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Correlative antimicrobial assay of various foreign toothpaste against oral microbial isolates

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Abstract

The *in-vitro* antimicrobial assay of four (4) foreign toothpastes sold in Nigeria viz; Sensodyne®, Raiya®, Mentadent® and Colgate® were carried out using agar well diffusion method against eight (8) oral clinical isolates which include the strains of *Streptococcus mutans*, *Brahamella catarrhalis*, *Veillonella* spp, *Micrococcus luteus*, *Lactobacillus* spp, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Candida albicans*. The statistical analysis was done using SPSS windows, by applying mean value, T-test, analysis of variance (ANOVA) with Post-hoc least significant differences (LSD) method ($\alpha = 0.05$). The result showed that based on the mean diameter of the zone of microbial inhibition produced by the toothpaste in agar well diffusion method, Mentadent® showed maximum zone of inhibition against the entire test organisms with *Streptococcus pneumoniae* being the most inhibited (sig< 0.01) compared to all other toothpaste formulations. Colgate® gave the least antimicrobial activity, whereas *Micrococcus luteus* was the least inhibited among the eight (8) isolates. Although all the toothpaste selected for this study had antimicrobial properties, Mentadent® distinguish itself because of its improved formulation of 2 % zinc citrate. There is an urgent need for toothpaste manufacturers to periodically review their formulations in order to improve the oral health of its users.

Keywords: Antimicrobial activity; Toothpaste; Oral microbes; Zone of inhibition

1 Introduction

Toothpaste is a gel or powder dentifrice used with toothbrush to clean and maintain the aesthetic and health of teeth [1]. It is used to promote oral hygiene and to prevent tooth decay as it aids in the removal of dental plaque and food particles from the teeth. It also assists in suppressing halitosis and delivers active ingredients such as xylitol and fluoride to the mouth that prevent tooth and gum disease (Gingivitis). Toothpaste has been used since antiquity [2] and is one of the main dispensable elements of Oral health care [3].

The production of toothpastes started in India and China about 300-500BC. Initially, crushed bone and crushed egg shells were formally used as an abrasive in tooth for Oral health care [4]. Modern toothpastes contain emulsifying agents such as sodium lauryl sulphate [5] and essentials oils, such as Eucapol, Menthol and Thymol, frequently used as flavour which also contribute to the antiseptic property of the products [6]. These substances kill microorganisms by disrupting their activity, prevents bacterial aggression and slows bacterial multiplication [7].

Toothpastes are designed and formulated to solve dental problems such as cavity plaque, gingivitis, tartar and to prevent fluorosis in children. They also ensure tooth whitening, tooth sensitivity and control fresh breath. They are classified as drug because they contain active ingredients that maintain a balance in each person's oral microbial population and prevent opportunistic microorganisms (which can proliferate when the balance is lost) and initiate

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disease process [8]. Toothpaste usually contains active ingredients, the first active ingredients included fluoride which improves the health of the teeth and helps the tooth enamel to resist cavities and decay [2]. It also helps the enamel to remain strong so that it can resist acid producing oral bacteria when sugary foods are eaten [4]. Other therapeutic agents that may be added to toothpaste includes Potassium nitrate, Pyrophosphate or zinc citrate, Triclosan and stannous fluoride. Potassium nitrate is used to treat dental hypersensitivity, Pyrophosphate or zinc citrate prevents tartar build up, and various abrasives or enzymes, to help whitening teeth while Triclosan and stannous fluoride reduce gingival inflammation [5].

The selection of appropriate toothpaste may provide a positive influence on the prevention of allergic reactions and tooth decay [3]. Since fluoride is not the only active ingredient in toothpaste, other important factors considered when selecting a toothpaste are the cleaning abilities of toothpastes as provided by abrasives [9], the antimicrobial qualities, which in turn are provided by a variety of substance with different abilities to inhibit the growth of germs in oral cavity [10], as well as a number of ingredients with specific purpose to solve specific problems. However, the wide selection of various ingredients make it difficult for patient to choose the proper toothpaste and complicated by the endorsement of dental product by professionals. Therefore, this study was aimed at determining the antimicrobial quality of different toothpaste to ascertain their effectiveness in reducing oral bacteria and destroying oral pathogenic microbes in order to improve dental health.

2   Material and methods

2.1   Sample collection

The toothpaste samples used for this study where purchased commercially from toothpaste vendors in New Benin market, Benin-City, Edo State, Nigeria. Four foreign toothpaste were used in all, these include: Colgate®, Mentadent®, Raiya®and Sensodyne®as shown in table 1. The samples were kept at room temperature and were analyzed within one week of collection.

