Flavonoid–Triterpenoid Synergy against *Staphylococcus aureus* Biofilms: Mechanistic Insights into Antivirulence Strategies

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Abstract

Staphylococcus aureus (S. aureus) is a major global health concern classified by the WHO as a high-priority pathogen due to its multidrug-resistant profile and virulence. Biofilm formation enhances bacterial tolerance to antimicrobials, highlighting the urgent need for anti-biofilm therapies as an alternative. Owing to their diverse modes of action and low resistance potential, natural compounds such as baicalin, quercetin, and 18β-glycyrrhetinic acid were evaluated individually and in combination for their antibacterial and antibiofilm activities against community-associated MRSA (CA-MRSA) isolates. Combination studies with thymoquinone and ciprofloxacin assessed synergistic effects. While none of the tested compounds exhibited strong bactericidal activity, baicalin and quercetin showed notable biofilm inhibition, whereas 18β-glycyrrhetinic acid demonstrated minimal effect. Quercetin showed potent, dose-dependent antibiofilm activity with the lowest $MBIC_{50}$ values and produced additive effects with baicalin or 18β-glycyrrhetinic acid, but antagonism with thymoquinone when combined. Sequential treatment with quercetin followed by ciprofloxacin markedly enhanced biofilm reduction, achieving nearly complete inhibition compared to either agent alone. None of the tested compounds eradicated pre-formed biofilms. These findings indicate that natural flavonoids, particularly quercetin-based combinations, can inhibit MRSA biofilm formation through non-bactericidal mechanisms, reducing resistance pressure and complementing existing antibiotics.

