

# Antibacterial Mechanism of *Patrinia scabiosaefolia* Against Methicillin Resistant *Staphylococcus epidermidis*

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**Purpose:** *Staphylococcus epidermidis* has become one of the most common causes of septicemia. Meanwhile, *S. epidermidis* has acquired resistance to many antibiotics. Among these, methicillin-resistant *S. epidermidis* (MRSE) were frequently isolated. Similar to methicillin resistant *Staphylococcus aureus* (MRSA), they also exhibited multi-resistance, which presented a danger to human health. *Patrinia scabiosaefolia* as traditional Chinese medicine had strong antibacterial activity against MRSE. However, the mechanism of *P. scabiosaefolia* against MRSE is not clear.

**Methods:** Here, the morphology of cell wall and cell membrane, production of  $\beta$ -lactamase and PBP2, energy metabolism, antioxidant system were systematically studied.

**Results:** The data showed that *P. scabiosaefolia* damaged the cell wall and membrane. In addition,  $\beta$ -lactamase, energy metabolism and antioxidant system were involved in mechanisms of *P. scabiosaefolia* against MRSE.

**Conclusion:** These observations provided new understanding of *P. scabiosaefolia* against MRSE to control MRSE infections.

**Keywords:** methicillin-resistant *Staphylococcus epidermidis*, *Patrinia scabiosaefolia*,  $\beta$ -lactamase, energy metabolism, antioxidant system

## Introduction

*Staphylococcus epidermidis* (*S. epidermidis*) is one of the major opportunistic pathogens associated with skin colonization.<sup>1</sup> With the widespread use of indwelling medical devices and implanted foreign bodies, *S. epidermidis* has become a significant threat to medical device associated infections (MDAI), including catheter-related bloodstream and surgical site infections, infective endocarditis, and so on.<sup>2–5</sup> Recently, several studies have reported that the distribution of *S. epidermidis* in human clinical samples were higher than infections caused by *Staphylococcus aureus* in Greece and India.<sup>6</sup> Antibiotics have been used as the most commonly used first-line treatment for *S. epidermidis*. Yet, a number of European health care outbreaks associated with multidrug-resistant *S. epidermidis* have also been described, among which more than 60% were resistant to methicillin (methicillin-resistant *S. epidermidis*, MRSE). The MRSE showed a broad drug resistance spectrum including  $\beta$ -lactam, quinolone, macrolide, aminoglycosides, and so on.<sup>7</sup> The emergence of MRSE infections emphasized the need for antimicrobial drug design to yield new treatments before the pathogens become resistant to the last-resort antibiotics.

Because of the safety and diversity, traditional Chinese medicine (TCM) has increased widespread interest in the search of alternative antibacterial agents.<sup>8</sup> In our previous study, *Patrinia scabiosaefolia* (PS) possessed antibacterial against MRSE (MIC: 5 mg/mL).<sup>9</sup> PS has a long application story in China, where it is also named “Bai Jiang Cao” recorded in the “Sheng Nong’ Herbal Classic”. Pharmacology researches showed that PS possessed good antibacterial activity in vitro.<sup>10</sup> The studies demonstrated that the extracts of PS had significant antibacterial activity against *S. aureus*, *Staphylococcus albus*, typhoid bacillus, *Streptococcus B*,

pneumococcus, *Escherichia coli*, dysentery bacillus, and *Pseudomonas aeruginosa*.<sup>10,11</sup> However, the antibacterial mechanism of PS against MRSE is still unknown.

In the present work, we systematically studied the antibacterial mechanism of PS against MRSE. The results showed that PS is a promising antibacterial agent and can be used to control MRSE infections.

## Materials and Methods

### Strains and Culture Conditions

In this study, the bacterial strain *S. epidermidis* ATCC12228 was obtained from American Type Culture Collection. The methicillin-resistant *S. epidermidis* (MRSE) was previously induced under methicillin from *S. epidermidis* ATCC12228 and preserved in our lab. The MRSE was cultured at 37°C in Tryptic Soy Broth (TSB). The strain has been identified by routine laboratory methods and stored in 20% (v/v) glycerol at -80°C.

### Antimicrobial Assessments

#### Preparation of *P. scabiosaefolia* Extracts

*P. scabiosaefolia* was purchased from Xi'an Yunyue Biotechnology Co., Ltd (20220106). Then *P. scabiosaefolia* was shade-dried, and then finely powdered. Next, 150 mL water was used to extract 30 g TMC for three times. Then the extracts were merged. The ointment was concentrated by rotary evaporator and dried by vacuum drying oven at 60°C. Finally, 3 g dry powder was received. Each acquired fractions are dissolved in distilled water for 80 mg/mL of final concentration and filtered through a 0.45 µm Millipore syringe filter.

#### Determination of the Antibacterial Capability of *P. scabiosaefolia*

Minimal bactericidal concentration (MBC) was evaluated as reported previously.<sup>12</sup> Briefly, the minimal inhibitory concentration (MIC) of *P. scabiosaefolia* was determined. Then all the MIC wells were then used for coating on agar plates for colony counting. The concentration of no growth (<5 CFU/mL) was determined as MBC.

Fractional inhibitory concentration index (FIC) was calculated using the following formula: FIC index (FICI)=(FIC of methicillin)+(FIC of *P. scabiosaefolia*). Among these, FIC of methicillin was MIC of methicillin in combination/MIC of methicillin alone. Similarly, FIC of *P. scabiosaefolia* was MIC of *P. scabiosaefolia* in combination/(MIC of *P. scabiosaefolia* alone). The combined effects were defined as follows: synergistic: FICI ≤ 0.5; additive: 0.5 ≤ FICI ≤ 1; and indifferent: 1 ≤ FICI ≤ 4. The above assays were repeated three times.

### Antimicrobial Mechanism

#### Effect of *P. scabiosaefolia* on Methicillin-resistant of *S. epidermidis* Membrane Integrity

Experiments were conducted by the technique of Ding et al.<sup>13</sup> Loss of 260 nm absorbing material released from bacteria was measured to detect the effect of *P. scabiosaefolia* on MRSE membrane integrity.<sup>14</sup> The MRSE was harvested at their logarithmic phase, washed and re-suspended in sterile physiological saline to 1×10<sup>6</sup> CFU/mL. Then, MIC of *P. scabiosaefolia* was mixed with the bacterial suspension. The mixtures were then incubated at 37°C for 0, 1, 2, 3, or 4 h. The mixtures were centrifuged at 9800 g for 2 min, and the supernatant was filtration using a 0.22 µm filter membrane. The filtrates were analyzed using a microplate reader at 260 nm.

