The Antibacterial Activity and Mechanism of Emilia Sonchifolia Against Methicillin Resistant Staphylococcus Epidermidis

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Research Article

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Abstract

Background: Bloodstream infections (BSIs) are a public health concern and cause substantial morbidity and mortality.The pathogen *Staphylococcus epidermidis* causes a significant number of BSIs. Antibiotics targeting *Staphylococcus epidermidis* have been the mainstay in BSIs. However, Conventional antibiotics have been eclipsed in combating with drug-resistant bacteria. Alternate ways of treating these antibiotic-resistant infections are thus urgently needed. Numerous studies have demonstrated that certain Chinese medicines exhibit notable antimicrobial activity and possess the ability to impede the development of bacterial resistance. Based on an extensive body of research in the field of traditional Chinese medicine(TCM), it has been determined that the Compositae plant exhibits a noteworthy anti-*Methicillin-Resistant Staphylococcus Epidermidis* (MRSE) effect.

Methods: Initially, the Compositae plant *Emilia sonchifolia* underwent a screening process, followed by crushing and extraction using water. Subsequently, the extract was concentrated based on a specific ratio, dried, partitioned, and subsequently prepared for utilization.Therefore, the objective of this study was to examine the antibacterial efficacy and underlying antibacterial mechanism of *Emilia sonchifolia* against *methicillin-resistant Staphylococcus epidermidis*(*MRSE*). The antibacterial activity of *Emilia sonchifolia* against *MRSE* was assessed through *in vitro* tests measuring minimum inhibitory concentration(MIC) and minimum bactericidal concentration(MBC). Furthermore, the antibacterial activity of *Emilia sonchifolia* against *MRSE* was evaluated *in vivo* using a mouse bloodstream infections model. Additionally, various aspects such as bacteria cell morphology and energy metabolism and defense mechanisms were investigated to explore the underlying antibacterial mechanisms of *Emilia sonchifolia*.

Results: The results showed that MIC and MBC values of *Emilia sonchifolia* against *MRSE* were 5mg/mL and 20mg/mL, respectively. Meanwhile, *Emilia sonchifolia* can effectively treat *MRSE* induced bloodstream infections. Furthermore, the utilization of scanning electron microscopy and transmission electron microscopy techniques unveiled that the administration of *Emilia sonchifolia* induced alterations in the cellular structure of *MRSE*, leading to the disruption of both cell wall and membrane integrity. Additionally, exposure to *Emilia sonchifolia* resulted in a decrease in the enzymatic activities of succinate dehydrogenase, NADP-malate dehydrogenase, catalase, and superoxide dismutase.

Conclusions: Thus, the aforementioned observations have contributed novel insights into the mechanistic understanding of *Emilia sonchifolia*'s efficacy against *MRSE*, thereby offering potential strategies for managing *MRSE* infections.

Introduction

Bloodstream infections (BSIs) represent a significant global public health concern, characterized by a substantial mortality rate. 1200 000 episodes of BSIs each year in Europe were estimated and had long-term sequelae.[1] BSIs were usually caused by catheter devices, including Hemodialysis catheter, central

venous catheters and so on.[2, 3] Bacterial or fungal colonization can initiate at the catheter, leading to the development of biofilms within the lumen that subsequently spread to the bloodstream. Catheter-related infections are frequently caused by skin commensals, with Gram-positive organisms accounting for 70% of cases.[3] Notably, *Staphylococcus epidermidis* (*S. Epidermidis*) is the predominant pathogen associated with both implanted devices and bloodstream infections (BSIs).[4] In recent times, S. epidermidis has emerged as a prevalent and significant nosocomial pathogen.[5, 6] Moreover, S. epidermidis infections frequently exhibit resistance to antibiotics, impeding the effective management of such infections.[7] Additionally, the occurrence of multi-resistance in nosocomial strains of *S. epidermidis* has been reported to reach as high as 70–85%.[8] Today, the great majority of nosocomial *S. epidermidis* isolates present resistance to methicillin (MRSE) and MRSE would induce the multi-resistant to β-lactam antibiotics.[8] *Methicillin-resistant Staphylococcus epidermidis* bloodstream infections (MRSE BSIs) are linked to substantial healthcare expenditures, morbidity, and mortality .[9] Vancomycin represents a restricted selection of available treatment options. However, it is important to note that vancomycin possesses limited biocidal properties, necessitating therapeutic drug monitoring, and may induce nephrotoxicity and/or ototoxicity in specific patient populations .[10, 11] Therefore, the current prevalence of MRSE BSIs has presented a significant challenge, prompting the necessity for ongoing research into more effective and secure therapeutic agents.[12]

“Traditional or herbal medicines”, derived from plants and historically employed in diverse cultures, are commonly perceived as safe when administered in appropriate dosages.[13] Consequently, there is a renewed focus on exploring traditional or herbal medicines as potential alternatives or complementary treatments for emerging and re-emerging multi-drug resistant bacteria .[14] Meanwhile, numerous traditional or herbal medicines have been studied for their antimicrobial activity.[15] The antibacterial activity of extracts derived from the leaves of Diospyros spp. (D. barteri and D. monbuttensis) against a diverse array of Gram-positive and Gram-negative bacteria was found to be potent.[16] Similarly, the aqueous extracts of Carum copticum and Albizia adianthifolia exhibited notable antibacterial effects against multidrug-resistant gram-negative human pathogenic bacteria, including E.coli, *Pseudomonas*, *K. pneumonia*, *Salmonella*, and Proteus species .[17, 18] More importantly, *Patrinia scabiosaefolia* had good antibacterial effect against MRSE in our previous study. [19] Furthermore the antibacterial activity of *Patrinia scabiosaefolia* on animal model of MRSE BSIs showed that *Patrinia scabiosaefolia* had good effect on MRSE BSIs *in vivo* .Therefore we speculated that the other traditional or herbal medicines may have good antibacterial activity against MRSE BSIs.

