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Antibacterial properties of *Polygonum cuspidatum* roots and their major bioactive constituents

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Abstract

Antibacterial activity, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of crude extract from *Polygonum cuspidatum* roots were assayed against five common foodborne bacteria (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella anatum*). The crude extract exhibited potent antibacterial properties. Major bioactive compounds in *P. cuspidatum* roots were identified as stilbenes (e.g., piceid, resveratroloside, and resveratrol) and hydroxyanthraquinones (e.g., emodin, emodin-1-*O*-glucoside, and physcion) by LC–ESI-MS. Both stilbenes and hydroxyanthraquinoines greatly contributed to the antibacterial properties. Additionally, scanning electron microscopy was used to observe morphological changes of the bacteria treated with the crude extract and its major antibacterial components. Possible mechanisms of the antibacterial action were also discussed. This study suggests that the roots of *P. cuspidatum* and its antibacterial components may have potential for use as natural preservatives.

Keywords: *Polygonum cuspidatum*; Antibacterial properties; Foodborne pathogenic bacteria; Phenolic compounds; Stilbenes; Hydroxyanthraquinones

1. Introduction

Foodborne illnesses are still a major problem and have a dramatic increase throughout the world in recent years (Mead et al., 1999). A variety of microorganisms may lead to food spoilage, so food poisoning is still a threat for both consumers and the food industry. Some synthetic chemicals have been made to control microbial growth and reduce the incidence of food poisoning and spoilage. Although these synthetic preservatives are effective, they might be detrimental to human health. Consumers are concerned about the safety of foods containing artificial preservatives. There has been a growing interest in new and effective antimicrobial substances from natural sources like plants to reduce cases of foodborne illnesses. Crude extracts of spices, herbs and medicinal plants rich in phenolic compounds are becoming increasingly important in food preservation because of their antimicrobial activity.

Polygonum cuspidatum Sieb. et Zucc. is a perennial of the genus *Polygonum* in the family Polygonaceae, which is distributed in China, Japan and Korea and also found growing throughout North America (Mexican bamboo) (Gao, Xu, & Li, 2000; Vastano et al., 2000). The dried root of *P. cuspidatum* is used as a well-known traditional Chinese medicine (called Huzhang) officially listed in the Chinese Pharmacopoeia (China Pharmacopoeia Committee, 1999), and also used for folk medicine in Korea and Japan (called Japanese knotweed or bamboo). It is often used as an analgesic, antipyretic, diuretic, expectorant, and antitussive agent and also used for the treatment of chronic bronchitis, infectious hepatitis, diarrhea, cancer, hypertension, atherosclerosis, hyperlipidemia, leucorrhoea, dysmenorrhea, trauma with blood stasis, burn, snake bites, and allergic inflammatory diseases (Gao et al., 2000; Kim, Kim, Yoo, & Shin, 2004; Yan et al., 1996).

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Kim et al. (2004) and Kim, Hwang, and Shin (2005) reported the antibacterial activity and bactericidal effect of volatile constituents from leaves of *P. cuspidatum* against foodborne microorganisms. Song et al. (2006) investigated *in vitro* inhibitory effects of the roots of *P. cuspidatum* on oral bacterial viability and virulence factors to improve oral hygiene. However, so far nobody has reported antibacterial properties of the roots of *P. cuspidatum* against foodborne microorganisms. Also, their corresponding antimicrobial components have not yet been investigated. Meanwhile, there have been few reports and discussion on the mechanisms of action of antimicrobial components.

As part of an ongoing study on natural antibacterial agents for food preservation and medicinal use, the objectives of the present study were: (1) to determine antibacterial properties of crude extract from *P. cuspidatum* roots against five foodborne pathogenic bacteria; (2) to identify and quantify 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 and its major components.

2. Materials and methods

2.1. Materials and chemicals/reagents

Compared with many other spices and herbs tested in our recent study, *P. cuspidatum* (root extract) exhibited much higher antibacterial activity (Shan, Cai, Brooks, & Corke, 2007a). Therefore, *P. cuspidatum* was screened and used in this study. *P. cuspidatum* roots were obtained from a well-known market for Chinese herbal medicines in Qichun, Hubei, China. 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 from BD (Sparks, NA), Mueller Hinton broth (MHB) medium from Difco (Sparks, MD), and trolox from Fluka Chemie AG (Buchs, Switzerland). Resveratrol, emodin, and physcion were purchased from Sigma (St. Louis, MO) and piceid was obtained from Guanyu Bio-technique (Xi'an, China). HPLC grade organic reagents were from BDH (Dorset, England).

