Keywords
Zanthoxylum acanthopodium; MRSA; *murA; mecA*; molecular docking; phytochemicals

Authors
Yumi Lindawati[1,2*]
Doctoral Study Program in Medical Science, Faculty of Medicine, Universitas Padjadjaran, Indonesia ; lindawatiyumi@gmail.com
Oral Biology Department, Faculty of Dentistry, Universitas Sumatera Utara, Indonesia ; yumi.lindawati@usu.ac.id
https://orcid.org/0000-0001-8176-0965

Mieke Hemiawati[3,4]
Departmeny of Oral Biology, Faculty of Dentistry, Universitas Padjadjaran, Indonesia
Faculty of Medicine, Universitas Islam Bandung, Indonesia;
satarimieke15@gmail.com
https://orcid.org/0000-0002-6021-9480

Ani Melani[3,5]
Departmeny of Oral Biology, Faculty of Dentistry, Universitas Padjadjaran, Indonesia
Department of Oral Biology and Biomedical, Faculty of Dentistry, Universitas Jenderal Achmad Yani, Indonesia;
ani.melani@fkg.unjani.ac.id
https://orcid.org/0000-0001-7707-0582

Ameta Primasari[2]
Department of Oral Biology, Faculty of Dentistry, Universitas Sumatera Utara, Indonesia
ameta.primasari@usu.ac.id
https://orcid.org/0000-0003-2604-2578

*Correspondence to :
Yumi Lindawati[1,2*]
Doctoral Study Program in Medical Science, Faculty of Medicine, Universitas Padjadjaran, Jl. Ir. Soekarno Km 21, Jatinangor, Sumedang, 45363, Jawa Barat, Indonesia ; lindawatiyumi@gmail.com
Department of Oral Biology, Faculty of Dentistry, Universitas Sumatera Utara, Jl. Alumni No.2 Kampus USU, Medan 20155, Sumatera Utara, Indonesia ; yumi.lindawati@usu.ac.id
https://orcid.org/0000-0001-8176-0965

Received:16-06-2026
Revised: 22-07-2026
Accepted: 30-07-2026
Doi:10.1922/ejprd.v34i3.1652

Multitarget Inhibition of MRSA Cell-Wall Biosynthesis by *Zanthoxylum acanthopodium*: Gene Expression, and Molecular Docking for Antibiotic-Resistant Oral Infections

Abstract
Objective: *Methicillin-resistant Staphylococcus aureus* (MRSA) has emerged as an important pathogen in persistent oral infections because of its resistance to β-lactam antibiotics. This study investigated the antibacterial potential of the methanolic fraction of *Zanthoxylum acanthopodium* against MRSA by evaluating its effects on *murA* and *mecA* gene expression and characterizing the molecular interactions of its major phytochemicals with *murA* and penicillin-binding protein 2a (PBP2a).
Methods: The methanolic fraction of *Z. acanthopodium* fruit, , was tested against MRSA ATCC 33591 at concentrations of 25, 50, and 100 mg/mL. Relative *murA* and *mecA* mRNA expression levels were quantified using quantitative real-time PCR and analyzed with the 2^-ΔΔCt method. Statistical analysis used the Shapiro-Wilk and Kruskal-Wallis tests. Molecular docking was conducted using AutoDock 4.2 to evaluate the binding interactions of the identified phytochemicals containing diosmetin, eucalyptol, and gallotannin with *murA* and PBP2a.
Results: The greatest biological suppression of *murA* and *mecA* expression occurred at 50 mg/mL, reducing relative expression to .84-fold and .15-fold, respectively, compared with untreated controls. Although the differences among treatment groups were not statistically significant (*murA*, p = .39; *mecA*, p = .42), the observed transcriptional trend suggested coordinated inhibition of bacterial cell-wall biosynthesis and methicillin resistance pathways. Molecular docking showed that diosmetin had the most favorable interactions with both *murA* and PBP2a, while eucalyptol and gallotannin also formed stable non-covalent interactions with both target proteins, which supports a multitarget antibacterial mechanism.
Conclusion: The methanolic fraction of *Z. acanthopodium* exhibits promising anti-MRSA activity through simultaneous modulation of *murA* and *mecA* and predicted interactions with *murA* and PBP2a. These findings indicate *Z. acanthopodium* as a potential source of adjunctive phytotherapeutic agents for managing antibiotic-resistant oral infections. Further functional, pharmacological, and clinical studies are needed to validate its translational application in evidence-based dental practice.

1. INTRODUCTION

Antimicrobial resistance (AMR) is now a significant challenge in managing oral infections because it lowers the effectiveness of empirical antibiotic therapy and raises the likelihood of treatment failure. A recent systematic review and meta-analysis that included 53 studies and 12,408 patients with oral and maxillofacial space infections found that Streptococcus continued to be the main pathogen (45.51%), while *Methicillin-resistant staphylococcus aureus* (MRSA) made up 19.18% of *all S. aureus* isolates, showing that resistant staphylococci form a considerable portion of oral pathogens needing clinical attention. Persistent bacterial infection can hinder pulp healing, slow periapical tissue repair, disrupt implant osseointegration, and raise the risk of prosthodontic treatment failure. Because of this, finding alternative antimicrobial agents that can suppress resistant oral pathogens has become a key research focus.[1] *Methicillin-resistant staphylococcus aureus* (MRSA) has increasingly been identified as an oral colonizer and opportunistic pathogen that may play a role in persistent oral infections and help spread infection in dental settings.[2] Data from Yemen indicated that 37% of *S. aureus* isolates collected from patients with oral infections were MRSA, while earlier global reports summarized in the same study estimated MRSA prevalence between 9.1% and 14.69% among patients with oral infections.[3] Molecular epidemiological research also showed that oral MRSA isolates are part of internationally distributed clones, such as ST45, ST30, and ST22, which suggests that genetically varied strains have adapted to the oral environment.[4,5] Clinical studies have confirmed MRSA carriage among dental healthcare workers, patients, and within the clinical environment, pointing to the potential for cross-transmission during routine dental procedures.[6] The multicentre European study found a relatively low prevalence of multidrug-resistant bacteria among undergraduate dental students, supporting the value of infection-control measures and highlighting the ongoing need for surveillance and the search for new plant-derived antibacterial agents.[7]

