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Rumex induratus Leaves: Interesting Dietary Source of Potential Bioactive Compounds

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The phenolic compounds of *Rumex induratus* leaves were determined by HPLC-DAD-MS/MS-ESI. The results revealed a profile composed of 19 compounds: caffeoyl-hexoside, two *p*-coumaroyl-hexoside isomers, feruloyl-hexoside, sinapoyl-hexoside, 6-*C*-hexosyl-quercetin, 8-*C*-hexosyl-luteolin, 6-*C*-hexosyl-luteolin, 6-*C*-hexosyl-apigenin, 3-*O*-hexosyl-quercetin, 3-*O*-rutinosyl-quercetin, 7-*O*-hexosyl-diosmetin, 3-*O*-rutinosyl-isorhamnetin, 7-*O*-(acetyl)-pento-hexosyl-diosmetin, 6-*C*-hexosyl-genkwanin, and four unidentified *O*-glycosyl-*C*-glycosylflavones. The quantification of the identified phenolics by HPLC-DAD showed 6-*C*-hexosyl-luteolin as the main compound. Organic acid composition was determined by HPLC-UV, revealing a high content of oxalic acid. *R. induratus* was also investigated for its capacity to act as a scavenger of DPPH and superoxide radicals. Good antioxidative results were obtained against both radicals. Unlike other species of the genus *Rumex*, *R. induratus* did not present any anthraquinone derivative.

KEYWORDS: ***Rumex induratus*; phenolic compounds; oxalic acid; antioxidant activity**

INTRODUCTION

The *Rumex* (Polygonaceae) genus comprises several species, of which leaves and roots have been used in traditional medicine for inflammation, blood purification, and constipation (*1*−*3*). Because of their high oxalic acid content, they have been implicated in oxalic intoxication, mainly in children (*2*, *3*). The growing interest in many *Rumex* species has led to the study of their biological activities, namely, the effect of *Rumex acetosa* in body weight and serum levels of amino acids and minerals (*4*), the psychopharmacological (*5*) and purgative (*6*) effects of *Rumex nepalensis*, the antioxidant and cytotoxic agents from *Rumex patientia* (*7*), the antifertility action of *Rumex steudelii* (*8*), the antimicrobial and anti-inflammatory activities of *Rumex nervosus* and *Rumex abyssinicus* (*9*), the antidiarrheal effect of *Rumex maritimus* (*10*), and the antiviral activity of *Rumex bequaertii* (*11*).

Rumex induratus Boiss and Reuter is an endemic Iberian herb, which fundamentally develops in the thermo-Mediterranean region. It grows spontaneously in northeast Portugal, where its leaves are highly consumed. This species is very appreciated in salads and, to attenuate its acidity, is dressed with olive oil and sometimes mixed with boiled potatoes. As far as we know, previous studies with *R. induratus* have only concerned its pollen allergenic action (*12*) and its mercury bioaccumulation capacity (*13*). Despite its high consumption, nothing has been reported about its nutritional value or biological potential, which could contribute for the expansion of its use in salads.

Phytochemicals, such as phenolic compounds and organic acids, are known to influence the organoleptic properties of plant foods, namely, fruits and vegetables (*14*), and have been successfully used in their quality control (*15*−*17*). It has been well-recognized that significant health risks and benefits are associated with dietary food choices. This association is often attributed to the antioxidants present in fruits and vegetables, such as phenolic compounds and organic acids, which prevent oxidative damage (*18*, *19*). Plant species are good sources of these antioxidants and may have relevance in health preservation, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation (*19*, *20*).

Several phenolic compounds, namely, phenolic acids and flavonoids (*21*−*24*), and organic acids (*1*−*3*, *24*) have been described in several *Rumex* species. In addition, *Rumex* species are known for the presence of anthraquinones, which may also act as antioxidants (*1*−*3*, *21*, *23*−*25*).

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Therefore, this work was developed to determine phenolics, by reversed-phase HPLC coupled to diode array (DAD) and mass spectrometry detectors (HPLC-DAD-MS/MS-ESI) and by HPLC-DAD analysis, organic acids (determined by HPLC-UV), and anthraquinone composition (investigated through the Bornträger reaction) of *R. induratus* leaves and to evaluate its antioxidant potential. This was accessed by DPPH and superoxide radical scavenging assays. *R. induratus* was also investigated for its effect on xanthine oxidase activity.

MATERIALS AND METHODS

Standards and Reagents. Caffeic, *p*-coumaric, ferulic, sinapic, oxalic, citric, malic, shikimic, and fumaric acids were from Sigma (St. Louis, MO), and 7-*O*-glucosyl-luteolin, 7-*O*-glucosyl-apigenin, 3-*O*-rutinosyl-quercetin, 3-*O*-rutinosyl-isorhamnetin, 3-*O*-galactosyl-quercetin, and 7-*O*-rutinosyl-diosmetin were from Extrasynthése (Genay, France). Methanol, acetic acid, hydrochloric acid, diethyl ether, chloroform, ferric chloride, and ammonia were obtained from Merck (Darmstadt, Germany), and sulfuric acid was from Pronalab (Lisbon, Portugal). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA). DPPH, xanthine, xanthine oxidase (XO) grade I from buttermilk (EC 1.1.3.22), β-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), and nitroblue tetrazolium chloride (NBT) were obtained from Sigma Chemical Co. (St. Louis, MO).

Plant Material. After harvesting in the Bragança region (Trás-os-Montes, Portugal), in May 2005, *R. induratus* aerial parts were immediately transferred to the laboratory and kept in a freezer at −20 °C, prior to their lyophilization in a Labconco 4.5 Freezone apparatus (Kansas City, MO). Then, the leaves from the dried material were separated and powdered.

Sample Preparation. For the identification of the phenolic compounds in *R. induratus* leaves, the lyophilized material (ca. 0.05 g) was thoroughly mixed with 1 mL of methanol/water (1:1), ultrasonicated, centrifuged, and filtered.

