

# Antibacterial Efficacy of Herbal Toothpaste Containing Bamboo Salt: A Randomized Controlled Clinical Trial

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## Research Article

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# Abstract

**Background:** The inclusion of herbal antibacterial agents in the composition of toothpastes is becoming increasingly popular, due to lower side effects. The present study intended to investigate the antibacterial efficacy of a herbal toothpaste containing Bamboo salt on cariogenic oral bacteria.

**Methods:** The present double-blinded parallel randomized controlled clinical trial was conducted on 60 dental students (age range: 18-30). Following the baseline saliva sampling, the participants were randomly assigned into the case and control groups, to use the Bamboo salt herbal toothpaste and conventional non-herbal toothpaste, respectively. They were instructed to brush their teeth twice a day using the Bass technique. Saliva sampling was repeated after four weeks. The salivary counts of *Streptococcus mutans* and *Lactobacillus* at baseline and 4-week follow-up were determined and presented as the logarithm of colony-forming units per milliliter (log CFU/mL). statistical analysis was performed using independent samples t-test and paired sample t-test ( $P < 0.05$ ).

**Results:** A significant decrease in salivary *Streptococcus mutans* and *Lactobacillus* was observed using both toothpastes ( $*P < 0.001$ ). The difference between the antibacterial efficacy of two toothpaste types on *Streptococcus mutans* and *Lactobacillus* was not statistically significant ( $P = 0.530$ , and  $P = 0.137$ , respectively).

**Conclusion:** Due to the comparable efficacy of the investigated herbal toothpaste with conventional toothpaste, it potentially qualifies as a complementary agent for self-care oral hygiene procedures.

**Trial registration:** This trial was registered in the “Iranian Registry of Clinical Trials” (IRCT20210414050964N1) on 21/06/2021.

## Background

Dental caries and periodontal problems are widespread oral bacterial diseases [1]. There is a rich ecosystem in the oral cavity, with a countless number of microorganisms [2]. Although both periodontal diseases and dental caries are multifactorial, dental plaque bacteria are the main factor in their onset and progression [3]. *Streptococcus mutans* is known as the main initiating pathogen of dental caries, colonizing on tooth surfaces by several mechanisms [4]. In the presence of surface-adsorbed salivary  $\alpha$ -amylase, carbohydrates are degraded by bacterial enzymes, producing organic acids that cause enamel demineralization [5]. *Lactobacilli* are Gram-positive bacteria that are transmitted to the oral cavity during the first years of life [6]. *Lactobacilli* are considered a major contributor to the caries progression [7]. Increased oral microbiota of *Streptococcus mutans* and *Lactobacillus* is associated with the onset and progression of tooth demineralization and caries [8].

The co-application of brushing and toothpaste containing certain chemical agents removes pathogenic biofilm and decreases the repopulation of bacteria on the enamel. Toothbrushing is associated with a decrease in oral bacterial levels and maintenance of a healthy balance of oral flora on tooth surfaces [9].

Conventional toothpastes contain triclosan and fluoride as the main antibacterial ingredients. These ingredients have been proven to be effective against cariogenic bacteria [10]. Recently, there is growing interest in therapeutic herbal ingredients worldwide, due to reduced side effects, leading to the increased popularity of herbal toothpastes [11]. Considering the numerous herbal toothpaste brands in the market, the efficacy of these toothpastes on oral bacteria is being widely investigated recently [12].

Bamboo salt, a popular herbal ingredient in Korean folk medicine, has been shown to exert anti-inflammatory, antimicrobial, and antioxidant properties [13, 14]. Bamboo salt is made by packing sea salt into bamboo tubes, plugging the ends with mud, and baking it on pine firewood. This method results in higher mineral content of calcium, potassium, copper, and zinc ions in Bamboo salt compared to sun-dried salts while maintaining its main ingredient, i.e., sodium chloride [14, 15]. The rich mineral content in Bamboo salt and its high alkalinity (pH = 11.4) makes it a potent antioxidant [16].

Toothpastes containing Bamboo salt are proposed to reduce plaque and gingivitis, whiten teeth, decrease demineralization, decrease tooth hypersensitivity, and strengthen tooth enamel [17, 18]. The antimicrobial efficacy of Bamboo salt on *Streptococcus mutans* has recently been observed in an in-vitro study [15]; however, its in-vivo efficacy on cariogenic microorganisms remains unknown.

