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Comparative study of the antibacterial effectiveness of copaiba oil versus sodium hypochlorite against endodontic biofilm on Gutta-Percha cones

Irene Victoria Cevallos Baque*, Kevin David Villota Trejo
Faculty of Health Sciences, Los Hemisferios University,Postgraduate Program in Endodontics, Quito, Ecuador
Corresponding Author: Irene Victoria Cevallos Baque

Abstract

Objective: To evaluate and compare the antibacterial efficacy of 10% copaiba oil and 5.25% sodium hypochlorite in disinfecting gutta-percha cones experimentally contaminated with *Enterococcus faecalis*, Streptococcus mutans, and *Streptococcus oralis.*

Materials and Methods: An in *vitro* comparative experimental study was conducted using 180 gutta-percha cones, randomly assigned to nine experimental subgroups (n = 20 each). The cones were contaminated with standardized bacterial strains and subsequently treated with 10% copaiba oil, 5.25% sodium hypochlorite, or distilled water (negative control). Antibacterial activity was assessed by counting colony-forming units (CFU). Data normality was tested using the Shapiro–Wilk test, and intergroup comparisons were performed with the Kruskal–Wallis test followed by Dunn's post hoc test, with a significance threshold of $p < 0.05$.

Results: Sodium hypochlorite exhibited the greatest antibacterial efficacy against all three bacterial species, with mean counts of 0.8 ± 2 CFU for E. faecalis, 1.4 ± 2.3 CFU for S. mutans, and complete inhibition of S. oralis. Copaiba oil also significantly reduced bacterial growth compared with the negative control. Statistically significant differences were observed among the three treatments for all bacterial species ($p < 0.005$).

Conclusions: 5.25% sodium hypochlorite demonstrated the highest antibacterial efficacy; however, 10% copaiba oil showed notable antimicrobial activity, particularly against S. oralis, suggesting its potential as a natural alternative and warranting further investigation in endodontics.

Keywords: Copaiba oil, sodium hypochlorite, gutta-percha cone, disinfection, endodontics, biofilm, *Enterococcus faecalis*, Streptococcus oralis, Streptococcus mutans

Introduction

Endodontic infections are among the primary causes of pulpal and periapical disease, with the persistence of microorganisms within the root canal system being a major factor contributing to treatment failure (Cabrera, Ruiz, & Farfan, 2021). Although mechanical instrumentation substantially reduces the microbial load, the anatomical complexity of the root canal system makes complete eradication of microorganisms challenging.

Therefore, chemical disinfection remains an essential component of endodontic treatment (Kowalski *et al*., 2024) [6].

Gutta-percha cones are the most widely used obturation material in endodontics due to their favorable physical and biological properties. However, they may become contaminated during manufacturing, storage, or clinical handling, making them a potential source of root canal reinfection. Because this material cannot be heat-sterilized without compromising its properties, immediate chemical disinfection before use is widely recommended (Rodríguez, 2021; Saavedra & Cabrera, 2022) [13, 14].

Sodium hypochlorite is considered the reference disinfectant owing to its strong antimicrobial efficacy and ability to dissolve organic tissue. Nevertheless, several studies have reported that it can induce surface alterations in gutta-percha cones, increasing roughness and potentially affecting the quality of the endodontic seal. These limitations have driven the search for alternative agents with sufficient antimicrobial activity, lower toxicity, and greater biocompatibility (Pedraza *et al*., 2025; Palacios, 2024; Marín, 2024; Galvis *et al*., 2024) [5, 7, 8, 9].

Among natural alternatives, copaiba oil stands out as an oleoresin derived from species of the genus Copaifera, recognized for its anti-inflammatory, wound-healing, and antimicrobial properties. Several studies have demonstrated its activity against microorganisms associated with oral infections; however, evidence regarding its effectiveness in disinfecting gutta-percha cones contaminated with endodontic bacteria remains scarce. This highlights the need for experimental studies to compare its performance with conventional disinfectants (Pedrinha *et al*., 2025; Pieri *et al*., 2012; Vanapatla *et al*., 2022; Rai *et al*., 2019) [10, 11, 12, 16].

