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ORIGINAL RESEARCH

Antimicrobial Activity and Metabolomic Analysis of Linalool Against Pathogenic Bacteria Methicillin-Resistant *Staphylococcus aureus*

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Purpose: The purpose of this study was to evaluate the antibacterial activity and mechanism of linalool against Methicillin-resistant *Staphylococcus aureus* (MRSA).

Methods: The determination of the antibacterial activity of linalool against clinically isolated MRSA strains was based on the minimum inhibitory concentration (MIC) and growth curve analysis. Finally, the inhibition mechanism of linalool was elucidated through metabolomic analysis and molecular docking.

Results: Among the isolated strains, penicillin resistance was found to be the highest, while resistance to daptomycin/quinupristin-dalfopristin, linezolid, vancomycin, tetracycline, telithromycin, and levofloxacin was not observed. The MIC range of linalool was 211.24–1.65 μg/mL, with MIC_{50} and MIC_{90} values of 13.2 μg/mL and 105.62 μg/mL, respectively. Metabolomic analysis revealed that linalool interferes with various substance metabolisms and energy metabolism in MRSA, with the glutathione pathway potentially being a key pathway affected by linalool. Molecular docking revealed that linalool exhibited good binding potential to the target glutathione.

Conclusion: This study suggests that linalool could be developed as a drug or preservative to inhibit MRSA growth.

Keywords: MRSA, linalool, antimicrobial resistance, metabolome, glutathione

Introduction

Staphylococcus aureus is a Firmicutes, Gram-positive bacterium, which is widely present in the symbiotic microbiota of the skin and nasal mucosa of the population.[1,2] When the skin and mucous membranes are damaged, such as skin diseases, physical wounds, and surgical wounds, *Staphylococcus aureus* will take the opportunity to enter the tissues and even the bloodstream, which will cause different degrees of infection, and even cause sepsis to be life-threatening.[3] It is estimated that the mortality rate from *S. aureus* bacteremia will rise, and the number of strains of microorganisms that are resistant to existing antibiotics is increasing.[4] Infections caused by drug-resistant bacteria increase the risk of poor clinical outcomes and even death. Methicillin-resistant *S. aureus* (MRSA) was first described in England in 1961,[5] soon after methicillin was introduced into clinical practice. Although antibiotics have been updated due to drug toxicity and other reasons, the term MRSA has been in use up to now. More seriously, different antibiotics introduce new resistances, including the penicillins, sulfonamides, tetracyclines, glycopeptides, and others, including plasmid-mediated horizontal transmission.[6] According to the WHO report on drug resistance, the most serious problems include the resistance of *Klebsiella pneumoniae* to third-generation cephalosporins and carbapenem, *Escherichia coli* to third-generation cephalosporins and fluoroquinolone, *Staphylococcus aureus* to methicillin, *Streptococcus pneumoniae* to penicillin, and *Salmonella sp*. to fluoroquinolones. The development of new antimicrobial drugs is urgent.

Essential oils (EOs) of aromatic plants and their major chemical compositions are considered as potential substitutes for conventional antibiotics because of their outstanding antimicrobial, antioxidant, and anti-

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inflammatory properties and the advantages of non-toxicity and low drug residue.[7,8] In food production, it is one of the key points of research to find antibacterial preservatives that are easy for the public to accepted. The addition of antibiotics and synthetic preservatives, such as benzoic acid, sorbic acid, and nitrite, has raised concerns about the safety of food, and as a result, safer natural antimicrobials may be more acceptable.[9] This may be another major application of EOs in addition to cosmetics and perfumes.[10] Linalool (2,6-dimethyl-2,7-octadien-6-ol) is an aromatic monoterpene alcohol that is widely found in EOs.[11] Many EOs with linalool as the main active ingredient (lavender, coriander, basil) exhibit excellent antimicrobial activity.[12–14] Linalool has also been shown to have antimicrobial activity when used alone and can also be used as an adjunct antimicrobial agent to increase antibiotic sensitivity.[15–18] In addition, linalool also exhibits antioxidant, anti-inflammatory, and neuroprotective effects, highlighting its potential applications in the fields of medicine, food, and cosmetics.[11] The activity of linalool against various microorganisms has been studied, including Pseudomonas aeruginosa E. coli and MRSA;[13,19] however, the inhibitory mechanism against MRSA remains to be elucidated. Most studies on antibacterial mechanisms involve cataloging organisms and/or genes using DNA or RNA sequencing methods to analyze changes in microbial communities. With the rise of metabolomics, it has evolved from biomarker discovery to the exploration of mechanisms. Due to the inherent sensitivity of metabolomics, subtle changes in biological pathways can be detected, providing deeper insights into the underlying mechanisms of various physiological conditions and pathological processes, including diseases.[20,21] The research focused on the inhibitory activity of linalool against MRSA, assessed through MIC measurements and growth curve analysis. Furthermore, a comprehensive metabolome analysis revealed the potential anti-MRSA molecular mechanisms of linalool. This study offers valuable theoretical insights for the development of linalool as an effective product for inhibiting MRSA.

Materials and Methods

Reagents and Strains

Linalool (≥98%) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd (Shanghai, China). Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) were purchased from OXOID International Ltd. (Basingstoke, United Kingdom). Polysorbate 80 was purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). A total of 87 MRSA strains from Dazhu County People's Hospital were used for measurements. These isolates were identified and subjected to antibiotic susceptibility testing using the automated VITEK2 system (Merieux Diagnostic Products (Shanghai) Co., Ltd, Shanghai, China). Bacterial strains were stored at −80°C as a stock solution in 20% glycerol until use.

Antibacterial Activity Evaluation

Determination of MIC (Minimum Inhibitory Concentration)

To further evaluate the antibacterial activity of linalool, MIC was measured using the double broth dilution method.[22] Briefly, linalool was dissolved in polysorbate 80 (final concentration 0.1%, 0.1% polysorbate 80 without antibacterial activity against MRSA) and diluted with MHB to obtain a series of concentrations (211.24, 105.62, 52.81, 26.40, 13.20, 6.60, 3.30, 1.65 μL/mL). Then, these solutions were incubated with the bacterial suspension (in the logarithmic phase, 1.5×10^6 CFU/mL) in 96-well micro-plates at 37°C for 24h. The blank group was with MHB without linalool. MIC was defined as the minimum linalool concentration at which no visible bacterial growth was noted. Each bacterium should be measured at least three times in repetition.

