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Original Article

Activities and mechanisms of eugenol and cinnamaldehyde against *Legionella pneumophila*

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Abstract: Background: *Legionella pneumophila* (*L. pneumophila*) is a primary human pathogenic gram-negative bacterium and is the causative agent of legionellosis, with an associated death rate of 5-10%. Eugenol and cinnamaldehyde were found to demonstrate antimicrobial properties, and are derived from aromatic plant-derived essential oils. Objectives: The aim of this study was to investigate the antibacterial activities and mechanisms of eugenol and cinnamaldehyde against *L. pneumophila*. Methods: Antibacterial activity was determined by broth microdilution method, and a time-kill curve plotted. The mechanism of antibacterial action was assessed by the measurement of release of 260 nm-absorbing material, SDS-PAGE and silver staining, and transmission electron microscopy (TEM). Results: The minimum inhibitory concentration (MIC) of eugenol to *L. pneumophila* was equal to its minimum bactericidal concentration (MBC) with a concentration of 0.0125% (v/v) or 133 μg mL^{-1} (w/v), whereas the MIC of cinnamaldehyde against *L. pneumophila* was 15-31 μg mL^{-1} and its MBC was 31-62 μg mL^{-1}. Eugenol completely inactivated *L. pneumophila* (6×10^5 CFU mL^{-1}) at a concentration of ≥ 8×MIC in 0.5 h. Cinnamaldehyde was demonstrated to have similar activity at a concentration of ≥ 32×MIC in 1.5 h. Eugenol kills *L. pneumophila* by the disruptive action on the bacterial cell envelope, while cinnamaldehyde has no effect on bacterial membrane. Conclusions: Our study showed that both eugenol and cinnamaldehyde can inhibit the growth of *L. pneumophila*, which provides the potential for preventing and treating *L. pneumophila* contamination and infection.

Keywords: Eugenol, cinnamaldehyde, *legionella pneumophila*, antimicrobial activity, antibacterial mechanism

Introduction

Legionella pneumophila (*L. pneumophila*), first identified in 1977, is a primary human pathogenic gram-negative bacterium that often causes a severe and life-threatening pneumonia [1, 2]. It is also the causative agent of legionellosis, also known as Legionnaire's disease, with an associated mortality of 5-10%. To date, there is no available vaccine for Legionnaires' disease. *L. pneumophila* occurs widely in rivers and lakes [3], and has the ability to grow in hot spring waters [4], hot water outlets of hospitals, hotels and apartments [5]. Therefore, there is a potential risk of legionella exposure in these areas, with associated health risks upon infection [6, 7]. Macrolides and fluoroquinolones are the recommended therapeutic agents to treat bacterial infection. However, some strains of *L. pneumophila* are resistant to erythromycin, ciprofloxacin, rifampin *in vitro* and also acquire resistance to fluoroquinolone *in vivo* [8-10]. Hence there is a significant clinical need to develop antimicrobial agents for the prevention and treatment of *L. pneumophila*.

In recent years, a variety of essential oils extracted from aromatic plants were found to have biocidal effects on bacteria, yeasts, filamentous fungi, and viruses [11], and, therefore, the antibacterial activity of essential oils has attracted a great deal of attention [12]. Eugenol and cinnamaldehyde are two bioactive components of aromatic plant-derived essential oils. Eugenol (4-allyl-2-methoxyphenol) is a naturally occurring phenol essential oil extracted from clove (*Eugenia caryophillis*). Cinnamaldehyde (3-phenyl-2-propenal), an active component of *Cassia oil*, is mainly isolated from the stem bark of *Cinnamomum cassia*. Both eugenol and cin-

namaldehyde have been of recent interest with respect to the development of antimicrobial agents due to their demonstrated activity against both gram-positive and gram-negative bacteria, including *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Clostridium botulinum*, *Staphylococcus aureus* and *Helicobacter pylori* [13, 14].

Previously, eugenol and cinnamaldehyde have been reported to exhibit *in vitro* antibacterial activity against *L. pneumophila* under various pH ranges [15, 16]. Although these two studies described eugenol and cinnamaldehyde as possessing antibacterial activity against *L. pneumophila*, their direct effects on *L. pneumophila* have not been thoroughly and systematically investigated. Therefore, the clarification of their antimicrobial mechanisms against *L. pneumophila* may play a pivotal role in the development of novel antimicrobial drugs. In this study, we evaluated the antibacterial activity of eugenol and cinnamaldehyde against *L. pneumophila* using broth microdilution and a time-kill curve. The mechanisms of their respective actions against *L. pneumophila* were assessed by the measurement of release of 260 nm-absorbing material, SDS-PAGE and silver staining, and transmission electron microscopy (TEM).

