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Short Communication

Synergistic Effects of Lupinifolin-Based Flavonoid Combinations on Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus, including methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains, is a major cause of community- and hospital-acquired infections. The rapid emergence of multidrug-resistant *S. aureus* has intensified the need for novel antibacterial agents and synergistic combinations. Lupinifolin, a prenylated flavanone, has shown promising activity against MSSA and MRSA, yet its interactions with other flavonoids remain underexplored. This study investigated the synergistic antibacterial effects of dual and triple combinations of lupinifolin with epigallocatechin gallate (EGCG) and/or baicalein against MSSA and MRSA. Two-dimensional and three-dimensional checkerboard assays were conducted to assess double- and triple-flavonoid combinations, respectively. The MICs of lupinifolin against MSSA and MRSA were 32 and 64 µg/mL, respectively, while EGCG and baicalein had MICs of >512 and >256 µg/mL against both bacterial strains. The lupinifolin–EGCG double combination demonstrated synergistic activity against MRSA, with a fractional inhibitory concentration index (FICI) of < 0.3125, and showed a close-to-the-synergy threshold against MSSA (FICI < 0.5625). Triple combinations of lupinifolin, EGCG, and baicalein produced synergistic effects against both MSSA and MRSA, with FICI values of < 0.5234 and < 0.3164, respectively. These results highlight the potential of flavonoid-based combinations as adjuncts to existing antibiotics or as leads for developing new phytochemical-derived therapeutics. Further studies are needed to validate their *in vivo* efficacy and elucidate the molecular mechanisms driving the observed synergism.

Keywords: *Staphylococcus aureus*, Lupinifolin, Epigallocatechin gallate, Baicalein, Synergism

Introduction

Staphylococcus aureus is a Gram-positive coccus and a major bacterial pathogen of global public health concern. It is responsible for a wide range of community- and hospital-acquired infections, including skin and soft tissue infections, endocarditis, osteomyelitis, and bacteremia.[1] Based on susceptibility to β-lactam antibiotics, *S. aureus* is classified as either methicillin-sensitive *S. aureus* (MSSA) or methicillin-resistant *S. aureus* (MRSA). The emergence and dissemination of antibiotic resistance in *S. aureus*, driven largely by antibiotic misuse and overuse, have significantly reduced therapeutic options and are associated with increased morbidity and mortality, particularly in hospitalized and immunocompromised patients.[2] Resistance in MSSA and MRSA arises through multiple mechanisms, including β-lactamase production, expression of penicillin-binding protein 2a (PBP2a), reduced drug uptake, active efflux, and protection or modification of antibacterial targets.[3-5] Consequently, treatment often relies on newer, more expensive agents with alternative mechanisms of action, underscoring the need for novel antibacterial strategies.

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Natural products, particularly plant-derived flavonoids, have attracted increasing interest as potential antibacterial agents and resistance-modifying adjuvants. Flavonoids from several subclasses—including flavanols, flavones, and flavanones—exhibit activity against both MSSA and MRSA through diverse mechanisms, such as disruption of bacterial membranes, inhibition of essential enzymes, induction of oxidative stress, and interference with DNA replication and transcription.[6] In addition to direct antibacterial effects, many flavonoids attenuate virulence by inhibiting biofilm formation, surface adhesion, quorum sensing, and toxin production, thereby enhancing bacterial susceptibility to antimicrobial agents.[6,7] Highly lipophilic flavonoids often exert their activity by integrating into bacterial membranes, increasing permeability and impairing membrane-associated functions.[8-10]

Lupinifolin, a prenylated flavanone predominantly isolated from *Derris reticulata* Craib, exemplifies this membrane-targeting mechanism and exhibits potent antibacterial activity against both MSSA and MRSA.[11-13] Beyond its intrinsic activity, lupinifolin has demonstrated synergistic effects when combined with conventional antibacterials, including β-lactam antibiotics, tetracycline, and streptomycin.[13-15] At sub-inhibitory concentrations, lupinifolin also significantly inhibits biofilm formation in both MSSA and MRSA and enhances the antibiofilm efficacy of protein synthesis inhibitors and β-lactam antibiotics, highlighting its potential as an antibacterial and antibiofilm adjuvant.[13,15-17]

