



Full Length Research

Antibacterial Efficacy of Different Dentifrice on Some Selected Bacteria Isolates from Mouth of Students of Ambrose Alli University, Ekpoma, Edo State.

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ABSTRACT

Dental diseases are a major health concern in Nigeria. This study was designed to investigate the *in vitro* inhibition of bacteria by different concentrations of different toothpastes on the bacterial isolates. The purpose of the study was to evaluate the antimicrobial activity of four dentifrices designated A, B, C and D and their efficacy against different bacteria species. Pure cultures of *Escherichia coli*, *Streptococcus mutaus* and *Lactobacillus acidophilus* were obtained from the Microbiology Laboratory of Irrua Specialist Teaching Hospital, Irrua Edo State. The agar well diffusion method was used for the antibacterial efficacy studies. Results obtained indicated that dentifrice B (contained triclosan and sodium fluoride), had highest inhibition zones (19.4) followed by dentifrice A (which contained sodium fluoride) and had inhibition zone diameter of 14.7mm. This is followed by dentifrices C and D with inhibition zone diameter of 12.7mm and 11.8mm respectively. Findings from this study also showed that *Lactobacillus acidophilus* posed more resistance to the antibacterial efficacy of these dentifrices. To achieve good oral health, there is need to balance up active ingredients to appropriate levels in all toothpastes so that in addition to providing protection against sensitivity, they can also protect the teeth from dental diseases caused by bacteria.

Keywords: Dentifrices, triclosan, isolates, antimicrobial, dental, antibacteria

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INTRODUCTION

Toothpaste is a dentifrice that functions to improve the aesthetic appearance and the health of the teeth. Toothpaste is mostly found and sold in flexible tubes or sachets for the appraisal of oral hygiene, removal of dental plaque and food debris from the mouth as well as the elimination of halitosis from the mouth. Toothpaste has also been used to promote oral hygiene and deliver active ingredients to protect the teeth from dental caries and other mouth diseases. Toothpaste is best classified as a drug and not as a cosmetic because they contain(s) components like sodium lauryl sulphate, sodium fluoride, *Mentha spicata*, *Curcuma longa* etc. to reduce microbial load (Gautam et al., 2017).

Abrasives, which are part of ingredients of toothpaste are the substances used for abrading, grinding or polishing. They constitute 8-20% of a typical toothpaste (Lippert, 2013). They remove substances adhering to the surface of the teeth without scratching it and bring out their natural luster. One of the major characteristics of these abrasives is hardness. The degree of abrasiveness is a function of the hardness of the abrasive, the morphology of its particles, and on the concentration of abrasive in the paste (Vranic et al., 2004).

Fluorides are synthetic high molecular weight polymers of acrylic acid cross-linked with polyalkenyl ethers of sugars or polyalcohols, which are produced in several grades characterized by the viscosity of a defined solution. They are white, hygroscopic powders that have a slight characteristic odor. They swell in water and in other polar solvents after dispersion and neutralization with sodium hydroxide solution (European Pharmacopoeia, 2002). They are also soluble in water, alcohol and

glycerol. Carbomers are used in toothpastes as a binder (thickener). Fluoride in various forms is the most popular and effective active ingredient in toothpaste to prevent cavities (Aspinall et al., 2021).

Triclosan is a non-ionic chlorinated phenolic agent with antiseptic properties. It has a broad-spectrum efficacy on Gram-positive and most Gram-negative bacteria and it is also effective against *Mycobacterium* and strictly anaerobic bacteria, and against the spores and fungi of the *Candida* species. The mechanism of its antiseptic action is by acting on the microbial cytoplasmic membrane, inducing leakage of cellular constituents and thereby results in lysis of the microorganisms (Vranic et al., 2004).

MATERIALS AND METHODS

Location of study

This study was carried out in Microbiology Laboratory, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State Nigeria.

Media Used/ Preparation

The media used included nutrient agar, blood agar, MacConkey agar and Mueller Hinton agar. They were prepared according to the manufacturer's instructions indicated on the media containers.