Growth Curve

The growth curve was plotted based on a previous method, with some modifications.[23] First, different concentrations of linalool (1/2MIC, 1/4MIC, 1/8MIC) were added to test tubes with logarithmic phase bacteria. The group with the MHB containing no linalool was designated as the blank. The absorbance of suspensions at different incubation times (0, 2, 4, 6, 8, 10, until 22 hours) was measured at 600 nm by using a spectral-scanning multi-template reader (Varioskan Flash, Thermo Fisher Scientific, Shanghai, China).

Metabolomics Analysis

The logarithmic phase MRSA strains was treated with linalool (1/4MIC) at 37 °C for 4h. The blank group was untreated with linalool at 37 °C for 4h. The precipitated cells were collected by centrifugation (6000 rpm for 5 min at 4 °C) and washed three times with PBS. Subsequently, the cells were snap-frozen in liquid nitrogen and stored at −80°C until required for experiments.

Sample Preparation and Extraction

According to previous study,[24] the bacterial cells were slowly thawed on ice. The sample was taken for protein quantification. Take another sample to a centrifuge tube, and mix it with 20% acetonitrile/methanol (1:5, V/V). Vortex for 3 minutes, centrifuge at 4°C (12000 rpm, 10 min). Transfer the supernatant into a new centrifuge tube and place it at −20°C for 30 minutes. Then centrifuge at 4°C (12000 rpm, 10 min). After centrifugation, transfer the supernatant to protein precipitation plate for further LC-MS analysis.

UPLC-MS Conditions

The sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC, ExionLC AD; https://sciex.com.cn/; MS, QTRAP® 6500+ System, https://sciex.com/). The analytical conditions were as follows: HPLC: column, ACQUITY BEH Amide (2.1 × 100 mm, 1.7 μm); solvent system, water with 2 mm ammonium acetate and 0.04% formic acid (A), acetonitrile with 2 mm ammonium acetate and 0.04% formic acid (B). The gradient started at 90% B (0–1.2 min), decreased to 60% B (9 min), then to 40% B (10–11 min), and finally ramped back to 90% B (11.01–15 min); flow rate, 0.4 mL/min; temperature, 40°C; injection volume, 2 μL. The AB 6500+ QTRAP® LC-MS/MS System, equipped with an ESI turbo ion-spray interface, operated in both positive and negative ion modes and was controlled by Analyst 1.6 software (AB Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature, 550°C; ion spray voltage (IS), 5500 V (positive) and −4500 V (negative); curtain gas (CUR), 35.0 psi; and DP and CE for individual MRM transitions were optimized further. A specific set of MRM transitions was monitored for each period, corresponding to the amino acids eluted during that period.

Data Processing and Statistical Analysis

For untargeted metabolomics, the acquired raw mass spectral data were imported into Analyst 1.6.3 for processing, which included peak extraction, retention time correction, adduct ion merging, missing value imputation, and background peak labeling. For targeted metabolomics, MultiQuant 3.0.3 was used to process the mass spectrometry data. The retention times and peak shapes of reference standards were used to construct standard curves for the targeted substances. The integrated peak area ratio of the samples is substituted into the standard curve's linear equation to calculate, the absolute content of metabolites. Identified metabolites were annotated using the KEGG compound database (http://www.kegg.jp/kegg/compound/), and the annotated metabolites were subsequently mapped to the KEGG Pathway database (http://www.kegg.jp/kegg/pathway.html). Pathways with significantly regulated metabolites were then analyzed using metabolite set enrichment analysis (MSEA), with significance determined by P-value from the hypergeometric test.

Principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) were performed. Differentially expressed metabolites (DEMs) were identified based on the following criteria: a variable importance in projection (VIP) score > 1 for the first principal component of the OPLS-DA model, a fold change ≥1.5 or ≤0.667 between the two groups, and a P-value ≤ 0.05 from the *t*-test.[25] Hierarchical cluster analysis and KEGG annotation of differential metabolites were then performed.

Glutathione and Superoxide Dismutase Measurement

The final concentration of linalool was determined in the MIC determination section, while 1×10^5 CFU/mL of MRSA cells were introduced into the treatment culture. The assay consisted of untreated MRSA cells, and linalool-treated MRSA cells (inoculum in MHB supplemented with 1/16 MIC, 1/8 MIC, 1/4MIC, respectively). The cells were incubated at 37°C with shaking at 200 rpm. Cells were collected by centrifugation at 10,000 rpm for 5 minutes, washed with PBS, and normalized to the same density. The samples were then homogenized in 1 mL of sterile water containing 50μg/mL of lysostaphin and incubation at 37°C for 30 minutes. The levels of superoxide dismutase (SOD) and glutathione (GSH) in

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MRSA were measured according to the manufacturer's instructions for the kits (Nanjing JianCheng Bioengineering Institute, China).

Molecular Docking of Linalool to Key Targets

The AutoDock software (https://vina.scripps.edu/) was used to perform molecular docking between key targets and linalool.[26] The crystal structures of the potential protein targets of Epicedium were retrieved from the RCSB Protein Data Bank (http://www.pdb.org/) and were subsequently modified using Autodock software. The target (PDB ID: 5VDN) was modified by removing ligands and water, adding hydrogens, and optimizing and patching amino acids. The modified structures were saved in PDBQT format. ChemBioDraw 3D was used to generate the 3D chemical structures and minimize their energy, with the results saved in MOL.2 format. The compounds were imported into Autodock, where all flexible bonds were set to be rotatable by default and saved in PDBQT format as docking ligands. Autodock Vina 1.1.2 was used for docking, and PyMOL was used to visualize the docking results. The Binding Affinity (kcal/mol) represents the binding free energy between linalool and target molecule (glutathione); the higher the absolute value of the binding free energy, the more stable the ligand-receptor interaction.

Statistical Analysis

All the experiments were repeated three times, and the results were statistically analyzed using GraphPad Prism 9. The significance of the difference was assessed using one-way ANOVA and Duncan's multiple comparisons ($p < 0.05$ was considered significant). The results are presented as means ± standard deviation (SD).

Results

Antibiotic Susceptibility of MRSA

A total of 87 MRSA strains (all resistant to methicillin) were clinically isolated and identified, and their antibiotic susceptibility was determined according to CLSI 2020 (Figure 1A). The highest resistance rate was observed for penicillin (97.66%). The tested strains were sensitive to dalfopristin/quinupristin, linezolid, vancomycin, tigecycline, and rifampicin. On the other hand, gentamicin, ciprofloxacin, and moxifloxacin showed more mediating values from sensitivity to excessive drug resistance. The separate tests for resistance screening against cefoxitin and clindamycin showed induction positivity rates of 34.88% and 7.81%, respectively (Figure 1B). Only 3 strains (2.34%) showed positive results for both tests.

