

Biological Interaction between *Sasa senanensis* Rehder Leaf Extract and Toothpaste Ingredients

HIROSHI SAKAGAMI¹, SHIGERU AMANO¹, TOSHIKAZU YASUI¹, KAZUE SATOH², SEIJI SHIODA²,
TAISEI KANAMOTO³, SHIGEMI TERAKUBO³, HIDEKI NAKASHIMA³, KOICHI WATANABE⁴,
TOMOKO SUGIURA⁵, MADOKA KITAJIMA⁵, HIROSHI OIZUMI⁵ and TAKAAKI OIZUMI⁵

¹Meikai University School of Dentistry, Sakado, Saitama, Japan;

²Department of Anatomy, School of Medicine, Showa University, Tokyo, Japan;

³Department of Microbiology, St. Marianna University School of Medicine, Kanagawa, Japan;

⁴Sampo Pharmaceutical Co., Ltd., Tokyo, Japan;

⁵Daiwa Biological Research Institute Co., Ltd., Kanagawa, Japan

Abstract. *Background:* Previous studies have shown antiviral, antibacterial, and anti-inflammatory activity of alkaline extract of the leaves of *Sasa senanensis* Rehder (SE). In order to manufacture an SE-containing toothpaste for combating oral diseases, we investigated the possible interaction between the candidate ingredients of toothpaste: SE, isopropyl methylphenol (IPMP, antibacterial agent) and charcoal prepared from *Sasa senanensis* Rehder. *Materials and Methods:* Cell viability of mock-infected, HIV-infected and UV-irradiated cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Superoxide radical scavenging activity was determined by electron-spin resonance spectroscopy. Antibacterial activity against *Porphyromonas gingivalis* 381 and *Streptococcus mutans* ATCC25175 was determined by the turbidity assay. *Results:* Exposure to less than 50% SE or less than 0.31 mM IPMP for 10 min scarcely damaged human cultured gingival and periodontal ligament fibroblasts. Both SE and IPMP showed bi-modal action, stimulating the bacterial growth at lower concentrations, but synergistically inhibiting it at higher concentrations. Addition of extremely high concentrations of charcoal enhanced both anti-HIV and anti-UV activity of SE. *Conclusion:* Practically, addition of charcoal may not be recommendable, since one or two orders higher concentrations of charcoal as compared with SE, are required to achieve the synergistic effect for anti-HIV and

anti-UV activity. Rather, addition of about one tenth of the amount of IPMP may be recommendable for enhancing the antibacterial activity.

Two bamboos, *Take* and *Sasa* (Japanese names) belong to grasses, but are not strictly distinguished from each other botanically. There are 70 genera of bamboos in the world and 14 genera (approximately 600 species) in Japan. They are distributed into Saghalien, the Kuriles, Hokkaido, Honshu, Shikoku and Kyushu in Japan. The dried leaves of *Sasa* plants have been used as herbal tea, functional food and medicines. Alkaline extract of the leaves of *Sasa senanensis* Rehder (SE) (SASA-Health[®]), which belong to Group III over-the-counter (OTC) drugs (1), is expected to be less hazardous, as compared with Kampo medicines, which belong to Group II. SE is recognized as being effective in treating fatigue, low appetite, halitosis, body odor and stomatitis, but there is no scientific evidence that demonstrates 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 (7-10), anti-UV (9-12) and radical-scavenging (5, 8-10, 13) activities, and synergistic action with vitamin C (7). SE exhibited several biological properties common to those of lignin-carbohydrate complex (LCC): prominent anti-HIV, anti-UV and synergistic activity with vitamin C (14), as expected from their extractability under alkaline condition. On gel filtration fractionation, we successfully concentrated the majority of the anti-UV activity into fraction (Fr.) I, which was eluted as a single major peak (SEE-1), using high-performance liquid chromatography (HPLC) (11). Structural analysis with UV absorption, ¹H-NMR and ¹³C-NMR spectral analyses identified SEE-1 as *p*-coumaric acid derivative(s), lignin precursor(s) (15). This was the first demonstration explaining why SE exhibits LCC-like properties.

Correspondence to: Hiroshi Sakagami, Division of Pharmacology, Meikai University School of Dentistry, Sakado, Saitama 350-0283, Japan. Tel: +81 492792758, Fax: +81 492855171, e-mail: sakagami@dent.meikai.ac.jp

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Three major products of bamboo leaf extract available as Group III OTC drugs are SE (pure alkaline extract of the leaves of *Sasa senanensis* Rehder) (7), Sunchlon® (approximately 80% of LCC removed by acid precipitation) (16), and Shojusen® (hot water extract of the leaves of *Sasa kريلensis* Makino et Sibata, supplemented with ethanolic extract of the leaves of *Pinus densiflora* Sieb et Zucc.) (17). We found that SE, which has the highest LCC content among these three products, exhibited the highest anti-HIV and anti-UV activity, and the least cytochrome *P-450* (CYP)-3A inhibitory activity (9). Similarly, LCCs prepared from various plants exhibited one or two-orders higher anti-HIV and anti-UV activity (18), as compared with Kampo medicines and constituent plant extracts (prepared by hot water extraction) (19), further confirming that the active principle in SE is LCC, and that alkaline extraction is superior to hot water extraction in isolating the anti-HIV and anti-UV substances.

All of these *in vitro* studies of SE suggest its potential applicability to treating virally-induced diseases (20). Due to the lack of well-designed clinical trials, however, the safety and efficiency of many alternative therapies are unclear. Accumulation of scientific evidence, based on component analysis by three-dimensional HPLC, pharmacokinetic-pharmacodynamic analyses and clinical trials are urgent to fill this gap. Our recent clinical pilot studies demonstrated that (i) the intake of LCC-vitamin C tablet significantly improved the symptoms of patients with herpes simplex virus infection (21, 22), and (ii) long-term treatment with SE progressively reduced symptoms of lichenoid dysplasia and the salivary concentration of interleukin-6 and -8 (23). These studies urged us to manufacture an SE-containing toothpaste for combating stomatitis, lichenoid dysplasia and periodontal diseases. As an initial step to explore such a toothpaste, we investigated how candidate toothpaste ingredients such as isopropyl-methylphenol (IPMP), a popular antibacterial agent, and charcoal produced from *Sasa senanensis* Rehder, a coloring material, affect the biological activity of SE.

Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: Dulbecco's modified Eagle's medium (DMEM): Gibco BRL, Grand Island, NY, USA; fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), hypoxanthine (HX), xanthine oxidase (XOD), diethylenetriaminepenta-acetic acid (DETAPAC), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO): Dojin, Kumamoto, Japan; RPMI-1640 medium, azidothymidine (AZT), 2',3'-dideoxycytidine (ddC): Sigma Chemical Co. St. Louis, MO, USA; dimethyl sulfoxide (DMSO), dextran sulfate (5 kDa): Wako Pure Chemical Ind., Ltd., Osaka, Japan; curdlan sulphate (79 kDa): Ajinomoto Co. Inc., Tokyo, Japan.

SE and *Sasa* charcoal were provided by Daiwa Biological Research Institute Co. Ltd., Kawasaki, Kanagawa, Japan. One

millilitre of SE was freeze dried to produce the powder (66.1 mg). Isopropyl methylphenol (IPMP) (MW 150) was provided by Sampo Pharmaceutical Co., Ltd., Tokyo, Japan.

Cell culture. Normal human oral cells, gingival fibroblast (HGF) and periodontal ligament fibroblast (HPLF) were established from the first premolar tooth extracted from the lower jaw of a 12-year-old girl as described previously (24). Since they stopped proliferating at approximately 40 population doubling level (PDL) (24), cells below 20 PDL were used in the present study. Human oral squamous cell carcinoma cell line HSC-2 was kindly provided by Professor Nagumo, Showa University. These cells were cultured in DMEM supplemented with 10% heat-inactivated FBS. Viability of these cells were determined by MTT method (24).

Assay for anti-HIV activity. Human T-cell leukemia virus I (HTLV-I)-bearing CD4⁺ positive human T-cell line, MT-4, was cultured in RPMI-1640 medium supplemented with 10% FBS and infected with HIV-1_{IIIB} at a multiplicity of infection of 0.01. HIV- and mock-infected (control) MT-4 cells (3×10^4 cells/96-microwell) were incubated for five days with different concentrations of samples and the relative viable cell number was determined by the MTT assay. The concentration that reduced the viable cell number of the uninfected cells by 50% (CC_{50}) and the concentration that increased the viable cell number of the HIV-infected cells to the 50% that of control (mock-infected, untreated) cells (EC_{50}) were determined from the dose-response curve with mock-infected and HIV-infected cells, respectively. The anti-HIV activity was evaluated by the selectivity index (SI), which was calculated using the following equation: $SI = CC_{50}/EC_{50}$ (25).

Assay of anti-UV activity. HGF and human oral squamous cell carcinoma HSC-2 cells were inoculated into 96-microwell plates (3×10^3 cells/well, 0.1 ml/well) and incubated for 48 h to allow cell attachment. The culture supernatant was replaced with 100 μ l phosphate-buffered saline without calcium and magnesium [PBS(-)] that contained different concentrations of sample, and cells were then placed at 21 cm distance from a UV lamp (wavelength: 253.7 nm) and exposed to UV irradiation (6 J/m²/min) for 1 min. The cells were then incubated for a further 48 h in DMEM containing 10% FBS to determine the relative viable cell number by the MTT assay. From the dose-response curve, the CC_{50} and the concentration that increased the viability of UV-irradiated cells up to 50% that of control (unirradiated, untreated) cells (EC_{50}) were determined. The SI was determined using the following equation: $SI = CC_{50}/EC_{50}$ (24, 26).

Assay for antibacterial activity. The minimum inhibitory concentration (MIC) was determined by serially-diluting SE or IPMP, according to the standard method of the Japan Society of Chemotherapy (27). For the measurement of MIC for *Streptococcus mutans* ATCC25175, Brain Heart Infusion (BHI) medium was used under aerobic conditions. For the measurement of MIC against *Porphyromonas gingivalis* 381, Gifu Anaerobic Medium (GAM) containing 5 μ g/ml hemin and 1 μ g/ml menadione was used under anaerobic conditions with mixed gas of nitrogen (83%), hydrogen (7%) and CO₂ (10%). Each bacteria strain (1×10^6 cfu/ml) was incubated for 24 h at 37°C in culture medium containing serially diluted SE or IPMP and the absorbance at 595 nm of the bacterial suspension was measured. From the dose-response curve, the MIC (99.9% bacteriostatic) was determined.

Radical-scavenging activity. The free radical intensity was determined at 25°C, using electron-spin resonance (ESR) spectroscopy (JEOL JES REIX, X-band, 100 kHz modulation frequency; JEOL Ltd., Tokyo, Japan) (28). The instrument settings were: centre field, 335.5±5.0 mT; microwave power, 16 mW; modulation amplitude, 0.1 mT; gain, 630; time constant, 0.03 s and scanning time, 2 min.

For the determination of the superoxide anion (in the form of DMPO-OOH), produced by the HX-XOD reaction (total volume: 200 µl) [2 mM HX in 0.1 M phosphate buffer (PB) (pH 7.4) 50 µl, 1 mM DETAPAC 10 µl, 10% DMPO 30 µl, test sample (in PB) 40 µl, PB 40 µl, XOD (0.5 U/ml in PB) 30 µl], the time constant was changed to 0.03 s (28).

Statistical treatment. Experimental values are expressed as the mean±standard deviation (SD). Statistical analysis was performed by using Student's *t*-test. A *p*-value <0.05 was considered to be significant.

