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Identification of Antioxidative Flavonols and Anthocyanins in *Sicana odorifera* Fruit Peel

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Ten flavonols and three anthocyanins were identified in the fruit peel of melón de olor (*Sicana odorifera*), and their structures were established by spectrometric and spectroscopic (ESI-MS and NMR) techniques. One of the identified flavonols, quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucopyranoside 4′-*O*-β-D-glucopyranoside, has not been reported before in the plant kingdom. Although quercetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside-4′-*O*-β-D-glucopyranoside had been reported before in literature and structure elucidation was done by comparison of NMR data with published data, to the best of our knowledge complete 1D and 2D NMR data have not been not delineated so far. Moreover, the antioxidant activity of pure compounds was measured by ABTS assay. It was established that quercetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, quercetin-3-*O*-(6″-malonyl)-glucopyranoside, quercetin-3-*O*-β-D-glucopyranoside, and quercetin-3-*O*-α-L-rhamno-pyranosyl-(1→6)-β-D-glucopyranoside-4′-*O*-β-D-glucopyranoside contribute significantly to the antioxidant activity exhibited by the fruit peel methanolic extract.

KEYWORDS: *Sicana odorifera*; flavonols; anthocyanins; antioxidant activity; quercetin glycosides

INTRODUCTION

Sicana odorifera (Vell.) Naudin, a plant belonging to the Cucurbitaceae family, is believed native to Brazil, but it has been spread throughout tropical America. This vine is perennial, herbaceous, fast-growing, and climbing trees to 15 m or more. Its fruit is known as melón de olor, melón melocotón, calabaza de olor or cassabanana, names that mostly refer to its strong, sweet, and pleasant sweet melon-like aroma. The shape is ellipsoid or nearly cylindrical, 30 to 60 cm in length, hard-shelled, purple with tinges of violet; smooth and glossy when ripe, the flesh is firm, orange-yellow or yellow, cantaloupe-like, tough, and juicy with a 3/4 in. (2 cm) thickness. The ripe flesh, finely sliced, is eaten raw, especially in the summer when it is appreciated as cooling and refreshing; however, it is mainly used in the kitchen for making jam or other preserves. The immature fruit is cooked as a vegetable or in soup and stews. The fruit is also kept around the house, especially in linen and clothes closets, because of its long-lasting fragrance, and it is believed to repel moths (*1*).

There are a few numbers of chemical studies on this fruit. Due to its intense aroma, the free and glycosidically bound volatiles of *S. odorifera* fruit were studied by Parada et al. (*2*). Thus, the free volatile extract obtained by liquid−liquid extraction was analyzed by GC and GC−MS, and 37 compounds were identified, among which 3-methyl-2-butanol, 3-hydroxy-2-butanone, ethyl 3-hydroxybutanoate, and (*Z*)-3-hexenol were found to be the major constituents. Regarding glycosidically bound volatiles, the 4-hydroxybenzyl methyl ether, 4-hydroxybenzyl alcohol, and 2-phenylethanol were identified as the major constituents. Interestingly, [4-(β-D-glucopyranosyloxy)benzyl] 2,3-dihydroxy-3-methylbutanoate and 4-(β-D-glucopyranosyloxy)benzyl alcohol were identified as precursors of 4-hydroxybenzyl alcohol, one of the major volatiles generated by enzymatic hydrolysis of the glycosidic fraction. In a later study, the two triterpenes cucurbita-5,23-diene-3β,25-diol and D:C-friedo-oleana-7,9(11)-diene-3α,29-diol dibenzoate (karounidiol dibenzoate) as well as (+)-taxifolin and quercetin were isolated from the seeds of this fruit (*3*).

As part of our current studies on tropical fruits with added value (*4, 5*), the methanolic extract of *Sicana odorifera* peel was submitted to a bioguided fractionation to isolate and identify the compounds responsible for its antioxidant activity. Depending on their chemical structure, a series of flavonol glycosides and anthocyanins were reported for the first time as the key antioxidants in *S. odorifera* peel.

MATERIALS AND METHODS

General. 1D and 2D NMR spectra were obtained at 303 K on a 400 MHz (DRX) and a 500 MHz Avance III spectrometer (Bruker, Rheinstetten, Germany) and a Jeol JNM LA-500 spectrometer in DMSO-d_6 and CD$_3$OD/CF$_3$COOD (19:1, v/v) with solvent peaks as references. The deuteriomethyl ^{13}C signal and the residual ^{1}H signal of the solvents were used as secondary references (for spectra recorded in CD$_3$OD, these peaks were located at δ 49.0 and δ 3.40 ppm; in DMSO-d_6, the solvent peaks were at δ 39.5 and δ 2.50 ppm). For structural elucidation and NMR

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signal assignment, 2D NMR experiments, like COSY-, DEPT-, TOCSY-, ROESY, *g*-HSQC-, and *g*-HMBC-spectroscopy, were carried out using the pulse sequences taken from the Bruker software library. Data processing was performed by using XWin-NMR software (version 3.5; Bruker, Rheinstetten, Germany) as well as Mestre-C (Mestrelab Research, A Coruña, Spain). HPLC separation was performed on an instrument consisting of a Rheodyne 7175 injection valve, a Merck-Hitachi L-6200A intelligent pump, a Merck Hitachi L-4500 diode array detector, and an interphase D-6000A. For preparative purposes a HPLC apparatus was used, consisting of a Merck-Hitachi L-6000A pump, a Rheodyne injection valve with a 500 μL loop, and a Merck-Hitachi UV−vis L-4250 detector. The CCC system was a multilayer coil countercurrent chromatograph series 521 (P.C. Inc., Potomac, MD, USA). A Bio Rad SmartSpect 3000 UV−vis spectrometer was used for monitoring MLCCC fractions and for measurement of antioxidant activity. Thin layer chromatography was developed on silica gel GF_{254} plates (0.25 mm, Merck, Darmstadt, Germany), and column chromatography was done over silica gel (0.040−0.063 mm, Merck, Darmstadt, Germany)

Chemicals. All solvents (analytical grade, Merck, Darmstadt, Germany) were redistilled before use. For LCMS analyses, acetonitrile, water and formic acid were purchased from Honeywell Burdick and Jackson (Muskegon, MI, USA). The following compounds were obtained commercially from the sources given in parentheses: hydrochloric acid, trifluoroacetic acid, DMSO-d_6, glucose, and KOH (Merck, Darmstadt, Germany); CD_3OD, trifluoroacetic acid-d_1 (Euriso-top, Saarbrücken, Germany); sodium hydroxide and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, Fluka Chemicals, Germany); potassium persulphate (Riedel-de Haën, Hannover, Germany); rhamnose (Fluka, Neu-Ulm, Germany); and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, Sigma-Aldrich, Steinheim, Germany). Water for chromatographic separation was purified by means of a Milli-Q water advantage A 10 water system (Millipore, Molsheim, France). Ascorbic acid was purchased from Merck (Darmstadt, Germany).

Plant Material. *Sicana odorifera* fruits were purchased from different local markets in La Mesa (Cundinamarca, Colombia). Fully ripe fruits, characterized by a pH of 6.2, a sugar-to-water ratio of 13.0 °Brix, and a completely purple peel, were selected. A voucher specimen was deposited at the Instituto de Ciencias Naturales, Universidad Nacional de Colombia under the code COL366758.

Extraction and Isolation. Peels of *S. odorifera* (3.5 kg) were homogenized and extracted at 18 °C with MeOH−AcOH (19:1) (10 L) over 20 h in the dark. The solvent was evaporated under vacuum, and the residue (ME, 187 g) was suspended in H_2O and partitioned successively with EtOAc (5 × 80 mL). After removing the solvent in vacuum, 4.5 g of the extract (OE) was obtained. The aqueous phase was applied in different portions to a 46 × 4.5 cm Amberlite XAD-7 resin open column (Aldrich Chemical Co., Milwaukee, WI, USA). The column was rinsed with water, and the adsorbed compounds each time were eluted with 1 L of methanol−acetic acid (19:1, v/v), according to the procedure described by Degenhardt et al. (*6*). The eluate was concentrated under vacuum; the residue was freeze-dried to get 24.8 g of AE (aqueous extract).

A part of OE (1.5 g) was repeatedly chromatographed over silica gel (100 g) with a discontinuous gradient of $CHCl_3$−MeOH (19:1 → 1:10) as eluent to get 150 fractions of 5 mL each. The combined fractions 81−95 (66 mg) were refractionated with $CHCl_3$−MeOH (1:5) over silica gel (6 g) to get five subfractions. The fourth fraction was finally purified by preparative HPLC using a Shimpack CLC-ODS C-18 (5 μm, 150 × 6.0 mm i.d., Shimadzu, Japan) column and MeOH−H_2O (1:1) as eluent to get 5.5 mg of a mixture of compounds **1** and **2**. This mixture was analyzed by LC−ESIMS, ^1H and ^{13}C NMR to elucidate the chemical structures of **1** and **2**.

