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Characterization and Quantitation of Polyphenolic Compounds in Bark, Kernel, Leaves, and Peel of Mango (*Mangifera indica* L.)

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The contents of secondary plant substances in solvent extracts of various byproducts (barks, kernels, peels, and old and young leaves) in a range of Brazilian mango cultivars were identified and quantitated. The results show that the profiles of secondary plant substances such as xanthone *C*-glycosides, gallotannins, and benzophenones in different byproducts vary greatly but are fairly consistent across cultivars. The free radical scavenging activity of the solvent extracts was evaluated using a high-performance liquid chromatography-based hypoxanthine/xanthine oxidase assay and revealed dose-dependent antioxidant capacity in all extracts. Four (mangiferin, penta-*O*-galloyl-glucoside gallic acid, and methyl gallate) of the major phenolic compounds detected were also evaluated in additional in vitro bioassay systems such as oxygen radical absorbance capacity, 2,2-diphenyl-1-picrylhydrazyl, and ferric reducing ability of plasma. Mangiferin in particular, detected at high concentrations in young leaves (Coite = 172 g/kg), in bark (Momika = 107 g/kg), and in old leaves (Itamaraka = 94 g/kg), shows an exceptionally strong antioxidant capacity.

KEYWORDS: Benzophenones; DPPH assay; FRAP assay; gallotannins; HPLC-ESI-MS; mangiferin; ORAC assay; cancer chemopreventive polyphenolic compounds; xanthones

INTRODUCTION

Mango (*Mangifera indica* L.), which belongs to the family Anacardiaceae, order Rutales, is one of the most important fruits marketed in the world with global production exceeding 26 million tons in 2004 (*1*). It is grown naturally or cultivated mainly in tropical and subtropical regions and has been reported to be the second largest tropical fruit crop in the world (*2*).

Extensive research in the area of plant breeding has generated hundreds of cultivars, the fruits of which show a pronounced diversity in size, color, flavor, seed size, and composition (*3*). In Brazil, mango is cultivated on a large scale in the Southeastern and Northeastern states. According to data published by the FAO, Brazil is the fifth largest producer of mangoes, although it represents just 2.7% of the total global production of 26 million tons.

Extracts of *M. indica* Linn have been reported to possess antiviral, antibacterial, analgesic, anti-inflammatory, and immunomodulatory activities (*4*); in vitro antiamoebic activity (*5*); interesting α-amylase and α-glucosidase inhibitory activities (*6*); and cardiotonic and diuretic properties (*7*). In traditional medicine, the use of mango extracts as herbal drugs is widespread. There are several reports available concerning the traditional uses of mango kernel in various parts of the world. In Fiji, fresh mango kernel is consumed as a cure for dysentery and asthma, while mango juice is used as a nose drop for sinus trouble (*8*). In India, dry seed power is applied to the head to remove dandruff and is also applied as an antidiarrheal agent (*9*). Kernel starch is eaten as a famine food (*10*), while hot water extracts of kernel are administered as anthelmintics, aphrodisiacs, laxatives, and tonics (*11*). The high carotenoid content, which is responsible for the yellow to orange color of ripe mango, provides a high provitamin A value and antioxidant capacity. Total carotenoid concentrations are usually in the range of 0.9−9.2 g/kg (*12*).

Phenolic compounds play an important role in the color and flavor of foods and beverages, and regular consumption is associated with beneficial effects for human health (*13*). Some phenolic compounds present in mango are antioxidants, contributing to a reduction in the risk of cardiovascular diseases, while others such as gallic acid and quercetin are claimed to have activity against allergies, inflammation, hypertension, arthritis, and carcinogenesis (*14, 15*).

Interest in the search for new natural antioxidants has grown dramatically over the past years because oxidative stress,

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mediated via the generation of reactive oxygen species (ROS), has been linked to age-related illnesses such as cancer. The fact that secondary plant metabolites, especially antioxidant phenolic compounds, can ameliorate this situation gave rise to a new scientific phenomenon, that is, cancer chemoprevention.

The pharmacology of phenolic secondary plant substances present in various products of *M. indica* L. is as yet in its infancy. However, a recent article has appeared in the literature (*16*) showing that mangiferin is bioavailable in preclinical models. Wang et al. (*16*) have shown that after oral administration of purified mangiferin (120 mg/kg) to Sprague−Dawley rats, followed by collection of both blood (over 24 h) and urine samples (over 48 h), the parent compound is bioavailable by detection in both of the biological matrices at a maximum of 24−25 μg/mL at 24 h.

Another important incentive for such research is the replacement with natural products of the potentially toxic synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene (*17*), which are currently used as food preservatives. The aim of this work was the identification and consolidation of the data of Berardini et al. (*18*) on polyphenolic compounds with antioxidant potentials in methanol extracts of byproducts from a range of Brazilian mango cultivars and comparison of their profiles, concentrations, and antioxidant capacities.

MATERIALS AND METHODS

Reagents. Acetic acid, dimethyl sulfoxide (DMSO), ethylenediaminetetraacetic acid, hypoxanthine, methanol, xanthine, and xanthine oxidase were purchased from Merck (Darmstadt, Germany); K_2HPO_4 and KH_2PO_4 were purcahsed from Serva (Heidelberg Germany); DPPH (2,2-diphenyl-1-picrylhydrazyl), $FeCl_3 \cdot 6H_2O$, salicylic acid, Trolox, and vitamin E were from Sigma-Aldrich Chemie (Steinheim, Germany); 2,4,6,-tripyridyl-*s*-triazine complex (TPTZ) was from Riedel de Haen (Seelze, Germany); ascorbic acid was from Sigma Chemie (Deisenhofen, Germany), while 3,3′-azo-bis(2-amidinopropane)dihydrochloride (AAPH) was obtained from Wako Chemicals (Neuss, Germany). Ellagic acid, gallic acid, methyl gallate, 3,4-dihydroxybenzoic acid, isoquercitrin (quercetin-3-*O*-glucopyranoside), and mangiferin were obtained from Extrasynthese (Lyon Nord, Genay, France). All solutions were made up in double-distilled water.

Mango Cultivars Studied. The 16 cultivars of mango studied were obtained from Embrapa (a Brazilian agricultural research company), and the samples were collected in the experimental field (quartz sandy soil) of the Curu, in Ceará, Brazil.

The cultivars studied were Amrapali, Coité, Fafá, Haden, Itamaraká, Keit, Kent, Mallika, Momiká, Primavera, Rosa, Tommy Atkins, Van Dyke, and cultivars resulting from plant breeding studies, Embrapa 141-roxa (Amrapali × Tommy Atkins), Embrapa 142-alfa (Mallika × Van Dyke), and CPAC 136/86.

Preparation of Plant Material. Mango leaves and barks were dried in an oven at 40 °C, whereas kernels and peels were freeze-dried (Christ, Gefriertrocknungsanlangen, Osterode, Germany) to constant weight. Dried samples were pulverized by blending to a fine homogeneous powder prior to extraction.

Extraction of Mango Byproducts. Byproducts of *M. indica* L. (2.5 g of freeze-dried material) from mango were extracted in duplicate for 3 h with hexane in a Soxhlet apparatus to remove lipids. After it was dried, the material was further extracted three times for 3 h with methanol, and the solutions were pooled and evaporated to dryness at 40 °C by rotary evaporation under reduced pressure.

The extracts were dissolved in methanol and analyzed by analytical high-performance liquid chromatography (HPLC), HPLC−electrospray ionization−mass spectrometry (ESI-MS), and in the hypoxanthine/xanthine oxidase HPLC-based assay. The major polyphenols were quantitated using analytical HPLC and were purified by semipreparative HPLC and further analyzed by HPLC-ESI-MS, nano-ESI-MS-MS, and NMR.

To compare our methods with those of Berardini et al. (*22*), 2.5 g of lyophylized kernel (Tommy Atkins) was worked-up in a similar manner to that described. The dried extract was suspended in methanol (5.0 mL) and analyzed by analytical HPLC and HPLC-ESI-MS as described below.

Analytical HPLC. Analytical HPLC was conducted on a Hewlett-Packard (HP) 1090 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a 250 mm × 4 mm i.d., 5 μm, C18, reversed-phase column (Latek, Eppelheim, Germany). The phenolic compounds were detected in the eluant with an UV diode-array detector (HP 1040M) set at 278 and 340 nm. Dried extracts of mango were suspended in methanol (5.0 mL for kernels, barks, and peels; 10.0 mL for leaves). Of these suspensions, 1.0 mL was placed in a plastic microfuge tube and spun at 14500 rpm. The supernatant (1.0 μL) was directly injected into the HPLC. The precipitate was suspended in DMSO (1.0 mL), and again, 1.0 μL was injected into the HPLC. The mobile phase used consisted of 2% acetic acid in water (solvent A) and methanol (solvent B), utilizing the following gradient over a total run time of 45 min: initially 95% A for 2 min; to 75% A in 8 min; to 60% A in 10 min; to 50% A in 10 min; to 0% A in 5 min; and continuing at 0% A until completion of the run. The amount of phenolic compounds in the extracts was calculated from standard curves in the range of 0.05−1.0 mM of either authentic commercial standards (gallic acid, 3,4-dihydroxybenzoic acid, mangiferin, methyl gallate, and isoquercitrin) or substances purified (>95%) by semipreparative HPLC, at the λ maximum of the phenolic class. Instrument control and data handling were performed with the HP Chemstation software on a PC.