Results

Cytotoxicity of SE. Considering that we usually spend only several minutes brushing our teeth in daily life, the effect of short-term treatment with SE on the viability of human oral normal cells was first investigated. To mimic saliva, phosphate-buffered saline without calcium and magnesium (PBS(-)) instead of normal culture medium was used to culture these cells. When HGF cells were exposed to 100% SE (equivalent to 66 mg dry material/ml), the viability did not change during the first 2 min, but declined thereafter with prolonged incubation (Figure 1A). When HGFs were exposed to 50% SE (33 mg/ml) [1:1 mixture of SE and PBS(-)], the viability did not significantly change until 9 min. HPLFs were more sensitive to SE as compared with HGFs. However, the treatment of HPLFs for 1 min with 50% of SE did not affect cell viability (Figure 1B). Since approximately 8 ml of saliva are produced and accumulated in the oral cavity by 5 min of tooth brushing (unpublished observation), the actual concentration of SE may be considerably reduced.

SE did not induce the growth-stimulation effect known as hormesis (29, 30) at lower concentrations (Figure 1).

Cytotoxicity of IPMP. IPMP was cytotoxic rather than cytostatic without the induction of hormetic stimulation of bacterial growth (Figure 2). Cytotoxicity of IPMP against HGFs and HPLFs was observed above 1.25 and 0.63 mM, respectively (Figure 2). Induction of cytotoxicity by IPMP was very rapid, reaching a near-maximum level of cytotoxicity within 1 min after contact of cells with IPMP [CC₅₀=0.98 mM (1-min contact), 0.94 mM (10-min contact), 0.41 mM (48-h contact), respectively] (Figures 2 and 3).

Cytotoxic activity of IPMP combined with Sasa charcoal. We investigated whether *Sasa* charcoal interferes with the cytotoxicity induction by IPMP. Since charcoal is insoluble

in DMSO, various amounts of charcoal were added to IPMP solution in DMSO, vortex-mixed and allowed to remain in contact for 1 h. After removal of precipitating charcoal by centrifugation, the supernatant was collected. We found that IPMP pre-incubation with charcoal did not change the cytotoxicity of IPMP [CC₅₀=0.94-0.99 mM (10-min treatment); 0.41-0.44 mM (48-h treatment), regardless of the mixing ratio (charcoal/IPMP=0, 0.03, 0.1, 0.3, 1 or 3) (Figure 3). Therefore, it is unlikely that charcoal adsorbs IPMP and reduces its cytotoxicity.

Anti-UV activity. We investigated whether the addition of IPMP modifies the anti-UV activity of SE, using HGF and HSC-2 as target cells. HGFs were relatively resistant to UV irradiation as compared with HSC-2 cells, slightly elevating the background levels of viable cells after UV irradiation (26).

UV irradiation (6 J/m²/min, 1 min) reduced the viability of HGFs after 48-h incubation to 35% that of the control (Figure 4). Addition of SE during UV irradiation protected the cells from UV-induced cell injury, with an EC₅₀ of 0.32 mM and CC₅₀ of 37.7 mM, yielding an SI of 118 (Figure 4). Slightly higher SI values, as compared with that observed for HSC-2 cells (SI=26.2-31.1, Table I), may be derived from the higher level of cell viability after UV irradiation. Addition of up to 0.3 mM IPMP did not reduce, but rather slightly increased the anti-UV activity of SE. However, addition of 1 mM IPMP resulted in the total loss of cell viability, with and without UV irradiation (Figure 4).

We next investigated whether addition of *Sasa* charcoal affected the anti-UV activity of SE. The addition of up to 100 mg charcoal to 1 ml SE did not affect the anti-UV activity of SE (compare 2 vs. 7; Table I). Interestingly, the addition of 300 mg charcoal significantly (*p*<0.01) increased the anti-UV activity (compare 2 vs. 8; Table I).

Antibacterial activity. Both SE and IPMP induced an apparent hormetic effect on the growth of *P. gingivalis* 381 (Figure 5A). Both of these agents stimulated or inhibited the growth of *P. gingivalis* 381 at lower and higher concentration ranges, respectively. The MIC of SE- and IPMP-alone was 1.25%, and 500 µM, respectively. Synergistic inhibition of bacterial growth was observed at 0.16-0.63% SE and 125 µM IPMP (Figure 5A).

On the other hand, SE, but not IPMP had a hormetic effect on *S. mutans* ATCC 25175 (Figure 5B). The MIC of SE-alone against *S. mutans* ATCC 25175 was 5%. Antibacterial activity of IPMP-alone was not observed below 500 µM. Synergistic inhibition of bacterial growth was observed at 2.5% SE and 500 µM IPMP (Figure 5B).

Anti-HIV activity. SE showed potent anti-HIV activity (SI=35.7±7.5), confirming our previous finding (7-10, 12),

