Title: ANTIFUNGAL AND CYTOTOXICITY EVALUATION OF Betula pendula GLYCOLIC EXTRACT ON Candida species

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Abstract:

Candida species have a great importance clinic to be present in several infections in humans. Thus, the aim of this study was to evaluate the antifungal activity and cytotoxicity of B. pendula glycolic extract for Candida spp. standard strains in planktonic growth and monotypic biofilm. The species tested were: C. albicans (ATCC 36801), C. dubliniensis (ATCC MYA 646), C. glabrata (ATCC 9030), C. guilliermondii (ATCC 6260), C. krusei (ATCC 6258) and C. tropicalis (ATCC 13803). Antimicrobial activity of B. pendula glycolic extract was evaluated by microdilution method based on Clinical and Laboratory Standards Institute in order to obtain the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Biofilms were formed in microtiter plates with suspensions of 10^7 cells/mL and incubation at 37°C under stirring for 48 h. Then the biofilms were treated with the extract for 5 min and 24 hours with different concentrations for each species. Saline was used as control. Biofilms were disaggregated and the decimal dilutions were plated on Sabouraud Dextrose agar by drop-plate technique, and incubated at 37 °C for 48 h. Next, the counting of colony forming units (CFU/mL) was performed. Data were compared by ANOVA and Tukey test (p ≤ 0.05). Cytotoxicity analyses was performed using the MTT colorimetric method after mouse macrophages cultures (RAW 264.7) were exposed to the extract and for 5 min and 24 h. MIC and MFC values demonstrated all strains had similar sensibility to B. pendula glycolic extract in planktonic growth, with MIC between 0.78 mg/mL (C. glabrata) and 3.13 mg/mL (C. tropicalis) and CFM between 3.13 mg/mL (C. albicans, C. glabrata, C. guilliermondii, and C. krusei) and 6.25 mg/mL (C. dubliniensis, and C. tropicalis). For biofilm growth, in 5 min of treatment, statistically significant reduction was obtained in extract concentration greater than 6.25 mg/mL for C. dubliniensis and C. glabrata, 25 mg/mL for C. albicans, C. krusei, and C. tropicalis, and 50 mg/mL for C. guilliermondii. In 24 h of treatment, 6.25 mg/mL eliminated more than 94% of all strains. In 5 min, the extract was not cytotoxic until the concentration of 10%. However, in 24 h, only concentrations below 1,56 mg/mL were not cytotoxic. It was concluded that B. pendula glycolic extract showed antifungal activity against the strains tested in both planktonic and biofilm growth and the antifungal concentrations were not cytotoxic (RAW 264.7) when in contact for 5 min.

Keywords: Antifungal activity; citotoxicity; Betula pendula