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Chemical Composition and Antibacterial Activity of the Essential Oil from Green Huajiao (*Zanthoxylum schinifolium*) against Selected Foodborne Pathogens

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ABSTRACT: Green huajiao, which is the ripe pericarp of the fruit of *Zanthoxylum schinifolium* Sieb. et Zucc, is widely consumed in Asia as a spice. In this work, the chemical composition of the essential oil from green huajiao was analyzed by gas chromatography (GC) and GC/mass spectrometry (MS), and the majority of components were identified. Linalool (28.2%), limonene (13.2%), and sabinene (12.1%) were found to be the major components. The antibacterial activity, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of the essential oil were evaluated against selected bacteria, including food-borne pathogens. The results showed that the sensitivities to the essential oil were different for different bacteria tested, and the susceptibility of Gram-positive bacteria tested was observed to be greater than that of Gram-negative bacteria. The antibacterial activity of the essential oil was particularly strong against *Staphylococcus epidermidis*, with MIC and MBC values of 2.5 and 5.0 mg/mL, respectively. A postcontact effect assay also confirmed the essential oil had a significant effect on the growth rate of surviving *S. epidermidis*. The antibacterial activity of the essential oil from green huajiao may be due to the increase in permeability of cell membranes, and the leakage of intracellular constituents, on the basis of the cell constituents' release assay and electron microscopy observations.

KEYWORDS: *green huajiao, zanthoxylum schinifolium Sieb. et Zucc, antibacterial activity, essential oil, foodborne pathogen*

INTRODUCTION

In recent years, food spoilage and food poisoning have been among 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.[3,4] 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. Recently, however, consumers have become increasingly concerned about the side effects of synthetic antimicrobial chemicals and want safer materials for preventing and controlling pathogenic microorganisms in foods.[5,6] Thus, development of safe and natural antibacterial compounds is becoming critically important.[7] Essential oils are the odorous, volatile products of the secondary metabolism of aromatic plants. A great number of plant essential oils have been tested for their antimicrobial activities, and some of them have been reported to possess strong antibacterial and antifungal effects.[5,6,8−11] Unlike the action of chemical antibiotics, an important characteristic of essential oil components is their hydrophobicity, which enables them to partition into the lipids of the bacterial cell membrane, disturbing the cell structures and rendering them more permeable. The ability of essential oils to disrupt the permeability barrier of the cell membrane and the accompanying loss of chemiosmotic control is the most likely cause of their lethal action.[12,13] In addition, it has been demonstrated that essential oils from various medicinal plants, spices, and herbs possess high volatility and toxicity to stored-grain insect pests while showing little harm to warm-blooded animals.[14,15] Considering their excellent antimicrobial function, natural essential oils possess great potential as antimicrobial agents to increase the safety and shelf life of foods. However, it might also be noted that some essential oils may be toxic if ingested, depending on the type and concentration of the oil.

Zanthoxylum schinifolium Sieb. et Zucc, found in China, Japan and Korea, is an aromatic shrub with protective thorns and culinary and pharmacological properties which has been cultivated in the southern provinces of China.[16−18] Green huajiao, which is the ripe pericarp of the fruits of *Z. schinifolium*, has strong anise, citrus, and pepper notes and is widely consumed in Asia as a spice.[18] Previous phytochemical studies indicated coumarins, alkaloids, triterpenoids, steroids, and flavonoids in the extract of *Z. schinifolium*.[17−19] It has been reported that the essential oil components from *Z. schinifolium* showed various biological activities, including antiplatelet aggregation, anti-inflammatory activity,[20] antioxidant[21] and anticancer activities,[22,23] and regulation of cardiac hormone secretion. [23] In addition, green huajiao possessed significant feeding deterrence against some insects (*Tribolium castaneum* and *Sitophilus zeamais*).[16,23] However, to the best of our knowledge, little information is available about the antibacterial effect and the mechanism of action of the essential oil from green huajiao on the growth and survival of food-borne pathogens. Therefore, the aim of the present study was to investigate the chemical composition and antibacterial activity of essential oil from green huajiao on several selected bacteria, including food-borne pathogens, and to further evaluate the possible mechanism of action of essential oil against sensitive strains by the postcontact effect assay, cell constituents' release

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test, and scanning electron microscopy as well as transmission electron microscopy observations.

