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Antibacterial Properties and Major Bioactive Components of Cinnamon Stick (*Cinnamomum burmannii*): Activity against Foodborne Pathogenic Bacteria

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Cinnamomum burmannii Blume (cinnamon stick) from Indonesia is a little-investigated spice. In this study, the antibacterial activity, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of cinnamon stick extract were evaluated against five common foodborne pathogenic bacteria (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella anatum*). Cinnamon stick extract exhibited significant antibacterial properties. Major compounds in cinnamon stick were tentatively identified by gas chromatography−mass spectrometry (GC-MS) and liquid chromatography (LC-MS) as a predominant volatile oil component ((*E*)-cinnamaldehyde) and several polyphenols (mainly proanthocyanidins and (epi)catechins). Both (*E*)-cinnamaldehyde and proanthocyanidins significantly contributed to the antibacterial properties. Additionally, scanning electron microscopy was used to observe morphological changes of bacteria treated with the crude extract of cinnamon stick and its major components. This study suggests that cinnamon stick and its bioactive components have potential for application as natural food preservatives.

KEYWORDS: Cinnamon stick; *Cinnamomum burmannii*; spices; antibacterial activity; pathogenic bacteria; phenolic compounds; cinnamaldehyde; proanthocyanidins

INTRODUCTION

In recent years, there has been a dramatic increase throughout the world in the number of reported cases of foodborne illness (*1*). A variety of microorganisms may lead to food spoilage, one of the most important concerns of the food industry (*2*). Many attempts, such as use of synthetic chemicals, have been made to control microbial growth and to reduce the incidence of food poisoning and spoilage with antimicrobial chemicals. Recently, however, consumers have grown concerned about the side effects and want safer materials for preventing and controlling pathogenic microorganisms in foods (*3*).

Some natural substances of plant origin have good antimicrobial properties and have been used as seasonings for centuries (*4*). Spices and aromatic vegetable materials have long been used in food not only for their flavor and fragrance qualities and appetizing effects but also for their preservative and medicinal properties (*5*). Since ancient times throughout the world, these have been used for preventing food spoilage and deterioration and also for extending shelf life of foods (*6*), while attempts to characterize these properties in the laboratory date back to the early 1900s. It has been extensively reported that the essential oils from many kinds of spices have shown antimicrobial functions against foodborne pathogens (*7*−*11*).

Cinnamomum is a genus in the family Lauraceae, many species of which are used for spices. Two major species are *Cinnamomum zeylanicum* Nees (cinnamon) and *Cinnamomum cassia* Blume (cassia) which are the source of the oldest spices known to man (*12*, *13*). *C. zeylanicum* is also known as *Cinnamomum verum* J.S. Presl. and *C. cassia* is also known as *Cinnamomum aromaticum* Nees. Their bark, leaves, and buds (especially bark) are used as spices or condiments. A closely related species is *Cinnamomum burmannii* Blume from Indonesia, also called Indonesian cassia; its commercial name is cinnamon stick. Cinnamon and cassia are rich in essential oils (mainly cinnamaldehyde and eugenol) which can inhibit microbial growth (*14*−*17*). Barks of *Cinnamomum* plants also contain condensed tannins, that is, dimeric, trimeric, and higher oligomeric polymeric proanthocyanidins (flavan-3-ols) (*18*, *19*). In contrast to the essential oils from *Cinnamomum* plants, little is known about their nonvolatile components (condensed tannins) and the related antibacterial activity. In comparison with cinnamon (*C. zeylanicum*) and cassia (*C. cassia*), cinnamon stick (*C. burmannii*) has been less studied. The bioactive components of cinnamon stick against foodborne pathogenic bacteria have been unclear.

The aims of the present investigation, therefore, were (1) to assay antibacterial properties of crude extract from cinnamon

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stick (*C. burmannii*) against five common pathogenic bacteria; (2) to identify and determine major bioactive components in the crude extract contributing to its antibacterial properties; and (3) to observe morphological changes of the bacteria treated with the crude extract of cinnamon stick and its major components. The ultimate goal of our study is to develop safe, effective, and inexpensive food formulations and processes by using natural compounds to reduce pathogens in foods and to extend the shelf life of foodstuffs.

