



The antibiotic activity and mechanisms of sugarcane (*Saccharum officinarum* L.) bagasse extract against food-borne pathogens



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ABSTRACT

Sugarcane bagasse contains natural compositions that can significantly inhibit food-borne pathogens growth. In the present study, the phenolic content in sugarcane bagasse was detected as higher than 4 mg/g dry bagasse, with 470 mg quercetin/g polyphenol. The sugarcane bagasse extract showed bacteriostatic activity against the growth of *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium*. Additionally, the sugarcane bagasse extract can increase the electric conductivity of bacterial cell suspensions causing cellular leaking of electrolytes. Results of sodium dodecyl sulfate polyacrylamide gel electrophoresis suggested the antibacterial mechanism was probably due to the damaged cellular proteins by sugarcane bagasse extract. The results of scanning electron microscopy and transmission electron microscopy showed that the sugarcane bagasse extract might change cell morphology and internal structure.

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1. Introduction

Chemical synthetic preservatives have always been used as antimicrobials to inhibit the growth of food-borne pathogens, which may generate chemical harm to human beings. Recent studies reported natural phytochemicals, such as phenolics, showed significant antibacterial, antiviral, and antiseptic activities (Romani, Vignolini, Isolani, Ieri, & Heimler, 2006).

Bacterial tolerance against polyphenols depends on the bacterial strain, phenolic structures, and content of phenolic compounds (Almajano, Carbó, Jiménez, & Gordon, 2008; Campos, Couto, & Hogg, 2003). Phenolic compounds including phenols, phenolic acids, coumarins, flavonoids, stilbenes, hydrolyzable and

condensed tannins, lignans, and lignins are rich in common dietaries like fruits, vegetables, nuts, seeds, flowers, barks, etc (Afaq & Katiyar, 2011). Epidemiological studies suggest that phenolic compounds in food may reduce the risk of some forms of cancer, cardiovascular disease, strokes, and chronic obstructive pulmonary disease (Rupasinghe, Wang, Huber, & Pitts, 2008).

Sugarcane (*Saccharum officinarum* L.) contains a high content of phenolic compounds (Zhao, Zhu, Yu, Han, & Song, 2009). Previous studies have evaluated the antioxidant activity (Duarte-Almeida, Salatino, Genovese, & Lajolo, 2011), anti-proliferative (Duarte-Almeida, Negri, Salatino, de Carvalho, & Lajolo, 2007), anti-mutation (Wang, Duh, Wu, & Huang, 2011), and DNA-damage-protecting (Abbas, Sabir, Ahmad, Boligon, & Athayde, 2014) activities of sugarcane, which showed positive results.

Interest in recovering bioactive substances from residual plant sources has significantly increased recently. Sugarcane bagasse is one of the most abundant by-products of agroindustry, with a

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production of more than 540 million tons per year. However, few literatures on the antimicrobial properties of sugarcane bagasse extract on pathogenic intestinal bacteria are reported.

The first objective of the present study is detecting the main phenolic acid compounds in sugarcane bagasse extract. The second objective is estimating the antimicrobial activities against food-borne pathogens of the sugarcane bagasse extract. And the third objective is studying the bacteriostatic mechanism.

2. Materials and methods

2.1. Plant material

Sugarcane bagasse was obtained from a sugar mill in HenFu Group (Zhanjiang, Guangdong, China). The sugarcane bagasse was dried, milled, filtered through a 0.5 mm sieve. The powder with particle size less than 0.5 mm was kept drily at -20°C .

2.2. Preparation of sugarcane bagasse extract

Briefly, 50 g dried sugarcane bagasse was extracted in 100 ml 70%v/v ethanol at 4°C , and stirred for 30 min. The extraction process was repeated 3 times. After centrifuged at 1000g for 10 min, the supernatant was combined and dried under vacuum at 45°C . The dried solid matter was weighed, re-dissolved with 10 ml 70% methanol, and stored at -40°C . Before use, the methanol was evaporated under a stream of nitrogen, and the extract was re-dissolved in an aqueous solvent.

2.3. Determination of polyphenols

Total polyphenol content of the sugarcane bagasse crude extract was determined as recently reported (Wolfe et al., 2008). Briefly, volumes of 0.5 ml of deionized water and 0.125 ml of the dilutions of extracts were added to a test tube. Folin–Ciocalteu reagent (0.125 ml) was added to the solution and allowed to react for 6 min. Then, 1.25 ml of 7% sodium carbonate solution was aliquoted into the test tubes, and the mixture was diluted to 3 ml with deionized water. The color was developed for 90 min, and the absorbance at 760 nm was measured. The results were expressed as gallic acid equivalents per gram of dried solid matter of sugarcane bagasse extract.

