193 Casbane Diterpene activity on mixed biofilms of *P. aeruginosa* and *Candida* species

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Pseudomonas aeruginosa and *Candida albicans* are two important opportunistic pathogens frequently associated to the most important nosocomial biofilm-associated infections. Moreover, recently, *Candida glabrata* has also been associated. The search for potential phytochemicals as anti-biofilm agents has become a compelling and active area of research. This study aimed to evaluate the antimicrobial features of Casbane Diterpene (CD), a secondary metabolite isolated from *Croton nepetaefolius*, in dual biofilms of *P. aeruginosa* and *C. albicans* or *C. glabrata*. Biofilms were developed in 96-well microtiterplates in the presence or absence of CD. After 24 hours of planktonic growth, 100 µL of cells suspensions of the yeasts and bacteria (1×10^6 cells ml^{-1} in Nutrient Broth) and 100 µL of solution of CD (500 - 31.5 µg/mL) were pipetted into each well and incubated for 24 h at 37°C in an orbital shaker at 120 rpm. Biofilms were characterized in terms of total biomass, through crystal violet (CV), and number of viable cells, expressed as log CFU/cm^2 (grown in selective medium for each microorganism). Additionally, Scanning Electron Microscopy (SEM) images of the un- and treated biofilms were recorded. CD showed to reduce biofilm biomass of dual species biofilms from a concentration of 62.5 µg/mL. However, CD presented low reduction of the CFU's for *P. aeruginosa* in mixed biofilms with both yeasts. Interestingly, CD reduced the number of CFUs of *C. albicans* (1.0 - 1.4 log) in all the concentrations. Moreover, the number of viable cells of *C. glabrata* in the dual-species biofilms was completely reduced in the presence of a 250 µg/mL of CD. For 125 and 62.5 µg/mL, CD reduces 1.8 and 1.1 log of CFU's respectively. The images of SEM showed *P. aeruginosa* cells placed firmly on hyphal elements of the *C. albicans* and on *C. glabrata* cells. Moreover, the use of CD, at 500 µg/mL, shows low reduction in the *P. aeruginosa* cells and but strong for the two *Candidas* species in mixed biofilms, confirming the results obtained. Data allowed to conclude that CD, a natural compound, has potential as anti-biofilm agent even against mixed biofilms involving *P. aeruginosa* and *Candidas* species.

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