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# Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*

Yunbin Zhang [a,*], Xiaoyu Liu [a], Yifei Wang [a], Pingping Jiang [a], SiewYoung Quek [b]

[a] *School of Perfume and Aroma Technology, Shanghai Institute of Technology, Shanghai 201418, China*
[b] *Food Science Programme, School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*

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ABSTRACT

Cinnamon essential oil (EO) exhibited effective antibacterial activity against foodborne spoilage and pathogenic bacteria in model systems using *Escherichia coli* and *Staphylococcus*. The minimum inhibition concentration (MIC) of cinnamon EO was similar for both bacteria (1.0 mg/ml) while the minimum bactericide concentration (MBC) were 4.0 mg/ml and 2.0 mg/ml for *E. coli* and *Staphylococcus aureus*. GC–MS analysis confirmed that cinnamaldehyde was the major constituent in cinnamon EO (92.40%). Much effort was focused on elucidating the mechanism of antibacterial action of cinnamon EO against *E. coli* and *S. aureus* by observing the changes of cell microstructure using scanning electron microscope, determination of cell permeability, membrane integrity and membrane potential. After adding cinnamon EO at MIC level, there were obvious changes in the morphology of bacteria cells indicating cell damage. When cinnamon EO were added at MBC levels, the cells were destroyed. Cinnamon EO led to leakage of small electrolytes, causing rapid increase in the electric conductivity of samples at the first few hours. The values for *E. coli* and *S. aureus* reached 60% and 79.4% respectively at 7 h. Moreover, the concentration of proteins and nucleic acids in cell suspension also rose with increased cinnamon EO. Bacterial metabolic activity was decreased 3–5 folds as reflected by the results of membrane potential. Overall, *S. aureus* was more susceptible to cinnamon EO than *E. coli*.

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## 1. Introduction

Food safety is critically related to public health and this area has received increasing attention in recent years. The emergence of new foodborne disease outbreaks caused by foodborne spoilage and pathogenic bacteria is one of the major food safety challenges (Arques, Rodriguez, Nunez, & Medina, 2008; Aslim & Yucel, 2007). Literature have reported that food contaminated with pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* represents a serious health risk to human being. *E. coli* presents in human intestines and causes urinary tract infection, coleocystitis or septicaemia. *S. aureus* is mostly accountable for food poisoning, toxic shock syndrome, endocarditis and post-operative wound infections (Doyle, 2013; Rahman & Kang, 2009; Singh, Chandra, Bose, & Luthra, 2000).

Chemical synthetic preservative has been used in food industry over the last few decades (Tian et al., 2014). However, the use of these artificial chemical compounds in controlling food spoilage and pathogenic bacteria has been a controversial topic. They have been reported to cause respiratory disease or other health risks (Fleming-Jones & Smith, 2003). As a result, there is a necessity to find novel and safe natural antibacterial agents to extent shelf-life of foods (Fisher & Phillips, 2008; Tajkarimi, Ibrahima, & Cliver, 2010).

Herbs and spices have been applied to improve the flavor of foods due to their well-documented sensory properties (Vallerdu-Queralt et al., 2014) and good preservative attributes since early years (Bakkali, Averbeck C, Averbeck D & Idaomar, 2008; Kivilompolo & Hyotylainen, 2007; Park, 2011). Essential oils (EOs) can be extracted from various aromatic plants including herbs and spices as they are synthesized by these plants (Tajkarimi et al., 2010). Spices and their EOs have been used as natural preservatives, to produce wholesome food products, for extension of shelf-life and to reduce pathogenic bacteria (Burt, 2004; Simitzis et al., 2008). Many researchers have stated the antibacterial

* Corresponding author. Shanghai Institute of Technology, Fengxian district, Haiquan Road 100, Shanghai, China.
*E-mail address:* ybzhangsit@163.com (Y. Zhang).

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activity of EOs extracted from spices and plants. For examples, carvacrol combined EOs show antibacterial activity against *Bacillus cereus* in milk; *Listeria monocytogenes* is sensitive to rosemary EO; clove oil exerts the inhibiting action on *Salmonella enteritidis* in diluted low-fat cheese; eugenol and coriander, oregano and thyme oils have good inhibiting effects on *L. monocytogenes* and *A. hydrophila* in meat products (Burt, 2004; Tajkarimi et al., 2010).

Cinnamon belongs to the Lauraceae family and the genus of *Cinnamomum* which comprises of about 250 species. Cinnamon is also a traditional herbal medicine that is widely distributed in China, India and Australia (Jayaprakasha, Rao, & Sakariah, 2003). It has been applied in food, seasonings, cosmetics and medical industries because of its antimicrobial, antioxidant and anticarcinogenic activities (Li, Kong, & Hong, 2013; Thomas & Kuruvilla, 2012; Todd, Friedman, Patel, Jaroni, & Ravishankar, 2013; Wang, Wang, & Yang, 2009; Yu, Lee, & Jang, 2007). The antimicrobial activity of both cinnamon EO and its major composition had been previously evaluated (Du et al., 2009; Matan et al., 2006; Pranoto, Salokhe, & Rakshit, 2005). However, to our knowledge, there was little data available explaining the mechanism behind the antibacterial action of cinnamon EO against foodborne spoilage and pathogenic bacteria in detail. Our study aimed to investigate the antibacterial property of cinnamon EO by adopting the mechanistic approach to provide fundamental understanding on the mode of antibacterial action.