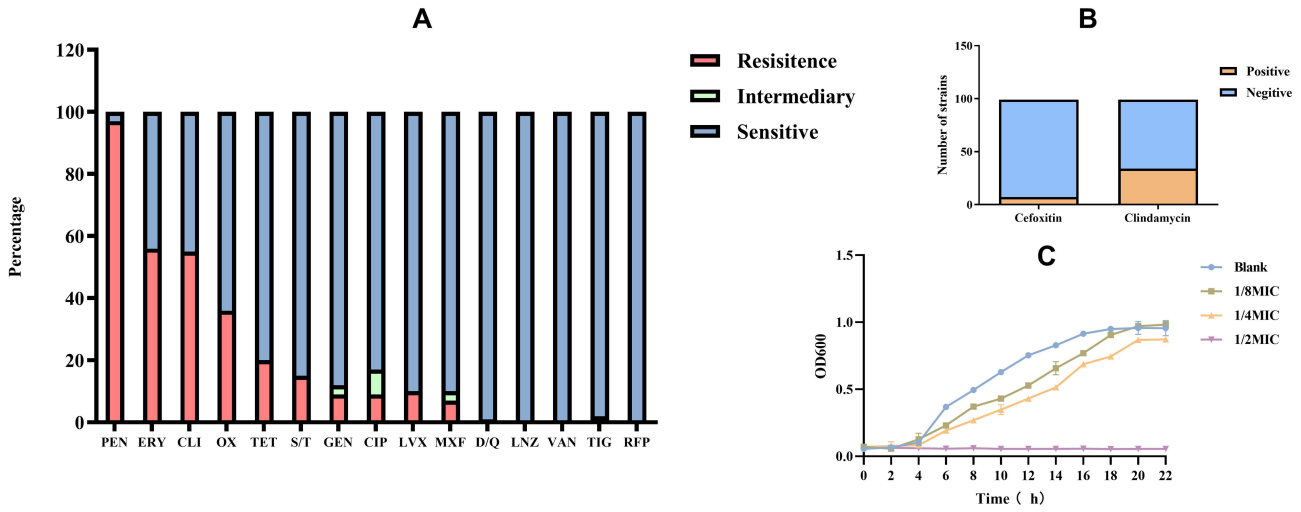

Figure 1 Susceptibility of MRSA. (**A** and **B**) antibiotic susceptibility of MRSA, (**C**) growth-time curve of MRSA.
Abbreviations: PEN, Penicillin; OX, Oxacillin; GEN, Gentamicin; CIP, ciprofloxacin; LVX, levofloxacin; MXF, Moxifloxacin; ERY, Erythromycin; CLI, clindamycin; D/Q, Dalfopristin/Quinupristin; LNZ, linezolid; VAN, Vancomycin; TET, Tetracyclines; TIG, Tigecycline; RFP, Rifampicin; SMZ/TMP, Sulfamethoxazole-Trimethoprim.

Table 1 The MIC of Linalool Against MRSA

MIC (μg/mL)	211.24	105.62	52.81	26.40	13.20	6.60	3.30	1.65
Number of strains	3	7	18	13	29	13	3	1
Percentage (%)	3.45%	8.05%	20.69%	14.94%	33.33%	14.94%	3.45%	1.15%

Antibacterial Activity of Linalool Against MRSA

The MIC distribution of linalool for MRSA is shown in Table 1. There were 29 MRSA strains identified with a MIC of 13.2 μg/mL, representing the highest number of strains at this concentration. However, the minimum concentration of 1.65 ug/mL was still able to inhibit the growth of one MRSA strain. The MIC_{50} and MIC_{90} of linalool were 13.2 μg/mL and 105.62 μg/mL, respectively, indicating that linalool possesses excellent antibacterial activity against MRSA.

To assess the antibacterial kinetics of linalool, a growth-time curve was plotted using the MRSA strain with the highest MIC for vancomycin (0.5 μg/mL). Among the other tested antibiotics, the strain showed resistance to penicillin, gentamicin, erythromycin, and tetracycline, while it exhibited intermediate resistance to gentamicin, levofloxacin, and moxifloxacin. The remaining antibiotics were found to be effective. As shown in Figure 1C, after 2 hours of incubation, growth was observed in the blank, 1/8MIC, and 1/4MIC groups; however, the growth rate of the latter two was slower than that of the blank group. After 4 hours, the blank group entered exponential growth phase, while the groups treated with linalool did not show significant exponential growth. No bacterial growth was observed in the 1/2MIC group during the re-observation period.

Effect of Linalool on the Metabolic Profile of MRSA

Changes in MRSA Metabolites After Linalool Treatment

To further investigate how linalool effects MRSA at the intracellular metabolism level, metabolites were extracted, and untargeted metabolomics analysis was conducted using UPLC-MS. After processing the metabolite peak data, OPLS-DA was performed (Figure 2A), revealing clear separation between the samples of the two groups. The high R^2 and Q^2 parameter values of OPLS-DA models (0.904 and 0.373, respectively), indicated good model fitness and high predictability. The PLS-DA permutation test (Figure 2B) showed that the intercept of the Q^2 regression line was less than 0, and the proportion of the substituted Y variable increased with a gradual decrease in substitution retention. This suggested

