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Research Article

IN-VITRO ANTIBACTERIAL ACTIVITY OF COMMONLY USED TOOTHPASTES IN NIGERIA AGAINST DENTAL PATHOGENS

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ABSTRACT

The in-vitro antibacterial activity of different brands of toothpaste on dental pathogens was investigated. A total of four different brands of toothpastes designated A, B, C and D were tested for their antibacterial effect on five dental pathogens namely, *Streptococcus mutans, Staphylococcus epidermidis*, *Lactobacillus acidophilus*, *Klebsiella pneumoniae* and *Enterobacter* species using agar well diffusion method at different concentrations. The result showed that all the toothpastes were effective against the test organisms. The diameter of zones of inhibition of brand A ranged from 18.0mm against *L. acidophilus and K. pneumonia at* 2:5 concentrations to 24.0mm against *S. mutans* at 4:5 concentrations, brand B ranged from 18.0mm (*L. acidophillus*) to 23.0mm (*S. mutans*), brand C ranged from 18.0mm (*K. pneumonia*) to 22.0mm (*S. mutans*) at 2:5 and 4:5 concentrations respectively while brand D zones of inhibition ranged from 18.0mm (*K. pneumonia*) to 23.0mm (*S. mutans*) at 2:5 and 4:5 concentrations respectively. On the average, brand D has the highest antibacterial effect against the test organisms (20.70mm) followed by brand C (20.60mm), then brand B (20.50mm) while brand A showed the least activity against the test organisms (20.30mm). The variations in the antibacterial activity of the various toothpastes compared favourably with the broad spectrum antibiotics, ampicillin, tetracycline and chloramphenicol. The effectiveness of these toothpastes is directly related to the antibacterial components in their formulations. Therefore, these brands of toothpastes and others with the same formulations can be used to control dental infections associated with the test organisms.

Keywords: Dental pathogens, Antibacterial activity, Zone of inhibition, Toothpastes, Infection.

1. INTRODUCTION

Dental caries is one of the most common chronic infectious diseases in the world [1, 2]. Dental caries results from the interaction of specific bacteria with constituents of the diet within a biofilm termed "dental plaque" [3]. Bacterial plaque accumulated on dental surfaces and composed of native oral flora is the primary etiologic agent of dental caries. Despite great improvements in the global oral health status, dental caries still remains one of the most prevalent diseases [4]. The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are bye products of carbohydrate metabolism by *Streptococcus mutans,* a cariogenic bacterium [5]. Colonization of teeth by cariogenic bacteria is one of the most important risk factors in the development of dental diseases [5]. *Staphylococcus* (*S. aureus and S. epidermidis*) as a major human pathogen, responsible for a number of hospital-acquired infections initially colonizes several locations in the human body, but the mouth and hands are the main reservoirs for propagation of this pathogen in the hospital environment, [6-8]. Individuals heavily colonized by cariogenic bacteria are considered to be at high risk for dental caries. Hence eradication of these microorganisms is important for dental treatment [9].

Cariogenic bacteria interact by various recognized ways including co-aggregation [10], metabolic exchange, cell-cell communication [11], and exchange of genetic material, [12]. These mechanisms benefit bacterial survival and can make dental biofilms difficult therapeutic targets in dental diseases. Dental caries cause destruction of enamel, dentin or cementum of teeth due to bacterial activities. Dental caries affect 60 to 90 per cent of school children and the vast majority of adults in most industrialized countries [13]. Among five- to 17-year-olds, dental decay is five times as common as asthma and seven times as common as hay fever [14]. An equally significant threat to health is periodontal disease, also known as gum disease, which is also caused by oral bacteria. Gum disease can be extremely serious. There is also a growing body of scientific research suggesting that a relationship exists between periodontal disease and a number of serious health conditions [14].

The burden of dental caries is still a major health problem in most industrialized countries as it affect 60%-90% of school-aged children and the vast majority of adult and this is largely due to the increasing consumption of sugar and

inadequate exposure to fluorides [15]. Tooth decay has been present throughout human history, from early hominids millions of years ago, to modern humans [16]. The prevalence of caries increased dramatically in the 19th century, as the Industrial Revolution made certain items, such as cane sugar and refined flour, readily available [17]. The diet of the "newly industrialized English working class then became centered on bread, jam, and sweetened tea, greatly increasing both sugar consumption and caries [17].