2.4. Determination of flavonoids

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Jia, Tang, & Wu, 1999). The appropriate dilutions of extracts with a concentration of 3 mg/ml were reacted with sodium nitrite, followed by reaction with aluminum chloride to form a flavonoid-aluminum complex. Absorbance at 510 nm was measured against a prepared reagent blank.

2.5. Quantification of phenolic acid compounds

Phenolic acid compounds were quantified by reversed-phase high-performance liquid chromatography (RP-HPLC) (Kim, Ro, Kim, Kim, & Chung, 2012). RP-HPLC analyses were performed in a Waters 600E HPLC system (Waters Technologies, Milford, MA, USA) equipped with a quaternary pump, an on-line degasser, a column temperature controller, a photodiode array detector (Model 2998), and Waters Empower 2 software.

Chromatographic separations were performed on a Waters Xbridge™ C18 (250 mm \times 6 mm ID, 5.0 μm). Injection volume was 10 μl , the detection wavelengths were set at 280 and 320 nm, with a flow rate of 0.7 ml/min. The column temperature

was maintained at 28°C . The mobile phase was a mixture of methanol-acetic acid-water (10:2:88, v/v, solvent A) and acetic acid-methanol-water (2:90:8, v/v, solvent B). The following linear gradient was used: 0–45 min, 0–50% A eluent; 45–70 min, 50–100% A eluent. All samples were filtered through a 0.45 μm syringe filter before HPLC analyses.

Identification and quantitative analyses were carried out by comparison with the retention time and HPLC peak area of standards of gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid, syringic acid, coumaric acid, ferulic acid, and sinapic acid. The amount of each phenolic compound was expressed as mg per gram of extract.

2.6. Microbial strains and cultures

Staphylococcus aureus ATCC 6538, *Listeria monocytogenes* ATCC 19115, *Escherichia coli* ATCC 8089, and *Salmonella typhimurium* ATCC 14028 (obtained from the China Center of Industrial Culture Collection, Beijing, China) were used for evaluating the antimicrobial activity of the sugarcane bagasse extract. All strains were inoculated on nutrient agar (NA) or nutrient broth (NB) media and incubated at 37°C for 16–18 h. Subsequently, the cultures were diluted in sterile 0.8% saline solution to 10^6 – 10^7 CFU/ml (colony forming units) for further study.

2.7. Determination of antibacterial effect with the Oxford cup method

Antibacterial inhibitory were measured using the Oxford cup method (Wang, Lu, Wu, & Lv, 2009) with some modifications. Diluted inoculum (0.4 ml, 10^5 CFU/ml) was uniformly spread onto individual NA plates using sterile cotton swabs. Sterile oxford cups (6 mm in diameter) were then placed on the agar medium and filled with 200 μl of sugarcane bagasse extract (20 mg/ml). Sterile water was used as negative control. The plates were kept in the refrigerator for 6 h at 4°C and then incubated at 37°C for 20 h. The antibacterial effect of the extract was evaluated by measuring and comparing the diameters of the transparent inhibition zones. All tests were repeated in triplicate.

2.8. Minimum inhibitory concentrations (MICs)

MICs were determined using standard inoculums of 1×10^5 CFU/ml with the two fold serial dilution method of Nedorostova, Kloucek, Kokoska, Stolcova, and Pulkrabek (2009) and Andrews (2001) with some modifications. Sugarcane bagasse extract was serially diluted in sterile water to final concentrations of 0, 0.1562, 0.315, 0.625, 1.25, 2.5, 5, and 10 mg/ml. After incubation for 18–24 h at 37°C with shaking at 150 rpm, the lowest concentration of the bagasse extract required to visibly inhibit growth was taken as the MIC. Sterilized distilled water was used as negative control.

2.9. Cell membrane permeability

The permeability of bacterial membranes, expressed as the electrical conductivity, was determined according to Lee, Choi, and Cho (1998) with some modifications. After incubation at 37°C overnight, bacteria were harvested by centrifugation at 8000g for 10 min, washed three times with 10 mM with phosphate buffered saline (PBS), pH 7.0, and diluted with PBS to about 10^5 CFU/ml. Sugarcane bagasse extract was then added to bacterial cell suspensions to a final concentration of 25 mg/ml. The samples were mixed and incubated at 37°C in a well shaken (150 rpm), and the conductivity was measured at 0, 1, 2, 3, 4, 5, 6, and 7 h.

