Title: EFFECT OF *Rosmarinus officinalis* (ROSEMARY) EXTRACT ON POLYMICROBIAL BIOFILM FORMED BY *Candida albicans* AND *Staphylococcus aureus*

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Abstract:

Both *S. aureus* and *C. albicans* may be responsible for numerous infectious conditions alone, but in polymicrobial infections can act in conjunction. Thus, this study evaluated the association of *C. albicans* and *S. aureus* in polymicrobial biofilm undergoing treatment with plant extract of rosemary. Therefore, the reference strains *C. albicans* (ATCC 18804) and *S. aureus* (ATCC 6538) were grown on solid medium and in liquid medium subsequently for 24 h at 37°C in each crop being used Sabouraud-dextrose agar (SD) and Yeast Nitrogen Base broth (YNB) for yeast and Heart Brain Infusion (BHI) agar and broth for bacterium. Then, a centrifugation was performed (2000 rpm/10 min), where there was discarded the supernatant and suspend the pellet in saline (0.9% NaCl) twice in succession. Subsequently, the suspension from each microorganism was adjusted in a spectrophotometer to 1 x 10^7 CFU/mL (colony forming units per milliliter) and was added into microtiter plate wells, with a part (100 µL) of *C. albicans* and another (100 µL) of *S. aureus*. After preincubation (37°C/90 min) under stirring (75 rpm), the supernatant was discarded and added culture medium (BHI+YNB, 1: 2) that was replaced after 24 h incubation. The polymicrobial biofilm formed for 48 h was subjected to treatment with glycolic extract of rosemary (200 mg/ml) for 5 min (n=10) and in the control group was added saline (n=10). Then, the biofilms were broken by sonication (25% power for 30 s). After that, a serial dilutions of suspensions were made and seeded on selective agars as SD agar with chloramphenicol (1%) for *C. albicans* and BHI agar with NaCl (NaCl 75 mg/ml) for *S. aureus*. After 48 h incubation, the CFU were counted and the data were statistically analyzed by ANOVA and Tukey Test ($p \leq 0.05$). It was observed that in polymicrobial biofilm the population of *C. albicans* was reduced in 89.28% (± 14.72) after treatment, since in the control group had 1.38 x 10^7 CFU/ml (± 0.46 x 10^7) and the treated group had 0.15 x 10^7 CFU/mL (± 0.2 x 10^7) ($p<0.05$) and 83.51% (± 4.81) of the population of *S. aureus*, since in the control group was formation of 8.27 x 10^8 CFU/ml (± 0.6 x 10^8) and after treatment was observed 1.35 x 10^8 CFU/ml (± 0.37 x 10^8) ($p<0.05$). Thus, it was found that the population of *C. albicans* in polymicrobial biofilm was suppressed by the population of *S. aureus*. However, both microorganisms were controlled with the application of rosemary extract.

Keywords: *Candida albicans*; Polymicrobial biofilm; *Rosmarinus officinalis*; *Staphylococcus aureus*;