Do natural ingredients in a dentifrice contribute to prevention of plaque and gingivitis?

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The data that support the findings of this study are available from the corresponding author upon reasonable request.
Abstract

Objective: To test the effectiveness of a dentifrice containing the turmeric and licorice extract compared to a control for preventing plaque and gingivitis over a four-month period.

Material and methods: Ninety (non-dental) participants with moderate gingival inflammation (≥40%) were selected. The triple blind study consisted of two phases, namely at first a 3-week pre-experimental phase of using an oxygenating and chlorhexidine (CHX) mouthrinse. Secondly, a 4-months experimental period in which participants were randomly assigned to a test or control group. All were instructed to brush their teeth twice daily for 2 minutes with their assigned dentifrice. Gingival bleeding (BI), plaque (PI) and gingivitis (GI) were assessed.

Results: Eighty participants completed the protocol. At the first assessment in the pre-experimental phase, the mean scores of all indices showed no differences for the two groups. At the second session, the values of all three parameters had decreased significantly (p<0.001). At the last session the BI values were 0.52(0.25) for the test group and 0.56(0.25) for the control, the mean GI was 0.27(0.17) for the test group and 0.31(0.16) for the control, and for PI the scores were 1.89(0.46) for the test group and 1.98(0.43) for the control group. Statistical comparison of the scores for the two groups at each stage of the study showed no significant difference for any of the parameters.

Conclusion: Within the limits of the current study design, dentifrice formulation and concentration of turmeric/licorice extracts the results show that the adjuvant effect of the natural ingredients in the test dentifrice was not evident on clinical parameters of gingivitis and plaque.

Key words: natural ingredients, dentifrice, gingivitis, gingival bleeding, dental plaque, clinical trial

Running head: natural ingredients in a dentifrice
Introduction

Early morphological and histopathological research showed that an intimate spatial relationship existed between dental plaque and the gingiva and periodontal tissues.\(^1\) Subsequent studies have provided confirming evidence that optimal control of supragingival plaque is a prerequisite for periodontal health.\(^{1-3}\) Although it is generally recognized that mechanical cleaning is potentially useful for controlling of supra-gingival plaque, the expectation that each person should maintain a high standard appears to be beyond most people's capabilities. Few people can sustain the dedication required to consistently perform a suitable daily tooth-cleaning ritual.\(^4\)

The use of dentifrice and a toothbrush is an integral part of most oral hygiene regimes.\(^5\) The widespread use of fluoride in dentifrices and the decreased prevalence of caries indicate that therapeutic agents can successfully be incorporated into dentifrice formulations, with no extra effort required by the user.\(^6\)

The additional daily use of anti-inflammatory compounds may be beneficial in oral care products to prevent the development of gingivitis or periodontitis.\(^7\) Various medicinal herbs have been used for centuries in traditional medicine\(^8\) and there have been many reports about the use of traditional plants and natural products for treating oral diseases.\(^9\) Amongst these herbs are turmeric root and licorice. Beneficial actions attributed to turmeric are analgesic, antibacterial, anti-inflammatory, anti-tumor, anti-allergic, antioxidant, and astringent.\(^10,\,11\) In vitro and vivo studies have indicated the possible potential of licorice and its bioactive constituents for the management of oral diseases.\(^12,\,13\) Licorice has been reported to inhibit plaque formation\(^14\) and to inhibit anticariogenic properties\(^15,\,16\).

Dentifrice manufactures are looking for additives that can further enhance the effectiveness of their products. At the same time consumers are seeking oral hygiene products with natural as part of a healthier lifestyle.\(^8,\,17\) These changes, along with marketing of herbal products, has led to increased use of natural compounds in foods, cosmetics, and pharmaceutical products.\(^8\) However, "natural" products are not undoubtedly somehow safer, better, or healthier. With regard to combining herbal products and consumer and marketing trends, it was of interest to explore whether the combination of turmeric and licorice extracts in dentifrice may provide a promising oral-care product to prevent gingivitis and periodontitis.

A clinical trial was performed to evaluate the clinical efficacy of these two compounds in an experimental dentifrice formulation. In order not to change the brushing habits of participants and
to achieve an improvement in gingival health in a short period of time to a level from which deterioration can be measured, rinsing with antimicrobial in a pre-experimental phase was chosen as a research model.\textsuperscript{18} 19 20 The aim of the present study was to evaluate the potential of a dentifrice with natural ingredients to inhibit gingivitis development over the subsequent four months in healthy participants.
Materials and methods
The recommendations for strengthening the reporting were followed, as suggested by the guidelines outlined in Consolidated Standards of Reporting Trials (CONSORT) and the checklist of the Template for Intervention Description and Replication (TIDieR) were used.

Ethical procedures
This study followed the Good Clinical Practice (CPMP/ICH/135/95) guidelines, in agreement with the ethical principles of the Declaration of Helsinki (October 2013, Brazil) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and applicable local regulations. The study was approved by the medical ethical committee at Amsterdam Medical Centre (MEC 07/021) and was registered at the Dutch Trial Register (NL1170). The study was conducted at the Department of Periodontology Academic Centre for Dentistry of Amsterdam (ACTA), in the Netherlands.

Before enrolment, all volunteers were provided with verbal and written information regarding the aim, rationale and duration of the study. The investigator explained the details of the trial and the potential risks involved. Prior to the study, an informed consent form was signed by all eligible participants who agreed to participate.

Sample size
To detect a 0.19 difference in scores for bleeding on marginal probing scores between two groups, at an 80% power level with an alpha of 0.05, a sample size of 40 was required. This number was calculated using a standard deviation (SD) of 0.31 based on earlier research. To allow for dropouts, a sample size of ≥45 participants per group was chosen. This study design was also able to discern a difference in plaque scores between two groups of 0.26, with an expected standard deviation of 0.43, an alpha of 0.05 and power of 80%.

