



Sub-Inhibitory Concentrations of Sodium Houttuynonate in Combination with Erythromycin Inhibit Biofilm Formation and Expression of IcaA in *Staphylococcus epidermidis*

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Abstract

Background: *Staphylococcus epidermidis* is an important opportunistic pathogenic bacterium causing infections on implanted medical devices by biofilm formation. Sodium houttuynonate (SH), which is the active modified compound of *Houttuynia cordata* is commonly applied for treating chronic bronchitis, purulent skin infections, and respiratory tract infections in China.

Objectives: We aimed to investigate the in vitro effects of SH in combination with erythromycin on the production of IcaA and expression of *icaA* to shed the light on the mechanism of SH and erythromycin in inhibiting biofilm formation of *S. epidermidis*.

Methods: The morphology of the biofilm of *S. epidermidis* was examined under a scanning electron microscope. Furthermore, inhibitory effects of SH and erythromycin on the production of IcaA and the expression of *icaA* were detected by Western blotting and quantitative reverse transcription PCR, respectively.

Results: First, the morphological results indicated that combining SH and erythromycin at sub-MIC concentrations can effectively inhibit biofilm formation of *S. epidermidis*. Furthermore, our presented results showed that combining SH and erythromycin at sub-MIC concentration can significantly downregulate the expression of *icaA* and repress the protein production of IcaA at irreversible attachment and maturation stages of biofilm formation of *S. epidermidis*.

Conclusions: Therefore, our findings suggest that the potent antibiofilm activity of SH in combination with erythromycin against *S. epidermidis* may be partially due to inactivation of *icaA*.

Keywords: Natural Product, Sodium Houttuynonate, Biofilm, *Staphylococcus epidermidis*, IcaA

1. Background

Staphylococcus epidermidis is an abiding Gram-positive bacterium of the normal human microbiota, usually found on mucosal membranes and skin (1). *Staphylococcus epidermidis* can establish a lifelong commensal relationship with its host that begins from born by adhering to tissue surface moieties of the mucosal membranes and skin via specific adhesions. Although commensal *S. epidermidis* strains display high rates of antibiotics resistance, their features as commensal bacteria render this phenomenon largely unconcerned with the normal healthy host (2). However, *S. epidermidis* has become an important opportunistic pathogen, causing infections on medical implants such as contact lenses, urinary catheters, prosthetic heart valves, central venous catheters, and orthopedic devices in specific persons, which is hard to cure (3-5).

The first step in biofilm formation is the capability of adhering to the surface of the implant, commonly believed to be the most dominant virulence factor of *S. epidermidis*. Biofilm formation of *S. epidermidis* facilitates resistance against the host immune system (6-8) and confers resistance to antibiotic (6, 9). Medical and surgical treatment protocols of implant medical device are also complicated by biofilm formation due to remove the biofilm. Biofilms of microorganism are defined as intricate communities of adherent microorganism cells enclosed in a matrix of self-biosynthesized extracellular polymeric substances (10). The adhesion and maturation of the *S. epidermidis* biofilm occur by several mechanisms.

Polysaccharide intercellular adhesin (PIA), synthesized by *icaADBC* coding enzymes (11) is responsible for biofilm formation in most *S. epidermidis* strains and was proved to

be the most common macromolecule associated with the adhesion and maturation of *S. epidermidis* biofilm (12). This was demonstrated by the finding that the *ica* operon was absent in most non-pathogenic *S. epidermidis* isolates (13, 14). IcaA, which is an N-acetylglucosaminyltransferase that synthesizes PIA oligomers from UDP-N-acetylglucosamine, exerts a primary role in the PIA synthesis to form biofilm (12). Thus, IcaA is a potential candidate for targeting anti-biofilm agents against *S. epidermidis*.

