



Clinical Effect of a Mouth Rinse containing *Ocimum gratissimum* on Plaque and Gingivitis Control

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ABSTRACT

Aim: The effect of *Ocimum gratissimum* (*Og*) on the reduction of dental plaque and gingivitis was evaluated in a randomized, parallel and double-blind clinical trial.

Materials and methods: Subjects were randomly allocated to the control group (n=10)—mouth rinse with no antiseptic agents; CLX group (n=10)—mouth rinse containing chlorhexidine digluconate or *Og* group (n=10)—mouth rinse containing *Ocimum gratissimum*. Plaque (PLI) and bleeding (BI) indexes were assessed at days 0 and after 3 months. Subjects were asked to brush their teeth with a fluoridated dentifrice, three times a day, during a 90-day period. After each brushing they rinsed with one of the three mouth rinses during 1 minute.

Results: There was a significant reduction on plaque and gingivitis in tests groups ($p < 0.05$), but no statistically significant difference was observed among them ($p > 0.05$).

Conclusion: Mouth rinse containing *Og* was effective as antiplaque and antigingivitis agent, in a similar manner that chlorhexidine digluconate.

Clinical significance: Research in treatment of chronic oral diseases using natural agents must be encouraged to verify which would be a useful addition to the current range of chemotherapeutic treatment options.

Keywords: Dental plaque, Gingivitis, *Ocimum gratissimum*, Randomized controlled clinical trial.

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INTRODUCTION

Gingivitis is one of the most prevalent infectious oral diseases in humans associated with dental plaque.¹ Afterward, the removal of bacterial biofilm is a crucial component in the prevention and treatment of this disease.²

Mechanical plaque control is a simple and cost-effective method that has been showed to be efficient in gingivitis control;²⁻⁴ however, its effectiveness is influenced by the individual's manual ability and motivation.² For the reason, there is a great interest to search antimicrobial agents in order to replace or to be adjuncts to the mechanical approaches. These chemicals, mainly triclosan and chlorhexidine, have been used to avoid plaque formation and development of gingivitis⁵⁻⁸ and are often recommended in situations in which oral hygiene is difficult, compromised or impossible.⁹ As some of these substances may have undesirable side effects, such as tooth staining and taste alteration, herbal agents with antimicrobial and anti-inflammatory properties have been investigated and showed varied results.¹⁰⁻¹⁵

Higher and aromatic plants have traditionally been used in folk medicine, showing inhibition against several group of microorganisms.¹⁶ Plants from Brazil biomes have also been used as natural medicines by local populations in the treatment of several tropical diseases, including fungal and bacterial infections.¹⁶

Among the various available herbal agents, *Ocimum gratissimum* (*Og*), originating in the Orient, is widely distributed in tropical and warm temperature regions, including Brazil, where it is popularly known as 'alfavacraço'.¹⁷ *Og* belongs to the group of plants known as spices and it is an erect small plumb with many barnacles usually not more than 1 m high.¹⁸ It is of the family Labiatea, genus *Ocimum* and species *gratissimum*,¹⁸ and it is commonly used in folk medicine to treat different diseases, e.g. skin diseases, pneumonia and also as a treatment for cough, fever and conjunctivitis.^{19,20} However, genetic material, culture conditions and environment are important factors to its effectiveness as therapeutic agent.¹⁶

Laboratorial previous studies showed that *Og* presents antimicrobial,^{18,19} antinociceptive and anti-inflammatory activities,²⁰ showing that this herbal agent can be effective against acute and chronic oral diseases.

At the time of the present work, there is no reported controlled trial evaluating the efficacy of *Og* in the control of plaque and gingivitis. Thus, the aim of the present study was to assess the antiplaque and antigingivitis effects of this phytotherapeutic agent in comparison to digluconate chlorhexidine.

MATERIALS AND METHODS

Subjects

Thirty adult subjects from the University of Fortaleza (15 females and 15 males aged 27 to 42 years) were enrolled in this double-blind, parallel, controlled clinical trial. All randomly screened participants were informed about the nature of the study and signed an informed consent form in compliance with the guidelines of the Brazilian National Health Council. The protocol was approved by the Institutional Ethics Committee (Report Coética/Unifor: 161/2009).