Table 1 Sample of selected toothpaste with their active ingredients

Toothpaste sample	Foreign toothpaste	Active ingredient
Sample 1	Sensodyne®	Sodium fluoride 0.315% w/w
Sample 2	Mentadent®	Sodium fluoride 0.32% and zinc citrate 2%
Sample 3	Raiya®	Sodium monofluorophosphate 0.84% w/w
Sample 4	Colgate®	Sodium monofluorophosphate 1.1% w/w

2.1.1   Collection of test organisms

Strains of *Streptococcus mutans, Branhamella catarrhlis, Veillonella spp, Micrococcus lutteus, Lactobacillus spp, Staphylococcus aureus, Streptococcus pneumoniae* and *Candida albicans* were used for these study. The pure isolates of these organisms were obtained from the oral cavities of healthy young informed-consenting adults and confirmed using standard cultural, morphological and biochemical characteristics [11] [12]. They were kept and sustained in the Microbiology Laboratory unit of Auchi Polytechnic, Auchi, Edo State in appropriate agar slants.

2.2   Sample preparation

1 g of each foreign toothpaste sample was weighed and dissolved in 10 ml of sterile water to make the stock solution.

2.3   Culture media

General purpose nutrient media, enrichment medium and other appropriate selection media were employed in the sub-culturing and conformation of specific organisms used in the study which include: Nutrient agar, Blood agar, Mannitol salt agar, Muller Hilton (LAB. M. England), peptone water (Oxoid, England) etc. They were all prepared according to manufacturer's specifications.

2.4 Assessment of antimicrobial effect using agar well method

The test organisms were sub-cultured into their appropriate broth cultures for 3 hours. The turbid broth was diluted and standardized to be equivalent to 0.5 McFarland standard. Thereafter, 0.02 ml of each of the test organism were immediately transferred into the Petri-dish before 20 ml of Muller Hilton agar was added, mixed by swirling and allow to solidify. A standard cork borer measuring 6 mm diameter was used to make the agar well. Thereafter, 0.1 ml of the prepared dilution of each foreign toothpaste sample was introduced onto the seeded plates in triplicates using a syringe, the plates were labelled accordingly and allowed to stand for 30 minutes to allow for diffusion. The plates were incubated at 37°C for 24 hours and their inhibition zone diameters were obtained in cm using ruler.

3 Results and discussion

Table 2 Confirmatory biochemical characterization of the microbial isolates

	Biochemical characteristic								Oral clinical isolates
Gram rxn	Mot.	Cat.	Coag.	Oxid.	Indole	Methylred	Ur	Nitrate	
+	-	+	+	-	-	-	-	ND	*Streptococcus mutans*
-	-	+	ND	+	ND	ND	ND	ND	*Branhamella catarrhalis*
-	-	-	ND	+	-	ND	-	+	*Veillonella* spp
+	-	+	-	ND	ND	ND	-	-	*Micrococcus luteus*
+	-	-	ND	-	-	-	-	-	*Lactobacillus* spp
+	-	+	+	-	-	+	+	+	*Staphylococcus aureus*
+	-	-	ND	-	ND	ND	-	ND	*Streptococcus pneumoniae*
ND	ND	ND	ND	ND	ND	ND	ND	ND	*Candida albicans*

Keys: + = present, - = Absent, Mot.= Motility, Oxid= Oxidase,Ur= Urase, Cat.=Catalase, Coag.=Coagulase ND = not determined
Ur = Urease

Table 3 Sample analysis of variance

		Value Label	N
Experimental sample	1.00	Sample 1	8
	2.00	Sample 2	8
	3.00	Sample 3	8
	4.00	Sample 4	8
Organisms	1.00	*Streptococcus mutans*	4
	2.00	*Branhamella catarrhalis*	4
	3.00	*Veillonella* spp	4
	4.00	*Micrococcus luteus*	4
	5.00	*Lactobacillus* spp	4
	6.00	*Staphylococcus aureus*	4
	7.00	*Streptococcus pneumoniae*	4
	8.00	*Candida albicans*	4

Dependent variable: inhibition zone diameter (cm³)

Table 4 ANOVA Result

Source	Type I Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Sample	.182	3	.061	.572	.640	.076
Organism	8.018	7	1.145	10.777	.000	.782
Error	2.232	21	.106			
Corrected Total	10.432	31				

R Squared = .786 (Adjusted R Squared = .684

The ANOVA shows that the organisms are not statistically significant at sig greater 0.01 at 1%. R-Square measures the accuracy of the experiment. The value of R-square is 0.786 indicating the samples experiment and organism for inhibition diameter is accurate 78.6 %. Adj r-square value of 0.684 implies the inhibition zone diameter explained the samples and organisms chosen for the experiment.