For the antioxidant capacity assays, an aqueous extract was used to avoid interference with the systems used. The extract was prepared by putting ca. 3.0 g of dried leaves in 500 mL of boiling water. The mixture was boiled for 30 min and then filtered over a Büchner funnel. The resulting aqueous extract was frozen at −20 °C and then lyophilized (ca. 1 g yield). The lyophilized extract was kept in an exsicator, in the dark. For phenolic compounds and organic acids analysis, the lyophilized extract was redissolved in water and in 0.01 N sulfuric acid, respectively.

HPLC-UV Analysis of Organic Acids. A total of 20 μL of the redissolved extract was analyzed as previously reported (*26*), in a system consisting of an analytical HPLC unit (Gilson) in conjunction with a column heating device set at 30 °C, with an ion exclusion column, Nucleogel Ion 300 OA (300 mm × 7.7 mm). Briefly, elution was carried out isocratically at a solvent flow rate of 0.2 mL min^{-1}, with 0.01 N sulfuric acid. The detection was performed with an UV detector, at 214 nm. Organic acid quantification was achieved by the absorbance recorded in the chromatograms relative to external standards, and the peaks in the chromatograms were integrated using a default baseline construction technique. The average regression equation for oxalic acid was $y = 1.79 \times 10^6x$. The detection limit was calculated as the concentration corresponding to three times the standard deviation of the background noise and corresponded to 17.7 μg mL^{-1}. The recovery value was 98%.

HPLC-DAD-MS/MS-ESI Qualitative Analysis of Phenolic Compounds. Chromatographic separations were performed with a 250 mm × 4 mm, 5 μm particle size, RP-18 LiChroCART (Merck, Darmstadt, Germany) column protected with a 4 mm × 4 mm LiChroCART guard column. Elution was developed using acetic acid 1% (A) and methanol (B) as solvents, starting with 15% B and using a gradient to obtain 30% B at 20 min and 55% B at 40 min. The flow rate was 1 mL min^{-1}, and the injection volumes varied between 10 and 50 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 software (Agilent, v. 08.03). Spectroscopic data from all peaks were accumulated in the range of 240−400 nm, and chromatograms were recorded at 330 nm. The mass detector was a G2445A Ion-Trap Mass Spectrometer equipped with an electrospray ionization (ESI) system and controlled by LCMSD software (Agilent, v. 4.1.). Nitrogen was used as a nebulizing gas at a pressure of 65 psi, and the flow was adjusted to 11 L min^{-1}. The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. The full scan mass covered the range from *m*/*z* 90 up to *m*/*z* 2000. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage ramping cycles from 0.3 up to 2 V. MS data were acquired in the negative ionization mode. MS^n data were achieved in the automatic mode on the more abundant fragment ion in MS^{n-1}. **Tables 1**−**3** show the R_t, UV data, and most frequent ions that characterize the fragmentation of the compounds. Other ions were found, but they have not been included due to their low significance on the MS behavior ions.

HPLC-DAD Quantitative Analysis of Phenolic Compounds. The aqueous lyophilized extract (20 μL) was analyzed using an analytical HPLC unit (Gilson), with a Spherisorb ODS2 (25.0 cm × 0.46 cm; 5 μm particle size) column and a solvent mixture of water−formic acid (19:1) (A) and methanol (B) (*16*). Elution was carried out at 0.9 mL min^{-1}, and the gradient was as follows: 5% B at 0 min, 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min, 55% B at 47 min, 70%

Table 1. R_t, UV, and MS^n Data of Cinnamoyl-Glycoside Derivatives

			MS^n, *m*/*z* (%)		
				MS^2[M − H]$^-$	
compounds[a]	R_t (min)	UV (nm)	[M − H]$^-$	[M − H − 162]$^-$	[M − H − 180]$^-$
1 Caff-Hex	7.1	300sh, 331	341	179 (100)	161 (42)
2 *p*.Coum-Hex	10.4	315	325	163 (80)	145 (100)
3 *p*.Coum-Hex isom	11.0	315	325	163 (100)	145 (80)
4 Fer-Hex	12.3	301sh, 329	355	193 (100)	175 (30)
5 Sinp-Hex	13.3	299sh, 325	385	223 (100)	205 (50)

[a] Caff-Hex: caffeoyl-hexoside, *p*.Coum-Hex: *p*-coumaroyl-hexoside, *p*.Coum-Hex isom: *p*-coumaroyl-hexoside isomer, Fer-Hex: feruloyl-hexoside; and Sinp-Hex: sinapoyl-hexoside.

Table 2. R_t, UV, and MS^n Data of *C*-Glycosylflavone Derivatives

			MS^n, *m*/*z* (%)			
				MS^2[M − H]$^-$		
compounds[a]	R_t (min)	UV (nm)	[M − H]$^-$	[M − H − 18]$^-$	[M − H − 90]$^-$	[M − H − 120]$^-$
7 6-*C*-Hex-Querc	21.7	255, 265sh, 300sh, 367	463	445 (19)	373 (65)	343 (100)
9 8-*C*-Hex-Lut	24.3	256, 267, 293sh, 349	447		357 (44)	327 (100)
11 6-*C*-Hex-Lut	26.2	255, 266, 292sh, 349	447	429 (5)	357 (75)	327 (100)
13 6-*C*-Hex-Apig	29.9	269, 337	431	413 (5)	341 (30)	311 (100)
19 6-*C*-Hex-Genk	38.9	272, 333	355	427 (1)	355 (25)	325 (100)

[a] 6-C–Hex-Querc: 6-*C*-hexosyl-quercetin; 8-*C*-Hex-Lut: 8-*C*-hexosyl-luteolin; 6-*C*-Hex-Lut: 6-*C*-hexosyl-luteolin; 6-*C*-Hex-Apig: 6-*C*-hexosyl-apigenin; and 6-*C*-Hex-Genk: 6-*C*-hexosyl-genkwanin.