Materials and methods

Bacterial strains and media

L. pneumophila serogroup 1 ATCC 33152 was used to investigate the antibacterial effect and mechanism of eugenol and cinnamaldehyde. N-(2-Acetamido)-2-aminoethanesulfonicacid (ACES; Sigma, St Louis, MO, USA)-buffered yeast extract broth (BYE broth) supplemented with 0.4 mg mL^{-1} L-cysteine, 0.1 mg mL^{-1} thymidine, and 0.135 mg mL^{-1} ferric nitrate was used as a liquid medium (supplemented BYE broth). Legionella CYE agar base (OXOID; Basingstoke, UK; CM0655) with added to Legionella BCYE growth supplement (OXOID; SR0110A) and used as a solid medium (BCYE agar).

Antibacterial agents

Eugenol (99%, v/v) and cinnamaldehyde (99%, w/w) standards were purchased from Shanghai Yuanye Biotechnology Ltd. Co. (Shanghai, China). Eugenol was in a liquid form but did not contain any solvents, while cinnamaldehyde was dissolved in ethanol to a concentration of 200 mg mL^{-1} as a stock solution.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays

The MIC and MBC of eugenol or cinnamaldehyde against *L. pneumophila* were determined by the broth microdilution method (Clinical Laboratory Standardization Institute, CLSI, 2000). Bacteria were incubated on BCYE agar for 4 days at 37°C in an atmosphere of 5% CO_2. Single colonies (10-15) were each transferred to 3 mL of fresh supplemented BYE broth, and then incubated at 37°C with constant shaking (160 rpm) for 20 h. The concentration of bacteria in broth was determined by measuring the optical density at 600 nm ($OD_{600\ nm}$). By diluting the bacterial suspension with supplemented BYE broth, the final concentration of approximately 6×10^5 CFU mL^{-1} was achieved for testing.

Serial two-fold dilutions of eugenol and cinnamaldehyde in supplemented BYE broth were prepared in 1.5 *mL* EP centrifuge tubes (Axygen, Tewskbury, MA, USA) and inoculated (100 µL/well) in a 96-well plate. An equal volume of diluted bacterial suspension was added to each well. The plate was incubated for 48 h at 37°C in 5% CO_2. From all wells not showing visible growth, 10 µL culture medium was plated onto BCYE agar, and the number of colonies counted following 72 h incubation at 37°C in 5% CO_2. The MIC was defined as the lowest concentration of eugenol or cinnamaldehyde without visible growth in the broth, and the MBC was defined as the lowest concentration, at which no growth was observed on BCYE agar. Positive, negative and solvent control (5% ethanol) was set up. Experiments were performed in triplicate in three independent experiments.

Determination of killing rate

The killing rate of eugenol and cinnamaldehyde against *L. pneumophila* was evaluated as described previously [17]. Briefly, an *L. pneumophila* suspension of 6×10^5 CFU mL^{-1} was prepared. Eugenol and cinnamaldehyde solutions were added at final concentrations of 32 to 1/2 MIC by serial two-fold dilutions (0.1% to 0.006% v/v (1064 µg mL^{-1} to 66.6 µg mL^{-1});

1000 to 15 μg mL^{-1}, respectively). Control tubes were prepared without drugs. Samples were incubated at 37°C in 5% CO_2, and at 0, 0.5, 1.5, 3, 6, 9 and 24 h, 100 μL was removed from each tube and the number of CFU mL^{-1} was determined by plating serial dilutions on BCYE agar. All determinations were carried out in triplicate.

