Original Research Article

EVIDENCE ON ANTIMICROBIAL EFFICACY OF COMMERCIAL TOOTHPASTE

ABSTRACT

This study aims to determine the antimicrobial activity of different brand of commercially available toothpaste in Malaysian outlet, namely Colgate-Total, Mu'min, Tesco and Safi. All the different toothpastes brand was tested for their antimicrobial activity against five oral pathogens namely *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumonia* and *Escherichia coli* by using agar well diffusion method. Colgate-Total brand has the highest mean zone of inhibition (Z.O.I) on the test organisms (30.7 mm) followed by Safi brand (29.2 mm) and Tesco brand (12.5 mm) while Mu'min brand showed the least activity on the test micro-organisms (2.4 mm). The present work has found to be that Colgate-total brand toothpaste was more effective in controlling pathogenic oral microflora as compared to other brand of toothpaste.

Keywords: Colgate-total, Mu'min, Tesco, Safi, antimicrobial, oral pathogens, zone of inhibition.

1. Introduction

Oral diseases remain as a major health problem worldwide [1]. Oral health survey of adults conducted by Ministry of Health Malaysia, Malaysia indicated that the number of periodontal disease is increasing continuously from 1990 to 2010 [2]. There is high correlation between oral disease and growth of microorganism [3]. The microbial infection may cause destruction of dental tissue leading to periodontal disease [4]. Periodontal diseases which is caused by plaque formation is defined as bacterial infections that cause disturbance of the supporting structure of the teeth such as gingival, cementum, periodontal membrane and alveolar

bone [3]. Plaque formation results from the accumulation of dietary carbohydrates on the surface of teeth that interact with bacteria. Bacterial metabolic products formed in plaque constantly react with salivary constituents on tooth surface, thus lead to dental caries [5].

There are numbers of microorganism which are associated with formation of dental carries such as *Staphylococcus aureus, Proteus mirabilis, Salmonella typhi, Klebsiella pneumonia and Escherichia coli*. Amongst them; *E. coli* is the most common microorganism responsible for the formation of dental caries due to oral cavity [4].

Recently, huge numbers of the oral hygiene products were introduced to public that claimed to provide maximum protection against periodontal disease. Basically, the efficacy of each toothpaste lies on it capability to retard growth of pathogenic oral microflora. Many oral toothpaste companies also highlighted that addition of chemical agents with antiplaque or antimicrobial activity into dental products served as a potential prophylactic method of reducing plaque mediated disease. This present investigation aimed to investigate antimicrobial efficacy of different commercial toothpastes by using a standard procedure. It is noteworthy that, the present result obtained from this research will provide useful evidence to the public to support the benefit of daily usage of current toothpaste to prevent oral disease.

2. Materials and Methods

2.1 Bacterial strains and growth media

The bacteria used in this study were wild type isolates of *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumonia* and *Escherichia coli* which were donated by Mr. Dhana Raj from Asian Institute of Medical Sciences and Technology University (AIMST), Sungai Petani, Malaysia. Microorganisms were maintained using Mueller Hinton Agar (Oxoid,

UK)and were sterilized at 121°C and 15 psi for 20 minutes before use. Suspension of each microbial isolates (*S. aureus*, *P. mirabilis*, *S. typhi*, *K. pneumonia* and *E. coli* were prepared accordingly using 0.5 McFarland standards (approx., 10^8 cfu/mL) for further use in antibacterial assay.

2.2 Preparation of toothpaste

Toothpaste used in this study were Mu'min premium (Al-Meswak Mu'min, Malaysia), Tesco Pro-tech (Tesco, Malaysia), Safi complete care (Wipro Unza, Malaysia) and Colgate-Total (Colgate Palmolive, Malaysia). All toothpastes were prepared as 2:5 and 4:5 stock concentration by mixing 2 g or 4 g of each various toothpastes in 5 ml of sterile distilled water. As for 100% concentration, toothpastes were directly inserted in the media wells without any dilution.