In this context, the objective of the present study was to evaluate and compare the antibacterial efficacy of 10% copaiba oil and 5.25% sodium hypochlorite in disinfecting gutta-percha cones experimentally contaminated with *Enterococcus faecalis*, Streptococcus mutans, and *Streptococcus oralis.*

Materials and Methods

An *in vitro* comparative experimental study was carried out to evaluate and compare the antibacterial efficacy of 10% copaiba oil and 5.25% sodium hypochlorite in disinfecting gutta-percha cones experimentally contaminated with bacteria associated with endodontic infections. The study was conducted at the General Microbiology Laboratory of

Los Hemisferios University under controlled biosafety conditions, following standardized experimental protocols and microbiological procedures.

Experimental Sample

The sample consisted of 180 new gutta-percha cones from the same commercial brand and manufacturing batch, randomly allocated into nine experimental subgroups (n = 20). The groups were defined according to the microorganism tested (*Enterococcus faecalis*, Streptococcus mutans, and Streptococcus oralis) and the disinfectant agent applied (10% copaiba oil, 5.25% sodium hypochlorite, or distilled water as a negative control).

The standard strains *Enterococcus faecalis* ATCC 29212, Streptococcus mutans ATCC 25175, and Streptococcus oralis ATCC 6249 were used. The microorganisms were cultured on blood agar and incubated at 37 °C. The bacterial concentration was then adjusted to a 0.5 McFarland standard and diluted to obtain a suspension of approximately 5×10^5 CFU/mL.

Experimental Procedure

The gutta-percha cones were contaminated by immersion in the bacterial suspension for 10 minutes and subsequently dried on sterile absorbent paper. They were then exposed for five minutes to one of the following treatments: 10% copaiba oil, 5.25% sodium hypochlorite, or distilled water (negative control). After the exposure period, the cones were transferred to brain heart infusion (BHI) broth, vortexed for one minute, and inoculated onto blood agar plates. The plates were incubated under anaerobic conditions at 37 °C for 24 hours. The number of colony-forming units (CFU) was determined and served as the primary study outcome.

Statistical Analysis

The data were analyzed using descriptive statistics, expressed as means and standard deviations. Normality was assessed with the Shapiro–Wilk test. Since the variables did not follow a normal distribution, intergroup comparisons were performed using the Kruskal–Wallis test, followed by Dunn's multiple-comparisons analysis. Statistical significance was set at $p < 0.05$.

Results

The antibacterial efficacy of 10% copaiba oil, 5.25% sodium hypochlorite, and distilled water was evaluated by quantifying colony-forming units (CFU) of *Enterococcus faecalis*, Streptococcus mutans, and *Streptococcus oralis.* The Shapiro–Wilk test confirmed that the data were not normally distributed; therefore, nonparametric tests were applied for comparative analysis. Sodium hypochlorite exhibited the highest antibacterial activity against all three bacterial species. For E. faecalis, the mean count was 0.8 ± 2 CFU, whereas copaiba oil yielded 31.3 ± 9.9 CFU, and the control group showed the highest bacterial growth. Similarly, for S. mutans, sodium hypochlorite produced a mean of 1.4 ± 2.3 CFU, significantly lower than that observed with copaiba oil (58 ± 25.4 CFU) and the negative control. In the case of S. oralis, sodium hypochlorite completely inhibited bacterial growth, while copaiba oil yielded a mean of 7.4 ± 3.3 CFU, demonstrating considerably greater antimicrobial activity than the control group (Table 1).

Table 1: Colony-forming unit (CFU) count by microorganism and treatment

Microorganism	10% Copaiba oil Mean ± SD	Distilled water	5.25% Sodium hypochlorite Mean ± SD
Enterococcus faecalis	31,3 ± 9,9	Increased bacterial growth	0,8 ± 2,0
Streptococcus mutans	58,0 ± 25,4	Increased bacterial growth	1,4 ± 2,3
Streptococcus oralis	7,4 ± 3,3	Increased bacterial growth	0

Source: Created by the author

Analysis using the Kruskal-Wallis test revealed statistically significant differences among the three treatments for the three bacterial species evaluated ($p < 0.005$) (Table 2).

Table 2: Comparison of Response Across Substances (Kruskal-Wallis)

Bacteria	Kruskal-Wallis	p
Enterococcus faecalis	49.8	<0.005
Streptococcus mutans	55.15	<0.005
Streptococcus oralis	54.65	<0.005

Source: Created by the author

Multiple comparisons using Dunn's test confirmed significant differences among sodium hypochlorite, copaiba oil, and the negative control; this demonstrates that both agents had an antibacterial effect. However, sodium hypochlorite showed the highest efficacy against all the microorganisms analyzed. Statistically significant differences were observed among the three treatments evaluated (Table 3).

