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Limited Benefit of Copaifera Oil on Gingivitis Progression in Humans

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Abstract

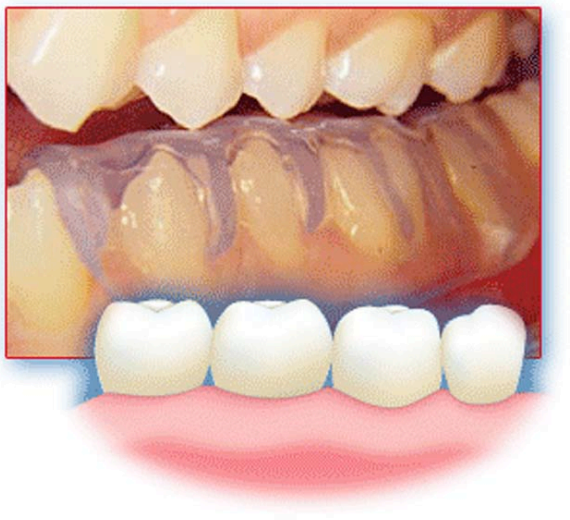
Aim: The aim of this *in vivo* study was to evaluate the antiplaque and antigingivitis effect of Copaifera sp (Cp).

Methods and Materials: Twenty-three subjects participated in a randomized controlled clinical trial using a 21-day, partial-mouth experimental model of gingivitis. A custom tooth shield was fabricated for each subject to prevent the brushing of the four experimental posterior teeth in the lower-left quadrant. The subjects were randomly assigned to either the control group (using a placebo gel) or the test group (using the test gel containing 10% *Cp*).

Results: The clinical results showed statistically significant differences for plaque (PLI), bleeding (BI), and gingival (GI) indexes at days 0 and 21 in both groups ($p<0.05$). However, on day 21 there was no statistically significant difference between groups for all indexes ($p>0.05$).

Conclusions: The test gel containing 10% *Cp* did not prevent plaque formation and development of gingivitis.

Clinical Significance: Several medicinal herbs are used empirically by persons in the treatment and prevention of oral conditions. Research in this area must be encouraged to determine which herbal agents would be a useful addition to the current range of chemotherapeutic periodontal treatment options.



Keywords: Dental plaque, gingivitis, *Copaifera sp*, randomized controlled clinical trial.

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Introduction

Plaque-induced gingivitis is one of the most frequent periodontal diseases, affecting more than 90% of the population, regardless of age, sex, or race. Epidemiologic studies have shown a high

prevalence of gingival inflammation, although the mean individual percentage of gingival bleeding varies from 28% to 35%.[1]

Mechanical plaque control is the most effective method of controlling plaque and gingivitis.[2,3] However, the lack of knowledge and ability among the normal adult population to adequately brush and floss their teeth has led to the search for chemotherapeutic agents in order to improve plaque control.[4] Such chemicals, mainly triclosan and chlorhexidine, have been used as mouth rinses or added to dentifrices to prevent plaque formation and development of gingivitis.[4,5,6,7] As some of these substances may have undesirable side effects, such as tooth staining and taste alteration, phytotherapeutic agents with antimicrobial and anti-inflammatory properties have been investigated.[8,9,10]

The use of natural products in the prevention and treatment of oral conditions has increased recently and could be beneficial for patients in low-socioeconomic-level urban and rural communities.[11] *Copaifera sp* (*Cp*) oil (Copaiba) that is extracted from a large tree commonly found in the northeast region of Brazil, Venezuela, and Colômbia is among the various herbal agents currently available.[12] The oil presents antimicrobial, anti-inflammatory, and healing properties and is used for diverse diseases, such as throat and stomach ailments.[12,13]

Biocompatibility of *Cp* was evaluated in the subcutaneous tissue of rats, showing that this phytotherapeutic was well tolerated[14,15] and animal studies have demonstrated favorable results in the healing process using this herbal agent.[16,17] At the time of the present study, there is no reported controlled trial evaluating the efficacy of a gel containing Cp in the control of plaque and gingivitis. Thus, the purpose of the present study was to assess the antiplaque and antigingivitis effects of this phytotherapeutic agent.