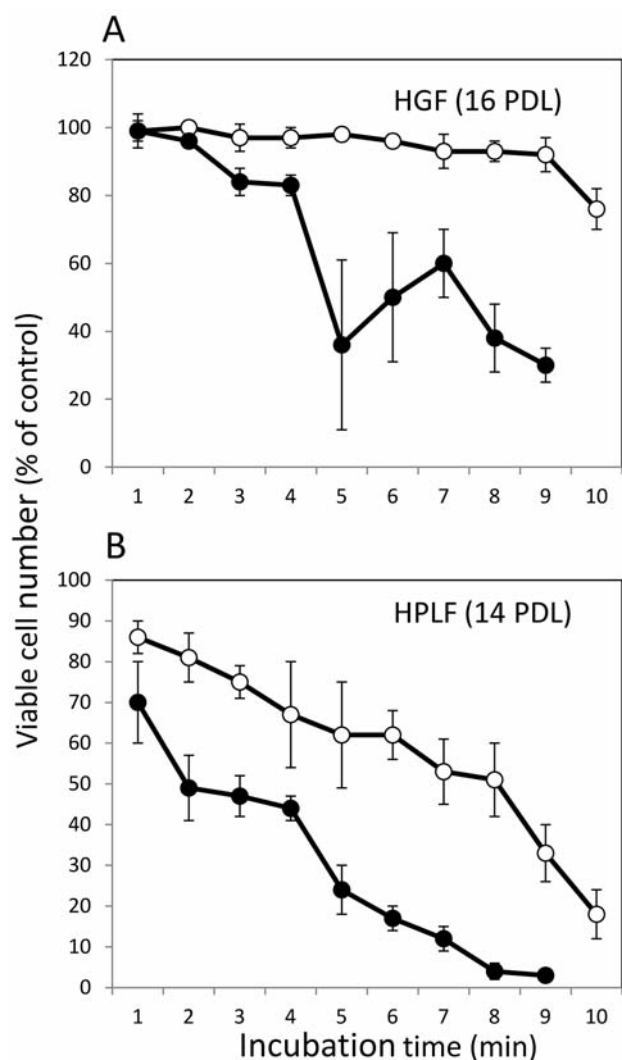


Figure 1. Determination of optimum exposure time to alkaline extract of the leaves of *Sasa senanensis* Rehder (SE). Near-confluent human gingival fibroblast (HGF) (16 population doubling level, PDL) (A) and periodontal ligament fibroblast (HPLF) (14 PDL) (B) were incubated for the indicated times with 50% SE (1:1 mixture of SE and PBS(-)) (○) or SE (●). After replacing the medium with fresh culture medium (DMEM+10% FBS), the cells were incubated for 48 h to determine the viable cell number. Each value represents the mean±S.D. of triplicate assays.

although the anti-HIV activity of SE was 6- to 600-fold lower than that of popular anti-HIV agents (dextran sulfate SI=211, curdian sulfate SI=3471, azidothymidine SI=20132, 2',3'-dideoxycytidine SI=2813) (Table II). Addition of 10 µg/ml charcoal did not affect the anti-HIV activity of SE, but addition of 200 µg/ml charcoal resulted in 2- to 3-fold increase in the anti-HIV activity of SE. On the other hand, IPMP at 1-20 µg/ml (6~133 µM) did not influence the anti-HIV activity of SE (Table II).

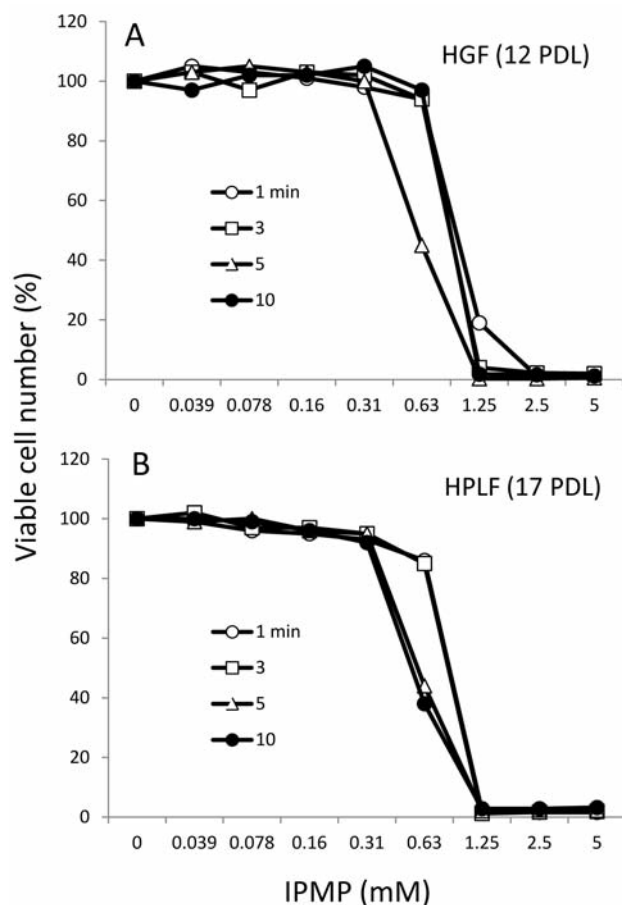


Figure 2. Determination of optimum exposure time to isopropylmethylphenol (IPMP). Near-confluent human gingival fibroblast (HGF) (12 PDL) (A) and periodontal ligament fibroblast (HPLF) (17 PDL) (B) were incubated with the indicated concentrations of IPMP for 1 min (○), 3 min (□), 5 min (△) or 10 min (●). After replacing the medium with fresh culture medium (DMEM+10%FBS), the cells were incubated for 48 h to determine the viable cell number. Each value represents the mean±S.D. of triplicate assays.

Radical-scavenging activity. SE dose-dependently scavenged the superoxide anion (detected as DMPO-OOH) generated by HX and XOD reaction, with an IC₅₀ of 0.15% (0.099 mg/ml) (Figure 6A). Charcoal (2 mg/ml) also exhibited comparable superoxide scavenging activity with SE (0.1%=0.066 mg/ml), whereas IPMP (2 mM=0.3 mg/ml) was inactive (Figure 6B). Combination of SE with IPMP, charcoal or IPMP plus charcoal did not enhance the superoxide radical-scavenging activity of SE (Figure 6B).

