Emulsions based on copaiba essential oil: a source of treatment for infectious diseases

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INTRODUCTION AND OBJECTIVE

Copaiba oil (CO) is used in folk medicine due to its antimicrobial and anti-inflammatory activities (Sousa 2011). This antimicrobial activity has been demonstrated as a valuable alternative to treat cutaneous infections caused by fungi and bacteria. Nevertheless, the lipophilic nature of CO impairs its use as a topical antimicrobial medicine (Xavier 2012).

Copaiba essential oil (CEO), a volatile fraction extracted from the CO, usually presents better pharmacological activities since it concentrates the sesquiterpenes components (Sousa 2011). Thus, the CEO is an important biodegradable and natural source for the treatment of infectious diseases caused by microorganisms resistant to current therapies (Souza 2011).

The low solubility and organoleptic characteristics of these natural products impeaches their use. In this context, these products may be incorporated into delivery systems for improving their pharmacokinetic properties (Donsì 2012). Emulsions are traditional and viable thermodynamically unstable drug delivery systems that contains an aqueous and an oily phase, one dispersed in the other (Xavier 2012).

The aim of this study was to evaluate the antibacterial and antifungal activities of a CEO emulsion (CEOE), for future use as an antimicrobial medicine to treat cutaneous infections.

MATERIAL AND METHODS

Chemicals - The CO-resin 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 - The following strains were used: *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC27853, *Candida albicans* ATCC 90027, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC 2001, *C. krusei* ATCC 6258, *C. tropicalis* ATCC 13803. Additionally, six clinical strains (CS) of bacteria and five of yeasts were provided by LMMM (Laboratório de Micologia Médica e Molecular), UFRN.

Extraction and characterization of CEO - The CO was subjected to hydrodistillation with deionized water for 4 hours at 100 ° C using a Clevenger apparatus. The generated CEO was posteriorly characterized by Gas Chromatography coupled to a Mass Spectrometer.

Emulsion preparation - The emulsion was prepared according to phase inversion technique. The Span 80® (0.44 %) was added 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. The obtained emulsion was characterized by pH, conductivity, zeta potential, polidispersity and droplet size.

Antimicrobial Screening – The antibacterial and antifungal activities were initially performed using a susceptibility test with Muller-Hinton Agar for bacteria and Mueller-Hinton Agar + 2 % Glucose and 0.5 µg/mL Methylene Blue Dye for yeasts. 10µL of the CEO or the emulsion were added to the wells. The CEO was prepared in DMSO (1 %) and chloramphenicol and ketoconazole were used as synthetic antimicrobial controls.

Minimum inhibitory concentration (MIC) - A microdilution broth assay with serial dilutions of the samples was performed with sterile Muller-Hinton Broth in a 96-well microplate from 5 to 1000 mg/L. The suspension of each microorganism was prepared in NaCl solution 0.9 % adjusted to the 0.5 McFarlandand's scale and 100 µL of the suspension was then added to each well. The plates containing bacteria were incubated for 24h at 37°C while the fungi were incubated for 48h at 37°C in a rotary shaker (Tecnal) at 150 rpm.

Statistical analysis - The results are presented as the mean ± S.D. Statistical significance between 3 groups was performed by ANOVA followed by Tukey's test. Students t-test was used between 2 unpaired groups. P values less than 0.05 (p<0.05) were used as significant.

RESULTS AND DISCUSSION

The Gas Chromatography showed a predominance of sesquiterpenes, showing the basic composition of the CEO, evidencing the volatile composition of this oil.

The emulsion showed a pH of 3.48 and a conductivity of 226.20µS. The droplet size was 0.28 ± 0.01 µm with a polidispersity of 0.14 ± 0.02 and a zeta potential of - 27.08 ± 0.89.

The antimicrobial screening to strains of bacterial and yeast revealed that the CEO showed antimicrobial activity against some microorganisms (Table 1). This fact is important for the treatment of infectious diseases affected by these microorganisms that are resistant to traditional antimicrobial and antifungal agents.

Table 1: Inhibition halos measures (mm) of CEO.

Microorganisms	CEO
***S. aureus* ATCC 29213**	9.88 ± 2.14
***S. epidermidis* ATCC 12228**	14.00 ± 2.39
***S. epidermidis* CS1**	11.89 ± 1.96
***S. epidermidis* CS2**	13.11 ± 3.14
***C. glabrata* ATCC 2001**	12.89 ± 6.03
***C. glabrata* 15V3C (CS)**	9.88 ± 0.78
***C. krusei* ATCC 6258**	13.67 ± 3.42
***C. krusei* LMM54 (CS)**	13.78 ± 3.23

It was also observed that the strain *S. epidermidis* ATCC 12228 showed a great susceptibility to CEO. This fact is promising for the treatment of infections, since there is a prevalence of methicillin-resistant *S. epidermidis* and the emergence of vancomycin-resistant contributes significantly to morbidity and mortality in hospitalized patients (O'Gara 2001).

Table 2: MIC (mg/L) of CEO and CEOE

Microorganisms	CEO	CEOE
***S. aureus* ATCC 29213**	55437.5 ± 0.0	> 249250.0 ± 0.0
***S. epidermidis* ATCC12228**	221715.0 ± 0.0	> 249250.0 ± 0.0
***S. epidermidis* CS1**	221715.0 ± 0.0	> 249250.0 ± 0.0
***S. epidermidis* CS2**	221715.0 ± 0.0	> 249250.0 ± 0.0
***C. glabrata* ATCC 2001**	108.3 ± 76.6	15578.1 ± 0.0
***C. glabrata* 15V3C (CS)**	108.3 ± 38.3	973.6 ± 0.0
***C. krusei* ATCC 6258**	34648.8 ± 0.0	15578.1 ± 0.0
***C. krusei* LMM54 (CS)**	34648.8 ± 0.0	3894.5 ± 0.0

The MIC results based on CEOE presented better activity for the strains of *C. krusei* and *C. glabrata*, showing that the emulsions have an antifungal activity against resistant and low sensitive strains to azoles, respectively, with only 5 % oil at low concentrations. The composition and hydrophobicity of the oil are important characteristics that confer antimicrobial activity by its interaction with the fungal membrane lipids becoming permeable to other cellular constituents (Galv 2012) and their interaction with the cellular metabolic mechanism (Marino 2001) (Table 2). This suggests that the oil droplets present in the system directs the active components for the target site by improving the action of natural products (Xavier 2012).

CONCLUSIONS

It may be concluded that the CEOE preserved and increased the pharmacological activity of CEO, showing a significant activity against some strains, especially against *C. krusei* ATCC 6258 and *C. krusei* LMM54 (CS). The results demonstrate that CEOE may be a promising candidate as antimicrobial product against some bacteria and fungi that causes superficial infections.

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