Emilia sonchifolia has a well-documented history of use in China, as recorded in the "Lingnan Medicine Collection." It is primarily utilized as a traditional Chinese medicine and is known for its anti-inflammatory, analgesic, antibacterial, antiviral, antitumor, hypoglycemic, and immune-regulating properties.[20] Ugwoke further reports that *Emilia sonchifolia* has demonstrated significant antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Neisseria gonorrhoeae in vitro*. [20] However, its effectiveness against MRSE remains unexplored.

The current study focuses on a systematic investigation of the *in vitro* and *in vivo* antibacterial activity of *Emilia sonchifolia* against *MRSE*. Additionally, the study explores the underlying antibacterial mechanisms. In conclusion, *Emilia sonchifolia* exhibits promising potential as an antibacterial agent and may be utilized for the management of MRSE bloodstream infections.

Materials and Methods

1. Materials

Methicillin-resistant Staphylococcus epidermidis (MRSE) was previously cultured and maintained in our laboratory. Male C57BL/6J mice, aged 6–8 weeks, were procured from Changsha Tianqin Biotechnology Co., Ltd.. The ethanol extract of *emilia sonchifolia* (10g crude drug/ 1g extract) was purchased from Shanxi Xibolan Biotechnology Co., Ltd. (20220315).. The assay kits for succinate dehydrogenase, NADP-Malate dehydrogenase, catalase, and superoxide dismutase activities were acquired from Beijing Solarbio Science & Technology Co., Ltd. Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) were procured from Oxoid.

2. Methods

2.1 Culture conditions

The MRSE strains were stored at a temperature of -80℃ in Tryptic Soy Broth (TSB) supplemented with 20% (vol/vol) glycerol. Before each experiment, approximately 20 μL aliquots of the frozen cultures were transferred to tubes containing 5 mL of TSB and incubated overnight at 37℃. Subsequently, the cultures were inoculated onto Tryptic Soy Agar (TSA) and stored at 4℃ until they were needed for further experimentation

.2.2 The Antibacterial Activity of Emilia Sonchifolia In Vitro

2.2.1 Determination of Minimum Inhibitory Concentration (MIC)

According to the Clinical and Laboratory Standards Institute of the United States of America, the MIC of *Emilia sonchifolia* was determined using the broth dilution method. In brief, a bacterial suspension cultured to the logarithmic phase was diluted to a concentration of 1×10^6 colony-forming units per milliliter (CFU/mL). Then, 100 μL of the adjusted inoculum (1×10^6 CFU/mL) was added to each well of a 96-well Microtiter plate. Subsequently, 100 μL of serial twofold dilutions of Emilia sonchifolia with tryptic soy broth (TSB) were dispensed in the wells, ranging from a concentration of 80 mg/mL to 0.0195 mg/mL. After incubation for 18 hours at 37℃, the MIC was defined as the lowest concentration of luteo.

2.2.2 Determination of Minimum Bactericidal Concentration (MBC)

The dilution in broth method was employed to determine the MBC for *emilia sonchifolia* [22]. The culture suspensions (100 L) of MRSE treated with emilia sonchifolia at concentrations of 5 and 40 mg/mL, where no bacterial growth was observed, were evenly spread on tryptic soy agar (TSA) plates and incubated at 37℃ for 18 hours. The surviving colonies were subsequently observed. The MBC was defined as the lowest concentration of antimicrobial agent capable of inactivating more than 99.99% of the bacterial population (<10 CFU/mL). The assay was performed in triplicate.

2.3 The Antibacterial Mechanism of Emilia Sonchifolia In Vitro

2.3.1 Morphological characterizations

Morphological characterizations of MRSE treated with *emilia sonchifolia* were explored using Scanning electron microscopy (SEM) and Transmission Electron Microscopy (TEM).

The scanning electron microscopy (SEM) was conducted using a modified method that had been previously employed. In summary, emilia sonchifolia (1 MIC) was introduced to a logarithmic phase bacterial suspension. The control group received an equal volume of phosphate buffer saline (PBS). All groups were incubated at 37℃ for a duration of 4 hours. Subsequently, the treated bacterial cells were harvested, washed, and subsequently fixed in a 3% glutaraldehyde solution overnight at 4℃. Next, the bacterial cells underwent dehydration through the application of ethanol with varying concentrations, followed by replacement with tertiary butyl alcohol. Subsequently, the samples were subjected to freeze-drying, sputter coated with gold, and examined through microscopic observations utilizing a scanning electron microscope. For the transmission electron microscopy (TEM) analysis, the MRSE were treated in a manner consistent with the SEM procedure. As per the report, the specimens were affixed to a fibrous carbon film and observed using TEM [21].

2.3.2 Effect of Emilia Sonchifolia on Energy Metabolism

In order to assess the impact of Emilia sonchifolia on the activity of SDH and NADP-MDH in MRSE, the SDH activity in bacterial cells was measured. A bacterial suspension in logarithmic phase was treated with Emilia sonchifolia (1 MIC), while the control group received an equivalent volume of PBS. Subsequently, all groups were incubated at 37℃ for a duration of 4 hours. Following the incubation period, the cultures were individually centrifuged at 12000r/min for 5 minutes at 4℃.The bacterial cells that had been collected were resuspended in 1 mL of phosphate-buffered saline (PBS) and subjected to ultrasonication at a strength of 30% for a duration of 30 minutes with an interval of 5 seconds. Subsequently, the supernatant was obtained through centrifugation at a speed of 12,000 revolutions per minute for 5 minutes at a temperature of 4 ºC. The activity of succinate dehydrogenase (SDH) and NADP-dependent malate dehydrogenase (NADP-MDH) was determined using the SDH and NADP-MDH assay kit.

