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Antibacterial Activity of Essential Oils Extracted From the Unique Chinese Spices Cassia Bark, Bay Fruits, and Cloves

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Abstract

Spices are widely used in daily life such as diet and have certain activity. Especially in China, spices have been mainly used as condiments for thousands of years in order to improve the sensory quality of food; in addition, they and their derivatives can also be used as preservatives. In this study, three species with Chinese characteristics unique and widely used by the public were selected: cassia bark (bark of Cinnamomum camphoraPresl), bay fruits (Laurus nobilis), and cloves (Syzygiumaromaticum). The main components and antibacterial ability of these three spices were analyzed by simulated extraction method. Through headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) analysis, it was determined that the main active compounds in the essential oils of cassia bark, bay fruits and cloves were cinnamaldehyde (78.11%), cinnamaldehyde (61.78%), and eugenol (75.23%), respectively. The agar plate diffusion test and the simulated food culture medium experiment confirmed that the essential oils extracted from the three flavors have antibacterial effects on *Vibrio parahaemolyticus*, *Listeria monocytogenes* and 4 kinds of *Listeria*. The antibacterial activity of different strains has different optimal extraction conditions. Generally speaking, cinnamon essential oil has the strongest antibacterial activity, while laurel fruit has the lowest antibacterial activity. The study proved the antibacterial activity of these three Chinese-specific spices, and provided some new ideas and methods for the subsequent research and preparation of natural food additives and food antibacterial agents.

Introduction

Many minimally processed foods are added with preservatives to extend their shelf lives, but consumers have questioned the safety of chemical additives and demanded for the removal of chemical additives from foods(Masri et al., 2021). This has resulted in an increasing interest in searching for preservatives from natural sources that can be used in foods to improve quality and safety(Calvo et al., 2021; da Silva et al., 2021; Pandey et al., 2021).

Vibrio parahaemolyticus is a Gram-negative bacterium which causes acute gastroenteritis in humans consuming contaminated seafoods(Yang et al., 2019). Since a great number of food-poisoning incidents were caused by *V. parahaemolyticus*, efforts are needed to enhance the safety of seafood(Rui et al., 2019; Zhiwei et al., 2019). *Listeria monocytogenes* is widely present in the environment owing to its versatile physiological adaptation mechanisms. Due to it high tolerance to adverse environmental conditions, *L. monocytogenes* is frequently found in food-processing environments and minimally processed foods(Pasonen et al., 2019; A et al., 2019; Wu et al., 2020). Moreover, its ability to grow at refrigeration temperatures increases the risk of foodborne illnesses caused by refrigerated foods contaminated with *L.monocytogenes*. Therefore, it is important to control the growth *L. monocytogenes* in refrigerated minimally processed foods.

The development of bacterial resistance to antibiotics is a serious problem. Reports of antibiotic-resistant bacteria causing increased morbidity and mortality have been published in the past two decades(Noman et al., 2021; Lv et al., 2021). There is a great concern of the overuse of antibiotics and the dissemination of multi-resistant bacteria through the food chain, trade and human migration(Ding et al., 2021; Mahros et al., 2021). Spices and herbs can be considered essential and natural components of the human diet; they not only impart food taste and flavor but also have considerable beneficial physiological effects on human health, such as antimicrobial(Mendes et al., 2017; Otunola and Afolayan, 2018; Qing et al., 2017), anti-inflammatory(Serafini and Peluso, 2016; Zhang et al., 2016; Ashktorab et al., 2019; Chin, 2016), and antioxidant effects(Mendes et al., 2017; Sepahpour et al., 2018; Yashin et al., 2017).The use of spices or their derivatives as antimicrobials in food products provide an alternative to the use of chemical preservatives in foods for shelf life and safety purposes(Tshabalala et al., 2021; El-Sayed and Youssef, 2019).

