



Fluoride Concentration, Antibacterial Effect, and Cytotoxicity in Children's Toothpaste: *In Vitro* Study

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Abstract

Objectives The aim of this study is to evaluate the effect on cell viability, antibacterial activity against cariogenic bacteria, and total fluoride availability of commercially available children's toothpastes in Thailand.

Materials and Methods Seven toothpaste slurries were prepared from seven commercial toothpastes for children. Total fluoride concentration was determined. The agar diffusion method was used to examine the antibacterial effect of toothpaste against *Streptococcus mutans*. The viability of L929 mouse fibroblasts exposed with different concentrations of each toothpaste slurry was tested by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay.

Statistical Analysis One way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests were used for zone inhibition analysis. Cell viability data were analyzed using Student's *t*-test.

Results Fluoride concentration in fluoride-containing toothpastes ranged from 521.36 to 1,377.83 ppm. Two toothpastes exhibited a similar level of fluoride concentration compared between the product labels. Other toothpastes showed a difference or slight difference in fluoride concentration between the companies' information and our data. The zone of inhibition ranged from 0 to 2.08 cm. A significantly higher zone of inhibition was observed in toothpastes with sodium lauryl sulfate. The concentration of toothpaste that reduced cell viability to less than 50% of the control was 0.8, 3.1, 6.3, 6.3, 25, and 100% for Fluocaril Deli Fruity, Oral-B Junior 6+, Kodomo Kids, CUdent Stevia, SunStar GUM, and Kindee Organic, respectively.

Conclusion The examined children's toothpastes can inhibit the growth of *S. mutans*, which did not correlate with fluoride concentration. The antibacterial effects could be the results of other ingredients, for example, sodium lauryl sulfate.

Keywords

- ▶ children's toothpaste
- ▶ fluoride concentration
- ▶ antibacterial effect
- ▶ cytotoxicity
- ▶ stevia fluoride
- ▶ SLS

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Introduction

Dental caries is one of the most common dental diseases found in childhood. It is formed by the communication of cariogenic bacteria, in addition to the host factors, such as dental tissue, saliva, and nutrients.¹ To control the disease, several approaches have been used. Among them, a combination of using toothbrushes and toothpaste is recommended. This method is simple and can be applied routinely. Due to the brushing action, dental plaque is physically removed, while toothpaste enhances the caries preventive effect through its composition.²

In Thailand and worldwide, fluoride toothpaste has been recommended for many decades. Fluoride compounds have been included in toothpaste to decrease demineralization and, meanwhile, increase remineralization effects.^{3,4} Some studies reported that fluoride influences antibacterial activity. However, it is unclear if the antibacterial effect in toothpaste is related to fluoride. Nowadays in Thailand, the demand for fluoride-free organic products is on the rise, particularly in the realm of children's toothpaste. A growing number of parents believe that organic items confer superior health advantages. However, to date, no comprehensive investigation has been conducted to assess the antibacterial attributes of children's toothpaste variants, comparing those containing fluoride with their fluoride-free counterparts in the Thai context. Nevertheless, it is important to note that the composition of children's toothpaste typically extends beyond just fluoride, but there are also abrasive particles, detergents, preservatives, disinfectants, sweeteners, artificial colors, odors, and flavors.⁵ Each of these elements is strategically incorporated to enhance diverse aspects and outcomes of the toothpaste's performance. For instance, xylitol is added as a sweetener, which may have a bacterial inhibitory effect.⁶ Sodium lauryl sulfate (SLS) is added to be a surfactant.⁷ Although the manufacturers informed the ingredients and qualities of their products, neither the exact antibacterial effect of these toothpastes nor the true fluoride concentration is known. In addition, the cytotoxicity of these kinds of toothpaste was not elucidated.

This aim of this study was to evaluate the effect of children's commercial fluoride toothpaste on cell viability, antibacterial activity against cariogenic bacteria, and total fluoride availability of fluoride toothpaste.

Materials and Methods

Toothpaste Preparation

Seven commercial toothpastes for children were purchased. The ingredients informed by the manufacturer are described in **Table 1**. The toothpaste solution was prepared according to ISO 11609:2017. Briefly, 1 g of each sample was dissolved in 3 mL of deionized water. The sample was mixed and 3 mL of the solution was transferred to the centrifuge tubes. The solution was centrifuged at a speed of 4,000 rpm for 15 minutes. The supernatant was collected and used for other experiments immediately.

Measurement of Total Fluoride Concentration

Total ionic strength adjustment buffer (TISAB IV) and 70% perchloric acid solution were purchased from Merck (Darmstadt, Germany). TISAB was dissolved in 1 L of distilled water. The standard fluoride solution was prepared as an internal standard at a concentration of 1,000, 500, 250, 125, and 62.5 ppm. Seven commercial children's toothpastes were prepared as mentioned earlier. For the solution of toothpastes containing sodium monofluorophosphate (Kodomo Kids and Fluocaril Deli Fruity), 4 mL of toothpaste solution was thoroughly mixed with 1 mL of perchloric acid solution and left at room temperature for 24 hours before testing. To measure fluoride concentration, 1 mL of prepared toothpaste solution was mixed with 1 mL of TISAB IV. A fluoride ion selective electrode (Versa Star; Thermo Fisher Scientific Inc., Waltham, MA, United States), calibrated using the external standard of the fluoride solution at a concentration of 1,000, 100, 10, and 1 ppm, was immersed in the solution for 1 minute. The procedure was repeated three times. The average total fluoride (ppm) was calculated and compared with the manufacturer's information.

