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ANTIBACTERIAL ACTIVITY OF SELECTED DENTIFRICES AGAINST STREPTOCOCCUS PYOGENES

Jackie Kpeinzeh Obey
University of Eastern Africa, Baraton, P. O. Box 2500-30100 Eldoret, Kenya
Email address: jackiekobey@gmail.com

Different formulations of dentifrices (toothpaste and mouthwashes) have been produced and are used to maintain oral hygiene. This study was carried out to determine the *in-vitro* antibacterial activity of seven brands of commercial dentifrices (A-G) against *Streptococcus pyogenes*. The dentifrices consisted of five toothpastes and two mouthwashes. The agar well diffusion method was used to determine the zones of inhibition caused by each dentifrice at five different dilutions against *S. pyogenes*. The results showed that the zones of inhibition (mm) of the dentifrices at 1:1 dilution ranged from 26±0.00 to15.67±0.33, 1:2 dilution from 24.50±0.288 to 12.00±0.00, 1:4 dilution was from 22.17±0.167 to 9.17±0.73, 1:8 dilution was 20.00±0.000 to 9.33±0.167 and 1:16 dilution was 17.00± to 0.00±0.000. Among the toothpastes, the 1:1 dilution of D, showed the highest zone of inhibition (24.00±0.00). Among the mouthwashes, F showed the largest zone of inhibition (26.00 ±0.000).
The common active ingredient found in the toothpaste brands was sodium fluoride in various formulations. Only toothpaste C did not contain sodium fluoride, yet possessed antibacterial activity. Another study also demonstrated a positive biological activity of toothpaste that contain propolis, hence, indicating that propolis can be added to toothpastes as a natural additive to mouthwashes (Morawiec et al., 2013). This research has demonstrated that toothpastes with or without sodium fluoride formulations can control the growth of *Streptococcus pyogenes in vitro.* The mouthwash formulation containing chlorhexidine was more active than the one without chlorhexidine against *S. pyogenes.*

Keywords: Antibacterial activity, dentifrice, mouthwash, toothpaste, sodium fluoride, chlorhexidine

Introduction

Microorganisms that reside in the oral cavity can be the source of major human diseases. Their mode of pathogenesis is primarily due to adhesion of cell surface proteins to host cells or tissues. S. pyogenes causes sore throat, impetigo, scarlet fever, glomerulonephritis, mastitis, rheumatic fever and necrotising fasciitis (Nobbs, Lamont, & Jenkinson, 2009). The maintenance of good oral hygiene is crucial in the colonization and establishment of streptococci in the oral cavity. Several studies have focused on the destruction of microorganisms that cause dental plagues, dental caries and periodontal diseases by dentifrices. Both herbal and non-herbal dentifrices have been studied for their antimicrobial activity against bacteria like *Streptococcus mutans, S. pyogenes, Escherichia coli, Pseudomonas aeruginosa* and other microorganisms. Chemical agents incorporated into the formulation of toothpastes and mouthwashes have been compared for their activity against different kinds of microorganism. Some of these agents are triclosan, sodium fluoride, sodium lauryl sulphate, sodium monofluoro phosphate, sorbitol, calcium carbonate and sodium benzoate. Mouthwashes mainly contain chlorhexidine, ethyl alcohol, sodium fluoride, potassium nitrate and zinc chloride (Prasanth,2011).

Very many dentifrices are being produced recently and claim to have antimicrobial activity, yet much work have not been done to confirm these claims in Kenya. The aim of this study was to determine the antibacterial efficacy of seven commercial dentifrices (A-F) in Kenya against the alpha-hemolytic Lancefield Group A bacterium S. pyogenes.

Materials and Methods

Study Area

The study was carried out at the Department of Medical Laboratory Science, University of Eastern Africa, Baraton. The University is located at about 50 km from Eldoret Town, Rift Valley Province, Kenya. Geographically, it is located at coordinates 0015'20"N 35004'57"E. It is a private Seventh-day Adventist university.

Study Design

The study was a laboratory-based experimental study. It included selection of five toothpastes from several brands of toothpastes purchased from local supermarkets and street vendors. Mouthwashes were purchased from a local supermarket.

Microorganism

Laboratory isolation of pure culture of S. pyogenes was obtained from the medical laboratory. It was first isolated on Blood agar. One colony was transferred to Mueller Hinton Broth and incubated overnight. The broth was diluted in distilled water and subjected to a UV-Vis Spectrophotometer until it reached a concentration of 1x108 cfu/ml. This was achieved at 625nm and 0.1A. This preparation was equivalent to a 0.5 McFarland's standard. This suspension was used for the experiment.