The increasing incidence of antimicrobial resistance has accelerated the search for plant-derived compounds as alternative therapeutic agents for oral infections. Medicinal plants are recognized as valuable sources of bioactive metabolites that exhibit antibacterial, anti-inflammatory, and antioxidant activities, making them attractive candidates for the management of infections caused by multidrug-resistant bacteria. Among these plants, Andaliman (*Zanthoxylum acanthopodium*), an endemic species from North Sumatra, Indonesia, contains abundant flavonoids, alkaloids, terpenoids, phenolic compounds, and essential oils that have demonstrated broad-spectrum antimicrobial properties against gram-positive bacteria, including *Staphylococcus aureus*.[8] Recent investigations have also shown that the essential oil of *Z. acanthopodium* possesses potent antibacterial activity against *S. aureus*, primarily through disruption of bacterial membrane integrity and inhibition of cellular metabolism.[9] Its direct antibacterial activity, *Z. acanthopodium* has been reported to exhibit immunomodulatory properties and contains endophytic bacteria capable of producing metabolites that inhibit the growth of *S. aureus*, further supporting its therapeutic potential.[10,11] Fractionation of crude extracts is expected to concentrate bioactive constituents responsible for antibacterial activity, thereby enhancing their efficacy against resistant bacteria such as MRSA while facilitating the identification of active compounds. This integrated approach may accelerate the discovery of plant-derived antibacterial candidates for the management of MRSA-associated oral infections and improve treatment strategies in restorative dentistry and prosthodontics.[8-11]

The identification of new antibacterial targets has become increasingly important to overcome methicillin resistance in *Staphylococcus aureus*. Among the essential bacterial targets, UDP-N-acetylglucosamine enolpyruvyl transferase (*murA*) catalyzes the first committed step in peptidoglycan biosynthesis by transferring an enolpyruvate group from phosphoenolpyruvate to UDP-N-acetylglucosamine. This reaction initiates the synthesis of peptidoglycan precursors that are indispensable for bacterial cell wall formation and structural integrity.[12] Since *murA* functions upstream of peptidoglycan assembly, its inhibition disrupts cell wall biosynthesis at an early stage, resulting in impaired bacterial growth and eventual cell lysis.[13,14] *murA* has emerged as an attractive molecular target for the development of novel antibacterial agents that act through mechanisms distinct from those of conventional β-lactam antibiotics.[12,13] MRSA is primarily mediated by the *mecA* gene, which encodes the low-affinity penicillin-binding protein PBP2a and enables bacterial survival despite exposure to β-lactam antibiotics.[15,16] Although *murA* and PBP2a act at different points in peptidoglycan biosynthesis, both are necessary for preserving the integrity of the bacterial cell wall and thus serve as complementary therapeutic targets in efforts to combat MRSA. Recent studies have demonstrated that phytochemicals can inhibit bacterial growth through simultaneous modulation of *murA* activity and suppression of *mecA* expression, highlighting their potential as alternative anti-MRSA agents.[12,15] Based on this rationale, the present study investigated the antibacterial activity of Andaliman (*Z.acanthopodium*) fractions by evaluating their effects on *murA* and *mecA* mRNA expression in MRSA using in vitro assays, followed by molecular docking analysis to characterize the interactions between the major phytochemical constituents and both target proteins.

2. METHODS

Methicillin-resistant staphylococcus aureus (MRSA) ATCC 33591 was used as the experimental model because this reference strain harbors SCCmec type III, a genotype that has also been identified among MRSA isolates recovered from the oral cavity. The shared SCCmec background enhances the clinical relevance of this strain for evaluating alternative therapeutic agents against oral MRSA infections.[17] To prepare the bacterial cultures, we grew them in sterile Nutrient Broth (Oxoid, UK) and incubated them at 37 °C for a period of 16 to 24 hours.

Fresh fruits of *Z. acanthopodium* were collected from Dairi, North Sumatra, Indonesia, fractionated using methanol by reflux method at the Integrated Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. The phytochemical profile of this fraction was characterized

using high-resolution liquid chromatography–tandem mass spectrometry (LC–MS/MS) to contain a range of major phytochemical constituents, including flavonoids (diosmetin), terpenoids (eucalyptol), and tannins (gallotannin) at Universitas Brawijaya Central Life Sciences Laboratory.

The study comprised descriptive and experimental phases. Initially, the antibacterial activity of *Z. acanthopodium* fraction (100 mg/mL, 50 mg/mL and 25 mg/mL) was evaluated the underlying molecular mechanism at the Molecular Genetics and Microbiology Laboratories, Faculty of Medicine, Universitas Padjadjaran. MRSA cells exposed to the extract were subjected to RNA isolation, we used the Quick-RNA MiniPrep Kit (Zymo Research, USA), and then quantified the relative expression of *murA* and *mecA* mRNA with SensiFAST SYBR® Master Mix. Gene expression levels were normalized against a validated reference gene and analyzed using the 2^−ΔΔCt method. Experimental data were analyzed using appropriate statistical Saphiro Wilk and Kruskal Wallis tests, with a p-value < 0.05 considered statistically significant.

The computational analyses were carried out at the Medicinal Chemistry Laboratory, Universitas Indonesia. The *murA* protein structure of *Methicillin-resistant staphylococcus aureus* (MRSA) was unavailable in the Protein Data Bank (RCSB PDB) and UniProt databases. Therefore, a three-dimensional *murA* model was generated using AlphaFold. The predicted structure was validated using a Ramachandran plot in SWISS-MODEL to assess its stereochemical quality. The model showed 98.57% of residues in the favored regions, indicating that it was suitable for molecular docking. The crystal structure of the *mecA* protein from the MRSA strain can be found in the Protein Data Bank (PDB ID: 1MWT), eliminating the need for modeling and validation. Molecular docking was performed using AutoDock 4.2.