HPLC-ESI-MS. HPLC-ESI-MS was conducted on an Agilent 1100 HPLC, coupled to an Agilent single quadrupole mass-selective detector (HP 1101; Agilent Technologies, Waldbronn, Germany). Chromatographic separation of methanolic extracts was conducted using a column of the same type and dimension as for analytical HPLC (Latek, Eppelheim, Germany). The mobile phase consisted of 2% acetic acid in water (solvent A) and acetonitrile (solvent B) with the following gradient: initially 95% A for 10 min; to 90% A in 1 min; to 60% A in 9 min; to 80% A in 10 min; to 60% A in 10 min; to 0% A in 5 min; and continuing at 0% A until completion of the run. Detection of phenolic compounds was by means of UV absorbance (A) at 278 and 340 nm at room temperature. Mass spectra in the negative-ion mode were generated under the following conditions: fragmenter voltage, 100 V; capillary voltage, 2500 V; nebulizer pressure, 30 psi; drying gas temperature, 350 °C; and mass range, 100−1500 D. Instrument control and data handling were performed with the same software as for analytical HPLC.

Quantitation of Polyphenols. The quantitation of polyphenolic compounds was determined from the analytical HPLC chromatograms, and the amounts were calculated based on the calibration curves generated from either standard or purified compounds.

HPLC Analysis of Dihydroxyphenols in the HPLC-Based Antioxidant Assay. This was conducted as described above except that the UV detector was set at 325 and 278 nm for the detection of the products produced by ROS attack on salicylic acid (2,5-dihydroxybenzoic acid and 2,3-dihydroxybenzoic acid) and hypoxanthine (uric acid), respectively.

Semipreparative HPLC. Semipreparative HPLC was conducted on a HP 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a similar C-18 column (10 mm i.d.) as for analytical HPLC. Peaks eluting from the column were collected on a HP 220 Microplate Sampler and subsequently freeze-dried. For the separation of individual compounds in the extracts, the mobile phase consisted of 0.2% trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B), utilizing the following solvent gradient over a total run time of 50 min: initially 95% A for 1 min; to 90% A in 9 min; to 85% A in 10 min; to 80% A in 10 min; to 0% A in 5 min; and continuing at 0% A until completion of the run. The flow rate of the mobile phase was 3 mL/min. Peaks eluting from the column were collected on an Agilent HP 220 Microplate Sampler. Each purified fraction was pooled, and the solvent was removed by lyophilization.

Nano-ESI-MS. Samples were dissolved in methanol, and ESI mass spectra were recorded on a Finningan MAT TSQ 7000 triple-quadrupole

mass spectrometer (Finningan, San Jose, CA) equipped with a nano-electrospray source (EMBL, Heidelberg, Germany) using both the positive- and negative-ion modes. Argon was used as collision gas at a nominal pressure of 2.5 mTorr (1 Torr = 133.3 Pa). Samples were sprayed from gold-plated glass capillaries prepared in-house with a type-87-B microcapillary puller (Sutter Instruments, Novato, CA). The applied voltage was 400−700 V with a scan range of *m/z* 20−2600.

Hypoxanthine-Xanthine Oxidase HPLC-Based Antioxidant Assay. The hypoxanthine/xanthine oxidase HPLC-based assay, conducted according to the methods of Owen et al. (*19–21*), was used to assess the antioxidant capacities of the raw methanolic extracts of mango byproducts and purified compounds therefrom.

Methanolic extracts of peels, kernels, barks, and old and young leaves were tested using stock solutions of 2 mg/mL. For the hypoxanthine/xanthine oxidase assay, the relevant sample concentration range (0−1000 μg) in methanol was added to 2 mL plastic microfuge tubes in duplicate, and the solvent was removed under a stream of nitrogen. The dried residues were suspended in phosphate buffer (1.0 mL), and 5.0 μL of a 1:5 dilution of xanthine oxidase in $(NH_4)_2SO_4$ (3.2 M) was added to initiate the reaction. The tubes were incubated at 37 °C until reaction completion in 3 h. After incubation, 20 μL of the reaction mixtures was analyzed by analytical HPLC. The amounts of dihydroxyphenol (2,5-dihydroxybenzoic acid and 2,3-dihydroxybenzoic acid) and uric acid produced were determined from the calibration standard curves generated for authentic standards at the relevant wavelength.

DPPH Assay. The free radical scavenging capacity of the substances was determined by using the DPPH radical discoloration method of Silva et al. (*22*),. The substances were diluted in methanol at concentrations between 0.01 and 1.0 mM. Twenty microliters of the different concentrations was placed in 96 well plates in duplicate. The reaction was initiated by adding 180 μL of DPPH solution (20 μg/mL in methanol). The absorbance was read at 515 nm over 45 min with an Elx 800 universal Micro plate reader (Bio-Tek Instruments, Winooski, VT) vs a control (20 μL of methanol). The concentration of DDPH radical was calculated from a standard curve of DPPH between 1 and 100 μg/mL measured simultaneously.

Steady-state levels were reached within the first 15 min. For comparison, the IC_{50} values for each substance (concentration of each substance where 50% of the DPPH radical is scavenged) were calculated with the DPPH values at 15 min for different concentrations using a table curve program (Jandel Scientific, Chicago, IL).

Ferric Reducing Ability of Plasma (FRAP) Assay. The dilution of the different substances was identical to that described for the DPPH assay. Ten microliters of substance was incubated with 30 μL of water and 300 μL of FRAP reagent, consisting of 25 mL of acetate buffer (300 mM sodium acetate buffer, pH 3.6), 2,5 mL of TPTZ (10 mM TPTZ in 40 mM HCl), and 2.5 mL of $FeCl_3$ solution (20 mM $FeCl_3 \cdot 6H_2O$ in water) at 37 °C. All reagents were freshly prepared and warmed to 37 °C before measurement. A calibration curve of ferrous sulfate (0.01−1.0 mM) was used, and the results are expressed in mM Fe^{2+}/L.

The reaction was measured every 1 min for 10 min. The reaction of all isolated substances reached a steady-state level after 5 min. A linear regression curve was generated at the 5 min reaction time point for different concentrations of the isolated substances with the Microcal Origin 5.0 program. Using these regression curves, the EC_1 values were calculated as the concentrations of antioxidant (μM) giving an absorbance equivalent to a 1 mM Fe(II) solution according to Pulido et al. (*23*).

Oxygen Radical Absorbance Capacity (ORAC) Assay. 3,3′-Azo-bis-(2-amidinopropane)dihydrochloride (AAPH) (Wako Chemicals) was used as a peroxyl radical generator, and fluorescein (0.21 μM in ORAC buffer) was used as a redox-sensitive fluorescent indicator.

Aliquots of 20 μL of a 10 mM stock solution of Trolox in DMSO were stored at −20 °C and melted and diluted with ORAC buffer to a final concentration of 20 μM immediately before use. The ORAC buffer contained 75 mM potassium hydrogen phosphate/potassium dihydrogen phosphate at pH 7.4. Freshly prepared sample solutions (1.0 mM) in methanol or DMSO were diluted in ORAC buffer to a final concentration of 20 μM. Ten microliters of the sample, buffer, or Trolox solution together with 170 μL of fluorescein solution were placed in quadruplate in a 96 well plate and incubated at 37 °C for 10 min. The exterior wells of the plates were not used for experimental determinations. The reaction was initiated by addition of 20 μL of APPH solution (103.5 mg/2 mL buffer) and freshly mixed on ice immediately before use.

The decline of fluorescence was measured at 37 °C every 2 min until completion at 122 min using a Cytoflour 4000 fluorescent microplate reader, excitation wavelength Ex 530/25 nm, and emission wavelength Em 585/30 nm (Perspeptive Biosystems, MN). The net area under the curve (AUC) of the standards and samples was calculated. The final ORAC values were calculated using the regression equation between the Trolox concentration and the net AUC and are expressed as relative ORAC units, where 1 ORAC unit equals the inhibition of the declining fluorescence produced by 1 μM Trolox.

RESULTS AND DISCUSSION

The structures of the secondary plant metabolites isolated and identified in byproducts of mango fruits (*M. indica* L.) are given in **Figure 1**. HPLC-ESI-MS and nano-ESI-MS-MS data for the compounds purified from the methanol extracts of the various mango byproduct are given in **Table 1**, and the characteristic fragmentations observed by nano-ESI-MS-MS are now described.

