Clinical effect of a mouthwash containing Anacardium occidentale Linn. on plaque and gingivitis control: A randomized controlled trial

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**ABSTRACT**

**Background:** Plaque-associated gingivitis is a prevalent disease and research in its treatment using herbal agents must be encouraged to verify which would be a useful addition to the current range or chemotherapeutic treatment options.

**Aims:** The aim of this study was to evaluate the clinical effect of a mouth rinse containing 10% Anacardium occidentale (AO) Linn., a typical plant commonly found in the Northeast Region of Brazil, on the reduction of plaque and gingivitis in comparison to a gold-standard chemotherapeutic agent.

**Materials and Methods:** Thirty normosystemic adult volunteers of both genders, who had a minimum of twenty natural teeth, aging between 18 and 32 years, were enrolled in this crossover, controlled, examiner-blind clinical study. They were randomly allocated into three groups: 10% AO Linn. (n = 10); 0.12% chlorhexidine digluconate (CLX, n = 10); or placebo (PB, n = 10). All volunteers were instructed to brush their teeth with a fluoridated dentifrice two times a day (12/12 h) and to rinse for 1 min with one of the mouthwashes (AO, CLX, or PB) 30 min after tooth brushing for 1 month. Plaque index (PLI) and gingival bleeding index (BLI) were recorded on days 0 and 30. Nonparametric Kruskal–Wallis and Wilcoxon tests (α = 0.05) were performed to evaluate statistical differences among groups.

**Results:** There was a significant reduction (P < 0.05) on plaque and gingivitis at day 30 just in CLX ([PLI = 0.47 ± 0.16; -30%]; [BLI = 0.15 ± 0.09; -55.8%]) and AO ([PLI = 0.49 ± 0.21; -31%]; [BLI = 0.13 ± 0.10; -56.6%]) groups, but no statistically significant difference was observed among them (P > 0.05).

**Conclusion:** Mouthwash containing 10% AO was effective as an antiplaque and antigingivitis agent, in a similar manner that 0.12% CLX.

**Key words:** Anacardium occidentale Linn., chlorhexidine, dental plaque, gingivitis, oral hygiene

The role of dental plaque in the etiology of periodontal diseases, mainly gingivitis, is well recognized.[1‑4] Regular plaque removal by effective mechanical tooth cleaning is the main goal of the prevention of gingival inflammation and a crucial component in its treatment.[2,5] In spite of mechanical cleaning of teeth being a simple and efficient method in control of gingivitis, its effectiveness is influenced by the individual’s manual ability and motivation.[5,6]

Therefore, in addition to mechanical oral hygiene, the use of antiseptics is strongly recommended.[1‑3] In this scope, chlorhexidine digluconate (CLX) is considered the...
gold-standard chemical agent and has been used in the
treatment of inflammatory diseases associated with dental
plaque accumulation.\textsuperscript{[1,2,7–9]} However, due to undesirable
effects after prolonged use, such as pigmentation and taste
disturbance,\textsuperscript{[1,3]} herbal agents had been researched recently
showing encouraging results.\textsuperscript{[3,5,10–12]}

\textit{Anacardium occidentale} (AO) Linn. (cashew), a member of
the family \textit{Anacardiaceae} and presently cultivated in many
regions of the world, is a tropical tree originally indigenous
and commonly found in the Northeast of Brazil.\textsuperscript{[13–15]} The
fruits, stem bark, and leaves extracts have been traditionally
used in folk medicine for the treatment of mouth and peptic
ulcers, intestinal disturbances, dyspepsia, asthma, diabetes,
sore throat, bronchitis, and inflammatory diseases.\textsuperscript{[13–15]}
Its antiinflammatory, anti-inflammatory, and antimicrobial
potentials are highlighted in clinical and laboratorial
studies.\textsuperscript{[15,16]}

The previous studies indicated that AO Linn. had shown \textit{in vitro}
potent antimicrobial activity against fungi and \textit{Streptococcus mutans}, \textit{Streptococcus mitis}, \textit{Streptococcus sanguis}, \textit{Streptococcus sobrinus}, and \textit{Lactobacillus casei}
that are the first settlers in dental plaque.\textsuperscript{[17,18]} Recently, a
dentifrice containing this herbal agent showed antimicrobial
action against \textit{Candida tropicalis}, \textit{Candida stellatoidea}, and
\textit{Lactobacillus acidophilus}.\textsuperscript{[19]}

There are no \textit{in vivo} studies about the clinical effect of AO
Linn. on dental plaque and gingivitis control. Thus, a trial
in humans was conducted to evaluate the antiplaque and
antigingivitis effects of a mouth rinse containing this herbal
agent in comparison to CLX.

