JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 5873−5878 **5873**

Analysis of Polyphenolic Antioxidants from the Fruits of Three *Pouteria* Species by Selected Ion Monitoring Liquid Chromatography−Mass Spectrometry

JUN MA,[†] HUI YANG,[†] MARGARET J. BASILE,[‡] AND EDWARD J. KENNELLY*,[†]

Department of Biological Sciences, Lehman College, and The Graduate Center, The City University of New York, 250 Bedford Park Boulevard West, Bronx, New York 10468, and Department of Neurology, University of Miami School of Medicine, 1501 N.W. 9th Avenue, Miami, Florida 33136

Pouteria campechiana, *Pouteria sapota*, and *Pouteria viridis* are tropical plants in the Sapotaceae family that bear edible fruits. The fresh fruits of these three *Pouteria* species were each extracted, and activity-guided fractionations were performed to identify the antioxidant constituents. Seven polyphenolic antioxidants, gallic acid (**1**), (+)-gallocatechin (**2**), (+)-catechin (**3**), (−)-epicatechin (**4**), dihydromyricetin (**5**), (+)-catechin-3-*O*-gallate (**6**), and myricitrin (**7**), were isolated and identified. Extracts of the three *Pouteria* fruits were analyzed by a selected ion monitoring liquid chromatography−mass spectrometry method to quantify their polyphenolic antioxidants. The highest level of the seven measured polyphenols was found in *P. sapota*, the second highest in *P. viridis*, and the lowest in *P. campechiana*. The levels of the seven polyphenols corresponded with the results of the 1,1-diphenyl-2-picrylhydrazyl assay, by which *P. sapota* had the highest antioxidant activity, *P. viridis* the second highest, and *P. campechiana* the lowest.

KEYWORDS: ***Pouteria*; Sapotaceae; antioxidants; polyphenols; 1,1-diphenyl-2-picrylhydrazyl (DPPH); selected ion monitoring liquid chromatography−mass spectrometry (SIM LC-MS)**

INTRODUCTION

Sapotaceae is a large, mostly tropical, plant family with 107 genera and >1000 species (*1*). There are many Sapotaceae genera that produce edible fruits, such as *Chrysophyllum*, *Manilkara*, *Mimusops*, and *Pouteria* (*1*). Sapotaceae species grow well in tropical and subtropical areas. In the United States, Sapotaceae species are grown in California, southern Florida, and Hawaii, and some are sold commercially. In our research, we have found Sapotaceae species to be a rich source of polyphenolic antioxidants. From *Manilkara zapota* (L.) P. Royen cv. Tikal, we have identified 10 polyphenolic antioxidants, including two new chlorogenic acid derivatives (*2*), and from *Chrysophyllum cainito* L., we found nine known polyphenolic antioxidants (*3*). These polyphenols may be important in the prevention of diseases in humans such as cancer and coronary heart disease (*2*, *4*). In the present study, we examine the antioxidant content of three edible fruits in the Sapotaceae: *Pouteria campechiana* (Kunth) Baehni, *Pouteria sapota* (Jacq.) H. E. Moore & Stern, and *Pouteria viridis* (Pittier) Cronquist.

P. campechiana, known commonly as canistel, is a native of Central America, and in the United States, it is grown in California, southern Florida, and Hawaii (*5*, *6*). The fruit is nearly round, oval, ovoid, or spindle-shaped, 7.5−12.5 cm long and 5−7.5 cm wide. When unripe, the skin is green, and upon ripening turns yellow. The pulp is yellow with one to four hard seeds (*5*), and the flavor is sweet, like that of a baked sweet potato (*7*). The fruit can be eaten fresh or after baking and also can be used in making custards, ice creams, milkshakes, jam, and marmalade (*7*). The bark has been used to treat fevers and skin eruptions. The seeds have been used to treat ulcers (*7*). No phytochemical studies have been published on this species.

P. sapota, known commonly as sapote, or mamey sapote, is indigenous to southern Mexico and Central America and is cultivated in California, southern Florida, and Hawaii in the United States (*5*, *6*). The fruit is round, ovoid, or elliptic, brown and scurfy, 8−23 cm long, 8−12 cm wide (*5*), with a thick rind and red, orange, or yellow, soft, sweet, pumpkin-like in flavor pulp, enclosing one to four large, hard seeds (*7*). The sapote fruits are typically eaten by spooning out the flesh. They are used in milkshakes and can be found incorporated into ice creams. The fruits and ice creams are both sold commonly in southern Florida supermarkets. Sapote fruit pulp has been used to treat gastric ulcers and dysentery (*8*), and the seed shell has been used to treat coronary diseases, kidney stones, and rheumatism (*7*, *8*). There has been one phytochemical study of *P. sapota*, and three benzenoids, lucumin, lucuminamide, and lucuminic acid, were identified from the seeds (*9*).

* Author to whom correspondence should be addressed [telephone (718) 960-1105; fax (718) 960-8236; e-mail kennelly@lehman.cuny.edu].

[†] Lehman College and The Graduate Center, The City University of New York.

[‡] University of Miami School of Medicine.

10.1021/jf049950k CCC: $27.50
Published on Web 08/25/2004

P. viridis, commonly called green sapote, also originated from Central America and is cultivated in California, Florida, and Hawaii in the United States (*10*, *11*). The fruit is nearly round or ovoid, 9−13 cm long and 6−8 cm wide, with a thin, green skin dotted with red-brown (*7*). The flesh is pale red-brown, of fine texture, juicy, and sweet, with one or two seeds (*10*). The fruit is picked while hard and stored until soft. The flesh is generally eaten raw, spooned from the skin. The fruits are sold commonly in Central America for food. No phytochemical studies have been published on this species. Growers in southern Florida have begun to consider *P. viridis* for further commercial development because it is similar in flavor to the popular *P. sapota* but has a superior shipping characteristic in terms of size (personal communication, Chris Rollins, Director, Metro-Dade County Fruit and Spice Park, Homestead, FL).