Fractionation of Aqueous Extract (AE). A portion of the freeze-dried powder (4 g) was fractionated by MLCCC (multilayer countercurrent chromatography) in portions of 1 g. The solvent system was a mixture of TBME−*n*-butanol−acetonitrile−water (2:2.1:5, v/v/v/v, acidified with 0.1% TFA, v/v). A single coil (75 × 2.6 mm i.d. PTFE tubing, total volume approximately 410 mL) was used, and the revolution speed was set to 800 rpm. The less dense layer was always used as the stationary phase, and the flow rate of the mobile phase was 1.0 mL/min. One hundred fractions (5 mL each) were collected and pooled as follows: F1 (1−10), F2 (11−19), F3 (20−32), F4 (33−44), F5 (45−51), F6 (52−60), F7 (61−75),

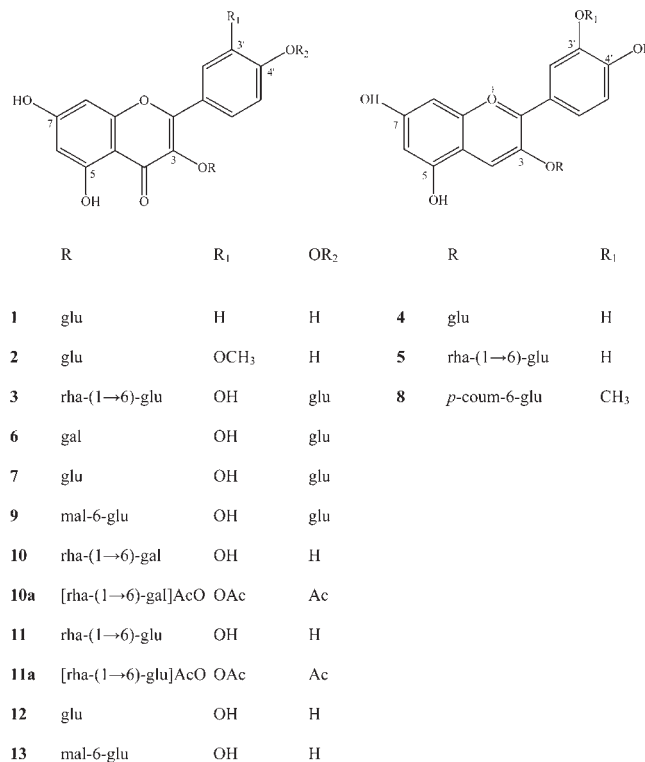|	R	R$_1$	OR$_2$		R	R$_1$
1	glu	H	H	**4**	glu	H
2	glu	OCH$_3$	H	**5**	rha-(1→6)-glu	H
3	rha-(1→6)-glu	OH	glu	**8**	*p*-coum-6-glu	CH$_3$
6	gal	OH	glu			
7	glu	OH	glu			
9	mal-6-glu	OH	glu			
10	rha-(1→6)-gal	OH	H			
10a	[rha-(1→6)-gal]AcO	OAc	Ac			
11	rha-(1→6)-glu	OH	H			
11a	[rha-(1→6)-glu]AcO	OAc	Ac			
12	glu	OH	H			
13	mal-6-glu	OH	H			

Figure 1. Chemical structures of the flavonols and anthocyanins identified in *S. odorifera* fruit peel. Flavonols: kaempferol 3-*O*-β-glucopyranoside (**1**), isorhamnetin 3-*O*-β-glucopyranoside (**2**), quercetin-3-*O*-(6′′-α-L-rhamnosyl-β-D-glucopyranoside)-4′-*O*-β-D-glucopyranoside (**3**), quercetin 4-*O*-β-D-glucopyranosyl-3-*O*-β-D-galactopyranoside (**6**), quercetin 4-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranoside (**7**), quercetin-4-*O*-β-D-glucopyranosyl-3-*O*-(6′′-malonyl)-β-D-glucopyranoside (**9**), quercetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**10**), quercetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**11**), quercetin-3-*O*-β-D-glucopyranoside (**12**), and quercetin-3-*O*-(6′′-malonyl)-β-D-glucopyranoside (**13**). Anthocyanins: cyanidin-3-*O*-β-D-glucopyranoside (**4**), cyanidin-3-*O*-rutinoside (**5**), and peonidin-3-*O*-(6′′-*O*-*p*-coumaroyl)-glucopyranoside (**8**).

F8 (76−91), F9 (92−98), and F10 (stationary phase). All fractions were monitored at 350 and 520 nm, searching for flavonols and anthocyanins, respectively.

HPLC-PDA. Characterization of the flavonols and anthocyanins present in the AE and each fraction was done by HPLC using a LUNA C-18 5 μm column (250 × 4.6 mm i.d., Phenomenex, USA), and detection was carried out using a diode array detector. The solvent system was a mixture of water/formic acid (90:10, v/v, solvent A) and water/formic acid/acetonitrile (9:1:90, v/v/v, solvent B) and the flow rate was 0.8 mL/min. A linear gradient from 8 to 25% B at 0−35 min, 25 to 50% B at 35−45 min, 50 to 8% B at 45−55 min was used.

The quantification of anthocyanins and flavonols was carried out relative to the external standards cyanidin-3-*O*-β-D-glucopyranoside (0.09−0.5 μM) and quercetin-3-*O*-β-D-glucopyranoside (0.07−0.28 μM), respectively.

Preparative HPLC. Eleven compounds (**3**−**13**, **Figure 1**) were purified by preparative HPLC from MLCCC fractions. The separation was carried out on a LUNA C18 5 μm column (250 × 10 mm i.d., Phenomenex, USA) using water/formic acid/acetonitrile (84:9:7, v/v/v) for F2 and (9:1:90, v/v/v) for F4, F6, F8, and F10, as the mobile phase. The flow rate was 5 mL/min. From F2 pure flavonol **3** (8.5 mg) was obtained; from F4 anthocyanins the **5** (11 mg) and **8** (1 mg) and the flavonols **6** (23.3 mg) and **7** (19.7 mg) were obtained; from F6 flavonols **7** (7.2 mg) and **9** (4.1 mg) were isolated; from F8 the flavonol **9** (28 mg) and the anthocyanin **4** (3.2 mg) were purified; and from F10 the flavonols **10** (5.6 mg), **11** (13.7 mg), **12** (11.6 mg), and **13** (6.6 mg) were obtained. Purified compounds were identified on the basis of their LC−ESI-MS and NMR spectra.

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Table 1. Chromatographic, Spectroscopic, and Spectrometric Data of the Flavonols and Anthocyanins from Melón de Olor (*S. odorifera*) Fruit Peel

compd[a]	fraction (MLCCC)	$t_R{}^a$ (min) (HPLC)	fragment ions (*m/z*)	$\lambda_{max}{}^b$ (nm)
1	OE[c]	—[d]	487 [M + K]$^+$, 471 [M + Na]$^+$, 287 [M − 162 + H]$^+$	349, 265
2	OE[c]	—	517 [M + K]$^+$, 501 [M + Na]$^+$, 317 [M − 162 + H]$^+$	349, 265
3	F2	16.54	773 [M + H]$^+$, 611 [M − 162 + H]$^+$, 465 [M − 162 − 146 + H]$^+$, 303 [M − 162 − 146 − 162 + H]$^+$	345, 243
4	F4, F8	9.62	449 [M]$^+$, 287 [M − 162]$^+$	511, 281
5	F4	13.16	595 [M]$^+$, 287 [M − 162 − 146]$^+$	511, 281
6	F4	15.95	665 [M + K]$^+$, 649 [M + Na]$^+$, 465 [M − 162 + H]$^+$, 303 [M − 162 − 162 + H]$^+$	351, 268, 257, 208
7	F4, F6	17.13	665 [M + K]$^+$, 649 [M + Na]$^+$, 465 [M − 162 + H]$^+$, 303 [M − 162 − 162 + H]$^+$	350, 268, 208
8	F4	18.74	609 [M]$^+$, 301 [M − 162−146]$^+$	511, 336, 279
9	F4, F6, F8	21.54	751 [M + K]$^+$, 735 [M + Na]$^+$, 713 [M + H]$^+$, 649 [M − 86 + Na]$^+$, 551 [M − 162 + H]$^+$, 465 [M − 162 − 86 + H]$^+$, 303 [M − 162 − 86 − 162 + H]$^+$	347, 265
10	F10	24.98	649 [M + K]$^+$, 633 [M + Na]$^+$, 303 [M − 162 − 146 + H]$^+$	363, 302, 258, 207
11	F10	25.82	649 [M + K]$^+$, 633 [M + Na]$^+$, 303 [M − 162−146 + H]$^+$	361, 259, 207
12	F10	27.19	503 [M + K]$^+$, 487 [M + Na]$^+$, 303 [M − 162 + H]$^+$	359, 259
13	F10	30.03	573 [M + Na]$^+$, 551 [M + H]$^+$, 529 [M + Na − 44]$^+$, 303 [M − 86 − 162 + H]$^+$	361, 258, 207

[a] Compound numbers and retention times refer to the numbers given in **Figures 1** and **3**. [b] Measured in acetonitrile. [c] These compounds were detected in the organic phase as a mixture. [d] —, not determined.