Benzophenone Derivatives and Related Compounds. Compound **1** gave a $[M - H]^-$ ion at *m/z* 423 with fragment ions at *m/z* 303 and 333 in the MS/MS experiment caused by the loss of 120 and 90 Da fragments from the glucose moiety, representing the characteristic fragmentation of *C*-glycoside substances. The resulting fragments at *m/z* 303 and 333 provided product ions at *m/z* 193 and 223, respectively, corresponding to the loss of 110 Da. The loss of H_2O provided a fragment ion at *m/z* 405. This compound was unambiguously identified as maclurin 3-*C*-β-D-glucoside after both 1D and 2D NMR (600 MHz) experiments.

Compound **2** showed a $[M - H]^-$ ion at *m/z* 575 in the ESI-MS mode and a loss of 272 Da in the ESI-MS/MS experiment resulting in a prominent ion (base peak) at *m/z* 303. The loss of 272 Da ($[M - H - 120-152]^-$) indicated the presence of a galloylated benzophenone as described by Berardini et al. (*22*). This was substantiated by a further prominent ion at *m/z* 333 due to the loss of 242 Da ($[M - H - 90 - 152]^-$). Compound **2** was unambiguously identified as maclurin mono-*O*-galloyl-glucoside after both 1D and 2D NMR (600 MHz) experiments.

Compound **3** gave a $[M - H]^-$ ion at *m/z* 727 in the nano-ESI mode with fragment ions at *m/z* 575 ($[M - H - 152]^-$) and at *m/z* 557 ($[M - H - 170]^-$), indicating the loss of a galloyl and a gallic acid moiety, respectively. Further prominent ions at *m/z* 423 and *m/z* 405 due to the loss of 152 Da ($[575 - 152]^-$ and $[557 - 152]^-$) suggested the presence of a further gallic acid moiety. These data were again suggestive of a digalloyl benzophenone derivative. Compound **3** was unambiguously identified as iriflophenone 3-*C*-(2,6-di-*O*-galloyl)-β-D-glucoside after both 1D and 2D NMR (600 MHz). The above three compounds also showed common fragmentation patterns from the loss of 110 Da, which is in accordance with the postulated fragmentation pathways of maclurin *C*-glycosides as described by Berardini and colleagues (*18*). Three further compounds were very similar to the above substances; the only difference appeared to be one less hydroxyl group in the B ring. Compound **4** gave a $[M - H]^-$ ion at *m/z* 407 and fragments at *m/z* 287 and 317 in the MS experiment again caused by the loss of 120 and 90 Da fragments from the glucose moiety and was unambiguously identified as iriflophenone 3-*C*-β-D-glucoside after both 1D and 2D NMR (600 MHz) experiments.

Compound **5** gave a $[M - H]^-$ ion at *m/z* 527, and the presence of a *p*-hydroxybenzoyl moiety was clear due to the

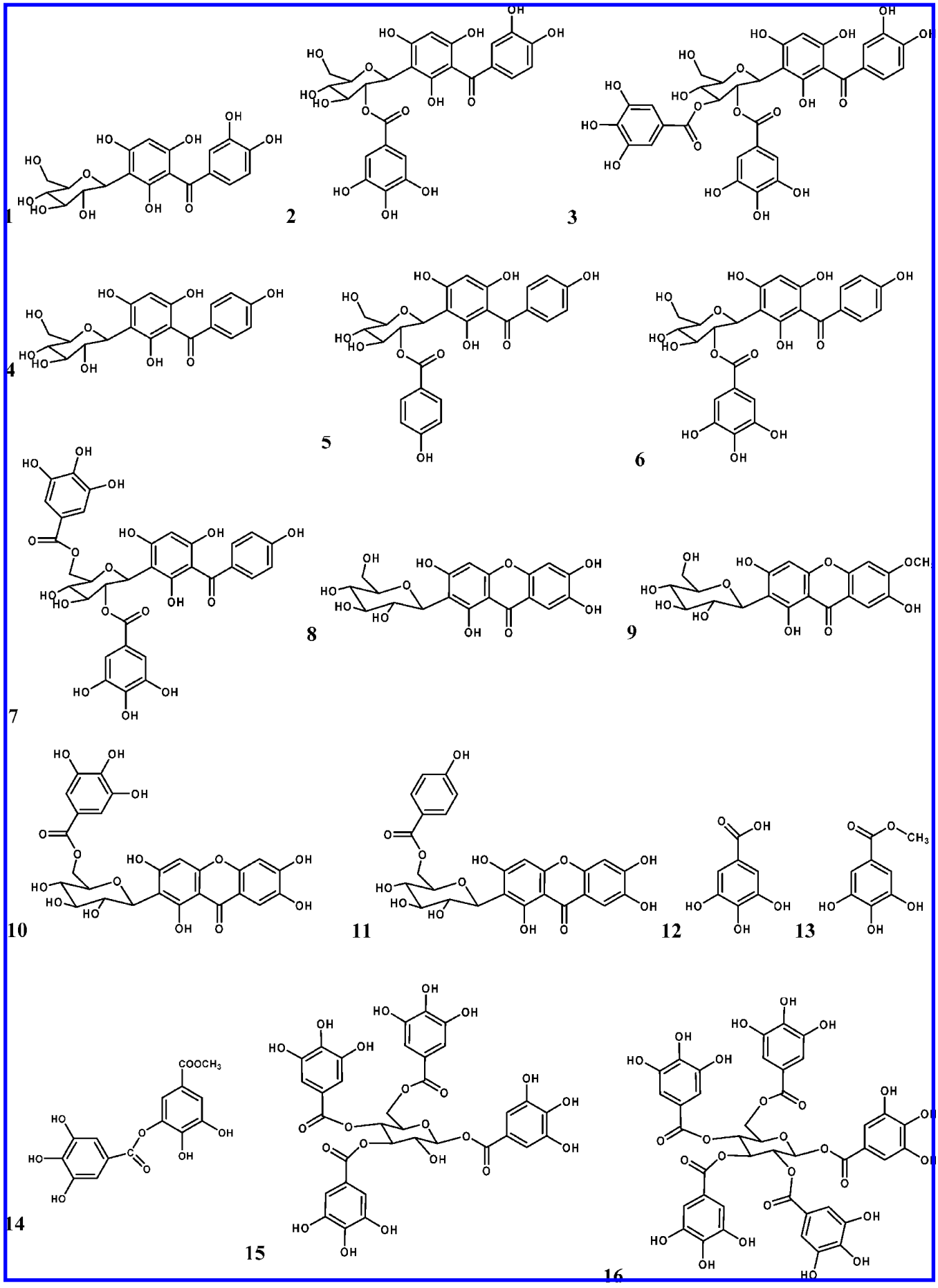

Figure 1

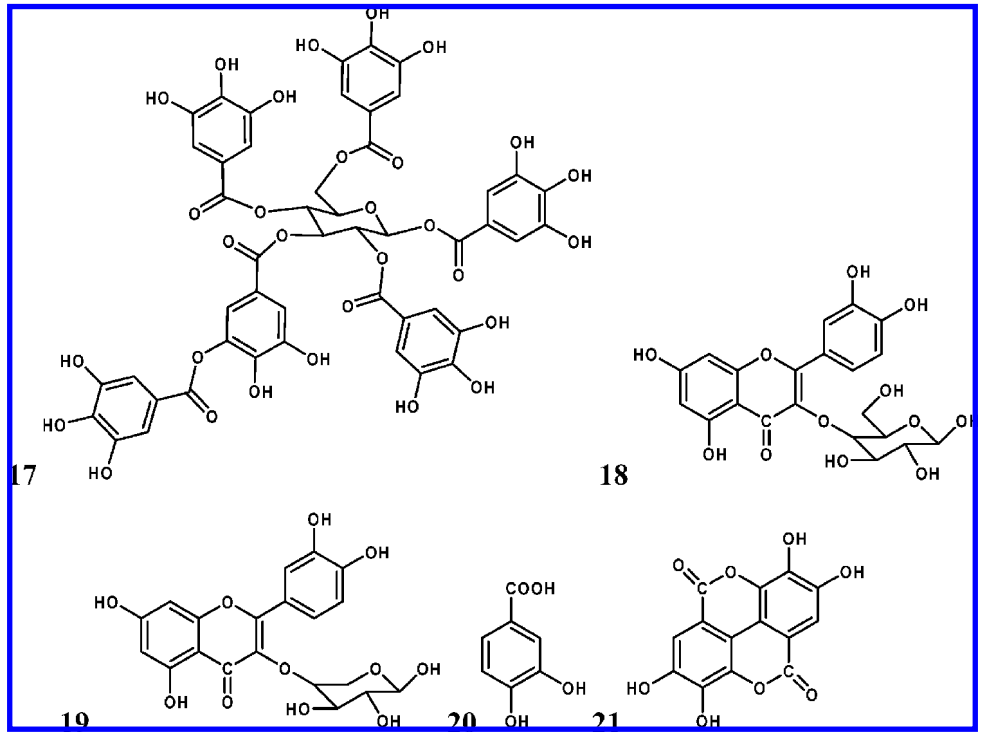

Figure 1. Structures of the phenolic compounds purified from methanol extracts of leaves, peels, kernels, and barks of different cultivars of *M. indica* L. from Brazil. Key: **1**, maclurin 3-*C*-β-D-glucoside; **2**, maclurin 3-*C*-(2-*O*-galloyl)-β-D-glucoside; **3**, maclurin 3-*C*-(2,3-di-*O*-galloyl)-β-D-glucoside; **4**, iriflophenone 3-*C*-β-D-glucoside; **5**, iriflophenone 3-C-(2-*O*-*p*-hydroxybenzoyl)-β-D-glucoside; **6**, iriflophenone 3-*C*-(2-*O*-galloyl)-β-D-glucoside; **7**, iriflophenone 3-*C*-(2,6-di-*O*-galloyl)-β-D-glucoside; **8**, mangiferin; **9**, homomangiferin; **10**, 6-*O*-galloyl-mangiferin; **11**, 6-*O*-(*p*-hydroxybenzoyl)mangiferin; **12**, gallic acid; **13**, methyl gallate; **14**, gallic acid/methyl gallate ester; **15**, tetra-*O*-galloyl-glucoside; **16**, penta-*O*-galloyl-glucoside; **17**, hexa-*O*-galloyl-glucoside; **18**, isoquercitrin; **19**, quercetin pentoside; **20**, 3,4-dihydroxy benzoic acid; and **21**, ellagic acid.