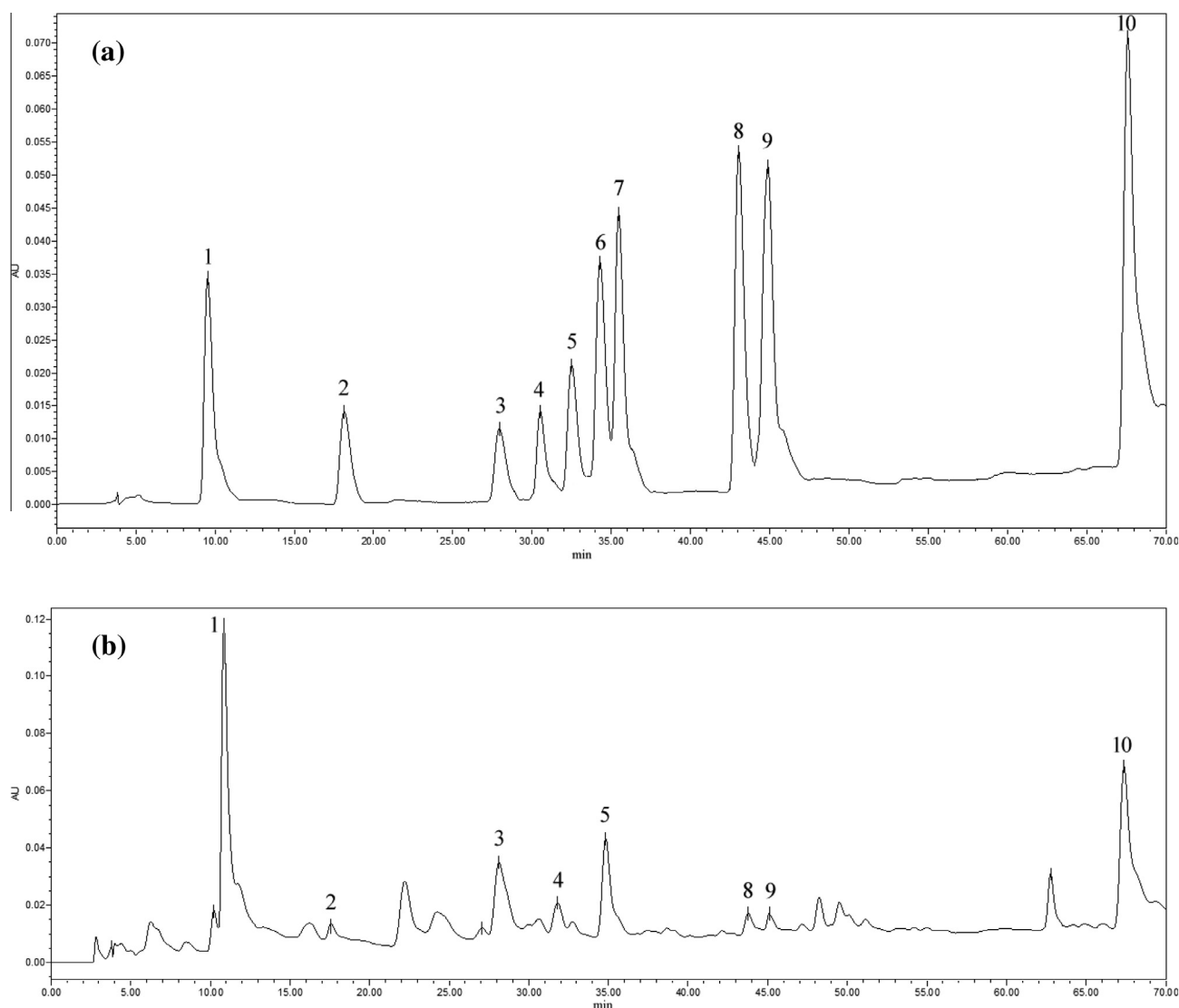


Fig. 1. RP-HPLC chromatograms of ten phenolic acids at 280 nm. (a) Phenolic acids standards. (b) Phenolic acids in the extract. 1. Gallic acid, 2. protocatechuic acid, 3. *p*-hydroxybenzoic acid, 4. vanillic acid, 5. chlorogenic acid, 6. caffeic acid, 7. syringic acid, 8. coumaric acid, 9. ferulic acid and 10. sinapic acid.

Table 1
Content of phenolic acid compounds on dry-weight basis in sugarcane bagasse extract.