3. RESULTS

Table 1. Relative *murA* and *mecA* mRNA Expression in MRSA ATCC 33591 Following Treatment with Methanolic Fraction of Zanthoxylum acanthopodium Fruit

Treatment	Concentration (mg/mL)	Relative Expression Level	
		murA	***mecA***
Methanolic fraction of Zanthoxylum acanthopodium fruit	100	2,43	5.30
	50	0,84	0.15
	25	1,19	0.34
Fosfomycin (Positive Control)	1	2,01	194.01
Untreated Control (Negative Control)	-	1,00	1,00

Table 2. Comparative Analysis Between Andaliman (AZanthoxylum acanthopodium) Fruit Extract Treatment with *murA* and *mecA* Gene Expression in MRSA ATCC 33591

Relationship	p-value
Andaliman extract concentration versus *murA* mRNA expression	0.39
Andaliman extract concentration versus *mecA* mRNA expression	0.42

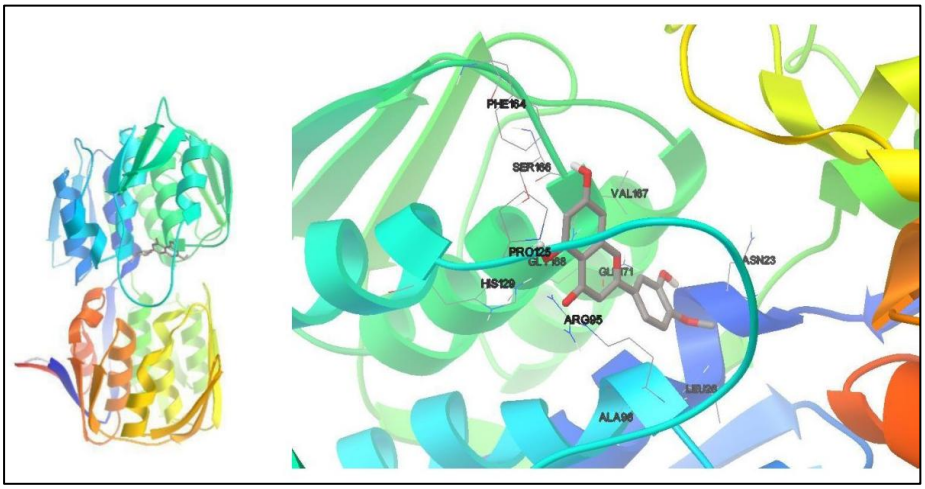

Figure 1. Binding Interactions of *murA* with Diosmetin (flavonoid)

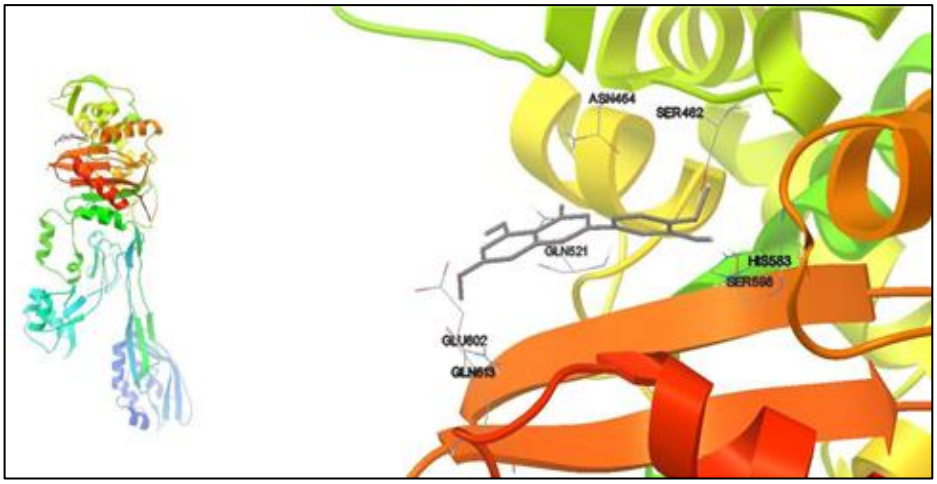

Figure 2. Binding Interactions of *mecA* (PBP2a) with Diosmetin (flavonoid)

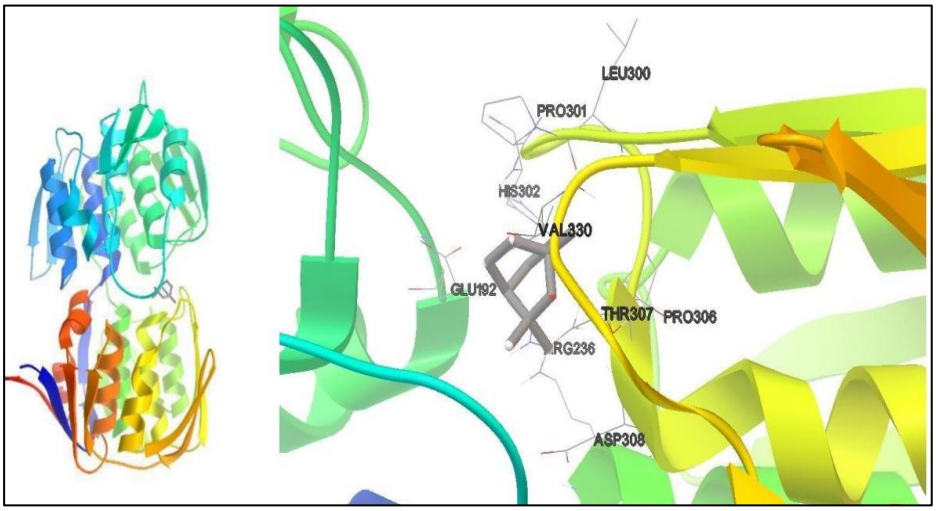

Figure 3. Binding Interactions of *murA* with Eucalyptol (terpenoid)

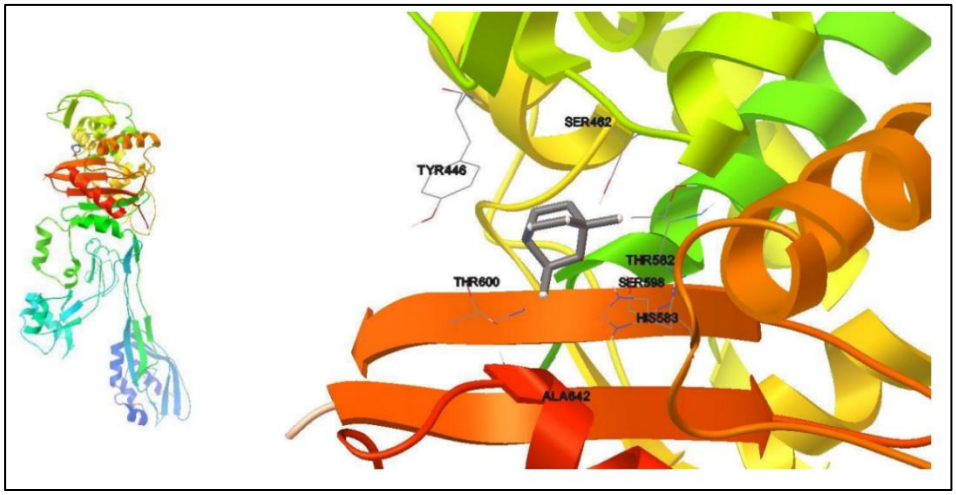

Figure 4. Binding Interactions of *mecA* (PBP2a) with Eucalyptol (terpenoid)

