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Antioxidative Phenolic Compounds Isolated from Almond Skins (*Prunus amygdalus* Batsch)

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Nine phenolic compounds were isolated from the ethyl acetate and *n*-butanol fractions of almond (*Prunus amygdalus*) skins. On the basis of NMR data, MS data, and comparison with the literature, these compounds were identified as 3′-*O*-methylquercetin 3-*O*-β-D-glucopyranoside (**1**); 3′-*O*-methylquercetin 3-*O*-β-D-galactopyranoside (**2**); 3′-*O*-methylquercetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**3**); kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**4**); naringenin 7-*O*-β-D-glucopyranoside (**5**); catechin (**6**); protocatechuic acid (**7**); vanillic acid (**8**); and *p*-hydroxybenzoic acid (**9**). All of these compounds have been isolated from almond skins for the first time. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities for compounds **1**−**9** were determined. Compounds **6** and **7** show very strong DPPH radical scavenging activity. Compounds **1**−**3**, **5**, **8**, and **9** show strong activity, whereas compound **4** has very weak activity.

KEYWORDS: Almond skins; *Prunus amygdalus*; phenolic constituents; DPPH; antioxidant activity

INTRODUCTION

In recent studies, a growing interest in biology and medicine has been focused on oxidative stress from the viewpoint of its participation in several diseases such as arteriosclerosis (*1*), cancer (*2*), and aging (*3*). Research has pointed out that the most effective method to reduce oxidative stress is antioxidant supplementation. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (*4*). When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life (*5*). However, possible toxicity as well as general consumer rejection led to decreasing use of synthetic antioxidants. Much work has been done to find safe and potent natural antioxidants from various plant sources (*6*). As one potential source, plant phenolics have primary (chain-breaking) antioxidant activity (*7*). Phenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (*8*). Crude extracts of fruits, herbs, vegetables, cereals, nuts, and other plant materials rich in phenolics are increasingly of interest in the food industry. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers (*9*).

Food plants including fruits, vegetables, nuts, and spices are the primary sources of naturally occurring antioxidants for humans. The antioxidative activity of many plants has been evaluated, and their antioxidants have been identified. Almonds (*Prunus amygdalus* Batsch), which belong to the Rosaceae family that also includes apples, pears, prunes, and raspberries (*10*), are one of the most popular tree nuts on a worldwide basis and rank number one in tree nut production. They are typically used as snack foods and as ingredients in a variety of processed foods, especially in bakery and confectionery products. The peach-like almond fruit consists of the edible seed or kernel, the shell, and the outer hull. At maturity the hull splits open. When dry, it may be readily separated from the shell. The almond pit, containing a kernel or edible seed, is the nut of commerce. Shelled almonds may be sold as whole natural almonds or processed into various almond forms. The whole natural almonds have had their shells removed but still retain their brown skins; blanched whole almonds have had both their shells and skins removed (*10*, *11*). Usually, the removed skins will be discarded. However, much study has shown that peanut skins are a rich source of phenolic compounds (*12*, *13*). This prompted us to investigate the chemical composition of almond skins. In this paper, we described the isolation and structure elucidation of nine phenolic compounds (**1**−**9**) from the skins

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of almond (*P. amygdalus*). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities for these compounds were also determined.

MATERIALS AND METHODS

General Procedures. ^{1}H (400 and 600 MHz), ^{13}C (100 and 150 MHz), and 2D NMR spectra were obtained on Varian AM-600 and AM-400 NMR spectrometers and with TMS as internal reference. Atmospheric pressure chemical ionization mass spectra (APCI MS) were obtained on a Fisons/VG Platform II mass spectrometer. Preparative thin-layer chromatography was performed on Sigma-Aldrich TLC plates (1000 μm thickness, 2−25 μm particle size). Thin-layer chromatography was performed on Sigma-Aldrich TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in ethanol solution.

Plant Material. Almond skin samples were supplied by the Almond Board of California. A voucher specimen (HS17) was deposited in the Department of Food Science, Cook College, Rutgers University.

