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EXPERIMENTAL ARTICLES

Chemical Constituents, Antibacterial Activity and Mechanism of Action of the Essential Oil from *Cinnamomum cassia* Bark against Four Food-Related Bacteria[1]

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Abstract—The majority of components of the essential oil from *Cinnamomum cassia* bark were identified by gas chromatography and mass spectrometry (GC-MS) in this study. The *trans*-cinnamaldehyde (68.52%) was found to be the major compound. The antibacterial activity of essential oil against four food-related bacteria was evaluated. The results showed it was stronger effect against *Staphylococcus aureus* with both the largest ZOI of 27.4 mm and the lowest minimum inhibitory concentration (MIC) of 2.5 mg/mL and minimum bactericidal concentration (MBC) of 5.0 mg/mL respectively. Postcontact effect (PCE) assay also confirmed the essential oil had a significant effect on the growth rate of surviving *S. aureus* and *Escherichia coli*. The mechanism against *S. aureus* and *E. coli* may be due to the increase in permeability of cell membranes, and the leakage of intracellular constituents based on cell permeability assay and electron microscopy observations.

Keywords: *Cinnamomum cassia* bark, antibacterial activity, essential oil, mechanism of action, food-related bacteria

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In recent years, food spoilage and food poisoning are amongst the most important issues facing the food industry [1, 2], and there has been a dramatic increase throughout the world in the number of reported cases of foodborne illness, even in developed countries [1, 3]. The essential oils of aromatic plants and their components have a wide range of applications in ethnomedicine, preservation, food flavoring and fragrances and in the perfume industries [4, 5]. In addition, it has been demonstrated that essential oils from various medicinal plants, spices and herbs possess high volatility, toxicity to stored-grain insect pests while have little harm to warm-blooded animals [6, 7]. There are a lot of reports about antimicrobial activity of spice extracts and its essential oils, and use of natural essential oils as antimicrobial agents in food systems may be considered as additional intrinsic determinant to increase the safety and shelf life of foods [8, 9].

Cinnamomum cassia Presl., also named Chinese cinnamon belonging to the family *Lauraceae*, is a type of deciduous evergreen tree that grows in Southeast Asia, and is one of the most important spices and medicinal materials in the world. *Cinnamomum cassia* bark or cortex is a natural spice and herb commonly used in traditional medicine to treat dyspepsia, gastritis, blood circulation disturbances, and inflammatory diseases [10–12]. Due to its industrial value, the constituents of the essential oil obtained from the bark and the young branches of this plant have been investigated widely for medicine, food and cosmetic additives [10, 11, 13]. It has been reported that the essential oil from *Cinnamomum cassia* bark showed various biological activities including antioxidant [14], antifungal [15–17] and antibacterial [18–20] activities. In addition, products containing *Cinnamomum cassia* oil may also be used as potential repellents and antitumor agents [21–24]. However, little work has been reported on the mechanism of action of essential oil from *Cinnamomum cassia* bark on the growth of microorganisms. Therefore, the aim of the present study was conducted to investigate antibacterial activity of essential oil on several food-related bacteria and to further evaluate the possible mechanism of action by the postcontact effect, cell membrane permeability assays and scanning electron microscopy as well as transmission electron microscopy observations.

MATERIALS AND METHODS

Plant materials and chemicals. *Cinnamomum cassia* trees grow in Deqing (Guangdong Province, China) and their dry bark were obtained as a commercial product from the Tongren-Tang Chinese pharmacy in

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Linfen, Shanxi on 2012. Dimethyl sulfoxide (DMSO) was purchased from Sigma (USA). Nutrient agar (NA), nutrient broth (NB) and tryptone soy agar were from Beijing Aoboxing Bio-tech Co. Ltd. (Beijing, China). Other chemicals used were all of analytical grade.

Microbial strains and culture. The antimicrobial activities of essential oil were tested against four different bacteria. Two strains of gram-positive bacteria were *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6051. Two strains of gram-negative were *Salmonella typhimurium* ATCC 19430 and *Escherichia coli* ATCC 25922. The strains were provided by the College of Life Science, Shanxi Normal University, and cultured at 37°C on NA or NB.

Extraction of essential oil. The dried *Cinnamomum cassia* bark was ground with a micro plant grinding machine (FZ102; Tianjin Taisite Instruments, Tianjin, China) to a powder and then hydrodistilled for 6 h using a Clevenger-type apparatus. The oil was separated from water and dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until use. The essential oil was obtained as a light yellow transparent liquid.

