

TITLE: INHIBITORY EVALUATION ACTIVITY OF THE COMBINATION BETWEEN CLOTRIMAZOLE AND ESSENTIAL OIL OF *M. ALTERNIFOLIA* AGAINST *CANDIDA ALBICANS* BIOFILMS

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ABSTRACT:

Candida species are capable of forming biofilms, a factor aggravated by the increasing resistance to conventional antifungals. Therapeutic options can be enhanced by the combination with other substances, such as the essential oil of *M. alternifolia*. This study evaluated the combined action of clotrimazole and *M. alternifolia* essential oil on inhibition of formation (MBIC) and eradication (MBEC) of *Candida albicans* biofilms. The clinical vaginal isolate of *C. albicans* (SV) and *C. albicans* ATCC 90028, and the broth microdilution methodology were used to determine the minimum inhibitory concentration (MIC), MBIC and MBEC, in 96-well flat-bottom microplates. A verification technique performed for the plaque tests between clotrimazole and essential oil. The final concentration of cells in the yeast in each of the wells was 0.5×10^3 to 1×10^3 CFU/mL. The plates were incubated at 35°C for 48 hours for the three tests, performed in triplicate. For MBIC and MBEC, after the incubation period, the wells were washed with PBS buffer, and MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide) and phytomenadione (2-methyl-3-[(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-enyl]naphthalene-1,4-dione) cell viability of *Candida* spp. The formed formazan was dissolved in DMSO (Dimethyl sulfoxide), and the optical density of each well was determined at 490nm. The MIC, MBIC and MBEC of *M. alternifolia* against *C. albicans* SV and ATCC 90028 were 4000 µg/mL, while the MIC, MBIC and MBEC values for clotrimazole were 0.125, 0.5, 1 µg/mL, 0.25, 1, 1 µg/mL, respectively. The combined substances showed MIC, MBIC and MBEC, respectively, for *M. alternifolia* x clotrimazole of >4000 x 0.0625; 2000 x 0.25 and 4000 x 0.5 for *C. albicans* SV and 2000 x 0,0625; 4000 x 0,5 e 4000 x 0,5 against *C. albicans* ATCC 90028. The combined inhibitory concentrations presented were lower than those found for the isolated substances, suggesting future applications as a potential active component on different stages of the development of the biofilm formed by *Candida* spp.

Keyword: *Candida* spp.; Clotrimazole; Synergy, Biofilm.

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