Discussion

The present study demonstrated that the anti-*P. gingivalis* activity of IPMP is detectable above 0.125 mM (0.019 mg/ml), but a four-fold higher concentration (0.5 mM=0.075

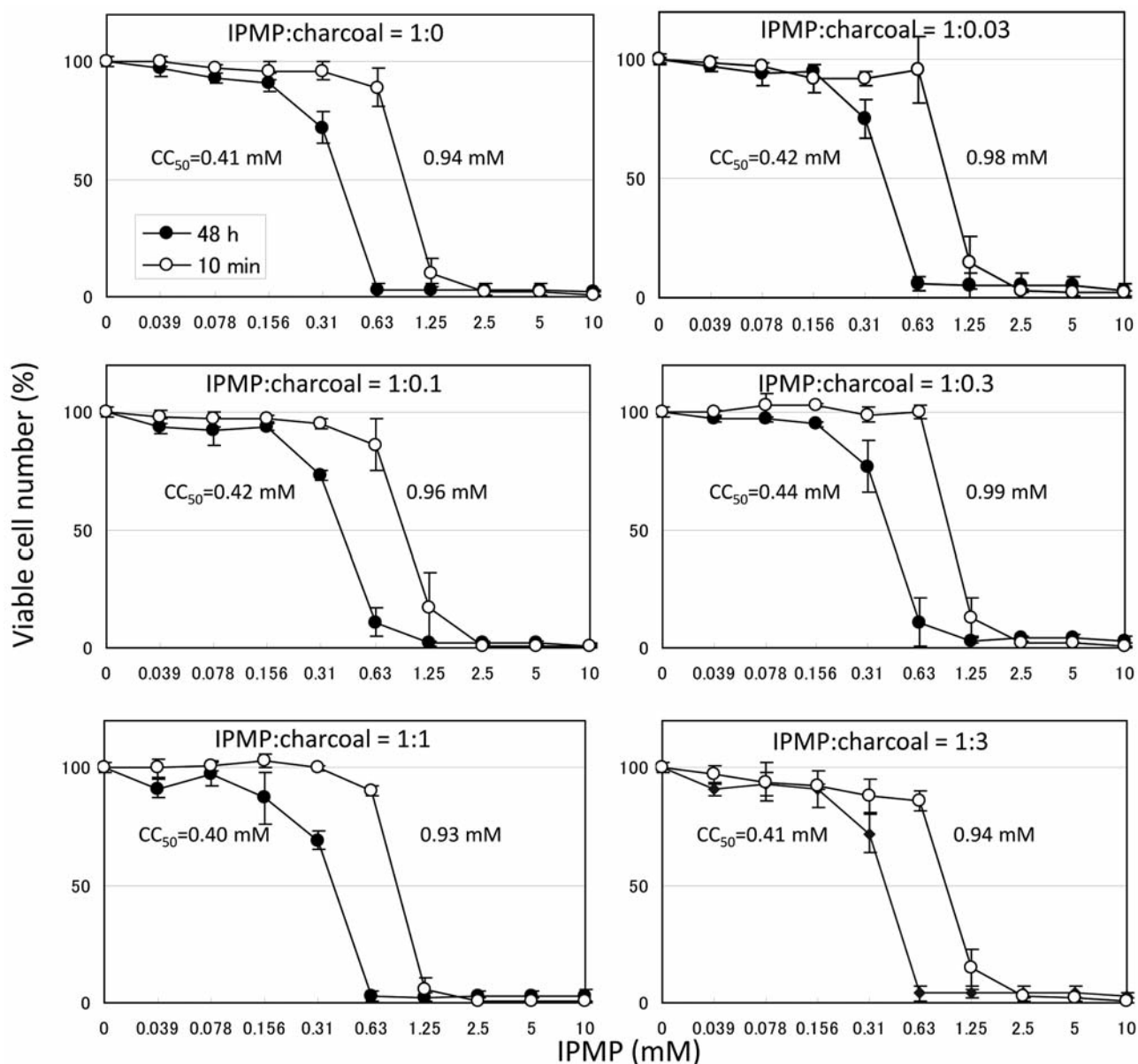


Figure 3. Cytotoxic activity of isopropyl-methylphenol (IPMP) combined with *Sasa charcoal*. Prior to experiment, 1 M IPMP solution (in DMSO) (0.8 ml=120 mg) was mixed with 0, 3.6, 12, 36, 120 or 360 mg *Sasa charcoal* (at a ratio of IPMP:charcoal=1:0, 1:0.03, 1:0.1, 1:0.3, 1:1, 1:3) and stood for 30 min at room temperature (vortex mixing four times). After centrifugation at 20,000 \times g for 1 min, the supernatant was collected. Near confluent HGFs (18 PDL) were incubated for 10 min in the IPMP-charcoal mixture, and then incubated in fresh drug-free culture medium for 47 h and 50 min (○) while aliquots of the cells were incubated with IPMP-charcoal mixture for 48 h (●). The determination of viable cell number by the MTT method. Each value represents the mean \pm S.D. of triplicate assays.

mg/ml) is required to achieve the complete elimination of *P. gingivalis* (Figure 5A). The anti-*P. gingivalis* activity of SE was detectable above 0.16% (0.11 mg/ml) of SE, but a 16-fold higher concentration (2.5%=1.7 mg/ml) was required for complete elimination. However, the combination of 0.25 mM (0.038 mg/ml) IPMP and 1.25% (0.83 mg/ml) SE achieved complete elimination of *P. gingivalis* (Figure 5A).