#### Effect of *P. scabiosaefolia* on Microcosmic Morphology of Methicillin-resistant *S. epidermidis*

MRSE in their logarithmic phase were harvested and re-suspended in sterile physiological saline to 1×10<sup>6</sup> CFU/mL. Furthermore, the bacterial suspensions with and without MIC *P. scabiosaefolia* were incubated at 37°C for 4 h. Meanwhile, the bacterial suspensions with MIC vancomycin were as positive control. Then, according to Ding WY, the mixtures were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).<sup>13</sup>

#### Evaluation of the Inhibitory Production of *P. scabiosaefolia* on β-Lactamase

The inhibition of *P. scabiosaefolia* on β-lactamase production was measured as follows. The MRSE were harvested at their logarithmic phase, washed and re-suspended in sterile physiological saline to 1×10<sup>6</sup> CFU/mL. Then MIC *P. scabiosaefolia* were mixed with the bacterial suspensions. Meanwhile, the bacterial suspensions with tazobactam were

as positive control. The mixtures were then incubated at 37°C for 4 h. Then, the mixture were centrifuged at 9800 g for 5 min at 4°C. Finally, the samples dealt with sonication were measured by ELISA Kit. Each treatment was repeated at least three times.

### Evaluation of the Inhibitory Production of *P. scabiosaefolia* on PBP2a

The inhibition of *P. scabiosaefolia* on PBP2a production was measured as follow. The MRSE were harvested at their logarithmic phase, washed and re-suspended in sterile physiological saline to  $1 \times 10^6$  CFU/mL. Then MIC *P. scabiosaefolia* were mixed with the bacterial suspensions. Meanwhile, the bacterial suspensions without *P. scabiosaefolia* were a negative control. The mixtures were then incubated at 37°C for 4 h. To this cultured broth, 10 mg/mL cefradine was added to induce PBP2a for 2 h. Then the mixtures were centrifuged at 9800 g for 5 min at 4°C. Finally, the samples dealt with sonication were measured by ELISA Kit. Each treatment was repeated at least three times.

### Key Enzymes in Energy Metabolism Process Analysis

The MRSE in the logarithmic phase was washed and re-suspended in sterile physiological saline to  $1 \times 10^6$  CFU/mL. Then MIC *P. scabiosaefolia* were mixed with the bacterial suspensions. The mixtures were then incubated at 37°C for 4 h. A negative control was also prepared, containing distilled water instead of *P. scabiosaefolia*. Next, the succinate dehydrogenase activity (SDH) and NADP-malate dehydrogenase activity (NADP-MDH) activity of MRSE with and without MIC *P. scabiosaefolia* were measured by using kits. Then the mixtures were centrifuged at 9800 g for 5 min at 4°C and the supernatant were removed. The cells were rinsed and suspended in physiological saline solution. Next, the samples dealt with sonication were centrifuged at 9800 g for 5 min at 4°C. Finally, the SDH and NADP-MDH activity in the supernatant were assayed according to directions of test kit from Nanjing Jiancheng Institute. Each treatment was repeated at least three times.

### Antioxidant System Analysis

The MRSE in the logarithmic phase was washed and re-suspended in sterile physiological saline to  $1 \times 10^6$  CFU/mL. Then MIC *P. scabiosaefolia* were mixed with the bacterial suspensions. The mixtures were then incubated at 37°C for 4 h. A negative control was also prepared, containing distilled water instead of *P. scabiosaefolia*. The mixtures were centrifuged 9800 g for 5 min at 4°C and and the supernatant were removed. The cells were rinsed and suspended in physiological saline solution. Next, the samples dealt with sonication were centrifuged 9800 g for 5 min at 4°C. Finally, The catalase (CAT) and superoxide dismutase (SOD) activities in the supernatant were estimated by using kits from Nanjing Jiancheng Institute. Each treatment was repeated at least three times.

## Antibacterial Activity of *P. scabiosaefolia* Against Bloodstream

### Establishment of Mouse Immunosuppressive Models

Male ec57bl/6j mice between six and eight-weeks-old were used in this study. 120 mg/kg cyclophosphamide (CP) treatment regimen was used to induce immunosuppressive model.<sup>15</sup> Briefly, the mice were injected intraperitoneally with CP doses of 120 mg/kg of body weight for four days. Control mice were received phosphate-buffered saline (PBS). Then, thymus and spleen were isolated from the immunosuppressive and control mice. The thymus and spleen index were calculated. The above mouse experiments were approved by the University Committee on Use and Care of Animals at the Guizhou University of Traditional Chinese medicine and done as Laboratory Animal – Guideline for Ethical Review of Animal Welfare (GB/T 35892—2018).

### Treatment of *P. scabiosaefolia* Against MRSE-induced Bloodstream Infection

The mice were randomly separated into four groups, including immunosuppressive group, bloodstream infection group, *P. scabiosaefolia* group and blank group. *P. scabiosaefolia* (8 g/kg) was administered by oral at seven days before infection in *P. scabiosaefolia* group. Then immunosuppression model was performed at three days before infection as the above method in immunosuppressive group, bloodstream infection group and *P. scabiosaefolia* group. Further, bloodstream infection were done in bloodstream infection group and *P. scabiosaefolia* group as follows. MRSE were cultured overnight at 37°C. Then, the bacteria were diluted into  $1 \times 10^8$  cfu/mL. The immunosuppressed mice were injected with 0.1 mL of the bacterial suspension at above doses by tail vein. At 24 h post-infection, the blood of the mice in each group was obtained. These samples were serially diluted in PBS and plated on TSB agar plates for 24 h at 37°C. Finally, the number of clones

were counted in order to evaluate the mouse BSI models.<sup>16</sup> The above mouse experiment was approved by the University Committee on Use and Care of Animals at the Guizhou University of Traditional Chinese Medicine and done as Laboratory Animal – Guideline for Ethical Review of Animal Welfare (GB/T 35892—2018).