2.3 Antibacterial assay

Microorganisms were exposed to the different toothpaste concentration (2:5 and 4:5) using an agar based assay [6, 7]. 100 µl of each isolates were spread uniformly over Mueller Hinton Agar (MHA) medium by using a cotton swab. Five 5mm wells was formed on the agar plates using a sterile cork borer and aliquots 100 µL of each toothpaste at different concentration were deposited into the well (Figure 1). The well at the center was placed with tap water which acts as a control. Each experiment for different toothpastes was done in triplicate. Inhibition activities against microbial strains were determined by measuring the zones of inhibition formed around the well in millimeter (mm) after 24 h of incubation at 37°C.

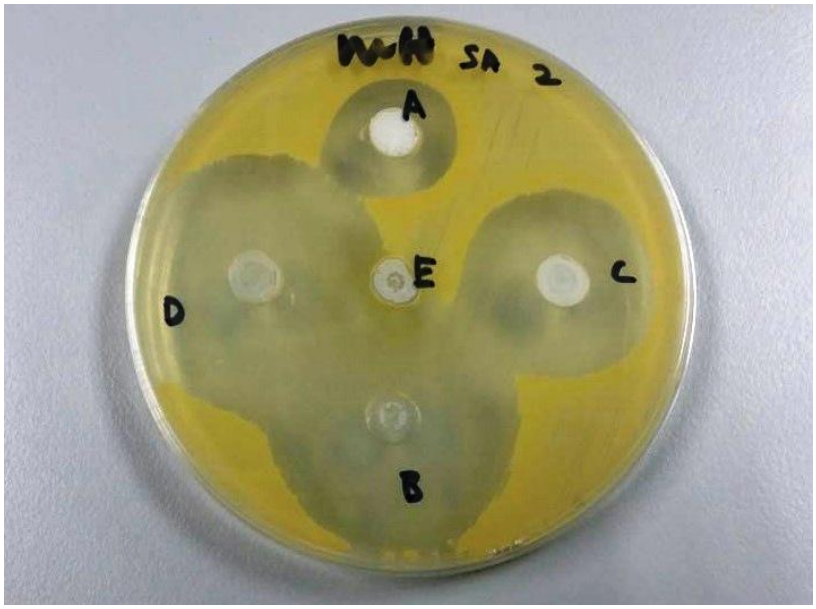

Figure 1. Bacterial isolates on MHA agar exposed to different toothpaste of same concentrations

2.4 Statistical Analysis

Statistical analyses were performed using GraphPadPrism 5 (GraphPad Software Inc., San Diego, CA, EUA) by applying one way ANOVA to assess the significance of change between experimental groups and control (tap water). The data were expressed as mean ± Standard Deviation (SD) and p-value <0.05 was considered as statistically significance.

3. Results

The composition on the label of the different toothpaste brands used in this study is shown in Table 1. All the toothpaste brands contain the same ingredients which were sodium lauryl sulfate, sorbitol and flavour. Both Colgate-total and Safi brand contain triclosan.

Table 1. Composition of toothpaste as per stated in the packaging.

Toothpaste	Composition
Colgate-Total	Sodium fluoride, water, triclosan, sorbitol, hydrated silica, sodium lauryl sulfate, flavor, carrageenan, sodium hydroxide, sodium fluoride, sodium saccharin, triclosan
Safi	Dicalcium phosphate dihydrate, water, sorbitol, glycerin, sodium lauryl sulfate, flavour, sodium fluoride, xanthan gum, sodium saccharin, triclosan, tetrasodium pyrophosphate, hydroxyethylcellulose, calcium lactate, piper betle leaf extract, salvadora persica (sugi) bark/root extract.
Tesco	Aqua, sorbitol, hydrated silica, glycerin, sodium lauryl sulfate, aroma, cellulose gum, sodium bicarbonate, zinc citrate, sodium fluoride, sodium saccharin, allantoin, hydroxyethylcellulose, limonene, sodium fluoride.
Mu'min	Calcium carbonate, water, hydrated silica, sorbitol, glycerin, sodium lauryl sulfate, flavor, dicalcium phosphate dihydrate, cellulose gum, mentha piperita (peppermint) leaf extract, calcium phosphate, sodium saccharin, sodium

	benzoate, xylitol.