MATERIALS AND METHODS

Plant Materials and Chemicals/Reagents. Cinnamon stick (*Cinnamomum burmannii* Blume), originally produced in Indonesia, was obtained from a local supermarket in Hong Kong and was authenticated by botanical experts. Potassium phosphate, penicillin G (16 mg/mL), and gentamicin solution (10 mg/mL) were purchased from Sigma/Aldrich (St. Louis, MO). Sodium dihydrogen phosphate monohydrate was from Merck (Darmstadt, Germany), plate count agar (PCA) medium was from BD (Sparks, NA), Mueller Hinton broth (MHB) medium was from Difco (Sparks, MD), and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was from Fluka Chemie AG (Buchs, Switzerland). Authentic standards, such as cinnamaldehyde and proanthocyanidins, were purchased from Extrasynthese (Genay, France), and catechin was from Sigma. HPLC grade organic reagents were from BDH (Dorset, United Kingdom).

Microorganisms and Culture. A total of five foodborne pathogenic or fecal indicator bacteria were kindly provided by the Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong. They are *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia* coli ATCC25922, and *Salmonella anatum*. The strains were cultured at 37 °C on PCA medium.

Preparation of Crude Extract. Cinnamon stick sample was further air-dried in a ventilated oven at 40 °C for 24 h and then was ground into a fine powder and was passed through a sieve (24 mesh). Powdered sample was extracted with 80% methanol (w/v: 1/25) at room temperature (~23 °C) for 24 h in a shaking water bath (Shaking Bath 5B-16) (Techne, Cambridge, United Kingdom). The extract was filtered by a Millipore filter with a 0.45 μm nylon membrane under vacuum at 23 °C. The filtrates were vaporized by Rotavapor (R-114) (Buchi, Flawil, Switzerland) and then were freeze-dried by a Heto FD3 freeze-dryer (Heto-Holten A/S, Allerod, Denmark). The freeze-dried sample of the crude extract was stored at 4 °C until use.

Fractionation and Preparation of Proanthocyanidins−(Epi)-catechins. The crude cinnamon stick extract was fractionated, and the mixture of proanthocyanidins and catechins was collected according to the published procedure (*20*) with some modifications. The preparative high-performance liquid chromatography (HPLC) apparatus was a Hewlett-Packard HPLC System (HP 1100 series, Waldbronn, Germany), consisting of a binary pump and a diode-array detector (DAD), and was equipped with a 250 × 9.4 mm i.d., 5-μm, Zorbax SB-C18 column (Agilent Technologies, Palo Alto, CA). The mobile phase consisted of solvent A (0.1% formic acid in H_2O) and solvent B (100% MeOH). The gradient was as follows: 0 min, 15% B; 0−10 min, 15−50% B; 10−20 min, 50−100% B; 20−23 min, 100% B. The injection volume was 100 μL, and the flow rate was 3.5 mL/min. Detection was at 280 nm for isolation of proanthocyanidins and (epi)-catechins. Because proanthocyanidins and (epi)catechins have similar polarities, it was not easy to separate them well under reversed-phase liquid chromatography in this study. The peaks of proanthocyanidins and catechins were collected together, were freeze-dried, and were stored at 4 °C until use.

Headspace SPME-GC-MS Analysis of Cinnamon Stick Powder. Gas chromatography−mass spectrometry (GC-MS) analysis was performed on a GCMS-QP2010 system with an AOL-5000 headspace/liquid autoinjector (Shimadzu, Japan). SPME (solid-phase microextract) (SUPELCO, Bellefonte, PA) was used for extraction of the essential oils from cinnamon stick powder. A conditioned SPME fiber coated with DVB-CAR-PDMS (50/30 μm) was exposed to the headspace of the sample at 50 °C for 10 min (including 3 min preincubation time). The SPME fiber absorbed with the volatile components was automatically inserted into the injection port (splitless injection) for 2 min for sample desorption. A CP-WAX 52 CB column (50 m × 0.20 mm, ø with 0.2 μm film thickness) was used with helium as a carrier gas at a flow rate of 1 mL/min, and the injection volume was 1 μL. The GC oven temperature was kept at 40 °C for 0.5 min and was programmed to 150 °C at a rate of 6 °C/min, to 250 °C at a rate of 8 °C/min, and was kept constant at 250 °C for 8 min. Splitless injections were done with both headspace and liquid injection methods. The MS was operated in electron ionization mode (70 eV) with a scan range of *m*/*z* 40−500. The interface and ion source temperatures were 260 °C and 200 °C, respectively. A library search was carried out using NIST and SZTERP libraries. Relative percentage amount of volatile compounds was calculated from TIC (total ions chromatograms).