Synergistic combinations based on natural products represent an attractive therapeutic approach, as they may enhance antibacterial efficacy, reduce required dosages, and mitigate the development of resistance. While numerous studies have examined flavonoid–antibiotic combinations, investigations into interactions among multiple flavonoids remain limited.[18,19] To date, no studies have evaluated synergistic interactions between lupinifolin and flavonoids from other major subclasses. Epigallocatechin gallate (EGCG), a flavan-3-ol abundant in *Camellia sinensis*, and baicalein, a flavone derived from *Scutellaria baicalensis*, exhibit antibacterial activity against *S. aureus* through mechanisms that differ from lupinifolin. EGCG disrupts cell

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wall integrity through direct interaction with peptidoglycan and interferes with intracellular metabolic and transcriptional processes, while baicalein has been reported to inhibit protein synthesis and alter membrane permeability.[20-22] Both compounds have also demonstrated synergistic activity when combined with selected antibiotics against *S. aureus*.[22-24]

Given the mechanistic diversity of these flavonoids, combining compounds that target distinct or complementary bacterial structures may enhance antibacterial activity, particularly against resistant strains such as MRSA. We hypothesized that lupinifolin would exhibit enhanced antibacterial interactions when combined with EGCG and/or baicalein, with distinct interaction patterns observed among individual, dual, and triple flavonoid combinations. Accordingly, this study aimed to evaluate the synergistic antibacterial effects of dual (lupinifolin + EGCG or baicalein) and triple (lupinifolin + EGCG + baicalein) flavonoid combinations against MSSA and MRSA. Antibacterial activity was assessed by minimum inhibitory concentration (MIC) determination, while synergistic interactions were evaluated using two-dimensional and three-dimensional checkerboard assays. By characterizing these interaction patterns, this work provides new insights into flavonoid-based synergy and explores the potential of multi-flavonoid combinations as alternative or adjunct strategies for managing antibiotic-resistant *S. aureus* infections.

Materials and Methods

Isolation of lupinifolin from D. reticulata Craib. stems

Lupinifolin, extracted from *Derris reticulata* Craib. stems, was obtained from our previous investigation.[13] In the prior investigation, thin-layer chromatography (TLC) was performed primarily to authenticate lupinifolin, followed by nuclear magnetic resonance (1H-NMR and 13C-NMR) and mass spectrometry. The process of lupinifolin isolation and authentication was described in full in our previous study.[25] Before being used in the experiment, the pure lupinifolin was stored at -20°C.

Determination of the MIC

The Clinical and Laboratory Standards Institute (CLSI) guidelines were followed while determining the minimum inhibitory concentration (MIC) using a modified microbroth dilution technique.[26] Stock solutions of lupinifolin, EGCG (Sigma-Aldrich, E4143), and baicalein (Sigma-Aldrich, 465119) were made in two-fold serial dilutions using the proper vehicle (0.1 M NaOH for lupinifolin, sterile deionized water for EGCG, and 50% DMSO for baicalein). MSSA (DMST 8013) and MRSA (DMST 20645) were acquired from the Department of Medical Sciences, Ministry of Public Health, Thailand. MSSA and MRSA suspensions at a concentration of $1.5x10^6$ CFU/mL were made in Tryptic Soy Broth (TSB; HiMedia Laboratories, Mumbai, India). Each well of a 96-well microplate was filled with 20 µL of the test agent or its vehicle, 50 µL of bacterial suspension, and 130 µL of TSB. The MIC was the lowest concentration of the test agent that prevented any discernible bacterial growth after 24 hours of incubation at 37°C. Vehicle controls (0.1 M NaOH and DMSO) at the highest concentrations used in the assays were tested in parallel and showed no inhibitory effects. MIC determinations were performed in at least three independent experiments, and results are reported as median values.

