©IDOSR PUBLICATIONS
International Digital Organization for Scientific Research ISSN: 2579-0781
IDOSR JOURNAL OF EXPERIMENTAL SCIENCES 2(1): 1-20, 2017.

Antimicrobial Effects of Toothpastes on some Oral Organisms of Dental Caries Origin

Tasie, Florita O., Anunike, Irene A. and Nwankwo, J. I.

Department of Applied Microbiology and Brewing, Faculty of Applied and Natural Sciences, Enugu State University of Science and Technology, PMB 01660, Enugu Nigeria.

ABSTRACT

This work was carried out to assess the antimicrobial activities of thirteen commercialized toothpastes against some oral organisms of dental caries origin. Staphylococcus aureus, Escherichia coli, Streptococcus mutans, Lactobacillus acidophilus and Candida albicans were used as test organisms. The antimicrobial activity was carried out using Kirby-Bauer disc diffusion method and was evaluated by measuring the diameter of zones of inhibition in mm. Minimum inhibitory concentration (MIC), minimum, bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were evaluated using the broth tube dilution method. Colgate (Herbal) had the lowest mean pH of 6.41 while Pepsodent had the highest mean pH of 7.70. Of all the toothpastes used, only Sensodyne was sensitive to all the test organisms. All the toothpastes were active against S.aureus with sensodyne having the highest mean zone of inhibition diameter of 25.0 ± 1.92mm and MIC/MBC of 1.25mg/ml and 2.50mg/ml respectively. Pepsodent had the lowest mean zone of inhibition diameter of 6.25 ± 0.64mm with MIC and MBC of 2.50mg/ml and 5.00mg/ml respectively. Escherichia coli, Lactobacillus acidophilus and Streptococcus mutans showed total resistance to Close-up, Biosalt, Holdent, Dabur, Mymy, Oral B, Pepsodent and Colgate. Regular cleaning of the teeth with toothpaste containing fluoride and a diet low in sugar is recommended to maintain healthy teeth.

Keywords: Antimicrobial, Toothpaste, Oral organism and Dental caries.

INTRODUCTION

Dental caries is a microbial disease that results in destruction of the mineralized tissue of the teeth initiated by lactic acid, butyric acid and other organic acids such as acetic acid, propionic acid, glutamic acid and aspartic acid which are produced as a result of bacterial fermentation of carbohydrates on the tooth surface. The bacteria responsible for dental caries include: *Actinomyces odontolyticus, Corynebacterium spp; Lactobacillus acidophilus, Streptococcus mutans, Escherichia coli, Staphilococcus aureus, Candida albicans* [1]. The acids produced by these bacteria reduce the pH of the mouth to less than 5.5 at which decalcification and loss of phosphate from the enamel occur resulting to dental decay [2]. This loss of calcium and phosphate is called demineralization [2].

The early manifestation of the caries process is a small patch of demineralised enamel at the tooth surface, often hidden from sight in the fissures of teeth or in between the teeth. The destruction spreads into the softer, sensitive part of the tooth beneath the enamel. The weakened enamel then collapses to form a cavity and the tooth is progressively destroyed [3]. Lactose and galactose are less cariogenic but sugars such as sucrose, fructose, glucose and maltose commonly found in human foods are capable of causing caries because they are readily fermented by bacteria in the mouth [4]. Modern toothpaste was invented to aid in the removal of foreign particles and food substances in addition to cleaning of tooth. The use of plant extracts and phytochemicals, both with known antimicrobial properties can be of great significance in therapeutic treatments [5]. The fluoride ion (F-) inhibits the bacterial enzyme enolase, thereby interfering with production of phosphoenol pyruvate (PEP). PEP is a key intermediate of the glycolytic pathway, and in many bacteria, it is a source of energy and phosphate needed for sugar uptake. The presence of 10 – 100ppm of fluoride ions, inhibits acid production by most plaque bacteria [6]. Dental floss is a cord of thin filaments used to remove food and dental plaque from between the teeth especially where tooth paste is not available [7]. As the build-up of plaque is the primary cause of dental disease, such as gingivitis and dental caries, the use of floss prevents these conditions from developing [8]. Chewing gum with xylitol prevent caries because it cannot be fermented by *Streptococcus mutans* [9]. Xylitol is non-cariogenic when substituted for sucrose in either foods or chewing gum. Silvertone, (1983) [10] reported that consumption of cheese provides phosphates which prevents demineralization of the enamel.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

