

## Research Article

# Effects of Black Pepper (*Piper nigrum L.*) Chloroform Extract on the Enzymatic Activity and Metabolism of *Escherichia coli* and *Staphylococcus aureus*

Wenxue Chen <sup>1</sup>, Lan Zou,<sup>2</sup> Weijun Chen <sup>1</sup>, Yueying Hu,<sup>3</sup> and Haiming Chen <sup>1</sup>

<sup>1</sup>College of Food Science and Technology, Hainan University, Haikou, Hainan 570228, China

<sup>2</sup>Qianxinan Institution for Food and Drug Inspection Center, Xingyi, Guizhou 562400, China

<sup>3</sup>College of Material and Chemical Engineering, Hainan University, Haikou, Hainan 570228, China

Correspondence should be addressed to Haiming Chen; hmchen168@126.com

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The chemical composition and antimicrobial mechanism of action of black pepper chloroform extract (BPCE) were investigated, as well as the potential antibacterial activities of BPCE against *Escherichia coli* and *Staphylococcus aureus*. The results showed that 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1 $\alpha$ ,4 $\alpha$ ,7 $\beta$ ,7 $\alpha$ , $\beta$ ,7 $\beta$  $\alpha$ .)]- (8.39%) and 2-methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane (6.92%) were identified as the two primary components of BPCE. The release of intracellular transaminases from bacteria after being incubated with BPCE revealed that the bacterial cell walls and membranes were degraded and that protein synthesis was inhibited to some extent. The inhibition of bacterial Na<sup>+</sup>/K<sup>+</sup>-ATPase activity upon the addition of BPCE also indicated an enhanced permeability of bacterial cell membranes. Moreover, an analysis of hexokinase and pyruvate kinase activities showed that BPCE affected the metabolic rate of glycolysis and disrupted the normal metabolism of bacteria. This phenomenon was supported by an observed accumulation of lactic acid (LA) in the treated bacterial cells. Overall, our results indicated that BPCE damaged bacterial cell walls and membranes, which was followed by a disruption of bacterial cell respiration.

## 1. Introduction

Natural antimicrobial agents are increasingly being used in food industries because of the serious health risks that chemical preservatives pose [1]. Plant essential oils exhibit various antibacterial activities. For instance, essential oils from oregano, thyme, and marjoram contain many active compounds that have good antimicrobial activities against gram-positive and gram-negative bacteria [2, 3]. China is very rich in endogenous aromatic and medicinal plants, which are used as natural health care products in traditional medicine. A number of these plants have been investigated for their biological and antibacterial activities, such as *Buddleia officinalis Maxim*, *Osmanthus fragrans*, and clove [4, 5]. Since ancient times, black pepper has been commonly used as spice in cooking. Moreover, black pepper is highly valued in folk medicine because of its antibacterial and physiological

benefits, particularly in treating pain, the flu, muscle aches, and rheumatism [6–8].

Recent studies have shown that black pepper extracts can inhibit food spoilage and food pathogens [9–13]. We previously explored the optimum extraction process for black pepper using chloroform and investigated the inhibitory effects and minimal inhibition concentration (MIC) of black pepper chloroform extract (BPCE) against *Escherichia coli* and *Staphylococcus aureus*. However, the antibacterial mechanisms of action of BPCE against these strains remain unclear.

Therefore, in the present study, we evaluated the antibacterial mechanism of action of BPCE against *E. coli* and *S. aureus*. Furthermore, we analyzed the modulation of key metabolic enzyme activities and assessed the leakage of the intracellular constituents of bacterial cells after being incubated with BPCE. Finally, to analyze the respiration of

bacterial strains, we examined the lactic acid (LA) content in bacterial cells.

## 2. Materials and Methods

**2.1. Materials and Chemicals.** The black pepper used in this study was purchased from Nanguo Supermarket (Haikou, China), grown in Wanning, Hainan province, China, and picked in June 2016 (nine months after flowering). Hexokinase (HK), pyruvate kinase (PK) and  $\text{Na}^+/\text{K}^+$ -ATPase assay kits were purchased from Nanjing JianCheng Bio-engineering Institute (Nanjing, China). LA was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), and 2,4-dinitrophenylhydrazine (DNP) was purchased from Aladdin Industrial Corporation. All other chemicals used in this study were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

**2.2. Bacterial Strains.** *E. coli* (ATCC8739) and *S. aureus* (ATCC6538) were provided by our laboratory. Both strains were cultured at 37°C for 24 h on nutrient agar medium, which contained 3.0 g of beef extract, 10.0 g of peptone, 5.0 g of NaCl, and 15.0 g of agar in 1000 mL of deionized water, with the pH adjusted to 7.2–7.3. Next, the bacteria were washed twice with 0.9% sterile NaCl and resuspended in 9 mL of the same NaCl solution. Bacterial suspensions were prepared at a density of  $1\text{--}2 \times 10^7$  cfu/mL in 0.9% sterile NaCl, after which 1.0 mL of an *E. coli* or *S. aureus* suspension was added to 48 mL of nutrient broth medium. BPCE (predissolved in ethanol) was added to the treatment group to determine the final MIC concentrations. The control group was provided an equivalent volume ethanol without BPCE. Both groups were incubated at 37°C and shaken at 130 rpm.