Cassia, which is called Chinese cinnamon (*Cinnamomum cassia* Presl), is a small evergreen tree native to southern China. The bark and twigs of *Cinnamomum* plant have long been used as a source of aromatic spices worldwide (HelmyAbdou et al., 2019; Zhang et al., 2019). Laurus nobilis is an evergreen tree that can grow up to 8 m in height and widely cultivated in southern China. Bay leaf extracts have been investigated for their wound healing, cytotoxic, and trypanocidal properties (Fidan et al., 2019), and bay fruits are commonly used as a folk medicine in China. Cloves are the aromatic dried flower buds of *Syzygiumaromaticum* in the family *Rhodomyrtus tomentosa*. It is one of the most used and known spices in the world. Although its use in the Western countries is mainly limited to the flavoring and preservation of foods, cloves have been long used for its drug-like properties in Asian countries(Kheawfu et al., 2018; Juan et al., 2019; Tshabalala et al., 2021). The aim of this work was to determine the antimicrobial activity of essential oils extracted from the three spices, which are widely cultivated in China, and identify the active antimicrobial compounds in the essential oils.

Materials And Methods

Plant materials and chemicals culture media.

Cassia bark, bay fruits, and cloves, which were harvested in Guiping Country of Guangxi Province, Chuxiong Country of Yunnan Province and Qingyun Country of Shandong Province, respectively. The three spices were obtained as a commercial product in the market of Shanghai. Samples were kept in dark at 25℃. Brain heart infusion broth (BHIB) was purchased from Oxoid (Basingstoke,UK). Tryptone soy broth (TSB), tryptic soy agar (TSA), and nutrient agar (NA) were obtained from Beijing Land Bridge Technology Company (Beijing, China).

Microorganisms.

The antimicrobial activities of the essential oils were tested against six different bacteria; *L. monocytogenes* ATCC19117 (LM), *L. innocua* ATCC33090 (LI), *L. welshimeri* ATCC43548 (LW), *L. ivanovii* ATCCBAA-678 (LL), *L. grayi* ATCC25400 (LG) and *V. parahaemolyticus* ATCC33847 (VP). These strains were maintained in glycerol broth at −80℃. Frozen cultures of *Listeria* spp. and *V. parahaemolyticus* were revised in 8 ml of BHIB and TSB added with 2% NaCl (2% NaCl TSB), respectively, and incubated overnight at 37℃ in a rotary shaker (180 rpm) (Huang et al., 2020). Subculture the culture again by inoculating 0.1 ml of overnight culture in BHI or 2% NaCl TSB, and incubate overnight at 37°C in a rotary shaker (180 rpm) to a final population of approximately 108 CFU/ml.

Extraction of Essential Oil and Optimal Extraction Parameters.

Four hundred grams of the three spices were washed with tap water twice, air-dried in a ventilated oven at 50℃ for 24 h, and then ground into fine powder using a commercial blender (Waring Laboratory, Torrington, CT, USA). Spice powder was screening by passing through a sieve (60-mesh) and stored in polythene bags at 4℃(Bajalan et al., 2017).

The extracts of the spices were obtained with the aid of ethanol solutions and an ultrasound machine. The extraction parameters were examined to find an optimal extract conditions that yielded extracts that had the highest antimicrobial activity against *L. monocytogenes* and *V. parahaemolyticus*, respectively. The extract parameters included the ratio of spice to ethanol, ethanol concentration, extraction temperature, extraction time, and the power level and time of the ultrasound extraction. Table 1 summarizes the ranges of variables investigated. Five grams of each powdered spice were added to 125 ml of 50% ethanol in a 250 ml Erlenmeyer flask. The flask was sealed with a polypropylene cap and the extraction was performed on at 40℃ for 3 h in a rotary shaker (180 rpm). Ultrasound-assisted extraction was conducted in an ultrasonic processor (Ningbo Scientz Biotechnology Co., Ningbo, China) with