Two-dimensional checkerboard assay for double flavonoid combinations

The antibacterial activity of lupinifolin in combination with EGCG or baicalein was assessed using the checkerboard assay, as described by Orhan *et al.*[27] In short, 50 µL of bacterial solution ($1.5x10^6$ CFU/mL) was combined with 130 µL of TSB containing various concentrations of lupinifolin (10 µL) and EGCG or baicalein (10 µL). While the concentrations of EGCG or baicalein used for testing were twofold serially diluted along the ordinate, the lupinifolin concentrations were serially diluted similarly along the abscissa. After a 24-hour incubation period at 37 °C, the minimum inhibitory concentrations (MICs) for each combination of lupinifolin and EGCG or baicalein were determined. The fractional inhibitory concentration index (FICI) for double flavonoid combinations was computed as $\Sigma FIC = FIC_A + FIC_B$, where A was lupinifolin and B was either EGCG or baicalein. The FIC of each flavonoid was obtained by dividing the MIC of the flavonoid in combination by the MIC of the flavonoid alone. A combination is classified as "antagonism," "no interaction," or "synergy" if its FIC index is >4.0, >0.5-4.0, or ≤0.5, respectively.[28] The MIC of flavonoid alone was divided by the MIC of flavonoid in combination to determine the dose reduction index (DRI). The results of at least three separate experiments were represented using the median.

Three-dimensional checkerboard assay for triple flavonoid combinations

The triple combination of lupinifolin, EGCG, and baicalein was tested against MSSA and MRSA using a modified three-dimensional checkerboard method, as described by Samaddar et al. (2024).[29] Two-fold serial dilutions of lupinifolin and EGCG were prepared as described for the dual assay, while baicalein was added at fixed concentrations of 0.5, 1, or 2 µg/mL. These concentrations were selected based on the MIC of baicalein observed in dual flavonoid combinations and to assess its modulatory effect without exerting dominant antibacterial activity. Each well of the microtiter plates containing 30 µL of the triple flavonoid combination was inoculated with 50 µL of the bacterial inoculum suspension and 120 µL of TSB. The MICs for each combination of flavonoids were established following a 24-hour incubation period at 37 °C. The FICI for triple combinations was expressed as $\Sigma FIC = FIC_A + FIC_B + FIC_C$, where A, B, and C represented lupinifolin, EGCG, and baicalein, respectively. The FIC of a single flavonoid was determined by dividing its MIC in the combination by its MIC on its own. According to Samaddar *et al.* (2024), a triple combination is classified as "synergism," "additive," "indifference", or "antagonism" if its FICI is ≤0.75, >0.75–3, >3–4, or >4, respectively.[29] By dividing the MIC of flavonoid alone by the MIC of flavonoid in combination, the dose reduction index (DRI) was computed. The results from at least three separate studies were represented by the median.

Statistical analysis

IBM SPSS Statistics Version 29 (IBM, USA) was used to evaluate the MIC and FICI data, and the results were reported as a median. This approach is consistent with CLSI recommendations and standard practice in antimicrobial susceptibility and checkerboard synergy studies.

Results and Discussion

The antibacterial activities of lupinifolin, EGCG, and baicalein—alone and in combination—were evaluated against MSSA (DMST 8013) and MRSA (DMST 20645) to characterize interaction patterns among flavonoids from different structural subclasses. Among the tested flavonoids, lupinifolin exhibited the strongest intrinsic antibacterial activity against both MSSA and MRSA, with greater potency against MSSA (Tables 1 and 2). In contrast, EGCG and baicalein showed weak intrinsic activity, with MIC values exceeding 512 µg/mL and 256 µg/mL, respectively. These findings are consistent with previous reports indicating that EGCG and baicalein generally display limited standalone antibacterial effects against *S. aureus*, with considerable strain-to-strain variability.[22,23,30-33] Collectively, the data confirm lupinifolin as the most active compound in this study and justify its selection as the core agent for combination experiments.

Dual-combination checkerboard assays were conducted with lupinifolin as the central compound. The combination of lupinifolin and EGCG demonstrated synergistic antibacterial activity against both MRSA and MSSA. Against MRSA, the interaction yielded an FICI value of <0.3125, accompanied by fourfold and eightfold reductions in the MICs of lupinifolin and EGCG, respectively. In MSSA, a synergistic trend was observed (FICI <0.5625), with twofold and 16-fold MIC reductions, respectively. These findings extend previous observations that lupinifolin can synergize with antibiotics targeting protein synthesis or cell wall biosynthesis, suggesting that lupinifolin is particularly amenable to combination strategies.[13-15,17]

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Table 1: MIC, DRI, FIC and FIC index of lupinifolin, EGCG and baicalein against MRSA

Test agent	MIC alone (μg/mL)	MIC in combination (μg/mL)	DRI	FIC	FIC index	N
Lupinifolin	64	16	4	0.25	<0.3125	3
EGCG	>512	32	8	0.0625		
Lupinifolin	64	64	1	1	<1.0078	3
Baicalein	>256	2	128	0.0078		
Lupinifolin	64	16	4	0.25	<0.3164	3
EGCG	>512	32	16	0.0625		
Baicalein	>256	1	128	0.0078		

Data is expressed as median from independent experiments.
FIC = Fractional Inhibitory Concentration
DRI = Dose Reduction Index
Note: FIC = MIC of drug in combination/ MIC of drug alone; DRI = MIC of drug alone/ MIC of drug in combination.