Table 3. R_t, UV, and MS^n Data of *O*-Glycosylflavone Derivatives

			MS^n, *m*/*z* (%)	
				MS^2[M − H]$^-$
compounds[a]	R_t (min)	UV (nm)	[M − H]$^-$	[A − H]$^-$
14 3-*O*-Hex-Querc	31.3	255, 267sh, 300sh, 350	463	301 (100)
15 3-*O*-Rut-Querc	31.5	coelution **14** + **15**	609	301 (100)
16 7-*O*-Hex-Diosm	34.5	255, 267, 349	461	299 (100)
17 3-*O*-Rut-Isorhamn	36.1	255, 267sh, 295sh, 355	623	315 (100)

[a] 3-*O*-Hex-Querc: 3-*O*-hexosyl-quercetin; 3-*O*-Rut-Querc: 3-*O*-rutinosyl-quercetin; 7-*O*-Hex-Diosm: 7-*O*-hexosyl-diosmetin; and 3-*O*-Rut-Isorhamn: 3-*O*-rutinosyl-isorhamnetin.

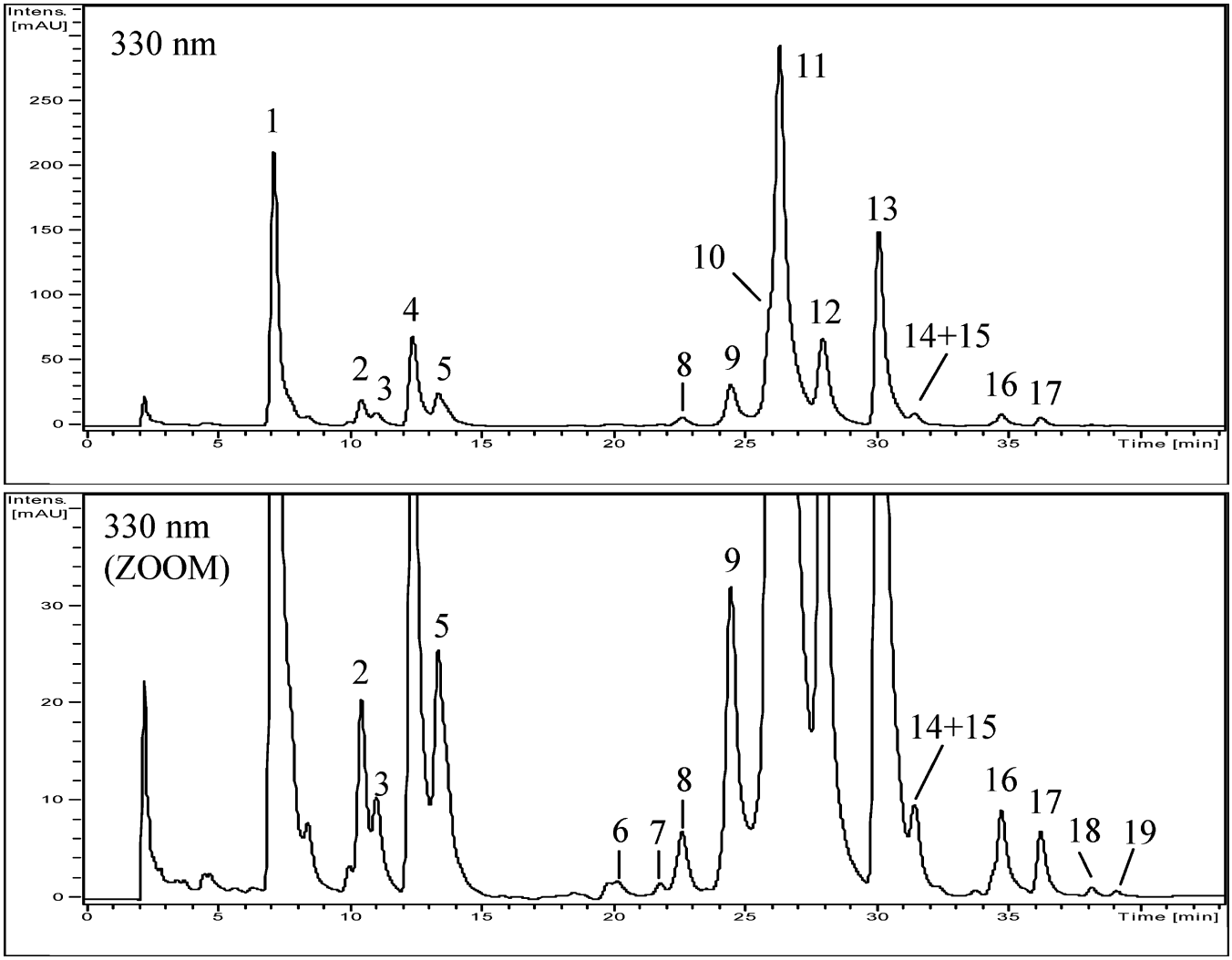

Figure 1. HPLC-DAD phenolic profile of *R. induratus* leaves. Detection at 330 nm. Peaks: (1) caffeoyl-hexoside; (2) *p*-coumaroyl-hexoside isomer; (3) *p*-coumaroyl-hexoside isomer; (4) feruloyl-hexoside; (5) sinapoyl-hexoside; (6) unidentified *O*-glycosyl-*C*-glycosylflavone; (7) 6-*C*-hexosyl-quercetin; (8) unidentified *O*-glycosyl-*C*-glycosylflavone; (9) 8-*C*-hexosyl-luteolin; (10) unidentified *O*-glycosyl-*C*-glycosylflavone; (11) 6-*C*-hexosyl-luteolin; (12) unidentified *O*-glycosyl-*C*-glycosylflavone; (13) 6-*C*-hexosyl-apigenin; (14) 3-*O*-hexosyl-quercetin; (15) 3-*O*-rutinosyl-quercetin; (16) 7-*O*-hexosyl-diosmetin; (17) 3-*O*-rutinosyl-isorhamnetin; (18) 7-*O*-(acetyl)-pento-hexosyl-diosmetin; (19) 6-*C*-hexosyl-genkwanin.

