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Anti-Halitosis Effect of Toothpaste Supplemented with Alkaline Extract of the Leaves of *Sasa senanensis* Rehder

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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.*

Alkaline extract of the leaves of *Sasa senanensis* Rehder (SE) ("SASA-Health"), which is a Group III over-the-counter drug in Japan (1), is expected to be less harmful, compared to Kampo medicines, which belong to Group II. SE is recognized as being effective in treating fatigue, loss of appetite, halitosis, body odor and stomatitis by oral administration, but there is no scientific evidence to demonstrate these phenomena due to the lack of appropriate biomarkers. SE has shown *in vitro* antiseptic (2), membrane-stabilizing (3), anti-inflammatory (4-6), antibacterial (7, 8), antiviral (7, 8), anti-UV (9, 10) and radical-scavenging (5, 10, 11) activities, and synergistic action with vitamin C (7). SE has several biological properties in common with lignin-carbohydrate complex (LCC), which is also extracted by alkaline solution: the prominent anti-HIV, anti-UV and synergistic activity with vitamin C (12). We have identified the anti-UV substances of SE as *p*-coumaric acid derivative(s), a lignin precursor (13). Both SE and LCC were found to have prominent antiviral activity, one to two orders higher than that of tannins and flavonoids (7, 8, 14), suggesting their possible application for the treatment of virally-induced oral diseases. We recently reported that long-term treatment of SE progressively reduced both the area of white streaks in oral mucosa and the base-line levels of salivary interleukin-6 and -8 (15). Likewise, oral intake of LCC–vitamin C tablet significantly improved the symptom of patients infected with herpes simplex virus (16, 17).

In order to further pursue the possibility of dental application of SE, we have manufactured toothpaste that contains 26.2 (w/v%) of SE (SETP) (Figure 1A). We investigated here whether brushing the teeth with SETP can actually suppress halitosis and bacterial number on the surface of the tongue at the same time.

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 no Hamigaki®") 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.

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Table I. *Components of alkaline extract from leaves of Sasa senanensis Rehder (SE) and placebo toothpastes. Circles are components included in the toothpastes.*

Components	Function	SE toothpaste	Placebo toothpaste
Alkaline extract of the leaves of *Sasa senanensis* Rehder	Base material	○	
Hydroxyapatite	Base material	○	○
Calcium carbonate	Cleaning agent	○	○
Water	Cleaning agent	○	○
Silica	Cleaning agent	○	○
Glycerine	Humectant	○	○
Polyethylene glycol	Humectant	○	○
Xylitol	Flavoring substance	○	○
Menthol	Flavoring substance	○	○
Saccharin sodium	Sweetening agent	○	○
Sucrose palmitate	Stabilizer	○	○
Sodium copper chlorophyllin	Stabilizer	○	○
Cellulose gum	Binding agent	○	○
Sodium Laurate	Washing soap	○	○
Isopropylmethyphenol (IPMP)	Antibacterial agent	○	○

Components included in the toothpaste we indicated by circles.

Assay for halitosis and bacterial number on the tongue. 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)] (Figure 1B, right) (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 Sasasumi (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.*

