Original Article

Effect of Herbal Toothpaste Containing *Piper betle, Psidium guajava* and *Garcinia mangostana* on Dental Plaque and Gingivitis

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Abstract

The aim of this randomized controlled clinical study was to compare the efficacy of herbal toothpaste containing *Piper betle, Psidium guajava* and *Garcinia mangostana* with fluoridated toothpaste in the reduction of dental plaque and gingival inflammation. Fifty one healthy participants were randomly allocated to one of two groups, the control group (fluoridated toothpaste) and the test group (herbal toothpaste). The enclosed label toothpastes and new soft bristled toothbrushes were distributed to each participant according to their groups. All participants were instructed to brush their teeth with assigned toothpaste for 2 minutes, 2 times a day for 4 weeks. The plaque index and gingival index were examined and recorded on the first day (baseline), 14th day and 28th day (endpoint). The plaque index and gingival index scores were decreased statistically significant in both groups at the end of the study period ($p < 0.05$). However, these parameters were not statistically significant different between the groups.

Key words: Gingivitis; Herbal toothpaste; Plaque control

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Introduction

Plaque-induced gingivitis is an inflammation of the gingival tissues resulting from dental biofilm located at the gingival margin. If it left untreated with time, the inflammation may progress and eventually involve the entire periodontal attachment apparatus of the affected teeth.[1-3] Although, it has been shown that gingivitis is reversible and the progression to be periodontitis is not predictable, the prevention of gingivitis in the population is still the first step toward preventing periodontitis.[4]

The mechanical plaque control is an effective method of controlling supragingival dental biofilm and gingival inflammation. To achieve sufficiently low levels of the dental biofilm, the antimicrobial toothpastes as an adjunct to tooth brushing are being used to improve the efficacy of self-performed mechanical tooth brushing method.[5] In addition, various herbal toothpastes are being marketed and have been used as adjunctive agents to prevent the gingival inflammation and dental biofilm accumulation.[6-8]

In Thailand, the local herbal ingredients are used in commercial toothpaste such as *Piper betle, Psidium guajava* and *Garcinia mangostana*. The microbiologic and experimental studies demonstrated that *Piper betle* extract can inhibit the adherence of dental plaque by reduction of dental pellicle formation on the tooth surface. It also increases hydrophobicity of the bacterial cell membrane resulting in decreased adherence to the acquired pellicle[9,10] Furthermore, the experimental study of *Piper betle* and *Psidium guajava* extract revealed that the biological activities possessed by these extracts collectively contribute to their positive antimicrobial effects on the early plaque bacteria as *S. sanguinis, S. mitis* and *Actinomyces sp.*[11] According to the clinical trial of the gel form of *Garcinia mangostana*, there was a significant reduction in theperiodontal pocket depth in periodontitis patients.[12] Besides, the herbal mouthwash containing pericarp of *Garcinia mangostana* demonstrated the reduction of volatile sulfur compound (VSC) in gingivitis patients.[13] Anyway, there is unavailable scientific document about the effect of herbal toothpaste containing *Piper betle, Psidium guajava* and *Garcinia mangostana* in plaque control and reduction of gingival inflammation.

The primary purpose of this study was to compare the efficacy of herbal toothpaste containing *Piper betle, Psidium guajava* and *Garcinia mangostana* and fluoridated toothpaste in the plaque control. The secondary purpose was to compare the efficacy of the herbal toothpaste and fluoridated toothpaste in the reduction of gingival inflammation.

Materials and methods

The ethical approval of the study was provided by the Ethical Committee Board of Rangsit University (RSEC 04/2556). Participants' age between 18 - 35 years, non-smoker and in good general health were enrolled in this study. All of the participants were given both verbal and written information of the study and signed a consent form. The participants were selected on the basis of having mild to moderate gingival

inflammation. Thus, the inclusion criteria were defined as a whole mouth pre-brushing plaque index scores[14] > 1.95 and gingival index[15] > 0.95 at the baseline. Participants must have no fixed or removable orthodontic appliance or removable partial denture. They all had at least 20 teeth which no large dental caries. The exclusion criteria included a medical condition with the history of antibiotic therapy or anti-inflammatory medications less than 1 month before the study. The participants had to refrain from any non-emergency dental care including prophylaxis during the study.

Prior to start this clinical trial, a single examiner who had an experience in periodontal investigation performed a pilot examination of periodontal indices used in this study. During the study period, the random allocation of participants was generated and blinded from the examiner. Every one of ten participants was repeated the periodontal examination in each time point. The intra-class correlation coefficient test was calculated for overall. The following indices were recorded from the buccal and lingual aspects of each tooth.