Extraction and Isolation Procedures. The dried almond skins (1.5 kg) were extracted with 95% ethanol (4 L) at 50 °C for 1 day. The extract was concentrated to dryness under reduced pressure, and the residue was suspended in water (250 mL) and partitioned successively with hexane (3 × 250 mL), ethyl acetate (3 × 250 mL), and *n*-butanol (3 × 250 mL). The ethyl acetate fraction (4 g) was subjected to silica gel column chromatography with an ethyl acetate/MeOH/H_2O (20:1:1−5:1:1) solvent system to give fractions 1−11. Fraction 1 eluted by ethyl acetate/MeOH/H_2O (20:1:1) was subjected to Sephadex LH-20 column chromatography with 95% EtOH to give compounds **6** (60 mg) and **7** (100 mg). Fraction 2, also eluted with ethyl acetate/MeOH/H_2O (20:1:1), was isolated by a preparative TLC plate eluted with $CHCl_3$/MeOH/H_2O (7:1:0.1) to give compounds **8** (20 mg) and **9** (15 mg). Fraction 3, eluted by ethyl acetate/MeOH/H_2O (10:1:1), was subjected to Sephadex LH-20 eluted by 95% EtOH to afford three subfractions (I−III). Thirty milligrams of compound **5** was obtained from subfraction I using a silica gel column eluted with $CHCl_3$/MeOH/H_2O (4:1:0.1). Subfraction II was rechromatographed on a silica gel column eluted by $CHCl_3$/MeOH/H_2O (4:1:0.1) to give four subfractions (A−D). Further chromatography of subfractions B and C on Sephadex LH-20 eluted by 95% EtOH yielded compound **1** (20 mg) and compound **2** (10 mg), respectively. Fractions 10 and 11 eluted with ethyl acetate/MeOH/H_2O (5:1:1) were combined together and rechromatographed on a silica gel column eluting with $CHCl_3$/MeOH/H_2O (3:1:0.15) to give compounds **3** (100 mg) and **4** (10 mg).

The *n*-butanol fraction (800 mg) was subjected to silica gel column chromatography with $CHCl_3$/MeOH/H_2O (4:1:0.15−3:1:0.15) solvent system to give fractions 1−3. Fraction 3, eluted by $CHCl_3$/MeOH/H_2O (3:1:0.15), was subjected to Sephadex LH-20 column chromatography with 95% EtOH to give compound **3** (50 mg).

Spectral Identification of Known Compounds. *3′-O-Methylquercetin 3-O-β-D-glucopyranoside (**1**):* yellow powder; APCI-MS, *m*/*z* 477 [M − H]$^-$, 315 [M − 162 − H]$^-$; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.96 (H-2′, d, J = 1.8 Hz), 7.59 (H-6′, dd, J = 8.0, 1.8 Hz), 6.92 (H-5′, d, J = 8.0 Hz), 6.39 (H-8, d, J = 1.8 Hz), 6.20 (H-6, d, J = 1.8 Hz), 5.40 (glc H-1, d, J = 7.2 Hz), 3.92 (OCH_3, s), 3.20−3.80 (6H, glc H-2 - H-6, m); ^{13}C NMR (CD_3OD) δ 179.2 (C-4, s), 166.9 (C-7, s), 162.9 (C-5, s), 158.5 (C-2, s), 158.5 (C-9, s), 150.8 (C-3′, s), 148.4 (C-4′, s), 135.2 (C-3, s), 123.8 (C-6′, d), 123.1 (C-1′, s), 116.1 (C-5′, d), 114.3 (C-2′, d), 105.5 (C-10, s), 103.6 (glc C-1, d), 100.2 (C-6, d), 94.9 (C-8, d), 78.5 (glc C-3, d), 78.1 (glc C-5, d), 75.9 (glc C-2, d), 71.4 (glc C-4, d), 62.5 (glc C-6, t), 56.7 (OCH_3, q).