Preparation of Dentifrices

Each toothpaste solution was made by mixing 2.0g in 2ml of sterile distilled water to obtain a 1:1 dilution. They were diluted by double dilution to obtain dilutions of 1:2, 1:4, 1:8 and 1:16 (w/v) concentrations. The mouthwashes were also diluted by mixing 2ml with 2ml of sterile distilled water and double diluted to obtain similar concentrations like the toothpastes. Mueller Hinton Agar plates were made to test the activity of the dentifrices against S. pyogenes.

Table 1

Antimicrobial Assay

The experiment was carried out using the agar well diffusion method. Mueller Hinton agar were inoculated with 0.5ml of the 24-hour broth cultures of S. pyogenes. After drying, the agar plates were punched with a sterile 6-mm improvised borer. Each plate contained three wells at the same distance from each other on the plate. The wells were filled with either distilled water as a control or an extract dilution. The culture plates were incubated at 370C for 24 hours. The antimicrobial activity was assessed by zones of inhibition measured in millimetres (mm).

Statistical Analysis

The statistical analysis was carried out using SPSS version 20. Significant differences among the mean was measured by analysis of variance (ANOVA). The Tukey's Honestly Significant Difference test was done for multiple comparisons among the dentifrice dilutions.

Results

The results from this study showed that all dilutions of the dentifrices and mouth rinses showed activity of various degrees against S pyogenes.

Composition of Toothpastes (A-E) and Mouth rinses (F-G)

Dentifrice	Composition
A	Sodium Fluoride, Sorbitol, Aqua, Hydrated Silica, Sodium Lauryl Sulfate, Aroma, Cellulose Gum, Cocomidopropyl Betaine, Sodium Saccharin, Hydroxypropyl Methylcellulose, Menthol, Eugenol, Limonene
B	Sodium Bicarbonate, Purified Water, Glycerine, Cocamidopropyl Betaine, Rhatany Tincture, Peppermint Oil, Commint Oil Terpeneless #2B, Coneflower Expressed Juice Stabilized, Xanthan Gum, Myrrh Tincture, Tincture of Chamomile, Sodium Fluoride, Oil of Sage, Sodium Saccharin, Iron Oxide Red E172,

C	Calcium Carbonate, Sorbitol, Silica, Sodium Lauryl Sulphate, Sodium Carboxyl Methyl Cellulose, Sodium Silicate, Sodium Benzoate, Sodium Saccharin, Glycerine, Treated Water
D	Sorbitol, Aqua, Hydrated Silica, Sodium Fluoride, Zinc Sulfate, Calcium Gum, Sodium Saccharin, Sodium Hydroxide, Limonene
E	Sodium monofluorophosphate, Lauryl sulphate, calcium carbonate, aqua, sorbitol, hydrated silica, aroma, cellulose gum, potassium citrate, sodium silicate, sodium saccharin, phenyl carbinol
F	Chlorhexidine gluconate, alcohol, FD &C Blue No. 1, glycerin, PEG-40 sorbitan diisostearate, peppermint flavour, sodium saccharin, purified water
G	Thymol, Alcohol, Sodium Benzoate, Benzoic Acid

Key: A-E=Toothpastes; F,G= Mouthwashes

Analysis of variance showed that there were significant differences among the zones of inhibition of different dentifrice formulations at different dilutions ($p<0.0001$).

Table 2

Antimicrobial Activity (Zone of Inhibition, mm±S.E.) of Selected Dentifrices Against Streptococcus Pyogenes

Dilution / Dentifrice	1:1 Dilution	1:2 Dilution	1:4 Dilution	1:8 Dilution	1:16 Dilution
A	20.00±0.000	17.67±0.330	15.00±0.000	14.33±0.333	11.33±0.333
B	18.00±0.000	15.33±0.167	13.67±0.333	11.83±0.170	9.00±0.000
C	17.83±0.440	15.33±0.333	12.00±0.000	10.00±0.000	7.00±0.577
D	24.00±0.000	21.00±0.000	18.33±0.333	15.00±.577	10.33±0.882
E	16.00±0.000	12.00±0.000	9.17±0.730	6.83±0.440	0.00±0.000
F	26.00±0.000	24.50±0.288	22.17±0.167	20.00±0.000	17.00±0.577
G	15.67±0.330	14.00±0.000	11.50±0.288	9.33±0.167	8.00±0.00

Tukey's honestly significant difference showed that zones of inhibition for different dentifrice formulations when compared for the dentifrices separately, were mostly significantly different ($p<0.01$). Significant differences in zones of

Table 3

Tukey's Honestly Significant Difference Comparisons of the Zones of Inhibition Among Dilutions of Dentifrice Formulations