HPLC−MS Analysis. LC−MS analyses of fractions were performed using a Shimadzu LCMS-2010 System (Shimadzu, Tokyo, Japan) equipped with a UV/vis detector (SPD-10A) and two pumps (LC-10AD) coupled online with a MS-2010 mass spectrometer. UV and MS data were acquired and processed using Shimadzu LCMS Solution software. The equipment also included an online DGU-14A degasser and a Rheodyne injection valve with a 5 μL loop. A LUNA RP-18 5 μm column (150 × 2.0 mm i.d., Phenomenex, USA) was used for the analysis of the constituents present in each fraction. The gradient used was identical to the one described for the analytical HPLC but at a flow rate of 0.2 mL/min. The electrospray ionization (ESI) probe was operated in the positive mode: CDL, 300 °C; block at 240 °C; flow gas (N$_2$) at 4.5 L/min; CDL voltage, 150.0 kV; Q array voltage RF 150 V; detector voltage, 1.5 kV; and scan range *m/z* 100−800.

For the HRESIMS measurements of pure compounds, a Shimadzu LC−MS-IT-TOF liquid chromatograph mass spectrometer (Kyoto, Japan) was used. The sample solutions were injected directly into the system. The MS/MS parameters were as follows: positive mode; CDL temperature 200 °C; heating block at 200 °C; detector voltage, 1.55 kV; flow nebulizing gas (N$_2$), 1.5 L/min; ion accumulation 20 ms; and scan range *m/z*, 200−1600. The energy of the collision gas (argon) was fixed at 15% for the anthocyanins and 10% for the flavonols. The LC−MS Solution software (Shimadzu, Tokyo, Japan) was used for data collection and analysis.

For compound identification of **3**, mass and product ion spectra were acquired on an API 4000 Q Trap triple quadrupole/linear ion trap mass spectrometer (Applied Biosystems, Darmstadt, Germany). The isolated fraction was dissolved in a mixture of methanol/water (70/30, v/v) and directly introduced into the mass spectrometer by flow infusion using a syringe pump. The mass spectrometer was activated in full-scan mode under an electrospray ionization (ESI) device running in positive ionization mode with a spray voltage of +5500 V and −4500 V in the negative mode. The MS/MS parameters were optimized for each substance. Data acquisition and instrumental control was completed with the Analyst 1.4.2 software (Applied Biosystems, Darmstadt, Germany).

Sugar Analyses. For compound **3**, the analysis of glycosidically bound carbohydrates was done by means of high performance ion chromatography (HPIC). The compound was analyzed by means of anion exchange chromatography using an ICS-2500 ion chromatography system (Dionex, Idstein, Germany) consisting of a GS 50 gradient pump, an AS 50 autosampler, an AS 50 thermal compartment, and an ED 50 electrochemical detector operating in pulsed amperometric detection mode. The detector was equipped with a gold working electrode operating with a standard carbohydrate quadrupole waveform supplied by manufacturer. Data acquisition and instrumental control was completed with the Chromeleon software (version 6.80, Dionex). Chromatographic separation was performed at 30 °C on a CarboPac PA-20 column (150 × 3 mm, Dionex) connected with a CarboPac PA-20 guard column (30 × 3 mm, Dionex), using an isocratic gradient of sodium hydroxide solution (2.5 mM) for 20 min. After each sample, the column was washed with a sodium hydroxide solution (200 mM) and equilibrated with sodium hydroxide solution (2.5 mM) for 10 min prior to injection. Moreover chromatography was performed with an injection volume of 10 μL and a flow rate of 0.5 mL/min. For qualitative analysis, glycosidically bound carbohydrates were identified by comparison of retention times and cochromatography of the following reference compounds: glucose and rhamnose. For sample preparation an aliquot (1 mg) of the target compound, dissolved in aqueous hydrochloric acid (2 mol/L; 0.5 mL), was placed into a closed glass vial, and then heated at 110 °C for 120 min. After cooling to room temperature 150 μL of potassium hydroxide solution (4 N) and 50 mL water were added. Each sample was transformed into autosampler vials for injection into the HPIC system.

To confirm the presence of galactose and glucose in compounds **6** and **7** (respectively), 3 mg of each compound separately was hydrolyzed with 4 mL of TFA 2 M under stirring at 18 °C during 2 h. The aglycons were extracted with AcOEt, and the aqueous phases were freeze-dried to be derivatized. The TMS derivates of sugars were obtained according to the procedure reported by Sanz et al. (7). Briefly, 5 mg of each sample was treated with 100 μL of anhydrous pyridine (Merck), 100 μL of trimethyl-silylimidazole (Sigma), and 100 μL of trimethylchlorosilane (Sigma) at room temperature (18 °C), under N$_2$ atmosphere. After derivatization, 0.1 mL of hexane and 0.2 mL of water were added to the mixture, and 1 μL of the upper layer was injected into the GC−MS equipment. The analysis was performed on a QP 5050 mass selective detector coupled to a Shimadzu GC17A gas chromatograph with a DB-1 methyl silicone column (30 m × 0.25 mm i.d., 0.50 μm film thickness). The temperature of the injector was 290 °C, and the column oven was programmed from 110 to 270 °C at a rate of 10 °C/min. Carrier gas was 1.0 mL of He/min, make up gas was nitrogen at 30 mL/min flow rate, and injections were made in the split mode, with a split ratio of 1:10. Mass spectra were recorded between 40−350 u, and processed by Class 5000 v 2.2 MS-Workstation software. The identity of each silylated carbohydrate was confirmed by comparison of retention time and mass spectra obtained with those exhibited by standards of glucose and galactose from Merck.

Spectroscopic Data. In this section the spectral data of compounds **1−13** are reported. Numbering of compounds refers to **Figure 1**.

Kaempferol 3-O-β-Glucopyranoside (**1**). EIMS [*m/z*, (%, interpretation)]: 286 (7.9, [M − 162]$^+$). UV−vis and ESIMS data in **Table 1**. ^{1}H NMR (400 MHz, CD$_3$OD; COSY) δ/ppm: δ 3.24 [m, 1H, H−C(5″)], 3.33 [m, 1H, H−C(4″)], 3.46 [m (o), 2H, H−C(2″, 3″)], 3.56 [dd, 1H, ^2J = 11.4 Hz, ^3J = 2.8 Hz, H−C(6″b)], 3.72 [m, 1H, H−C(6″a)], 5.28 [d, 1H, ^3J = 5.0 Hz, H−C(1″)], 6.22 [brs, 1H, H−C(6)], 6.42 [brs, 1H, H−C(8)], 6.91 [d, 2H, ^3J = 7.2 Hz, H−C(3′, 5′)], 8.08 [d, 2H, ^3J = 7.4 Hz, H−C(2′, 6′)]. ^{13}C NMR: data in **Table 2**.

Isorhamnetin 3-O-α-Glucopyranoside (**2**). EIMS [*m/z*, (%, interpretation)]: 316 (4.3, [M − 162]$^+$). UV−vis and ESIMS: data in **Table 1**. ^{1}H NMR (400 MHz, CD$_3$OD; COSY) δ/ppm: δ 3.28 [m, 1H, H−C(5″)], 3.37 [m, 1H, H−C(4″)], 3.46 [m, 1H, H−C(3″)], 3.48 [m, 1H, H−C(2″)], 3.60 [m, 1H, H−C(6″b)], 3.78 [m, 1H, H−C(6″a)], 3.97 [s, 3H, OCH$_3$], 5.44 [d, 1H, ^3J = 1.1 Hz, H−C(1″)], 6.22 [brs, 1H, H−C(6)], 6.42 [brs, 1H, H−C(8)], 6.91

Table 2. ^{13}C NMR Data of Flavonols Isolated from *S. odorifera* Fruit Peel (δ in ppm)

