

Antimicrobial Efficacy of Commercially Available Low-fluoride and Fluoride-free Dentifrices for Children

Divya Reddy¹, Arul Selvan², Santhosh T Paul³, Umme Azher⁴

ABSTRACT

Aim and objective: The objective of this study was to evaluate and compare the antimicrobial efficacy of low-fluoride and fluoride-free dentifrices against *Streptococcus mutans*.

Materials and methods: The antimicrobial efficacy of four commercially available low-fluoride child formula dentifrices and four fluoride-free dentifrices against *S. mutans* was determined using the agar diffusion test. Fifty microliters of various dilutions (1:1, 1:2, 1:4) of each dentifrice were inoculated on the assigned plates under aseptic conditions. Saline was taken as negative control and 0.2% chlorhexidine was considered as a positive control. The plates were incubated at 37°C for 24 hours and the zone of inhibition around the wells was measured.

Results: All the tested low-fluoride dentifrices showed varying degrees of antimicrobial activity against *S. mutans* with F2 (Pediflor®) and F4 (Cheerio™) showing greater zones of inhibition when compared to F1 (Colgate®kids) and F3 (Kidodent). When the mean zones of inhibition produced by non-fluoridated dentifrices were compared with that of fluoridated dentifrices, no statistically significant difference was noted between NF1, NF3, NF4, and F2, F4. The antibacterial activity of F1 and F3 was significantly lower when compared to others. However, no antibacterial activity was noted with NF2.

Conclusion: Both low-fluoride and fluoride-free formulations tested in the study exhibited antimicrobial activity against *S. mutans*. In very young children where the risk of fluorosis is of concern, fluoride-free formulations can be considered as safe alternatives to fluoride formulations.

Clinical significance: Several dentifrices, both fluoride-free and low-fluoride formulations, are being aggressively marketed for young children. Though these toothpastes are being very commonly used by young parents for their infants and toddlers, there is very little published literature available on their antimicrobial activity and this study focuses on addressing this.

Keywords: Antimicrobial efficacy, Child formula dentifrice, *Streptococcus mutans*.

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INTRODUCTION

Dental caries is the most common oral disease characterized by demineralization of inorganic portions and destruction of the organic substance of the tooth. Although the etiology of dental caries is multifactorial in nature, high counts of cariogenic bacteria form one of the principal factors in the initiation and progression of the disease. Of the species implicated in dental caries, a large body of epidemiologic evidence links *Streptococcus mutans* to the initiation of dental caries.¹

Various preventive protocols are practiced today for the prevention of dental caries. The effectiveness of fluoridated dentifrices in the prevention of dental caries and reversal of enamel demineralization is well documented. Holt and Murray² identified over a hundred clinical trials in their review which showed that brushing with fluoridated dentifrices reduced the incidence of dental caries significantly. Levy³ has stated that dentifrice is the most common source of fluoride for young children. However, young children may accidentally swallow enough amount of fluoridated dentifrice to produce levels of fluoride consumption associated with a risk of developing dental fluorosis.⁴ Previous studies have estimated that approximately 0.5 mg of fluoride may be ingested by children when a 1,000 ppm fluoride dentifrice is used twice daily which establishes the potential of fluoridated dentifrices to be a significant source of fluoride intake.⁵

Two approaches can be recommended to reduce the hazard of fluorosis from ingested dentifrices:⁴

- By reducing the amount of toothpaste dispensed on the toothbrush.

^{1,3,4}Department of Pediatric and Preventive Dentistry, Sri Rajiv Gandhi College of Dental Sciences and Hospital, Bengaluru, Karnataka, India

²Department of Microbiology, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, Karnataka, India

Corresponding Author: Divya Reddy, Department of Pediatric and Preventive Dentistry, Sri Rajiv Gandhi College of Dental Sciences and Hospital, Bengaluru, Karnataka, India, Phone: +91 9886651419, e-mail: divyacreddy@yahoo.com

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- By reducing the concentration of fluoride in the toothpastes used by children.

Based on these recommendations, several child formula dentifrices are available in the Indian market with low concentrations of fluoride ranging from 458 to 500 ppm. Even though the antimicrobial potential of high fluoride concentration dentifrices is well established, very few studies have been documented testing the antimicrobial potential of these low-fluoride child formula dentifrices.

Another alternative toothpaste in the market for use in infants and young children includes the ones which are fluoride-free. These fluoride-free dentifrices are popular among parents as

they are easily available in most baby stores and are attractively marketed as being fluoride-free focusing on the ill effects of fluoride when ingested in young children. Though these toothpastes are being very commonly used by young parents for their infants and toddlers, there is no published literature available on their antimicrobial activity. Therefore, the present study was carried out to evaluate and compare the antimicrobial efficacy of low-fluoride and fluoride-free dentifrices against *S. mutans*.

