

## **Isolation of Antimicrobial Proteins From Pomegranate (*Punica granatum L.*) Seeds With Activity Against *Staphylococcus aureus***

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Since its discovery during the 1880s, *Staphylococcus aureus* has emerged as an important pathogenic Gram-positive bacterium. This gram-positive bacterium is the main causer of a wide range of life-threatening infections, including toxic shock syndrome, necrotizing pneumonia, endocarditis and osteomyelitis. For all these problems, novel classes of antibiotic agents are requested to control *S. aureus* infections and a wide searching for new antimicrobial compounds from plant sources have been stimulated. Pomegranate (*Punica granatum*) seeds show high protein content and no reports about antimicrobial proteins among them were published. In order to isolate such proteins, Pomegranate seeds were macerated with a solution containing 0.6M NaCl and 0.1% HCl and further precipitated with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (100%). After dialysis (*cut off*= 3.5 kDa), the protein rich fraction demonstrated activity against *S. aureus* (17%). Protein rich fraction was applied onto a cationic-hydrophobic chromatography Blue-Sepharose, previously equilibrated with 20mM Phosphate buffer (pH 7.2). Non-retained proteins were eluted with the same buffer and retained proteins were eluted with 20mM Phosphate buffer (pH 7.2) containing 2M NaCl, and collected as fraction B1 and B2, respectively. Both fractions showed considerable inhibitory activity against *S. aureus* (11 and 23%, respectively). SDS-PAGE indicates proteins ranging between 7 kDa and 120 kDa.. B2 fraction was applied onto RP-HPLC (Vydac 218MS C18 – Grace), generating five major fractions, which were collected and will be tested against *S. aureus*. In short, data here reported indicated that, in a near future, these proteins could be utilized in the development of novel antibiotics against multidrug-resistant *S. aureus*.

Key words: *Punica granatum* seeds, Antimicrobial proteins