C	1^a	2^a	3^{a,b}	6^c	7^c	9^c	10^c	11^c	12^a	13^c
					Aglycon					
2	159.1	158.5	158.6	155.3	155.2	155.8	156.5^h	156.6	159.0	156.7
3	135.5	135.4	136.1	134.0	133.7	133.5	133.3	133.3	135.6	133.2
4	179.5	179.4	179.5	177.5	177.4	177.3	177.3	177.3	179.5	177.4
5	163.1	163.1	163.1	161.2	161.1	161.1	161.2	161.2	163.0	160.9
6	99.9	99.9	100.1	98.9	98.8	98.7	98.6	98.7	99.9	98.6
7	166.1	166.1	166.3	164.9	164.9	164.2	164.1	164.2	166.0	163.9
8	94.8	94.8	94.9	93.7	93.7	93.6	93.5	93.6	94.7	93.6
9	158.5	158.5	158.5	156.4	156.4	156.3	156.4^h	156.4	158.5	156.1
10	105.7	105.8	105.8	103.9	103.8	103.9	103.9	103.9	105.7	103.9
1'	122.8	123.1	126.6	124.5	124.4	124.2	121.1	121.6	123.1	121.1
2'	132.3	114.4	118.6	116.3	116.4	116.5	116.2	115.2	117.5	116.1
3'	116.1	148.4	147.4	146.3	146.1	146.1	144.7	144.8	145.9	144.6
4'	161.6	−	149.1	147.5	147.4	147.4	148.4	148.4	149.9	148.3
5'	116.1	116.0	117.1	115.5	115.4	115.3	115.2	115.3	116.0	115.1
6'	132.6	123.8	122.6	121.1	120.8	120.7	121.5	121.1	123.2	121.6
O—CH$_3$	−	56.8	−	−	−	−	−	−	−	−
					3-*O*-Sugar					
1''	104.1	103.7	104.4	101.8	100.7	100.8	101.1	101.2	104.3	100.9
2''	75.7	75.9	75.7	71.2	74.1	73.9^f	75.9	74.1	75.7	73.8
3''	78.1	78.1	77.6	73.2^d	76.4	76.1	74.0	76.4	78.1	75.9
4''	71.4	71.5	72.2	67.9	69.9	69.5^g	70.3^i	70.5^j	71.2	69.4
5''	78.4	78.5	78.2	75.8^e	77.6	73.8^f	76.4	75.9	78.4	73.8
6''a	62.7	62.6	68.5	60.1	60.9	63.4	67.0	67.0	62.5	63.6
6''b										
					4'-*O*-Sugar					
1'''			103.2	101.6	101.4	101.4	100.7	100.7		
2'''			74.9	73.3^d	73.2	73.2	70.5	70.4		
3'''			77.2	75.9^e	75.8	75.7	70.0i	70.0j		
4'''			71.5	69.7	69.7	69.6^g	71.8	71.8		
5'''			78.4	77.2	77.2	77.1	68.2	68.2		
6'''			62.5	60.6	60.6	60.6	17.7	17.7		
					6''-*O*-Sugar					
1''''			102.4							
2''''			72.0							
3''''			71.4							
4''''			73.9							
5''''			69.8							
6''''			17.9							
					Malonyl					
C=O						166.6				166.5
CH$_2$						41.2				40.6
COOH						167.7				167.5

^{a} CD$_3$OD, 100 MHz. ^{b} CD$_3$OD, 125 MHz. ^{c} DMSO-d_6, 100 MHz. $^{d–j}$ Values followed by the same letter are interchangeable.

[d, 1H, ^3J = 7.2 Hz, H−C(5')], 7.60 [dd, 1H, ^3J = 6.4 Hz, ^4J = 0.8 Hz, H−C(6')], 7.95 [brs, 1H, H−C(2')]. ^{13}C NMR: data in **Table 2**.

Quercetin 3-O-α-L-Rhamnopyranosyl-(1→6)-β-D-glucopyranoside-4'-O-β-D-glucopyranoside (**3**). HRESIMS 773.2145 [calcd for C$_{33}$H$_{41}$O$_{21}$ [M + H]$^+$: 773.2140]; 795.1958 [calcd for C$_{33}$H$_{40}$O$_{21}$Na [M + Na]$^+$: 795.1960]. UV−vis and ESIMS: data in **Table 1**. ^{1}H NMR (500 MHz, CD$_3$OD; COSY) δ/ppm: δ 1.09 [d, 3H, ^3J = 6.5 Hz, H−C(6'''')], 3.23−3.28 [m (o), 2H, H−C(4''', 4'''')], 3.33−3.47 [m (o), 2H, H−C(3''', 3'''')], 3.38−3.47 [m, 4H, H−C(4''', 5''', 5''''', 6b'')], 3.47−3.56 [m, 5H, H−C(2'', 3'', 2''', 3''', 5''')], 3.60 [dd, 1H, ^3J = 1.6 Hz, ^3J = 3.5 Hz, H−C(2'''')], 3.73 [dd, 1H, ^3J = 5.6 Hz, ^2J = 12.1 Hz, H−C(6b''')], 3.83 [dd, 1H, ^3J = 1.2 Hz, ^2J = 10.5 Hz, H−C(6a'')], 3.93 [dd, 1H, ^3J = 2.6 Hz, ^2J = 12.0 Hz, H−C(6a''')], 4.51 [d, 1H, ^3J = 1.4 Hz, H−C(1'''')], 5.00

[d, 1H, ^3J = 7.4 Hz, H−C(1''')], 5.18 [d, 1H, ^3J = 7.6 Hz, H−C(1'')], 6.22 [d, 1H, ^4J = 2.1 Hz, H−C(6)], 6.41 [d, 1H, ^4J = 2.1 Hz, H−C(8)], 7.27 [d, 1H, ^3J = 8.8 Hz, H−C(5')], 7.65 [dd, 1H, ^4J = 2.2 Hz, ^3J = 8.6 Hz, H−C(6')], 7.78 [d, 1H, ^4J = 2.1 Hz, H−C(2')]. ^{13}C NMR: data in **Table 2**.

Cyanidin-3-O-β-D-glucopyranoside (**4**). HRESIMS 449.1078 [calcd for C$_{21}$H$_{21}$O$_{11}$ [M]$^+$: 449.1084]. UV−vis and ESIMS: data in **Table 1**. ^{1}H and ^{13}C NMR data were in agreement with those published in the literature previously (*5*, *8*).

Cyanidin-3-O-rutinoside (**5**). HRESIMS 595.1631 [calcd for C$_{27}$H$_{31}$O$_{15}$ (M)$^+$: 595.1663]. UV−vis and ESIMS: data in **Table 1**. ^{1}H and ^{13}C NMR data were in agreement with those published in the literature previously (*5*, *9*).

Quercetin 3-O-β-D-Galactopyranoside-4'-O-β-glucopyranoside (**6**). HRESIMS 649.1366 [calcd for C$_{27}$H$_{30}$O$_{17}$Na [M + Na]$^+$: 649.1381]. UV−vis and ESIMS: data in **Table 1**. ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ/ppm: δ 3.21 [t, 1H, ^3J = 8.8 Hz, H−C(4''')], 3.31 [brd, 1H, ^2J = 9.1 Hz, H−C(6''a)], 3.34 [m (o), 3H, H−C(5''', 2''', 3''')], 3.37 [dd, 1H, ^3J = 9.0 Hz, ^3J = 3.0 Hz, H−C(3'')], 3.39 [m, 1H, H−C(5''')], 3.48 [dd, 1H, ^2J = 8.6 Hz, ^3J = 3.9 Hz, H−C(6')], 3.50 [dd, 1H, ^2J = 11.6 Hz, ^3J = 6.1 Hz, H−C(6''b)], 3.55 [dd, 1H, ^3J = 9.2 Hz, ^3J = 7.8 Hz, H−C(2'')], 3.66 [d, 1H, ^3J = 2.9 Hz, H−C(4')], 3.73 [brd, 1H, ^2J = 10.8 Hz, H−C(6''a)], 4.86 [d, 1H, ^3J = 7.1 Hz, H−C(1''')], 5.40 [d, 1H, ^3J = 7.7 Hz, H−C(1'')], 6.21 [brs, 1H, H−C(6)], 6.44 [brs, 1H, H−C(8)], 7.18 [d, 1H, ^3J = 8.7 Hz, H−C(5')], 7.63 [d, 1H, ^4J = 2.1 Hz, H−C(2')], 7.67 [dd, 1H, ^3J = 8.7 Hz, ^4J = 2.1 Hz, H−C(6')]. ^{13}C NMR: data in **Table 2**.

Quercetin 3,4'-Di-O-β-D-glucopyranoside (**7**). HRESIMS 649.1326 [calcd for C$_{27}$H$_{30}$O$_{17}$Na [M + Na]$^+$: 649.1381]. UV−vis and ESIMS: data in **Table 1**. ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ/ppm: δ 3.08 [m, 2H, H−C(4'', 5'')], 3.20 [m, 1H, H−C(4''')], 3.21 [m, 2H, H−C(2'', 3''-H)], 3.32 [m, 1H, H−C(6''b)], 3.33 [m, 1H, H−C(3''')], 3.34 [dd, 1H, ^3J = 9.1 Hz, ^3J = 7.1 Hz, H−C(2''')], 3.38 [ddd, 1H, ^3J = 8.0 Hz, ^3J = 5.5 Hz, ^3J = 1.9 Hz, H−C(5''')], 3.59 [brd, 1H, ^2J = 11.4 Hz, H−C(6''a)], 3.50 [dd, 1H, ^2J = 11.8 Hz, ^3J = 5.5 Hz, H−C(6'''b)], 3.73 [brd, 1H, ^2J = 10.5 Hz, H−C(6''a)], 4.87 [d, 1H, ^3J = 7.1 Hz, H−C(1''')], 5.50 [d, 1H, ^3J = 7.3 Hz, H−C(1'')], 6.20 [d, 1H, ^4J = 1.7 Hz, H−C(6)], 6.43 [d, 1H, ^4J = 1.6 Hz, H−C(8)], 7.20 [d, 1H, ^3J = 8.7 Hz, H−C(5')], 7.64 [d, 1H, ^4J = 2.1 Hz, H−C(2')], 7.61 [dd, 1H, ^3J = 8.6 Hz, ^4J = 2.2 Hz, H−C(6')]. ^{13}C NMR: data in **Table 2**.

