Effect of mouthrinse containing *Streblus asper* leaf extract on gingivitis and plaque formation


Abstract

The aim of this single blind and crossover design study was to describe a 4-day no oral hygiene model to assess the pattern of *de novo* plaque formation and gingivitis in the human. Thirty-five subjects were recruited for the trial. Each participant received a professional tooth cleaning at the preparatory period. Following baseline clinical examination for plaque and gingival index, the saliva and plaque samples were taken at the beginning of experimental period. The participants were subsequently given a professional tooth cleaning, asked to rinse twice daily for 60 s with 10 ml distilled water or *Streblus asper* leaf extract (80 mg/ml) and told to abstain from mechanical plaque control efforts for the next 4 days. On day 5, the volunteers were exposed to a new clinical examination, the gingival and plaque index were recorded and the saliva and plaque samples were taken. Then they were given a professional tooth cleaning and asked to exercise proper self performed plaque control during the next 10 days. A new test period was then initiated. The results of the study revealed that *S. asper* leaf extract significantly reduced the gingival index compared with distilled water. However, the mean difference from baseline of plaque index was not significantly different between rinsing with *S. asper* leaf extract and distilled water. In addition, no significant changes in mutans streptococci and total salivary bacterial counts were observed. It is concluded that mouthrinse containing *S. asper* leaf extract has effect only on gingival health.

Key words: gingivitis; mouthrinse; plaque; *Streblus asper*

Introduction

Mouthrinses are generally considered as adjunctive to oral hygiene and widely used in the delivery of active agents to the teeth and gum. Of the numerous types of mouthrinses currently available, there are relatively few which have been shown unequivocally to reduce both plaque and gingivitis. Although the bactericidal activity of some antiseptic mouthrinses has received the most attention in explaining their clinical efficacy, it has been suggested that antiseptics may have additional effects on plaque accumulation by changing the colonizability of the tooth and plaque surface (1). The ability of mouthrinses to influence plaque formation and to alter the course of gingival inflammation has been extensively studied (2-11).

The use of natural products in all types of cosmetics and in health-care preparations continues to be popular. A wide variety of plant extracts have been reported to have anti-inflammatory properties, antibacterial and astringent effects (12-23). Therefore, several herbal extracts have been...
added to some commercial mouthwashes as antigingivitis and astringent agents.

*Streblus asper* Lour, Moraceae, is a medicinal plant which has been used for several pharmaceutical purposes, e.g. the bark extract has been used in fever, dysentery, relief of toothache and antigingivitis (24). The leaf extract has been shown to possess insecticidal activity towards mosquito larvae (25). The branch of the plant has been used as a toothbrush for strengthening teeth and gums (26). The root has been applied to unhealthy ulcers, sinuses and locally as antidote to snake bite; and the milky juice has been used as antiseptic and astringent applied to chapped hands and sore heels (27).

There have been several reported studies on the antibacterial actions of *Streblus asper* leaf extract (SAE) towards *Streptococcus mutans* in vitro and in vivo (28-30). Recently, our study found that SAE possessed antibacterial activity towards *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* which are important periodontal pathogens (31). However, no reported study on the ability of mouthrinse containing SAE to influence gingivitis and plaque formation has been found. The aim of this study was therefore to provide more information on the antigingivitis and plaque inhibitory properties of SAE by studying the agent in mouthwash form.

**Materials and Methods**

**Subjects**

Thirty-five volunteers, ranging in age from 16-60 years, participated in this single blind, crossover design study. They were dentate, without any oral appliances and had no relevant medical history. In particular, no volunteer had received systemic antimicrobials in the preceding 3 months and did not receive such pharmacotherapy during the study. All participants were selected on the basis of having moderate gingival inflammation but were free from signs of destructive periodontal disease. Moderate gingivitis was defined as having at least 50% of the test sites showing bleeding on probing. The project was approved by the Human Ethics Committee of Khon Kaen University. The procedures, possible discomforts or risks, as well as possible benefits were fully explained to the volunteers, and their informed consent was obtained prior to entry into the trial. All subjects were requested to follow the study protocol to ensure accuracy of the data obtained.