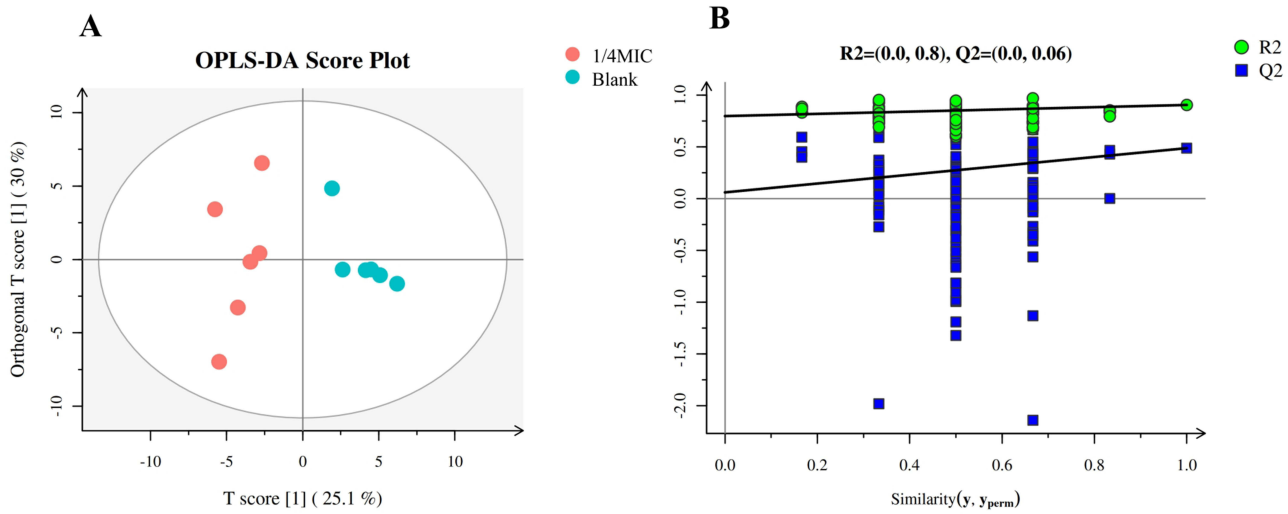

Figure 2 Untargeted metabolomics statistical analysis. Orthogonal partial least-squares discriminant analysis (OPLS-DA) score plots (**A**). Partial least squares-discriminate analysis (PLS-DA) permutation plots in the (**B**).

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that the models were reliable and robust, without overfitting. These results confirmed a significant difference between the two groups, indicating that linalool had a significant impact on metabolism in MRSA.

Differentially Expressed Metabolites (DEMs) Screening and Analysis

In this study, a total of 1,582 putative metabolites were identified based on LC-MS retention time, peak area, total ion chromatography, and molecular weights. Then, under the criteria of VIP > 1, FC > 1.5 or <0.667, and $p < 0.05$, 28 and 9 DEMs were selected from the positive and negative modes, respectively. The expression level of DEMs were visualized using volcano plots (Figure 3C and D). In total, 7 DEMs were upregulated and 21 were downregulated in the positive ion mode, while 4 DEMs were downregulated and 5 were upregulated in negative the ion mode (Figure 4A and B). DEMs in the negative ion mode were classified into benzene compounds, organic acids and their derivatives, organic oxygen compounds, organic heterocyclic compounds, phenylpropanoids, and polyketides. DEMs in the positive ion mode were divided into 6 categories, including benzenoids, lipids and lipid-like molecules, organic acids and derivatives, organic nitrogen compounds, organoheterocyclic compounds, phenylpropanoids, and polyketides. The hierarchical clustering results of DEMs are shown using heatmaps (Figure 3A and B). There was a correlation between these metabolites (Figure 4C–F). These results indicated that linalool treatment caused intracellular metabolic disorder in MRSA. Under linalool treatment, metabolic pathways such as amino acids biosynthesis, valine, leucine, and isoleucine biosynthesis, the pentose phosphate pathway, 2-oxocarboxylic acid metabolism, D-Alanine metabolism, and insulin secretion were

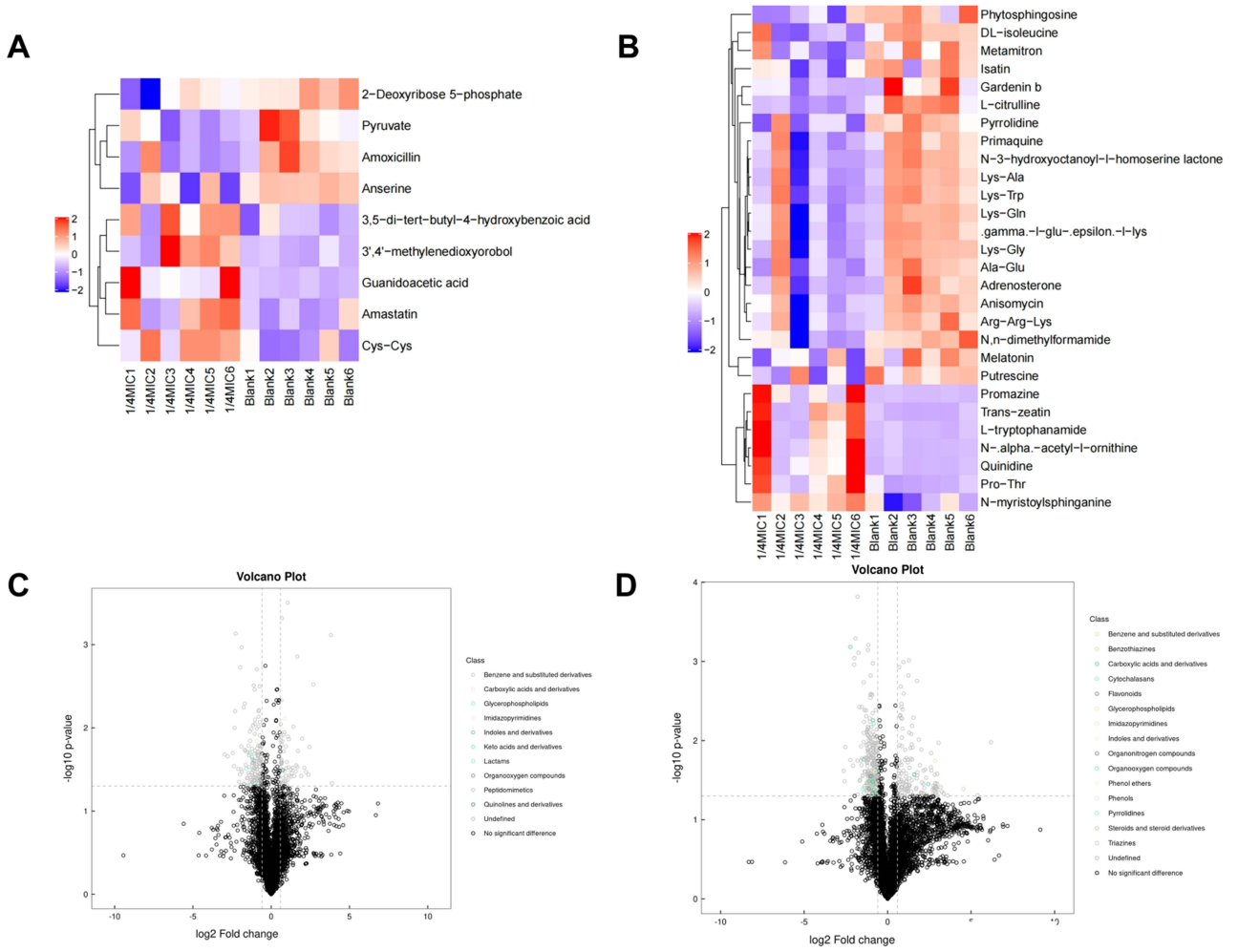

Figure 3 Screening and cluster analysis of differential metabolites. Cluster heatmaps in the (**A**) negative ion mode and (**B**) positive ion mode. Rows: metabolites, Columns: samples. (**C** and **D**) Visual volcano plots of differential metabolites in the two ion modes.