We report the identification of a total of seven polyphenolic antioxidants from these three species. Using a selected ion monitoring liquid chromatography−mass spectrometry (SIM LC-MS) technique, the levels of the seven antioxidants were quantified in these *Pouteria* species.

MATERIALS AND METHODS

Plant Material. Fruits of three *Pouteria* species were collected from the Fruit and Spice Park (Homestead, FL). Frozen fruits were shipped to New York City by overnight courier and stored at −20 °C until extracted. Herbarium voucher specimens of three *Pouteria* species were prepared, identified, and deposited at the Steere Herbarium of The New York Botanical Garden (Bronx, NY).

Instrumentation. ^{1}H and ^{13}C NMR spectra were recorded using a JEOL GX-400 MHz, operating at 400 and 100 MHz, respectively. All compounds were measured in CD_3OD. HPLC was carried out on a Waters 2690 separations module equipped with a Waters 996 photodiode array (PDA) detector and Millenium^{32} software using a 250 × 4.6 mm i.d., 5 μm, Aqua C18 column (Phenomenex, Torrance, CA). The wavelength was set at 280 nm.

LC-MS was performed on a Thermo Finnigan LCQ mass spectrometer (San Jose, CA) in the negative mode with a Waters 2690 separations module and a Waters 2487 dual-wavelength absorbance detector. The instrument was equipped with an electrospray ionization (ESI) source and controlled by Xcalibur software. The capillary temperature was 230 °C. Nitrogen was used as the sheath gas and the auxiliary gas at flow rates of 80 and 30 units, respectively. The capillary voltage was 10 V, the spray needle voltage was 4.5 kV, and the tube lens offset was 0 V. A mass range of 75−500 was scanned in the negative full or selected ion monitoring (SIM) scan type. Compounds were also monitored simultaneously using the dual-wavelength absorbance detector at 280 nm. Compounds were separated using the Aqua C18 column as for HPLC.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay was conducted on the 96-well microtiter plate (Nalge Nunc International, Rochester, NY). The absorbance of sample in the DPPH assay was detected on a *VERSA*$_{max}$ microplate reader (Molecular Devices, Sunnyvale, CA).

Thin-layer chromatography (TLC) analysis was performed on RP-18 F_{254} plates (Merck, Darmstadt, Germany), with compounds visualized by spraying with vanillin in 10% (v/v) H_2SO_4 in 95% EtOH.

Sephadex LH-20 (25−100 μm) (Pharmacia Fine Chemicals, Piscataway, NJ) and reversed-phase C18 (RP18) silica gel (40 μm) (J. T. Baker, Phillipsburg, NJ) were used for column chromatography (CC). All solvents for chromatographic isolation were of analytical grade. HPLC grade MeCN, MeOH, and H_2O were used for HPLC and LC-MS. Gallic acid, (+)-catechin, (−)-epicatechin, and DPPH were obtained from Sigma Chemical Co. (St. Louis, MO). (+)-Gallocatechin, dihydromyricetin, myricitrin, and (+)-catechin-3-*O*-gallate were isolated and identified by MS and NMR in our laboratory.

Extraction and Isolation Procedures. The fresh frozen fruits (2.6 kg) of *P. sapota* were macerated with MeOH twice, at room temperature for 1 h each time. After the MeOH was removed in vacuo, the resulting aqueous extract was partitioned with hexane and EtOAc, respectively. The EtOAc fraction displayed antioxidant activity (IC_{50} = 12.8 μg/mL) in the DPPH assay. The EtOAc fraction was concentrated in vacuo to give 13.8 g of a residue, of which 12.9 g was separated by Sephadex LH-20 CC with an isocratic solvent system of 100% MeOH. The resulting fractions were recombined according to their RP18 TLC profiles to give seven fractions in total (SA−SG). All fractions were tested in the DPPH assay, and fractions SC (621.3 mg), SD (296 mg), SE (252.2 mg), SF (109.4 mg), and SG (954.9 mg) showed antioxidant activity (IC_{50} = 2.8, 4.7, 7.7, 7.3, and 6.8 μg/mL, respectively).

Fraction SC was separated by LH-20 CC with an isocratic solvent system of 100% MeOH to yield gallic acid (**1**) (78.5 mg). Fraction SD was separated by RP18 CC (from 1:4 to 2:3 MeOH/H_2O) to yield (+)-gallocatechin (**2**) (174 mg), (+)-catechin (**3**) (52.7 mg), and (−)-epicatechin (**4**) (5.4 mg). Fraction SE was separated by RP18 CC (from 1:4 to 2:3 MeOH/H_2O) to yield (+)-gallocatechin (**2**) (127.4 mg), (+)-catechin (**3**) (20.8 mg), and dihydromyricetin (**5**) (12.9 mg). Fraction SF was separated by RP18 CC (from 3:7 to 11:14 MeOH/H_2O) to yield dihydromyricetin (**5**) (14.1 mg). Subfraction SF-1 was purified by LH-20 CC eluting with an isocratic solvent system of 100% MeOH to yield (+)-gallocatechin (**2**) (14.5 mg). Fraction SG was separated by RP18 CC (from 3:7 to 1:1 MeOH/H_2O). Subfraction SG-3 was purified by LH-20 CC eluting with an isocratic solvent system of 100% MeOH to yield (+)-catechin-3-*O*-gallate (**6**) (52.3 mg).