LC-MS Analysis of Crude Extract from Cinnamon Stick. The LC-MS-2010EV system consisted of an LC-20AD binary pump, an SIL-20AC autosampler, a photodiode-array detector, a central controller, and a single quadrupole MS detector with electrospray ionization (ESI) interface (Shimadzu, Japan). The column was a VP-ODS C_{18} column (250 × 2.0 mm, 4.6 μm) (Shimadzu, Japan). LC elution conditions were as follows: the mobile phase was solvent A (0.1% formic acid in H_2O) and solvent B (100% MeOH with 0.1% formic acid). A gradient elution used was 0−5 min, 5% B; 5−15 min, 5−30% B; 15−40 min, 30−40% B; 40−60 min, 40−50% B; 60−65 min, 50−55% B; 65−90 min, 55−100% B; 90−95 min, 100% B; 95−96 min, 100−5% B; 96−100 min, 5% B. The flow rate was 0.2 mL/min, the injection volume was 5 μL, and the detection was at 280 nm. The LC elute was introduced directly into the ESI interface without flow splitting. The scan range of ESI-MS was *m*/*z* 120−1200 (120−700 and 700−1200). The ESI voltage was 4.5 kV in positive ion mode and 3.5 kV in negative ion mode. A nebulizing gas of 1.5 L/min and a drying gas of 10 L/min were applied for ionization using nitrogen in both cases. Relative percentage amount of major components isolated in the crude extract of cinnamon stick was calculated according to individual peak area and total peak area of LC chromatogram.

Determination of Antibacterial Activity. Agar-well diffusion method was employed for determination of antibacterial activity (*21*). The freeze-dried crude extract sample and standard or isolated samples (major components of cinnamon stick) were dissolved in the phosphate buffer saline (PBS, pH 7.0−7.2) to the final concentration of 100 mg/mL and were sterilized by filtration through 0.22 μm sterilizing Millipore express filter (Millex-GP, Bedford, OH). All bacteria were suspended in sterile water and were diluted to ~10^6 CFU/mL. The suspension (100 μL) was "flood-inoculated" onto the surface of PCA medium. Wells (4.6 mm in diameter) were cut from the agar, and 60 μL sample solutions were delivered into them. Negative controls were prepared using PBS solution. Penicillin G (960 μg/well) and gentamicin (600 μg/well) were used as positive reference standards to determine the sensitivity of each microbial species tested. The inoculated plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters. Tests were performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC). Two-fold microdilution broth method (*22*) was used to determinate the MIC value. Dilutions were used to dispense 0.1 mL into each of the sterile 96 wells of a standard tray. Each well contained 5 × 10^5 CFU/mL of test bacteria, serially diluted test samples, and respective MHB medium. The positive control well contained inoculated growth medium without test samples. The negative control well included uninoculated medium only. Microdilution trays were incubated at 37 °C for 20 h in an ambient air incubator. Growth inhibition of the bacteria in microdilution wells was detected by the unaided eyes. The amount of growth in the wells containing test samples was compared with the amount of growth in the control wells when determining the growth end points. The acceptable growth (≥2 mm button or definite turbidity) must occur in the positive control well. When a single skipped well occurred, the highest MIC was read. Triplicate samples were performed for each test concentration.

Table 1. Antibacterial Activity (DIZ), Minimum Inhibitory Concentrations (MIC), and Minimum Bactericidal Concentrations (MBC) of Crude Extract from Cinnamon Stick (*C. burmannii*) and Its Major Bioactive Components[a]

