Evaluation of antibiofilm activity of copaiba essential oil emulsion

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INTRODUCTION AND OBJECTIVE

Biofilms are produced by microorganisms that adhere in surfaces and produce an extracellular matrix of polymers. This structure has a protective function for the microorganisms to various environmental conditions, including the presence of antibiotics. The bacterial and fungal resistance to traditional antimicrobial and antifungal products, reflects the need to search new strategies for the control of biofilms (Simões 2010).

The copaiba oil (CO), extracted from *Copaifera langsdorffii* trees, is widely used in popular medicine due to its antimicrobial and anti-inflammatory activities (Santos 2008). The copaiba essential oil (CEO), a volatile fraction of CO, presents large amount of sesquiterpenes compounds that confer great pharmacological activity (Gelmini 2013). Additionally, the use of natural products as a source for treatment of infectious diseases could present advantages such as less toxicity and biodegradable property (Xavier 2012).

Given the broad pharmacological activity, delivery systems containing CEO in their formulation are becoming more relevant. Emulsions are delivery systems containing a mixture of one or more phase dispersed in another and are used to carry water-insoluble drugs and natural assets, improving its bioavailability (Cui 2006).

The aim of this study was to evaluate the antibiofilm activity of the CEO and the CEO-emulsion (CEO-emul) in order to prevent skin infections caused by biofilm formation.

MATERIAL AND METHODS

Chemicals - The Copaiba oil was obtained from Flores & Ervas (Piracicaba, SP, Brazil), Span 80® was purchased from Sigma Aldrich Inc (St Louis, MO, USA) and Tween 20® from VETEC (Rio de Janeiro, RJ, Brazil). Deionized water was used throughout the experiments.

Microorganisms - Strains of *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *C. glabrata* ATCC 2001, *C. krusei* ATCC 6258, and two clinical strains (CS) of bacteria and two of yeasts were provided by LMMM (Laboratório de Micologia Médica e Molecular), UFRN. The bacteria were maintained in Nutrient Agar medium and the yeasts were maintained in Sabouraud Dextrose Agar medium at - 80 °C until the experiment.

Extraction of CEO - The CO-resin was subjected to hydrodistillation with deionized water for a period of 4 hours at 100 ºC using Clevenger apparatus. The essential oil obtained after extraction was dried with anhydrous sodium sulfate and filtered with a 0,22 µm porous membrane.

Emulsion preparation - The emulsion (CEO-emul) was prepared according to phase inversion technique. The Span 80® (0.44 %) was dispersed in the CEO (5 %) (phase 1). Tween 20® (1.56 %) was dispersed in the water (93 %) (phase 2). Both phases were heated separately at 70 °C and then mixed using an Ultra-Turrax® T 25 homogenizer (IKA, Germany) at 13,000 rpm for 10 minutes.

Antibiofilm assay - The strains were used after 24 h and 48 h of incubation for bacteria and fungi, respectively. Then, they were inoculated on Mueller-Hinton broth adjusted to the McFarland 0.5 Standard. The CEO was diluted (99:1) with DMSO 1 %. Subsequently, the samples were added (12.5 %) in the wells containing the Mueller-Hinton broth and posteriorly the microorganisms. The microplates were incubated at 37 °C for 24 h and 48 h for bacteria and fungi, respectively, at 150 rpm. Then, the supernatants were drained and the wells were washed with sterile water to remove non-adherent cells. The biofilm content was stained with crystal violet, washed after 20 minutes, then, 200 µL of absolute ethanol was added and the optical density (OD) was read by ELISA reader (BioTek, µQuant) at 570 nm. Ketoconazole and Chloramphenicol were used as synthetic antimicrobial references for fungi and bacterial strains, respectively.

Statistical analysis - Statistical significance between groups was performed by Analyses of Variance (ANOVA) followed by Tukey's test. P values less than 0.05 ($p < 0.05$) were considered significant. The results are presented as the mean ± S.D.

RESULTS AND DISCUSSION

The antibiofilm assay for the tested strains showed that the CEO-emul presented better activity than the CEO for some microorganisms, probably because the oil droplets interact with the membrane of the microorganisms preventing its adhesion at the surface,

and consequently, preventing the development of some pathologies (Trombetta 2005). Furthermore, the systems containing only 5% of oil presented greater activity when compared to the pure oil, showing the importance of the delivery systems for natural oils (Figure 1).

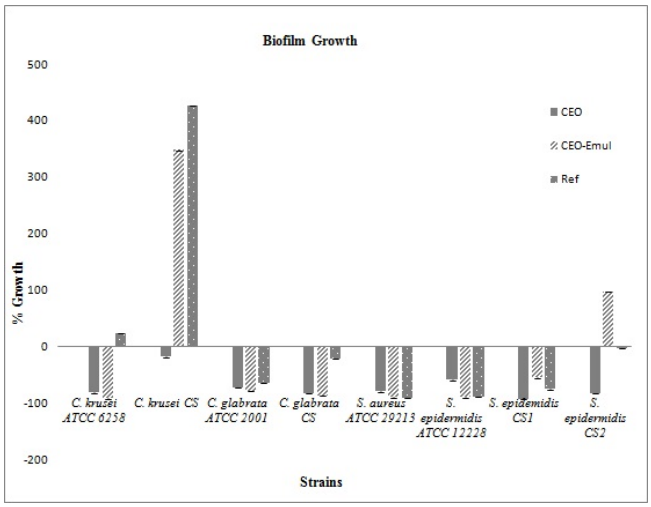

Figure 1: Biofilm growth in the presence of CEO and CEO-emul. CEO: Copaiba essential oil; CEO-Emul: Copaiba essential oil emulsion; Ref: Antimicrobial reference (Ketoconazole or Chloramphenicol).

In addition, the CEO-emul showed better adhesion inhibition of strain *S. epidermidis* ATCC 12228 compared to the CEO, showing that even the small percentages of CEO in the emulsion provided the best antibiofilm activity compared to larger amounts of CEO. This fact is relevant as a source of alternative therapy, since these microorganisms may exhibit antimicrobial resistance to produce biofilm (O'Gara 2001).

Concerning the strains of *C. krusei* ATCC 6258 and *C. glabrata* ATCC 2001, the CEO-emul showed a better antibiofilm activity than Ketoconazole. This result may be explained by the cross-resistance against these antifungal agents, since the azoles have very similar chemical structures (Kanafani 2008).

The *C. krusei* ATCC 6258 and *S. epidermidis* CS2 biofilm inhibition by the COE-emul was not satisfactory, showing a probable resistance of these microorganisms to the production of biofilm since both strains also showed great resistance to the reference antimicrobial agents.

Thus, the activity of CEO-emul against strains investigated in this study may be an alternative therapy for the treatment of infectious diseases, since many microorganisms, which produce biofilms, are resistant to traditional antimicrobial treatment.

CONCLUSION

The results showed a better antibiofilm activity of the CEO-emul compared to the CEO, mainly against strains of *S. epidermidis* ATCC 12228, *C. krusei* ATCC 6258 and *C. glabrata* ATCC 2001. This information is important due to the wide range of resistant strains to antimicrobial and antifungal agents. Thus, the use of this natural product along with delivery systems may become a potential alternative for the treatment of infectious skin diseases triggered by biofilm formation.

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