Analysis of 260 nm-absorbing materials

The release of UV-absorbing material by bacterial cells is used as an indicator of cell lysis and was measured as described previously [18]. Briefly, broth cultures of *L. pneumophila* were grown for 20 h and reached an $OD_{600\ nm}$ of 1.5 corresponding to approximately 6×10^9 CFU mL^{-1}. After centrifugation at 400 × g for 15 min, cells were collected. The cell pellet was washed twice and then resuspended in PBS (pH 7.4). Different concentrations of eugenol (0.0125%, 0.4% and 0.8%, v/v, 133 μg mL^{-1}, 4256 μg mL^{-1} and 8512 μg mL^{-1}) and cinnamaldehyde (1 mg mL^{-1}) were added to the cell suspension. Levofloxacin (500 ng mL^{-1}) was used as a positive control. 5% ethanol was used as a solvent control. After samples were incubated at 37°C for 60 min, the cell suspension was centrifuged at 13,400 × g for 15 min and the $OD_{260\ nm}$ value of the supernatant was measured by a multifunctional microplate reader (TECAN, Infinite M200 PRO).

SDS-PAGE and silver staining

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining was used to investigate membrane damage of bacteria as described by Devi *et al.* [18]. Briefly, 1 ml aliquots of *L. pneumophila* culture ($OD_{600\ nm}$ = 1.5) were pipetted into a series of centrifuge tubes. After centrifugation at 9,300 × g for 5 min at room temperature, cells were collected, washed twice and resuspended in 1 mL PBS (pH 7.4). Eugenol (0.4%, 0.8%, v/v, 4256 μg mL^{-1}, 8512 μg mL^{-1}), cinnamaldehyde (1 mg mL^{-1}) or levofloxacin (500 ng mL^{-1}) were added to cell suspensions and samples were incubated at 37°C for 30 min. Cell suspensions was then centrifuged at 9300 × g for 5 min. Control samples were prepared similarly with the corresponding solvents. 80 μL of supernatant in a centrifuge tube was mixed with 20 μL of sample buffer (5 ×; 10% SDS, 50% glycerol, 5% β-mercaptoethanol and 0.5% bromophenol blue). Samples were mixed and heated at 95°C for 5 min and then electrophoresed on a 12% SDS-PAGE gel. Finally, silver staining was conducted to assess any proteins released due to membrane damage.

Transmission electron microscopy

After 20 h incubation, broth cultures of *L. pneumophila* reached about $OD_{600\ nm}$ = 1.5 (approximately 6×10^9 CFU mL^{-1}), and then centrifuged at 6,000 × g for 10 min. The pellet was washed twice with 0.9% sodium chloride (NS), resuspended in normal saline containing 0.4% (4256 μg mL^{-1}) eugenol and 1 mg mL^{-1} cinnamaldehyde, and the resuspension incubated at 37°C for 60 min. After centrifugation (6-10 min), the pellet was fixed with 2.5% glutaraldehyde, dissolved in 0.1 M sodium cacodylate and sent to the Electron Microscopy Center of China Medical University (Shenyang, China). The morphology of bacterial cells was observed by TEM (JEM-1200EX, JEOL Company, Tokyo, Japan).

Statistical analysis

Results were expressed as the mean ± standard deviation (SD) of individual experiments, performed in triplicate. The comparison of multiple means used analysis of variance, after the equal check of variance, the two-two comparisons among the means were done by bonferroni method. Results with $P<0.05$ were considered statistically significant.

Results and discussion

MICs and MBCs of eugenol and cinnamaldehyde against L. pneumophila

L. pneumophila ATCC 33152, belonging to serogroup 1, was used to evaluate the antimicrobial activities of eugenol and cinnamaldehyde. The MIC of eugenol against *L. pneumophila* ATCC 33152 was 0.0125% (133 μg mL^{-1}), equal to its MBC, whereas the MIC and MBC of cinnamaldehyde against *L. pneumophila* ATCC 33152 was 15-31 μg mL^{-1} and 31-62 μg mL^{-1}, respectively.

MIC and MBC of eugenol against *L. pneumophila* was 0.0125% (133 μg mL^{-1}), which was lower than the previously published data of 0.06% and 0.12% against *S. pneumoniae*, respectively [19]. Eugenol had the same MIC against *L.*