the selected powder and sonicated for 25 min. The extractives were centrifuged at 3500×g for 10 min and the supernatant was recovered after filtering through a 0.45μm filter paper (Whatman No. 1).The supernatant solvent in the supernatants was evaporated in a rotary evaporator (Shanghai SENCO Technology Co., Shanghai, China) under vacuum (30 mmHg) at room temperature to recover essential oils. The essential oil was dissolved in sterile Milli-Q water to achieve the essential oil concentration at 100 mg/ml. The essential oils were stored in airtight, sterile vials at 4℃. The antimicrobial activity of essential oils obtained from different extraction parameters were evaluated using agar-disc diffusion assay (with some modifications. *Listeria* spp. and *V. parahaemolyticus* (0.1ml at 108 CFU/ml) was spread with a sterile swab on TSA (for *Listeria* spp.) or 2% NaCl TSA (for *V. parahaemolyticus*) plates and allowed to absorb for 15 minutes. Three disks of Whatman No. 1 sterile filter paper, 6 mm in diameter, impregnated with each essential oil (100 mg /ml) were placed on the inoculated agar surfaces. Disks with Milli-Q water were used as control. The plates were incubated at 37℃ for 24 h and examined for zones of inhibition. A positive antibacterial activity was recorded when an inhibition zone was greater than 6 mm(Chen et al., 2019).

Table 1
The ranges of variables investigated.

Parameters	Ranges
Spice to ethanol ratio	1:5,1:10,1:15,1:20,1:25
Ethanol concentration (%, v/v)	10%,30%,50%,70%,95%
Extraction temperature	40℃, 50℃, 60℃, 70℃, 80℃
Extraction time (h)	0, 2, 4, 6, 8
Ultrasonic power (W)	200, 250, 300, 350,400
Ultrasonic time (min)	15, 30, 45, 60, 75

Antimicrobial Activity of Essential Oils

To confirm the antimicrobial activity of the essential oils in a medium that simulated food products, the essential oils were tested against the six bacterial strains in TSB. Each essential oil at 0.5 ml (100 mg /ml) was added into each test culture of 9.5 ml (10^8 CFU/ml) (1:10, corresponding to 10 mg essential oil/ml). The control consisted of 0.5 ml Milli-Q water and 9.5 ml cultures. The cultures were incubated at 37℃ for 16 h in a rotary shaker (180 rpm). After incubation, 1 ml of culture was serially diluted in 9 ml sterile 0.1% peptone water, and 0.1ml of appropriate dilutions was spread in duplicate onto TSA, 2%TSA, PALCAM and TCBS to enumerate viable *Listeria* spp., viable *V. parahaemolyticus*, uninjured *Listeria* spp. and *V. parahaemolyticus*, respectively. The experiment was performed three times.

Isolation and analysis of the volatile compounds of the spice essential oil by headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS).

After several preliminary tests to optimize the extraction system, 4 ml of essential oil and 100μl of 250 g/L NaCl (to favor the transfer of the analytes from the aqueous solution to the headspace) were placed in a 15 ml glass vial with a polypropylene screw-on cap and a polytetrafluoroethylene/silicone septum (Supelco, Bellefonte, PA, USA) to create an airtight seal. The volume ratio of the essential oil to headspace was approximately 1:3. The glass jar was placed in a water bath with temperature control and agitated with a stir bar. The headspace in glass vials were equilibrated with the volatile compounds at 55℃ for 5 min in the bath. Headspace volatiles of crude extraction were isolated

using a 50/30 µm Divinylbenzene/CARBOXEN/Polydimethylsiloxane fiber (Supelco) inserted through the septum and exposed in the headspace for 30 min at 55℃ to allow absorption of the volatile compounds. The fibers were selected for its high capacity of trapping volatile compounds and were conditioned for 30 min at 250℃ before use. After sampling, desorption of the volatile compounds from the fiber coating was carried out in the injection port of a gas chromatography-mass spectrometry (GC-MS) during 5 min in split mode set at 250℃(Bajpai et al., 2013).