2.3.3 Effect of Emilia Sonchifolia on Antioxidant system

The activities of CAT and SOD were evaluated in MRSE under *emilia sonchifolia* stress. MRSE in the logarithmic growth phase was introduced to emilia sonchifolia at a concentration of 1 minimum inhibitory concentration (MIC). Subsequently, the mixtures were incubated at 37°C for 4 hours. A negative control was also prepared, consisting of phosphate-buffered saline (PBS) instead of emilia sonchifolia. The mixtures were then subjected to centrifugation at 12000 revolutions per minute for 5 minutes at 4°C, and the resulting supernatant was discarded. The cells were subsequently washed and suspended in PBS. Subsequently, the samples were subjected to ultrasonication at a concentration of 30% for a duration of 30 minutes (with an interval of 5 seconds) and then centrifuged at a speed of 12000 revolutions per minute for 5 minutes at a temperature of 4℃. Ultimately,the supernatant was divided into aliquots and used for the CAT and SOD assays by kits from Beijing Solarbio Science & Technology Co.,Ltd..

2.4 The Antibacterial Activity of Emilia Sonchifolia In Vivo

2.4.1 Animals conditioning

The mice were assigned to individual cages in a pathogen-free facility, with a maximum occupancy of six mice per cage. Prior to their utilization, the mice were subjected to a 12-hour light-dark cycle at a temperature of 22 ± 2°C for a duration of one week. Additionally, they were provided unrestricted access to both food and water.

2.4.2 Establishment of mouse immunosuppressive models

Based on the existing literature, a mouse immunosuppressive model was established through intraperitoneal injection of CP doses at 120 mg/kg of body weight for a duration of 4 days. Control mice were administered PBS. Subsequently, the thymus and spleen were extracted from both the immunosuppressive and control mice, and the thymus and spleen index was computed.

2.4.3 Treatment of Emilia Sonchifolia against MRSE-induced BSIs

The animals were randomly assigned to different groups prior to the experiment, including the immunosuppressive group, bloodstream infection group, Emilia sonchifolia group, and blank group. In the Emilia sonchifolia group, *Emilia sonchifolia* was administered orally at a dosage of 8g/kg, 7 days prior to infection. The other three groups received an equal volume of PBS.

The immunosuppression model was implemented three days prior to infection, following the aforementioned method, in the immunosuppressive group, bloodstream infection group, and Patrinia scabiosaefolia group, and the blank group were dealt with PBS. Furthermore, bloodstream infection group, *Emilia sonchifolia* group were infected at 7 d after *Emilia sonchifoli* oral administration by tail vein injection with 1×10^{8}cfu/mL MRSE, and the immunosuppressive group,blank group were dealt with PBS. The way of tail veins were consistent with the procedure outlined in Liu et al.[24] After that, at 12 h post infection (pi), blood samples were collected from tail veins in all groups. Meanwhile the above mouse

were euthanized by 100mg/kg Pentobarbitol Sodium (IP). Subsequently, the aforementioned samples underwent serial dilution in phosphate-buffered saline (PBS) and were subsequently plated on tryptic soy broth (TSB) agar plates for a duration of 24 hours at a temperature of 37◦C. Ultimately, the enumeration of clones was conducted to assess the efficacy of the mouse bloodstream infection (BSI) models.[25]

The aforementioned mouse experiments were authorized by the University Committee on Use and Care of Animals at the Guizhou University of Traditional Chinese Medicine, in accordance with the Laboratory Animal – Guideline for Ethical Review of Animal Welfare (GB/T 35892—2018).

2.5 Statistical analysis

The values were reported as means ± standard deviations (SDs). To assess the statistical differences among the various groups, a one-way analysis of variance (ANOVA) was conducted. Significant differences between means were determined using Tukey's Honest Significant Difference test, with a significance level set at $p < 0.05$.

Results

1. The Antibacterial Activity of Emilia Sonchifolia In Vitro

The antibacterial activities of *Emilia sonchifolia* against MRSE were mainly indicated by MIC and MBC. After 18 h of incubation at 37°C, turbidity was noticed in the well 2.5 to 0.039 mg/ml of *Emilia sonchifolia*. In concentrations ranging from 40 to 5 mg/ml, the absence of turbidity was observed, indicating the inhibitory effect on bacterial proliferation. According to CLSI, the the MIC value of *Emilia sonchifolia* against MRSE was 5 mg/mL (Table 1).

Table 1
MIC value of *Emilia sonchifolia* against MRSE isolates

Bacteria	Sample	MIC (mg/mL)	Concentration of antimicrobial agent (mg/mL)
MRSE	*Emilia sonchifolia*	5	160-0.078125

Meanwhile, MRSE showed resistance to oxacillin, confrming the efective resistance of MRSE. Furthermore, the suspension ranging from 40 to 5 mg/mL was introduced onto a TSA agar plate and subjected to an 18-hour incubation period. The resulting observation of less than 10 CFU/mL in the concentration range of 40 to 20 mg/mL serves as conclusive evidence of its bactericidal properties. So, the MBC value of *Emilia sonchifolia* was 20 mg/mL (Table 2).

Table 2
MBC of *Emilia sonchifolia* value against MRSE

Bacteria	Samples	CFU/ml
MRSE	Control (TSB)	TNTC
MRSE	5mg/mL *Emilia sonchifolia*	TNTC
MRSE	10mg/mL*Emilia sonchifolia.*	7.3×10^4
MRSE	20mg/mL *Emilia sonchifolia*	Nil
MRSE	40mg/mL *Emilia sonchifolia*	Nil
MRSE	80mg/mL *Emilia sonchifolia*	Nil
MRSE	160mg/mL *Emilia sonchifolia*	Nil

2. The Antibacterial Mechanism of Emilia sonchifolia In Vitro

2.1 SEM Analysis

In order to examine the alterations in cellular morphology of MRSE following treatment with *Emilia sonchifolia*, we conducted observations of MRSE utilizing scanning electron microscopy (SEM). For the control group (Fig. 1A), the cells of MRSE presented regular shape, smooth surface, and intact surfaces. After subjecting the bacteria to an incubation period of 4 hours with a concentration of 5 mg/mL of Emilia sonchifolia, notable alterations in the morphology of the bacterial cells were observed, as depicted in Fig. 1B. The surface of MRSE with Emilia sonchifolia displayed irregular and rugged characteristics, with certain areas exhibiting extensive fractures that resulted in the release of contents.

