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Emilia sonchifolia (L.) DC. inhibits the growth of Methicillin-Resistant Staphylococcus epidermidis by modulating its physiology through multiple mechanisms

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Bloodstream infections (BSIs) are a public health concern, causing substantial morbidity and mortality. *Staphylococcus epidermidis* (*S. epidermidis*) is a leading cause BSIs. Antibiotics targeting *S. epidermidis* have been the mainstay of treatment for BSIs, however their efficacy is diminishing in combating with drug-resistant bacteria. Therefore, alternative treatments for antibiotic-resistant infections are urgently required. Studies have demonstrated that certain traditional Chinese medicine (TCM) exhibit notable antimicrobial activity and can help mitigate bacterial resistance. Among these, The ethanol extract of *Emilia sonchifolia (L.) DC* (*E. sonchifolia*) (10 g crude drug/1 g extract) exhibits a noteworthy anti-methicillin-resistant *S. epidermidis* (MRSE) effect. This study explores antibacterial activity and underlying mechanisms of *E. sonchifolia* against MRSE. The antibacterial activity of *E. sonchifolia* against MRSE was assessed in vitro by measuring the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MRSE-induced mouse BSIs model was used to evaluate the antibacterial activity of *E. sonchifolia* in vivo. Proteomic and transcriptomic analyses were performed to elucidate the underlying antibacterial mechanisms. The MIC and MBC values of *E. sonchifolia* against MRSE were 5 mg/mL and 20 mg/mL, respectively. In vivo, *E. sonchifolia* effectively treated MRSE-induced BSIs. Additionally, proteomic and transcriptomic analyses revealed considerable down-regulation of purine metabolism, that were associated with oxidative stress and cell wall synthesis. The enzyme linked immunosorbent assay(ELISA) results showed decreased levels of inosine monophosphate (IMP), Adenosine monophosphate(AMP) and guanine monophosphate (GMP), indicating inhibited purine metabolism. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysis confirmed bacterial cell wall damage. *E. sonchifolia* exerts antibacterial effects by inhibiting purine metabolism, promoting bacterial oxidative stress, and impairing cell wall synthesis. These findings provide novel insights into the mechanistic understanding of *E. sonchifolia*'s efficacy against MRSE, offering potential strategies for managing MRSE infections.

Keywords Methicillin-resistant *Staphylococcus epidermidis*, Bloodstream infections, *Emilia sonchifolia*, Purine metabolism

Abbreviations

BSIs	Bloodstream infections
Emilia sonchifolia (L.) DC.	*E. sonchifolia*
S. Epidermidis	Staphylococcus epidermidis
MRSE	Methicillin-resistant Staphylococcus epidermidis
CAT	Catalase
SOD	Superoxide dismutase

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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
TCM	Traditional Chinese medicine
GMP	Guanosine
AMP	Adenosine monophosphate
IMP	Inosine monophosphate

Bloodstream infections (BSIs) represent a significant global public health concern, characterized by a high mortality rates[1]. In Europe, an estimated 1,200,000 BSI episodes occur annually, leading to long-term sequelae[2]. BSIs are often caused by catheter devices, including Hemodialysis catheterand central venous catheters[3,4]. These catheter-related infections are frequently caused by skin commensals, with gram-positive organisms accounting for 70% of cases[4]. Among these, *Staphylococcus epidermidis* (*S. epidermidis*) is the predominant pathogen associated with both implanted device infections and BSIs[5]. Alarmingly, *S. epidermidis* infections frequently exhibit antibiotic resistance, with multiple resistance in nosocomial strains reported in 70–85% of cases[6,7]. Furthermore, a large proportion of *S. epidermidis* isolates are methicillin-resistant (methicillin-resistant *Staphylococcus Epidermidis*; MRSE), and demonstrating extensive-resistance to β-lactam antibiotics[7]. Vancomycin is a restricted selection of available treatment options[8]. However, it has limited biocidal efficacy, necessitates therapeutic drug monitoring, and may induce nephrotoxicity and/or ototoxicity in specific patient populations[9,10]. Therefore, the current prevalence of MRSE-associated BSIs presents a significant therapeutic challenge, emphasizing the need for research on more effective and safer therapeutic agents[11].

"Traditional Chinese medicines (TCM)", derived from plants and historically used across diverse cultures, are commonly safe when administered in appropriate dosages[12]. Consequently, there is a renewed focus on exploring traditional or herbal medicines as potential alternatives or complementary treatments for emerging and re-emerging multidrug-resistant bacteria[13]. Numerous traditional and herbal medicines have been studied for their antimicrobial activity[14–17]. Aqueous extracts of *Carum copticum* and *Albizia adianthifolia* exhibited notable antibacterial effects against multidrug-resistant gram-negative human pathogenic bacteria, including *Escherichia coli*, *Pseudomonas* species, *Klebsiella pneumoniae Salmonella* species, and *Proteus* species[18,19]. Similarly, in our previous study, *Patrinia scabiosaefolia* exhibited strong antibacterial effects against MRSE both in vitro and in vivo[20]. Therefore, we speculate that other traditional or herbal medicines may also have effective antibacterial activity against MRSE-associated BSIs.

Emilia sonchifolia (L.) DC(*E. sonchifolia*) has a well-documented history of use in China, as recorded in the "Lingnan Medicine Collection." It is primarily used in TCM for its anti-inflammatory, analgesic, antibacterial, antiviral, antitumor, hypoglycemic, and immune-regulating properties[21]. Notably, *E. sonchifolia* has demonstrated significant antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Neisseria gonorrhoeae in vitro*[21]. However, its antibacterial activity and mechanisms of action against MRSE remain unknown.

Contemporary research increasingly employs multi-omics approaches to study microorganisms[22–24]. Proteomics is employed in antibacterial research to elucidate antimicrobial mechanisms, screen potential drugs, and facilitate vaccine development[23]. Similarly, transcriptomics is applied to explore antibacterial mechanisms, identify therapeutic targets, and support the development of novel drugs by analyzing of gene expression profiles[25]. The application of proteomics and transcriptomics has remarkably advanced our understanding of bacterial mechanisms[26]. Hence, integrating these techniques in antibacterial research enables a comprehensive understanding of changes in bacterial protein expression and gene regulation. This approach aids in elucidating antibacterial mechanisms and identifying novel antibacterial targets.

Therefore, this systematic study in vitro and in vivo antibacterial activities of *E. sonchifolia* against MRSE. Additionally, transcriptomic and proteomic approaches were employed to elucidate the potential mechanisms underlying its antibacterial effects on MRSE.

Materials and methods
Materials
Methicillin-resistant *Staphylococcus Epidermidis*(MRSE) strains were cultured and maintained in our laboratory. The ethanol extract of *E. sonchifolia* (10 g crude drug/1 g extract) was purchased from Shanxi Xibolan Biotechnology Co., Ltd. (20,220,315). Assay kits for AMP, GMP, and IMP were acquired from Beijing APPLYGEN Gene Technology Co., Ltd.. Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) were obtained from Beijing Solarbio Science & Technology Co., Ltd.. Forty male C57 mice were used: C57BL/6 J mice (n = 40, both from Changsha Tianqin Biotechnology Co., Ltd.) were used. At the time of the test the mice were 6–8 weeks old and had body weights of 20.17 ± 1.21 g, respectively (mean ± SEM).

Methods
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.

The antibacterial activity of E. Sonchifolia in vitro

Determination of minimum inhibitory concentration (MIC)   The MIC of *E. sonchifolia* extract was determined using the broth dilution method according to the Clinical and Laboratory Standards Institute of the United States of America (CLSI). 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 *E. sonchifolia* with TSB were dispensed in the wells, ranging from a concentration of 80 mg/mL to 0.0195 mg/mL. After incubation for 18 h at 37℃, the MIC was defined as the lowest concentration of luteo.