B at 50 min, 75% B at 56 min, and 80% B at 60 min. Detection was achieved with a Gilson diode array detector. Spectral data from all peaks were accumulated in the 200−400 nm range, and chromatograms were recorded at the wavelength corresponding to the higher absorption of each compound: 320 nm for phenolic acid derivatives and 350 nm for flavonoidic compounds. The data were processed on Unipoint system software (Gilson Medical Electronics, Villiers le Bel, France), and peak purity was checked by means of the software contrast facilities.

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. Since standards of the compounds identified in the *R. induratus* leaves were not commercially available, caffeic, *p*-coumaric, ferulic, and sinapic acid derivatives were quantified as caffeic, *p*-coumaric, ferulic, and sinapic acids, respectively, luteolin derivatives as 7-*O*-glucosyl-luteolin, the apigenin derivative as 7-*O*-glucosyl-apigenin, 6-*C*-hexosyl-quercetin as 3-*O*-galactosyl-quercetin, and diosmetin derivatives as 7-*O*-rutinosyl-diosmetin. The 3-*O*-hexosyl-quercetin and 3-*O*-rutinosyl-quercetin were quantified together as 3-*O*-rutinosyl-quercetin. The average regression equations for caffeic, *p*-coumaric, ferulic, and sinapic acids, 3-*O*-galactosyl-quercetin, 7-*O*-glucosyl-luteolin, 7-*O*-glucosyl-apigenin, 3-*O*-rutinosyl-quercetin, 7-*O*-rutinosyl-diosmetin, and 3-*O*-rutinosyl-isorhamnetin were $y = 1.94 \times 10^9x$, $y = 2.46 \times 10^9x$, $y = 2.11 \times 10^9x$, $y = 1.78 \times 10^9x$, $y = 7.96 \times 10^8x$, $y = 1.14 \times 10^9x$, $y = 1.10 \times 10^9x$, $y = 6.68 \times 10^8x$, $y = 1.34 \times 10^8x$, and $y = 8.42 \times 10^8x$, respectively. The detection limit values were calculated as the concentration corresponding to three times the standard deviation of the background noise and ranged from 20.5 to 87.4 μg mL^{-1}. The recovery values varied between 95 and 99%.

Anthraquinone Compounds. For the extraction of aglycone compounds, ca. 0.1 g of *R. induratus* leaves was thoroughly mixed with 2.5 mL of diethyl ether. The extract was filtered, 0.5 mL of ammonia was added, and the coloration of the ammonia phase was registered.

Glycosidic derivatives in *R. induratus* leaves (ca. 0.1 g) were extracted with water (25 mL). The 2.5 mL of hydrochloric acid was added, and the mixture was boiled for 15 min. After cooling, the obtained solution was shaken twice with 20 mL of diethyl ether. The ether fractions were gathered and shaken with 5 mL of ammonia, and the coloration of the ammonia phase was registered.

To the remaining acidic aqueous solution, ferric chloride (10 mL) was added, and the resultant mixture was boiled for 30 min. This solution, after cooling, was then shaken with 10 mL of chloroform; the chloroformic fraction was separated and agitated with ammonia (5 mL), and the coloration of the ammonia phase was registered (adapted from ref *32*).

DPPH Radical Scavenging Activity. The antiradical activity was determined spectrophotometrically in an ELX808 IU Ultra Microplate Reader (Bio-Tek Instruments, Inc.), by monitoring the disappearance of DPPH at 515 nm, according to a described procedure (*26*). The reaction mixtures in the sample wells consisted of 25 μL of aqueous

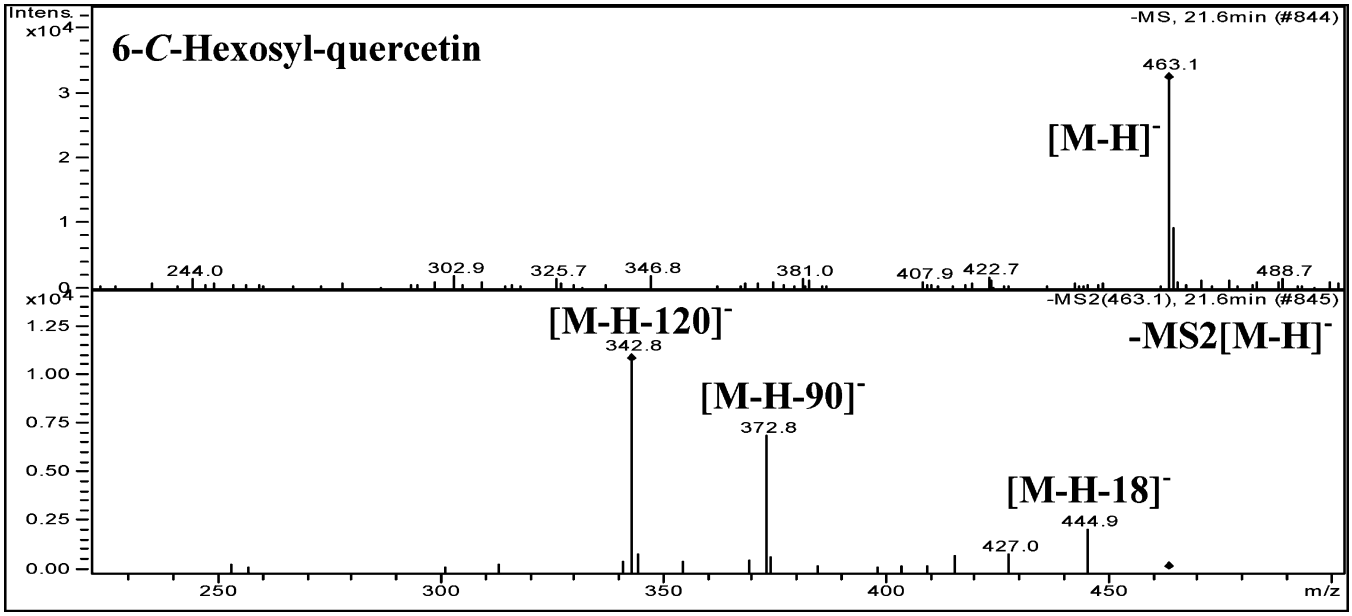

Figure 2. MS^n analysis of 6-*C*-hexosyl-quercetin (peak 7).

extract (five different concentrations) and 200 μL of DPPH• dissolved in methanol. The reaction was conducted at room temperature for 30 min. Three experiments were performed in triplicate.