Recruitment and inclusion
A total of 90 healthy participants were enrolled. They were non-dentistry students who had moderate gingivitis and fitted the inclusion criteria; they were recruited from universities and colleges in and around Amsterdam. They were informed about the study in a recruitment letter and at the first appointment and were given a written explanation of the background of the study, its objectives, and their involvement. Participants agreed to participate in the study. They were numbered consecutively according to their arrival in the study.
The participants had been screened by a dental hygienist (NAMR). To qualify for inclusion, the participants were required to be 18 years or older, systemically healthy, and non-smokers. They were also required to have at least five teeth per quadrant and to display moderate gingivitis (≥ 40% bleeding on marginal probing). Excluded were those who presented with an orthodontic appliance or a removable (partial) denture, any pathological alterations of the oral mucosa, or overt caries. Also excluded were those:
- who were pregnant or breastfeeding;
- with any relevant allergies or on relevant medications;
- who were participating in professional dental cleaning during the study period or who had participated in a clinical study within the previous 30 days;
- who were having concomitant therapy;
- with current periodontitis with periodontal pocketing ≥ 5mm
- with non-physiological tooth mobility.

All participants completed a medical questionnaire. A necessary concomitant medication or therapy was permitted as long as it was not in the exclusion criteria. All changes in health and use of medication during the study were documented.

Study Products

Product 1:
This dentifrice was the test or experimental one. It contained 0.01% Glycyrrhiza inflata root extract; 0.1% tetrahydrocurcumin (THC), 1400 ppm F (NaF), GABA International AG.

Product 2:
This was the control dentifrice. It contained 1.400 ppm fluoride from sodium fluoride, GABA International AG.

Other ingredients in both products were as follows (qualitative): aqua, sorbitol, hydrated silica, hydroxyethylcellulose, PEG-40 hydrogenated castor oil, cocamidopropyl betaine, titanium dioxide, aroma, sodium citrate, disodium lauryl sulfosuccinate, sodium fluoride, alumina, saccharin, methylparaben, tocopherol, polyaminopropyl biguanide, propylparaben, phenoxyethanol, benzoic acid, dehydroacetic acid, and stearic acid.

Both products were packed in identical tubes.

Randomization
Allocation to the test and control group was assigned by the means of a sealed opaque envelope containing a code derived from a computer-generated randomized list. The randomization list was limited to the persons of the sponsor responsible for creation of the randomization list, preparation of the random code envelopes, and preparation of the study products until final examination of the last participant and completion of the case report forms. Copies of the randomization list were kept in sealed envelopes in case of emergency that would require knowledge of the specific treatment.

Clinical assessments
Throughout the study all examinations were performed by the same trained examiner (NAMR) under the same conditions at the dental faculty, ACTA Amsterdam. The examiner was blinded to the treatment randomization. At every examination, data were recorded on a case record form (CRF). Records of earlier examinations were not available at the time of re-examinations.

The indices were scored in two randomly chosen contra-lateral quadrants of the mouth, either quadrants I and III or quadrants II and IV. (That is, either upper right and lower left quadrants, or upper left and lower right). Once chosen, the selected quadrants stayed the same throughout the study for each individual participant.

Primary study parameter
Bleeding Index (BI) as Bleeding on Marginal Probing (BOMP; acc. to van der Weijden et al. 1994). The absence or presence of bleeding was scored within 30 s of probing, on a scale from 0 to 2 (0 = no bleeding, 1 = pinprick bleeding, 2 = excess bleeding). The gingival margin was probed at an angle of approximately 60° to the longitudinal axis of the tooth.

Secondary study parameters
Modified Gingival Index (GI; visual aspect only; Lobene et al. 1986). The gingival condition was assessed using visual signs of inflammation as scored on a scale from 0 to 4 (0 = pale pink, 4 = reddish-blue and enlarged).

Plaque Index (PI). Plaque was assessed after disclosing with Mira-2-Ton® (Hager & Werken GmbH & Co. KG. Duisburg, Germany) according to the Quigley & Hein plaque index, as described in detail by Paraskevas et al. (2007). The results were scored on a scale of 0 to 5.

Study design
The study was designed as a single-center, randomized, parallel group, placebo controlled and consisted of two phases, a pre-experimental phase of three weeks and an experimental phase of four months. The participants were blinded to the product and the examiner was blinded to treatment randomization. Text messages (short message service, SMS) were sent to remind each participant before the visits concerning the study procedures and appointments.

**Pre-experimental phase**
At the start of the pre-experimental phase, participants were instructed to brush their teeth 2 to 3 hours prior to their appointment to avoid the risk of increased bleeding from tooth brushing.\(^{31, 32}\) Clinical parameters of BI, GI, and PI were assessed. Participants received professional and written instructions in the use of a manual toothbrush (Aronal® öko-dent soft, GABA International AG) according to the Bass-technique.\(^{33}\) Furthermore, a combination of Bocasan® (Oral-B Laboratories, Cincinnati, OH, US) and chlorhexidine 0.20% (Corsodyl®, GSK, Zeist, The Netherlands) was used to rinse twice daily for 1 minute during the three weeks of the pre-experimental phase before the experimental period.\(^{20}\) The purpose of the pre-experimental phase was to motivate participants to follow an oral hygiene regime capable of achieving and maintaining healthy gingivae. As a check for compliance, participants were asked to register the time of using the products onto a calendar record chart. During the pre-experimental phase, the participants brushed with Everclean® dentifrice (HEMA, Amsterdam, The Netherlands) and a standard toothbrush. Participants also received a stopwatch as a timer to control their total brushing time of 2 min.