**Preparation of Streblus asper leaf extract (SAE)**

Leaves of *S. asper* were locally collected in Khon Kaen province. The leaves were washed, air-dried and pulverized. The dried-pulverized *Streblus asper* leaves were extracted by the method previously described (30). Approximately 5 g of dark brown sticky material was obtained from 100 g of the dried-pulverized leaves. The material was dissolved in distilled water at 250 mg/ml, centrifuged at 10,000 rpm (9410 x g) at 4°C for 20 min and passed through a 0.2 µm filter (Acrodisc® PF, Gelman Sciences, USA). The filtrate was used as the starting material for subsequent studies.

**Experimental design**

All subjects were arbitrarily divided into two groups so that one group used SAE rinse and one group used distilled water (DW) rinse during the first experimental period. The 27-day trial included an initial preparation period of 7 days, two sequential “experimental” periods, each of 5 days, and an interim recovery period of 10 days between experimental periods 1 and 2 (Fig. 1). The participants were asked to exercise proper self performed plaque control during the 7-day initial preparation period and during the 10-day interim recovery period.

At the start of the initial preparation period, the teeth of all participants were thoroughly scaled and polished with a non-fluoride pumice paste to remove all calculus and plaque. On day 1 and 5, i.e. at the start of the experimental periods 1 and 2, respectively, unstimulated whole saliva (1.5 ml) was collected from each subject and examined for the numbers of mutans streptococci and total salivary bacteria per ml of saliva as described later.

The gingival index (GI) was recorded on the teeth (16,21,24,36,41,44) identified as representative of the whole dentition (32, 33). Four gingival areas (distal, facial, mesial, lingual) were examined systematically for each tooth using the following criteria:

- **0** = absence of inflammation
- **1** = Mild inflammation – slight change in color, slight edema. No bleeding on probing.
- **2** = Moderate inflammation – redness, edema, and glazing. Bleeding on probing.
- **3** = Severe inflammation – marked redness and edema. Ulceration. Tendency to spontaneous bleeding.

GI for the individual was determined by totalling the
**Fig. 1** Experimental protocol of the mouthrinse study in human volunteers.

In the study, participants were divided into two groups: one that received an experimental rinse and another that received a placebo rinse. The protocol involved a baseline period of oral hygiene followed by rinsing with SAE on days 1, 5, and 15. The interim period involved no oral hygiene, followed by rinsing with DW on days 15 and 20.

Prophylaxis and oral hygiene instruction included:

1. Collection of unstimulated whole saliva
2. G.I.
3. P.I.
4. Plaque weight
5. Bacterial enumeration
6. Removal of all supragingival deposits

**Fig. 2** Plaque score for individual tooth. **A.** Plaque is assessed by dividing a tooth into 5 subdivisions, each of which is scored 1 when plaque is shown to be present after use of basic fuchsin. **B.** Example of plaque score of 3. Shaded portion represents plaque stained by basic fuchsin. **C.** Example of plaque score of 1. (24)
scores for each area and divided by 24. Suggested nominal scale for evaluation of scores by Loe and Silness are as follows:

<table>
<thead>
<tr>
<th>Scores</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Excellent (healthy tissue)</td>
</tr>
<tr>
<td>0.1-1.0</td>
<td>Good</td>
</tr>
<tr>
<td>1.1-2.0</td>
<td>Fair</td>
</tr>
<tr>
<td>2.1-3.0</td>
<td>Poor</td>
</tr>
</tbody>
</table>

The plaque index (PI) was recorded on the teeth (16,11,26,36,31,46), after staining the teeth with basic fushin (3%) according to a method described by Podshadley & Haley. The facial surfaces of incisors and maxillary molars and the lingual surfaces of mandibular molars were examined. Briefly, each tooth surface to be evaluated was subdivided (mentally) into 5 sections (Fig. 2) as follows: 1) Vertically. Three divisions – mesial, middle, and distal. 2) Horizontally. The middle third was subdivided into gingival, middle, and occlusal or incisal thirds. Each of the 5 subdivisions was scored for the presence of stained plaque as follows:

0 = No plaque (or questionable).
1 = Plaque definitely present.