The fresh frozen fruits (11.7 kg) of *P. viridis* were extracted with MeOH twice at room temperature for 1 h each time. After the MeOH was removed in vacuo, the resulting aqueous extract was partitioned with hexane and EtOAc, respectively. The EtOAc fraction (IC_{50} = 52.6 μg/mL in the DPPH assay) was concentrated in vacuo to give 9.6 g of a residue, of which 9.5 g was separated by Sephadex LH-20 CC with an isocratic solvent system of 100% MeOH. The resulting fractions were combined according to their RP18 TLC profiles to give six combined fractions in total (VA−VF). All fractions were tested in the DPPH assay, and fractions VD (294.7 mg), VE (134 mg), and VF (546.2 mg) showed antioxidant activity (IC_{50} = 20.1, 17.2, and 22.2 μg/mL, respectively).

Fraction VD was separated by RP18 CC (from 100% H_2O to 13:37 MeOH/H_2O) to yield gallic acid (**1**) (115 mg). Fraction VE was separated by RP18 CC (from 1:19 MeOH/H_2O to 100% MeOH) to yield gallic acid (**1**) (22.6 mg). Fraction VF was separated by RP18 CC (from 1:3 to 11:9 MeOH/H_2O) to yield (+)-gallocatechin (**2**) (31.3 mg). Subfraction VF-3 was purified by RP18 CC eluting with a gradient of 1:4 to 3:7 MeOH/H_2O to yield (+)-gallocatechin (**2**) (32.8 mg), (+)-catechin (**3**) (3.3 mg), and VF-3-4, and then VF-3-4 was purified repeatedly by RP18 CC eluting with a gradient of 1:4 to 2:3 MeOH/H_2O to yield (−)-epicatechin (**4**) (6.3 mg). Subfraction VF-5 was purified by LH-20 CC eluting with an isocratic solvent system of 100% MeOH to obtain VF-5-3, and then VF-5-3 was purified repeatedly by preparative RP18 TLC (3:2 MeOH/H_2O) to yield myricitrin (**7**) (4.8 mg).

The fresh frozen fruits (3.1 kg) of *P. campechiana* were also extracted with 80% acetone three times, at room temperature for 1 h each time. After the acetone was removed in vacuo, the resulting aqueous extract was fractionated by HP-20 CC eluting with H_2O, MeOH/H_2O (1:1), MeOH, and acetone. The MeOH/H_2O (1:1) fraction (IC_{50} = 35.2 μg/mL in the DPPH assay) was concentrated in vacuo to give 10.6 g of a residue, of which 10.0 g was separated by Sephadex LH-20 CC with an isocratic solvent system of 100% MeOH. The resulting fractions were combined according to their RP18 TLC profiles to give seven combined fractions in total (CA−CG). All fractions were tested in the DPPH assay, and fractions CA (2002.7 mg) and CC (770.0 mg) showed antioxidant activity (IC_{50} = 90.7 and 11.2 μg/mL, respectively). Fraction CC was separated by RP18 CC (from 1:9 MeOH/H_2O to 100% MeOH) to yield gallic acid (**1**) (12.0 mg) and (+)-catechin (**3**) (16.5 mg).

The fresh frozen fruits (114 g) of *P. campechiana* were extracted at room temperature with MeOH twice, for 1 h each time. After the MeOH was removed in vacuo, the resulting aqueous extract was partitioned with hexane and EtOAc, respectively. The EtOAc fraction was concentrated in vacuo to give 163.6 mg of a residue. It displayed low

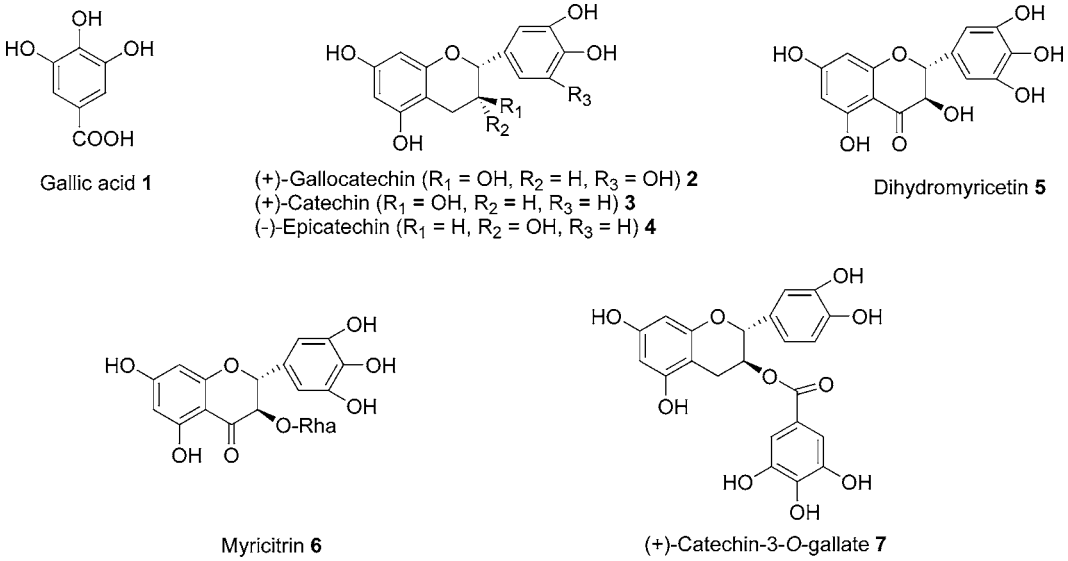

Figure 1. Polyphenolic antioxidants from *P. sapota*, *P. viridis*, and *P. campechiana*.

antioxidant activity (IC_{50} = 146.3 μg/mL) in the DPPH assay and was kept for SIM LC-MS analysis.