The thirteen different toothpastes were purchased from open markets in Oko, Ekwulobia and Awka, in Anambra State, Nigeria.

COLLECTION OF TEST ORGANISMS

The test organisms were all obtained from Microbiology Laboratory, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

MEDIA USED

The media used were prepared according to manufacturer's specifications and autoclaved at 121°C for 15mins. The media include:- Mueller Hinton agar, Nutrient agar, Nutrient broth, Sabourand Dextrose agar, Sabourand Dextrose broth, Eosin Methylene Blue (EMB) agar and Manitol Salt agar (MSA).

CONFIRMATION OF TEST ORGANISMS

Confirmatory tests were carried out on the test organisms according to the methods of Ciancio, (2003) [11]. These include Gram staining, inoculation of *E.coli* test organism on Eosin Methylene Blue (EMB) agar. *Staphylococcus aureus* on Manitol Salt Agar (MSA). *Streptococcus mutans* was inoculated on blood agar. Lactophenol wet mount of the yeast isolate was performed and examined under the microscope using X 10 and X 40 objective lens. The organism was also streaked on Candida Chromogenic agar. The stock cultures were stored at 4°C in Nutrient Agar and Sabourand Dexttrose Agar slants. For bacteria and yeast respectively.

PREPARATION OF INOCULUM

The test organisms were inoculated into nutrient broth and sabourand dextrose broth and incubated at 37°C for 24 and 48 hours respectively.The resulting turbidity was adjusted to 0.5 McFarland turbidity standards using the same broth medium. The broth cultures were diluted 1:200 by mixing 0.1ml of the inoculum in 19.9ml of the broth respectively. This gave working inocula that contained 10^{-5}-10^{-6} organisms/ml which were used within 30minutes.

PREPARATION OF PAPER DISCS

Discs were prepared by cutting out 6mm size from Whatman no.1 grade filter paper using a mechanical perforator. The discs were sterilized in a glass petri dish using hot air oven at 100ºC for 1hour.

PREPARATION OF TOOTHPASTES FOR SENSITIVITY TEST

Ten gramme (10g) of each of the selected toothpaste was dissolved in 10ml sterile distilled water to obtain a concentration of 1:1, this was further diluted serially to obtain concentration of 1:2, 1:4, 1:8, 1:16 respectively.

STERILITY OF THE TOOTHPASTES

Solutions of the different toothpastes were prepared and streaked on sterile nutrient agar contained in Petri plates and incubated for 24 hours at 37°C.

SENSITIVITY TESTS

Sensitivity tests were carried out using disc diffusion sensitivity test (Kirby-Bauer disc-diffusion method). The test organisms were swabbed uniformly across the culture plates using a glass spreader. Filter paper disc, impregnated with 0.1ml of the toothpastes at different concentrations dried at room temperature were placed on the surface of the agar using a sterile forcep. The different concentrations were marked with a marker. Discs that were not impregnated were placed in the center of the plates to serve as control and incubated at 37ºC for 24hours for bacteria and 48 hours for yeast. The clear zone where there were no colonies was regarded as the zones of inhibition. Thus, the sizes of the zones of inhibition were a measure of the toothpaste effectiveness against the test organisms and were measured in millimeter (mm)[12].

