Comparative Efficacy of 4,5,7-Trihydroxyflavanone (THF) and THF-Mediated Silver Nanoparticles against *Enterococcus gallinarum*: Insights from *in vitro*, *in vivo*, and *in silico* Analysis

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Abstract

Enterococcus gallinarum is an opportunistic and intrinsically vancomycin-resistant bacterium increasingly associated with severe infections such as bacteremia, meningitis, peritonitis, and infective endocarditis. Its ability to form biofilms and withstand conventional antibiotics complicates therapeutic management and promotes persistent infections. These challenges highlight the need for novel, multi-targeted strategies that can simultaneously target planktonic cells, biofilms, and host inflammatory damage. The present work comparatively evaluates 4,5,7-trihydroxyflavanone (THF) and THF-mediated silver nanoparticles (THF-AgNPs) as alternative therapeutics against *E. gallinarum*-associated infections. The aim of this study was to delineate the relative efficacy of THF and THF-AgNPs, with emphasis on antimicrobial activity, biofilm disruption, anti-inflammatory potential, and tissue recovery in zebrafish infected with *E. gallinarum*. THF alone demonstrated significant antibacterial activity, inhibiting over 80% of bacterial growth at >150 µg/ml and effectively suppressed Hemolysin, a key virulence factor. The In silico studies of molecular interactions revealed the strong binding affinity between THF and Hemolysin. Furthermore, THF exhibited anti-inflammatory activity, as confirmed by BSA denaturation assay, and in silico analysis revealed strong inhibition of matrix metalloproteinases MMP-2 and MMP-9, which are critical for extracellular matrix degradation and inflammation. In contrast, THF-AgNPs displayed superior efficacy, achieving approximately >91% bacterial growth inhibition and >90% biofilm reduction at ~128µg/ml, while showing non-toxicity to human red blood cells. Histopathological analysis demonstrated that THF-AgNPs promoted higher tissue recovery rates compared to THF alone in *E. gallinarum* infected zebrafish. In conclusion, THF functions as a dual anti-virulence and anti-inflammatory agent, while its nanoparticle formulation substantially amplifies antimicrobial and tissue-healing outcomes. Future work includes dose translation of THF-AgNPs into advanced delivery systems for managing endocarditis and other biofilm-associated infections.

Keywords: *Enterococcus gallinarum,* 4,5,7-Trihydroxyflavanone (THF), Anti-inflammatory, Matrix-Metalloproteinases, Histopathology, Endocarditis