Triclosan, a chlorophenol derivative is a major constituent of most toothpastes and oral rinses. It kills germs by interfering with the enzymes required for fatty acid synthesis. Similar to triclosan, the fluorinated products were also found to possess marked antibacterial activities. These active compounds were reducing cariogenic bacteria to strengthen the teeth by reducing demineralization and increasing re-mineralization of teeth [18].

Biofilms are surface-adherent population of microorganisms consisting of cells, water and extra cellular matrix material [19]. *Streptococcus mutans*, the principle cariogen for dental caries, co-exist with over 500 other species of bacteria as an interactive community known as the dental biofilm [20]. A quorum sensing signaling system is essential for genetic competence to function optimally in Biofilms [21]. Dental caries is a biofilm-dependent oral disease, and fermentable dietary carbohydrates are the key environmental factors involved in its initiation and development. Sucrose is considered the most cariogenic dietary carbohydrate, because it is fermentable, and also serves as a substrate for the synthesis of extracellular (EPS) and intracellular (IPS) polysaccharides in dental plaque [2, 22]. Enamel integrity is disrupted secondary to the formation of a dental biofilm and the caries process occurs along the interface between the dental biofilm and the enamel surface [23, 24]. Cariogenicity of sucrose has been associated with its frequency of exposure and concentration [25, 26]. Depending on the extent of tooth destruction, various treatments can be used to restore teeth to proper form, function, and aesthetics, but there is no known method to regenerate large amounts of tooth structure. Instead, dental health organizations advocate preventive and prophylactic measures, such as regular oral hygiene and dietary modifications, to avoid dental caries [27]. The aim of this work was to determine the antimicrobial activity of toothpaste brands (A, B, C and D) on five isolated bacterial cariogen.

2. MATERIAL AND METHODS

2.1. Collection of samples and test organisms

Four toothpastes brands (A, B, C and D) commonly used in Nigeria were purchased from the Uselu market, Benin City, Nigeria and were immediately taken to the Laboratory, Microbiology Department, Faculty of Life sciences, University of Benin, Benin City Nigeria for analysis. The dental organisms (*S. mutans, S. epidermidis, lactobacillus acidophilus, Klebsiella pneumonia* and *Enterobacter* sp.) used for this work were collected from the Department of Medical Microbiology, University of Benin Teaching Hospital (UBTH), Benin City, Nigeria.

2.2. Identification and maintenance of test organisms

The various test organisms were screened, identified and purified by series of sub-culture on specific media such as Brain Heart Infusion Agar, BHI (*S. mutans*), Manitol Salt Agar (*S. epidermidis*), Chocolate agar (*Lactobacillus acidophilus)*, MacConkey agar (*Klebsiella* pneumonia) and blood agar *(Enterobacter* sp.), and were incubated aerobically at 37^{O}C for 24hours. The identification of all the microbes was confirmed by standard biochemical and staining methods, [28-30]. All the pure cultures were stored and maintained in nutrient broth at 4°C for further use.

2.3. Antibacterial Assay

The antibacterial activity of the different concentrations, 2:5 and 4:5 (prepared by mixing 2g and 4g each of the toothpastes in 5 mL of sterile distilled water respectively) of the various toothpaste brands (A, B, C and D) was determined by modified agar well diffusion method as described by, [31]. In this method nutrients agar plates were seeded with 0.5ml of 0.5 McFarland standards (approx., 10^8 cfu/mL) of each isolate (*S. mutans, S. epidermidis, Lactobacillus acidophilus, Klebsiella pneumonia* and *Enterobacter* sp.). The plates were allowed to solidfy for 1hour. A sterile 8mm cork-borer was used to cut one central and five wells at equidistance of the plates. 0.2ml of the toothpaste dilutions was introduced into each of the five wells while the same amount of sterile distilled water was introduced into the first well as control. The efficacy of extracts against bacteria was compared with the broad spectrum antibiotics ampicillin, tetracycline and chloramphenicol (positive control). The same procedure was used for the broad spectrum antibiotics and the plates were incubated at 37°C for 24hours. The antimicrobial activity was evaluated by measuring the diameters of zones of inhibition (in mm). All plates were made in triplicate and the experiments repeated three times.