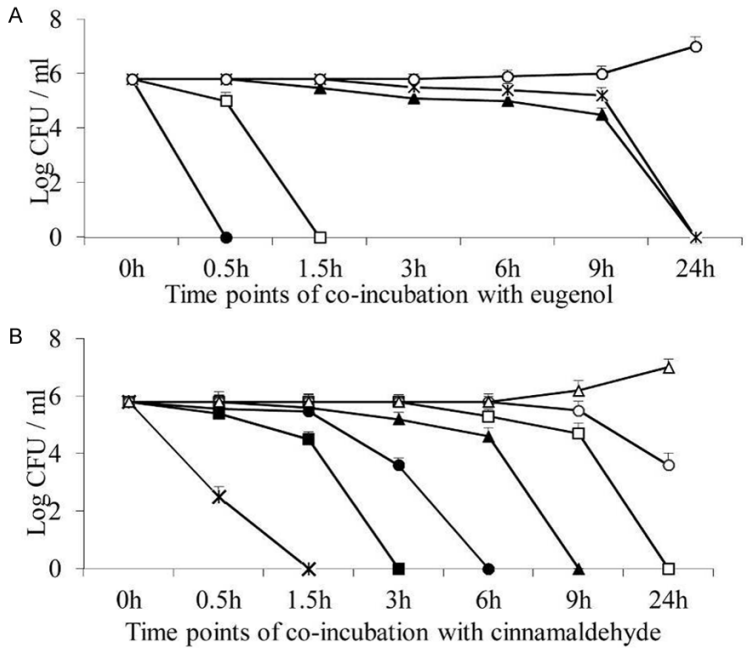

Figure 1. Time-kill curves of eugenol and cinnamaldehyde against *L. pneumophila*. A. Time-kill curve of eugenol against *L. pneumophila*. ●, 0.1% (1064 μg mL^{-1}, 8×MIC). □, 0.05% (532 μg mL^{-1}, 4×MIC). ▲, 0.025% (266 μg mL^{-1}, 2×MIC). *, 0.0125% (133 μg mL^{-1}, 1×MIC). ○, 0.0062% (66.5 μg mL^{-1}, 1/2×MIC). B. Time-kill curve of cinnamaldehyde against *L. pneumophila*. *, 1000 μg mL^{-1} (32×MIC). ■, 500 μg mL^{-1} (16×MIC). ●, 250 μg mL^{-1} (8×MIC). ▲, 125 μg mL^{-1} (4×MIC). □, 62.5 μg mL^{-1} (2×MIC). ○, 31 μg mL^{-1} (1×MIC). Δ, 15 μg mL^{-1} (0.5×MIC).

pneumophila and *S. typhi* (0.0125%), but the MBC of eugenol to *L. pneumophila* was lower than that previously found for *Salmonella typhi* (0.025%) [18]. Singh *et al*. [20] investigated the effect of eugenol on the growth of gram-positive (*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) bacteria using the agar well diffusion method, and demonstrated that 0.031% (2,000 ppm) eugenol completely inhibited the growth of *P. aeruginosa*, while the growth of other bacteria were only partially inhibited. Gill and Holley [13] reported that the MIC of eugenol against *L. monocytogenes* was 0.077% (5 mmol L^{-1}), while its MIC against *L. sakei* was 0.092% (6 mmol L^{-1}) in tryptic soy broth with yeast extract (TSB-YE) broth. Thus, the inhibitory activity of eugenol against *L. pneumophila* was more effective than those to the aforementioned bacteria.

Kim *et al*. [21] reported that the MIC of cinnamaldehyde against both *E. coli* O157:H7 and O26 was 250 μg mL^{-1}, and its MIC to *E. coli* ATCC11105 and O111 was 500 μg mL^{-1}. However, Helander *et al.* [22] found that the MIC of cinnamaldehyde to *E. coli* and *Salmonella typhi* was 396 μg mL^{-1}. Additionally, Chang *et al*. [15] also found that the MIC of cinnamaldehyde against *E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus* was 250-1000 μg mL^{-1}. Based on our experimental data, the MIC of cinnamaldehyde against *L. pneumophila* was 15-31 μg mL^{-1}. Clearly, the antibacterial effect of cinnamaldehyde on *L. pneumophila* was higher than those the aforementioned bacteria. Therefore, *L. pneumophila* was more sensitive to cinnamaldehyde than *E. coli*, *S. typhi*, *P. aeruginosa*, *E. faecalis* and *S. aureus*.

