TECHNICAL NOTE

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A Comparative Study of the Antimicrobial Activity of Selected Herbal Toothpastes and their Non-Herbal Counterparts Against Oral Isolates in Sagamu, Nigeria.

F. O. ODELEYE[A-F], *M.O. AKPOTU[A-F] and A. E. ADEBESIN[BCDEF]

Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: In recent years in Nigeria, there has been an upsurge in the marketing and utilization of herbal toothpastes alongside their non–herbal counterparts that had been in use to improve oral health.

Objective: This study was carried out to compare the antimicrobial activity of herbal toothpastes and their non-herbal counterparts against oral isolates.

Methodology: Microorganisms were isolated from early morning mouth rinses of volunteer subjects and identified using standard protocols. A total of four brands of toothpastes (four herbal and their non-herbal counterparts) making a total of eight toothpastes labeled A, C, E, G (herbal toothpastes) B, D, F, H (non-herbal) were procured, diluted into three different concentrations of 1:1, 1:2 and 1:4 by two-folds serial dilution and tested for their antimicrobial activity against oral isolates using agar well diffusion method. Inhibition zone diameters (IZDs) were measured in millimeters after 24h incubation at 25^0C (fungal isolates) and 37^0C (bacterial isolates). Result analysis was done by SPSS using one way analysis of variance (ANOVA) and significance taken at $p<0.05$.

Results: The isolates were identified as *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA) and *Candida albicans* (CA). The undiluted Paste A gave the highest IZD of 22 mm against SA and the least IZD was 0.0 mm by paste A against CA at 1:2 dilution, paste C at 1:2 dilution against CA and paste E at 1:1 dilution against CA and thereby making CA the least responsive to all the concentrations of toothpaste evaluated. On the whole, the herbal toothpastes gave a better IZD than their non- herbal counterparts. At $p<0.05$, there was no significant difference in the overall activity between the herbal toothpastes and their non-herbal counterparts.

Conclusion: The result shows that the herbal toothpastes were marginally better in the inhibition of the isolates than their non-herbal counterparts, though their overall difference is not significant, necessitating further studies.

Keywords: Antimicrobial activity, Herbal toothpaste, Non-herbal toothpaste, Oral isolates.

INTRODUCTION

Dentifrices are agents produced in the form of powder, paste, gel or liquid used to improve oral health usually in conjunction with a toothbrush, the most common dentifrice used to improve oral health in Nigeria being toothpastes. These toothpastes are now produced in the herbal and non-herbal forms to meet the demands of the teaming population in search of a healthy oral cavity. It has been reported that the brushing of teeth using toothpaste is the commonest form of oral hygiene in the world (Sadeghi and Assar, 2009). This method provides the needed abrasion required to remove food particles and preventing the formation of dental plaques, which usually precedes tooth decay and other dental problems in most instances (Prasanth, 2011). It has also been shown to suppress or eliminate halitosis (Nwankwo and Ihesiulo, 2014). The benefits of oral health, however, do not lie squarely in brushing with tooth paste, but on a number of factors. Such factors include the active ingredients in the pastes, that is, whether it contains the relevant antimicrobial agents to mitigate the proliferation of pathogenic microbial flora that could hamper oral health and certain anti plaque and anti-gingivitis agents like triclosan and fluorides (Nogueria-Filho *et al*, 2002). However, some of these anti-plaque agents had been shown to elicit undesirable side effects such as tooth staining or altered taste in some instances (De Olivera *et al*, 2008; Barnes *et al*, 2010), leading to an increase in the use of natural ingredients in herbal dentifrices. Researches had shown quite a number of benefits inherent in the contents of some herbal toothpaste. For instance, chamomile, contained in some herbal toothpastes had been shown to have anti-inflammatory effect, echinacea has immune-stimulatory property, sage and rhatany have anti-haemorrhagic properties, myrrh is a natural antiseptic, and peppermint oil has analgesic, antiseptic, and anti-inflammatory properties (Radafshar *et al*, 2010).

There are limited studies available comparing the antimicrobial activity between herbal and non-herbal tooth paste in Sagamu, Nigeria; hence, the present study was undertaken to assess the antimicrobial activity of both herbal and non-herbal toothpastes and whether there exist any significant difference in the elicitation of their antimicrobial activities.

MATERIALS AND METHODS

Materials

Four herbal toothpastes and their corresponding non-herbal brands were procured from supermarkets in Sagamu, Ogun State, Nigeria and labeled: A, C, E, G (herbal toothpastes) and B, D, F, H (non-herbal).

Method

Collection and dilution of the toothpastes

From each of the eight (8) toothpastes, 2g was weighed out and diluted with 2mL of sterile distilled water to give 1:1 dilution; further dilutions were done to obtain 1:2 and 1:4, respectively. The compositions of each of the toothpastes are as shown in Table 1.

