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Phytochemicals of Apple Peels: Isolation, Structure Elucidation, and Their Antiproliferative and Antioxidant Activities

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Bioactivity-guided fractionation of Red Delicious apple peels was used to determine the chemical identity of bioactive constituents, which showed potent antiproliferative and antioxidant activities. Twenty-nine compounds, including triterpenoids, flavonoids, organic acids and plant sterols, were isolated using gradient solvent fractionation, Diaion HP-20, silica gel, and ODS columns, and preparative HPLC. Their chemical structures were identified using HR-MS and 1D and 2D NMR. Antiproliferative activities of isolated pure compounds against HepG2 human liver cancer cells and MCF-7 human breast cancer cells were evaluated. On the basis of the yields of isolated flavonoids (compounds **18**−**23**), the major flavonoids in apple peels are quercetin-3-*O*-β-D-glucopyranoside (compound **20**, 82.6%), then quercetin-3-*O*-β-D-galactopyranoside (compound **19**, 17.1%), followed by trace amounts of quercetin (compound **18**, 0.2%), (−)-catechin (compound **22**), (−)-epicatechin (compound **23**), and quercetin-3-*O*-α-L-arabinofuranoside (compound **21**). Among the compounds isolated, quercetin (**18**) and quercetin-3-*O*-β-D-glucopyranoside (**20**) showed potent antiproliferative activities against HepG2 and MCF-7 cells, with EC_{50} values of 40.9 ± 1.1 and 49.2 ± 4.9 μM to HepG2 cells and 137.5 ± 2.6 and 23.9 ± 3.9 μM to MCF-7 cells, respectively. Six flavonoids (**18**−**23**) and three phenolic compounds (**10**, **11**, and **14**) showed potent antioxidant activities. Caffeic acid (**10**), quercetin (**18**), and quercetin-3-*O*-β-D-arabinofuranoside (**21**) showed higher antioxidant activity, with EC_{50} values of <10 μM. Most tested flavonoids and phenolic compounds had high antioxidant activity when compared to ascorbic acid and might be responsible for the antioxidant activities of apples. These results showed apple peel phytochemicals have potent antioxidant and antiproliferative activities.

KEYWORDS: Apples; phytochemicals; antioxidant; structure identification; antiproliferative activities; antioxidant activities

INTRODUCTION

Epidemiologic studies have consistently shown that diets rich in fruits and vegetables have been associated with reduced risk of developing chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, and age-related functional declines (*1*−*3*).

Apples (*Malus domestica* Borkh) are a very significant part of the diet in humans and are rich in phenolic compounds (*4*). The total phenolic content ranges from 110 to 357 mg/100 g of fresh apple (*5*−*8*). Twenty-two percent of fruit phenolics consumed in the United States are from apples, which are the largest source (*4, 8, 9*). Apples also have a high content of flavonoids (*8, 9*). In Finland, apples are the top contributors of flavonoids along with onions (*10*).

Consumption of apples has been linked to the prevention of various chronic diseases. Apple intake has been reported to reduce lung cancer incidence (*10, 11*), cardiovascular disease (*12*), symptoms of chronic obstructive pulmonary disease (*13*), and the risk of thrombotic stroke (*14*).

Some research on apple peels has been carried out in our group. Apple phytochemical extracts have been shown to have potent antioxidant activity (*5, 8*) and antiproliferative activity against human cancer cells (*8, 15*) and to prevent mammary tumors in rats (*16*). Apple peels had exhibited higher antioxidant activity and antiproliferative activity than apple flesh (*5, 17*−*19*), suggesting apple peels provided the major portion of bioactive phytochemicals.

However, the bioactive compounds of apple peels that may be responsible for antioxidant and antiproliferative activities are still not clear. In continuing efforts to seek bioactive components

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from fruits, vegetables, and other natural products (*20*−*22*), bioactivity-guided fractionation of apple peels was used to determine the identity of bioactive compounds that inhibit tumor cell growth and antioxidant activities. Thirteen bioactive triterpenoids had been isolated and identified in our previous paper (*19*). The objectives of this paper were further to isolate and identify phytochemicals of apple peels with potent antioxidant and antiproliferative activities.