Phenolic acid	(mg/g)
Gallic acid	4.36 ± 0.12
Protocatechuic acid	0.23 ± 0.15
<i>p</i> -Hydroxybenzoic acid	1.42 ± 0.16
Vanillic acid	0.62 ± 0.09
Chlorogenic acid	1.63 ± 0.12
Caffeic acid	–
Syringic acid	–
Coumaric acid	1.66 ± 0.14
Ferulic acid	1.87 ± 0.13
Sinapic acid	1.12 ± 0.09

ND, not detected.

Table 2
Diameters of inhibition zones and minimum inhibitory concentrations of sugarcane bagasse extract against food-borne pathogens.

Strain	Diameter of inhibition zone (mm)	MIC (mg/ml)
<i>E. coli</i> (G–)	15.6 ± 0.21	2.50
<i>S. aureus</i> (G+)	19.4 ± 0.15	0.625
<i>L. monocytogenes</i> (G+)	16.7 ± 0.15	1.25
<i>S. typhimurium</i> (G–)	14.0 ± 0.18	2.50

at 8000g for 10 min at 4 °C, and re-suspended in 0.1 M PBS (10 ml, pH 7.0). Proteins were separated by SDS–PAGE (Laemmli, 1970) using a 5% stacking gel and a 15% separating gel. Gels were dyed with 0.1% Coomassie Brilliant Blue R250 and destained with glacial acetic acid and methanol in distilled water.

2.10. SDS–PAGE of bacterial proteins

The *S. aureus* was incubated in NB overnight at 37 °C to approximately 10⁷ CFU/ml. Sugarcane bagasse extract was added at 1 × MIC and 3 × MIC concentrations, and incubated at 37 °C. Samples of 5 ml were withdrawn every 3 h up to 12 h, centrifuged

2.11. Scanning electron microscopy (SEM)

The *E. coli* and *S. aureus* (approximately 10⁷ CFU/ml) were incubated with sugarcane bagasse extract at 1 × MIC for 6 h at 37 °C in the broth. Cells were collected by centrifugation at 8000g for 10 min at 4 °C, washed three times with 0.1 M PBS, pH 7.0, and

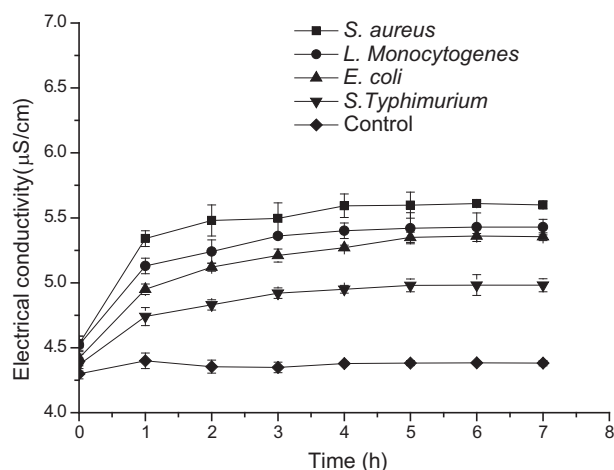


Fig. 2. Effects of sugarcane bagasse extract (25 mg/l) on cellular leakage of *S. aureus*, *L. monocytogenes*, *E. coli*, and *S. typhimurium*.

fixed with 2.5%v/v glutaraldehyde in 0.1 M PBS for 2 h. Subsequently, cells were dehydrated in a graded series of ethanol, ranging from 50–100%, followed by rinsing with tertiary butyl alcohol for 25 min. After centrifugation, cells were dried by CO₂ critical point drying (HCP-2, Hitachi, Tokyo, Japan), and observed by SEM (JSM-6360, JEOL, Japan).

2.12. Transmission electron microscopy (TEM)

Bacteria (approximately 10⁷ CFU/ml) were incubated in the broth with sugarcane bagasse extract at the MIC for 6 h at 37 °C. Cells were collected by centrifugation at 8000g for 10 min at 4 °C, the sediment was washed 3 times with 0.1 M PBS (pH 7.0), and fixed with 3%v/v glutaraldehyde at 4 °C for 4 h. The cells were washed in the same buffer and post-fixed for at 4 °C for 2 h with 1% osmic acid in 0.1 M PBS. Next, samples were embedded, solidified, sectioned, dyed, and observed by TEM (JEM-1230, JEOL, Japan). The untreated cell culture was used as a control.

2.13. Statistical analysis

All data were expressed as the mean ± standard deviation. Analysis of variance (ANOVA) was carried out to determine any significant differences ($p < 0.05$) among the applied treatments using the SPSS software package (SPSS 10.0 for Windows).