Peonidin-3-O-(6''-O-p-Coumaroyl)-β-D-glucopyranoside (**8**). UV−vis and ESIMS: data in **Table 1**.

Quercetin 3-O-(6''-O-Malonyl)-β-D-glucopyranoside 4'-O-β-D-Glucopyranoside (**9**). HRESIMS 735.1336 [calcd for C$_{30}$H$_{32}$O$_{20}$Na [M + Na]$^+$: 735.1385]. UV−vis and ESIMS data in **Table 1**. ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ/ppm: δ 3.11 [s, 2H, CH$_2$- malonyl], 3.16 [t, 1H, ^3J = 8.9 Hz, H−C(4'')], 3.21 [t, 1H, ^3J = 9.2 Hz, H−C(4''')], 3.23 [dd, 1H, ^3J = 8.7 Hz, ^3J = 7.3 Hz, H−C(2'')], 3.25 [t, 1H, ^3J = 8.9 Hz, H−C(3'')], 3.33 [m, 1H, H−C(3''')], 3.34 [dd, 1H, ^3J = 9.0 Hz, ^3J = 7.0 Hz, H−C(2''')], 3.34 [ddd, 1H, ^3J = 9.2 Hz, ^3J = 6.2 Hz, ^3J = 2.8 Hz, H−C(5'')], 3.39 [ddd, 1H, ^3J = 8.1 Hz, ^3J = 5.3 Hz, ^3J = 2.4 Hz, H−C(5''')], 3.50 [dd, 1H, ^2J = 11.7 Hz, ^3J = 5.6 Hz, H−C(6''b)], 3.73 [brd, 1H, ^2J = 10.7 Hz, H−C(6''a)], 4.01 [dd, 1H, ^2J = 11.8 Hz, ^3J = 5.7 Hz, H−C(6'b)], 4.22 [brd, 1H, ^2J = 10.6 Hz, H−C(6'a)], 4.87 [d, 1H, ^3J = 7.0 Hz, H−C(1''')], 5.42 [d, 1H, ^2J = 7.3 Hz, H−C(1'')], 6.22 [d, 1H, ^4J = 1.8 Hz, H−C(6)], 6.44 [d, 1H, ^4J = 1.8 Hz, H−C(8)], 7.21 [d, 1H, ^3J = 8.7 Hz, H−C(5')], 7.52 [dd, 1H, ^3J = 8.6 Hz, ^4J = 2.0 Hz, H−C(6')], 7.60 [d, 1H, ^4J = 2.1 Hz, H-(2')], 12.52 [brs, 1H, 5-OH]. ^{13}C NMR: data in **Table 2**.

Quercetin 3-O-α-L-Rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**10**). HRESIMS 633.1378 [calcd for C$_{27}$H$_{30}$O$_{16}$Na [M + Na]$^+$: 633.1432]. UV−vis and ESIMS: data in **Table 1**. ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ/ppm: δ 0.99 [d, 3H, ^3J = 6.2 Hz, H−C(6''')], 3.07 [t, 1H, ^3J = 9.2 Hz, H−C(4''')], 3.20−3.40 [m (o), 4H, H−C(3'', 4'', 5'', 3''')], 3.23 [dd, 1H, ^3J = 6.9 Hz, ^3J = 4.5 Hz, H−C(2'')], 3.25 [m, 1H, H−C(5''')], 3.28 [dd, 1H, ^2J = 10.6 Hz, ^3J = 3.0 Hz, H−C(6b'')], 3.39 [m, 1H, H−C(2''')], 3.71 [m, 1H, ^2J = 10.3 Hz, H−C(6''a)], 4.38 [brs, 1H, H−C(1''')], 5.34 [d, 1H, ^3J = 7.3 Hz, H−C(1'')], 6.19 [d, 1H, ^4J = 1.8 Hz, H−C(6)], 6.38 [d, 1H, ^4J = 1.8 Hz, H−C(8)], 6.84 [d, 1H, ^3J = 8.2 Hz, H−C(5')], 7.53 [brs, 1H, H-(2')], 7.54 [dd, 1H, ^3J = 7.8 Hz, ^4J = 2.1 Hz, H−C(6')]. ^{13}C NMR: data in **Table 2**.

Peracetyl Derivative of Quercetin 3-O-α-L-Rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**10a**). ^{1}H NMR (400 MHz, CDCl$_3$; COSY) δ/ppm:

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δ 1.10 [d, 3H, ^3J = 6.7 Hz, H−C(6‴)], 1.99−2.43 [10 s, 30 H, 10 CH_3CO], 3.20 [dd, 1H, ^2J = 10.5 Hz, ^3J = 6.4 Hz, H−C(6″ b)], 3.41 [dd, 1H, ^2J = 10.0 Hz, ^3J = 3.4 Hz, H−C(6″a)], 3.61 [1H, dq, ^3J = 10.6 Hz, H−C(5‴)], 3.70 [m, 1H, H−C(5″)], 4.49 [brs, 1H, H−C(1‴)], 4.96 [t, 1H, ^3J = 10.0 Hz, H−C(4‴)], 5.06 [m, 1H, H−C(3‴)], 5.09 [m, 1H, H−C(2‴)], 5.25 [d, 1H, ^3J = 8.1 Hz, H−C(1″)], 5.33 [dd, 1H, ^3J = 8.3 Hz, ^3J = 3.2 Hz, H−C(3″)], 5.36 [d, 1H, ^3J = 2.4 Hz, H−C(4″)], 5.38 [1H, dd, ^3J = 7.8 Hz, ^3J = 4.6 Hz, H−C(2″)], 6.54 [d, 1H, ^4J = 0.5 Hz, H−C(6)], 6.81 [d, 1H, ^4J = 0.7 Hz, H−C(8)], 7.32 [d, 1H, ^3J = 9.0 Hz, H−C(5′)], 7.88 [d, 1H, ^4J = 2.2 Hz, H−C(2′)], 7.94 [dd, 1H, ^3J = 8.5 Hz, ^4J = 2.2 Hz, H−C(6′)].

Quercetin 3-O-α-L-Rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**11**). HRESIMS 633.1381 [calcd for $C_{27}H_{30}O_{16}$Na [M + Na]$^+$: 633.1432]. UV−vis and ESIMS: data in **Table 1**. ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ/ppm: δ 0.99 [d, 3H, ^3J = 6.2 Hz, H−C(6‴)], 3.07 [t, 1H, ^3J = 9.2 Hz, H−C(4‴)], 3.20−3.40 [m (o), 6H, H−C(3″, 4″, 5″, 6″b, 3‴, 5‴)], 3.20 [m, 1H, H−C(2″)], 3.39 [m, 1H, H−C(2‴)], 3.70 [d, 1H, ^2J = 10.3 Hz, H−C(6″a)], 4.38 [brs, 1H, H−C(1‴)], 5.34 [d, 1H, ^3J = 7.4 Hz, H−C(1″)], 6.19 [brs, 1H, H−C(6)], 6.38 [brs, 1H, H−C(8)], 6.84 [d, 1H, ^3J = 8.1 Hz, H−C(5′)], 7.53 [brs, 1H, H-(2′)], 7.54 [dd, 1H, ^3J = 7.8 Hz, ^4J = 2.1 Hz, H−C(6′)]. ^{13}C NMR: data in **Table 2**.

Peracetyl Derivative of Quercetin 3-O-α-L-Rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**11a**). ^{1}H NMR (400 MHz, CDCl$_3$; COSY) δ/ppm: δ 1.06 [d, 3H, ^3J = 6.4 Hz, H−C(6‴)], 1.94−2.43 [10 s, 30 H, 10 CH_3CO], 3.25 [dd, 1H, ^2J = 11.2 Hz, ^3J = 6.1 Hz, H−C(6″ b)], 3.50 [dd, 1H, ^2J = 11.0 Hz, ^3J = 3.0 Hz, H−C(6″a)], 3.55 [ddd, 1H, ^3J = 9.0 Hz, ^3J = 6.1, ^3J = 3.0 Hz, H−C(5‴)], 3.64 [m, 1H, H−C(5″)], 4.51 [brs, 1H, H−C(1‴)], 4.92 [t, 1H, ^3J = 9.3 Hz, H−C(4″)], 4.94 [t, 1H, ^3J = 10.0 Hz, H−C(4‴)], 5.07 [dd, 1H, ^3J = 8.5 Hz, ^3J = 3.4 Hz, H−C(3‴)], 5.10 [brs, 1H, H−C(2‴)], 5.16 [dd, 1H, ^3J = 9.8 Hz, ^3J = 8.0 Hz, H−C(2″)], 5.27 [t, 1H, ^3J = 9.5 Hz, H−C(3″)], 5.38 [d, 1H, ^3J = 8.0 Hz, H−C(1″)], 6.53 [d, 1H, ^4J = 0.7 Hz, H−C(6)], 6.79 [d, 1H, ^4J = 0.5 Hz, H−C(8)], 7.32 [d, 1H, ^3J = 8.6 Hz, H−C(5′)], 7.88 [d, 1H, ^4J = 2.2 Hz, H−C(2′)], 7.91 [dd, 1H, ^3J = 8.6, ^4J = 2.2 Hz, H−C(6′)].