Considering the increasing popularity of herbal toothpastes, and with the aim of providing evidence-based recommendations to the patients [12], the present randomized controlled clinical trial was performed to determine the antimicrobial efficacy of Tiger Herb® toothpaste (containing Bamboo Salt) in comparison to Crest Complete® toothpaste as control, on *Streptococcus mutans* and *Lactobacillus*.

## Methods

### Participants

The present double-blinded parallel randomized controlled clinical trial was conducted at Shahid Beheshti School of Dentistry, after it was approved by the committee for ethics in research, school of dentistry, Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1399.033). The study design was in accordance with the Helsinki Declaration of Human Rights and was also registered in the “Iranian Registry of Clinical Trials” (IRCT20210414050964N1) on 21/06/2021. Each participant was included in the study after reading, understanding, and completing the written informed consent document.

Approximately 200 dental students (18-30 years old) were screened by routine dental examination and finally, 60 participants were chosen for the study. All participants had a history of routine daily toothbrushing with fluoride-containing toothpastes. The exclusion criteria included the following: having a periodontal pocket depth of more than three millimeters, receiving antibiotic or anti-inflammatory drugs during the past month prior to the study, history of systemic diseases, allergic reaction to the toothpaste, smoking, having orthodontic appliances, or presence of untreated dental caries.

### Sample size

The sample size was calculated based on previous studies to be 26 in each group ( $\alpha = 5\%$  and power=80%), which was increased to 30 to improve the validity of the study and compensate for possible sample loss during the follow-up period [19, 20].

**Clinical procedure:** The participants were randomly assigned into the control and case groups (allocation ratio= 1:1), to use the conventional non-herbal toothpaste (Crest® Complete, Procter & Gamble Plaza, Cincinnati, United States), and the Bamboo salt toothpaste (Tiger Herb®, LG, Korea), respectively. Randomization was performed using a random table number by Microsoft Excel (Microsoft®, Washington, US). The composition of examined toothpaste types is listed in Table 1.

The information labels on all toothpaste tubes were removed and their packages were all designed similarly, to ensure the blinding of participants to the contents of toothpaste tubes.

All participants were instructed to brush their teeth with a medium bristled brush (Oral B Pro Expert Brush, Procter & Gamble, United States) for two minutes using the Bass technique twice a day (after breakfast and before bedtime) for four weeks [21], using the designated toothpaste type. They were also instructed to use non-fluoridated dental floss (Oral B essential floss, Procter & Gamble, United States) every night before going to bed, and refrain from using other fluoride-containing products.

### Saliva sampling

Saliva sampling in both groups was performed at baseline and after four weeks of toothpaste use. At both time points, samples were collected before breakfast and one hour after toothbrushing with a medium-bristled brush (Oral B Pro Expert Brush, Procter & Gamble, United States) without any toothpaste, followed by drinking a cup of water. The participants were asked to sit for five minutes while the saliva was being accumulated in their oral cavity. Two milliliters of unstimulated resting saliva were then collected using disposable syringes (Ulticare insulin syringe, Ultimed, Minnesota, USA) and transferred into sterile microtubes (SPL life sciences, Korea). Thereafter, the microtubes were coded, stored in ice containers, and immediately transferred to the laboratory of microbiology.

### Microbiological studies

The saliva samples were diluted by 1/10 and then they were transferred to the Mitis Salivarius Agar (MSA) and MRS-Agar (MRS-A) containing plates (Merck KGaA, Darmstadt, Germany). All plates were then incubated at 37 °C for 24-48 hours (Memmert, Hong Kong) (Table 2). Colonies of *Streptococcus mutans* and *Lactobacillus* were counted after gram staining, using an automatic colony counter (Scan® 500, Interscience, Saint Nom, France) by two experienced microbiologists who were “blinded” to the administered toothpaste type. The inter-examiner reliability was determined using the Kappa agreement coefficient ( $k=0.9$ ).

### Statistical analysis

Statistical analysis was performed using SPSS software version 25 (SPSS Inc., Chicago, IL, USA) ( $\alpha = 0.05$ ) (confidence interval=95%). The logarithm of colony counts was calculated. Numerical data were presented as the mean and standard deviation (Mean  $\pm$  SD). The normal distribution of data was assessed by the Kolmogorov-Smirnov test. Independent samples t-test and Paired samples t-test were used for inter-group and intra-group comparisons, respectively.