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) USING BROTH TUBE DILUTION METHOD

The MIC values were determined by broth tube dilution method. Two fold serial dilution of the toothpastes were prepared to get concentration ranges of 10mg/ml to 0.156mg/ml. Then 0.1ml of the standardized culture (test organism) was added to the different tubes and incubated at 37°C for 24 hours for bacterial cultures and 48hours for yeast culture. The tubes were checked for turbidity after incubation. The lowest or least concentration of the toothpaste that inhibited the growth (no growth or no turbidity) of the test organism is the MIC [13]. Negative controls were set up with sterile distilled and test organisms in the absence of the toothpaste.

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC) AND MINIMUM FUNGICIDAL CONCENTRATION (MFC)

Minimum Bactericidal Concentration and Minimum Fungicidal Concentration were determined from MIC. From those tubes showing no visible growth or turbidity in MIC, 0.1ml of the sample (tubes that showed no sign of growth) were inoculated on the sterile nutrient agar and SDA using streak plate method. The plates were incubated at

37ºC for 24 and 48hrs respectively. The least concentration that did not show any growth of the test organism was evaluated as the MBC or MFC [14].

STATISTICAL ANALYSIS

Collected data were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS), where P-values <0.05 were considered statistically significant.

RESULTS

Table 1: Toothpastes Composition, Batch No, Manufacture Date and Expiry Date.

S/N	TOOTH PASTE	COMPOSITION	BATCH NO	MANUFACTURE DATE	EXPIRY DATE
1.	Close up (Deep Action)	Sodium fluoride, sorbitol, aqua, sodium saccharine, hydrated silica, sodium lauryl sulphate, PEG-32, aroma, cellulose, gum, zinc sulphate, mica, sodium hydroxide, glycerine, Eugenol	140733	12/08/14	12/08/16
2.	Close up (Herbal)	Sodium fluoride, sorbitol, aqua, sodium saccharine, hydrated silica, sodium lauryl sulphate, PEG-32, aroma, cellulose, gum, zinc sulphate, mica, sodium hydroxide, glycerine, aloe barbadensis leaf extract, limonene, color, eucalyptus, peppermint, sage, thyme, leaf extract.	135699	03/06/14	03/06/16
3.	Close up (Fire Freeze)	Sodium fluoride, sorbitol, aqua, sodium saccharine, hydrated silica, sodium lauryl sulphate, PEG-32, aroma, cellulose, gum, zinc sulphate, mica, sodium hydroxide,	162327	01/03/14	01/03/16

		glycerine, eugenol			
4.	Colgate (Herbal)	Sodium monofluorophosphate, calcium carbonate, water, sorbitol, hydrated silica, sodium lauryl sulphate, flavour, cellulose gum, magnesium aluminium silicate, sodium saccharin, sodium carbonate, benzyl alcohol sodium bicarbonate, eugenol.	25612	06/08/14	06/08/16
5.	Dabur	Basil oil, herbal extract, chalk, sodium lauryl sulphate, ginger, peppermint, spearmint, Eucalyptus and lemon oils.	1230	08/14	08/16
6.	Macleans	Sodium fluoride, sorbitol, aqua, sodium saccharine, hydrated silica, sodium lauryl sulphate,PEG-32, aroma, cellulose gum.	0031AT	29/10/13	29/10/15
7.	Oral-B	Sodium fluoride, sorbitol, aqua, hydrated silica, sodium lauryl sulphate, aroma, cellulose gum, trisodium phosphate, sodium phosphate, sodium saccharine, carbomer.	401603 86CAF	01/14	01/16
8.	Pepsodent	Sodium monofluorophosphate, calcium carbonate, aqua, sorbitol, hydrated silica, sodium lauryl sulphate, aroma, cellulose gum, potassium, citrate,	142063	05/14	05/16

