

TITLE: TERPENES COMBINATIONS INHIBIT BIOFILM FORMATION IN *Staphylococcus aureus* BY INTERFERING WITH INITIAL ADHESION

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ABSTRACT:

The biofilm formed by *Staphylococcus aureus* contributes to chronic infections and its elimination becomes complicated given its resistance to traditional antibiotics. This is why efforts have been increased in the search for new therapeutic options, either to prevent its formation or to eliminate the biofilm already formed. This work aimed to evaluate the effect of the terpenes (-) - trans-caryophyllene, S- (cis) -Verbenol, (S) - (-) - Limonene, (R) - (+) - Limonene and Linalool, individually as in combinations, on growth, biofilm formation and associated gene expression. Two clinical isolates ST30-t019 and ST5-t311 were used for the trials, determined to be responsible for approximately 90% of pediatric infections caused by *S. aureus* in Paraguay. The determination of the minimum inhibitory concentration (MIC) was carried out by assay with resazurin. For the biofilm formation inhibition assay, the polystyrene plate assay and crystal violet staining were used. The most effective combination to inhibit biofilm formation was used to evaluate the relative expression of genes associated with this phenomenon. All the combinations of terpenes tested were able to inhibit the formation of biofilm in a percentage higher than 50%, without inhibiting bacterial growth. The most effective combination was (-) - trans-caryophyllene and Linalool, with an inhibition percentage of 88%. This combination significantly decreased the expression levels of the *sdrD* and *spa* genes, associated with the initial cell adhesion stage. Individually, terpenes did not have a significant effect on biofilm formation or even induced their formation at certain concentrations. The individual terpenes also did not affect bacterial growth significantly. The ability to inhibit biofilm without affecting bacterial growth is a desirable characteristic, since the development of resistance phenomena is avoided. Synergistic interactions would be responsible for the ability of terpene combinations to inhibit bacterial biofilm. It is postulated that the mechanism through which the combination of (-) - trans-caryophyllene and Linalool inhibits its formation is by decreasing the adhesion capacity of cells in the early stages of biofilm.

Keywords: biofilm, *Staphylococcus aureus*, terpenes