MATERIALS AND METHODS

Plant Materials and Chemicals/Reagents. Green huajiao, which grows in Jinyang County of Sichuan Province, was obtained as a commercial product in the market of Chengdu in 2012. The sample was identified as a single fruit and the dry and the ripe pericarp of Jiuye Green huajiao by Professor Xuezhi Wei. Dimethyl sulfoxide (DMSO) was purchased from Sigma (USA). Nutrient agar (NA), nutrient broth (NB), and tryptone soy agar were from Beijing Aoboxing Biotech Co. Ltd. (Beijing, China). Other chemicals used were all of analytical grade.

Microorganisms and Culture. The antimicrobial activities of the essential oil were tested against seven different bacteria. Three Gram-positive strains were *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 8799, and *Bacillus subtilis* ATCC 6051. Four Gram-negative strains were *Salmonella typhimurium* ATCC 19430, *Pseudomonas aeruginosa* ATCC 9027, *Shigella dysenteriae* CMCC (B) 51252, 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.

Essential Oil Extraction. The green huajiao was again air-dried at 40 °C for 6 h, and its moisture content was 8.92%. The dried green huajiao was ground with a micro plant grinding machine (FZ102; Tianjin Taisite Instruments, Tianjin, China) to a powder and then hydrodistilled for 4 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 and had a distinct sharp odor with a 3.6% yield.

GC−FID Analysis. The essential oil was analyzed using a Hewlett-Packard 5890 II GC apparatus equipped with a FID detector and HP-5 MS capillary column (30 m × 0.25 mm; film thickness, 0.25 μm). Injector and detector temperatures were set at 250 and 200 °C, respectively. The oven temperature was programmed from 40 °C for 2 min, raised to 80 °C at a rate of 3 °C/min, held isothermal for 2 min, and finally raised to 240 °C at 5 °C/min for 10 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 1:100. The percentages of the constituents were calculated by electronic integration of the FID peak areas without the use of a 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; film thickness, 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 50 to 550 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 250 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 1:100 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.

Antimicrobial Activity. The essential oil was first dissolved in DMSO to a concentration of 50% (v/v), and then sterilized by filtration through 0.22 μm Millipore filters. Antimicrobial tests were carried out by the Oxford cup method using 100 μL of suspension containing 10^8 CFU/mL of bacteria spread on nutrient agar (NA) medium. Oxford cups (6 mm in diameter) were placed on the inoculated agar, and 100 μL of essential oil was added. The diameter of the zones of inhibition (ZOI) was measured after 24 h of incubation at 37 °C. DMSO was used as the negative control. Tests were 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 et al.[24] with minor modifications. Briefly, 50% (v/v) essential oil in DMSO as stock solutions was prepared. 2-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 ∼2×10^6 CFU/mL. A control test containing inoculated broth supplemented with only DMSO was also performed. The maximum final concentration of DMSO in each medium was 2%, which did not affect the growth of the test strains. 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 drug-free 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.[25] The bacterial cultures of the exponential phase (approximately 10^8 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. Viable counts were immediately measured in tryptone soy agar and 1−24 h (one time per hour) after incubation. PCE was determined using the equation PCE = $t - c$, where t is the time needed for a log increase in counts in treated cultures and c is the time needed for a log increase in counts in untreated cultures. All experiments were performed in triplicate.

Cell Constituents' Release. The release of cell constituents into the supernatant was examined according to the method described by Rhayour et al.[9] with some modifications. Cells from the 100 mL working culture of tested bacteria were collected by centrifugation at 5000g for 15 min, washed three times with 0.1 M phosphate buffer solution (PBS, pH 7.4), and resuspended in 0.1 M PBS. One hundred milliliters of cell suspension was incubated at 37 °C under agitation for 1 h in the presence of essential oil at three different concentrations (control, MIC, and 2 × MIC), then 5 mL samples were collected and centrifuged at 11000g for 5 min. To determine the concentration of the constituents released, 2 mL supernatant was used to measure UV absorption at 260 nm. Correction was made for the absorption of the suspension with PBS containing the same concentration of essential oil after 2 min of contact with the tested strains. The untreated cells (control) were corrected with pH 7.4 PBS.