Determination of minimum bactericidal concentration (MBC)   The broth dilution method was used to determine the MBC of *E. sonchifolia*[27]. The culture suspensions (100 L) of MRSE treated with *E. sonchifolia* at concentrations of 5 and 40 mg/mL, where no bacterial growth was observed, were evenly spread on TSA plates and incubated at 37℃ for 18 h. Surviving colonies were subsequently observed. The MBC was defined as the lowest concentration of an antimicrobial agent capable of inactivating > 99.99% of the bacterial population (< 10 CFU/mL). The assay was performed in triplicates.

The antibacterial activity of E. Sonchifolia in vivo

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-h light–dark cycle at a temperature of 22 ± 2 °C for a duration of one week. They were provided unrestricted access to food and water.

Establishment of mouse immunosuppressive models   Based on the existing literature, a mouse immunosuppressive model was established by intraperitoneal injection of Cyclophosphamide solution at 120 mg/kg body weight for 4 days. Control mice were administered phosphate-buffered saline(PBS). Subsequently, the thymus and spleen were extracted from both immunosuppressed and control mice, and the thymus and spleen indices were computed.

Treatment of *E. Sonchifolia* against MRSE-induced BSIs   The animals were randomly assigned to different groups prior to the experiment, including group A(Immunosuppressive, no BSI, no treatment), group B(Immunosuppressive, BSI, no treatment), group C (Immunosuppressive, BSI, *E. sonchifolia*), and group D(Non- Immunosuppressive, no-BSI, no treatment). In the group C, *E. sonchifolia* was administered orally at a dose of 8 g/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 group A, group B, and group C, the group D was treated with PBS. Furthermore, the group B and group C were infected 7 days after *E. sonchifolia* oral administration by tail vein injection with 1×10^8 cfu/mL MRSE, and the group A and group D were treated with PBS. The tail vein method was consistent with that described by Liu et al.[28]. At 12 h post infection (pi), blood samples were collected from the tail vein of all groups. The mice were euthanized with 100 mg/kg pentobarbitol sodium (IP). Subsequently, the aforementioned samples underwent serial dilution in PBS and were subsequently plated on TSA agar plates for a duration of 24 h at a temperature of 37 °C. Ultimately, the enumeration of clones was conducted to assess the efficacy of the mouse BSI models[29].

The aforementioned mice experiments were approved by the University Committee on Use and Care of Animals at the Guizhou University of Traditional Chinese Medicine in accordance with the Laboratory Animal Guidelines for Ethical Review of Animal Welfare (GB/T 35892—2018). All experiments were performed in accordance with relevant guidelines and regulations.

The study is reported in accordance with ARRIVE guidelines.

Proteomics study

TMT-based quantitative proteomic analyses were performed as described[20].

Proteins were extracted from the treated (using *E. sonchifolia*) and untreated cultures of MRSE with 1/2 MIC (2.5 mg/mL) for 4 h and were prepared which were sent to LC-BIO Technologies Co. Ltd. (Hangzhou, Zhejiang, China) for proteomic analyses. Protein identification, quantification, classification, and interaction prediction were performed as previously described[23]. The raw files generated by AQ Exactive Plus were converted using Proteome Discoverer 2.1 (Thermo Fisher Scientific) and sent to OmicStudio tools (Lc-bio Technologies Co., Ltd.) for analysis[30]. Differentially expressed proteins were defined as those with a fold change (FC) ≥ 1.2 or $\leq 1/1.2$ -fold and a P-value < 0.05. Differentially expressed proteins were functionally classified using Gene Ontology (GO) terms (http://www.omicsbean.com). We used the KEGG database (www.kegg.jp/feedback/copyright.html) for the pathway enrichment analysis[31].

Transcriptomics study

Transcriptome sequencing was performed as Cheng described[32]. Proteins were extracted from the treated (using *E. sonchifolia*) and untreated cultures of MRSE with 1/2 MIC (2.5 mg/mL) for 4 h and were prepared which were sent to LC-BIO Technologies Co. Ltd. (Hangzhou, Zhejiang, China) for transcriptome sequencing.

After estimating the expression levels of all transcripts and analyzing the expression levels for mRNAs, the differentially expressed mRNAs were selected with fold change > 2 or fold change < 0.5 with p < 0.05, by DESeq2, GO, and KEGG enrichment of the differentially activated pathways were analyzed. We used the KEGG database (www.kegg.jp/feedback/copyright.html) for the pathway enrichment analysis[31].

Transcriptomic and proteomic integrative analysis methods
Transcriptomic and proteomic analyses were performed to identify the expression of genes and potential antibacterial mechanisms of *E. sonchifolia* against MRSE.

Effect of E. sonchifolia on purine metabolism
The activities of IMP, GPM, and AMP were evaluated in MRSE under *E. sonchifolia* stress. MRSE in the logarithmic growth phase was introduced to *E. sonchifolia* at a MIC. The mixture was then incubated at 37 °C for 4 h. A negative control consisting of PBS was prepared. The mixtures were then subjected to centrifugation at 12,000 revolutions per minute for 5 min at 4 °C, and the resulting supernatant was discarded. The cells were then washed and resuspended in PBS. Subsequently, the samples were subjected to ultrasonication at a concentration of 30% for a duration of 30 min (with an interval of 5 s) and then centrifuged at a speed of 12,000 revolutions per minute for 5 min at a temperature of 4℃. Finally, the supernatant was divided into aliquots and used for IMP, GPM, and AMP assays using kits from Beijing APPLYGEN Gene Technology Co., Ltd.

Effect of E. sonchifolia on antioxidant system
The activities of superoxide dismutase (SOD), catalase (CAT) were evaluated in MRSE under *E. sonchifolia* stress. MRSE in the logarithmic growth phase was introduced to *E. sonchifolia* at a MIC. The mixture was then incubated at 37 °C for 4 h. A negative control consisting of PBS was prepared. The mixtures were then subjected to centrifugation at 12,000 revolutions per minute for 5 min at 4 °C, and the resulting supernatant was discarded. The cells were then washed and resuspended in PBS. Subsequently, the samples were subjected to ultrasonication at a concentration of 30% for a duration of 30 min (with an interval of 5 s) and then centrifuged at a speed of 12,000 revolutions per minute for 5 min at a temperature of 4℃. The supernatant was divided into aliquots and used for CAT and SOD assays using kits from Beijing Solarbio Science and Technology Co.,Ltd.

Effect of E. sonchifolia on morphological characterizations of MRSE
Morphological characterization of MRSE treated with *E. sonchifolia* was performed using Scanning Electron Microscopy (SEM) Analysis Transmission Electron Microscopy (TEM) Analysis. SEM was performed using a modified version of a previously reported method. In summary, *E. sonchifolia* (1 × MIC) was introduced into a logarithmic phase bacterial suspension. The control group received an equal volume of PBS. All groups were incubated at 37℃ for a duration of 4 h. Subsequently, the treated bacterial cells were harvested, washed, and subsequently fixed in a 3% glutaraldehyde solution overnight at 4℃. Next, the bacterial cells were dehydrated by applying varying concentrations of ethanol, followed by replacement with tertiary butyl alcohol. Subsequently, the samples were freeze-dried, sputter-coated with gold, and examined using a scanning electron microscope. For TEM, the MRSE was treated in a manner consistent with the SEM procedure. As per a previous report, the specimens were affixed to a fibrous carbon film and observed using TEM[33].

Statistical analysis
Values are reported as means ± standard deviation (SDs). One-way analysis of variance (ANOVA) was conducted to assess statistical differences among various groups. Significant differences between means were determined using Tukey's Honest Significant Difference test, with the significance level set at $p < 0.05$.

