

Comparative Analysis of the Antibacterial Potentials of Some Brands of Toothpaste Commonly Used In Umuahia Abia State

Nwankwo, I. U. And Ihesiulo, S. C.

*Department Of Microbiology, College Of Natural Sciences Michael Okpara University Of Agriculture Umudike
P.M.B 7267 Umuahia Abia State*

Abstract: *This In- vitro study was carried out to determine the antibacterial efficacy of six different toothpastes against the pathogens isolated from the mouth (Escherichia coli, Staphylococcus aureus and Streptococcus spp) by disc diffusion method. Holdent toothpaste showed maximum zone of inhibition against S. aureus, E. coli and Streptococcus spp (14.00 ± 1.00, 12.33±0.57 and 14.00 ± 1.00 respectively). There was no significant difference (p>0.05) between the zones of inhibition for Staphylococcus aureus in both holdent and oral B toothpaste. There was a significant difference (p>0.05) between the zones of inhibition for Streptococcus species in close up herbal, oral B and holdent toothpaste B. This study demonstrated that triclosan containing toothpaste formulation e.g. holdent is more effective in the control of oral micro flora compared to non triclosan containing toothpaste e.g. 2080. This work therefore suggest that toothpaste effectively reduced the total bacterial load of the mouth.*

Keywords: *Antibacterial potentials, toothpaste, comparative analysis, Oral bacteria,.*

I. Introduction

The American Dental Association (ADA) describes toothpaste as a paste or dentifrice used with the aid of a toothbrush to cleanse and maintain the aesthetic and well being of the buccal cavity (tooth). Brushing of teeth using toothpaste is the most commonly practiced form of oral hygiene in most countries (Pannuti et al, 2003). It serves as an abrasive which helps in removing dental plaque and food particles from the teeth, assist in suppressing halitosis and release active ingredients mainly fluoride (Itthagarum and Wei, 1996). The success of any toothpaste, in part, lies on its ability to eliminate pathogenic oral microflora (Degiam, 2010).

In Nigeria, as in other developing countries, a very significant proportion of dental problems are due to microbial infections. Dental problems are of three types, formation of dental plaques, dental caries and periodontal diseases (Manupati, 2011). A wide range of chemicals mainly antimicrobial agents have been added to toothpaste in order to produce a direct inhibitory effect on plaque formation (Fine et al, 2006, Pannuti et al, 2003). The addition of antimicrobial agents such as triclosan, fluoride and zinc chloride to toothpaste has been suggested as one possible method to improving the efficacy of mechanical tooth-cleaning procedures (Fine et al, 2006), aiding the periodontal diseases (Ozaki et al, 2006; White et al, 2006).

Bacteria forms an important group of microorganisms seen in both healthy and diseased mouth, this may include the following: Streptococcus spp, Lactobacillus spp, Klebsiella spp Staphylococcus spp, Escherichia coli, Proteus spp etc. (Robert, 2005).

Staphylococcus mutans, a bacterium seen in the mouth is known to be one of the major causes of dental plaque and may also cause dental caries (Williams and Cummins, 2003).

The accumulation and development of dental plaque is dependent on the result of the interactions between the adhesiveness of plaque to the tooth surface and physical shear forces that helps to remove or dislodge the dental plaque (Robert, 2005). Toothpaste and brushes are among the physical forces that remove plaque. Active agents such as surfactants, antibacterial agents, baking soda, peroxide, metal salts herbal and natural extracts and chloride dioxide (Williams and Cummins, 2003) are incorporated into treatment forms. When these substances are added to oral products, they kill microorganisms by disrupting their cell walls and inhibiting their enzymatic activity. They prevent bacterial aggregation, slow multiplication and release endotoxins (Bon-Chacra et al, 2005, Ozaki et al, 2006).

Microorganisms enter the mouth with food, water and air. Contaminated toothbrushes can also be a source for oral bacteria growth. Toothbrushes which are used regularly become contaminated with microorganisms that colonize the teeth and the oral cavity. Under the usual conditions of storage, a toothbrush can therefore serve as a vector for the re-introduction of potential pathogens into the oral cavity, and also for the introduction of other microbial species originating from the bathroom environment (Otoikhian and Okoror, 2012).