Antibacterial Inhibition Test

Streptococcus mutans from -80°C stock was cultured in sheep blood agar (SBA) and incubated at 37°C with 5% CO_2 for 20 to 24 hours. A single colony was transferred to 5 mL of Mueller Hinton Broth (MHB; HIMEDIA, India) and incubated at 37°C with 5% CO_2 for 24 hours. The turbidity suspension was adjusted to 0.5 McFarland (1.0×10^8 CFU/mL). The optical density was measured at a wavelength of 600 nm using a spectrophotometer. Adjusted standard turbidity suspension of 50 μL was spread on MHB (HIMEDIA, India) with 5% sheep blood using sterile cotton swab. The cotton swab was spread at the edge of the agar plate at the last step. A 6-mm-diameter paper disk was soaked with 20 μL of prepared toothpaste solution. Deionized water was used for negative control. The Vancomycin Antimicrobial Susceptibility disk (OXOID, Hampshire, UK) was used for positive control. The disk was placed and gently pressed on the surface of the agar. The center of each disk was apart at least 24 mm. The number of disks was limited to four disks being placed on a 100-mm Petri dish. To ensure accurate results, the disk remained stationary upon placement on the agar to prevent solution dispersion. Additionally, care was taken to position the disk at an appropriate distance from the agar plate's edge, as proximity could lead to an incomplete inhibition zone formation. After that, the plate was incubated at 37°C with 5% CO_2 for 24 hours. The experiment was repeated three times. The result was interpreted by measuring the diameter of the complete inhibition zone with a digital scale (Digital Vernier Caliper).

Cell Viability Test

L929 cell line (NCTC clone 929; CCL-1, American Type Culture Collection, ATCC, Lot number: 70026472) was cultured in a medium consisting of 88% Eagle's Minimum Essential Medium (EMEM), 10% fetal bovine serum (FBS), 1% L-glutamax, 1% L-glutamine/antimicrobial in humidified 5% CO_2 at 37°C until it reached 80 to 90% confluency. After passaging,

Table 1 Ingredients in children's toothpastes commercially available in Thailand

	Toothpaste	Brand	Ingredient	Company of manufacturer	Company of distributor
A	Baybee Milk Enzyme Baby Toothpaste Organics	Baybee	Water, sorbitol, xylitol, glycerin, hydrated silica, sodium carboxymethylcellulose, PEG-40, hydrogenated castor oil, flavor, sodium benzoate, calcium lactate, potassium sorbate, <i>Musa sapientum</i> (Banana) fruit extract, citric acid, lactoperoxidase (and) glucose oxidase (and) sorbitol, <i>Aloe barbadensis</i> leaf juice, ascorbic acid, dipotassium glycyrrhizate, <i>Pyrus malus</i> (apple) fruit extract, <i>Chamomilla recutita</i> (<i>Matricaria</i>) flower extract	Milott Laboratories Co., Ltd., Samutprakan, Thailand	Intervision Supplies Co., Ltd., Bangkok, Thailand
B	Kindee Organic Toothpaste	Kindee	Aqua, sorbitol, acrylates/c10–30 alkyl acrylate crosspolymer, xylitol, propanediol, cellulose gum, sodium benzoate, xanthan gum, sodium lauroyl sarcosinate, flavor, PEG-40 hydrogenated castor oil, sodium saccharin, potassium sorbate, disodium EDTA, sodium fluoride, glycerin, calcium phosphoryl oligosaccharides, <i>Vitis vinifera</i> (grape) seed extract, <i>Aloe barbadensis</i> (aloe vera) leaf juice, CI 14700, CI 42090	Surathin International Co., Ltd., Samutprakan, Thailand	Kindee Kiddee Kids Co., Ltd., Bangkok, Thailand
C	SunStar GUM Toothpaste	Sunstar	Water, sorbitol, silica, isomalt, cellulose gum, disodium phosphate, aroma, sodium lauryl sulfate, lauryl glucoside, sodium fluoride, sodium saccharin, sodium phosphate, methylparaben, CI 77891	Sunstar Inc, Osaka, Japan	Sunstar Singapore Pte., Ltd., Singapore
D	Kodomo Kids Toothpaste Super Guard	Kodomo	Water, sorbitol, hydrated silica, PEG-8, CI77891, sodium lauryl sulfate, sodium citrate, flavor, sodium monofluorophosphate, xanthan gum, carrageenan, menthol, sodium saccharin, calcium glycerophosphate, sodium hydroxide, xylitol, methylparaben, dipotassium glycyrrhizate, o-cymen-5-ol, citric acid, butylparaben, CI17200	Lion (Thailand) Co., Ltd., Chonburi, Thailand	Saha Pathanapibul Public Co., Ltd., Bangkok, Thailand
E	Fluocaril Deli Fruity Toothpaste	Fluocaril	Water, sorbitol, hydrated silica, glycerin, sodium lauryl sulfate, flavor, cellulose gum, cocamidopropyl betaine, sodium monofluorophosphate, titanium dioxide, xylitol, sodium fluoride, potassium acesulfame, sucralose, CI42051	Meiyume Manufacturing (Thailand) Co., Ltd., Pathum Thani, Thailand	DKSH (Thailand) Co., Ltd., Bangkok, Thailand
F	CUdent Stevia Fluoride (Melon Flavor)	CUdent	Water, sorbitol, hydrated silica, PEG-8, aroma (flavor), cellulose gum, lauryl glucoside, PPG-26-buteth-26, PEG-40 hydrogenated castor oil, sodium benzoate, rebaudioside A, sodium fluoride, CI19140, CI42090, CI17200	Meiyume Manufacturing (Thailand) Co., Ltd., Pathum Thani, Thailand	C.U.Dent Products Co., Ltd., Bangkok, Thailand
G	Oral-B Junior 6+ Years	Oral-B	Water, hydrated silica, sorbitol, sodium lauryl sulfate, cellulose gum, flavor, trisodium phosphate, sodium fluoride, carbomer, sodium saccharin, polysorbate 80, CI 77891	Procter & Gamble Guangzhou Co., Ltd., Guangdong, China	Procter & Gamble Trading (Thailand) Co., Ltd., Bangkok, Thailand