		Gram-positive bacteria			Gram-negative bacteria	
antibacterial properties	extract or compounds	*B. cereus*	*L. monocytogenes*	*S. aureus*	*E. coli*	*S. anatum*
DIZ (mm) (mean ± SD)[b]	crude extract of cinnamon stick	15.4 ± 0.3^{D}	11.5 ± 0.2^{C}	15.7 ± 0.4^{C}	8.7 ± 0.4^{B}	12.1 ± 0.4^{B}
	(*E*)-cinnamaldehyde	43.3 ± 0.8^{A}	50.2 ± 0.4^{A}	68.6 ± 0.8^{A}	41.4 ± 0.7^{A}	22.3 ± 0.2^{A}
	proanthocyanidins-(epi)catechins	17.8 ± 0.5^{C}	16.6 ± 0.4^{B}	20.8 ± 0.8^{B}	9.2 ± 0.6^{B}	10.0 ± 0.4^{B}
	procyanidin B2	21.4 ± 0.3^{B}	18.4 ± 0.7^{B}	21.5 ± 0.6^{B}	9.8 ± 0.4^{B}	11.4 ± 0.4^{B}
	(+)-catechin[c]	NA	NA	NA	NA	NA
MIC (μg/ mL)	crude extract of cinnamon stick	625	>2500	>2500	>2500	>2500
	(*E*)-cinnamaldehyde	312.5	500	156.3	156.3	125
	proanthocyanidins-(epi)catechins	78.1	312.5	312.5	625	2500
	procyanidin B2	39.1	625	625	1250	2500
	(+)-catechin[c]					
MBC (μg/ mL)	crude extract of cinnamon stick	2500	>2500	>2500	>2500	>2500
	(*E*)-cinnamaldehyde	2500	500	625	312.5	250
	proanthocyanidins-(epi)catechins	78.1	312.5	1250	2500	>2500
	procyanidin B2	78.1	625	1250	1250	2500
	(+)-catechin[c]					

[a] Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. The DIZ value of negative control for each bacterium was 4.6 mm (i.e., the bored well diameter in the agar plate). The concentrations of DIZ test were 100 mg/mL for crude extract of cinnamon stick and 50 mg/mL for (*E*)-cinnamaldehyde, proanthocyanidins, and catechins. [b] DIZ value = mean ± SD. Means with the same letter were not significantly different ($p < 0.05$). [c] NA, no activity. (+)-Catechin did not have any antibacterial activity against any of the tested bacteria (DIZ = 4.6 mm, like negative control). Therefore, its MIC and MBC values were not determined.

Determination of Minimum Bactericidal Concentration (MBC). MBC was determined by a modification of the method in ASM pocket guide to clinical microbiology (*23*). Briefly, the samples (50 μL) for the MBC assays were taken from the wells of the MIC assays in which any visible turbidity (growth) was not observed. They were spread on freshly prepared PCA plates and were incubated at 37 °C for 24 h so as to determine the MBC. The MBC was regarded as the lowest concentration of the samples that allowed less than 0.1% of the original inoculum treated with the extract or compound samples to survive and grow on the surface of the medium used. Triplicate samples were performed for each test concentration.

Scanning Electron Microscope (SEM) Observations. The bacteria cells were incubated for 12 h in nutrient broth at 37 °C. The suspension was divided into two portions. To one portion was added suitable concentrations of the crude extract sample of cinnamon stick and standard or isolated samples (major components of cinnamon stick). The other portion was left untreated as a control. The resuspension was incubated at 37 °C for 2 h, and then the cells from both tubes were harvested by centrifugation and were prefixed with a 2.5% glutaraldehyde solution overnight at 4 °C. After this, the cells were again harvested by centrifugation and 0.1 M Na-cacodylate buffer solution was added. A drop of each resuspension was filtered by 30%, 50%, 70%, 90%, and 100% ethanol, respectively, through a polycarbonate isopore membrane with 0.8 μm pore size (Millipore, Tullagreen, Ireland). Then, cells were dried at "critical point" (Balzers CPD 030) in liquid CO_2 under 95 bar pressure. The samples were gold-covered by cathodic spraying (Edwards S 150 B). Finally, morphology of the bacterial cells was observed on a scanning electronic microscope (Steroilsscann 440, Cambridge).

Statistical Analysis. The results of all DIZ values were calculated as mean ± standard deviation (SD) in this study. Differences between means of data were compared by least significant difference (LSD) calculated using the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Antibacterial Activity, MIC, and MBC of Crude Extract from Cinnamon Stick. The DIZ (diameter of inhibition zone), MIC, and MBC values (antibacterial properties) of crude extract from cinnamon stick (*C. burmannii*) are presented in **Table 1**. The DIZ values showed that the crude extract had a wide range of antibacterial activities against both Gram-positive and Gram-negative bacteria. Compared with many other spices tested in our recent study (*24*), this crude extract possessed much higher antibacterial activity. Of the three Gram-positive bacteria, *S. aureus* was the most sensitive (DIZ 15.7 mm) to the crude extract, followed by *B. cereus* (15.4 mm), with *L. monocytogenes* being the most resistant (11.5 mm). Of the two Gram-negative bacteria, *S. anatum* was more sensitive (DIZ 12.1 mm) than *E. coli* (8.7 mm) to the crude extract. Generally, the Gram-positive bacteria were more sensitive than the Gram-negative ones to the crude extract. To some extent, this was consistent with previous studies on antibacterial activity of the essential oils from other spices (*7*−*9*, *11*).