Table 2 shows the inhibition zone (mm) of the various toothpastes used against the test micro-organisms. The result revealed that Colgate-Total brand has the highest mean zone of inhibition (Z.O.I) on the test organisms (30.7 mm) followed by Safi brand (29.2 mm) and Tesco brand (12.5 mm), while Mu'min brand showed the least activity on the test micro-organisms (2.4 mm). Tap water does not showed any antibacterial against any of the tested microbial species (Table 2).

Table 2. Zone of inhibition (mm) of different toothpastes brand.

Toothpaste brands	Test organism	Z.O.I at 2:5 concentration (mm)	Z.O.I at 4:5 concentration (mm)	Z.O.I at 100% concentration (mm)	Average Z.O.I (mm)
Mu'min	*S. aureus*	17.0 ± 1.4	18.0 ± 1.3	19.0 ± 1.2	12.0
	P. mirabilis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
	K. pneumoniae	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
	S. typhi	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
	E. coli	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
				Mean	**2.4**
Safi	*S. aureus*	42.0 ± 1.4*	40.0 ± 0.0*	44.0 ± 0.0*	42.0
	P. mirabilis	21.0 ± 1.6	31.0 ± 2.1*	30.0 ± 2.2*	27.3
	K. pneumoniae	21.0 ± 1.7	25.0 ± 2.3	28.0 ± 1.8*	24.7
	S. typhi	25.0 ± 1.1	15.0 ± 1.6	31.0 ± 2.0	23.7
	E. coli	27.0 ± 1.2	27.0 ± 1.4	31.0 ± 2.4	28.3
				Mean	**29.2**
Tesco	*S. aureus*	29.0 ± 1.6*	27.0 ± 1.4*	20.0 ± 1.3	25.3
	P. mirabilis	15.0 ± 1.2	16.0 ± 1.3	16.0 ± 1.2	15.7
	K. pneumoniae	11.0 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	3.7
	S. typhi	15.0 ± 1.2	14.0 ± 1.3	0.0 ± 0.0	9.7
	E. coli	13.0 ± 1.3	11.0 ± 0.6	0.0 ± 0.0	8.0
				Mean	**12.5**
Colgate-Total	*S. aureus*	40.0 ± 2.1*	42.0 ± 2.4*	44.0 ± 2.0*	42.0
	P. mirabilis	27.0 ± 1.6	28.0 ± 1.4	30.0 ± 2.1	28.3
	K. pneumoniae	24.0 ± 2.0	25.0 ± 1.2	29.0 ±0.0	26.0
	S. typhi	30.0 ± 1.9*	29.0 ± 1.4*	31.0 ± 1.8*	30.0
	E. coli	27.0 ± 1.7*	26.0 ± 1.6	29.0 ± 1.5*	27.3
				Mean	**30.7**
Tap water	*S. aureus*			0.0 ± 0.0	0.0
	P. mirabilis			0.0 ± 0.0	0.0
	K. pneumoniae			0.0 ± 0.0	0.0
	S. typhi			0.0 ± 0.0	0.0
	E. coli			0.0 ± 0.0	0.0

Z.O.I; zone of inhibition; n=3. *p<0.05

Figure 2 depicts that Mu'min brand toothpaste showed antibacterial activity against *S. aureus* in dose dependent manner. Mu'min brand toothpaste does not possess any antibacterial against other microorganisms tested. Figure 3 showed that Colgate-total brand possessed an antibacterial activity against all the tested microbial strains in dose dependent manner. The most

prominent activity of Colgate-total brand was against *S. aureus.* Tesco brand toothpaste brand showed antimicrobial effect against some of the tested microorganisms. As depicted in Figure 4, Tesco brand toothpaste brand showed statistically significant antimicrobial against *S. aureus* at 2:5 (Z.O.I of 29 mm). However, the antibacterial activity was found to be reduced with the increase in toothpaste concentration (4:5; Z.O.I: 27 mm; 100%; Z.O.I: 20 mm) (Table 2). Figure 3 also indicated that Tesco brand toothpaste showed the highest Z.O.I at 2:5 as compared to 4.5 mg/ml and 100% against the entire microorganisms tested except *P. mirabilis*. Tesco brand toothpaste showed maximum zone of inhibition against *P. mirabilis* at 4:5 (16 mm) as compared to 2:5 (15 mm). However, there is no increase in antimicrobial effect (Z.O.I; 16 mm) of Tesco brand toothpaste against *P. mirabilis* with the increase in concentration (100%). Safi brand toothpaste also possessed promising antimicrobial activity against the entire tested microorganism especially *S. aureus* with average zone of inhibition of 29.2 mm (Figure 5).