It was unexpected that lower concentrations (0.00049-0.0625 mM=0.0000735-0.00938 mg/ml) of IPMP would slightly enhance the bacterial growth, and the same is true for lower concentrations of SE (0.08-0.16%=0.053-0.11 mg/ml). On the other hand, the complete elimination of *S. mutans* required 0.5 mM (0.075 mg/ml) IPMP and 2.5% (1.7 mg/ml) SE. These concentrations did not injure normal

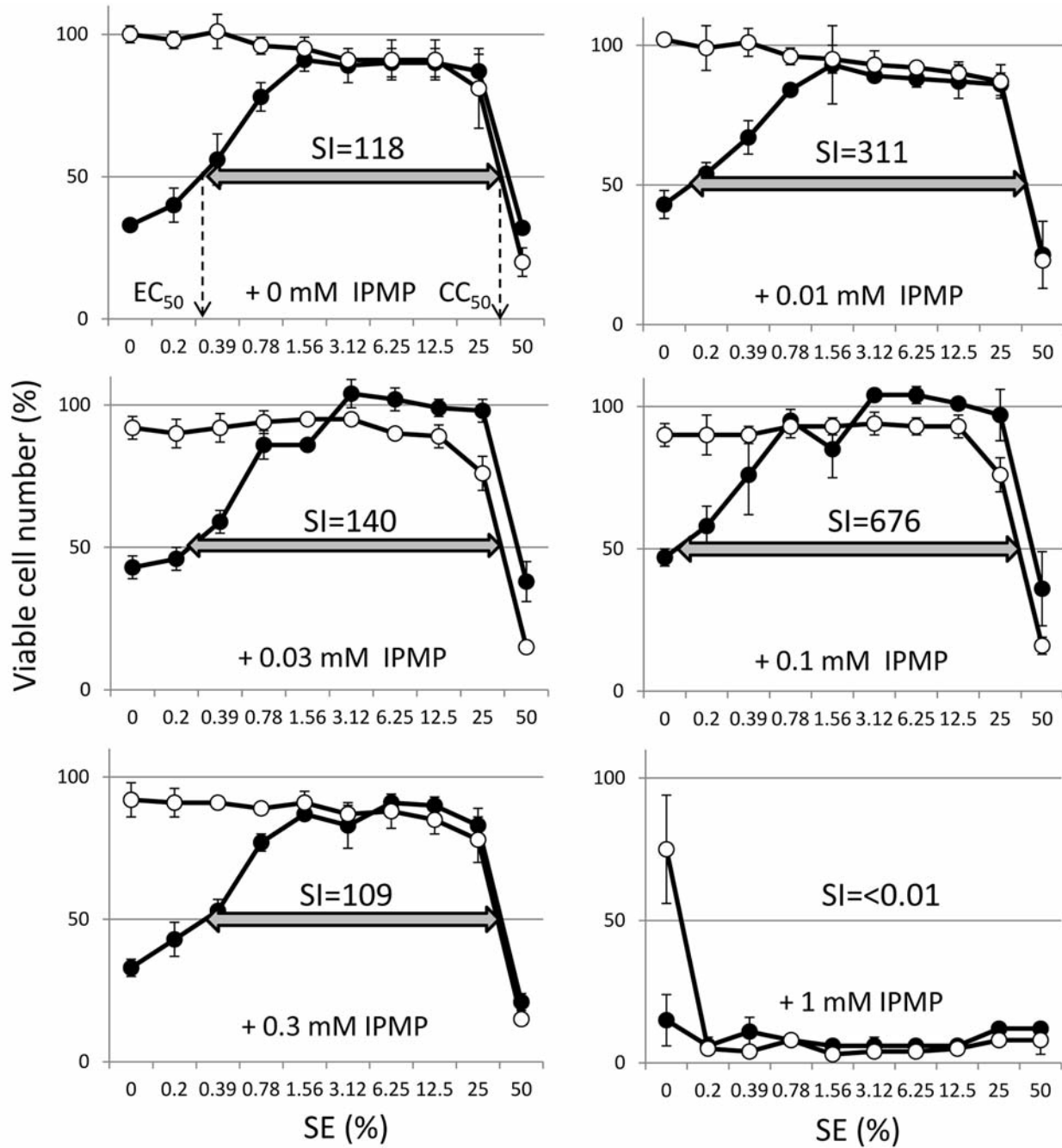


Figure 4. Anti-UV activity of alkaline extract of the leaves of *Sasa senanensis* Rehder (SE) plus isopropyl-methylphenol (IPMP). Near confluent HGFs (13 population doubling level, PDL) were replaced with PBS(-) containing the indicated concentrations of SE in the presence of 0, 0.01, 0.03, 0.1, 0.3 or 1 mM IPMP, and exposed to UV irradiation for 1 min. After replacing the medium with fresh medium, the cells were further incubated for 48 h to determine the viable cell number. Anti-UV activity was then determined as described in the Materials and Methods. Viable cells were determined by the MTT method. Each value represents mean±S.D. (n=3). CC₅₀: 50% Cytotoxic concentration, EC₅₀: 50% effective concentration; selectivity index, SI=CC₅₀/EC₅₀.

oral cells, when the contact time was reduced to 3 min (Figure 2). IPMP-alone did not have any anti-HIV activity (Table II), anti-UV (not shown) nor radical-scavenging activity (Figure 6B).

The present study also demonstrated that addition of 200 mg/ml charcoal resulted in three-fold increase of anti-HIV of SE (from an SI value of 35.7±7.5 to 92) (Table II), and that addition of 100-300 mg/ml charcoal resulted in a