**2.3. Preparation of Black Pepper Chloroform Extract (BPCE).** Black pepper powder (100 g) was immersed and stirred in 2 L of 80% ethanol at 50°C for 12 h. This process was repeated three times according to our previous study. The mixture was then filtered, and the solvent of the combined extracts was vacuum evaporated using a rotary vacuum evaporator (RV 10, IKA, Germany) (0.098 MPa, 50°C). The remaining water was evaporated at 50°C in a thermostat-controlled water bath. BPCE was prepared according to our previous study [14]. Briefly, 100 mL of distilled water was added to the dried extract to obtain a turbid suspension. Next, 100 mL of petroleum ether was added to the suspension in a separatory funnel. The lower turbid suspension was transferred into another separatory funnel, after which 100 mL of chloroform was added to perform the extraction. Finally, the black pepper dissolved in chloroform was collected and concentrated, and the BPCE was obtained and stored at 4°C for further analysis.

**2.4. Determination of Transaminase Activities.** The activities of alanine transaminase (ALT) and aspartate transaminase (AST) in soluble fractions of cell lysates were measured. The treatment and control group samples were centrifuged at 4°C (21,621g, 10 min). Supernatants were obtained and collected in ice until transaminase measurements were made using the

classical Reitman-Frankel colorimetric endpoint reaction (Lv, 1967). Subsequently, 0.5 mL of ALT substrate [containing L-alanine (0.2 mol/L),  $\alpha$ -ketoglutarate (2.0 mmol/L), and phosphate buffer (0.1 mol/L, pH 7.4)] and AST substrate [containing L-aspartate (0.2 mol/L),  $\alpha$ -ketoglutarate (2.0 mmol/L), and phosphate buffer (0.1 mol/L, pH 7.4)] were transferred into test tubes and incubated in a water bath at 37°C for 5 min. Supernatants (0.1 mL) were added to the test tubes, mixed, and incubated in a water bath at 37°C for 30 or 60 min for ALT and AST assays, respectively. Next, 0.5 mL DNP (0.1 mmol/L) was added and the reactions were mixed and incubated for another 20 min at 37°C. Subsequently, 5 mL of NaOH (0.5 mol/L) was added as color developer and mixed after heating the mixture in a water bath at 37°C for 10 min. The absorbance was measured at 505 nm after the mixture was cooled at room temperature for 10 min. The ALT and AST activities were calculated using a pyruvic acid calibration curve.

**2.5. ATPase Activity and Key Enzymes Activities in Glycolysis Pathway.** The  $\text{Na}^+/\text{K}^+$ -ATPase, HK, and PK activities were assessed using the appropriate assay kits. Bacterial cells were collected by centrifugation at 4°C (21,621g, 10 min), then washed thrice, and resuspended in phosphate-buffered saline solution (0.1 mol/L, pH 7.4). Bacterial cells were lysed with lysozyme (2 g/L) in a water bath for 10–20 min at 37°C until the bacteria aggregated, after which samples were immediately placed in an ice bath and 1 mL of Tris-SDS was added. Cell debris was removed by centrifugation at 21,621g for 10 min at 4°C. Supernatants were collected and stored on ice prior to ATPase, HK, and PK measurements.

**2.6. LA Content Determination.** LA was measured by high-performance liquid chromatography (HPLC) according to the methodology described by Soria and Audisio [15]. Cell free supernatants were centrifuged at 4°C (17,297g, 10 min), filter sterilized (0.22  $\mu\text{m}$ ), and maintained at 4°C until used. The supernatants were deproteinized prior to the assay [16]. Samples (1 mL) were added to 2 mL of  $\text{Ba}(\text{OH})_2$  (1.8%) and 2 mL of  $\text{ZnSO}_4$  (2%). Next, the mixture was centrifuged, and each supernatant was filter sterilized (0.22  $\mu\text{m}$ ) before the HPLC analyses. HPLC was performed using a Waters Alliance 2695 system (USA) equipped with a 2489 UV detector (Milford, MA, USA) connected in series with a Thermo Betasil  $\text{C}_{18}$  chromatography column (300  $\times$  7.8 mm). The HPLC conditions used were as follows: column temperature 30°C; ratio of 0.1% orthophosphoric acid and methanol used for the mobile phase 99:1; and flow rate 0.5 mL/min. The injection volume was 20  $\mu\text{L}$ .

