and *O*-glycosyl-*C*-glycosyl derivatives (*19*), without their previous isolation and cleanup. The presence of a deoxyhexose as a sugar moiety of C-glycosylation is unusual in *C*-glycosyl flavonoid derivatives (*20*). To the best of our knowledge, a study of the structures of *C*-deoxyhexosyl derivatives by HPLC–MS ion trap has not been conducted, with the exception of the description of two di-*C*-glycosyl derivatives (6-*C*-rhamnosyl-8-*C*-hexosyl luteolin and 6-*C*-hexosyl-8-*C*-rhamnosyl luteolin) in sweet pepper (*21*). On the other hand, Pereira and co-workers (*22*) used HPLC–MS (OA-TOF and CID) to differentiate pairs of orientin and isoorientin isomers and vitexin and isovitexin isomers in some *Passiflora* species, including *P. edulis*.

The search for new natural products with antioxidant properties is a very active domain. Antioxidant supplements or antioxidant-containing foods may be used to help the organism in the reduction of the amount of oxidative damage at different levels and by specific actions on physiological pathways. Among

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phytochemicals, flavonoids may contribute to the protection against various diseases in which oxidative species are involved, due to the antioxidant potential that they possess (*23*, *24*). In this respect, few data on *P. edulis* leaves are available. As far as we know, only the antioxidative capacity of the hydroethanolic extract has been estimated by the TRAP assay and for the prevention of lipid peroxidation (*25*).

In this study, we characterized *P. edulis* flavonoids by HPLC–DAD–MS and detected some *O*-glycosyl-*C*-deoxyhexosyl flavones. The antioxidant capacity of *P. edulis* leaf aqueous extract, the main form of consumption of this matrix, was also assessed against DPPH radical and reactive oxygen species (superoxide radical, hydroxyl radical, and hypochlorous acid).

MATERIALS AND METHODS

Standards and Reagents. Methanol and acetic acid were obtained from Merck (Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA). DPPH$^{\bullet}$, xanthine, xanthine oxidase (XO) grade I from buttermilk (EC 1.1.3.22), β-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitroblue tetrazolium chloride (NBT), anhydrous ferric chloride ($FeCl_3$), ethylenediaminetetraacetic acid disodium salt (EDTA), ascorbic acid, trichloroacetic acid, thiobarbituric acid, deoxyribose, a sodium hypochlorite solution with 4% available chlorine (NaOCl), and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were obtained from Sigma Chemical Co. (St. Louis, MO).

Plant Material and Sample Preparation. *P. edulis* leaves were collected in Viseu, Portugal, in March 2006 and dried in a stove, at 40 °C, for 4 days.

For the identification of the phenolic compounds in *P. edulis*, the dried powdered material (ca. 0.05 g) was thoroughly mixed with 1 mL of a methanol/water mixture (1:1), ultrasonicated (60 min), centrifuged, and filtered.

For the antioxidant activity assays, an aqueous extract was prepared by boiling ca. 2.0 g of powdered leaves with 400 mL of water, for 15 min, and then the mixture was filtered through a Büchner funnel. The resulting extract was lyophilized, and a yeld of 0.71 g was obtained. The lyophilized extract was kept in a desiccator, in the dark.

HPLC–DAD–MS/MS-ESI Analysis of Phenolic Compounds. Chromatographic separations were carried out on a 250 mm × 4 mm, 5 μm, RP-18 LiChroCART column (Merck) protected with a 4 mm × 4 mm LiChroCART guard column, with 1% acetic acid (A) and methanol (B) as solvents, starting with 20% B and using a gradient to obtain 50% B at 40 min and 80% B at 55 min. The flow rate was 1 mL/min, and the injection volumes varied between 10 and 90 μL. The HPLC system was equipped with an Agilent 1100 Series diode array and a mass detector in series (Agilent Technologies, Waldbronn, Germany). It consisted of a G1312A binary pump, a G1313A autosampler, a G1322A degasser, and a G1315B photodiode array detector, controlled by ChemStation, version 08.03 (Agilent). Spectroscopic data from all peaks were accumulated in the range of 240–400 nm, and chromatograms were recorded at 340 nm. The mass detector was a G2445A ion-trap mass spectrometer equipped with an electrospray ionization (ESI) system and controlled by LCMSD, version 4.1 (Agilent). Nitrogen was used as the nebulizing gas at a pressure of 65 psi, and the flow rate was adjusted to 11 L/min. The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. The full scan mass covered the range from *m*/*z* 200 to 1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 to 2 V. MS data were acquired in the negative ionization mode. The classical nomenclature (*26*) for glycoconjugates was adopted to designate the fragment ions. The ions $^{k,l}\mathbf{X}_j$, $\mathbf{Y}^n_j$, and $\mathbf{Z}^n_j$ represent those fragments still containing the flavonoid aglycone, where **j** is the number of the interglycosidic bonds broken, counted from the aglycone, ***n*** represents the position where the oligosaccharide is attached to the aglycone, and ***k*** and **l** denote the cleavage within the carbohydrate rings.