Many different products are currently marketed that promise to provide consumers with fresh breath, Dentifrices need to contain various antimicrobial agents in order to reduce, control and prevent different kinds of dental diseases. Many dentifrices claim to have antimicrobial properties but very little research has been

conducted to investigate those claims. Based on these facts, the present study aimed at investigating the antibacterial efficacy of different toothpaste against some bacterial isolates from the mouth.

II. Materials and Methods

Collection of Specimen: Early morning mouth wash with water were collected from volunteers, which were mainly Students of Michael Okpara University of agriculture, Umudike. Subjects were each given sterile bottle and were instructed not to swallow their saliva as soon as they wake up in the morning.

Isolation Method: Using a streak plate method (Collee et al, 1996), the collected specimen was aseptically streaked on the surface of sterile solid agar (Macconkey agar, Eosin Methylene Blue and Nutrient agar) with the aid of sterile wire loop. The culture plates were incubated at the temperature of 37°C for 24h to 48h.

Characterization and Identification of Bacteria Isolates:

The isolates were characterized and identified following standard microbiological techniques such as gram staining catalase test, coagulase test, oxidase test, indole test, urease test and sugar fermentation test

Antibacterial Assay: All tooth pastes samples were diluted in sterile water. The antibacterial activity of different concentrations 500mg/ml, 250mg/ml and 62.5mg/ml, of the toothpaste was determined by agar disc diffusion method. In this method, Mueller-Hinton agar plates were seeded with 0.5ml of 24h broth cultures of each isolate, the plates were allowed to dry for 1h. A sterile forceps was used to place the paper disc impregnated with 0.2ml of the respective toothpaste, on the surface of the agar. Paper disc impregnated with sterile distilled water was used as negative control.

Plates were incubated at 37°C for 24h. The antibacterial activity was evaluated by measuring the diameter zone of inhibition in millimeter.

The various concentrations mentioned above were used to determine the minimum inhibitory concentration using Mueller- Hinton broth. 9ml of a sterile Mueller-Hinton broth was dispensed into each tube, then 1ml of each of the mixed in a test tube. 0.1ml of inoculums was added to each test tube. The tubes were incubated aerobically at 37°C for 24h. A broad spectrum antibiotic (Gentamycin) was used as positive control. The lowest concentration of the toothpaste that produced no visible growth when compared with the negative control was regarded as the MIC (Kadar, 2005).

Some Commonly Used Toothpaste and their Compositions

Toothpaste Ingredients as listed in Packages

Macleans Sodium fluoride. Aqua, Hydrated Silica, Sorbitol Glycerine.PEG6,Sodium Lauryl Sulphate, Flavour, Xanthan Gum, sodium saccharin, C1-73360,C1-7418.

2080 Sodium monofluorophosphate, sodium carboxymethyl Cellulose. sodium lauryl sulphate sodium saccharin, sorbitol. PEG-32, titanium dioxide, flavour, tocopheryl acetate.

Holdent Sodium fluoride, triclosan, sorbitol, silica, water, flavour, cellulose gum. sodium saccharin, food grade red color.

Dabur-Herbal Basil oil, herbal extracts, sodium lauryl sulphate, calcium carbonate. Ginger, eucalyptus and lemonade, sodium silicate glycerin, gum cariacen, sodium monofluorophosphate. sodium saccharin.

Close up Herbal Sodium fluoride, sorbitol, aqua, sodium lauryl sulphate, sodium saccharin, PEG-32, aloe vera, barbadensis leaf extract, lemon, C1 73360, C1 74260. C1 772681.

Oral B Sodium fluoride, sorbitol, aqua, hydrated silica, sodium lauryl sulphate, aroma, cellose gum, triclosan phosphate, sodium saccharin, polyethylene, limonene, C1 19140, Eugenol, C1 42090, Carboner

III. Statistical Analysis

Statistical analysis was performed using a statistical package, SPSS windows version is by applying mean values using analysis of variance (ANOVA) with post-hoc Duncan method.