*3′-O-Methylquercetin 3-O-β-D-galactopyranoside (**2**):* yellow powder; APCI-MS, *m*/*z* 477 [M − H]$^-$, 315 [M − 162 − H]$^-$; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.04 (H-2′, d, J = 2.0 Hz), 7.59 (H-6′, dd, J = 8.4, 2.0 Hz), 6.91 (H-5′, d, J = 8.4 Hz), 6.39 (H-8, d, J = 1.8 Hz), 6.20 (H-6, d, J = 1.8 Hz), 5.32 (gal H-1, d, J = 8.0 Hz), 3.97 (OCH_3, s), 3.40−3.90 (6H, gal H-2 - H-6, m); ^{13}C NMR (CD_3OD) δ 179.3 (C-4, s), 167.3 (C-7, s), 162.9 (C-5, s), 158.4 (C-2, s), 158.4 (C-9, s), 150.8 (C-3′, s), 148.4 (C-4′, s), 135.3 (C-3, s), 123.6 (C-6′, d), 123.0 (C-1′, s), 115.9 (C-5′, d), 114.4 (C-2′, d), 105.3 (C-10, s), 104.4 (gal C-1, d), 100.3 (C-6, d), 95.0 (C-8, d), 77.2 (gal C-5, d), 74.9 (gal C-3, d), 73.1 (gal C-2, d), 70.0 (gal C-4, d), 62.1 (gal C-6, t), 56.9 (OCH_3, q).

*3′-O-Methylquercetin 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**3**):* yellow powder; APCI-MS, *m*/*z* 623 [M − H]$^-$, 477 [M − 146 − H]$^-$, 315 [M − 146 − 162 − H]$^-$; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.95 (H-2′, d, J = 2.0 Hz), 7.61 (H-6′, dd, J = 8.4, 2.0 Hz), 6.92 (H-5′, d, J = 8.4 Hz), 6.41 (H-8, d, J = 2.0 Hz), 6.20 (H-6, d, J = 2.0 Hz), 5.25 (glc H-1, d, J = 7.2 Hz), 4.54 (rha H-1, brs), 3.96 (OCH_3, s), 3.20−3.90 (10H, m), 1.11 (rha H-6, d, J = 6.0 Hz); ^{13}C NMR (CD_3OD) δ 179.2 (C-4, s), 166.4 (C-7, s), 162.9 (C-5, s), 158.6 (C-2, s), 158.4 (C-9, s), 150.8 (C-3′, s), 148.3 (C-4′, s), 135.3 (C-3, s), 123.8 (C-6′, d), 123.0 (C-1′, s), 116.1 (C-5′, d), 114.4 (C-2′, d), 105.6 (C-10, s), 102.6 (glc C-1, d), 100.1 (rha C-R_1, d), 100.0 (C-6, d), 94.9 (C-8, d), 78.2 (glc C-3, d), 77.3 (glc C-5, d), 75.9 (glc C-2, d), 74.0 (rha C-4, d), 72.1 (glc C-4, d), 72.0 (rha C-3, d), 71.6 (rha C-2, d), 69.8 (rha C-5, d), 68.7 (glc C-6, t), 56.7 (OCH_3, q), 17.9 (rha C-6, q).

*Kaempferol 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**4**):* yellow powder; APCI-MS, *m*/*z* 593 [M − H]$^-$, 447 [M − 146 − H]$^-$, 285 [M − 146 − 162 − H]$^-$; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.07 (H-2′,6′, d, J = 8.8 Hz), 6.90 (H-3′,5′, d, J = 8.8 Hz), 6.40 (H-8, d, J = 1.8 Hz), 6.21 (H-6, d, J = 1.8 Hz), 5.15 (glc H-1, d, J = 7.6 Hz), 4.54 (rha H-1, s), 3.20−3.90 (10H, m), 1.14 (rha H-6, d, J = 5.8 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 179.5 (C-4, s), 166.4 (C-7, s), 163.0 (C-5, s), 161.6 (C-4′, s), 159.5 (C-9, s), 158.6 (C-2, s), 135.6 (C-3, s), 132.5 (C-2′,6′, d), 122.9 (C-1′, s), 116.2 (C-3′,5′, d), 105.7 (C-10, s), 104.8 (glc C-1, d), 100.4 (rha C-1, d), 100.2 (C-6, d), 95.1 (C-8, d), 78.3 (glc C-3, d), 77.3 (glc C-5, d), 75.9 (glc C-2, d), 74.0 (rha C-4, d), 72.4 (glc C-4, d), 72.2 (rha C-3, d), 71.6 (rha C-2, d), 69.8 (rha C-5, d), 68.7 (glc C-6, t), 18.0 (rha C-6, q).