Table 3: Multiple comparisons of the behavior of the three bacteria in response to the three substances in the study

Bacteria	Comparison	p
Enterococcus faecalis	Copaiba vs. Hypochlorite	<0.005
	Copaiba vs. Distilled Water	0.011
	Hypochlorite vs. Distilled Water	<0.005
Streptococcus mutans	Copaiba vs. Hypochlorite	<0.005
	Copaiba vs. Distilled Water	<0.005
	Hypochlorite vs. Distilled Water	<0.005
Streptococcus	Copaiba vs. Hypochlorite	<0.005
	Copaiba vs. Distilled Water	<0.005
	Hypochlorite vs. Distilled Water	<0.005

Source: Created by the author

Sodium hypochlorite at 5.25% was found to be effective against the three microorganisms evaluated, while copaiba oil showed similar efficacy against S. oralis (Table 4).

Table 4: Antibacterial efficacy observed against the microorganisms tested

Treatment	E. faecalis	S. mutans	S. oralis
5.25% Sodium Hypochlorite	Very high	Very high	Complete
10% Copaiba Oil	Moderate	Moderate	High
Distilled water	Ineffective	Ineffective	Ineffective

Source: Prepared by the author based on experimental results

Discussion

The present study compared the antibacterial efficacy of 10% copaiba oil and 5.25% sodium hypochlorite in disinfecting gutta-percha cones contaminated with *Enterococcus faecalis*, *Streptococcus mutans*, and *Streptococcus oralis.* The results demonstrated that sodium hypochlorite exhibited the highest antimicrobial activity against all three bacterial species. The superior efficacy of sodium hypochlorite observed in this study is consistent with previous research identifying it as the endodontic irrigant and disinfectant with the broadest antimicrobial spectrum. Its ability to oxidize cellular components, denature bacterial proteins, and dissolve organic tissue explains the markedly low number of colony-forming units detected across the three bacterial groups. Several authors have also reported that concentrations of 5.25% enable rapid and effective disinfection of gutta-percha cones prior to clinical use, findings that align with the present results. These outcomes are, in agreement with those reported by Galvis *et al*. (2024), Pedraza *et al*. (2025), Aucinaitė *et al*. (2025) [2, 9], and Pedrinha *et al*. (2025), who consistently demonstrated high efficacy of sodium hypochlorite against microorganisms associated with endodontic infections.

Nevertheless, copaiba oil significantly reduced bacterial growth compared with the negative control, confirming its antibacterial activity, particularly against *S. oralis*. These findings suggest that, although sodium hypochlorite remains the reference disinfectant for gutta-percha cones, copaiba oil represents a promising natural alternative with potential applications in endodontics. Its antimicrobial effect has been primarily attributed to the presence of diterpenes and sesquiterpenes, bioactive compounds capable of disrupting bacterial cell membranes and interfering with mechanisms essential for microbial growth.

(Pedrinha *et al*., 2025) [10]. Although the bacterial reduction was lower than that achieved with sodium hypochlorite, the observed behavior confirms that copaiba oil possesses antimicrobial properties and supports the growing interest in the use of natural products as a complement to endodontic disinfection. This mechanism has been previously described by Pieri *et al*. (2012) [11], who demonstrated inhibitory activity against Streptococcus mutans, and by Diefenbach *et al*. (2018) [4], who reported activity against various microorganisms associated with oral biofilms.

A particularly notable finding was the greater activity of copaiba oil against *Streptococcus oralis*, for which a considerably lower number of colony-forming units was recorded compared with *E. faecalis* and *S. mutans*. This difference may be related to the varying susceptibility of each bacterial species to the metabolites present in the oleoresin, as well as structural differences in the cell wall and in the mechanisms involved in adaptation to antimicrobial stress. Such variations in bacterial response to natural compounds have been highlighted in previous studies, including those of Pieri *et al*. (2012) and Diefenbach *et al*. (2018) [4, 11], reinforcing the potential of copaiba oil as a natural antimicrobial agent in dentistry.