MATERIALS AND METHODS

The antimicrobial efficacy of four commercially available low-fluoride child formula dentifrices and four fluoride-free dentifrices against *S. mutans* was determined using the agar diffusion test.

Selection of Toothpastes

Before the start of the study, a small survey was conducted among parents, pharmacies, and popular baby stores in the area to find various popular or commonly preferred toothpaste brands in both low-fluoride and fluoride-free categories. Based on the findings, four low-fluoride and four fluoride-free dentifrices were selected for the study. The details of toothpastes and their compositions are listed in Tables 1A and 1B.

Dentifrice Slurry Preparation

Dilutions of selected dentifrices were prepared by mixing the measured amount of toothpaste (1 g) in the calculated volume of distilled water (1 mL) to give a 1:1 dilution (dentifrice:distilled water). Further dilutions of 1:2 and 1:4 were made using sterile distilled water.

Isolation of *S. mutans*

Specimens were obtained from the carious lesions of a patient, transferred to 1 mL of sterile thioglycollate broth, and was transported to the laboratory. The broth was incubated for 1 hour at 37°C and vortex mixed for 30 seconds to allow the dispersion of bacteria into the medium. One hundred microliters of this

broth were transferred to 1 mL of brain heart infusion (BHI) broth and vortexed for 30 seconds. Fifty microliters of diluted broth were transferred onto Mitis Salivarius Bacitracin agar and streaked for isolation. The plate was incubated at 37°C in a candle extinction jar for 24 hours. Mucoid colonies were identified as *S. mutans* using standard cultural, morphological, and biochemical characteristics.

Agar Well Diffusion Test

One colony of *S. mutans* was inoculated into 5 mL of BHI broth, incubated at 37°C for 4 hours. The growth was adjusted to McFarland 0.5 opacity standard to obtain a growth equivalent to 1.5×10^8 CFU/mL. Twenty-five microliters of the adjusted culture were inoculated into freshly prepared BHI agar (cooled to 50°C), mixed well, and poured into sterile Petri plates and allowed to set (Pour plate method).

Using sterile templates, five wells of 6 mm diameter were made in each of the agar plates. Fifty microliters of various dilutions (1:1, 1:2, 1:4) of each dentifrice were inoculated in three wells on the assigned plates under aseptic conditions. Saline was taken as negative control and 0.2% chlorhexidine was considered as a positive control.

The plates were then incubated at 37°C for 24 hours and the zone of inhibition around the wells was measured using Vernier calipers.

Statistical Analysis

The experiment was conducted in triplicates for each dentifrice for all the dilutions. Mean and standard deviation of the zones of inhibition of the three replicates were calculated and subjected to statistical analysis using Statistical Package for Social Sciences version 24 software (SPSS Inc, Chicago, IL, USA). A one-way ANOVA analysis method was carried out to identify statistical differences in inhibition zones. The Tukey HSD *post hoc* test was run for multiple comparisons. Statistically significant differences among the groups were set at $p < 0.05$.

Table 1A: Low-fluoride child formula dentifrices

| Code | Brand name | Composition |
|------|----------------|--|
| F1 | Colgate® kids | Sorbitol, silica, polyethylene glycol 600, sodium lauryl sulfate, sodium carboxymethyl cellulose, tetrasodium pyrophosphate, flavor, sodium saccharin, sodium fluoride (max 500 ppm), CI 17200, CI 16035, titanium dioxide-coated mica, d-limonene, in aqueous base |
| F2 | Pediflor® Kidz | Sorbitol, silica, xylitol 10%, glycerin, purified water, sodium lauryl sulfate, sodium carboxymethyl cellulose, sodium monofluorophosphate (458 ppm), saccharin sodium, sodium methyl hydroxybenzoate, flavors, mica particles, sodium propyl hydroxybenzoate, Ponceau 4R (coloring agent) |
| F3 | Kidodent | Sodium monofluorophosphate (500 ppm), xylitol, flavored gel base, Ponceau 4R (coloring agent) |
| F4 | Cheerio™ gel | Sodium monofluorophosphate (458 ppm), flavored gel base, coloring agent Ponceau 4R, titanium dioxide IP |