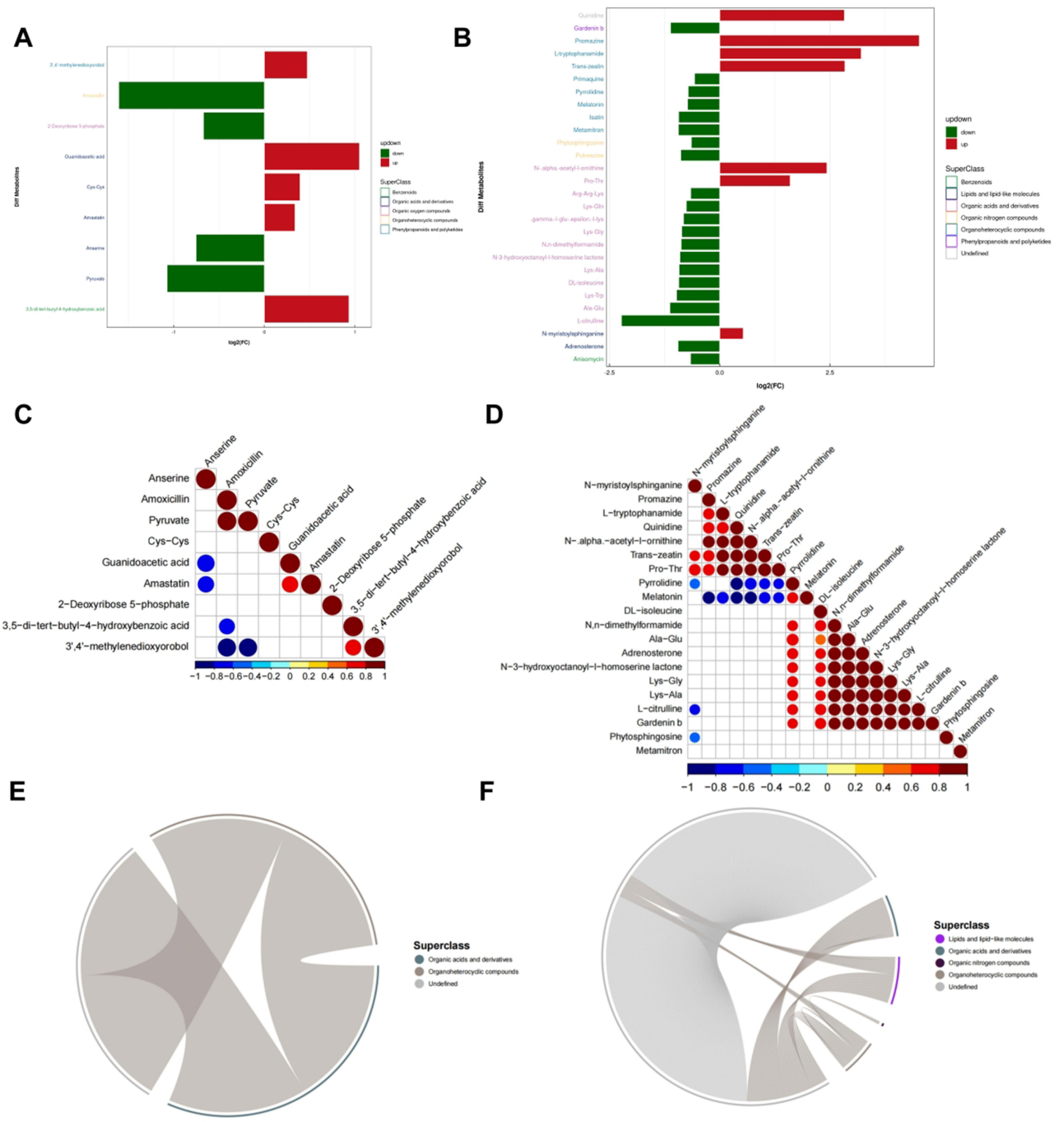

Figure 4 DEMs changes and correlation analysis. Fold change of DEMs in negative ion mode (**A**), heat map of DEMs association (**C**), and circlize (**E**). Fold change of DEMs in positive ion mode (**B**), heat map of DEMs association (**D**), and circlize (**F**).

downregulated. On the other hand, organoheterocyclic compounds, organic acids and derivatives, lipids and lipid-like molecules, phenylpropanoids, and polyketides showed upregulation.

Targeted Metabolomics Was Used to Verify the Effect of Linalool on MRSA Metabolites

After processing the metabolite peak data, PCA was performed. According to the PCA score plots (Figure 5A), the blank group was completely separated from the linalool-treated group. A total of 75 metabolites were detected, and metabolites with a fold change ≥ 2 or ≤ 0.5 were selected as significant DEMs, including 6 DEMs (5 upregulated and 1 down-regulated) (Figure 5B). After the quantitative analysis of the enriched DEMs, the Z-value was calculated through