Sterilization of equipment

All glass were properly washed with detergents, rinsed in distilled water and sterilized at 160°C in hot air oven for 1 hour before usage, media were sterilized by autoclaving at 121°C for 15 minutes, while wire loops were sterilized by passing them through Bunsen burner flame until red hot.

Bacterial culture

Pure cultures of *Escherichia coli*, *Streptococcus mutans* and *Lactobacillus acidophilus* were obtained from Microbiology Laboratory of Irrua Specialist Teaching Hospital, Irrua, Edo State. The choice of choosing these bacterial cultures over all the numerous bacterial cultures was because from past research, these are the common frequent bacteria from the oral cavity that causes dental infections around Edo state. Following methods by Paula et al. (2020), these cultures were subsequently screened and identified by sub culturing them in Microbiology Laboratory in Ambrose Alli University, using Brain heart infusion agar for the *Streptococcus mutans*, Chocolate agar for *Lactobacillus acidophilus* and Nutrient agar for *Escherichia coli* and they were incubated at 37°C for 24 hours, characterization and identification of the bacterial isolates were done using standard biochemical tests and staining methods.

Evaluation of dentifrices (tooth paste)

This study was aimed at determining the different brands of dentifrices that are commonly used in Ekpoma community and as a result of this, four (4) different dentifrices were purchased from a supermarket in Ekpoma, for an *in vitro* determination of their antibacterial efficacy. The composition of these four dentifrices were listed out in Table 1. The four different dentifrices were labeled A, B, C and D respectively. Thereafter, selected solutions of each dentifrice were made by mixing 2g of each paste with 2ml of distilled water to give a 1:1 dilution factor. From the 1:1 dilution, different concentration of 1:2, 1:4 and 1:8 dilution factors were made on all the dentifrice by increasing the distilled water.

Antimicrobial assessment of the different concentrations of dentifrices on the bacterial isolates.

The antimicrobial assessment of the different concentrations of the four different dentifrices used was determined using the agar well diffusion method as described by Paula et al. (2020). Here, Nutrient agar plates were flooded with a 0.5ml of 0.5 McFarland standardized broth of each bacterial isolates freshly prepared. The plates were allowed to dry within a period of one hour, then a sterile 8mm Cork-borer was used to bore five wells in each of the Nutrient agar plates seeded with bacterial isolates. Thereafter, 0.2ml of the different concentrations of dentifrices (1:2, 1:4, 1:8) were introduced into 4 wells respectively while 0.2ml of distilled water was introduced into a 5th well to serve as control. The plates were incubated at 37°C for 24 hours.

After 24 hours incubation, plates were removed and assessed for Zone of clearance which were also measured, using the control well as a guide.

RESULT

Table 1 presents the compositions of the four dentifrices labeled A through D: A-Oral B, B -Colgate Total, C-Bentodent, and D-Toms. Among the antibacterial constituents, dentifrice A contains only sodium fluoride as its primary active agent. Dentifrice B combines sodium fluoride with triclosan, offering a dual antibacterial approach. Dentifrice C features propolis, while dentifrice D utilizes xylitol as its antibacterial component.

Table 1: Composition of dentifrices used

Dentifrices	Ingredients listed on packs
Oral B (A)	Phosphoric acid, Sorbitol, Aqua, hydrated silica, Sodium Lauryl, Sulfa, Aroam, Xanthangum, Sodium saccharin, Cocamidopropyl Betaine, sodium fluoride, mica, sodium Benzoate.
Colgate Total (B)	Triclosan, Aqua, sorbitol, Hydrated silica, glycerin, strontium acetate, sodium methyl cocoyl, Taurate, Xanthan gum, Aroma, Titanium dioxide, sodium saccharin, sodium fluoride, sodium propylparaben, Limonene.
Bentodent (C)	Aqua, Bentonite clay, Stevia extract, Tea tree oil, Mint oil, Clove oil, Natural glycolipids, Salt, Propolis, Humectants, Orange extracts.
Toms (D)	Calcium carbonate, Aqua, hydrated silica, Sodium Lauryl, Zinc citrate, Benzyl alcohol, Natural flavors, Sodium sulphate, Corrageenan, Xylitol, Glycerine, sodium Benzoate.