2.2 TEM Analysis

In order to examine the ultrastructural modifications in MRSE cells following treatment with Emilia sonchifolia, we utilized transmission electron microscopy (TEM). The control group exhibited an intact cell wall, wherein the cytoplasm remained undisturbed in conjunction with the bacterial cell membrane (Fig. 2A). Nevertheless, notable alterations in MRSE morphology were observed subsequent to interaction with Emilia sonchifolia. Figure 2B exhibited the occurrence of cell wall lysis and the presence of a discontinuous cell membrane, resulting in the release of numerous intracellular components from the MRSE cells. Moreover, a small proportion of cells underwent dissolution.

2.3 Effect of Emilia sonchifolia on Energy Metabolism

Under aerobic conditions, pyruvic acid undergoes complete oxidation to carbon dioxide and water through the tricarboxylic acid cycle (TCA), thereby serving as the primary energy source for bacterial metabolic processes. The enzymes succinate dehydrogenase (SDH) and NADP-dependent malate

dehydrogenase (NADP-MDH) play crucial roles in the TCA. SDH acts as a pivotal link between oxidative phosphorylation and electron transport, while NADP-MDH catalyzes the conversion of malic acid to oxaloacetic acid, which is vital for cellular growth. Consequently, the activities of SDH and NADP-MDH are commonly employed to assess the functionality of the TCA. As depicted in Fig. 3, the activities of SDH and NADP-MDH exhibit a decrease in the MRSE treatment with *Emilia* sonchifolia for 4 h.

In comparison to the control group, the inclusion of 1MIC *Emilia sonchifolia* led to a respective decrease of 37% and 85% in the activities of SDH and MDH. These findings provide evidence that *Emilia sonchifolia* possesses the ability to impede the enzymatic activity crucial for the proliferation of MRSE.

2.4 Effect of Emilia sonchifolia on antioxidant system

The levels of antioxidant can alleviate the harmful ROS.[26] In contrast, high antioxidant level is considered as the mechanism of antibacterial agents. The levels of antioxidant molecules, such as CAT and SOD played important role in antioxidant system. In this study, we found that we found that the activities of CAT and SOD respectively decreased by 73%and 90 after MRESE exposed to MIC *Emilia sonchifolia* for 4h (Fig. 4).Such findings indicated that *Emilia sonchifolia* can inhibit the activities of SOD and CAT in MRSE.

3.The Antibacterial Activity of Emilia Sonchifolia In Vivo

Emilia Sonchifolia exhibited superior *in vitro* antibacterial activity against MRSE, as demonstrated by previous research. To further assess its antibacterial efficacy *in vivo*, Emilia Sonchifolia treatment was administered against MRSE-induced bloodstream infection. Prior to the establishment of MRSE-induced bloodstream infection, an immunosuppression model was employed. Meanwhile, the spleen and thymus index were used to evaluate the immunosuppression model. The Figs. 5A and B demonstrate a significant reduction in the spleen and thymus indices of the immunosuppressive group, bloodstream infection group, and Emilia Sonchifolia group, when compared to the blank group, which demonstrated that model was successfully established. Based on the immunosuppression model, a bloodstream infection was induced by MRSE. Additionally, the quantification of bacterial presence in the blood served as a standard measure to assess the antibacterial efficacy of Emilia Sonchifolia. In Fig. 5C, a notable decrease in the number of clones in the blood was observed in the Patrinia scabiosaefolia group compared to the bloodstream infection group, indicating a significantly superior *in vivo* antibacterial activity of Patrinia scabiosaefolia.

Discussion

The incidence of Coagulase-negative staphylococci -induced bloodstream infections has significantly increased.[4] *S. epidermidis* has emerged as the most prominent species in this context.[9] Currently, the emergence of antibiotic-resistant strains, particularly MRSE, has posed a significant challenge in the treatment of S. epidermidis infections.The theory of traditional Chinese medicine (TCM) believes that clearing heat and detoxifying TCM, which is cold in nature, can alleviate or eliminate the infection of

pathogenic microorganisms such as bacteria and viruses.[27] Pharmacology researches showed that clearing heat and detoxifying TCM such as *Lonicera japonica Thunb., Forsythia suspensa, Scutellaria baicalensis Georgi, Coptis chinensis Franch.* had good antibacterial activity.[28] Meanwhile, *Patrinia scabiosaefolia* belonged to clearing heat and detoxifying TCM had strong antibacterial activity against MRSE in our previous study.[19] In addition, the previous study showed that *Emilia sonchifolia* extracts had better antibacterial effect to *Staphylococcus aureus, Pseudomonas aeruginosa* and *Neisseria gonorrhoeae.*[20]

The findings of this study demonstrated that Emilia Sonchifolia exhibited a minimum inhibitory concentration (MIC) of 5mg/mL and a minimum bactericidal concentration (MBC) of 20mg/mL against MRSE, as presented in Table 1. It is worth noting that there is currently no established standard for TCM in terms of its antibacterial efficacy. Consequently, we conducted a comprehensive review of existing literature on the antibacterial properties of TCM, revealing a range of effective antibacterial activity for TCM crude extracts, spanning from 3.9 mg/mL to 125mg/mL [29, 30]. So, *Emilia Sonchifolia* had potential antibiotic sources for MRSE infections.