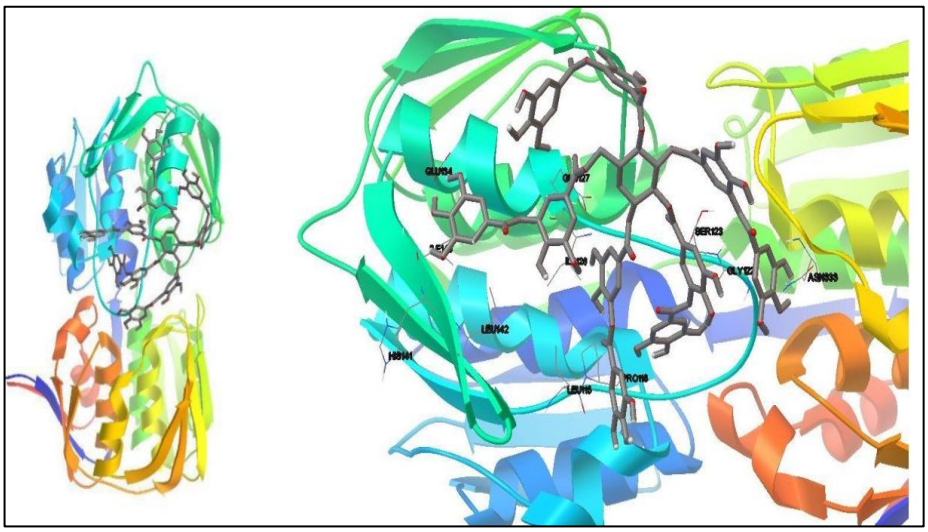

Figure 5. Binding Interactions of *murA* with Gallotannin (tannin)

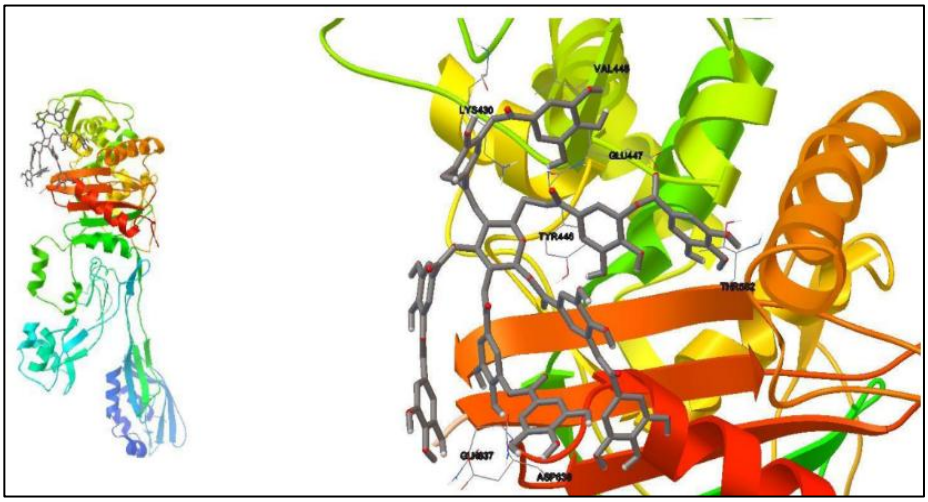

Figure 6. Binding Interactions of *mecA* (PBP2a) with Gallotannin (tannin)

4. DISCUSSION

Table 1 shows that the methanolic fraction of *Zanthoxylum acanthopodium* produced a concentration-dependent but non-linear effect on the expression of *murA* and *mecA* genes in MRSA ATCC 33591. The strongest suppression of both genes appeared at 50 mg/mL, where *murA* and *mecA* mRNA levels dropped to .84-fold and .15-fold of the untreated control, respectively. At 25 mg/mL, *mecA* expression was still greatly reduced (.34-fold), while *murA* expression was only slightly above the control (1.19-fold). In contrast, treatment with 100 mg/mL led to increased expression of both genes, and fosfomycin strongly induced *mecA* expression (194.01-fold) even though it only moderately increased *murA* expression. In line with these findings, the Kruskal-Wallis analysis (Table 2) found no statistically significant differences among treatment concentrations for either *murA* (p = .39) or mecA (p = .42). From a molecular standpoint, the biological response at 50 mg/mL is notable because simultaneous downregulation of *murA* and *mecA* could interfere with two key steps of peptidoglycan biosynthesis. The *murA* gene encodes UDP-N-acetylglucosamine enolpyruvyl transferase, which catalyzes the first committed cytoplasmic step in the synthesis of peptidoglycan precursors. Reduced *murA* expression is therefore expected to lower the production of UDP-N-acetylmuramic acid precursors, limiting the supply of building blocks needed for bacterial cell wall assembly. At the same time, suppression of *mecA* may decrease the production of penicillin-binding protein 2a (PBP2a), the low-affinity transpeptidase that maintains peptidoglycan cross-linking in the presence of β-lactam antibiotics. Simultaneous impairment of precursor synthesis and transpeptidation is likely to weaken cell wall integrity, which then restricts bacterial growth and survival. Recent studies have shown that *murA* is an attractive antibacterial target because inhibition of this enzyme disrupts the earliest stage of peptidoglycan biosynthesis, while suppression of *mecA* undermines the main resistance mechanism of MRSA against β-lactam antibiotics.[18-22]