*Naringenin 7-O-β-D-glucopyranoside (**5**):* yellow power; APCI-MS, *m*/*z* 433 [M − H]$^-$, 271 [M − 162 − H]$^-$; ^{1}H NMR (CD_3OD, 600 MHz) δ 7.32 (H-2′,6′, d, J = 8.4 Hz), 6.82 (H-3′,5′, d, J = 8.4 Hz), 6.22 (H-8, brs), 6.20 (H-6, brs), 5.38 (H-2, brd, J = 13.2 Hz), 4.98 (glc H-1, d, J = 7.2 Hz), 3.88 (glc H-6b, brd, J = 12.0 Hz), 3.70 (glc H-6a, m), 3.30−3.50 (4H, glc H-2 − H-5, m), 3.17 (H-3b, dd, J = 13.2, 16.8 Hz), 2.75 (H-3a, brd, J = 16.8 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 198.6 (C-4, s), 167.1 (C-7, s), 165.0 (C-5, s), 164.7 (C-9, s), 159.2 (C-4′, s), 130.9 (C-1′, s), 129.2 (C-2′,6′, d), 116.4 (C-3′,5′, d), 103.5 (C-10, s), 101.3 (glc C-1, d), 98.1 (C-6, d), 97.0 (C-8, d), 80.8 (C-2, d), 78.3 (glc C-3, d), 77.9 (glc C-5, d), 74.7 (glc C-2, d), 71.2 (glc C-4, d), 62.4 (glc C-6, t), 44.2 (C-3, t).

*Catechin (**6**):* white powder; APCI-MS, *m*/*z* 289 [M − H]$^-$; ^{1}H NMR (CD_3OD, 600 MHz) δ 2.53 (H-4a, dd, J = 16.1, 8.2 Hz), 2.87 (H-4b, dd, J = 16.1, 5.2 Hz), 4.02 (H-3, m), 4.60 (H-2, d, J = 7.3 Hz), 5.87 (H-6, d, J = 2.2 Hz), 5.96 (H-8, d, J = 2.2 Hz), 6.74 (H-6′, dd, J = 1.5, 8.4), 6.78 (H-5′, d, J = 8.4), 6.85 (H-2′, d, J = 1.5 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 28.4 (C-4, t), 68.8 (C-3, d), 82.8 (C-2, d), 95.7 (C-8, s), 96.5 (C-6, d), 101.0 (C-4a, s), 115.3 (C-2′, d), 116.3 (C-5′, d), 120.2 (C-6′, d), 132.2 (C-1′, s), 146.2 (C-3′, 4′, s), 156.9, 157.5, 157.8 (C-5, C-7, C-8a, s) [identical with the literature (*14*)].

*Protocatechuic acid (**7**):* white powder; APCI-MS, *m*/*z* 153 [M − H]$^-$; ^{1}H NMR (CD_3OD, 600 MHz) δ 6.75 (H-5, d, J = 8.0 Hz), 7.39 (H-6, dd, J = 2.0, 8.0 Hz), 7.43 (H-2, d, J = 2.0 Hz) [identical with the literature (*15*)].

*Vanillic acid (**8**):* white powder; APCI-MS, *m*/*z* 167 [M − H]$^-$; ^{1}H NMR (CD_3OD, 200 MHz) δ 6.85 (H-5, d, J = 9.0 Hz), 7.56 (H-2, H-6, m), 3.92 (OCH_3, s) [identical with the literature (*16*)].

*p-Hydroxybenzoic acid (**9**):* white powder; APCI-MS, *m*/*z* 137 [M − H]$^-$; ^{1}H NMR (CD_3OD, 600 MHz) δ 6.74 (H-4,6, d, J = 8.4 Hz), 7.83 (H-3,7, d, J = 8.4 Hz) [identical with the literature (*17*)].