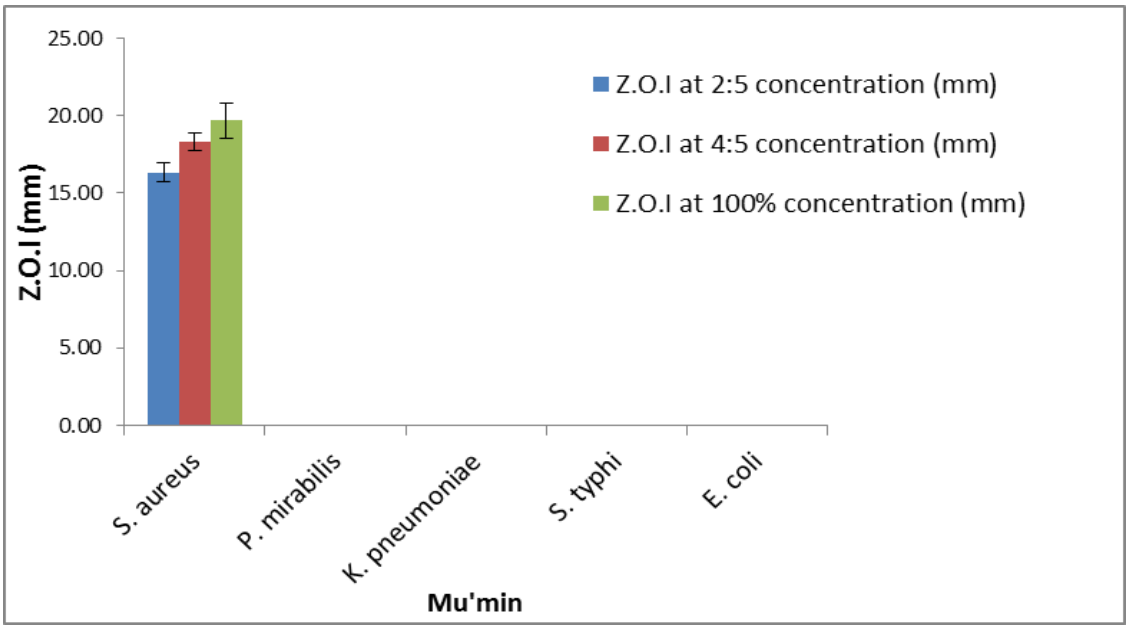

Figure 2. Zone of inhibition of Mu'min brand toothpaste against microorganisms.