Collection of sample

10mL of distilled water was dispensed into universal bottles and sterilized in an autoclave at 121^{o}C for 15mins. The bottles were distributed to volunteers who were not on antibiotics therapy in the last two weeks, who were not pregnant, and who did not brush the teeth the night before gargling with the sterile distilled water. The sample was taken to the laboratory within one hour of collection and kept in the refrigerator just for few minutes to allow for the preparation of the peptone water. The waiting period was less than 30 minutes.

Preparation and inoculation of samples

Exactly 1mL of the sample was inoculated into 5mL peptone water (Lab M), thoroughly mixed and incubated at 37^{0}C for 24 hours. This was followed by the inoculation of the overnight culture with sterile inoculating loop onto Nutrient agar (Titan Biotech Ltd, India) and various selective media such as Blood Agar (Titan Biotech Ltd, India), Mannitol Salt Agar (Lab M), Cetrimide Agar (Lab M) and Sabouraud's Dextrose Agar (Lab M). All the media were incubated at 37^{0}C for 24hours, except Sabouraud's dextrose agar that was incubated at 25^{0}C for 72 hours.

Identification of the oral isolates

Table 1: The composition of the toothpastes evaluated

Toothpastes	Ingredients
A	Natural lemon extract, flavor containing natural blend of mint, Eucalyptus, Rosemary, chamomile, sage, myrrh and other natural oil, sorbitol, silica, treated water, polyethylene glycol 1500, sodium lauryl sulphate, sodium carboxyl methylcellulose, sodium saccharin, tri-sodium orthophosphate, citric acid.
B	Paung pudina, Tomar, mint, natural calcium base, sodium saccharin, citric acid
C	Calcium carbonate, Sorbitol, Hydrated silica, Sodium lauryl sulphate, Sodium monofluorophosphate, Cellulose gum, Magnesium aluminium silicate, Sodium carbonate, Benzyl alcohol, Sodium saccharin, Sodium bicarbonate, *Eucalyptus globulus* leaf oil, *Mentha piperita* (Peppermint) oil.
D	Sorbitol, Aqua, Hydrated silica, Sodium lauryl sulphate, Aroma, PEG 12, Cellulose gum, Cocamido propyl betaine, Sodium fluoride, Sodium saccharin, Hydroxypropyl methylcellulose, Menthol, Glycerine, Limonene.
E	Aqua, Hydrated Silica, Sorbitol, Glycerin, PEG-6, Sodium Lauryl Sulfate, Aroma, Titanium Dioxide, Xanthan Gum, Chondrus Crispus (Carrageenan), Sodium Fluoride, Sodium Saccharin, Limonene, Contains Sodium Fluoride 0.306% w/w (1400 ppm Fluoride).
F	Sodium fluoride 0.306%w/w, Aqua, Hydrated silica, sorbitol, Glycerin, PEG-6, Sodium lauryl sulphate, Xanthan gum, Sodium saccharin, Cl73360, Cl74160
G	Sodium fluoride (1450 ppm fluoride), Herbal extracts (Eucalyptus, peppermint, Sage, thyme, Aloe barbadensis leaf extract, limonene, Cl73360, Cl74260, Cl77268:I, Cl77492, Cl7789I
H	Sorbitol, Aqua, Hydrated silica, Sodium lauryl sulphate, PEG 32, Aroma, Cellulose gum, Sodium saccharin, Sodium fluoride, Zinc sulphate, Mica, Sodium hydroxide, Glycerin, Eugenol.

Primary isolation of the required microbes was done on selective media. Yellow colonies on mannitol salt agar were taken to be *Staphylococcus aureus* while colonies that produced green pigment on cetrimide agar were taken as *Pseudomonas aeruginosa*. The isolates were further characterized using Gram staining technique and standard biochemical tests such as mannitol, catalase, coagulase and oxidase tests in a modified method described by Ngwai and co-workers (Ngwai *et al.*, 2010). Primary isolation of *Candida albicans* was done on Sabouraud's Dextrose Agar.

Determination of the antimicrobial activity of the herbal toothpastes and their non-herbal counterparts

The agar-well diffusion method was employed in this determination. First, the bacterial isolates were inoculated into tubes containing 5mL of peptone water and incubated at 37^0C for 5hrs, while the fungal isolate was inoculated into Sabouraud's dextrose broth and incubated at 25^0C for 72hrs. The isolates were then diluted to 0.5 McFarland's standard. Next, the entire surfaces of the Mueller Hinton agar plates were swabbed aseptically with the standardized culture, four (4) holes were dug out using a 6mm cork borer and 20µL from each of the four concentrations of toothpastes were dispensed into the holes and this was done in triplicates. The plates were allowed to stand for 1hr to allow for diffusion and subsequently incubated at 25^0C for the fungal isolate and at 37^0C for the bacterial isolates respectively. The inhibition zone diameters (IZDs) were then measured to the nearest millimetre.

