DOI:10.1021/jf901621m

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Antimicrobial Activity of Gallotannins Isolated from Mango (*Mangifera indica* L.) Kernels

Christina Engels,[†] Matthias Knödler,[§] Yuan-Yuan Zhao,[†] Reinhold Carle,[§] Michael G. Gänzle,[†] and Andreas Schieber*,[†]

[†]Department of Agricultural, Food and Nutritional Science, University of Alberta, 410 Ag/For Centre, Edmonton, Alberta T6G 2P5, Canada, and [§]Institute of Food Science and Biotechnology, Chair Plant Foodstuff Technology, Hohenheim University, Garbenstrasse 25, D-70599 Stuttgart, Germany

Gallotannins were extracted from mango (*Mangifera indica* L.) kernels with aqueous acetone (80%, v/v) and purified using liquid−liquid extraction and two-step low-pressure liquid chromatography (LPLC) on Sephadex LH-20. Analytical high-performance liquid chromatography and mass spectrometry confirmed the presence of hydrolyzable tannins with a degree of galloylation ranging from 4 to 9 and additionally revealed the presence of deca-, undeca-, and dodeca-*O*-galloylglucose. Further purification using two-step semipreparative HPLC resulted in three pure hydrolyzable tannins, penta-, hexa-, and hepta-*O*-galloylglucose, with antibacterial activity, as evidenced from the agar spot and critical dilution assays. Although the growth of lactic acid bacteria was not inhibited, the proliferation of Gram-positive food spoilage bacteria was prevented and the growth of Gram-negative *Escherichia coli* was reduced. Because bacterial growth could be restored by the addition of iron to the medium, this study strongly supports the view that the inhibitory effects of hydrolyzable tannins are due to their iron-complexing properties.

KEYWORDS: Mango (*Mangifera indica* L.) kernel; gallotannins; HPLC; mass spectrometry; antimicrobial activity

INTRODUCTION

Mangoes (*Mangifera indica* L.) are one of the most popular and economically important tropical fruits marketed worldwide and are often referred to as "the king of fruits" (*1*). Between 1961 and 2004, global mango production has increased from approximately 11 million tons to over 26 million tons (*2*). Mangoes are consumed either as fresh fruits or processed into a wide range of products such as purees, nectars, chutneys, fruit slices, bars, and powders. For this purpose, the peels and kernels are usually removed, which creates large amounts of byproduct.

Although these byproducts have often been considered waste, more recent studies have shown that the peels and kernels are promising sources of valuable components such as peel pectin (*3*, *4*), kernel fat (*5*), and phenolic compounds (*3*, *6*−*8*). The polyphenol fractions from peels and kernels are interesting from various points of view because they display, for example, antioxidant and anti-inflammatory activities (*3*, *6*, *7*, *9*, *10*). Kabuki et al. (*11*) reported antimicrobial activities of an ethanolic extract from mango kernels against 18 bacterial species including foodborne pathogens. It was found that the extract was composed primarily of polyphenols, but the antibacterial principles were not characterized. In a subsequent study we demonstrated that mango kernel extracts contain high amounts of hydrolyzable tannins (*8*) and hypothesized that the antimicrobial activity could be due to the presence of gallotannins, especially because the HPLC elution profiles of the extracts were very similar to those reported by Kabuki et al. (*11*). Moreover, gallotannins from *Galla chinensis*, a plant also belonging to the Anacardiaceae family, were very recently shown to exhibit antibacterial activity (*12*, *13*). Therefore, the objective of this investigation was to identify antimicrobially active polyphenols present in mango kernels and to characterize their inhibitory spectrum. The crude extract was fractionated by liquid−liquid extraction, low-pressure liquid chromatography, and semipreparative high-performance liquid chromatography to isolate gallotannins with antimicrobial activity.

*Corresponding author [telephone (780) 492-2912; fax (780) 492-4265; e-mail schieber@ualberta.ca].

MATERIALS AND METHODS

Solvents and Reagents. Ethanol was obtained from Brenntag (Toronto, ON, Canada). All other solvents were purchased from Fisher Scientific (Ottawa, ON, Canada) and were of HPLC grade. Deionized water was used throughout.

Sample Preparation. Lyophilized mango kernels (cultivar 'Kaew') obtained from Chiang Mai, Thailand, were ground finely with a type A10 knife mill (IKA-Werke GmbH & Co. KG, Stauffen, Germany). Aliquots of 10 g were defatted by extraction with hexane (3 × 50 mL) under stirring for 25 min in a nitrogen atmosphere. The extracts were filtered, and the solvent was discarded. The dried material was weighed into amber bottles, and 10 g portions were extracted with 40 mL of aqueous acetone (80%, v/v) under stirring with a magnetic stirrer for 6 h in a nitrogen atmosphere at ambient temperature. During extraction, the mixture was sonicated several times for 3 min. The extract was filtered, and the residue was re-extracted with 20 mL of aqueous acetone (80%, v/v) for 1 h. The remaining solid was discarded; the organic solvent was removed from the combined supernatants by evaporation at 30 °C in vacuo.

 Published on Web 08/05/2009 © 2009 American Chemical Society

After lyophilization, aliquots of 10 g were weighed into a separatory funnel and dissolved with 150 mL of distilled water. The solution was extracted with dichloromethane (4 × 150 mL). The remaining aqueous phase was then extracted with ethyl acetate (3 × 150 mL). All extractions were performed under a nitrogen atmosphere to prevent oxidation. The ethyl acetate fractions were transferred into a round-bottom flask. The solvents were evaporated to dryness at 30 °C in vacuo, and the residue was dissolved in 10 mL of aqueous acetone (80%, v/v).