MATERIALS AND METHODS

Plant Material. Apples (*M. domestica* Borkh) of the Red Delicious variety were purchased from Cornell Orchard (Cornell University, Ithaca, NY). They were harvested in October 2005.

Reagents. All chemicals used in the study, such as methanol, acetone, hexane, ethyl acetate, dichloromethane, and *n*-butanol, were of analytical grade and were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). All deuteriated solvents, chloroform-*d*, dimethyl-d_6 sulfoxide, and pyridine-d_5 for NMR measurement were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). 2,2′-Azobis(amidinopropane) (ABAP) was purchased from Wako Chemicals (Richmond, VA). Dichlorofluorescein diacetate was purchased from Sigma-Aldrich.

Chromatographic Materials. Silica gel for column chromatography, 230−400 mesh, and precoated silica gel 60 TLC plates were purchased from Merck KGaA (Darmstadt, Germany). Precoated Rp-18 TLC plates were obtained from Macherey-Nagel (Düren, Germany). Diaion HP-20 was purchased from Supelco, Inc. (Bellefonte, PA). Octadecylsilane (ODS) for open column chromatography was a product of Aldrich Chemical Co., Inc. (Milwaukee, WI). The 100 × 7.8 mm i.d., 5 μm, XTera MS C_{18} semipreparative HPLC column was purchased form Waters Corp. (Milford, MA). The 250 × 22 mm i.d., 5 μm, Alltima C_{18} preparative HPLC column was purchased from Alltech Associates, Inc. (Deerfield, IL).

Instrumentation. HPLC analysis and purification were performed on a Millennium HPLC system composed of two 515 pumps with a 2480 UV−visible detector (Waters Corp.). All NMR spectra were obtained on a Varian INOVA 400 NMR spectrometer (Varian Inc., Palo Alto, CA) operating at 400 MHz for proton and at 100 MHz for carbon (*20*). Mass spectra were obtained on a Bruker Esquire 3D ion trap ESI mass spectrometer (Bruker, Switzerland), and optimal ESI conditions were as follows: capillary voltage, 3000 V; source temperature, 110 °C; cone voltage, 55 V. The ESI gas was nitrogen. Data were acquired in continuum mode until acceptable averaged data were obtained (*20*).

Extraction, Isolation, and Purification Procedures of Bioactive Constituents from Apple Peels. Fresh apples of the Red Delicious variety (105 kg) were peeled by an apple peeler as described previously (*15, 19*). The apple peels (10.8 kg, 10.3% of whole apples as fresh weight) were homogenized for 5 min with chilled 80% acetone (1:2, w/v) using a chilled Waring blender as reported previously from our laboratory (*8, 17, 23*). Samples were then homogenized further using a Polytron homogenizer for an additional 3 min. The homogenates were filtered, and the filtrate was evaporated under vacuum at 45 °C until approximately 90% of the filtrate had been evaporated. The residue (1792 g, 16.6% of apple peels) was then resuspended in 4000 mL of water, extracted three times with the same volume of ethyl acetate, and then extracted three times with water-saturated *n*-butanol.