DPPH$^{\bullet}$ Assay. The antiradical activity of the extracts was determined spectrophotometrically in a Multiskan Ascent microplate reader (Thermo Labsystems), by monitoring the disappearance of DPPH$^{\bullet}$ at 515 nm, according to a described procedure (*27*). The reaction mixtures in the sample wells consisted of 25 μL of lyophilized extract and 200 μL of 150 μM DPPH$^{\bullet}$ dissolved in methanol. Three experiments were performed in triplicate.

Superoxide Antiradical Scavenging Assay. Antiradical activity was assessed spectrophotometrically in a Multiskan Ascent microplate reader (Thermo Labsystems), by determining the effect of the lyophilized extract on the superoxide radical-induced reduction of NBT at 562 nm.

Nonenzymatic Assay. Superoxide radicals were generated by the NADH/PMS system, according to a described procedure (*28*). All components were dissolved in phosphate buffer (19 mM, pH 7.4). Three experiments were performed in triplicate.

Enzymatic Assay. Superoxide radicals were generated by the xanthine/xanthine oxidase system, following a described procedure (*28*). Briefly, xanthine was dissolved in NaOH (1 μM) and subsequently in phosphate buffer (50 mM) with EDTA (0.1 mM, pH 7.8), xanthine oxidase in EDTA (0.1 mM), and the remaining components in phosphate buffer (50 mM) with EDTA (0.1 mM, pH 7.8). Three experiments were performed in triplicate.

Effect on Xanthine Oxidase. The effect of the lyophilized extract on xanthine oxidase activity was evaluated by measuring the extent of formation of uric acid from xanthine, in a Helios α double-beam spectrophotometer (Unicam), at room temperature. The reaction mixtures contained the same proportion of components as in the enzymatic assay for superoxide radical scavenging activity, except NBT, in a final volume of 600 μL. The absorbance was measured at 295 nm for 2 min. Three experiments were performed in triplicate.

Hydroxyl Radical Scavenging Assay. The deoxyribose method for determining the effect on hydroxyl radicals was used as previously reported (*29*), in a Helios α double-beam spectrophotometer (Unicam). Reaction mixtures contained ascorbic acid, $FeCl_3$, EDTA, H_2O_2, deoxyribose, and lyophilized extract. All components were dissolved in KH_2PO_4-KOH buffer (10 mM, pH 7.4). This assay was also performed, either without ascorbic acid or without EDTA, in an attempt to evaluate the extracts' prooxidant or metal chelation potential, respectively. In each case, three experiments were performed in triplicate.

Hypochlorous Acid Scavenging Assay. The inhibition of the HOCl-induced oxidation of 5-thio-2-nitrobenzoic acid (TNB) to DTNB was performed as described previously (*29*), in a Helios α double-beam spectrophotometer. Hypochlorous acid and TNB were prepared immediately before use. The amount of TNB unchanged after incubation was calculated and expressed as a percentage of the initial value. Three experiments were performed in triplicate.

RESULTS AND DISCUSSION

The HPLC–DAD–MS/MS screening of the aqueous methanolic extract of *P. edulis* (**Figure 1**) showed the presence of flavonoids [apigenin or luteolin (**Figure 2**)], mono-*C*-glycosides (**1**, **3**, **15**, and **16**), and *O*-glycosyl-*C*-glycosyl (**2**, **4**, **6–10**, and **13**) and *O*-glycosyl derivatives (**5**, **11**, **12**, and **14**) (**Figure 1** and **Table 1**). Compounds **1** and **3** provide the typical MS fragmentation of mono-*C*-hexosyl flavones (**Table 1**), with the characteristic aglycone ions Aglycone+41 and Aglycone+71 (*18*), which correspond to $^{0,2}X_0^-$ and $^{0,3}X_0^-$ in mono-*C*-glycosyl derivatives: *C*-hexosyl luteolin (**1**) and *C*-hexosyl apigenin (**3**), respectively. With regard to the mono-*C*-deoxyhexosyl derivatives **15** and **16**, the ions Aglycone+41 {$^{0,2}X_0^-$, [(M − H) − 104]$^-$} and Aglycone+83 {[$^{0,4}X_0$ − 18]$^-$, [(M − H) − 62]$^-$} were observed (**Table 1** and **Figure 3A**), which are consistent with *C*-deoxyhexosyl apigenin (**15**) and *C*-deoxyhexosyl apigenin isomer (**16**). To the best of our knowledge, the occurrence of [$^{0,4}X_0$ − 18]$^-$ in the MS fragmentation of mono-*C*-pentosyl and mono-*C*-hexosyl derivatives has not been observed as a characteristic. The ion Aglycone+83 is characteristic of di-*C*-glycosyl flavones (*18*), but not of mono-*C*-hexosyl/pentosyl