Results
The antibacterial activity of *E. Sonchifolia* in vitro
The antibacterial activities of *E. sonchifolia* against MRSE were primarily indicated by MIC and MBC. After 18 h of incubation at 37℃, turbidity was noticed in the well 2.5 to 0.039 mg/ml of *E. sonchifolia*. Turbidity was absent at concentrations ranging from 40 to 5 mg/ml, the absence of turbidity was observed, indicating an inhibitory effect on bacterial proliferation. According to CLSI, the MIC of *E. sonchifolia* against MRSE was 5 mg/mL (Table 1).

MRSE was resistant to *E. sonchifolia*, confirming its efficacy. Suspensions ranging from 40 to 5 mg/mL were cultured onto TSA agar plates and subjected to an 18 h incubation period. An observation of less than 10 CFU/mL in the concentration range of 40–20 mg/mL served as conclusive evidence of its bactericidal properties. Therefore, the MBC value of *E. sonchifolia* was 20 mg/mL (Table 1).
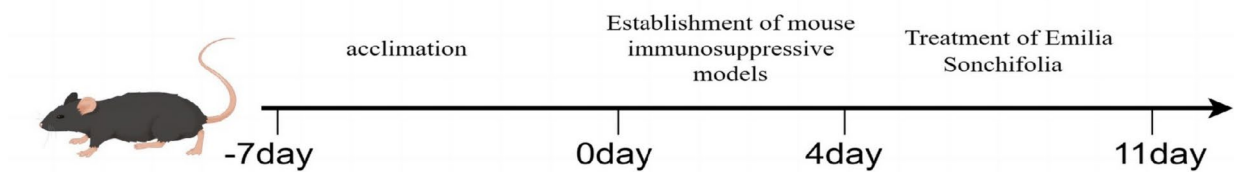
The antibacterial activity of *E. sonchifolia* in vivo
E. sonchifolia exhibited superior in vitro antibacterial activity against MRSE, as demonstrated in previous studies. To further assess its antibacterial efficacy in vivo, *E. Sonchifolia* was administered to treat MRSE-induced bloodstream infection**s** (Fig. 1a). An immunosuppression model was used prior to the establishment of MRSE-induced BSIs. Spleen and thymus indices were used to evaluate the immunosuppression model. Figures 1b and c demonstrate a significant reduction in the spleen and thymus indices of the group A, group B, and group C when compared to the group D , that demonstrated that the model was successfully established. Based on the immunosuppression model, BSIs were induced by MRSE. Additionally, the quantification of bacteria in the blood served as a standard measure to assess the antibacterial efficacy of *E. Sonchifolia*. As shown in Fig. 1d, a

Bacteria	Sample	MIC (mg/mL)	MBC (mg/mL)
MRSE	*E. sonchifolia*	5	20

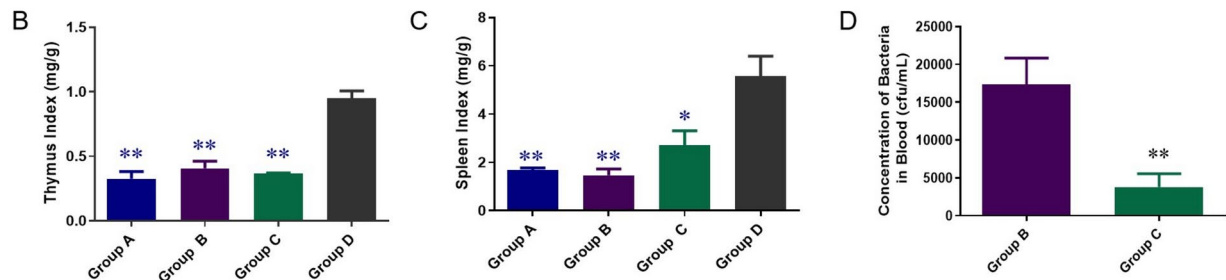**Table 1**. MIC and MBC value of *E. sonchifolia* against MRSE isolates.

Fig. 1. Investigation of the antibacterial activity of *Emilia sonchifolia* in vivo. (**a**) Experimental scheme determining the MRSE-induced BSIs in mice treated with *E. sonchifolia*. (**b**) Determination of spleen index in the group A, group B, group C, and group D. (**c**) Determination of the thymus index in the group A, group B, group C, and group D. (**d**) Bacterial load in blood 24 h post-infection. The x-axis indicates the mice treated with or without *E. sonchifolia*. Data are expressed as mean (±SD) of eight replicates (compared with the control, * $p < 0.05$, ** $p < 0.01$).

notable decrease in the number of clones in the blood was observed in group C compared to that of the group B, indicating the significantly superior in vivo antibacterial activity of *E. sonchifolia*.

Proteomic analysis of MRSE following *E. sonchifolia* treatment

The effect of *E. sonchifolia* extract on MRSE at the protein level was evaluated using a TMT-based quantitative method. A total of 1684 proteins were identified in MRSE in this study. Differentially expressed proteins (DEPs) were defined as follows: fold-change (FC) > 1.20 or < 0.80 and $p < 0.05$. The comparison of control and *E sonchifolia*-treated MRSE revealed 113 significant DEPs (64 upregulated proteins and 49 downregulated proteins), volcano plots of FC values against p-values (two-tailed Student's t-test), and the number of differentially expressed proteins and genes (Fig. 2a–d). GO functional enrichment analysis of the DEPs revealed that the 15 most significantly enriched GO terms were mainly BPs and MFs, including the oxidation–reduction process (GO:0055114),integral component of the membrane (GO:0016021), and cytoplasm (GO:0005737) ($p < 0.001$; Fig. 2e. KEGG functional enrichment analysis of the DEPs showed that the most significantly enriched metabolic pathways were biosynthesis of secondary metabolites, biosynthesis of antibiotics, microbial metabolism in diverse environments, and carbon metabolism.

Biosynthesis of amino acids, purine metabolism is shown in Fig. 2f, Moreover, KEGG pathway analysis revealed components of 21 KEGG pathways among the 33 downregulated proteins, the majority of which were associated with urine metabolism, cationic antimicrobial peptide (CAMP) resistance, two-component system (TCS), and metabolic pathways (Fig. 2g), and upregulated KEGG pathways were associated with ribosome and nitrogen metabolism (Fig. 2h).

Transcriptomic analysis of MRSE upon *E. sonchifolia* treatment

The effects of *E. sonchifolia* treatment on the MRSE transcriptome were determined by RNA-seq experiments. A total of 2353 mRNA were identified in this study Differentially expressed genes (DEGs) were defined as follows: corrected p-value < 0.05 and |log2 FC| ≥ 1. Comparison of the control and *E. sonchifolia* -treated MRSE revealed 511 DEGs (299 upregulated and 212 downregulated genes) (Fig. 3a–c).