In China, *Houttuynia cordata* Thunb (Saururaceae family) is an edible medicinal vegetable, which has been widely applied in traditional Chinese medicine for centuries to cure plenty of inflammatory and infectious diseases (15, 16). Sodium houttuynonate [SH, $\text{CH}_3(\text{CH}_2)_8\text{COCH}_2\text{CHOHSO}_3\text{Na}$], is a product of addition reaction of sodium bisulfite and houttuynin [decanoyl acetaldehyde, $\text{CH}_3(\text{CH}_2)_8\text{COCH}_2\text{CHO}$], which is the most important active constituent of the volatile oil derived from *H. cordata* (17). Thus, SH is commonly used for treating chronic bronchitis and respiratory tract and purulent skin infections (17). Recently, SH are found to inhibit LPS-induced mastitis in mouse via the NF- κ B signaling pathway (18) and alleviate post-infarct remodeling in rats depending on MAP-activated protein kinase pathway (19). However, despite its effective and widespread medical applications especially antimicrobial application, the detailed mechanism of antimicrobial effects of SH remains unknown.

Previously, our group found that SH is promising and effective at repressing pathogenic related biofilm formation and motility of *Pseudomonas aeruginosa* (20-22), and can inhibit *S. epidermidis* and *Candida albicans* (20, 23), and act synergistically with $\text{Na}_2\text{-EDTA}$ and levofloxacin against biofilm formation of *P. aeruginosa*, *S. aureus* and *C. albicans* (23, 24). In addition, we found that SH is a possible natural analog of N-acyl-homoserine lactone, and can effectively inhibit the Las quorum sensing (QS) system and regulated virulence factors of *P. aeruginosa* (25). However, owing to the high resistance of antimicrobial agents of biofilm, we found that SH alone cannot effectively inhibit the biofilm formation of *S. epidermidis*. Thus, we have chosen the commonly used macrolide antibiotic, erythromycin to combine with SH to inhibit the biofilm formation of *S. epidermidis*.

Interestingly, our previous results indicate that SH alone can only mildly inhibit the biofilm formation of *S. epidermidis*, and SH in combination with erythromycin can significantly inhibit the biofilm formation of *S. epidermidis*, and up-regulate the transcription of *luxS*, and down-regulate the expression of *agr*/RNA III, which are members of regulatory QS system involved in biofilm formation of *S. epidermidis* (26, 27). However, the mechanism of SH in

combination with erythromycin against biofilm development cycle and PIA biosynthesis, especially the adhesion and maturation stages of *S. epidermidis* remains unclear.

2. Objectives

Therefore, we investigated in vitro effects of SH in combination with erythromycin on the production of IcaA and expression of *icaA* to shed the light on the mechanism of SH and erythromycin in inhibiting biofilm formation of *S. epidermidis* in this research. Our results may explore the mechanism of anti-biofilm effect of SH and erythromycin against *S. epidermidis*.

3. Methods

3.1. Bacterial Strains and Cultures

Staphylococcus epidermidis strain ATCC 35984, which was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China), was normally cultured in tryptone soy broth (TSB) (Aoboxing Bio-tech, Beijing, China) and grown in a shaker under normal condition (37°C, 5.4 g) unless otherwise mentioned. Minimum inhibitory concentrations (MIC) of SH and erythromycin against *S. epidermidis* were determined by CLSI standard method according to our previous research (24, 25).

The concentrations of different drug treatments were set as follows: 64 $\mu\text{g SH mL}^{-1}$ ($1 \times \text{MIC}$), 32 $\mu\text{g SH mL}^{-1}$ ($1/2 \times \text{MIC}$), 16 $\mu\text{g SH mL}^{-1}$ ($1/4 \times \text{MIC}$), 8 $\mu\text{g SH mL}^{-1}$ ($1/8 \times \text{MIC}$), 8 $\mu\text{g erythromycin mL}^{-1}$ ($1 \times \text{MIC}$), 4 $\mu\text{g erythromycin mL}^{-1}$ ($1/2 \times \text{MIC}$), 2 $\mu\text{g erythromycin mL}^{-1}$ ($1/4 \times \text{MIC}$), 1 $\mu\text{g erythromycin mL}^{-1}$ ($1/8 \times \text{MIC}$), 32 $\mu\text{g SH mL}^{-1}$ ($1/2 \times \text{MIC}$) in combination with 4 $\mu\text{g erythromycin mL}^{-1}$ ($1/2 \times \text{MIC}$), 16 $\mu\text{g SH mL}^{-1}$ ($1/4 \times \text{MIC}$) and 2 $\mu\text{g erythromycin mL}^{-1}$ ($1/4 \times \text{MIC}$), 8 $\mu\text{g SH mL}^{-1}$ ($1/8 \times \text{MIC}$) and 1 $\mu\text{g erythromycin mL}^{-1}$ ($1/8 \times \text{MIC}$). Moreover, $1 \times \text{MIC}$ of erythromycin was set as the positive control for its promising anti-biofilm formation activity. Erythromycin was dissolved in sterile deionized water as store liquor of 16 mg mL^{-1} to dilute working concentrations. Based on the method of Shao (18), preparation for the SH were as follows: one drop of polyoxyethylene sorbitan fatty acid esters and glycerol added to 102.4 mg SH, and then 1 mL deionized sterile water was added to the SH, and the solution was heated to dissolve the SH drugs as store liquor for diluting working concentrations.