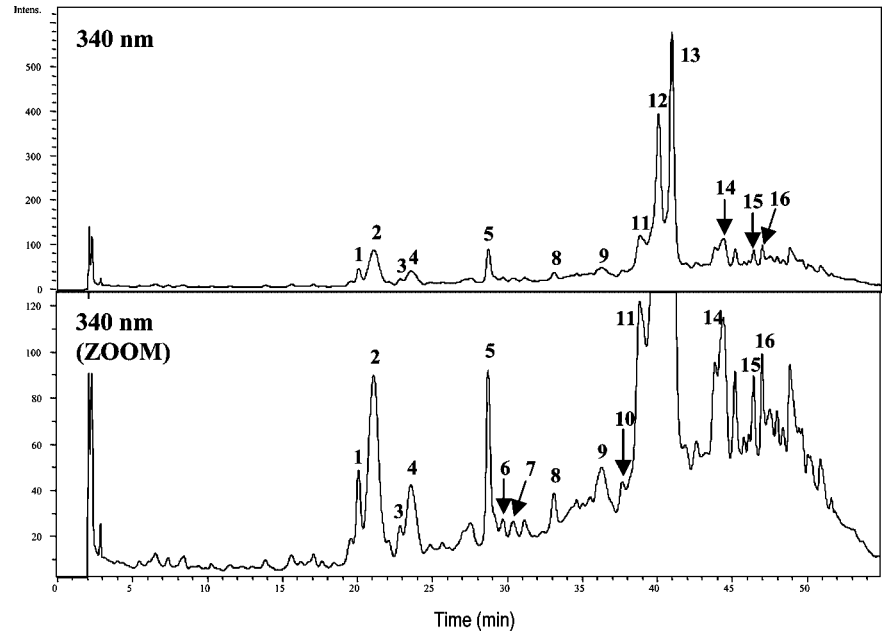

Figure 1. HPLC phenolic profile of *P. edulis* leaf extract. Detection was at 340 nm: (**1**) *C*-hexosyl luteolin, (**2**) 2″-*O*-deoxyhexosyl-*C*-hexosyl luteolin, (**3**) *C*-hexosyl apigenin, (**4**) 2″-*O*-deoxyhexosyl-*C*-hexosyl apigenin, (**5**) 7-*O*-deoxyhexosyl (1→2)hexosyl luteolin, (**6**) 7-*O*-hexosyl-*C*-deoxyhexosyl apigenin, (**7**) 2″-*O*-deoxyhexosyl-*C*-pentosyl luteolin, (**8**) 7-*O*-hexosyl-*C*-deoxyhexosyl apigenin isomer, (**9**) 2″-*O*-deoxyhexosyl-*C*-deoxyhexosyl luteolin, (**10**) 7-*O*-hexosyl-*C*-deoxyhexosyl apigenin isomer, (**11**) unidentified X-*O*-deoxyhexosyl-144 luteolin, (**12**) unidentified X-*O*-deoxyhexosyl-144 luteolin isomer, (**13**) 4′-*O*-hexosyl-*C*-deoxyhexosyl apigenin, (**14**) unidentified 7-*O*-deoxyhexosyl-144 apigenin, (**15**) *C*-deoxyhexosyl apigenin, and (**16**) *C*-deoxyhexosyl apigenin isomer.

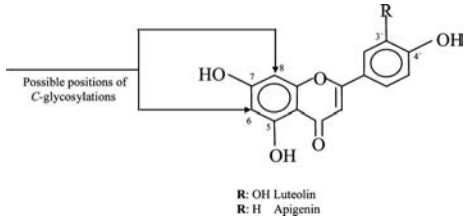

Figure 2. Structures of the aglycones apigenin and luteolin with an indication of the possible attachments of C-glycosylation.

flavones. In sweet pepper (*21*), two di-*C*-glycosyl derivatives (6-*C*-rhamnosyl-8-*C*-hexosyl luteolin and 6-*C*-hexosyl-8-*C*-rhamnosyl luteolin) have been identified. Losses of −104 and −74 amu, corresponding to MS rhamnose fragmentation ($^{0,2}X_0^-$ and $^{0,3}X_0^-$, respectively), and the ions Aglycone+83 and Aglycone+113 that characterize the aglycone in di-*C*-glycosides, were observed for the two compounds. Thus, $[^{0,4}X_0 - 18]^-$ and Aglycone+83 characterize the mono-*C*-deoxyhexosyl derivatives.