The lower concentration of the crude extract could fully inhibit the growth or could almost kill *B. cereus* (MIC 625 μg/mL and MBC 2500 μg/mL). However, both MIC and MBC of the crude extract for the other four bacteria were more than 2500 μg/mL. There were certain differences between this study and previous studies (*16*). This was mainly due to the differences of the samples used. The crude methanolic extract from cinnamon stick (methanol was removed during sample preparation) was used in the present study for MIC and MBC assays, while the essential oils distilled from spices including cinnamon and cassia were widely used in many previous studies for MIC and MBC assays (*17*, *25*−*27*). The essential oils distilled from spices normally contained higher levels of volatile components (e.g., cinnamaldehyde) than the crude extract containing both volatile and nonvolatile components. Therefore, it was reasonable for the crude extract to have higher MIC and MBC values than the essential oils. In addition, different definitions and methodologies of MIC and MBC may significantly influence the assay results. Some researchers considered MIC as the lowest concentration resulting in maintenance or reduction of inoculum viability (*16*), whereas others defined it as the lowest concentration resulting in a significant decrease (>90%) (*28*) or complete destruction of the inoculum viability (*29*). Some researchers defined MBC as concentration where 99.9% or more of the initial inoculum was killed; others considered MBC as the lowest concentration at which no growth was observed after subculturing into fresh broth or as the smallest concentration of

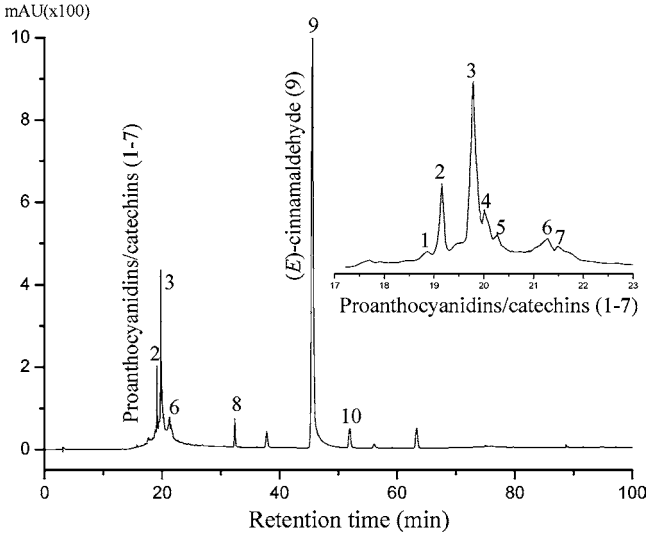

Figure 1. LC chromatogram (280 nm) of crude extract from cinnamon stick. For all peak assignments, see **Table 2**. Major peaks were identified by ESI-MS: 1–7, proanthocyanidins–(epi)catechins; 9, (*E*)-cinnamaldehyde.

antimicrobial agent that, on subculture, either failed to show growth or resulted in 99.9% decrease in the initial inoculum (*16*). So, the comparison of data from different reports must be approached with caution.

Identification of Major Bioactive Components from Cinnamon Stick. Little information about major components from *C. burmannii* bark (cinnamon stick) has been published. LC-MS and GC-MS chromatograms and the associated analytical data of major compounds in cinnamon stick showed that major constituents of cinnamon stick were volatile (essential) oils (mainly (*E*)-cinnamaldehyde) and condensed tannins (proanthocyanidins) (**Figure 1** and **Table 2**).

The essential oils obtained from barks, leaves, root barks, and buds of *Cinnamomum* plants vary significantly in chemical composition. In this study, headspace SPME-GC-MS analysis clearly indicated that (*E*)-cinnamaldehyde was a predominant volatile component and accounted for 83.6% of the total peak area in the essential oils extracted from cinnamon stick power. Eugenol was not detected in the essential oils from cinnamon stick powder. This was similar to the essential oils from *C. cassia* which contained 80−90% cinnamaldehyde and no or little eugenol, which was different from *C. zeylanicum*. The essential oils from the barks of *C. zeylanicum* contained 60−80% cinnamaldehyde and also about 2% eugenol, and those from its leaves were rich in eugenol (70−75%) (*12*, *13*).

