

Effect of extracts from bark of *Azadirachta indica* A. Juss against planktonic cells and bacterial biofilms related to infections

MALVEIRA, E.A.¹; MOURA, A.L.P.¹; LIMA, A.J.G.¹; PEREIRA, A.L.²; MORAIS, M.B.¹; ALBUQUERQUE, C.C.¹; TEIXEIRA, E.H.²; VASCONCELOS, M.A.^{1,2}.

¹Departamento de Ciências Biológicas, Faculdade de Ciências Exatas e Naturais Universidade do Estado do Rio Grande do Norte, (CEP: 59610-210, Mossoró – RN, Brazil); ²Laboratório Integrado de Biomoléculas (LIBS), Universidade Federal do Ceará, Departamento de Patologia e Medicina Legal, (CEP: 60430-160, Fortaleza – CE, Brazil).

Microorganisms are found in a wide range of ecosystems as highly structured species communities, termed biofilms. Biofilms are surface associated microbial communities embedded within a self-produced extracellular matrix and adhered on inert and biotic surfaces. These microbial communities can form on medical devices and be involved in many chronic diseases, such as chronic otitis, tonsillitis, cystic fibrosis, periodontal diseases, and urinary tract infections. Thus, the discovery of new compounds that are able to eradicate such biofilms is critical. The purpose of this work was to evaluate the antimicrobial and antibiofilm activity of extracts from bark of *Azadirachta indica* A. Juss against Gram-positive and Gram-negative bacteria. The bacterial species used in this study were *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 700698, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus epidermidis* ATCC 35984 and *Pseudomonas aeruginosa* ATCC 10145. The ethanolic and methanolic extract were tested in concentrations ranging from 7,8 to 500 µg/mL. The antibacterial activity was determined by microdilution methods: minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The activity of extracts on biofilm formation was evaluated by quantification of biomass total (crystal violet staining), presence of metabolically active cells by XTT (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium- 5-carboxanilide sodium salt) assay and enumeration of biofilm-entrapped viable cells. Both extracts showed antimicrobial activity against Gram-positive bacteria with MIC values ranging from 250 to 31.25 µg/mL and showed MBC only to *S. epidermidis* ATCC 12228 (125 µg/mL). Regarding effect on biofilm, in general, the extracts reduced significantly the biomass, number of viable cells and metabolic activity of bacterial biofilms. The bacterium Gram-negative *P. aeruginosa* and its biofilm showed less sensitivity to the action of extracts. Therefore, the ethanolic and methanolic extract of *A. indica* contain important compounds with antibacterial activity. Furthermore, the present study highlights the importance of natural products in discovery of new antibacterial and antibiofilm compounds.

Keywords: antibacterial, biofilm, plant extracts, *Azadirachta indica*

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