

TITLE: OPTIMIZATION OF THE SESQUITERPENES EXTRACTION FROM THE *ZINGIBER OFFICINALE* ROSCOE RHIZOMES AND EVALUATION OF THEIR ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY IN PATHOGENIC BACTERIA

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ABSTRACT

Zingiber officinale Roscoe (ginger) is an herb known thousand of years for its therapeutic properties, attributed, mainly, to the essential oil present in the rhizomes. In addition to the various monoterpenes present in the essential oil, sesquiterpenes: α -curcumene, α -zingiberene, α -farnasene, β -sesquifelandreno and β -bisabolene are important bioactive secondary metabolites of this species. The objective of this work was optimize sesquiterpenes extraction and evaluate their antimicrobial and antibiofilm potential against *Staphylococcus aureus*, *Streptococcus mutans*, *S. agalactiae*, *Escherichia coli* and *Pseudomonas aeruginosa*. Different extraction techniques were used to obtain the sesquiterpene-enriched fraction (SEF), however, microwave assisted extraction was selected to perform the optimization of the extraction using a factorial design 2^3 . The analyzes of extracted compounds were performed by GC-MS. The antimicrobial and biofilm formation assays were performed by the microdilution technique in 96-well plates. In addition to evaluating biofilm inhibition, the hydrophobicity index was also determined as an indirect way of assessing the adhesion capacity of the microorganisms to the host tissues and abiotic surfaces. The best conditions to obtain the SEF were: extraction time: 2 min, plant/solvent ratio: 1:20 (m/v) and microwave power: 400 W. The SEF presented a minimum inhibitory concentration (MIC) of 2000 $\mu\text{g/mL}$ against *S. mutans*. In spite of the low antimicrobial activity, SEF (250 $\mu\text{g/mL}$) inhibited the formation of biofilms of *S. aureus*, *S. agalactiae* and *E. coli* in 50%, 80% and 80%, respectively, which can contribute to the control of these microorganisms, since biofilm is an important virulence factor used as strategy of survival of these bacteria. In addition, FES (250 $\mu\text{g/mL}$) reduced the hydrophobicity index in 58%, 73.4% and 49% of the *S. aureus*, *S. agalactiae* and *E. coli*, respectively, which may be related to the reduction of initial adhesion, one of the first steps in biofilm formation. The results showed that the SEF presented potential in the combat of producing biofilms microorganisms. In this way, these substances represent good candidates on drug prototypes used in the treatment of infections caused by multidrug resistant bacteria. To the best of our knowledge, this is the first time that the antimicrobial activity and antibiofilm of the FES was studied.

Keywords: extraction; ginger; sesquiterpenes; antimicrobial; antibiofilm.

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