A total of seven polyphenolic antioxidants were isolated from the fruits of these *Pouteria* species (**Figure 1**). The properties of the compounds are presented below.

*Gallic acid (**1**)* was obtained as a white powder; negative ESIMS *m*/*z* 169 $[M - H]^-$; ^1H and ^{13}C NMR consistent with previously published data (*12*).

*(+)-Gallocatechin (**2**)* was obtained as a white powder; negative ESIMS *m*/*z* 305 $[M - H]^-$; ^1H and ^{13}C NMR consistent with previously published data (*13*). The identification was further supported by RP18−HPLC comparison (retention time and UV spectrum) with authentic compound.

*(+)-Catechin (**3**)* was obtained as a white powder; negative ESIMS *m*/*z* 289 $[M - H]^-$; ^1H and ^{13}C NMR consistent with previously published data (*13*, *14*). The identification was further supported by RP18−HPLC comparison (retention time and UV spectrum) with authentic compound (Sigma).

*(−)-Epicatechin (**4**)* was obtained as a white powder; negative ESIMS *m*/*z* 289 $[M - H]^-$; ^1H and ^{13}C NMR consistent with previously published data (*12*, *15*). The identification was further supported by RP18−HPLC comparison (retention time and UV spectrum) with authentic compound (Sigma).

*Dihydromyricetin (**5**)* was obtained as a yellow powder; negative ESIMS *m*/*z* 319 $[M - H]^-$; ^1H and ^{13}C NMR consistent with previously published data (*14*). The identification was further supported by RP18−HPLC comparison (retention time and UV spectrum) with authentic compound.

*(+)-Catechin-3-O-gallate (**6**)* was obtained as a white powder; negative ESIMS *m*/*z* 441 $[M - H]^-$; ^1H and ^{13}C NMR consistent with previously published data (*16*).

*Myricitrin (**7**)* was obtained as a yellow powder; negative ESIMS *m*/*z* 463 $[M - H]^-$; ^1H and ^{13}C NMR consistent with previously published data (*14*). The identification was further supported by RP18−HPLC comparison (retention time and UV spectrum) with authentic compound.

TLC Analysis. RP18 TLC was performed on all LH-20 fractions with a mobile phase composed of 3:7 MeOH/H_2O. Fractions were pooled according to their TLC profiles to give combined fractions for further activity-guided fractionation.

DPPH Assay. The DPPH assay was performed on the extracts as previously described (*17*, *18*). Reaction mixtures containing extracts (dissolved in DMSO) and ethanolic solution of DPPH were placed in a 96-well microtiter plate. After incubation at 37 °C for 30 min, absorbance was read at 515 nm with a microplate reader. Antioxidant activity was determined as a percent inhibition by sample treatment by comparison with DMSO-treated controls. IC_{50} values were obtained that signify the concentration of sample necessary to scavenge 50% of DPPH free radicals. The lower the IC_{50} value, the higher the antioxidant activity.

Preparation of Samples for HPLC and LC-MS Analyses. The EtOAc fractions of *P. sapota*, *P. viridis*, and *P. campechiana* were redissolved in HPLC grade MeOH to make 10 mg/mL sample solutions. All samples were filtered with a 0.45 μm nylon microfilter (Phenomenex) before HPLC and LC-MS analyses.

Preparation of Standards for HPLC Analysis. Stock solutions (1 mg/mL) for the seven standards were prepared by dissolving individual standards in HPLC grade MeOH. The seven standard stock solutions were mixed in equal amounts to make a standard mixture. Concentrations of individual polyphenolic standards in the standard mixture were 0.1 mg/mL.

Preparation of Standards for LC-MS Analysis. Stock solutions (2 mg/mL) for the seven standards were prepared by dissolving individual standards in HPLC grade MeOH. To decrease the number of runs required to generate the calibration curves, four group stock solutions were prepared: group 1, gallic acid (**1**), (+)-gallocatechin (**2**), and (+)-catechin-3-*O*-gallate (**6**); group 2, (+)-catechin (**3**) and (−)-epicatechin (**4**); group 3, dihydromyricetin (**5**); group 4, myricitrin (**7**). Concentrations of individual standards in the group stock solutions were 0.5 mg/mL. The group stock solutions were diluted with HPLC grade MeOH into six 2-fold serial solutions, respectively. The concentrations of individual standards in the six two-fold serial solutions were 0.016, 0.031, 0.063, 0.125, 0.25, and 0.5 mg/mL. $[M - H]^-$ ions for the seven standards were monitored in SIM mode and the peak areas calibrated versus the concentrations of individual standards. Standard curves were generated for individual standards after runs of the six two-fold serial solutions.

HPLC Analysis. For HPLC analysis, a sample or standard (10 μL) was separated over a Phenomenex Aqua C18 column, using elution conditions according to a previously published method (*19*). The mobile phase consisted of 3% aqueous acetic acid (A) and MeOH (B) with a flow rate of 1 mL/min. The mobile phase composition began with 100% A, which was maintained for 1 min, followed by a linear increase to 63% B in 27 min, and then returned to the initial condition in 5 min for the next run.