Determination of the DPPH Radical Scavenging Capacity. This method was adapted from that of Chen and Ho (*18*). DPPH radicals were prepared in ethanol as a 2.0 × 10^{-4} M solution. This DPPH solution (final concentration = 1.0 × 10^{-4} M) was mixed with different tested compounds (final concentrations were 100, 50, 20, and 2 μM) and kept in a dark area for 0.5 h. The absorbance of the samples was measured on a spectrophotometer (Milton Roy, model 301) at 517 nm against a blank of ethanol without DPPH. All tests were run in triplicate and averaged.

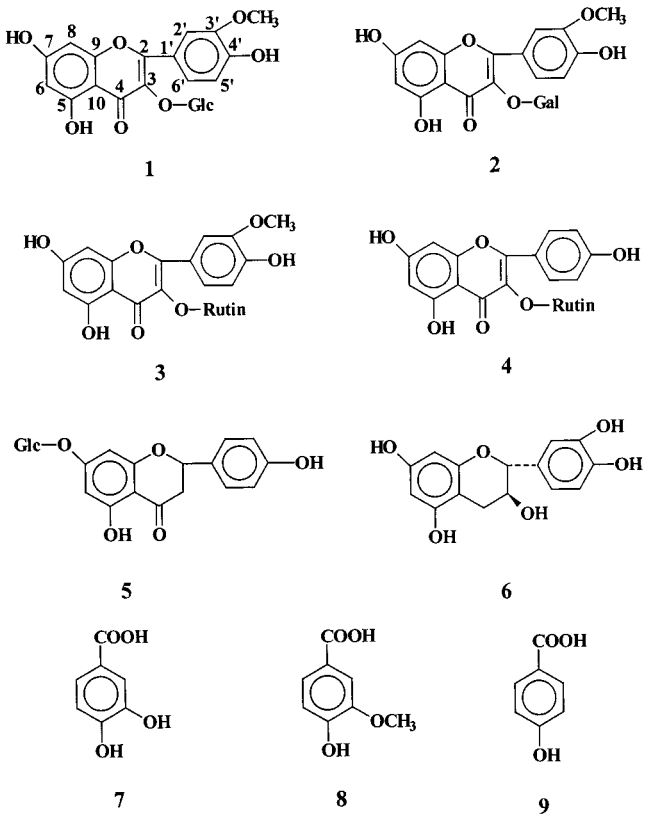

Figure 1. Structures of compounds **1**–**9**.

RESULTS AND DISCUSSION

The ethyl acetate and *n*-butanol fractions of almond skin extract were chromatographed successively on silica gel, Sephadex LH-20, and preparative TLC plate to afford compounds **1**−**9** (**Figure 1**). Their structures were established by interpretation and full assignments of 1D and 2D NMR spectroscopic data, APCI-MS data, and comparison with literature data.

Among these nine compounds, compounds **1**−**5** are flavonol glycosides. The molecular formulas $C_{22}H_{22}O_{12}$ for **1**, $C_{22}H_{22}O_{12}$ for **2**, $C_{28}H_{32}O_{16}$ for **3**, $C_{27}H_{30}O_{15}$ for **4**, and $C_{21}H_{22}O_{10}$ for **5** were determined by negative APCI-MS and ^{13}C NMR analysis. Their ^{1}H and ^{13}C NMR spectra indicated that compounds **1**−**3** had quercetin as aglycon, compound **4** had kaempferol, and compound **5** had naringenin.

The ^{1}H NMR spectrum of **1** showed one methoxyl group at δ 3.92, one anomeric proton at δ 5.40 (glc H-1, d, $J = 7.2$ Hz), and the signals of quercetin at δ 7.96 (H-2′, d, $J = 1.8$ Hz), 7.59 (H-6′, dd, $J = 8.0$, 1.8 Hz), 6.92 (H-5′, d, $J = 8.0$ Hz), 6.39 (H-8, d, $J = 1.8$ Hz), and 6.20 (H-6, d, $J = 1.8$ Hz). This was confirmed by the ^{13}C NMR spectrum, which exhibited 22 carbon signals, with 15 representing the aglycon, 1 for the methoxyl group (δ 56.7), and 6 for the glucopyranose unit [δ 103.6 (glc C-1, d), 78.5 (glc C-3, d), 78.1 (glc C-5, d), 75.9 (glc C-2, d), 71.4 (glc C-4, d), 62.5 (glc C-6, t). The β-anomeric configuration for the glucose was judged from its large $^{3}J_{H1,H2}$ coupling constants ($J = 7.6$ Hz) (*19*). The NMR data of **1** were identical with those of 3′-*O*-methylquercetin 3-*O*-β-D-glucopyranoside (*20*), which has been confirmed by the 2D NMR. Thus, the structure 3′-*O*-methylquercetin 3-*O*-β-D-glucopyranoside was assigned to **1**.

