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Evaluation of Toothpaste Effectiveness in Reducing Oral Bacteria

Ebelechukwu Oluchukwu Nwakalor*, Gideon Chijioke Okpokwasili

Department of Microbiology, Faculty of Sciences, University of Port Harcourt, Choba, Nigeria

Email address

ebelenwakalor@gmail.com (E. O. Nwakalor), gidsilman@yahoo.com (G. C. Okpokwasili)

*Corresponding author

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Abstract

This *in vitro* study was undertaken to determine which toothpaste represses oral bacteria (*Streptococcus mutans* and *Lactobacillus* species) growth most effectively. The toothpaste samples used were nine-fluoride-containing toothpastes (A, B, C, D, F, G, H, I and J) and one non-fluoride containing toothpaste (E). Disc diffusion tests were performed to examine the mean inhibition zones for each toothpaste/bacteria sample using 0.5g/ml and 0.4g/ml concentrated toothpaste slurries. Toothpaste samples B and D showed the highest mean zone of inhibition on exposure to *S. mutans* and *Lactobacillus* sp. respectively while the least inhibition zone and no observable zones were exhibited by toothpaste sample I. The challenge test done to determine their behavioural profile at set time intervals of 0.5, 2, 5, 10 and 15 minutes revealed the dentifrices to be intensively bactericidal on *Streptococcus mutans* while a fluctuative antibacterial activity was obtained with respect to *Lactobacillus* species. The toothpastes were significantly different ($P < 0.05$) from each other showing that their effectiveness varies. These results show that the mere presence of any of the toothpastes is capable of repressing the growth of oral bacteria, which is also affected by the toothpaste's components and the components' concentration.

Keywords

Inhibition, Oral Bacteria, *Lactobacillus* Species, *Streptococcus Mutans*, Toothpastes

1. Introduction

Oral health has a strong correlation with the overall health of an individual. The oral cavity is the primary entry for dietary and respiratory elements [1]. Although dental plaque forms naturally on teeth, in the absence of adequate oral hygiene, it can accumulate beyond levels that are compatible with dental health and at a susceptible site, dental caries or periodontal diseases can occur [2]. Consequently, the incorporation of chemical agents with anti-plaque or antimicrobial activity into dental products has been proposed as a potential prophylactic method of reducing plaque-mediated disease [2-4].

It is common for toothpastes to contain different main ingredients and this leads to an inquiry concerning the efficacy of these various ingredients and the toothpaste as a whole [1]. Also, an effective oral antibacterial according to Otten *et al*., [5], should have a broad spectrum of antibacterial activity but at the same time it should not disturb the oral microbiome at health and should be compatible with other toothpaste ingredients.

An oral hygiene product should be tested against microorganisms related to dental caries and periodontal diseases [3]. Such microorganisms include *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus* and *Lactobacillus* species. However, to make the right choice from so many different products now available in the local market is a very daunting task, and it have been found that most of the people make choice in accordance to the most persuasive advertisement and the various claimed benefits provided by the product. This paper, presents an *in vitro* evaluation of various toothpaste brands available in the Nigerian market to assess their effectiveness in reducing oral

bacteria such as *Streptococcus mutans* and *Lactobacillus* species by exploring their behavioral profile within utilization period.

2. Materials and Methods

2.1. Source of Samples

2.1.1. Toothpastes

A total of ten toothpaste brands namely Aquafresh, Close Up (tingly red), Colgate (advanced whitening), Crest 5 complete, Dabur herbal, Florish gel, Holdent, Macleans, Pearl drops smokers' and Sensodyne designated as toothpaste sample A, B, C, D, E, F, G, H, I and J respectively (Table 1) which were a representation of different types of toothpastes in the Nigerian market were used. These were both local and foreign formulated and packaged brands that were obtained from supermarkets in Port Harcourt city.