Superoxide Radical Scavenging Activity. Antiradical activity was determined spectrophotometrically in a 96-well plate reader (ELX808 IU Ultra Microplate Reader, Bio-Tek Instruments, Inc.) by monitoring the effect of the lyophilized extract on the reduction of NBT by the superoxide radical, at 562 nm.

Nonenzymatic Assay. Superoxide radicals were generated by the NADH/PMS system according to a described procedure (*27*). All components were dissolved in phosphate 19 mM buffer, pH 7.4. The reaction was conducted at room temperature for 2 min and initiated by the addition of PMS. Three experiments were performed in triplicate.

Enzymatic Assay. Superoxide radicals were generated by the xanthine/xanthine oxidase (X/XO) system following a described procedure (*27*). Xanthine was dissolved in 1 μM NaOH and subsequently in a phosphate 50 mM buffer with 0.1 mM EDTA, pH 7.8, xanthine oxidase in 0.1 mM EDTA, and the other components in phosphate 50 mM buffer with 0.1 mM EDTA, pH 7.8. The reaction was conducted at room temperature for 2 min and initiated by the addition of XO. Three experiments were performed in triplicate.

Effect on Xanthine Oxidase Activity. The effect of the lyophilized extracts on XO activity was evaluated by measuring uric acid formation from xanthine in a double beam spectrophotometer (Heλios α, Unicam) at room temperature (*27*). The reaction mixtures contained the same proportion of components as in the enzymatic assay for superoxide radical scavenging activity, except NBT. The absorbance at 295 nm was measured for 2 min. Three experiments were performed in triplicate.

RESULTS AND DISCUSSION

Identification and Quantification of *R. induratus* Phenolic Compounds. The screening of the hydromethanolic extract of *R. induratus* leaves by HPLC coupled to DAD and MS^n ion-trap electrospray ionization detection showed, in the first part of the chromatogram (**Figure 1**), several compounds with UV spectra characteristic of hydroxycinnamic acid derivatives (compounds **1**−**5**) (**Table 1**). The remaining compounds (compounds **6**−**19**) exhibited UV spectra of polyhydroxyflavones (**Tables 2** and **3**). In the MS study of the first group of compounds (**Table 1**), it was possible to observe the fragmentation of the deprotonated molecular ion (MS^2 [M − H]$^-$) with losses of 162 u (hexosyl radical) and 180 u (hexosyl radical plus water), which characterizes this type of compound as cinnamoyl-hexoside derivatives: caffeoyl-hexoside (**1**), *p*-coumaroyl-hexoside isomers (**2** and **3**), feruloyl-hexoside (**4**), and sinapoyl-hexoside (**5**).

Among the compounds with flavone-like UV spectra, two groups of compounds can be noticed on the basis of their MS^n fragmentation: *C*-glycosylflavones (compounds **7**, **9**, **11**, **13**, and **19**) and *O*-glycosylflavones (compounds **14**−**17**).

In the MS^2 of the studied *C*-glycosylflavones (**Table 2**), losses of 90 and 120 u from the [M − H]$^-$ ion could be observed, which characterize a C-glycosylation with a hexose linked to the flavone nucleus. The presence of the ion [M − H − 18]$^-$ in compounds **7**, **11**, **13**, and **19** indicates the position of the C-glycosylation over carbon 6 (**Figure 2**). The absence of this latter ion in compound **9**, as well as the lower abundance of the [M − H − 90]$^-$ ion relative to that noticed for compound **11**, points to the C-glycosylation of carbon 8 in compound **9** (*28*). On the other hand, the aglycone of compound **19** corresponds to that of a dihydroxy-methoxyflavone (genkwanin/acacetin), but its UV spectrum (*29*) agrees better with the substitution of genkwanin (5,4′-dihydroxy-7-methoxyflavone). For the previous reasons, we tentatively consider that the detected *C*-glycosylflavones are 6-*C*-hexosyl-quercetin (**7**), 8-*C*-hexosyl-luteolin (**9**), 6-*C*-hexosyl-luteolin (**11**), 6-*C*-hexosyl-apigenin (**13**), and 6-*C*-hexosyl-genkwanin (**19**). It must be noticed that the occurrence of *C*-glycosylflavones presenting a hydroxyl in the 3 position (flavonols) is not common in nature.

With respect to the *O*-glycosylflavones, and according to their UV and MS spectra (**Table 3**), the compounds detected are hexosides or rutinosides of quercetin, isorhamnetin, and diosmetin: 3-*O*-hexosyl-quercetin (**14**), 3-*O*-rutinosyl-quercetin (**15**), 7-*O*-hexosyl-diosmetin (**16**), and 3-*O*-rutinosyl-isorhamnetin (**17**). Another detected *O*-glycosylflavone, compound **18** (UV: 253, 267, 350 nm, MS: 635 ([M − H]$^-$); MS^2[M − H]$^-$: 593 (50%, [M − H − 42]$^-$), 575 (100%, [M − H − 42 − 18]$^-$), 299 (10%, [A − H]$^-$); MS^3[(M − H) → 575]$^-$: 299 (100%, [A − H]$^-$), 284 (45%, [A − H − 15]$^-$)) shows, in its MS^2 fragmentation, a loss of 42 u, pointing to the presence of acetylation (**Figure 3**). In MS^3, practically only one ion at *m*/*z* 299 ([A − H]$^-$) is observed, which indicates the nature of its aglycone (trihydroxy-methoxyflavone) and that the two sugars (hexose and pentose) are linked to only one phenolic hydroxyl