Shimizu *et al*. [16] studied the antibacterial effect of aromatic substances on *L. pneumophila* standard strain JCM7571 (Philadelphia, No. 1), and concluded that the MICs of eugenol and cinnamaldehyde were 0.063% and 82 μg mL^{-1}, respectively, which were both higher than those determined in our study. Previously, essential oil compounds extracted from *Cinnamomum osmophloeum* exhibited strong anti-Legionella activity, where cinnamaldehyde was confirmed as the main effective ingredient. 10^4 CFU mL^{-1} of *L. pneumophila* were completely inactivated by 1000 μg mL^{-1} cinnamaldehyde for 10 min at 42°C [15]. In comparison, we found that after using a cinnamaldehyde concentration of 1000 μg mL^{-1} at 37°C, treatment for 1.5 h was required to completely kill 10^5 CFU ml^{-1} of *L. pneumophila*. We hypothesized that increase in incubation time with the compounds was possibly associated with the increased amount of bacteria and low temperature used in our study.

Time-kill kinetics of eugenol and cinnamaldehyde against L. pneumophila

Although the MIC and MBC reflect the antimicrobial activity of antibiotics at specific concentrations, these values do not reveal the dynam-

Table 1. $OD_{260\ nm}$ of *L. pneumophila* broth treated with eugenol and cinnamaldehyde

	Solvent control	Cin-1 mg mL^{-1}	Lev-0.5 μg mL^{-1}	E-0.0125%	E-0.4%	E-0.8%
$OD_{260\ nm}$ (mean ± SD)	0.016 ± 0.004	0.023 ± 0.005	0.025 ± 0.006	0.034 ± 0.011	0.268 ± 0.062**	0.462 ± 0.079**

Abbreviations: Cin, cinnamaldehyde. Lev, levofloxacin. E, eugenol. **p<0.001.

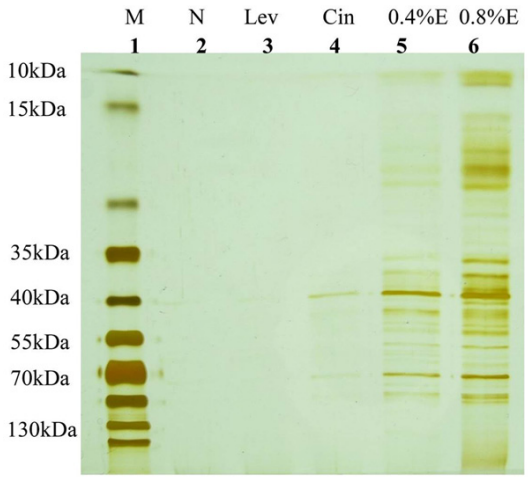

Figure 2. SDS-PAGE and silver staining analysis of *L. pneumophila* broth treated with eugenol and cinnamaldehyde. 1, Protein Marker. 2, Control (5% ethanol). 3, Levofloxacin (0.5 μg mL^{-1}). 4, Cinnamaldehyde (1mg mL^{-1}). 5, Eugenol (0.4%, 4256 μg mL^{-1}). 6, Eugenol (0.8%, 8512 μg mL^{-1}).

ic process involved in the inactivation by antibacterial drugs at different concentrations. Time-kill curve methods can make up for these shortfalls, allowing dynamic changes in antibacterial activity to be observed through determining the rate of killing [23].

As shown in **Figure 1**, eugenol completely eliminated *L. pneumophila* at a concentration of ≥ 0.1% (1064 μg mL^{-1}, 8×MIC) in 0.5 h, and killed *L. pneumophila* at a concentration of 0.05% (532 μg mL^{-1}, 4×MIC) in 1.5 h. However, at a concentration of 0.0125% (133 μg ml^{-1}, 1×MIC), eugenol was bactericidal, as demonstrated by the gradient of the line in **Figure 1A**. On the contrary, cinnamaldehyde required 1.5 h to completely kill *L. pneumophila* at concentration of 1000 μg mL^{-1} (32×MIC) and 24 h at 2×MIC, which also indicated that cinnamaldehyde was bactericidal (**Figure 1B**). However, our findings demonstrated that the anti-bactericidal efficiency of cinnamaldehyde was inferior to that of eugenol at the same > 2×MIC.

In our study, eugenol completely killed *L. pneumophila* at a concentration ≥ 0.1% (1064 μg mL^{-1}, 8×MIC) in 0.5 h, however, any further increase in concentration did not contribute to any further increase in the slope of the curve. This result indicated that the killing effect of eugenol on *L. pneumophila* was time-dependent, but not concentration-dependent, which was in accordance with Devi's study [18], where a concentration of 0.05% eugenol (532 μg mL^{-1}, 4×MIC) completely inactivated 10^9 CFU mL^{-1} of *S. typhi* in 1 h.