Compound **2** has the same molecular formula as **1**. The spectral data of **2** showed that it possessed the same aglycon as that of **1** but differed from the saccharide structure of **1**. From a comparison of the NMR data with the literature (*19*), the sugar of **2** is β-galactopyranose instead of the β-glucopyranose of **1**. Therefore, **2** was identified as 3′-*O*-methylquercetin 3-*O*-β-D-galactopyranoside.

The spectral data of **3** showed that it possessed the same aglycon as that of **1** but differed from the saccharide structure of **1**. The molecular weight of **3** was 146 mass units greater than that of **1**, indicating that **3** had one more rhamnopyranosyl unit than **1**. This was confirmed by its ^{1}H [δ5.25 (glc H-1, d, $J = 7.2$ Hz), 4.54 (rha H-1, brs), 1.11 (rha H-6, d, $J = 6.0$ Hz)] and ^{13}C [δ102.6 (glc C-1, d), 100.1 (rha C-1, d)] NMR data, respectively**.** The rhamnose was attached to the hydroxyl group at the C-6 position of glucose as judged from the downfield shift (6.6 ppm) of the C-6 signal. Furthermore, the NMR data of **3** were identical with those of 3′-*O*-methylquercetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (*20*), which have been confirmed by the 2D NMR. Thus, the structure 3′-*O*-methylquercetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside was assigned to **3**.

The spectral data of **4** showed that it possessed the same saccharide structure as that of **3** but differed from the aglycon part. The aglycon of **4** is the very common flavonol kaempferol. Therefore, the structure kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside was assigned to **4**.

Compound **5** was a flavanone glycoside. Its ^{1}H NMR data indicated the presence of one methylene group [δ 3.17 (dd, $J = 13.2$, 16.8 Hz), 2.75 (brd, $J = 16.8$ Hz)] and one oxygen-substituted methine group at δ 5.38 (brd, $J = 13.2$ Hz). The proton spectrum also showed six aromatic protons, two of which

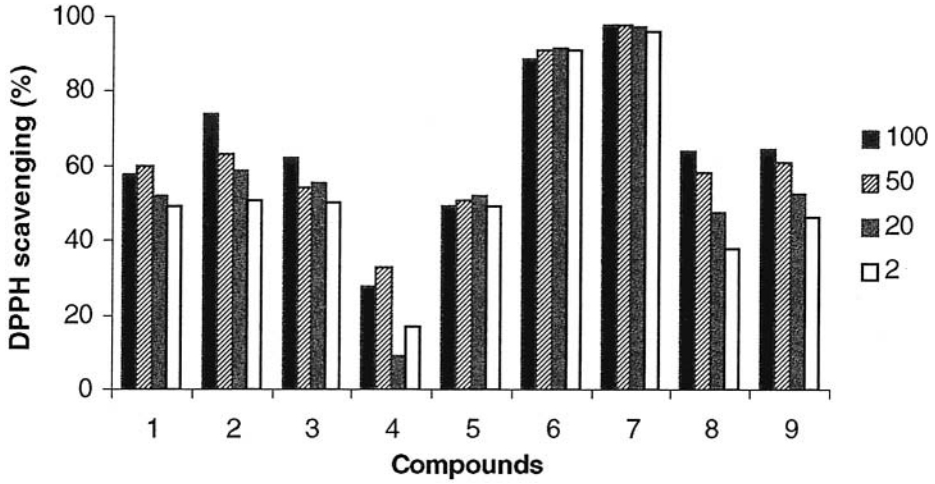

Figure 2. DPPH scavenging effects (percent) of compounds **1**–**9** in four different concentrations (100, 50, 20, and 2 μM).