GO annotation analysis revealed 1421 functional terms, including 308 MFs, 28 CCs, and 197 BPs. GO functional enrichment analysis of the DEGs showed that most of the top-enriched terms were BPs and MFs, including phosphorylation (GO:0016310), integral components of the membrane (GO:0016021), cytoplasm (GO:0005737), and metal ion binding (GO:0046872) (Fig. 3d). KEGG pathway annotation and enrichment analysis indicated that the DEGs were enriched in several pathways including energy metabolism, amino acid metabolism, biosynthesis of other secondary metabolites, carbohydrate metabolism, and metabolism of cofactors and vitamins (Fig. 3e).
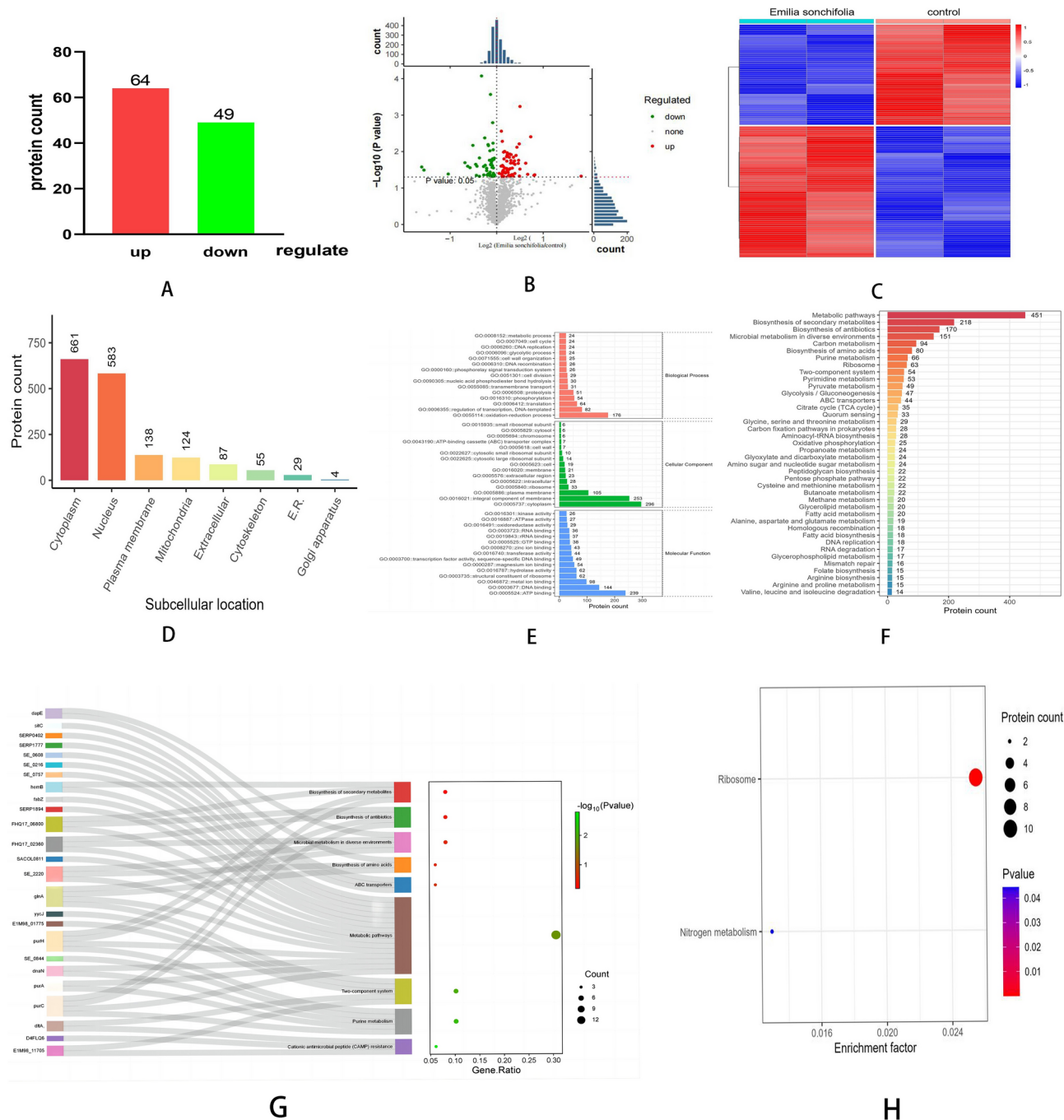
Fig. 2. Significantly differential proteins of MRSE in 5 mg/mL *E. sonchifolia* stress using TMT-based quantitative proteomics. (**a**) The number of DEPs red represent up-regulated proteins, and green represent down-regulated proteins. (**b**) The horizontal axis is the relative quantitative protein value after Log2 logarithm conversion, and the vertical axis is the difference significance test p-value value after -Log10 logarithm conversion. The red dots indicate up-regulated proteins, and blue dots indicate down-regulated proteins. (**c**) Hierarchical cluster analysis of differentially expressed proteins between MRSE from control and treated with *E. sonchifolia*. (**d**) The number of proteins in different subcellular localization in the proteomics between MRSE from control and treated with *E. sonchifolia*. (**e,f**) Go annotation and KEGG pathway of DEPs. (**g**) Enrichment plot of connectivity between differentially expressed down-regulated proteins on the left and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway on the right. Each line represents overlap between pairwise comparisons, based on gene set enrichment analysis. (**h**) KEGG pathway analysis of the up-regulated of KEGG.
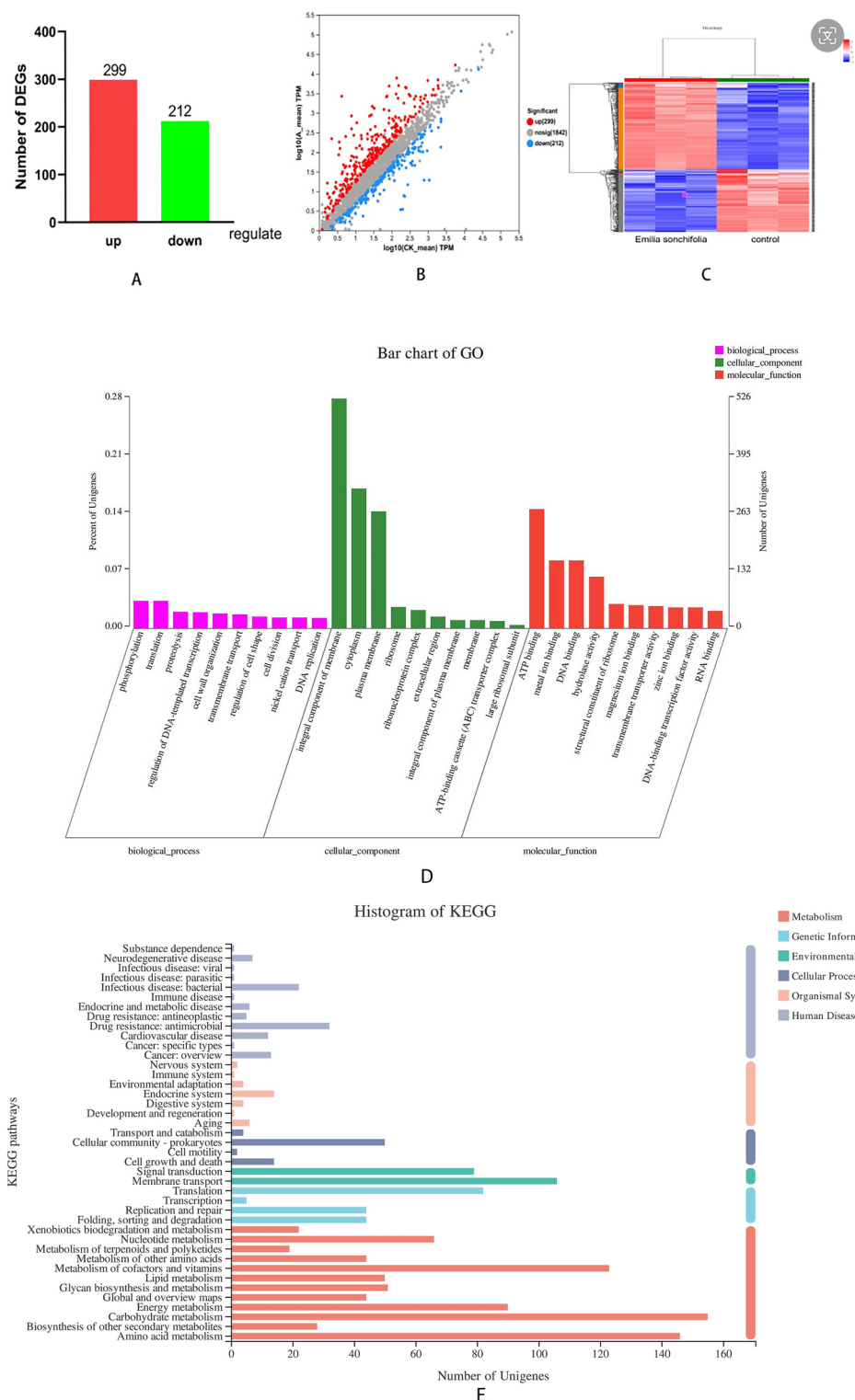
Fig. 3. Remarkably differential proteins of MRSE in 5 mg/mL *E. sonchifolia* stress using Transcriptomic. (**a**) Number of DEPs: red represents up-regulated proteins, and green represents down-regulated proteins. (**b**) Horizontal axis depicts the relative quantitative protein value after Log2 logarithm conversion, and the vertical axis presents the difference significance test p-value value after -Log10 logarithm conversion. The red dots indicate up-regulated proteins, and blue dots indicate down-regulated proteins. (**c**) Hierarchical cluster analysis of differentially expressed proteins between MRSE from control and those treated with *E. sonchifolia*. (**d,e**) GO annotation and KEGG pathway of DEPs. (**f**) Energy metabolism down-regulates energy metabolism-related pathways in mRNA. (**g**) KEGG pathway analysis of the down-regulated.