The ethyl acetate fraction (90.0 g, 5.0% of the extracts) was further purified by silica gel chromatography (230−400 mesh, 430 × 75 mm) and eluted with a CH_2Cl_2/MeOH gradient elution (the ratios of CH_2Cl_2/MeOH were from 100:0 to 0:100). The CH_2Cl_2 eluant (3.70 g) was further subjected to silica gel column chromatography (260 × 30 mm) and eluted with hexane/ethyl acetate (the ratios of hexane/ethyl acetate were from 100:0 to 0:100), and compounds **27** (10.2 mg), **28** (5.8 mg), **29** (7.6 mg), and **9** (14.1 mg) were purified from the hexane/ethyl acetate (20:1) fraction. Compounds **15** (2.3 mg), **16** (1.8 mg), and **24** (15.2 mg) were obtained from the hexane/ethyl acetate (10:1) fraction. Compound **25** (12.3 mg) was purified from the hexane/ethyl acetate (5:1) fraction. The CH_2Cl_2/MeOH (200:1) fraction (0.80 g) of the ethyl acetate fraction was further subjected to silica gel column chromatography (200 × 15 mm) and eluted with hexane/acetone (the ratios of hexane/acetone were from 100:0 to 0:100), and compounds **12** (3.1 mg) and **13** (1.5 mg) were purified from the 20:1 and 10:1 fractions, respectively. The CH_2Cl_2/MeOH (100:1) eluant (1.0 g) of the ethyl acetate fraction was further subjected to silica gel column chromatography (220 × 20 mm) and eluted with hexane/ethyl acetate, then followed by semipreparative HPLC using the XTera C_{18} column eluted isocratically with 90% methanol in water at a flow rate of 4.0 mL/min; compounds **1** (1.5 mg), **2** (1.2 mg), **3** (3.1 mg), **4** (2.1 mg), **5** (1.1 mg), and **6** (1.3 mg) were purified, respectively. The CH_2Cl_2/MeOH (20:1) elution (5.75 g) of the ethyl acetate fraction was further isolated using silica gel column (420 × 25 mm) eluted with CH_2Cl_2/MeOH. The CH_2Cl_2/MeOH (20:1) eluant (0.7 g) was purified with HPLC (250 × 22 mm, 5 μm, Alltima C_{18} preparative HPLC column) using 80% methanol (containing 0.1% CF_3COOH, pH 2.0) as mobile phase. Compounds **7** (2.3 mg) and **8** (2.5 mg) were obtained. Compounds **18** (20.3 mg), **19** (1.88 g), **20** (9.1 g), **21** (1.2 mg), and **26** (612.7 mg) were obtained from the CH_2Cl_2/MeOH (10:1) elution of the ethyl acetate fraction.

The butanol fraction (262.0 mg) of apple peel extracts was dissolved in water and then subjected to the Diaion HP-20 column (55 × 550 mm). The Diaion HP-20 column was eluted with 4000 mL of water, 10% MeOH (v/v), 30% MeOH (v/v), and MeOH. The 30% MeOH eluant (5.1 g) was subjected to a silica gel column (330 × 30 mm) and eluted with CH_2Cl_2/MeOH (the ratios of CH_2Cl_2/MeOH were from 100:1 to 0:100). The CH_2Cl_2/MeOH (10:1) eluant (1.1 g) was purified by HPLC (250 × 22 mm, 5 μm, Alltima C_{18} preparative HPLC column), and compounds **22** (5.2 mg) and **23** (3.8 mg) were obtained from 50% methanol elution. Compounds **10** (7.1 mg), **11** (3.1 mg), **14** (6.2 mg), and **17** (8.5 mg) were obtained from CH_2Cl_2/MeOH (10:1) elution.

Measurement of Inhibition Activity against Tumor Cell Proliferation. Antiproliferative activities against HepG2 human liver cancer cells (*15, 24*) and MCF-7 human breast cancer cells (*25*) of the pure compounds isolated from apple peels were measured by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay as described previously.

HepG2 cells (American Type Culture Collection, ATCC, Rockville, MD) were maintained in Williams medium E, containing 10 mM Hepes, 5 μg/mL insulin, 2 μg/mL glucagon, 0.05 μg/mL hydrocortisone, and 5% fetal bovine serum (Gibco, Life Technologies, Grand Island, NY) and 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin (*26, 27*). HepG2 cells were maintained at 37 °C in 5% CO_2 in an incubator. A total of 2.5×10^4 HepG2 cells in growth media were placed in each well of a 96-well flat-bottom plate. After 4 h of incubation at 37 °C in 5% CO_2, the growth medium was replaced by media containing different concentrations of the fractions of apple extracts or purified compounds. Control cultures received the same solution without the extracts or purified compounds, and blank wells contained 100 μL of growth medium with no cells. After 96 h of incubation, cell proliferation was determined by colorimetric MTS assay. Cell proliferation (percent) was determined at 96 h from the MTS absorbance (490 nm) reading for each concentration compared to the control (*20*). At least three replications for each sample were used to determine the cell proliferation.

MCF-7 human breast cancer cells (ATCC) were maintained at 37 °C with 5% CO_2 in minimum essential medium (MEM) α medium containing 10% fetal bovine serum (Gibco, Life Technologies), and 10 mM Hepes, 10 μg/mL insulin, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin as described previously (*20, 25*). MCF-7 cells in growth media were placed in each well of a 96-well flat-bottom plate at a concentration of 2.5×10^4 cells/well. Antiproliferative activities to MCF-7 cell line of all pure compounds were measured using the same procedures described above. At least three replications for each sample were used to determine the cell proliferation.