Although mechanistic investigations were beyond the scope of this study, the observed synergy may arise from complementary antibacterial actions. Lupinifolin primarily disrupts bacterial membrane function, whereas EGCG is known to interact with cell wall components and affect multiple intracellular processes.[11,12,20,23] Such complementary targeting could plausibly enhance antibacterial efficacy, although this interpretation remains hypothetical and warrants experimental validation. In contrast, the combination of lupinifolin and baicalein produced indifferent interactions against both MSSA and MRSA, with FICI values ≥1.0. Notably, co-administration with lupinifolin markedly reduced the MIC of baicalein (up to 128-fold), whereas the MIC of lupinifolin remained unchanged. This asymmetric effect suggests that while lupinifolin may facilitate baicalein activity, baicalein does not reciprocally enhance lupinifolin's antibacterial potency. This lack of synergy may reflect overlapping antibacterial mechanisms—particularly membrane-associated effects—which could limit further potentiation of lupinifolin. Since lupinifolin primarily acts against bacteria at the bacterial membrane,[11,12] it is possible that when lupinifolin is used alone, its binding sites at the membrane are already saturated, leaving little space for baicalein to increase its activity. Moreover, both compounds may rely on lipophilicity to interact with and disrupt bacterial membranes. Their shared physicochemical behavior may result in overlapping mechanisms of membrane interference, thereby limiting the potential for potentiation of lupinifolin by baicalein. Structure–activity relationship (SAR) data further support this interpretation. Hydroxyl substitution at the C-5 position of the A-ring is critical for anti-MRSA activity in both flavanones and flavones,[34,35] and this functional group is present in both lupinifolin and baicalein. However, lupinifolin possesses additional structural features that more strongly favor antibacterial activity. As a prenylated flavanone, lupinifolin contains (1) a saturated C2–C3 bond, a characteristic associated with stronger antibacterial activity in flavanones compared with flavones; (2) hydroxyl groups at C-5 and C-4′; and (3) a prenyl group at C-8—features known to enhance membrane interaction and lipophilicity.[35] Prenylated flavonoids are notably more lipophilic than other flavonoid classes, enabling efficient membrane insertion and disruption. Taken together, these SAR characteristics indicate that lupinifolin is structurally more optimized for membrane-targeting antibacterial activity than baicalein, which may explain why co-administration of baicalein does not enhance the antibacterial potency of lupinifolin.

Table 2: MIC, DRI, FIC and FIC index of lupinifolin, EGCG and baicalein against MSSA

Test agent	MIC alone (μg/mL)	MIC in combination (μg/mL)	DRI	FIC	FIC index	N
Lupinifolin	32	16	2	0.5	<0.5625	3
EGCG	>512	32	16	0.0625		
Lupinifolin	32	32	1	1	<1.0073	3
Baicalein	>256	2	128	0.0073		
Lupinifolin	32	16	2	0.5	<0.5234	3
EGCG	>512	8	64	0.0156		
Baicalein	>256	2	128	0.0078		

Data is expressed as median from independent experiments.
FIC = Fractional Inhibitory Concentration
DRI = Dose Reduction Index
Note: FIC = MIC of drug in combination/ MIC of drug alone; DRI = MIC of drug alone/ MIC of drug in combination

The triple combination of lupinifolin, EGCG, and baicalein produced consistent synergistic antibacterial activity against both MSSA and MRSA. FICI values were <0.3164 for MRSA and <0.5234 for MSSA, satisfying the accepted synergy threshold for three-agent combinations (FICI ≤0.75).[29] In MSSA, the triple combination reduced the MICs of lupinifolin, EGCG, and baicalein by twofold, 64-fold, and 128-fold, respectively. In MRSA, MIC reductions of fourfold, 16-fold, and 128-fold were observed, respectively. A notable finding was the markedly greater enhancement of EGCG activity in the triple combination compared with the lupinifolin–EGCG dual combination. This pattern suggests that baicalein contributes indirectly to the overall synergistic effect, even though it did not enhance lupinifolin activity in dual assays. While speculative, this may reflect cooperative effects among flavonoids acting on distinct but converging bacterial targets. Importantly, such mechanistic interpretations are presented as hypotheses, as no direct assays of membrane integrity, intracellular accumulation, or gene expression were performed.