\textbf{MATERIALS AND METHODS}

\textbf{Subjects}

Thirty adult patients from the University of Fortaleza
(15 female and 15 male, age 18–32 years) were enrolled in
this examiner-blinded, crossover, controlled clinical trial
from August 2012 to November 2012. All participants were
randomly screened, informed about the nature of the study,
and provided written informed consent for participation, in
compliance with the guidelines of the Brazilian National
Health Council and Declaration of Helsinki. The study
protocol was approved by the Institutional Research Ethics
Committee (Coetica/Unifor Report No. 200/2011).

The criteria for inclusion were a bleeding index (BLI)\textsuperscript{[20]}
≥20%, the presence of at least twenty natural teeth, and the
absence of plaque-retentive factors such as restoration
excess, dental caries, or calculus. Smokers, pregnant
women, subjects with medical disorders, and those under
antimicrobial therapy or using mouth rinses containing
chemical agents for the last 3 months were excluded from
the trial. Volunteers with a history of allergy to mouth rinses
or toothpaste and those with a probing depth ≥3 mm were
not included in this clinical study.

\textbf{Essential oil extraction, preparation, and
composition}

AO Linn. essential oil was prepared from stem bark samples
collected in September 2012 of the medicinal herb Garden
at the University of Fortaleza, Ceará, Brazil. Essential oil
was extracted using a modified Clevenger apparatus by the
hydrodistillation technique.\textsuperscript{[5,10]} The volume of essential oil
obtained was measured and then it was stored in hermetically
sealed glass receptacles with rubber stoppers, covered
with aluminum foil to protect the contents from light, and
kept under refrigeration at 8°C until use.\textsuperscript{[5,10]} The chemical
composition was determined by high-performance thin layer
chromatography. The major constituents of the essential oil
were, respectively, tannins, cardol, and anacordol.

\textbf{Preparation of the mouth rinses}

Initially, 1 ml of essential oil from AO Linn. was
diluted in 9 ml of ethyl alcohol (1:9), preparing a 10%
mixture (V/V) (AO group).\textsuperscript{[3]} A mouth rinse containing just
distilled water (placebo [PB] group) and other containing
0.12% CLX (CLX group) were formulated too. In all groups,
the following substances were in common: Deionized
water (q.s.), 0.2% nipagin (24 g), 2.5% glycerin (300 ml), 5%
sorbitol (600 ml), 0.2% saccharine (24 g), and flavoring (q.s.)
and color agents (q.s.). Due to the incompatibility
between CLX and saccharine, this was replaced by 0.2%
aspartame (24 g). These values were calculated for the
production of 600 ml of each solution.

First, nipagin was dissolved in deionized water at room
temperature, and after this, saccharine or aspartame
was dissolved in hot deionized water because it was not
completely solubilized at room temperature. In sequence,
glycerin and sorbitol were added. In the CLX group, 0.12%
CLX (144 ml) was added; in the AO group, 10% AO Linn.
(600 ml) was added, and in the PB group, just distilled
water was used (600 ml). Then, the color and flavoring
agent was added in the PB and CLX groups until reaching
a color similar to AO group that had a natural color. The
solutions were shaken for 10 min and the sedimentation
and organoleptic characteristics were observed to verify its
acceptability.

Finally, the mouth rinses were packed into bottles,
previously cleaned with 70° alcohol in the Pharmacetics’
Laboratory at the University of Fortaleza. The bottles
were coded to warrant that neither the examiner nor the
participants knew their content, which was revealed by the
pharmacist just after the study was completed.