		trisodium phosphate, sodium saccharine, calcium.			
9.	Biosalt	Tocopheryl acetate, sodium chloride, hydrated silica, sodium fluoride, herbal extract, lonicera japonica, taraxacum green tea.	176	05/15	05/17
10.	Olive	Abrasive silica, Thickening silica, sorbitol, sodium carboxy methyl cellulose, sodium saccharine, sodium lauryl sulphate, PEG, Monosodium Phospahte, sodium fluoride, aqua, color, flavour.	B717	11/14	11/16
11.	Hodent	Sodium fluoride, triclosan, sorbitol, silica, water, flavour, sodium lauryl sulphate, cellulose gum, sodium saccharine, color.	SM101	11/13	11/15
12.	MyMy (Herbal)	Sodium monofluorophosphate, sorbitol, silica, sodium lauryl sulphate, binder, sodium saccharine, aqua, calcium carbonate, color, flavour, preservative.	13814	08/14	08/16
13.	Sensodyne	Potassium nitrate, sodium fluoride, fluoride ion, sodium monofluorophosphate, baking soda, zinc, zinc nitrate, flavour, vitamin E, vitamin B5	204BC	02/14	02/16

Table 2: Mean pH Reading of the Different Toothpastes

S/N	Toothpaste	First reading	Second reading	Mean
1.	Close up (Deep Action)	6.98	7.12	7.05
2.	Close up (Herbal)	6.9	7.10	7.0
3.	Close up (Fire Freeze)	7.0	7.1	7.09
4.	Colgate (Herbal)	6.36	6.45	6.41
5.	Dabur (Herbal)	7.65	6.99	7.32
6.	Macleans	6.46	6.47	6.47
7.	Oral-B	6.55	6.56	6.56
8.	Pepsodent	7.69	7.70	7.70
9.	Biosalt	6.88	6.89	6.89
10.	Olive	6.43	6.50	6.47
11.	Holdent	7.54	7.55	7.55
12.	MyMy (Herbal)	6.60	6.61	6.61
13.	Sensodyne	7.1	7.0	7.05

Table 3: Anti-microbial Activity of Toothpaste Formulations Against *Staphylococcus aureus* in (mm)

Toothpastes	1:1 Dilution Mean value ± std deviation	1:2 Dilution Mean value ± std deviation	1:4 Dilution Mean value ± std deviation	1:8 Dilution Mean value ± std deviation	1:16 Dilution Mean value ± std deviation
Biosalt	12 ± 1.63	11.5 ± 1.30	10.5 ± 1.30	8.5 ± 1.92	-
Colgate (Herbal)	12.25 ± 1.04	10.75 ± 0.91	10.25 ± 1.26	8.0 ± 0.62	6.5 ± 0.57
Close up (Fire Freeze)	19.75 ± 1.04	16.5 ± 0.90	12.75 ± 1.0	7.5 ± 0.82	-
Olive	14.5 ± 0.57	11.25 ± 0.99	10.25 ± 1.30	7.5 ± 0.57	6.6 ± 0.57
Sensodyne	25.0 ± 1.92	24.25 ± 1.66	21.0 ± 0.82	16.5 ± 1.0	8.5 ± 2.24
Holdent	12.75 ± 0.95	11.25 ± 0.95	10.5 ± 1.0	9.25 ± 0.71	7.5 ± 0.82
Close up (Herbal)	15.0 ± 0.99	12.0 ± 0.37	9.5 ± 0.58	-	-
Dabur (Herbal)	14.0 ± 0.82	12.5 ± 0.82	-	-	-
Macleans	15.75 ± 0.82	14.75± 0.82	12.5 ± 0.72	9.5 ± 0.70	-
MyMy (Herbal)	12.0 ± 0.82	10.5 ± 0.57	8.25 ± 1.0	7.5 ± 0.57	-
Oral-B	18.0 ± 0.82	14.0 ± 0.82	11.0 ± 0.82	10.0 ± 0.82	8.0 ± 0.82
Pepsodent	11.0 ± 1.15	9.5 ± 1.29	8.5 ± 0.89	7.0 ± 0.50	6.25 ± 0.64
Close up (Deep action)	19.25 ± 0.98	13.5 ± 0.92	10.25 ± 1.0	7.5 ± 0.84	-

Where std = Standard

Table 4: Antimicrobial Activity of Toothpastes Formulation Against *Candida albicans* n (mm)