GC-FID analysis. The essential oil was analyzed using Hewlett-Packard 5890 II GC equipped with flame ionization detector (FID) and HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm). Injector temperature was set at 230°C. The oven temperature was programmed from 120°C for 5 min, raised to 150°C at a rate of 5°C/min, held isothermal for 6 min, and finally raised to 220°C at 5°C/min for 5 min. Helium was the carrier gas, at a flow rate of 1 mL/min. A sample of 0.2 μL of essential oil was injected manually, and the GC split ratio used was 1 : 50. The percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction.

GC-MS analysis. The analysis of the essential oil was performed using a Hewlett-Packard 5890 II GC, equipped with a HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm) and a HP 5972 mass selective detector for the separation. The mass selective detector was operated in electron-impact ionization (EI) mode with a mass scan range from m/z 10 to 450 at 70 eV. Helium was the carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 230 and 200°C, respectively. The oven temperature was programmed as in the GC-FID analysis. A sample of 0.2 μL of essential oil was injected manually using a 1 : 50 split ratio. The components were identified by comparing their GC retention indices, NIST mass spectral search program (version 2.0, National Institute of Standards and Technology), and mass spectra with published data.

Agar disc diffusion assay. The agar disc diffusion method described by Viuda-Martos et al. [25] with some modifications was used to determine the antibacterial capacity of the essentials oils. Briefly, a suspension (0.1 mL of 10^6 CFU/mL) of each microorganism was spread on the solid medium plates. Whatman no. 1 sterile filter paper discs (6 mm diameter) were impregnated with 25 mL of the oil dissolved in 5% dimethylsulphoxide (DMSO) and placed on the inoculated plates; these plates were incubated at 37°C for 24 h. Negative controls were prepared using DMSO. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition (ZOI) against the tested bacteria. Each assay was performed in triplicate.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay. MIC and MBC were determined according to the method described by Kubo, Fujita, Kubo, Nihei, and Ogura [26] with minor modifications. Briefly, 50% (v/v) essential oil in DMSO as stock solutions was prepared. Two fold serial dilutions of essential oil were prepared in sterile NB medium ranging from 0.625 to 20 mg/mL. To each tube 100 μL of the exponentially growing bacterial cells was added to give a cell concentration of approximately 1×10^6 CFU/mL. A control test was also performed containing inoculated broth supplemented with only DMSO. The tubes were incubated at 37°C for 24 h and then examined for evidence of the growth. The MIC was determined as the lowest concentration of the essential oil that demonstrated no visible growth. The MBC was determined as follows. After the determination of the MIC, 100-fold dilutions with fresh NB from each tube showing no turbidity were incubated at 37°C for 48 h. The MBC was the lowest concentration of the essential oil that showed no visible growth in the drug-free cultivation. All experiments were performed in triplicate.

Evaluation of postcontact effect (PCE). The PCE of the essential oil on tested strains was evaluated according to the method as described previously with some modifications [27]. The bacterial cultures of the exponential phase (approximately 10^6 CFU/mL) were exposed to 1× and 2× MIC of essential oils at 37°C for 1 h. Exposed and control cultures were washed twice with PBS by centrifugation at 4000 rpm for 20 min and then diluted in fresh broth before incubation at 37°C. The bacteria number was determined by dilution method of plate counting in tryptone soy agar and 1–24 h (a time per hour) after incubation. PCE was determined using the equation $PCE = t - c$, where t was the time needed for a log increase in counts in treated cultures and c the time needed for a log increase in counts in untreated cultures.

Cell membrane permeability. The permeability of bacteria membrane is expressed in the relative electric conductivity and determined according to the method described by Kong et al. [28]. After incubated at 37°C for 4 h, cells were separated by centrifugation at 5000 rpm for 10 min. Then the bacteria were washed with 5% of glucose until their electric conductivities were near to that of 5% glucose, and they were the case for isotonic bacterial suspension. The essential oils at

two different concentrations (MIC, and 2× MIC) were added to 5% glucose and the electric conductivities of the mixtures were marked as L_1. Then different concentrations of essential oils were added into the isotonic bacteria solution. After completely mixed, the samples were incubated at 37°C for 4 h, and then the conductivities were measured and marked as L_2. The conductivity of bacteria in 5% glucose treated in boiling water for 5 min was served as the control and marked as L_0. The permeability of bacteria membrane is calculated according to the formula, the relative electric conductivity (REC)% = $100 \times (L_2 - L_1)/L_0$.