Flavonoids and other phytochemicals are now seen as multitarget antibacterial agents that can affect bacterial gene expression and also influence membrane integrity, oxidative balance, and key metabolic enzymes, resulting in a combined inhibitory effect on bacterial proliferation.[23-25] Although there was a clear biological reduction in *murA* and *mecA* expression at intermediate concentrations, these differences were not statistically significant ($p > 0.05$). This gap is likely due to a mix of biological and methodological factors rather than an absence of antibacterial activity. Bacterial transcriptional responses to phytochemicals are highly dynamic and often display biphasic or hormetic dose-response patterns instead of simple linear concentration-dependent effects. At moderate concentrations, bioactive compounds may suppress transcription of genes involved in cell wall synthesis; at higher concentrations, however, severe cellular stress can activate compensatory regulatory pathways that temporarily boost the expression of genes linked to cell wall repair and antibiotic resistance. These adaptive responses have been widely reported in MRSA exposed to cell wall-active agents and represent an attempt to maintain cell envelope integrity under stress.[21,22] The notable induction of *mecA* after fosfomycin treatment also supports this view, suggesting activation of a compensatory cell wall stress response rather than increased resistance directly caused by fosfomycin, whose main molecular target remains *murA*. Plant extracts contain multiple phytochemicals with different molecular targets and pharmacodynamic properties. Synergistic or antagonistic interactions among flavonoids, terpenoids, tannins, and other secondary metabolites may lead to varied transcriptional responses at different concentrations, increasing biological variability and reducing statistical power.[23,24] In addition, limited biological replicates, inter-sample variability, RNA stability, differences in qRT-PCR amplification efficiency, and the transient nature of mRNA expression may also contribute to the lack of statistical significance despite biologically meaningful trends. Similar results have been reported in recent studies examining phytochemical-mediated modulation of MRSA virulence and resistance genes, where measurable changes in gene expression did not always correspond to statistically significant differences because bacterial adaptive regulatory networks can partially compensate for antimicrobial stress.[22,26] At this time, no published meta-analysis has specifically assessed the effects of *Zanthoxylum acanthopodium* on *murA* and *mecA* expression in MRSA. Still, recent systematic reviews consistently report that plant-derived phytochemicals show anti-MRSA activity through several molecular targets, including inhibition of peptidoglycan biosynthesis, disruption of bacterial membranes, reduction of resistance determinants, and interference with quorum sensing, although substantial heterogeneity in extraction methods, phytochemical composition, bacterial strains, and experimental protocols remains a major limitation for quantitative evidence synthesis.[23-25]

The molecular docking visualization in Figure 1 shows that diosmetin sits within a *murA*-binding pocket, which is surrounded by residues Asn23, Ile94, Arg95, Ala98, Pro125, His129, Phe164, Ser166, Val167, Gly168, and Glu171. The hydroxyl and carbonyl groups on the diosmetin flavone scaffold may serve as hydrogen-bond donors or acceptors, allowing for possible interactions with polar or charged residues such as Arg95, His129, Ser166, Glu171, and Asn23. Its aromatic rings, on the other hand, are likely stabilized by hydrophobic and van der Waals interactions with Ile94, Ala98, Pro125, Phe164, and Val167. Diosmetin's close position to Arg95 and the Phe164-Glu171 region could be functionally important, since these residues are involved in substrate recognition and help stabilize the *murA* catalytic pocket. Consequently, diosmetin binding may restrict conformational flexibility of the enzyme or interfere with the access and appropriate positioning of phosphoenolpyruvate and UDP-N-acetylglucosamine within the catalytic site. Unlike fosfomycin, which irreversibly inhibits *murA* by forming a covalent bond with the catalytic cysteine residue, diosmetin lacks a strongly electrophilic group capable of comparable covalent modification and is therefore more likely to act as a reversible competitive or non-covalent inhibitor that stabilizes a less catalytically active *murA* conformation.[12,13,20,27] Inhibition of *murA* would reduce the formation of UDP-N-acetylglucosamine-enolpyruvate, an essential early precursor of peptidoglycan biosynthesis, potentially resulting in impaired cell-wall formation, defective bacterial division, increased susceptibility to osmotic stress, growth inhibition, and ultimately MRSA cell death. This proposed mechanism matches evidence that flavonoids show antibacterial activity through several targets, including enzyme inhibition, membrane disruption, interference with peptidoglycan synthesis, and modulation of bacterial virulence.[23-25] As a comparison, catechin-related flavonoids from *Uncaria gambir* have also been computationally predicted to bind to the *murA* active pocket through hydrogen bonding and hydrophobic interactions, supporting the potential of hydroxyl-rich plant polyphenols as natural *murA* inhibitors.[28] Similarly, resveratrol and curcumin, polyphenolic compounds associated with grapes and *Curcuma longa*, respectively, have been reported to alter the expression of MRSA virulence-associated genes, indicating that phytochemicals may inhibit bacterial pathogenicity through both direct protein interactions and modulation of bacterial gene regulation.[26]

Figure 2 illustrates the predicted binding mode of diosmetin with penicillin-binding protein 2a (PBP2a), the protein encoded by the *mecA* gene, indicating that diosmetin occupies a binding cavity within the transpeptidase domain of PBP2a. The flavonoid scaffold features several hydroxyl and carbonyl groups that can serve as hydrogen-bond donors or acceptors, forming stabilizing interactions with nearby polar amino acid residues. Its planar aromatic rings also contribute to hydrophobic and van der Waals interactions, which help stabilize the protein-ligand complex. These non-covalent interactions are thought to reduce the conformational flexibility of PBP2a and may interfere with substrate accommodation or the dynamics of the catalytic domain. Unlike β-lactam antibiotics, which are sterically excluded from the active site because of the intrinsically low affinity of PBP2a, flavonoids may inhibit PBP2a through

reversible occupation of either the catalytic cavity or neighboring allosteric regions, thereby perturbing the conformational changes required for transpeptidase activity and peptidoglycan cross-linking.[15,21,29] PBP2a is the principal determinant of methicillin resistance in MRSA, impairment of its catalytic function would be expected to weaken cell-wall biosynthesis, reduce peptidoglycan cross-linking efficiency, increase susceptibility to osmotic stress, and potentially restore bacterial sensitivity to β-lactam antibiotics.[21,29] This mechanism aligns with previous studies showing that plant-derived flavonoids display anti-MRSA activity by targeting PBP2a through stable hydrogen-bonding and hydrophobic interactions.[24,30] Supporting evidence was provided by Alhadrami et al., who experimentally and computationally demonstrated that several naturally occurring flavonoids interact with PBP2a and exhibit significant anti-MRSA activity, suggesting that PBP2a is a promising molecular target for flavonoid-based antibacterial development.[30] Likewise, Masumi et al. performed large-scale virtual screening and molecular dynamics simulations of 46 natural flavonoids against PBP2a and identified rutin and kaempferol 3-rutinoside-7-sophoroside as highly stable binders at both the catalytic and allosteric sites, highlighting the ability of polyphenolic compounds to modulate PBP2a conformation and inhibit MRSA through non-covalent interactions.[31] Collectively, these findings support the hypothesis that diosmetin may function as a reversible PBP2a inhibitor capable of disrupting the resistance mechanism mediated by *mecA*.