3.2. Biofilm Formation

Staphylococcus epidermidis with overnight culturing in liquid TSB was diluted to 0.05 of OD600 with fresh liquid

TSB, and then 100 μ L of diluted bacterial culture was cultured in six-well polystyrene plates within a coverglass and 2 mL fresh liquid TSB in each well for 3 days for biofilm formation. Then, the mature biofilm on cover glass was removed and washed gently with phosphate buffer saline (PBS), and then used to isolate RNA to perform gene expression analysis.

3.3. Scanning Electron Microscope Analysis of Biofilm Morphology

Basically, preparation of the carrier coverglass with biofilm was also performed as described above, and the planktonic bacteria were rinsed gently with PBS. The prepared biofilm samples of control, ($1/4 \times$ MIC) SH, ($1/4 \times$ MIC) erythromycin and $1/8 \times$ MIC SH in combination with $1/8 \times$ MIC erythromycin groups were stained with silver staining. The morphology of biofilm was observed by scanning electron microscope (SEM) (Sirion200, FEI, USA), using 500 kV and $\times 40,000$ or $\times 10,000$ magnifications.

3.4. Gene Expression Analysis

Total RNA was isolated by RNAPrep Bacteria Kit (Tiangen, Beijing, China), according to the manufacturer's protocols. The quality and quantity of extracted RNA were measured by the concentration and OD 260/280 of samples using Nanodrop One (Thermo Fisher, Waltham, USA). A FastQuant RT Kit (Tiangen, Beijing, China) was used to remove genomic DNA, and reverse transcript to cDNA. The resulting cDNA was separated by electrophoresis on 1% agarose gel and imaged. Quantitative RT-PCR (qRT-PCR) was performed using SYBR Premix ExTaq II (Tli Rnase H Plus) (TaKaRa, Kusatsu, Japan) under the conditions as follows: the initial step of 5 min at 95°C, followed by 40 cycles of 95°C for 15 s, 60°C for 15 s, and 72°C for 30 s.

The qRT-PCR reactions were performed in QuantStudio TM 6 Flex thermal cyclers (Applied Biosystems, Foster City, USA). The real-time PCR was validated by sequencing the PCR products of *icaA* and *gyrB*. The calculated cycle threshold (CT) of *icaA* was normalized to the CT for *gyrB* amplified from the corresponding sample. *GyrB* is a housekeeping gene in *S. epidermidis*, and commonly used as a control in qRT-PCR experiment of bacteria (27). Fold changes of *icaA* were calculated by relative quantification method, according to the $2^{-\Delta\Delta C_T}$ method (28). The oligonucleotide sequence of *icaA* and *gyrB* primers were listed in Table 1. The primers of *icaA* were designed in this study, and the primers of *gyrB* were synthesized based on our previous study (27).