Synergistic effects were generally more pronounced in MRSA than in MSSA, as reflected by lower FICI values. Lupinifolin alone exhibited stronger activity against MSSA, which may have limited the extent of further enhancement achievable through combination. In contrast, MRSA—characterized by altered membrane composition and a thicker cell wall—may be more susceptible to combination strategies that concurrently disrupt multiple bacterial structures.[36-39] The inclusion of EGCG, which is known to interact with peptidoglycan[23], may therefore be particularly advantageous in overcoming MRSA-associated barriers.

Several FICI values are reported as "<" rather than exact numerical values because the MICs of certain compounds exceeded the highest concentrations tested in the checkerboard assays. In such cases, FICI values represent upper-bound estimates based on assay limits, in accordance with established antimicrobial combination testing practices. This study is limited by its reliance on checkerboard assays to assess antibacterial interactions. Time–kill studies were not performed to confirm bactericidal synergy. In addition, mechanistic endpoints such as membrane integrity, intracellular accumulation, gene regulation, or biofilm inhibition by flavonoid combinations were not evaluated. The antibacterial efficacy of these combinations was also not assessed *in vivo*. These limitations highlight important directions for future research.

In conclusion, this study demonstrates that lupinifolin can synergize with EGCG in dual combinations and with EGCG and baicalein in triple combinations against both MSSA and MRSA. These findings provide new evidence that rationally designed flavonoid–flavonoid combinations can enhance antibacterial efficacy, particularly against resistant *S. aureus*. Further mechanistic and *in vivo* studies are required to validate these interactions and explore their translational potential.