To assess the *in vivo* antibacterial activity, the MRSE-induced BSIs of mouse was established. As the result of *S. epidermidis* being the opportunistic pathogen, the host immune response can eliminate them. So MRSE infection would occur in immunosuppression.

On the basis of the above gist, the mouse immunosuppressive model was built. Furthermore, *Emilia Sonchifolia* was used to demonstrate the therapy to BSIs in mouse. The results showed that *Emilia Sonchifolia* had good antibacterial activity *in vivo* (Fig. 5).

In order to offer sufficient evidence of *Emilia sonchifolia* used in clinical, the antibacterial mechanisms were explored. Literature data showed that there are three main antibacterial mechanisms by TCM: (1) destruction of bacteria cell morphology; (2) inhibition of energy metabolism; (3) inhibition of defense mechanisms.[21, 31, 32] Hence, the objective of this study was to investigate the antibacterial mechanisms of Emilia sonchifolia against methicillin-resistant Staphylococcus epidermidis (MRSE) from three different perspectives.

The cell wall and membrane serve as a physical barrier, effectively separating the internal cellular environment from the external surroundings and preventing the escape of intracellular components.[33] The findings depicted in Figs. 1 and 2 demonstrate that *Emilia sonchifolia* has the ability to disrupt the cell wall and membrane of MRSE, thereby compromising the stability of the internal environment. Similar outcomes were observed when Aronia melanocarpa (Michx.), Chaenomeles superba Lindl., and Cornus mas L. leaf extracts were tested against bacterial cells.[32] It is widely recognized that energy is a fundamental requirement for nearly all cellular processes, and enzymes play a crucial role in facilitating the catalysis of physiological metabolism in microorganisms.[21] SDH, an essential enzyme in the tricarboxylic acid (TCA) cycle, serves as a crucial link between oxidative phosphorylation and electron transport.[21]Additionally, MDH plays a vital role in catalyzing the conversion of malic acid to oxaloacetic

acid, which is indispensable for cellular growth. Consequently, the activities of SDH and NADH-MDH were examined. Figure 3 demonstrates that Emilia sonchifolia effectively inhibits the activities of SDH and NADH-MDH. These findings align with previous research, such as the study by Chen et al. [34], which observed significant suppression of SDH enzyme activity in Staphylococcus aureus when exposed to *Persimmon Tannin*.[34] We further detected changes in defense mechanisms. The disruption of microorganism homeostasis caused by the oxidative burst of reactive oxygen species (ROS) was counteracted by the cellular defense mechanisms of the antioxidant enzyme system[31]. This system comprises various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and others [35]. As seen in Fig. 4, the activity of SOD and CAT were decreased by *Emilia sonchifolia*, illustrating *Emilia sonchifolia* can inhibit the defense mechanisms of MRSE. Similarly, curcumin-based photodynamic inactivation can amplify the oxidative damage to bacteria by inhibiting the activity of SOD and CAT.[31]

In comparison to conventional antibiotics, *Emilia sonchifolia* exhibits the advantageous characteristic of targeting multiple sites against MRSE. This attribute may account for the reduced likelihood of drug resistance development in TCM. The present investigation aims to provide a preliminary elucidation of the antibacterial activity and mechanisms of *Emilia sonchifolia* against MRSE. Nevertheless, further research is warranted to comprehensively understand the specific mechanism of action employed by *Emilia sonchifolia* against MRSE.

Conclusions

In conclusion, the present study has demonstrated that *Emilia sonchifolia* exhibits a noteworthy antibacterial efficacy against *Methicillin-Resistant Staphylococcus epidermidis* (MRSE) both *in vitro* and *in vivo*. *Emilia sonchifolia* can exert possible antibacterial mechanism of antibacterial in several ways. First, *Emilia sonchifolia* treatment can destroy the morphology of MRSE cells. Secondly, *Emilia sonchifolia* treatment also inhibition of energy metabolism of MRSE cells. In addition, *Emilia sonchifolia* treatment could decrease the defense mechanisms of MRSE cells. The above results demonstrated that *Emilia sonchifolia* had great potential as antibacterial agent.

Abbreviations

BSIs: Bloodstream infections

S.Epidermidis: Staphylococcus epidermidis

MRSE: Methicillin-resistant Staphylococcus epidermidis

SDH: Succinate dehydrogenase activity

NADP-MDH: NADP-Malate dehydrogenase activity

CAT: catalase

SOD: Superoxide dismutase

TSB: Tryptic soy broth

TSA: Tryptic soy agar

MIC: Minimum inhibitory concentration

MBC: Minimum bactericidal concentration

SEM: Scanning electron microscopy

TEM: Transmission electron microscopy

PBS: Phosphate buffer saline

TCA: Tricarboxylic acid cycle

TCM: traditional Chinese medicine

Declarations

Ethics approval and consent to participate

This study was conducted after it is ethically reviewed and approved by the all participants.

Consent for publication

Not applicable

Availability of data and materials

The corresponding author can provide all data and materials of this work upon request.

Competing interests

The authors have explicitly stated that they do not possess any conflicting interests.