Scanning electron microscope (SEM). To determine the efficacy of the essential oil and the morphological changes of the bacteria, SEM observation was performed on the tested bacteria according to the method as described previously [29]. After 10 h incubation of a cell suspension of *S. epidermidis* of approximately 2×10^6 cfu/mL at 37°C in NB, essential oil was added at the MIC. The control culture was left untreated. All suspensions were incubated at 37°C for 4 h, and then centrifuged. The cells were washed twice with 0.1 M PBS (pH 7.4) and fixed with 2.5% (v/v) glutaraldehyde in 0.1 M PBS overnight at 4°C. After this, the cells were successively dehydrated using 30, 50, 70, 90, and 100% ethanol, and then the ethanol was replaced by tertiary butyl alcohol. Then, cells were dried at "critical point" in liquid CO_2 under 95 bar pressure, and samples were gold-covered by cathodic spraying. Finally, morphology of the bacterial cell was observed with a scanning electronic microscope (SEM) (JSM-7500F, JEOL Ltd., Japan).

Transmission electron microscope (TEM). The bacteria cells were incubated for 10 h in NB at 37°C, before addition of essential oil to give a final concentration of the MIC. All treatments and controls were incubated at 37°C for 4 h respectively, and then centrifuged. The cells were washed twice with 0.1 M PBS (pH 7.4) and fixed with 2.5% (v/v) glutaraldehyde in 0.1 M PBS overnight at 4°C. Then the cells were post-fixed with 1% (w/v) OsO_4 in 0.1 M PBS for 2 h at room temperature and washed three times with the same buffer, then dehydrated by a graded series of ethanol solutions (30, 50, 70, 90, and 100%). Stained bacteria were viewed and photographed with a transmission electron microscope (H-600, HITACHIL Ltd., Japan) operated at 75 kV, and were analyzed with digital imaging software (MEGAVIEW G2).

Statistical analysis. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant differences ($p < 0.05$) between the means by Data Processing System (DPS, version 7.05) and EXCEL program.

RESULTS

Chemical composition of the essential oil. The essential oil was obtained by hydrodistillation of air-dried sample with a yield of 2.76% yield (v/w), which was supported the previous study that reported yields of 2.7–3.11% of essential oil for the branch barks [13]. The chemical composition of essential oil was analyzed by GC and GC-MS and the result was listed in Table 1. In total, 34 components of the essential oil from the dried bark of *Cinnamomum cassia*, representing 92.18% of the total amount, were identified in this study. Results showed that *trans*-cinnamaldehyde (68.52%) was found to be the major compounds, followed by copaene (4.66%), benzenepropanal (3.67%), γ-cadinene (3.41%), *cis*-cinnamaldehyde (2.15%), α-cadinol (1.85%), cinnamyl alcohol (1.24%). Besides, other components were also found and to have lower content (<1.00%) in the essential oil of *Cinnamomum cassia* bark in the present study (Table 1). The *trans*-cinnamaldehyde can be used as chemical markers of the oil.

ZOI, MIC and MBC of the essential Oil. The ZOI, MIC, and MBC values of the essential oil from *Cinnamomum cassia* bark against four bacteria are presented in Table 2. The results showed that the essential oil had certain antibacterial effects on all of the tested bacteria (Table 2). The ZOI, MIC and MBC values for tested strains were in the range of 14.5–27.4 mm, 2.5–10 mg/mL and 5–20 mg/mL, respectively. Of these bacteria, the essential oil had the greatest antibacterial effect against *S. aureus*, with both the largest ZOI of 27.4 mm and the lowest MIC of 2.5 mg/mL and MBC of 5.0 mg/mL. Although there are no differences in ZOI and MBC for *B. subtilis* and *E. coli*, the MIC of the essential oil for *B. subtilis* was lower than that for *E. coli*, which indicated the sensitivity of *B. subtilis* was stronger than that of *E. coli* to the essential oil, while the essential oil had the poor antibacterial effect against *S. typhimurium* compared to other tested strains. Therefore, the antibacterial properties and mechanism of action of essential oil from *Cinnamomum cassia* bark against *S. aureus* will be further investigated, and the strain of gram-negative *E. coli* is also used as a reference in this study.