3. Results and discussion

3.1. Total phenolic content of sugarcane bagasse extract

The quantity of phenolic compounds is an important parameter to evaluate the biological potential of sugarcane bagasse extract. The antimicrobial activities of phenolic compounds are strongly dependent on the type of the concentration and polarity of the solvent, and the extraction process. Polyphenols in sugarcane tissues are often associated with proteins and/or polysaccharides, bound by hydrogen bonds and hydrophobic interactions. Therefore, a good solvent for phenolic compounds extraction is not only displaying high solvency, but also disrupting hydrogen bonds. Generally, 70%v/v ethanol in water is used in extracting phenolic compounds in natural materials with nontoxic, low-cost, etc.

The total polyphenol content of sugarcane bagasse extract is 4.3152 mg of GAE/g of dried sugarcane bagasse extract. And the total flavonoid content is 0.47 g of quercetin/g of polyphenol. The contents of gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid, syringic acid, coumaric acid, ferulic acid, and sinapic acid in sugarcane bagasse extract was detected by RP-HPLC (Fig. 1). Table 1 summarizes the detailed contents of phenolic acid compounds in dried sugarcane extract. The most abundant phenolic acids identified in the sugarcane bagasse extract were gallic acid (4.36 mg/g extract), ferulic acid (1.87 mg/g extract), coumaric acid (1.66 mg/g extract), and chlorogenic acid (1.63 mg/g extract), respectively. Generally, agro-industrial by-products are good sources of lignocellulosic materials, which are rich in phenolic compounds. For example, Brewer's spent grain is a by-product rich in ferulic and *p*-coumaric acids (Mussatto, Dragone, & Roberto, 2007). Onions and potato by-products can serve as natural sources of anti-inflammatory substances (Albishi, John, Al-Khalifa, & Shahidi, 2013). Lettuce and chicory by-products are also rich in antioxidant phenolic compounds that can be extracted to functionalize foodstuffs (Llorach, Tomás-Barberán, & Ferreres, 2004). This result showed that sugarcane bagasse is rich in phenolic compounds.

Although phenolic acids are main compositions resulting in color formation of juice during sugarcane processing (Bucheli & Robinson, 1994), it was found that phenolic acids showed higher antioxidant and antitumor capacities, with high antibacterial activity against *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* (Vaquero, Alberto, & de Nadra, 2007).

3.2. Inhibition zone diameters

The antibacterial effects of sugarcane bagasse extract (20 mg/ml) were studied by detecting the inhibition zone diameters of the Gram-positive and -negative food-borne pathogens and shown in Table 2. The sugarcane bagasse extract showed antibacterial activity on all of the tested food-borne pathogens. The maximum inhibition zone diameters for the Gram-positive *S. aureus* and *L. monocytogenes*, and the Gram-negative *E. coli* and *S. typhimurium* were 19.4 ± 0.15 mm, 16.7 ± 0.15 mm, 15.6 ± 0.21 mm, and 14.0 ± 0.18 mm, respectively. The average inhibition zone diameters for the Gram-positive bacteria were significantly larger than those of the Gram-negative bacteria ($p < 0.05$), demonstrated that *S. aureus* was the most significantly inhibited in this study.

3.3. MIC of food-borne pathogens

Table 2 showed the minimum concentrations of sugarcane bagasse extract inhibiting the growth of the four bacteria tested *in vitro* with MICs ranging from 0.1562 to 10 mg/ml. The lowest MIC for *S. aureus* was 0.625 mg/ml, while the highest MIC for both *E. coli* and *S. typhimurium* was 2.5 mg/ml, suggesting that the antibacterial activity of the sugarcane bagasse extract was higher for Gram-positive bacteria than for Gram-negative bacteria. This result is in agreement with the inhibition zones detected above.

Higher bacteriostatic activity to the Gram-positive bacteria may be due to the differences in the constituents and structures of the Gram-positive and -negative cell membranes, which is consistent with recent studies (Delgado Adamez, Gamero Samino, Valdés Sánchez, & González-Gómez, 2012; Kao et al., 2010; Si et al., 2006). In general, Gram-positive bacteria have a peptidoglycan layer in the cell wall that encases the cell membrane, which facilitates penetration of cell walls. The higher resistance of the Gram-negative bacteria by sugarcane bagasse extract may be due to their lipopolysaccharide outer membrane, which restricts diffusion of hydrophobic compounds.