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Figures

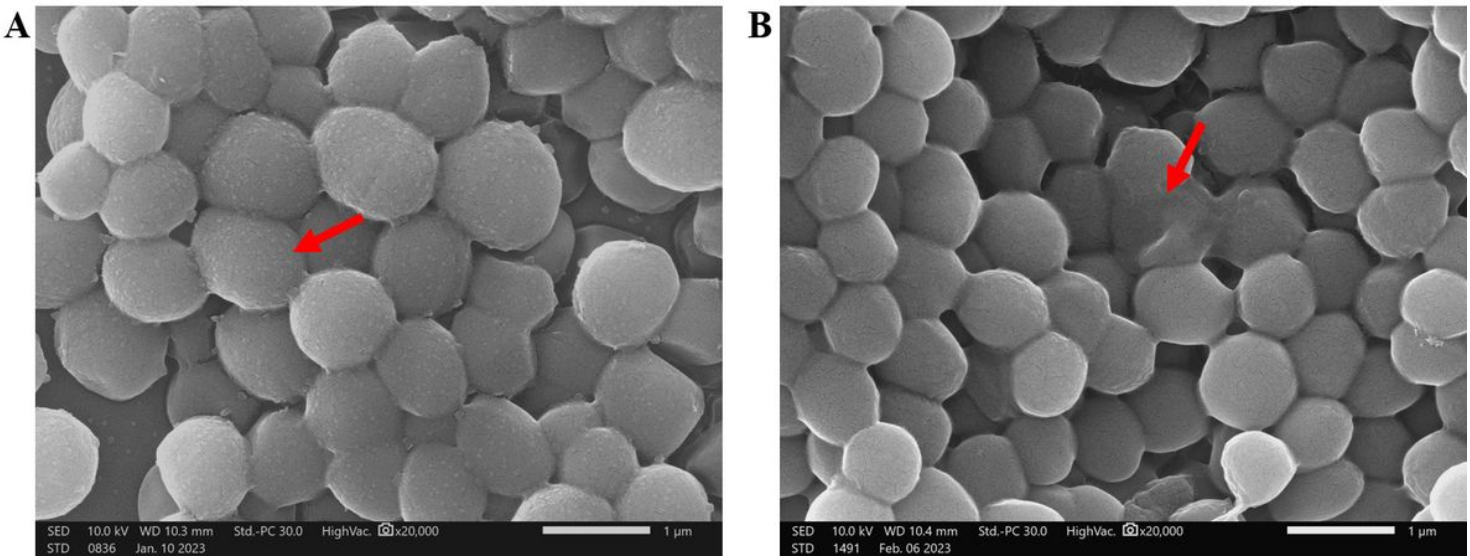

Figure 1

SEM observations of morphology changes in MRSE bacteria (A) MRSE bacteria untreated with *Emilia sonchifolia* (B) MRSE bacteria treated with *Emilia sonchifolia* for 4 hours. The red arrows indicate the regions of the lost cellular integrity.

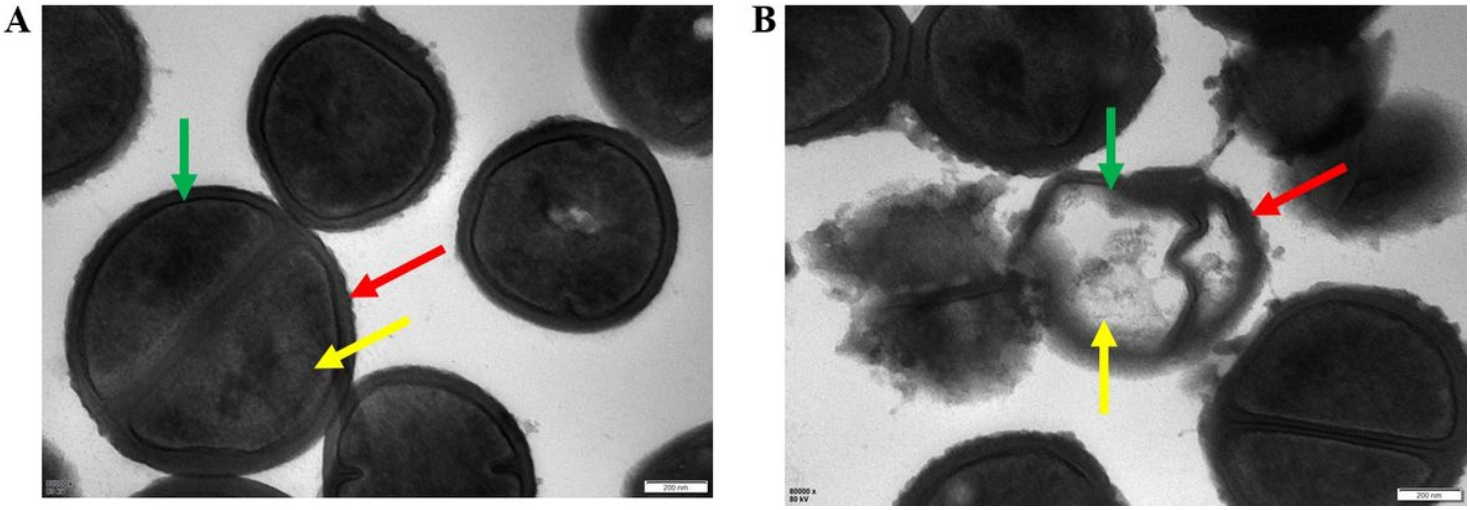

Figure 2

TEM observations of morphology changes in MRSE bacteria (A) MRSE bacteria untreated with *Emilia sonchifolia* (B) MRSE bacteria treated with *Emilia sonchifolia* for 4 hours. The red arrows indicate the cell wall. The green arrows indicate the cell membrane. The yellow arrows indicate the cytoplasmic contents.