Metabolic pathways

F

KEGG enrichment analysis

G
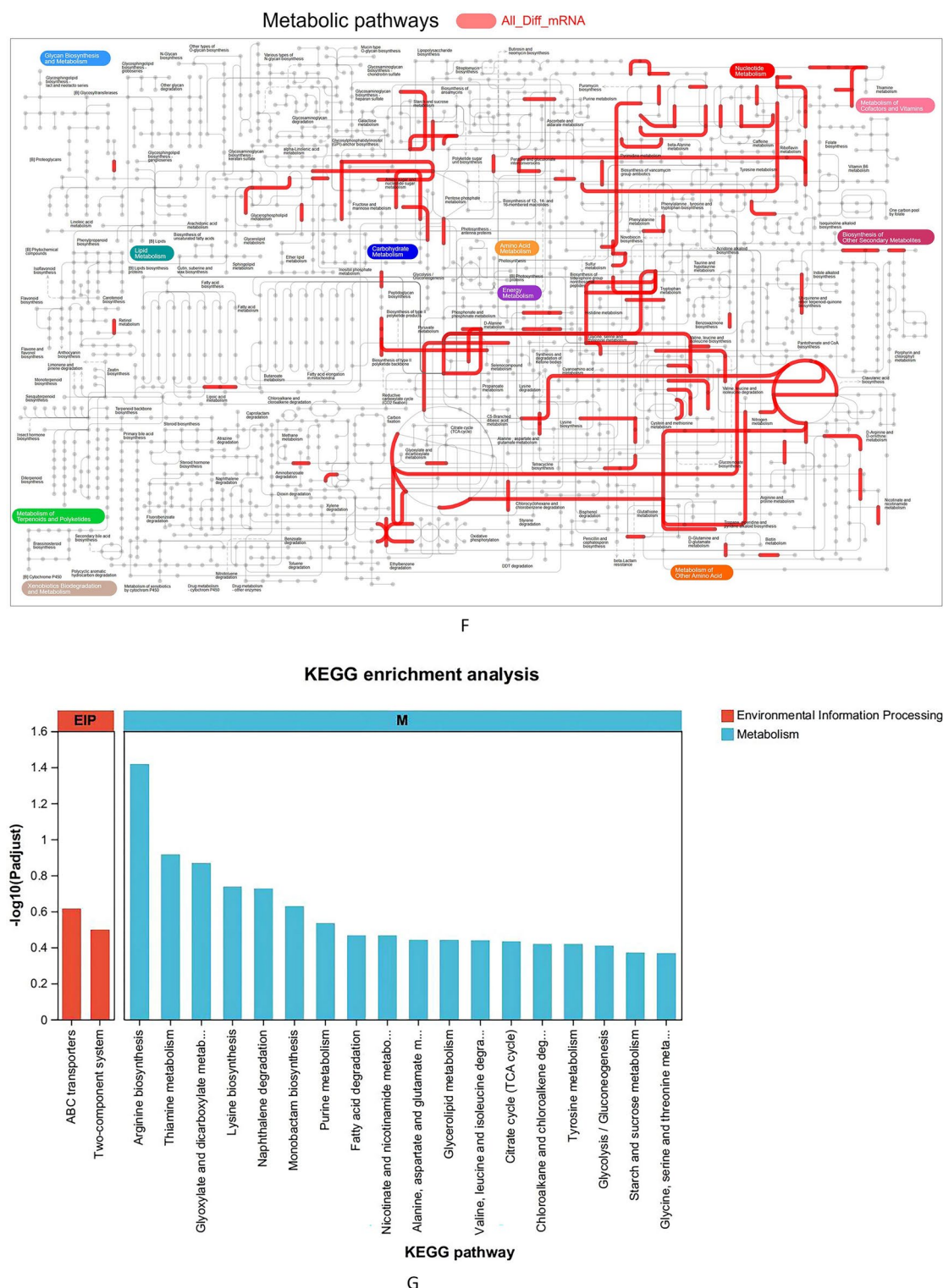
Figure 3. (continued)

Moreover, KEGG pathway analysis revealed components of the KEGG pathways among the 212 downregulated proteins, the majority of which were associated with metabolism, including arginine biosynthesis, thiamine metabolism, glyoxylate and dicarboxylate metabolism, lysine biosynthesis (Fig. 3f); and environmental Information Processing (ABC transporters) (Fig. 3g).
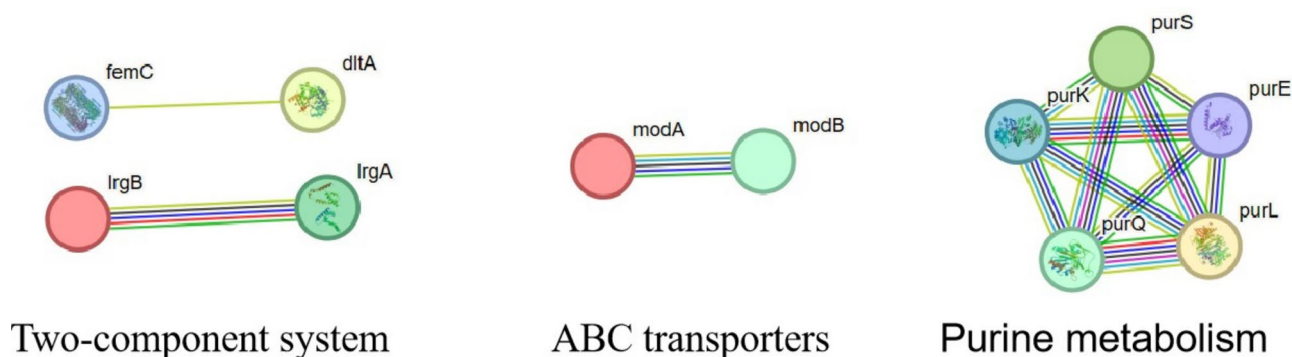
Fig. 4. Targeted transcriptomic data based on proteomic pathways were found to be related to pathways with string network of down-regulated proteins.
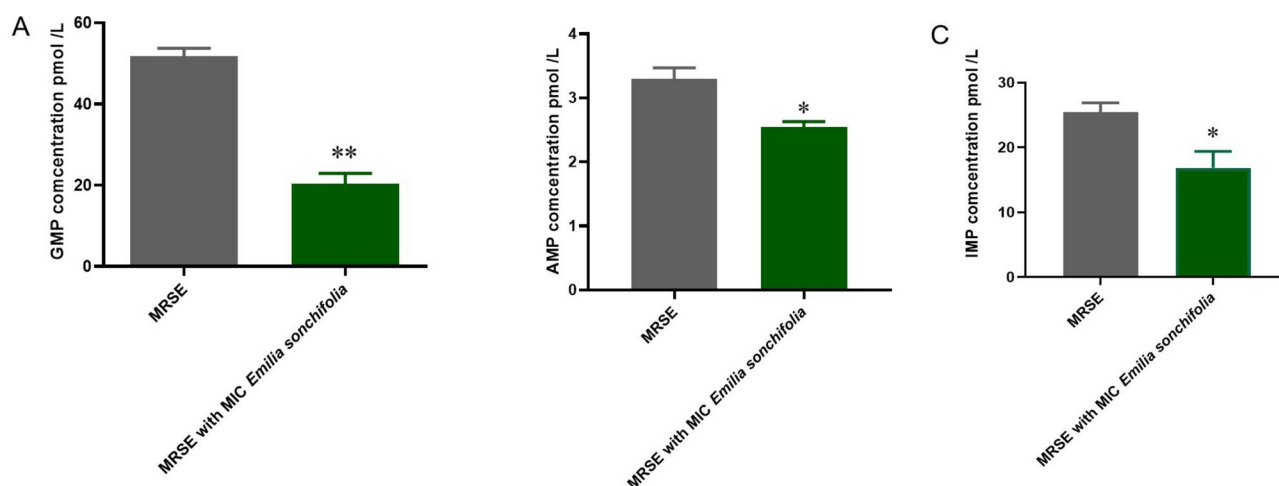
Fig. 5. Effect of *E. sonchifolia* on purine metabolism of MRSE. (**a**) Effect of *E. sonchifolia* on GMP concentration in MRSE. (**b**) Effect of *E. sonchifolia* on AMP concentration in MRSE. (**c**) Effect of *E. sonchifolia* on IMP concentration in MRSE. Data are presented as the mean ($\pm$ SD) of three replicates (compared with the control, $*p < 0.01$, $**p < 0.01$).

