

Prophylactic outcomes of Casbane Diterpene in *Candida albicans* and *Candida glabrata* biofilms

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Biofilms are surface associated communities of microorganisms embedded within a self-produced extracellular matrix and adhered on inert and biotic surfaces. These biological consortia are considered the most prevalent growth form of microorganisms. Biofilm formation is a potent virulence factor for a number of *Candida* species, as it confers significant tolerance to antimicrobial therapy, primarily by limiting the penetration of substances through the biofilm matrix. Casbane Diterpenes (CD) belongs to the class of diterpenoids isolated from few species of plants from Euphorbiaceae family with important anticancer and antibacterial activities.

So, the goal of this study was to assess the antibiofilm effect of a Casbane Diterpene isolated from the stalks of *Croton nepetaefolius* against *Candida albicans* and *Candida glabrata*. Biofilms were developed within the 96-well microtiterplates in the presence of the CD. After 24 hours of growth, 100 μL of cells suspensions (1×10^6 cells mL^{-1} in Nutrient Broth) and 100 μL of solution of CD (500 - 31.5 $\mu\text{g}/\text{mL}$) were pipetted into each well and incubated for 24 h at 37°C in an orbital shaker at 120 rpm. Biofilms formation was characterized by total biomass, through crystal violet (CV), and number of viable cells, expressed as log CFU per cm^2 .

CD showed to be able to reduce the biofilm formation of *C. albicans* and *C. glabrata*. CD reduced *C. albicans* biomass in ≈ 82 , 64, 57 and 27 % at the concentrations of 500, 250, 125 and 62.5 $\mu\text{g}/\text{mL}$, respectively. *C. glabrata* biomass was reduced in ≈ 68 and 26 % at 500 and 250 $\mu\text{g}/\text{mL}$. Regarding the number of viable cells embedded in the yeast biofilms, CD at 500 and 250 $\mu\text{g}/\text{mL}$ reduced ≈ 2 and ≈ 1 log of *C. albicans* biofilm CFUs, and ≈ 2.5 and ≈ 1 log for *C. glabrata*, respectively. Regarding the high resistance and recalcitrance of *Candida* biofilms to the traditional therapies, CD emerges as a good prophylactic alternative to be used alone or in combination with other traditional drugs.

Keywords: Casbane diterpene, natural antimicrobial, *Candida* biofilms, antibiofilm features, novel prophylactic strategies

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