Postcontact effect (PCE) of essential oil. The PCEs of the essential oil from *Cinnamomum cassia* bark on *S. aureus* and *E. coli* were evaluated at 1× MIC and 2× MIC concentrations respectively (Table 3). The PCE increased significantly with the increase of the concentration of essential oil ($p < 0.05$). At 1× MIC concentration, the PCEs were 5.2, 3.3 h, and increased to 7.6, 6.2 h at 2× MIC concentration ($p < 0.05$) for *S. aureus* and *E. coli*, respectively, which confirmed the antibacterial activity of essential oil from *Cinnamomum cassia* bark and showed a severe effect on recovery of surviving *S. aureus* and *E. coli* after treatment.

Cell membrane permeability. Further antibacterial mode of action of essential oil against the tested food-related bacteria were confirmed using the assay of cell membrane permeability determined by the measurement of the relative electric conductivity (REC) of the supernatant of tested strains. Table 3 showed the effect

Table 1. Chemical composition of essential oil from *Cinnamomum cassia* bark

RT[a]	Compounds	Peak area, %[b]	RT	Compounds	Peak area, %
2.94	Hexanal	0.15	6.08	*cis*-Cinnamaldehyde	2.15
3.02	Furfural	0.07	7.42	*trans*-Cinnamaldehyde	68.52
3.16	Styrene	0.14	9.13	Copaene	4.66
3.30	α-Pinene	0.26	10.71	2-Propen-1-ol,3-phenyl,acelale	0.45
3.37	Camphene	0.47	10.92	α-Caryophyllene	0.27
3.46	Benzaiolehyde	0.64	11.45	γ-Cadinene	3.41
3.59	α-Phellandrene	0.06	11.78	Dodecane	0.22
3.70	Limonene	0.65	11.89	*cis*-1,4-Dimethyladamantane	0.25
3.76	Eucalyptol	0.08	12.01	α-Muurolene	0.26
3.85	α,4-Dimethyl benzenemethanol	0.07	12.14	β-Bisabolene	0.07
4.06	Acetohpenone	0.21	12.57	δ-Cadinene	0.36
4.39	Fenchol	0.12	12.74	α-Famesene	0.05
4.78	Camphor	0.29	12.81	Cadinene-5,8-diene	0.16
4.92	Benzenepropanal	3.67	13.35	2-Propenal, 3-(2-methoxyphenyl)	0.44
4.98	Borneol	0.36	14.36	γ-Elemane	0.09
5.11	Terpinen-4-ol	0.32	15.26	Isoledene	0.17
5.76	Cinnamyl alcohol	1.24	16.42	α-Cadinol	1.85

[a] RT, Retention time (min); [b] peak area obtained by GC-FID.

of essential oil from *Cinnamomum cassia* bark on the membrane permeability of *S. aureus* and *E. coli* treated with different concentrations essential oil for 4 h respectively. The results showed that after adding the corresponding essential oil to strains, the RECs increased with the increased concentration of the essential oil ($p < 0.05$). Compared to the control, the REC of the suspensions increased from 0.54% to 17.26% ($p < 0.05$), then to 48.52% ($p < 0.05$) when *S. aureus* were treated with essential oil at 1× and 2× MIC, respectively; while it increased from 0.62% to 11.71% ($p < 0.05$), then to 44.35% ($p < 0.05$) for *E. coli*. It meant that the permeability of bacteria membrane would be increased correspondingly, which caused the leakage of intracellular ingredient, especially losses of electrolytes including K^+, Ca^{2+}, Na^+ and so on.

Table 2. Zone of inhibition (ZOI), antibacterial (MIC) and bactericidal (MBC) activities of the essential oil from *Cinnamomum cassia* bark against tested bacteria

Bacteria	ZOI[a] , mm	MIC, mg/mL	MBC, mg/mL
Gram-positive			
S. aureus	27.4 ± 0.6 a	2.5	5.0
B. subtilis	21.1 ± 0.56 b	5.0	10.0
Gram-negative			
S. typhimurium	14.5 ± 0.4 c	10.0	20.0
E. coli	20.7 ± 0.8 b	10.0	10.0

[a] Values represent means of three independent replicates ± SD. Different letters within a column indicate statistically significant differences between the means ($p < 0.05$) for ZOI.