Figure 3 shows that eucalyptol, also known as 1,8-cineole, is positioned inside a murA cavity surrounded by Glu192, Arg236, Leu300, Pro301, His302, Pro306, Thr307, Asp308, and Val330. Because eucalyptol is a compact bicyclic oxygenated monoterpene with only one ether oxygen and no hydroxyl group, its stabilization within this pocket is likely to depend mainly on hydrophobic and van der Waals contacts with Leu300, Pro301, Pro306, and Val330. Its ether oxygen might serve as a weak hydrogen-bond acceptor for polar or charged residues such as Arg236, His302, Thr307, Glu192, or Asp308. This interaction pattern differs from that of polyhydroxylated flavonoids, which usually form more extensive hydrogen-bond networks, and thus suggests that eucalyptol may act as a moderate and reversible *murA*-binding ligand rather than as a high-affinity covalent inhibitor. Nevertheless, occupancy of this cavity could sterically interfere with substrate accommodation or restrict local conformational movements required for the catalytic transfer of the enolpyruvyl group from phosphoenolpyruvate to UDP-N-acetylglucosamine. Because *murA* catalyzes the first committed cytoplasmic step of peptidoglycan biosynthesis, interference with its activity may reduce peptidoglycan-precursor formation, impair cell-wall assembly and bacterial division, increase osmotic vulnerability, and ultimately suppress MRSA growth.[12-14,20,27] The biological plausibility of targeting *murA* with plant terpenoids is supported by Funes Chabán et al., who demonstrated that diterpenes obtained from *Lepechinia meyenii* and their synthetic analogues inhibited *murA* enzymes from both *Escherichia coli* and *Staphylococcus aureus*, confirming that hydrophobic terpenoid scaffolds can interfere with this essential bacterial enzyme.32 However, eucalyptol probably acts through several antibacterial targets rather than *murA* inhibition alone. In an experimental study using MRSA ATCC 43300, Hartman et al. found that 1,8-cineole showed measurable antibacterial activity and participated in interactions with other essential-oil constituents, supporting its direct but relatively modest anti-MRSA potential.33 Recent mechanistic evidence in E. coli O157:H7 further showed that eucalyptol disrupted membrane integrity, altered cellular morphology, caused leakage of intracellular macromolecules, and affected the expression of genes associated with survival, adhesion, quorum sensing, and biofilm formation, indicating that membrane damage and transcriptional dysregulation may complement any predicted murA-directed effect.34 Therefore, the present docking result supports a hypothesis in which eucalyptol contributes to MRSA inhibition through partial, non-covalent interference with murA, together with broader membrane- and regulatory-level effects.

Figure 4 illustrates the predicted interaction of eucalyptol (1,8-cineole) with PBP2a, the protein product encoded by the mecA gene, within a cavity surrounded by Tyr446, Ser462, Thr582, His583, Ser598, Thr600, and Ala642. Because eucalyptol is a small oxygenated monoterpene containing only one ether oxygen, its binding is expected to be stabilized mostly by hydrophobic and van der Waals contacts, especially with Tyr446 and Ala642. Its ether oxygen might form a weak hydrogen bond or dipole interaction with nearby serine, threonine, or histidine residues. The closeness of eucalyptol to the His583-Thr600 region indicates that it may occupy a secondary cavity next to the PBP2a transpeptidase domain, rather than directly interacting with the catalytic Ser403 residue. As a result, its predicted mechanism aligns more with reversible, non-covalent modulation of local protein conformation than with direct catalytic inactivation. This type of binding could limit the flexibility of loops that control substrate access, disrupt peptidoglycan-substrate positioning, and partly interfere with the transpeptidase activity necessary for cell-wall cross-linking. Since PBP2a enables MRSA to maintain peptidoglycan synthesis in the presence of β-lactam antibiotics, its functional perturbation could weaken cell-wall integrity and potentially increase bacterial susceptibility to β-lactams.[21,29] This interpretation is consistent with evidence that natural compounds can interact with catalytic or allosteric regions of PBP2a and suppress its resistance-associated function.[30,31] Eucalyptol also shows membrane-disruptive and anti-biofilm effects.[33,34] Its anti-MRSA activity is likely multitarget and cannot be attributed solely to PBP2a binding. Molecular-dynamics simulation, purified PBP2a transpeptidase assays, peptidoglycan cross-linking analysis, and β-lactam combination testing are needed to confirm the functional significance of this docking pose.

Figure 5 shows that gallotannin occupies a relatively large region within the *murA* binding pocket, interacting with residues including Leu76, Tyr109, Gly121, Ser123, His141, Lys142, Glu194, Glu227, and Arg236. Owing to its highly polyhydroxylated structure and several galloyl groups, gallotannin has a strong ability to form many hydrogen bonds along with van der Waals, electrostatic,

and aromatic interactions inside the binding cavity. The simultaneous engagement of polar residues such as Glu194, Glu227, Lys142, Arg236, Ser123, and His141 suggests that gallotannin may form a stable protein-ligand complex capable of reducing the conformational flexibility of *murA* and partially hindering the accommodation of its natural substrates, phosphoenolpyruvate and UDP-N-acetylglucosamine. As *murA* catalyzes the first committed step in peptidoglycan biosynthesis, interfering with this catalytic process could impair cell-wall precursor formation, weaken peptidoglycan assembly, and ultimately inhibit the growth and survival of MRSA.[12-14,20,27] Although no previous study has specifically demonstrated that gallotannin binds directly to *murA*, the docking pattern observed in the present study is consistent with the well-established physicochemical behavior of hydrolysable tannins, whose multiple phenolic hydroxyl groups enable the formation of extensive hydrogen-bond networks with bacterial proteins and contribute to their broad-spectrum antibacterial activity.[24,25] This interpretation is further supported by recent reviews showing that tannins frequently exert antibacterial effects through interactions with essential bacterial proteins, membrane-associated proteins, extracellular enzymes, and metal ions, thereby disrupting multiple physiological pathways rather than acting through a single molecular target.[25,35] Consequently, the present findings suggest that gallotannin may act as a multitarget antibacterial compound, with murA being one plausible intracellular target predicted by molecular docking.