100 μ L of culture medium consisting of 3×10^4 cells was cultured in 96-well plates. The plate was incubated in humidified 5% CO₂ at 37°C. After 24 hours, the culture medium was discarded and washed with 100 μ L of phosphate buffered saline (PBS). The toothpaste solution was prepared as mentioned earlier, with the exception that the culture medium was employed to dilute the solution instead of distilled water. Each toothpaste solution of 100 mL was added to the cell plate and incubated for 2 minutes before cell viability testing. A culture medium without toothpaste was

used as a control. After being exposed to the toothpaste solution for a duration of 2 minutes, the morphology of L929 cells was captured using an inverted light microscope (Olympus CKX41, Japan) equipped with the Cell A Analysis program at a magnification of $10 \times$.

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) solution was prepared by dissolving 1 mg of MTT in 1 mL of PBS at pH 7.4. A 50- μ L MTT solution was added to each well and then the plate was incubated in humidified 5% CO₂ at 37°C for 2 hours. The MTT solution was

removed and 100 μ L of dimethyl sulfoxide (DMSO) was added to each well. The plates were swayed to dissolve the formazan crystal. The solution was pipetted into a new well plate. The absorbance was read at 570 nm using an ultraviolet (UV) spectrophotometer and calculated for the percentage of cell viability. The percentage of cell viability was calculated by comparing it with the control.

Statistical Analysis

Statistical analysis was conducted with IBM SPSS Statistic 26 (SPSS, Chicago, IL, United States). One way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests were used for zone inhibition analysis data and the significant difference was determined when $p < 0.01$. Cell viability data were analyzed using Student's *t*-test and the significance level was set when $p < 0.05$.

Results

Measurement of Total Fluoride Concentration

The measurement of fluoride concentration was shown in ►Fig. 1. Oral-B Junior 6+ provided the highest fluoride concentration among all types of toothpaste. Cudent Stevia Fluoride, SunStar GUM, Fluocaril Deli Fruity, Kodomo Kids, and Kindee Organic exhibited fluoride concentration in descending order. Baybee Milk Enzyme Baby expressed 1.64 ± 0.36 ppm fluoride concentration, which was a level similar to that of distilled water. The comparison of fluoride certification on the product label and the fluoride concentration measured from a fluoride ion selective electrode is shown in ►Table 2. Kindee Organic and SunStar GUM showed a similar level of fluoride concentration compared between the product label and those measured with a fluoride ion selective electrode. Kodomo Kids, Fluocaril

Deli Fruity, Cudent Stevia Fluoride, and Oral-B Junior 6+ showed slightly lower concentrations than those presented on the label of the product. SunStar GUM, Cudent Stevia Fluoride, Kindee Organic, Oral-B Junior 6+, Fluocaril Deli Fruity, and Kodomo Kids were found to exhibit the difference in fluoride concentration between the companies' information and our data ascendingly.

Antibacterial Inhibition Test

Antibacterial activity against *S. mutans* was examined using the zone of inhibition test (►Fig. 2). The exact number of inhibition zones is shown in ►Table 3. Vancomycin antimicrobial susceptibility disk was used as a positive control showing an inhibition zone of 2.33 ± 0.03 cm. Fluocaril Deli Fruity significantly provided the highest inhibition zone among all toothpastes (2.08 ± 0.03 cm). The inhibition zones of Kodomo Kids and Oral-B Junior 6+ were 1.93 ± 0.16 and 1.91 ± 0.04 cm, respectively, which were higher than those of SunStar GUM (1.75 ± 0.05 cm) and Cudent Stevia Fluoride (1.17 ± 0.06 cm, $p < 0.01$). The Baybee Milk Enzyme Baby and Kindee Organic exhibited an absence of an inhibition zone similar to that of distilled water (negative control). Images of the inhibition zone on Mueller Hinton agar and 5% sheep blood are shown in ►Fig. 3.