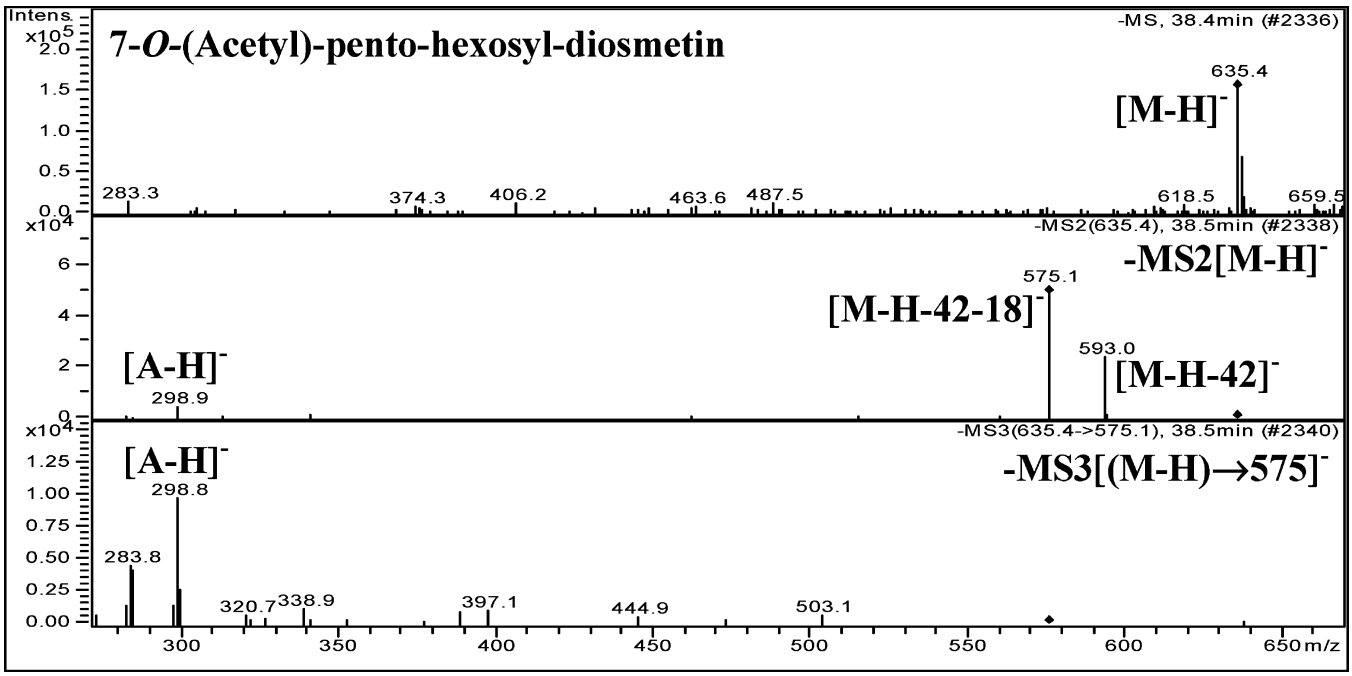

Figure 3. MS^n analysis of 7-*O*-(acetyl)-pento-hexosyl-diosmetin (peak 18).

(*30*). Its UV spectrum is more similar to that of a diosmetin derivative than of a chysoeryol one (*29*, *31*). So, we tentatively propose the structure 7-*O*-(acetyl)-pento-hexosyl-diosmetin for compound **18**.

Besides these compounds, another four were also detected (compounds **6**, **8**, **10**, and **12**), presenting UV spectra of flavones, in which MS do not correspond to *O*-glycoside derivatives and are not the typical ones described for *C*-glycosides (*28*). For these reasons, we consider them to be *O*-glycosyl-*C*-glycosylflavones, and their structures remain under study.

As far as we know, all these compounds are reported for the first time in *R. induratus*. Among the identified phenolics, only 3-*O*-rutinosyl-quercetin (**15**) has been previously described to occur in other *Rumex* species, namely, in *R. acetosa* (*24*) and in *R. chalepensis* (*21*).

Besides hydromethanolic extract, also methanolic and aqueous compounds were prepared. All of them presented the same qualitative composition, and the quantitative analysis showed similarities. However, as the assays for superoxide radical scavenging activity involved aqueous systems, we decided to use an aqueous extract to avoid interferences. To obtain a better characterization of the aqueous lyophilized extract of *R. induratus* leaves used in the antioxidant assays, its phenolic compounds were identified and quantified by HPLC-DAD (**Table 4**). This extract presented the same composition of the hydromethanolic one, exhibiting a high content of phenolic compounds (ca. 29.8 g/kg, dry basis). 6-*C*-Hexosyl-luteolin (**11**) was the compound present in highest amounts, representing ca. 40.8% of total phenolics. 7-*O*-(Acetyl)-pento-hexosyl-diosmetin (**18**) and 6-*C*-hexosyl-genkwanin (**19**) were the minor compounds.

Organic Acids in *R. induratus* Leaves. The HPLC-UV analysis of the aqueous lyophilized extract revealed only the existence of oxalic acid (**Figure 4**). The existence of other organic acids, namely, citric, malic, shikimic, and fumaric, was checked, but they were not found in the sample. Oxalic acid is reported for the first time in *R. induratus*. This compound was present in very high amounts in the lyophilized extract (51.7 ± 3.7 g/kg, dry basis), which is in accordance with the literature