Previous reports on *Cinnamomum* spices mainly focused on the essential oils, but fewer reports studied the nonvolatile components. In the present study, LC-MS analysis showed that the crude extract of cinnamon stick also contained high levels of nonvolatile compounds (mainly condensed tannins), that is, 23.2% proanthocyanidins and 3.6% (epi)catechins, in addition to cinnamaldehydes (64.1%) (**Table 2**). **Figure 1** clearly displays the predominant peaks isolated in the crude extract, that is, proanthocyanidins and (epi)catechins (peaks 1−7) and (*E*)-cinnamaldehyde (peak 9). Peaks 1−7, proanthocyanidins and (epi)catechins, were tentatively identified as procyanidin dimers (B1, B2), trimer, and tetramer, and (+)-catechin and (−)-epicatechin, according to the UV spectra and retention time (R_t) of available authentic standards and by comparison of our MS data and literature data (*19*, *30*, *31*). Peak 5 ($[M - H]^-$ at *m*/*z* 575, $[M + H]^+$ at *m*/*z* 577) might be A-type procyanidin dimer, different from peaks 1 and 2 belonging to B-type dimers (procyanidin B1 and B2) ($[M - H]^-$ at *m*/*z* 577, $[M + H]^+$ at *m*/*z* 579). Peak 3 was A-type procyanidin trimer ($[M - H]^-$ at *m*/*z* 863, $[M + H]^+$ at *m*/*z* 865), according to the literature (*18*, *31*). Gu and co-workers (*19*, *30*) reported that cinnamon contained very high levels of proanthocyanidins (about 2−10 mers), mainly A-type proanthocyanidins and certain B-type ones identified by normal-phase LC-MS. However, in this study, more than pentamers (>4 mers) of proanthocyanidins could not be isolated and detected by reversed-phase LC-MS.

Antibacterial Activity, MIC, and MBC of Major Bioactive Compounds from Cinnamon Stick. The antibacterial properties of the crude extract of cinnamon stick might be expected to be attributable to its major components. The above results indicated that cinnamon stick not only possessed high levels of cinnamaldehydes but also contained many proanthocyanidins/(epi)catechins. We assayed the antibacterial activity, MIC, and MBC values of these major compounds to determine their individual roles as antibacterial components of cinnamon stick. Our results clearly indicated that (*E*)-cinnamaldehyde exhibited potent antibacterial properties against the tested five bacteria and possessed a wide spectrum of inhibitory effects (the DIZ values: 22.3−68.6 mm; MIC: 125−500 μg/mL; MBC: 250−2500 μg/mL) (**Table 1**). Therefore, (*E*)-cinnamaldehyde was a predominant antibacterial component in cinnamon stick. This was consistent with the results of previous studies on other *Cinnamomum* spices (cinnamon and cassia) (*15*−*17*).

Antibacterial activities of cinnamaldehyde from the essential oils of cinnamon and cassia or from commercial sources had been widely studied. However, antibacterial properties of proanthocyanidins (condensed tannins) from *Cinnamomum* spices were little reported, because previous studies concentrated on the essential oil components distilled from the spices. Proanthocyanidins are mixtures of oligomers and polymers composed of flavan-3-ols and possess potent antioxidant capacity. The proanthocyanidins consisting exclusively of (epi)catechin are designated as procyanidins (*30*). Since proanthocyanidins and (epi)catechins have similar polarities, it is not easy to separate them well. Therefore, the mixed fractions of proanthocyanidins and (epi)catechins (peaks 1−7 in **Figure 1**) were collected using preparative HPLC for assaying their antibacterial properties in this study. Additionally, procyanidin B2 and (+)-catechin from commercial sources were used as the respective standards. The results showed that the proanthocyanidins−(epi)catechins from cinnamon stick also exhibited strong antibacterial properties (the DIZ values: 9.2−20.8 mm; MIC: 78.1−2500 μg/mL; MBC: 78.1−≥2500 μg/mL) (**Table 1**). The reference standard procyanidin B2 had similar antibacterial properties to the proanthocyanidins−(epi)catechins, but (+)-catechin did not have any antibacterial properties against any of the tested bacteria. This suggested that the antibacterial properties of the proanthocyanidin−(epi)catechin fractions collected in this study were fully from contribution of the proanthocyanidin components. All the proanthocyanidins accounted for 23.2% of total peak area in the crude extract of cinnamon stick (**Table 2**). This indirectly indicates that the proanthocyanidins were also important bioactive components contributing to its antibacterial properties.