Table 1. *Ingredients of the Various Toothpastes Tested for Antimicrobial Potential.*

Toothpaste	Toothpaste type on label	Ingredients as listed on packages
A	Anti-calculus & anti-plaque	Sodium fluoride, aqua, hydrated silica, sorbitol, glycerin, PEG-6, sodium lauryl sulfate, aroma, xanthan gum, titanium dioxide, carrageenan, sodium saccharin, limonene, Cl 73360, Cl 74160
B	Fluoride	Sodium fluoride (0.32%), triclosan (0.1%), sorbitol, hydrated silica, PEG-32, SLS, flavor, cellulose gum, sodium saccharin, trisodium phosphate, Cl 16035, Cl 17200
C	All in One	Aqua, hydrated silica, sorbitol, glycerin, PEG-6, sodium lauryl sulfate, tetrapotassium pyrophosphate, disodium pyrophosphate, tetrasodium pyrophosphate, aroma, carbomer, cellulose gum, limonene, sodium fluoride, sodium saccharin, triclosan, xanthan gum, Cl 74160, Cl 74260, Cl77891
D	Whitening	Sodium monofluorophosphate 1.1%, aqua, calcium carbonate, sorbitol, alumina, sodium silicate, carrageenan (chondrus crispus), sodium saccharin, methyl paraben
E	Herbal (non-fluoride)	Basil oil 0.01%, 5%herbal extract (obtained from bullet wood, acacia Arabica, lotur bark, pellitory root, bark of black berry), chalk (calcium carbaonate, SLS, blend of peppermint, spearmint, coriander, ginger, eucalyptus, lemon oil, sodium silicate, glycerin, purified water, gum carrageenan, chlorophyllin, sodium saccharin
F	Fluoride	0.76% sodium monofluorophosphate, sorbitol, water, silica, PEG, sodium lauryl sulphate, flavor, magnesium sulphate, cellulose gum, sodium saccharin, trisodium phosphate, methyl paraben, menthol, FD&C Red No. 40, D&C red No. 33
G	Calcium+Fluoride	Sodium monofluorophosphate, sorbitol, calcium carbonate, water, hydrated silica, sodium lauryl sulfate, cellulose gum, flavor, sodium saccharin, carrageenan
H	Fluoride	Sodium fluoride 0.306%w/w, aqua, hydrated silica, sorbitol, glycerin, PEG-6, sodium lauryl sulphate, flavor, titanium dioxide, xanthan gum, sodium saccharin
I	Smokers'	Sorbitol, aqua, hydrated silica, glycerin, tetrapotassium pyrophosphate, sodium lauryl sulfate, tetrasodium pyrophosphate, PEG-12, cellulose gum, sodium saccharin, sodium fluoride, Cl 42090, Cl 47005
J	Desensitivity	Aqua, hydrated silica, sorbitol, glycerin, pentasodium triphosphate, potassium nitrate, PEG-6, Aroma, titanium dioxide, cocamidopropyl betaine, sodium methyl cocoyl taurate, xanthan gum, sodium hydroxide, sodium fluoride, sodium saccharin

2.1.2. Microorganisms

The test organisms were isolated from the saliva samples which were collected in sterile bottles from ten healthy volunteers that were students of the University of Port Harcourt Rivers state and stored in the refrigerator until when needed.

The isolates were identified by observing their morphological characteristics and biochemical test results. The identified isolates were then subcultured onto nutrient agar plates and incubated for 24 hours at 37^{o}C. Colonies were picked and used to inoculate sterile nutrient broth medium. The cultures were grown overnight for 24 hours at 37^{o}C after which the bacterial suspension was standardized using the McFarland nephelometer. Standard inocula were used for the subsequent tests.

2.2. Determination of the Antimicrobial Activity of the Toothpaste Types Using Disc Diffusion Test

This was done using the Kirby-Bauer method [6]. The nutrient agar plates were inoculated using sterile swabs dipped in a suspension of the test organism whose turbidity has been standardized. Sterile discs (6mm in diameter) which were prepared by punching holes on individual filter paper (Whatman's No1) were placed one at a time on the centre of the inoculated plates. A 1g toothpaste per 2ml distilled water (0.5g/ml) and 2g toothpaste per 5ml distilled water (0.4gml) concentrated slurries were made and 0.04ml of it applied on the disc. The plates were incubated for 24h at 37^{o}C. The clear zones of inhibition that resulted were measured in millimeter. All plates were made in quadruplets and the experiment done twice. This was carried out on each toothpaste sample and analyzed using analysis of variance at 95% confidence level.