Quantitative LC-MS Analysis. For quantitative analysis, a sample or standard (10 μL) was separated over a Phenomenex Aqua C18 column with a flow rate of 1 mL/min. The LC conditions were modified on the basis of a previously published HPLC method (*19*). The mobile phase consisted of 0.1% aqueous acetic acid (A) and 0.1% acetic acid in MeOH (B) with a flow rate of 1 mL/min. The mobile phase composition began with 100% A, which was maintained for 1 min, followed by a linear increase of B to 56% B in 24 min, and then the mobile phase composition went back to the initial condition in 1 min

for the next run. Column elutant was monitored by an ESI probe in the negative ion SIM mode for the seven standard compounds.

RESULTS AND DISCUSSION

The EtOAc-soluble fractions of *P. sapota* and *P. viridis* displayed antioxidant activity (IC_{50} = 12.8 and 52.6 μg/mL, respectively) in the DPPH assay. Activity-guided fractionation was performed on both plants to isolate the antioxidant constituents. The EtOAc-soluble fractions of *P. sapota* and *P. viridis* were each separated by Sephadex LH-20 CC. Active fractions were further separated by CC (RP18 and LH-20) and preparative RP18 TLC. Six antioxidant compounds, gallic acid (**1**), (+)-gallocatechin (**2**), (+)-catechin (**3**), (−)-epicatechin (**4**), dihydromyricetin (**5**), and (+)-catechin-3-*O*-gallate (**6**), were isolated and identified from the fruits of *P. sapota*. Five antioxidant compounds, gallic acid (**1**), (+)-gallocatechin (**2**), (+)-catechin (**3**), (−)-epicatechin (**4**), and myricitrin (**7**), were isolated and identified from the fruits of *P. viridis*.

In initial work on a MeOH extract of *P. campechiana* fruits, we were not able to isolate any antioxidant constituents. Therefore, the *P. campechiana* fruits were extracted with 80% acetone in an attempt to enhance the extraction of polyphenols and tannins (*20*). The 80% acetone extract was fractionated by HP-20 CC eluting with H_2O, MeOH/H_2O (1:1), MeOH, and acetone. The MeOH/H_2O (1:1) fraction displayed antioxidant activity (IC_{50} = 35.2 μg/mL) in the DPPH assay. Similarly, activity-guided fractionation was performed on this fraction. Two antioxidant compounds, gallic acid (**1**) and (+)-catechin (**3**), were isolated and identified from the fruits of *P. campechiana*.

HPLC-PDA analysis was conducted on the mixture of seven standards and the EtOAc fractions of three *Pouteria* species using a previously published method (*19*). This method achieved baseline separation among the seven standard polyphenols (**Figure 2A**). In the three *Pouteria* extracts, however, the seven polyphenols could not all be resolved using this method (**Figure 2B**−**D**) because other compounds in the extracts coeluted with these polyphenols and interfered with their separation. Therefore, quantitative results of polyphenols using HPLC would have some inaccuracies. In this study, to achieve more accurate quantitative results, SIM LC-MS instead of HPLC-PDA was used to quantify the polyphenols in the plant extracts.

The antioxidant constituents of the three *Pouteria* species were quantified by LC-MS. Baseline separation of seven polyphenols was achieved by modifying the previously published HPLC-PDA method (*19*) for application by LC-MS. Compounds were detected using MS total ion current (TIC) and UV absorbance at 280 nm (**Figure 3A**,**B**). Each of the specific $[M - H]^-$ ions was detected by MS (**Figure 3C**).

The retention time of each standard was obtained from the SIM chromatograms of individual compounds. Peak areas for individual standards were measured from the SIM chromatograms and used for quantification. Individual calibration curves for each standard measured in the SIM mode were linear, with r^2 values of >0.99 in the range of calibrations of the standards tested. In this SIM LC-MS method, the limits of detection (LOD) were 250 pg for gallic acid and 500 pg for (+)-catechin; the limits of quantification (LOQ) were 1 ng for gallic acid and 2 ng for (+)-catechin.

In a spike−recovery experiment, 50 g of *P. campechiana* fruits spiked with 30 mg of dihydromyricetin was extracted with MeOH twice, at room temperature for 1 h each time. After the MeOH was removed in vacuo, the resulting aqueous extract was partitioned with hexane and EtOAc, respectively. The EtOAc fraction was concentrated in vacuo to dryness and dissolved with HPLC grade MeOH to prepare 1 mg/mL solution. This solution was analyzed for dihydromyricetin using SIM LC-MS, and a percent recovery was calculated. This experiment was repeated, and the average percent recovery was 85%.