Compounds **2**, **4**, and **7** exhibited a MS fragmentation typical of 2″-*O*-glycosyl-*C*-glycosyl flavones (*19*), where the loss of the sugar of O-glycosylation provides an ion Z_1^- ($[Y_1 - 18]^-$), which characterizes a deoxyhexose $[(M - H) - 146 - 18]^-$ in these compounds (**Table 1** and **Figure 3B**). In addition, the ion $^{0,2}X_0^-$ is observed from the sugar fragmentation of C-glycosylation $[(M - H) - 120]^-$ (**2** and **4**, hexose) and $[(M - H) - 90]^-$ (**7**, pentose). Other ions characteristic of the aglycone (Aglycone+41 and Aglcyone+71) obtained as the simultaneous loss of the sugar moiety of O-glycosylation (−146, deoxyhexose) and the sugar fragments of C-glycosylation to give $^{0,2}X_0^-$ (−120 and –90, hexose and pentose, respectively) and $^{0,3}X_0^-$ (−90 and –60, hexose and pentose, respectively), respectively, were detected (**Table 1**). According to what is described above, the following 2″-*O*-deoxyhexosyl derivatives have been characterized: 2″-*O*-deoxyhexosyl-*C*-hexosyl luteolin (**2**), 2″-*O*-deoxyhexosyl-*C*-hexosyl apigenin (**4**), and 2″-*O*-deoxyhexosyl-*C*-pentosyl luteolin (**7**).

After the MS^2 experiments with compounds **6**, **8–10**, and **13**, a loss of −104 amu was observed leading to $^{0,2}X_0^-$, which indicates the presence of *C*-deoxyhexose derivatives (**Figure 3B,C**). The occurrence of an abundant ion Y_0^- $[(M - H) - 162]^-$ for compounds **6**, **8**, **10**, and **13** indicates O-glycosylation with a hexose on the phenolic hydroxyl (**Table 1** and **Figures 3C** and **4**) (*19*). The UV spectra of compound **13** (269, 310, 335sh) showed the absence of a free hydroxyl at the 4′ position (*30*). Therefore, O-glycosylation occurs on this hydroxyl, while for compounds **6**, **8**, and **10**, it occurs at the 7 position. The loss of −164 amu from the deprotonated molecular ion of compound **9** $[(M - H) - 146 - 18]^-$, giving rise to Z_1^-, indicates an O-glycosylation with deoxyhexose at the 2″ position (*19*) (**Table 1** and **Figure 3B**). On the other hand, the occurrence of an abundant ion at *m/z* 269 in the MS^2 event of compounds **8** and **10** (coincident with the ion $[Aglycone - H]^-$) may point to these compounds being *O*-glycosyl derivatives instead of *C*-glycosyl derivatives. However, the absence of the cited ion in the $-MS^3$ $[(M - H) \rightarrow (M - H - 162)]^-$ (**Table 1**) confirms the *C*-glycosidic nature of those compounds. In addition, the ions Aglycone+41 and Aglycone+83 were also detected. As described above, they are generated as a simultaneous loss of the sugar moiety of O-glycosylation (−162, hexose for compounds **6**, **8**, **10**, and **13**; −146, deoxyhexose for compound **9**) and of the −104 and −62 fragments to provide the ions $^{0,2}X_0^-$ and $[^{0,4}X - 18]^-$, respectively. For the luteolin derivative (**9**), Aglycone+71 ion was also detected. However, this ion is much less abundant or absent in the apigenin derivatives (*19*). Therefore, the following *C*-deoxyhexosyl derivatives have been characterized: 7-*O*-hexosyl-*C*-deoxyhexosyl apigenin (**6**), 7-*O*-hexosyl-*C*-deoxyhexosyl apigenin isomer (**8**), 2″-*O*-deoxyhexosyl-*C*-deoxyhexosyl luteolin (**9**), 7-*O*-hexosyl-*C*-deoxyhexosyl apigenin isomer (**10**), and 4′-*O*-hexosyl-*C*-deoxyhexosyl apigenin (**13**).

With regard to *O*-glycosyl flavonoids, 7-*O*-deoxyhexosyl(1→2)hexosyl luteolin (**5**) has been detected (**Table 1**) (*31*).