Table 1B: Fluoride-free dentifrices

| Code | Brand name | Composition |
|------|------------|--|
| NF1 | Chicco® | Hydrogenated starch hydrolysate, aqua, hydrated silica, xylitol, glycerin, cellulose gum, sodium lauryl sarcosinate, flavor, sucralose, calcium gluconate, CI 16035 |
| NF2 | Pigeon | Water, dicalcium phosphate dehydrate, maltitol, xylitol, glycerin, carrageenan, citric acid, sodium citrate, sodium benzoate, sodium methyl cocoyl taurate, benzyl alcohol, trimagnesium phosphate, flavor |
| NF3 | Mee mee® | Triple calcium phosphate, purified water, glycerin, hydrated silica, sorbitol (humectant), sodium saccharin, sodium lauryl sarcosinate, flavor, cellulose gum, tetrasodium diphosphate, sodium benzoate (preservative), xylitol, calcium lactate (anti tartare), calcium pyrophosphate |
| NF4 | Aquawhite™ | Sorbitol, silica, glycerin, sodium lauryl sulfate, polyethylene glycol, sodium benzoate, sodium saccharin, tetrasodium pyrophosphate, sodium carboxymethyl cellulose (binder/thickener), flavor, permitted color, aqua |

RESULTS

Comparison of Mean Zones of Inhibition (mm) of Different Low-fluoride Toothpastes in the Concentration of 1:1, 1:2, 1:4 (Fig. 1)

All the tested low-fluoride dentifrices showed varying degrees of antimicrobial activity against *S. mutans* with F2 (Pediflor®) and F4 (Cheerio™) showing greater zones of inhibition of 12.83 ± 1.04 and 12.33 ± 0.577 , respectively, when compared to F1 (Colgate® kids) (10.33 ± 0.577) and F3 (Kidodent) (10.16 ± 0.288). A statistically significant difference was noted when the mean zones of inhibitions produced by F2 and F4 were compared with that of F1 and F3. It was also observed that the zone of inhibition decreased with the increase in dilution.

Comparison of Mean Zones of Inhibition (mm) of Different Non-fluoridated Toothpastes in the Concentration of 1:1, 1:2, 1:4 (Fig. 2)

It was observed that NF1 (Chicco®) showed the highest zone of inhibition (14.16 ± 0.763 mm) when compared to other fluoride-free dentifrices followed by NF4 (Aquawhite™) and NF3 (Mee mee®) with the mean zone of inhibition of 12.83 ± 1.04 and 12.66 ± 0.28 mm, respectively. No statistically significant difference was noted among the three dentifrices. However, NF2 (Pigeon) did not show any zone of inhibition against *S. mutans* in all the dilutions tested. It was also observed that the zone of inhibition decreased with the increase in dilution.

Comparison of Antibacterial Efficacy of Different Non-fluoridated and Low-fluoride Dentifrices at 1:1 Dilution (Table 2)

When the mean zones of inhibition produced by non-fluoridated dentifrices were compared with that of the fluoridated dentifrices, no statistically significant difference was noted between NF1, NF3, NF4, and F2, F4. The antibacterial activity of F1 and F3 was significantly lower when compared to others. However, no antibacterial activity was noted with NF2.

DISCUSSION

The mechanical and chemical forms of oral hygiene aids are one of the common tools for the prevention of common dental

diseases. Dental marketing is widespread and many toothpastes are extensively marketed including the ones specially formulated for children. These include the toothpastes with low-fluoride concentrations and those which are free of fluoride. All of these toothpastes are marketed highlighting their benefits. Assessing the antimicrobial activity of various commercially available dentifrices marketed for young children was the primary focus of this study.

All the low-fluoride dentifrices tested in the study exhibited varying antimicrobial activity against *S. mutans*. This is in accordance with the findings of Malhotra et al.⁶ and Lodaya et al.⁷ who observed antimicrobial activity of low-fluoride dentifrices against *S. mutans*.

Among the different fluoride-containing dentifrices tested, greater antimicrobial activity was observed in formulations with 458 ppm of sodium monofluorophosphate (MFP) when compared to other fluoridated toothpastes containing 500 ppm of sodium fluoride (NaF) and sodium MFP. In sodium MFP, the fluoride ions are compactly arranged and need enzymatic hydrolysis for their

Table 2: Comparison of mean zone of inhibition of various non-fluoride and low-fluoride dentifrices at 1:1 dilution

| Product | Inhibition zone (fluoride-free) | Comparison (low-fluoride) | p value |
|---------|---------------------------------|------------------------------|---------|
| NF1 | 14.1667 ± 0.76376 | F1 (10.3333 ± 0.57735) | 0.000** |
| | | F2 (12.8333 ± 1.04083) | 0.207 |
| | | F3 (10.1667 ± 0.28868) | 0.000** |
| | | F4 (12.3333 ± 0.57735) | 0.055 |
| NF2 | 0.0 ± 0.0 | F1 (10.3333 ± 0.57735) | 0.000** |
| | | F2 (12.8333 ± 1.04083) | 0.000** |
| | | F3 (10.1667 ± 0.28868) | 0.000** |
| | | F4 (12.3333 ± 0.57735) | 0.000** |
| NF3 | 12.6667 ± 0.28868 | F1 (10.3333 ± 0.57735) | 0.007* |
| | | F2 (12.8333 ± 1.04083) | 0.997 |
| | | F3 (10.1667 ± 0.28868) | 0.004* |
| | | F4 (12.3333 ± 0.57735) | 0.961 |
| NF4 | 12.8333 ± 1.04083 | F1 (10.3333 ± 0.57735) | 0.016* |
| | | F2 (12.8333 ± 1.04083) | 1.000 |
| | | F3 (10.1667 ± 0.28868) | 0.011* |
| | | F4 (12.3333 ± 0.57735) | 0.924 |