Introduction

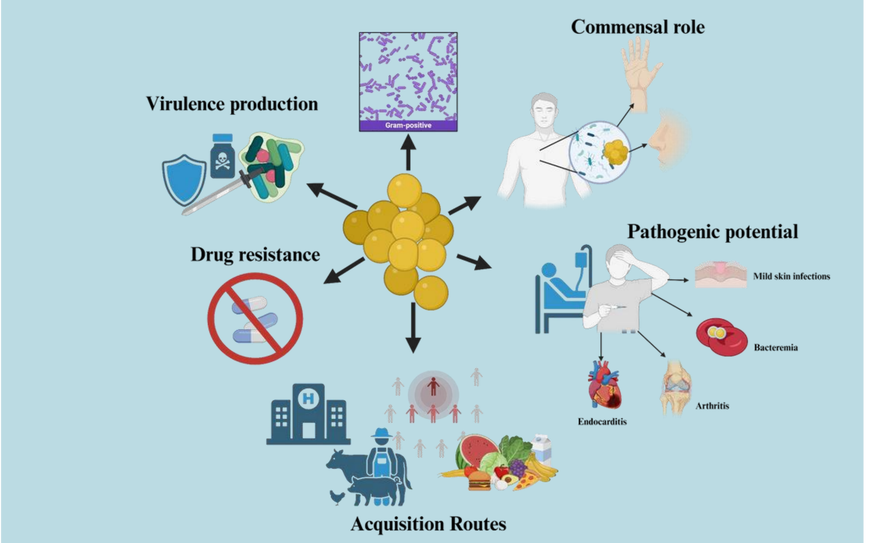

Figure 1: S. aureus overview

Hypothesis and methods

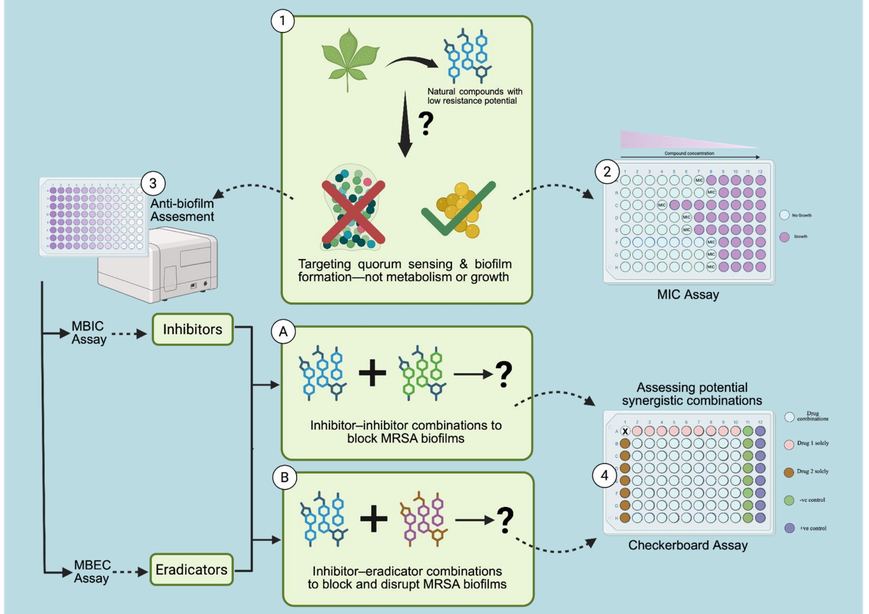

Figure 2: A Schematic overview of the study hypothesis and experimental workflow

Selected Results

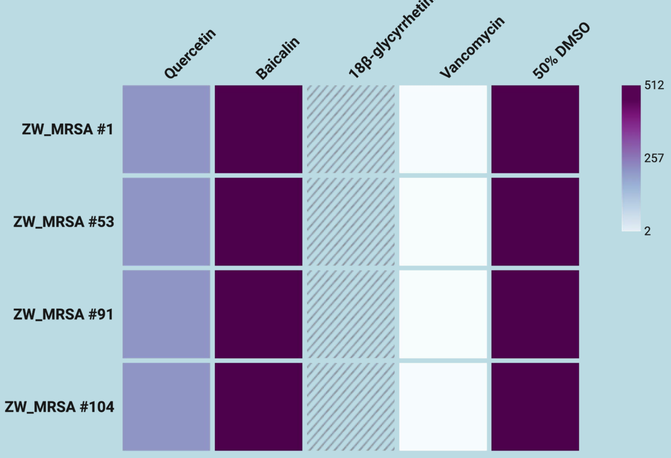

Figure 3: Heatmap showing MIC (µg/mL) values of natural compounds across four S. aureus isolates from different clonal lineages and agr groups, indicating weak or no growth-inhibitory effects.

Selected Results

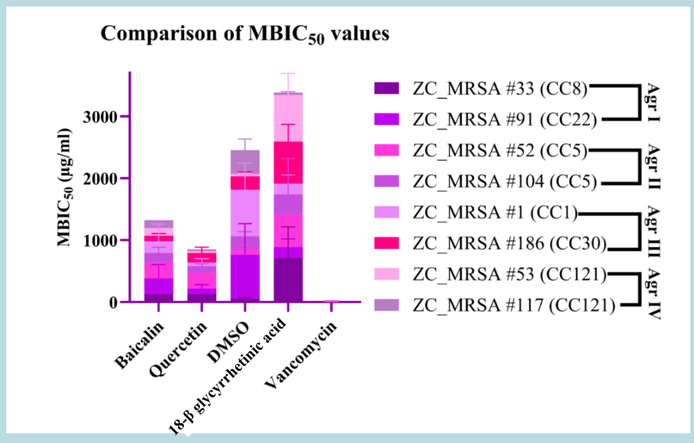

Figure 4: Stacked bar graph comparing the average $MBIC_{50}$ (µg/mL) values of natural compounds, indicating Q & B as the most potent inhibitors