The identification of volatile compounds was performed using an Agilent 6890N Network GC system combined with an Agilent 5975 B Inert MSD detector (quadruple) in the electron impact mode (70 eV). The GC-MS system was equipped with a SLB-5ms capillary column (Supelco), 95% dimethyl siloxane and 5% diphenyl (60m ×250µm i.d. ×0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1.0 ml/min with a pressure of 15.60 psi and the following program: (a) 65℃ for 5 min; (b) rate of 4.0 ml/min from 40 to 100℃ and hold for 5 min; (c) rate of 2 ml/min from 100 to 150℃ and hold for 2 min; (d) rate of 5 mL/min from 150 to 190℃ and hold for 2 min; (e) rate of 15 mL/min from 190 to 250℃ and hold for 2 min. Detector was held at 270℃.

The compounds were identified by comparing their Kovats indices (KI), GC retention times (authentic chemicals), NIST mass spectral search program (version 2.0, National Institute of Standards and Technology) and mass spectra of published data. For each compound, quantification was performed by measuring the corresponding peak area of the total ion chromatogram and expressed as relative (percent) areas by normalization.

Statistical analysis.

All experiments were repeated three times. Analysis of variance (ANOVA) was performed on zone of inhibition and cell counts using SPSS 15.0 (SPSS Inc., Chicago, Ill., U.S.A.). The level of significance of was set at $\alpha = 0.05$.

Results And Disscussion

Optimal Extraction Parameters.

The antimicrobial efficacy of the essential oils obtained from different extract conditions against *V. parahaemolyticus* and *Listeria* spp. are shown in Table 2. All of the essential oils showed antimicrobial activity. Generally, essential oil of cassia bark showed highest antimicrobial activity ($p<0.05$) followed by bay fruits and cloves. *V. parahaemolyticus* was more sensitive than *Listeria* spp. to the three essential oils. In particular, the inhibition diameters of cassia bark spice essential oils ranged from 9.1 to21.0 mm for *V. parahaemolyticus*. There were only 12 crude extracts, which were significantly different ($P < 0.05$) from the other essential oils as well as the control. The optimum extraction conditions for obtaining essential oils with the highest antimicrobial potentials are listed in Table 2. For the experimental parameters, we chose the value of an experimental variable that gave the maximal mean zones of inhibition against the tested strains, significantly different from other values. Furthermore, we considered the efficiency of different values of experimental parameters. Table 3 shows the optimal extraction conditions of the three spices against the tested strains.

Table 3
The optimum extraction conditions of the three spices to every tested strains

ID	Spice	Extraction conditions						Tested Strain
		Spice to Ethanolratio	Ethanol concentration	Extraction temperature	Extraction time	Ultrasonic power	Ultrasonic time	
LM1	cassia bark	100 ml	50%	40 °C	2 h	250 W	15 min	LM
LI1		100 ml	50%	40 °C	2 h	200 W	30 min	LI
LW1		100 ml	50%	40 °C	2 h	200 W	15 min	LW
LL1		100 ml	50%	40 °C	2 h	250 W	30 min	LL
LG1		100 ml	50%	40 °C	2 h	250 W	15 min	LG
VP1		50 ml	95%	60 °C	2 h	250 W	15 min	VP
LM2	bay fruits	75 ml	50%	40 °C	2 h	250 W	15 min	LM
LI2		100 ml	50%	70 °C	2 h	200 W	30 min	LI
LW2		75 ml	50%	40 °C	2 h	250 W	30 min	LW
LL2		100 ml	50%	40 °C	4 h	250 W	15 min	LL
LG2		100 ml	50%	40 °C	2 h	300 W	75 min	LG
VP2		50 ml	70%	40 °C	2 h	250 W	15 min	VP
LM3	cloves	50 ml	50%	40 °C	2 h	200 W	15 min	LM
LI3		75 ml	50%	40 °C	2 h	200 W	15 min	LI
LW3		75 ml	50%	40 °C	2 h	200 W	60 min	LW
LL3		100 ml	70%	40 °C	2 h	200 W	15 min	LL
LG3		125 ml	50%	40 °C	2 h	200 W	30 min	LG
VP3		125 ml	50%	40 °C	2 h	200 W	15 min	VP

Antimicrobial Activity of Essential Oils.