Table 1. HPLC-ESI-MS and Nano-ESI-MS-MS Data in the Negative-Ion Mode for Individual Phenolic Compounds Isolated from Methanolic Extracts of Mango Cultivar Byproducts by Semipreparative HPLC

		HPLC-ESI-MS		nano-ESI-MS	
compound no.	HPLC-DAAD λ_{max} (nm)	$[M-H]^-$	$[2M-H]^-$	$[M-H]^-$	major fragment ions (*m*/*z*, %)
1	232, 284, 320	423.2	847.3	423 (21)	333 (14), 303 (100), 223 (3), 193 (4)
2	238, 290, 336	575.2	1151.3	575 (35)	557 (1), 465 (2), 333 (19), 303 (100)
3	236, 288, 344	727.2	1455.7	727 (100)	575 (96), 557 (8), 423 (11), 405 (37), 333 (8), 315 (12)
4	230, 296, 340	407.2	815.4	407 (10)	317 (27), 287 (100), 245 (3)
5	238, 292, 340	559.1	1119.4	559 (26)	439 (2), 407 (96), 389 (11), 317 (21), 287 (100), 269 (61), 245 (13), 169 (32)
6	236, 266, 282, 316	527.1	1055.2	527 (12)	407 (22), 389 (38), 287 (6), 269 (100)
7	226, 280, 334	711.2	1423.4	711 (25)	559 (46), 541 (25), 317 (12), 287 (30), 271 (100)
8	240, 258, 276, 320, 368	421.1	843.3	421 (15)	403 (7), 331 (86), 301 (100), 271 (8)
9	220, 260, 270, 317, 365	435.1	n.d.[a]	435 (35)	345 (81), 331 (11), 315 (100)
10	236, 248, 266, 282, 324, 372	573.2	1147.4	573 (28)	421 (10), 403 (12), 331 (100), 301 (94)
11	232, 248, 264, 282, 324, 372	541.1	1083.3	541 (32)	403 (3), 331 (71), 301 (100)
12	226, 278	169.1	n.d.	169 (24)	125 (100)
13	226, 272	183.1	n.d.	183 (21)	168 (15), 124 (100)
14	220, 278	335.1	n.d.	335 (17)	183 (100), 124 (2)
15	226, 278	787.0	n.d.	787 (98)	635 (39), 617 (100), 465 (37)
16	226, 280	939.1	n.d.	939 (48)	787 (12), 769 (100), 617 (41)
17	226, 280	1091.1	n.d.	1091 (29)	939 (100), 787 (7), 769 (12), 617 (6)
18	232, 264, 300, 360	463.2	927.3	463 (67)	301 (100), 179 (4), 151 (3)
19	220, 263, 295, 350	433.2	867.3	433 (5)	301 (100)
20	225, 289	153.1	n.d.	153 (4)	109 (100)
21	256, 308, 354, 367	301.1	603.1	301 (41)	300 (62), 283 (56), 245 (20), 200 (55), 145 (100)

[a] n.d.=not detected.

fragment ions at *m*/*z* 407 (loss of 120 Da) and 389 (loss of 138 Da) besides the presence of a characteristic fragmentation of a *C*-glycosidic compound with fragment ions at *m*/*z* 287 and *m*/*z* 269 resulting from the loss of 120 Da from the glucose core. From these data, the compound was tentatively identified as iriflophenone 3-*C*-(6-*O*-*p*-hydroxybenzoyl)-β-D-glucoside, which was confirmed after both 1D and 2D NMR (600 MHz) experiments. Compound **6** gave a $[M-H]^-$ ion at *m*/*z* 559, with fragments ions at *m*/*z* 407 and 389 caused by the loss of galloyl (M − H − 152 Da) and gallic acid (M − H − 170 Da) moieties, respectively. Further fragments ions at *m*/*z* 287 and 317 resulting from the loss of 120 and 90 Da from the *C*-glucosidic moiety plus 152 Da from the galloyl moiety enabled tentative identification as iriflophenone mono-*O*-galloyl-glucoside, which was confirmed after both 1D and 2D NMR (600 MHz) experiments.

Table 2. Content (g/kg Dry Matter) of Total Polyphenolic Compounds in Methanol Extracts of Byproducts from Various Mango Cultivars[a]

	total secondary plant substances (g/kg)				
cultivar	peels	kernels	bark	old leaves	young leaves
Embrapa-141-Roxa	24.24	89.49	20.45	246.27	349.75
Momiká	ND	ND	122.53	154.75	250.70
Fafá	52.28	149.33	ND	104.26	ND
CPAC-136/86	ND	ND	6.56	196.98	262.23
Keit	ND	ND	18.07	245.07	309.31
Van Dyke	59.09	70.10	31.62	59.03	163.43
Tommy Atkins	25.13	200.05	73.34	199.22	60.59
Rosa	ND	ND	24.92	184.85	351.23
Embrapa-142-alfa	ND	ND	232.86	155.46	142.04
Primavera	19.68	ND	16.21	141.41	178.41
Itamaraka	ND	ND	82.39	157.52	356.78
Haden	ND	ND	21.60	319.24	186.10
Coite	9.18	165.45	137.73	356.34	298.17
Amrapali	18.12	ND	77.28	241.59	ND
Mallika	ND	ND	36.28	90.55	ND
Kent	91.21	191.25	20.01	ND	249.70
Mean	37.37	144.28	61.46	190.17	242.96

[a] Mean of two experiments (SD < 5); ND, not determined.

Compound **7** gave a $[M - H]^-$ ion at *m/z* 711 in the nano-ESI mode with fragment ions at *m/z* 559 ($[M - H - 152]^-$) and at *m/z* 541 ($[M - H - 170]^-$), indicating the loss of a galloyl and a gallic acid moiety, respectively. This was substantiated by further prominent ions at *m/z* 317 and *m/z* 287 due to the loss of 242 Da ($[M - H - 90 - 152]^-$) and 272 Da ($[M - H - 120 - 152]^-$), respectively. These data were suggestive of a digalloyl benzophenone derivative. Compound **7** was unambiguously identified as maclurin 3-*C*-(2,3-di-*O*-galloyl)-β-D-glucoside after both 1D and 2D NMR (600 MHz).

Mangiferin and Derivatives. Mass spectrometric analysis of Compound **8** gave a $[M - H]^-$ ion at *m/z* 421 and typical fragmentation behavior of a *C*-glycoside with fragment ions at 301 (M − H − 120 Da) and 331 (M − H − 90 Da). The structure was unambiguously identified as mangiferin after both 1D and 2D NMR (600 MHz) experiments.

The UV spectrum of compound **9** was very similar to mangiferin, and its ESI-MS fragmentaion patterns indicated the presence of an extra methyl group. This compound is tentatively assigned the structure of homomangiferin, but the exact location of the methyl group requires elucidation by NMR experiments.

Two other compounds with similar fragmentation patterns in the MS experiments were detected. Compound **10**, which gave a $[M - H]^-$ ion at *m/z* 573 besides the typical fragmentation pattern observed for mangiferin also showing loss of 170 Da (gallic acid) from the $[M - H]^-$ ion, was readily identified as mangiferin gallate. Compound **11** gave a $[M - H]^-$ ion at *m/z* 541, and the presence of a *p*-hydroxybenzoic acid moiety was evident, due to the fragment ion a *m/z* 403 caused by the loss of 138 Da. Further fragment ions characteristic of a mangiferin derivative at *m/z* 301 and 331 suggested the structure to be mangiferin 3-*C*-6-*O*-*p*-hydroxybenzoic acid. The structures of both compounds were confirmed unambiguously after both 1D and 2D NMR (600 MHz) experiments.

Gallates and Gallotannins. Compound **12** was identified as gallic acid showing a $[M - H]^-$ ion at *m/z* 169 and a major fragment ion at *m/z* 124 due to the loss of 59 Da. The structure was unambiguously confirmed after both 1D and 2D NMR (600 MHz) experiments.