The subjects were entered in the study if they had bleeding index (BI)²¹ $\geq 20\%$, presence of at least 20 natural teeth and absence of supragingival calculus and other plaque retentive factors, such as carious cavity and restoration excess. Participants with medical disorders and under antimicrobial therapy, as well as smokers, pregnant women and individuals presenting a probing depth ≥ 3 mm were excluded from the trial.

Tests and Control Products

The control and tests mouth rinses were formulated and packed into bottles in the Pharmaceutics' Laboratory at the University of Fortaleza. The bottles were previously coded to warrant that neither the examiner nor the participants knew their content, which was revealed by the pharmacist only after the study was completed. All subjects used just one of these mouth rinses, according to a parallel study.

The control mouth rinse was constituted by triethanolamine (q.s.p.), alcohol, water (q.s.p.), nipagin (0.2%), glycerin (2.5%), aspartame (q.s.p.), presenting color and taste similar to those used in the tests of mouth rinses. These had the same formulation, adding 0.12% digluconate chlorhexidine (CLX group) or *Og* (*Og* group).

Experimental Design

The participants were assigned to either control group (n = 10) or the tests groups—CLX group (n = 10) and *Og* group (n = 10)—by random permutation of three. The volunteers were examined for plaque and gingivitis at baseline and after 3 months. A single, previously calibrated

examiner scored the BI and the plaque index (PLI),²² which were recorded on the buccal, mesial, distal and lingual surfaces of all teeth. The values of four sites of each tooth were averaged to determine the BI and PLI for each subject. 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.

After the initial examination, all teeth of each subject were polished with pumice and flossed to eliminate plaque remnants. A personal 'kit' containing a new toothbrush (Leader[®], Facilit Odontológica e Perfumaria Ltda., Rio de Janeiro, RJ, Brazil), a commercial dentifrice with no anti-inflammatory properties (Sorriso[®], Kolynos do Brazil Ltda., Osasco, SP, Brazil) and tests or control mouth rinses was given to all participants. They were instructed to brush their teeth for 1 minute, three times a day, using their habitual technique. Additionally, after each brushing the participants rinsed with one of the formulations (10 ml, 1 min, 3×/day). Verbal and written instructions about the correct use of hygiene products were given to all subjects as well. In addition to verbal instructions, the students were given recommendations to follow at home. On the last day of experimental phase (90th day), the indexes were recorded and the teeth were polished with pumice.

Statistical Analysis

ANOVA and Student Newman-Keuls test were performed to evaluate statistical differences between control and tests groups on days 0 and 90 ($\alpha = 0.05$). In each group, the mean scores of all indexes were compared between baseline and the end of the trial by the paired t-test ($\alpha = 0.05$). However, for illustration, the results are presented as mean and standard deviation.

RESULTS

All the participants completed the trial. The tests mouth rinses had a good acceptance and did not show formation of abscess, ulcerations or allergic reactions. However, three volunteers from CLX group reported pigmentation and temporary taste disturbance.

At the beginning, there was no statistically significant difference between the control and tests groups with respect to PLI and BI ($p > 0.05$) means. These results indicated that all groups were well balanced at baseline (Tables 1 and 2), displaying around 25% of gingival bleeding. At the 90th day, there was a statistically significant difference between control and tests groups for the PLI and BI ($p < 0.05$) (Tables 1 and 2; Fig. 1).

Comparing the means between baseline and day 90 in each group, there was statistically significant difference in BI and PLI indexes for CLX and *Og* group ($p < 0.05$), but with no difference between them ($p > 0.05$) (Tables 1 and 2; Fig. 1).