Table 4. Phenolic Compounds in *R. induratus* Lyophilized Extract (mg/kg)[a]

compound	mean	SD
1 caffeoyl-hexoside	3105.4	1.4
2 + 3 *p*-coumaroyl-hexoside isomers	489.4	1.7
4 feruloyl-hexoside	1395.4	2.4
5 sinapoyl-hexoside	1451.3	82.5
7 6-*C*-hexosyl-quercetin	102.1	5.0
9 8-*C*-hexosyl-luteolin	1865.1	44.0
11 6-*C*-hexosyl-luteolin	12147.6	6.6
13 6-*C*-hexosyl-apigenin	5694.8	54.2
14 + 15 3-*O*-hexosyl-quercetin + 3-*O*-rutinosyl-quercetin	781.4	17.6
16 7-*O*-hexosyl-diosmetin	2368.2	19.3
17 3-*O*-rutinosyl-isorhamnetin	397.5	6.1
18 7-*O*-(acetyl)-pento-hexosyl-diosmetin	nq	nq
19 6-*C*-hexosyl-genkwanin	nq	nq
Σ	29798. 3	

[a] Results are expressed as the mean of three determinations. SD: standard deviation; nq: not quantified; and Σ: sum of the determined phenolic compounds.

that characterizes several *Rumex* species as having high contents of oxalic acid (*1*−*3*, *32*). The high oxalic acid level may be responsible for the acidity of the plant and must be taken into account since this acid is known to form insoluble calcium salts, which cause a disturbance in the calcium concentrations and affect the blood coagulation mechanism (*3*). In addition, the ingestion of large amounts of oxalates may result in gastrointestinal symptoms, and the systemic absorption may lead to kidney damage (*2*). On the other hand, the high oxalic acid content may be important for the protection of the plant against foraging animals, insects, and microbial pathogens, by affecting taste, texture, and calcium availability. Furthermore, this compound may act as a pH regulator and osmoregulator in the plant (*33*).

Anthraquinone Compounds in *R. induratus* Leaves. The existence of hydroxyanthraquinone derivatives in *R. induratus* leaves was checked by the Bornträger coloring reaction (*34*): quinones are dissolved in alkaline aqueous medium (ammonia), turning this solution red. This reaction is only positive for free anthraquinones. To verify the presence of heterosidic forms, the previous hydrolysis of bound compounds is required. In the present work, neither aglycones nor *O*- or *C*-glycosidic an-

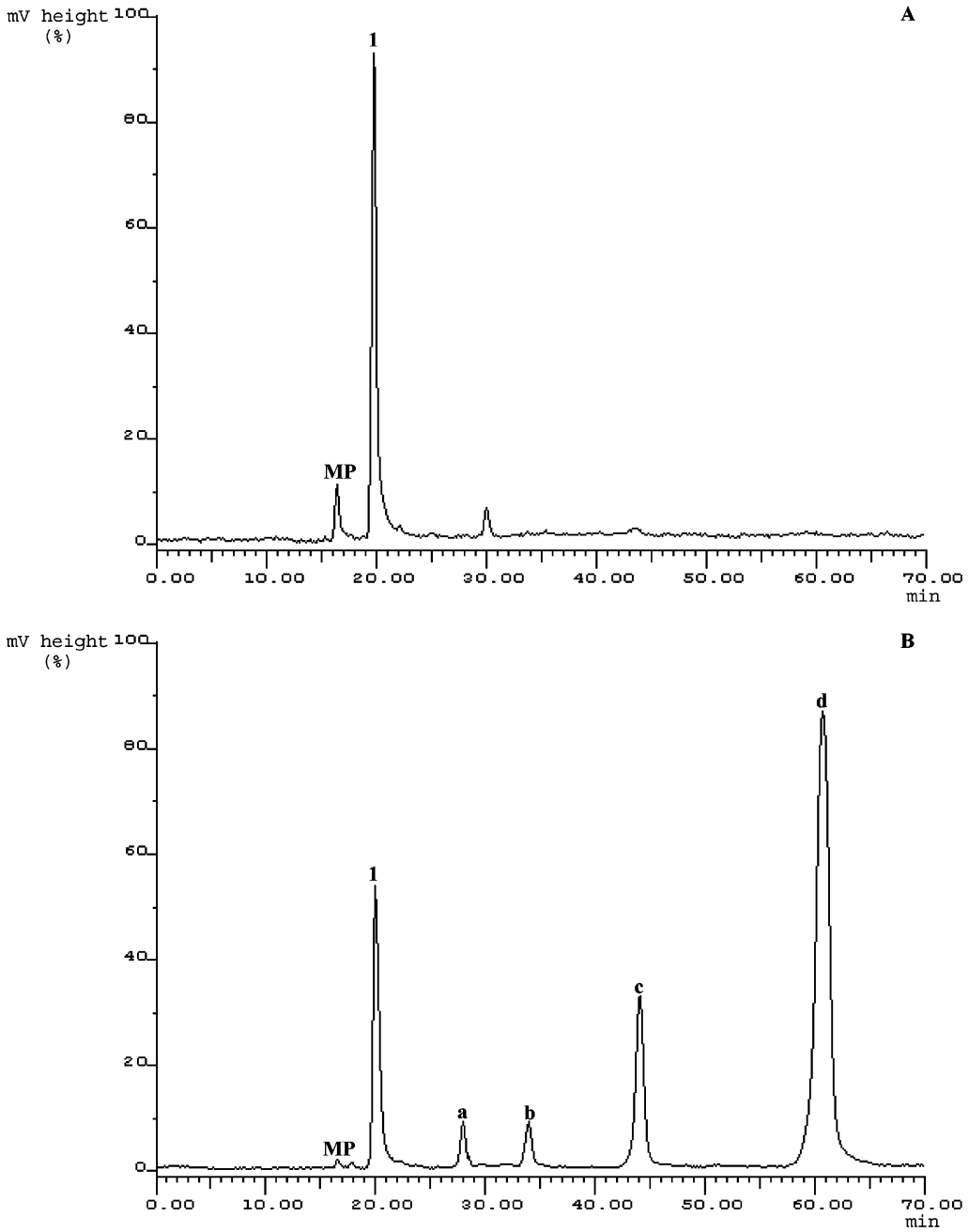

Figure 4. HPLC–UV chromatogram of organic acids in (**A**) *R. induratus* leaves and (**B**) standard mixture. Detection at 214 nm. Peaks: (MP) mobile phase; (1) oxalic acid; (a) citric acid; (b) malic acid; (c) shikimic acid; and (d) fumaric acid.

thraquinones were detected in *R. induratus* leaves, which turns this species different from other *Rumex* ones found to have anthraquinonic compounds (*1*−*3*, *21*, *23*−*25*).