The next appointment for the baseline assessment (Bleeding on Marginal Probing, Modified Gingival Index, Plaque Index,) was scheduled 3 weeks later and participants were instructed to return all remaining products received for the pre-experimental phase toothbrush to ensure no further use of these products.

**Experimental phase**
At the start of the experimental phase (baseline), participants were instructed to brush their teeth 2 to 3 hours prior to their appointment at the clinic in order to avoid the risk of increased bleeding as a result of toothbrushing.\(^{31, 32}\) Each participant was asked about any changes in medication, their general health, participation in other research, and dental treatment received other than in the study protocol.

All three parameters (BI, GI and PI) were assessed. To ensure that participants would enter the experimental phase of the study with equally clean teeth, a dental hygienist provided a
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professional dental scale and polish after the clinical assessment, spending up to a maximum of 30 min. Participants were then randomly assigned to one of the two groups, test and control. New study products were provided to last them until the next appointment, including new toothbrushes (Aronal® öko-dent soft).

All participants were instructed to brush their teeth with their assigned dentifrice for 2 min twice daily, using the timer. No further oral hygiene instruction was given at any stage during the remainder of the study. Participants were told to refrain from using mouthrinses but there was no other interference with their habitual interdental oral hygiene habits.

After two months participants were provided with a fresh exchangeable brush head for the manual toothbrush. At the end of the experimental phase (4 months), participants were instructed to brush their teeth two to three hours before their appointment at the clinic to avoid the risk of increased bleeding from tooth brushing.31,32 Participants returned all their study products, dentifrice tubes were weighed. Clinical examinations were performed. All parameters assessed at the start of the study were re-evaluated at this visit (BI, GI, PI).

Questionnaire
After completing the clinical assessment, participants were asked to complete a short questionnaire to assess their attitude to the assigned dentifrice. A visual analogue scale (VAS) was used in most questions to assess their opinions.34 Participants were requested to mark a point on a 10-cm uncalibrated line, with the two endpoints annotating extreme responses. The left side was an extreme negative response, and the right was the positive extreme. After the trial, participants resumed using their normal oral hygiene procedures.

Monitoring of compliance and adverse events
The dentifrice tubes were weighed in advance and after being returned to the clinic to assess compliance. If a participant discontinued the study, their reasons and circumstances of discontinuation were documented. Any adverse events reported by participants during the course of the study were appropriately recorded.

Statistical analysis
Computations were performed using R (https://www.r-project.org).35 The BI scores were used as the main response variable. Analyses comparing differences between the test and control groups were performed using Mann-Whitney tests. Wilcoxon tests were performed to analyze differences within the groups between sessions. An analysis of covariance (ANCOVA) was performed with the scores of session 1 as the covariate. P-values ≤ 0.05 were accepted as statistically
significant. Differences in mean plaque score reduction between dentifrices were expressed as a ratio and as a percentage reduction relative to the control dentifrice. The statistical analysis was performed blinded to product allocation.
RESULTS

The study flow proposed by Consort\textsuperscript{21}, is shown in Figure 1, which also provides details about drop-outs of the study. A total of 132 possible participants were recruited, of whom 90 participants were found to be eligible.

In total, 80 participants completed the protocol. Table 1 shows their general characteristics. About 80\% of participants were female and the mean age of all participants was 22.2 years (SD 2.50) with a range of 18-29 years. There was no statistically significant difference between the groups in relation to age (p = 0.07) and sex (p = 0.66).

Table 2a-2c show the mean scores of both groups, for all indices and at all sessions. At the first assessment (pre-experimental phase), the mean scores for all indices did not differ between the groups. At the second assessment (baseline), none of the values for any of the parameters of interest showed significant differences between groups.

At the third session, which was held after four months, the values of BI, GI and PI did not show any significant differences between the groups. No statistically significant differences (p < 0.05) were observed between the groups at any stage of the study, for any of the parameters or at the different measurement times. However, for both groups, all three parameters decreased significantly (p < 0.001) during the pre-experimental period – that is, between the first assessment and the baseline measurements.

Results from the questionnaire showed that a statistically significant difference (p = 0.003) could be observed regarding the strongness of the taste between the two dentifrices. Participants expressed their preference for the control dentifrice (Table 3).

Weighing of the dentifrice tubes indicated an average use of 226.33 g (SD 85.6) across the 78 participants during the four months of the study. Divided by groups, this resulted in a mean of 209.5 g (66.7) for the test group, whereas for the control group the mean was 241.6 g (98.0). This difference was not statistically significant (p = 0.093).

Adverse events

In the pre-experimental phase, eight participants complained about a burning sensation, alteration in taste and irritation of mucosa. Three of these eight participants stopped using the Bocasan mouthrinse. During the experimental phase, two adverse events in the control group...
were registered: one person dropped out after complaining of irritation of the gingiva because of
the use of the prescribed toothbrush; another dropped out because of irritation of the mouth, with
ulceration, bad taste, and warm and sour sensitivity. One adverse event registered for the test
group was a drop-out who complained about a burning feeling in the mouth. See Figure 1.
DISCUSSION

The design of the present study was based on a model published by Svatun et al.\textsuperscript{18, 37} The current study tested after gingival health was established through a prophylactic aid the concept whether a dentifrice can prevent deterioration of the gingival status. It is presumed that without the ongoing use of the prophylactic aid, improved gingival health tends to fade away over time and returns to its original values. It has been observed that the most marked deterioration occurs within the first three months following the pre-experimental phase, indicating a relatively rapid loss of the dedication required to maintain effective plaque control.\textsuperscript{6} In both the Svatun studies\textsuperscript{18, 37}, a moderately inflamed gingival condition in a group of young, health-conscious volunteers was brought to an excellent state of health by professional cleaning and oral hygiene instruction. This study model proved to be effective in testing dentifrice to suppress both plaque accumulation and the development of gingivitis.