Table 1: Plaque index (PLI) means and standard deviation on day 0 and day 90 for the control and test groups				
	Og	CLX	Control	95% CI
Day 0	1.08 ± 0.56 A,a	1.55 ± 0.79 A,a	1.59 ± 0.79 A,a	-0.0916 – 1.0296
Day 90	0.62 ± 0.26 A,b	0.55 ± 0.29 A,b	1.69 ± 0.83 B,a	-0.3016 – 0.1596

Notes: Means followed by the same uppercase letter (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 90 for the control and test groups				
	Og	CLX	Control	95% CI
Day 0	0.22 ± 0.04 A,a	0.27 ± 0.02 A,a	0.23 ± 0.07 A,a	-0.0592 – 0.0357
Day 90	0.08 ± 0.02 A,b	0.06 ± 0.03 A,b	0.24 ± 0.08 B,a	-0.2033 – 0.1187

Notes: Means followed by the same uppercase letter (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 ($p < 0.05$)

The relation between PLI and BI is shown in Figures 2 to 4. The linear regression coefficients were $R^2 = 0.21$, $R^2 = 0.84$ and $R^2 = 0.66$ for the Og, CLX and control groups respectively. A relation between both indexes was observed for the CLX and control groups but not for the Og group, indicating that its antimicrobial and anti-inflammatory actions occurred independently.

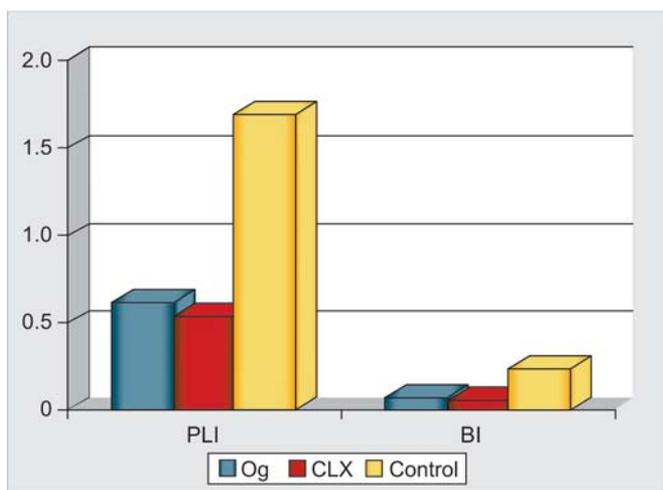


Fig. 1: PLI and BI scores at day 90 for the control and tests groups

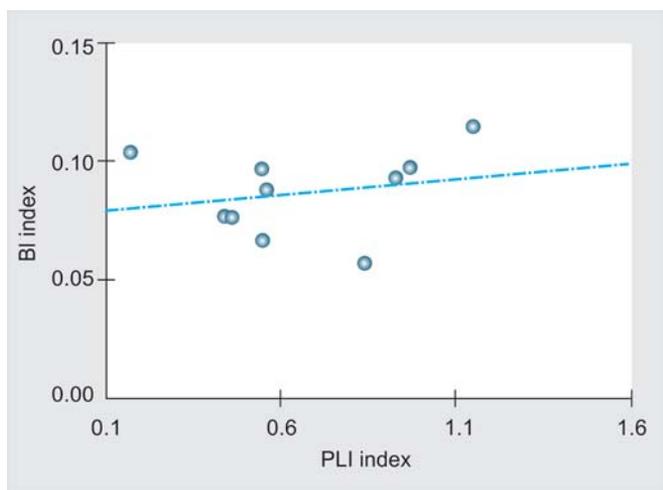


Fig. 2: Linear regression analysis between PLI and BI index for Og group at day 90

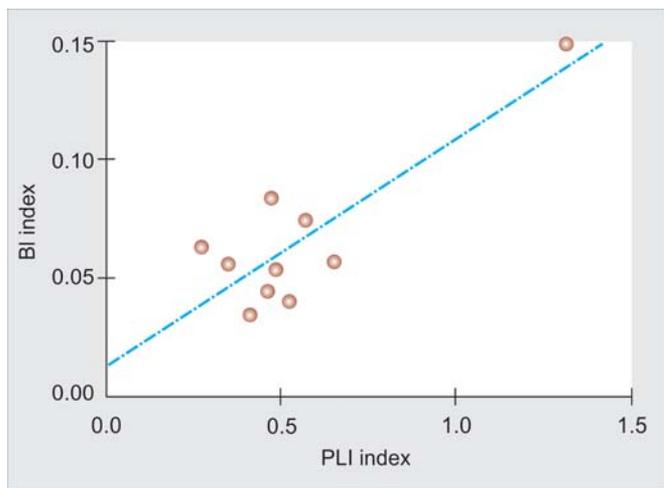


Fig. 3: Linear regression analysis between PLI and BI index for CLX group at day 90