Statistical analysis

Statistical analysis was performed using SPSS by one way analysis of variance (ANOVA) and significance taken at $p<0.05$.

RESULTS AND DISCUSSION

Table 2: Zones of inhibition of the toothpastes against the oral isolates

	Inhibition Zone diameter(mm)											
	Staphylococcus aureus				***Pseudomonas aeruginosa***				***Candida albicans***			
Pastes	**UD**	**1:1**	**1:2**	**1:4**	**UD**	**1:1**	**1:2**	**1:4**	**UD**	**1:1**	**1:2**	**1:4**
A	22.0±0.6	20.3±0.3	11.0±0.6	0.3±0.3	15.0±0.6	0.7±0.3	0.7±0.3	0.3±0.3	0.7±0.3	0.7±0.3	0.0±0.0	1.0±0.6
B	20.3±0.3	18.3±0.3	12.0±0.6	0.7±0.3	14.0±0.6	0.3±0.3	0.7±0.3	0.3±0.3	0.3±0.3	0.7±0.3	1.3±0.3	1.0±0.0
C	19.0±0.6	17.0±0.6	9.0±0.6	0.7±0.3	13.0±0.6	0.3±0.3	0.3±0.3	0.7±0.3	1.0±0.0	0.3±0.3	0.0±0.0	0.3±0.3
D	20.0±0.6	18.3±0.3	11.0±0.6	0.7±0.3	12.3±0.3	1.0±0.0	1.0±0.0	0.3±0.3	0.3±0.3	0.3±0.3	1.0±0.0	0.3±0.3
E	19.0±0.6	17.0±0.6	11.0±0.6	1.7±0.3	13±0.6	9.0±0.6	1.3±0.3	1.3±0.3	15.0±0.6	0.0±0.0	0.7±0.3	1.0±0.6
F	20.0±0.6	17.0±0.6	12.0±0.6	0.7±0.3	15.0±0.6	0.7±0.3	1.0±0.0	1.0±0.6	17.0±0.6	1.7±0.3	1.0±0.6	1.7±0.3
G	20.0±0.6	20.0±0.6	11.0±0.6	1.3±0.7	18.0±0.6	9.0±0.6	0.7±0.3	1.0±0.6	2.0±0.0	0.7±0.3	1.0±0.6	2.0±0.6
H	19.3±0.3	16.0±0.6	11.0±0.6	1.0±0.0	21.0±0.6	9.0±0.6	0.3±0.3	1.0±0.0	1.0±0.0	1.7±0.3	2.0±0.6	0.7±0.3

Key: Toothpastes A, C, E & G are Herbal; Toothpastes B, D, F & H are non-herbal; UD: Undiluted concentration.

Many types of colonies developed on the plates inoculated with the mouth rinses, but only three (3) were isolated and characterized. They are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans.* Following primary isolation on selective media, isolates on Mannitol salt agar that were Gram positive as well as mannitol salt $^{(+)}$, catalase $^{(+)}$, coagulase $^{(+)}$ and oxidase $^{(-)}$ were confirmed as *S. aureus* while those from Cetrimide agar that were Gram negative, rod-shaped, catalase $^{(+)}$, oxidase $^{(+)}$ and coagulase $^{(-)}$ were characterized as *P. aeruginosa.*

The isolates showed varying degrees of susceptibility to the toothpastes evaluated. The undiluted Paste A gave the highest IZD of 22 mm against *S. aureus* and the least IZD was 0.0 mm by paste A against *C. albicans* at 1:2 dilution, paste C at 1:2 dilution against *C. albicans* and paste E at 1:1 dilution against *C. albicans* as well (see Table 2 and Figure 1).

The inclusion of antimicrobial agents in dental care products is to ensure that oral organisms, which are part of the normal flora of the mouth, are kept at a level that would not jeopardize the normal oral health of individuals in the population, hence, preventing dental plaques that could lead to dental caries in the long run, as well as preventing gingivitis.

This study showed that the inclusion of antimicrobial agents in the toothpastes exhibited wide variations in their effectiveness in taming the population of the oral microorganisms in tandem with previous studies (Prasanth, 2011; Awah *et al*, 2016). It has been shown that the addition of antimicrobial substances to dentifrices enhance to a very great extent their ability to kill microorganisms by disrupting their cell walls, preventing the formation of biofilms, release endotoxins and inhibiting their enzymatic activity (Bou-Chacra *et al*, 2005). In addition, natural products which normally have antioxidant properties also exhibit antimicrobial properties which could also be the responsible for the antimicrobial properties the herbal toothpastes elicited (Akpotu *et al* 2015; Akpotu *et al*, 2017). The fluoride containing toothpastes had been shown to elicit antimicrobial activity by earlier workers, thereby preventing dental caries in the long run (Prasanth, 2011). All the toothpastes (that is herbal and their non-herbal