3. RESULTS

The composition on the label of the different toothpaste brands used in this study is show in Table 1. All the toothpaste brands contain sorbitol, sodium fluoride, hydrated silica, while triclosan and trisodium phosphate were only present in brand B and D respectively.

Table 1: Composition of the Toothpastes

Toothpaste Brand	Composition on the label
A	Sodium Fluoride 0.306%w/w, Aqua, Hydrated Silica, Sorbitol, Glycerin, PEG-6, Sodium Lauryl Sulphate, Flavor, Xanthan gum, Sodium Saccharin, Cl 73360, Cl 74160.
B	Aqua, Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, PVM/MA copolymer, Aroma, Carrageenan, Sodium hydroxide, Sodium fluoride, Sodium Saccharin, Triclosan, Limonene, Cl 77891
C	Sodium Fluoride, Sorbitol, Hydrated Silica, Aqua (water), Sodium lauryl sulfate, PEG-32, Aroma (Flavour), Cellulose Gum, Sodium Saccharin, Zinc sulfate, mica, Sodium hydroxide, Glycerin, Eugenol, Cl 12490, Cl 16035, Cl 17200, Cl77491 and Cl7789.
D	Sorbitol, Hydrated Silica, Aqua, Sodium lauryl sulfate, Aroma, Cellulose Gum, Trisodium phosphate, Sodium phosphate, Sodium Saccharin, Sodium Fluoride, Carbomer, polyethylene, Limonene, Cl 77891, Cl 42090

Table 2 shows the inhibition zone (mm) of the various toothpastes used against the test organisms. The result revealed that brand D has the highest mean inhibition zone on the test organisms (20.70mm) followed by brand C (20.60mm), brand B (20.50mm) while brand A showed the least activity on the test organisms (20.30mm).

The inhibition zone (mm) of the control (broad spectrum antibiotics) was shown in Table 3. Chloramphenicol was found to have the highest mean inhibition zone (27.00mm) followed by tetracycline (24.50mm) and then ampicillin (22.90mm).

The various toothpastes showed a marked antibacterial activity against the isolates and compared favourably with the various broad spectrum antibiotics as represented in Figures1-5.

Table 2: Minimum Inhibitory zone (mg/ml) of the toothpastes

Tooth pastes brands	Test Organisms	Inhibition at 2:5 conc.	Zone (mm) at 4:5 conc.	Average Inhibition Zone (mm)
A	*Streptococcus mutans*	20	24	22.0
	Staphylococcus epidermidis	19	21	20.0
	Lactobacillus acidophilus	18	22	20.0
	Enterobacter sp	20	21	20.5
	Klebsiella pneumonia	18	23	20.5
				Mean: 20.60
B	*Streptococcus mutans*	21	23	22.0
	Staphylococcus epidermidis	19	22	20.5
	Lactobacillus acidophilus	18	20	19.0
	Enterobacter sp	20	21	20.5
	Klebsiella pneumonia	19	22	20.5
				Mean: 20.50
C	*Streptococcus mutans*	20	22	21.0
	Staphylococcus epidermidis	20	21	20.5
	Lactobacillus acidophilus	19	21	20.0
	Enterobacter sp	19	22	20.5
	Klebsiella pneumonia	18	21	19.5
				Mean: 20.30
D	*Streptococcus mutans*	21	23	22.0
	Staphylococcus epidermidis	19	22	20.5
	Lactobacillus acidophilus	20	21	20.5
	Enterobacter sp	20	22	21.0
	Klebsiella pneumonia	18	21	19.5
				Mean: 20.70

Table 3: Minimum inhibition zone (mg/ml) of the control (broad spectrum antibiotics)

