

# Clinical effect of a gel containing *Lippia sidoides* on plaque and gingivitis control

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## ABSTRACT

**Objective:** This parallel controlled clinical trial evaluated the effect of a gel containing *Lippia sidoides* essential oil on plaque and gingivitis control.

**Methods:** Thirty patients (n=30) were randomly selected and allocated into three groups: *Lippia sidoides* (LS, n=10), chlorhexidine (CLX, n=10) or placebo (control, n=10). Plaque and bleeding index were recorded at baseline and after three months. All volunteers were instructed to brush with the gel three times a day throughout the experiment period.

**Results:** There was a significant reduction on plaque and gingivitis in the test groups ( $P < .05$ ), but no statistically significant difference was observed between them ( $P > .05$ ).

**Conclusion:** A gel preparation containing 10% *Lippia sidoides* essential oil was an efficient herbal antiplaque and antigingivitis agent. (Eur J Dent 2013;7:28-34)

**Key words:** Dental plaque; gingivitis; *Lippia sidoides*; chlorhexidine

## INTRODUCTION

Gingivitis is one of the most prevalent infectious oral diseases in humans associated with dental plaque.<sup>1</sup> The removal of bacterial biofilm is a crucial component in the prevention and treatment of this disease.<sup>2</sup> Regular plaque removal by effective mechanical cleaning of the teeth is a simple and cost-effective method that has proved efficient in the control of gingivitis,<sup>2,3</sup> but its effectiveness is influenced by the individual's manual ability and motivation.<sup>1,2,3</sup>

Therefore, the use of antiseptics in addition to mechanical oral hygiene is recommended.<sup>4,5</sup> For

this reason, there is great research interest in the discovery of antimicrobial agents that can replace or serve as adjuncts to mechanical hygiene methods. Such compounds, particularly chlorhexidine, have been used to prevent plaque formation and development of gingivitis,<sup>4,5,6,7</sup> and are often indicated in situations in which oral hygiene is difficult, compromised or even impossible.<sup>6,7</sup>

Chlorhexidine is considered the gold standard agent for chemical oral hygiene, and is known to produce positive results by inhibiting or slowing bacterial proliferation.<sup>4,8,9</sup> However, due to undesirable effects after prolonged use, such as pigmentation and taste disturbance, several phyto-pharmaceutical alternatives to chlorhexidine have been investigated.<sup>10,11,12,13,14,15</sup>

Higher plants and aromatics have traditionally seen widespread use in folk medicine, and many have inhibitory properties against several

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groups of microorganisms.<sup>16,17</sup> Plants from Brazilian biomes have been used extensively as natural medicines by local populations in the treatment of several tropical diseases, including fungal and bacterial infections.<sup>16</sup>

*Lippia sidoides* is a typical shrub commonly found in the Northeast Region of Brazil. Its camphoric foliage is indicated as a topical antiseptic agent for conditions of the skin and mucous membranes and also for throat infections.<sup>10,18</sup> The essential oil obtained from *L. sidoides* is composed mainly of thymol (56.7%-59.65%) and carvacrol (16.7%-19%). The other main compounds are caryophyllene (1.1%-10.6%), p-cymene (7.1%-9.08%) and myrcene (0.86%-5.46%).<sup>18,19</sup> The composition can vary greatly depending upon the geographic region of collection, variety, and age of the plant, as well as on the methods employed for drying and extraction of the oil.<sup>19</sup>

Previous studies have indicated that these major components of *L. sidoides* essential oil exhibit potent antimicrobial activity against oral pathogens<sup>18,20</sup> and reduce the severity of gingivitis, dental plaque and histological inflammatory infiltrate in dogs.<sup>21</sup> Recently, short-term clinical studies showed a positive effect of *L. sidoides*-based preparations as preventive agents.<sup>10,22</sup>

These initial studies notwithstanding, no published controlled trials have evaluated the efficacy of a *L. sidoides*-based gel in the control of plaque and gingivitis. Thus, a clinical study in humans was conducted to evaluate the antiplaque and antigingivitis effects of this phytopharmaceutical agent and compare them to those of chlorhexidine.

## MATERIALS AND METHODS

### Subjects

Thirty adult patients from the University of Fortaleza (15 female and 15 male, age 26-47 years) were enrolled in this examiner-blinded, parallel, controlled clinical trial. All participants were randomly screened, were 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. The study protocol was approved by the institutional Research Ethics Committee (Coética/Unifor report 156/2008)

The criteria for inclusion were a bleeding index (BI)<sup>23</sup>  $\geq 30\%$ , presence of at least 20 natural teeth,

and absence of supragingival calculus and other plaque-retentive factors, such as dental caries and restoration excess. Participants with medical disorders and those under antimicrobial therapy, as well as smokers, pregnant women, and those with a probing depth  $\geq 3$  mm, were excluded from the trial.