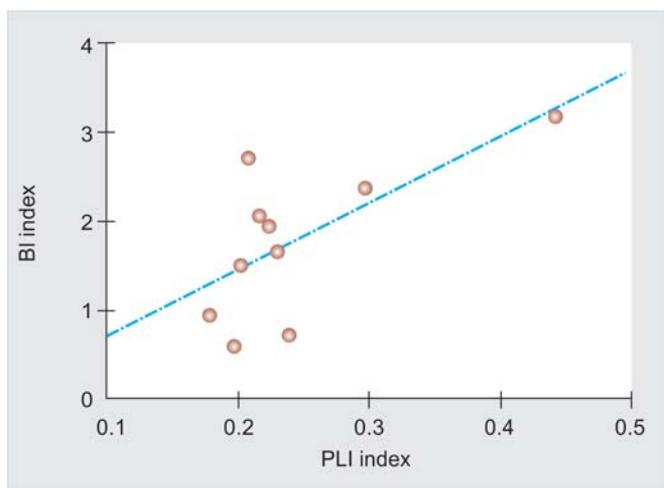


Fig. 4: Linear regression analysis between PLI and BI index for control group at day 90

DISCUSSION

This paper presents the data of a randomized, parallel, double-blind clinical study where a phytotherapeutic agent was used as an adjunct to tooth brushing in a group of patients with gingivitis, comparing it with chlorhexidine

digluconate. This study design was based on previous studies and it was chosen in order to generate the best possible clinical evidence.^{13,15}

In the control group, PLI and BI stayed in the same baseline levels at the end of the experiment, indicating the inability of this adult population to perform adequate tooth cleaning, which is still the gold standard for patients that have the capacity of performing it.^{2,5} In contrast with other studies, in which the patients were instructed to use Bass technique,^{15,23} the usual tooth brushing was not modified to avoid hide the test agents' actual effect.

Chlorhexidine mouth rinse is considered as the golden standard for oral antiseptics and its effectiveness was observed in the present and other studies.^{2,9,24,25} In terms of plaque accumulation, several studies did not find differences between 0.12 and 0.20% chlorhexidine concentrations.^{2,24,26,27} In the present work, it was used the 0.12%, that is the generally used in commercial mouth rinses. Lower concentrations of chlorhexidine should be prescribed since higher concentrations do not seem to generate lower plaque and gingivitis scores.²

Unfortunately, chlorhexidine, as most active antiseptics, has some disadvantages such as discoloration in proximal areas and tongue and reversible effect on the taste.^{9,23,24} In the present study, these aspects were observed in 30% of the participants and it is in accordance with Botelho et al.¹³

In recent times, report of a number of medicinal herbs used in the treatment and prevention of gingivitis have been published worldwide, presenting limited^{11,15,23,28} and encouraging results.^{13,14,29,30} Despite its commercial use in pharmaceutical industries,¹⁶ there is a lack of data to support the antigingivitis and antiplaque claims about *Og*. The absence of adverse effects using this herbal agent in the present study showed that it was well tolerated, supporting safety for the clinical use.

To the best of our knowledge, the present work is the first to evaluate the effect of a mouth rinse containing *Og* on plaque accumulation and gingivitis. The results showed that both tests groups were efficient on plaque reduction (64% in CLX group and 43% in *Og* group). This percent difference was not significant at the end of the trial. Conversely, the control group presented a higher percent increase of 12%, but not significant, on plaque accumulation.