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.[26] After 10 h incubation of a cell suspension of *S. epidermidis* of ∼2×10^6 CFU/mL at 37 °C in NB, the essential oil was added at either the MIC or 2 × MIC. The control culture was left untreated. All suspensions were incubated at 37 °C for 4 and 8 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. 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 the cells were dried at a "critical point" in liquid CO_2 under 95 bar pressure, and the samples were gold-covered by cathodic spraying. Finally, the morphology of the bacterial cell was observed with a scanning electronic microscope (SEM) (JSM-7500F, JEOL Ltd., Japan) operated at an accelerating voltage of 25−30 kV.

Transmission Electron Microscope (TEM). The bacteria cells were incubated for 10 h in NB at 37 °C before addition of the essential oil to give a final concentration of the MIC or 2 × MIC. All treatments and controls were incubated at 37 °C for 4 and 8 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 postfixed with 1% (w/v) OsO_4 in 0.1 M PBS for 2 h at room temperature and washed three times with the

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Table 1. Chemical Composition of Essential Oil from Green Huajiao

RI[a]	compd	peak area (%)[b]	RI	compd	peak area (%)
1030	α-thujene	0.48	1581	linalyl acetate	3.90
1033	α-pinene	1.50	1594	terpineol	0.31
1121	β-pinene	1.29	1600	hexadecane	0.25
1132	sabinene	12.1	1606	pinocarvone	0.06
1173	myrcene	6.12	1615	hotrienol	0.04
1176	α-phellandrene	0.17	1619	4-terpinenol	3.72
1196	α-terpinene	1.21	1620	β-caryophyllene	1.31
1217	limonene	13.2	1631	3-methylpentadecane	0.08
1226	1,8-cineole	1.32	1636	*cis*-*p*-2-menthen-1-ol	0.24
1227	β-phellandrene	3.38	1660	myrtenal	0.29
1244	*cis*-β-ocimene	0.49	1694	α-humulene	0.68
1257	6-methylheptanal	0.05	1712	α-terpineol	1.41
1261	γ-terpinene	2.32	1715	α-terpinyl acetate	0.18
1282	*p*-cymene	0.28	1733	neryl acetate	0.12
1299	terpinolene	0.61	1733	germacrene D	2.48
1400	tetradecane	0.13	1746	γ-cadinene	0.03
1423	nonanal	0.06	1764	bicyclogermacrene	0.65
1460	α-thujone	1.53	1769	carvone	0.06
1469	*trans*-linalool oxide	0.31	1781	δ-cadinene	0.13
1479	isodecanal	0.04	1789	benzeneacetic acid methyl ester	0.07
1480	β-thujone	0.92	1812	nerol	0.11
1487	*trans*-sabinene hydrate	1.12	1817	myrtenol	0.24
1498	*cis*-linalool oxide	0.24	1811	2,4-dimethylacetophenone	0.06
1500	pentadecane	0.11	1834	2-phenylethyl acetate	0.04
1507	citronellal	0.06	1842	*trans*-carveol	0.06
1530	6-methyl-1-heptanol	0.08	1857	geraniol	0.23
1566	linalool	28.2	2004	*trans*-nerolidol	0.04
1578	*cis*-sabinene hydrate	0.46	2144	spathulenol	0.06

[a]RI, match with reference GC retention index. [b]Peak area obtained by GC−FID.

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, Hitachi 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 an air-dried sample with a yield of 3.6% yield (v/w), which was consistent with most previous reports of yields of 3−4% of essential oil for the dried huajiao pericarp.[27] The chemical composition of the essential oil was analyzed by GC and GC/MS, and the result is listed in Table 1. In total, 56 components were identified, representing 94.63% of the total amount. Results showed that linalool (28.2%), limonene (13.2%), and sabinene (12.1%) were found to be the major compounds in the essential oil of green huajiao, followed by myrcene (6.12%), linalyl acetate (3.90%), 4-terpinenol (3.72%), and β-phellandrene (3.38%). In addition, other components were also found to be of lower content (0.04−2.48%) in the essential oil of green huajiao in the present study (Table 1).