2.2. Microorganisms and culture

Five foodborne pathogenic or faecal indicator bacteria tested in the present study were *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* ATCC25922, and *Salmonella anatum*, which were kindly provided by the Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong. The bacterial strains were cultured at 37 °C on PCA medium.

2.3. Preparation of crude extract

P. cuspidatum roots were air-dried in a ventilated oven at 40 °C for 24 h, then ground into a fine powder and passed through a sieve as (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, UK). The extract was filtered by a Millipore filter with a 0.45 μm nylon membrane under vacuum at 23 °C. The filtrates were concentrated and then 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.

2.4. LC–ESI-MS analysis

The LC–MS-2010EV system consisted of a LC-20AD binary pump, 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). Liquid chromatographic conditions followed our previous method (Cai, Xing, Sun, Zhan, & Corke, 2005). The mobile phase included solvent A (0.1% formic acid in H_2O) and solvent B (100% MeOH with 0.1% formic acid). The gradient elution 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, injection volume was 5 μL, and detection was at 280 nm. The scan range of ESI-MS was *m/z* 160–800. 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 of major components isolated in the crude extract of *P. cuspidatum* roots was calculated, according to individual peak area and total peak area of LC chromatogram.

2.5. Antibacterial activity assay

Antibacterial activity was assayed with standard agar-well diffusion method (NCCLS, 2000). Briefly, the bacterial suspension (10^6 CFU/mL, 100 μL) was "flood-inoculated" onto the surface of PCA medium and the wells (4.6 mm in diameter) were then cut from the agar. The freeze-dried crude extract sample and standard or isolated samples (major components of *P. cuspidatum* roots) were dissolved in the phosphate buffer saline (PBS, pH 7.0–7.2) to the final concentration of 100 mg/mL. The sample solutions were sterilized by filtration through 0.22 μm sterilizing Millipore express filter (Millex-GP, Bedford, OH) and 60 μL these sample solutions were delivered into the wells. Gentamicin (600 μg/well) was used as positive reference standards to determine the sensitivity of each microbial species tested and PBS solutions were applied as negative controls. The

inoculated plates were incubated at 37 °C for 24 h. The diameter of inhibition zone (DIZ) of the tested bacteria was measured and expressed in millimetres to evaluate the antibacterial activity of the samples. Tests were performed in triplicate.

2.6. Minimum inhibitory concentration (MIC) assay

MIC was assayed using two-fold microdilution broth method (NCCLS, 2003). 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 colony forming units (CFU)/mL of test bacteria, serially diluted test samples and respective growth medium. Negative controls were prepared with uninoculated medium and the positive control wells contained inoculated growth medium without test samples. After being incubated at 37 °C for 20 h in an ambient air incubator, the microdilution trays were checked with unaided eyes to detect the growth inhibition of the bacteria. The growth end points were determined by comparing the amount of growth in the wells containing test samples with that in the control wells. The acceptable growth ($\geqslant$2 mm button or definite turbidity) must occur in the positive control well. When a single skipped well occurred, the highest MIC was read. Tests were performed in triplicate for each test concentration.

2.7. Minimum bactericidal concentration (MBC) assay

A method in ASM Pocket Guide to Clinical Microbiology (Patrick, 1996) was slightly modified to determine MIC in this study. Briefly, 50 μL of the samples were taken from the wells of the MIC assays, where any visible turbidity (growth) was not observed, and spread on freshly prepared PCA plates. The plates were incubated at 37 °C for 24 h so as to determine the MBC. The MBC was defined as the lowest concentration of the samples which 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. Tests were performed in triplicate.

2.8. Scanning electron microscope (SEM) observation

The bacteria cells were suspended in nutrient broth and incubated at 37 °C for 12 h. The suspension was divided equally into two tubes. Suitable concentrations of the crude extract sample of *P. cuspidatum* roots and the major components identified in this study were added to one tube and incubated at 37 °C for 2 h. The other tube was used as control. After this, the cells from both tubes were harvested by centrifugation and prefixed with a 2.5% glutaraldehyde solution overnight at 4 °C. The cells were collected by centrifugation and suspended in 0.1 M Na-cacodylate buffer solution. The samples were dehydrated rapidly with ethanol series (30%, 50%, 70%, 90% and 100%), dried with liquid CO_2 at "critical point" (Balzers CPD 030) under 95 bar pressure and gold-covered by cathodic spraying (Edwards S 150 B), subsequently. Finally, morphology of the bacterial cells was observed on scanning electronic microscope (Steroilsscann 440, Cambridge).