\textbf{Experimental design}

Volunteers were designed initially to either the PB
group (n = 10), CLX group (n = 10) or the AO group (n = 10),
by random allocation using a computer-generated random table made by a person, not participant of the study. Participants were examined for plaque and gingivitis at baseline and after 30 days, in three experimental phases with a 1-month washout interval between them until all subjects had rinsed with each formulation. A single, previously calibrated examiner scored the plaque index (PLI) [21] and BLI [20] index, which were observed on the buccal and lingual surfaces of all teeth. The values of the two sites of each tooth were averaged to determine the BLI and PLI for each subject. In addition, the hard and soft tissues of the oral cavity were visually inspected for the presence of any adverse effect by the same examiner. Intraexaminer calibration was achieved by two records in ten patients, with a 24-h interval among them, obtaining 0.78 kappa coefficient. [5]

After the initial examination, a personal kit containing a new toothbrush (Leader, Facilit Odontológica e Perfumaria Ltda., Rio de Janeiro, RJ, Brazil) and a test or control mouth rinse (600 ml) was given to all participants. They were instructed to brush their teeth with a fluoridated dentifrice two times a day (12/12 h) for 1 min using their habitual technique. After 30 min, the participants were instructed to rinse for 1 min with one of the mouth rinses and then expectorate it. Verbal and written instruction about the correct use of oral hygiene products was given to all subjects as well. After 30 days, indexes were recorded by the same examiner and the teeth were polished with pumice.

Statistical analysis
Nonparametric Kruskal–Wallis analysis was performed to evaluate statistical differences among groups on days 0 and 30 (α = 0.05). In each group, the mean scores of all indexes were compared between baseline and the end of the trial with Wilcoxon test (α = 0.05). However, for illustrative purposes, the results are presented as means and standard deviations. The linear regression coefficients were realized to evaluate the association among PLI and BLI indexes as well.

RESULTS
Three patients were excluded from the study. None of the patients showed any adverse reaction with the different formulations. At baseline, there was no statistically significant difference among groups with respect to mean PLI and BLI indexes (P = 0.2531) [Tables 1 and 2]. These findings indicated that all groups were well balanced at baseline.

At day 30, there was no statistically significant difference among groups for the PLI (P = 0.0760) and BLI (P = 0.7692) indexes [Tables 1 and 2], but just AO and CLX groups significantly reduced the PLI and BLI indexes in a similar manner, when comparison of means between baseline and day 30 was performed (CLX = P = 0.00251; AO = P = 0.0417); [Tables 1 and 2].

The relation between PLI and BLI indexes is shown in Figures 1–3. The linear regression coefficients were R² = 0.23, R² = 0.12 e, R² = 0.32 for the CLX, AO, and PB groups, respectively.

DISCUSSION
The present work showed the data of a clinical study where a herbal agent in mouthwash was used by patients with gingivitis and compared with CLX. The design was based on previous studies and it was chosen to generate the best possible clinical evidence in a short-term period. [5,11]

In the PB group, PLI and BLI indexes remained at baseline levels at the end of the experiment, for example, the plaque accumulation was reduced just 5.8%, indicating the inability of this adult population to perform an effective mechanical plaque control. The tooth brushing was not modified to avoid concealment of the actual effect of the test agents, [5,11] in contrast with other studies, in which volunteers were instructed to use the Bass technique. [22,23]

CLX has been tested and its efficacy and safety have been confirmed in several in vivo studies. [1,2,5,7–9] Likewise, the absence of adverse effects with the use of the AO showed that it was well tolerated, supporting its safety profile for clinical use. These results were already expected as the biocompatibility of this herbal agent has been reported previously in animal studies. [15] Unfortunately, chlorhexidine has some disadvantages, such as discoloration in teeth and tongue, and a reversible effect on taste. [1,3,8] In a recent study, 30% of the patients using CLX showed these

### Table 1: Plaque index on day 0 and day 30, placebo and test groups (mean±standard deviation)

|          | CLX  | AO  | PB  |
|----------|------|-----|-----|
| Day 0    | 0.67±0.18   | 0.71±0.19   | 0.70±0.13   |
| Day 30   | 0.47±0.16   | 0.49±0.21   | 0.66±0.03   |