3.5. Western Blotting Assay

The western blotting assay of IcaA was performed according to the method of Hnasko and Hnasko (29). First,

Table 1. Oligonucleotide Primers used During qRT-PCR

Gene	Forward (5'-3')	Reverse (5'-3')
<i>icaA</i>	CAATGAGGGAATCAAACAAGCA	AAAGGCGCATCGTCATCAA
<i>gyrB</i> (25)	AGCGGTTCGTAAAAGACCGGTATG	CCTGCTAATGCCTCGTCAATAC

the customized IcaA polyclonal antibody (HuaAn Biotechnology Co., Ltd, Hangzhou, China) was prepared by standard polyclonal antibody preparation method of synesthetic IcaA immunizing rabbit and validated by western blotting assay. For protein isolation, bacterial planktonic cells treated by different concentration drugs were collected and adjusted to the same OD₆₀₀ value in PBS. Then, the PBS with bacterial cells was replaced by fresh liquid TSB with different concentrations of erythromycin and SH at 37°C for 24 h as mentioned before.

Bacterial cells were lysed in Lysis buffer (Sigma, St. Louis, MO) containing protease inhibitors to extract the protein. Same amounts (60 μ g) of proteins of each group were loaded in each lane and then subjected to electrophoresis on a 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA), and then blocked with non-fat dry milk (5%, w/v) at room temperature for 2 h.

The membrane was incubated at 4°C overnight with the primary antibody i.e., customized IcaA polyclonal antibody. After washing with PBS with 0.1% Tween, the membranes were individually incubated in the horseradish peroxidase-conjugated secondary goat anti-rabbit antibody (Santa Cruz Biotechnology, CA, USA) for 2 h at room temperature. The detection of IcaA protein was performed by ECL-Plus Western blotting reagents (GE Healthcare, CT, USA) and the image was captured and analyzed using Image Quant Software (Bio-Rad, CA, USA).

3.6. Statistical Analysis

One-way ANOVA followed by Dunnett's multiple comparisons test of several groups was analyzed by GraphPad Prism version 6.02 (GraphPad Software, La Jolla, USA). All individual experiments were carried out at least in triplicate.

4. Results

4.1. Sodium Houttuynonate in Combination with Erythromycin Affected the Morphology of *Staphylococcus epidermidis* Biofilm

Previously, we found that SH can effectively inhibit *S. epidermidis* (27). To demonstrate that any apparent reduction is genuinely a result of inhibition of biofilm formation and not reduction of bacterial growth of sub-MIC SH

and erythromycin, we perform a checkboard test containing alone and in combination treatments of $2 \times$, 1 MIC , $1/2 \times \text{MIC}$, $1/4 \times \text{MIC}$, $1/8 \times \text{MIC}$, $1/8 \times \text{MIC}$ of SH and $2 \times$, 1 MIC , $1/2 \times \text{MIC}$, $1/4 \times \text{MIC}$, $1/8 \times \text{MIC}$, $1/8 \times \text{MIC}$ of erythromycin. The results showed that alone and especially in combination of sub-MIC, SH and erythromycin cannot inhibit the growth of *S. epidermidis* strain (27). Here, we determined the effect of SH in combination with erythromycin against the biofilm formation of *S. epidermidis* by SEM. According to the SEM images, the morphology of bacterial biofilm was clearly different among drug treatment groups at 3 d of biofilm maturation stage of *S. epidermidis*.

In the control group (Figures 1A and 1E), the bacterial accumulation was dense, and form a thick biofilm structure by multiple layer cells and intracellular matrix. Compared with the control group, the dense biofilm structure of $1/4 \times \text{MIC}$ erythromycin group (Figures 1C and 1G) was dramatically damaged, and some of the mucosa properties remained. The biofilm structure of $1/4 \times \text{MIC}$ SH group (Figures 1B and 1F) is substantially damaged, and none of the mucosa properties remained. Notably, biofilm structure of the SH in combination with erythromycin group at sub-inhibitory concentration (Figures 1D and 1H) did not exist and bacteria were rare and shrinking with defective cell structure. Therefore, the morphology of the biofilm cells of *S. epidermidis* was significantly destroyed by SH in combination with erythromycin at sub-inhibitory concentration.