2.3. Determination of the Survival Ability of the Test Organisms in the Presence of the Toothpaste Types (Challenge Test)

This test was aimed at evaluating the product behaviour during the utilization period. One milliliter of 1g toothpaste per 2ml sterile distilled water concentrated slurry was added to 5ml sterile nutrient broth after which it was inoculated with 0.1ml of the standardized bacteria suspension. The culture was mixed thoroughly after which aliquots (0.1ml) from the culture was plated out at 0.5minutes, 2minutes,

5minutes, 10minutes and 15minutes intervals. The inoculated plates were incubated for 24hours at 37°C. This was done for each toothpaste sample and the test tube containing no toothpaste served as the negative control. The results were analyzed using linear regression method.

3. Theory

The prevalence of dental caries can be reduced if oral hygiene employed such as tooth brushing is complimented with toothpaste that has antimicrobial efficacy which can reduce the microbial count of the oral cavity when used on a daily basis [4, 7-8]. Over the years, toothpaste has evolved from a once simple form into many different types of toothpaste that are available in the market with the most popular type been those with whitening ability and/or those with herbal contents [9]. The question is now which one is the best to go for – Fluoride containing or Non-fluoride containing, and is capable of doing what toothpaste was originally designed for.

4. Results and Discussion

4.1. Results

4.1.1. Test Organisms

Based on the results obtained on the characterization index (Table 2) and with reference to *Bergey's Manual of Determinative Bacteriology* [10], the isolates were identified as belonging to the following genera: *Lactobacillus* species, *Actinomyces* species, *Staphylococcus* species, *Streptococcus mutans* and *Corynebacterium* species.

Table 2. *Morphological and Biochemical Properties of Bacteria Isolated.*

Isolate code	Cultural characteristics	Gram Reaction	Motility	Spore formation	Catalase	Haemolytic ability
S1	Gray smooth flat translucent	+ve rod	-	-	+	γ
S2	Gray rough entire opaque	+ve rod	-	-	-	α
S3	Yellow round convex translucent	+ve cocci	-	-	+	β
S4	White round convex opaque	+ve rod	-	-	+	α
S5	Cream round convex translucent	+ve cocci	-	-	-	α
S6	White round raised translucent	+ve cocci	-	-	-	α
S7	White round convex translucent	+ve cocci	-	-	-	α
S8	Cream raised undulated edge opaque	+ve rod	-	-	-	α

Table 2. *Continued.*

Isolate code	Cultural characteristics	Citrate	Urease	Indole	Methyl red	Voges proskeur	Maltose
S1	Gray smooth flat translucent	+	-	-	-	+	A
S2	Gray rough entire opaque	+	-	-	-	+	A
S3	Yellow round convex translucent	+	+	-	-	+	A
S4	White round convex opaque	+	-	-	+	-	A
S5	Cream round convex translucent	+	-	-	-	+	A
S6	White round raised translucent	+	-	-	-	+	A
S7	White round convex translucent	+	-	-	-	-	A
S8	Cream raised undulated edge opaque	+	-	-	-	+	A

Table 2. *Continued.*

Isolate code	Cultural characteristics	Sucrose	Glucose	Lactose	Mannitol	Suspected organism
S1	Gray smooth flat translucent	A	A	-	-	*Actinomyces* sp.
S2	Gray rough entire opaque	A	A	-	-	*Lactobacillus* sp.
S3	Yellow round convex translucent	A	A	A	A	*Staphylococcus* sp.
S4	White round convex opaque	-	A	-	-	*Corynebacterium* sp.
S5	Cream round convex translucent	A	A	-	A	*Streptococcus mutans*
S6	White round raised translucent	A	A	A	-	*Streptococcus* sp.
S7	White round convex translucent	A	A	-	-	*Streptococcus* sp.
S8	Cream raised undulated edge opaque	A	A	-	A	*Lactobacillus* sp.

Key: α - alpha, β - beta, γ - gamma, + - positive, - - negative, A – Acid.