Antibiotics	Test Organisms	Inhibition at 1.8grams	Zone (mm) at 2.4grams	Average Inhibition Zone (mm)
Ampicillin	*Streptococcus mutans*	23	26	24.5
	Staphylococcus epidermidis	21	25	23.0
	Lactobacillus acidophilus	23	24	23.5
	Enterobacter sp	20	22	21.0
	Klebsiella pneumonia	22	23	22.5
				Mean: 22.90
Tetracycline	*Streptococcus mutans*	26	30	28.0
	Staphylococcus epidermidis	23	25	24.0
	Lactobacillus acidophilus	24	25	24.5
	Enterobacter sp	22	24	23.0
	Klebsiella pneumonia	24	28	26.0
				Mean: 24.50
Chloramphenicol	*Streptococcus mutans*	28	33	30.5
	Staphylococcus epidermidis	24	28	26.0
	Lactobacillus acidophilus	22	27	24.5
	Enterobacter sp	23	28	25.5
	Klebsiella pneumonia	27	30	28.5
				Mean: 27.00

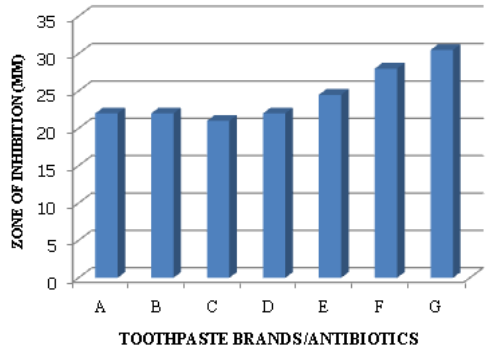

Fig 1: Comparison of the antibacterial activity of the different brands of toothpaste and antibiotics against *Streptococcus mutans*.
Key: A-D = different brands of toothpaste, E = Ampicillin, F = tetracycline, G = Chloramphenicol

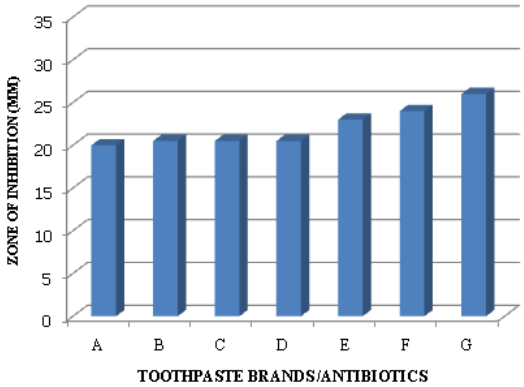

Fig 2: Comparison of the antibacterial activity of the different brands of toothpaste and antibiotics against *Staphylococcus epidermidis*.

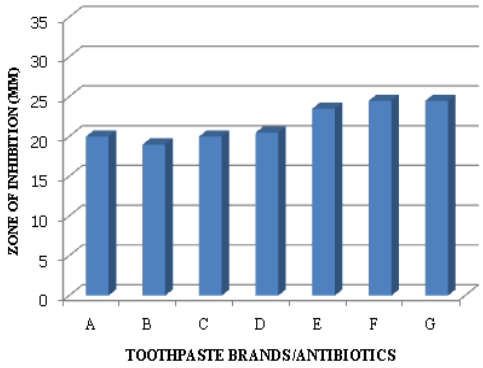

Fig 3: Comparison of the antibacterial activity of the different brands of toothpaste and antibiotics against *Lactobacillus acidophilus*

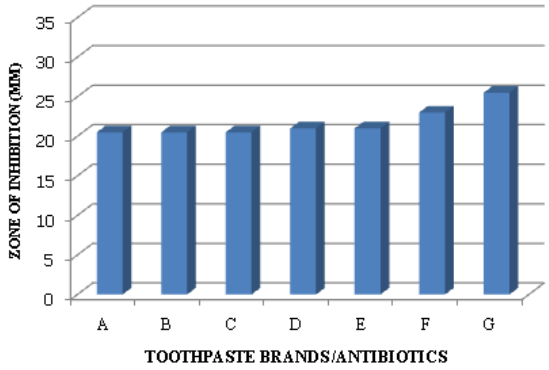

Fig 4: Comparison of the antibacterial activity of the different brands of toothpaste and antibiotics against *Enterococcus faecalis*

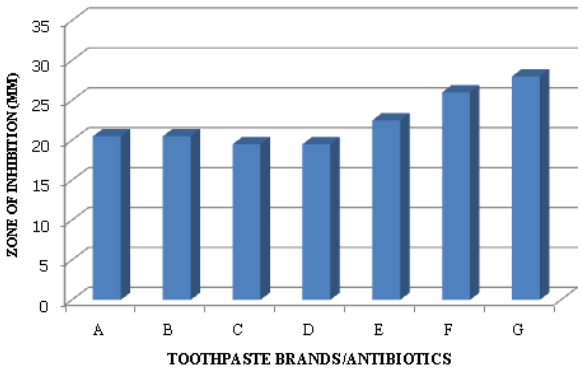

Fig 5: Comparison of the antibacterial activity of the different brands of toothpaste and antibiotics against *Klebsiella pneumoniae*.