Measurement of Antioxidant Activity Using the Peroxyl Radical Scavenging Capacity (PSC) Assay. The antioxidant activity of extracts and pure compounds was determined by using the PSC assay as described previously (*20, 28*). Just prior to use in the reaction, 107 μL of 2.48 mM dichlorofluorescein diacetate was hydrolyzed to dichlorofluorescein with 893 μL of 1.0 mM KOH for 5 min in a vial to remove the diacetate moiety and then diluted with 7 mL of 75 mM phosphate

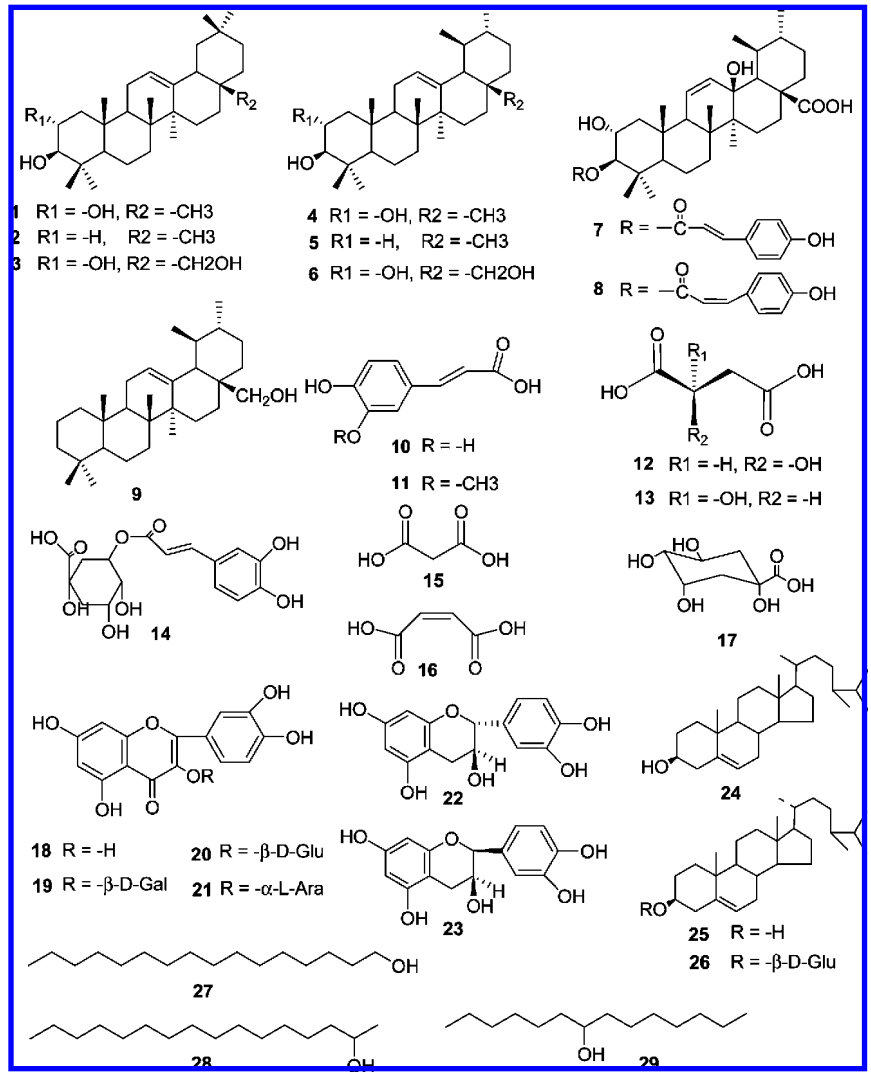

Figure 1. Chemical structures of the compounds isolated from apple peels.