The score for individual tooth ranged from 0 to 5. PI for the individual was determined by totalling the scores for the individual tooth and divided by the number of teeth examined. After recording the GI and PI, all supragingival plaque present on the selected teeth (16,11,26,36,31,46) was collected with a sterile curette. The plaque from each subject was immediately placed on a preweighed-sterile glass slide in a humidified box. The wet weight of the plaque sample was obtained and the numbers of mutans streptococci in plaque were determined as described later. Then the plaque deposition on all tooth surfaces of subjects was removed as completely as possible with scaling and polishing. After these procedures, the subjects rinsed with either 10 ml of SAE (80 mg/ml) or 10 ml DW for 60 s. They were instructed to abstain from all forms of active oral hygiene for the next 4 days (experimental period 1). Twice daily during this period, they rinsed with either 10 ml of SAE (80 mg/ml) or DW for 60 s. During the crossover experimental period 2, from day 15 to day 20, the group which had previously used SAE rinsed with DW, while the other group rinsed with SAE (Fig. 1). On day 5 and 20, i.e. at the end of experimental periods 1 and 2, respectively, 1.5 ml of unstimulated whole saliva was collected, the GI and PI were scored and plaque samples were collected and weighed as described above.

A questionnaire about any potential adverse side effects after rinsing with SAE including oral irritation, dysgeusia, burning sensation, numbness of tongue, increase or decrease salivary flow, or any other unusual occurrence was administered to each subject.

### Bacteriological examination of saliva and plaque

Samples of saliva were processed immediately on receipt. In each case, samples were mixed thoroughly on a vortex mixer and 1 ml volumes removed and serially diluted to 1:10⁵ in phosphate-buffered saline (PBS). Then, 25 μl portions from dilution 1:10 and 1:10² were spread using a glass spreader over mitis-salivarius-bacitracin agar (MSBA, Difco) in duplicate and 25 μl portions from dilution 1:10⁴ and 1:10⁵ were spread over plate count agar in duplicate as well. Following a 24 h incubation period on plate count agar and 48 h incubation period on MSBA, at 37°C, 5% CO₂, the number of microbial colonies on each plate was counted and compared.

The wet plaque sample from each individual was crushed in 2 ml of PBS using a homogenizer. To facilitate dispersion of the plaque, each sample was sonified for 15 s using the sonifier (Ultrasonic Quantrex model 210H) and 0.1 ml volumes removed and serially diluted to 1:10² in PBS. Then 25 μl portions from dilution 1:10 and 1:10² were spread using glass spreader over mitis-salivarius-bacitracin agar (MSBA) in duplicate. Following a 48 h incubation period, at 37°C, 5% CO₂, the number of microbial colonies on each plate was counted and compared. The CFU/1 mg plaque was calculated by dividing the CFU of the dispersed sample by the exact weight of the sample in the tube.

### Statistical analysis

Data were analyzed by the wilcoxon signed rank test, using the statistical package for social sciences (SPSS).

### Results

#### Effect of Streblus asper leaf extract on gingiva and plaque accumulation

Of the 35 volunteers who initially were recruited for the study, 5 failed to complete the entire 27-day period of trial. Two volunteers did not conform to the protocol and three
volunteers received systemic antibiotics during the trial. The remaining 30 volunteers, 18 males and 12 females (mean age 34.29 years), satisfactorily completed the rinsing regimens without any noted side effects except occasional complaints of taste. After 4 days of rinsing with SAE, the mean differences from baseline (mean ± SEM) of PI scores was not significantly different compared with DW. However, a significant decrease in mean difference from baseline of GI scores after rinsing with SAE compared with those after rinsing with DW was found (Table 1). In addition, differences in intraindividual GI scores before and after rinsing with SAE were also statistically significant (p < 0.001) (Table 2). The mean GI scores after rinsing with SAE showed a mean 10.62% reduction while the mean GI scores after rinsing with DW showed a mean 2.82% reduction compared with values from baseline. The difference in intraindividual GI scores before and after rinsing with DW were not statistically significant (Table 2).

Table 1 Comparison of the mean difference from baseline of gingival and plaque index after rinsing with *Streblus asper* leaf extract and distilled water.

<table>
<thead>
<tr>
<th>Mouthrinsing with</th>
<th>Mean difference from baseline ± SEM</th>
<th>GI</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>-0.033 ± 0.027</td>
<td>0.615 ± 0.083</td>
<td></td>
</tr>
<tr>
<td>SAE</td>
<td>-0.124 ± 0.028*</td>
<td>0.598 ± 0.093</td>
<td></td>
</tr>
</tbody>
</table>

DW, distilled water; SAE, *Streblus asper* leaf extract; GI, gingival index; PI, plaque index; *p < 0.05 compared with DW

Table 2 Comparison of gingival index before and after rinsing with *Streblus asper* leaf extract and distilled water.