Cell Viability Test

The cytotoxicity of all toothpastes examined was evaluated on the L929 cell line (►Fig. 4). Cell viability was examined after cells were exposed with an ascending concentration of each toothpaste for 2 minutes. The control showed cell viability without exposure to toothpaste. The concentration of toothpaste that reduced cell viability to be less than 50% of the control was 0.8, 3.1, 6.3, 6.3, 25, and 100% for Fluocaril Deli Fruity, Oral-B Junior 6+, Kodomo Kids, Cudent Stevia,

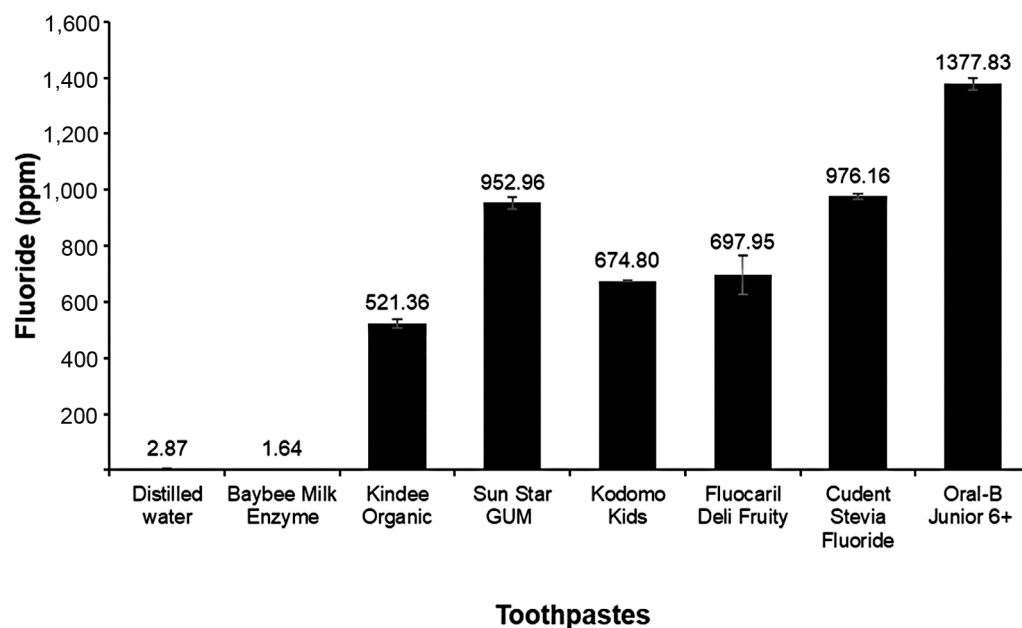
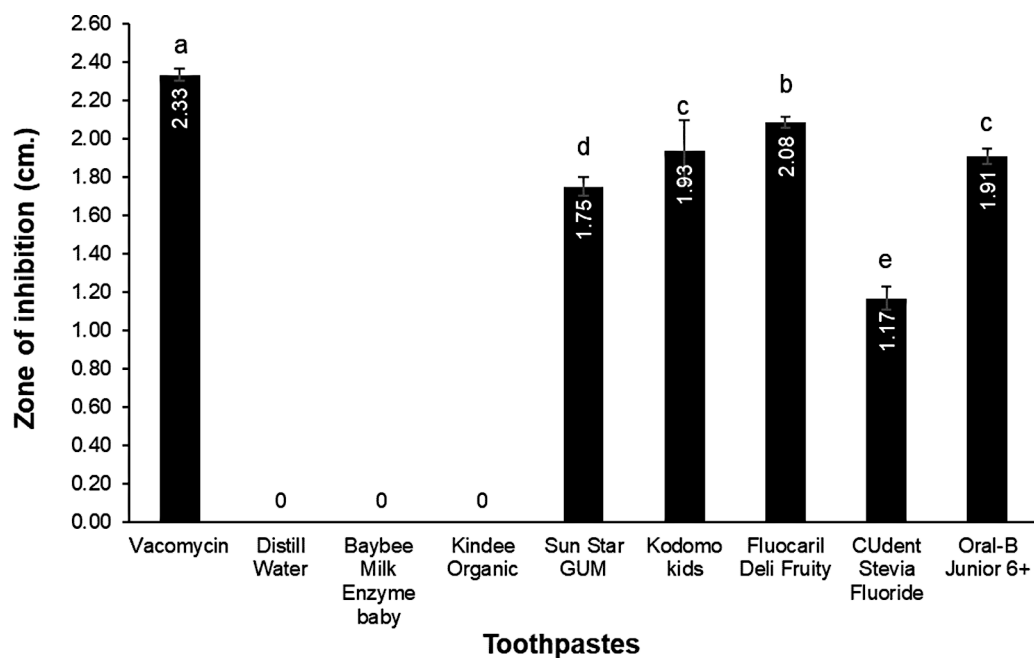


Fig. 1 Fluoride ion concentration (F^-) concentration measured in children's toothpastes. The bar represents the standard deviation of 3 replicates.

Table 2 Fluoride-ion (F-) concentration measured in children's toothpastes with standard deviation and the amount of fluoride certification from the manufacturers

Products	Fluoride certification (ppm)	Fluoride concentration (mean \pm SD), ppm
Distilled water	No fluoride contains	2.87 \pm 0.95
Baybee Milk Enzyme Baby	No fluoride contains	1.64 \pm 0.36
Kindee Organic	500	521.36 \pm 15.21
SunStar GUM	950	952.96 \pm 19.42
Kodomo Kids	1000	674.80 \pm 2.47
Fluocaril Deli Fruity	1000	697.95 \pm 69.62
CUdent Stevia Fluoride	1000	976.16 \pm 9.90
Oral-B Junior 6+	1450	1377.83 \pm 22.63