Essential oil extraction, preparation and composition

*L. sidoides* essential oil was prepared from leaves collected from the medicinal herb garden of the University of Fortaleza, Ceará, Brazil. Leaf essential oil was extracted using a modified Clevenger apparatus by the hydrodistillation technique.<sup>10,18</sup> The volume of essential oil obtained was measured and the essential oil 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.<sup>10,18</sup>

The chemical composition was determined by gas chromatography-mass spectroscopy. The constituents were identified by a computer-based library search, using retention indices and visual interpretation of the mass spectra<sup>10,18</sup>. The major constituents of essential oil were thymol (58.7%) and carvacrol (17.1%). Minor constituents included caryophyllene (10.3%) and p-cymene (8.98%).

### Preparation of the gels

After extraction, 1 mL of the essential oil was diluted in 9 mL of ethanol (1:9), thus preparing a 10% mixture. As much as 50 g of carboxymethylcellulose was added to the *L. sidoides* infusion (1000 mL) and the mixture was kept boiling until its complete dissolution to obtain the 10% gel concentration. A glycerin/ethanol mixture (50mL:50 mL) was added and the solution was vigorously stirred for 15 min until gel formation occurred.<sup>22</sup> Similar procedures were used to obtain a 2% chlorhexidine digluconate gel.

### Test and control gels

The inert control gel was compounded so as to have color and taste similar to the test gels, and contained triethanolamine (q.s.p.), ethanol, water (q.s.p.), methylparaben (0.2%), glycerin (2.5%), and aspartame (q.s.p.). The test gels had the same formulation, with 2% digluconate chlorhexidine or 10% *L. sidoides* essential oil added as appropriate.

The control and test gels were compounded and packaged into bottles in the Pharmaceuticals Laboratory of the University of Fortaleza. Bottles were pre-coded to ensure that neither the examiner nor the participants knew their content, which was revealed by the pharmacist only after the study was completed. In accordance with the parallel group design, subjects used only one of these gels throughout the study period.

### Experimental design

Participants were assigned to either the control group (placebo gel, n=10) or the test groups, CLX (chlorhexidine gel, n=10) and LS (*L. sidoides*-based gel, n=10), by random permutation of three. Participants were examined for plaque and gingivitis at baseline and after three months. A single, previously calibrated examiner scored the BI and the plaque index (PI)<sup>24</sup>, which were recorded on the buccal, mesial, distal and lingual surfaces of all teeth. The values of the four sites of each tooth were averaged to determine the BI and PI for each subject. In addition to this examination, the hard and soft tissues of the oral cavity were visually inspected for the presence of any adverse reaction by the same examiner.

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 gel was given to all participants. They were then instructed to apply the gel over all bristles of the toothbrush and brush their teeth for one minute, three times a day, using their habitual technique. Verbal and written instructions about the correct use of oral hygiene products were given to all subjects as well. In addition to verbal instructions, participants were given

recommendations to follow at home. On the last day of the experiment stage (day 90), indices were recorded and the teeth were polished with pumice.

### Statistical analysis

ANOVA and Student Newman-Keuls post-hoc analysis were performed to evaluate statistical differences between the control and test groups on days 0 and 90 ( $\alpha=.05$ ). In each group, the mean scores of all indices were compared between baseline and the end of the trial with the paired t-test ( $\alpha=.05$ ). However, for illustrative purposes, the results are presented as means and standard deviations.

## RESULTS

All participants completed the trial. Both test gels had good acceptance and did not produce any adverse effects, such as ulcerations or allergic reactions.

At baseline, there was no statistically significant difference between the control and test groups with respect to mean PI ( $P=.8376$ ) and BI ( $P=.3198$ ). These findings indicated that all groups were well balanced at baseline (Tables 1 and 2). At day 90, there was a statistically significant difference in PI and BI scores between the control and test groups ( $P<.05$ ) (Table 1).

Comparison of means between baseline and day 90 in each group showed a statistically significant difference in BI and PI scores for the CLX and LS groups in relation to the control group ( $P<.05$ ), but no difference between the CLX and LS groups ( $P>.05$ ) (Tables 1 and 2).

## DISCUSSION

The inability of the adult population to perform adequate mechanical tooth cleaning has stimu-

Table 1. Plaque index (PI) on day 0 and day 90, control and test groups (mean + standard deviation).