Table 5 Estimated marginal means

Experimental Sample	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Sample 1	1.156	.115	.917	1.396
Sample 2	1.204	.115	.964	1.443
Sample 3	1.129	.115	.889	1.368
Sample 4	1.000	.115	.760	1.240

Dependent variable: Inhibition zone diameters (cm^3)

The best sample against the test organism had the highest mean and confidence interval values respectively. Therefore from this study, among the four samples selected, sample 2 had the highest mean value of 1.204 and highest inhibition zone diameter.

Table 6 Homogeneous subset

	Experimental Sample	N	Subset
			1
	Sample 4	8	1.0000
	Sample 3	8	1.1288
Duncan[a,b]	Sample 1	8	1.1563
	Sample 2	8	1.2038
	Sig.		.265

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is mean square (Error) = .106.
a. Uses Harmonic Mean Sample Size = 8.000. b. Alpha = .05. Inhabitation zone diameters (cm^3)

Table 7 Subject factors

		Value Label	N
Tooth Paste	1.00	FPA	8
	2.00	FPB	8
	3.00	FPC	8
	4.00	FPD	8
Organism	1.00	*Streptococcus mutans*	4
	2.00	*Branhamella catarrhalis*	4
	3.00	*Veillonella* spp	4
	4.00	*Micrococcus luteus*	4
	5.00	*Lactobacillus* spp	4
	6.00	*Staphylococcus aureus*	4
	7.00	*Streptococcus pneumoniae*	4
	8.00	*Candida albicans*	4

Key: FP = Foreign Paste
Dependent Variable: Inhabitation zone diameters (cm³)

Table 8 Subject effects

Source	Type I Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Samples	3.383	3	1.128	3.924	.023	.359
Anti	4.020	7	.574	1.998	.104	.400
Error	6.035	21	.287			
Corrected Total	13.437	31				

R Squared = .551 (Adjusted R Squared = .337)

The ANOVA shows that the organisms are not statistically significant at sig greater 0.01 at 1%. This implies that the samples used on the experiment are different but the organism have the same inhibition diameter values. R-Square is measure the accuracy of the experiment the value of R-SQUARE is 0.551 indicates that the experiment of samples and organize for inhibition diameter is accurate 55.1%. Adj R-Square value of 0.337 implies the inhibition zone diameter explained 33.7% samples and organisms chosen for the experiment.

Table 9 Organisms

Test Organisms	Mean	Std. Error	95% confidence interval	
			Lower Bound	Upper Bound
Streptococcus mutans	1.158	.268	.600	1.715
Branhamella catarrhalis	.525	.268	-.032	1.082
Veillonella spp.	.558	.268	.000	1.115
Micrococcus luteus	.250	.268	-.307	.807
Lactobacillus spp.	1.170	.268	.613	1.727
Staphylococcus aureus	.838	.268	.280	1.395
Streptococcus pneumoniae	1.345	.268	.788	1.902
Candida albicans	.958	.268	.400	1.515

Streptococcus pneumoniae has the highest mean value of 1.345, indicating that among the eight (8) organisms *Streptococcus pneumoniae* had the highest inhibition.

Table 10 Post hoc tests

	(I) Tooth Paste	(J) Tooth Paste	Mean Difference(I-J)	Std. Error	Sig.	95% Confidence Interval
						Lower Bound
		FPB	.0963	.26803	.723	-.4611
	FPA	FPC	-.0063	.26803	.982	-.5636
		FPD	.7750*	.26803	.009	.2176
		FPA	-.0963	.26803	.723	-.6536
	FPB	FPC	-.1025	.26803	.706	-.6599
LSD		FPD	.6788*	.26803	.019	.1214
		FPA	.0063	.26803	.982	-.5511
	FPC	FPB	.1025	.26803	.706	-.4549
		FPD	.7813*	.26803	.008	.2239
		FPA	-.7750*	.26803	.009	-1.3324
	FPD	FPB	-.6788*	.26803	.019	-1.2361
		FPC	-.7813*	.26803	.008	-1.3386