Table 1  
List of ingredients in the two toothpaste types.

<b><i>Toothpaste type</i></b>	<b><i>Ingredients</i></b>	<b><i>Manufacturer</i></b>
Tiger Herb®	Bamboo Salt, Hydrated Silica, Dipotassium Glycyrrhizinate, Sodium Fluoride, Sorbitol Solution (70%), Cellulose Gum, Sodium Saccharin, Sodium Lauryl Sulfate, Sodium Bisulfate, Blue 1 (E142090), Yellow 10 (47005) Flavor, Water.	LG, Korea
Crest® Complete	Aqua, Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, Cellulose Gum, Aroma, Zinc Citrate, Chondrus Crispus Powder, Sodium Fluoride (1450 ppm), Sodium Saccharin, Hydroxyl Ethylcellulose, CI 77891, Sodium Citrate, Stannous Chloride, Silica, Glycerin and CI 74160.	Procter & Gamble Plaza, United States

## Results

Sixty participants with a mean age of 24.5 years were included in the study and allocated into two groups with a 1:1 ratio. No sample loss occurred until the 4-week follow up session, although its possibility was anticipated in sample size calculation. The flow chart of participants from baseline to 4-week follow-up is shown in Fig. 1.

Descriptive statistics for baseline logarithm of colony-forming units per milliliter (log CFU/mL) are presented in Table 3. According to the results of the independent samples t-test, there were no significant differences in the baseline salivary colony counts of *Streptococcus mutans* ( $P=0.811$ ) and *Lactobacillus* ( $P=0.829$ ) between the experimental groups (Table 3).

Independent samples t-test also revealed that after four weeks of toothpaste use, no significant differences were observed in the salivary colony counts of *Streptococcus mutans* ( $P=0.530$ ) and *Lactobacillus* ( $P=0.137$ ) between the experimental groups (Table 4).

Paired samples t-test revealed that in both groups, salivary colony counts of *Streptococcus mutans* and *Lactobacillus* decreased significantly after four weeks of toothpaste use ( $*P<0.001$  in Tiger Herb®, and  $*P=0.001$  in Crest®).

Furthermore, the mean amount of change was not significantly different between the groups for both *Streptococcus mutans* ( $P=0.737$ ) and *Lactobacillus* ( $P=0.126$ ), as shown by the independent samples t-test (Fig. 2).

Table 2  
List of examined microorganisms and their culture media.

Bacteria	Culture media	Manufacturer	Incubation
S. Mutans <sup>a</sup>	Mitis Salivarius Agar (MSA)	Merck KGaA, Darmstadt, Germany	24 h, in CO <sub>2</sub> - containing incubator
L. Casei <sup>b</sup>	MRS-Agar (MRS-A)	Merck KGaA, Darmstadt, Germany	48 h
<sup>a</sup> S. Mutans= <i>Streptococcus Mutans</i> , <sup>b</sup> L. Casei= <i>Lactobacillus Casei</i>			

Table 3  
*Mean ± SD <sup>a</sup> of baseline log CFU/mL <sup>b</sup> of salivary microorganisms among the experimental groups*

Bacteria	Groups	Mean ± SD	Pvalue <sup>c</sup>
<i>Streptococcus mutans</i>	Crest® (control)	7.6298± 0.57121	0.811
<i>Streptococcus mutans</i>	Tiger Herb® (test)	7.5970± 0.48328	
<i>Lactobacillus</i>	Crest® (control)	7.6733± 0.62726	0.829
<i>Lactobacillus</i>	Tiger Herb® (test)	7.7082± 0.62013	
<sup>a</sup> SD= Standard deviation			
<sup>b</sup> Log CFU/mL= logarithm of colony forming units per milliliter			
<sup>c</sup> Significance level=0.05, according to independent samples t-test.			

Table 4  
*Mean ± SD <sup>a</sup> of follow-up log CFU/mL <sup>b</sup> of salivary microorganisms among the experimental groups*

Bacteria	Groups	Mean ± SD	<i>P</i> value <sup>c</sup>
<i>Streptococcus mutans</i>	Crest® (control)	7.0761± 0.57172	0.530
<i>Streptococcus mutans</i>	Tiger Herb® (test)	6.9872± 0.51628	
<i>Lactobacillus</i>	Crest® (control)	7.1873± 0.51839	0.137
<i>Lactobacillus</i>	Tiger Herb® (test)	6.9687± 0.60092	
<sup>a</sup> SD= Standard deviation			
<sup>b</sup> Log CFU/mL= logarithm of colony forming units per milliliter			
<sup>c</sup> Significance level=0.05, according to independent samples t-test.			