Toothpastes	1:1 Dilution Mean value ± std deviation	1:2 Dilution Mean value ± std deviation	1:4 Dilution Mean value ± std deviation	1:8 Dilution Mean value ± std deviation	1:16 Dilution Mean value ± std deviation
Biosalt	20 ± 0.82	12.5 ± 1.30	-	-	-
Olive	18.0 ± 0.82	12.75 ± 0.96	9.0 ± 0.82	-	-
Sensodyne	20.6 ± 0.93	15.0 ± 0.98	10.5 ± 0.86	8.0 ± 0.57	-
Holdent	22.0 ± 0.82	16.0 ± 0.80	-	-	-
Close up (Herbal)	8.0 ± 0.70	-	-	-	-
Dabur (Herbal)	11.25 ± 0.71	10.0 ± 0.82	-	-	-
Macleans	10.25 ± 0.82	8.75 ± 0.57	-	-	-
MyMy (Herbal)	13.75 ± 0.71	11.5 ± 0.82	8.25 ± 0.71	-	-
Oral-B	13.0 ± 1.15	10.0 ± 1.63	-	-	-
Pepsodent	12.5 ± 1.60	11.75 ± 1.58	10.0 ± 1.38	-	-
Close up (Deep action)	11.5 ± 0.92	9.75 ± 0.88	7.75 ± 0.75	-	-
Close up (Fire Freeze)	-	-	-	-	-
Colgate	-	-	-	-	-

Where std = Standard

Table 5: Antimicrobial Activity of Toothpastes Formulations Against *Escherichia coli* in (mm)

TootHpastes	1:1 Dilution Mean value ± std deviation	1:2 Dilution Mean value ± std deviation	1:4 Dilution Mean value ± std deviation	1:8 Dilution Mean value ± std deviation	1:16 Dilution Mean value ± std deviation
Macleans	9.6 ± 0.98	-	-	-	-
Sensodyne	10.0 ± 1.41	9.25 ± 1.66	7.25 ± 0.25	-	-
Biosalt	-	-	-	-	-
Olive	-	-	-	-	-
Holdent	-	-	-	-	-
Close up (Herbal)	-	-	-	-	-
Dabur (Herbal)	-	-	-	-	-
Mymy (Herbal)	-	-	-	-	-
Oral B	-	-	-	-	-
Pepsodent	-	-	-	-	-
Close up (Deep action)	-	-	-	-	-
Close up (Fire freeze)	-	-	-	-	-
Colgate	-	-	-	-	-

Where std = Standard

Table 6: Antimicrobial Activity of Toothpaste Formulations Against *Lactobacillus acidophilus* in (mm)

Toothpastes	1:1 Dilution Mean value ± std deviation	1:2 Dilution Mean value ± std deviation	1:4 Dilution Mean value ± std deviation	1:8 Dilution Mean value ± std deviation	1:16 Dilution Mean value ± std deviation
Olive	7.25 ± 0.82	-	-	-	-
Sensodyne	10.25 ± 1.11	8.25 ± 0.75	7.25 ± 0.87	-	-
Macleans	-	-	-	-	-
Biosalt	-	-	-	-	-
Holdent	-	-	-	-	-
Close up (Herbal)	-	-	-	-	-
Dabur (Herbal)	-	-	-	-	-
Mymy (Herbal)	-	-	-	-	-
Oral B	-	-	-	-	-
Pepsodent	-	-	-	-	-
Close up (Deep action)	-	-	-	-	-
Close up (Fire freeze)	-	-	-	-	-
Colgate	-	-	-	-	-

Where std = Standard

Table 7: Antimicrobial Activity of Toothpaste Formulations Against *Streptococcus mutans* in (mm)