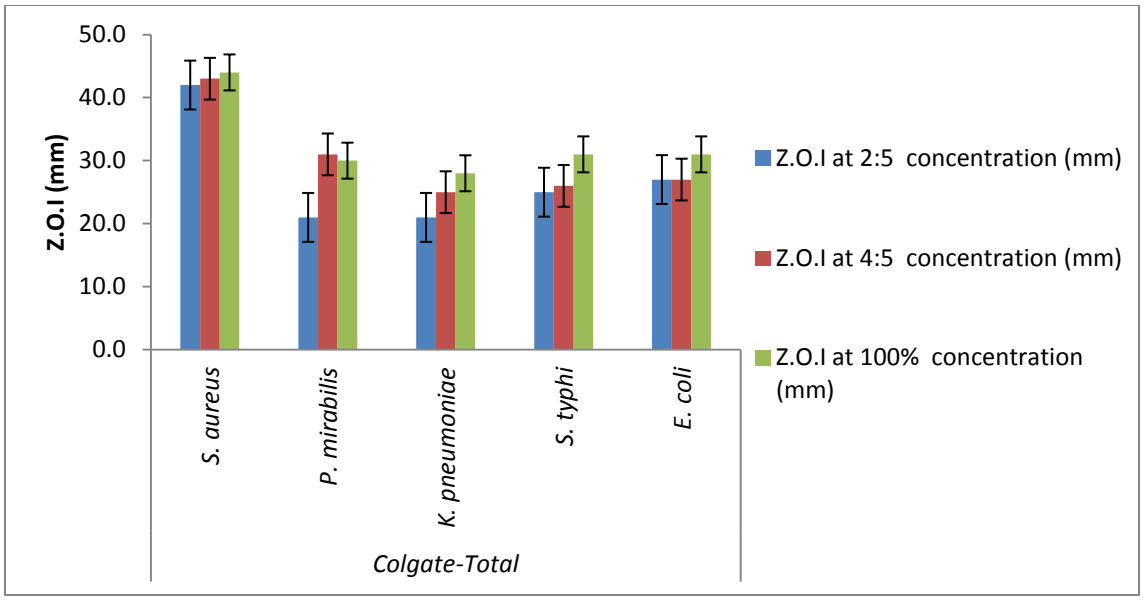

Figure 3. Zone of inhibition of Colgate-Total brand toothpaste against tested microorganisms.

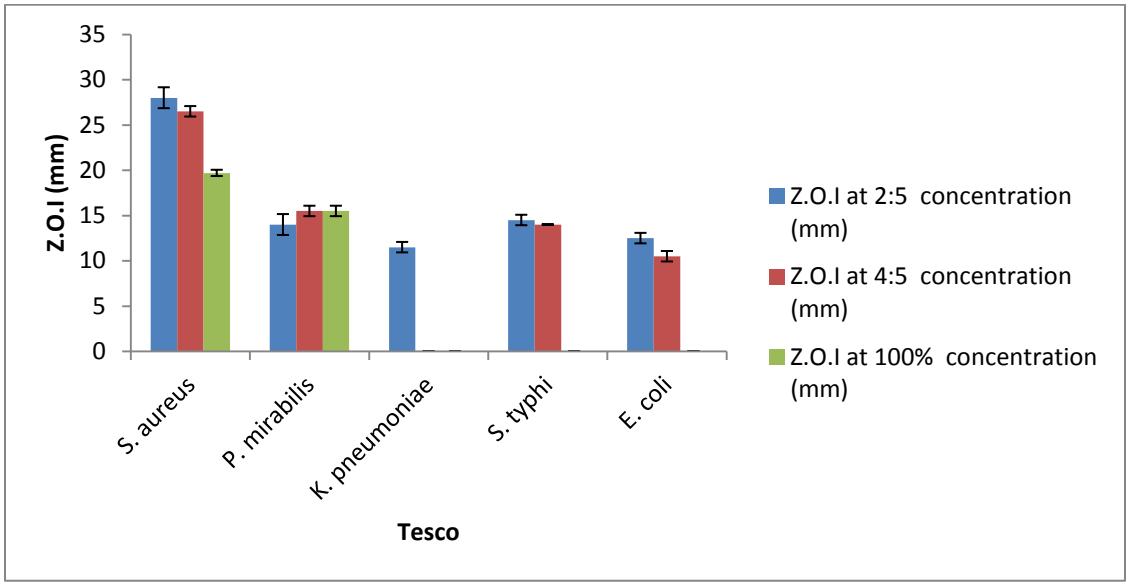

Figure 4. Zone of inhibition of Tesco brand toothpaste against tested microorganisms.

