

TITLE: SYNERGISTIC ANTIMICROBIAL ACTIVITIES OF *Melaleuca alternifolia* ESSENTIAL OIL WITH CHITOSAN AGAINST *S. aureus* and *E. coli*

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Abstract:

S. aureus and *E. coli* are representatives Gram positive and negative bacteria, respectively, which are associated with several diseases in humans. The *Melaleuca alternifolia* essential oil, (MAEO) and chitosan (CHI) have been reported as potential antimicrobial agents. Combination of two naturally occurring antimicrobial components may provide a system with enhanced antimicrobial properties. Incorporation of MAEO into the chitosan gel could reduce losses of active components due to evaporation and establish possibilities for synergic antimicrobial action. The present study has the aim to evaluate the synergistic antimicrobial activity of chitosan and MAEO against *S. aureus* and *E. coli* strains. Inoculum of *S. aureus* and *E. coli* adjusted to 5×10^8 colonies Forming Unity/mL, were used as test microorganisms. Chitosan with low and high molecular weight (1:1w/w), from Sigma, were solubilized in 1% acetic acid (20mg/mL). The *M. alternifolia* essential oil was diluted in Tween 80 (0.5% v/v) to obtained an initial concentration of $200 \mu\text{L} \cdot \text{mL}^{-1}$. The pHs of solutions were adjusted for 5.8 using NaOH. The antimicrobial assays were carried out by microdilution method in BHI broth, and subsequent incubation in BHI agar without substance test, for determine the Minimum Inhibitory Concentration (MIC), and the Minimum Bactericidal Concentration (MBC), respectively. Bacterial were incubated at 37°C/24h. To MIC was used resazurin staining, as a bacterial growth. The concentration of substance test varied: CHI ($12\text{-}0.5\text{mg} \cdot \text{mL}^{-1}$), MAEO ($120.0\text{-}5.0\mu\text{L} \cdot \text{mL}^{-1}$). The CHI solution of high e low molar weight, demonstrated MIC and MBC of $2.0 \text{mg} \cdot \text{mL}^{-1}$ for *S. aureus* and $3.0 \text{mg} \cdot \text{mL}^{-1}$ for *E. coli*. MAEO solubilized shows only bacteriostatic inhibition with MIC of $20\mu\text{L} \cdot \text{mL}^{-1}$ and $50\mu\text{L} \cdot \text{mL}^{-1}$ for *S. aureus* and *E. coli*, respectively. However, when associated MAEO with CHI presented MIC and MBC for both bacteria strains of $2.0 \text{mg} \cdot \text{mL}^{-1}$ for CHI and $20\mu\text{L} \cdot \text{mL}^{-1}$ for EOMA. The results show that the association of chitosan with *M. essential oil* exhibit synergic effect, raising the antimicrobial activity of melaleuca oil against *S. aureus* and *E. coli*. The synergic antimicrobial effect of CHI and MAEO associated was more effective for Gram positive bacteria.

Key-words: Biopolymer, Tea tree oil, Antimicrobial properties

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