**2.7. GC-MS Analysis.** The chemical composition of BPCE was determined by gas chromatography-mass spectrometry (GC-MS). GC-MS experiments were performed on an Agilent Technologies 7890A gas chromatograph (Santa Clara, CA) and an Agilent 7683B autoinjector coupled with a 240 Agilent Ion Trap mass spectrometer (MS/MS). The mass spectral scan rate was 2.86 scans/s. The GC was operated with a helium (ultrahigh purity) flow rate of 0.7 mL/min under a

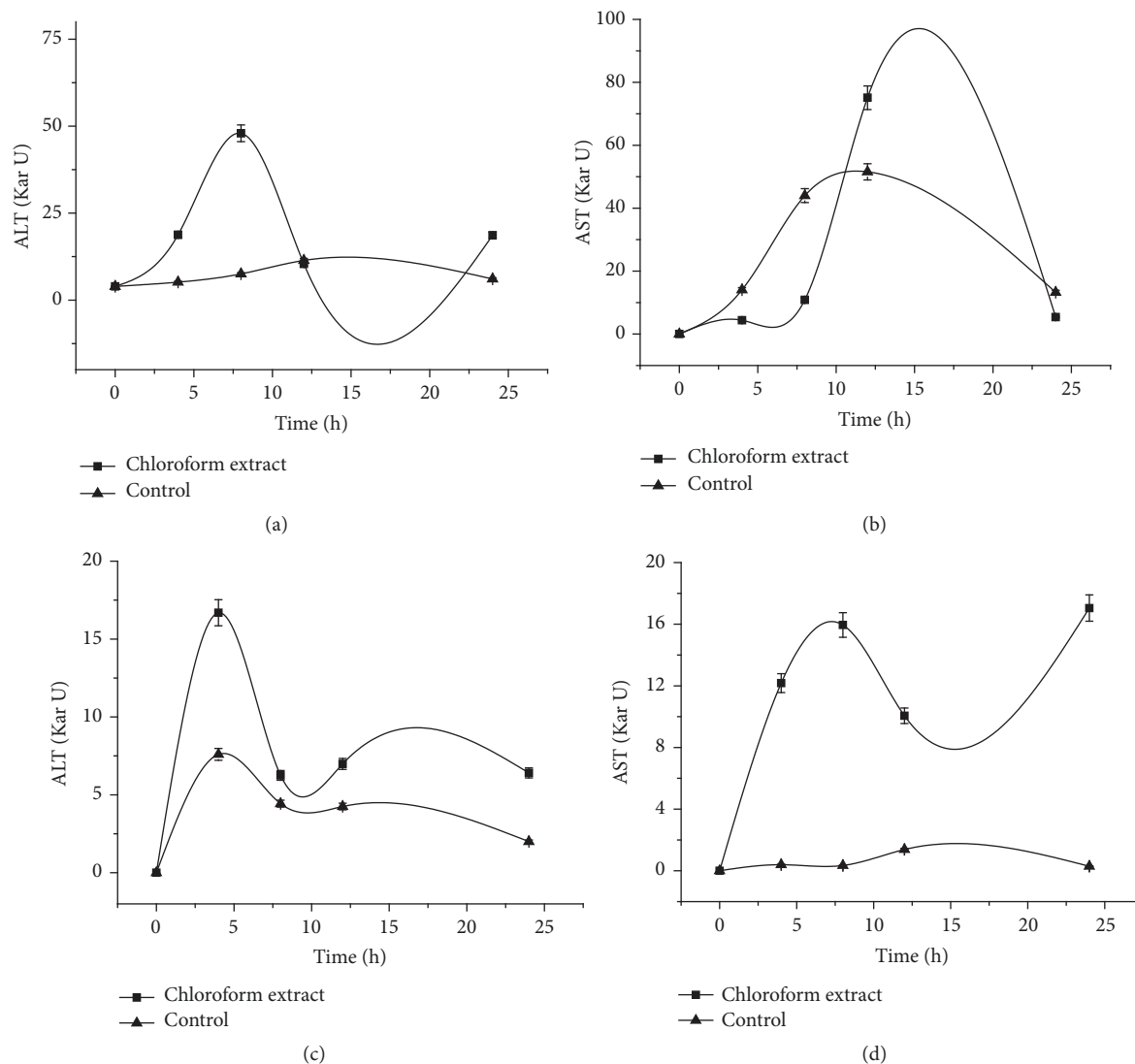


FIGURE 1: Transaminase activities in *E. coli* (a, b) and *S. aureus* (c, d).

head pressure of 10 psi, and the injection volume of  $1\ \mu\text{L}$ . The MS was operated in the electron ionization (EI) mode using an ionization voltage of 70 eV and a source temperature of  $230^\circ\text{C}$ . The scan type used was the automated method development function (AMD) and the optimum MS/MS excitation amplitude was 1.20 V. Relative percentages of the primary components were calculated by integrating the registered peaks.