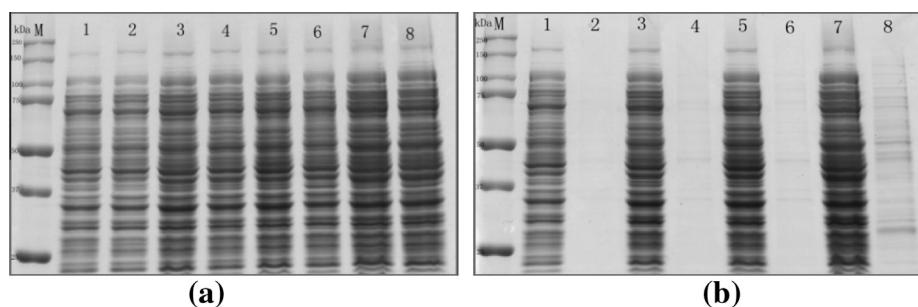


Fig. 3. SDS–PAGE analysis of *S. aureus* proteins treated with sugarcane bagasse extract at the concentration of $1 \times \text{MIC}$ (a) and $3 \times \text{MIC}$ (b). Lanes M: marker. Lane 1, 3, 5, 7: control for 3, 6, 9, 12 h respectively; Lane 2, 4, 6, 8: treated sample for 3, 6, 9, 12 h, respectively.

3.4. Cell membrane permeability

The conductivities of the *E. coli*, *S. aureus*, *L. monocytogenes* and *S. typhimurium* cell suspensions treated with 25 mg/l of sugarcane bagasse extract were detected to evaluate the relationship between antibacterial activity of sugarcane bagasse extract and membrane permeability of the tested bacteria. As shown in Fig. 2, samples from strains exposed to sugarcane bagasse extract had a higher conductivity than the control, and significantly increased in the first hour ($p < 0.05$).

The conductivities increased significantly with the duration of the treatment (1 vs. 4 h) from 5.34 $\mu\text{S}/\text{cm}$ to 5.59 $\mu\text{S}/\text{cm}$ for *S. aureus*, 5.13 $\mu\text{S}/\text{cm}$ to 5.40 $\mu\text{S}/\text{cm}$ for *L. monocytogenes*, 4.95 $\mu\text{S}/\text{cm}$ to 5.27 $\mu\text{S}/\text{cm}$ for *E. coli* and 4.74 $\mu\text{S}/\text{cm}$ to 4.95 $\mu\text{S}/\text{cm}$ for *S. typhimurium*. After 5 h treatment, the conductivities of all samples changed slightly ($p > 0.05$). Compared with the control, the conductivities of the *S. aureus*, *L. monocytogenes*, *E. coli*, and *S. typhimurium* suspensions increased 24.65%, 21.18%, 21.00%, and 13.97%, respectively, which suggested that the sugarcane bagasse extract affects bacterial membrane integrity, causing cellular electrolytes leaking.

The results are similar to those reported by Shen et al. (2015), who suggested that cinnamaldehyde damaged the cell membranes of the *E. coli* and *S. aureus*, and concluded that the degree of the damaged cell membrane was increased with the cinnamaldehyde concentration. Diao, Hu, Zhang, and Xu (2014) studied the effect of essential oil extracted from fennel seeds on membrane permeability of *Shigella dysenteriae* by measuring relative conductivity, and found that membrane permeability increased with treatment time and concentration of essential oil.

3.5. Protein patterns of bacteria treated with sugarcane bagasse extract

As shown in Fig. 3, the protein compositions changed after the addition of sugarcane bagasse extract. The duration of treatment and the increase of the sugarcane bagasse extract concentration significantly changed the protein profile. The bands of proteins from untreated *S. aureus* clearly appeared, and no significant shallow was shown after treating with $1 \times \text{MIC}$ of sugarcane bagasse extract for 3–12 h (Fig. 3(a)). However, with $3 \times \text{MIC}$ of sugarcane bagasse extract treatment, most of the bacterial protein bands disappeared (Fig. 3(b)). This result suggests that the sugarcane bagasse extract can kill bacteria by destructing its cellular proteins. Polyphenols play an important role in protein precipitation and enzyme inhibition (Fan, Chi, & Zhang, 2008). This interferes with energy metabolism by altering the structure of the cytoplasmic membrane due to interaction with membrane proteins, causing rapid loss of proteins (Ricke, 2003). Thus, the reason resulted in the disappearance of protein bands is probably caused from the

interfering with the protein synthesis and controlling the gene expression by the sugarcane bagasse extract.