2.9. 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

Table 1
Antibacterial activity (DIZ), minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of crude extract from *P. cuspidatum* roots and their major bioactive components[a]

Antibacterial properties	Extract or compounds	Gram-positive bacteria			Gram-negative bacteria	
		B. cereus	*L. monocytogenes*	*S. aureus*	*E. coli*	*S. anatum*
DIZ (mm) (mean ± SD)[b]	Crude extract of *P. cuspidatum*	16.4 ± 0.5^{E}	19.2 ± 0.5^{C}	20.2 ± 0.4^{E}	6.4 ± 0.9^{E}	12.8 ± 0.6^{E}
	Piceid	30.5 ± 0.4^{A}	27.6 ± 0.3^{A}	35.0 ± 0.5^{B}	31.6 ± 0.5^{B}	29.3 ± 0.8^{A}
	Resveratrol	25.3 ± 0.6^{B}	26.9 ± 0.6^{B}	48.3 ± 0.7^{A}	35.4 ± 0.5^{A}	25.3 ± 0.7^{D}
	Emodin	21.3 ± 0.5^{C}	15.2 ± 0.6^{E}	25.1 ± 0.4^{D}	24.5 ± 0.8^{C}	29.1 ± 0.7^{B}
	Physcion	20.2 ± 0.5^{D}	18.0 ± 0.5^{D}	26.6 ± 0.4^{C}	21.1 ± 0.7^{D}	27.2 ± 0.5^{C}
MIC (μg/mL)	Crude extract of *P. cuspidatum*	312.5	156.3	312.5	>2500	>2500
	Piceid	625	625	312.5	625	312.5
	Resveratrol	312.5	625	312.5	312.5	312.5
	Emodin	625	625	312.5	312.5	625
	Physcion	625	312.5	625	312.5	625
MBC (μg/mL)	Crude extract of *P. cuspidatum*	625	312.5	1250	>2500	>2500
	Piceid	>2500	625	625	1250	312.5
	Resveratrol	2500	625	625	625	625
	Emodin	>2500	1250	1250	625	1250
	Physcion	2500	625	1250	625	1250

[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 *P. cuspidatum* and 50 mg/mL for piceid, resveratrol, emodin, and physcion.

[b] DIZ value = mean ± SD. Means with the same letter were not significant different ($p < 0.05$).

difference (LSD) calculated using the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

3. Results

3.1. Antibacterial activity (DIZ), MIC and MBC of crude extract from P. cuspidatum roots

Antibacterial properties (DIZ, MIC and MBC) of crude extract from *P. cuspidatum* roots 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. Of the three gram-positive bacteria, *S. aureus* was the most sensitive (DIZ 20.2 mm) to the crude extract, followed by *L. monocytogenes* (19.2 mm), with *B. cereus* being the most resistant (16.4 mm). Of the two gram-negative bacteria, *S. anatum* was more sensitive (DIZ 12.8 mm) than *E. coli* (6.4 mm) to the crude extract. Three gram-positive bacteria (*B. cereus*, *L. monocytogenes*, and *S. aureus*) were obviously inhibited at the lower concentration of the crude extract than two gram-negative bacteria (*S. anatum* and *E. coli*). Therefore, generally, the gram-positive bacteria were more sensitive than the gram-negative ones to the crude extract.

In addition, the MIC and MBC values presented in Table 1 indicated that the lower concentration of the crude extract could fully inhibit the growth or almost kill three gram-positive bacteria (MIC = 156.3–312.5 μg/mL; MBC = 312.5–1250 μg/mL). However, both the MIC and MBC values of the crude extract against two gram-negative bacteria were more than 2500 μg/mL.