IV. Results

The cultural, morphological and biochemical examination of the growth on the culture plates reveals the isolates to belong to the genera Staphylococcus aureus, Streptococcus spp and Eschericharia coli. This is shown in Table 1.

The result of the antibacterial assay of the different toothpaste against staphylococcus aureus is shown in Table 2. As observed from the table, the mean diameter zone of inhibition produced by 2080 ranges from 8.33± 0.58 mm to 9.33± 0.58mm, for Darbur Herbal, it ranges from 8.67± 0.58 -13.00±1.00. That of Close-up herbal ranges from 9.00±0.00mm to 13.33±0.58. Macleans ranges from 8.33±0.58mm to 9.33±0.58mm, oral B ranges from 6.33± 0.58mm to 12.67± 0.58mm and Holdent ranges from 6.67±0.58mm to 14.67± 1.00mm. Oral

B and Holdent were more effective on the isolate, even at the lowest concentration the exhibited a slight inhibitory effect which was not observed with other toothpaste. Their MIC value was 62.5mg/ml respectively.

Table 3 shows the mean diameter zone of inhibition (mm) of the six toothpaste samples on *E. coli*. None of the toothpaste inhibited the growth of the organism at 62.5mg/ml concentration. Macleans exhibited a slight inhibitory effect only at 500mg/ml concentration. All the toothpaste except macleans were effective at the concentration of 250 -500mg/ml. The MIC value ranges from 125-500mg/ml.

As shown in Table 4, all the toothpaste samples inhibited the growth of streptococcus spp. at the concentration of 250mg/ml and 500mg/ml. The highest mean diameter zone of inhibition was recorded against Holdent, 14.00 ± 1.00 mm and 12.00 ± 1.00 mm at 500mg/ml and 250mg/ml concentration respectively. The MIC value ranges from 125-250mg/ml.

The result of the zone diameter of inhibiting the standard antibiotic (Gentamycin) is shown in table 5. The highest zone diameter of inhibition was recorded against *Streptococcus* spp (21.76mm) followed by *S aureus* (19.50mm) while the least was with *E coli* (9.20mm).

V. Discussion

Maintenance of good oral hygiene is the key to prevention of dental diseases (Gamboa et al, 2004). The activities of the oral micro flora are the cause for most oral diseases and mouth odour. The addition of antibacterial agents in the production of toothpaste aids in keeping these oral organisms to a level consistent with oral health.

The present study shows the antibacterial activity of different toothpaste against *E coli*, *S. aureus* and *Streptococcus* spp as observed from the results, Holdent and oral B emerged as the most effective based on their mean diameter zone of microbial inhibition values. The exceptional ability of these two toothpaste to inhibit the growth of the test organisms even at the least concentration (62.5mg/ml) might be due to the presence of Triclosan and Sodium fluoride. Triclosan is an antibacterial and antifungal agent used in the production of toothpaste. It works by inhibiting the enzyme enoyl-acyl carrier protein reductase thereby blocking the lipid biosynthesis (McMurry et al, 1998). Past studies has shown that toothpaste containing Triclosan significantly improves plaque control and periodontal health (Gullsolley, 2006).

Among the toothpaste used in this study, fluorinated toothpaste like Maclean and 2080 were not statistically significant. The effectiveness of fluoride toothpaste is concentration dependent (Fejerskov and Kik, 2003). Thus, the low activity of Macleans and 2080 may be due to the low concentration of the ingredient. These toothpaste contain sodium monofluorophosphate and sodium fluoride as active ingredients. Fluorides are widely used in many dental products including toothpaste which aids in many dental products including toothpaste which aids the prevention of dental caries (Marinho et al, 2007).