## Statistical Analysis

Values were expressed as means  $\pm$ SDs. The statistical differences among the different groups were compared by one-way ANOVA, significant means were separated using Tukey's Honest significant difference and  $p < 0.05$ .

## Results and Discussion

### Antimicrobial Assessments

#### Antimicrobial Activity of *P. scabiosaefolia* Against Methicillin-resistant *S. epidermidis*

More precise data on the antimicrobial properties were obtained through the determination MBC. Based on the above results, MIC of *P. scabiosaefolia* was determined as 5 mg/mL (Table 1). Since complete killing has occurred at 20 mg/mL, this concentration was determined as MBC (Table 2).

In the current study, there is no standard of TCM against bacteria. In this study, literature about the antibacterial activity of TCM was consulted and the results indicated that the effective antibacterial activity of TCM crude extract was from 3.9 mg/mL to 125 mg/mL.<sup>17,18</sup> Therefore, the results showed that *P. scabiosaefolia* may be a promising candidate for the treatment of MRSE infection.

#### Checkerboard Assays of *P. scabiosaefolia* and Methicillin

Beside antimicrobial activity of TCM, they may enhance the antibacterial effects of antibiotics in vitro.<sup>19</sup> So, FICs of *P. scabiosaefolia* and methicillin in vitro were determined using the standard checkerboard methodology. The FIC index of *P. scabiosaefolia* and methicillin against MRSE was 0.75 by reducing MICs of methicillin from 64  $\mu$ g/mL to 16  $\mu$ g/mL (Table 3). According to the standard, the FIC index which is interpreted as  $0.5 < \text{FIC} < 1$ , stand for additive effect. So, the

**Table 1** MIC Value Against MRSE Isolates

Bacteria	Sample	MIC (mg/mL)	Concentration of Antimicrobial Agent (mg/mL)
MRSE	<i>Thlaspi arvense</i> L.	5	160–0.078125

**Table 2** MBC Value Against MRSE Isolates

Bacteria	Samples	CFU/mL
MRSE	Control (TSB)	TNTC
MRSE	5 mg/mL <i>Thlaspi arvense</i> L.	TNTC
MRSE	10 mg/mL <i>Thlaspi arvense</i> L.	$8.1 \times 10^4$
MRSE	20 mg/mL <i>Thlaspi arvense</i> L.	Nil
MRSE	40 mg/mL <i>Thlaspi arvense</i> L.	Nil
MRSE	80 mg/mL <i>Thlaspi arvense</i> L.	Nil
MRSE	160 mg/mL <i>Thlaspi arvense</i> L.	Nil

**Table 3** The Effect of Combinations

Bacteria	Combination	FIC value	Effect	Reduction Fold of MICs of Methicillin by <i>Thlaspi arvense</i> L.
MRSE	<i>Thlaspi arvense</i> L. +methicillin	$0.5 < \text{FIC} < 1$ (0.750)	Additive	64 $\mu$ g/mL to 16 $\mu$ g/mL

combination antibacterial of *P. scabiosaefolia* and methicillin against MRSE was an additive effect. It demonstrated that *P. scabiosaefolia* can improve the sensitivity of methicillin against MRSE.

## Antimicrobial Mechanism

### Effect of *P. scabiosaefolia* on Methicillin-resistant *S. epidermidis* Membrane Integrity

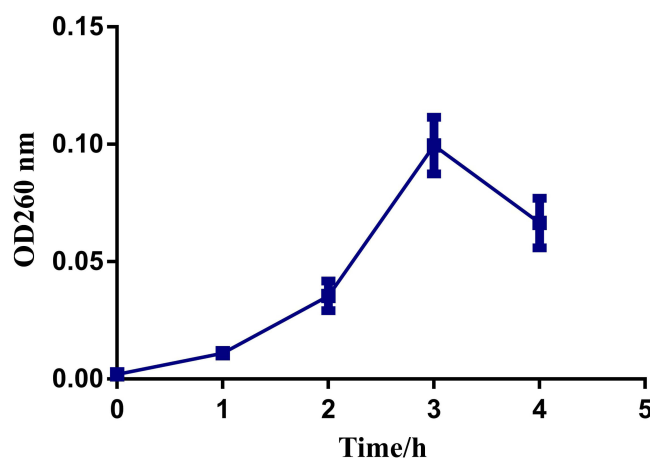
The above results showed that *P. scabiosaefolia* was a strong antibacterial. However, the mechanism of *P. scabiosaefolia* against MRSE was not understood. We investigated the antibacterial effect of *P. scabiosaefolia* to evaluate its possible mechanism. The previous study reported that some antimicrobial agents cause gross membrane damage.<sup>20</sup> So, in our study, membrane perturbation is involved in the antibacterial action. The intracellular components of cells are protected by cell membranes. Thus, the release of the intracellular components of bacterial cells can be used to monitor the membrane integrity.<sup>13</sup> Thus, the detection of DNA and RNA from the protoplasm of bacterial cells represented whether the cell membrane has been destroyed.<sup>14</sup> After treatment with *P. scabiosaefolia* at different times, the OD260 of filtrates of MRSE significantly changed during 4 h and the most remarkable increases occurred after 3-h treatment (Figure 1). The result suggested that *P. scabiosaefolia* damaged the cytoplasmic membrane, thereby causing the leakage of the DNA and RNA from the cells to kill MRSE. Interestingly, the absorbance at 4 h decreased. This was because of the release of RNA or DNA gradually degrading with the extension of time. Similar results were also reported as *Staphylococcus xylosus* and *E. coli* against CD-g-CS.<sup>21</sup>

