

Bacteriostatic Effect of Copaiba Oil (*Copaifera officinalis*) against *Streptococcus mutans*

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This study evaluated the inhibitory activity of copaiba oil (*Copaifera officinalis*) against the cariogenic microorganism, *Streptococcus mutans*. For such purpose, a minimum inhibition concentration test of copaiba oil against *S. mutans* was performed, using the serial dilution in broth technique, with a negative control, a positive control (0.12% chlorhexidine) and a 10% copaiba oil solution as a test. A minimum bactericidal concentration test with tubes presenting microbial inhibition was also conducted. In the minimum inhibitory concentration test, copaiba oil showed inhibition of bacterial growth at all concentrations tested up to 0.78 µL/mL of the 10% copaiba oil solution in the broth. In addition, the negative control had no inhibition, and the 0.12% chlorhexidine solution was effective up to 6.25 µL/mL in the broth. Copaiba oil showed a bacteriostatic activity against *S. mutans* at low concentrations, and could be an option of phytotherapeutic agent to be used against cariogenic bacteria in the prevention of caries disease.

Key Words: dentistry, cariology, antimicrobials, phytotherapies.

INTRODUCTION

Teeth have several important functions such as mastication and speech (1). Dental caries affects patients, especially young children, so often causing significant loss of tooth structure (2,3). *Streptococcus mutans*, is an important microorganism in the production of acid in the dental plaque (4,5). The reduction of bacteria associated with caries in the dental plaque is a major preventive strategy and is also used as treatment resource (6).

Chlorhexidine has been used for decades as an antimicrobial agent in dentistry because it reduces the number of certain microorganisms (2,4). However, its use should be restricted to a few days due to various side effects (7-9), and thus a substance with no collateral effects has been sought (10). Copaiba oil has been used for various purposes in traditional medicine therapies in Brazil (11,12). Many of its properties, such as its antibacterial action, have been studied to include this

phytotherapeutic agent as an option for the treatment of infections (13,14).

The aim of this study was to evaluate the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of copaiba oil against the cariogenic microorganism *S. mutans*.

MATERIAL AND METHODS

Three solutions were used. The negative control solution was prepared with 30 mL of alcohol 96°, 20 mL of propylene glycol, 20 mL of polyethylene glycol, 20 mL of glycerin and 10 mL of distilled water. The test solution consisted of negative control solution replacing distilled water by the same amount of copaiba oil. For the positive control solution, chlorhexidine was added to the negative control solution to reach a final concentration of 0.12%. The microorganism used was a standard strain of *S. mutans* (ATCC 25175; American Type Culture

Collection, Manassas, VA, USA).

The MICs of the solutions against *S. mutans* were obtained as follows. Using the method of successive dilutions of solutions in liquid culture medium (15), the MICs were prepared with a series of 9 culture tubes containing brain heart infusion (BHI) (Oxoid, Hampshire, UK) broth contaminated by *S. mutans* suspension in saline, adjusted to McFarland standard tube #1. The first 8 tubes of each series contained decreasing concentrations of one of the solutions (100 $\mu\text{L}/\text{mL}$ to 0.78 $\mu\text{L}/\text{mL}$) and the 9th tube had the control of bacterial growth without solution. The tubes were incubated at 35.5°C for 48 h, and the inhibition was analyzed visually, recognizing the microbial growth by turbidity of the medium. The MIC was considered as the lowest concentration of the solution required to inhibit microbial growth without turbidity of the medium compared with a negative control without bacterial contamination.

The MBCs of the solutions against *S. mutans* were obtained as follows. For all tubes where the bacterium was inhibited in the MIC assay, using the method described by Phillipps (16), 0.1 mL aliquots of the contents were added to Petri dishes with 30 mL of BHI agar and incubated at 35.5°C for 24 h. The dishes were analyzed visually to obtain the MBC, which was the highest dilution that killed 99.9% of bacteria, showing no growth on the Petri dish. All tests were performed in triplicate.