Compound **13** was identified as methyl gallate showing a $[M - H]^-$ ion at *m/z* 183 and major fragment ions at *m/z* 168 due to the loss of 15 Da and at *m/z* 124 due to the loss of 59 Da.

Table 3. Content (g/kg Dry Material) of Individual Polyphenolic Compounds in Methanol Extracts of Byproducts from the Mango Cultivar Van Dyke (Part A) and Embrapa-141-Roxa (Part B)[a]

phenolic compounds	peels	kernels	bark	old leaves	young leaves
	part A				
gallic acid	ND	ND	0.24	0.43	3.49
methyl gallate	15.46	12.68	0.34	2.01	17.95
gallic acid/methyl gallate dimer	2.30	ND	ND	ND	ND
3,4-dihydroxy benzoic acid	ND	ND	0.29	0.17	0.82
maclurin 3-*C*-β-D-glucoside	1.97	ND	0.19	0.17	0.63
iriflophenone 3-*C*-β-D-glucoside	2.05	ND	2.05	8.30	26.81
tetra-*O*-galloyl-glucoside	7.22	0.99	ND	ND	1.09
isomangiferin	ND	ND	0.79	ND	ND
penta-*O*-galloyl-glucoside	17.71	50.03	0.70	1.82	23.26
6-*O*-galloyl-mangiferin	ND	ND	1.67	ND	ND
mangiferin	4.94	6.40	18.33	36.9	58.12
isomangiferin	ND	ND	0.79	ND	ND
iriflophenone 3-*C*-(2-*O*-galloyl)-β-D-glucoside	ND	ND	3.05	2.68	7.45
iriflophenone 3-*C*-(2,6-di-*O*-galloyl)-β-D-glucoside	ND	ND	0.42	1.67	1.94
maclurin 3-*C*-(2-*O*-galloyl)-β-D-glucoside	4.05	ND	1.08	ND	ND
maclurin 3-*C*-(2,3-di-*O*-galloyl)-β-D-glucoside	3.37	ND	ND	ND	ND
isoquercitrin isomers	ND	ND	ND	2.12	11.42
quercetin pentosides	ND	ND	ND	1.33	4.93
iriflophenone 3-*C*-(2-*O*-*p*-hydroxybenzoyl)-β-D-glucoside	ND	ND	1.66	0.48	1.16
6-*O*-(*p*-hydroxybenzoyl)-mangiferin	ND	ND	0.81	0.95	4.38
total	59.07	70.10	31.62	59.03	163.45
	part B				
gallic acid	0.02	2.45	0.16	3.21	3.71
methyl gallate	0.87	29.10	0.26	15.98	19.29
3,4-dihydroxy benzoic acid	ND	ND	0.14	1.43	0.93
maclurin 3-*C*-β-D-glucoside	ND	ND	0.15	2.33	2.94
iriflophenone 3-*C*-β-D-glucoside	ND	ND	0.59	86.31	118.04
tetra-*O*-galloyl-glucoside	1.19	11.77	ND	ND	ND
homomangiferin	0.26	ND	0.26	ND	ND
penta-*O*-galloyl-glucoside	2.76	36.77	0.65	16.37	19.79
6-*O*-galloyl-mangiferin	0.30	ND	1.36	ND	ND
mangiferin	15.23	8.98	12.33	44.63	67.20
isomangiferin	ND	ND	0.51	ND	ND
iriflophenone 3-*C*-(2-*O*-galloyl)-β-D-glucoside	0.19	ND	1.03	14.62	12.38
iriflophenone 3-C-(2,6-di-*O*-galloyl)-β-D-glucoside	0.65	ND	0.29	6.17	6.89
maclurin 3-*C*-(2-*O*-galloyl)-β-D-glucoside	0.14	ND	0.94	ND	ND
maclurin 3-*C*-(2,3-di-*O*-galloyl)-β-D-glucoside	0.88	ND	ND	ND	ND
isoquercitrin isomers	0.69	ND	ND	16.72	29.78
quercetin pentosides	0.91	ND	ND	1.33	40.94
iriflophenone 3-*C*-(2-*O*-*p*-hydroxybenzoyl)-β-D-glucoside	0.15	ND	0.84	12.70	13.34
6-*O*-(*p*-hydroxybenzoyl)-mangiferin	ND	ND	0.94	11.60	14.52
mangiferin gallate isomer	ND	ND	1.36	ND	ND
ellagic acid	ND	0.42	ND	ND	ND
total	24.24	89.49	20.45	246.27	349.75

[a] Mean of two experiments (SD < 5); ND, not detected.

The structure was unambiguously confirmed after both 1D and 2D NMR (600 MHz) experiments.

The UV spectrum of compound **14** was suggestive of a gallate type compound, although the λ_{max} at 278 nm was quite broad. The mole peak $[M - H]^-$ was detected at *m/z* 335, with major fragment ions at *m/z* 183 and *m/z* 124, suggesting the presence of a methyl gallate moiety. Although no evidence of a gallic acid moiety could be detected, it is likely a *meta*-depside dimer of methyl gallate and gallic acid and probably represents a fragment of, for example, hexagallates, due to hydrolytic

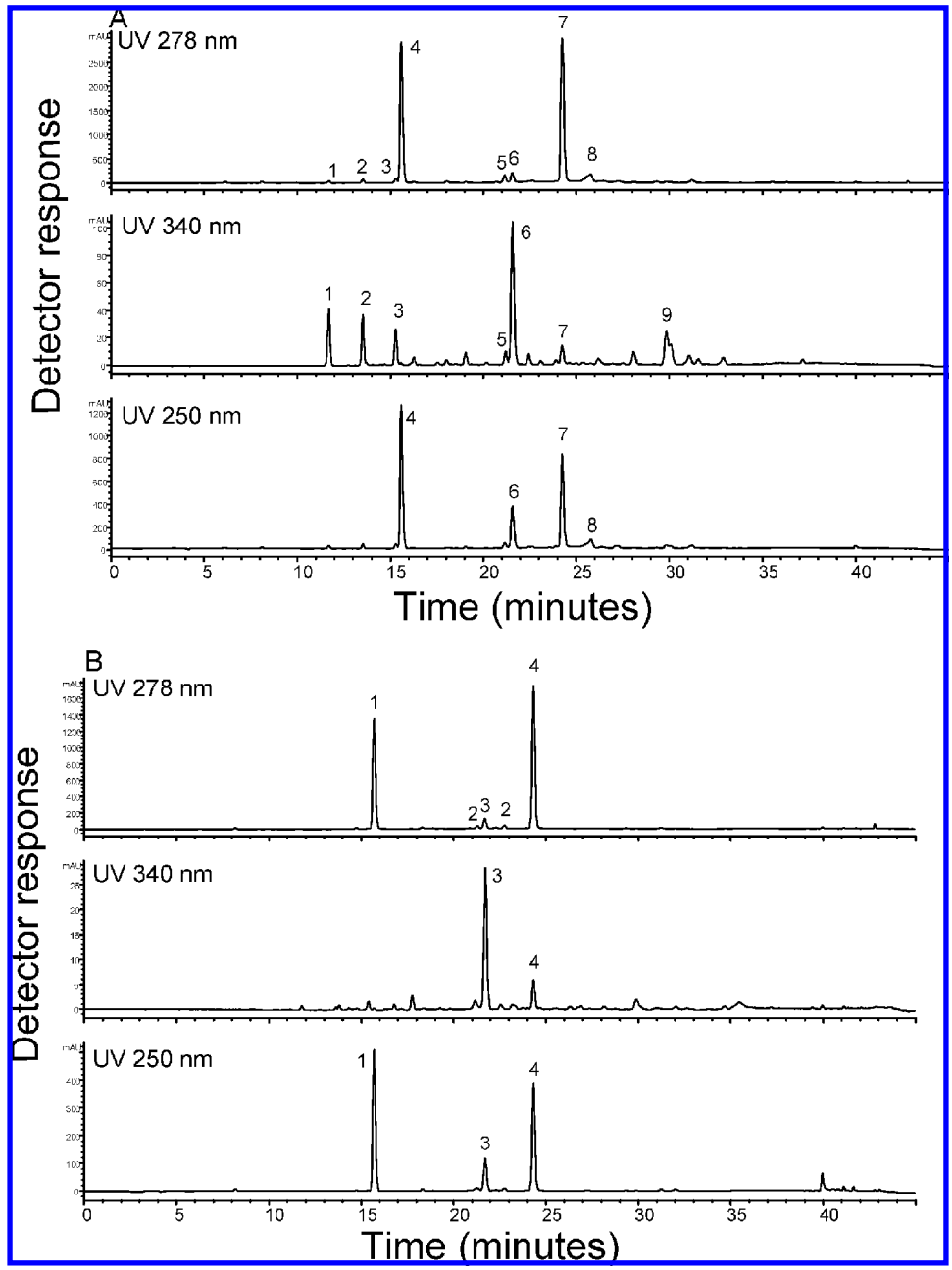

Figure 2. (**A**) Analytical HPLC chromatogram of a methanol extract of mango peel (cultivar, Van Dyke). Peaks: 1, maclurin 3-*C*-*β*-D-glucoside; 2, maclurin 3-*C*-(2-*O*-galloyl)-*β*-D-glucoside; 3, maclurin 3-*C*-(2,3-di-*O*-galloyl)-*β*-D-glucoside; 4, methyl gallate; 5, tetra-*O*-galloyl-glucoside; 6, mangiferin; 7, penta-*O*-galloyl-glucoside; 8, gallic acid/methyl gallate dimer; and 9, isoquercitrin isomers. (**B**) Analytical HPLC chromatogram of a methanol extract of mango kernels (cultivar, Van Dyke). Peaks: 1, methyl gallate; 2, tetra-*O*-galloyl-glucoside; 3, mangiferin; and 4, penta-*O*-galloyl-glucoside.

transesterification with methanol (also the source of methyl gallate) occurring during the extraction and column chromatography procedures. The structure, however, requires confirmation by NMR, and these studies are ongoing.