Key: FP= Foreign Paste

Toothpaste (multiple comparism) Dependent variable: inhibition zone diameters (cm)

Table 11 Homogeneous subset

Inhibition zone diameters **Organism** (cm).		N	Subset	
			1	2
	Micrococcus luteus	4	.2500	
	Branhamella catarrhalis	4	.5250	.5250
	Veillanella spp	4	.5575	.5575
	Staphylococcus aureus	4	.8375	.8375
Duncan[a,b]	*Candida albicans*	4	.9575	.9575
	Streptococcus mutans	4		1.1575
	Lactobacillus spp	4		1.1700
	Streptococcus pneumoniae	4		1.3450
	Sig.		.107	.070

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = .287.
a. Uses Harmonic Mean Sample Size = 4.000. b. Alpha = .05.

4 Discussion

The *in-vitro* antimicrobial assay of four (4) toothpaste (Sensodyne®, Raiya®, Mentadent® and Colgate®) was tested against eight oral clinical isolates which includes strains of *Streptococcus mutans*, *Brahamella catarrhalis*, *Veillonella* spp, *Micrococcus luteus*, *Lactobacillus* spp, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Candida albicans*. These microbes are commonly found inhuman mouth (oral cavity) as microbial flora or pathogenic microbes before brushing the mouth [13].

The results of the antimicrobial assay of the different foreign toothpaste revealed that they were active against all the tested organisms. This agrees with the findings of Nascimento *et al* which stated that the active substance present in toothpastes kill microorganisms by disruptive activity, preventing bacterial aggression and slow bacterial multiplication [7]. However, among the selected toothpaste, Mentadent® had the highest zone of inhibition against the test organisms followed by Sensodyne® as shown in Table 6. Mentadent® toothpaste formulations includes zinc citrate and other additives in addition to fluoride, which is one of the major active component that helps to increase the

antimicrobial activity of toothpaste. Scully and Bagan, reported that zinc citrate, triclosan and stannous fluoride reduce gingival inflammation and enhances the antimicrobial effect of toothpaste hence, can reduce the oral microbial load when used to wash or brush the mouth [5].

The result obtained from this research also revealed that Colgate® had the least antimicrobial activity against the tested organisms. This could be as a result of the continuous use of the toothpaste over a long period of time that has allowed the organisms to develop resistance against the active ingredients of the toothpaste. This was in agreement with [7] which revealed that microorganisms have the ability to develop resistant against antimicrobial substance when used for over a long period of time through genetic mutation or transfer of resistant gene and become resistant or less susceptible to the substance.

Among the eight organisms tested as shown in Table 9, *Micrococcus luteus* was the least inhibited having the lowest diameter of inhibition while *Streptococcus pneumoniae* had the highest mean value of 1.345 cm. *Streptococcus mutans* which is one of the main opportunistic pathogens of dental caries [14], has an inhibition mean value of 1.158 cm. This pathogen play central roles in fermenting carbohydrates resulting in acid production which could lead to demineralization of the tooth enamel [15]. In addition, *Candida albicans* which is usually associated with active caries lesions in immuno-compromised patients has an inhibition mean value of 0.958 cm. Poor oral hygiene is also one of the reasons responsible for the growth of this microbe as well as their harmful activities. According to the research conducted by Ledder and his colleagues, the fungus (*Candida albicans*) was the most common yeast isolated from the oral cavity, infected root canals, showing resistance to inter-canal medication [16]. It causes oral thrush in which curd-like white patches formed inside the mouth, on the tongue and palate and around the lips of immuno-compromised persons, it may also cause cracked, red, moist areas of skin at the corners of the mouth [17]. Therefore, toothpaste as a mouth cleaning agent should be carefully selected and used twice daily as recommended by Dental Association in order to effectively maintain oral hygiene and prevent tooth decay.

5 Conclusion

The correlative *in-vitro* antimicrobial assay of various foreign toothpaste against oral microbial isolates showed that all the toothpaste selected for this study had antimicrobial properties but, Mentadent distinguish itself by having the highest zone of inhibition against the tested organisms because of its improved formulation of 2% zinc citrate. Therefore, Mentadent appears to be more effective than other selected toothpastes. There is an urgent need for toothpaste manufacturers to periodically review their formulations in order to improve the oral health of its users.

6 Compliance with ethical standards

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Disclosure of conflict of interest

The authors hereby declared that there was no conflict of interest in carrying out this research work.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

Statement of informed consent

The present research work does not contain any information about any individual.

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