Various extracts of *Og* have been tested *in vitro* and shown to be active against some bacteria and fungal isolates.^{18,31,32} Nakamura et al¹⁹ found that the essential oil of *Og* has antibacterial activity against *S. flexineri*, *E. coli* and *Proteus mirabilis*. Silva et al³³ demonstrated that this phytotherapeutic exhibited antifungal activities against dermatophytes, in concordance with the study of Lemos et al.³⁴

In the susceptibility test *in vitro*, *Og* inhibited the growth of oral microorganisms^{32,35} which allows us to deduce that this phytotherapeutic could be used as antiplaque agent. When prepared as components of mouth rinses, *Og* completely inhibited the growth of all the organisms, *Streptococcus viridans* and *S. albus* respectively implicated for gingivitis and dental caries.³⁵ Despite these studies had been *in vitro* and the likelihood of possibility of change in activity of microflora of a patient cannot be ruled out as it function *in vivo*, the antimicrobial action was confirmed in the present clinical study.

Volatile oils constitute a group of plant secondary metabolites which can best be obtained through hydrodistillation and some has potent antimicrobial effects.³⁵ The major constituents found in *Og* and previously known for its antimicrobial activity are eugenol and thymol.¹⁶

The action mechanism of eugenol occurs in plasmatic membrane level and is attributed to cellular lipids alterations; lost of intracellular material and inhibition of nucleic acid synthesis.³⁶ Helander et al³⁷ attributed the thymol antimicrobial action to its phenolic character, which can cause membrane-disturbing activities. These data support the antiplaque effect of *Og* founded in the present work and are in agreement with others studies that investigated other herbal products with similar constituents.^{13,14}

The test groups reduced gingivitis significantly at the end of the trial, in which the *Og* group presented just 8% of the sites with bleeding and CLX group 6%. Nevertheless, this percent difference was not significant showing that this herbal agent had potential similar to chlorhexidine as antiseptic agent. The BI is a generally used dichotomous index to evaluate gingivitis,^{11,13,15} but it does not access the severity of gingival inflammation. Studies evaluating the reduction of gingivitis by a grading index could be interesting to complement these results, as used in other works evaluating herbal agents.^{14,30} However, color change used as parameter in this grading index cannot be necessarily an accurate indicator of gingivitis.¹⁵

The anti-inflammatory activity demonstrated by *Og* might be due to the presence of flavonoids in its composition.²⁰ This component inhibits phosphodiesterases which are involved in cell activation, and their effects depend upon the biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to the sites of injuries.²⁰ In addition, extracts of *Og* appeared to improve the phagocytic function without affecting the humoral or cell-mediated immune system, showing its immunobiological activity.¹⁷ In spite of these explanations, the exact mechanism of its anti-inflammatory action is hidden yet and more studies are necessary.²⁰

Home-use mouth rinses studies are often influenced by a number of factors which can mask the superiority of a test agent over the controls. Participants in clinical trials may experience some improvement associated not specifically to the therapeutic properties of the test agent but rather related to a behavior change—Hawthorne effect.¹⁵ Subjects enrolled in oral hygiene studies usually improve their tooth brushing, irrespective of the product they receive.¹⁵

Although the volunteers of the present study were not conscious of which mouth rinse they were using, another main factor is the Novelty effect which is the motivation on oral hygiene practice by the use of a new substance. In contrast, lack of compliance in the correct use of mouth rinse can occur as well.¹⁵ In order to minimize its event, the participants were asked to bring the bottle at the end of the trial, so we could evaluate indirectly subject compliance. Reduction on gingivitis in both test groups showed that they used the mouth rinse correctly.

Finally, the results showed that *Og* was an effective antiplaque and antigingivitis herbal agent in a similar manner of chlorhexidine digluconate and it should be advantageous in cases where patients have little motor skills and tooth brushing is compromised. However, laboratorial analysis, such as immunoenzymatic assays of gingival crevicular fluid, would be needed to a better understanding of the role of *Og* as an effective agent on gingivitis control. Further clinical studies must be performed to evaluate the action of this herbal agent in other oral diseases, such as chronic periodontitis.

CONCLUSION

Within the limits of this clinical study, it may be concluded that the mouth rinse containing *Og* was effective in the plaque and gingivitis control, comparable to chlorhexidine digluconate.

CLINICAL SIGNIFICANCE

Research in treatment of chronic oral diseases using natural agents must be encouraged to verify which would be a useful addition to the current range of chemotherapeutic treatment options.

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