Transcriptomic and proteomic integrative analysis

Targeted transcriptomic data based on proteomic pathways were related to pathways such as mapko00230 (purine metabolism), mapko02020 (TCS), and mapko01100 (metabolic pathways; Fig. 4).

The antibacterial mechanism of *E. sonchifolia* in vitro

Effect of E. sonchifolia on purine metabolism

The purine metabolism pathway plays an important role in bacterial survival and adaptation to the environment. Recently, researchers have discovered a number of new enzymes and metabolic pathways involved in bacterial purine metabolism such as GMP, AMP and IMP played important role in purine metabolism. In this study, we found that the activities of GMP, AMP, IMP decreased after exposure of MIC *E. sonchifolia* for 4 h (Figs. 5a–c). These findings indicate that *E. sonchifolia* can inhibit GMP, AMP, and IMP activities in MRSE.

Effect of E. sonchifolia on antioxidant system

Antioxidant levels can alleviate harmful reactive oxygen species (ROS)[34]. In contrast, high antioxidant levels are considered to be the mechanism of action of antibacterial agents. Levels of antioxidant molecules, such as CAT and SOD, play important roles in the antioxidant system. In this study, we found that the activities of CAT and SOD decreased by 73% and 90%, respectively, after exposure of MIC *E. sonchifolia* for 4 h (Fig. 6a and b). These findings indicate that *E. sonchifolia* inhibits SOD and CAT activity in MRSE.

SEM analysis

To examine the alterations in the cellular morphology of MRSE following treatment with *E. sonchifolia*, we conducted MRSE observations SEM. In the control group (Fig. 7a), MRSE cells presented a regular shape, smooth surface, and an intact surface. After subjecting the bacteria to an incubation period of 4 h with 5 mg/mL
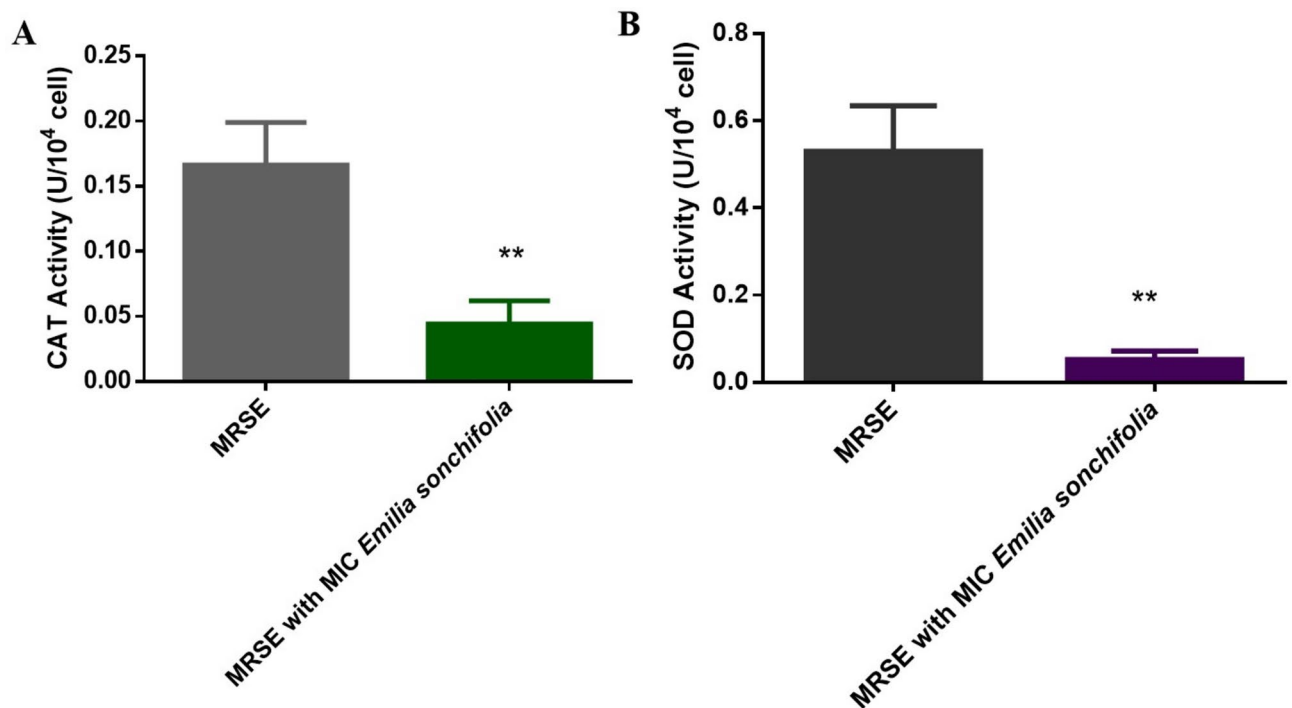
Fig. 6. Effect of *Emilia sonchifolia* on defense mechanisms of MRSE. (**a**) Effect of *E. sonchifolia* on CAT activity in MRSE. (**b**) Effect of *E. sonchifolia* on SOD activity in MRSE.
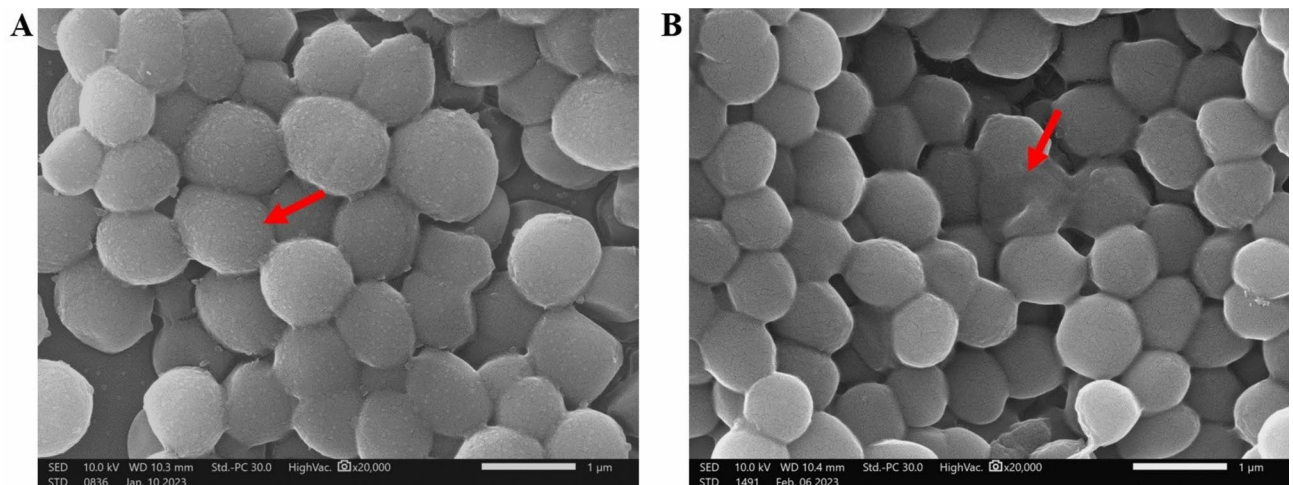

Fig. 7. Scanning electron microcopy observations of morphology changes in MRSE. (**a**) MRSE untreated with *Emilia sonchifolia*. (**b**) MRSE treated with *E. sonchifolia* for 4 h. Red arrows indicate regions of the lost cellular integrity.