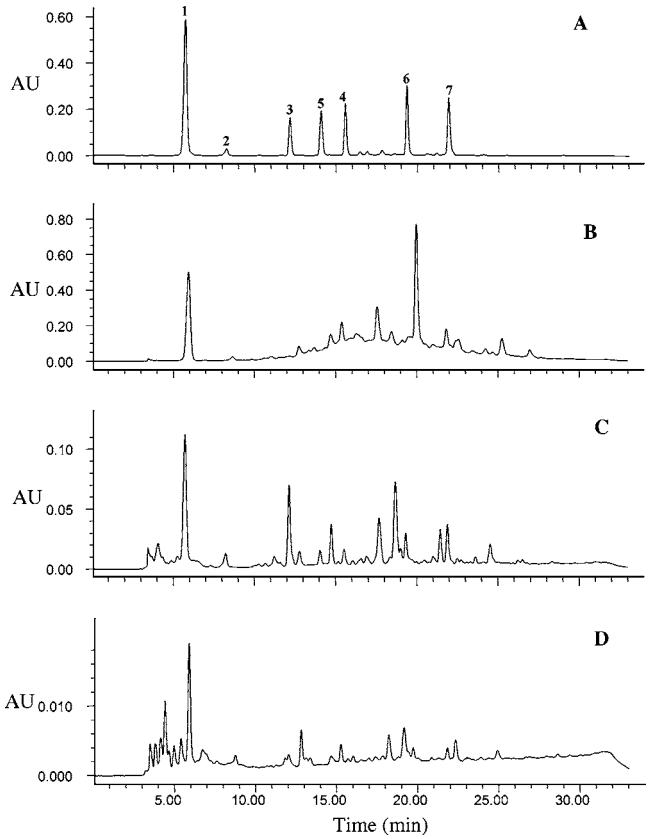

Figure 2. HPLC chromatograms at 280 nm of the mixture of seven standards (**A**), EtOAc extracts of *P. sapota* (**B**), *P. viridis* (**C**), and *P. campechiana* (**D**): **1**, gallic acid; **2**, (+)-gallocatechin; **3**, (+)-catechin; **4**, (–)-epicatechin; **5**, dihydromyricetin; **6**, (+)-catechin-3-*O*-gallate; **7**, myricitrin.

By SIM LC-MS, all seven polyphenols (**1**−**7**) were detected from *P. sapota* and *P. viridis*, and four polyphenols (**1**−**3** and **7**) were detected from *P. campechiana*. All of these polyphenols have antioxidant activity (IC_{50} < 40 μM) (**Table 1**). By activity-guided fractionation, we were not able to isolate myricitrin (**7**) from *P. sapota*, dihydromyricetin (**5**) and (+)-catechin-3-*O*-gallate (**6**) from *P. viridis*, nor (+)-gallocatechin (**2**) and myricitrin (**7**) from *P. campechiana*, despite the fact that we could detect them by SIM LC-MS. Our inability to isolate these compounds by activity-guided fractionation may result from their low content and/or interference of coeluting compounds.

Of these three *Pouteria* species, the fruits of *P. sapota* contain the highest level of total polyphenolic antioxidants; the contents of individual polyphenolic antioxidants range from 24.42 ± 0.97 ppm for (−)-epicatechin (**4**) to 200.77 ± 11.73 ppm for dihydromyricetin (**5**). *P. sapota* contains high amounts of gallic acid (**1**) (170.91 ± 0.53 ppm), (+)-gallocatechin (**2**) (172.85 ± 2.21 ppm), and dihydromyricetin (**5**) (200.77 ± 11.73 ppm). These three polyphenolic antioxidants likely contribute significantly to the high antioxidant activity of its EtOAc fraction (IC_{50} = 12.8 μg/mL in the DPPH assay), the highest antioxidant activity of the three *Pouteria* species studied. The fruits of *P. viridis* contain the second highest level of total polyphenolic antioxidants in this study. The contents of individual polyphenolic antioxidants range from 2.73 ± 0.21 ppm for (+)-catechin-