Electron microscope observations. *S. aureus* and *E. coli* were treated with the essential oil at 1× MIC for 4 h respectively, and then the morphological and physical changes of treated strains were observed by SEM and TEM. Figure 1 showed the SEM images of the treated and untreated bacteria. The surfaces of the treated strains underwent some morphological changes compared with the untreated controls. Untreated *S. aureus* cells were spherical, regular, intact and showed a smooth surface (Fig. 1, A1), while bacterial cells treated with the essential oil became irregular, pitted, and shriveled (Fig. 1, A2); Untreated *E. coli* cells were rod shaped, regular, and intact (Fig. 1, B1), while some bacterial cells treated with the essential oil became deformed, pitted, shriveled, adhesive to each other and cell plasmolysis were found

Table 3. Postcontact effect (PCE) and effect of the essential oil on the permeability of cell membrane of tested strains

Concentrations, mg/mL	*S. aureus*		*E. coli*	
	PCE, h	REC, %	PCE, h	REC, %
Control	0	0.54 ± 0.10 c	0	0.62 ± 0.13 c
1× MIC	5.2 ± 0.3 b	17.26 ± 1.25 b	3.3 ± 0.1 b	11.71 ± 1.21 b
2× MIC	7.6 ± 0.5 a	48.52 ± 2.40 a	6.2 ± 0.4 a	44.35 ± 1.86 a

Values represent means of three independent replicates ± SD. Different letters within a column indicate statistically significant differences between the means ($p < 0.05$).

(Fig. 1, B2). These changes supported the results of PCE study and the permeability of cell membrane assay, and indicated that the essential oil may have severe effects on the cell wall and cytoplasmic membrane.

Figure 2 showed the TEM images of the *S. aureus* and *E. coli* after treatment with the essential oil. It was observed from the TEM photographs that untreated *S. aureus* and *E. coli* bacteria remained intact and clearly discernible cell membrane with uniformly distributed cytoplasm and electron dense material inside the cell (Fig. 2, A1, and B1). However, the bacterial cell wall and cytoplasmic membrane after treatment became uneven. In treated *S. aureus* cells, loosening of cell wall at some parts was found (Fig. 2, A2), while some treated *E. coli* cells turned from normal shape into irregular shapes and parts of the cell wall were broken which may give rise to the leaching out of cell contents (Fig. 2, B2), which was consistent with the result of SEM and supported the results of the PCE and cell membrane permeability assay.

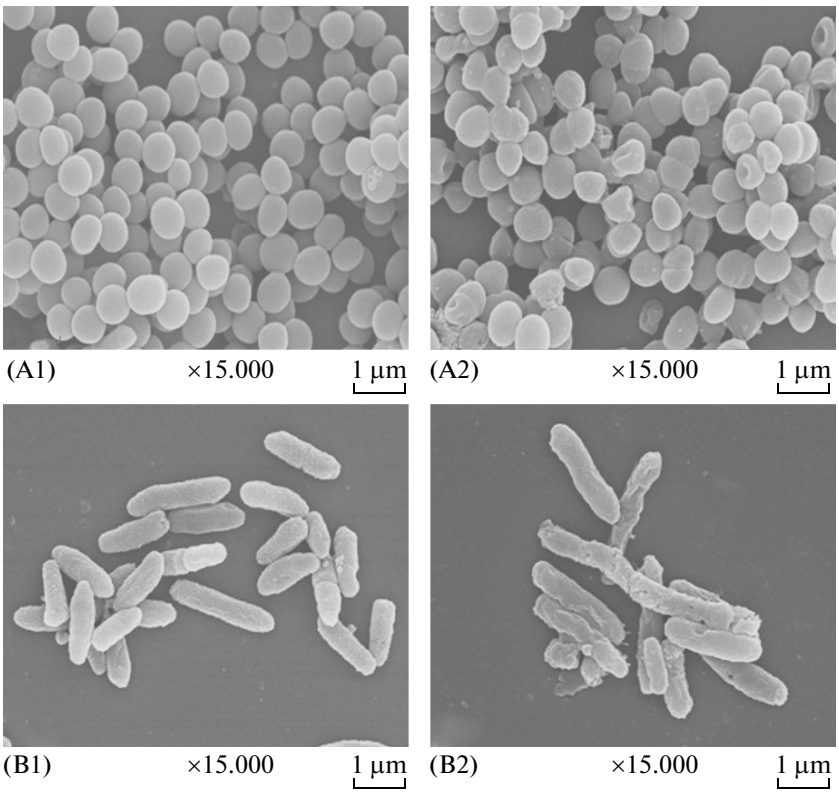

Fig. 1. The SEM photography of tested strains for A1 and B1, untreated *S. aureus* and *E. coli*, respectively; A2 and B2, *S. aureus* and *E. coli* treated with the essential oil, respectively.