Copaifera leaves

Methods and Materials

Subjects

Twenty-five dental students from the University of Fortaleza (12 female and 13 male aged 19 to 25 years) were enrolled in this study. All subjects had at least 20 natural teeth, among which were the four posterior teeth in the lower left quadrant (experimental teeth). All students, randomly screened, were informed about the nature of the study and signed an informed consent form in compliance with the guidelines of the Brazilian Health Council. Participants with medical disorders and under antimicrobial therapy, as well as smokers, pregnant women, and individuals presenting a probing depth ≥3 mm associated with any mandibular teeth were excluded from the trial. Subjects with local plaque retentive factors, such as carious lesions and bulky restorations in the test area, also were excluded. The protocol was approved by the university's Ethics Committee (Report Coética no. 144/2007, University of Fortaleza).

Tooth Shield Fabrication

An alginate impression of the experimental teeth was taken and die stone poured in to obtain casts. A 0.3-mm-thick spacer was then fabricated on each stone cast, using thermoplastic mouthguard material and a vacuum former. Upon that spacer, an individual toothshield was made of a 2-mm-thick thermoplastic mouthguard material using the same vacuum former. The toothshield was trimmed 2 mm beyond the gingival margin to assure that gel would be in contact with the gingival margin of the experimental teeth during toothbrushing of the remaining teeth (Figures 1 and 2).

Test and Control Products

The control and test gels were formulated and packed into tubes in the Pharmaceutics Laboratory at the University of Fortaleza. The tubes were

previously coded to ensure that neither the examiner nor the participants knew the content until it was revealed by the pharmacist after the completion of the study. All subjects used both gels in alternate periods consistent with a crossover study. The test gel consisted of trietanolamine (q.s.p.), alcohol, water (q.s.p.), nipagin (0.2%), 10% *Cp* oil, a very small amount of menthol (flavoring), and a preservative. The control gel had the same formulation except for the omission of the *Cp* oil.

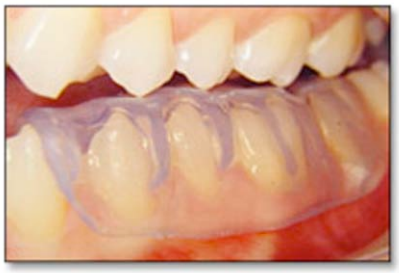


Figure 1. Toothshield *in situ*, facial view.

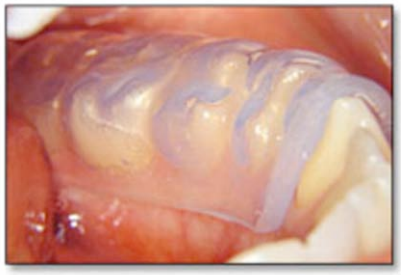


Figure 2. Toothshield *in situ*, lingual view.

Clinical Design

This study was a double-blind, randomized, controlled clinical trial of two crossover groups of dental students performed in two experimental phases of 21 days each with a one-month washout interval between them. A partial mouth experimental model was used.[18] To standardize the groups, the participants were submitted to a meticulous evaluation (pre-experimental phase) to score the plaque index (PLI),[19] the gingival index (GI),[20] and the bleeding index (BI)[21] of each tooth. All teeth of each subject were polished and flossed by the examiner to eliminate plaque remnants. The importance of oral hygiene was strongly reinforced.

Thirty days after the initial phase, the volunteers were randomly assigned to two groups by random