**Fig. 2** Zone of inhibition of children's toothpastes against *Streptococcus mutans*. The bar represents the standard deviation. a–f: No significant differences between the same letters (analysis of variance [ANOVA], Tukey's honestly significant difference [HSD] test, $p < 0.01$, $n = 3$).**Table 3** Mean and standard deviation (SD) of the zone of inhibition of children's toothpastes against *Streptococcus mutans*

Samples	Zone of inhibition (mean \pm SD), cm
Vancomycin	2.33 \pm 0.03 ^a
Distill Water	0.00 \pm 0.00 ^f
Baybee Milk Enzyme Baby	0.00 \pm 0.00 ^f
Kindee Organic	0.00 \pm 0.00 ^f
SunStar GUM	1.75 \pm 0.05 ^d
Kodomo Kids	1.93 \pm 0.16 ^c
Fluocaril Deli Fruity	2.08 \pm 0.03 ^b
CUdent Stevia Fluoride	1.17 \pm 0.06 ^e
Oral-B Junior 6+	1.91 \pm 0.04 ^c

^{a–f}No significant differences between the same letters (ANOVA, Tukey's HSD test, $p < 0.01$, $n = 3$).

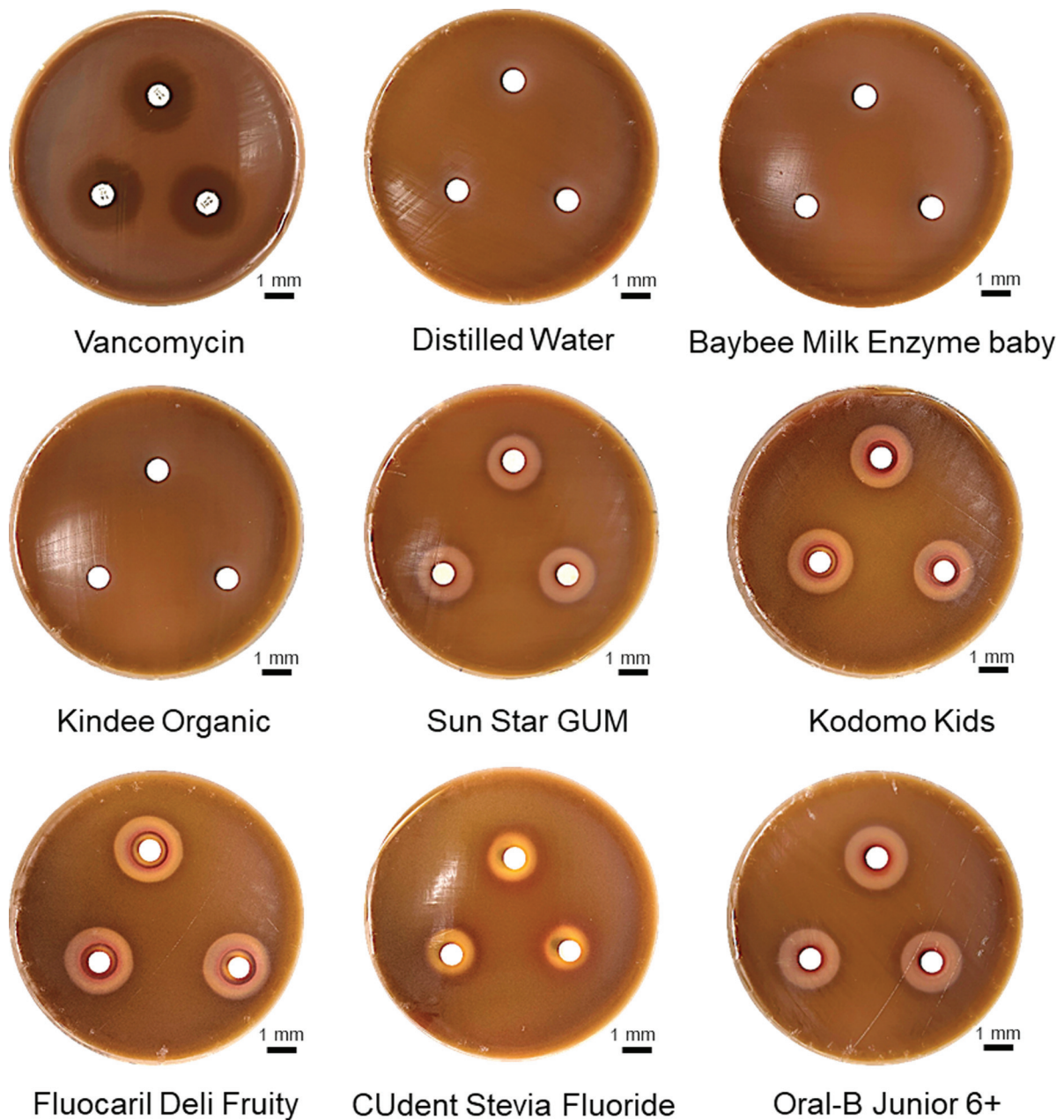


Fig. 3 Zone of inhibition of children's toothpastes against *Streptococcus mutans*. Culture on Mueller–Hinton agar with 5% sheep blood.

SunStar GUM, and Kindee Organic, respectively. For Baybee Milk Enzyme Baby, cell viability was greater than 50% of the control at all concentrations. The raw data on cell viability are shown in ►Table 4. Images of L929 cells treated with different concentrations of each toothpaste are shown in ►Supplementary Fig. S1 (online only).