3.2. Identification of major bioactive components in P. cuspidatum roots

LC–ESI-MS analysis results of the crude extract of *P. cuspidatum* roots are shown in Table 2 and Fig. 1. According to the UV–vis spectra, chromatographic profiles (retention time, R_t) and MS data and by comparison with authentic standards and literature data (Vastano et al., 2000; Xiao, Xuan, Xu, Bai, & Zhong, 2002; Ye, Han, Chen, Zheng, & Guo, 2007; Zhao, Liu, & Zhou, 2005), peaks 3 and 5–7 were identified as stilbenes, i.e., reseveratrol-4′-*O*-glucose (resveratroloside), reseveratrol-3-*O*-glucoside (piceid), reseveratrol-galloyl-glucoside, and reseveratrol, respectively. Peaks 8–10, 12 and 13 were identified as hydroxyanthraquinones, i.e., emodin-8-*O*-glucoside, emodin-1-*O*-glucoside, emodin-8-*O*-(6′-*O*-malonyl)-glucoside, emodin, and physcion, respectively. Peaks 1, 2 and 4 were identified as proanthocyanidins/catechins, i.e., procyanidin dimer gallates and catechin gallate. Peak 11 was an unknown compound.

Quantitative analysis (percentage of total peak area) (Table 2) showed that stilbenes (mainly piceid and resveratrol) and hydroxyanthraquinones (mainly emodin, emodin-1-*O*-glucoside, and physcion) were major components in the crude extract of *P. cuspidatum* roots, totally accounting for 46.62% and 43.90% of the total peak area, respectively. Fig. 1 indicates that piceid (5) and emodin (12) are two dominant peaks, accounting for 29.60% and 24.85%, respectively. Furthermore, the crude extract of *P. cuspidatum* roots also contained low levels of proanthocyanidins/catechins, i.e., procyanidin dimer gallate and its isomer (3.24%) and catechin gallate (1.75%).

3.3. Antibacterial activity (DIZ), MIC and MBC of major bioactive compounds in P. cuspidatum roots

The antibacterial properties of the crude extract of *P. cuspidatum* roots would be expected to be due to the major chemical components existing in the crude extract. The above results indicated that *P. cuspidatum* roots not only possessed high levels of piceid (5) and emodin (12) but also contained low concentrations of resveratroloside (3), resveratrol (7), emodin-1-*O*-glucoside (9), and physcion (13). Authentic standards of piceid, emdoin, resveratrol, and physcion were commercially available and so were used for assays of their antibacterial properties. The results clearly indicated that most of them exhibited good antibacterial properties against the tested five bacteria and

Table 2
LC–MS analysis of major compounds in crude extract of *P. cuspidatum* roots

Peak no.	Tentative names of compounds	R_t (min)	λ_{max} (nm)	Mass of observed ions (*m*/*z*)			Peak area (%)
				$[M-H]^-$	$[M+H]^+$	$[M+Na]^+$	
1	Procyanidin dimer gallate (isomer)	20.73	276	729	730	753	1.58
2	Procyanidin dimer gallate	21.74	276	729	730	753	1.66
3	Resveratrol-4′-*O*-glucoside (resveratroloside)	24.75	303	389	391	413	7.04
4	Catechin gallate	25.79	277	441	443	465	1.75
5	Resveratrol-3-*O*-glucoside (piceid)	27.83	306	389	391	413	29.60
6	Resveratrol-galloyl-glucoside	33.93	294	541	543	565	3.56
7	Resveratrol	38.77	305	227	229	–	6.42
8	Emodin-8-*O*-glucoside	52.36	282, 420	431	432	455	0.97
9	Emodin-1-*O*-glucoside	67.23	272, 426	431	432	455	11.92
10	Emodin-8-*O*-(6′-*O*-malonyl)-glucoside	75.77	280, 424	517	519	541	3.27
11	Unknown	81.23	266, 341	245	247	265	1.63
12	Emodin	86.88	287, 439	269	271	–	24.85
13	Physcion	91.26	285, 435	283	285	–	2.89