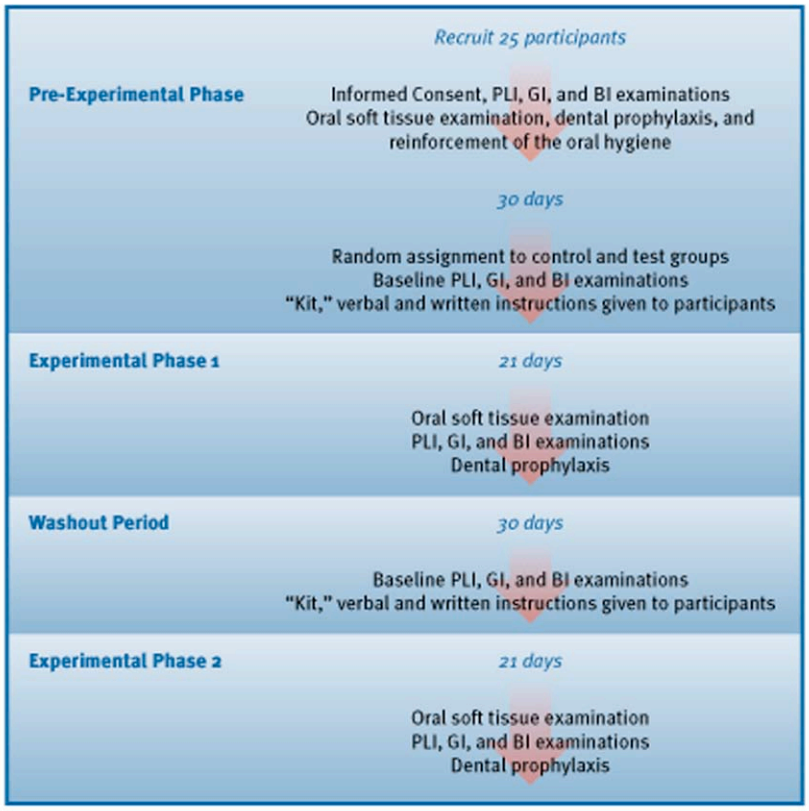


Figure 3. Schematic outline of the study design.

permutation of three and the experimental phase began. On day 0 of both experimental periods, PLI, GI, and BI were recorded. A personal "kit" was given to each student containing a tooth shield, a tube with 90g of either the control or test gel, and a commercial dentifrice with no anti-inflammatory properties (Sorriso®, Kolynos do Brazil Ltda., Osasco, SP, Brazil). During each 21-day experimental period, the participants were instructed to fill the toothshield with the gel prior to insertion in the mouth and seat it over the experimental teeth three times a day for at least one minute. The students refrained from brushing the test quadrant, while the other teeth were normally brushed three times a day using the commercial dentifrice. In addition to verbal instructions, the students were given written recommendations to follow at home. On the last day of each period (21st day), the indexes were recorded and the teeth were polished with pumice (Figure 3).

Clinical Assessment

All indices were recorded by the same examiner on the mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual surfaces of the experimental teeth. The values of six sites of each tooth were recorded to obtain the PLI, GI, and BI means. Then, the means for the four experimental teeth were calculated to determine index means of each volunteer. Intra-examiner agreement for all indexes was calculated by repeating the measurements on 10 patients with an interval of at least one hour. The Kappa coefficient was used to verify the agreement between the examinations. PLI, BI, and GI means were 0.72, 0.81, and 0.85, respectively. In addition to this examination, the hard and soft oral tissues were visually inspected for the presence of any adverse reaction by the same examiner.

Statistical analysis

The Mann-Whitney nonparametric test was used to estimate the difference between the control and test groups on days 0 and 21. In each group, the mean scores of all indexes were compared between baseline and the end of the trial by the Wilcoxon test. Confidence intervals of 95% were calculated for the difference in PLI, BI, and GI scores between groups with p-values ≤0.05 considered to be statistically significant.

Results

Twenty-three subjects completed the clinical trial. Two students were excluded from the study during the experimental phase due to third molar extraction. The test gel had good acceptance and showed no adverse effects, such as the formation of an abscess, ulcerations, or any allergic reactions.

On day 0, there was no statistically significant difference between the control and test groups with respect to PLI (p=0.9650), BI (p=0.6445), and GI (p=0.5904) means. These results indicated that both groups were well balanced at baseline (Tables 1, 2, and 3). On the 21st day, plaque (p=0.3619), gingival bleeding (p=0.8605), and gingivitis (p=0.5828) were present in both groups, but the difference between them was not statistically significant (Tables 1, 2, and 3; Figure 4).

Discussion

The use of antimicrobial mouth rinses may be considered a useful adjunct to oral hygiene. However, because of their cost, the majority of

Table 1. Plaque index (PLI) means and standard deviation on day 0 and day 21 for the control and test groups and the 95% confidence interval (CI) of the differences.

