Abstract. Background: Previous studies have shown activity against viruses, bacteria, inflammation and oral lichenoid dysplasia of alkaline extract of the leaves of Sasa senanensis Rehder (SE), suggesting its possible application to oral diseases. In the present study, we performed a small-scale clinical test to investigate whether SE is effective against halitosis and in oral bacterial reduction. Materials and Methods: A total of 12 volunteers participated in this study. They brushed their teeth immediately after meals three times each day with SE-containing toothpaste (SETP) or placebo toothpaste. Halitosis in the breath and bacterial number on the tongue were measured by commercially available portable apparatuses at a specified time in the morning. Results: Some relationship was observed between halitosis and bacterial number from each individual, especially when those with severe halitosis were included. Repeated experiments demonstrated that SETP significantly reduced halitosis but not the bacterial number on the tongue. Conclusion: The present study provides for the first time the basis for anti-halitosis activity of SE.

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Key Words: Sasa senanensis Rehder leaf, alkaline extract, toothpaste, halitosis, antibacterial activity, small-scale clinical test.

Materials and Methods

Materials. SE was manufactured by Daiwa Biological Research Institute Co., Ltd., Kawasaki, Kanagawa, Japan. Lyophilized SE contained 66 mg of solid materials/ml SETP (“SASA-Health®”) and placebo toothpaste (Table I) were manufactured by Sampo Pharmaceutical Co., Ltd., Tokyo, Japan. The components of the SETP and placebo toothpaste (which otherwise lacks only SE) are listed in Table I.
Twelve healthy volunteers (11 males and one female) were enrolled in this small-scale clinical study. They brushed their teeth with SETP or placebo toothpaste immediately after meals three times each day, and their halitosis and bacterial count were recorded at 11:00 AM four to five times a week, according to the guideline of the Intramural Ethic Committee (approval no. A1219).

For the assay of halitosis, breath was collected for 45 sec through a mouthpiece, and the concentration of volatile sulfur compounds (VSCs) in the breath was measured by the commercially available Breathtron [150 mm (W) × 150 mm (H) × 230 mm (D)] (Yoshida Co., Ltd., Tokyo, Japan) (Figure 1B, left) and recorded in parts per billion, according to the instructions of the manufacturer.

For the assay of bacterial number, bacteria on the center of the dorsum of the tongue were gently scraped off by cotton swab (three strokes) and immersed in 5 ml of water in a disposable cup, and the number of bacterial was determined by bacteria counter [144 mm (W) × 147 mm (H) × 189 mm (D)] (Panasonic Healthcare Co. Ltd., Tokyo, Japan), according to the instructions of the manufacturer.

Since the data for VSCs and bacterial numbers fluctuated considerably, each volunteer was subjected to repeated measurements: 5 times (Table II), or 10 to 15 times (Table III) for the calculation of mean value.

Statistical treatment. Each experimental value is expressed as the mean±standard deviation (SD). Statistical analysis was performed by using Student’s t-test and non-parametric analysis. A value of p<0.05 was considered significant.

Results

Manufacturing of STEP. We have previously reported that (i) contact with SE (50% concentration) and isopropylmethylphenol (IPMP) (0.31 mM) for 10 min did not significantly affect the viability of human gingival fibroblast and human periodontal ligament fibroblast; and (ii) SE and IPMP synergistically inhibited the growth of Porphyromonas gingivalis 381 when the concentration of SE and IPMP were higher than 0.63% and 0.25 mM, respectively (18). Based on these data, when formulating SETP, we set the concentration of SE and IPMP at 26.2% and 0.1%, respectively. Since Sasa-sumi (charcoal of SE) did not affect the bacterial growth (18), it was omitted from SETP.

Relationship between halitosis and bacterial number. When halitosis levels (VSCs) and bacterial numbers on the tongue were plotted, a smoothed curve was generated. When data from individuals with heavy halitosis were included in the plot, a good correlation coefficient was obtained (y=−0.1331x^2 + 16.344x + 210.35, R^2=0.4908) (Figure 1C).

Effect of SETP. Compared to placebo toothpaste, SETP did not aggravate halitosis nor increase the bacterial number on the tongue. Prolonged use of SETP rather more efficiently reduced halitosis, but the effect of SETP on the number of bacterial on the tongue was not so apparent (Table II).

We repeated similar experiments many times (10 to 15 times) with larger number of volunteers to evaluate this tendency. We found that SETP significantly reduced halitosis (p=0.046, non-parametric analysis), but not the number of bacterial on the tongue (p=0.60) (Table III).
Table II. Effect of alkaline extract of the leaves of Sasa senanensis Rehder (SE) and placebo toothpastes on halitosis and number of bacteria on the tongue. Participants brushed their teeth three times immediately after meals three times each day with SE or placebo toothpaste and measured their halitosis and number of bacteria at 11:00 AM. The measurements were performed usually 4-5 times a week. Each value represents the mean±S.D. of five trials performed on different days.