4.2. Sodium Houctuyfonate in Combination with Erythromycin Repressed the Expression of *icaA*

Here, we determined the effects of SH on the expression of *icaA* of *S. epidermidis* that was responsible for biofilm formation at adhesion and mature stages of biofilm development (Figure 2A). The amplification curve of qRT-PCR is presented in Figure 2B, and the melting curves of *icaA* and *igrB* are presented in Figures 2C and 2D. At 6 h of the irreversible attachment stage of biofilm formation, the gene expression results (Figure 2A) indicated that the expression of *icaA* was downregulated by SH in combination with erythromycin.

Compared with the control group, SH at $1 \times \text{MIC}$ concentration and erythromycin at $1 \times \text{MIC}$, $1/2 \times \text{MIC}$ and $1/4 \times \text{MIC}$ concentrations alone could significantly repress the expression of *icaA* ($P < 0.001$). Notably, SH in combination with erythromycin groups at all three sub-inhibitory concentrations could significantly repress the expression of *icaA* ($P < 0.001$). At 24 h of the maturation stage of biofilm formation, all SH and erythromycin alone and in combination treatments could significantly downregulate the gene expression *icaA*. According to the results (Figure 2A), downregulation of *icaA* expression was dose-dependent with SH

in combination with erythromycin. Thus, the gene expression *icaA* of *S. epidermidis* was significantly repressed by SH alone and in combination with erythromycin.

Interestingly, the different effects between 6 h and 24 h drug treatment were intriguing as the action of SH alone, flipping from minimally inhibitory effect (or promotional at low concentration) at 6 h to substantially inhibitory at 24 hours of all concentrations. Thus, SH appeared to provide protection against the inhibitory action of erythromycin in all combination treatments at 6 h. This effect was reversed at 24 h that the *icaA* expression of erythromycin alone treatment vs. erythromycin ($1/2 \times \text{MIC}$) in combination with SH were statistically significant ($P < 0.05$). Therefore, these findings might suggest that SH counteracted the inhibitory action of erythromycin at the 6 h of the adhesion stage of biofilm formation, but amplified the inhibition at the 24 h of the mature stage of biofilm formation.

4.3. Sodium Houctuyfonate in Combination with Erythromycin Repressed the Production of *IcaA*

Furthermore, we performed a western blotting assay to determine the production changes of IcaA after combining SH and erythromycin treatments. At 6 h of adhesion stage of biofilm, our presenting results (Figure 3) indicated that the production of IcaA was not significantly reduced by SH and erythromycin alone but significantly reduced SH in combination with erythromycin groups. At 24 h of the mature stage of biofilm formation, the production of IcaA (Figure 3) was significantly reduced by SH and erythromycin alone in a concentration-dependent manner and was substantially repressed by SH in combination with erythromycin in all three sub-inhibitory groups. Therefore, the production of IcaA was significantly inhibited by SH alone and in combination with erythromycin, especially at the mature stage of biofilm formation of *S. epidermidis*.

5. Discussion

Staphylococcus epidermidis is an important conditional pathogen involved in device-related infections due to biofilm formation activity (1). The formation of epidermal *Staphylococcus* biofilm is a dynamic process (30). Firstly, the bacteria surface hydrophobic protein or polysaccharide adhesion to the host organism. The initial attachment of the material forms the bacterial community; then the bacterial cells gather together to build biofilm structure (30). Among them, PIA is a bacterial biofilm material which is necessary for the aggregation stage (30). IcaA is the first gene of the operon and plays a decisive role in the biosynthesis of PIA, and could be a possible target for antibiofilm