1. The gingival index[14] (Löe and Silness, 1963): score 0 - 3

2. The plaque index[15] (Tureskey modification of the Quigley-Hein index, 1970): score 0 - 5

A randomized controlled clinical trial was performed in parallel group for 28 days home use protocol. Fifty one participants who fulfilled the entry criteria were randomly allocated in 2 groups by simple random sampling method. Each participant used the soft bristled toothbrush (Systema®, the original, Lion Corporation) and they were also allowed to use dental floss during the study. The assigned toothpastes with enclosed label were distributed to each participant as following:

- Control group: fluoridated toothpaste (containing active ingredients as 1,000 ppm sodium monofluorophosphate 0.76 %, Colgate®, Colgate-Palmolive)

- Test group: herbal toothpaste (containing active ingredients as *Piper betle, Psidium guajava* and *Garcinia mangostana*, Abhaibhubejhr®)

All of the participants were instructed to brush for 2 minutes twice a day, in the morning and before bedtime. They were demonstrated to squeeze 1 cm (approximate 1.2 g) of toothpaste across the bristle of the toothbrush. During the 28 days of this study, the participants were asked to refrain from using other tooth cleaning procedures or oral hygiene product. Instruction brochure and appointment card with contact number were given to all participants in case of any doubt or if they experienced any adverse effect from the toothpaste

Data Analysis

The indices were recorded for each participant in 3 phases.

1. Baseline: day 0

2. After the initial use of the toothpaste: 14th day

3. End point: 28th day

A whole mouth gingival index and plaque index, a buccal and a lingual score were calculated. The normality of data distribution was assessed with Kolmogorov-Smirnov test and the homogeneity of variance was tested with

Levene's test. The paired *t*-test and the independent sample *t*-test were used for the comparisons within the groups and between groups in all three phases.

Results

Fifty one (29 males and 22 females) of the initial 66 participants completed the 28 days study period. There were fifteen participants drop-out throughout this trial. Three individuals were contacted but declared, they were "too busy" or "didn't have time" to come to the 28th day exam, seven had discomfort using the herbal toothpaste, one had sinus tract opening in his mouth due to pulpal disease and four discontinued using the toothpaste by themselves. The drop-out subjects were examined and received a prophylaxis, but the pertaining data were not included in the statistical analyses.

Table 1 shows the mean age and gender in the test group (N = 25) and control group (N = 26). The mean age of the control group was 25.27 ± 5.33 and 27.16 ± 7.05 in test group. There was no significant difference between the groups with regard to age (p = 0.46) and gender (p = 0.284).

Table 1 *Gender and means age of the participants in both groups*

Groups	Gender		Mean age
	Female (N)	Male (N)	
Test group	12	13	27.16 ± 7.05
Control group	10	16	25.27 ± 5.33

The mean PI of the control group and the test group at baseline, 14th and 28th days were shown in Table 2. The whole mouth PI significantly decreased from baseline toward the endpoint in both groups (p < 0.05). However, there was no significant difference between the groups.

Table 2 *Mean of Whole mouth plaque index score and comparisons within and between the groups at baseline, 14th day, and endpoint*

PI score Whole mouth (Mean ± SD)	Herbal toothpaste N = 25	Fluoridated toothpaste N = 26	*p (***)*
Baseline	2.61 ± 0.71	2.74 ± 0.80	0.54
14th day	2.17 ± 0.67*	2.30 ± 0.65*	0.50
p (*)	< 0.001	< 0.001	

Table 2 *Mean of Whole mouth plaque index score and comparisons within and between the groups at baseline, 14th day, and endpoint (continue)*

PI score Whole mouth (Mean ± SD)	Herbal toothpaste N = 25	Fluoridated toothpaste N = 26	*p* (***)
Endpoint	2.05 ± 0.67**	2.14 ± 0.63**	0.62
p (**)	< 0.001	< 0.001	0.50

Significant $p < 0.05$: * = pair sample *t*-test at baseline and 14th day, ** = pair sample *t*-test at baseline and endpoint, *** = independent sample *t*-test

Table 3 presented means of gingival index score and comparisons between the groups at baseline, 14th and 28th day (endpoint). The gingival index scores significantly decreased in the test and the control groups, but the comparison between the groups revealed no statistical difference.