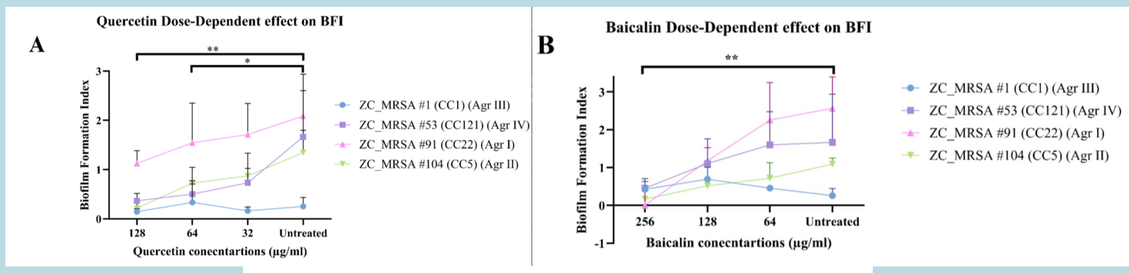

Figure 5: A collective figure of scatter plots for dose-dependent inhibition effects of natural compounds on biofilm formation, showing strong inhibition by quercetin, significant reduction by baicalin, and minimal effect by 18β-glycyrrhetinic acid.

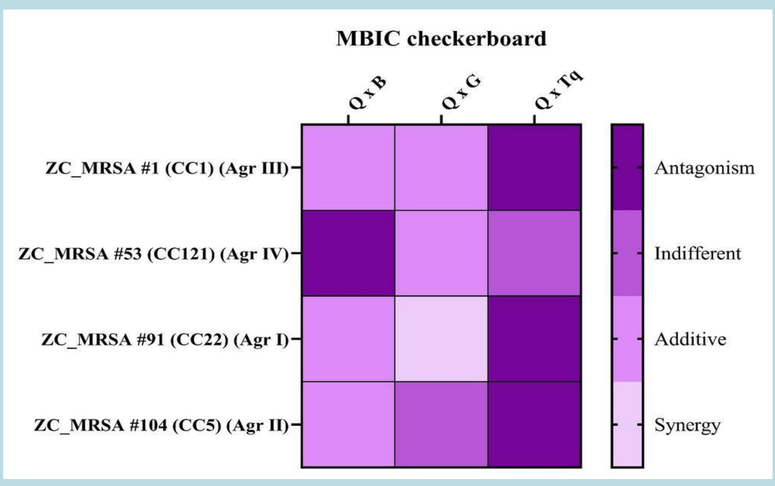

Figure 6: Heatmap indicating the outcome of the MBIC combination of natural compounds, showing mostly promising synergistic and additive effects

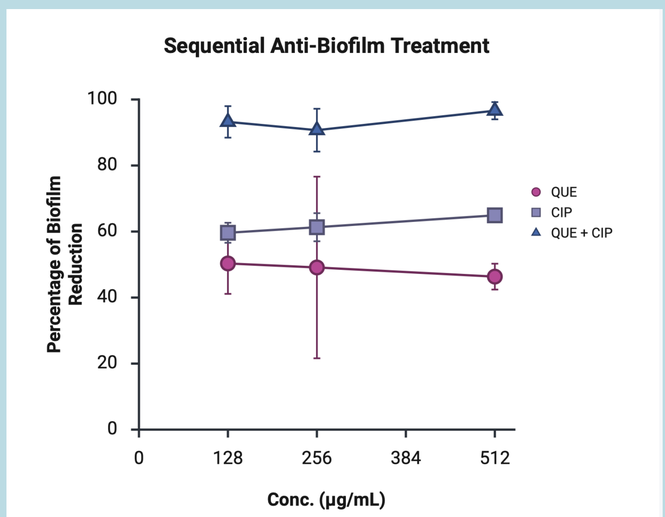

Figure 7: Sequential treatment with QUE (quercetin) and CIP (ciprofloxacin) shows enhanced biofilm reduction in S. aureus compared to individual treatments, suggesting potential synergistic interactions.

Figure 8: Heatmap representing the biofilm eradication efficacy of natural compounds, indicating a lack of eradicating activity

Future recommendation

Further investigation into the molecular effects the compounds exert, shedding light on their regulatory pathways to inhibit biofilm, their impact on bacterial virulence production. Developing advanced delivery systems, such as nanoparticle-encapsulated formulations, would overcome the limitation of biofilm penetration and could enhance biofilm inhibition and eradication effects. Besides that, assessing cytotoxicity using mammalian cells or 3D tissue models to confirm safety. Additionally, conducting In vivo infection models, such as catheter- or wound-associated infections, to investigate insights into therapeutic efficacy under realistic conditions.

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