This result was consistent with a previous report of Wang, Chang, Yang, and Cui (2015), who observed that bacterial protein bands of *Salmonella*, *E. coli*, and *Listeria* became slighter, and even disappeared after exposure to lactic acid, suggesting that lactic acid decreased the content of cellular soluble protein content by permeating and disrupting the cell membrane.

3.6. Effect of sugarcane bagasse extract on bacterial morphology

SEM and TEM were used to evaluate morphological properties of *E. coli* and *S. aureus* treated with sugarcane bagasse extract for 6 h at 37 °C (Fig. 4). The untreated *E. coli* and *S. aureus* cells displayed regular morphology, with a smooth and rounded surface (Fig. 4). However, after incubating with the sugarcane bagasse extract at the MIC for 6 h, cells were of irregular shape with a wrinkled surface, and cell debris and adhesion were visible. As shown in Fig. 4(b), partial cell wall and membrane of *E. coli* cells disappeared after treated by sugarcane bagasse extract. Fig. 4(d) shows that *S. aureus* cells treated with sugarcane bagasse extract showed an irregular wrinkle on their surface, with fragmentation, adhesion and aggregation of damaged cells or cellular debris. All the changes indicated that the MIC of sugarcane bagasse extract damaged the external structure of *E. coli* and *S. aureus*, causing leaking of cytoplasmic components such as proteins, nucleic acids, etc.

The ultra-structures of *E. coli* and *S. aureus* cells before and after treated with sugarcane bagasse extract was observed by the TEM. As shown in Fig. 4(e), the TEM of the untreated *E. coli* cells showed a similar morphology as SEM. After incubation with the sugarcane bagasse extract at the MIC, cells exhibited abnormal properties, which included separation of the cytoplasmic membrane from the cell wall, leaking the cytoplasmic content, and distorting the cells (Fig. 4(f)).

The TEM images also showed remarkable interior damage of *S. aureus* cells treated with sugarcane bagasse extract. Normal *S. aureus* cells exhibit a homogeneous microstructure (Fig. 4(g)). While, cells treated with the sugarcane bagasse extract showed a clear-cut edge, with dark mass aggregates appearing in the cell, forming a cavity, and plasmolysis (Fig. 4(h)). Both *E. coli* and *S. aureus* showed cell wall degradation, envelope disruption, and leaking of cytoplasmic substance. Apparently, the sugarcane bagasse extract affects growth and function of strains by inducing dysfunctions of the cell membrane. Similar TEM observations were reported in recent works on *E. coli* and *S. aureus* treated with MICs of cinnamaldehyde (Shen et al., 2015), on *Trichophyton rubrum* by treated with onion extracts (Ghahfarokhi, Goodarzi, Abyaneh, Al-Tiraihi, & Seyedipour, 2004), and on *E. coli* treated with tea tree oil (Gustafson et al., 1998). Additionally, *Cassia spectabilis* extract