buffer (pH 7.4). ABAP (200 mM) was prepared fresh in the buffer and was kept at 4 °C between runs. In an assay, 100 μL of pure compounds or extracts was diluted in 75 mM phosphate buffer (pH 7.4) and then transferred into reaction cells on a 96-well plate, and 100 μL of dichlorofluorescein was added. The 96-well plate was loaded into the Fluoroskan Ascent fluorescence spectrophotometer (Thermo Labsystems, Franklin, MA), and the solution in each cell was mixed by shaking at 1200 rpm for 20 s. The reaction was then initiated by adding 50 μL of ABAP from the autodispenser on the equipment. Each set of dilutions for a replicate and control was analyzed three times in adjacent columns. The reaction was carried out at 37 °C, and fluorescence was monitored at 485 nm excitation and 538 nm emission with the fluorescence spectrophotometer. The buffer was used for control reactions. Data were acquired with Ascent software, version 2.6 (Thermo Labsystems). The areas under the average fluorescence-reaction time kinetic curve (AUC) for both control and samples (up to 36 min) were integrated and used as the basis for calculating antioxidant activity according to the equation

$$\text{PSC unit} = 1 - (\text{SA}/\text{CA})$$

where SA is the AUC for sample or standard dilution and CA is the AUC for the control reaction using only buffer. Compounds inhibiting the oxidation of dichlorofluorescein produced smaller SA and higher PSC units. The median effective concentration (EC_{50}) was defined as the dose required to cause a 50% inhibition (PSC unit = 0.5) for each pure compound or extracts. Results were expressed as micromoles of vitamin C equivalents per micromoles of pure compound or gram of sample extracts ± standard deviation (SD) for triplicate analysis (*28*).

RESULTS AND DISCUSSION

Structure Identification of the Purified Compounds. The chemical structures of compounds **1**−**26** are shown in **Figure 1**.

Compound **1** was obtained as a white powder. The ESI-MS of compound **1** gave the ion $[M - H]^-$ at 441, and the molecular formula was inferred as $C_{30}H_{50}O_2$ from analysis of ^{1}H NMR, ^{13}NMR, and DEPT spectra. In the ^{1}H NMR spectra, there were eight methyl groups in the molecule, showing characteristic peaks at high field. The signals at 145.3 (C-13) and 123.0 (C-12) were two olefinic carbons, suggesting that there was a carbon−carbon double bond in the molecule. On the basis of the analysis of spectra, compound **1** was identified as olean-12-en-2α,3β-diol (*29*).

Compound **2** was obtained as a white amorphous powder. The molecular formula was inferred as $C_{30}H_{50}O$ according to its ESI-MS, ^{1}H and ^{13}C NMR, and DEPT spectra. In the lower field of ^{1}H NMR, it had an olefinic proton signal at 5.40 ppm (H, br d). It showed the characteristic signals of triterpenoids in the higher field. Comparison of the ^{13}C NMR data to those of compound **1** showed that they were almost the same except for the A-ring. Compound **1** had an additional hydroxy group as compared with compound **2** from its molecular weight. From the above analysis, compound **2** was identified as olean-12-en-3β-ol (β-amyrin) (*30, 31*).

The molecular formula of compound **3** was inferred as $C_{30}H_{50}O_3$from ESI-MS and ^{13}C NMR. In the high field of ^1H NMR, there were seven methyl groups. There were two olefinic carbons at 145.0 (C-13) and 122.7 (C-12), suggesting that there was a carbon−carbon double bond in the molecule. There were three hydroxylated carbons in the ^{13}C NMR. The carbon data of compound **3** were very similar to those of compound **1** when compared to their ^{13}C NMR, except compound **3** had an additional hydroxylated carbon and some slight shifts of some carbons. From the above analysis and reported literature (*32, 33*), compound **3** was identified as olean-12-en-2α,3*β*,28-triol.

Compounds **4**−**6** were three ursane-type triterpenoids. They had the same substituted modes of hydroxyl group as compounds **1**−**3**, respectively. Compounds **4**, **5**, and **6** were identified as urs-12-ene-2α,3*β*-diol, urs-12-ene-3*β*-ol, and urs-12-ene-2α,3*β*,28-triol, respectively, according to their ESI-MS and ^1H and ^{13}C NMR data, as well as reported literature (*31, 32, 34*).