In contrast, *Enterococcus faecalis* exhibited marked resistance to treatment with copaiba oil. This behavior is consistent with the literature, which identifies this bacterium as one of the principal microorganisms implicated in endodontic treatment failure due to its ability to survive in nutrient-deprived environments, penetrate dentinal tubules, and form biofilms resistant to multiple antimicrobial agents. These characteristics explain why *E. faecalis* remains a microorganism of major interest in endodontic research and support the superior performance of sodium hypochlorite against this species. This finding is consistent with the systematic review by Ardila *et al*. (2026) [1], which emphasized that *E. faecalis* continues to represent the greatest microbiological challenge in endodontics. The authors also noted that several natural products exhibit promising antimicrobial activity; however, no evidence indicates that any of these substances surpass sodium hypochlorite in eradicating mature biofilms. Consequently, the results of the present study are in line with the available evidence and reinforce the need for continued research into complementary alternatives capable of enhancing the elimination of this microorganism without increasing the toxicity associated with clinical procedures.

Another important finding relates to the clinical implications of using sodium hypochlorite for the disinfection of gutta-percha cones. Although its antimicrobial efficacy was clearly superior to that of copaiba oil, several authors have reported that this agent may cause surface alterations to gutta-percha cones, which may subsequently promote the formation of crystalline deposits, increased surface roughness, and potential changes in the adaptation of root canal sealers. In this regard, the systematic review by Silva *et al*. (2024) [15] on the influence of disinfectant solutions on the surface topography of gutta-percha cones concluded that, although sodium hypochlorite maintains the best microbiological performance, some unfavorable effects on the physical properties of the material remain a concern. Therefore, further investigation of biocompatible alternatives

remains relevant to achieve an appropriate balance between antimicrobial efficacy and preservation of the surface integrity of gutta-percha.

From a clinical perspective, the results suggest that copaiba oil could represent an option of interest for the development of new disinfection protocols, not as a replacement for sodium hypochlorite as a disinfectant, but rather as a potential basis for the development of future disinfectant formulations of natural origin. Its antimicrobial, anti-inflammatory, and biocompatibility, along with its anti-inflammatory and antioxidant properties, are benefits that endorse additional research to optimize its concentration, contact time, and delivery systems. Several studies have indicated that the efficacy of plant extracts depends on both the concentration used and the exposure time. Diefenbach *et al*. (2018) [4], for example, identified significant antimicrobial activity of copaiba oil against various oral pathogens, while Pedrinha *et al*. (2025) [10] concluded that natural compounds represent an emerging strategy for complementing endodontic disinfection because of their biological potential and lower toxicity. These factors could be optimized in future studies to enhance the activity of copaiba oil.

Nevertheless, the evidence obtained in this study is insufficient to recommend replacing sodium hypochlorite in clinical practice, as the latter demonstrated significantly greater efficacy against all microorganisms evaluated. Consequently, copaiba oil should, at present, be regarded as an agent with potential for future research rather than as a replacement for the conventional disinfectant.

The present study has several strengths, including the application of a controlled experimental design, standardized ATCC strains, random allocation of samples, and the inclusion of three microorganisms of clinical relevance in endodontics. These characteristics add to the internal validity of the results and allow for an objective comparison of the treatments evaluated. However, care should be taken when extrapolating the results since they were obtained in an *in vitro* model under controlled experimental conditions. However, care should be taken when extrapolating the results since they were obtained in an *in vitro* model under controlled experimental conditions.

Conclusions

Sodium hypochlorite 5.25% was the most effective disinfectant against *Enterococcus faecalis*, Streptococcus mutans and Streptococcus oralis contaminated gutta-percha cones confirming its role as a reference disinfectant in endodontics.

The 10% concentration of copaiba oil demonstrated significant antimicrobial activity against all three tested microorganisms, with the highest activity against *Streptococcus oralis.* While not as effective as sodium hypochlorite, the results support its potential as a natural alternative for future research to develop more biocompatible disinfection protocols.

The results obtained contribute to increasing the available evidence regarding the use of copaiba oil in endodontics and serve as a basis for designing preclinical and clinical studies to confirm its applicability in clinical conditions.

Limitations

The present study has several limitations that should be considered when interpreting the results. First, this was an *in vitro* study; therefore, the experimental conditions do not fully reproduce the biological environment of the root canal system. Second, only three standardized bacterial strains were evaluated, which limits the extrapolation of the results to multispecies endodontic biofilms present under clinical conditions. Likewise, the study focused exclusively on antibacterial efficacy and did not assess potential changes in the physical or surface properties of the gutta-percha cones following exposure to the disinfecting agents. Finally, variables related to cytotoxicity, biocompatibility, and clinical performance were not evaluated. These aspects should be addressed in future research to establish the true therapeutic potential of copaiba oil.

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