Purification of Gallotannins by Low-Pressure Liquid Chromatography (LPLC). Purification by LPLC was performed using a Bio-Rad Econo gradient pump, a model 2128 fraction collector, and acetone-resistant Chemfluor tubings, i.d. $^1/_{16}$ in., o.d. $^1/_8$ in. (Sigma-Aldrich, St. Louis, MO). The column used was packed with Sephadex LH-20 (Sigma-Aldrich) and had a bed volume (BV) of 150 mL. For the packing of the column, Sephadex LH-20 was swollen in distilled water for 3 h and poured into the column in one continuous motion. The resin was pressed tightly and then equilibrated with ethanol.

The extract obtained as described above was applied onto the column at a flow rate of 2.0 mL/min and fractionated using mobile phases consisting of ethanol (eluent A), distilled water (eluent B), and acetone (eluent C). The following gradient program was used: 100% A; 90% A and 10% B; 70% A and 30% B; 50% A, 30% B, and 20% C; 20% A, 30% B, and 50% C; and 50% B and 50% C. Six fractions of 150 mL each were collected. Fractions 3 and 4, which contain the gallotannins according to Nishizawa et al. (*14*), were combined, and the solvents were evaporated at 30 °C in vacuo. The residue was dissolved in 10 mL of aqueous acetone (80%, v/v) and subjected to a second purification step using the same LPLC system as described above. The following gradient program was used: 100% A; 90% A and 10% B; 70% A and 30% B; 50% B and 50% C; and 30% B and 70% C. Fractions were collected in portions of 150 or 25 mL. The solvents were evaporated at 30 °C in vacuo, and the residues were dissolved in 2 mL of aqueous acetone (80%, v/v) and purified using semipreparative HPLC.

Purification with Semipreparative High-Performance Liquid Chromatography (HPLC). Semipreparative HPLC was performed using a Varian HPLC system equipped with Galaxie Chromatography software, an HP autosampler series 1050, a Varian 9010 gradient pump, and an LDC UV detector Spectro Monitor III, model 1204 A (Varian, Mississauga, ON, Canada) set at 280 nm. A 25 cm × 10 mm, 5 μm Supelcosil LC-18-DB semipreparative column (Supelco, St. Louis, MO) with a 4.0 × 2.0 mm i.d. C18 ODS guard column (Phenomenex, Torrance, CA) was used as the stationary phase and operated at ambient temperature. The gallotannins were eluted with 2% (v/v) acetic acid in water (eluent A, pH 2.6) and 0.5% acetic acid in water/acetonitrile (50:50, v/v, eluent B, pH 3.6) at a flow rate of 4.0 mL/min.

Isolation of the gallotannins by semipreparative HPLC included two steps. The first step was based on the following gradient system: 20% B to 35% B (25 min), 35% B (2 min), 35% B to 100% B (0.5 min), 100% B (3.5 min), and 100% B to 20% B (0.5 min). The injection volume was 100 μL. The predominant peaks were collected, and the solvents from 20 repeated runs were evaporated at 30 °C in vacuo and dissolved in 0.5 mL of aqueous acetone (80%, v/v). The second purification step was performed using an isocratic solvent system (70% A). The injection volume was 100 μL. Compounds from five repeated chromatographic runs were collected, and the solvents were evaporated at 30 °C in vacuo. The residues were dissolved in 200 μL of aqueous acetone (80%, v/v) and used for mass spectrometric characterization and antimicrobial assays.

Characterization of Gallotannins by Analytical HPLC and Mass Spectrometry. Analytical HPLC was performed to assess the purity of the isolated compounds. For this purpose, the same Varian HPLC system and stationary phase as described above were used. Aliquots of 10 μL were injected, and the compounds were eluted with 2% (v/v) acetic acid in water (eluent A, pH 2.6) and 0.5% acetic acid in water/acetonitrile (50:50, v/v, eluent B, pH 3.6) using the following gradient: 20% B to 35% B (25 min), 35% B to 40% B (25 min), 40% B to 80% B (20 min), 80% B (2 min), 80% B to 20% B (0.5 min) according to the method of Berardini et al. (*8*). The flow rate was 0.5 mL/min. Monitoring was performed at 280 nm.

Mass spectrometry experiments were performed on a QSTAR Elite System model 1017064/N with a turbo spray ion source (Applied Biosystems, Streetsville, ON, Canada), a series 1200 G1329A autosampler, and a series 1200 G1312A pump (Agilent Technologies, Santa Clara, CA). The compounds were dissolved in methanol and characterized using flow injection with injection volumes of 2 μL at a flow rate of 400 μL/min. Negative ion mass spectra were recorded in the range from m/z 100 to 2050 at an accumulation time of 1.000/s and a voltage of −4000 V. The ion source temperature was set at 400 °C. Nitrogen was used as the drying gas at 30 psi (gas 1) and 50 psi (gas 2), respectively. The declustering and focusing potentials were −50 and −150 V, respectively. Gallotannins were identified by their mass spectrometric data on the basis of the findings of Berardini et al. (*8*).