The similarity of the antibacterial activities of the procyanidin B2 reference standard and the proanthocyanidins−(epi)catechins is clearly visible in the DIZ values for all tested bacterial strains. However, there were some significant differences in measured MIC and MBC values, which varied by factors of up to 2×. Although the antibacterial activity (DIZ values) of the proan-

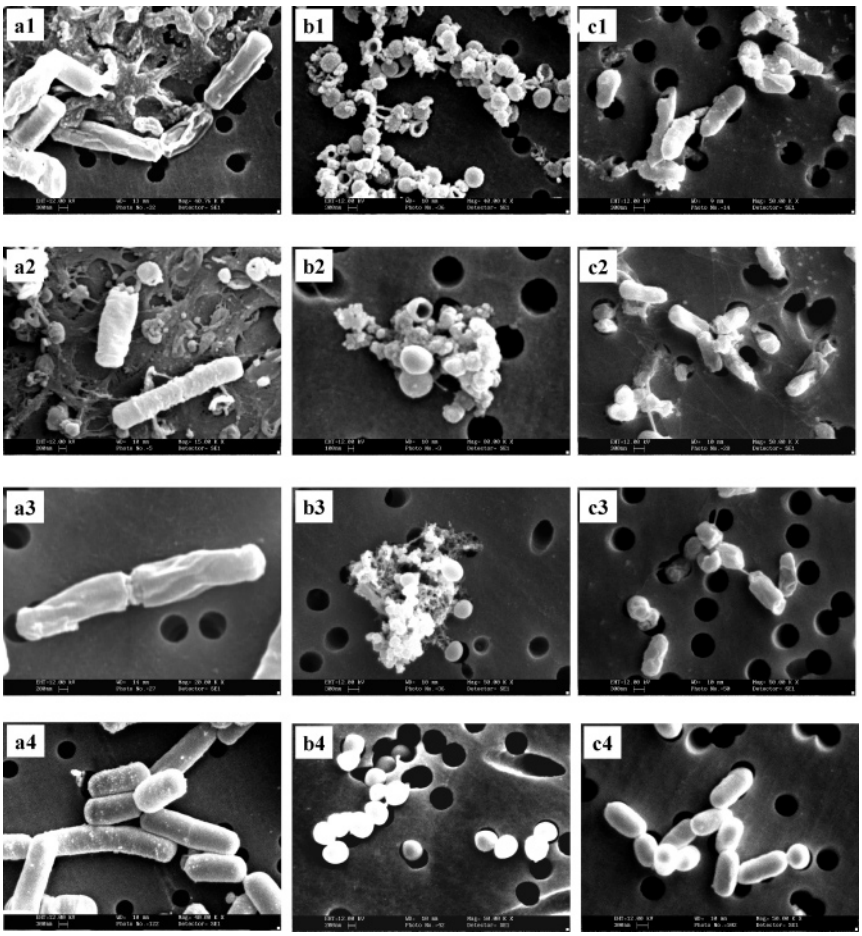

Figure 2. Scanning electron microscope observations of three selected pathogenic bacteria (**a**, *B. cereus*; **b**, *S. aureus*; **c**, *S. anatum*) treated with the samples (**1**, the crude extract of cinnamon stick; **2**, (*E*)-cinnamaldehyde; **3**, proanthocyanidins) and untreated bacterial cells (**4**, control).

Table 2. Tentative Identification of Major Compounds (Proanthocyanidins/Catechins and Cinnamaldehyde) in Crude Extract of Cinnamon Stick (*C. burmannii*) by LC-MS

peak	R_t (min)	tentative names of compounds	MS (*m/z*) $[M-H]^-$	MS (*m/z*) $[M+H]^+$	peak area (%)
1	18.9	dimer (procyanidin B1)	577	579	0.70
2	19.2	dimer (procyanidin B2)	577	579	5.12
3	19.8	procyanidin trimer	863	865	13.76
4	20.1	(+)-catechin	289	290	2.61
5	20.4	procyanidin dimer	575	577	1.27
6	21.3	procyanidin tetramer	1153	nd[a]	2.35
7	21.6	(−)-epicatechin	289	290	1.02
8	32.4	(*E*)-cinnamic acid	147	149	2.69
9	45.6	(*E*)-cinnamaldehyde	131	133	62.18
10	51.9	(*S*)-cinnamaldehyde	131	133	1.95

[a] nd, not detected.

thocyanidins was weaker than that of (*E*)-cinnamaldehyde, there were significant differences between their MIC and MBC values against the various tested bacteria. The proanthocyanidins possessed lower MIC and MBC values against *B. cereus* and *L. monocytogenes* but higher MIC and MBC values against *S. aureus*, *E. coli*, and *S. anatum* than (*E*)-cinnamaldehyde (**Table 1**).