Using the Nephelometer, *Streptococcus mutans* had a cell density of 3.0 x 10^8 cfu/ml while *Lactobacillus* species had a cell density of 3.73 x 10^8 cfu/ml both falling within the McFarland scale of number one.

4.1.2. Antibacterial Potential of the Toothpaste Samples Using Disc Diffusion

Results obtained from the evaluations of the antimicrobial activity of 0.4g/ml and 0.5g/ml of the ten toothpaste samples on *Streptococcus mutans* and *Lactobacillus* sp. are shown in Table 3 and Table 4 respectively. No observable inhibitory effect was produced by distilled water which served as the negative control.

Against *Streptococcus mutans* (Table 3), the highest mean zone of inhibition of 25.75mm at 0.5g/ml and 24.75mm at 0.4g/ml was produced by toothpaste sample B while toothpaste sample I gave the lowest mean zone of inhibition

of 7mm at 0.5g/ml and no observables zone of inhibition at 0.4g/ml. The non-fluoride containing toothpaste sample, E, produced a mean zone of inhibition of 16.3mm at 0.5g/ml and 16mm at 0.4g/ml. The results obtained from the ANOVA test at 95% confidence level showed that there was a statistically significant difference among the ten toothpastes.

Against *Lactobacillus* species (Table 4), the highest mean inhibition zones of 24.5mm at 0.5g/ml and 23.5mm at 0.4g/ml was produced by toothpaste sample D while toothpaste sample I still produced the lowest mean inhibition zone of 8mm at 0.5g/ml and no observable inhibition at 0.4g/ml. The non-fluoride containing toothpaste sample produced mean zones of inhibition of 19.67mm and 18.25mm at 0.5g/ml and 0.4g/ml respectively. There was statistically significant difference among the ten toothpastes based on the ANOVA test result.

Table 3. *Antibacterial Activity of the Toothpaste Samples against Streptococcus mutans.*

Toothpastes	0.5g/ml Mean Value ± Standard Deviation*	0.4g/ml Mean Value ± Standard Deviation*
A	22.25 ± 0.838	19.75 ± 1.09
B	25.71 ± 0.85	24.75 ± 0.83
C	20.5 ± 1.5	20 ± 1
D	21.25 ± 1.29	18.75 ± 1.3
E	16 ± 1.58	16± 1.14
F	20.5 ± 1.12	20.5 ± 2.5
G	17.5 ± 2.06	14 ± 1.87
H	18.75 ± 0.829	12 ± 1.22
I	7 ± 0	0 ± 0
J	16 ± 1.58	± 1.66

*= Mean zone of inhibition (mm) n = 4.

Table 4. *Antibacterial Activity of the Toothpaste Samples against Lactobacillus species.*

Toothpastes	0.5g/ml Mean Value ± Standard Deviation*	0.4g/ml Mean Value ± Standard Deviation*
A	18.75 ± 0.829	17.75 ± 0.83
B	19.75 ± 1.479	19.5 ± 1.13
C	19.75 ± 1.479	18.75 ± 1.09
D	24.5 ± 2.29	23.5 ± 2.06
E	19 ± 1.58	18.75± 1.48
F	19.5 ± 1.6	18.5 ± 1.12
G	15.5 ± 2.278	12.5 ± 2.06
H	19.5 ± 2.278	12 ± 1.48
I	8 ± 0	0 ± 0
J	22.75 ± 1.08	18 ± 1.41

*= Mean zone of inhibition (mm) n = 4.

4.1.3. The Survival Ability of the Test Organisms in the Presence of the Toothpaste Samples

All tested toothpastes were active against *Streptococcus mutans* but it was not possible to detect differences among them since viable organisms were not observed in all samples that underwent counting after 0.5minutes.

The behaviour of *Lactobacillus* species on exposure to the various toothpastes at the various time intervals is illustrated in Figures 1 and 2. On contact with toothpaste samples E, H, I and J, the counts obtained were fluctuated initially and then increased (Figure1). This was similar to the behaviour exhibited by the negative control. These four toothpastes also showed a low correlation coefficient (Table 3). On the other hand, the counts of the test organisms decreased steadily on contact with toothpaste sample A, B, C, D, F and G (Figure 2). They also demonstrated high correlation coefficient (Table 5).

