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Antimicrobial Efficacy of Toothpastes Containing Fluoride against Clinically Isolated *Streptococci mutans*

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Authors' contributions

This work was carried out in collaboration among all authors. Author EEA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CUE, AJD, ALO, EEA and JIU managed the analyses of the study. Author EEA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Dental caries is one of the prevalent oral diseases which affect all ages. Many chemical substances with antimicrobial properties, are effective against microorganisms causing dental caries. The objective of this study was to check the antimicrobial efficacy of some toothpastes found in Enugu metropolis, Nigeria more specifically against clinically isolated *Streptococci mutans*. The antimicrobial efficacy of different brands of toothpastes marketed in Enugu metropolis was assayed against *Streptococci mutans* using the agar well diffusion method. The clinical *Streptococci mutans* used for this study were taken from carious lesions of patients that attended the clinic at Federal College of Dental Therapy and Technology, Trans Ekulu, Enugu, Nigeria and were isolated and cultivated using traditional laboratory methods. The present study has shown that toothpaste which contains mint extract as herbal constituent and sodium

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fluoride had the highest antimicrobial activity, followed by toothpastes containing only sodium monofluorophosphate. Herbal toothpaste with mint can be a safe and effective alternative in the prevention of dental caries.

Keywords: Dental caries; Streptococci mutans; toothpaste; fluoride; herbal.

1. INTRODUCTION

The oral cavity has a large number of gram-positive and gram-negative microorganisms. Growing as a biofilm, pathogens responsible for tooth decay, gingivitis, and periodontitis may find protection in this biofilm [1,2]. Dental problems from microbial infection are mainly of the following three types: formation of dental plaques, dental caries, and periodontal diseases [3].

Microorganisms play a significant role in the development of dental caries which is a multifactorial disease. Many bacteria are present in dental plaque. Based on traditional culture techniques it has been estimated that nearly 700 different types of bacteria exist in dental plaque. One of these is *S. mutans* which is responsible for the initiation and development of dental caries. Therefore to maintain a safe oral environment; the dental plaque must be removed regularly. This is achieved through chemical and mechanical means of oral hygiene. Most common in many homes is tooth brushing. Since brushing is not enough hence some antimicrobial agents like fluoride are incorporated into toothpastes to prevent diseases like dental carries and periondontitis [4,5]. Although these toothpastes containing fluoride are very active against bacteria like *S. mutans*, there is limited information on its efficacy against these microbes. The objective of this study therefore was to evaluate the antimicrobial efficacy of toothpastes containing fluoride against clinically isolated *S. mutans*.

2. MATERIALS AND METHODS

2.1 Toothpastes

Seven different brands of toothpaste were collected from the open market in Enugu Metropolis, Nigeria. Their identity profile is shown in Table 1.

2.2 Isolation and Identification of Bacteria Associated with Dental Caries Sample

Clinical isolates of *S. mutans* were obtained from carious lesions of patients that attended the clinic at Federal College of Dental Therapy and Technology, Trans Ekulu, Enugu. All reagents used for the studies were prepared and stored in a refrigerator. Subsequently, brain heart blood agar and infusion broth were prepared and autoclaved at 121^0C for 15 minutes. 20 ml of blood agar and brain infusion broth were dispensed into sterile bijou bottles and Petri dishes respectively. Sterile bijou bottles holding 20 ml of brain infusion were taken down to the surgery rooms. The tooth of patients that presented with a carious lesion was extracted by the dental surgeon under strict aseptic condition. Teeth extracted by the dental surgeon were introduced into sterile bijou bottles holding 20 ml of brain heart infusion broth and labelled accordingly. After 5 hours sterile swab sticks were inserted into the bijou bottles containing the extracted tooth and they were spread onto solid blood agar plates. The plates were developed at 37^0C for 48 hours aerobically and at 37^0C for 72 hours anaerobically using a gas pack for each patient sample. Colonies that grew were respectively subcultured into prepared blood agar using a streaking method for isolation of pure colonies. The plates were incubated aerobically and anaerobic culture conditions. Pure colonies were identified by macroscopic examination of colony growth on agar plates, morphological characteristics, motility tests, and other biochemical tests (catalase, coagulase, sugar fermentation, indole, oxidase, and haemolytic) according to standard microbiological procedures. All the organisms that were isolated and identified were stabbed in nutrient agar slant, labeled appropriately, and kept in the refrigerator [6,7].

2.3 Formulation of the Dentifrices

1 g of the toothpaste was mixed with 1 ml of sterile water (1:1 dilution), followed by serial dilutions of 1:2, 1:4, 1:6, and 1:8.

2.4 Antimicrobial Screening of the Dentifrices

The efficacy of dentifrices was evaluated using the agar well diffusion and zones of inhibition method against *S. mutans*. Organisms were re-activated from nutrient agar slant by