In the present study, the Svatun\textsuperscript{18} model was adapted so that during the three-week pre-experimental phase, oral hygiene instruction was combined with the use of oxygenating and chlorhexidine (CHX) rinse. The use of these mouthrinses was added to enable participants to enter the experimental phase with the healthiest possible gingival condition. This would assist the researchers in discerning the maximum differences in gingivitis levels between Day 0 and baseline. The same adaptation was used previously and was effective for reducing the mean score of an inflammatory parameter.\textsuperscript{4}

From a systematic review, there is moderate evidence that a combination of CHX and an oxygenating rinse (H\textsubscript{2}O\textsubscript{2}) reduces tooth staining without interfering with the plaque growth inhibition of CHX.\textsuperscript{38} This might be why no participants dropped out during the pre-experimental phase due to CHX staining. One of the most widely used detergents in dentifrice is sodium lauryl sulphate (SLS). There has been a lengthy discussion regarding whether SLS and CHX counteract each other. To date, the general recommendation by CHX manufacturers is to rinse with CHX 30 min after brushing or to use an SLS-free dentifrice. This is in contrast to the findings of a systematic review that showed that the combination of CHX and a SLS dentifrice is not contraindicated.\textsuperscript{39} Therefore, the regular dentifrice in the pre-experimental phase could be used without any specific instruction regarding CHX effectivity.

At session 1 (pre-experimental), no significant differences between groups were observed, indicating that the groups were comparable. Again, at session 2 (baseline), no significant
differences were observed. However, as expected, significant decreases in all measured variables were noted after the participants received the instructions and both mouth rinses. Although no statistically significant differences between groups were observed at session 3, there might have been an effect of the test dentifrice; if so, it was not large enough to be detected with the statistical method for analyzing a two-group parallel design. A Wilcoxon test to check for differences within groups over time indicated a significant increase for the control group regarding mean BOMP scores. The test group, however, did not exhibit a significant increase. See Table 2. As we expected based on the Svatun et al. 18 paper the bleeding scores in the control group did not return to approximately the baseline level. This in contrast to the PI. Why this is the case cannot be explained based on the present findings. We have reported what we observed from the analysis of the collected data. Fortunately, this was a controlled study so that both groups were affected by any effect that may have resulted in the present findings.

In this study, licorice, and turmeric root (also known as glycyrrhiza glabra and curcuma xanthorrhiza respectively) were the active ingredient in the dentifrice under investigation. Both additives were employed medicinally by ancient civilizations such as the Romans and Chinese. For many years, the capacities of these plants have been used to improve human health.40 Licorice is also used as an important sweetening and flavoring agent in food products, beverages, medicines and dentifrice.41, 42 In modern medicine, isolated components of licorice have been shown to inhibit the growth and cytopathology of viruses - such as hepatitis A43 and C,44, 45, herpes zoster,46 HIV,47, 48 herpes simplex,49, 50, and cytomegalovirus.51 Evidence for a therapeutic application of licorice in oral diseases has also been reported.12, 42, 52 Similarly, curcuma is widely investigated, and numerous studies have reported medicinal benefits from its components, the curcuminoids. 53, 54

Although both licorice and turmeric are often suggested to be effective in oral care products, the proposed mechanism is hardly ever mentioned. It has also been reported that the mechanism by which licorice may inhibit dental plaque formation is not fully understood14 Licorice however has shown to have antibacterial activity against S.mutans15, 16 and also anti-adhesive properties against P.gingivalis.55 Curcumin is most commonly reported as an antioxidant with antibacterial and anti-inflammatory effects 54, 56, 57,

The combination of licorice and turmeric is well known in food, tea and skin products. It is presumed that it may also be a promising adjective for dentifrice products. This combination
promotes strong anti-oxidative activity, which supports the action of preventing gingivitis. Before the trial, the efficacy of an enriched licorice and turmeric root extract was investigated in vitro. The model used is well established for testing anti-inflammatory effects of compounds as it examines various inflammatory mediators.\textsuperscript{58, 59} Parameters such as PGE2 and IL-1 are known to play a crucial role in gingivitis. The in vitro results showed that the tested extracts potently prevented both PGE2 and IL-1 release. However, the statistical comparison between groups at each stage of the in vivo study, for all parameters, showed no significant differences.

The reason for the incremental differences between sessions not being reflected in the parallel analyses between groups is unclear and requires speculation. Apparently, the effect is detectable but small. Maybe a pre-trial phase without the use of both mouthrinses would have made difference between both test and control groups in time clearer. It is conceivable that the effect of the pre-trial phase might have lasted throughout the four-month session. However, Van Leeuwen et al.\textsuperscript{60}, demonstrated limited residual effects from a rinsing treatment after four months.