However, if the bacterial load is too high, it is not possible for it to inhibit the growth completely. This is in agreement with the work done by Davies et al, (2004) where they observed that toothpaste containing triclosan and copolymer provide more effective level on plaque control and periodontal health than conventional fluoride toothpaste. The appeared to be equally effective as fluoride toothpaste, Darbur herbal and close up herbal are the herbal products used in this study. This two showed moderate effective as fluoride toothpaste, but not superior to them (Amrutesh et al, 2010). Secondary metabolites such as alkaloik, flauroids and polyphenols present in the herbs used for the production are responsible for the antibacterial activity of these toothpaste (Fatima et al, 2000). The synergistic interaction between the main component of these herbs is seen as a vital part of their effectiveness. However, this synergistic activity needs to be established. This however, is in agreement with a work carried out by Moran et al, (1991) where they observed that herbal toothpaste have rarely been shown to have significantly greater antiplague activity than other non- herbal toothpaste.

More so, as observed from the results, *E coli* exhibited moderate resistance potentials to the various toothpaste used than the other test isolate. This however could be as a result of the gram negative nature of *E coli*, complexity of its cell wall and possession of antimicrobial resistance plasmids (Kadar, 2005).

VI. Conclusion

This work revealed that toothpaste containing triclosan was more effective in inhibiting the growth of the bacteria isolates from the oral cavity compared to toothpaste containing sodium fluoride and plant extracts. This suggests that the use of toothpaste made of iriclosan will go a long way to control cases of dental caries and oval diseases. Hence manufacture types of toothpaste for the dental benefit of the general public.

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Table1: Test isolates from Oval Cavity Samples

Gram positive
Staphylococcus aureus
Streptococcus spp
Gram negative
Escherichia coli

Table 2: Mean Diameter Zone of Inhibition (mm) in the Six Toothpaste Samples Against Staphylococcus aureus And Their MIC (mg/ml)

Sample	Concentration (Mg/Ml)				MIC
	500	250	125	62.5	
2080	9.33±0.58	8.33±0.58	0.00	0.00	250
Darbur herbal	13.00±1.00	11.33±0.58	8.67±0.58	0.00	125
Close-up herbal	13.33±0.58	12.00±1.00	9.00±0.00	0.00	125
Macleans	9.33±0.58	8.33±0.58	0.00	0.00	250
Oral B	12.67±0.58	11.00±1.00	8.67±0.58	6.33±0.58	62.5
Holdent	14.00±100	11.67±0.58	9.00±1.00	6.67±0.58	62.5

Table 3: Mean diameter zone of inhibition (mm) in the six toothpaste samples against Escherichia coli and their MIC (mg/ml)

Sample	Concentrations (Mg/M)				MIC
	500	250	125	62.5	
2080	8.33±0.58	6.67±0.58	0.00	0.00	250
Darbur herbal	9.33±0.58	7.67±0.58	0.00	0.00	250
Close-up herbal	9.33±0.58	7.67±0.58	0.00	0.00	250
Macleans	7.67±0.58	0.00	0.00	0.00	500
Oral B	9.50±1.38	8.33±0.58	6.33±0.57	0.00	250
Holdent	12.33±0.57	10.66±0.57	0.00	0.00	125

Table 4: Mean diameter zone of inhibition (mm) in the six toothpaste sample against Streptococcus spp and their MIC (mg/ml)

Sample	Concentration (Mg/M)				MIC
	500	250	125	62.5	
2080	7.33±0.57	6.66±0.57	0.00	0.00	250
Darbur herbal	9.67±0.57	8.33±0.57	0.00	0.00	250
Close-up herbal	10.33±0.57	8.67±0.57	6.66±0.57	0.00	125
Macleans	9.33±0.57	7.66±0.57	0.00	0.00	250
Oral B	10.33±0.57	8.67±0.57	6.66±0.57	0.00	125
Holdent	14.00±1.00	12.00±1.00	9.67±1.00	0.00	125

Table 5: Antibacterial diameter zones of inhibition (mm) of Gentamycin

Test organism				Zone of inhibition (mm)	
S. aureus				19.50	
E. coli					9.20
Streptococcus spp				21.76	