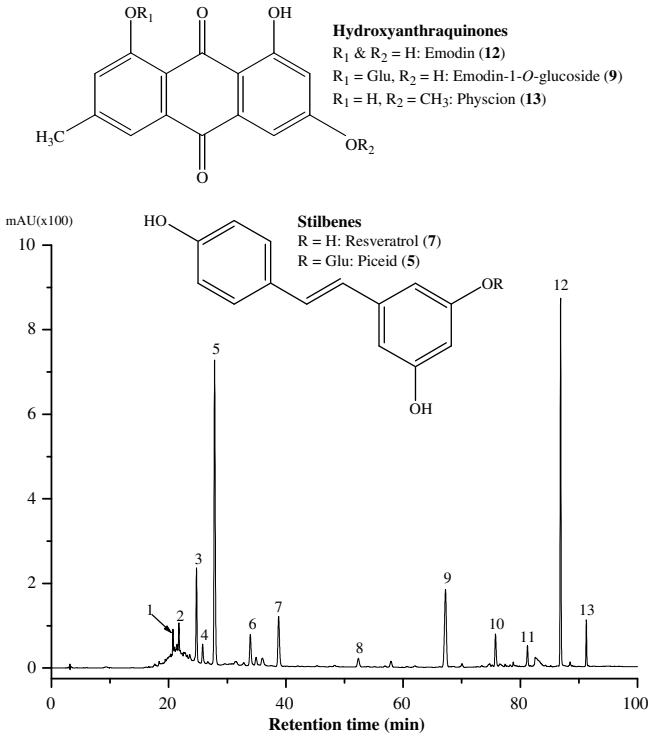

Fig. 1. LC chromatogram (280 nm) of crude extract from *P. cuspidatum* roots and chemical structures of major phenolic compounds identified in the tested sample. For all peak assignments, see Table 2. Major peaks were identified by LC–ESI-MS: 1, 2, and 4, proanthocyanidins and catechins; 3, 5–7, stilbenes; 8–10, 12, and 13, hydroxyanthraquinones.

possessed a wide spectrum of inhibitory effects (the DIZ values: 15.2–48.3 mm; MIC: 312.5–625 μg/mL; MBC: 312.5– $\geqslant$ 2500 μg/mL) (Table 1). The results also suggested that lower concentrations of the tested stilbenes (piceid and resveratrol) and hydroxyanthraquinones (emodin and physcion) could inhibit or even kill most of the tested five foodborne bacteria. They were important bioactive components contributing to the antibacterial properties of *P. cuspidatum* roots.

3.4. Scanning electron microscope observation

All five tested bacteria were treated with the crude extract of *P. cuspidatum* roots and its major bioactive components (piceid and emodin), and then were observed by SEM to investigate any physical changes in the appearance of the cells. The SEM images of three treated species, *B. cereus*, *S. aureus*, and *S. anatum* (Fig. 2), illustrates the destructive effect of the samples on the tested bacteria. The crude extract and bioactive component samples could cause physical damage (considerable morphological alteration and damage) to all the treated bacteria. In contrast to the control, many bacterial cells were damaged by the crude extract, piceid and emodin. Non-treated bacterial cells (control) remained intact and showed a smooth surface (Fig. 2, a4, b4, and c4). For the treated bacterial cells, some cells presented damage as pores or deformities in the cell membranes (Fig. 2, a1–a3, b1–b3, and c1–c3), and some cell structures appeared to be empty and the remains were flaccid (Fig. 2, a2, b1–b3, and c2).

4. Discussion

The crude extract of *P. cuspidatum* roots possessed much stronger antibacterial activity against five foodborne bacteria than many other spices and herbs tested in our recent study (Shan et al., 2007a). Song et al. (2006) reported the inhibitory effects (MIC and MBC) of *P. cuspidatum* roots against 20 bacterial strains from mouth and teeth. In contrast to the results reported by Song et al. (2006), this study showed that the crude extract of *P. cuspidatum* roots exhibited much stronger antibacterial activity (quite lower MIC and MBC values) against five foodborne bacteria, although different bacterial strains were tested in our and their studies. The present study also showed that the gram-positive bacteria were more sensitive than the gram-negative ones to the crude extract of *P. cuspidatum* roots. To some extent, this was consistent with previous study (Kim et al., 2005) about antibacterial activity of volatile oils from *P. cuspidatum* leaves against similar foodborne bacteria (both gram-positive and gram-negative ones). However, major antibacterial components of *P. cuspidatum* roots were completely different from those of *P. cuspidatum* leaves. The former possessed high levels of stilbenes and hydroxyanthraquinones, while the latter only contained bioactive volatile oils.