	Control	LS	CLX
Day 0	2.87 + 1.00 A, a	3.10 + 0.68 A, a	2.94 + 0.53 A, a
Day 90	2.10 + 0.56 B, a	1.55 + 0.79 A, b	1.43 + 0.79 A, b

Different uppercase letters (A, B) in the same row denote a statistically significant difference ( $P<.05$ ).

Different lowercase letters (a, b) in the same column denote a statistically significant difference ( $P<.05$ ).

Table 2. Bleeding index (BI) on day 0 and day 90, control and test groups (mean ± standard deviation).

	Control	LS	CLX
Day 0	0.48 + 0.08 A, a	0.36 + 0.10 A, a	0.52 + 0.13 A, a
Day 90	0.38 + 0.12 B, a	0.23 + 0.09 A, b	0.25 + 0.16 A, b

Different uppercase letters (A, B) in the same row denote a statistically significant difference ( $P<.05$ ).

Different lowercase letters (a, b) in the same column denote a statistically significant difference ( $P<.05$ ).

lated the search for chemotherapeutic agents that can improve plaque control and gingivitis.<sup>4,5,6,7</sup> This paper presents the data of a clinical study where a phytopharmaceutical agent in gel dentifrice formulation was used in a group of patients with gingivitis and compared with chlorhexidine digluconate. The design was based on previous studies and it was chosen to generate the best possible clinical evidence.<sup>14,25</sup>

Chlorhexidine digluconate has been tested for many years and its long-term efficacy and safety have been confirmed in several in vivo studies.<sup>4,7,8,9</sup> Likewise, the absence of adverse effects with use of the herbal agent tested herein showed that it was well tolerated, supporting its safety profile for clinical use. These results were already expected, as the biocompatibility of *L. sidoides* essential oil has been reported previously,<sup>26</sup> although mild, transient burning had been noted after use of a mouth rinse containing this natural agent.<sup>10</sup> Unfortunately, chlorhexidine has some disadvantages, such as discoloration in proximal areas and the tongue and a reversible effect on taste.<sup>4,8,9</sup> In the present study, these aspects were indeed observed in some participants, in accordance with the findings of Botelho et al.<sup>10</sup>

In this study, the Turesky index<sup>24</sup> was used due its sensitivity for detection of small plaque deposits.<sup>2,22</sup> However, as the cutoff between scores can be difficult to assess and could interfere with results, calibration of examiners was performed to address this issue and ensure the reliability of results.<sup>2</sup> Other studies have recorded plaque accumulation using a similar plaque index.<sup>2,14,25</sup>

In the control group, PI and BI remained at baseline levels at the end of the experiment, indicating the inability of this adult population to perform adequate tooth cleaning. In contrast with other studies, in which patients were instructed to use the Bass technique,<sup>14,25</sup> habitual tooth brushing was not modified to avoid concealment of the actual effect of the test agents.

Recently, reports of a number of medicinal herbs used in the treatment and prevention of gingivitis have been published worldwide, with limited<sup>13,14,15,25</sup> and encouraging results.<sup>10,11,12,22,27,28</sup> Despite its commercial use for pharmaceutical purposes, there is a lack of data to support claims of an antigingivitis and antiplaque effect of *L. sidoides*. To the best of our knowledge, the present

work is the first to evaluate the effect of a gel containing *L. sidoides* essential oil in the treatment of gingivitis. The results showed that both test formulations were efficient for plaque reduction (52% in the CLX group and 50% in the LS group). This percent difference was not significant at the end of the trial. Conversely, the control group presented a higher, but not significant, percent increase in plaque buildup (12%).

In vitro studies have shown that *L. sidoides* extract was effective in inhibiting the growth of oral pathogens,<sup>18,20</sup> which led us to deduce that this phytopharmaceutical could be used as an antiplaque agent. This hypothesis was confirmed in this study and is in agreement with the previous findings of Botelho et al.<sup>10</sup> The concentration of *L. sidoides* essential oil used in this trial was based in a previous in vitro study, in which 2%, 5% or 10% *L. sidoides* preparations showed inhibition rates similar to those of chlorhexidine.<sup>20</sup> Because in vitro conditions do not fully reproduce the oral environment and part of the gel could be lost by expectoration or other factors,<sup>22,29</sup> the highest concentration was used. Nevertheless, another clinical study showed an antiplaque effect of 1% *L. sidoides*-based mouth rinse using a 7-day treatment regimen.<sup>10</sup> However, it is interesting to note that this study did not include a negative control group, which may have biased results.