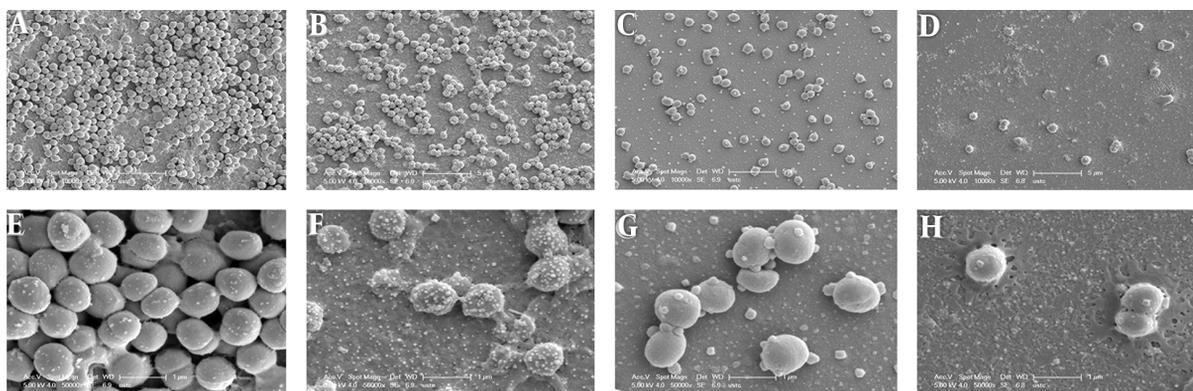


Figure 1. Microscopic morphology changes of *Staphylococcus epidermidis* biofilm treated by SH in combination with erythromycin. A and E, Negative control group without drug treatment; B and F, $1/4 \times$ MIC SH treatment group; C and G, $1/4 \times$ MIC erythromycin treatment group; D and H, $1/8 \times$ MIC SH in combination with $1/8 \times$ MIC erythromycin group. The A, B, C, D figures were magnified 10,000 times, and the E, F, G, H figures were magnified 50,000 times. The morphology of *S. epidermidis* biofilm in the different groups was detected at 24 h after drug treatments.

formation of *S. epidermidis*. Blocking the expression of the biofilm formation-related gene requires the inhibition of QS mechanism and biosynthesis of polysaccharides and extracellular proteins of biofilm compositions (31).

Currently, macrolide antibiotics, including erythromycin and vancomycin are common anti-staphylococci agents in the clinic by repressing protein biosynthesis of bacterial cell; however, staphylococci easily get resistant to the antibiotics (32), for example, medical devices-related infections by *S. epidermidis* strains. Therefore, it is important to seek effective anti-biofilm drugs or multi-drug combination application in traditional alternative medicines in order to achieve high drug efficiency against *S. epidermidis* biofilm and reduce the toxicity of drug treatments. Because the commonly used antibiotic, erythromycin, has lower adverse reaction and price than the third line antibiotics, like vancomycin (32). Thus we used erythromycin as the positive control of antimicrobial agents and selected combination agents in this research.

Recently, SH was found to inhibit the biofilm formation of *P. aeruginosa*, *S. aureus*, and *C. albicans* in vitro (20-25), but the detail of mechanisms remains unclear. Previously, we found that SH in combination with erythromycin groups at sub-inhibitory concentrations could effectively inhibit the biofilm formation of *S. epidermidis* at adhesion and maturation stages (26, 27). Furthermore, we have explored the effect of SH in combination with erythromycin on QS system, which regulates biofilm system of *S. epidermidis*. The results (27) indicate that SH in combination with erythromycin can quickly upregulate the expression of *luxS* at adhesion stage, and significantly reduce the expression of *agr* and *RNAIII* at adhesion and maturation stages.

These three genes encode the protein and RNA, which are key members of QS system of *S. epidermidis*. Notably, *LuxS* can inhibit biofilm formation of *S. epidermidis* via inactivation of gene expression of *icaADBC* operon by an *icaR*-activation pathway (33, 34). Therefore, our previous results suggested that SH may affect QS system to repress the biofilm formation of *S. epidermidis*.

Here, our results indicated that the morphology of the biofilm cells of *S. epidermidis* were significantly destroyed by SH alone and in combination with erythromycin at sub-inhibitory concentrations. Furthermore, the qRT-PCR data showed that the gene expression *icaA* of *S. epidermidis* was significantly repressed by SH alone and in combination with erythromycin at sub-inhibitory concentrations because SH has been proved to affect QS system in bacteria (25). Therefore, the inhibitory effects of SH against *S. epidermidis* may be due to the cell density and anti-QS dependent of promotion in the early stage and repression in the maturation stage to cause the intriguing effects of SH against transcript levels of *icaA* at 6 and 24 hours of exposure. Further, SH interestingly counteracts the inhibitory action of erythromycin at the time point of 6 h but amplifies the inhibition at the 24 h, which may be due to the QS system.