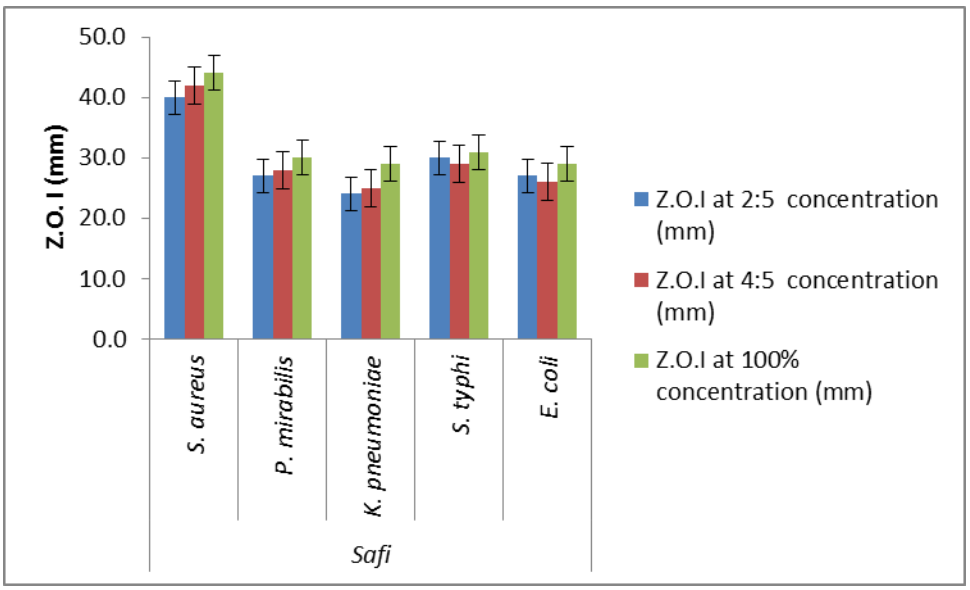

Figure 5. Zone of inhibition of Safi brand toothpaste against tested microorganisms.

4. Discussion

The main step towards a healthy and good looking tooth is maintenance of good oral hygiene. A good oral hygiene will prevent growth of microbial species that can eventually cause mouth odor and dental plaque [4]. Hence, there is a massive need to minimize the growth of microorganism by using antimicrobial agent [8]. In current scenario, there is huge number of companies promoting their toothpaste by claiming the presence of special active ingredient which could protect the teeth against decay. The active ingredients in oral products act by different mechanism to show their bactericidal effect. It may act via disruption of microorganism's cell wall, inhibition of microbial enzyme activity or by slowing multiplication process of microbial species [9]. The well diffusion antimicrobial assay served as an established method to determine antimicrobial activity of herbs, commercial oral product and cosmetic product [10].

As showed in Figure 3, Colgate-Total brand toothpaste showed highest inhibition zone in average (30.7 mm) as compared to other toothpaste brand against all the tested microbial with most promising activity against *S. aureus.* The presence of fluoride as the active ingredient in this toothpaste might reduce the number of streptococcal colony forming unit that is responsible for dental plaque formation [11]. Safi brand toothpaste also possessed promising antimicrobial activity against the entire tested microorganism with average zone of inhibition of 29.2 mm. The presence of triclosan and sodium fluoride in the Colgate-total brand and Safi toothpaste might contribute to the significant antimicrobial activity of Colgate-total and Safi brand toothpaste (Table 1). It was reported that triclosan and sodium fluoride could reduce 20% of oral bacteria formation [12]. In addition, triclosan has been used over 30 years in oral hygiene product industry for toothpaste and mouth-rinses formulation due to its antibacterial and antifungal effect [13]. In the present study, it was revealed that the presence of piper betle leaf extract and *Salvadora persica* (sugi) bark/root extract might promote the antibacterial effect of Safi brand toothpaste, but it is not equally effective as Colgate-Total brand formulation. However, Safi brand toothpaste showed better antimicrobial effect as compared to Mu'min and Tesco brand toothpaste. This was in accordance with the review data by Moran et al that indicated the efficacy of herbal toothpaste in reducing plaque formation [14].

Figure 4 indicated that 2:5 mg/mL concentration of Tesco brand toothpaste showed the highest Z.O.I at 2:5 as compared to 4.5 mg/ml and 100% against the entire microorganisms tested except *P. mirabilis*. This result indicated that the antimicrobial activity of this toothpaste against all the tested microorganisms except *P. mirabilis* is at maximum in diluted form (2:5).

As shown in Table 2, Mu'min brand toothpaste showed the least inhibitory effect (mean Z.O.I; 2.4) against all the tested microorganisms as compared to other brand toothpaste. Mu'min

brand toothpaste showed dose dependent zone of inhibition (2:5; 17 mm; 4:5; 18 mm and 100%; 19 mm). There was no significant zone of inhibition was obtained for Mu'min brand toothpaste against *P. mirabilis, K. pneumoniae, S. typhi* and *E. coli*.

5. Conclusion

The present work has shown that Colgate-total brand toothpaste was more effective in controlling oral microflora as compared to other brand of toothpaste. This result will provide useful evidence to the public to support the benefit of daily usage of current toothpaste to prevent oral disease.

Conflict of interest statement

We declare that we have no conflict of interest.

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