Toothpastes	1:1 Dilution Mean value ± std deviation	1:2 Dilution Mean value ± std deviation	1:4 Dilution Mean value ± std deviation	1:8 Dilution Mean value ± std deviation	1:16 Dilution Mean value ± std deviation
Sensodyne	12.75 ± 0.95	11.25 ± 0.95	10.5 ± 1.0	8.0 ± 0.82	-
Macleans	-	-	-	-	-
Biosalt	-	-	-	-	-
Olive	-	-	-	-	-
Holdent	-	-	-	-	-
Close up (Herbal)	-	-	-	-	-
Dabur (Herbal)	-	-	-	-	-
Mymy (Herbal)	-	-	-	-	-
Oral B	-	-	-	-	-
Pepsodent	-	-	-	-	-
Close up (Deep action)	-	-	-	-	-
Close up (Fire freeze)	-	-	-	-	-
Colgate	-	-	-	-	-

Where std = Standard

Table 8: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) of the Toothpastes Against the Test Organisms in mg/ml.

Toothpastes	Organisms	MIC	MBC	MFC
Sensodyne	*Staphylococcus aureus* *Escherichia coli* *Streptococcus mutans* *Lactobacillus acidophilus* *Candida albicans*	1.25 2.50 5.00 5.00 1.25	2.50 5.00 - - - 2.50	- - - -
Dabur (herbal)	*S. aureus* *C. albicans*	2.50 5.00	5.00 -	- -
Close up (deep action)	*S.aureus* *C. albicans*	2.50 -	5.00 -	- -
Oral-B	*S.aureus* *C. albicans*	5.00 5.00	- -	- -
Biosalt	*S.aureus* *C. albicans*	2.50 2.50	5.00 - 5.00	-
Colgate	*S.aureus*	2.50.	5.00	-
Olive	*S.aureus* *C. albicans* *L. acidophilus*	5.00 5.00 0.625	- - 1.25	- - -
Holdent	*S.aureus* *C. albicans*	2.50 2.50	5.00 - 5.00	-
MyMy	*S.aureus* *C. albicans*	5.00 5.00	- -	- -
Pepsodent	*S.aureus* *C. albicans*	2.50 -	5.00 -	- -
Macleans	*S.aureus* *C. albicans* *E. coli*	2.50 2.50 -	5.00 - 5.00 -	- -
Close up (Herbal)	*S.aureus* *C. albicans*	2.50 2.50	5.00 - 5.00	-
Close up (Freeze)	*S.aureus* *C. albicans*	5.00 2.50	- - 5.00	-

Table 1showed the composition, batch number, manufacture and expiry dates of the different toothpastes used in this work. Table 2 showed the mean pH readings of the different toothpastes. Pepsodent had the highest mean pH value of 7.7 while Colgate herbal had the least mean pH value of 6.41.Table 3 showed the antimicrobial activity of the toothpaste formulations against *Staphylococcus aureus*. Sensodyne had the highest mean zone of inhibition of 25.0 $\pm$ 1.92mm and Pepsodent had the lowest mean zone of inhibition of 6.25 $\pm$ 0.64mm at 1:16 dilution. Table 4 showed the antimicrobial activity of the toothpastes formulations against *Candida albicans*. All the toothpastes had activity against *C. albicans* except Close up (Fire freeze) and Colgate (herbal). Based on the findings, only Sensodyne and Macleans were active against *E. coli* in table 5. In table 6, only Olive and Sensodyne were sensitive to *Lactobacillus acidophilus*. Finally, in table 7 only Sensodyne showed activity against *Streptococcus mutans*. Table 8 showed the MIC/MFC in mg/ml of the test organisms.

STERILITY TEST OF THE TOOTHPASTES

No growths were seen on the nutrient agar plates after 24 hours incubation at 37°C. This confirmed the sterility of the toothpastes.