ZOI, MIC, and MBC of the Essential Oil. The ZOI, MIC, and MBC values of the essential oil from green huajiao against different bacteria are presented in Table 2. The results showed that the essential oil of green huajiao had certain antibacterial effects on all of the tested bacteria (Table 2). The ZOI, MIC, and MBC values for Gram-positive bacterial strains were in the range of 13.8−18.2 mm, 2.5−10 mg/mL, and 5−10 mg/mL, respectively; they were in the range of 8.5−11.6 mm, 10−20 mg/mL, and ≥20 mg/mL for Gram-negative bacterial strains, respectively. Of these bacteria, the essential oil of green huajiao had the greatest antibacterial effect against *S. epidermidis*, with both the lowest MIC of 2.5 mg/mL and MBC of 5.0 mg/mL. The MIC and MBC values of the essential oil for *S. typhimurium* and *Shigella dysenteriae* strains were greater than 20 mg/mL which was the highest concentration of essential oil tested in this study.

Table 2. Zone of Inhibition (ZOI), Antibacterial (MIC), and Bactericidal (MBC) Activities of the Essential Oil from Green Huajiao against Tested Bacteria

bacteria	ZOI[a] (mm)	MIC (mg/mL)	MBC (mg/mL)
	Gram-positive		
S. aureus	13.8 ± 0.5 b	10	10
S. epidermidis	17.5 ± 0.4 a	2.5	5.0
B. subtilis	18.2 ± 0.3 a	5.0	5.0
	Gram-negative		
S. typhimurium	8.8 ± 0.4 d	>20	NT[b]
P. aeruginosa	10.6 ± 0.6 c	20	>20
S. dysenteriae	8.5 ± 0.8 d	>20	NT
E. coli	11.6 ± 0.5 c	10	20

[a]Values represent means of three independent replicates ± SD. [b]NT, not tested. Different letters within a column indicate statistically significant differences between the means ($p < 0.05$) for ZOI.

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Postcontact Effect (PCE) of Essential Oil. The PCEs of the essential oil from green huajiao on *S. epidermidis* were evaluated at 1 × MIC and 2 × MIC concentrations (Table 3).

Table 3. Postcontact Effect (PCE) and Effect of the Essential Oil on Cell Constituents' Release of Tested *S. epidermidis*

concn (mg/mL)	PCE (h)	cell constituents' release (OD_{260nm})
control	0	0.065 ± 0.006 c
1 × MIC	5.5 ± 0.2 b	0.153 ± 0.011 b
2 × MIC	9.2 ± 0.3 a	0.292 ± 0.014 a

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

The PCE increased significantly with an increase in the concentration of the essential oil ($p < 0.05$). At 1 × MIC concentration, the PCE was 5.2 h, and increased to 9.2 h at 2 × MIC concentration ($p < 0.05$), which confirmed the antibacterial activity of the essential oil from green huajiao and showed a severe effect on the recovery of surviving *S. epidermidis* after treatment.

Cell Constituents' Release. The cell constituents' release was determined by the measurement of the absorbance at 260 nm of the supernatant of tested *S. epidermidis* strains. Table 3 showed the results when *S. epidermidis* was treated with different concentrations of essential oil for 1 h. The results showed that after adding the corresponding essential oil to the strains, the cell constituents' release increased with the increased concentration of the essential oil compared with the control group ($p < 0.05$). The cell constituents' release increased from 0.065 to 0.153 ($p < 0.05$), then to 0.292 ($p < 0.05$) when *S. epidermidis* was treated with essential oil at 1 × and 2 × MIC, respectively, which implied that cell constituents of *S. epidermidis* were released into the buffer. The results indicated that the permeability of cell membranes might be increased, even damaged, which led to the loss of cell constituents such as protein and some essential molecules.