In addition, the professional cleaning and polishing likely contributed to the improved oral health scores. Both of these interventions have a known short-term effect on all parameters.\textsuperscript{20, 61, 62} This gives researchers the opportunity to observe an effect of a participant returning to their habitual level for the parameters assessed at the end of the trial. The process was described by Svatun et al.\textsuperscript{18} The combination of oral hygiene instruction, professional prophylaxis, and the use of the combination of both CHX and the oxygenating rinse may have resulted in an overwhelming effect, which would limit the potential effect of the test dentifrice. Other possible explanations for the lack of adjuvant efficiency may have been insufficient concentrations of the natural ingredients and/or chemical incompatibility in the dentifrice formulation which also contains a mixture of anionic detergents and organic antibacterial surface agent which may have counteracted with the presumed active ingredients.\textsuperscript{63}
CONCLUSION
Within the limits of the current study design, dentifrice formulation and concentration of turmeric/licorice extracts the results show that the adjuvant effect of the natural ingredients in the test dentifrice was not evident on clinical parameters of gingivitis and plaque. A modified set up of protocol, formulation and concentration could be subject to future research.
CLINICAL RELEVANCE

Scientific rationale for the study
When new oral hygiene products are developed, it is of important to assess their effectiveness and safety. Such studies can inform dental professionals regarding a dentifrice’s inhibitory effects on plaque and its effect on gingivitis parameters.

Principal findings
After four months of using the test dentifrice with a specific formulation and concentration of turmeric and licorice, no adjuvant effect of these medicinal herbal extracts was found in comparison with the control dentifrice for the employed study design.

Practical implications
A practical implication of the study is that a design with a pre-experimental phase in which a combination of both CHX and an oxygenating agent as prophylactic aid is used could be of influence to detect differences in four-month studies.
References

1. Löe H. Oral hygiene in the prevention of caries and periodontal disease. Int Dent J. 2000;50:129-139.
2. Löe H, Theilade E, Jensen SB. Experimental Gingivitis in Man. J Periodontol. 1965;36:177-187.
3. Axelsson P, Lindhe J. Effect of controlled oral hygiene procedures on caries and periodontal disease in adults. J Clin Periodontol. 1978;5:133-151.
4. Rosema NA, Timmerman MF, Versteeg PA, van Palenstein Helderman WH, Van der Velden U, Van der Weijden G. Comparison of the use of different modes of mechanical oral hygiene in prevention of plaque and gingivitis. J Periodontol. 2008;79:1386-1394.
5. Hioe KP, Van der Weijden GA. The effectiveness of self-performed mechanical plaque control with triclosan containing dentifrices. Int J Dent Hyg. 2005;3:192-204.
6. Saxton CA. A combination of therapeutic agents for the control of dental plaque and gingivitis in man. [Academic Thesis]. Catholic University, Nijmegen; 1993. p. 126.
7. Van der Weijden GA, Slot DE. Oral hygiene in the prevention of periodontal diseases: the evidence. Periodontol 2000. 2011;55:104-123.
8. Vyas S, Kulkarni S. Patanjali Dant Kanti: Is it worth all the hype!? Comparative evaluation with other herbal dentifrices for efficacy against S. mutans. IJAR. 2018;4:212-215.
9. Palombo EA. Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases. Evid Based Complement Alternat Med. 2011;2011:680354.
10. Chaturvedi TP. Uses of turmeric in dentistry: an update. Indian J Dent Res. 2009;20:107-109.
11. Hugar SS, Metgud R. Turmeric in dentistry. Indian J Sci Res. 2015;4:2553-2557.
12. Messier C, Epifano F, Genovese S, Grenier D. Licorice and its potential beneficial effects in common oro-dental diseases. Oral Dis. 2012;18:32-39.
13. Tharakan AP, Pawar M, Kale S. Effectiveness of licorice in preventing dental caries in children: A systematic review. J Indian Soc Pedod Prev Dent. 2020;38:325-331.
14. Segal R, Pisanty S, Wormser R, Azaz E, Sela MN. Anticariogenic activity of licorice and glycyrrhizine I: Inhibition of in vitro plaque formation by Streptococcus mutans. J Pharm Sci. 1985;74:79-81.
15. Sela MN, Steinberg D, Segal R. Inhibition of the activity of glucosyltransferase from Streptococcus mutans by glycyrrhizin. Oral Microbiol Immunol. 1987;2:125-128.
16. He J, Chen L, Heber D, Shi W, Lu Q-Y. Antibacterial Compounds from Glycyrrhiza uralensis. J Nat Prod. 2006;69:121-124.
17. Janakiram C, Venkitachalam R, Fontelo P, Iafolla TJ, Dye BA. Effectiveness of herbal oral care products in reducing dental plaque & gingivitis—a systematic review and meta-analysis. BMC Complement Med Ther. 2020;20:1-12.
18. Svatun B, Saxton CA, Van der Ouwerka F, Rölla G. The influence of a dentifrice containing a zinc salt and a nonionic antimicrobial agent on the maintenance of gingival health. J Clin Periodontol. 1987;14:457-461.
19. Lie MA, Timmerman MF, van der Velden U, van der Weijden GA. Evaluation of 2 methods to assess gingival bleeding in smokers and non-smokers in natural and experimental gingivitis. J Clin Periodontol. 1998;25:695-700.
20. Gründemann L, Timmerman M, Ijzerman Y, van Der Velden U, van Der Weijden G. Stain, plaque and gingivitis reduction by combining chlorhexidine and peroxyborate. J Clin Periodontol. 2000;27:9-15.
21. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMC medicine. 2010;8:18.
22. Hoffmann TC, Glasziou PP, Boutron I, Milne R, Perera R, Moher D, et al. Better reporting of interventions: template for intervention description and replication (TIDieR) checklist and guide. BMJ. 2014;348:g1687.
23. Van der Weijden GA, Danser MM, Nijboer A, Timmerman MF, van der Velden U. The plaque-removing efficacy of an oscillating/rotating toothbrush. A short-term study. J Clin Periodontol. 1993;20:273-278.
24. Van der Weijden GA, Timmerman MF, Nijboer A, Lie MA, van der Velden U. A comparative study of electric toothbrushes for the effectiveness of plaque removal in relation to toothbrushing duration. Timerstudy. J Clin Periodontol. 1993;20:476-481.
25. Van der Weijden GA, Timmerman MF, Reijerse E, Danser MM, Mantel MS, Nijboer A, et al. The long-term effect of an oscillating/rotating electric toothbrush on gingivitis. An 8-month clinical study. J Clin Periodontol. 1994;21:139-145.
26. Bentley CD, Disney JA. A comparison of partial and full mouth scoring of plaque and gingivitis in oral hygiene studies. J Clin Periodontol. 1995;22:131-135.
27. Van der Weijden GA, Timmerman MF, Saxton CA, Russell JI, Huntington E, Van der Velden U. Intra-/inter-examiner reproducibility study of gingival bleeding. J Periodontal Res. 1994;29:236-241.
28. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in clinical trials. Clin Prev Dent. 1986;8:3-6.
29. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. J Am Dent Assoc. 1962;65:26-29.
30. Paraskevas S, Rosema NA, Versteeg P, Timmerman MF, van der Velden U, van der Weijden GA. The additional effect of a dentifrice on the instant efficacy of toothbrushing: a crossover study. J Periodontol. 2007;78:1011-1016.
31. Abbas F, Voss S, Nijboer A, Hart AA, Van der Velden U. The effect of mechanical oral hygiene procedures on bleeding on probing. J Clin Periodontol. 1990;17:199-203.
32. Van der Weijden GA, Timmerman MF, Piscaer M, YI J, van der Velden U. A clinical comparison of three powered toothbrushes. J Clin Periodontol. 2002;29:1042-1047.
33. Bass CC. An effective method of personal oral hygiene. J La State Med Soc. 1954;106:57-73.
34. Mottola CA. Measurement strategies: the visual analogue scale. Decubitus. 1993;6:56-58.
35. R Core Team. R: A Language and environment for statistical computing. 3.6.2 ed: R Foundation for Statistical Computing, Vienna, Austria.; 2019.
36. Ghassemi A, Vorwerk LM, Hooper WJ, Putt MS, Milleman KR. A four-week clinical study to evaluate and compare the effectiveness of a baking soda dentifrice and an antimicrobial dentifrice in reducing plaque. J Clin Dent. 2008;19:120-126.
37. Svatun B, Saxton CA, Rolla G, van der Ouderaa F. A 1-year study on the maintenance of gingival health by a dentifrice containing a zinc salt and non-anionic antimicrobial agent. J Clin Periodontol. 1989;16:75-80.
38. Van Maanen-Schakel NW, Slot DE, Bakker EW, Van der Weijden GA. The effect of an oxygenating agent on chlorhexidine-induced extrinsic tooth staining: a systematic review. Int J Dent Hyg. 2012;10:198-208.
39. Elkerbout TA, Slot DE, Bakker EW, Van der Weijden GA. Chlorhexidine mouthwash and sodium lauryl sulphate dentifrice: do they mix effectively or interfere? Int J Dent Hyg. 2016;14:42-52.
40. Shah B, Seth A, Maheshwari K. A review on medicinal plants as a source of anti-inflammatory agents. Res J Med Plant. 2011;5:101-115.