Compound **7** was obtained as a white amorphous powder. The molecular formula was inferred as $C_{39}H_{54}O_7$ according to its ESI-MS, ^1H and ^{13}C NMR, and DEPT spectra. In the ^1H NMR, it had two olefinic protons, which resonated at δ 5.62 (dd, J = 10.4, 3.0 Hz) and 6.01 (br d, J = 10.4 Hz). It showed the characteristic signals of triterpenoids in the higher field. The signals at δ 7.90 (2H, d, J = 8.4 Hz) and 7.12 (2H, d, J = 8.4 Hz) showed that there was a 1,4-disubstituted benzene fragment in the molecule. The signals at δ 8.10 (H, d, J = 15.6 Hz) and 6.70 (H, d, J = 15.6 Hz) were characteristic signals of *trans*-olefinic protons. When the ^{13}C NMR was compared to that of the authentic compound, 2α,3*β*,13*β*-trihydroxy-urs-11-en-28-oic acid, there were nine additional carbon signals, which were all olefinic protons and appeared at lower fields. Except for the signals of the A-ring, the carbon signals of the skeleton of them were almost the same. The additional nine olefinic carbons were identified as a *trans-p*-coumaroyloxy group. The signal at C-3 of compound **7** was shifted downfield from 84.2 to 85.2 ppm. Therefore, the *trans-p*-coumaroyloxy group was linked at C-3. On the basis of the above analysis, compound **7** was identified as 3*β*-*trans-p*-coumaroyloxy-2α,3*β*,13*β*-trihydroxy-urs-11-en-28-oic acid. This compound was newly identified and has not been reported previously in the literature.

The ^1H NMR of compound **8** was similar to that of compound **7** and also had a *p*-coumaroyloxy unit in the molecule. The difference between these two compounds was the configuration of the carbon−carbon double bond of cinnamic acid. The *cis-p*-coumaroyloxy configuration was drawn from the coupling constant of 12.6 Hz for the carbon−carbon double bond in **8** with olefinic signals at 6.10 and 6.90 ppm. Therefore, compound **8** was identified as 3*β*-*cis-p*-coumaroyloxy-2α,3*β*,13*β*-trihydroxy-urs-11-en-28-oic acid. This compound was newly identified and has not been reported previously in the literature.

Compound **9** was obtained as colorless needle crystals. The ESI-MS showed the ion $[M - H]^-$ at *m*/*z* 425, corresponding to the molecular formula $C_{30}H_{50}O$, in combination with its ^1H and ^{13}C NMR. In the ^1H NMR, there was an olefinic proton at 5.35 (H, d, J = 3.3 Hz). There were 30 carbons in the ^{13}C NMR. The two sp^2-type carbons at 140.9 (C-13) and 121.9 (C-12) suggested that there was a carbon−carbon double bond in the molecule. According to its ^1H and ^{13}C NMR, compound **9** was identified as urs-12-en-28-ol, which was almost the same as the reported data (*31, 32*).

Compounds **10**−**17** were eight organic acids, which were identified as caffeic acid (**10**), ferulic acid (**11**), 2(*R*)-hydroxybutanedioic acid (**12**), 2(*R*)-hydroxybutanedioic acid 1-methyl ester (**13**), chlorogenic acid (**14**), malonic acid (**15**), maleic acid (**16**), and D-(−)-quinic acid (**17**), respectively, through their MS and ^1H and ^{13}C NMR data.