Table 1. Identity profiles of the toothpastes used in the study

S/N	Toothpaste	Net wt.	Batch or Lot No.	Active Ingredient	Country of origin
1	Crest Fluoride Anticavity toothpaste	3.5 Oz (99 g)	4135CA	Sodium fluoride – 0.243% (0.15% w/v fluoride ion)	-
2	Colgate fluoride toothpaste (Baking soda sparkling white)	2.8 Oz (79 g)	4068MX1119	Sodium monofluorophosphate 0.76% (0.14% w/v fluoride ion)	Mexico
3	Ultra brite anticavity fluoride toothpaste	6.0 Oz (170 g)	4068MX111E	Sodium monofluorophosphate 0.76% (0.14% w/v fluoride ion)	Mexico
4	Crest fluoride anticavity toothpaste (Baking soda whitening)	3.5 Oz (99 g)	4159CA	Sodium fluoride 0.243% (0.16% w/v fluoride ion)	-
5	Colgate fluoride toothpaste (cavity protection)	2.8 Oz (79 g)	4086MX1119	Sodium monofluorophosphate 0.76% (0.15% w/v fluoride ion)	Mexico
6	Sensodyne	20 ml	40212KWC	Sodium fluoride 0.315% w/w (1450 ppm fluoride)	-
7	Oral-B: pro-health toothpaste (Herbal Mint Gel)	140 gr	42280386EA	Sodium fluoride (1100 ppm fluoride)	China

sub-culturing onto blood agar plates. Mueller Hinton agar was prepared and allowed overnight to check for sterility. From a 24 hours culture plate, two colonies of each microorganism were aseptically transferred using a sterile inoculation loop into a test tube filled with 10 ml sterile normal saline, to be further and standardized by comparing with 0.5 Mac-farland turbidity solution [8]. A sterile swab stick was used to spread the resulting solution uniformly on the surface of Mueller Hinton agar, after which it was allowed to stand for 30 minutes. A sterile cork borer, 6 mm in diameter, was used to bore six wells on the agar plates. 0.5 ml of each dentifrice were seeded into the wells. Gentamicin (10 µg/ml) was used as positive control and distilled water as a negative control. The plates were incubated at 37ºC for 24 hours. The inhibition zone diameters in millimeters were measured and recorded after incubation. The growth inhibition of the test organism around the extracts indicated the antimicrobial activity of a specific dentifrice against the test organism. The diameters of these zones were measured diagonally in millimeters with a meter rule and recorded.

3. RESULTS AND DISCUSSION

It is obvious that mechanical and chemical aids of oral hygiene are the most common tools in the fight against some diseases like dental caries and periodontal diseases. The issue still remains the effectiveness of these methods. Toothpastes are widely available in different dental markets with a regular advertisement for newer toothpastes, and with claims of antimicrobial activity. Some consumers are even not aware of the type of toothpaste they use [2,3]. In this study, we want to provide consumers in Nigeria and the world at large with the crucial knowledge of the effectiveness of their day to day use of toothpaste against *S. mutans* responsible for dental caries. *S. mutans* was chosen as the only microorganism in this study since it has been well established for its responsibility in the initiation of the carious process [5,9]. The antimicrobial activity was determined *in vitro* for

Table 2. Mean zones of inhibition of all toothpastes

Toothpastes	1:1 Dilution	1:2 Dilution	1:4 Dilution	1:6 Dilution	1:8 Dilution
1	34±0.17	34±0.23	34±0.11	34±0.06	34±0.11
2	35±0.09	35±0.16	35±0.11	35±0.23	27±0.09
3	35±0.16	35±0.17	35±0.31	35±0.11	33±0.31
4	26±0.23	25±0.06	21±0.09	19±0.16	0±0.0
5	35±0.11	35±0.16	35±0.31	27±0.17	20±0.06
6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
7	35±0.06	35±0.23	35±0.11	35±0.17	35±0.09
Gentamicin (10 µg/ml)	22±0.20	-	-	-	-
Water	0±0.0	-	-	-	-

S. mutans since it is current practice to use this microorganism for *in vitro* antimicrobial screening of dentifrices prior to randomized controlled trials [10,11]. Although the disk diffusion method is especially appropriate for fluids like water, it is also applicable for antimicrobial screening of semi-solid materials, like toothpaste mixed with saliva or water [11-16].

The results in this study (Table 2) show that toothpaste 7 containing mint extract and sodium fluoride had the highest antimicrobial activity, followed by toothpastes 3, 2, 5 containing only sodium monofluorophosphate. Toothpaste 1 and 4 containing sodium fluoride also gave a promising antimicrobial activity against *S. mutans*, having a nearly comparable activity with toothpaste 7. Toothpaste 7 was even more active than the positive control. Toothpaste containing mint extract herbal component together with fluoride was thus more effective than those with only fluoride. In a mixture mint extract and fluoride may act synergistically. The high efficacy of toothpaste with mint extract is probably due to the presence of antimicrobial components in this extract (e.g. menthol) [17-20], although metabolites like lectins, flavonoids, polyphenols, and alkaloids also may have antimicrobial properties [5,21].

The lack of efficacy of toothpaste 6 which contains only sodium fluoride confirms its usefulness in case of teeth sensitivity [22], but not for antimicrobial efficacy against *S. mutans*. Toothpaste 1, 4 and 6 contain sodium fluoride, but toothpaste 6 did not inhibit the growth of *S. mutans* in vitro. This is because toothpaste 1 and 4 contain both sodium fluoride and sodium lauryl sulfate (SLS), but toothpaste 6 does not. Earlier reports indicate that fluoride and SLS (inactive ingredient) function synergistically to inhibit the growth of *S. mutans* [23]. Dental professionals warn for excessive use of fluoridated toothpastes for reasons of dental fluorosis [5,24].

4. CONCLUSION

The higher efficacy of herbal toothpaste (with mint extract) as demonstrated in this study shows that it can be a safe and effective alternative for traditional fluoridated toothpastes. There is need for further studies *in vitro* antimicrobial screening of all toothpastes sold in the open market in Enugu Metropolis, as well as to define the substance responsible for the antimicrobial activity of these toothpastes.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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