**2.8. Statistical Analysis.** All experiments were performed in triplicate. Data are presented as the means  $\pm$  SD. Data were analyzed with SAS 9.0, and significance was assessed using Duncan's one-way multiple comparison. Differences between groups were considered significant at  $p \leq 0.05$ .

### 3. Results and Discussion

**3.1. Transaminase Activities.** Transaminase is an endoenzyme that catalyzes a reaction between an amino acid and  $\alpha$ -ketoglutaric acid and is often used as a general indicator

of bacterial injury [17]. The ALT activity in the treatment group was higher than that of the control within the initial 12 h. However, the AST activity in the treatment group was lower than that of control at 12 h for *E. coli* (Figures 1(a) and 1(b)). The activities of transaminases for *S. aureus* samples were lower in the control culture without the BPCE treatment (Figures 1(c) and 1(d)). By contrast, the control group slightly changed, particularly for AST. Under normal physiological conditions, the intracellular enzymes will not release from the cell. It illustrates the cell wall and cell membrane are injured, once the intracellular enzyme was determined in the extracellular fluid [18, 19]. The current results indicated that the overall cellular structure was damaged by the leakage of transaminase, which may have been caused by BPCE. Recent studies have shown that a number of essential oils affect fungal cell permeability by directly interacting with the cytomembrane [20–22]. Using extracellular ALT and AST activities as indicators of membrane damage, the membrane structure of the bacteria was observed to be significantly

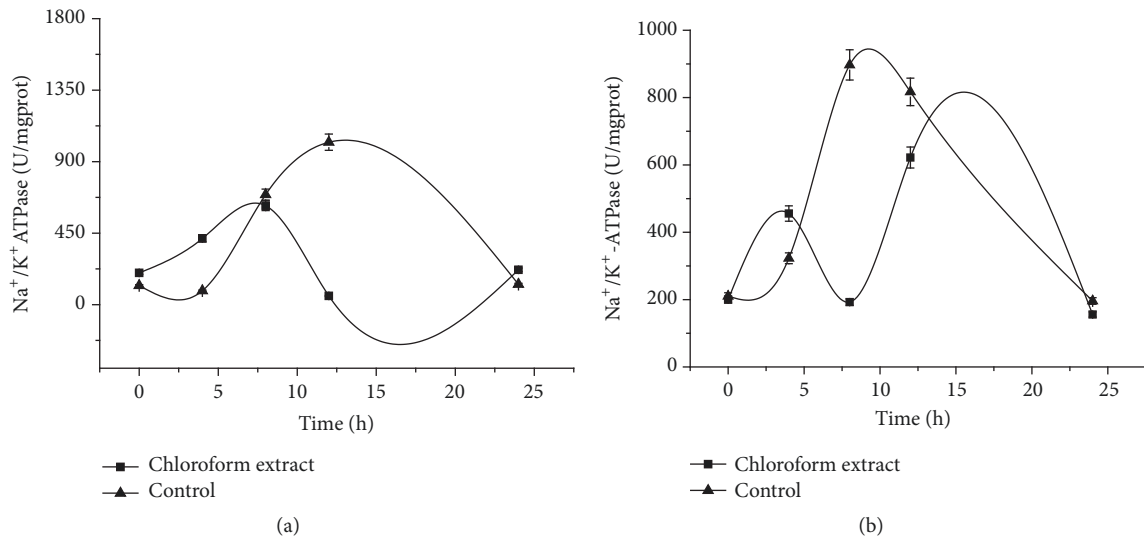


FIGURE 2: ATPase activity in *E. coli* (a) and *S. aureus* (b).

damaged by BPCE, especially for *S. aureus*. The MICs of BPCE for *E. coli* and *S. aureus* were 2.5 mg/mL. In addition, transaminases disrupt the normal degradation and synthesis of cell peptides and proteins.

**3.2. Na<sup>+</sup>/K<sup>+</sup>-ATPase Activity.** Na<sup>+</sup>/K<sup>+</sup>-ATPase is a common sodium pump in the membrane of eukaryotic cells and is important for establishing and maintaining high K<sup>+</sup> and reducing Na<sup>+</sup> concentrations in the cytoplasm [23]. An electrochemical gradient for Na<sup>+</sup> across the plasma membrane is essential for diverse cellular functions, such as the loss of DNA, polysaccharide, and ions, inhibition of nutrient absorption, and pH regulation [24, 25]. Figure 2(a) shows that the Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in the control increased for 12 h, whereas ATPase activity increased from 0 to 8 h and then decreased from 8 to 12 h before increasing again from 12 to 24 h when treated with BPCE. In the control groups, the high activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase observed may be due to bacterial growth during the initial 12 h, at which time cells require more nutrition and energy. In addition, a certain amount of time is required for ethanol to kill bacterial cells, which cannot affect the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase. When the bacterial culture was in the stationary phase and the death phase, most of the bacteria gradually died, and the Na<sup>+</sup>/K<sup>+</sup>-ATPase activity decreased, with the same rationale applying to the test groups. Moreover, the decreased ATP level is also probably due to excessive cell apoptosis and excessive ATP consumption, because apoptosis is an ATP-dependent process [26]. Figure 2(b) illustrates that the control and treatment groups demonstrated similar changing trends compared with Figure 2(a). ATPase activity was reduced when BPCE was added from 8 to 24 h. These results indicated the degradation of the cell membrane and inhibition of ATPase in the presence of BPCE. Liu et al. [27] observed the effects of  $\epsilon$ -polylysine and nisin on the changes in Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in *Bacillus subtilis* when

