

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

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Abstract:

The search for alternative therapies against resistant bacteria has led to demand for antimicrobial substances mainly of natural origin. *S. aureus* and *E. coli* are representatives Gram positive and negative bacteria, which are associated with several diseases in humans. *C. martini*. (CMEO) and chitosan (CHI) have been reported as potential antimicrobial agents. The present study has the aim to evaluate the synergistic antimicrobial activity of chitosan and *Cymbopogon martini* essential oil (CMEO) against *S. aureus* and *E. coli* strains. Inoculum of *S. aureus* and *E. coli* adjusted to 5×10^8 colony 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^{-1}). The pHs of solutions were adjusted for 5.8 using NaOH. The *Cymbopogon martini* essential oil was diluted in Tween 80 (0.5% v/v) to obtained an initial concentration of $200 \mu\text{L.mL}^{-1}$. 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 $37^\circ\text{C}/24\text{h}$. To MIC was used resazurin staining, as a bacterial growth. The concentration of substance test varied: CHI ($12\text{-}0.5\text{mg.mL}^{-1}$), CMEO ($120.0\text{-}5.0\mu\text{l.mL}^{-1}$). The CHI solution of high e low molar weight, demonstrated MIC of 2.0mg.mL^{-1} and CBM of 5.0mg.mL^{-1} for *S. aureus* and *E. coli*. CMEO shows only bacteriostatic inhibition with MIC of $40\mu\text{L.mL}^{-1}$ for *S. aureus* and *E. coli*. However, when associated CMEO with CHI presented MIC of 4.0mg.mL^{-1} and 6.0mg.mL^{-1} for CHI and 40.0mg.mL^{-1} and 60.0mg.mL^{-1} for EOMA, and CBM of 2.0mg.mL^{-1} and 4.0mg.mL^{-1} for CHI and 60.0mg.mL^{-1} and 80.0mg.mL^{-1} for EOMA, to *E. coli* and *S. aureus*, respectively. This probably occurs because the incorporation of CMEO into the chitosan gel could reduce losses of active components due to evaporation. The results show that the association of chitosan with *Cymbopogon martini* 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 CMEO associated was more effective for Gram positive bacteria.

Key-words: Biopolymer, Natural products, Antimicrobial properties

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