Conclusion

This study provides the first *in vitro* evidence that lupinifolin-based flavonoid combinations exhibit synergistic antibacterial activity against both methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. Using MIC determination and two- and three-dimensional checkerboard assays, we demonstrated that the dual combination of lupinifolin and EGCG and the triple combination of lupinifolin, EGCG, and baicalein enhanced antibacterial efficacy relative to individual flavonoids, with more pronounced synergism observed in MRSA than in MSSA. Although EGCG and baicalein displayed weak intrinsic antibacterial activity, their co-administration with lupinifolin substantially reduced effective concentrations, particularly within the triple-flavonoid combination. These findings suggest that combining flavonoids with complementary physicochemical and antibacterial properties may enhance activity against *S. aureus* in an *in vitro* setting. Importantly, the present results are limited to *in vitro* assays and do not directly establish therapeutic efficacy. Nevertheless, this work highlights flavonoid–flavonoid synergy as a promising exploratory strategy for identifying antibacterial adjuvants or lead compounds for further development. Future studies should focus on elucidating the molecular mechanisms underlying the observed interactions, evaluating bactericidal effects using time–kill kinetics, assessing antibiofilm activity of flavonoid combinations, and validating efficacy and safety in relevant *in vivo* infection models.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 2015;28(3):603–661.
2. Kong EF, Johnson JK, Jabra-Rizk MA. Community-associated methicillin-resistant *Staphylococcus aureus*: an enemy amidst us. PLoS Pathog 2016;12(10):e1005837. Doi: 10.1371/journal.ppat.1005837.
3. Guo Y, Song G, Sun M, Wang J, Wang Y. Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. Front Cell Infect Microbiol 2020;10:107. Doi: 10.3389/fcimb.2020.00107.
4. Mlynarczyk-Bonikowska B, Kowalewski C, Krolak-Ulinska A, Marusza W. Molecular mechanisms of drug resistance in *Staphylococcus aureus*. Int J Mol Sci 2022;23(15):8088. Doi: 10.3390/ijms23158088.
5. Pantosti A, Sanchini A, Monaco M. Mechanisms of antibiotic resistance in *Staphylococcus aureus*. Future Microbiol 2007;2(3):323-334.
6. Cushnie TPT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents 2011;38(2):99-107.
7. Borges A, Saavedra M, Simoes M. Insights on antimicrobial resistance, biofilms and the use of phytochemicals as new antimicrobial agents. Curr Med Chem 2015;22(21):2590-2614.
8. Coppo E, Marchese A. Antibacterial activity of polyphenols. Antibiotics (Basel) 2021;11(1):46. Doi: 10.3390/antibiotics11010046.
9. Daglia M. Polyphenols as antimicrobial agents. Opin Biotechnol 2012;23(2):174-181.
10. Radulovic NS, Blagojevic PD, Stojanovic-Radic ZZ, Stojanovic NM. Antimicrobial plant metabolites: structural diversity and mechanism of action. Curr Med Chem 2013;20(7):932-952.
11. Yusook K, Panvongsa P. Antibacterial activity of lupinifolin from *Derris reticulata* and its effect on cytoplasmic membrane of methicillin resistant *Staphylococcus aureus*. Walailak J Sci Technol 2020;17(10):1104–1112.
12. Yusook K, Weeranantanapan O, Hua Y, Kumkrai P, Chudapongse N. Lupinifolin from *Derris reticulata* possesses bactericidal activity on *Staphylococcus aureus* by disrupting bacterial cell membrane. J Nat Med 2017;71(2):357–366.
13. Kwaengmuang P, Chaiyawong K, Warong T, Rattanakiat S, Pulbutr P. Antibiofilm and antibacterial activities of lupinifolin in combination with protein synthesis inhibitors against methicillin-resistant *Staphylococcus aureus*. J Herbmed Pharmacol 2023;12(4):549–559.
14. Rattanakiat S, Kaewchang K, Thongsang S, Jaruchotikamol A, Pulbutr P. Synergistic activity of lupinifolin in combinations with antibiotics against *Staphylococcus aureus*. Pak J Biol Sci 2021;24(6):656–662.
15. Khwankaew S, Promthong C, Joycharat N, Yingkajorn M, Sianglum W. Synergistic antibacterial and antibiofilm properties of lupinifolin in combination with antibiotics against pathogenic bacteria. Arch Microbiol 2026;208(1):34. https://link.springer.com/10.1007/s00203-025-04585-9
16. Pulbutr P, Thongrak K, Thitprapai A, Rattanakiat S, Mudjupa C, Jaruchotikamol A. Inhibitory activity of lupinifolin isolated from *Derris reticulata* stem against biofilm formation of *Streptococcus mutans* and *Staphylococcus aureus*. Pharmacognosy Res. 2020;12(4):403-408.
17. Rattanakiat S, Taensantia A, Jaemamporn K, Khamnuanin S, Mudjupa C, Jaruchotikamol A, Pulbutr P. Antibiofilm formation activity of lupinifolin against methicillin-resistant *Staphylococcus aureus*. Pak J Biol Sci 2022;25(11):961–970.
18. Amin MU, Khurram M, Khattak B, Khan J. Antibiotic additive and synergistic action of rutin, morin and quercetin against methicillin resistant *Staphylococcus aureus*. BMC Complement Altern Med 2015;15:59. Doi: 10.1186/s12906-015-0580-0.
19. Betts JW, Sharili AS, Phee LM, Wareham DW. *In vitro* activity of epigallocatechin gallate and quercetin alone and in combination versus clinical isolates of methicillin-resistant *Staphylococcus aureus*. J Nat Prod 2015;78(8):2145–2148.
20. Kitichalermkiat A, Kurahachi M, Nonaka A, Nakayama M, Shimatani K, Shigemune N, Tsugukuni T, Hitomi J, Sato J,