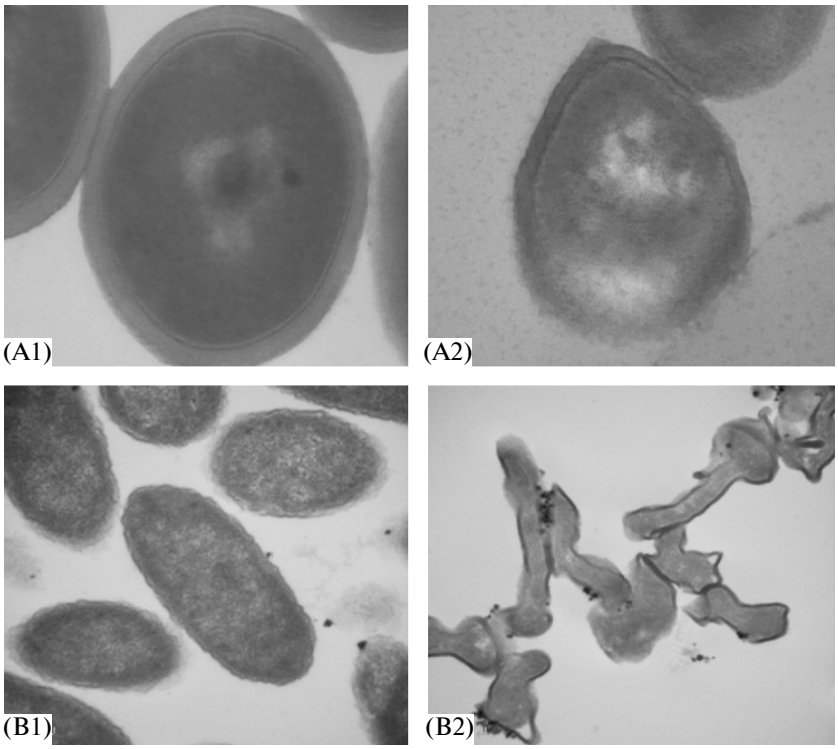

Fig. 2. The TEM photography of tested strains for A1 and B1, untreated *S. aureus* and *E. coli*, respectively; A2 and B2, *S. aureus* and *E. coli* treated with the essential oil, respectively.

DISCUSSION

In this study, *trans*-cinnamaldehyde was found to be the major component of the essential oil from *Cinnamomum cassia* bark by GC and GC-MS analysis, which is supported by previous studies [10, 13, 16]. However, as a volatile product of the secondary metabolism of aromatic plants, the composition of essential oils is variable [11, 30]. Wang et al. [11] identified 27 components in the essential oil of *Cinnamomum cassia* and found that cinnamaldehyde (30.67%), copaene (27.71%), 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1S-*cis*)-naphthalene (13.55%) were the major compounds of the essential oil. Tao, Huang, and Mai [31] identified 38 components in the essential oil from *Cinnamomum cassia* bark, and the content of cinnamaldehyde reached to 90.74%. Giordani, Regli, Kaloustian, and Portugal [15] reported that *trans*-cinnamaldehyde (92.20%) was the majority component of the essential oil from *Cinnamomum cassia* bark, followed by *o*-methoxy cinnamaldehyde (4.67%) and *trans*-cinnamyl acetate (3.13%). Geng et al. [13] studied variations in essential oil yield and composition during *Cinnamomum cassia* bark growth, and found the essential oil from bark observed at different stages displayed a different chemical profile. They reported that the main compounds were *trans*-cinnamaldehyde (33.95–76.4%), cinnamyl alcohol acetate (0.09–49.63%), 2'-methoxycinnamaldehyde (0.09–6.69%) and copaene (1.09–14.3%) during all stages, while the essential oil compositions at 11 growth stages were characterized by high levels of *trans*-cinnamaldehyde. Although we can find some differences in the contents and components of essential oil from *Cinnamomum cassia* in the literature studies, it is difficult to compare because the components and contents of the essential oil are affected by many factors, such as different growing seasons or months [32], growth years [13], sampled parts [13, 33], geographical origins [34] as well as extraction methods [31] and analysis conditions of the essential oil.