Table 5. *R^2 Value, Equation and Correlation Coefficient of Lactobacillus species for the Challenge Test.*

Toothpaste	R Square	Equation	Correlation coefficient
A	0.669772767	Y=-0.0327X + 5.7047	-0.818396
B	0.869069242	Y=-0.038X + 6.4924	-0.932239
C	0.415599405	Y=-0.0281X + 1287	-0.64467
D	0.961594495	Y=-0.0137X + 4.9113	-0.980609
E	0.093492234	Y=-0.008X + 5.4417	0.305765
F	0.970658033	Y=-0.0163X + 5.2181	-0.98522
G	0.952159305	Y=-0.0168X + 6.6913	-0.975787
H	0	Y=5.808	-1.44E-15
I	0.063258296	Y=0.0045X + 6.2989	0.251512
J	0.462903988	Y=0.0328X + 5.9366	0.68037

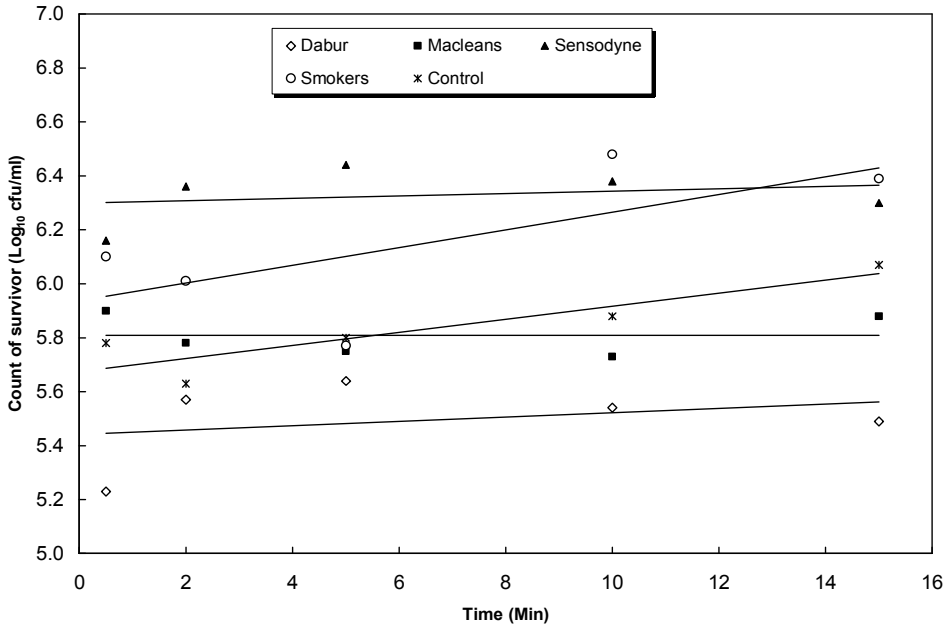

Figure 1. *Behaviour Profile of Lactobacillus species on exposure to the various toothpastes at set time intervals.*