Discussion

Tooth brushing with toothpaste is accepted to be one of the most effective ways to prevent dental caries.^{8,9} Fluoride is included in the toothpaste to increase the effectiveness of dental caries prevention,¹⁰ although 500 to 1,500 ppm fluoride toothpaste was recommended to be used, the bioavailable fluoride, which was exactly released in the commercial

product, was not known. In this study, we revealed the concentration of fluoride ions contained in each toothpaste. Most of them showed the same amount as labeled by the manufacturer. Baybee Milk Enzyme Baby showed a small concentration of fluoride ions that was assumed to be none of the fluorides, as this small number was also shown in distilled water. Kodomo Kids, Fluocaril Deli Fruity, and Oral-B Junior 6+ displayed fluoride concentrations that were below the manufacturer's stated levels. This finding is in line with several prior studies that have similarly identified variations between tested fluoride levels and the manufacturer's labeling.^{11,12}

S. mutans is an important gram-positive bacterial species implicated in dental caries,¹³ so it is interesting to study the potency of the anticavity effect by inhibiting *S. mutans* of

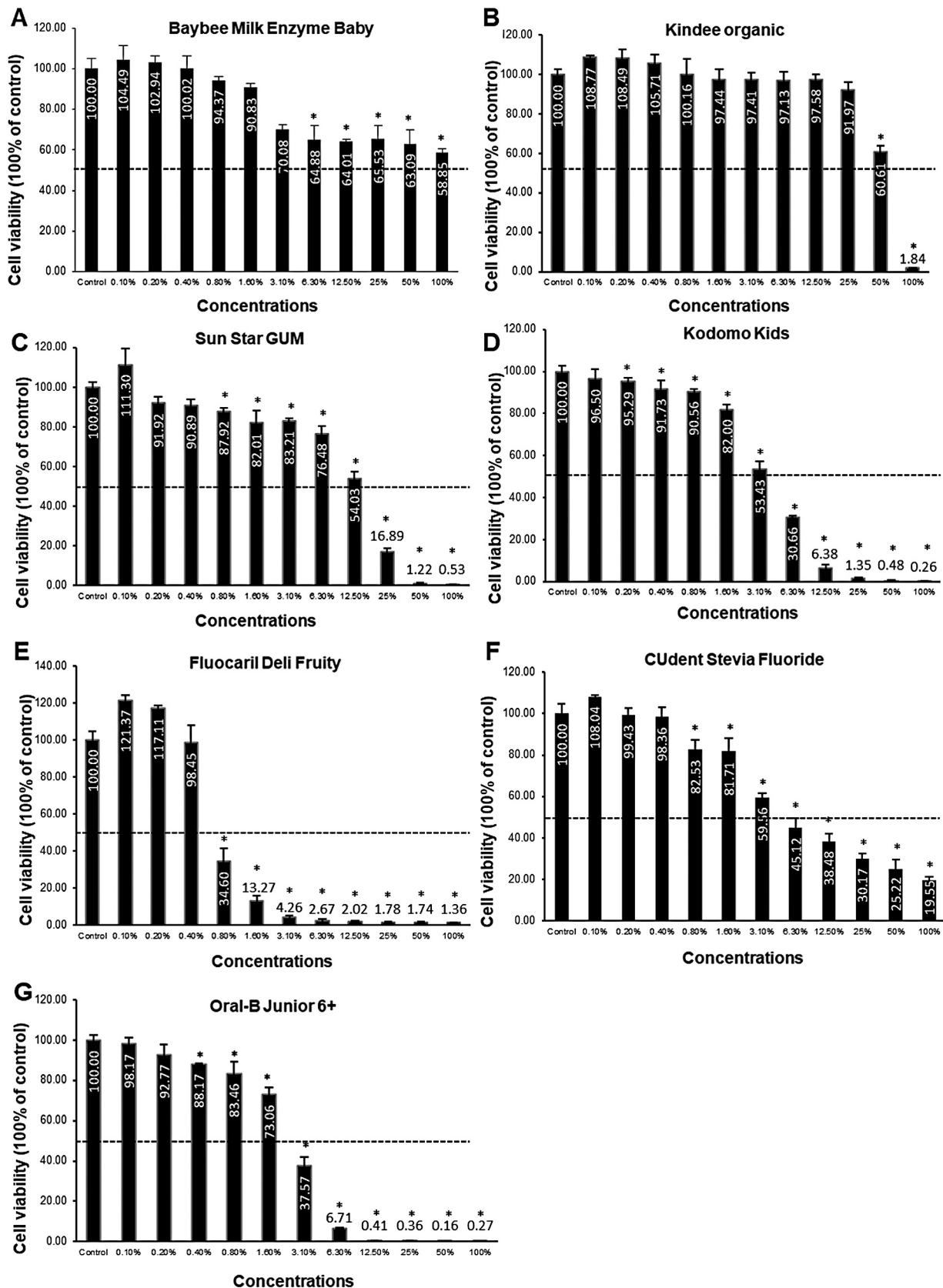


Fig. 4 (A–G) Cell viability of L929 cells after a 2-minute exposure to different concentrations of children's toothpastes. (A) Baybee Milk Enzyme Baby. (B) Kindee organic. (C) SunStar GUM. (D) Kodomo Kids. (E) Fluocaril Deli Fruity. (F) CUDent Stevia Fluoride. (G) Oral-B Junior 6+. The dotted lines indicate the cell viability at 50% of control. The bars represent the standard deviation. The asterisks represent the statistically significant findings from the control ($p < 0.05$).