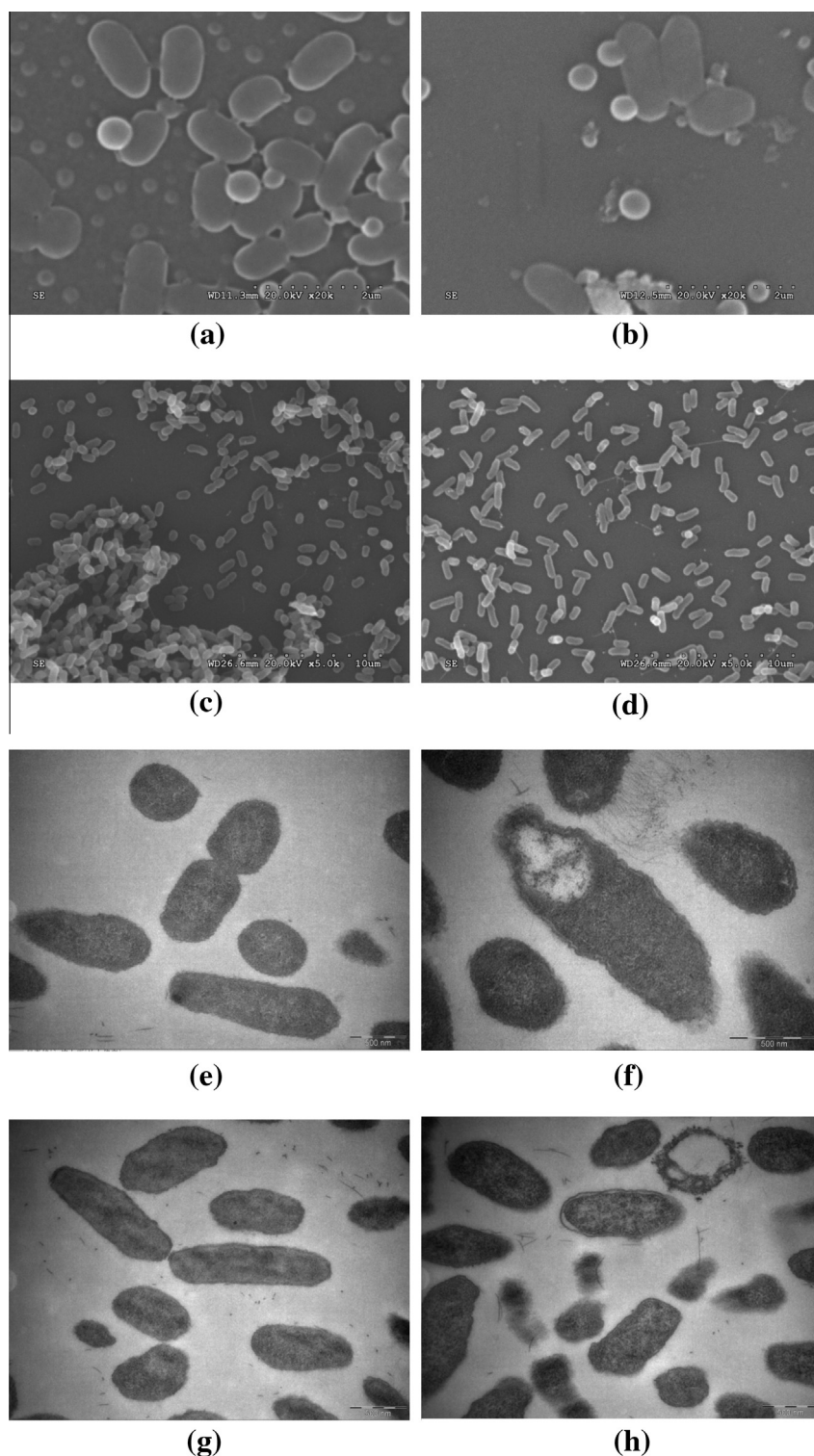


Fig. 4. SEM images of *E. coli* cells (20,000 \times) (a) control; (b) treated with MIC concentration and *S. aureus* cells (5000 \times) (c) control; and (d) treated with MIC concentration. TEM images of *E. coli* cells (80,000 \times): (e) control; (f) treated with MIC concentration and *S. aureus* cells (50,000 \times) (g) control; and (h) treated with MIC concentration.

can prevent *Candida albicans* biofilm formation (Sangeetha, Zuraini, Suryani, & Sasidharan, 2009).

Generally, the position of hydroxyl groups in the phenolic structure can significantly influence the antimicrobial activities (Taguri, Tanaka, & Kouno, 2004). Phenolic acids such as gallic acid and cinnamic acid can cause irreversible changes of membrane through altering hydrophobicity and local rupture or pore formation in

the cell membranes, resulting in leaking intracellular constituents (Borges, Ferreira, Saavedra, & Simoes, 2013). The bacteriostatic mechanism of flavonoids may be explained by their ability to form complexes with the bacterial cell wall (Cowan, 1999) to inhibit the growth of the bacteria. The bacteriostatic mechanism of quercetin is partly due to the inhibition of DNA gyrase (Brvar, Perdih, Oblak, Masic, & Solmajer, 2010). Therefore, the bacteriostatic

milt-mechanism is probably attributed to different compounds in the sugarcane bagasse extract. Among them, the primary mechanism is the penetrating the cell membrane and destroying membrane integrity. The second antimicrobial mechanisms might be caused by complexes formation between metal ions and essential substrates for bacteria growth, changing the metabolism of bacteria (Serrano, Puupponen-Pimia, Dauer, Aura, & Saura-Calixto, 2009), and inactivating the protein (Mason & Bruce, 1987).

4. Conclusion

The results of the present study indicated that sugarcane bagasse was rich in phenolic compounds, flavonoids, and phenolic acid compounds, which showed significant antibacterial activities against the foodborne pathogens *S. aureus*, *L. monocytogenes*, *E. coli*, and *S. typhimurium*. The bacteriostatic mechanism of the sugarcane bagasse extract was probably due to the toxicity of polyphenolic compounds to microorganisms. The results of the present study indicated that sugarcane bagasse extract may be used as a high added-value natural bioactive material for antimicrobial applications in the food industry.

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