## Discussion

The main pathogenic mechanism of dental caries initiation is lactic acid production by dental biofilm bacteria [22]. Acidogenic *Lactobacilli* spp. and *Streptococcus mutans* are the main pathogens of dental plaque biofilm [23]. Although plaque removal during toothbrushing is mainly achieved by the mechanical action of toothbrushes, further antibacterial efficacy is achieved when using proper toothpaste [24]. The aim of the present randomized controlled clinical trial was to compare the antibacterial efficacy of herbal toothpaste containing Bamboo salt with a non-herbal toothpaste as control, and it was observed that both toothpaste types similarly decreased the *Streptococcus mutans* and *Lactobacillus* counts of saliva.

According to previous in-vitro studies, Bamboo salt is proposed to exert various therapeutic effects, making it a potentially suitable ingredient for natural toothpastes [25]. Among them is the remineralization potential of Bamboo salt on incipient enamel lesions [17, 26]. In an in vitro study by Choi et al., a significant improvement in surface hardness of artificial caries-like enamel lesions was observed using the Bamboo salt-sodium fluoride toothpaste [17]. A synergic remineralization potential of Bamboo salt and sodium fluoride was later confirmed in an in vitro study by Kim et al., showing a higher increase in surface hardness using both agents, compared to using them separately [26].

Another therapeutic property of Bamboo salt as a toothpaste ingredient is its inhibitory effect on cytokines related to gingival inflammation. In a molecular study on gingival fibroblasts, a reduction in IL-1 $\beta$ , IL-8, TNF- $\alpha$  was observed following the application of Bamboo salt and sodium fluoride, increasing the gingival resistance to inflammation [27]. The anti-gingivitis effect of Bamboo salt was further confirmed in a randomized controlled clinical trial comparing a Bamboo salt toothpaste with another herbal toothpaste containing Centella Asiatica [28].

Regarding the antimicrobial efficacy of Bamboo salt toothpaste, a recent in-vitro study by Lee et al. (2019) investigated the efficacy of Bamboo salt combined with sodium fluoride on *streptococcus mutans*. Various properties attributed to the cariogenesis of this microorganism including its growth, acid production, adherence to glass beads, and expression of *gtfB*, *gtfC*, *gtfD*, and *ftf* genes were significantly restrained by the Bamboo salt and sodium fluoride mixture. It was concluded that Bamboo salt and sodium fluoride can synergically act in inhibiting the cariogenic potential of *Streptococcus mutans* [15], which is in line with the results of the present in-vivo study.

To the best of our knowledge, no clinical studies have ever investigated the antimicrobial efficacy of Bamboo salt on cariogenic oral bacteria before. Nowadays, dentistry has evolved from a practice-based medical discipline to an evidence-based one. A great number of herbal extracts have been incorporated into oral hygiene products such as toothpaste or mouthwash, due to their proposed antimicrobial effects. Although the antimicrobial efficacy of some of them have already been demonstrated in clinical studies, evidence regarding this issue remains limited [29].

Patil et al. (2010) compared the anti-microbial efficacy of Himalaya (herbal) and Cheerio gel (fluoridated) toothpaste in an in vivo study and observed both toothpaste types to be effective on salivary

*Streptococcus mutans* in children, with no significant difference between them[30]. Pradeep et al. (2012) evaluated the anti-microbial efficacy of aloe vera (herbal) toothpaste in comparison with triclosan (conventional) toothpaste as a placebo in a randomized controlled clinical trial and observed a comparable reduction in microbial counts between them [20].

The present study was similarly designed as a double-blind, parallel, randomized, controlled, clinical trial (RCT). The potential of RCTs to minimize the bias improves their level of evidence, making them the gold standard for the investigation of health care outcomes [31]. The results of this study indicated the promising antibacterial efficacy of toothpaste containing Bamboo salt, which may suggest its administration in self-care oral hygiene procedures. However, further clinical studies on other therapeutic effects of Bamboo salt toothpaste, with a larger sample size, and more extended follow-up period could obtain more definitive conclusions regarding its efficacy for routine daily use.