Strains and Culture Conditions. The following strains were used to assess the antimicrobial activity of mango extracts: *Bacillus subtilis* FAD 110 and *Bacillus amyloliquefaciens* FAD 82, both isolated from ropy bread (*15*); *Staphylococcus warneri* FUA 3136 isolated from the vagina of dairy cows; *Staphylococcus aureus* ATCC 6538, *Escherichia coli* AW1.7 and GGG10, *Listeria monocytogenes* CDC 7762, and *Pediococcus acidilactici* FUA 3072. *S. aureus* and *L. monocytogenes* were propagated overnight in Luria−Bertani broth (LB) (Difco, Becton, Dickinson & Co., Sparks, MD) at 30 °C, and bacilli and *E. coli* were grown in LB at 37 °C. MRS broth (Difco) was used to cultivate *P. acidilactici* under anaerobic conditions (BBL GasPak System, Becton, Dickinson & Co). Stock cultures were maintained at −70 °C in 30% glycerol.

Characterization of the Antimicrobial Activity of Gallotannins. The antimicrobial screening was performed with the agar spot method and the critical dilution assay on microtiter plates. For the agar spot method, aliquots of 10 μL of the samples were applied on agar plates. The solvents were completely evaporated in the air flow of a sterile bench for 3 h. The plates were covered with aluminum foil to prevent degradation of the compounds by light. Overnight cultures (100 μL) of the strains were spread onto the plates with a sterile Drigalski spreader. The plates were incubated overnight at 30 or 37 °C. The diameter of the inhibition zone was measured, and all experiments were performed in duplicate.

Critical dilution assays were performed by preparing serial 2-fold dilutions of the samples with LB medium on 96-well cell microtiter plates. Solvents were evaporated by placing the microtiter plate under a laminar flow hood for 3 h. LB medium was inoculated with overnight cultures of the indicator strain, and 50 μL of inoculated medium was added to the dilutions. The microtiter plates were incubated overnight at 30 or 37 °C. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration of the substances to prevent the growth of microbial strains and were expressed in grams of dry matter (dm) per liter.

The antimicrobial activity of fractions obtained during the bioassay-guided fractionation of mango kernel extract was determined by the agar spot test and the critical dilution assay. The determination of the inhibitory spectrum of purified penta-, hexa-, and heptagalloylglucose was performed with the agar spot assay only.

CAS Agar Diffusion Assay and Effect of Fe^{2+} and Fe^{3+} Ions on Antibacterial Activity of Mango Kernel Extract. The iron chelating capacity of the tannin-rich ethyl acetate fraction was determined with the Chrome azurol S (CAS) agar diffusion assay (*16*) and compared to tannic acid and EDTA. The ethyl acetate fraction was diluted to six concentrations ranging from 24 mg/L to 24 g/L. Tannic acid and EDTA were used at concentrations ranging from 25 μM to 10 mM. Pipes buffer was replaced with Tris-HCl in the same molar concentration. The effect of iron on the antibacterial activity was determined with the critical dilution assay and *B. subtilis* as an indicator strain. The Fe^{2+} or Fe^{3+} (ferrous sulfate or ferric sulfate) concentration in the assay medium was adjusted to 0−5 mM.

RESULTS AND DISCUSSION

Fractionation of Antimicrobial Polyphenols by Liquid−Liquid Extraction. All fractions obtained from bioassay-guided fractionation of the mango kernel crude extracts were characterized by HPLC and MS. Because initial experiments demonstrated that *B. subtilis* FAD110 was sensitive to antimicrobial compounds in the acetone extract of mango kernels, this strain was chosen to identify active fractions. Their antimicrobial activity was determined using the agar spot assay as well as the critical dilution assay, and active fractions were further purified.

The mango kernel crude extract was partitioned by liquid−liquid extraction into dichloromethane and ethyl acetate fractions.

Table 1. Antibacterial Activity against *Bacillus subtilis* of Mango Kernel Crude Extracts and the Fractions Obtained after Liquid−Liquid Extraction

fraction (concn,[a] g/L)	inhibition zone[b] (cm)	MIC[c] (g/L)
crude extract (66.7)	1.1 ± 0.1	1.4
dichloromethane (12.7)	±	2.1
ethyl acetate (354.7)	1.7 ± 0.1	0.9
aqueous phase (260.4)	1.0 ± 0.3	21.7

[a] Concentration was determined gravimetrically. [b] Diameters of inhibition zones were determined. Means ± standard deviations are shown. [c] MIC, minimum inhibitory concentration. No standard deviations are shown because in all tests identical results were obtained from duplicate determinations.

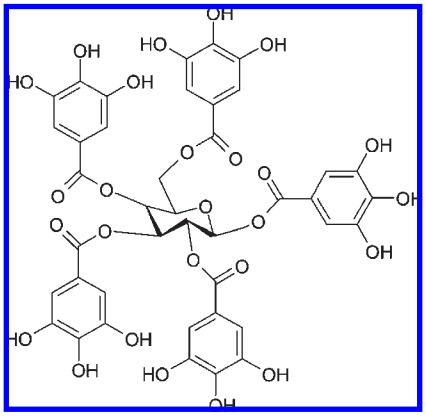

Figure 1. Structure of penta-*O*-galloyl-β-D-glucose.

The aqueous phase obtained after extraction with ethyl acetate was also tested for the presence of antimicrobial components. Preliminary tests and HPLC analyses (results not shown) revealed that the ethyl acetate soluble fraction contained the highest level of tannins and also showed the highest antimicrobial activity against *B. subtilis* (**Table 1**). The antimicrobial activities of the dichloromethane and aqueous fractions were less than 50 and 10%, respectively, compared to the ethyl acetate fraction. Therefore, only the ethyl acetate fraction was considered in subsequent investigations.