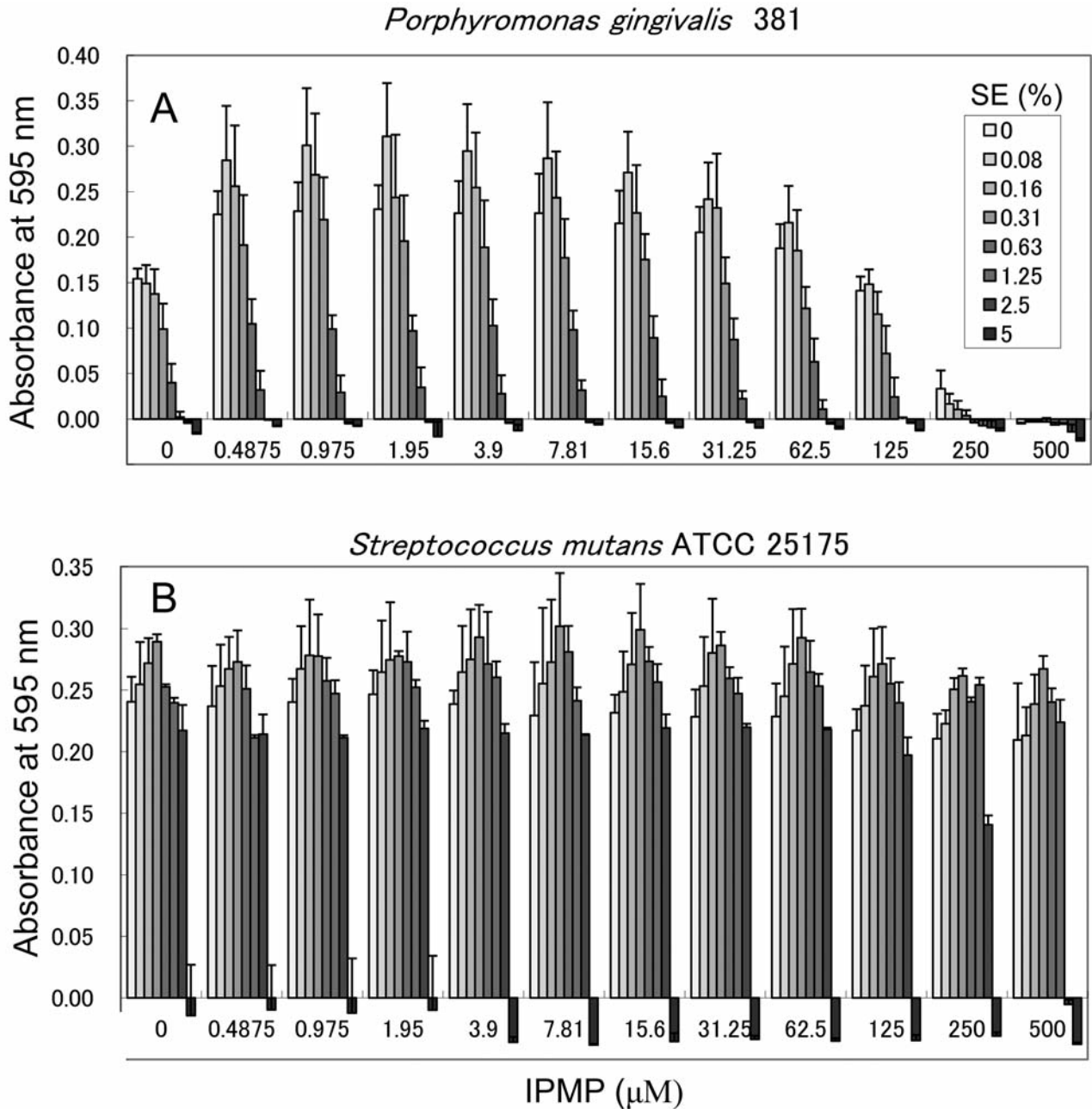


Figure 5. Antibacterial activity of alkaline extract of the leaves of *Sasa senanensis* Rehder (SE) combined with isopropyl-methylphenol (IPMP). Antibacterial assay was performed as described in the Materials and Methods. Each value represents the mean \pm S.D. of three independent experiments, each performed in triplicate. * $p < 0.01$ compared with control.

10-44% increase of anti-UV activity of SE. However, lower concentrations (1-30 mg/ml) of charcoal did not lead to such enhancing effects. Practically, addition of charcoal cannot be recommended, since more than 60- to 180-fold higher amounts of charcoal, as compared with SE are required to achieve the synergistic effect with SE. Charcoal-alone had no detectable anti-HIV activity (SI<1),

and very weak superoxide scavenging activity (1/30 of that of SE) (Figure 6B).

It has been reported that many toxic substances, environmental hormones, inorganic compounds, and even irradiation can modulate the growth of cultured cells in a bi-phasic fashion, stimulating or inhibiting the growth of cultured cells at lower and higher concentrations,

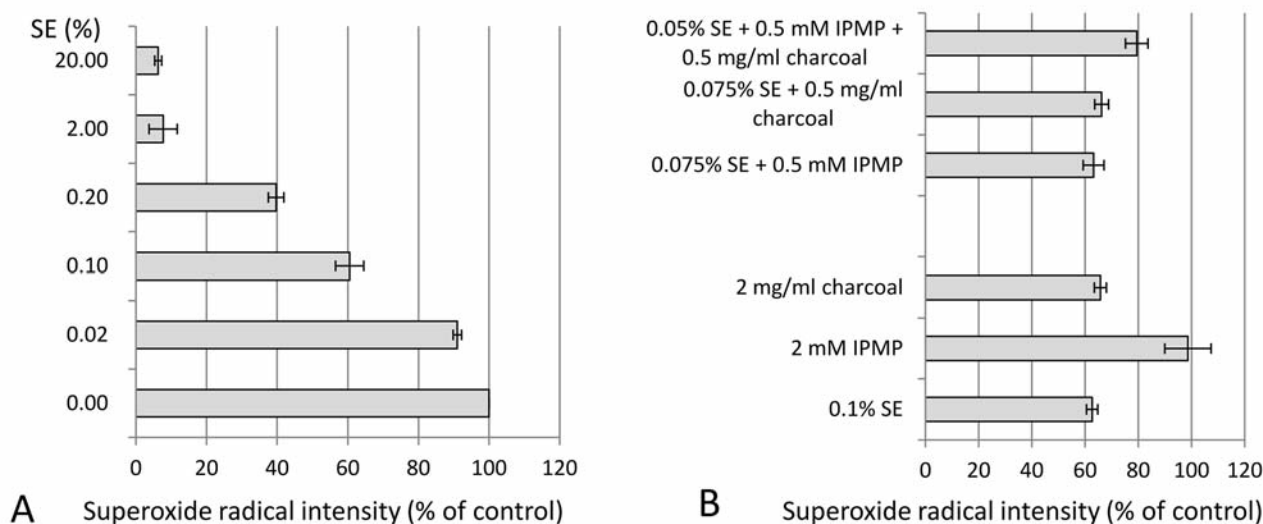


Figure 6. Effect of isopropyl-methylphenol (IPMP) and charcoal on superoxide scavenging activity of alkaline extract of the leaves of *Sasa senanensis* Rehder (SE). A: Superoxide scavenging activity as a function of SE. B: Effect of IPMP and charcoal. Each value represents the mean±S.D. (n=3).