Electron Microscope Observations. *S. epidermidis* was treated with the essential oil at 1 × and 2 × MIC for 4, 8 h, and then the morphological and physical changes of treated *S. epidermidis* were observed by SEM and TEM. Figure 1 shows 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 cells were spherical, regular, and intact and showed a smooth surface (Figure 1a, b), whereas the bacterial cells treated with the essential oil became irregular, pitted, and shrivelled (Figure 1A4, A8, B4, and B8), which might result in the leakage of the contents of the cells.

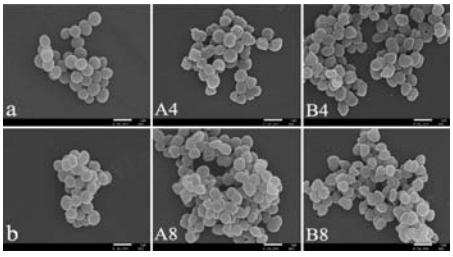

Figure 1. The SEM photography of *S. epidermidis*. (a, b) Untreated bacteria cultured for 14, 18 h, respectively; (A4, A8) bacteria treated with the essential oil at 1 × MIC for 4, 8 h, respectively; (B4, B8) bacteria treated with the essential oil at 2 × MIC for 4, 8 h, respectively.

Figure 2 shows the TEM image of the *S. epidermidis* after treatment with the essential oil at different concentrations. It was observed from the TEM photographs that untreated *S. epidermidis* bacteria after being cultured for 14 and 18 h remained intact and had a clearly discernible cell membrane with uniformly distributed cytochylema and electron-dense material inside the cell (Figure 2a, b). However, the bacterial cell wall and cytoplasmic membrane after treatment became uneven and appeared thick, and some lysis was seen (Figure 2A4, A8, B4, and B8). Some cells turned from the normal round shape into irregular shapes, and parts of the cell wall were broken, which may give rise to the leaching out of cell contents, and the changes were more evident with an increase in the concentration and treatment time of the essential oil, which was consistent with the result of the SEM and supported the results of the PCE and cell constituents' release assays.

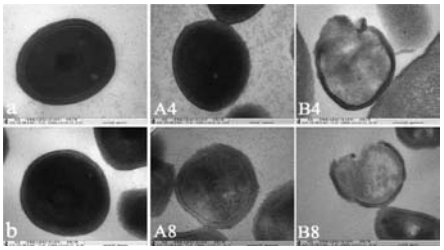

Figure 2. TEM photography of *S. epidermidis*. (a, b) untreated bacteria cultured for 14, 18 h, respectively; (A4, A8) bacteria treated with the essential oil at 1 × MIC for 4, 8h, respectively; (B4, B8) bacteria treated with the essential oil at 2 × MIC for 4, 8h, respectively.

DISCUSSION

In this study, linalool, limonene, and sabinene were found to be the major components of the essential oil from green green huajiao by GC and GC/MS analysis. This is supported by previous studies.[18,19] However, the huajiao composition is variable. Yang[18] identified 88 components in the essential oil of green huajiao. Jia et al.[19] identified 63 components and also found that linalool (40.15%), limonene (18.81%), and sabinene (14.53%) were the major compounds of the essential oil from Hanyuan green huajiao. The major components of the supercritical CO_2 extract of green huajiao from Chongqing were reported as linalool (59.24%) and limonene (11.28%).[28] Iseli et al.[17] identified 20 components of the essential oil of green huajiao obtained in China and found that limonene (21%) was the main component, followed by 4-terpineol and γ-terpinene. Although we can find some differences in the contents and components of the essential oil from green huajiao in the literature studies, it is difficult to compare because the species, geographic regions, and extraction methods used are different among studies.[17−19,27,28] Moreover, the same species may also present different chemotypes.[29] It has been reported that the antimicrobial activity of essential oils can be attributed to the presence of a number of low-molecular-weight phenols, terpenes, and aldoketones, which also have been shown to exhibit antimicrobial activity in the pure form.[30] These findings laid the foundations for later study because the essential oil of green huajiao was also found to contain these components. It is expected that the essential oil of green

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huajiao may also show antimicrobial effects and might thereby perform as a natural antimicrobial agent in a food context.