41. Fu Y, Chen J, Li YJ, Zheng YF, Li P. Antioxidant and anti-inflammatory activities of six flavonoids separated from licorice. Food Chem. 2013;141:1063-1071.

42. Sidhu P, Shankargouda S, Rath A, Ramamurthy PH, Fernandes B, Singh AK. Therapeutic benefits of liquorice in dentistry. J Ayurveda Integr Med. 2018.

43. Crance JM, Biziagos E, Passagot J, van Cuyck-Gandre H, Deloine R. Inhibition of hepatitis A virus replication in vitro by antiviral compounds. J Med Virol. 1990;31:155-160.

44. Su X, Chen H, Wang L, Jiang C, Liu J. Clinical and laboratory observation on the effect of glycyrrhin in acute and chronic viral hepatitis. J Tradit Chin Med. 1984;4:127-132.

45. Van Rossum TG, Vulto AG, Hop WC, Brouwer JT, Niesters HG, Schalm SW. Intravenous glycyrrhin for the treatment of chronic hepatitis C: a double-blind, randomized, placebo-controlled phase I/II trial. J Gastroenterol Hepatol. 1999;14:1093-1099.

46. Baba M, Shigeta S. Antiviral activity of glycyrrhin against varicella-zoster virus in vitro. Antiviral Res. 1987;7:99-107.

47. Ito M, Sato A, Hirabayashi K, Tanabe F, Shigeta S, Baba M, et al. Mechanism of inhibitory effect of glycyrrhin on replication of human immunodeficiency virus (HIV). Antiviral Res. 1988;10:289-298.

48. Hattori T, Ikematsu S, Koito A, Matsushita S, Maeda Y, Hada M, et al. Preliminary evidence for inhibitory effect of glycyrrhin on HIV replication in patients with AIDS. Antiviral Res. 1989;11:255-261.

49. Pompei R, Flore O, Marccialis MA, Pani A, Loddo B. Glycyrrhizic acid inhibits virus growth and inactivates virus particles. Nature. 1979;281:689-690.

50. Partridge M, Poswillo DE. Topical carbenoxolone sodium in the management of herpes simplex infection. Br J Oral Maxillofac Surg. 1984;22:138-145.

51. Numazaki K, Umetsu M, Chiba S. Effect of glycyrrhin in children with liver dysfunction associated with cytomegalovirus infection. Tohoku J Exp Med. 1994;172:147-153.