<table>
<thead>
<tr>
<th>Gender, age (years)</th>
<th>VSC (ppb)</th>
<th>Bacterial-count (×10⁶/tongue surface)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ordinary toothpaste 1 Week</td>
<td>Placebo toothpaste 1 Week</td>
</tr>
<tr>
<td>M. 61</td>
<td>542±175</td>
<td>435±169</td>
</tr>
<tr>
<td>M. 53</td>
<td>403±89</td>
<td>371±80</td>
</tr>
<tr>
<td>F. 52</td>
<td>626±237</td>
<td>702±416</td>
</tr>
<tr>
<td>M. 24</td>
<td>248±60</td>
<td>312±144</td>
</tr>
<tr>
<td>M. 28</td>
<td>369±125</td>
<td>341±148</td>
</tr>
<tr>
<td>M. 24</td>
<td>268±98</td>
<td>319±141</td>
</tr>
<tr>
<td>M. 24</td>
<td>495±206</td>
<td>472±139</td>
</tr>
<tr>
<td>M. 24</td>
<td>388±118</td>
<td>375±65</td>
</tr>
</tbody>
</table>

M: Male; F: female.

Table III. Effect of alkaline extract of the leaves of Sasa senanensis Rehder (SE) and placebo toothpastes on halitosis and number of bacteria on the tongue. Participants brushed their teeth three times immediately after meals three times each day with SE or placebo toothpaste and measured their halitosis and number of bacteria at 11:00 AM. The measurements were performed usually 4-5 times a week. Each value represents the mean±S.D. of 10-15 trials performed on different days. Statistical analysis was non-parametric.

<table>
<thead>
<tr>
<th>Gender, age (years)</th>
<th>VSC (ppb)</th>
<th>Change (%)</th>
<th>Bacterial-count (×10⁶/tongue surface)</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo toothpaste</td>
<td>SE toothpaste</td>
<td>Change (%)</td>
<td>Placebo toothpaste</td>
</tr>
<tr>
<td>M. 26</td>
<td>75.2</td>
<td>51.5</td>
<td>31.5</td>
<td>5.2</td>
</tr>
<tr>
<td>M. 26</td>
<td>211.6</td>
<td>87</td>
<td>58.9</td>
<td>9.37</td>
</tr>
<tr>
<td>M. 32</td>
<td>153.5</td>
<td>147.9</td>
<td>3.6</td>
<td>5.24</td>
</tr>
<tr>
<td>M. 26</td>
<td>80.8</td>
<td>45.1</td>
<td>44.2</td>
<td>7.88</td>
</tr>
<tr>
<td>M. 62</td>
<td>134.2</td>
<td>104.3</td>
<td>22.3</td>
<td>7.56</td>
</tr>
</tbody>
</table>

p=0.046  p=0.600
Discussion

The present study demonstrated, for the first time, that SETP significantly reduced halitosis, but not the number of bacteria on the tongue. The suppression of halitosis by SETP may result from the combinational antibacterial activity of SE and IPMP (18). It is conceivable that the active component(s) of SE may have higher affinity for oral mucosa, then kill or inactivate more efficiently the bacteria on it, resulting in unnoticeable effects on tongue surface bacterial number.

It has been reported that sinusitis (19) and acute pharyngitis (20) are virally but not bacterially caused. Association of oral lichen planus (21) and stomatitis (22) with viral infection have been reported. SE, as well as alkaline extracts from green tea (23) and licorice root (24), had potent anti-HIV activity. Therefore, alkaline extracts can be applicable for the treatment of virally induced oral diseases. In this sense, it would be beneficial to explore portable apparatus for monitoring and evaluating the antiviral effect of SE instantly at the chairside.

SE was found to have potent radical-scavenging activity, in a synergistic manner with vitamin C (7) and inhibited cyclooxygenase-2 protein expression and prostaglandin E₂ production by interleukin-1β-stimulated human gingival fibroblast (Sakagami et al., data not shown). SE also inhibited mouse macrophage activation (5) and osteoclastogenesis (17). These results suggest the potent anti-inflammatory activity of SE. It has been recently suggested that the inflammation involving nuclear factor-κB (NF-κB) activation is related not only to carcinogenesis, but also to aging (25). It remains to be investigated whether SE inhibits the NF-κB signaling pathway that stimulates aging.

Conflicts of Interest

The first author (HS) was supported by Daiwa Biological Research Institute Co., Ltd., Kanagawa, Japan. We wish to confirm that such financial support has not influenced the outcome or the experimental data.

Acknowledgements

The Authors thank Dr. H. Watanabe, Division of Fixed Prosthodontics, Meikai University School of Dentistry, for performing the non-parametric analysis.

References


Received November 25, 2015
Revised December 25, 2015
Accepted January 7, 2016