Figure 6 illustrates the predicted interaction of gallotannin with penicillin-binding protein 2a (PBP2a), the resistance protein encoded by the mecA gene, within a broad binding region involving residues Lys430, Glu447, Tyr446, Val448, Thr582, Gly587, and Asp638. The numerous phenolic hydroxyl and carbonyl groups distributed across the galloyl moieties serve as hydrogen-bond donors and acceptors, which allows for simultaneous polar interactions with Lys430, Glu447, Thr582, Gly587, and Asp638. Its aromatic rings, on the other hand, may provide hydrophobic, van der Waals, and π-mediated contacts with Tyr446 and Val448. This combination of interactions could help stabilize gallotannin inside the protein cavity and may limit the local conformational changes needed for substrate entry and transpeptidase activity. Because PBP2a preserves peptidoglycan cross-linking when other penicillin-binding proteins are inhibited by β-lactams, interference with its catalytic or adjacent regulatory region may weaken cell-wall formation and potentially increase MRSA susceptibility to β-lactam antibiotics.[21,29,31] However, the present docking result should not be interpreted as evidence that gallotannin has previously been proven to inhibit PBP2a; rather, it is consistent with the general protein-binding behavior of hydrolysable tannins, whose multiple phenolic groups enable extensive hydrogen bonding with bacterial proteins and contribute to membrane disruption, enzyme inhibition, and other multitarget antibacterial effects.[25,35] As a botanical comparison, polyphenolic constituents of sappan wood (*Caesalpinia sappan*), including brazilin and protosappanins, have been evaluated by molecular docking against PBP2a and were predicted to interact with its binding region, supporting the broader feasibility of plant-derived polyphenols as non-covalent PBP2a modulators.[36]

5. CONCLUSION

The methanolic fraction of *Zanthoxylum acanthopodium* demonstrated promising anti-MRSA activity by targeting two essential components of bacterial cell-wall biosynthesis. The greatest biological suppression of *murA* and *mecA* expression was observed at 50 mg/mL, while molecular docking predicted that diosmetin, supported by eucalyptol and gallotannin, forms favorable interactions with *murA* and PBP2a, suggesting a multitarget mechanism involving inhibition of peptidoglycan precursor synthesis and interference with the methicillin-resistance determinant. Although the transcriptional changes were not statistically significant, the consistent biological trend together with the in silico findings indicates that Andaliman (*Z. acanthopodium)* phytochemicals possess meaningful antibacterial potential that warrants further mechanistic and functional validation.

These findings have important implications for clinical dentistry because MRSA is increasingly implicated in persistent endodontic infections, peri-implantitis, odontogenic abscesses, and biofilm-associated oral infections that frequently respond poorly to conventional antibiotics. Andaliman-derived phytochemicals therefore represent promising candidates for the development of adjunctive antimicrobial agents that could enhance the management of antibiotic-resistant oral infections while reducing reliance on conventional antibiotics. Although immediate clinical application cannot yet be recommended, this study provides a strong preclinical rationale for advancing Andaliman into translational research, including molecular dynamics simulations, enzyme inhibition studies, anti-biofilm evaluation, pharmacological safety assessment, and clinical trials, to establish its future role as a novel phytotherapeutic strategy in evidence-based dental practice.

REFERENCES

1. Lai Y, Ye L, Wu C. Antimicrobial resistance patterns and microbial epidemiology of oral and maxillofacial space infections: a systematic review and meta-analysis. Microb Drug Resist. 2026;32(7):233-246. doi:10.1177/10766294261442220.
2. Donkor ES, Kotey FCN. Methicillin-resistant Staphylococcus aureus in the oral cavity: implications for antibiotic prophylaxis and surveillance. Infect Dis (Auckl). 2020;13:1178633720976581. doi:10.1177/1178633720976581.
3. Al-Hammadi S, Al-Shehari WA, Edrees WH, Al-Kaf AG, Pan S, et al. Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) among patients with oral infections in Sana'a City-Yemen. BMC Oral Health. 2025;25:1047. doi:10.1186/s12903-025-06247-0.
4. Kawayanagi T, Kawada-Matsuo M, Kusaka S, Yasutomi Y, Suzuki Y, Nishihama S, et al. Clinical