52. Madan S, Kashyap S, Mathur G. Glycyrrhiza glabra: An efficient medicinal plant for control of periodontitis—A randomized clinical trial. J Int Clin Dent Res Organ. 2019;11:32-35.
53. Ramachandran C, You W. Differential sensitivity of human mammary epithelial and breast carcinoma cell lines to curcumin. Breast Cancer Res Treat. 1999;54:269-278.
54. Nagpal M, Sood S. Role of curcumin in systemic and oral health: An overview. J Nat Sci Biol Med. 2013;4:3-7.
55. Sidhu P, Shankargouda S, Rath A, Hesarghatta Ramamurthy P, Fernandes B, Kumar Singh A. Therapeutic benefits of liquorice in dentistry. J Ayurveda Integr Med. 2020;11:82-88.
56. Hwang JK, Shim JS, Pyun YR. Antibacterial activity of xanthorrhizol from Curcuma xanthorrhiza against oral pathogens. Fitoterapia. 2000;71:321-323.
57. Kim JE, Kim HE, Hwang JK, Lee HJ, Kwon HK, Kim BI. Antibacterial characteristics of Curcuma xanthorrhiza extract on Streptococcus mutans biofilm. J Microbiol. 2008;46:228-232.
58. Schmitter T, Fiebich BL, Fischer JT, Gajfulin M, Larsson N, Rose T, et al. Ex vivo anti-inflammatory effects of probiotics for periodontal health. J Oral Microbiol. 2018;10:1502027.
59. Zemouri C, Jakubovics NS, Crielaard W, Zaura E, Dodds M, Schelkle B, et al. Resistance and resilience to experimental gingivitis: a systematic scoping review. BMC Oral Health. 2019;19:212.
60. Van Leeuwen M, Rosema N, Versteeg P, Slot D, Hennequin-Hoenderdos N, Van der Weijden G. Effectiveness of various interventions on maintenance of gingival health during 1 year—a randomized clinical trial. Int J Dent Hyg. 2017;15:e16-e27.
61. Tan HH, Saxton CA. Effect of a single dental health care instruction and prophylaxis on gingivitis. Community Dent Oral Epidemiol. 1978;6:172-175.
62. Dona BL, Grundemann LJ, Steinfort J, Timmerman MF, van der Weijden GA. The inhibitory effect of combining chlorhexidine and hydrogen peroxide on 3-day plaque accumulation. J Clin Periodontol. 1998;25:879-883.
63. Goultschin J, Palmon S, Shapira L, Brayer L, Gedalia I. Effect of glycyrrhizin-containing toothpaste on dental plaque reduction and gingival health in humans. A pilot study. J Clin Periodontol. 1991;18:210-212.
### Table 1
Study demographic characteristics

|                  | Test group N = 39 | Control group N = 41 | p-value (between groups) |
|------------------|-------------------|-----------------------|--------------------------|
| Gender           |                   |                       |                          |
| Female           | n (%)             | 32 (82%)              | 32 (78%)                 | 0.655*                   |
| Male             | n (%)             | 7 (18%)               | 9 (22%)                  |                           |
| Age (years)      | Mean (SD)         | 21.64 (2.15)          | 22.66 (2.74)             | 0.069**                  |
| Range (years)    | min-max           | 18-26                 | 18-29                    |                           |

* Chi square test

** T-test
### TABLES 2a-c

Table 2a. Mean bleeding scores (BOMP) for both groups at all sessions.

| Bleeding (BOMP) | N  | Session 1 (pre-experimental) | Session 2 (baseline) | Session 3 (end) | Diff (Session 1-2) | Diff (Session 2-3) |
|-----------------|----|-----------------------------|----------------------|-----------------|-------------------|-------------------|
| Group Test      | 39 | 1.11 (0.23)                 | 0.42 (0.18)          | 0.52 (0.25)     | -0.68 (0.22); < 0.001; -62.2% | 0.10 (0.26); 0.08; 23.8% |
| Group Control   | 41 | 1.13 (0.22)                 | 0.43 (0.18)          | 0.56 (0.25)     | -0.69 (0.23); < 0.001; -61.9% | 0.13 (0.19); < 0.001; 30.2% |
| **P-values analysis between groups** | | | | | 0.44*; 0.83*; 0.97* | 0.57*; 0.97* |
| **95% CI**      | | -0.12; 0.07                | -009; 0.07           | -0.15; 0.07     | -0.08; 0.13        | -0.16; 0.03       |
| **Ratio; % diff.** | | | | | 0.99x**; -1.43%** | 0.77x**; -23.1%** |

Table 2b. Mean gingivitis scores (MGI) for both groups at all sessions.

| Gingivitis (MGI) | N  | Session 1 (pre-experimental) | Session 2 (baseline) | Session 3 (end) | Diff (Session 1-2) | Diff (Session 2-3) |
|------------------|----|-----------------------------|----------------------|-----------------|-------------------|-------------------|
| Group Test       | 39 | 0.61 (0.38)                 | 0.23 (0.12)          | 0.27 (0.17)     | -0.38 (0.31); < 0.001; -62.3% | 0.04 (0.14); 0.12; 17.4% |
| Group Control    | 41 | 0.63 (0.26)                 | 0.27 (0.11)          | 0.31 (0.16)     | -0.36 (0.23); < 0.001; -57.1% | 0.03 (0.12); 0.09; 14.8% |
| **P-values analysis between groups** | | | | | 0.32*; 0.11*; 0.09* | 0.80*; 0.09* |
| **95% CI**       | | -0.18; 0.06                | -0.09; 0.01          | -0.10; 0.02     | -0.10; 0.12        | -0.06; 0.07       |
| **Ratio; % diff.** | | | | | 1.06x**; 5.6%** | 1.0x**; 0%** |

Table 2c. Mean plaque scores (Q&H) for both groups at all sessions.