Quercetin 3-O-β-D-Glucopyranoside (**12**). HRESIMS 487.0811 [calcd for $C_{21}H_{20}O_{12}$Na [M + Na]$^+$: 487.0853]. UV−vis and ESIMS: data in **Table 1**. ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ/ppm: δ 3.24 [m, 1H, H−C(5″)], 3.37 [t, 1H, ^3J = 10 Hz, H−C(4″)], 3.45 [t, 1H, ^3J = 8.9 Hz, H−C(3″)], 3.51 [t, 1H, ^3J = 8.3 Hz, H−C(2″)], 3.60 [dd, 1H, ^2J = 11.7 Hz, ^3J = 5.3 Hz, H−C(6″a)], 3.73 [dd, 1H, ^2J = 11.9 Hz, ^3J = 1.2 Hz, H−C(6″b)], 5.28 [d, 1H, ^3J = 7.2 Hz, H−C(1″)], 6.23 [brs, 1H, H−C(6)], 6.42 [brs, 1H, H−C(8)], 6.89 [d, 1H, ^3J = 8.1 Hz, H−C(5′)], 7.61 [d, 1H, ^3J = 8.0 Hz, H−C(6′)], 7.73 [brs, 1H, H−C(2′)]. ^{13}C NMR: data in **Table 2**.

Quercetin 3-O-(6″-Malonyl)glucopyranoside (**13**). HRESIMS 573.0796 [calcd for $C_{24}H_{22}O_{15}$Na [M + Na]$^+$: 573.0857]. UV−vis and ESIMS: data in **Table 1**. ^{1}H and ^{13}C NMR data were consistent with the previously published data (*10*).

Antioxidant Activity Assay. Antioxidant activity of ME, OE, AE, fractions and pure compounds was evaluated according to the modified TEAC assay published by Re et al. (*11*). For this purpose, an ABTS$^{•+}$ solution was prepared by dissolving 38.4 mg of ABTS (7.0 mM) and 6.6 mg of potassium persulfate (2.45 mM) in 10 mL of demineralized water. The stock solution was diluted with ethanol until the absorbance of the solution was finally at 0.7 (λ = 734 nm). An aliquot of 10 μL of each sample was added to 1 mL of the above-mentioned ABTS solution, and the absorbance was measured spectrophotometrically at 734 nm after exactly 6 min. The activities of the extracts were estimated within the range of the dose−response curve of Trolox (0.5−2.0 mM) and expressed as the "Trolox-equivalent antioxidant capacity" (TEAC). The antioxidant activity of the extracts was determined after dilution (depending on their activity) and expressed as μmol of Trolox /g of fruit or solid. All experiments were made by triplicate, and solvent blanks were run in each assay.

Statistical Analyses. Analysis of variance (ANOVA) and Tukey test were performed using the MINITAB v. 15 software. Differences at $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Identification of Flavonols and Anthocyanins. The methanolic extract (ME) of *S. odorifera* fruit peel showed antioxidant activity under ABTS assay. This extract was partitioned between ethyl acetate and water to obtain the corresponding flavonoid-enriched extracts (OE and AE). These extracts showed a comparable and higher antioxidant activity than the crude methanolic extract (**Table 3**).

The organic extract (OE) was fractionated by column chromatography, and the fractions with the highest antioxidant activity were selected for further purification by preparative HPLC. In this way, a mixture of compounds **1** and **2** was isolated and submitted to EIMS, ESIMS and NMR analyses for their identification. From MS data (**Table 1**), the molecular weight of major compound **1** was determined as 448 u, and its structure was constituted by one hexose and one aglycon with a molecular mass of 286 u. In a similar way, compound **2** was identified as a flavonoid constituted by one hexose and one aglycon with 316 u, with a molecular weight of 478 u. ^{1}H NMR data of compound **1** and **2** evidenced the presence of aromatic signals (δ 6.0−8.5 ppm), two anomeric protons (δ 5.28 and 5.44 ppm), one methoxyl group at δ 3.97 ppm, and the signals of oxymethines from the sugars (δ 3.2−3.8 ppm). The integration of anomeric proton signals (5.28 and 5.44) led us to conclude that compounds **1** and **2** were present in a 5:2 mixture, respectively. The more intense signals (from compound **1**) were consistent with the presence of one para-disubstituted aromatic ring, two aromatic protons in meta-position, and one sugar moiety. The careful analysis of H−H COSY spectra led us to differentiate the sugar signals. The analysis of ^{13}C NMR signals (**Table 2**) and their correlations with protons in the HMQC experiment indicated that the sugar was a glucopyranose (*12*). The carbonyl group at $δ_C$ 179.5 ppm indicated the presence of a flavonol with a hydroxyl group at C-5 (*13*). The HMBC experiment evidenced that C-3 ($δ_C$ 135.5 ppm) was glucosylated and gave the information to complete the assignment of all signals. Thus, compound **1** was elucidated as kaempferol 3-O-β-D-glucopyranoside (**Figure 1**), a ubiquitous compound in nature (*14*). Regarding compound **2**, the difference of 30 u in the molecular weight in comparison to compound **1** suggested the presence of a methoxyl group, which was confirmed by the signal at $δ_H$ 3.97 ppm and $δ_C$ 56.8 ppm. The analysis of ^{1}H NMR and H−H COSY spectra revealed that the structural differences between compounds **1** and **2** was placed in the B-ring of flavonol because the C-3 of **2** holds the methoxyl group. The chemical structure of compound **2** was elucidated as isorhamnetin 3-O-α-L-glucopyranoside, which has been isolated from several fruits and vegetables (*14*).

Aqueous extract (AE) was fractionated by an all-liquid technique of countercurrent chromatography to get one hundred fractions. This technique has the advantages of avoiding the loss of polar compounds by adsorption or artifact formation and allowing the complete recovery of sample on a preparative scale (*15*). Based on their UV−vis absorption at λ 520 nm (for anthocyanins) and 350 nm (for flavonols), nine subfractions were obtained. The profiles at both wavelengths were different, indicating that AE contained polyphenols different from anthocyanins (**Figure 2**). All of these fractions were submitted to antioxidant assay (**Table 3**), and those with the highest antioxidant value were purified by preparative HPLC to get eleven pure compounds (**3−13**), which structures were elucidated by spectroscopical means.

From F2, a glycosylated flavonol **3** was isolated by preparative HPLC (**Figure 3A**). The structure of this compound could be determined by means of UV/vis, LC−MS/MS, LC−TOF-MS, and 1D/2D NMR experiments. Compound **3** showed typical UV−vis absorption maxima at 243 and 345 nm expected for a quercetin glycoside. Furthermore compound **3** showed a pseudomolecular ion [M + H]$^+$ with m/z 773 in the ESI$^+$ mode, thus suggesting a molecular mass of 772 u. The exact mass of this compound corresponded with the molecular formula $C_{33}H_{40}O_{21}$. Additional HPLC−MS/MS measurements led to the identification

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Table 3. Antioxidant Activity of Fractions, Flavonols, and Anthocyanins from *S. odorifera* Fruit Peel

sample	amount[a] (g)	antioxidant activity	
		mmol of Trolox/g	μmol of Trolox/100 g of fruit peel
Fractions			
methanolic extract (ME)	187	0.21 ± 0.01 b[b]	—[c]
organic extract (OE)	4.7	1.01 ± 0.06 c	—
aqueous extract (AE)	24.8	1.14 ± 0.04 d	—
F1	35.3	0.68 ± 0.02 e	—
F2	24.1	0.51 ± 0.01 f	—
F3	12.2	0.75 ± 0.01 g	—
F4	23.8	1.14 ± 0.06 d	—
F5	14.8	0.54 ± 0.01 h	—
F6	7.9	0.86 ± 0.02 i	—
F7	18.7	0.40 ± 0.02 j	—
F8	8.5	0.76 ± 0.03 k	—
F9	3.4	0.66 ± 0.02 e	—
F10	169.4	1.21 ± 0.02 l	—
Pure Compounds			
3	30	1.78 ± 0.12 m	53.4
4	1	1.62 ± 0.06 m	1.6
5	3	3.62 ± 0.15 n	10.9
6	40	0.97 ± 0.04 o	38.8
7	40	1.06 ± 0.08 oq	42.4
9	50	0.35 ± 0.04 p	17.5
10	37	1.12 ± 0.03 q	41.4
11	307	1.51 ± 0.02 r	463.7
12	66	1.35 ± 0.11 s	89.1
13	66	2.31 ± 0.10 t	152.5