### Effect of *P. scabiosaefolia* on Microcosmic Morphology of Methicillin-resistant *S. epidermidis*

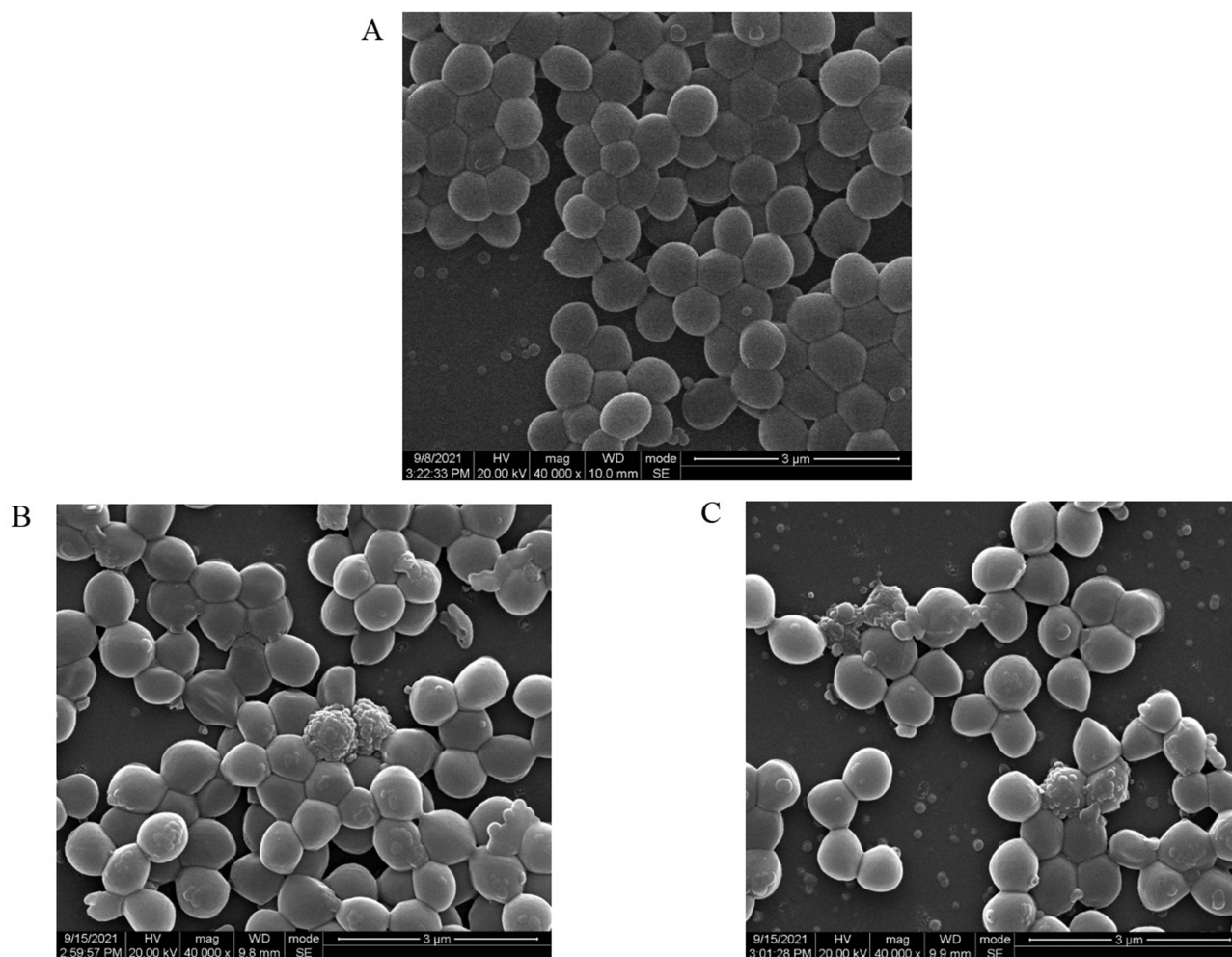
The cell wall and cytoplasmic membrane play a very important role in protecting cells from external damage, and the integrity of the structure and morphology are vital to the normal functioning of cells.<sup>22</sup> Therefore, the cell wall and cytoplasmic membrane of MRSE would be good target.

To understand the mode of action of *P. scabiosaefolia*, morphological changes of MRSE cells were observed using SEM. SEM images of MRSE included the untreated group (Figure 2A), positive-treated (vancomycin) group (Figure 2B), and *P. scabiosaefolia*-treated group (Figure 2C). Figure 2A showed that MRSE cells had smooth, rounded shapes and intact cell membranes. However, MRSE treated with vancomycin showed extracellular changes (Figure 2B). At the same time, Figure 2C showed that the outermost layer of the MRSE had disappeared and the cells had lost a lot of protection which can cause cell death. The results demonstrated that *P. scabiosaefolia* may have severe effects on the cell wall and cytoplasmic membrane. However, more detailed observations were still needed.

To further elucidate the effect of *P. scabiosaefolia* against MRSE membrane, the above groups were imaged with TEM. TEM allowed higher magnification and observation of intracellular structures of the cells. As shown in Figure 3A, MRSE cells without *P. scabiosaefolia* were normal and surrounded by cell membranes with a compact



**Figure 1** Release of intracellular components of methicillin-resistant *S. epidermidis* treated with MIC *P. scabiosaefolia*. Data were expressed as mean  $\pm$  standard deviation (n=3).