Table 4 Mean and standard deviation (SD) of cell viability of L929 cells after a 2-minute exposure to different concentrations of children's toothpastes

Toothpaste/ concentration (%)	Cell viability (100% of control)						
	Baybee Milk Enzyme Baby	Kindee Organic	SunStar GUM	Kodomo Kids	Fluocaril Deli Fruity	CUdent Stevia Fluoride	Oral-B Junior 6+
Control	100.00 ± 4.89	100.00 ± 2.61	100.00 ± 2.61	100.00 ± 2.61	100.00 ± 4.89	100.00 ± 4.89	100.00 ± 2.61
0.1	104.49 ± 6.70	108.77 ± 0.87	111.30 ± 8.35	96.50 ± 4.65	121.37 ± 2.64	108.04 ± 0.93	98.17 ± 2.87
0.2	102.94 ± 3.51	108.49 ± 4.04	91.92 ± 3.04	95.29 ± 1.53 ^a	117.11 ± 1.71	99.43 ± 3.26	92.77 ± 4.96
0.4	100.02 ± 6.21	105.71 ± 4.49	90.89 ± 2.97	91.73 ± 3.98 ^a	98.45 ± 9.61	98.36 ± 4.68	88.17 ± 0.37 ^a
0.8	94.37 ± 1.99	100.16 ± 7.93	87.92 ± 1.78 ^a	90.56 ± 1.00 ^a	34.60 ± 6.86 ^a	82.53 ± 4.57 ^a	83.46 ± 5.67 ^a
1.6	90.83 ± 1.82	97.44 ± 5.34	82.01 ± 6.05 ^a	82.00 ± 2.26 ^a	13.27 ± 2.71 ^a	81.71 ± 6.51 ^a	73.06 ± 3.51 ^a
3.1	70.08 ± 2.17	97.41 ± 3.35	83.21 ± 1.26 ^a	53.43 ± 4.00 ^a	4.26 ± 1.06 ^a	59.56 ± 1.82 ^a	37.57 ± 4.37 ^a
6.3	64.88 ± 7.27 ^a	97.13 ± 4.23	76.48 ± 4.04 ^a	30.66 ± 0.62 ^a	2.67 ± 0.90 ^a	45.12 ± 4.38 ^a	6.71 ± 0.43 ^a
12.5	64.01 ± 1.13 ^a	97.58 ± 2.30	54.03 ± 3.18 ^a	6.38 ± 1.81 ^a	2.02 ± 0.29 ^a	38.48 ± 3.64 ^a	0.41 ± 0.02 ^a
25	65.53 ± 6.30 ^a	91.97 ± 4.03	16.89 ± 1.76 ^a	1.35 ± 0.43 ^a	1.78 ± 0.40 ^a	30.17 ± 2.24 ^a	0.36 ± 0.06 ^a
50	63.09 ± 6.95 ^a	60.61 ± 3.27 ^a	1.22 ± 0.10 ^a	0.48 ± 0.03 ^a	1.74 ± 0.38 ^a	25.22 ± 4.49 ^a	0.16 ± 0.15 ^a
100	58.85 ± 1.78 ^a	1.84 ± 0.14 ^a	0.53 ± 0.02 ^a	0.26 ± 0.06 ^a	1.36 ± 0.16 ^a	19.55 ± 1.57 ^a	0.27 ± 0.04 ^a

^aStatistically significant findings from the control ($p < 0.05$).

commercial toothpaste for children's dental care. The toothpaste compound was expected to inhibit the growth of *S. mutans*. Fluoride is known to hinder demineralization and promote remineralization.¹⁴ However, the antibacterial activity of fluoride in toothpaste is not clear. From our result, the children's toothpaste containing exact fluoride less than 500 ppm (Baybee Milk Enzyme Baby and Kindee Organic) did not exhibit an inhibitory effect on the growth of *S. mutans*. For toothpaste containing greater than 500 ppm fluoride, there was no relation between fluoride concentration and inhibitory effect, as seen in the toothpaste with the highest fluoride concentration (Oral-B Junior 6+), which did not display the highest inhibitory effect. Thus, other ingredients in the toothpaste formula could also play a role in inhibiting *S. mutans*.

Fluoride was explained in a mode of antimicrobial action that when the pH of the extracellular environment was decreased, H^+ caught with F^- easily. Hydrogen fluoride (HF) diffuses into bacterial cells and dissociates to the proton (H^+) and fluoride ion (F^-) in the cytoplasm.¹⁵ Intracellular F^- and H^+ can directly or indirectly affect enzymatic activities and physiological processes in the cell, leading to lower acid production, acid tolerance, and adherence of *S. mutans* to tooth surfaces. F^- inhibited enolase, which is involved in glycolysis. Enolase also indirectly inhibits the cytoplasm caused by the accumulation of H^+ . Moreover, enolase contains the ability to catalyze the production of phosphoenolpyruvate (PEP) for glucose uptake through the PEP-dependent phosphotransferase system (PTS). Therefore, inhibition of enolase activity by F^- also has a negative effect on glucose uptake.¹⁶ However, the previous report revealed that fluoride could bring about antibacterial effects when a high concentration was used.¹⁷ Besides, a study by Randall et al found that sodium fluoride did not show any growth inhibition against *S. mutans* at fluoride standard concentrations

(2,000, 4,000, 8,000, and 10,000 ppm).¹⁸ Thus, it was assumed that the inhibitory effects of *S. mutans* may not relate to the availability of fluoride in these toothpastes.