[a] For fractions: amount obtained from 1 g of AE fractionated. For pure compounds: mg/100 g of fruit peel FW. Values are expressed as means $\pm$ SE from three measurements. [b] Values followed by the same letter within the antioxidant activity column are not significantly different ($p < 0.05$) according to Tukey's multiple range test. Ascorbic acid was used as positive control in agreement with literature (1.10 ± 0.03 TEAC) (37). [c] —, Not determined.

of daughter ions at *m/z* 611, 465 and 303, which indicates the presence of two hexoses and one hexose-methylpentose moiety. The identification of glycosidically bound glucose and rhamnose was confirmed by means of acid hydrolysis followed by HPIC. To further confirm the structure of the aglycon and the type of sugar linkage to the aglycon, 1D and 2D NMR experiments like COSY, DEPT, TOCSY, HMQC, and HMBC spectra were performed. The ^{1}H NMR spectrum of compound **3** showed five characteristic, aromatic protons, thus confirming quercetin as the aglycon and in addition the protons of two hexosyl and one methyl pentose moiety. Two β-D-glucopyranosyl groups with anomeric protons at δ 5.00 ppm (1H, d, *J* = 7.4 Hz, H−C(1‴)) and δ 5.18 ppm (1H, d, *J* = 7.6 Hz, H−C(1″)) showed coupling constants of 7.4 and 7.6 Hz, thus indicating a β-configuration. However the anomeric proton of the rhamnosyl moiety showed a coupling constant of 1.4 Hz, which is well in agreement with the data reported recently for other α-configured rhamnosides (16, 17). The determination of conjugation of the sugar chain was performed by HMBC and TOCSY experiments. In the HMBC spectrum, long-range correlations from H−C(1″) to C(3), H−C-(1⁗) to C(6″) and H−C(1‴) to C(4′) indicated that α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl and β-D-glucopyranosyl moieties were linked at C(3) and C(4′) of quercetin,

respectively. Thus, the structure of **3** was identified as quercetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside-4′-*O*-β-D-glucopyranoside. Although this compound had been reported in *Paliurus spina-christi* (18), *Prunus avium* leaves (19, 20), and Geraniaceae species (21), to the best of our knowledge, complete 1D and 2D NMR data were here characterized for the first time.

Three anthocyanins (**4**, **5** and **8**) were identified on the basis of their UV−visible spectral characteristics (λ_{max} = 511 and 281 or 279 nm). The presence of characteristic fragments of the anthocyanidins in the ESI-MS spectra evidenced that compounds **4** and **5** were derivatives of cyanidin (*m/z* 287) and compound **8** from peonidin (*m/z* 301). Compound **4** was identified as cyanidin-3-*O*-β-D-glucopyranoside and compound **5** as cyanidin-3-*O*-rutinoside by comparison of their NMR spectra with those published in the literature (8, 9) and their chromatographic properties with samples belonging to our lab (5). These anthocyanins are common in fruits and vegetables. The number of organic acids attached to an anthocyanidin could be estimated from UV−vis spectra according to the relation $A_{max\text{-}acyl}/A_{max\text{-}vis}$ ($A_{max\text{-}acyl}$ appears between 310 and 340 nm and $A_{max\text{-}vis}$ between 510 and 520 nm) (22). For the case of anthocyanin **8**, the value of 0.6 indicated that this compound is acylated. Additionally, the relation $A_{440}/A_{max\text{-}vis}$ for this compound was 0.40, which suggested that it is acylated only in the C-3. The loss of 308 u from molecular ion in the ESIMS of compound **8** was in agreement with a *p*-coumaroyl moiety (23), so this compound was tentatively identified as peonidin-3-*O*-(6″-*O*-*p*-coumaroyl)-β-D-glucopyranoside. This anthocyanin has been isolated from *Vitis vinifera*, *Olea europaea*, and *Citrus sinensis*, among others species (24).

From fraction F4 (**Figure 3B**), the isomeric flavonols **6** and **7** were isolated exhibiting identical ESIMS spectra. The ions at *m/z* 665 [M + K]$^+$ and 649 [M + Na]$^+$ were concordant with a molecular weight of 626 u for both compounds. The fragment ions at *m/z* 465 and 303 correspond to the subsequent losses of two hexose units. Fragment at *m/z* 303 corresponds to quercetin and this observation was supported by UV−vis (absorption maxima in λ 350 and 268 nm), ^{1}H and ^{13}C NMR spectra of compounds **6** and **7** (25). In contrast, some differences were present in the region of sugar oxymethines for these compounds. For compound **6**, two anomeric protons were present (δ 5.40 and 4.86 ppm) exhibiting diaxial coupling constant (7.7 and 7.1 Hz, respectively). The most remarkable difference was the presence of a doublet signal at 3.66 ppm (*J* = 2.9 Hz) which correlates with the signal at 3.37 ppm (dd, *J* = 9.0, 3.2 Hz) in the ^{1}H−^{1}H COSY experiment. This signal also correlates with the double doublet at 3.55 ppm (*J* = 9.2, 7.8 Hz), which was assigned to the C-2 position of a hexose, based on its coupling to the anomeric proton at δ 5.40 ppm. These data suggested that this hexose is galactose (26). All the ^{1}H and ^{13}C NMR signals were assigned on the basis of HSQC, HMBC, and TOCSY experiments. The signals that correlate with the other anomeric proton at δ 4.86 ppm were in agreement with those of glucose (13). The identity of two hexoses was confirmed by comparison of the retention time and spectral pattern of their TMSi derivatives, after hydrolysis of compound **6**, with those exhibited by authentical samples in GC−MS analyses. According to DeJongh et al. (27), in the spectra of 1,2,3,4,6-penta-*O*-trimethylsilyl-glucopyranose the fragment ion at *m/z* 204 [TMSiO − CHCH − OTMSSi]$^{+\bullet}$ is the most intense; in contrast, the ion at *m/z* 217 [TMSiO − CH=CHCH − OTMSi]$^+$ predominates in the spectrum of 1,2,3,4,6-penta-*O*-trimethylsilyl-galactofuranoside. The glycosidic linkages were determined by the correlations in the HMBC spectra between the anomeric proton at δ 5.40 ppm and the signal in δ_C 134.0 ppm (C-3), and the other anomeric proton at δ 4.87 with the carbon at δ_C 147.5 ppm (C-4′). These correlations were confirmed by

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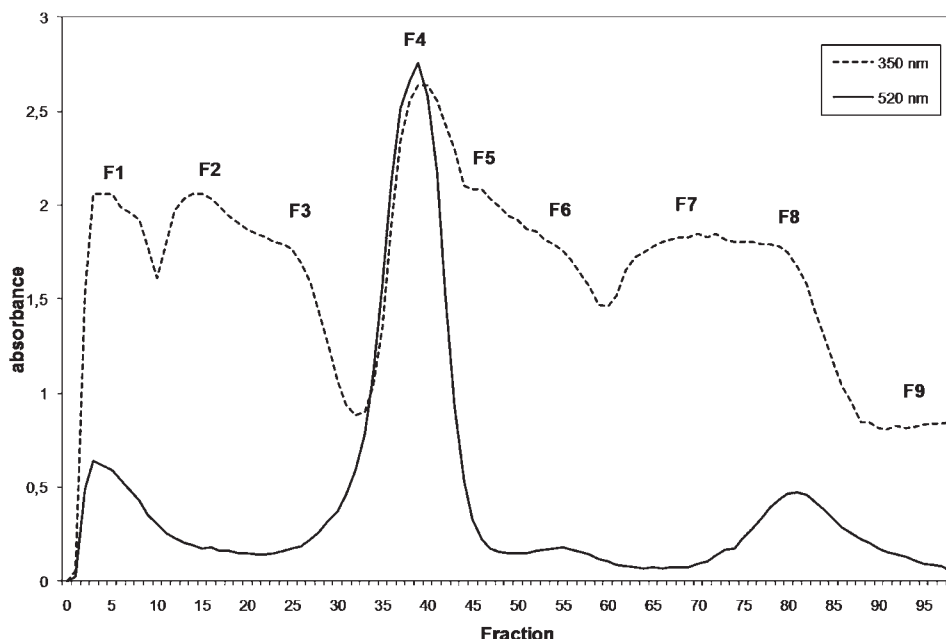*J. Agric. Food Chem.,* Vol. 59, No. 3, 2011 **981**

Figure 2. MLCCC separation of flavonols (λ = 350 nm) and anthocyanins (λ = 520 nm) from *S. odorifera* fruit peel. Solvent system: TBME–*n*-butanol–acetonitrile–water (2:2:1:5, v/v/v/v, acidified with 0.1% TFA, v/v), flow rate 1.0 mL/min.
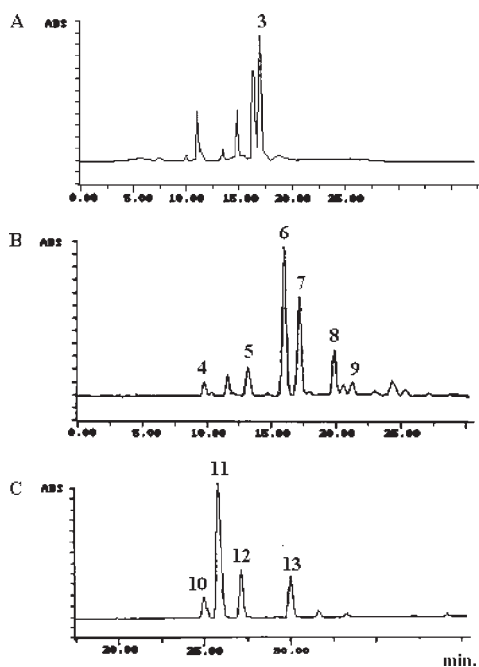

Figure 3. HPLC analyses (λ = 350 nm) of *S. odorifera* fruit peel fractions F2 (**A**), F4 (**B**), and F10 (**C**). Peak numbers correspond to the compound numbers in **Figure 1**.