$\epsilon$ -polylysine and nisin were added, where Na<sup>+</sup>/K<sup>+</sup>-ATPase activity decreased. ATPase was inhibited when added in the presence of the  $\epsilon$ -polylysine and/or nisin. In addition, Wang et al. [24] demonstrated that chlorine dioxide can inhibit the ATPase activity of *Nosema bombycis* spores, which destroyed the inner structure of the spores. Thus, cell membrane dysfunction induces the depolarization of the cytoplasmic membrane and leads to a rapid termination of all biosynthetic processes [28].

**3.3. Key Enzymatic Activities in Glycolysis Pathway.** PK and HK are key enzymes in glycolysis, which is an important pathway for cellular energy metabolism and biosynthesis. The utilization of glycolytic enzymes also enables the controlled production of pyruvate and acetaldehyde, which are vital for synthesizing other biomolecules [29]. The effects of BPCE on the activities of HK and PK in *E. coli* and *S. aureus* cells are shown in Figure 3. For *E. coli*, although the HK activity of the treatment group was higher than that of the control, the final HK activity in treatment group was blocked when BPCE was added (Figure 3(a)). A similar variation was observed in the PK groups (Figure 3(b)). HK activity in the *S. aureus* treatment group slightly changed during the first 8 h, whereas the HK activity in the control group significantly increased within the initial 4 h and then decreased. Subsequently, the groups exhibited similar changing trends (Figure 3(c)). The PK activity slightly changed in the treatment group, whereas remarkable variations were observed in the control group. For bacterial growth, PK activity reached 424.2 U/g protein. A lower HK activity resulted in an impaired oxidation of glucose via glycolysis and decreased ATP production. The observed decline in PK activity in bacteria was responsible for the reduced glycolysis and amplified gluconeogenesis, indicating that these two pathways were disrupted [30]. These results suggested that BPCE affected glycolysis, which decreased the synthesis of energetic substances and vital intermediate materials, such as ATP and pyruvate.