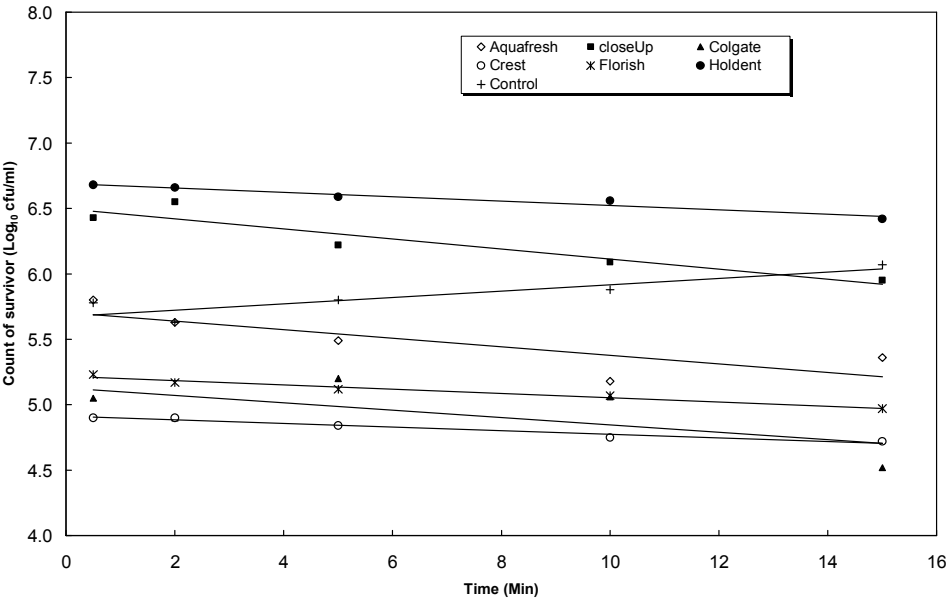

Figure 2. *Behaviour Profile of Lactobacillus species on exposure to the various toothpastes at set time intervals.*

4.2. Discussion

The effectiveness of the different toothpastes was determined in this study by the evaluation of their antibacterial activity against oral *Streptococcus mutans* and *Lactobacillus* species. These two bacteria were chosen because of their involvement in the initiation and progression of dental disease [1, 7, 11-12]. In this study, dilutions of the toothpaste samples were used in the disc diffusion test and the challenge test in order to mimic the dilution by saliva when used *in vivo* [11]. All the ten toothpaste brands were found to effectively repress the growth of the two test organisms in the two experiments carried out on them. The herbal toothpaste was also found to be at bar with most of the fluoride containing toothpastes in terms of antibacterial activity in both tests conducted.

There was no single dentifrice tested which showed the same mean zone inhibition diameter on the two microorganisms at the two concentrations employed. The reason could be attributed to the differences in interaction between the two bacteria and the dentrifrice [1]. In addition, one should keep in mind that the mean inhibition zone of one dentrifrice may not be directly comparable with that of another dentrifrice because dentrifrices may diffuse at different rates. Therefore, the dentrifrice that was identified as having the largest microbial inhibition zone may not necessarily be superior to those found to have smaller diameter inhibition zones [11, 13].

The bactericidal effect of the toothpaste samples were

more evident in the challenge test results where no viable microorganism was observed after 0.5minute exposure of *Streptococcus mutans* to the dentifrices. This could be due to their sensitivity to the antimicrobial agents in the toothpaste. However, this hindered the detection of differences that may have existed in the activities of the various toothpastes. This suggests that there would have been the observance of distinct differences if more diluted samples were employed. On the contrary, differences in the behaviour of *Lactobacillus* species when exposed to the toothpastes were observed distinctively. Aquafresh (A), Close Up (B), Colgate (C), Crest (D), Florish (F) and Holdent (G) were consistent in repressing the growth of *Lactobacillus* sp. with increase in exposure time while Dabur (E), Macleans (H), Sensodyne (I) and Smokers' (J) toothpastes repressed the test organism within the first five minutes but recorded an increase in their viable counts within the last five minutes of exposure. Furthermore, bacteria in biofilms forms such as plaque have decreased sensitivity to antibacterial agents [14]. Hence, formulations for topical oral use such as mouthrinse and dentifrices must be able to penetrate the biofilm matrix and deliver the active agents quickly because exposure times are limited under actual use conditions [11]. The data generated from this study show the importance of analyzing the antimicrobial activity of products such as toothpaste by applying linear regression since this test provides the profile for reducing the bioburden that may be analyzed according to the product use needs [15]. However, the use of specific media type and positive control in further tests would aid in the reduction of human error and give room for more robust statistical analysis. In addition, further tests to validate the effectiveness of these toothpastes including more herbal forms on the two test organisms and more oral bacteria and fungi would be necessary which should be both *in vitro* and *in vivo*.

5. Conclusion

It is obvious through the results obtained in this study that both fluoride containing and non-fluoride containing toothpastes can effectively repress oral bacteria. The media used in analysis may have impact on the results obtained. Again, one should never underestimate the role ones diet, health condition and general oral hygiene play in ensuring that one achieves good value for the money spent on the purchase of good dentifrice.

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