Table 1. t_R, UV, MS, and MS^2 Data of *C*-Glycosyl, *O*-Glycosyl-*C*-glycosyl, and *O*-Glycosyl Flavonoid Derivatives[a]

compound[b]		t_R (min)	UV (nm)	$[M-H]^-$ (*m*/*z*)	$-MS^2$ $[M-H]^-$ (*m*/*z*) (%)								
					−90[c]	−104[d]	−120[e]	−146[f]	−162[g]	−164[h]	AGly+83[i]	AGly+71[i]	AGly+41[i]	AGlyH[i]
							C-Glycosyl Flavonoids							
1	*C*-Hex-Lut	20.0	257, 269, 347	447								357 (39)	327 (100)	
3	*C*-Hex-Ap	22.7	269, 339	431								341 (4)	311 (100)	
15	*C*-Deoxyhex-Ap[j]	46.3		415							353 (18)		311 (100)	
16	*C*-Deoxyhex-Ap[j]	46.8		415							353 (20)		311 (100)	
							O-Glycosyl-*C*-glycosyl Flavonoids							
2	2″-*O*-Deoxyhex-*C*-hex-Lut	21.0	257, 267, 349	593			473 (65)			429 (25)		357 (29)	327 (100)	
4	2″-*O*-Deoxyhex-*C*-hex-Ap	23.5	269, 339	577			457 (9)			413 (100)			311 (14)	
6	7-*O*-Hex-*C*-deoxyhex-Ap	29.6	269, 339	577		473 (5)			415 (40)		353 (27)		311 (100)	
7	2″-*O*-Deoxyhex-*C*-pent-Lut	30.3	256, 267, 348	563	473 (100)					399 (30)		357 (38)	327 (100)	
8	7-*O*-Hex-*C*-deoxyhex-Ap[k]	33.0	267, 339	577		473 (14)			415 (70)		353 (7)		311 (49)	269 (100)
9	2″-*O*-Deoxyhex-*C*-deoxyhex-Lut	36.2	255sh, 269, 346	577		473 (45)				413 (35)	369 (7)	357 (41)	327 (100)	
10	7-*O*-Hex-*C*-deoxyhex-Ap[k]	37.5	269, 339	577		473 (6)			415 (100)		353 (5)		311 (37)	269 (63)
13	4′-*O*-Hex-*C*-deoxyhex-Ap	40.8	269, 310, 335sh	577		473 (43)			415 (94)		353 (11)		311 (100)	
							O-Glycosyl Flavonoids							
5	7-*O*-Deoxyhex(1→2)hex-Lut	28.6	256, 266, 347	593				447 (43)						285 (100)
11	X-*O*-Deoxyhex-144-Lut	38.7	257sh, 269, 349	575						411 (100)			301 (83)	285 (44)
12	X-*O*-Deoxyhex-144- Lut	39.9	255sh, 269, 347	575						411 (100)			301 (80)	285 (35)
14	7-*O*-Deoxyhex-144-Ap	44.3	269, 339	559						395 (100)				269 (18)

[a] Main observed fragments. Other ions were found, but they have not been included. [b] Hex, hexose; Deoxyhex, deoxyhexose; pent, pentose; Ap, apigenin; Lut, luteolin. [c] $^{0,2}X_0^-$ (pentose). [d] $^{0,2}X_0^-$ (deoxyhexose). [e] $^{0,2}X_0^-$ (hexose). [f] Y_1^- (deoxyhexose). [g] Y_0^- (hexose). [h] Z_1^- (deoxyhexose). [i] AGly, aglycone. [j] Compounds hidden by others. Their UV spectra have not been observed properly. [k] **8**: MS^3 $[(M-H) \rightarrow (M-H-162)]^-$ 353 (62), 311 (100). **10**: MS^3 $[(M-H) \rightarrow (M-H-162)]^-$: 353 (88), 311 (100).