Sonoda T, Masuda Y, Honjoh K, Miyamoto T. Effects of epigallocatechin gallate on viability and cellular proteins of *Staphylococcus aureus*. Food Sci Technol Res 2019;25(2):277–285.
21. Mu L, Xie K, Chen R, Cao J, Shu Y, Huang X. The antibacterial activity, action mechanisms and prospects of baicalein as an antibacterial adjuvant. World J Microbiol Biotechnol 2025;41(8):304. Doi: 10.1007/s11274-025-04508-2.
22. Moulick S, Roy DN. Bioflavonoid baicalein modulates tetracycline resistance by inhibiting efflux pump in *Staphylococcus aureus*. Microb Drug Resist 2024;30(9):363-371.
23. Zhao WH, Hu ZQ, Okubo S, Hara Y, Shimamura T. Mechanism of synergy between epigallocatechin gallate and β-lactams against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2001;45(6):1737–1742.
24. Fujita M, Shiota S, Kuroda T, Hatano T, Yoshida T, Mizushima T, Tsuchiya T. Remarkable synergies between baicalein and tetracycline, and baicalein and beta-lactams against methicillin-resistant *Staphylococcus aureus*. Microbiol Immunol 2005;49(4):391–396.
25. Pulbutr P, Nantana P, Suksabai S, Mudjupa C, Denchai R, Rattanakiat S, Dhammaraj T. Inhibitory actions of lupinifolin isolated from *Derris reticulata* stem against carbohydrate digesting enzymes. Pharmacognosy Res 2020;10:24–30.
26. CLSI. Performance standards for antimicrobial susceptibility testing. 30th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
27. Orhan G, Bayram A, Zer Y, Balci I. Synergy tests by E test and checkerboard methods of antimicrobial combinations against *Brucella melitensis*. J Clin Microbiol 2005;43(1):140–143.
28. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother 2003;52(1):1.
29. Samaddar A, Mendonsa JM, Sudhaharan S, S N, Kindo AJ, Shetty A, Pamidimukkala U. *In vitro* evaluation of antifungal combinations against neurotropic dematiaceous fungi associated with primary cerebral phaeohyphomycosis. Microbiol Spectr 2024;12(8). https://Doi.org/10.1128/spectrum.00781-24
30. Blanco AR, Sudano-Roccaro A, Spoto GC, Nostro A, Rusciano D. Epigallocatechin gallate inhibits biofilm formation by ocular staphylococcal isolates. Antimicrob Agents Chemother 2005;49(10):4339–4343.
31. Yoda Y, Hu ZQ, Zhao WH, Shimamura T. Different susceptibilities of *Staphylococcus* and Gram-negative rods to epigallocatechin gallate. J Infect Chemother 2004;10(1):55-58.
32. Chen Y, Liu T, Wang K, Hou C, Cai S, Huang Y, Du Z, Huang H, Kong J, Chen Y. Baicalein inhibits *Staphylococcus aureus* biofilm formation and the quorum sensing system *in vitro*. PLoS One 2016;11(4):e0153468. Doi: 10.1371/journal.pone.0153468.
33. Muniyasamy R, Manjubala I. Synergistic combination of baicalein and rifampicin against *Staphylococcus aureus* biofilms. Front Microbiol 2024;15:1458267. Doi: 10.3389/fmicb.2024.1458267.
34. Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhtar S, Alsharif MA, Parveen H, Zakaria ZA. Antibacterial effects of flavonoids and their structure-activity relationship study: a comparative interpretation. Molecules 2022;27(4):1149. Doi: 10.3390/molecules27041149.
35. Osorio M, Carvajal M, Vergara A, Butassi E, Zacchino S, Mascayano C, Montoya M, Mejías S, Martín MC, Vásquez-Martínez Y. Prenylated flavonoids with potential antimicrobial activity: synthesis, biological activity, and *in silico* study. Int J Mol Sci 2021;22(11):5472. Doi: 10.3390/ijms22115472.
36. Shireen T, Singh M, Dhawan B, Mukhopadhyay K. Characterization of cell membrane parameters of clinical isolates of *Staphylococcus aureus* with varied susceptibility to alpha-melanocyte stimulating hormone. Peptides (NY) 2012;37(2):334–339.
37. Nikolic P, Mudgil P, Harman DG, Whitehall J. Untargeted lipidomic differences between clinical strains of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. Infect Dis 2022;54(7):497–507.
38. Mishra NN, Yang SJ, Sawa A, Rubio A, Nast CC, Yeaman MR, Bayer AS. Analysis of cell membrane characteristics of in vitro-selected daptomycin-resistant strains of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2009;53(6):2312-2318.
39. García AB, Viñuela-Prieto JM, López-González L, Candel FJ. Correlation between resistance mechanisms in *Staphylococcus aureus* and cell wall and septum thickening. Infect Drug Resist 2017;10: 353–356.