The results from the Oxford cup method, followed by measurements of MIC and MBC, indicated that the essential oil from green huajiao had different inhibitory effects against different bacteria. *S. epidermidis* was found to be the most sensitive microorganism tested, showing the largest inhibition zone and the lowest MIC and MBC values. The results show that the Gram-positive bacteria were more sensitive than the Gram-negative ones to the essential oil from green huajiao ($p < 0.05$). To some extent, this is consistent with previous studies on antibacterial activity of the essential oils,[4,12,26,29] and is likely due to the significant differences in the outer layers between Gram-negative and Gram-positive bacteria. 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 and almost impermeable to lipophilic compounds, thus presenting a barrier to the penetration of numerous antibiotic molecules,[12,13,31] whereas because of the absence of this barrier in Gram-positive bacteria, some antibacterial substances can target the bacterial cell wall and further destroy the cytoplasmic membrane, which could result in a leakage of the cytoplasm or vital intracellular constituents or impairment of the bacterial enzyme systems. However, comparing Gram-positive and Gram-negative bacteria, some studies[32,33] reported that Gram-positive bacteria were less or equally sensitive. Kim et al.[34] suggested that the antibacterial activity did not depend on the type of Gram reaction. Dorman et al.[35] suggested that antibacterial activity depended on the type of essential oil. Therefore, as a mixture of numerous molecules, the activity of the essential oils may be attributed to the molecular structure and molecular weight of their major and minor components, and the antimicrobial mechanism of the essential oil from green huajiao, like other plant essential oils, still needs to be further investigated. Furthermore, the effects of the essential oil on the growth of *S. epidermidis* were investigated by measuring the viable cell counts. The results revealed that exposure to the essential oil had a significant effect on the cell viability of the tested *S. epidermidis*.

Some studies have 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.[12,29,36] Subsequently, the active components can lead to disruption of the synthesis of some macromolecules, such as DNA, RNA, protein, or polysaccharides, causing the death of the cells.[2,8] Some authors have suggested that the distortion of the cell wall and cytoplasmic membrane would cause the expansion and destabilization of the membrane and increase membrane permeability, resulting in a leakage of various vital intracellular constituents, which lead to cell death.[2,8,34] Measurement of specific cell leakage markers, such as proteins and 260 nm absorbing materials, is an indicator of membrane permeability and integrity to specific antimicrobial agents in relationship to unexposed cells.[36] Our experimental results showed that the essential oil of green huajiao caused rapid losses of the cell constituents from the treated bacteria, indicating an increase in the permeability of the cell membranes occurred, which was supported by the results of SEM and TEM. These changes in the cell membranes may be attributed to the antibacterial activity of the essential oil from green huajiao against *S. epidermidis* bacteria; however, whether the essential oil influenced the synthesis of vital intracellular constituents to cause the cell death needs to be further studied.

The SEM and TEM micrograph of *S. epidermidis* cells treated with the essential oil of green huajiao 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.[26,29,36] The changes in the morphology of bacterial cells might be due to the effect of the essential oil on the permeability of the membrane, which could result in the lysis of the bacterial cell wall, followed by the loss of intracellular dense materials on the surface of the treated cells.

Results from this study indicated that the essential oil from green huajiao possesses antibacterial activities against three Gram-positive bacteria and four Gram-negative bacteria to various extents. The essential oil was more effective against Gram-positive bacteria, in particular against *S. epidermidis*, as compared wtih Gram-negative bacteria. Although the exact mode of action of the essential oil on the bacteria is still not clear, the result of PCE, cell constituents' release assays, and ultrastructural analysis revealed that the loss of integrity of the cell membranes and loss of vital intracellular constituents could be one of the mechanisms of action of the essential oil from green huajiao against *S. epidermidis*. The essential oil from green huajiao and its major antibacterial components have potential for application as natural food preservatives. However, further research on the mechanisms of action and the toxicological and 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 green huajiao in foods.

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Notes

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ABBREVIATIONS USED

DMSO, dimethyl sulfoxide; NA, nutrient agar; NB, nutrient broth; ZOI, the zone of inhibition; MIC, minimum inhibitory concentration; MBC, minimum bactericide concentration; PCE, postcontact effect; SEM, scanning electron microscope; TEM, transmission electron microscope

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