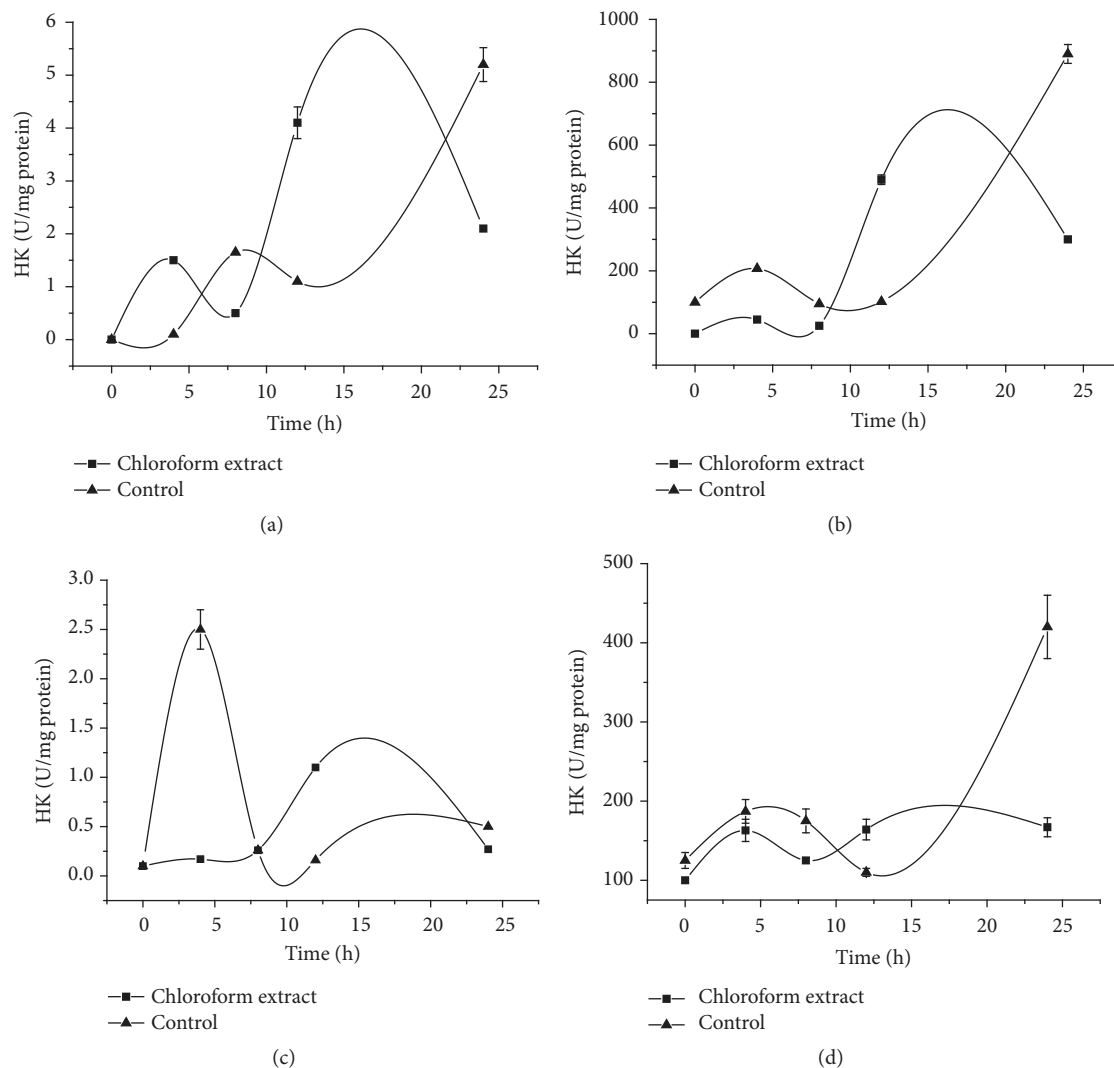


FIGURE 3: Activities of key enzymes in *E. coli* (a, b) and *S. aureus* (c, d).

**3.4. Lactic Acid Content.** LA is produced by glucose oxidation when available oxygen is insufficient for a microorganism to perform aerobic respiration. The LA content in the treatment group slightly changed when BPCE was added to both bacteria (Figures 4(a) and 4(b)). In comparison, the LA content in the control group increased initially before severely decreasing in *E. coli* cells (Figure 4(a)), which was similarly observed in *S. aureus*. However, the LA content of *S. aureus* decreased (Figure 4(b)). The large amount of LA in the treatment cells indicated that aerobic respiration was inhibited in the presence of BPCE, indicating that the TCA cycle was hindered. Thus, the bacterial cells obtained energy through anaerobic respiration to maintain their growth. Additionally, LA cannot normally accumulate in cells. However, LA only slightly decreased in our experiments. Moreover, high LA concentration inhibited bacterial cell growth, resulting in bacterial cell death [31, 32]. These findings were consistent with our previous report, in which BPCE treatment of cells led to the accumulation of pyruvate and inhibited the TCA cycle in bacterial cell respiration [14].

**3.5. GC-MS Analysis of the Chemical Composition of BPCE.** The results of a GC-MS analysis of the constituents of BPCE identified 152 chemical constituents (presented in Figure 5), and the 41 primary substances are presented in Table 1. The results revealed that 1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1 $\alpha$ ,4 $\alpha$ ,7 $\beta$ ,7 $\alpha$ , $\beta$ ,7 $\beta$ .)]- (8.39%) and 2-methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane (6.92%) were the two primary components of BPCE. In addition, BPCE was rich in olefinic acids [n-hexadecanoic acid (5.12%), trans-2-octadecenoic acid (4.90%), 10,13-octadecadienoic acid (4.89%), 6-octadecenoic acid (4.88%), 9,12-octadecadienoic acid (Z,Z)- (3.11%), 4-hexadecenoic acid (1.34%), cis-13-octadecenoic acid (0.90%), 13-eicosenoic acid (0.58%), and cis-2-dicarboxylic acid (0.84%)], alkenes, or oxygenated alkenes [cyclohexene (0.69%),  $\alpha$ -copaene (0.98%), caryophyllene oxide (4.11%), and alloaromadendrene oxide (3.53%)], esters [ethyl 6,9,12-hexadecatrienoate (0.81%), hexadecanoic acid methyl ester (0.91%), octadecanoic acid ethyl ester (0.92%), ethyl oleate (3.64%), ethyl cis-9, trans-11-octadecadienoate