Many different phenolic compounds had been isolated and identified from the roots of *P. cuspidatum*, such as stilbenes and hydroxyanthraquinones, flavonoids (catechins and its derivatives), lignans, and phenolic compounds (gallic acid) (Chu, Sun, & Liu, 2005; Vastano et al., 2000; Xiao et al., 2002; Zhao et al., 2005). In the present study and chromatographic conditions, based on the UV–vis spectra, R_t values, MS data and by comparison with authentic standards and literature data (Vastano et al., 2000; Xiao et al., 2002; Ye et al., 2007; Zhao et al., 2005), 12 major peaks were tentatively identified as 5 hydroxyanthraquinones, 4 stilbenes, 2 proanthocyanidins, and 1 catechin gallate, and just one major peak (peak 11) could not be identified (Table 2). Among them, procyanidin dimer gallates, catechin gallate, reseveratrol-galloyl-glucoside, and emodin-8-*O*-(6′-*O*-malonyl)-glucoside were firstly detected in the roots of *P. cuspidatum*. Nobody reported the presence of these phenolic compounds in the roots of *P. cuspidatum* before. Also, gallic acid (a minor peak, $R_t = \sim 9.3$ min) was isolated and identified in this study. However, some phenolic compounds reported previously (Chu, Peng, & Ye, 2004), such as some hydroxyanthraquinones (rhein and chrysophanol) and lignans (lignan sulfates), were not detected in the crude extract of *P. cuspidatum* roots in this study. This was likely due to the differences in the methods and instruments for isolation and identification of the phenolic compounds between our and previous studies. In this study, the crude extract of

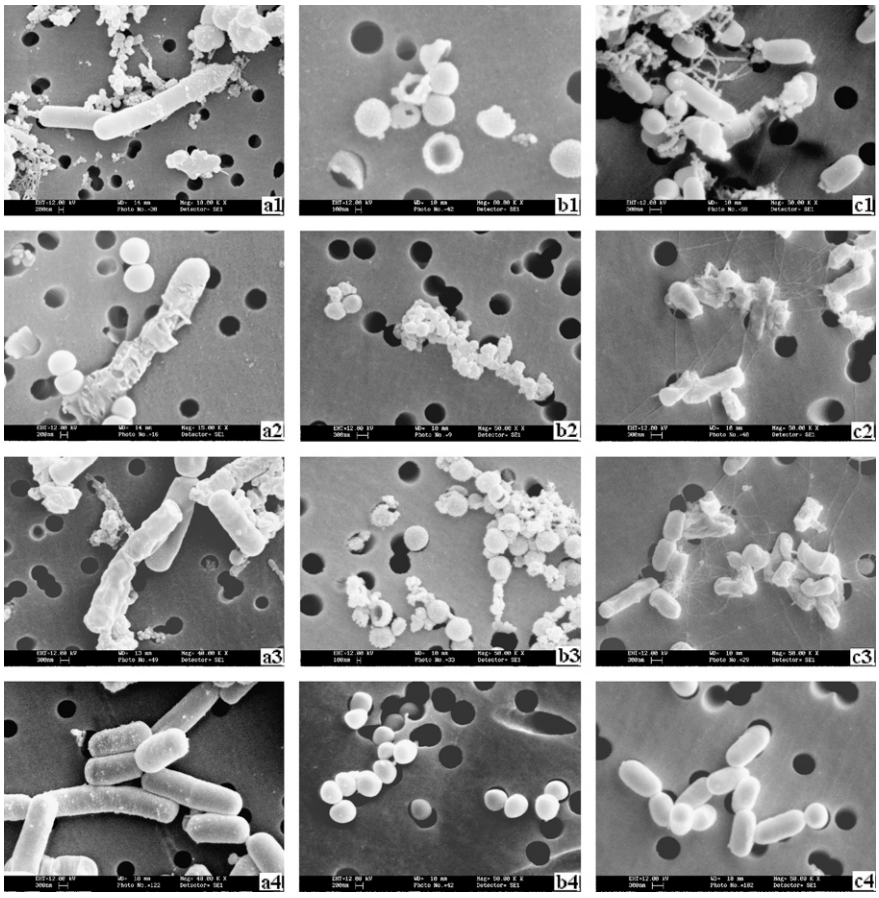

Fig. 2. Scanning electron microscope observations of three selected pathogenic bacteria (a, *B. cereus*; b, *S. aureus*; c, *S. anatum*) treated with the samples (1, the crude extract of *P. cuspidatum* roots; 2, piceid; 3, emodin) and untreated bacterial cells (4, control).

P. cuspidatum roots was not semi-prepared and not purified. It was directly used for simultaneous determination of the phenolic compounds by LC–ESI-MS.