Toothpastes commonly contain several ingredients, including abrasives, binders, preservatives, flavors, and color agents. All experimental toothpaste formulations included these components.¹⁹ Abrasives, such as silica, essential for grinding and polishing, were found in all toothpastes except Kindee Organic Toothpaste. Binders, like sodium carboxymethylcellulose, xanthan gum, cellulose gum, and carrageenan, used to control viscosity, were a consistent feature across all toothpaste types. Flavors and color agents, contributing to an attractive taste and appearance, were found in all toothpastes, enhancing the sensory aspect.

Furthermore, preservatives such as sodium benzoate, potassium sorbate, and methylparaben, commonly employed in pharmaceutical products for their mold-controlling, yeast-inhibiting, and bacterial protection properties, were consistently found across all toothpaste formulations.²⁰ In our study, these preservatives were present in both toothpastes with inhibition zones and those without any inhibition zones. This indicates that the observed inhibition zones cannot be solely attributed to the effects of these preservatives. Therefore, it is apparent that other contributing factors within the toothpaste formulations play a significant role in the presence or absence of antibacterial effect.

In this study, the bacterial inhibitory effect was observed in toothpastes containing SLS. SLS was known to reduce lactate production, glucosyltransferase activity, and extracellular polysaccharide formation by *S. mutans*.²¹ Besides that, it retarded protein adsorption in the tooth structure to hinder protein pellicle formation, which is the initial stage of biofilm formation.²² Every toothpaste containing SLS demonstrated an inhibitory effect on *S. mutans*. The exception to

this was CUdent Stevia Fluoride, which lacks SLS and still displayed an inhibitory effect, even though it had a milder impact compared to toothpaste products containing SLS. Although the absolute concentration of SLS was not known, SLS is incorporated mainly as detergent/foaming agents at a concentration of 0.5 to 2% (5,000–20,000 ppm)²³; this concentration exceeded the growth inhibition dose studied in previous reports,²⁴ and this effect of SLS may explain the antibacterial activity in these children's toothpaste.

Apart from SLS, a toothpaste that exhibited *S. mutans* inhibitory effect is CUdent Stevia Fluoride. The attractive ingredient of this toothpaste is stevia extract (rebaudioside A). Stevia was reported to inhibit *S. mutans*' total growth and biofilm formation, especially when stevia was extracted in alcoholic extracts compared with aqueous extract.^{20,25} Although the extraction method of stevia in CUdent Stevia Fluoride is not known, it can be considered that stevia is an important ingredient in exhibiting the bacterial inhibitory effect. The limitation of this study is that the exact amount of each ingredient was not revealed. Thus, it is difficult to derive at a definite result of the active ingredients of the toothpaste.

Nowadays, organic products have come to signify improved health benefits. Thus, organic toothpaste became more popular as a product of choice for parents. However, there was no investigation into the effect of children's toothpaste available in Thailand on the vitality of fibroblast cells. In this study, L929 mouse fibroblast was used, as it is routinely used for the testing of the cytotoxic properties of dental materials due to their reproducible growth rates and biological responses.^{26,27} From the result of this study, L929 was exposed for 2 minutes to various concentrations diluted from the original toothpaste preparation at 100, 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8, 0.4, 0.2, and 0.1%. It revealed that the concentration of toothpaste that decreased cell viability to less than 50% of the control was 100, 25, and 0.8% for Kindee Organic Toothpaste, SunStar GUM Toothpaste, and Fluocaril Deli Fruity Toothpaste, respectively. Kodomo Kids Toothpaste Super Guard, CUdent Stevia Fluoride (Melon Flavor), and Oral-B Junior 6+ Years exhibited vitality less than 50% of control at 6.30% concentration, while Baby Milk Enzyme Baby Toothpaste Organics showed no vitality reduction of less than 50% at any level of concentration. Although organic toothpaste such as Kindee Organic Toothpaste and Baby Milk Enzyme Baby Toothpaste Organics had lower toxicity on fibroblast cells, the result cannot be interpreted clinically. In the clinical situation, salivary flow, protective effects of the tissue barrier, and the immunological aspect must be considered to play a role in the safety of toothpastes. Therefore, they may be suitable for children who are allergic to some active ingredients in ordinary toothpastes. Nevertheless, it is still necessary to strictly control the amount of one-time use by parents and split out the remaining toothpaste as much as possible to avoid adverse effects of toothpaste use.

Conclusions

The examined children's toothpastes can inhibit the growth of *S. mutans*, which did not correlate with fluoride concentration.

The antibacterial effects could be the results of other ingredients, for example, SLS. Moreover, the evaluation of *in vitro* cytotoxicity revealed that organic toothpaste displayed reduced cytotoxicity. However, it should be in caution when interpreting these results, as the experimental conditions may not readily infer the clinical scenario. Additional factors must be taken into consideration for a comprehensive assessment.

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Conflict of Interest

Thanaphum Osathanon is on the board of directors of C.U. Dent Product Co., Ltd. The rest of the authors have no conflict of interest to declare.

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