Consistent with the gene expression results, the production of IcaA is also significantly inhibited by SH alone and in combination with erythromycin at sub-inhibitory concentrations, especially at maturation stage of biofilm formation of *S. epidermidis*. Based on our previous results of repressing of QS system of *S. epidermidis* by SH in combination with erythromycin (27), the down-regulation of *icaA* may be due to the repressing of QS system of *S. epidermidis* by combining SH and erythromycin. Furthermore, reducing the production of IcaA may lead to the decreasing

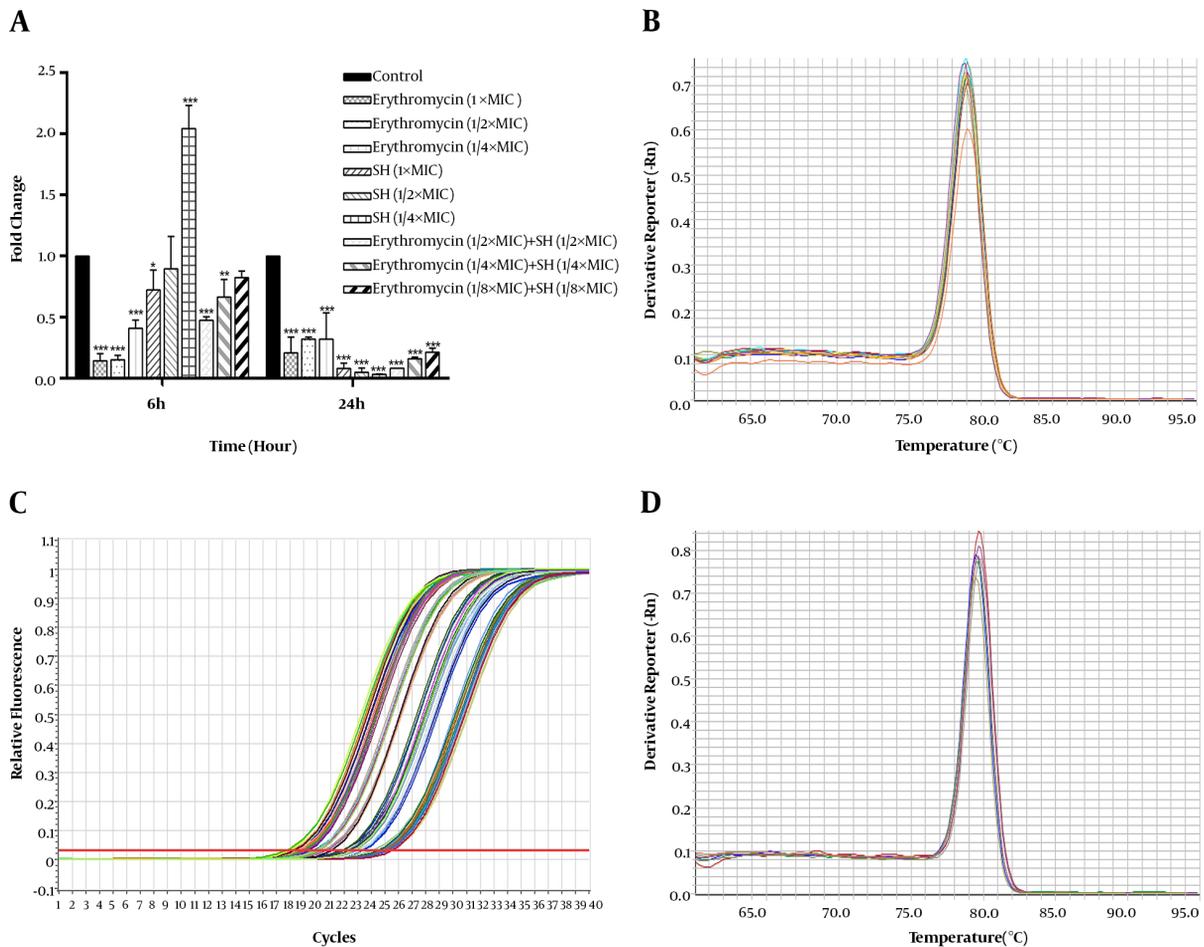


Figure 2. Different expression levels of the *icaA* of *Staphylococcus epidermidis* treated by SH in combination with erythromycin. A, Expression of *icaA* was monitored in response to SH in combination with erythromycin treatments by qRT-PCR assay. Housekeeping gene, *gyrB*, was set as the internal reference gene for each sample. The concentrations of different drug treatments were as follows: negative control group (without any drug), $1 \times \text{MIC}$ SH ml, $1/2 \times \text{MIC}$ SH mL⁻¹, $1/4 \times \text{MIC}$ SH, $1/8 \times \text{MIC}$ SH, $1 \times \text{MIC}$ erythromycin, $1/2 \times \text{MIC}$ erythromycin, $1/4 \times \text{MIC}$ erythromycin, $1/8 \times \text{MIC}$ erythromycin, $1/2 \times \text{MIC}$ SH and $1/2 \times \text{MIC}$ erythromycin, $1/4 \times \text{MIC}$ SH and $1/4 \times \text{MIC}$ erythromycin, $1/8 \times \text{MIC}$ SH mL⁻¹ and $1/8 \times \text{MIC}$ erythromycin. The expression of *icaA* in the different groups was detected at 6 h and 24 h after drug treatments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n = 4; B, amplification curve of the real time PCR; C, melting curve of *icaA* gene; D, melting curve of *gyrB* gene.

of biofilm matrix PIA, and result in decreasing of biofilm formation. Additional in vivo experiments will be required to assess the future applicability of SH in combination with erythromycin, and effective activity of combining SH and erythromycin against biofilm formation of bacteria may provide a promising approach.

5.1. Conclusions