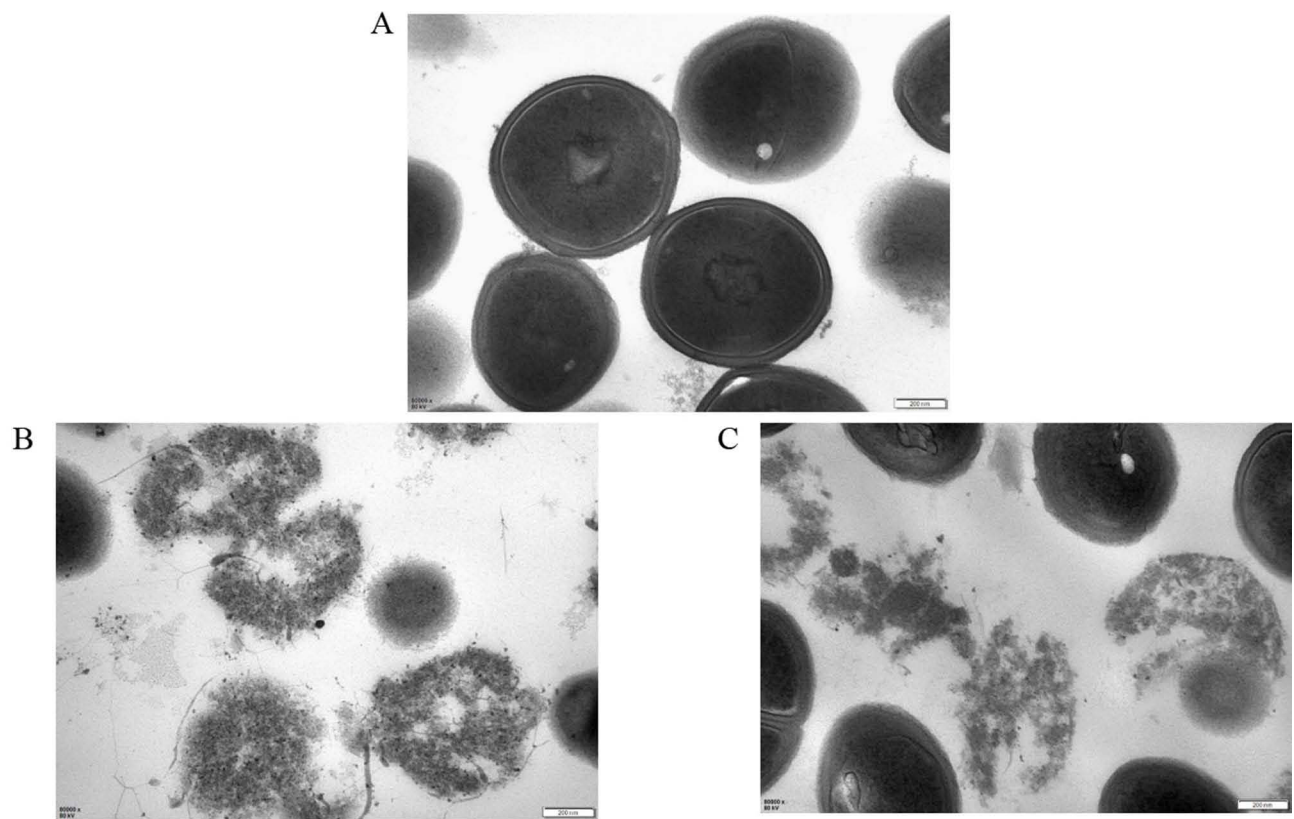
**Figure 2** SEM micrographs of methicillin-resistant *S. epidermidis*. (A) MRSE untreated with *P. scabiosaefolia*. (B) Methicillin-resistant *S. epidermidis* treated with MIC vancomycin. (C) Methicillin-resistant *S. epidermidis* treated with MIC *P. scabiosaefolia*.

surface, which showed a well-defined cell membrane and a uniform cytoplasm region, without the release of intracellular components. However, most MRSE cells with *P. scabiosaefolia* (Figure 3C) appeared the lysis of cell wall, discontinuous cell membrane, and a few cells had dissolved. Meanwhile, the similar breakage also appeared in positive-treated (vancomycin) group (Figure 3B). In short, among the three samples, only the morphological structure of the bacteria in untreated group was relatively normal. In the *P. scabiosaefolia* and vancomycin groups, different degrees of necrosis were found.

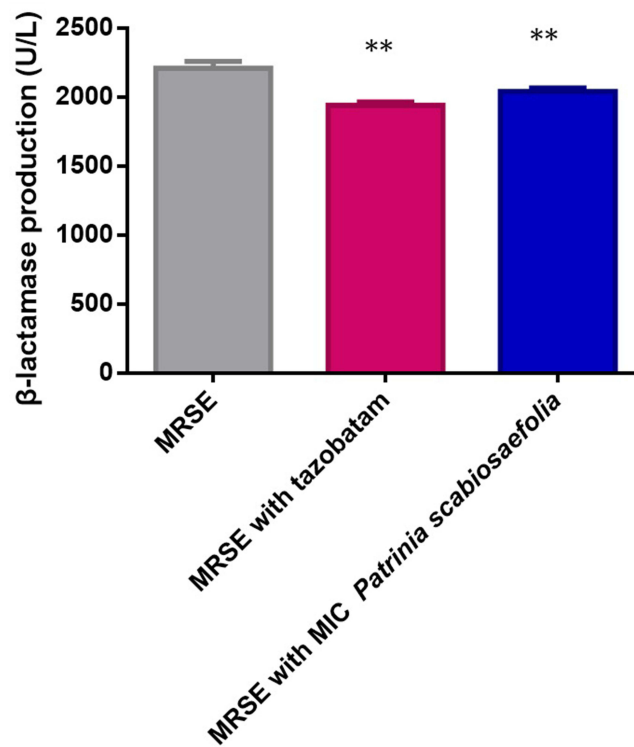
So, the both images suggested that the antimicrobial mechanism of *P. scabiosaefolia* was related to wall and membrane disruption. Meanwhile, The similar results occurred in *Aronia melanocarpa* (Michx.), *Chaenomeles superba* Lindl. and *Cornus mas* L. leaf extracts against bacteria cells.<sup>23</sup>

#### Effect of *P. scabiosaefolia* on $\beta$ -Lactamase and PBP2a

It is well known that the production of PBP2a and  $\beta$ -lactamase is attributed to  $\beta$ -lactam resistance.<sup>24</sup> So, the antimicrobial mechanism of *P. scabiosaefolia* against MRSE may relate to them. In order to explore relationship of  $\beta$ -lactamase and *P. scabiosaefolia*,  $\beta$ -lactamase production was tested in MRSE untreated or treated with *P. scabiosaefolia*. Figure 4 showed that the positive control (tazobactam) reduced  $\beta$ -lactamase production. Since  $\beta$ -lactamase production was significantly reduced by MIC *P. scabiosaefolia*, compared to the untreated group. The results instructed that the antimicrobial mechanism of *P. scabiosaefolia* against MRSE was related to  $\beta$ -lactamase.



**Figure 3** TEM micrographs of methicillin-resistant *S. epidermidis*. (A) MRSE untreated with *P. scabiosaefolia*. (B) Methicillin-resistant *S. epidermidis* treated with MIC vancomycin. (C) Methicillin-resistant *S. epidermidis* treated with MIC *P. scabiosaefolia*.