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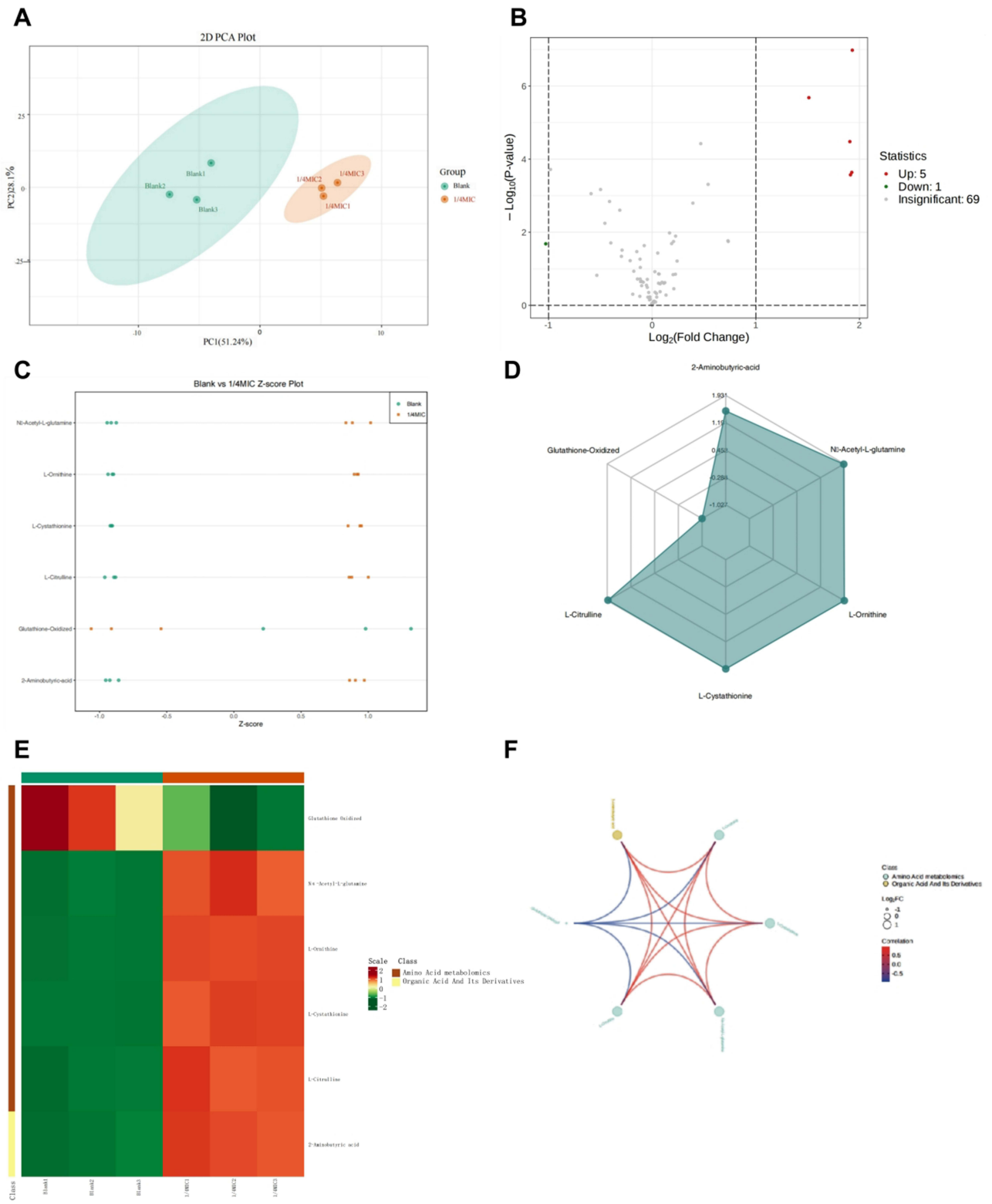

Figure 5 Targeted metabolite analysis. Principal component analysis (PCA) score plots of untreated (blank) and linalool treated MRSA (1/4MIC) (**A**). Visual volcano plots of DEMs (**B**). Z-value plot of DEMs (**C**), the x axis represented the normalized value of the substance, and the y axis represented the name of the metabolite. Radar map of DEMs (**D**), the value of the grid line corresponds to the fold of change of DEMs. Heatmap of clusters of significantly dDEMs (**E**), and chord plots (**F**).

normalization (Figure 5C). The differential fold changes of the DEMs across different samples were compared, and a radar chart of the DEMs was generated (Figure 5D). The six DEMs were completely separated between the blank group and the 1/4MIC group. Except for glutathione oxidized, which was downregulated after 1/4MIC linalool treatment, the other five metabolites (2-Aminobutyric acid, L-Citrulline, L-Ornithine, L-Cystathionine, Nα-Acetyl-L-glutamine) were upregulated (Figure 6B). Clustering heat maps were created for the significantly differentially DEMs distributed in amino