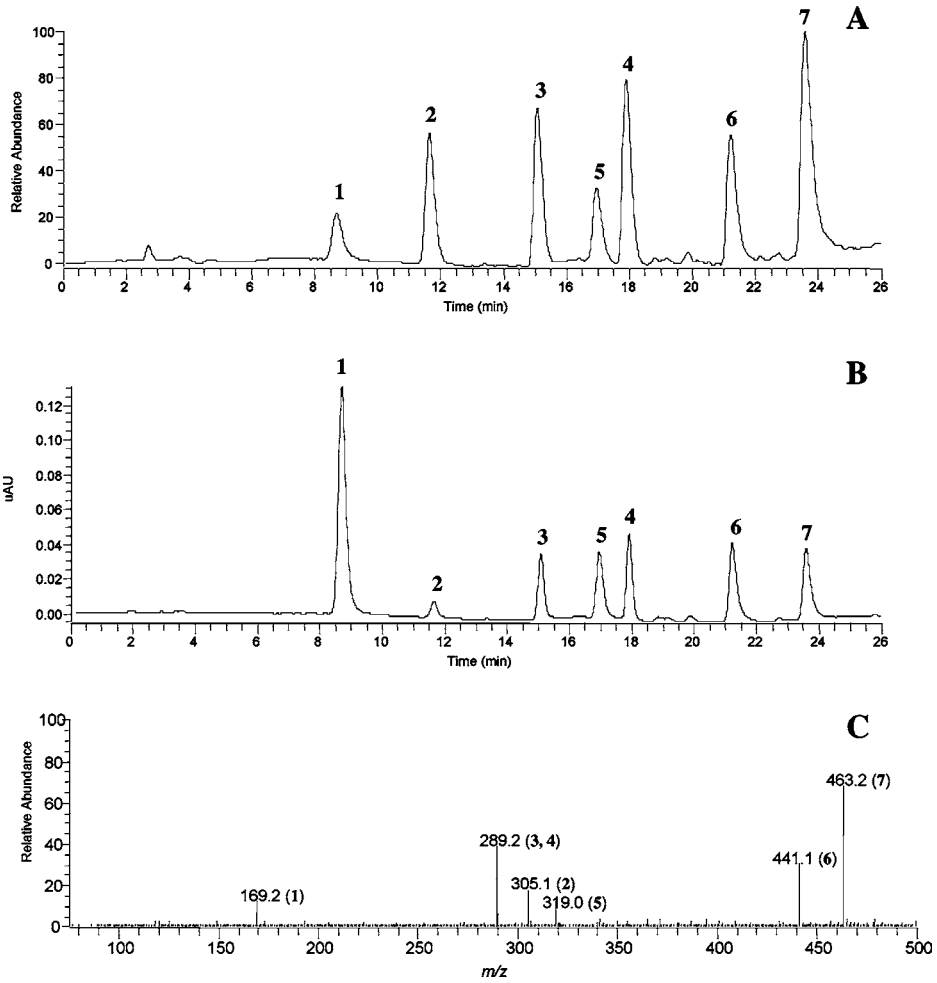

Figure 3. (**A**) Total ion current (TIC) chromatogram from the HPLC separation of the mixture of seven standards: **1**, gallic acid; **2**, (+)-gallocatechin; **3**, (+)-catechin; **4**, (–)-epicatechin; **5**, dihydromyricetin; **6**, (+)-catechin-3-*O*-gallate; **7**, myricitrin. (**B**) HPLC-UV–vis chromatogram at 280 nm of the mixture of seven standards. (**C**) Mass spectrometric profile of the mixture of seven standards.

Table 1. Contents of Polyphenolic Antioxidants by SIM LC-MS in the Fruits of Three *Pouteria* Species and Their DPPH Scavenging Activity[a]

compound	*P. sapota* (ppm)	*P. viridis* (ppm)	*P. campechiana* (ppm)	DPPH activity IC_{50}
gallic acid (**1**)	170.91 ± 0.53	2.82 ± 0.17	16.85 ± 0.09	20.9 μM
(+)-gallocatechin (**2**)	172.85 ± 2.21	47.26 ± 1.46	5.62 ± 0.13	20.7 μM
(+)-catechin (**3**)	75.01 ± 2.67	27.32 ± 0.93	1.04 ± 0.05	34.7 μM
(–)-epicatechin (**4**)	24.42 ± 0.97	5.91 ± 0.13	ND[b]	38.3 μM
dihydromyricetin (**5**)	200.77 ± 11.73	4.68 ± 0.29	ND	30.8 μM
(+)-catechin-3-*O*-gallate (**6**)	80.50 ± 0.81	2.73 ± 0.21	ND	19.0 μM
myricitrin (**7**)	25.48 ± 3.70	7.34 ± 0.80	2.82 ± 0.02	26.4 μM
P. sapota EtOAc fraction				12.8 μg/mL
P. viridis EtOAc fraction				52.6 μg/mL
P. campechiana EtOAc fraction				146.3 μg/mL

[a] Replication, 4. [b] ND, not detected by SIM LC-MS.

3-*O*-gallate (**6**) to 47.26 ± 1.46 ppm for (+)-gallocatechin (**2**). Its EtOAc fraction showed the second highest antioxidant activity (IC_{50} = 52.6 μg/mL in the DPPH assay). In this study, the lowest level of total polyphenols measured was found in the fruits of *P. campechiana*, and this corresponds to the lowest antioxidant activity of the three plant extracts (**Table 1**). Only four polyphenols were detected in *P. campechiana*, and the contents of individual polyphenolic antioxidants range from 1.04 ± 0.05 ppm for (+)-catechin (**3**) to 16.85 ± 0.09 ppm for gallic acid (**1**). The EtOAc fraction of *P. campechiana* has an IC_{50} = 146.3 μg/mL in the DPPH assay.

(+)-Gallocatechin (**2**), (+)-catechin (**3**), (−)-epicatechin (**4**), and (+)-catechin-3-*O*-gallate (**6**) belong to the catechin, or flavan-3-ol, class of flavonoids, and they have been studied intensively because they are important components of edible foods, such as tea (*16*). They are all strong antioxidants with IC_{50} values in the DPPH free radical assay ranging from 19.0 to 38.3 μM (**Table 1**). The tea catechins have been shown to prevent the oxidation of plasma low-density lipoprotein (LDL) in vitro (*4*). Because LDL oxidation is a key step in the pathogenesis of cardiovascular disease (*21*), catechins are thought to be important cardioprotective agents. Epidemiological

studies have shown that the intake of tea catechins can decrease the risk of cardiovascular disease (*22*). Gallic acid (**1**), dihydromyricetin (**5**), and myricitrin (**7**) are found in many edible plants (*2*, *3*) and also have strong antioxidant activity (IC_{50} from 20.9 to 30.8 μM) (**Table 1**). The fruits of *P. sapota* and *P. viridis* are rich sources of polyphenolic antioxidants.

In our study, we used a SIM LC-MS method rather than an HPLC-PDA method to quantify polyphenolic antioxidants. SIM LC-MS was useful in this study because LC provided an initial separation of the mixture of compounds based on their polarities, and then SIM MS was used to select specific ions corresponding to specific antioxidants. This method allowed the major polyphenolic constituents of the three *Pouteria* species to be distinguished and quantified. The SIM LC-MS method used in this study provides lower LOD and LOQ than HPLC-PDA methods previously published (*23*). For example, in the HPLC-PDA method reported by Donovan et al. (*23*), the LOD for (+)-catechin is 22 ng, 44 times higher than in the SIM LC-MS method (500 pg). The SIM LC-MS method employed in this study is a quick, sensitive, and accurate way to quantify polyphenolic antioxidants in plant samples. In addition to the *Pouteria* species we examined, other species may be analyzed for polyphenolic antioxidant contents by this method. This method may also be extended to measure other polyphenolic antioxidants.

By SIM LC-MS, we found that different *Pouteria* species have significantly different levels of polyphenolic antioxidants. These results provide additional nutrition information for three lesser used tropical fruits that are now grown commercially in the United States and other subtropical/tropical areas.