In accordance with our previous findings (*8*), hydrolyzable tannins with four (mass-to-charge ratios $[M - H]^-$ = 787 and $[M - 2H]^{2-}$ = 393), five (939 and 469) (**Figure 1**), six (1091 and 545), seven (1243 and 621), eight (1395 and 697), and nine (1547 and 773) galloyl groups were found. In addition, three highly galloylated tannins, that is, deca-, undeca-, and dodeca-*O*-galloylglucose with $[M - H]^-$ ions of 1699, 1851, and 2003 and $[M - 2H]^{2-}$ ions of 849, 925, 1001, respectively, were detected in the kernel extract.

Fractionation of Antimicrobial Gallotannins by Two-Step LPLC. The ethyl acetate extract was further purified by LPLC. The first run was fractionated into eight fractions of 150 mL each. HPLC and MS analyses showed that tannins eluted according to their degree of galloylation; fractions 3−8 exhibited antimicrobial activity with MICs ranging from 6.2 g/L (fraction 4) to 21 g/L (fraction 7). Fractions 3 and 4 were combined, concentrated, and subjected to a second fractionation on the same column to obtain fractions of 150 mL (fractions 1−3) or 25 mL (fractions 4−17). Fractions 8−15 exhibited antimicrobial activity, and analysis by analytical HPLC or MS revealed that these fractions contained gallotannins with various degrees of galloylation rather than individual compounds. Fraction 9 was composed of a mixture of tetra- and pentagalloylglucose, and fraction 12 consisted of a mixture of penta-, hexa-, and heptagalloylglucose (data not shown).

Purification of Gallotannins by Semipreparative HPLC. The compounds identified in fractions 9 and 12 were purified by semipreparative HPLC. Subsequent analytical HPLC and mass spectrometric investigations confirmed that three gallotannins, that is, penta-*O*-galloylglucose, hexa-*O*-galloylglucose, and hepta-*O*-galloylglucose, were purified (**Figure 2**). The TOF mass spectra show the single- and double-charged negative ions ($[M - H]^-$ and $[M - 2H]^{2-}$).

Antimicrobial Activity of Penta-*O*-galloylglucose, Hexa-*O*-galloylglucose, and Hexa-*O*-galloylglucose. Fractions containing pentagalloylglucose, hexagalloylglucose, and heptagalloylglucose were evaporated to dryness and dissolved in aqueous acetone (80%) to concentrations of 41, 84, and 17 g/L, respectively. The antimicrobial activities of the three isolated compounds were tested against eight strains of five bacterial genera (**Table 2**). *Listeria monocytogenes* was the most sensitive indicator strain and inhibited by all three compounds. The Gram-positive indicator strains *B. subtilis* and *S. aureus* were also inhibited by all three gallotannins, although the concentration of the heptagalloylglucose solution (17 g/L) was close to the MIC. The two strains of *E. coli* were only slightly inhibited by any of the three gallotannins, and *P. acidilactici* was resistant to all three compounds.

Iron Chelating Properties of Mango Gallotannins and Effect of Fe^{2+} and Fe^{3+} Ions on Their Antibacterial Activity. The selective inhibitory spectrum indicates that iron chelating properties contribute to antimicrobial activity (*17*). To determine whether the selective inhibitory spectrum of mango gallotannins is attributable to their iron complexing properties, the iron chelating capacity of the tannin-rich ethyl acetate fraction was compared to those of EDTA and tannic acid. The formation of orange halos in the CAS assay proved that the components in the tannin-rich ethyl acetate fraction from mango kernels sequestered Fe^{3+} from the blue complex, whereas the chelating agent EDTA had no effects when applied in the same concentration. The fraction had a dry matter content of 24 g/L and exhibited an iron chelating capacity that was equivalent to a solution of 42.5 g/L (25 mM) tannic acid. In agreement with the strong iron chelating properties of mango polyphenols, Fe^{2+} and Fe^{3+} in concentrations of 0.5−2 mM antagonized the antibacterial activity of mango gallotannins against *B. subtilis* (**Figure 3**). The better soluble, biologically active ferrous ions had a greater impact on the bacterial growth than the ferric irons. Concentrations exceeding 2 mM showed no additional effects.

Gallotannins in Mango Kernel Extracts. Plant extracts are recognized as a source of natural antimicrobials such as essential oils (*18*) and phenolic compounds with potential for application in food preservation and medicine (*19*−*21*). Hydrolyzable tannins are known to exhibit antimicrobial activity (*12*, *13*, *22*−*24*). Mango kernel extracts contain hydrolyzable tannins (*8*) and have been demonstrated to inhibit the growth of various bacterial species including foodborne pathogens (*11*, *25*). However, so far the antibacterial activity could not be assigned to distinct compounds present in the kernel extracts. This study confirms, and extends, previous investigations on the composition of the gallotannin fraction from mango kernels, in which the presence of gallotannins up to a degree of galloylation of 10 has been reported (*6*, *8*). Whereas undecagalloylglucose has been detected in mango leaves (*26*), to our knowledge hydrolyzable tannins with a degree of galloylation exceeding 10 have so far not been found in mango kernels. This is important because structure−activity relationships, that is, the influence of the degree of galloylation on the antimicrobial and other activities of hydrolyzable tannins, are still not very well understood.

Inhibitory Spectrum. Through bioassay-guided fractionation, three compounds with antimicrobial activity, that is, penta-, hexa-, and heptagalloylglucose, were successfully identified.

