TITLE: ANTIMICROBIAL ACTIVITY OF GUAVA AGRO-INDUSTRIAL WASTES EXTRACTS AGAINST BIOFILM-FORMING FOODBORNE PATHOGENS

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In Brazil, the annual production of guava (*Psidium guajava* L.) is around 424,000 tones. Its processing produces a considerable amount of wastes, as seeds and peel, and several studies have been demonstrated its biological activity. This work aimed to prospect natural antimicrobials from guava seeds against biofilm-forming bacteria. The freeze-dried seeds were ground in mechanical mill, immersed (3:1 v/w) in different solvents 70% $v.v^{-1}$ (ethanol, methanol and acetone), and kept under agitation (150 rpm) at 50 ºC for 60 min. The mixture was centrifuged at 2000 × *g* for 15 min, filtered and the solvent was removed using a rotary evaporator at 40 ºC. The *screening* was performed as follow: 25 mL of agar inoculated with *Enterobacter cloacae* (isolated from raw milk), *Staphylococcus aureus* FRI 722 (from contaminated foods) and *S. aureus* EMBRAPA 4018 (from cows with mastitis) (10^5 $UFC.mL^{-1}$) were transferred to Petri dishes, in which wells of 8 mm in diameter were produced and filled with 40 µL of each extract. The solvent 70 % $v.v^{-1}$ concentrated in rotary evaporator and Kanamycin 750 $\mu g.mL^{-1}$ were used as negative and positive controls, respectively. The dishes were incubated at 30°C/37°C for 24 hours. Inhibition zones diameters were measured using a digital caliper and the results were presented in mm. For MIC determination, the dilution in 96-well microplates method was used. The concentrations of the extracts ranged from 25% to 0.78%. After incubation at 35°C for 24 hours, all wells received 30 mL of resazurin (0.01% w/v) to verify changes in the color related to the bacterial growth. For the MBC determination, 10 mL of broth were removed from the wells considered inhibitory and sown in Petri dishes containing BHI agar, incubated at 37°C for 24 hours. *E. cloacae* did not show susceptibility to any of the extracts. For *S. aureus* FRI 722, the inhibition zones ranged from 8.54 ± 0.26 mm (methanolic extract) to 10.47 ± 0.29 mm (acetonic extract); for *S. aureus* EMBRAPA 4018, from 8.96 ± 0.19 mm (methanolic extract) to 9.67 ± 0.36 mm (acetone extract). Only methanolic extract inhibited both *S. aureus* strains at 6.25%. Bactericidal activity was observed at 25% for *S. aureus* FRI 722 and at 12.5% for *S. aureus* EMBRAPA 4018, considering the same extracts. These results suggest that guava seeds present antimicrobial activity against biofilm-forming microorganisms, and could be used to prospect compounds to control pathogenic bacteria in the food industry.

Keywords: agro-industrial wastes, biofilm, natural antimicrobials, *Enterobacter cloacae*, *Staphylococcus aureus*.

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