**Figure 4** Inhibition of  $\beta$ -lactamase production in methicillin-resistant *S. epidermidis*. X axis indicated methicillin-resistant *S. epidermidis* treated with or without *P. scabiosaefolia*. Difference analysis between methicillin-resistant *S. epidermidis* with different drugs and MRSE. \*\*Indicated a significant difference ( $p \leq 0.01$ ). Data were expressed as mean  $\pm$  standard deviation ( $n=3$ ).

In addition, PBP2a production was also tested. In order to explore relationship between PBP2a and *P. scabiosaefolia*, PBP2a production was tested in MRSE untreated or treated with *P. scabiosaefolia*. Differently, [Supplementary Figure 1](#) showed that PBP2a production was not reduced by MIC *P. scabiosaefolia*, compared to the untreated group. So, the results indicated that the antimicrobial mechanism of *P. scabiosaefolia* against MRSE was not related to PBP2a.

### Effect of *P. scabiosaefolia* on Energy Metabolism Enzyme of MRSE

The TCA cycle is the vital pathway to provide energy for cell activity, which not only provides energy for organisms, but also serves as the hub for the transformation of three major substances (sugar, lipid, and protein).<sup>25</sup> In TCA cycle, MDH can catalyze malic acid to form oxaloacetic acid, which is essential for cell growth. In addition, SDH is also a key enzyme in the TCA cycle and one of the key links between oxidative phosphorylation and electron transport. So, the activities of SDH and NADP-MDH were investigated. Compared with the control group, [Figure 5A](#) and [B](#) showed that the activities of SDH and NADP-MDH of MRSE under *P. scabiosaefolia* stress for 4 h significantly decreased. The above results were consistent with previous research reported by Chen et al, that the SDH enzyme activity of *S. aureus* was significantly suppressed under persimmon tannin.<sup>26</sup> Therefore, the *P. scabiosaefolia* had a strong effect on the two key enzymes in energy metabolism.

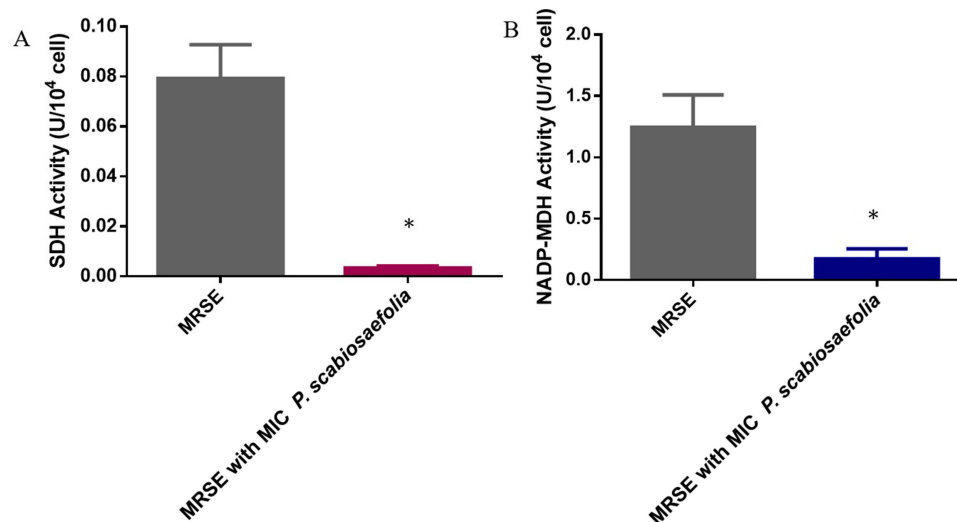
### Effect of *P. scabiosaefolia* on Antioxidant Enzyme System of MRSE

Defense mechanisms of cells including SOD, CAT and other antioxidant enzymes have the ability to counteract the killing effect of ROS, which is one of their mechanisms of antibacterial activity.<sup>27</sup> To explore the impact of MRSE by *P. scabiosaefolia*, the activity of ROS related enzymes (SOD and CAT) were determined. As seen in [Figure 6A](#) and [B](#), CAT and SOD treated with *P. scabiosaefolia* significantly reduced ( $p < 0.05$ ) when compared with control group. Thence, we believed that the *P. scabiosaefolia* had stronger effect on the two key enzymes in antioxidant enzyme system.

The production of ROS inevitably caused oxidative stress into bacteria death.<sup>28</sup> On the contrary, antioxidant enzyme system can withstand the killing effect.<sup>29</sup> The research demonstrated the plant extracts can decrease of SOD and CAT activities.<sup>23</sup> Similarly, the activity of antioxidant enzyme system decrease in our study, demonstrating that *P. scabiosaefolia* can promote the effect of oxidative stress.

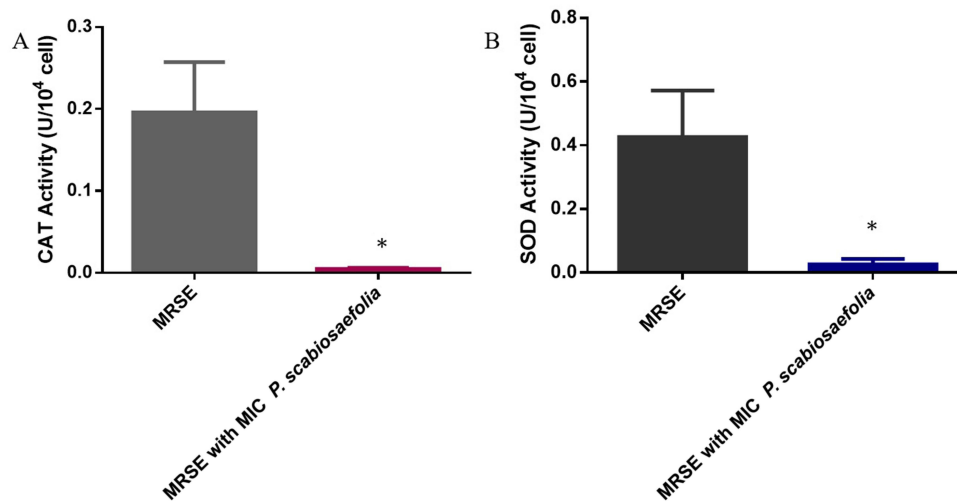
### Effect of *P. scabiosaefolia* Against MRSE-induced Bloodstream Infection