*minor clinical significance

**low clinical significance

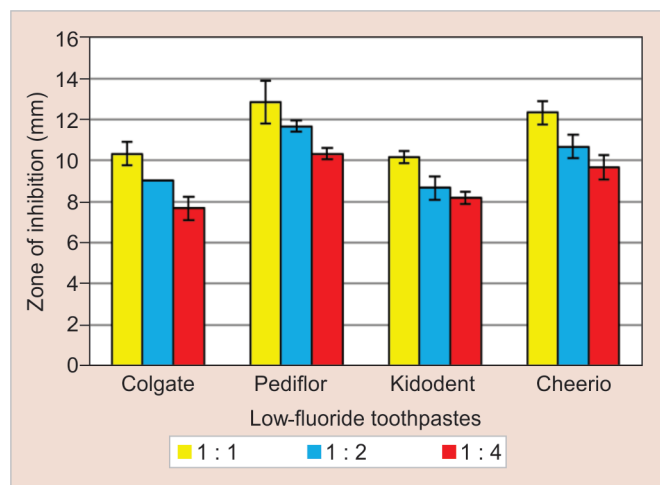


Fig. 1: Comparison of antimicrobial activity of low-fluoride dentifrices at different dilutions

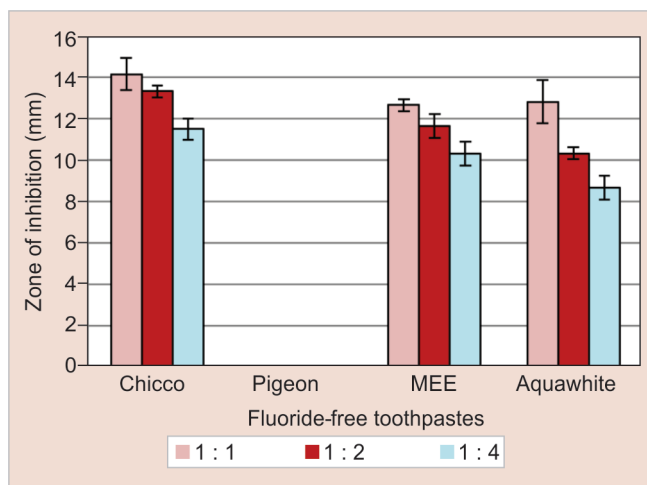


Fig. 2: Comparison of antimicrobial activity of fluoride-free dentifrices at different dilutions

release. Thus, a large number of free active fluoride is obtainable on the surface of the tooth. On the contrary, NaF combinations interact with the filler particles, and thereby, the availability of active fluoride is reduced.^{6,7} This could explain the effectiveness of sodium MFP observed in this study.

Pertaining to the observation of 458 ppm of MFP being more effective than 500 ppm, Malhotra et al.⁶ conducted a study where they compared different concentrations of MFP and found 0.38% showed a less effective response in inhibiting *S. mutans* when compared to 0.35%, which is similar to our findings. This could be due to the chemical interaction with the other ingredients that could render fluoride unavailable on the tooth surface. Therefore, it may be perceived that the uptake of free F ion for MFP relies on the interaction with other ingredients in the dentifrice and not on the entire concentration of fluoride present. Reed⁸ demonstrated that the clinical effectiveness of dentifrice was proportional to the total concentration of fluoride while using NaF. However, this proportionality is not the same for dentifrices containing MFP.⁹

All the fluoride-free dentifrices tested in this study except NF2 showed significant antimicrobial activity against *S. mutans*. This could be attributed to various ingredients present in these formulations such as sodium lauryl sulfate,¹⁰ xylitol,¹¹ sorbitol.¹² No clear explanation could be given for the absence of antimicrobial activity of NF2 except that it is fluoride-free, sodium lauryl sulfate-free, and could also be due to the inhibition of active compounds by other ingredients.

In the comparison of low-fluoride and fluoride-free toothpastes, similar antimicrobial activity was exhibited by both groups.

CONCLUSION

Both low-fluoride and fluoride-free formulations tested in the study exhibited antimicrobial activity against *S. mutans*. In very young children where the risk of fluorosis is of concern, fluoride-free formulations can be considered as safe alternatives to fluoride formulations. However, clinical studies especially those testing the remineralization potential of these extensively marketed and commercially available dentifrices are needed.

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