Table 2: Inhibition zones (mm) of the different concentrations

Dentifrices	Test bacteria	Inhibition zone (mm) of the different concentrations			Average zone of inhibition (mm)
		1:2	1:4	1:8	
A	<i>Escherichia coli</i>	22	14	9	14.6
	<i>Streptococcus mutans</i>	21	16	11	18.3
	<i>Lactobacillus acidophilus</i>	18	13	10	17
				Mean value	14.7
B	<i>Escherichia coli</i>	28	22	9	23
	<i>Streptococcus mutans</i>	22	18	15	18.3
	<i>Lactobacillus acidophilus</i>	23	17	11	17
				Mean value	19.4
C	<i>Escherichia coli</i>	19	16	9	14.6
	<i>Streptococcus mutans</i>	17	12	8	12.3
	<i>Lactobacillus acidophilus</i>	19	10	5	11.3
				Mean value	12.7
D	<i>Escherichia coli</i>	19	12	8	13
	<i>Streptococcus mutans</i>	16	11	9	12
	<i>Lactobacillus acidophilus</i>	16	8	7	10.3
				Mean value	11.8

Dentifrices A–D represent the average zones of inhibition observed across different formulations: A -Oral, B-Colgate Total, C- Bentodent, and D -Toms.

In Table 2, the three bacterial isolates (*Escherichia coli*, *Streptococcus mutans* and *Lactobacillus acidophilus*) were tested for their resistant ability to different concentrations (1:2, 1:4 and 1:8) of the dentifrices A to D and it was shown from the average zone of inhibition that dentifrice B had the highest average zone of clearance (19.4mm) which shows it has the best antibacterial efficacy while, dentifrice D had the lowest average zone of clearance (11.8mm) in diameter, showing it has the least antibacterial efficacy.

A comparison of the three test bacteria, *Escherichia coli*, *Streptococcus mutans*, and *Lactobacillus acidophilus* across the four dentifrices (A–D) based on their average

zones of inhibition reveals notable differences. *Lactobacillus acidophilus* exhibited the lowest inhibition zone (11.3 mm), indicating greater resistance and suggesting it may be more challenging to eradicate. In contrast, *Escherichia coli* showed the highest zone of inhibition (23 mm), implying greater susceptibility to the dentifrices tested (Table 3).

Figure 1 depicts the average mean zone of inhibition of the different dentifrices (A- Oral, B- Colgate total, C- Bentodent and D- Toms) on each bacterial isolates where it shows that dentifrice A had a mean value of 14.7mm, B had 19.4mm, C had 12.7mm and D had 11.8mm respectively.

Table 3: Comparison of the test bacteria on the dentifrices used based on their average zone of inhibition

Dentifrices	Lowest zone of inhibition (mm)	Highest zone of inhibition (mm)
A	<i>Escherichia coli</i> (14.6)	<i>Streptococcus mutans</i> (18.3)
B	<i>Lactobacillus acidophilus</i> (17)	<i>Escherichia coli</i> (23)
C	<i>Lactobacillus acidophilus</i> (11.3)	<i>Escherichia coli</i> (14.6)
D	<i>Lactobacillus acidophilus</i> (10.3)	<i>Escherichia coli</i> (13)

Dentifrices A–D represent the average zones of inhibition observed across different formulations: A -Oral, B- Colgate Total, C- Bentodent, and D -Toms.

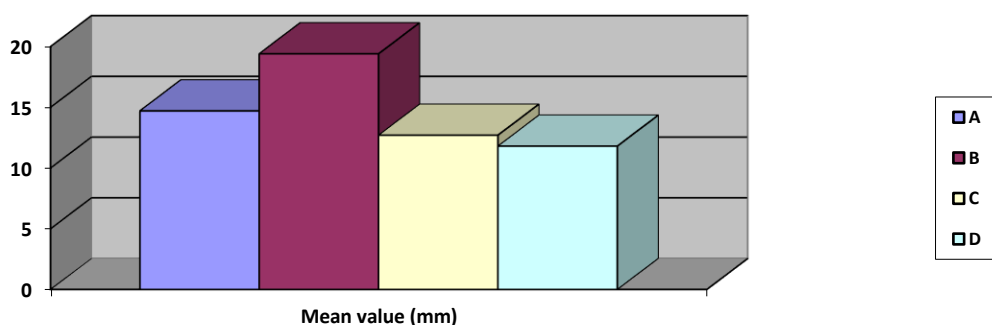


Figure 1: Mean Zones of inhibition of different dentifrices used on the test isolates