## 2. Materials and methods

### 2.1. Microorganisms and chemicals

The Gram-positive bacteria *S. aureus* ATCC 25923 and Gram-negative bacteria *E. coli* ATCC 25922 were obtained from Shanghai Institute of Technology and maintained in slants of nutrient agar (NA) at 4 °C. All microorganisms were cultured in nutrient broth (NB) at 37 °C for 24 h. Tween 80, n-alkanes (C5–C28) and Rhodamine123 were purchased from Sigma–Aldrich (Shanghai) Trading Co. Ltd. NA and NB were purchased from Qing Dao Hope Bio-Technology Co. Ltd. (China). All other chemicals used in the study were of analytical grade.

### 2.2. Essential oil

Cinnamon EO was purchased from Pulan Trading Co. Ltd. (Shanghai, China), which was produced by steam distillation. The oil was stored at 4 °C before analysis.

### 2.3. Gas chromatography-mass spectrometry analysis of cinnamon EO

The GC–MS analysis of cinnamon EO was performed on an Agilent 7890A GC apparatus with a 5975C mass spectrometer detector. A HP-INNOWax 19091N-136 silica capillary column (60 m × 250 μm; film thickness 0.25 μm) was used. The operating conditions were as follows: carrier gas: helium, at a flow rate of 1 ml/min; split ratio 1:10; injector and detector temperatures: 250 °C; sample size: 1 μL of cinnamon EO, manual injection; oven temperature program: 40 °C as an initial temperature, 1 min isothermal raised to 230 °C at a rate of 4 °C/min, then isothermal at 230 °C for 10 min; ion source temperature: 230 °C; energy ionization: 70 eV; electron ionization spectra with a mass scan range of 20–350. The compounds of cinnamon EO were identified by comparing their retention indices (RI) relative to series of n-alkanes (C5–C28), retention times (RT) and mass spectra with National Institute of Standards and Technology (NIST 08) library data. Compounds were expressed as percentages of the peak area to the total oil peak area.

### 2.4. Antibacterial assays

#### 2.4.1. Agar diffusion

The antibacterial activity of cinnamon EO was described using agar diffusion method according to Goni et al. (2009) with modifications. Each sterile petri plate (90 mm) was prepared with 20 ml NB medium. After solidifying, 100 μl of bacterial suspension ($1 \times 10^7$ cfu/ml) was spread onto the plates. After 5 min, a sterile filter paper disc (6 mm) containing 5 μl of cinnamon EO was placed in the surface of plate. Afterwards, the plate was incubated at 37 °C for 24 h. The antibacterial activity of cinnamon EO was expressed by measuring the diameter of inhibition zone (DIZ). Tween 80 was used as control. Values are described as mean ± SD of assays performed in triplicate.

#### 2.4.2. Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC)

MIC was defined as the lowest concentration of EO with no visible (no turbidity) bacterial growth. MBC was defined as the lowest concentration of EO with initial inoculum bacteria killed. MIC and MBC were determined according to the method used by Silva, Ferreira, Queiroz, and Domingues (2011) with minor modifications. Firstly, the stock of cinnamon EO was dissolved in Tween 80 and added in the 10 ml sterile NB medium to achieve a concentration of 16 mg/ml. Then a serial two fold diluted to obtain final concentrations ranging from 8 to 0.25 mg/ml. Finally, 50 μl of bacterial suspensions (adjusted to $1 \times 10^7$ cfu/ml) were added in each test tube. All tubes were incubated at 37 °C for 24 h. MBC was measured by subculture of 50 μl from each tube with no visible bacterial growth on a NA plate followed by incubation at 37 °C for 24 h. Each test was performed in triplicate.

### 2.5. Antibacterial mechanism

#### 2.5.1. Scanning electron microscopy (SEM)

SEM was used to observe the morphological changes according to the method as described by Bajpai, Sharma, and Baek (2013) with modifications. The bacteria were incubated in NB medium at 37 °C (approximately $1 \times 10^7$ cfu/ml). The suspensions were treated with different concentrations of cinnamon EO (control, MIC and MBC) based on the MIC and MBC concentrations obtained from section 2.4.2. All samples were incubated at 37 °C for 3 h. After incubation, the suspensions were centrifuged at 1500 g for 10 min and washed twice with 0.1 M phosphate buffer solution (PBS, pH 7.4). The bacteria cells were fixed in 2.5% glutaraldehyde for 4 h at 4 °C. The samples were dehydrated in a sequential graded ethanol (30%, 50%, 80%, 90%, 100%) and the ethanol was then replaced by 100% tertiary butyl alcohol. Finally, all samples were sputter-coated with gold in an ion coater for 2 min, followed by microscopic examinations by using a scanning electron microscope (Quanta200 FEG; FEI; USA).

#### 2.5.2. Permeability of cell membrane

The permeability of cell membrane was studied according to the method of Diao, Hu, Zhang, and Xu (2014). Relative electric conductivity was examined to express the change of permeability. Bacterial was separated by centrifugation at 1500 g for 10 min after incubated for 12 h at 37 °C. The cells were washed with 5% glucose until the electric conductivities of the cells were near to that of the 5% glucose. The electric conductivity was measured with an electrical conductivity meter. These washed cells were isotonic bacteria. 5% glucose was added with different concentrations of cinnamon EO (control, MIC and MBC) and measured their electric conductivities. Those values were denoted as $L_1$. Different concentrations of cinnamon EO were also added into the isotonic bacteria,

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