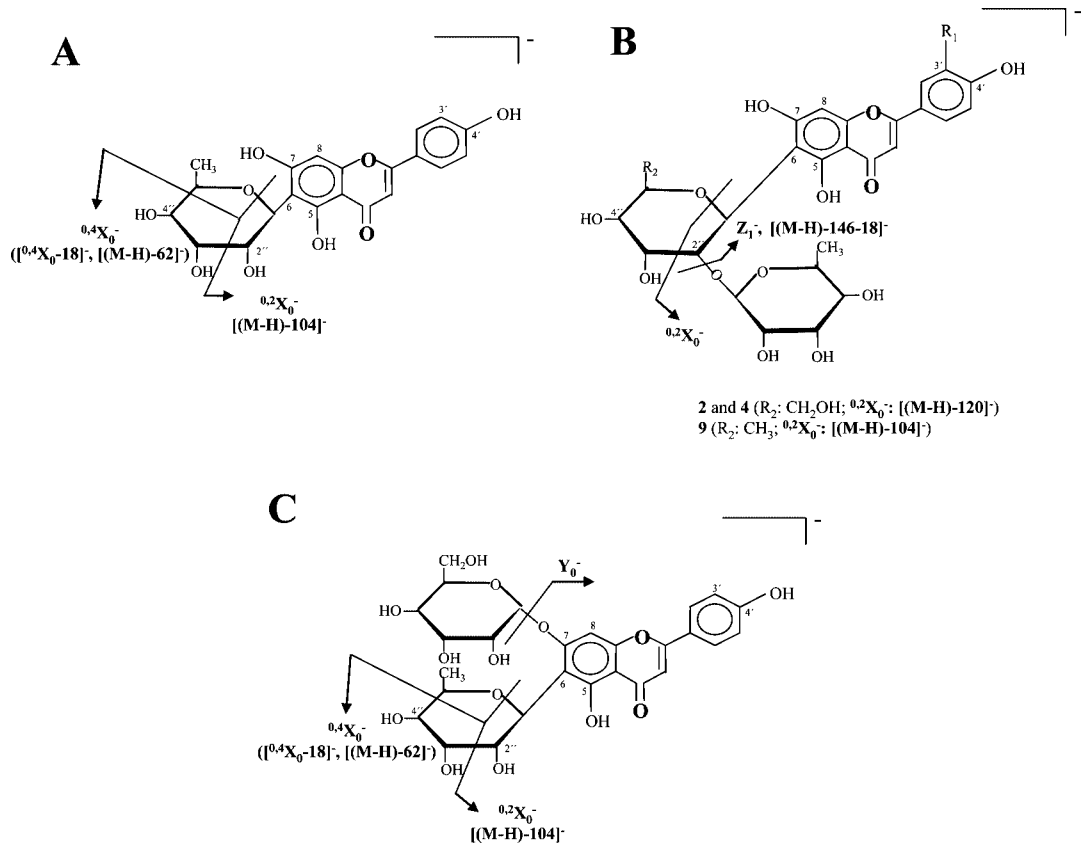

Figure 3. (**A**) General fragmentation of mono-*C*-deoxyhexosyl flavones: compounds **15** and **16**. (**B**) Fragmentation of 2″-*O*-deoxyhexosyl-*C*-glycosyl flavones: compounds **2** and **9** (R_1 = OH for luteolin) and **4** (R_1 = H for apigenin). (**C**) Fragmentation of 7-*O*-glycosyl-*C*-deoxyhexosyl flavones: compounds **6**, **8**, and **10**.

Moreover, the structural characterization of compounds **11**, **12**, and **14** was not totally accomplished and remains under study. The presence in all of them of the ion Z_1^- {[(M – H) – 146 – 18]$^-$} identified a deoxyhexose as the terminal sugar, not directly bound to the aglycone. The intermediate molecule between the possible aglycone (**11** and **12** [Aglycone – H]$^-$: 285; **9** [Aglycone – H]$^-$: 269) and the deoxyhexose shows a value 2 amu lower than that of deoxyhexose (compounds **11** and **12**, 575 = 285 + 144 + 146; compound **14**, 559 = 269 + 144 + 146). On the other hand, the UV spectra of

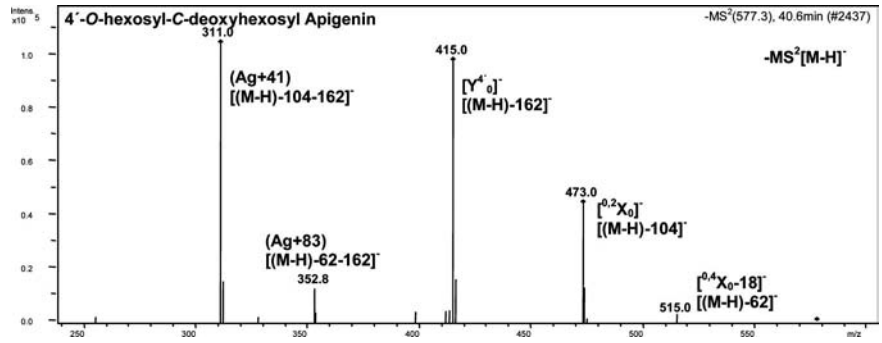

Figure 4. MS^2 analysis of compound **13** (4′-*O*-hexosyl-*C*-deoxyhexosyl apigenin).