RESULTS

Regarding the MIC of solutions against *S. mutans*, the copaiba oil showed antimicrobial action against the bacterial strain at all tested concentrations, as indicated by the inhibition of growth and turbidity of the medium in all tubes. The MIC obtained in this test was 0.78 $\mu\text{L}/\text{mL}$ of solution in the culture medium. For the tubes with different concentrations of 0.12% chlorhexidine solution, inhibition of growth was observed in the first five tubes and the MIC obtained in this test was 6.25 $\mu\text{L}/\text{mL}$ of solution in the culture medium. For other concentrations, microbial growth was identified by turbidity of the medium. The negative control solution had no inhibitory activity against *S. mutans* in the concentrations used.

Regarding the MBC of the solutions against *S. mutans*, all the dishes showed microbial growth after 24 h, with no bactericidal action of any concentration of solutions against *S. mutans*. This indicates that the antimicrobial activity reported in the MIC test was bacteriostatic for both the copaiba oil and the positive

control. The inoculum used for MIC was obtained by counting of viable bacteria in the Petri dishes, and the result was 1.6×10^6 colony forming units.

DISCUSSION

The antimicrobial activity of the copaiba oil was demonstrated in the MIC test, as this solution interfered with the microbial growth at all tested concentrations. This antimicrobial activity has been described by Pieri et al. (13) against other oral microorganisms such as *S. pyogenes* and *S. salivarius*. Copaiba oil has advantages over chlorhexidine because it is composed by several different substances that may have different interactions with the bacterial cell, reducing the development of resistant *S. mutans* strains. This justifies the use of only the ATCC strain in the present study, as field isolates are expected to have similar susceptibility to the antimicrobial effect of the Copaiba oil compared to the standard strain.

This inhibition, however, seems to be bacteriostatic in nature, as none of the concentrations caused 99.9% of bacterial destruction in the MBC test. According Souza et al. (17), this bacteriostatic activity may be attributed to the copalic acid, a component of this oil. Packer and Luz (18) did not find microbial inhibition by copaiba oil against other bacterial strains as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with some strains having resistance to the oil's components.

Bacteriostatic activity of 0.12% chlorhexidine solution was found at concentrations between 100 and 6.75 $\mu\text{L}/\text{mL}$. Swerts et al. (19) obtained similar results but also found inhibitory activity at lower chlorhexidine concentration.

The findings of the present study suggest that the copaiba oil has great potential for use against the growth of *S. mutans*, the main etiological agent of dental caries. In addition, even at low concentration, the copaiba oil was as effective as 0.12% chlorhexidine against this pathogen. However, further studies should be done involving analysis of the ideal concentration in the use of copaiba oil and the possible collateral effects of this phytotherapeutic agent in long-term treatment.

RESUMO

Este estudo avaliou a atividade inibitória do óleo de copaíba (*Copaifera officinalis*) contra o microrganismo cariogênico, *Streptococcus mutans*. Para isso, foi realizado um teste de concentração mínima inibitória do óleo de copaíba contra *S.*

mutans, utilizando a técnica de diluição seriada em caldo, com um controle negativo, um controle positivo (clorexidina a 0,12%) e uma solução de óleo de copaíba 10% como teste. Também foi conduzido um teste de concentração mínima bactericida com os tubos que apresentaram inibição microbiana. No teste de concentração inibitória mínima, o óleo de copaíba mostrou inibição do crescimento bacteriano em todas as concentrações testadas até 0,78 µL/mL da solução a 10% do óleo de copaíba no caldo. Além disso, o controle negativo não teve nenhuma inibição, e a solução de clorexidina 0,12% foi eficaz até 6,25 µL/mL no caldo. O óleo de copaíba mostrou uma atividade bacteriostática contra *S. mutans* em baixas concentrações, apresentando-se assim como uma opção de fitoterápico a ser utilizado contra bactérias cariogênicas na prevenção de cáries.

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