STATISTICAL ANALYSIS

The result of the statistical analysis carried out showed that there was significant difference ($P < 0.05$) in comparing the sensitivity of each test organism to the toothpastes using the one-way analysis of variance (one-way ANOVA) as shown in the appendices I, II, and IIIb. Whereas there was no siginificant different ($P \geq 0.05$) in the zones of inhibition produced by the two toothpastes compared on appendix IIIa. The two toothpastes compared had equal strength in inhibiting the test organism, *E. coli.*

DISCUSSION

Maintenance of good oral hygiene is the key to the prevention of dental diseases. The activities of oral micro flora being responsible for mouth odour and most oral diseases are not in doubt. The need to keep these oral organisms to a level consisted with oral health by antimicrobial agent inclusion in dentifrices has been stressed. When these substances are added to oral products in right proportions, they kill micro-organisms by disrupting their cell walls and inhibiting their enzymatic activities. Almost all the toothpastes used in this work would expire in 2016 except Biosalt toothpaste which will expire in 2017.

Colgate (Herbal) had the lowest mean pH value of 6.41 as against Pepsodent with the mean pH value of 7.70. Pepsodent contains calcium carbonate which must have brought about its high pH value. The range of pH values agrees partly with the work of Manupati (2011) who determined the pH of mouth washes and tooth-whitening products and reported that the pH range varied from 3.08 – 8.13. Driessens *et al.*, (1986) also reported that enamel demineralization can occur at a pH lower than 5.2 to 5.8. The need to keep the pH of dentifrices at alkaline range is important as that will affect the survival of these oral organisms which thrive well at acidic pH and cause more harmful effects of producing acids that demineralize the tooth enamel. Alkaline toothpastes will also help to neutralize the effects of acids produced by these oral organisms. The mean values $\pm$ standard deviation of zone of inhibition of the toothpaste formulations used at different dilutions were given for the test organisms in tables 3-7.

All the toothpastes used showed activity against *Staphylococcus aureus*. This could be as a result of the antimicrobial agents contained in them. Sensodyne showed maximum efficacy against the *S.aureus* with mean zone of inhibition of 25.0 $\pm$ 1.92mm with MIC and MBC of 1.25mg/ml and 2.50mg/ml respectively, while Pepsodent had the least mean zone of inhibition of 6.2 5 $\pm$ 0.64mm with MIC and MBC of 2.50mg/ml and 5.00mg/ml respectively. The zones of inhibition reduced significantly at higher dilutions.

In the antimicrobial activity of toothpastes formulations against *Candida albicans*, only Close up (fire freeze) and Colgate (herbal) had no activity on *C. albicans*.

Only Sensodyne at dilutions of 1:1, 1:2, and 1:4 and Macleans at dilution of 1:1 were sensitive to *E. coli*. Also only Olive at 1:1 dilution and Sensodyne at 1:1, 1:2 and 1:4 dilutions were sensitive to *Lactobacillus acidophilus*. Of all the toothpastes used only Sensodyne at dilutions of 1:1 to 1:8 showed activity against *Streptococcus mutans*. The other toothpastes formulations showed no activity. Among the toothpastes used in this study, only sensodyne contained Zinc nitrate in its formulations and had activity on all the test organisms. From this study, it was shown that Zinc nitrate posses strong antimicrobial activities against all the test organisms.

Herbal based products used exhibited appreciable effectiveness as other formulations. This may be due to the ingredients present. The herbal formulation studied appeared to be equally effective as the fluoride formulations which is in line with the works of George *et al.*, (2009), Amrutesh *et al.*, (2010)[15], who reported the effectiveness of herbal based toothpastes in control of plaque and gingivitis. Also the awareness created by the study of Fatima *et al.*, (2000)[16], who studied antibacterial activity possessed by medicinal plants used in tooth power and the efficacy of herbal-based toothpastes in the control of plaque and gingivitis respectively.

The sterility test performed on the toothpastes showed that all the toothpastes were sterile, this is in line with the work done by Okpalugo *et al.*, (2009)[17], who worked on the efficacy of toothpaste formulations in reducing oral flora. The test was important to ascertain quality assurance of these products. No visible growth was observed in any of the toothpastes indicating that manufacturers claims on quality can be trusted from this study.