	VSC (ppb)						
Gender, age (years)	Ordinary toothpaste 1 Week	Placebo toothpaste 1 Week	SE toothpaste 1 Week	SE toothpaste 2 Week	SE toothpaste 3 Week	SE toothpaste 4 Week	SE toothpaste 5 Week
M, 61	542±175	435±169	513±79	582±128	634±250	233±114	188±63
M, 53	403±89	371±80	399±106	352±130	401±185	226±191	175±42
F, 52	626±237	702±416	628±450				
M, 24	248±60	312±144	307±182	157±44	84±54		
M, 28	369±125	341±148	323±88	474±494	304±183	215	
M, 24	268±98	319±141	324±147	219±36	164±213		
M, 24	495±206	472±139	362±147	319±270	105±84	124±66	
mean	388±118	375±65	371±77	351±158	282±211	200±51	182±9
(%)	100	97	96	90	73	52	47
	Bacterial-count ($\times 10^6$/tongue surface)						
Gender , age (years)	Ordinary toothpaste 1 Week	Placebo toothpaste 1 Week	SE toothpaste 1 Week	SE toothpaste 2 Week	SE toothpaste 3 Week	SE toothpaste 4 Week	SE toothpaste 5 Week
M, 61	16.0±11.5	4.71±2.20	7.82±3.94	18.39±31.04	15.41±5.28	10.9±4.27	5.31±2.21
M, 53	5.64±4.41	7.14±3.97	12.99±4.46	13.0±1.53	8.25±5.45	8.53±3.68	7.46±4.20
F, 52	32.4±21.1	65±33.3	34.8±37.5				
M, 24	1.68±1.19	0.962±0.077	1.22±0.65	1.80±1.62	1.27±0.89		
M, 28	15.8±9.62	15.99±7.86	12.6±4.68	10.55±1.41	14.5±5.46	13.8	
M, 24	3.51±3.88	5.63±4.16	1.90±0.42	2.27±1.31	8.30±9.80		
M, 24	7.89±5.73	6.26±4.59	5.49±2.35	3.22±2.52	4.33±3.93	3.04±0.37	
mean	8.42±6.15	6.78±4.99	7.00±5.09	8.21±6,83	8.68±5,54	9.07±4.56	6.39±1.08
(%)	100	81	83	98	103	108	86

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.*

	VSC (ppb)			Bacterial-count ($\times 10^6$/tongue surface)		
Gender, age (years)	Placebo toothpaste	SE toothpaste	Change (%)	Placebo toothpaste	SE toothpaste	Change (%)
M, 26	75.2	51.5	31.5	8.31	6.86	17.4
M, 26	211.6	87	58.9	36.53	27.21	25.5
M, 32	153.5	147.9	3.6	9.37	14.54	–55.2
M 31	54.9	55.2	–0.5	5.24	5.32	–1.5
M, 26	80.8	45.1	44.2	7.88	5.80	26.4
M, 62	134.2	104.3	22.3	7.56	7.65	–1.2
		p=0.046			p=0.600	

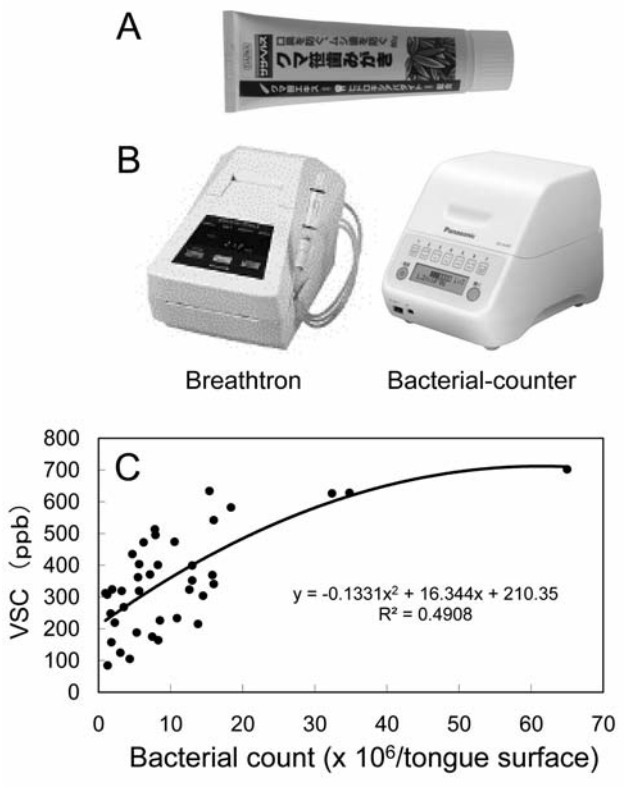

Figure 1. *A: Alkaline extract of the leaves of Sasa senanensis Rehder (SE)-containing toothpaste. B: Breathtron (left) and bacterial counter (right). C: Correlation of halitosis and bacterial number on the tongue. Each data point (mean of five measurements) was derived from Table II.*

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 E_2 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.

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