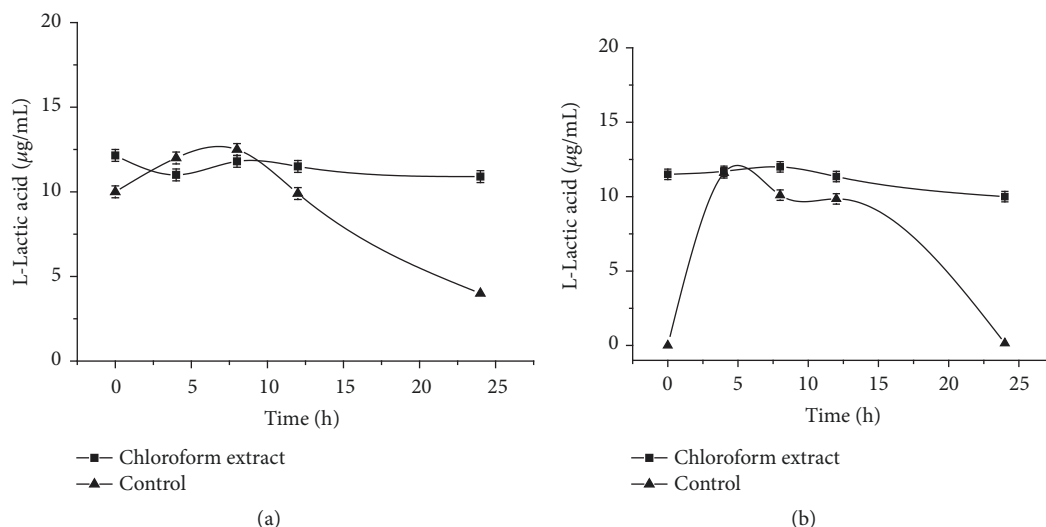


FIGURE 4: Lactic acid (LA) content in *E. coli* (a) and *S. aureus* (b).

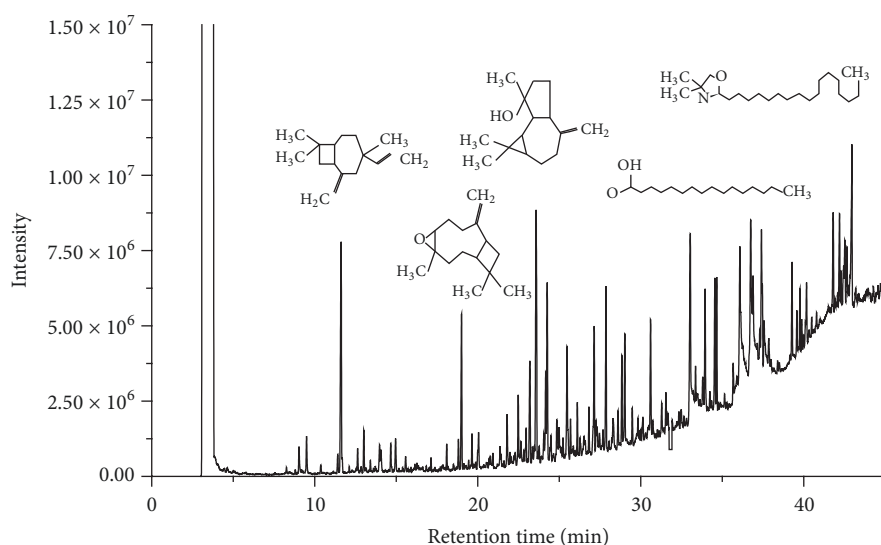


FIGURE 5: GC-MS profile of BPCE.

(4.00%), ethyl 9,12,15-octadecatrienoate (1.91%), isonipecotic acid, N-acryloyl-, undecyl ester (0.93%), and furan-2-carboxylic acid 2,2,6,6-tetramethyl-4-(2,2,2-trifluoro-acetylamino)-piperidin-1-yl ester (1.52%) and piperonal (3.06%). A previous study assessed the antimicrobial activity of the volatile oil from *Fusarium tricinctum*, containing 12.0% 2-methylene-4,8,8-trimethyl-4-vinyl bicyclo[5.2.0]nonane, against eight bacteria and two fungi [33]. In addition, some alkenes, such as caryophyllene oxide [34], alloaromadendrene oxide [35], and  $\alpha$ -copaene [36], showed strong antibacterial activity.

#### 4. Conclusion

The results of this study showed that 1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1 $\alpha$ ,4 $\alpha$ ,7 $\beta$ ,7 $\alpha$ , $\beta$ ,7 $\beta$ .)]- (8.39%) and 2-methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane (6.92%) were the two primary

components of BPCE. The antibacterial activity of BPCE primarily occurred via two pathways. The first pathway was the alteration of membrane potential and inhibition peptide or protein synthesis. The second pathway involved the hindering of metabolic pathways, such as glycolysis and the TCA cycle. Based on the results, the following antibacterial mechanism of BPCE is proposed: BPCE rapidly destroys bacterial cell walls and membranes and decreases the ATPase level. This phenomenon resulted in a remarkably rapid loss in cell contents, such as transaminase proteins, polysaccharides, and ions. The active ingredients of BPCE entered the cells and interacted with key glycolysis enzymes, eventually hindering and disrupting cell metabolism. Furthermore, an accumulation of LA indicated that bacteria obtained ATP through anaerobic respiration, probably because BPCE blocked the TCA cycle and weakened the electron transport chain to generate ATP. The results of the current investigation will facilitate the development of antibacterial agents targeting

TABLE 1: The chemical components of BPCE.