Scanning Electron Microscope Observations. Treated samples of bacteria were observed by SEM to investigate any physical changes in the appearance of the cells. SEM observations confirmed the physical damage and considerable morphological alteration to all five tested bacteria treated with the crude extract of cinnamon stick, and its major bioactive components ((*E*)-cinnamaldehyde and proanthocyanidins−catechins) were observed. **Figure 2** shows the SEM images of treated and control samples for three selected bacterial species (*B. cereus*, *S. aureus*, and *S. anatum*). These images directly illustrate the destructive effects of the extracts on the tested bacteria. Nontreated cells were intact and showed a smooth surface (**Figure 2**, **a4**, **b4**, and **c4**), while bacterial cells treated with the extracts underwent considerable damage (**Figure 2**, **a1**−**a3**, **b1**−**b3**, and **c1**−**c3**). Although the samples were not prepared in a quantitative manner, it was clearly observed that the number of the damaged cells was significantly greater in the treatments (the crude extract of cinnamon stick, cinnamaldehyde, and proanthocyanidins−catechins) than in the control.

There are many possible explanations for the observations. The literature suggests that the active components of the extracts

might bind to the cell surface and then penetrate to the target sites, possibly the phospholipid bilayer of the cytoplasmic membrane and membrane-bound enzymes. The effects might include the inhibition of proton motive force, inhibition of the respiratory chain and electron transfer, and inhibition of substrate oxidation. Uncoupling of oxidative phosphorylation, inhibition of active transport, loss of pool metabolites, and disruption of synthesis of DNA, RNA, protein, lipid, and polysaccharides might follow (*32*−*34*).

Some cells present damage as pores or deformity in the cell walls (**Figure 2, a1**, **a3**, **b1**, **b2**, **b3**, **c1**, **c2**, and **c3**). Some authors have suggested that the damage to the cell wall and cytoplasmic membrane was the loss of structural integrity and the ability of the membrane to act as a permeability barrier (*35*−*37*). The distortion of the cell physical structure would cause the expansion and destabilization of the membrane and would increase membrane fluidity, which in turn would increase passive permeability (*38*) and manifest itself as a leakage of various vital intracellular constituents, such as ions, ATP, nucleic acids, and amino acids (*39*−*41*).

Some cells in the SEM images (**Figure 2**, **a1**, **b1**, **b2**, **b3**, and **c2**) even appeared to be empty, and the remains were flaccid. Cell death may have been the result of the extensive loss of cell contents, the exit of critical molecules and ions, or the initiation of autolytic processes (*33*). From all the SEM observations, it seemed that the extracts caused severe damage to the bacteria.

In addition, the modes of action of bacterial agents depend on the type of microorganisms and are mainly related to their cell wall structure and to the outer membrane arrangement. This study and many previous studies (*9*, *11*, *26*, *42*) indicated that most spice extracts were more active against Gram-positive bacteria than Gram-negative bacteria. This is likely due to the significant differences in the outer layers of Gram-negative and Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique periplasmic space not found in Gram-positive bacteria (*43*, *44*). The resistance of Gram-negative bacteria toward antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules, and is also associated with the enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from outside (*45*, *46*). Gram-positive bacteria do not have such an outer membrane and cell wall structure. Antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm and its coagulation (*10*).

In summary, this study showed that cinnamon stick and its major components ((*E*)-cinnamaldehyde and proanthocyanidins) possessed significant in vitro antibacterial properties against five common foodborne pathogenic or fecal indicator bacteria. It is the first report to extensively investigate the antibacterial properties of cinnamon stick from *C. burmannii* bark and its major bioactive components. The antibacterial properties of cinnamon stick were not only from contribution of the essential oils (cinnamaldehydes) but also from contribution of the nonvolatile components (proanthocyanidins). Cinnamon stick and its major antibacterial components have great potential for application as natural food preservatives. However, further research, particularly on the interactions with other food ingredients, is still necessary.

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