The antimicrobial activity of the three essentials oils obtained with the optimum extraction parameters against the six bacterial are listed in Fig. 1.Generally, the antimicrobial activity of essential oil of cassia bark was more significant than the essential oils of bay fruits and cloves. The inhibitory values were different; that were evaluated with non-selective agar (TSA) and selective agar (TCBS and PALCAM). The different results of non-selective agar and selective agar showed there were some injured bacteria that might be repaired on non-selective agar. The essential oil of cassia bark with VP1 extraction condition had the highest antimicrobial activity against *V. parahaemolyticus* and can reduce 8.3 log CFU/ml evaluated with TCBS agar. The essential oil of bay fruits with VP2 extraction condition had the lowest antimicrobial activity against *L. innocua* and only reduced the bacterial count by 0.13log CFU/ml when evaluated with TSA agar.

Most of the antimicrobial activity of the essential oil using the six extraction conditions against each strain was significantly different. As seen in Table 4, the extraction condition had more of an effect on the antimicrobial activity of cassia bark and cloves extracts against bacteria. The strains *L. grayi* ATCC25400 and *V. parahaemolyticus* ATCC33847 are more sensitive to all of the extracts, even with different extraction conditions. The results of in vitro susceptibility of crude extracts of the three spices have antibacterial activity against *V. parahaemolyticus* and *Listeria* spp..

Table 4
Analysis of the common volatile compounds of ethanol extract of the spices with optimal extraction conditions against six bacterial as determined using headspace solid-phase microextraction (HS-SPME) and gas chromatography coupled to mass spectrometry (GC–MS) methods[a]

No	Name of the compound		
	Cassia bark	Bay fruits	Cloves
11 1	Benzenepropanal	2-Propenal, 3-phenyl-	Phenol, 4-(2-propenyl)-
2	2-Propenal, 3-phenyl-	Copaene	2-Propenal, 3-phenyl-
3	2-Propen-1-ol, 3-phenyl-	Caryophyllene	Eugenol
4	2-Propen-1-ol, 3-phenyl-, acetate	2H-1-Benzopyran-2-one	Phenol, 2-methoxy-4-(1-propenyl)-, (E)-
5	eugenol,	2-Propenoic acid, 3-phenyl-, ethyl ester	à-Caryophyllene
6	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1 -(1-methylethyl)-, (1à,4aà,8aà)-	à-Farnesene
7	Isolongifolene, 4,5,9,10-dehydro-	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
8	à-Calacorene	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1à,4aà,8aà)-	2,3,3a,4,5,6,7,7a-Octahydro-1H-cyclopenta[a]pentalen-7-ol
9	(-)-Spathulenol	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	Caryophyllene oxide
10	Cubenol	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1à,4aá,8aà)]-	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-
11	tau.-Muurolol	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	Cyclooctasiloxane, hexadecamethyl-
12	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1à,4á,4aá,8aá)]-	à-Calacorene	Tetracyclo[6.3.2.0(2,5).0(1,8)] tridecan-9-ol, 4,4-dimethyl-
13	à-Cadinol	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	Caryophyllene oxide
14	3-Cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	Murolan-3,9(11)-diene-10-peroxy	Benzyl Benzoate
15	à-Bisabolol	Caryophyllenyl alcohol	
16		(-)-Spathulenol	

No	Name of the compound		
	Cassia bark	Bay fruits	Cloves
17		Isoaromadendrene epoxide	
18		Globulol	
19		3,7-Cycloundecadien-1-ol, 1,5,5,8-tetramethyl-	
20		Cubenol	
21		4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	
22		à-Cadinol	
23		1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1à,4á,4aá,8aá)]-	
24		2-Furanmethanol, tetrahydro-à,à,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2à,5á(R*)]]-	
25		3-Cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	
26		Longiverbenone	
27		à-Bisabolol	
28		Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	

[a]The table listed the common components of each spice. The peak area, peak (%) and RT(retention time) (min) of the same components of each spice with different extraction conditions were different.

Volatile compounds in Essential Oils.