E. sonchifolia, notable alterations in the morphology of the bacterial cells were observed, as depicted in Fig. 7b. The surface of the MRSE with *E. sonchifolia* exhibited irregular and rugged characteristics, with certain areas exhibiting extensive fractures that resulted in the release of the contents.

TEM analysis
To examine the ultrastructural modifications in MRSE cells following treatment with *E. sonchifolia*, we utilized TEM. The control group exhibited intact cell walls, where in the cytoplasm remained undisturbed in conjunction with the bacterial cell membrane (Fig. 8a). Notable alterations in MRSE morphology were observed following the interaction with *E. sonchifolia*. Figure 8b shows the occurrence of cell wall lysis and the presence of a discontinuous cell membrane, resulting in the release of numerous intracellular components from MRSE cells. Moreover, a small proportion of the cells underwent dissolution.
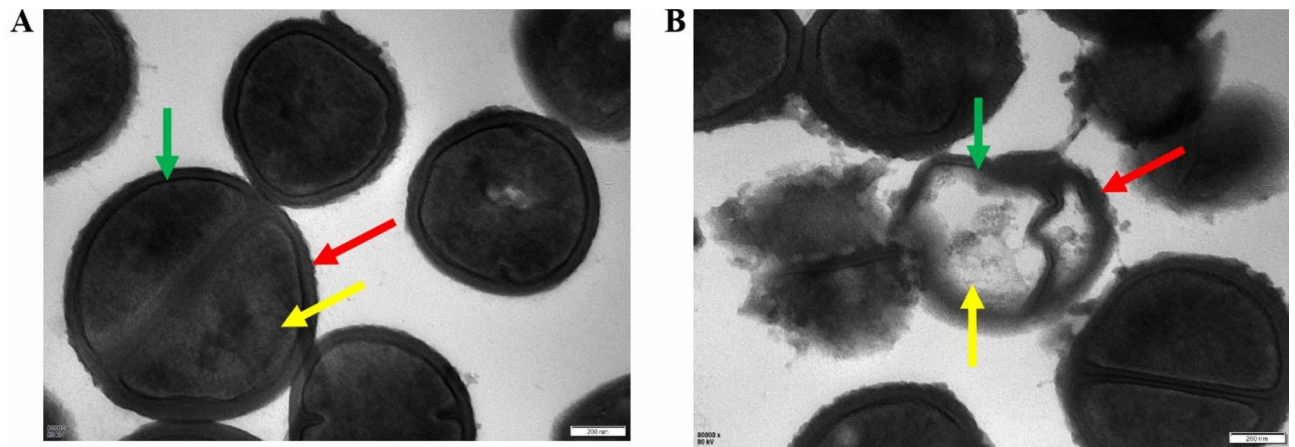
Fig. 8. Transmission electron microscopy observations of morphological changes in MRSE. (**a**) MRSE untreated with *E. sonchifolia*. (**b**) MRSE treated with *E. sonchifolia* for 4 h. Red arrows indicate cell walls. Green arrows indicate cell membranes. Yellow arrows indicate the cytoplasmic contents. Data are presented as mean ($\pm$ SD) of three replicates (compared with the control, **$p < 0.01$).

Discussion

S. epidermidis is one of the leading causes of BSIs[35]. The emergence of antibiotic-resistant strains, particularly MRSE, poses a significant challenge in the treatment of BSI. TCM posits that "clearing heat and detoxifying" TCM, which is cold in nature, can alleviate or eliminate infection caused by pathogenic microorganisms such as bacteria and viruses[36]. Pharmacological studies have demonstrated that clearing heat and detoxifying TCM, such as *Lonicera japonica Thunb., Forsythia suspensa, Scutellaria baicalensis Georgi,* and *Coptis chinensis Franch.* exhibit good antibacterial activity[37]. Similarly, in our previous study, *Patrinia scabiosaefolia,* classifie as a "clearing heat and detoxifying" TCM showed strong antibacterial activity against MRSE[20]. Additionally, a previous study revealed that *E. sonchifolia* extracts demonstrated strong antibacterial effects against *S. aureus, P. aeruginosa* and *N. gonorrhoeae*[21]. Therefore, the present study examined the in vitro and in vivo antibacterial activities of *E. Sonchifolia* against MRSE.

The MIC and MBC results demonstrated that *E. Sonchifolia* exhibits strong antibacterial efficacy in vitro, as presented in Table 1. To assess its antibacterial activity in vivo, the mouse model of MRSE-induced BSIs was established. As *S. epidermidis* is an opportunistic pathogen, the host immune response can eliminate it. Therefore, MRSE infections may occur in immunosuppressed patients. Based on these findings, a an immunosuppressive mouse model was developed. Subsequently, *E. Sonchifolia* was tested to evaluate its therapeutic efficacy against BSIs in mice. The results revealed that *E. sonchifolia* exhibited good antibacterial activity in vivo (Fig. 1). These findings confirm that *E. sonchifolia* possesses good antimicrobial activity.

Recently, a deeper understanding of antibacterial mechanisms has necessitated the integration of multi-omics data[38]. Proteomic and transcriptomic analyses revealed that proteins involved in purine metabolism were notably downregulated in the energy metabolic pathway. Purine metabolites play a vital role in bacterial growth[39,40]. In this study, proteomics and transcriptomics analyses were performed on MRSE cultured with *E sonchifolia*. The results indicated that proteins associated with purine metabolism were notably downregulated. These findings align with those of a previous report showing that tannic acid downregulated purine metabolism proteins in *S. aureus*[26].

The downregulation of purine metabolism suggests inhibition of bacterial growth, providing evidence that *E. sonchifolia* exhibits antibacterial properties by inhibiting purine metabolism.