<table>
<thead>
<tr>
<th>Mouthrinsing with</th>
<th>GI (group mean ± SD)</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After mouthrinsing</td>
</tr>
<tr>
<td>DW</td>
<td>1.171 ± 0.151</td>
<td>1.138 ± 0.131</td>
</tr>
<tr>
<td>SAE</td>
<td>1.164 ± 0.174</td>
<td>1.040 ± 0.146*</td>
</tr>
</tbody>
</table>

DW, distilled water; SAE, *Streblus asper* leaf extract; GI, gingival index; *p < 0.001 compared with baseline

Table 3 Comparison of the mean difference from baseline of total salivary bacteria and mutans streptococci in plaque and saliva after rinsing with *Streblus asper* leaf extract and distilled water.

<table>
<thead>
<tr>
<th>Mouthrinsing with</th>
<th>Mean difference from baseline ± SEM</th>
<th>Mutans streptococci in plaque x 10⁶ CFU/1 mg plaque</th>
<th>Mutans streptococci in saliva x 10⁵ CFU/ml</th>
<th>Total salivary bacteria x 10⁸ CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>19.366 ± 11.901</td>
<td>1.067 ± 0.667</td>
<td>2.271 ± 1.232</td>
<td></td>
</tr>
<tr>
<td>SAE</td>
<td>1.019 ± 5.044</td>
<td>2.556 ± 1.276</td>
<td>8.022 ± 2.723</td>
<td></td>
</tr>
</tbody>
</table>

DW, distilled water; SAE, *Streblus asper* leaf extract.
Effect of Streblus asper leaf extract on mutans streptococci in plaque and saliva and total salivary bacterial count

Following 4-day regimen of SAE mouthrinse, the mean differences from baseline of mutans streptococci, both in plaque and saliva, and total salivary bacterial count were not significantly different compared with those of DW (Table 3), although a numerically lower mean difference from baseline of mutans streptococci counts in plaque compared with those of DW mouthrinse was found.

Discussion

Our previous study on the effect of a mouthrinse containing SAE at a concentration 80 mg/ml in human volunteers, revealed that this concentration can reduce the number of mutans streptococci in stimulated saliva without changing the oral ecology (30). Therefore, this concentration of SAE was used as a mouthrinse in all subjects in the present study. This clinical trial, which examined the effect of SAE mouthrinse on gingivitis and plaque formation, was designed so that each subject acted as his own control and the 4-day plaque regrowth model was used (35).

Following 4-day regimen of DW mouthrinse, a reduction of GI was found but not significantly different compared with the baseline. This result was probably due to an effect of plaque removal by scaling and polishing before rinsing. However, after rinsing with SAE, the reduction of GI was significantly different compared with the baseline. This finding indicated that SAE mouthrinse can reduce gingivitis. In addition, 24 (80%) of the 30 volunteers mentioned a feeling of freshness and cleanness after rinsing with SAE although they abstained from all forms of active oral hygiene. Moreover, one subject who had recurrent aphthous ulceration at mucobuccal fold indicated that the lesion resolved more rapidly than previous time after rinsing with SAE.

S. asper is considered to have antigingivitis and astringent effects (24, 27), and has been used for a long time in traditional herbal medicine to counter gingivitis and for relief of toothache. An underlying mechanism of the GI reduction detected in the present study could be due to the antigingivitis and astringent effects of S. asper. Another possibility is that SAE may have a direct effect on gingivitis by reducing mediators of inflammation which could reduce inflammation independent of any effect at reducing plaque since no clinical effect on visible plaque was observed in this study. Additional studies are necessary to further determine the mechanism of S. asper on gingivitis.

In the present study, mouthrinsing with SAE reduced neither the CFU of total salivary bacteria nor of mutans streptococci in saliva. This finding is consistent with our previous study that SAE has no effect on total salivary bacterial count and can significantly reduce mutans streptococci counts only within 1 h after rinsing (30). However, mouthrinsing with SAE may alter microorganisms in the plaque, particularly mutans streptococci. A considerable reduction of mutans streptococci in the plaque was observed with increasing amounts of mutans streptococci in saliva. Removal of mutans streptococci from the plaque may further alter the adhesion of other oral bacteria to the plaque and hence more bacteria were found in saliva. In contrast, higher numbers of mutans streptococci were detected in the plaque after rinsing with DW. This may assist other oral bacteria to adhere to the plaque and could be responsible for the lower number of bacteria found in saliva.