Table 3 *Means of Gingival index score and comparisons within and between the groups at baseline, 14th day and endpoint*

GI score Whole mouth (Mean ± SD)	Herbal toothpaste N = 25	Fluoridated toothpaste N = 26	*p* (***)
Baseline	1.76 ± 0.38	1.81 ± 0.39	0.63
14th day	1.52 ± 0.25*	1.53 ± 0.23*	0.81
p (*)	< 0.001	< 0.001	
Endpoint	1.46 ± 0.23*	1.44 ± 0.26*	0.87
p (**)	< 0.001	< 0.001	

Significant $p < 0.05$: * = pair sample *t*-test at baseline and 14th day, ** = pair sample *t*-test at baseline and endpoint, *** = independent sample *t*-test

Discussion

Nowadays, the interest in alternative toothpaste based on plant extracts has increased. Previous *in vitro* studies shown the antimicrobial properties of several of herbal extracts.[9-13] However, there are limited clinical studies available regarding the efficacy of herbal toothpastes. This randomized controlled clinical trial aimed to compare the efficacy of containing *Piper betle*, *Psidium guajava* and *Garcinia mangostana* with fluoridated toothpaste on reduction of dental plaque and gingival inflammation. The results demonstrated that plaque index and gingival index scores at baseline, 14th day and 28th day (endpoint) were statistically significant decreased in both groups of herbal toothpaste and the conventional fluoridated toothpaste, but the statistical difference between groups was not found. This is in accordance with the previous clinical study by Ozaki *et al.*[16], which assessed the efficacy of herbal toothpaste on the reduction of plaque and gingivitis for 28 days. The herbal toothpaste containing active ingredients as chamomile, Echinacea, sage, myrrh and peppermint oil (Parodontax®) was compared to the positive control group usage the fluoridated toothpaste with tricosan.[16] They reported a significant reduction in plaque index and gingivitis in both groups, but there was no statistically significant difference between the groups. This is in agreement with the studies by Saxer *et al.*[17], Mullaly *et al.*[18] that the plaque index and gingival index were significantly reduced in the herbal-based toothpaste (Parodontax®), as well as in the conventional toothpaste group, but there were no statistically significant difference was found between the groups. Pannuti *et al.*[19] performed a 21-day clinical study to evaluate the effect of herbal toothpaste (Parodontax®) and the standard toothpaste. Although, the result showed no significant reduction in plaque index within groups, but the herbal toothpaste product provided a significant reduction in gingivitis. Another herbal toothpaste study by George *et al.*[20], presented that the herbal toothpaste containing eucalyptus and lemon extracts (Colgate® herbal) to be as effective as the non-herbal toothpaste in the reduction of gingivitis. Jayashankar *et al.*[21] compared the efficacy between the mixture of Indian herbal toothpaste (Sudantha®) and placebo in terms of reduction on plaque index, bleeding on probing and probing depth. The result demonstrated that the evaluated parameters were decreased significantly within herbal toothpaste group at 4, 8 and 12 weeks when compared to baseline, but the placebo group did not show any statistically significant improvement in all parameters.

Various clinical trials demonstrated the comparable effect of herbal toothpaste to conventional fluoridated toothpaste as mentioned above. The active ingredients in herbal toothpaste are claimed as anti-inflammatory effects, while the major property of fluoridated toothpaste is to prevent dental caries. As the reason of standard oral care, the control group in this study was allocated to use the fluoridated toothpaste. At the end of this study, the plaque index and gingival index score were decreased statistically

significant within both groups. The reason might be the benefit of mechanical plaque control combined with toothpaste. Thus the reduction of gingival inflammation was the consequence from the mechanical plaque control resulting in reduction of dental plaque.

In this study, seven participants had some discomfort after brushing with the assigned herbal toothpaste. They were advised to disuse the product at once and the oral examination were performed by the investigator. The oral sign and symptom were recorded including mucosal redness, dry mouth and alteration of taste sensation. After cessation of toothpaste, the unpleasant effects disappeared. These effects might be the allergic effects from the toothpaste ingredients. The review study by Zirwas and Otto[22] demonstrated that the most common allergen was the flavoring agents, especially cinnamon, spearmint, peppermint carvone and anethole. The fifth most common allergen is the parabens which be used as preservatives in many over the counter products. The herbal toothpaste used in this study contains methylparaben and propylparaben, which might cause the allergic reactions. While, the fluoridated toothpaste is paraben free. Nevertheless, these participants were not received the allergic test, thus the conclusion of allergy was still unclear.

Many clinical trials used multiple examiners to help collect data. In those situations, examiners need to be trained systematically and calibrated with each other to be a standard examiner. The reason that this study was carried out by one examiner was to avoid inter-examiner variable. Repetition measurement and intra-class correlation coefficient test (ICC) were also performed to assess the intra-examiner reliability. The present study demonstrated an overall ICC as 0.78 which is acceptable, but the ICC in each time point was not presented. The lacking of inter-observational due to single examiner might be the weakness point of present study. Moreover, using only one examiner is time consume and difficult in schedule arrangement.

In conclusion, the herbal toothpaste and the fluoridated toothpaste are effective in reduction of dental plaque and gingivitis, although there is no additional benefit of the herbal toothpaste over the fluoridate toothpaste could be observed. However, with the limitations of this clinical study, the more sample size and long-term study may be required to prove the effectiveness of this herbal toothpaste in reduction of plaque and gingivitis.

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