Purine metabolism involves metabolic pathways, such as synthesis, salvage, and regulation of purine synthesis[41]. The key components of purine metabolism include the aspartate and glutamate pathways. The aspartate pathway facilitates the synthesis of inosine monophosphate (IMP) from aspartic acid, followed by the synthesis of other nucleotides from IMP. Conversely, glutamate pathway involves the synthesis of GMP (guanine monophosphate) from glutamate, followed by the synthesis of other nucleotides from GMP. The purine nucleotide salvage pathway is primarily facilitated by three enzymes: adenosine kinase (ADK), adenine phosphoribosyl transferase (APRT), and hypoxanthine–guanine phosphoribosyl transferase (HGPRT). The complementary salvage pathway generally fulfills the cellular requirements for purines[42]. In the guanine nucleotide pathway, two enzymes facilitate the conversion of IMP to GMP[43]. Purines are essential organic compounds in bacterial cells, serving as fundamental components of nucleic acids, including DNA and RNA. They are integral to storage and dissemination of genetic material[26]. Additionally, purines play a role in bacterial energy metabolism by producing Adenosine triphosphate (ATP), that is essential for sustaining biological activities[44]. Studies have demonstrated that IMP is an important intermediate in de novo purine biosynthesis, because it can be converted to either GMP or AMP (Adenosine monophosphate) via two parallel pathways. The enzymes involved in the GMP pathway include IMP dehydrogenase (IMPDH) and GMP synthase (GMPS)[45]. Thus, in this study, the activities of GMP, AMP, and IMP were examined. Figure 6 demonstrates that *E. sonchifolia* effectively inhibited the activities of GMP, AMP, and IMP. These findings align with previous research[46,47].
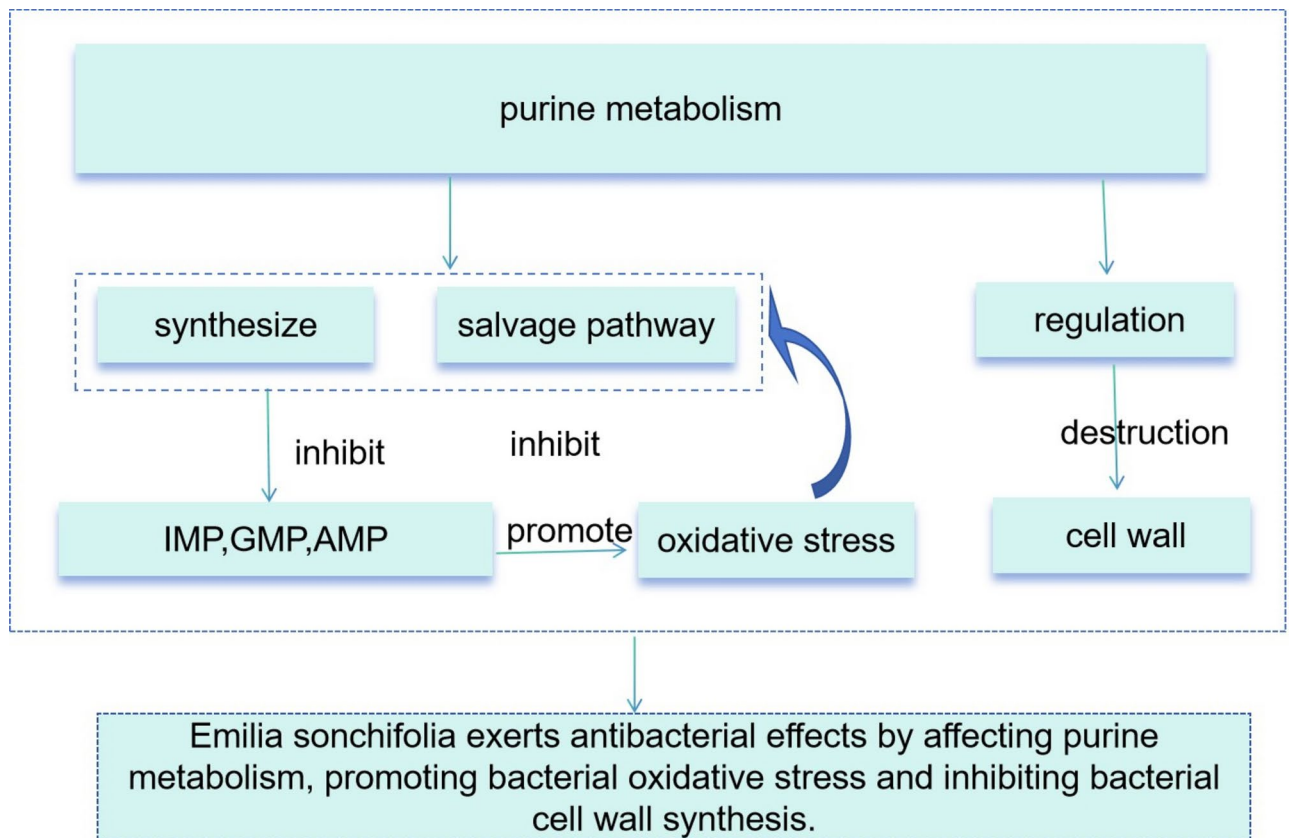
Fig. 9. Mechanistic hypothesis diagram of the purine metabolism mechanism of *E. sonchifolia*.

Research has revealed that inhibition of three enzymes, GMP, AMP, and IMP, correlates with the inhibition of bacterial growth (Fig. 9). Experimental results confirmed that the activities of these enzymes were downregulated, validating that *E. sonchifolia* inhibits the three enzymes involved in both the synthesis and salvage pathways, thereby inhibiting purine metabolism and exerting an antibacterial effect.

The regulatory mechanism of bacterial purine metabolism relies on a TCS that precisely modulates the expression of pertinent genes in response to purine levels, ensuring coordinated regulation of the entire purine metabolic pathway, is the primary signal transduction system in bacteria. When external stimuli diminish, bacteria experience stress, leading to downregulation of corresponding proteins.. Proteomics and transcriptomic analyses revealed that *E. sonchifolia* treatment downregulated dltA proteins, that are involved in the TCS in bacterial cell wall synthesis. The cell wall and membrane serve as physical barriers, separating the internal cellular environment from the external surroundings and preventing the loss of intracellular components[48]. As presented in Figs. 1 and 2, *E. sonchifolia* disrupted the cell walls and membranes of MRSE, thereby compromising the stability of the bacterial internal environment. Similar outcomes were observed with leaf extracts of *Aronia melanocarpa* (Michx.), *Chaenomeles superba* Lindl., and *Cornus mas* L. tested against bacterial cells[49].

Some studies have indicated that the utilization of AMP, IMP, and GMP can lead to increased levels of Uric acid , the final product of the purine metabolism pathway and is associated with increased oxidative stress[50,51]. We further examined changes in the defense mechanisms of MRSE. Disruption of microorganism homeostasis caused by an oxidative burst of ROS is counteracted by the cellular defense mechanisms of the antioxidant enzyme system[52]. This system comprises various antioxidant enzymes, including SOD, CAT, and glutathione peroxidase (GSH-Px)[53]. As presented in Fig. 6, the activity of SOD and CAT decreased following treatment with *E. sonchifolia,* demonstrating *E. sonchifolia* can inhibit the defense mechanisms of MRSE. Similarly, curcumin-based photodynamic inactivation can amplify oxidative damage in bacteria by inhibiting SOD and CAT activity[52].

Data exhibited that *E. sonchifolia* inhibits both the purine metabolic synthesis and salvage pathways by inhibiting AMP, IMP, and GMP, leading to enhanced oxidative stress and ultimately exerts an antibacterial effect.

Compared with conventional antibiotics, *E. sonchifolia* exhibits the advantage of targeting multiple sites against MRSE. This may account for the reduced likelihood of drug resistance development in TCM. This investigation provides a preliminary elucidation of the antibacterial activity and mechanisms of *E. sonchifolia* against MRSE, by inhibiting purine metabolism, thereby affecting oxidative stress and damaging cell membranes and cell walls. However, further research is warranted to comprehensively understand the specific mechanism of action of *E. sonchifolia* against MRSE.

Conclusion

In conclusion, this study demonstrated that *E. sonchifolia* exhibits noteworthy antibacterial efficacy against MRSE both in vitro and in vivo. Furthermore, proteomic and transcriptome analyses predicted several antibacterial mechanisms, including inhibition of purine metabolism, increase in bacterial oxidative stress, and inhibition bacterial cell wall synthesis. Furthermore, enzymatic assays related to purine metabolism, antioxidant activity, and cell morphology analyses confirmed *E. sonchifolia* inhibits purine metabolism, induces oxidative stress and damages the bacterial cell wall. These findings suggest that *E. sonchifolia* holds great potential as an antibacterial agent.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Lili An, Xin Liu and and Wei Peng wrote the main manuscript text and Gongzhen Chen, Luo, Qian Tonghan, Meng Ni, Xuebing Wang , Fu yufeng, Yonghui Zhou, YuqiYang prepared figures and tables. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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