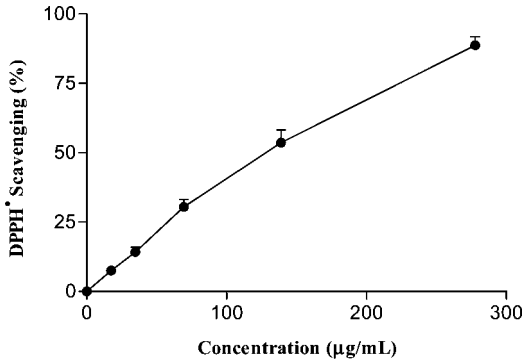

Figure 5. Effect of *P. edulis* leaf extract on DPPH• reduction. Values are means ± the standard error from three experiments performed in triplicate.

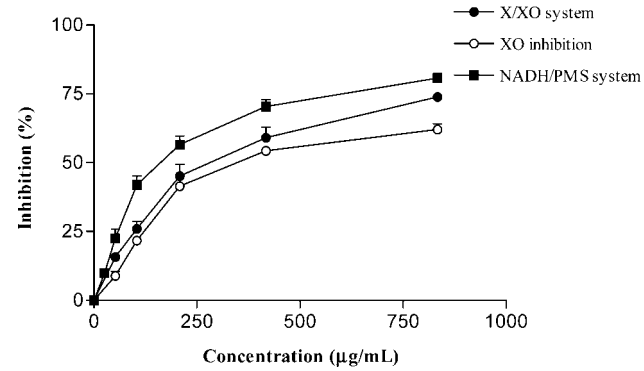

Figure 6. Effect of *P. edulis* leaf extract on superoxide radical generated in enzymatic (X/XO) and chemical (NADH/PMS) systems and on XO activity. Values are means ± the standard error from three experiments performed in triplicate.

compounds **11**, **12**, and **14** (*30*) lead us to think of a glycosylation at ring A.

As detailed above, the number of *C*-deoxyhexosyl flavonoids reported is very scarce (*20*). In *P. edulis*, the *C*-chinovoside and 6-*C*-fucoside of luteolin have been described (*17*), not taking into account the possible existence of other *C*-deoxyhexosyl flavonoids in *Passiflora*. With regard to other *Passiflora* species, rhamnose has been detected as the deoxyhexose moiety (*5*, *13–16*). To the best of our knowledge, and considering previous reports on these compounds, no information about their MS fragmentation has been reported. The occurrence of the ions $[^{0,4}X_0 - 18]^-$ and Aglycone+83 is underlined since they are not detected in mono-*C*-pentosyl and mono-*C*-hexosyl derivatives. As far as we know, this is the first time these compounds have been described in *P. edulis* leaves.

Antioxidant Activity. The DPPH• assay provides basic information about the antiradical activity of extracts. In this study, the aqueous lyophilized extract of *P. edulis* leaves exhibited a strong concentration-dependent antioxidant potential, exhibiting an IC_{50} value of 128 μg/mL (**Figure 5**).

A concentration-dependent scavenging activity was observed against superoxide radicals generated in the enzymatic system (IC_{25} at 99 μg/mL), as shown in **Figure 6**. The metabolic conversion of xanthine to uric acid was monitored to determine whether the lyophilized extract might inhibit xanthine oxidase, since an inhibitory effect on the enzyme itself would also lead to a decrease in the level of NBT reduction (*28*). The results revealed an effective xanthine oxidase inhibitory activity (**Figure 6**), which was concentration-dependent (IC_{25} at 121 μg/mL). This finding may be important for the prevention of gout, which is related to the occurrence of high levels of uric acid (*32*). As it was not possible to show a clear-cut scavenging effect, we also evaluated the scavenging activity against NADH/PMS-generated superoxide radical, which indicated an IC_{25} of 59 μg/mL (**Figure 6**). According to these results, it may be inferred that *P. edulis* leaf lyophilized extract has antioxidant activity achieved by both the scavenging of superoxide radicals and xanthine oxidase inhibition.

P. edulis lyophilized extract also appeared to be a scavenger of hydroxyl radical generated by a Fenton system (**Figure 7A**), in a concentration-dependent manner (IC_{10} at 5.8 μg/mL). As some compounds are capable of redox cycling the metal ion required for hydroxyl generation, thus increasing the level of radical production and, consequently, deoxyribose degradation (*33*), the assay was performed by omitting ascorbic acid. Indeed, we found that *P. edulis* was a very effective substitute for ascorbic acid, exhibiting a concentration-dependent pro-oxidant capacity (**Figure 7B**). It seems that, at the tested concentrations, *P. edulis* leaves have both antioxidant and pro-oxidant effects, the first being more pronounced than the latter. The assay performed in the absence of EDTA provides an indication of the potential of the extract to chelate iron ions, once Fe^{3+} chelators decrease the amount of thiobarbituric-reactive substances formed from deoxyribose as a result of removal of Fe^{3+} from the sugar (*34*). However, no chelating capacity was found for *P. edulis* lyophilized extract (**Figure 7A**).