There were 15, 29 and 14 common compounds isolated and identified from the essential oils of cassia bark, bay fruits, and cloves, respectively (Table 4). Only 3-Phenyl-2-propenal (cinnamaldehyde) was the common compound among the three spices.

The peak area, peak (%) and RT (min) of same compound using different extraction conditions were different for each spice (Fig. 2). The main common compounds were found in the same spice with different extraction conditions. The major components in essential oil of cassia bark (*Cinnamomum camphora*Presl) were 3-phenyl-2-propenal (cinnamaldehyde), 3-phenyl-2-propen-1-ol acetate, eugenol, cubenol and tau.-muurolol. The relative amounts in LG1 extracts were 78.11%± 2.65%, 4.11% ± 2.53%, 2.73% ± 0.28%, 2.55%± 1.42% and 1.31% ± 0.30%, respectively. The main common compounds of bay fruits (Laurus nobilis) extracts were 3-phenyl-2-propenal (cinnamaldehyde), cubenol, à-Cadinol and 1,2,4a,5,6,8a-Hexahydro-4,7-dimethyl-1-(1-methylethyl) naphthalene. The content in LW2 extracts were 61.78% ± 15.58%, 2.73% ± 0.85%, 2.65% ± 0.38% and 2.24% ± 1.65%, respectively. The main common compounds of cloves extracts were eugenol, eugenol acetate[Phenol, 2-methoxy-4-(2-propenyl)-acetate] and caryophyllene. The content in LM3 extracts were 75.23% ± 4.55%, 17.01% ± 1.33% and 4.33% ± 3.15%, respectively.

Conclusion

In this study, essential oils were extracted from China's unique spices, cassia bark, bay fruits and cloves, which are widely grown and used as condiments. This study extracted the essential oils from cassia bark, bay fruits, and cloves, which are widely cultivated and used as condiments. The results show that all of these have certain antibacterial activity on *Vibrio parahaemolyticus*, *Listeria monocytogenes*, and 4 types of *Listeria*. Among the three spices, cassia bark showed the greatest antimicrobial activity against *V. parahaemolyticus* and *Listeria* spp., while bay fruits had the lowest antimicrobial activity. Although the major component of both cassia bark and bay fruits essential oils was cinnamaldehyde, the antimicrobial activity of the extracts was significantly different between the two spices. The antimicrobial activity of cinnamaldehyde and eugenol have been previously demonstrated (Dhara and Tripathi, 2013; Anna et al., 2017; Liu et al., 2014).Therefore, extracts of cassia bark, bay fruits, and cloves could be an effective antimicrobials for controlling the growth of pathogenic microorganisms in foods.

Each spice has its own beneficial property to human health and the synergic effects of different spices can increase the potential properties (Yashin et al., 2017; Alan, 2019).Studies have reported the synergistic effects of using several essential oils in combination improves antimicrobial effects without raising their concentrations(Hussein et al., 2016; Bulent and W, 2017).While the antimicrobial activities of crude extracts of various spices have been studied, it is necessary to subdivide and purify the contents of the crude extracts, and determine the main active component as well as the underlining antimicrobial mechanism. Additional studies are needed to identify the active compounds mode of action and toxicity.

Declarations

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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Table

Table 2 is available in the Supplemental Files section.