Table I. Effect of *Sasa* charcoal on the anti-UV activity of alkaline extract of the leaves of *Sasa senanensis* Rehder (SE). Prior to the experiment, the indicated amounts of *Sasa* charcoal (1, 3, 10, 30, 10 or 300 mg) were added to 0.01 ml of 100% SE (66 mg/ml), mixed and remained for 1 h at room temperature, with occasional swirlings with a vortex mixer. After centrifugation at 20,000 ×g, the supernatant was collected. Near-confluent HSC-2 cells in a 96-microwell plate were replenished with PBS(-) containing different concentrations of the supernatant, and were exposed to UV irradiation for 1 min. After incubation for 48 h in fresh culture medium, the viable cell number was determined, and the anti-UV activity was calculated. Each value represents the mean from triplicate assays. *p<0.05 compared with control (SE-alone).

	Exp	CC ₅₀ (%)	EC ₅₀ (%)	SI	mean±S.D.
1 SE + 0 mg/ml charcoal (Not centrifuged)	I	25.0	1.09	22.9	26.2±3.3
	II	34.7	1.18	29.4	
	III	30.3	1.15	26.3	
2 SE + 0 mg/ml charcoal (Centrifuged) Group 2	I	37.9	1.00	38.1	31.1±6.8
	II	33.5	1.37	24.5	
	III	32.7	1.06	30.8	
3 SE + 1 mg/ml charcoal (Centrifuged)	I	23.6	1.10	21.5	25.1±4.5
	II	24.4	1.03	23.7	
	III	36.2	1.20	30.2	
4 SE + 3 mg/ml charcoal (Centrifuged)	I	25.5	1.11	23.0	26.8±4.7
	II	24.8	0.98	25.3	
	III	36.5	1.14	32.0	
5 SE + 10 mg/ml charcoal (Centrifuged)	I	37.8	1.15	32.9	29.9±9.1
	II	28.9	1.08	26.8	
	III	35.3	1.18	29.9	
6 SE + 30 mg/ml charcoal (Centrifuged)	I	36.1	1.21	29.8	30.6±1.7
	II	28.2	0.96	29.4	
	III	36.8	1.13	32.6	
7 SE + 100 mg/ml charcoal (Centrifuged)	I	42.2	1.12	37.7	34.3±3.4
	II	33.7	1.09	30.9	
	III	39.0	1.14	34.2	
8 SE + 300 mg/ml charcoal (Centrifuged)	I	50.0	1.04	48.1	44.9±2.8*
	II	48.6	1.13	43.0	
	III	50.0	1.15	43.5	

CC₅₀: 50% Cytotoxic concentration, EC₅₀: 50% effective concentration; selectivity index, SI=CC₅₀/EC₅₀. *p<0.05 compared 2.

Table II. Anti-HIV activity of alkaline extract of the leaves of *Sasa senanensis* Rehder (SE) combined with *Sasa* charcoal and isopropyl-methylphenol (IPMP).

	SE		
	CC ₅₀ (%)	EC ₅₀ (%)	SI
SE + 200 µg/ml charcoal + 20 µg/ml IPMP	0.939	0.019	48
SE + 200 µg/ml charcoal + 1 µg/ml IPMP	1.055	0.019	57
SE + 200 µg/ml charcoal	1.244	0.013	97
SE + 10 µg/ml charcoal + 20 µg/ml IPMP	0.876	0.026	33
SE + 10 µg/ml charcoal + 1 µg/ml IPMP	1.035	0.026	41
SE + 10 µg/ml charcoal	1.132	0.036	32
SE + 20 µg/ml IPMP	0.987	0.028	36
SE + 1 µg/ml IPMP	0.986	0.032	31
SE (Exp. I)	1.042	0.029	36
SE (Exp. II)	1.241	0.029	43
SE (Exp. III)	1.098	0.039	28
IPMP (µg/ml)	187.78	>1000	<1
Charcoal (µg/ml)	5348.75	>10000	<1
Dextran sulfate (µg/ml)	112.16	0.532	211
Curdlan sulfate (µg/ml)	501.61	0.144	3,471
Azidothymidine (µM)	257.19	0.013	20,132
2',3'-Dideoxycytidine	1961.48	0.697	2,813

Anti-HIV activity of SE-alone and that supplemented with charcoal (200, 10 µg/ml) and/or IPMP (20 or 1 µg/ml=133, or 6.7 µM) was assayed in triplicate. CC₅₀: 50% Cytotoxic concentration, EC₅₀: 50% effective concentration; selectivity index, SI=CC₅₀/EC₅₀.

respectively (29, 30). We found that both SE and IPMP showed bi-phasic hormetic effects on bacterial cell growth. Lower concentrations of SE enhanced growth of both *P. gingivalis* 381 and *S. mutans* ATCC 25175, whereas IPMP showed such an effect on only *P. gingivalis* 381 (Figure 5). This indicates that use of lower concentrations of SE and IPMP should be avoided for the treatment of bacterially-induced oral diseases. Careful selection of the dose of therapeutic agents should be recommended.

In conclusion, we propose that the best mixing ratio (w/w) of SE to IPMP should be 22.7 (1.7/0.0075) for manufacturing an SE-containing toothpaste. Search for compounds that further enhance the antibacterial activity of SE is underway.

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