Antibacterial properties of the main components of *P. cuspidatum* roots have been little reported. In the present study, the antibacterial activity (DIZ), MIC and MBC values of major phenolic compounds (mainly stilbenes and hydroxyanthraquinones, e.g., piceid and emodin) from *P. cuspidatum* roots were assayed to evaluate their individual antibacterial roles (Table 1). It was found that most of them exhibited good antibacterial properties. From Table 2 and Fig. 1, all the identified stilbenes and hydroxyanthraquinones accounted for 90.52% of total peak area in the crude extract of *P. cuspidatum* roots. This indicated that stilbenes and hydroxyanthraquinones (piceid, resveratrol, emodin, and physcion) were major antibacterial components in the crude extract of *P. cuspidatum* roots. The presence of these phenolic compounds was directly related to the antibacterial properties of the tested crude extract. In addition, our recent study showed that the proanthocyanidins from cinnamon stick also had good antibacterial activity (Shan, Cai, Brooks, & Corke, 2007b). However, only 3.24% of proanthocyanidins were detected in the crude extract of *P. cuspidatum* roots, and so contributed less to its antibacterial properties.

The SEM observations proved that the crude extract of *P. cuspidatum* roots and its major components (piceid and emodin) could cause considerable morphological alteration and damage of the treated bacteria so as to exert their bacteriostatic or bactericidal effect. Several possible mechanisms of action were proposed. The active components in the crude extract of *P. cuspidatum* roots might bind to the cell surface, and then penetrate to the target sites, which would sensitize the phospholipid bilayer of the cytoplasmic membrane and inhibit membrane-bound enzymes. After combining to the target sites, the active components might inhibit proton motive force, the respiratory chain and electron transfer, substrate oxidation, and/or energy transport processes in the bacteria. As a result, such inhibitions could lead to the uncoupling of oxidative phosphorylation, restraining of active transport, loss of pool metabolites, and disruption of synthesis of DNA, RNA, protein, lipid, and polysaccharides (Denyer, 1990; Kim et al., 2004; Nychas, 1995).

The damage to the bacterial cell wall and cytoplasmic membrane might indicate loss in structural integrity and in the membrane's ability to act as a permeability barrier (de Billerbeck, Roques, Bessiere, Fonvieille, & Dargent, 2001). The distortion of the cell physical structure would cause the expansion and destabilisation of the membrane

and increase membrane fluidity, which in turn would increase passive permeability and leak various vital intracellular constituents including ions, ATP, nucleic acids, and amino acids (Cox et al., 1998; Helander et al., 1998; Ultee, Bennik, & Moezelaar, 2002). Cell death might be the result of the extensive loss of cell contents, the exit of critical molecules and ions or the initiation of autolytic processes (Denyer, 1990).

In addition, there may be relationships between the antibacterial activity and the chemical structures of the major phenolic compounds in the tested extract. The chemical structure of the major phenolic components (hydroxyanthraquinones and stilbenes) is shown in Fig. 1. They all have aromatic nucleus containing polar functional groups (e.g., hydroxyl groups). The presence and position of the hydroxyl (–OH) groups in phenolic compounds might influence their antimicrobial effectiveness. Certain hydroxyl groups in the phenolic compounds might bind to the active site of enzymes, form hydrogen bonds with enzymes and alter their metabolism, and also the lipid solubility and the degree of steric hindrance of the phenolic compounds might determine their antimicrobial activity (Beuchat & Golden, 1989; Ceylan & Fung, 2004). Phenolic compounds might be involved in other modes of action for their antimicrobial activity. These compounds might interact with the cell membrane and attack the cytoplasmic membrane, thereby destroying its permeability and releasing intracellular constituents, and also causing membrane dysfunction in respect of electron transport, nutrient uptake, nucleic acid synthesis, and ATPase activity (Denyer & Hugo, 1991; Rico-Munoz, Bargiota, & Davidson, 1987).

In summary, this study showed that the crude extract from roots of *P. cuspidatum* and its major components possessed significant *in vitro* antibacterial properties against five common foodborne bacteria. Major phenolic compounds in the crude extract of *P. cuspidatum* roots were identified as five hydroxyanthraquinones, four stilbenes, two proanthocyanidins, and one catechin derivative by LC–ESI-MS. The antibacterial properties of *P. cuspidatum* roots were mostly from contribution of both the stilbenes (mainly piceid) and hydroxyanthraquinones (mainly emodin) in the crude extract. The roots of *P. cuspidatum* and its major components may have potential as natural antibacterial agents for food preservation and medicinal use. It should be further researched for use in the food sector to improve food safety by the control or elimination of foodborne pathogenic bacteria.

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