In our study, cinnamaldehyde completely killed *L. pneumophila* at 2×MIC (62 μg mL^{-1}) in 24 h. After the concentration of cinnamaldehyde was increased to 4×MIC, 8×MIC, 16×MIC and 32×MIC, the slope of the curve also increased, indicating that the bactericidal activity of cinnamaldehyde was concentration-dependent. Shimizu *et al. [16]* has previously demonstrated that cinnamaldehyde could almost completely kill *L. pneumophila* (JCM7571, Philadelphia 1) at 2×MIC in 3 h, and *L. pneumophila* decreased below detectable levels at 1×MIC in 12 h. Our study showed that treatment with 2×MIC cinnamaldehyde for 24 h killed *L. pneumophila* to below detectable levels, and levels of *L. pneumophila* decreased 4-log. The MIC of cinnamaldehyde determined by Shimizu et al. was 82 μg mL^{-1}, whereas our study demonstrated an MIC of 15-31 μg mL^{-1}, which may be related to the use of different strains of Legionella between studies.

Membrane disruption of eugenol and cinnamaldehyde

To investigate the possible mechanism of the antibacterial action of eugenol and cinnamaldehyde, the determination of $OD_{260\ nm}$, SDS-PAGE and silver staining was performed to evaluate their damage to the membrane of *L. pneumophila*.

UV-absorbing materials release was measured as an index of lysis [24]. Levofloxacin was applied as a control, which acts as a bactericidal agent by antagonism of DNA gyrase, leading to no damage to the cell membrane and no effects on cellular permeability. The $OD_{260\ nm}$ of

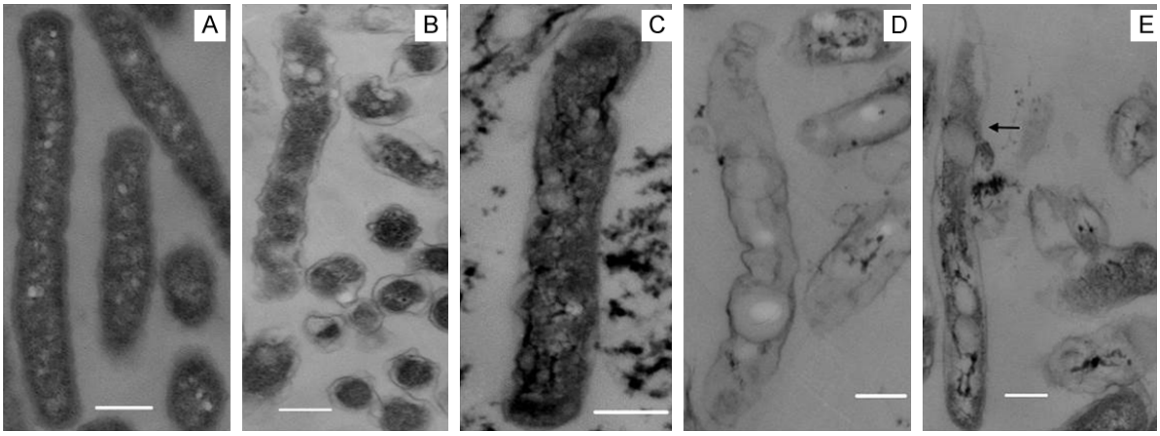

Figure 3. TEM morphology analysis of *L. pneumophila* treated with eugenol and cinnamaldehyde. A. *L. pneumophila* grown on plate. B. *L. pneumophila* after 20 h broth culture. C. Cinnamaldehyde (1 mg mL^{-1}) treated group. D, E. eugenol (0.4%, 4256 μg mL^{-1}) treated groups. Arrow, cytoplasm leakage from damaged membrane. × 10000. Bar: 500 nm.