## Conclusion

Within the limitations of this study, it can be concluded that due to the observed in vivo antimicrobial efficacy of herbal toothpaste containing Bamboo salt on oral bacteria responsible for dental caries, it potentially qualifies as a complementary agent for self-care oral hygiene procedures.

## Declarations

**Ethics approval and consent to participate:** The present study was conducted after being approved by the committee for ethics in research, school of dentistry, Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1399.033). The study design was in accordance with the Helsinki Declaration of Human Rights and was also registered in the “Iranian Registry of Clinical Trials” (IRCT20210414050964N1) on 21/06/2021. Each participant was included in the study after reading, understanding, and completing the written informed consent document.

**Consent for publication:** Not applicable.

**Availability of data and materials:** All data generated or analysed during this study are included in this published article [and its supplementary information files].

**Competing interests:** The authors declare that they have no competing interests

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**Authors' contributions:** M.B. and Y.R. conceptualized and designed the methodology. A.R. performed the saliva sampling. R.R. and P.I. performed the formal analysis and data curation. A.R., Y.R., and R.R. prepared the original draft of the manuscript. M.B. and P.I. edited the manuscript and prepared the final version. All authors read and approved the final manuscript.

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Figures

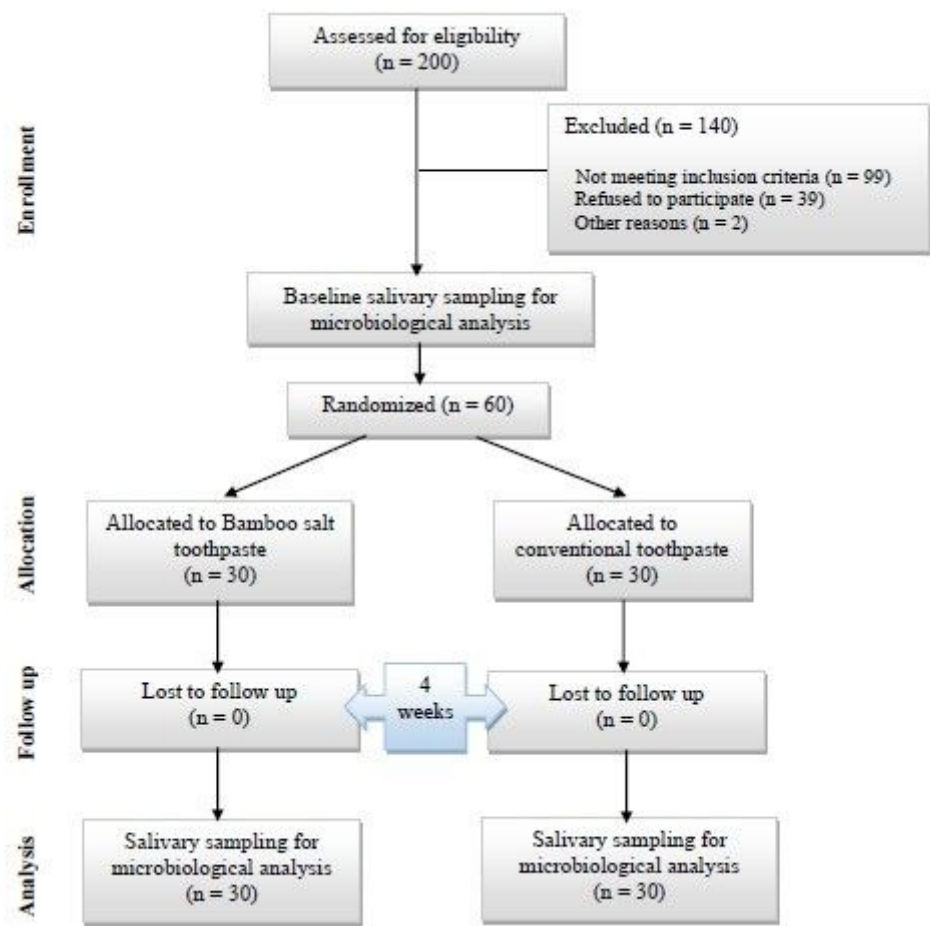
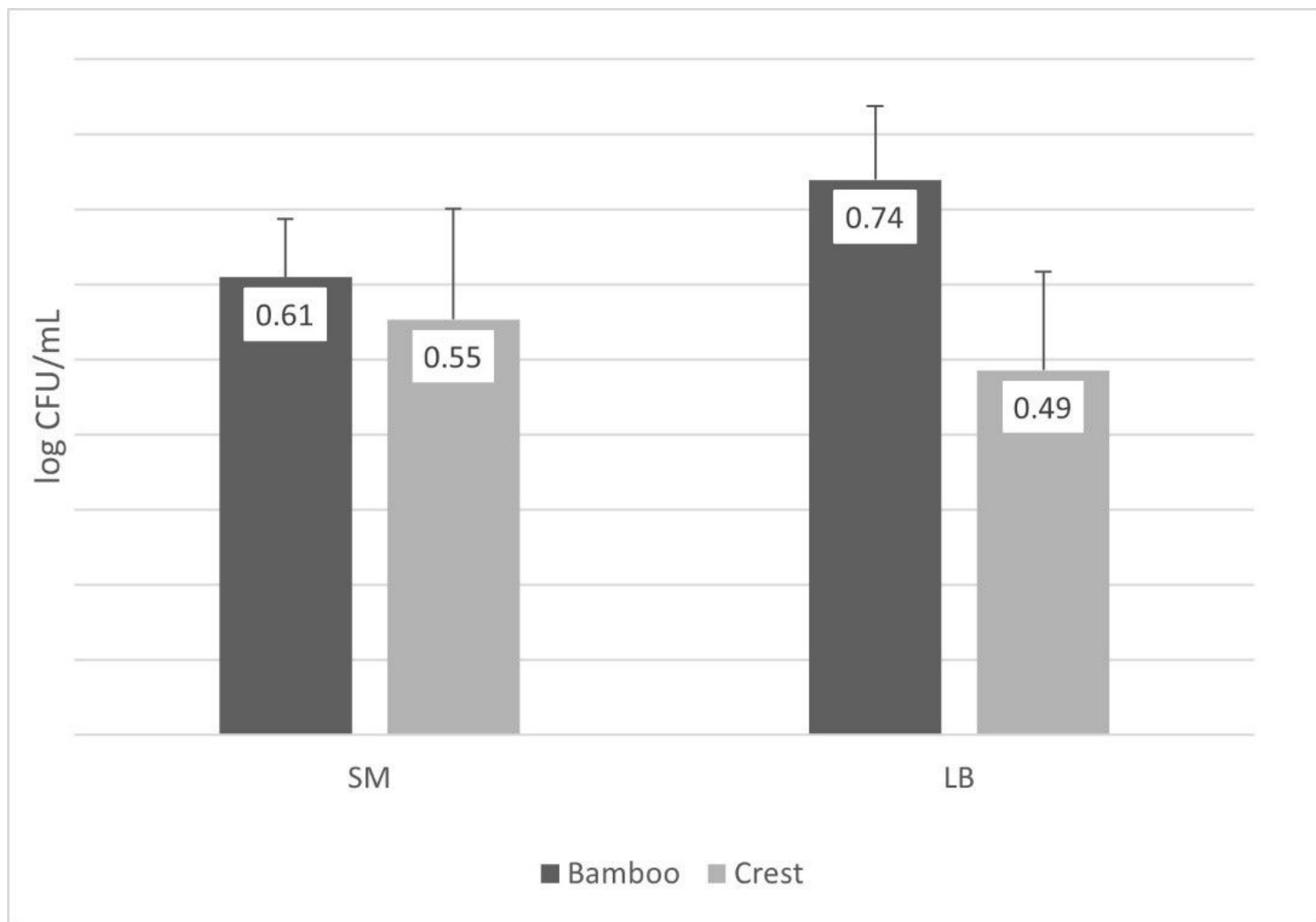


Figure 1  
Flowchart of participants from baseline to 4-week follow up.



**Figure 2**

Mean amount of change in the logarithm of colony-forming units per milliliter (log CFU/mL) of *Streptococcus mutans* (SM) and *Lactobacillus* (LB) in case (Tiger Herb<sup>®</sup>, containing Bamboo salt) and control (Crest<sup>®</sup>) groups, during the 4-week follow-up period.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Rawdata.xlsx](#)