Evaluation of Hexyl Gallate as a candidate for a novel antimicrobial agent

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The increase in bacterial resistance to conventional antibiotics has driven the search for new compounds with inhibitory activity against microorganisms of interest. Among these compounds, phenolic derivatives have shown antimicrobial potential, with hexyl gallate, derived from the esterification of gallic acid, standing out. Although previous studies have reported antimicrobial activity for compounds of this class, data against certain bacterial species remain scarce, justifying further investigation.

The aim of this study was to evaluate the antimicrobial activity of hexyl gallate against Gram-positive and Gram-negative bacteria of clinical and environmental relevance, as well as to determine whether the compound exhibits bacteriostatic or bactericidal effects.

Initially, the Resazurin Microtiter Assay (REMA) was performed using hexyl gallate concentrations ranging from 100 to 0.78 µg/mL against Gram-negative bacteria (*Pseudomonas aeruginosa, Pseudomonas putida, Escherichia coli, Salmonella typhi,* and *Xanthomonas perforans*) and Gram-positive bacteria (*Enterococcus faecium, Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Listeria* sp., and *Cutibacterium acnes*). REMA results were based on the reduction of resazurin to resorufin by metabolically active cells, indicated by a color change from blue (growth inhibition) to pink (growth). All assays included growth controls, standard antibiotic controls, and solvent controls (DMSO).

Based on growth inhibition results, Minimum Bactericidal Concentration (MBC) assays were performed in microtubes using effective concentrations, followed by incubation under agitation for 24 h, plating on solid medium, and growth observation for up to 48 h to determine bactericidal or bacteriostatic activity.

In the REMA assay, no growth inhibition was observed for *P. aeruginosa, E. faecium, C. acnes,* and *Listeria* sp. at the concentrations tested. For the remaining species, the following Minimum Inhibitory Concentrations (MICs) were observed: 25 µg/mL for *B. subtilis, P. putida,* and *E. faecalis*; and 100 µg/mL for *S. typhi, E. coli, X. perforans,* and *S. aureus*. In the MBC assay, effective concentrations identified in REMA still resulted in bacterial growth, indicating bacteriostatic effects for all susceptible species. Higher concentrations were then evaluated, revealing bactericidal effects for *E. coli* and *X. perforans* at 200 µg/mL and for *S. typhi* and *E. faecalis* at 300 µg/mL. For *B. subtilis, P. putida,* and *S. aureus*, no bactericidal effect was observed at the concentrations tested, confirming only bacteriostatic activity.

The results demonstrate that hexyl gallate exhibits concentration-dependent antimicrobial activity, with

predominantly bacteriostatic effects at lower doses and bactericidal effects at higher concentrations. Variation in sensitivity among species suggests possible differences in cell permeability or molecular targets of the compound.

In conclusion, hexyl gallate shows potential as a candidate for a novel antimicrobial agent, justifying further studies to elucidate its mechanism of action and evaluate its activity against additional bacterial strains. Additionally, confocal fluorescence microscopy analyses will be performed to determine whether the bactericidal mechanism of hexyl gallate involves damage to the cytoplasmic membrane or interaction with bacterial DNA.

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