The results from the agar disc diffusion assay, followed by measurements of MIC and MBC indicated that the essential oil from *Cinnamomum cassia* bark had different inhibitory effects against different bacteria. *S. aureus* was found to be the most sensitive microorganism tested, showing the largest inhibition zone and the lowest MIC and MBC values. Some studies reported that the essential oil extracted from *Cinnamomum cassia* bark exhibited antibacterial effect against food-borne pathogens such as *Staphylococcus aureus, Listeria monocytogenes, Streptococcus oralis, Streptococcus anginosus*, *Escherichia coli,* and so on [18, 19], which supported our results in the present study, indicating that essential oil of *Cinnamomum cassia* bark was a potent bacterial inhibitor with a broad antibacterial spectrum. The antimicrobial properties of the essential oil from *Cinnamomum cassia* bark may come

from cinnamaldehyde because of it is major volatile and divers constituent present in it and also has variety of active components [18]. Furthermore, the effects of essential oil on the growth of *S. aureus* and *E. coli* were investigated by measuring the viable cell counts and the results revealed that exposure of essential oil had a significant effect on the cell viability of the tested strains.

Some studies reported that the active components of the essential oil might bind to the cell surface and then penetrate to the phospholipid bilayer of the cytoplasmic membrane and membrane-bound enzymes [35–37], which enables them to accumulate in cell membranes, to disturb the structures and cause an increase of permeability, resulting in a leakage of various vital intracellular constituents, and leading to cell death [2, 38]. The bacterial plasma membrane provides a permeability barrier to the passage of small ions such as K^+, Na^+ which are necessary electrolytes, facilitate cell membrane functions and maintain proper enzyme activity. Increases in the leakage of electrolytes will indicate a disruption of this permeability barrier. A relatively slight change to the structural integrity of cell membranes can detrimentally affect cell metabolism and lead to cell death [39]. Our experimental results showed that the relative electric conductivity of the suspension increased rapidly with the increasing concentration of essential oil, indicating the increase in permeability of cell membranes occurred, causing the leakage of electrolytes and leading to cell death, which was supported by the results of SEM and TEM.

The SEM and TEM micrograph of *S. aureus* and *E. coli* cells treated with the essential oil of *Cinnamomum cassia* bark showed that morphological alterations appeared in the cell wall and membrane, which have also been observed for various kinds of tested organisms when treated with different essential oils [29, 36, 37]. The changes in morphological of bacterial cells might be due to the effect of essential oil on the destruction of cell membranes (especially, in *E. coli*), which could result in the destruction of bacterial cell wall, followed by the losses of intracellular dense materials on the surface of treated cells. This mode of action of the essential oil from *Cinnamomum cassia* bark is the same both to *S. aureus* and to *E. coli* in this study, though there are some significant differences in the outer layers between gram-negative and gram-positive bacteria. The results showed that the essential oil from *Cinnamomum cassia* bark have broad-spectrum antibacterial activity, indicating nonspecific effect of the essential oil on different bacteria, which may be attributed to the complex composition of the essential oil. Interestingly, the signs of destructive changes caused by essential oil are much more prominent in *E. coli* cells than *S. aureus* (Fig. 2) in contrast to the power of action as estimated from MIC and MBC (Table 2), which may be related to their cell structure.

In summary, results from this study indicated that the essential oil from *Cinnamomum cassia* bark possesses antibacterial activities against four food spoilage bacteria to various extents. The essential oil was more effective against *S. aureus* as compared to other bacteria. Though the exact mode of action of the essential oil on bacteria is still not clear, the result of PCE, cell membrane permeability assays, and ultrastructural analysis revealed that the loss of permeability and integrity of cell membranes and losses of vital intracellular constituents could be one of the mechanisms of action of essential oil from *Cinnamomum cassia* bark against *S. aureus* and to *E. coli*. The essential oil from *Cinnamomum cassia* bark and its major antibacterial components have potential for application as natural food preservatives. However, further research on mechanisms of action, the toxicological, sensory effects as well as the effect on other food spoilage and poisoning bacteria is still necessary to fully evaluate the potential of the essential oil of *Cinnamomum cassia* bark in foods.

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