When the protection by antioxidants against damage by hypochlorous acid was evaluated, *P. edulis* leaves exhibited an effective concentration-dependent protective activity, with an IC_{25} of 932 μg/mL (**Figure 8**). These results are interesting, considering the use of *P. edulis* leaves as an anti-inflammatory and the fact that hypochlorous acid is produced in the organism at sites of inflammation, by the oxidation of Cl^- ions, catalyzed by neutrophil-derived myeloperoxidase, in the presence of H_2O_2 (*35*).

The phenolic composition of the aqueous extract of *P. edulis* leaves used in the antioxidant activity assays revealed to be similar to that of the aqueous methanolic one (HPLC–UV). This allows us to suggest that the identified flavonoids might

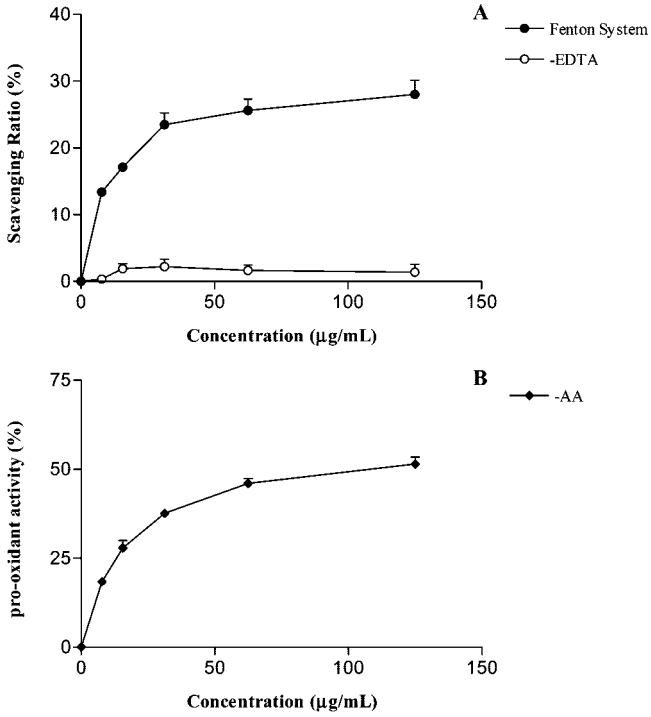

Figure 7. *P. edulis* leaf extract (**A**) nonspecific hydroxyl radical scavenging activity and specific hydroxyl radical scavenging (–EDTA) and (**B**) pro-oxidant activity (–AA). Values are means ± the standard error from three experiments performed in triplicate.

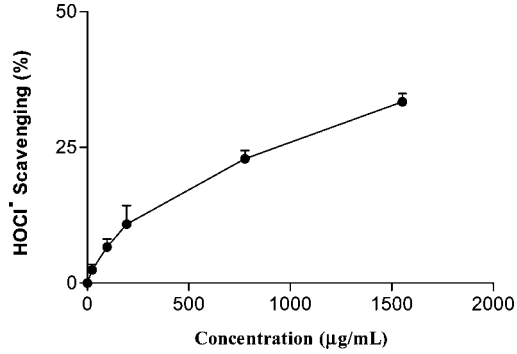

Figure 8. Effect of *P. edulis* leaf extract on the oxidation of TNB by HOCl. Values are means ± the standard error from three experiments performed in triplicate.

contribute to the observed effects. In fact, the antioxidative properties of luteolin (*36*, *37*) and apigenin (*37*) glycosides are well-known. Flavonoids can be absorbed in the gastrointestinal tube as the free aglycone and glycoside and have been detected in blood and urine in the nonesterified form (*38*, *39*) In addition, the deconjugation of luteolin monoglucuronides from free luteolin has been described at the inflammation sites by glucuronidase from neutrophils in rats (*40*). These data point to the protective role that the intake of *P. edulis* aqueous extract might exert in the organism. However, clinical trials should be carried out for the intake of a serving of *P. edulis* infusions to establish the levels of aglycone and conjugates with glucuronic acid and sulfate of these types of flavones as circulating metabolites at a systemic level (*41*). In conclusion, *P. edulis* leaves are identified as a source of *C*-deoxyhexosyl derivatives of luteolin and apigenin, which has not been described previously and is rather uncommon in nature.

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