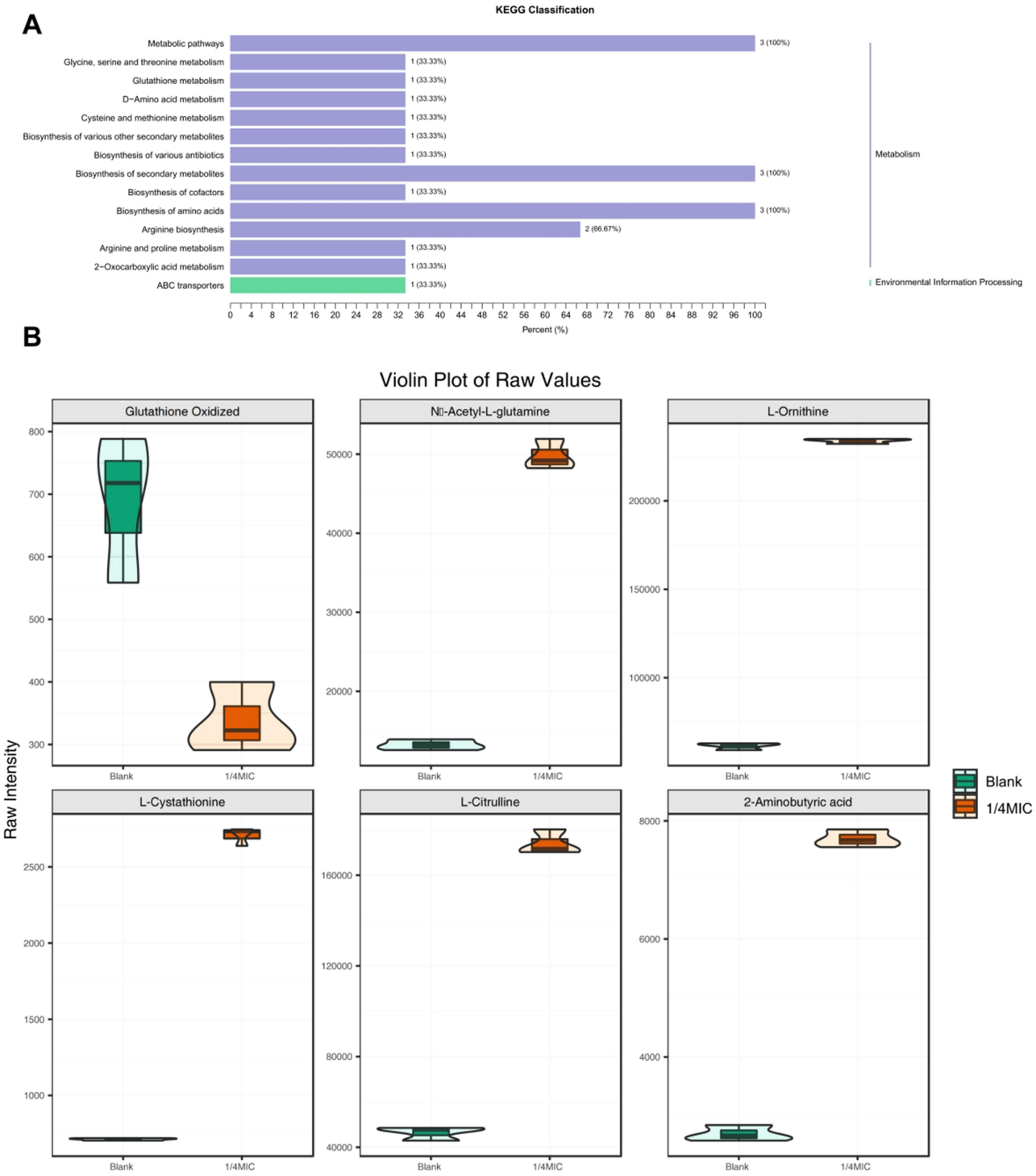

Figure 6 DEMs content and metabolic pathway enrichment. KEGG classification diagram of DEMs (**A**). Violin diagram of DEMs (**B**), the black horizontal line in the middle is the median, and the outer outline indicates the data distribution density.

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acid metabolism, organic acid and its derivatives, correlation analysis found that glutathione oxidized had a low correlation with other metabolites, whereas a high correlation between other metabolites (Figure 5E and F).

To further understand the underlying mechanism of metabolite changes, KEGG pathway enrichment analysis was performed. Significantly differentially expressed DEMs were enriched in 14 metabolic pathways and 2 pathways related to environmental information processing (Figure 6A). The pathways most involved with DEMs included metabolic pathways, biosynthesis of secondary metabolites, and biosynthesis of amino acids.

Linalool Causes MRSA Superoxide Dismutase (SOD) and Glutathione (GSH) Alterations

Linalool efficiently reduced intracellular GSH and SOD levels in MRSA at different concentrations (1/16, 1/8, 1/4 MIC). The results demonstrated that the intracellular SOD concentration in MRSA was significantly reduced after the addition of 1/8 MIC and 1/4 MIC linalool to the bacterial cultures for 4 to 12 hours ($p < 0.01$). At 4 hours, the GSH concentration in the blank group was lower than the group with linalool. However, with continued culture, the GSH content in the blank group increased, while the decrease in GSH levels was negatively correlated with the concentration of linalool (Figure 7).

Molecular Docking

The molecular docking results between linalool and glutathione are shown in Figure 8. The crystal structures of targets were obtained from the PDB database. The results demonstrate that linalool binds well to glutathione, with a binding affinity of −14.98 KJ/mol. Molecular docking suggests that linalool may exert an anti-MRSA effect by targeting this substance.

Discussion

As the biological activity of linalool was gradually reported, the inhibition of microorganisms has also become increasingly well-documented, such as *Salmonella Typhimurium* (MIC of 0.5%), *Salmonella Senftenberg* (MIC of 2%), and *Aeromonas hydrophila* (MIC of 0.3125%).[27–29] After testing, the MIC of linalool against MRSA was found to be between 211.24 and 1.65 μg/mL (0.25% to 0.002%), with an MIC_{50} of 13.2 μg/mL (0.016%), demonstrating good inhibitory activity. Several antibiotics were used to treat MRSA infections and target major bacterial processes, including cell wall synthesis, translation, transcription, and DNA synthesis.[30] Although resistance has been observed in most antibiotics currently used in clinical practice, not all strains are resistant to every drug. More commonly, some strains exhibit both resistant and susceptible characteristics. Furthermore, with increasing duration of treatment, a slight increase in MIC for vancomycin is observed, for example, in the MRSA used in the study (the MIC for vancomycin was 0.5 μg/mL). Strains of *S. aureus* that are susceptible to vancomycin (VSSA) have MICs of less than 2 μg/mL, while vancomycin-intermediate strains (VISA) exhibit MICs ranging from 4 to 16 μg/mL. For resistant strains, the MIC is

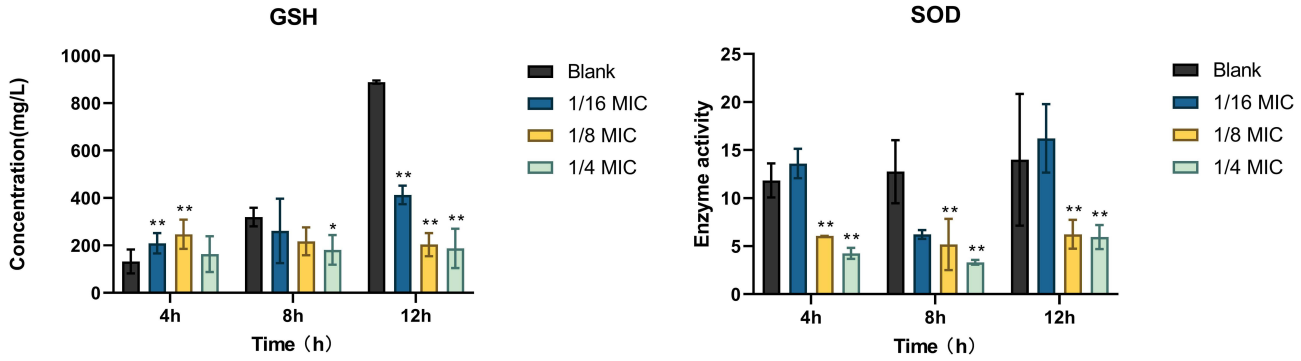

Figure 7 Effect of linalool on MRSA GSH and SOD. Compared with the blank group at the same time point, *$p < 0.05$, **$p < 0.01$.