In the above work, we found that *P. scabiosaefolia* had better antibacterial activity against MRSE in vitro. In order to further evaluate the antibacterial activity in vivo, the antimicrobial efficacy of *P. scabiosaefolia* treating



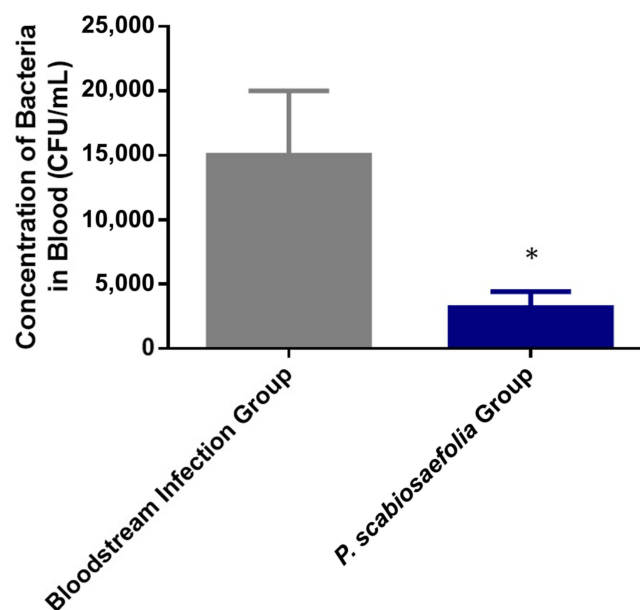
**Figure 5** The activity of SDH and NADP-MDH of MRSE with and without MIC *P. scabiosaefolia*. X axis indicated methicillin-resistant *S. epidermidis* treated with or without *P. scabiosaefolia*. Difference analysis between methicillin-resistant *S. epidermidis* with different drugs and MRSE. \*Indicated a significant difference ( $p \leq 0.01$ ). Data were expressed as mean  $\pm$  standard deviation ( $n=3$ ). (A) The activity of SDH of MRSE with and without MIC *P. scabiosaefolia*. (B) The activity of NADP-MDH of MRSE with and without MIC *P. scabiosaefolia*.





**Figure 6** The activity of CAT and SOD of MRSE with and without MIC *P. scabiosaefolia*. X axis indicated methicillin-resistant *S. epidermidis* treated with or without *P. scabiosaefolia*. Difference analysis between methicillin-resistant *S. epidermidis* with different drugs and MRSE. \*Indicated a significant difference ( $p \leq 0.01$ ). Data were expressed as mean  $\pm$  standard deviation ( $n=3$ ). (A) The activity of CAT of MRSE with and without MIC *P. scabiosaefolia*. (B) The activity of SOD of MRSE with and without MIC *P. scabiosaefolia*.

MRSE-induced bloodstream infection was done. Because *S. epidermidis* is one of the major opportunistic pathogens, the immunosuppression model should be established. [Supplementary Figure 2](#) showed that compared to blank group, the thymus and spleen index of immunosuppressive group, bloodstream infection group, and *P. scabiosaefolia* group significantly reduced. The results illustrated the immunosuppression model was successfully established. The MRSE-induced bloodstream infection model was established following the immunosuppression model. Based on the bloodstream infection model, the validity of *P. scabiosaefolia* was studied. The [Figure 7](#) showed that after treatment of *P. scabiosaefolia*, the the number of clones of blood from *P. scabiosaefolia* group were significantly reduced, when compared with bloodstream infection group. The results illustrated *P. scabiosaefolia* had better antibacterial activity.



**Figure 7** Bacterial loads in the blood at 24 h post-infection. X axis indicated the mice treated with or without *P. scabiosaefolia*. \*Indicated a significant difference ( $p \leq 0.05$ ). Data were expressed as mean  $\pm$  standard deviation ( $n=3$ ).

## Conclusion

*P. scabiosaefolia* showed strong antimicrobial activity against MRSE in vitro and vivo. Furthermore, based on cell membrane integrity tests, SEM, TEM observation, enzyme activity determined that the antimicrobial mechanisms of *P. scabiosaefolia* were due to membrane disruption,  $\beta$ -lactamase reduction, inhibition of energy metabolism and antioxidant system. The findings of this study can help us understand the antimicrobial mechanism of *P. scabiosaefolia*, and may become a promising candidate for the treatment of MRSE infection.

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## Disclosure

All authors declare no competing interests.