DISCUSSION

Proper oral hygiene is the main key to preventing dental diseases. These diseases such as dental plaque, dental caries, are majorly caused by adherence and multiplication of some bacteria and other etiological agents such as fungi. Once present in the saliva and gum, they multiply and in turn destroy the hard enamel causing dental diseases (Aspinall et al., 2021). The need to keep these oral health organisms to a level consistent with oral health by including antimicrobial agent to the dentifrices so as to eliminate or inhibit microbes in the oral cavity (Bowen et al., 2018). In this present study, four different brands of dentifrices designated A, B, C and D (brand names in table 1) were used to test for their antimicrobial activity against 3 dental pathogens viz; *Escherichia coli*, *Lactobacillus acidophilus* and *Streptococcus mutans*, obtained from Irrua Specialist Teaching Hospital Laboratory. It was found that all the four different dentifrices showed antibacterial activity against the three bacterial isolates revealing varying zones of inhibition for any given concentration/dilution.

The results of the incidence of the inhibition of bacteria by different concentrations of different dentifrices (toothpaste) sold in Ekpoma revealed that dentifrice B had the best antibacterial effect (19.4mm) on these bacterial isolates giving the highest inhibition zone diameter. A quick review from table 1, showing the different compositions of these dentifrices (A-D) showed that only dentifrice B had Triclosan and sodium fluoride as part of its chemical Composition. Triclosan [5-Chloro-2-(2, 4-dicloro phenoxy) phenol] has

been used for more than 50 years as a general antibacterial and anti-fungal agent. It has recently been suggested that triclosan acts on bacteria by blocking lipid biosynthesis through inhibition of the enzyme enoyl-acyl carrier protein reductase (Zhao et al., 2020).

Table 2 presented a holistic view of the different level of efficacy (zone of clearance) of the dentifrices (A to D) tested against the three bacterial isolates (*Escherichia coli*, *Streptococcus mutans* and *Lactobacillus acidophilus*) based on the different concentrations and table 3 showed the average zone of clearance (mm), calculated from the different concentrations in table 2 to depict the level of efficacy of each dentifrice. Dentifrice A, (14.7mm) in its composition has the presence of sodium fluoride, which according to Simon-soro et al. (2022), is also an antimicrobial agent. It has also been shown by Fernando et al. (2015) that the presence of sodium fluoride on dentifrices impacts a good antimicrobial characteristic to the products, while the zones of inhibition of dentifrices C and D were 12.7mm and 11.8mm respectively. Figure 1 briefly showed the different average zone size (mm) of the dentifrices and also, with a quick glance through table 1, in, their chemical composition dentifrice C and D, both lack triclosan and sodium fluoride but also has some other antibacterial constituents. It was also shown from this research that *Lactobacillus acidophilus* had the lowest zone of clearance to almost all the dentifrices used in this study, while *Escherichia coli* always had the highest zone of clearance to almost all the dentifrices used. It is therefore worthy of the note that the presence of

Lactobacillus acidophilus in the oral cavity (tooth, saliva, gum) could pose a greater threat than other bacteria used for this study. This consistent lower zone of clearance could also be due to the emergence of resistant genes to these dentifrices used for this study (especially to the antimicrobial constituents in the dentifrices).

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CONCLUSION

Results from this study has shown that dentifrices containing a mixture of triclosan and sodium fluoride formulation were more effective in controlling the oral bacterial pathogens compared to dentifrices containing only sodium fluoride or other antimicrobial constituents and has also revealed that the presence of *Lactobacillus acidophilus* in the oral cavity could prove more resistant to eradicate with some dentifrices.