Our results indicate that the potent antimicrobial activity of natural plant products, SH, in combination with erythromycin may be partially due to its inhibitory effect on biofilm formation in *S. epidermidis* via inactivation of *icaA*. Therefore, combining SH and erythromycin may provide a new possible option for the treatment of medical

devices-related infections by *S. epidermidis* biofilm. Furthermore, the above results also imply that IcaA could be a potent drug target to explore new treatment application against biofilm formation of *S. epidermidis*.

Footnotes

Authors' Contribution: Yan Guan and Daqiang Wu conceived and designed the experiments and are guarantors. Daqiang Wu, Qiangjun Duan, and Ganfei Xu performed the experiments and data analysis. Daqiang Wu, Qiangjun Duan, and Yan Guan wrote the paper. All authors in this study have read and approved the final manuscript.

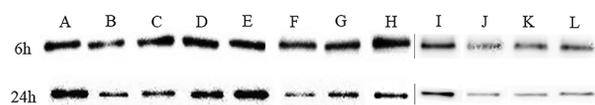


Figure 3. Repressive effects of SH in combination with erythromycin on the production of IcaA of *Staphylococcus epidermidis*. The production of IcaA was monitored in response to SH in combination with erythromycin treatments by Western blotting assay. Distinct drug concentrations of treatments representing different bands were as below: negative control group (without any drug), A, $1 \times \text{MIC SH mL}^{-1}$; B, $1/2 \times \text{MIC SH mL}^{-1}$; C, $1/4 \times \text{MIC SH mL}^{-1}$; D, $1/8 \times \text{MIC SH}$; E, $1 \times \text{MIC erythromycin}$; F, $1/2 \times \text{MIC erythromycin}$; G, $1/4 \times \text{MIC erythromycin}$; H, $1/8 \times \text{MIC erythromycin}$; I, $1/2 \times \text{MIC SH and } 1/2 \times \text{MIC erythromycin}$; J, $1/4 \times \text{MIC SH and } 1/4 \times \text{MIC erythromycin}$; K, $1/8 \times \text{MIC SH and } 1/8 \times \text{MIC erythromycin}$; L, the gels of A-H and I-L were grouped together. The production of IcaA in different groups was detected at 6 h and 24 h after drug treatment.

Ethical Approval: This is an in vitro study and ethical approval is not required.

Conflict of Interests: The authors declare that this research was conducted without any commercial or financial relationships.

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