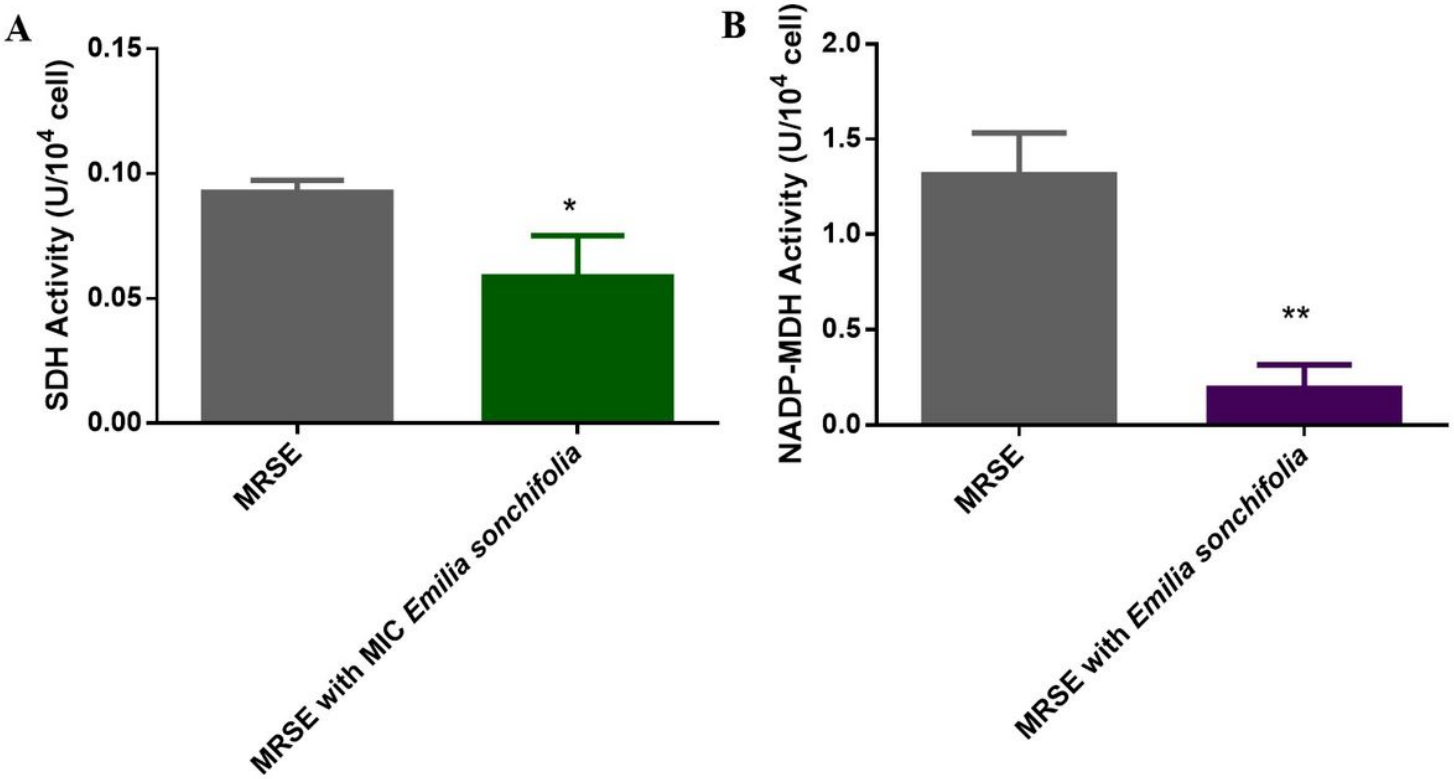

Figure 3

Effect of *Emilia sonchifolia* on energy metabolism of MRSE (A) Effect of *Emilia sonchifolia* on the SDH activity in MRSE (B) Effect of *Emilia sonchifolia* on the NADP-MDH activity in MRSE. Data are presented as mean (± SD) of three replicates (compared with the control, *p< 0.05, ** p < 0.01).