Ideally, supragingival plaque control should prevent periodontal tissue inflammation and breakdown. However, since complete plaque removal is unrealistic, prevention may be achieved by (1) reducing the quantity of plaque below the individual’s threshold for disease or (2) changing the quality of plaque to a more tissue friendly composition (36). In the present study, although a reduction of plaque was not observed, qualitative changes may have occurred since a numerically, but not statistically, lower mean difference from baseline of mutans streptococci counts in plaque after rinsing with SAE compared with DW was found.

There is evidence, primarily from in vitro studies that extracts from plants could exert an effect on the composition and accumulation of bacteria. Even inhibitory effects on potential virulence factors of P. gingivalis, P. intermedia, and T. denticola have been demonstrated with extracts from plants commonly used as chewing sticks (37). These antimicrobial properties were not substantiated in a clinical test in which the chewing stick was compared to a nylon toothbrush (38). Another in vitro study established the ability of sanguinarine, an alkaloid extract from the blood root plant, Sanguinarine canadensis, to inhibit growth of 98% of oral isolates at a relatively low concentration (39). In two long-term studies of the product in a dentifrice form, no significant reduction in plaque or gingivitis occurred (40, 41). However, a significant reduction in plaque and gingivitis was shown when patients used both the mouthrinse and dentifrice for 6 months, sug-
gesting that combination usage may be of therapeutic value (42, 43).

In conclusion, the results of the present study have shown that SAE when used in a mouthrinse has a clinically measurable effect on gingival health without significant effect on plaque growth. Additional usage of SAE mouthrinse to routine mouth cleaning may enhance the protective value to oral hygiene. Since herbal extracts are natural products, the complexity of the constitution makes impossible the identification of one or other specifically active ingredient, but clearly such recipes are worthy of further attention.

Acknowledgements

This work was supported by a research grant of Faculty of Dentistry, Khon Kaen University.

References


29. Sowiseth C, Thanasarnthavee W, Phantumvanit P. Effectiveness of extract from *Koi* (Streblus asper) to *Streptococcus mutans* in vivo. In: 7th Scientific Meeting of Asia Pacific Dental Student Association Congress; 1991 7-15 April; Bangkok; 1991.


บทวิจารณ์
ผลของการใช้น้ำบ่วนปากผสมสารสกัดจากใบช่วยด้วยการอักเสบของ
เหงือกและการเกิดครบุคคลที่ยืน

สุวิทย์ ทริปสิทธิ์ โพธิ์ วงศ์ค้า อารีวี วัฒนทองค้า ฉัตรภรณ์ ศิริภา เทียมทวี ชุพย์ สราวุธ สุขวิทย์

บทคัดย่อ
การวิจัยนี้มีวัตถุประสงค์เพื่ศึกษาผลของการใช้น้ำบ่วนผสมสารสกัดจากใบช่วยด้วยการอักเสบของเหงือกและการเกิดครบุคคลที่ยืน ซึ่งเป็นสารสกัดจากใบช่วยที่มีความชัดเจนและมีความเหมาะสมในการใช้ในร่างกาย ผลการวิจัยนี้พบว่าการใช้น้ำบ่วนผสมสารสกัดจากใบช่วยมีผลลดการอักเสบของเหงือกและมีผลลดการเกิดครบุคคลที่ยืนได้ผลในระดับที่มีความมั่นคงและมีความเหมาะสมในการใช้ในร่างกาย.

* ภาควิชาวิจิตรศิลป์ช่างปาก คณะสุนัขแพทยศาสตร์ มหาวิทยาลัยยะลา ถนน จ.ยะลา 40002
** ภาควิชาวิจิตรศิลป์ คณะสุนัขแพทยศาสตร์ มหาวิทยาลัยยะลา ถนน จ.ยะลา 40002
*** ภาควิชาวิชวกรรมช่างปาก คณะสุนัขแพทยศาสตร์ มหาวิทยาลัยยะลา ถนน จ.ยะลา 40002