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 ≤ 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 10^8 CFU/ml (± 0.46 x 10^7) and the treated group had 0.15 x 10^7 CFU/mL (± 0.2 x 10^6) (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;