	Control	Test	95% CI
Day 0	1.81 ± 0.53 A,a	1.80 ± 0.60 A,a	-0.3285–0.3502
Day 21	3.09 ± 0.49 A,b	2.83 ± 0.90 A,b	-0.0983–0.7627

Notes: Means followed by the same uppercase letters (A) in the same line do not differ statistically (p<0.05).

Means followed by different lowercase letters (a,b) in the same column differ statistically (p<0.05).

Table 2. Bleeding index (BI) means and standard deviation on day 0 and day 21 for the control and test groups and the 95% confidence interval (CI) of the differences.

	Control	Test	95% CI
Day 0	0.010 ± 0.023 A,a	0.008 ± 0.027 A,a	-0.0125–0.0177
Day 21	0.127 ± 0.115 A,b	0.130 ± 0.135 A,b	-0.0771–0.0719

Notes: Means followed by the same uppercase letters (A) in the same line do not differ statistically ($p<0.05$).

Means followed by different lowercase letters (a,b) in the same column differ statistically ($p<0.05$).

Table 3. Gingival Index (GI) means and standard deviation on day 0 and day 21 for the control and test groups and the 95%confidence interval (CI) of the differences.

	Control	Test	95% CI
Day 0	0.045 ± 0.074 A,a	0.096 ± 0.234 A,a	-0.1545–0.0519
Day 21	0.327 ± 0.301 A,b	0.360 ± 0.293 A,b	-0.2091–0.1439

Notes: Means followed by the same uppercase letters (A) in the same line do not differ statistically ($p<0.05$).

Means followed by different lowercase letters (a,b) in the same column differ statistically ($p<0.05$).

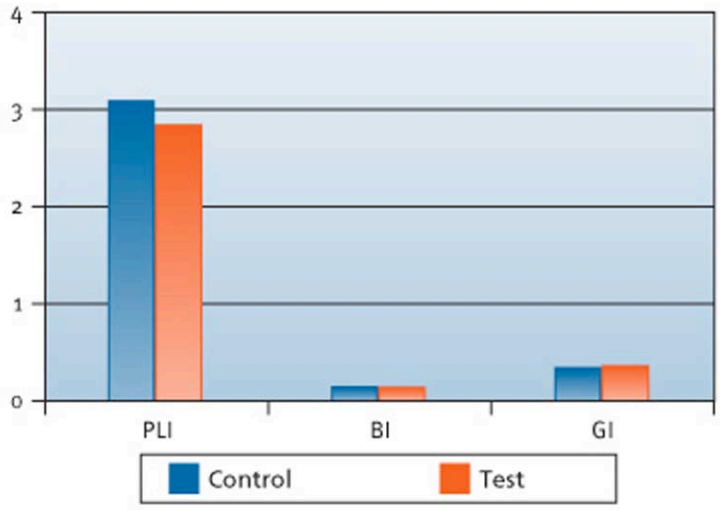


Figure 4. PLI, BI, and GI scores at day 21 for the control and test groups.

these agents are out of reach for patients in developing countries. Therefore, there is a need to develop new agents that are effective, safe, and inexpensive. Reports of several medicinal herbs used empirically in the prevention of gingivitis have been published worldwide.[11,22–24] However, in Brazil due to the lack of adequately controlled studies, the effectiveness of these natural products and extracts remains undetermined.

Since the sixteenth century *Cp* oil has been used as an antiseptic, analgesic, and anti-inflammatory and for wound healing because it is rich in diterpens, sesquiterpen, and polylactic acid, which are compounds known for their biological properties.[12,13,15,16] Despite its popular use, there is a lack of data to support the antigingivitis and antiplaque claims about this phytotherapeutic agent.

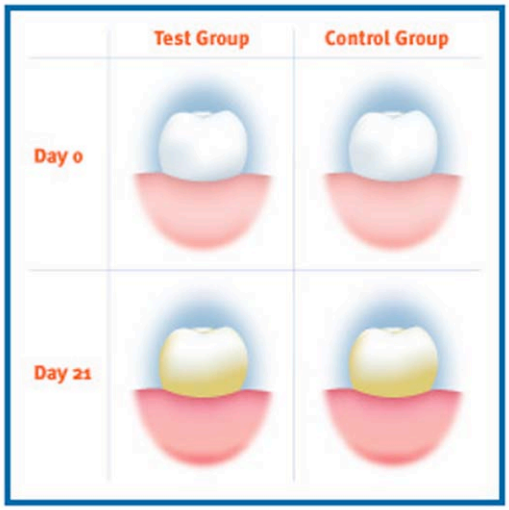
To the best of our knowledge, this is the first to report the effect of a gel containing *Cp* on gingivitis. The absence of adverse effects using the test gel showed that it was well tolerated, supporting its safety for clinical use. These results were not surprising since the biocompatibility of *Cp* had been reported previously.[14,15]