Compound **15** gave a $[M - H]^-$ ion at *m*/*z* 787 and fragments at *m*/*z* 635 and 617 caused by the loss of galloyl (152 Da) and gallic acid (170 Da) moieties, respectively. Further loss of galloyl and gallic acid moieties revealed a fragmentation pattern typical of tetra-*O*-galloyl-glucoside. The structure, however, requires confirmation by NMR, and this is ongoing.

Compound **16** gave a $[M - H]^-$ ion at *m*/*z* 939 and fragment ions at *m*/*z* 787 and 769 caused by the loss of galloyl (152 Da) and gallic acid (170 Da) moieties, respectively. Further loss of galloyl and gallic acid moieties revealed a fragmentation pattern typical of penta-*O*-galloyl-glucoside. Again, the structure of this compound was unambiguously identified after both 1D and 2D NMR (600 MHz) experiments.

Compound **17** gave a $[M - H]^-$ ion at *m*/*z* 1091 and fragment ions at *m*/*z* 939 and 787 caused by the loss of two successive galloyl (152 Da) moieties, respectively. Further loss of galloyl and gallic acid moieties revealed a fragmentation pattern typical of hexa-*O*-galloyl-glucoside. Again, the structure of this compound was unambiguously identified after both 1D and 2D NMR (600 MHz) experiments.

Flavonoids. Compound **18** was isolated as a mixture of two isomers with an UV spectrum typical of quercetin derivatives (**Table 2**). The isomers gave a $[M - H]^-$ ion at *m*/*z* 463 with fragment ions at *m*/*z* 301 and 151, typical of quercetin glycosides and probably representing isoquercitrin and quercetin galactoside, respectively, as described by Berardini et al. (*18*).

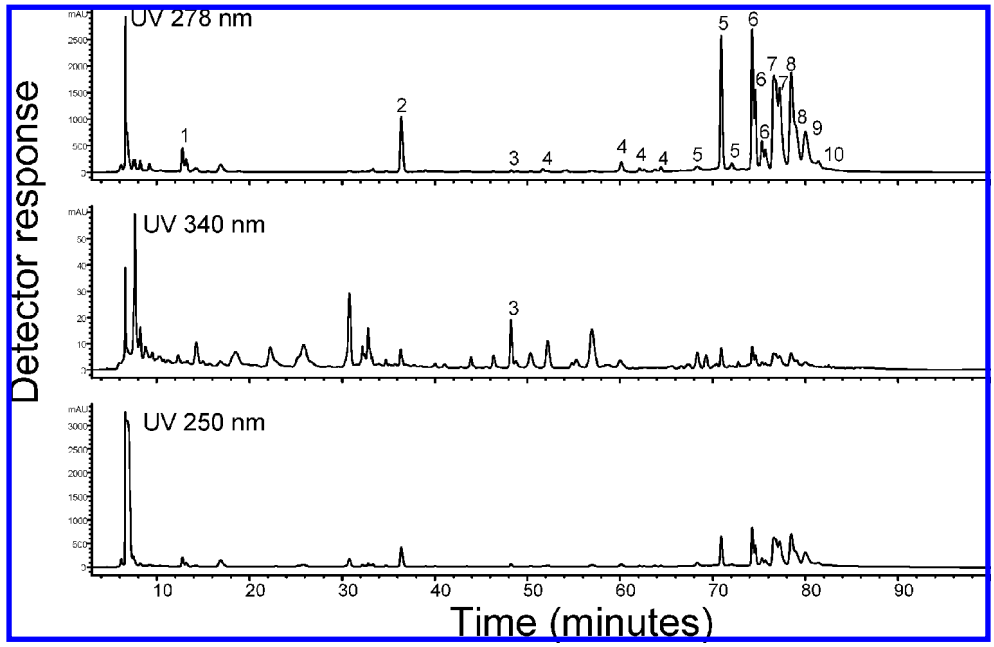

Figure 3. Analytical HPLC chromatogram of a 70% acetone (aqueous) extract of mango kernels (cultivar, Van Dyke). Peaks: 1, gallic acid; 2, methyl gallate; 3, mangiferin; 4, tetra-*O*-galloyl-glucosides; 5, penta-*O*-galloyl-glucosides; 6, hexa-*O*-galloyl-glucosides; 7, hepta-*O*-galloyl-glucosides; 8, octa-*O*-galloyl-glucosides; 9, nona-*O*-galloyl-glucosides; and 10, deca-*O*-galloyl-glucosides.

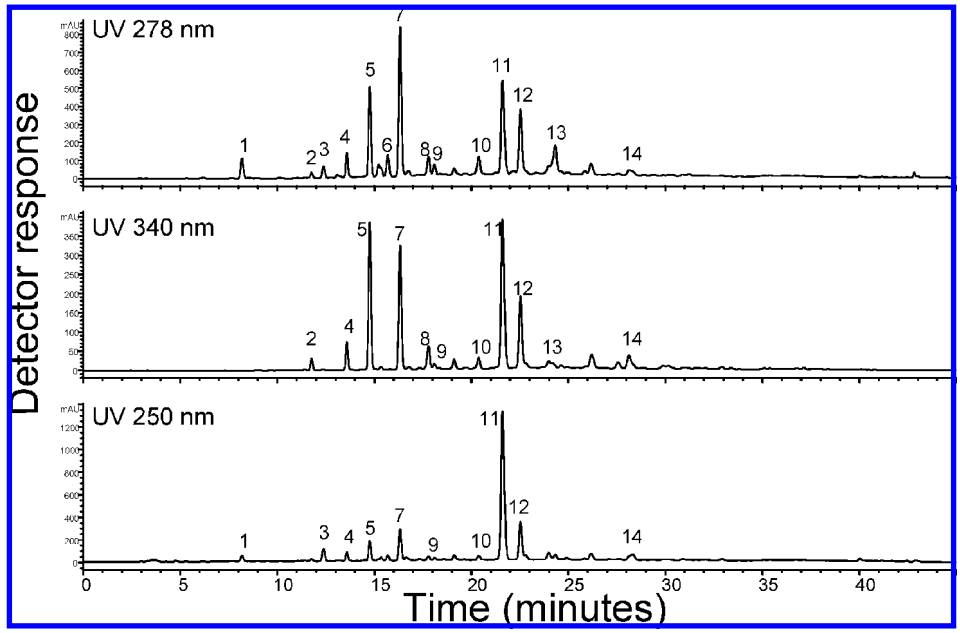

Figure 4. Analytical HPLC chromatogram of a methanol extract of mango bark (cultivar, Van Dyke). Peaks: 1, gallic acid; 2, maclurin 3-*C*-β-D-glucoside; 3, 3,4-dihydroxybenzoic acid; 4, maclurin 3-*C*-(2-*O*-galloyl)-β-D-glucoside; 5, iriflophenone 3-*C*-β-D-glucoside; 6, methyl gallate; 7, iriflophenone 3-*C*-(2-*O*-galloyl)-β-D-glucoside; 8, isomangiferin; 9, iriflophenone 3-*C*-(2,6-di-*O*-galloyl)-β-D-glucoside; 10, 6-*O*-galloyl-mangiferin; 11, mangiferin; 12, iriflophenone 3-*C*-(2-*O*-*p*-hydroxybenzoyl)-β-D-glucoside; 13, penta-*O*-galloyl-glucoside; and 14, 6-*O*-(*p*-hydroxybenzoyl)mangiferin.

Compound **19** was isolated as a mixture of three isomers, again with an UV spectrum typical of quercetin derivatives (**Table 2**). The isomers gave a $[M - H]^-$ ion at *m*/*z* 433 with a major fragment ion at *m*/*z* 301, typical of quercetin glycosides, and probably represent quercetin xyloside, quercetin arabinopyranoside, and quercetin arabinofuranoside, respectively, as described by Berardini et al. (*18*),.