ROESY experiment. By irradiation of H-1″ of galactose (δ 5.40 ppm) an enhancement in the signal of OH at C-5 of quercetin was observed. Additionally, by irradiation of anomeric proton of glucose (δ 4.87 ppm), a strong increase of the H-5′ (δ 7.18 ppm) of quercetin appeared. Thus, compound **6** was identified as quercetin 3-*O*-β-D-galactopyranoside-4′-*O*-β-glucopyranoside. This compound has been only reported as constituent of the leaves of *Culcitium reflexum* HBK (*28*). A similar analysis had been performed with compound **7**, finding that two hexoses were glucose; so, the structure of this compound was elucidated as quercetin 3,4′-di-*O*-β-D-glucopyranoside, a common flavonol glycoside in nature (*25, 29*).

The HRESI-MS spectrum in positive mode for compound **9** showed an adduct ion in m/z 735.1336 which was in agreement with the formula $C_{30}H_{32}O_{20}Na$ [M + Na]$^+$. The molecular ion at m/z 712 was confirmed also by the fragments in low resolution ESI-MS spectrum: m/z 751 [M + K]$^+$, 735 [M + Na]$^+$, and 713 [M + H]$^+$. Quercetin as aglycon was suggested because of the ion in m/z 303 and UV–vis data (λ 347 and 265 nm) of this compound. The fragment ions at m/z 649 [M − 86 + Na]$^+$, 465 [M − 162−86 + H]$^+$, and 303 [M − 162 − 86 − 162 + H]$^+$ evidenced the presence of two hexoses and one malonyl group (86 u) (*30*). The ^{1}H and ^{13}C NMR data of compound **9** resembled those of compound **7**, with the exception of the signals at δ_C 166.6 ppm (C=O), 167.7 ppm (COOH), 41.2 ppm (CH$_2$), and δ_H 3.11 ppm (s), which are in agreement with that the oxymethylene protons of one malonyl group. It is remarkable that the protons of oxymethylene of glucose attached to C-3 of quercetin suffered a *ca.* 0.6 ppm lowfield shift due to malonylation in comparison to the data exhibited by compound **7**. This fact indicates that the malonyl group is attached to C-6 of 3-*O*-glucosyl moiety, and was confirmed by the correlation between the carbonyl (δ_C 166.6) from the malonyl group and H-6″ (δ_H 4.22) of the β-glucopyranosyl in the HMBC spectra. The other long-range correlations in this spectrum, between H-1″ (δ_H 5.42 ppm) and C-3 (δ_C 133.5 ppm) and H-1‴ (δ_H 4.87 ppm) and C-4′ (δ_C 147.4 ppm), confirmed the bonds between quercetin and the two glucose moieties. All the ^{1}H and ^{13}C NMR signals were assigned on the basis of HSQC, HMBC, and TOCSY experiments. Thus, compound **9** was determined to be quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucopyranoside 4′-*O*-β-D-glucopyranoside. To our knowledge this is the first time that this compound is reported in nature.

HPLC profile of fraction F10 is presented in **Figure 3C**. The ESI-MS spectra of compounds **10** and **11** were identical (649 [M + K]$^+$, 633 [M + Na]$^+$), indicating that these compounds are isomeric quercetin derivatives with a MW of 610 u. The fragment ion at m/z 303 [M − 162 − 146 + H]$^+$ suggested that quercetin was attached to one hexose and one deoxyhexose. The presence of deoxyhexose was confirmed by NMR data. The signal at δ_C 17.7 ppm (characteristic of rhamnose) correlated with the signal

at δ_H 0.99 ppm in the HMQC spectra. By comparison of NMR data of compounds **10** and **11** with those of compounds **6**, **7**, and **9**, it was suggested that sugar moiety was attached to C-3 of quercetin. For the case of compound **10**, the correlation between the anomeric carbon of deoxyhexose (δ_C 100.7 ppm) and H−C-(6″) (δ_H 3.71 ppm), and the correlation between the anomeric proton of deoxyhexose (δ_H 4.38 ppm) and the C-6 of hexose (δ_C 67.0 ppm) in the HMBC spectra evidenced the 1→6 linkage of the two sugars. With the aim to stablish the identity of hexoses and deoxyhexoses in compounds **10** and **11**, these compounds were acetylated with acetic anhydride and pyridine to obtain the compounds **10a** and **11a**, respectively. The ^{1}H NMR spectra of these compounds showed a better resolution of sugar signals. The presence of signals at δ_H 5.36 ppm (d, $J = 2.4$ Hz, H−C(4″)) and 5.33 ppm (dd, $J = 8.3$ and 3.2 Hz, H−C(3″)) in the spectra of acetylated compound **10a** confirmed that the hexose was galactose (*31*). The comparison of ^{1}H NMR data of **10a** and **11a** with those reported in the literature (*32*) allow us to conclude that deoxyhexose was rhamnose. Based on the spectroscopic analysis of compound **10**, its structure was established as quercetin 3-*O*-α-ʟ-rhamnopyranosyl-(1→6)-β-ᴅ-galactopyranoside. For the case of **11**, the hexose was defined as glucose by comparison with literature (*32*, *33*). Thus, its structure was defined as quercetin 3-*O*-α-ʟ-rhamnopyranosyl-(1→6)-β-ᴅ-glucopyranoside. In a similar way, the structures of compounds **12** and **13** were elucidated to be quercetin-3-*O*-β-ᴅ-glucopyranoside (isoquercitrin) and quercetin-3-*O*-(6″-*O*-malonyl)-β-ᴅ-glucopyranoside, respectively (*14*).

Antioxidant Activity. Pure compounds (**3**−**7** and **9**−**13**) were submitted to ABTS assay in order to evaluate their antioxidant activity. The other compounds were not evaluated because they were characterized as part of a mixture. ABTS assay was used because this radical reacts rapidly with not only hydrophilic but also lipophilic antioxidants, and also it can be used over a wide pH range (*34*). The results of these analyses are shown in **Table 3**. Here, the compounds that more contributed to the antioxidant activity of *S. odorifera* fruit peel were quercetin 3-*O*-α-ʟ-rhamnopyranosyl-(1→6)-β-ᴅ-glucopyranoside (**11**), quercetin-3-*O*-(6″-malonyl)-glucopyranoside (**13**), quercetin-3-*O*-β-ᴅ-glucopyranoside (**12**), and quercetin-3-*O*-α-ʟ-rhamnopyranosyl-(1→6)-β-ᴅ-glucopyranoside-4′-*O*-β-ᴅ-glucopyranoside (**3**). Regarding antioxidant activity of pure compounds, the anthocyanins exhibited higher values in comparison to the corresponding glycosidated flavonoids (compound **4** vs compound **12**, and compound **5** vs compound **11**). The cyanidin rutinoside (**5**) exhibited a higher antioxidant activity than cyanidin glucoside (**4**) according to the data published by Rice-Evans et al. (*35*).

Among flavonols, the quercetin-3-*O*-(6″-malonyl)-glucopyranoside (**13**) presented the highest antioxidant activity following by quercetin-3-*O*-α-ʟ-rhamnopyranosyl-(1→6)-β-ᴅ-glucopyranoside-4′-*O*-β-ᴅ-glucopyranoside (**3**) and quercetin 3-*O*-α-ʟ-rhamnopyranosyl-(1→6)-β-ᴅ-glucopyranoside (**11**). It is important to point out that the simultaneous glycosidation of C-3 and C-4′ of quercetin in compounds **6** and **7** reduced the antioxidant activity in comparison to compound **12**. Quercetin is a typical flavonoid ubiquitously present in fruits as glycosides; taking into account the possible benefits on human health, their bioavailability has been studied (*36*).

In conclusion, flavonol glycosides and anthocyanins present in fruit peel of *S. odorifera* exhibited *in vitro* antioxidant activity. The use of appropriate separation techniques allows the isolation of different isomeric flavonol glycosides. These results showed the potential of fruit peel of *S. odorifera*, a waste material, as innovative source of antioxidant compounds.

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