

**Título: ANTIMICROBIAL ACTIVITY OF ETHYL ACETATE FRACTION OF *Punica granatum* L. LEAF**

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**Resumo:**

The increasing incidence of drug-resistant pathogens related with nosocomial infections has drawn the attention of the scientific communities towards studies on the potential antimicrobial activity of plant-derived substances. The aim of this study was to investigate the antimicrobial activity of the ethyl acetate fraction from *Punica granatum* L. leaves against *Staphylococcus aureus* (ATCC<sup>®</sup> 25923), *Pseudomonas aeruginosa* (ATCC<sup>®</sup> 27853) and *Klebsiella pneumoniae* (ATCC<sup>®</sup> 10031). The hydroalcoholic extract was prepared from fresh leaves and the ethyl acetate fraction (EtOAcF) was obtained after the partitioning of the methanol/water solution with different polarity solvents (hexane, chloroform and then ethyl acetate). Agar well diffusion and the microbroth dilution (resazurin reduction microtiter) assays according to Clinical Laboratory Standards Institute (CLSI) were used to test the antimicrobial activity. In the case of microbroth dilution, the used concentrations of the EtOAcF ranged from 1.024 to 0.004 mg/ml and the highest dilution that showed no visible bacterial growth per spot and presented in blue after to add resazurin was considered as Minimum Inhibitory Concentration (MIC). To determine minimum bactericidal concentration (MBC), 10µl of Mueller-Hinton (MH) broth from each well of microbroth assay was cultured on MH agar plates and after 24h, the bacterium colony growth were observed. The lowest concentration of EtOAcF that showed no bacterial growth was considered as MBC. Experiments were performed in quadruplicate in two different days. The highest inhibitory effect of EtOAcF was observed against *Pseudomonas aeruginosa* with MICs 0.256mg/ml, MBCs 0.512mg/ml and a zone of inhibition of 17 mm. Likewise, The EtOAcF also affected the *Staphylococcus aureus* with MICs 0.512mg/ml, MBCs 0.512mg/ml and a zone of inhibition of 18 mm around the EtOAcF solution. On the other hand, the extract was not able to inhibit the growth of *K. pneumoniae* (no inhibition zone was formed). In conclusion, our results clearly reveal that the EtOAcF from *Punica granatum* L. leaves possesses antimicrobial activity against bacteria related with nosocomial infections.

**Palavras-chaves:** Antimicrobial activity, *Punica granatum* L., Nosocomial infections

**Agencia de fomento:** FAPEMA