Other Compounds. Compounds **20** and **21** were easily identifiable from their UV spectra and ESI-MS-MS fragmentation patterns (**Table 1**) as 3,4-dihydroxy benzoic acid and ellagic acid, respectively, which was confirmed with reference to standard compounds by HPLC-ESI-MS experiments.

Quantity of Polyphenolic Compounds in Mango Byproducts. Total amounts of secondary plant substances detected in waste products of the mango cultivars are given in **Table 2**, while detailed profiles and amounts for the cultivars Van Dyke and Embrapa-141-Roxa are given in **Table 3A**,**B**, respectively.

Peels. Mango peels from eight cultivars were studied. An analytical HPLC chromatogram of a methanol extract of the cultivar Van Dyke is shown in **Figure 2A** as a general example of mango peels. Substantial differences in the profiles were not detected between the cultivars, but there was considerable variation in the amounts of the major polyphenolic compounds. The predominant compound de-

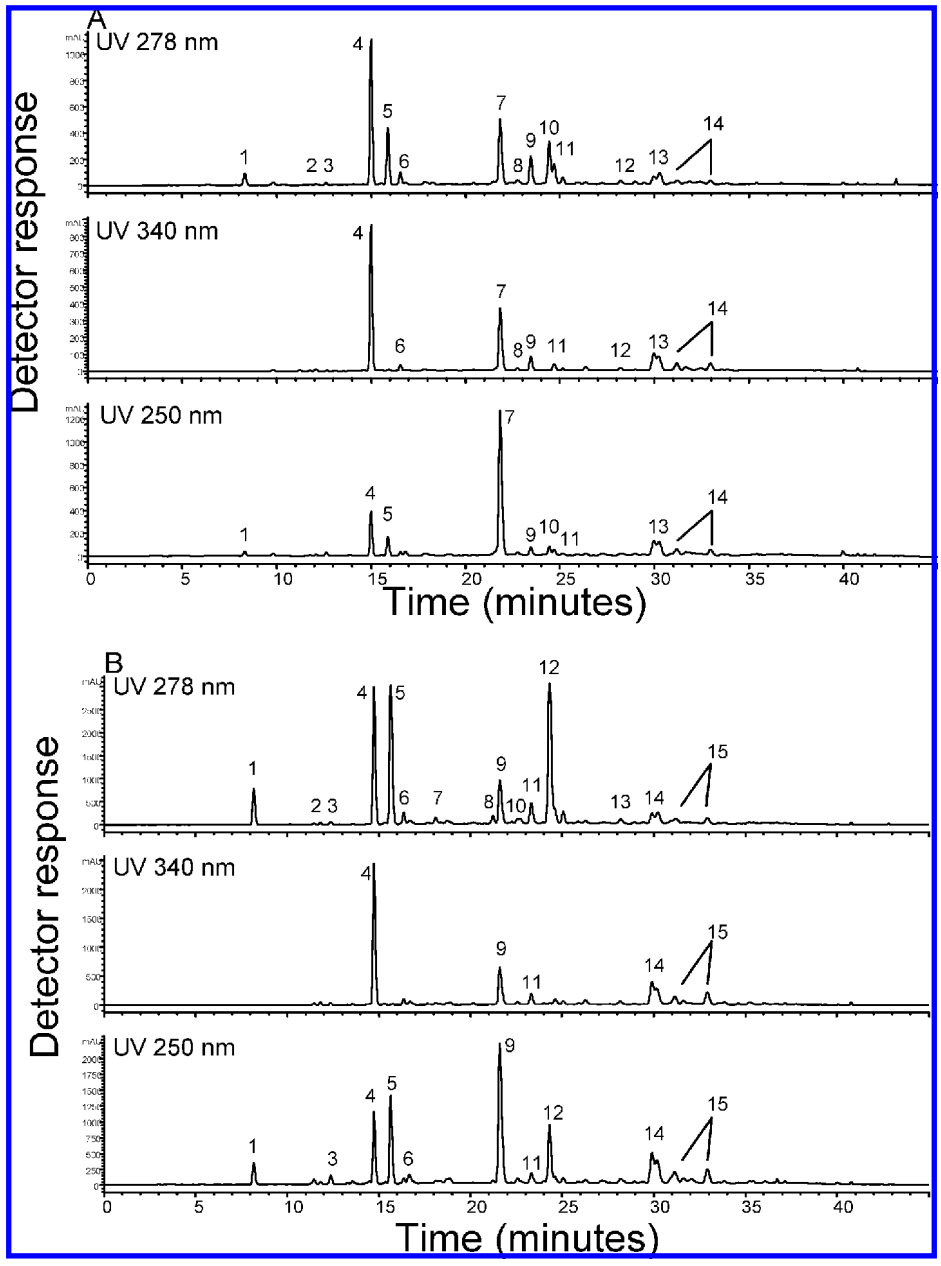

Figure 5. (**A**) Analytical HPLC chromatogram of a methanol extract of old mango leaves (cultivar, Van Dyke). Peaks: 1, gallic acid; 2, maclurin 3-*C*-β-D-glucoside; 3, 3,4-dihydroxybenzoic acid; 4, iriflophenone 3-*C*-β-D-glucoside; 5, methyl gallate; 6, iriflophenone 3-*C*-(2-*O*-galloyl)-β-D-glucoside; 7, mangiferin; 8, iriflophenone 3-*C*-(2-*O*-*p*-hydroxybenzoyl)-β-D-glucoside; 9, iriflophenone mono-*O*-galloyl-glucoside; 10, penta-*O*-galloyl-glucoside; 11, iriflophenone 3-*C*-(2,6-di-*O*-galloyl)-β-D-glucoside; 12, 6-*O*-(*p*-hydroxybenzoyl)mangiferin; 13, isoquercitrin isomers; and 14, quercetin pentosides. (**B**) Analytical HPLC chromatogram of a methanol extract of young mango leaves (cultivar, Van Dyke). Peaks: 1, gallic acid; 2, maclurin 3-*C*-β-D-glucoside; 3, 3,4-dihydroxybenzoic acid; 4, iriflophenone 3-*C*-β-D-glucoside; 5, methyl gallate; 6, iriflophenone 3-*C*-(2-*O*-galloyl)-β-D-glucoside; 7, iriflophenone di-*O*-galloyl-glucoside; 8, tetra-*O*-galloyl-glucoside; 9, mangiferin; 10, iriflophenone 3-*C*-(2-*O*-*p*-hydroxybenzoyl)-β-D-glucoside; 11, iriflophenone mono-*O*-galloyl-glucoside; 12, penta-*O*-galloyl-glucoside; 13, 6-*O*-(*p*-hydroxybenzoyl)mangiferin; 14, isoquercitrin isomers; and 15, quercetin pentosides.

tected was penta-*O*-galloyl-glucoside, and the amounts of this compound varied from 3.51 (Amrapali) to 45.39 (Kent) g/kg dry matter. The second major compound was methyl gallate ranging from 0.25 (Tommy) to 45.82 (Kent) g/kg followed by mangiferin ranging from 3.37 (Amrapali) to 21.53 (Embrapa 141-Roxa) g/kg dry matter. Only traces of compounds such as tetra-*O*-galloyl-glucoside, maclurin di-*O*-galloyl-glucoside, and isoquercitrin were detected in the majority of extracts, but isoquercitrin, at 14.8 g/kg dry, was present at a high concentration in the cultivar Fafá.

Kernels. Mango kernels from six cultivars were studied. An analytical HPLC chromatogram of the methanol extract of the cultivar Van Dyke is shown in **Figure 2B** as a general example of mango kernels. Substantial differences in the profiles were again not detected, but there was considerable variation in the amounts of the major polyphenolic compounds.

The predominant compound detected was penta-*O*-galloyl-glucoside, and the amounts of this compound varied from 31.45 (Fafá) to 153.57 (Tommy) g/kg dry matter. The second major compound was methyl gallate ranging from 28.36 (Embrapa 141-roxa) to 54.79 (Fafá) g/kg dry matter. Traces of mangiferin, tetra-*O*-galloyl glucoside, and gallic acid were also detected in some cultivars.