counterpart) evaluated against *S. aureus* at the undiluted concentration gave the highest IZD and these IZDs tend to decrease down the concentration gradient. This is in agreement with earlier studies that apart from the addition of antimicrobial agents in a toothpaste, the concentration of the agent is also paramount to achieving the desired outcome of reducing oral microorganisms (Sadeghi and Assar, 2009; Tatikonda *et al*, 2014). *C. albicans* was the least responsive to all the toothpastes evaluated. This could be as a result of the inclusion at very low concentration anti-fungal agents in formulating the toothpastes or it may be absent all together. An earlier observer could not derive any significant difference in the antimicrobial capacity between the herbal and their non-herbal counterparts (George et al, 2009) and this seems to be the outcome of this study.

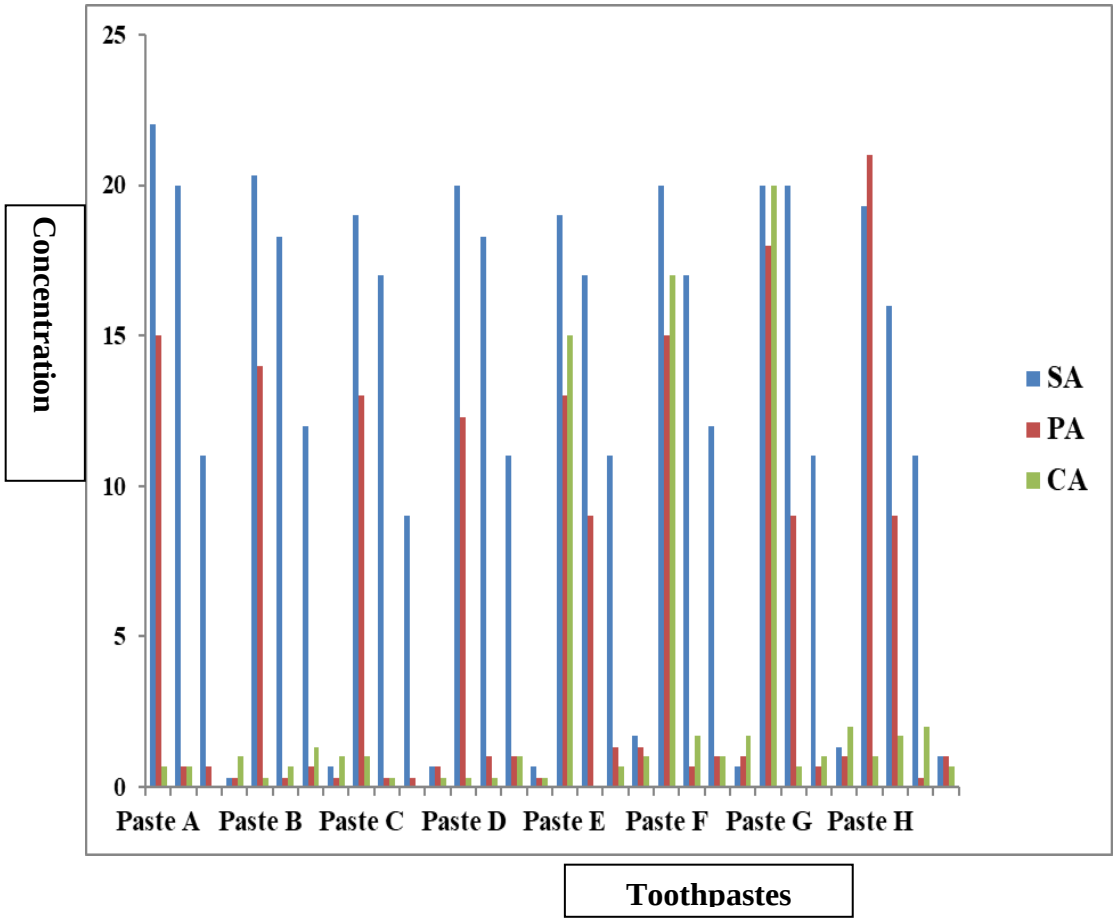

Figure 1: Antimicrobial activity of the toothpastes against the oral isolates

KEY- SA: *Staphylococcus aureus*; PA: *Pseudomonas aeruginosa*; CA: *Candida albicans*

CONCLUSION

The result of this study showed that the herbal toothpastes were marginally better in the inhibition of the oral isolates than their non-herbal counterpart, though their overall difference is not significant, necessitating further studies.

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*Address for correspondence: Mark O. Akpotu
Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University Sagamu Campus, Ogun State, Nigeria.
Telephone: +234 7068104700
E-mails: markakpotu@yahoo.com

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