4. DISCUSSION

Maintenance of good oral hygiene is the key to the prevention of dental diseases. The activities of oral microflora being responsible for mouth odor and most oral disease are not in doubt. Hence the need to keep these organisms to a level consistent with oral health by antimicrobial agent inclusion in dentifrices has been stressed [32].

In the present study, four different brands of toothpastes designated A, B, C and D was tested for antibacterial activity against five dental pathogens. All the four toothpastes were found to be effective against the five tested dental bacterial pathogens with brand D having the highest inhibition zone on the average (20.70mm) followed by brand C (20.60mm), brand B (20.50mm) while brand A showed the least activity (20.30mm). Several clinical studies have demonstrated the inhibitory effects antimicrobial dentifrice on oral bacteria and gingival [33]. Data from the present study is in support of this assertion as all the investigated dental care products exhibited wide variation in their effectiveness against the five test microorganisms, a feature that may have been largely due to their antimicrobial active ingredients such as sodium fluoride and triclosan. These reports corresponds with the work of Okpalugo and co-workers [34] who reported that toothpastes containing two antibacterials, sodium fluoride plus Triclosan had a 20% more reduction in oral bacterial flora than non triclosan containing toothpastes. Also the result of the present study is in consistent with a 3 years trial in the United Kingdom which shows that dental decay was reduced by as much as one third by regular use of Colgate containing fluoride [35] and the report of [36] that fluoridated toothpaste is associated, on average, with a 24% reduction in tooth decay.

It is known that a balance exists in a person's oral microbial population. If this balance is lost, opportunistic microorganisms can proliferate, enabling the initiation of disease processes. Therefore, the toothpaste identified as having the largest microbial inhibition zone and thus probably the strongest antibacterial properties may not be necessarily superior to those found to have smaller diameter of inhibition zones because a toothpaste used in vivo is likely to be diluted by saliva, the level to which antimicrobial properties are buffered or lost in dilution *in vitro* is of interest [37]. In addition, it should be borne in mind that the mean average inhibition zone of a toothpaste brand may not be directly compared with that of other toothpaste because different toothpaste constitutes different active ingredients and may diffuse at different rates. The test was conducted *in vitro*, so it cannot be assumed that the results of antimicrobial efficacy could be proportional or transferable to the oral cavity and translated into clinical effectiveness. Studies have demonstrated the effectiveness of rinsing with antimicrobial toothpaste and mouthwash in significantly reducing salivary [38-40] and mucosal [41, 42] levels of bacteria. Thus, from the overall results obtained, it is evident that various toothpastes have several active and not active ingredients that presented different levels of antimicrobial activities. This is probably due to differences in formulations, the active product concentration and its interaction with other constituents. However, this observation justifies the antimicrobial claims of the mouthwashes, made by earlier workers [43- 45].

The result of the antibacterial activity of the different brands of toothpastes in the present study compared favorably with the broad spectrum antibiotics with little differences in the diameter zone of inhibition across the organisms under study. All the toothpastes showed their highest activity against *Streptococcus mutans* ranging from 21.0mm to22.0mm as well as the antibiotics ranging from 24.5mm to 30.5mm. Howerver, chloramphenicol was observed to be more effective on the average (27.00mm) followed by tetracycline (24.50mm) and then ampicillin (22.90mm). Hence, these antibiotics can be used in treatment of infections associated with the test organisms.

5. CONCLUSION

The present study has shown that the various brands of toothpaste (A, B, C and D) demonstrated marked antibacterial activity against the test organisms (*S. mutans, S. epidermidis, lactobacillus acidophilus, Klebsiella pneumonia* and *Enterobacter* sp.) *in vitro* and compared favourable with broad spectrum antibiotics. Therefore, these brands of toothpastes and others with the same formulations shown above can be used to control dental infections caused by these microorganisms.

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