Comparison of our method of extraction with that of Berardini et al. (*18*) showed substantial differences, in that in our experiments, the major gallotannin was penta-*O*-gallate glucoside as compared

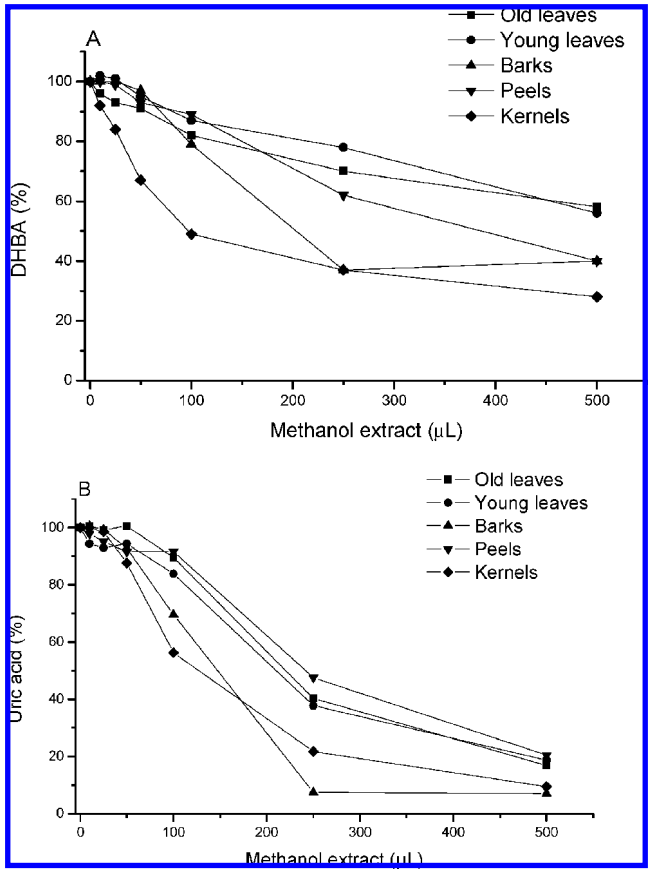

Figure 6. (**A**) Inhibition of reactive oxygen species attack on salicylic acid by methanol extracts from leaves (old and young), barks, peels, and kernels of the mango cultivar Tommy Atkins in the hypoxanthine/xanthine oxidase HPLC-based assay. (**B**) Inhibition of the xanthine oxidase activity by methanol extracts from leaves (old and young), barks, peels, and kernels of the mango cultivar Tommy Atkins in the hypoxanthine/xanthine oxidase HPLC-based assay.

Table 4. Antioxidant Capacities of Major Individual Phenolic Compounds Purified from Byproducts of Various Mango Cultivars in the HX/XO, DPPH, FRAP, and ORAC Assay Systems[a]

	assay			
substance	HX/XO (mM) (IC_{50})	DPPH (μM) (IC_{50})	FRAP (μM) (EC_1)	ORAC (units)
mangiferin	0.47	0.59	1.03	12.85
methyl gallate	4.00	4.28	5.60	0.79
gallic acid	pro-oxidant	3.20	5.01	1.94
penta-*O*-galloyl-glucoside	0.19	4.63	3.84	1.20
ascorbic acid	pro-oxidant	50.74	81.82	1.07
Trolox	2.30	65.96	62.00	1.00

[a] Mean of two experiments (SD < 5).

to a range (penta- to decagallates) of such products using a cold 70% acetone extraction procedure. This indicates that *meta*-depside gallotannins are extremely susceptible to hydrolysis under the conditions used for Soxhlet extraction. However, this facilitates their quantitation by analytical HPLC. An analytical chromatogram of the 70% acetone extract of kernals dissolved in methanol (5.0 mL) of the cultivar Van Dyke is shown in **Figure 3** for comparison with **Figure 2B**.

Barks. Fifteen cultivars of mango barks were studied, and a much greater diversity of polyphenols was detected in these extracts. An analytical HPLC chromatogram of a methanol extract of the cultivar Van Dyke is shown in **Figure 4** as a general example of mango barks.

The major compound detected in all extracts was mangiferin, varying from 4.77 (CPAC 136/86) to 107.18 (Momiká) g/kg dry matter. Maclurin or iriflophenone derivatives were also detected in some cases, sometimes as the second major compound or otherwise as traces.

Old Leaves. Fifteen cultivars of old leaves were analyzed, and no large differences were found across each cultivar, except in their relative concentrations. An analytical HPLC chromatogram of a methanol extract of the cultivar Van Dyke is shown in **Figure 5A**. The predominant compound for the majority of the cultivars was iriflophenone 3-*C*-β-D-glucoside. The observed amounts ranging from 6.30 (Mallika) to 120.95 (Keit) g/kg dry matter. The second major compound was mangiferin, which was present in all cultivars, the amounts varying from 3.71 (Fafá) to 93.62 (Itamaraká) g/kg dry matter.

Young Leaves. Young leaves from 13 mango cultivars were studied. An analytical HPLC chromatogram of a methanol extract of the cultivar Van Dyke is shown in **Figure 5B** as a general example of young mango leaves. Again, no significant differences among cultivars were found in the profiles of these extracts, only in the relative concentration. Mangiferin was the major compound detected in these extracts, with amounts ranging from 11.11 (Tommy) to 171.67 (Coité) g/kg dry matter.

Antioxidant Capacity of Mango Byproduct Extracts and Selected Pure Compounds. The methanolic extracts of each mango byproduct of all cultivars were evaluated in the hypoxanthine/xanthine oxidase assay with regard to their antioxidant capacities. The antioxidant potential of methanolic extracts of the byproduct of the cultivar Tommy Atkins is depicted in **Figure 6A**. In addition to their direct antioxidant capacity, extracts of kernels were also potent inhibitors of xanthine oxidase activity (**Figure 6B**). This can be attributed to the high content of penta-*O*-galloyl-glucoside and mangiferin, which when tested individually were potent inhibitors of xanthine oxidase (**Table 4**).

Four major phenolic compounds, namely, mangiferin, gallic acid, methyl gallate, and penta-*O*-galloyl-glucoside, purified from these extracts, were tested in the hypoxanthine/xanthine oxidase HPLC-based antioxidant assay as well as by the DPPH, FRAP, and ORAC assays and were potent antioxidants in comparison to the classical in vivo and in vitro free radical scavengers ascorbic acid and Trolox (synthetic form of vitamin E). The IC_{50} values are given in **Table 4**.

Penta-*O*-galloyl-glucoside has the higher antioxidant capacity in the hypoxanthine/xanthine oxidase assay, because it is a potent inhibitor of xanthine oxidase, but mangiferin has exceptionally high activity in all antioxidant test systems in comparison to the other substances (**Table 4**). The relative ORAC unit of 12.85 assigned to mangiferin is greater than that reported for any other secondary plant substance.

Penta-*O*-galloyl-glucoside was the major compound detected in the peels and kernels of the mango byproduct, the concentration of which was higher in kernels than in peels. In the bark and young leaves, the predominant compound detected was mangiferin, an important molecule with potential pharmacologic activities. From a phytochemical point of view, detection of galloylated benzophenone derivatives in mango peels provides evidence that iriflophenone and maclurin glucosides known as biosynthetic precursors of mangiferin and isomangiferin (*24*) are galloylated prior to cyclization of benzophenones and may be an important means of clarifying the biosynthetic pathways of xanthone derivatives. In a recent report, Berardini and colleagues (*18*) quantitated mangiferin in mango peels of the cultivar Tommy

Atkins at 1.7 g/kg dry matter. In our study, we detected 7.2 g/kg. Only the cultivar Tommy Atkins was common to both studies (*18*). Berardini et al. (*18*) also identified several higher molecular weight gallotannins in 70% acetone (aqueous) extracts of mango kernels, such as hepta, octa, and nona gallates. In our study, we detected very high amounts of penta-*O*-galloyl-glucoside, which we believe is due to hydrolysis of the higher gallates during the elevated temperatures of the Soxhlet extraction process. This was proven by a comparison of both methods.

A direct comparison of our data with regard to the antioxidant capacity of purified compounds, as opposed to extracts of mango peels of the cultivar Tommy Atkins (*25*), is difficult. However, Beradini et al. (*25*) showed that lyophilized extracts displayed potent antioxidant capacity, which is in agreement with our results.

The results presented in this work are in accordance with the data reported by Saleh and El-Ansari (*26*), who detected, but did not quantitate, mangiferin as the major component of mango leaves and barks, with gallotannins as the major components of flesh and seeds. There are, however, no literature citations on the quantitation of individual polyphenols in mango byproduct, other than peels (*18*) and barks (*26*), to compare with our data, which reveal a very high concentration of phenolic compounds in all byproducts. With regard to phenolic compounds present in the bark of *M. indica* L., a great deal of information has emanated from Cuba. Nuno-Sallez et al. have reported (*27*) that Vimang, a commercially prepared extract of the bark of certain *M. indica* L. cultivars grown in Cuba, contains the following compounds in ascending amounts (g/kg dry material): benzoic acid (1.99), gallic acid (2.08), 3,4-dihydroxybenzoic acid (2.26), propyl benzoate (3.99), methyl gallate (4.45), propyl gallate (4.76), (−)-epicatechin (8.07), (+)-catechin (13.08), and mangiferin (71.40), giving a total of around 112 g/kg. Although the profiles of the cultivars from Brazil and Cuba have substantial differences (e.g., bark from Brazilian cultivars do not contain benzoic acid derivatives and only trace amounts of catechins), the reasons for this are currently unclear.

The results presented in this study show that mango byproducts may be an invaluable source of mangiferin (a compound with high cancer chemopreventive potential) and related compounds and uphold the widespread utilization of extracts and decoctions in traditional medicine worldwide. These data warrant extensive future studies on the utilization of xanthone derivatives from mango byproducts, especially in the possible prevention of oncogenesis.

ABBREVIATIONS USED

ROS, reactive oxygen species; DMSO, dimethyl sulfoxide; FRAP, ferric reducing ability of plasma; ORAC, oxygen radical absorbance capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

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