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| Plaque (Q&H) | N  | Session 1 (pre-experimental) | Session 2 (baseline) | Session 3 (end) | Diff (Session 1-2) | Diff (Session 2-3) |
|--------------|----|-------------------------------|----------------------|----------------|--------------------|--------------------|
| Group Test   | 39 | 1.84 (0.42)                   | 0.76 (0.62)          | 1.89 (0.46)    | -1.08 (0.54); < 0.001; -58.7% | 1.12 (0.54); < 0.001; 148.7% |
| Group Control| 41 | 1.91 (0.40)                   | 0.80 (0.50)          | 1.98 (0.43)    | -1.11 (0.46); < 0.001; -58.1% | 1.18 (0.47); < 0.001; 147.5% |
| P-values analysis between groups | | 0.35* | 0.40*; 0.93* | 0.33*; 0.53* | 0.75*; 0.93* | 0.99*; 0.58* |
| 95% CI      |    | -0.26; 0.10                   | -0.26; 0.12          | -0.29; 0.12    | -0.18; 0.25        | -0.23; 0.20        |
| Ratio; % diff. ** | |                           |                      |               | 0.97x**; -2.70%** | 0.96x**; -4.24%** |

Session 1 = pre-experimental phase (3 weeks), Session 2 = baseline, Session 3 = end (4 months).
Standard deviation in parentheses.
* from Mann-Whitney test
# P-value from Wilcoxon test between sessions within each group
* P-value from ANCOVA analyses between groups with Session 1 as covariate
** Difference in reduction mean scores between dentifrices is expressed as a ratio and percentage of reduction score for the reference dentifrice. A positive value indicates greater plaque reduction in favor of brushing with the test dentifrice as compared to the reference dentifrice.
ratio = (test dentifrice mean plaque reduction)/ (reference dentifrice mean plaque reduction)
% difference in score reduction = 100% x (test dentifrice mean plaque reduction - reference dentifrice mean plaque reduction) / reference mean score reduction

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1. Ghassemi A, Vorwerk LM, Hooper WJ, Putt MS, Milleman KR. A four-week clinical study to evaluate and compare the effectiveness of a baking soda dentifrice and an antimicrobial dentifrice in reducing plaque. J Clin Dent. 2008;19:120-126.
Table 3 Questionnaire related to the attitude of the participants to the assigned dentifrice using a Visual Analogue Scale (VAS) presented as mean and standard deviation (SD) with negative extremes on the left and positive extremes on the right (from 0 to 10).

| Question                                                                 | Extreme          | Dentifrice |
|--------------------------------------------------------------------------|------------------|------------|
|                                                                          | From        | To         | TEST N = 39 | CONTROL N = 41 | P *          |
| What is your opinion of the taste of the dentifrice?                      | very bad    | very nice  | 5.26 (2.10) | 5.66 (2.14)   | 0.405        |
| What is your opinion of the freshness of the dentifrice?                 | not fresh   | very fresh | 4.63 (2.11) | 5.12 (1.85)   | 0.268        |
| Do you consider this                                                      | not fresh at all | too fresh  | 3.40 (1.55) | 3.81 (1.28)   | 0.195        |
| What is your opinion of the strengthness of the taste of the dentifrice?  | not strong at all | too strong | 3.25 (1.51) | 4.30 (1.53)   | 0.003        |
| If yes, for how long does this fresh/clean feeling persist after brushing? | very short  | very long  | 32(Yes)/ 7(No) | 39(Yes)/ 1(No) | 0.057**     |
|                                                                         | 4.03 (1.64) | 3.57 (1.36) |              |               | 0.191        |
| What is your opinion of the taste of food and drinks after brushing?      | changed negatively | changed positively | 4.71 (1.23) | 4.51 (1.36)   | 0.500        |
| What is your opinion of the foaming effect of the dentifrice?             | does not foam | too much foam | 4.76 (1.41) | 4.45 (1.59)   | 0.360        |
| Would you use this dentifrice in the future?                              | absolutely not | for sure  | 4.07 (2.64) | 4.44 (2.52)   | 0.529        |
| Do you consider this dentifrice is improving your gums?                   | changed negatively | changed positively | 4.53 (1.70) | 4.98 (2.00)   | 0.288        |
| What is your opinion about the brush?                                    | very bad    | very nice  | 5.78 (2.17) | 6.05 (2.15)   | 0.579        |
| What is your opinion about the stiffness of the filaments of the brush?   | too soft    | too stiff  | 4.27 (1.61) | 4.25 (1.46)   | 0.958        |
| Do you have the feeling that the brush cleans well?                      | does not clean at all | cleans very well | 5.69 (2.13) | 5.44 (2.07)   | 0.598        |

* T-test
** Chi-square statistic with continuity correction
Experimental phase (4m)

Pre-experimental phase (3w)

Screening

Enrollment
N=90

Session 1
BI, GI, PI assessment
N=90

Screening for eligibility
N=132

Excluded by not meeting the criteria
(N=42)
<40% bleeding (N=21)
Pockets ≥ 5mm (N=9)
Caries (N=7)
Bridge (N=1)
Smoking (N=3)
Antibiotics (N=1)

Allocation products

Randomization
N=90

Session 2 (baseline)
BI, GI, PI assessment
Oral Prophylaxis
N=90

3 weeks use of
2 daily TB + RDF
CHX + H₂O₂
N=90

Session 3
BI, GI, PI assessment
Survey and weighed product
N=39

Test DF
N=45
Drop outs
N=6
No time N=4
Caries N=1
Irritation N=1

Control DF
N=45
Drop outs
N=4
No time N=2
Irritation N=2

Session 3
BI, GI, PI assessment
Survey and weighed product
N=41

Analyzed