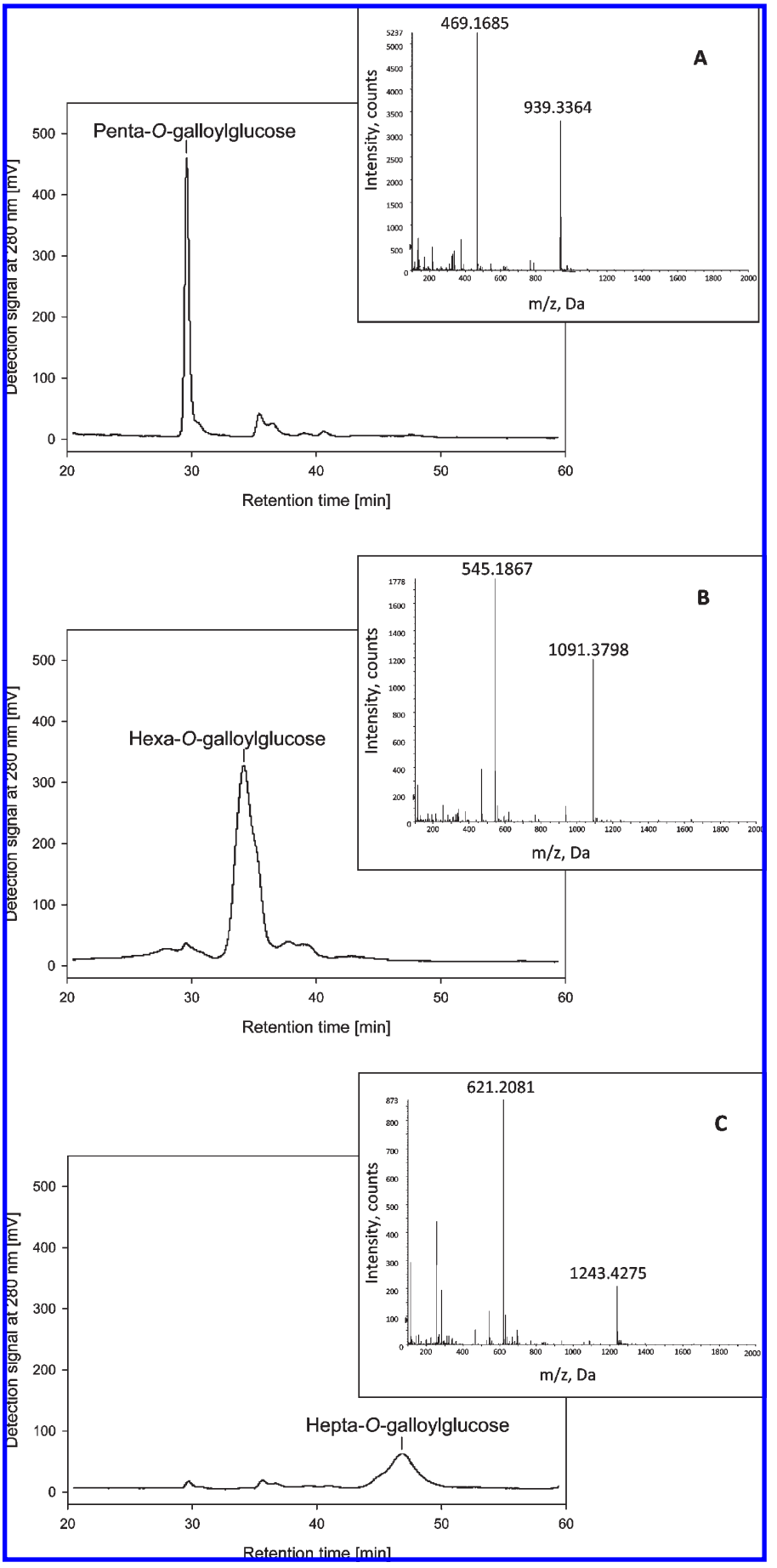

Figure 2. Liquid chromatographic runs (280 nm) and TOF mass spectra of penta-*O*-galloylglucose, 81% purity (**A**), hexa-*O*-galloylglucose, 93% purity (**B**), and hepta-*O*-galloylglucose, 86% purity (**C**) isolated from mango kernels.

Table 2. Antimicrobial Activity of the Hydrolyzable Tannins Isolated from Mango Kernels

	penta-*O*-galloylglucose (41.2 g/L)[a]	hexa-*O*-galloylglucose (83.7 g/L)[a]	hepta-*O*-galloylglucose (17.0 g/L)[a]
Bacillus subtilis	0.9 ± 0.0	0.9 ± 0.0	±
Bacillus amyloliquefaciens	0.8 ± 0.1	0.9 ± 0.1	nd
Escherichia coli AW 1.7	±	±	±
Escherichia coli GGG 10	±	nd	nd
Listeria monocytogenes	1.1 ± 0.1	1.5 ± 0.1	0.8 ± 0.0
Pediococcus acidilactici	−	−	−
Staphylococcus aureus	0.8 ± 0.1	1.1 ± 0.0	±
Staphylococcus warneri	±	±	±

[a] Concentration was determined gravimetrically. Diameters of the inhibition zones (nd, not determined; ±, partial growth inhibition; −, no inhibition). Shown are means ± standard deviations of two replicate experiments.