Compounds **18**−**21** were flavonoids with different sugar moieties. Compound **18** was obtained as a pale yellow powder. It was identified as quercetin by its ^1H and ^{13}C NMR. Compound **19** was obtained as a pale yellow powder. The ESI-MS showed the ion $[M + H]^+$ at 465, and the molecular formula of $C_{21}H_{20}O_{12}$ was inferred from ^1H and ^{13}C NMR and DEPT. In the ^1H NMR spectra, an ABX spin-coupling system was ascribed to the three protons of the B-ring of a flavone, with signals at δ 7.68 (H, dd, J = 8.4, 2.1 Hz), 7.54 (H, d, J = 2.1 Hz), and 6.82 (H, d, J = 8.4 Hz), respectively. The signals at δ 6.41 (H, d, J = 2.4 Hz) and 6.20 (H, d, J = 2.4 Hz) were meta-substituted protons of the A-ring. The signal at 12.60 ppm (H, s) was the characteristic signal of the hydroxyl at C-5 of a flavone. Comparison of the NMR data with those of compound **18**revealed that the aglycone of compound **19** was quercetin. There was an anomeric proton at δ 5.40 (H, d, J = 7.5 Hz). The signals in the ^{13}C NMR showed 21 carbons in the molecule. The sugar unit was linked at C-3 according to its ^1H, ^{13}C, and literature data (*20*). Compound **19** was identified as 3,5,7,3′,4′-pentahydroxy-flavonol-3-*O*-*β*-D-galactopyranoside, which was identical to the reported data in the literature (*20, 35*). Compound **20** had the same aglycone, quercetin, as compound **19**. There were 21 carbons in the molecule. The difference between them was the sugar unit. The sugar was glucose in compound **20**. Therefore, **20** was identified as 3,5,7,3′,4′-pentahydroxy-flavonol-3-*O*-*β*-D-glucopyranoside (*20, 36*). Compound **21** also had the same aglycone, quercetin, as compound **19**. The ESI-MS gave the ion $[M + H]^+$ at 435 corresponding to the molecular formula of $C_{20}H_{18}O_{11}$. There were 20 carbon signals in the ^{13}C NMR, which showed that the sugar of compound **21** was pentose instead of hexose. By comparison to the literature, compound **21** was identified as 3,5,7,3′,4′-pentahydroxy-flavonol-3-*O*-α-L-arabinofuranoside (*20*). Compounds **22** and **23** were identified as (−)-catechin and (−)-epicatechin, respectively, by comparison of their ^1H and ^{13}C NMR with standard samples.

On the basis of the yields of isolated flavonoids (compounds **18**−**23**), the major flavonoids in apple peels are quercetin-3-*O*-*β*-D-glucopyranoside (compound **20**, 82.6%), then quercetin-3-*O*-*β*-D-galactopyranoside (compound **19**, 17.1%), followed by trace amounts of quercetin (compound **18**, 0.2%), (−)-catechin (compound **22**), (−)-epicatechin (compound **23**), and quercetin-3-*O*-α-L-arabinofuranoside (compound **21**).

Compounds **24**−**26** were plant sterols. They were identified as stigmast-5-en-3*β*-ol, *β*-sitosterol, and *β*-sitosterol-3-*O*-*β*-D-glucoside, respectively, according to their MS and ^1H and ^{13}C NMR. Compounds **27**−**29** were identified as 1-hexadecanol, 2-hexadecanol, and tetradecan-7-ol, respectively, on the basis of the analysis their MS and NMR data.

Antiproliferative Activities. All isolated compounds were evaluated for antiproliferative activities against MCF-7 human breast cancer cells and HepG2 human liver cancer cells. Among the pure compounds isolated from apple peels, compounds **18** and **20** showed potent antiproliferative activities against HepG2 cell growth in a dose-dependent manner (**Figure 2**), with EC_{50} values of 40.9 ± 1.1 and 49.2 ± 4.9 μM, whereas they did not display any cytotoxicity at the concentrations applied in the experiments (data not shown). Compounds **18** and **20** also showed potent inhibitory activities against the proliferation of MCF-7 cells (**Figure 3**), with EC_{50} values 137.5 ± 2.6 and 23.9 ± 3.9 μM, respectively.

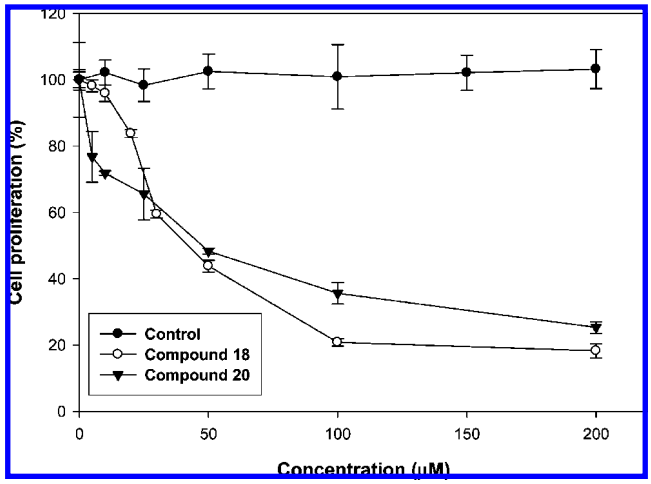

Figure 2. Dose−response curve of antiproliferative activity against HepG2 cells of compounds **18** and **20** (mean ± SD, $n = 3$).