Dentifrice / P-value	A	B	C	D	E	F	G	Comparison
1:1 vs 1:2	0.000	0.000	0.004	0.011	0.000	0.000	0.002	
1:1 vs 1:4	0.000	0.000	0.000	0.000	0.003	0.000	0.000	
1:1 vs 1:8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
1:1 vs 1:16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
1:2 vs 1:4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
1:2 vs 1:8	0.000	0.001	0.000	0.000	0.000	0.000	0.000	
1:2 vs 1:16	0.000	0.000	0.000	0.022	0.000	0.000	0.000	
1:4 vs 1:8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
1:4 vs 1:16	0.000	0.000	0.018	0.005	0.010	0.000	0.000	
1:8 vs 1:16	0.000	0.000	0.001	0.000	0.000	0.000	0.008	

Discussion

The production of various dentifrice formulations is important in the control of microorganisms cause dental caries, periodontal diseases general decay, and general oral hygiene. The aim of this to compare the efficacy of seven commercial dentifrices used in Kenya. The dentifrices composed of five toothpastes found in supermarkets and open markets and two mouthwashes. dentifrices labelled TD showed significantly higher inhibition were obtained mainly for forulations of 1:1 vs 1:2 dilution for Dentifrice D, 1:2 vs 1:16 dilutions for D and 1:2 vs 1:8 dilutions for dentifrice C, with the higher dilution having significantly larger zones of inhibition than the lower dilution (Table 3).

In a study by Prasanth (2011) toothpaste formulation A showed the highest zone of inhibition against *Escherichia coli* ($P<60$; 0.001) compared to the other formulations. The zones of inhibition that against *Streptococcus mutans* and *Candida albicans* tooth were low but higher at higher dilutions for toothstudy was paste formulation A. The same study also showed that toothpaste with calcium carbonate as the main ingredient showed maximum zone of inhibition. A

bactericidal activity against total anaerobic bacteria ($p<0.05$) in a study by Forbes, Latimer, Sreenivasan, & McBain, 2016). The same study showed that the streptococci, when incubated aerobically, were inhibited with very low concentrations of the dentifrices (3.1-8.3 mg/ml). Toothpastes that contain triclosan, A, have shown increase in percent reduction of *S. mutans* at 1:1000w/v concentration from 0 minute (94.68%) to 15 minutes (94.84%), while other toothpastes in the study had reduced percent reduction (Munir, Umar, Hameed, & Ahmed, 2005). In another study, all toothpastes exhibited activity against gram positive bacteria except Paradontax (De Rossi et. al., 2014).

This result was similar to ours since most of the dilutions were active against *S. pyogenes.* Another study showed that toothpastes containing natural extracts, chlorhexidine or trilosan were active against gram positive bacteria and yeast. The natural substance present in Paradontax, Echinacea, has shown activity against microbial organisms (Harastzy, Zambon, & Sreenivasan, 2010). In a study where most of the compositions of the toothpastes were similar, toothpaste D showed the highest zone of inhibition and in addition, toothpaste D had limonene, Carbomer and Trisodium phosphate. The main zone of inhibition for the study was 20.70 for toothpaste D, similar to but slightly higher than the other toothpastes (Inetianbor, Ehiowemwenguan, Yakubu, & Ogodo, 2014).

A study on a selection of toothpastes sold in the open market in Abuja showed that they met the drug standard of the government (Okpalugo, Ibrahim, & Inyang, 2009). A study on triclosan, herbal and homeopathy toothpastes showed that the zones of inhibition of triclosan-containing toothpaste were statistically significant ($p<0.05$) for against all the four test organisms. Three herbal dentifrices had antimicrobial activity but were not statistically significant except for one formulation. Statistical significance was recorded only against *Streptococcus mutans* for the three herbal dentifrices while the homeo-based dentifrices had the least efficacy among the formulations (Anushree, Alimullah, Narahari, Whahela, & Syed, 2015). A polyherbal toothpaste tested against four bacterial organisms revealed that the highest zone of inhibition was obtained for Staphylococcus aureus (10-15 mm). Zones for *Escherichia coli* was 9-12mm, *Bacillus cereus* 7-12mm and P*seudomonas aeruginosa* 9-11mm (Sekar & Abdullah, 2016). Intense studies have also been conducted to determine the role of various ingredients of toothpastes and mouth washes. The study showed that chlorhexidine is regarded as the main or most important ingredient for oral hygiene. It kills bacteria by cell membrane damage and intracellular coagulation. Fluoride is a composition of almost all toothpastes and it inhibits topic demineralization and promotes remineralization of dental caries lesion. Potassium nitrate is added to get rid of hypersensitivity and benzydamine hydrochloride is included for antiinflammation and antimicrobial properties. Sodium lauryl sulphate is a synthetic cleansing agent but may cause skin dermatitis and desquamation of the oral mucosa (Iqbal et al., 2011).

Conclusion

This study has shown that several dentifrice showed activity against S. pyogenes, regardless of composition. It also showed that the higher the dilution of the dentifrice, the larger the zones of inhibition. Dentifrice dilutions showed zones of inhibitions that were generally significantly different. Mouth rinse F and toothpaste D at 1:1 dilutions give the overall best results for the study.

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