NO.	Name of compound	RT (min)	Area%
(1)	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-	8.959	0.69
(2)	$\alpha$ -Copaene	9.406	0.98
(3)	2-Methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane	11.622	6.92
(4)	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	13.010	0.97
(5)	1-Piperidinecarboxaldehyde	14.959	0.93
(6)	Caryophyllene oxide	19.006	4.11
(7)	Ethyl 6,9,12-hexadecatrienoate	20.051	0.81
(8)	1,3,3-trimethyl-2-Oxabicyclo[2.2.2]octan-6-ol	22.482	1.60
(9)	Hexadecanoic acid, methyl ester	22.968	0.91
(10)	Piperonal	23.193	3.06
(11)	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a $\alpha$ ,4a $\alpha$ ,7 $\beta$ ,7a, $\beta$ ,7b $\alpha$ .)]-	23.584	8.39
(12)	4,4-dimethyl-tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol	24.167	1.29
(13)	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	24.262	3.94
(14)	Alloaromadendrene oxide-(1)	25.477	3.53
(15)	(+)-1-Isopropylcyclopropane-trans-1,cis-2-dicarboxylic acid	25.689	0.84
(16)	Octadecanoic acid, ethyl ester	26.821	0.92
(17)	Ethyl oleate	27.144	3.64
(18)	Ethyl 9.cis.,11.trans.-octadecadienoate	27.871	4.00
(19)	Vanillin lactoside	28.302	0.83
(20)	n-Tetracosanol-1	28.600	0.83
(21)	Ethyl 9,12,15-octadecatrienoate	28.840	1.91
(22)	Phytol	29.020	2.45
(23)	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7beta.,7a.beta.,7b.alpha.)]-	30.599	2.81
(24)	n-Hexadecanoic acid	33.037	5.12
(25)	Naphthalene, decahydro-2,2-dimethyl-	33.36	0.63
(26)	1-(1-(2-Thienyl)cyclohexyl)pyrrolidine	33.948	2.85
(27)	(8R,Z)-8-Methyl-6-((R)-2-methylpentylidene)octahydroindolizine	34.544	3.17
(28)	2-Ethyl-5-undecyl-. $\delta$ .1- -pyrroline	34.662	3.02
(29)	6-Octadecenoic acid	36.080	4.88
(30)	9,12-Octadecadienoic acid (Z,Z)-	36.744	3.11
(31)	(3S,5R,7aS)-3-(But-3-en-1-yl)-5-(hex-5-en-1-yl)hexahydro-1H-pyrrolizine	36.889	1.12
(32)	Piperidine, 1-(1-oxo-3-phenyl-2-propenyl)-	37.403	1.62
(33)	2-Cyclohexen-3-ol-1-one, 2-[1-iminotetradecyl]-	39.279	2.21
(34)	Isonipecotic acid, N-acryloyl-, undecyl ester	39.574	0.93
(35)	4-Hexadecenoic acid, pyrrolidide	39.763	1.34
(36)	cis-13-Octadecenoic acid, 4,4-dimethyloxazoline derivative	40.171	0.90
(37)	trans-2-Octadecenoic acid	41.804	4.90
(38)	13-Eicosenoic acid, pyrrolidide	42.444	0.58
(39)	Furan-2-carboxylic acid 2,2,6,6-tetramethyl-4-(2,2,2-trifluoro-acetyl-amino)-piperidin-1-yl ester	42.520	0.88
(40)	4,5,6,7-Tetrahydrobenz[z]isoxazole-5-ol-4-one, 3-[9-tridecenyl]-	42.650	1.52
(41)	10,13-Octadecadienoic acid	42.950	4.89

bacterial energy metabolism. However, further studies are needed to determine whether antibacterial agents affect the transcriptome. The experimental results also provided a basis to develop promising natural antimicrobial agents with potential applications in manufacturing industries.

### Additional Points

**Highlights.** (1) The antimicrobial mechanism of action of black pepper chloroform extract (BPCE) was investigated. (2) Bacterial intracellular transaminases and  $\text{Na}^+/\text{K}^+$ -ATPase

activity were inhibited by BPCE. (3) Bacterial cell walls and membranes were degraded in response to BPCE. (4) The normal metabolism of bacteria was disrupted by BPCE.

## Disclosure

The authors alone are responsible for the content and writing of the paper.

## Conflicts of Interest

The authors report no conflicts of interest.

## Authors' Contributions

Wenxue Chen and Haiming Chen conceived and designed the experiments; Lan Zou performed the experiments; Yueying Hu and Weijun Chen analyzed the data; Wenxue Chen and Haiming Chen wrote the paper.

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