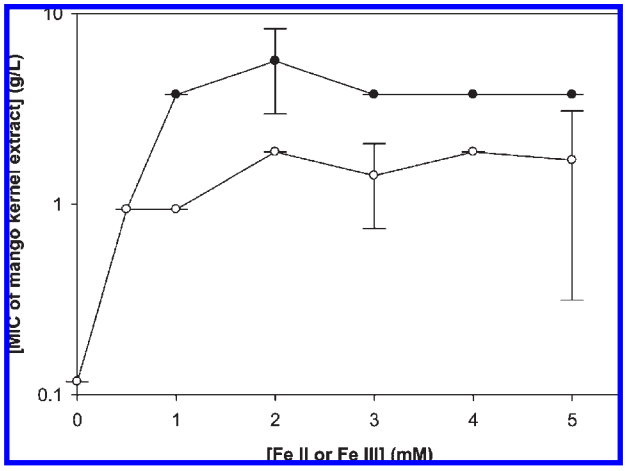

Figure 3. Effects of Fe^{2+} (●) and Fe^{3+} (○) on the MIC of mango kernel extracts.

The MIC of the ethyl acetate extract, the most active fraction obtained by LPLC, and the purified gallotannins increased from 0.9 to 6.2 to >10 g/L, which indicates the presence of other antibacterial compounds in the crude extracts or synergistic activity of the different compounds.

The inhibitory spectrum of gallotannins observed in our study indicates a remarkable selectivity. Bacilli, *S. aureus* and *L. monocytogenes*, were highly sensitive to gallotannins, whereas growth of *E. coli* and *S. warneri* was only slightly inhibited and *P. acidilactici* was resistant. The limited quantity of gallotannins necessitated the selection of a few indicator strains to determine the inhibition spectrum. The MIC of eight strains, varying in their sensitivity toward the purified gallotannins, is, however, well in agreement with the activity and inhibitory spectrum of an ethanolic extract of mango seed kernel extract (MKE) (*11*). The ethyl acetate fraction obtained in this study inhibited *B. subtilis* at a concentration of 0.9 g/L, compared to a MIC of 0.5 g/L reported for MKE (*11*). Moreover, the chromatographic profile of the ethanolic MKE matches the profile observed in this study, indicating a comparable composition of the extracts. Of the 43 bacterial strains tested by Kabuki et al. (*11*), strains of *S. aureus*, *Clostridium* spp., and *L. monocytogenes* were most sensitive to MKE. *B. cereus*, *B. subtilis*, and *B. licheniformis* exhibited intermediate sensitivity, whereas the MIC against lactobacilli, *P. acidilactici*, enterococci, *Salmonella enterica*, and *E. coli* exceeded the MIC against *L. monocytogenes* by factors of 10−25. In our study, penta- and hexa-*O*-galloylglucose were more effective against *S. aureus* than against *S. warneri*. A large strain-to-strain variation in sensitivity to MKE was previously reported for *S. aureus* (*11*). Most strains were inhibited at concentrations of 50 mg/L, but one strain of *S. aureus* type A tolerated 20-fold higher concentrations of the extract.

The marked selectivity of hydrolyzable tannins is in agreement with reports on extracts from other plant materials. Kamijo et al. (*24*) demonstrated that hydrolyzable tannins from *Rosa rugosa* Thunb. inhibited *E. coli*, *S. aureus*, *B. cereus*, and *Salmonella* spp., whereas little or no effects on *Bifidobacterium breve* and *Lactobacillus salivarius* were observed. Likewise, Chung et al. (*27*) showed growth of *Lactobacillus acidophilus* and *Bifidobacterium infantis* was unaffected by tannic acid.

The selective inhibitory activity of gallotannins may enable food applications to eliminate foodborne pathogens while retaining growth and metabolic activity, respectively, of beneficial lactic acid bacteria. Virtually all Gram-positive foodborne pathogens, *B. cereus*, *C. botulinum*, *C. perfringens*, *L. monocytogenes*, and *S. aureus*, as well as the Gram-negative pathogens *Campylobacter jejuni*, *Vibrio parahemolyticus*, and *Yersinia enterocolitica* were found to be sensitive to gallotannins. In contrast, lactic acid bacteria, which are applied in food as starter cultures, protective cultures, or probiotics, were not inhibited, as shown by Kabuki et al. (*11*) and in this study. However, the efficacy of gallotannins in food matrices that are rich in proteins and divalent ions, respectively, remains to be determined.

Antimicrobial Activity of Gallotannins. The antimicrobial properties of hydrolyzable tannins have been attributed to various modes of action, in particular to their ability to interact with proteins and to inhibit enzyme activities (*28*, *29*). Furthermore, hydrolyzable tannins were shown to damage lipid bilayer membranes in *Helicobacter pylori* (*23*). A third mode of action by which hydrolyzable tannins may inhibit bacterial growth is related to the complexation of metal ions (*22*, *30*, *31*).

The inhibitory profile of gallotannins observed in our study and comparison with previous reports allow a preliminary assessment of their mode of action. Lactic acid bacteria are unique among bacteria as they do not require iron for growth (*17*, *32*) and their metabolic activity relies neither on a respiratory chain with iron-dependent NADH dehydrogenases nor on Fe-S hydrogenases that are present in the strict anaerobic clostridia (*33*, *34*). These unique physiological characteristics of lactic acid bacteria in combination with their exceptional resistance to gallotannins conform with a role of iron or iron-containing dehydrogenases as a target of the antimicrobial activity of gallotannins. The resistance of *E. coli* to penta-, hexa-, and hexagalloylglucose may be attributable to the formation of enterobactin, a cyclic triester of 2,3-dihydroxy-*N*-benzoyl-L-serine, or other siderophores that bind Fe^{3+} or Fe^{2+} with high affinity (*35*).