Another clinical study found that a gel containing 10% *L. sidoides* essential oil was not a good antiplaque agent.<sup>22</sup> This study used a 21-day partial-mouth experimental model of gingivitis, in which the test gel was placed undiluted on a toothshield. It is possible that solubilization in saliva or the mechanical action of a toothbrush may be necessary for an antibacterial effect to occur,<sup>13,22</sup> which could explain the difference between this and the present study. We might also infer that, in our trial, tooth brushing released the volatile active components from the test gel, thus enabling these compounds to exert their biological actions.

This study did not test the bioavailability or half-life of *L. sidoides* essential oil in the formulated gel. By inference, a study showed that, after a single oral dose, thymol can be detected for 24 h in urine and 41 h in plasma, which shows its high systemic availability in humans.<sup>30</sup> Moreover, use of *L. sidoides* essential oil did not induce any significant acute toxicological changes as evaluated

by biochemical or hematological parameters, and this product is considered safe for use in vivo.<sup>19</sup>

The composition of *L. sidoides* essential oil was similar to that reported in other studies, with the phenolic compounds thymol and carvacrol being the major constituents.<sup>18,19</sup> These volatile oils constitute a group of plant secondary metabolites that are best obtained through hydrodistillation and have potent antimicrobial activity, as has been well documented in the literature.<sup>10,18,19,28</sup>

Thymol and carvacrol have a similar molecular formula (C<sub>10</sub>H<sub>14</sub>O), with only a minor structural difference in the position of the hydroxyl group.<sup>18</sup> The antimicrobial action of these compounds is attributed to their phenolic character and is similar to that of eugenol. Its antimicrobial action occurs at the cell membrane level and is attributed to cellular lipid changes, loss of intracellular material, and inhibition of nucleic acid synthesis.<sup>31</sup> These data support the antiplaque effect of *L. sidoides* found in the present trial and are in agreement with other studies that investigated agents with similar compounds.<sup>10,18,20,28</sup>

The test groups showed significant reductions on gingivitis at the end of the trial (LS, 40%; CLX, 52%); this is consistent with previous studies.<sup>10,21</sup> Nevertheless, this percent difference was not significant, showing that LS had a potential similar to chlorhexidine as an antigingivitis agent. In spite of insufficient data in the literature about the anti-inflammatory action of *L. sidoides* preparations, this property has been reported previously.<sup>10,21,22</sup>

This anti-inflammatory action could be due an indirect action on plaque reduction or to a direct effect on the cyclooxygenase cycle (COX-2).<sup>22</sup> Similarly to those found in other herbal products, the flavonoids contained in *L. sidoides* essential oil inhibit phosphodiesterases, which are involved in cell activation, and their effect depends on the biosynthesis of protein cytokines that mediate adhesion of circulating white blood cells to sites of injury.<sup>28,32</sup> Despite these potential explanations, the exact mechanism of the anti-inflammatory action of *L. sidoides* is still unknown, and further studies are required.

The BI is a generally used dichotomous index for evaluation of gingivitis<sup>10,13,14</sup>, but it does not assess 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.<sup>12,22,33</sup> However, color change, used as a parameter in this grading index, is not necessarily an accurate indicator of gingivitis.<sup>33</sup> Furthermore, laboratory tests such as enzyme immunoassays of gingival crevicular fluid would be required for a better understanding of the role of *L. sidoides* preparations as effective agents for gingivitis control.

Home-use studies are often influenced by a number of factors which can mask the superiority of a test agent over controls. Participants of clinical trials may experience some improvement not specifically associated to the therapeutic properties of the test agent, but rather related to a behavioral change; this is known as the Hawthorne effect.<sup>14,34</sup> Subjects enrolled in oral hygiene studies usually improve their tooth brushing irrespective of the product they receive.<sup>14,34</sup>

Although the volunteers in the present study were not aware of which gel they were using, another main factor is the so-called novelty effect, which is the motivation to improve oral hygiene practices induced by the use of a new substance. In contrast, lack of compliance with correct use of the gel can occur as well.<sup>14,34</sup> In order to minimize this potential, participants were asked to bring the bottle at the end of the trial, so we could perform an indirect assessment of compliance. The reduction in gingivitis observed in both test groups showed that participants used the product correctly, at least to some extent.

Finally, the results showed that LS was an effective herbal antigingivitis agent, with performance similar to that of chlorhexidine digluconate, and could be advantageous in cases where patients spend little time on toothbrushing. Further clinical studies are required to evaluate the action of this herbal agent in other oral diseases, such as chronic periodontitis.

## CONCLUSION

A gel formulation containing 10% *L. sidoides* essential oil was an efficient antiplaque and antigingivitis agent.

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