CONCLUSION

As biofilm accumulation represents the principal etiologic agent for oral diseases including caries and periodontal diseases, the use of toothpaste with adequate/proper formulations is paramount. Since oral hygiene is claimed to be the major factor influencing the degree of dental caries and periodontal disease control.

This work revealed that toothpastes containing fluoride were more effective in inhibiting the growth of the test organisms compared to toothpastes containing plant extracts (herbal toothpastes) and toothpaste containing triclosan. This suggests that the use of fluoridated toothpastes will go a long way to control cases of dental caries and periodontal diseases.

REFERENCES

1. Touger – Decker. R. and VanLoveren, C. (2003). "Sugars and dental caries". *The American Journal of Clinical Nutritio,* ***78*** (4): 8815-8925.

2. Oztan, M.D., Klyan, M. and Gerceker, D. (2006). Antimicrobial effect, *in vitro* of guttan-perchia points containing root canal medications against Yeast and *Enterococcus faecalis. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontology,* ***102*** (3): 410 – 6.

3. Silk, H. (2014). "Disease of the mouth.". *Primary care*, ***41*** (1): 75 – 90.

4. Yao, L., Jeremanus, C., Barbetta, B., Choi, C. and Verbeke, P. (2010). Porphyromonas gingivitis infection. *Molecular Oral Microbiology,* ***25***: 89–101.

5. Tyagi, A.K. and Malik, A. (2011). Antimicrobial potential and chemical composition of *Eucalyptus globules* oil in liquid and vapour phase against food spoilage microorganisms. *Food Chemistry*, ***126*** (1) :228 – 235.

6. Anderson, T. (2004). "Dental Treatment In Medieval England". *British Dentistry Journal,****197*** (7): 419 – 25.

7. ADA (2010). American Dental Association. "Floss and other interdental cleanes". *TheJournal of the American Dental Association*, **9**(3): 139 – 144.

8. Hujoel, P.P., Cunha-Cruz, J., Banting, D.W. and Loesch, W.J. (2006). "Dental Flossing and Inter-proximal Caries: a systematic review". *Journal of Dental Research,* ***85***(4): 298 – 305.

9. Selwitz, R.H., Ismail, A. I. and Pitts, N.B. (2007). "Dental Caries". *Lancet,* ***369*** (9555): 51 – 9.

10. Silverstone, L. M. (1983). "Remineralization and enamel caries: new concepts". *Dental Updat,* ***10***(4): 261 – 73.

11. Ciancio, S. (2003). Improving oral health: Current considerations. *Journal of Clinical Periodontology,* **30** (5): 4-6.

12. Bauer, A.W., Kirby, M.D.K., Sherris, J.C. and Truck, M. (1966). Antibiotic susceptibility testing by standard single disc diffusion method. *American Journal of Clinical Pathology,* ***45***:486 – 493.

13. Bhttacharya, S., Haider, P.K. and Ghosh, A.K. (2009). *In vitro* effects of *Trichosanthes dioica* leaves on annelids and nematodes. *Pharmacology on Line*, ***2***:242 – 248.

14. Madigan, M.T., Martinko, J. M. and Parker, J. (1997). Biology of Microorganisms. In: Brook Biology of Microorganisms. 8th Edition. Prentice Hall International Inc. New York. pp.891-921.

15. Amrutesh, S., Malini, J., Tandur, P. S. and Patki, P.S. (2010). Clinical evaluation of novel herbal dental cream in plaque formation: a double blind, ramdomnized controlled clinical trial. *Journal of Experimental Pharmacolog,* ***2***:105-9.

16. Fatima, S., Farrooqi, A.H., Kumar, R., Kumar, T.R. and Khanuja, S.P. (2000). Antibacterial activity possessed by medicinal plants used in tooth powder. *Journal of Medicinal Aromatic Plants Science*, **22**: 187 – 189.

17. Okpalaugo, J., Ibrahim, K. and Inyang, U.S. (2009). Toothpaste formulation efficacy in reducing oral flora. *Tropical Journal of Pharmaceutical Research,* ***8***(1): 71-77.