and genetic analysis of oral and nasal Staphylococcus aureus isolates in dental patients. Sci Rep. 2025;15:13149. doi:10.1038/s41598-025-93773-0.
5. Kwapisz E, Garbacz K, Kosecka-Strojek M, Schubert J, Bania J, Międzobrodzki J. Presence of egc-positive major clones ST45, ST30 and ST22 among methicillin-resistant and methicillin-susceptible oral Staphylococcus aureus strains. Sci Rep. 2020;10:18889. doi:10.1038/s41598-020-76009-1.
6. Khairalla AS, Wasfi R, Ashour HM. Carriage frequency, phenotypic, and genotypic characteristics of methicillin-resistant Staphylococcus aureus isolated from dental health-care personnel, patients, and environment. Sci Rep. 2017;7:7390.
7. Volgenant CMC, Hoogenkamp MA, Dahlén G, Kalfas S, Petti S, De Soet JJ. Low prevalence of multi-resistant bacteria in undergraduate dental students: an observational case-control multi-centre study in Europe. J Oral Microbiol. 2021;13(1):1889898. doi:10.1080/20002297.2021.1889898.
8. Adrian, Syahputra RA, Juwita NA, et al. Andaliman (Zanthoxylum acanthopodium DC.), a herbal medicine from North Sumatera, Indonesia: phytochemical and pharmacological review. Heliyon. 2023;9(5):e16159. doi:10.1016/j.heliyon.2023.e16159.
9. Septama AW, Rahmi EP, Tasfiyati AN, Khairunnisa NA, Nasution HR, Nirmal N, et al. Chemical profiles and antibacterial actions of Zanthoxylum acanthopodium DC. essential oil growing in Indonesia. Fitoterapia. 2025;180:106300. doi:10.1016/j.fitote.2024.106300.
10. Hutapea DB, Susilawati Y, Muhaimin M, et al. The potential of Zanthoxylum acanthopodium DC. as immunomodulators: formulation, activity testing, and extract profiling. Pharmaceuticals (Basel). 2025;18(7):1001. doi:10.3390/ph18071001.
11. Rizqoh D, Amelia A, Kumala WO, et al. Novel endophytic bacteria isolates from Zanthoxylum acanthopodium DC. which potentially inhibit Escherichia coli and Staphylococcus aureus. J Biomed Transl Res. 2024;10(1):26–34. doi:10.14710/jbtr.v10i1.21740.
12. Liu Q, Luo A, Jin H, Si X, Li M. Machine learning-based discovery of a novel noncovalent murA inhibitor as an antibacterial agent. Chem Biol Drug Des. 2025;105(3):e70084. doi:10.1111/cbdd.70084.
13. Boulhissa I, Chikhi A, Bensegueni A, Ghattas MA, Mokrani EH, Alrawashdeh S, et al. Investigation of new inhibitors of UDP-N-acetylglucosamine enolpyruvyl transferase (murA) by virtual screening with antibacterial assessment. Curr Comput Aided Drug Des. 2021;17(2):214–224.
14. Garbinski LD, Rosen BP, Yoshinaga M. Organoarsenicals inhibit bacterial peptidoglycan biosynthesis by targeting the essential enzyme murA. Chemosphere. 2020;254:126911. doi:10.1016/j.chemosphere.2020.126911.
15. Mohammed MM, Isa MA, Abubakar MB, Dikwa ASB, Kappo AP. Molecular detection of mecA gene from methicillin-resistant Staphylococcus aureus isolated from clinical and environmental samples and its potential inhibition by phytochemicals using in vitro and in silico approaches. Silico Pharmacol. 2025;13(1):1–25. doi:10.1186/s40580-025-00429-3.
16. Ahmed MY, Ali HA, Gorish BMT, Ali SO, Abdalrhim SA, Mergani MH, et al. Molecular detection of staphylococcal enterotoxins and mecA gene products in selected food samples collected from different areas in Khartoum State. Int J Microbiol. 2021;2021:6652328. doi:10.1155/2021/6652328.
17. Alewi AAM, Al-Ruaby KJW. Prevalence of SCCmec-encoded resistance genes in oral staphylococcal isolates. J Wasit Sci Med. 2025;18(4):24–31.
18. Liu Q, Luo A, Jin H, Si X, Li M. Machine learning-based discovery of a novel noncovalent MurA inhibitor as an antibacterial agent. Chem Biol Drug Des. 2025;105(3):e70084.
19. Boulhissa I, Chikhi A, Bensegueni A, Ghattas MA, Mokrani EH, Alrawashdeh S, et al. Investigation of new inhibitors of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) by virtual screening with antibacterial assessment. Curr Comput Aided Drug Des. 2021;17(2):214–224.
20. Raina D, Kumar C, Kumar V. Potential inhibitors targeting Escherichia coli UDP-N-acetylglucosamine enolpyruvyl transferase (MurA): an overview. Indian J Microbiol. 2022;62(1):11–22.
21. Nusrat S, Aliyu M, Zohora FT. Mechanisms of antimicrobial resistance: From genetic evolution to clinical manifestations. AIMS Microbiol. 2025;11(4):1007–1034.
22. Mohammed MM, Isa MA, Abubakar MB, Dikwa ASB, Kappo AP. Molecular detection of mecA gene from methicillin-resistant Staphylococcus aureus isolated from clinical and environmental samples and its potential inhibition by phytochemicals using in vitro and in silico approaches. In Silico Pharmacol. 2025;13:1–25.
23. Liang M, Ge X, Xu H, Ma K, Zhang W, Zan Y, et al. Phytochemicals with activity against methicillin-resistant Staphylococcus aureus. Phytomedicine. 2022;100:154073.
24. Shamsudin NF, Ahmed QU, Mahmood S, Shah SAA, Khatib A, Mukhtar S, et al. Antibacterial effects of flavonoids and their structure–activity relationship study: A comparative interpretation. Molecules. 2022;27:1149.
25. Jubair N, Rajagopal M, Chinnappan S, Abdullah NB, Fatima A. Review on the antibacterial mechanism of plant-derived compounds against multidrug-resistant bacteria. Evid Based Complement Alternat Med. 2021;2021:3663315.
26. El-Mahdy AM, Alqahtani M, Almukainzi M, Alghoribi MF, Abdel-Rhman SH. Effect of resveratrol and curcumin on gene expression of methicillin-resistant Staphylococcus aureus toxins. J Microbiol Biotechnol. 2024;34(1):141–148.
27. de Oliveira MVD, Furtado RM, da Costa KS, Vakal S, Lima AH. Advances in UDP-N-

acetylglucosamine enolpyruvyl transferase (MurA) covalent inhibition. Front Mol Biosci. 2022;9:889825. doi:10.3389/fmolb.2022.889825.
28. Kurniawan I, Rohmatika AU. Molecular docking, QSAR, and bioactivity prediction of Uncaria gambir flavonoids as antibacterial agents targeting MurA enzyme. Biointerface Res Appl Chem. 2026;16(1):32. doi:10.33263/BRIAC161.032.
29. Wong MTY, Chiang YW, Mabanglo MF, Smyth MS. Molecular dynamics simulations of antibiotic ceftaroline at the allosteric site of penicillin-binding protein 2a (PBP2a). Isr J Chem. 2020;60(9–10):848–857. doi:10.1002/ijch.202000012.
30. Alhadrami HA, Hamed AA, Hassan HM, Belbahri L, Rateb ME, Sayed AM. Flavonoids as potential anti-MRSA agents through modulation of PBP2a: a computational and experimental study. Antibiotics (Basel). 2020;9(9):562. doi:10.3390/antibiotics9090562.
31. Masumi M, Noormohammadi F, Kianisaba F, et al. Methicillin-resistant Staphylococcus aureus: docking-based virtual screening and molecular dynamics simulations to identify potential penicillin-binding protein 2a inhibitors from natural flavonoids. Int J Microbiol. 2022;2022:9130700. doi:10.1155/2022/9130700.
32. Funes Chabán M, Hrast M, Frlan R, Graikioti DG, Athanassopoulos CM, Carpinella MC. Inhibition of MurA enzyme from Escherichia coli and Staphylococcus aureus by diterpenes from Lepechinia meyenii and their synthetic analogs. Antibiotics (Basel). 2021;10(12):1535. doi:10.3390/antibiotics10121535.
33. Hartman K, Świerczyńska M, Wieczorek A, Baszuk P, Wojciechowska-Koszko I, Garbacz K, et al. Preliminary evaluation of the synergistic antibacterial effects of selected commercial essential oil compounds against methicillin-resistant Staphylococcus aureus ATCC 43300. Antibiotics (Basel). 2025;14(7):733. doi:10.3390/antibiotics14070733.
34. He Y, Wang Y, He Y, Liu Z, et al. Unraveling the antibacterial mechanism of eucalyptol against Escherichia coli O157:H7 through transcriptomics and its application in beef broth. Int J Food Microbiol. 2026;448:111580. doi:10.1016/j.ijfoodmicro.2025.111580
35. Farha AK, Yang QQ, Kim G, Li HB, Zhu F, Liu HY, et al. Tannins as an alternative to antibiotics. Food Biosci. 2022;46:101615. doi:10.1016/j.fbio.2021.101615.
36. Gondokesumo ME, Kurniawan IM. Molecular docking study of sappan wood extract to inhibit PBP2A enzyme on methicillin-resistant Staphylococcus aureus (MRSA). J Basic Clin Physiol Pharmacol. 2020;30(6). doi:10.1515/jbcpp-2019-0282.