Mila et al. (*36*) demonstrated that the bacterium *Erwinia chrysanthemi* is inhibited by polyphenols with several chelating *o*-dihydroxyphenyl groups such as ellagitannins and pentagalloylglucose and that growth was restored by the addition of Fe^{3+} ions. The growth-inhibiting activity of tannic acid on *E. coli* was also antagonized by the addition of iron (*27*). The authors attributed the resistance of *B. infantis* and *L. acidophilus* to tannic acid to their ability to grow in the absence of iron. This study demonstrated that the MIC of the ethyl acetate extracts from mango increased >20-fold after the addition of iron, confirming that the chelating effect of gallotannins is related to their antibacterial activity. Mangiferin, a xanthone glucoside present in mango kernels, has also been demonstrated to possess iron-complexing activity (*37*). Therefore, its presence in the crude extracts might contribute synergistically to the inhibition of

bacterial growth, as can be deduced from the increasing MIC of purified gallotannins compared to the ethyl acetate extract. It also becomes evident that there is a potential for optimizing the efficacy of antibacterial preparations through combinations of hydrolyzable tannins and xanthone derivatives.

In conclusion, this study demonstrated that the antibacterial activity of mango kernel extracts is mediated by hydrolyzable tannins. Penta-, hexa-, and heptagalloylglucose have been identified as the most active components, which is in agreement with reports on hydrolyzable tannins from *Galla chinensis* (*12*, *13*), another genus of the Anacardiaceae family. Our study strongly supports the hypothesis that iron chelation by tannins is highly relevant in plant defense systems and human nutrition (*31*). The high selectivity of action of penta-, hexa-, and heptagalloylglucose may enable their application as biopreservatives in foods, especially because the growth of several pathogenic bacteria is inhibited, whereas the growth of nonpathogenic lactic acid bacteria is not affected. Further studies on the isolation of larger amounts of hydrolyzable tannins are currently being performed to obtain a better understanding of structure−function relationships of phenolic compounds and to examine their mode of action.

ACKNOWLEDGMENT

Thai mango peels were kindly provided by subproject E 2.2 of SFB 564 (Special Research Program of Deutsche Forschungsgemeinschaft (DFG) "Research for Sustainable Land Use and Rural Development in Mountainous Regions of Southeast Asia").

LITERATURE CITED

(1) Tharanathan, R. N.; Yashoda, H. M.; Prabha, T. N. Mango (*Mangifera indica* L.), "The king of fruits"—an overview. *Food Rev. Int.* **2006**, *22*, 95–123.

(2) FAO. http://faostat.fao.org/, accessed April **2006**.

(3) Berardini, N.; Knödler, M.; Schieber, A.; Carle, R. Utilization of mango peels as a source of pectin and polyphenolics. *Innovative Food Sci. Emerg. Technol.* **2005**, *6*, 442–452.

(4) Sirisalkuwat, S.; Nagel, A.; Sruamsiri, P.; Carle, R.; Neidhart, S. Yield and quality of pectins extractable from the peels of Thai mango cultivars depending on fruit ripeness. *J. Agric. Food Chem.* **2008**, *56*, 10727–10738.

(5) Moharram, Y.; Moustafa, A. M. Utilization of mango seed kernel (*Mangifera indica* L.) as a source of oil. *Food Chem.* **1982**, *8*, 269–276.

(6) Barreto, J. C.; Trevisan, M. T. S.; Hull, W. E.; Erben, G.; De Brito, E. S.; Pfundstein, B.; Würtele, G.; Spiegelhalder, B.; Owen, R. W. Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). *J. Agric. Food Chem.* **2008**, *56*, 5599–5610.

(7) Masibo, M.; He, Q. Major mango polyphenols and their potential significance to human health. *Compr. Rev. Food Sci. Food Saf.* **2008**, *7*, 309–319.

(8) Berardini, N.; Carle, R.; Schieber, A. Characterization of gallotannins and benzophenone derivates from mango (*Mangifera indica* L. cv. 'Tommy Atkins') peels, pulp and kernels by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2208–2216.

(9) Soong, Y.-Y.; Barlow, P. J. Antioxidant activity and phenolic content of selected fruit seeds. *Food Chem.* **2004**, *88*, 411–417.

(10) Knödler, M.; Conrad, J.; Wenzig, E. M.; Bauer, R.; Lacorn, M.; Beifuss, U.; Carle, R.; Schieber, A. Anti-inflammatory 5-(11*Z*-heptadecenyl)- and 5-(8*Z*,11*Z*-heptadecadienyl)-resorcinols from mango (*Mangifera indica* L.) peels. *Phytochemistry* **2008**, *69*, 988–993.

(11) Kabuki, T.; Nakajima, H.; Arai, M.; Ueda, S.; Kuwabara, Y.; Dosako, S. Characterization of novel antimicrobial compounds from mango (*Mangifera indica* L.) kernel seeds. *Food Chem.* **2001**, *71*, 61–66.

(12) Tian, F.; Li, B.; Ji, B.; Yang, J.; Zhang, G.; Chen, Y.; Luo, Y. Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*: the polarity affects the bioactivities. *Food Chem.* **2009**, *113*, 173–179.

(13) Tian, F.; Li, B.; Ji, B.; Zhang, G.; Luo, Y. Identification and structure−activity relationship of gallotannins separated from *Galla chinensis*. *LWT−Food Sci. Technol.* **2009**, *42*, 1289–1295.

(14) Nishizawa, M.; Yamagashi, T.; Nonaka, G.; Nishioka, I. Structure of gallotannins in *Paeonia radix*. *Chem. Pharm. Bull.* **1980**, *28*, 2850–2852.

(15) Röcken, W.; Spicher, G. Fadenziehende Bakterien—Vorkommen, Bedeutung und Gegenmassnahmen. *Getreide Mehl Brot* **1993**, *47*, 30–35.