Antioxidant Activity. The DPPH assay provides basic information on the antiradical activity of extracts: the decrease in absorbance at 515 nm occurs due to the reduction of DPPH by the antioxidant (*35*). The lyophilized extract of *R. induratus* leaves exhibited a potent concentration-dependent antioxidant potential (IC_{50} = 149.9 μg mL^{-1}) (**Figure 5**).

Reactive oxygen species produced in vivo include a superoxide radical and hydrogen peroxide, which can interact in the presence of certain transition metal ions to yield a highly reactive oxidizing species, the hydroxyl radical (*36*). In the present work, *R. induratus* leaf extract strongly scavenged the superoxide radical generated by the enzymatic system in a concentration-dependent manner (IC_{50} = 67.5 μg mL^{-1}), as shown in **Figure 6A**. Attending to the fact that an inhibitory effect on xanthine

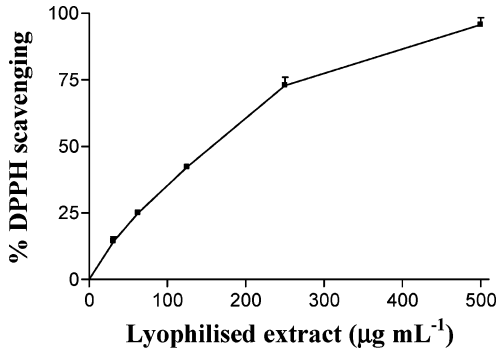

Figure 5. Effect of *R. induratus* on DPPH reduction. Values show mean ± SE from three experiments performed in triplicate.

oxidase would also lead to a decrease in NBT reduction (*27*), the effect of the extract on the enzyme activity was checked by

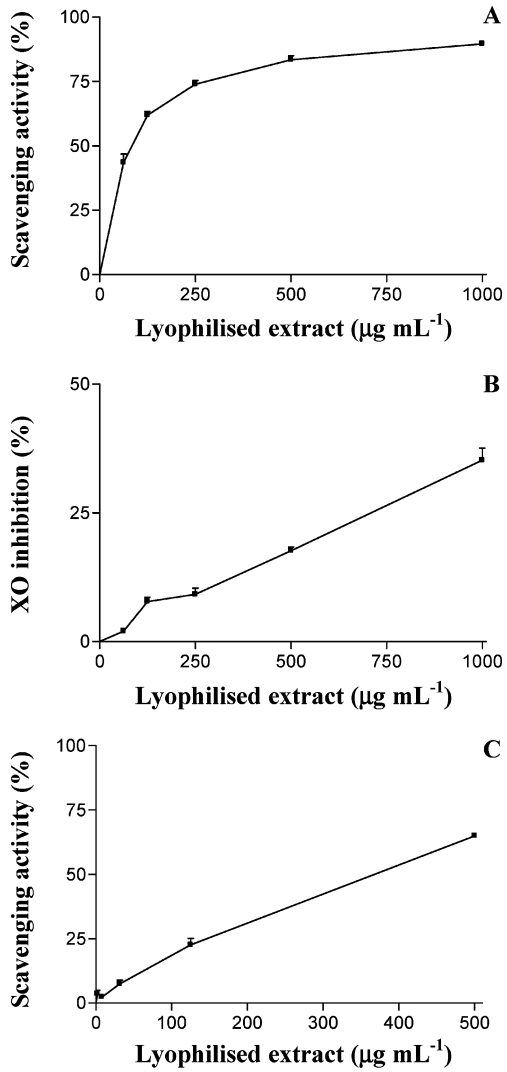

Figure 6. Effect of *R. induratus* on (**A**) NBT reduction induced by a superoxide radical generated in an X/XO system, (**B**) XO activity, and (**C**) NBT reduction induced by a superoxide radical generated in a NADH/PMS system. Values show mean ± SE from three experiments performed in triplicate.

monitoring the metabolic conversion of xanthine to uric acid. The results demonstrated that the extract exerts an inhibitory effect on the enzyme, with an IC_{25} of 708.8 μg mL^{-1} (**Figure 6B**). According to these data, it was not possible to show a clear-cut scavenging effect on the superoxide radical. The capacity of the extract to effectively scavenge superoxide radicals in a concentration-dependent way was confirmed when this radical was generated by a nonenzymatic system, and an IC_{50} at 336.9 μg mL^{-1} was found (**Figure 6C**). Thus, *R. induratus* leaves exhibit antioxidant activity, achieved by its capacity to act as both superoxide radical scavenger and XO inhibitor.

Obviously, the chemical composition of *R. induratus* leaf lyophilized extract determines the observed activity. Certainly, the phenolic compounds and the oxalic acid present in the extract contribute to its antioxidant capacity to an important extent. In fact, the antioxidant capacity of hydroxycinnamic acid derivatives (*37*−*39*) and of luteolin, apigenin, quercetin, and diosmetin glycosides (*38*, *40*, *41*) in several models is well-known, such as that of oxalic acid (*42*).

Conclusion. On the basis of the data obtained in this study, *R. induratus* leaves constitute a promising dietary source of biologically active compounds for the consumer, namely, phenolic compounds. In general, it may provide nutritional and health benefits associated with the consumption of fruits and vegetables. Thus, due to its agreeable taste and high phenolic content, it can be a good alternative in the preparation of salads, which nowadays are mainly composed of tomatoes and/or lettuce. However, due to its high oxalic acid content, some care must be taken, and no high doses can be consumed because oxalic acid has a predicted LD_{50} value of 375 mg/kg body weight. Further studies with more *R. induratus* samples, from different origins, should be performed, to check the possible use of the phenolic compound profile in the quality control of the species.

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