the *L. pneumophila* broth treated with levofloxacin was very low, at a value 0.025 ± 0.006. The $OD_{260\ nm}$ of the *L. pneumophila* broth treated with 0.4% (4256 μg mL^{-1}, 0.268 ± 0.062) or 0.8% (8512 μg mL^{-1}, 0.462 ± 0.079) eugenol was significantly higher than those of the solvent control group (0.016 ± 0.004), 0.5 μg mL^{-1} levofloxacin (0.025 ± 0.006), and 0.0125% (133 μg mL^{-1}) eugenol (0.034 ± 0.011) (**Table 1**). These data indicated that eugenol may act on the bacterial envelope of *L. pneumophila*, resulting in membrane damage and cytoplasm leakage. In contrast, the $OD_{260\ nm}$ of *L. pneumophila* broth treated with 1 mg mL^{-1} cinnamaldehyde was 0.023 ± 0.005 (**Table 1**), which was comparable to that of the solvent control group (0.016 ± 0.004), 0.5 μg mL^{-1} levofloxacin (0.025 ± 0.006), and 0.0125% eugenol (0.034 ± 0.011). These findings suggest that cinnamaldehyde does not disrupt the bacterial envelope of *L. pneumophila* or result in membrane damage. Furthermore, protein analysis by SDS-PAGE and detection by silver staining (**Figure 2**) also demonstrated that eugenol resulted in non-selective protein leakage due to the membrane damage; however these effects were not apparent with cinnamaldehyde treatment. These findings suggest that eugenol and cinnamaldehyde may exhibit anti-*L. pneumophila* effects by different antibacterial mechanisms.

Transmission Electron Microscopy studies

Transmission Electron Microscopy (TEM) of plate-grown *L. pneumophila* displayed rod morphologies and typical gram-negative envelopes, consisting of clearly defined outer and inner membranes of equal widths. Cytoplasmic regions were rich in ribosomes and uniform in electron density (**Figure 3A**). After 20 h broth culture, *L. pneumophila* entered a post-exponential growth phase with a more evident, wavy outer membrane. Moreover, inclusions appeared in the cytoplasm, as indicated by the multiple empty holes shown by TEM (**Figure 3B**). The outer membranes of 1 mg mL^{-1} (32×MIC) cinnamaldehyde-treated *L. pneumophila* were still visible, and treated bacteria showed a high cytoplasmic density (**Figure 3C**), which was nearly identical to the non-treated control (**Figure 3A**). After treatment with 0.4% eugenol (4256 μg mL^{-1}, 32×MIC), *L. pneumophila* lost the wavy outer membrane, developed a thin and lower cytoplasmic density, and had visible leakage from damaged cytoplasmic membrane evident (**Figure 3D**, **3E**).

The above findings demonstrated that eugenol acted on the bacterial envelope, leading to membrane damage, cytoplasm leakage and eventual cell death. Eugenol is a lipophilic molecule and unstable in aqueous solution, which can actively penetrate the cell membrane lipid bilayer [25]. Eugenol has a phenol group, which was previously demonstrated to be a functional group possessing the ability to destroy the cell membrane [26]. Furthermore, such a phenolic group is actually widely utilized in the extraction of lipopolysaccharide (LPS) from bacteria [27]. Thus, we speculated that the damaging effects

of eugenol on the membrane of *L. pneumophila* may be related to its phenolic group.

In contrast, cinnamaldehyde was found to have no effect on the structure of *L. pneumophila* membrane. This may be accounted for by the following two reasons: cinnamaldehyde does not possess any chemical groups which have the ability to damage membrane function, such as phenol or hydroxyl groups. These groups interact with the cell membrane to cause disruption sufficient to disperse proton motive force by leakage of small ions without leakage of larger cell components [13]. The other reason for the lack of effects exhibited by cinnamaldehyde is that it may be pumped out from the periplasm at a rate exceeding its penetration rate [28], resulting in its inability to access the cell. The specific mechanisms of cinnamaldehyde against *L. pneumophila* require further study.

Conclusions

In conclusion, our study demonstrated that both eugenol and cinnamaldehyde possessed significant anti-legionella activities. Eugenol primarily acted on the bacterial envelope of *L. pneumophila*, leading to cell membrane damage, cytoplasm leakage and bacterial death, however, cinnamaldehyde did not significantly alter the permeability of the envelope of *L. pneumophila*, suggesting that cinnamaldehyde did not directly act upon the bacterial membrane. Our findings provide considerable evidence for the promising application of these compounds in the prevention and treatment of *L. pneumophila*.

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Disclosure of conflict of interest

None.

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