Figures

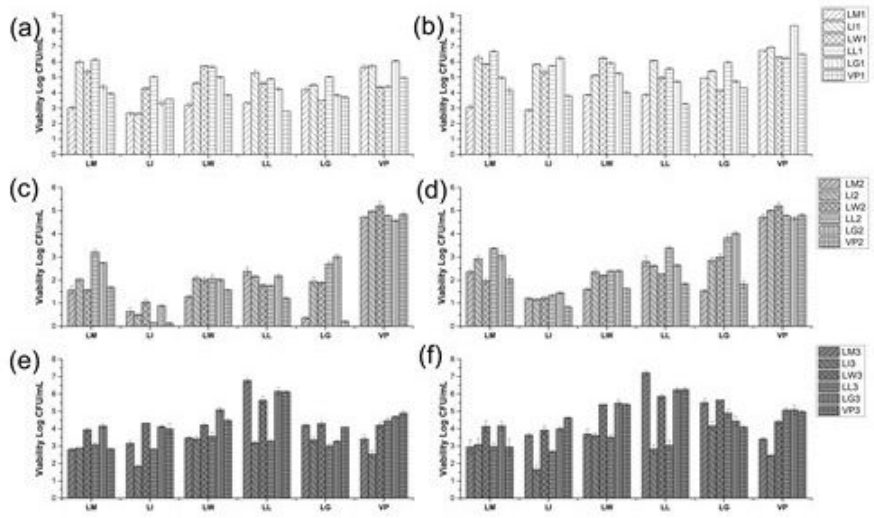

Figure 1

Effects of essentialoils on the viability of *V. parahaemolyticus* and *Listeria* spp. (a) strains cultured on nonselective medium with Cassiabark; (b) strains cultured on selective medium with Cassiabark; (c) strains cultured on nonselective medium with bay fruits; (d) strains cultured on selective medium with bay fruits; (e) strains cultured on nonselective medium with cloves; (f) strains cultured on selective medium with cloves.

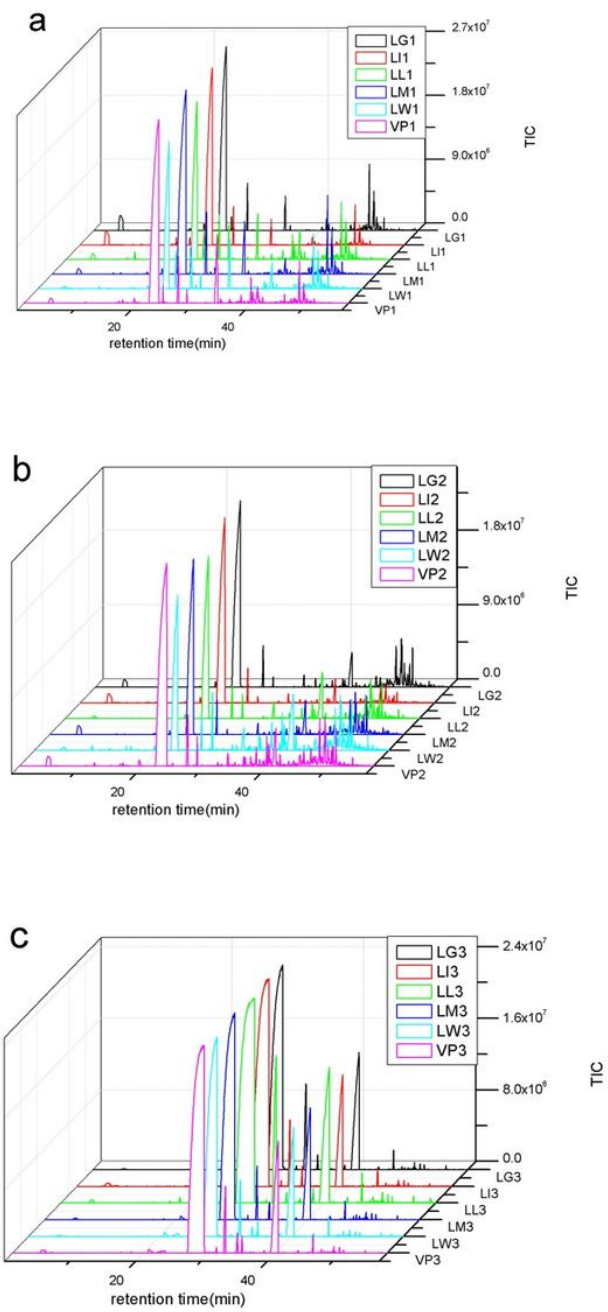

Figure 2

GC/MS spectrogram for the three spices extractedby the optimal extraction conditions against six tested strains. a, spectrogram of the cassia bark extracts; b,spectrogram of the bay fruits extracts;c,spectrogram of the cloves extracts.

Supplementary Files

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- Table2.docx