ACKNOWLEDGMENT

Chris Rollins (Metro-Dade County Fruit and Spice Park, Homestead, FL), Dr. Richard Campbell (Fairchild Tropical Garden), Murray Corman (Montgomery Botanical Center), Dr. Jonathan Crane (University of Florida Tropical Research Education Center), and Larry Schokman (The Kampong of the National Tropical Botanical Garden) are thanked for their help in procuring the fruits used in this study. Dr. Lining Cai, Ming Chang, and Kurt Reynertson are thanked for their helpful comments on the manuscript. Dr. Michael Blumenstein (Hunter College, The City University of New York) is acknowledged for assistance with NMR experiments.

LITERATURE CITED

(1) Mabberley, D. J. *The Plant-Book: A Portable Dictionary of the Higher Plants*; Cambridge University Press: New York, 1993; p 520.

(2) Ma, J.; Luo, X. D.; Protiva, P.; Yang, H.; Ma, C.; Basile, M. J.; Weinstein, I. B.; Kennelly, E. J. Bioactive novel polyphenols from the fruit of *Manilkara zapota* (Sapodilla). *J. Nat. Prod.* **2003**, *66*, 983−986.

(3) Luo, X. D.; Basile, M. J.; Kennelly, E. J. Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L. (Star Apple). *J. Agric. Food Chem.* **2002**, *50*, 1379−1382.

(4) Tijburg, L. B.; Mattern, T.; Folts, J. D.; Weisgerber, U. M.; Katan, M. B. Tea flavonoids and cardiovascular disease: A review. *Crit. Rev. Food Sci. Nutr.* **1997**, *37*, 771−785.

(5) Balerdi, C. F.; Shaw, P. E. Sapodilla, sapote and related fruit. In *Tropical and Subtropical Fruits*; Shaw, P. E., Chan, H. T., Jr., Nagy, S., Eds.; AgScience, Inc.: Auburndale, FL, 1998; pp 78−136.

(6) Facciola, S. *Cornucopia II: A Source Book of Edible Plants*; Kampong Publications: Vista, CA, 1998; pp 228−229.

(7) Morton, J. F. *Fruits of Warm Climates*; Miami, FL, 1987; pp 398−405.

(8) Morton, J. F. *Atlas of Medicinal Plants of Middle America*; Thomas: Springfield, IL, 1981; pp 660−661.

(9) Takeda, T.; Gonda, R.; Hatano, K. Constitution of lucumin and its related glycosides from *Calocarpum sapota* Merrill. *Chem. Pharm. Bull.* **1997**, *45*, 697−699.

(10) Kennard, W. C.; Winters, H. F. *Some Fruits and Nuts for the Tropics*; U.S. Department of Agriculture: Washington, DC, 1960; pp 40−80.

(11) Thomson, P. H. The green sapote. *CRFG Yearb.* **1973**, *5*, 41−48.

(12) Gottlieb, H. E.; Kumar, S.; Sahai, M.; Ray, A. B. Ethyl brevifolin carboxylate from *Flueggea microcarpa*. *Phytochemistry* **1991**, *30*, 2435−2438.

(13) Foo, L. Y.; Newman, R.; Waghorn, G.; McNabb, W. C.; Ulyatt, M. J. Proanthocyanidins from *Lotus corniculatus*. *Phytochemistry* **1996**, *41*, 617−624.

(14) Chung, T. Y.; Kim, A. A.; Jones, A. D. Antioxidant activity of flavonoids isolated from jindalrae flowers (*Rhododendron mucronulatum* Turcz.). *Agric. Chem. Biotechnol.* **1996**, *39*, 320−326.

(15) Watanabe, M. Catechins as antioxidants from buckwheat (*Fagopyrum esculetum* Moench). *J. Agric. Food Chem.* **1998**, *46*, 839−845.

(16) Davis, A. L.; Cai, Y.; Davies, A. P.; Lewis, J. R. ^{1}H and ^{13}C NMR assignments of some green tea polyphenols. *Magn. Reson. Chem.* **1996**, *34*, 887−890.

(17) Blois, M. S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, *181*, 1199−1200.

(18) Smith, R. C.; Reeves, J. C.; Dage, R. C.; Schnettler, R. A. Antioxidant properties of 2-imidazolones and 2-imidazolthiones. *Biochem. Pharmacol.* **1987**, *36*, 1457−1460.

(19) Cabrera, C.; Gimenez, R.; Lopez, M. C. Determination of tea components with antioxidant activity. *J. Agric. Food Chem.* **2003**, *51*, 4427−4435.

(20) Escribano-Bailón, M. T.; Santos-Buelga, C. Polyphenol extraction from foods. In *Methods in Polyphenol Analysis*; Williamson, G., Santos-Buelga, C., Eds.; Royal Soiety of Chemistry: London, U.K., 2003; pp 1−16.

(21) Upston, J. M.; Kritharides, L.; Stocker, R. The role of vitamin E in atherosclerosis. *Prog. Lipid Res.* **2003**, *42*, 405−422.

(22) Miyazawa, T. Absorption, metabolism and antioxidative effects of tea catechin in humans. *Biofactors* **2000**, *13*, 55−59.

(23) Donovan, J. L.; Luthria, D. L.; Stremple, P.; Waterhouse, A. L. Analysis of (+)-catechin, (−)-epicatechin and their 3′- and 4′-O-methylated analogs. A comparison of sensitive methods. *J. Chromatogr. B* **1999**, *726*, 277−283.

Received for review January 8, 2004. Revised manuscript received July 6, 2004. Accepted July 7, 2004. This work was supported by funds from the National Institutes of Health−National Institute of General Medical Sciences SCORE Award S06GM008225 and the Professional Staff Congress of The City University of New York Award 669662.

JF049950K