(16) Shin, S. H.; Lim, Y.; Lee, S. E.; Yang, N.; Rhee, J. H. CAS agar diffusion assay for the measurement of siderophores in biological fluids. *J. Microbiol. Methods* **2001**, *44*, 89–95.

(17) Weinberg, E. D. The lactobacillus anomaly: total iron abstinence. *Perspect. Biol. Med.* **1997**, *40*, 578–583.

(18) Burt, S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253.

(19) Puupponen-Pimiä, R.; Nohyne, K.; Meier, C.; Kähkönen, M.; Hopia, A.; Oksman-Caldentey, K. M. Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol.* **2001**, *90*, 494–507.

(20) Heinonen, M. Antioxidant activity and antimicrobial effect of berry phenolics—a Finnish perspective. *Mol. Nutr. Food Res.* **2007**, *51*, 684–691.

(21) Wu, V. C. H.; Qui, X.; de los Reyes, B. G.; Lin, C.-S.; Pan, Y. Application of cranberry concentrate (*Vaccinium macrocarpon*) to control *Escherichia coli* O157:H7 in ground beef and its antimicrobial mechanism related to the downregulated *slp, hdeA* and *cfa*. *Food Microbiol.* **2009**, *26*, 32–38.

(22) Chung, K.-T.; Wei, C.-I.; Johnson, M. G. Are tannins a double-edged sword in biology and health?. *Trends Food Sci. Technol.* **1998**, *9*, 168–175.

(23) Funatogawa, K.; Hayashi, S.; Shimomura, H.; Yoshida, T.; Hatano, T.; Ito, H.; Hirai, Y. Antibacterial activity of hydrolyzable tannins derived from medicinal plants against *Helicobacter pylori*. *Microbiol. Immunol.* **2004**, *48*, 251–261.

(24) Kamijo, M.; Kanazawa, T.; Funaki, M.; Nishizawa, M.; Yamagishi, T. Effects of *Rosa rugosa* petals on intestinal bacteria. *Biosci., Biotechnol., Biochem.* **2008**, *72*, 773–777.

(25) Abdalla, A. E. M.; Darwish, S. M.; Ayad, E. H. E.; El-Hamahmy, R. M. Egyptian mango by-product 2: antioxidant and antimicrobial activities of extract and oil from mango seed kernel. *Food Chem.* **2007**, *103*, 1141–1152.

(26) Tanaka, T.; Sueyasu, T.; Nonaka, G.-I.; Nishioka, I. Tannins and related compounds. XXI. Isolation and characterization of galloyl and *p*-hydroxybenzoyl esters of benzophenone and xanthone *C*-glucosides from *Mangifera indica* L. *Chem. Pharm. Bull.* **1984**, *32*, 2676–2686.

(27) Chung, K. T.; Lu, Z.; Chou, M. W. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. *Food Chem. Toxicol.* **1998**, *36*, 1053–1060.

(28) Konishi, K.; Adachi, H.; Ishigaki, N.; Kanamura, Y.; Adachi, I.; Tanaka, T.; Nishioka, I.; Nonaka, G.-I.; Horikoshi, I. Inhibitory effects of tannins on NADH dehydrogenases of various organisms. *Biol. Pharm. Bull.* **1993**, *16*, 716–718.

(29) Cannell, R. J. P.; Farmer, P.; Walker, J. M. Purification and characterization of pentagalloylglucose, an α-glucosidase inhibitor/antibiotic from the freshwater green alga *Spirogyra varians*. *Biochem. J.* **1988**, *255*, 937–941.

(30) Scalbert, A. Antimicrobial properties of tannins. *Phytochemistry* **1991**, *30*, 3875–3883.

(31) Scalbert, A.; Mila, I.; Expert, D.; Marmolle, F.; Albrecht, A.-M.; Hurrell, R.; Huneau, J.-F.; Tomé, D. Polyphenols, metal ion complexation and biological consequences. In *Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology*, 1st ed.; Gross, G. G., Hemingway, R. W., Yoshida, T., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999; *Vol. 66*, p 545.

(32) Imbert, M.; Blondeau, R. On the iron requirement of lactobacilli grown in chemically defined medium. *Curr. Microbiol.* **1998**, *37*, 64–66.
(33) Meyer, J. Clostridial iron−sulphur proteins. *J. Mol. Microbiol. Biotechnol.* **2000**, *2*, 9–14.
(34) Vignais, P. M. Hydrogenases and H^+-reduction in primary energy conservation. *Results Probl. Cell Differ.* **2007**, *45*, 223–252.
(35) Grass, G. Iron transport in *Escherichia coli*: all has not been said and done. *BioMetals* **2006**, *19*, 159–172.
(36) Mila, I.; Scalbert, A.; Expert, D. Iron withholding by plant polyphenols and resistance to pathogens and rots. *Phytochemistry* **1996**, *42*, 1551–1555.
(37) Andreu, G. P.; Delgado, R.; Velho, J. A.; Curti, C.; Vercesi, A. E. Iron complexing activity of mangiferin, a naturally occurring glucosylxanthone, inhibits mitochondrial lipid peroxidation induced by Fe^{2+} citrate. *Eur. J. Pharmacol.* **2005**, *513*, 47–55.

Received May 14, 2009. Revised manuscript received July 3, 2009. Accepted July 07, 2009. C.E. acknowledges stipends from HRH Carl Herzog von Württemberg and the Deutscher Akademischer Auslandsdienst (DAAD). A.S. and M.G. acknowledge funding from the Research Chairs of Canada.