appeared as broad singlets at δ 6.22 and 6.20 and four as doublets at δ 7.32 (2H) and 6.82 (2H). The ^{13}C NMR spectrum showed 12 aromatic carbons between δ 97.0 and 167.1, a carbonyl carbon at δ 198.6, 1 oxygen-substituted methine at δ 80.8, and 1 methylene in the aliphatic region at δ 44.2. All of these data are consistent with naringenin (*21*, *22*). In addition, the ^{1}H and ^{13}C NMR spectra of **5** also showed the existence of one glucopyranoyl unit. The proton NMR showed one anomeric proton at δ 4.98 (d, J = 7.2 Hz). In the ^{13}C NMR spectrum, among the 21 carbon signals, 6 were for the glucopyranose unit [δ 103.6 (glc C-1, d), 78.5 (glc C-3, d), 78.1 (glc C-5, d), 75.9 (glc C-2, d), 71.4 (glc C-4, d), 62.5 (glc C-6, t)]. The β-anomeric configuration for the glucose was judged from its large $^3J_{H1,H2}$ coupling constants (J = 7.2 Hz) (*19*). HMBC correlation between C-7 (δ 167.1) and glc H-1 (δ 4.98 d, J = 7.2 Hz) suggested that the β-glucopyranose unit was attached at the hydroxyl group of the C-7 position of the aglycon. Thus, compound **5** was identified as naringenin 7-*O*-β-D-glucopyranoside (prunin). Its NMR data were identified with those in the literature (*22*).

Compounds **6**−**9** were identified as catechin (**6**), protocatechuic acid (**7**), vanillic acid (**8**), and *p*-hydroxybenzoic acid (**9**) by comparison of their NMR and MS data with those reported in the literature (*14*−*17*).

According to this paper, almond skins are also a natural source of phenolic compounds. The antioxidative activities of isolated compounds were measured. The scavenging effect of these compounds is shown in **Figure 2**. Compounds **6** and **7** show very strong DPPH radical scavenging activity. Compounds **1**−**3**, **5**, **8**, and **9** show strong activity, whereas compound **4** has very weak activity. Phenolic compounds are widely distributed in nature. The antioxidant potential of phenolic compounds depends on the number and arrangement of the hydroxyl groups and the extent of structural conjugation (*23*, *24*). For flavonoid compounds, *o*-dihydroxy groups in the B-ring, the presence of a C2−3 double bond in conjunction with 4-oxo in the C-ring, and 3- and 5-hydroxy groups and the 4-oxo function in the A- and C-rings are associated with antioxidant activity (*25*−*27*). For phenolic acids, the activity improves as the number of hydroxyl and methoxyl groups increases, the number of hydroxyl groups being more important (*28*, *29*). Therefore, it is not a surprise that most of the phenolic compounds from almond showed some antioxidative activity.

Compounds **1**−**9** were isolated as phenolic antioxidants of almond skins. These compounds were divided into flavonoids and phenolic acid. Plant flavonoids have been shown to be powerful antioxidants in vitro (*30*). Studies on the absorption, metabolism, and antioxidation actions in vivo of flavonoids such as quercetin and its glycosides have been developed (*31*−*33*). Catechin is the flavonoid that is the most widely distributed in edible plants and in foodstuffs derived from plants. Many in vitro and animal studies have demonstrated the high antioxidant activity of catechin and its inhibitory effect on numerous enzymes, which may result in a protective activity against cancer, cardiovascular, and inflammatory diseases (*34*−*41*). Phenolic acids are shown to have antioxidant activity in different lipid systems (*42*−*44*). It was reported that the simple phenolic acid, protocatechuic acid (PA), is one of the major benzoic acid derivatives from vegetables and fruits with a strong antioxidative effect, 10-fold higher than that of α-tocopherol (*45*). PA, even at 100 ppm in a diet, shows potent chemopreventive effects on colon and oral carcinogenesis in rats (*46*). Thus, consumption of almond skins would be a good source of dietary antioxidants.

LITERATURE CITED

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