*Same uppercase letters (A) in the same row did not denote a statistically significant difference (P>0.05). **Different lower case letters (a,b) in the same column denote a statistically significant difference (P<0.05). CLX=Chlorhexidine digluconate, AO=Anacardium occidentale, PB=Placebo

### Table 2: Bleeding index on day 0 and day 30, placebo and test groups (mean±standard deviation)

|          | CLX  | AO  | PB  |
|----------|------|-----|-----|
| Day 0    | 0.34±0.10   | 0.30±0.08   | 0.20±0.04   |
| Day 30   | 0.15±0.09   | 0.13±0.10   | 0.14±0.08   |

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The previous in vitro studies showed that AO presented high inhibition action on microorganisms from supragingival dental plaque, such as S. mutans, S. mitis, S. sanguis, S. sobrinus, and L. casei. In spite of the fact that, in-vitro conditions do not fully reproduce the oral environment, this fact led us to deduce that AO could be used as an antiplaque agent. This hypothesis was confirmed in this study, in which the plaque accumulation was significantly reduced similarly to CLX; however, the comparison between other studies is difficult because, to the best of our knowledge, this study is the first to evaluate the effect of a mouthwash containing AO in the plaque control.

The antimicrobial activity of AO can be explained by the presence of phenolic compounds that causes disturbances in the bacterial membrane and inhibits the enzymatic system for its formation. Indeed, anacardic acids, which are present, namely, in the nut and fruit juice, were also found in the stem bark in the present study, even though in low concentrations. The concentration of AO essential oil used in this trial was based on a previous study, in which S. mutans and S. sanguis were sensitive to AO. Moreover, other studies evaluating herbal agents used similar concentrations.

In the present work, the mouthwash containing AO reduced gingivitis in a similar manner that CLX did (56.6% vs. 55.8%), agreeing with previous studies, in which other herbal agents were tested, and disagreeing with others that did not show gingivitis reduction in comparison to PB group. In the PB group, it had a reduction of 30% of sites with bleeding, so AO almost duplicated the reduction of gingivitis, showing that it has potential as an antigingivitis agent.

The anti-inflammatory effect of AO could be explained by the presence of tannins that act by inhibiting the cyclooxygenase and arachidonic acid metabolic pathways, blocking prostaglandins formation, which is responsible for the inflammatory events. The stem bark is astringent and rich in tannins, as observed in the present study, which possibly supports its popular use as an anti-inflammatory agent.

Other studies pointed that AO inhibits other enzymes, such as acetylcholinesterase and tyrosinase. By inference, studies showed antinociceptive and antiedematogenic effects in rats and clinical trials presented anti-inflammatory and healing effects in skin and oral lesions after use of compounds containing AO.

The data of regression analysis presented a weak association among PLI and BLI indexes, namely, in the AO group, indicating that its antimicrobial and anti-inflammatory adverse effects after 3 months. A factor that could explain this difference is the time of the experiment, in spite of other studies presented this intercorrence in a short-term period.

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Figure 1: Linear regression analysis between plaque index and bleeding index for CLX group at day 30

Figure 2: Linear regression analysis between plaque index and bleeding index for AO group at day 30

Figure 3: Linear regression analysis between plaque index and bleeding index for placebo group at day 30
actions occurred independently, because just 34% of the sites with gingivitis can be attributed to plaque accumulation, while in the CLX and PB groups were 48% and 56%, respectively. These data agree with the results of a recent work, in which another herbal agent was tested as an antiseptic agent, in comparison to CLX.[11]

Home-use mouth rinse studies can be influenced by the Hawthorne and Novelty effects[6,11,22] that can mask the superiority of a test agent over the control. To minimize this event, the participants were asked to bring the bottle at the end of the trial so that we could evaluate indirectly patient compliance. Significant reduction on gingival inflammation in CLX and AO groups showed that they used the mouthwash correctly. Finally, the results revealed that AO was effective on plaque and gingivitis control comparatively with CLX and it should be advantageous in situations, in which tooth brushing is compromised, such as physically challenged and hospitalized patients. However, long-term studies must be performed to evaluate if these positive results are maintained over time and if this herbal agent is effective in other more complex infect-inflammatory oral diseases, for example, chronic periodontitis.

CONCLUSION

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

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Conflicts of interest

There are no conflicts of interest.

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