Interest in plants with antimicrobial properties has been revived as a consequence of current problems associated with the use of chemotherapeutic agents.[9] *In vitro* studies showing that 10% *Cp* was effective in inhibiting the growth of oral pathogens, mainly *Streptococcus mutans* and *Streptococcus sanguis*,[25] raises the possibility that this phytotherapeutic could be used as an antiplaque agent. It should be highlighted, however, that *in vitro* studies do not reproduce exact oral conditions.[26] This fact was confirmed in the present study in which both groups did not avoid plaque accumulation. The test group presented less plaque at day 21, but not significantly as compared to the control group. This showed the antibacterial rather than an anti-inflammatory activity of the *Cp*.

Although the composition of the gel was different with the presence of *Cp*, it can be inferred that this phytotherapeutic agent had no significant effect on bacterial growth *in vivo*. The test gel was placed on the tooth shield in an undiluted state. Therefore, it is possible that solubilization by saliva or mechanical action by toothbrushing could be necessary in order to produce an antibacterial effect.[4] The experimental model used in this present study discarded other factors that could jeopardize the clinical results, such as the mechanical plaque control that could hide the herbal agent effect. However, the efficiency of other dentifrices has been shown using this clinical design.[4]

In the present study, the Turesky et al.[19] index was used to assess plaque because of its sensitivity in detecting small deposits of plaque. However, the cutoff between the scores can be difficult to assess and could interfere with the results. Therefore, the calibration of the examiner was performed to solve this problem to ensure the confidence of the results.

Some studies have shown that *Cp* oil inhibited edema and granulation tissue formation by



reduction of vascular permeability, but its exact mechanism of action is unknown.[15–17] In both experimental and control groups in the present study there was a significant increase in marginal bleeding, indicating that the herbal agent did not prevent the development of gingivitis. This result is in agreement with Salgado et al.,[9] which evaluated the antigingivitis effect of a gel containing pomegranate extract.

The hydrocarbon sesquiterpenes present in *Cp* are responsible for the anti-inflammatory action and if these substances are missing, this could account for the lack of an anti-inflammatory effect.[17] However, the sample of commercial *Cp* oil was analyzed prior to the onset of the present study to confirm its chemical composition for pharmacological use.[16,17]

There is no "gold standard" index for assessing the degree of gingivitis. Since the GI has been the most widely used index in studies investigating oral hygiene products, it was included in this study. This index uses a scale in which color changes in the gingival tissue are assessed prior to evaluating gingival bleeding on probing; however, this parameter is not necessarily an accurate indicator of gingivitis.[27] Thus, the gingival inflammation was evaluated in conjunction with the bleeding index as well.

Unfortunately the majority of commercially available antiplaque agents are out of reach for patients in developing countries. Hence, the outcomes of the present study may have an important preliminary impact on the development of effective and inexpensive oral health

interventions, especially in low-socioeconomic communities and in regions where *Cp* oil is a culturally accepted herbal agent and used empirically.[11]

Therefore, other controlled studies using different concentrations of *Cp* oil are necessary to verify its action on supragingival microflora *in vivo* and established gingivitis. Further studies will be required to identify the real benefits of *Cp* as a therapeutic and preventive agent on gingivitis, in addition to its common use in contemporary medicine.

Conclusion

Within the limits of this clinical study, it may be concluded that the test gel containing 10% *Cp* was not efficient in preventing supragingival dental plaque formation and gingivitis.

Clinical Significance

Several medicinal herbs are used empirically by persons in the treatment and prevention of oral conditions. Research in this area must be encouraged to determine which herbal agents would be a useful addition to the current range of chemotherapeutic periodontal treatment options.

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