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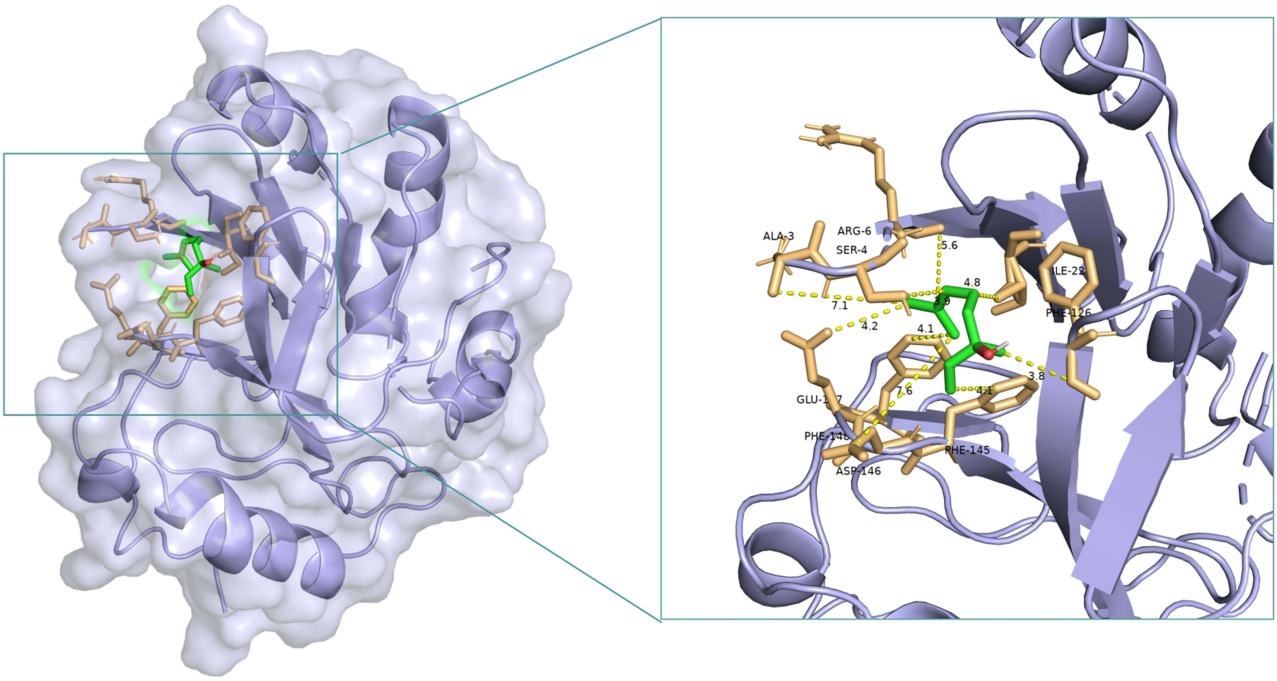

Figure 8 Linalool-GSH partial diagram of molecular docking.

≥16 μg/mL. Although sensitivity has not reached the level of resistance, the increase in MIC is still noteworthy as it relates to the first-line treatment for MRSA infection.[31]

Many studies, including our own, have demonstrated that EOs exhibited strong antibacterial activities.[7,32–34] The antimicrobial mechanisms of EOs are also being investigated in depth, primarily focusing on the disruption of cell structures, biofilms, metabolic pathways, and other factors.[35–37] Linalool inhibits bacterial growth by disrupting cell membranes and preventing biofilm formation. In P*seudomonas aeruginosa*, it disrupts cell membrane integrity and interferes with the respiratory chain, leading to cell death.[38] In *Escherichia coli*, linalool binds to plasmid DNA and alters its structure, reducing plasmid transfer between strains and thereby decreasing the spread of resistance.[39] Biofilm is a polymer that forms on the surface where bacteria aggregate, and it is a significant factor contributing to antibiotic resistance and persistent infections, often leading to treatment failure.[40] Linalool can inhibit the formation of various microbial biofilms by disrupting quorum sensing systems or inhibiting hyphal formation.[41–43] As research progresses, there is increasing focus on the differences between the transcriptome and metabolome, shedding light on the antibacterial mechanism of linalool. This research provides evidence of cell wall and membrane damage in *Streptococcus mutans* by detecting changes in amino acids and fatty acids.[22] Central metabolic pathways control virulence and antibiotic resistance, making them potential targets for antibacterial drugs. The impact of linalool on the metabolism of MRSA cells appears to be complex. The downregulation of amino acid biosynthesis pathways suggests that linalool interferes with amino acid synthesis in MRSA cells, potentially affecting protein synthesis and other vital biological functions.[44] This has been validated through targeted metabolomics. While we identified many amino acid metabolites, the ones with significant changes include glutathione oxidized, Nα-Acetyl-L-glutamine, L-Ornithine, L-Cystathionine, L-Citrulline, and 2-aminobutyric acid, with only glutathione oxidized showing a decrease. In the correlation analysis, glutathione oxidized showed weak correlations with other differentially expressed metabolites. Glutathione and glutathione-dependent enzymes help protect organisms from harmful environmental factors and play a crucial role in bacterial resistance.[45] This suggests that the glutathione metabolism pathway involving glutathione oxidized may be a key route through which linalool inhibits MRSA. Additionally, the decrease in metabolites from the pentose phosphate pathway indicates that linalool could impact the energy metabolism in MRSA cells', potentially affecting their survival and proliferative capacity.[46] Simultaneously, the upregulation of metabolites associated with organic acids, lipids, and lipid-

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like molecules may indicate MRSA cell's response mechanism to linalool. These changes may reflect an adaptive regulatory response to the external environment, potentially serving as a survival strategy against linalool. The results were further validated through assays (GSH and SOD level) and simulations (Auto dock). These findings offer insights into the antibacterial mechanism of linalool against MRSA, but further research is needed to elucidate the detailed mechanisms involved.

Conclusion

The study demonstrates that linalool exhibits potent antibacterial activity against methicillin-resistant *Staphylococcus aureus*, with MIC_{50} and MIC_{90} values of 13.2 μg/mL and 105.62 μg/mL, respectively. Growth curves further confirm this inhibitory effect, as MRSA showed no significant growth during the observation period when the concentration reached 1/2 MIC. Further metabolomic analysis reveals that linalool disrupts the metabolism of MRSA, with core amino acid metabolism consistently being affected. The glutathione metabolism pathway may play a key role in linalool's inhibition of MRSA growth. This study provides a theoretical foundation for the further development and application of linalool, while also identifying new targets for the research and development of antimicrobial drugs.

Abbreviations

MRSA, Methicillin-resistant Staphylococcus aureus; MIC, minimum inhibitory concentration; MIC_{50}, Inhibits the concentration of 50% bacteria; MIC_{90}, Inhibits the concentration of 90% bacteria; Eos, Essential oils; SOD, Superoxide dismutase; GSH, Glutathione.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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