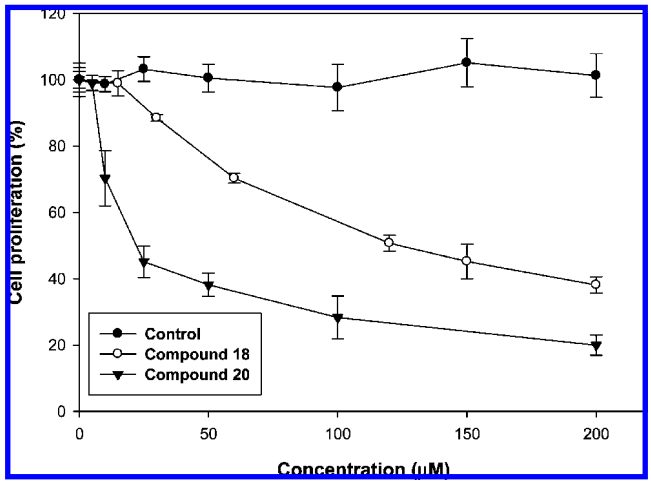

Figure 3. Dose−response curve of antiproliferative activity against MCF-7 cells of compounds **18** and **20** (mean ± SD, $n = 3$).

Apple peel phytochemical extracts have been shown to have potent antioxidant and antiproliferative activities (*5, 17, 18*). Most triterpenoids isolated from apple peels showed very potent antiproliferative activities against HepG2 human liver cancer cells, MCF-7 human breast cancer cells, and Caco-2 human colon cancer cells. Triterpenoids were also the main constituents of apple peels (*19*). Previously, quercetin was always thought to be the major bioactive compound that was responsible for the antiproliferative activity of apples; however, quercetin was only a minor component in the apple peels. This research suggests that triterpenoids, especially ursolic acid (*19*), and quercetin-3-*O*-β-D-glucopyranoside (compound **20**) were mainly responsible for the antiproliferative activities of apple peels against cancer cells.

Antioxidant Activities of the Pure Compounds Isolated from Apple Peels. The antioxidant activities of the purified compounds were evaluated using the PSC assay (*28*). Six flavonoids (compounds **18**−**23**), as well as three phenolic compounds (compounds **10**, **11**, and **14**), showed potent antioxidant activity (**Table 1**). Compound **10** (caffeic acid) had the highest antioxidant activity with an EC_{50} of 5.71 ± 0.70 μM, followed by **17** (EC_{50} = 7.63 ± 0.93 μM) and **18** (EC_{50} = 7.95 ± 0.40 μM). The antioxidant activity of compound **10** is 2.33 times higher than that of ascorbic acid.

All six flavonoids (compounds **18**−**23**) isolated from apple peels showed high antioxidant activities to scavenging peroxyl radicals. Apple peels contain large amounts of quercetin-3-*O*-β-D-glucopyranoside (compound **20**) and quercetin-3-*O*-β-D-galactopyranoside (compound **19**) and many other phytochemicals. Therefore, the combination of phytochemicals may be responsible for the antioxidant activity of whole apples (*5, 37*).

Table 1. Antioxidant Activity of Selected Compounds Isolated from Apple Peels (Mean ± SD, $n = 3$)

compd	amount isolated (mg)	EC_{50} (μM)	PSC value (μmol of vitamin C equiv/μmol of compd)
10	7.1	5.71 ± 0.70	2.33 ± 0.27
11	3.1	43.43 ± 0.71	0.30 ± 0.01
14	6.2	10.94 ± 1.30	1.22 ± 0.14
18	20.3	7.95 ± 0.40	1.66 ± 0.11
19	1880	10.26 ± 0.16	1.28 ± 0.01
20	9100	10.15 ± 0.69	1.30 ± 0.09
21	1.2	7.63 ± 0.93	1.73 ± 0.05
22	5.2	19.34 ± 1.44	0.68 ± 0.05
23	3.8	27.96 ± 0.41	0.47 ± 0.01

Research should be done to determine the cellular antioxidant activities of isolated compounds using the newly developed cellular antioxidant activity assay (*38*). Further testing is needed to determine the in vivo bioactivity of isolated compounds.

LITERATURE CITED

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