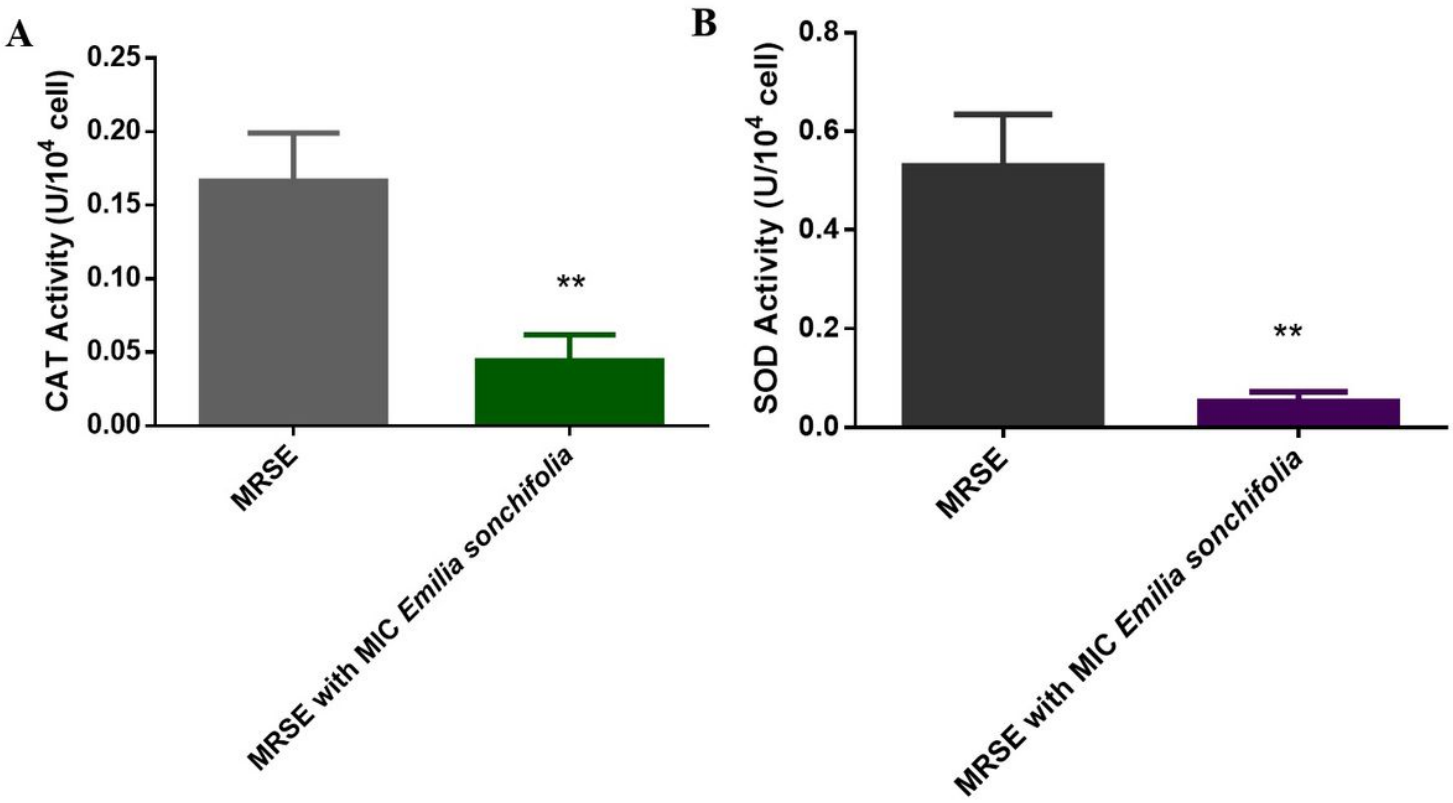

Figure 4

Effect of *Emilia sonchifolia* on defense mechanisms of MRSE (A) Effect of *Emilia sonchifolia* on the CAT activity in MRSE (B) Effect of *Emilia sonchifolia* on the SOD activity in MRSE. Data are presented as mean (± SD) of three replicates (compared with the control, ** p< 0.01).

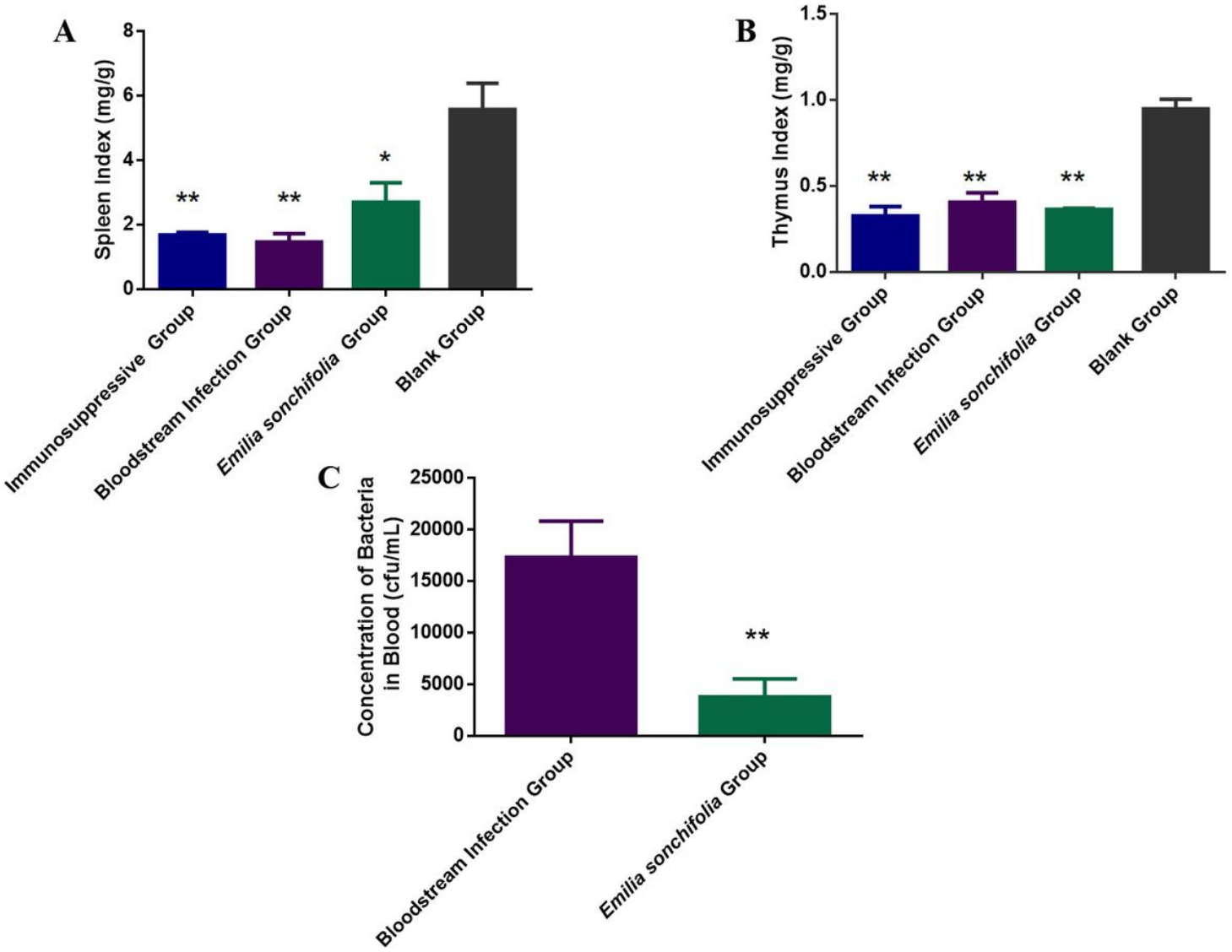

Figure 5

Exploration of antibacterial activity of *Emilia sonchifolia in vivo.* (A) The determination of spleen index in immunosuppressive group, bloodstream infection group, *Emilia sonchifolia* group and blank group. (B) The determination of thymus index in immunosuppressive group, bloodstream infection group, *Emilia sonchifolia* group and blank group.(C) The bacterial loads in the blood at 24 h post-infection. X axis indicated the mice treated with or without *Emilia sonchifolia*. Data were expressed as mean (±SD) of eight replicates.(compared with the control, * $p < 0.05$, ** $p < 0.01$).