

TITLE: EVALUATION OF ANTIBACTERIAL, SYNERGISTIC AND ANTI-VIRULENCE ACTIVITIES OF *Portulaca elatior* ROOT LECTIN AGAINST *Staphylococcus aureus*

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ABSTRACT:

Plants contain bioactive compounds, such as lectins, proteins that interact with carbohydrates. This ability results in several biological activities, including antimicrobial action. PeRoL is a lectin present in the roots of *Portulaca elatior* active against pathogenic bacteria. In this work, it was evaluated the antibacterial activity of PeRoL against susceptible (8325-4) and methicillin-resistant (MRSA; LAC USA300) *Staphylococcus aureus* isolates. Root extract of *P. elatior* in saline solution (0.15 M NaCl) was prepared and PeRoL was isolated by chitin column affinity chromatography. The bacteria were inoculated in Müller-Hinton Agar and incubated at 37°C for 18 h. After that, a bacterial suspension in NaCl was performed to obtain approximately 10⁵ CFU/mL and 80 µL of that suspension was inoculated into microplates wells containing culture medium and lectin or antibiotic (cefotaxime, cefoxitin or cefuroxime) at different concentrations. The minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) were determined. Synergistic effects between PeRoL and antibiotics against MRSA isolate were evaluated by determining the fractional inhibitory concentration index (FICI), as follows: $FICI = (MIC \text{ of PeRoL in combination} / MIC \text{ of PeRoL alone}) + (MIC \text{ of antibiotic in combination} / MIC \text{ of antibiotic alone})$. The combinations were classified as synergistic ($FICI \leq 0.5$), additive ($0.5 < FICI \leq 1$), indifferent ($1 < FICI \leq 2$) or antagonistic ($FICI > 2$). In addition, it was evaluated the gene expression of the virulence factors δ -hemolysin (*hla*), RNA III (*rnalIII*), and protein A (*spa*) by *S. aureus* reporter strains (PC322, *hla::lacZ*; PC203, *spa::lacZ*, and SH101F7, *rnalIII::lacZ*) treated or not with the lectin (26.25–210 µg/mL). PeRoL presented bactericidal activity against both isolates, with MIC and MBC of 20 µg/mL for susceptible strain and MIC and MBC of 5 and 40 µg/mL, respectively, for MRSA strain. PeRoL showed synergistic effect together with cefotaxime (FICI: 0.12), cefoxitin (FICI: 0.12) and cefuroxime (FICI: 0.25). Incubation with PeRoL did not alter the expression of the virulence factors investigated. In conclusion, PeRoL presented antibacterial activity against *S. aureus* strains as well as synergistic effect with cefotaxime, cefoxitin and cefuroxime on MRSA strain.

Keywords: plant lectin, synergistic activity, antimicrobial activity, multidrug-resistant bacteria

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