## References

1. Xu Z, Cave R, Chen LQ, et al. Antibiotic resistance and molecular characteristics of methicillin-resistant *Staphylococcus epidermidis* recovered from hospital personnel in China. *J Global Antimicrob Resist*. 2020;22:195–201. doi:10.1016/j.jgar.2020.02.013
2. Watanabe S, Kawakami Y, Kimura H, Murakami S, Yamaguchi T. Association between daptomycin susceptibility and teicoplanin resistance in *Staphylococcus epidermidis*. *Sci Rep*. 2019;9(1). doi:10.1038/s41598-019-55149-z
3. Nguyen TH, Park M, Otto M. Host Response to *Staphylococcus epidermidis* Colonization and Infections. *Front Cell Infect Microbiol*. 2017;7:90. doi:10.3389/fcimb.2017.00090
4. Rice C, Lam AK, Foxley MA, Moen EL, Pusavat J, Wouters CL. Cationic Branched Polyethylenimine (BPEI) disables antibiotic resistance in methicillin-resistant *Staphylococcus epidermidis* (MRSE). *ChemMedChem*. 2018;13(20):2240–2248. doi:10.1002/cmdc.201800433
5. Yang M, Wang YW, Zhang YH, Li YJ, Li QF, Tan JT. Role of interleukin-33 in *Staphylococcus epidermidis*-induced septicemia. *Front Immunol*. 2020;11:534099. doi:10.3389/fimmu.2020.534099
6. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. *Clin Microbiol Rev*. 2014;27:870–926. doi:10.1128/CMR.00109-13
7. Ran FU, Fang-mei FU, Han-mei H, et al. Multi-drug resistance phenotype analysis of methicillin resistant *Staphylococcus epidermidis* and screening of antibacterial Chinese medicine. *J Med Inform*. 2022;35(3):1–8.
8. Paolo MG. Traditional phytotherapy in central Italy (Marche, Abruzzo, and Latium). *Fitoterapia*. 2005;76(1):1–25. doi:10.1016/j.fitote.2004.09.006
9. Liu X, An LL, Ren SJ, Zhou YH, Peng W. Comparative proteomic analysis reveals antibacterial mechanism of *Patrinia scabiosaefolia* against methicillin resistant *Staphylococcus epidermidis*. *Infect Drug Resist*. 2022;15:883–893. doi:10.2147/IDR.S350715
10. He XR, Luan F, Zhao ZF, Ning N, Li MX, Jin L. Chemical constituents and pharmacological effects of genus *patrinia*: a review. *Curr Pharmacol Rep*. 2020;6(6):1–35.
11. Chao T, Sun Z, Zhou K, Xu J. Studies on chemical constituents and sedation and antibacterial effect of *Patrinia scabiosaefolia* Fisch. *J Trad Chin Vet Med*. 2003;22(4):3–5.
12. Hashizume H, Takahashi Y, Masuda T, et al. In vivo efficacy of  $\beta$ -lactam/triprropeptin C in a mouse septicemia model and the mechanism of reverse  $\beta$ -lactam resistance in methicillin-resistant *Staphylococcus aureus* mediated by tripropeptin C. *J Antibiot*. 2017;71(1):79–85.
13. Ding WY, Zheng SD, Qin Y, Yu F, Bai JW, Cui WQ. Chitosan grafted with  $\beta$ -cyclodextrin: synthesis, characterization, antimicrobial activity, and role as absorbent and solubilizer. *Front Chem*. 2019;6. doi:10.3389/fchem.2018.00657
14. Devi KP, Nisha SA, Sakthivel R, Pandian SK. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *J Ethnopharmacol*. 2010;130(1):107–115. doi:10.1016/j.jep.2010.04.025
15. Subashchandrabose S, Sara S, Valerie D, et al. *Acinetobacter baumannii* Genes required for bacterial survival during bloodstream infection. *MSphere*. 2015;1(1). doi:10.1128/mSphere.00013-15
16. Chen X, Lou WY, Liu JX, Ding BS, Fan WM, Hong J. A novel antimicrobial polymer efficiently treats multidrug-resistant MRSA-induced bloodstream infection. *Biosci Rep*. 2019;39(10). doi:10.1042/BSR20192354
17. Zhou Y, Li FH, Guo JJ, Ren H, Wang QH, Chen XQ. Screening of antibacterial activity in vitro of 25 kinds of Chinese medicinal materials from guizhou. *Chin J Ethnomed Ethnopharm*. 2020;29(9):7.

18. Yi GJ. *Study on the Pharmacodynamics and Mechanism of Anti-Drug-Resistant Pseudomonas Aeruginosa Biofilm of Qingrejiedu Traditional Chinese Medicine*. Chengdu University; 2020.
19. Kirmusaoglu S. Sensitizing of  $\beta$ -lactam resistance by tannic acid in methicillin-resistant *S. aureus*. *World J Microbiol Biotechnol*. 2019;35(4). doi:10.1007/s11274-019-2637-6
20. Chovanová R, MikuláOvá M, Vaverková Š. In vitro antibacterial and antibiotic resistance modifying effect of bioactive plant extracts on methicillin-resistant *Staphylococcus epidermidis*. *Int J Microbiol*. 2013;2013:1–7. doi:10.1155/2013/760969
21. Kpadonou D, Kpoviessi S, Bero J, et al. Chemical composition, in vitro antioxidant and antiparasitic properties of the essential oils of three plants used in traditional medicine in Benin. *J Med Plant Res*. 2019;13(6):384–395.
22. Wang R, Zhang Y, Jia Y, et al. Persimmon oligomeric proanthocyanidins exert antibacterial activity through damaging the cell membrane and disrupting the energy metabolism of *Staphylococcus aureus*. *ACS Food Sci Technol*. 2021;1(1):35–44. doi:10.1021/acsfoodscitech.0c00021
23. Efenberger-Szmechtyk M, Nowak A, Czyowska A, Niadowska M, Otlewska A, Yelewicz D. Antibacterial mechanisms of *Aronia melanocarpa* (Michx.), *Chaenomeles superba* Lindl. and *Cornus mas* L. leaf extracts. *Food Chem*. 2021;350:129218. doi:10.1016/j.foodchem.2021.129218
24. Tajima Y. Effects of tungstosilicate on strains of methicillin-resistant *Staphylococcus aureus* with unique resistant mechanisms. *Microbiol Immunol*. 2003;47(3):207–212. doi:10.1111/j.1348-0421.2003.tb03388.x
25. Mla B, Mf C, Ky A, et al. Transcriptomic and metabolomic analyses reveal antibacterial mechanism of astringent persimmon tannin against Methicillin-resistant *Staphylococcus aureus* isolated from pork - ScienceDirect. *Food Chem*. 2020;309:125692.
26. Chen J, Tang C, Zhang R, Ye S, Yang D. Metabolomics analysis to evaluate the antibacterial activity of the essential oil from the leaves of *Cinnamomum camphora* (Linn.) Presl. *J Ethnopharmacol*. 2020;253:112652. doi:10.1016/j.jep.2020.112652
27. Chai Z, Zhang F, Liu B, Chen X, Meng X. Antibacterial mechanism and preservation effect of curcumin-based photodynamic extends the shelf life of fresh-cut pears. *LWT Food Sci Technol*. 2021;142(23):110941. doi:10.1016/j.lwt.2021.110941
28. Chen Z, Chen Z, Gan Y, Li T, Kexin GU, Yin L. Antibacterial photodynamic gold nanoparticles for skin infection. *ACS Appl Bio Mater*. 2021;4(2):1461–1469. doi:10.1021/acsabm.0c01334
29. Kim J, Kim S, Lee K, Kim RH, Hwang KT. Antibacterial photodynamic inactivation of fagopyrin f from tartary buckwheat (*Fagopyrum tataricum*) flower against *Streptococcus mutans* and its biofilm. *Int J Mol Sci*. 2021;22(12):6205.

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