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Antibacterial activity of chewing stick, dental powder and toothpastes sold in Umuahia, Abia State, Nigeria

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Abstract

Aims: To determine and compare the antimicrobial activity of chewing stick *Salvadora persica* (Miswak), dental powder and toothpastes sold in Umuahia, Abia state Nigeria against selected oral bacteria (*Streptococcus spp*, *Staphylococcus spp*, *Porphyromona spp* and *Lactobacillus spp*).

Method: The antimicrobial activity of chewing stick *Salvadora persica* (Miswak) extracts (ethanol and aqueous), two dental powder (Agnes nwamma and N-sol) and five toothpastes brand (Close-Up, Oral-B, Colgate, MacClean and Pepsodent) was investigated against selected test organisms (*Streptococcus spp*, *Staphylococcus spp*, *Porphyromona spp* and *Lactobacillus spp*) that cause dental caries. This was carried out at different concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml).

Results: Among the chewing stick extracts, the ethanol extract showed more antimicrobial efficacy than the aqueous extract. Agnes nwamma dental powder showed more efficacy than N-sol dental powder. Among the toothpastes, Close-Up showed more efficacy than the other toothpastes. The antimicrobial activity observed majorly depend on the concentrations; the higher the concentration, the higher the efficacy. In the overall comparison between the chewing stick, dental powder and toothpastes, the toothpastes with the exception of MacClean tend to be a bit more efficacious than the dental powder and chewing stick. The dental powder and chewing stick extracts also showed a good antimicrobial activity.

Conclusion: This work suggests that the toothpastes, dental powder and chewing stick are effective against oral pathogens. Chewing stick and dental powder can be used by families that cannot afford toothpastes.

Keywords: Oral bacteria; Chewing Stick; Dental Powder; Toothpastes; Antibacterial activity.

1. Introduction

Good and adequate oral hygiene is an indicator for good body health, poor oral hygiene not only affect the oral cavity but also a risk factor for initiation of many systemic diseases. Presence of dental plaque is an indicator of poor oral hygiene and if not treated properly can change into dental calculus which will further deteriorate the situation. Environmental factors such as culture, socioeconomic status, life style and diet pattern have a great influence on maintaining good oral hygiene [1]. Oral health is an essential component of a person's health. According to World Health

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Organization (WHO), about 60 percent to 90 percent of children and nearly every adult in the world have dental cavities [2]. According to American Dental Association (ADA), "oral health is a functional, structural, aesthetic, physiologic, and psychosocial state of well-being and is essential to an individual's general health and quality of life" [3]. Poor oral health is related to significant morbidity and mortality [4]. According to a study done in the USA, it was concluded that the frequency of emergency department visits because of preventable dental conditions has increased by 16 percent since 2006 [5]. Poor oral hygiene has a significant impact on general health and is associated with various systemic diseases.

The human mouth is a suitable environment for the growth of characteristic microorganisms found there. It provides a source of water and nutrients; from food debris as well as a moderate temperature. The oral cavity is inhabited by an indigenous normal micro flora that is composed of more than 500 species, the majority of which still remain uncultivable, of which the number of micro-organisms in an infected root canal may be anywhere between 10^2 -10^8 [6, 7, 8]. The major genera of microorganisms inhabiting the oral cavity are *Streptococcus, Eubacteria, Fusobacterium, Capnocytophaga, Eubacteria, Staphylococcus, Eikenella, Porphyromona, Leptotrichia, Prevotella, Peptostreptococcus, Treponema, Actinomyces* genera [9].
Dental caries and periodontal diseases are the most prevalent infectious diseases of the oral cavity and are responsible for more than 50% cases of tooth mortality [10, 11]. Dental caries is a multifactorial disease causing irreversible loss of dental tissue and microorganisms have a critical role in its etiopathogenesis, initiation and progression which has been established by many studies done worldwide [12, 13, 14]. Most microorganisms involved in dental caries belong to the *Streptococcus, staphylococcus, Veillonella, porphyromona, Actinomyces, Bi-fidobacterium, Bacillus and Lactobacillus* genera [15, 16, 17].

The tooth with its unique structure is a non-shedding surface. Thus, it is suitable for the colonization of oral microbes as it allows large masses of microbes to accumulate to form the oral biofilm. Each tooth is made up of the pulp, dentine, cementum and enamel [18, 19]. The enamel being the outer layer of the tooth is the only part that is exposed to the oral environment under normal condition. In addition to the varying intrinsic biological properties, the tooth provides several distinct surfaces such as pits and fissures that influence the colonization and growth of different populations of microbes. The tooth surface normally encourages the residence of aerobic, facultative and anaerobic micro flora [20, 21, 22].

Oral health status has a major impact on the general feature of life and well-being. With the increasing rate of oral diseases, the global necessity of effective and economical products for prevention and treatment has intensified [23]. This calls for an understanding of traditional practices and oral health beliefs [24]. Dentifrices (toothpastes and tooth powder) are used almost universally in the developed world but, in some groups and cultures, people still practice traditional tooth brushing without dentifrice with, for example, a Miswak or salt [25]. A toothpaste may be classed as either a cosmetic or a medicine depending on the claims that are made and the level of certain constituents. The primary function of a toothpaste is to clean the teeth which is considered to be a cosmetic benefit. The use of words such as 'protects', 'cleans', 'freshens breath', 'fights bacteria which may cause gum problems', 'whitens' or 'fights tartar' are considered to be cosmetic claims. Toothpastes that contain up to 1500 ppm F can make claims such as, 'cavity protection', 'helps prevent tooth decay' and 'fights tooth decay' all of which are cosmetic claims. Cosmetic products can be marketed without clearance from any regulatory body but the manufacturer has an obligation to ensure that such products are safe and do not cause damage to health under normal conditions of use [26]. Toothpastes contain active ingredients or additives that perform specific functions. These additives are abrasives, fluorides, desensitizing agents, antiplaque agents, and antitartar ingredients. Toothpastes also contain detergents, humectants, thickeners, preservatives, flavoring agents, sweeteners, and coloring agents [27].

Tooth powders are simple and cheap to be prepared locally. These are expected to fulfill the functions such as cleansing of tooth, prevention of formation/removal of dental plaque/ calculus, polishing of tooth, reduction of the occurrence of tooth decay, reduction of periodontal diseases, prevention or reduction of mouth odour and freshening of breath etc. As one ingredient cannot fulfill the desired criteria, various ingredients such as abrasive, surfactant or detergent, sweetening agent, flavour, colour etc. are added to obtain the desired goal. In herbal tooth powders, different crude drugs are added that help in cleansing the oral cavity. As all of these are composed of fine particles, the physiochemical properties of allopathic and herbal powders may depend upon micromeritics of particles. These preparations should be suspended/solublized in oral cavity content to form the foam for showing the cleansing effect and it will depend upon the nature of ingredients [28].

Use of modern toothbrushes and inter-dental cleaners has ignored the most effective primitive oral hygiene tool, that is, the chewing sticks also known as Miswak [29]. Even with the many toothbrushes being invented nowadays, chewing stick is being used by many people all over the world, especially among Muslims because it has religious and customary values. Chewing stick is also practiced by many people in developing countries because of the availability, low cost,

simplicity and/or traditional culture. Chewing sticks of plants were prehistorically used by the early Arabs, Babylonian, Greek, and Roman societies for cleaning teeth. Chemical examinations have revealed a new era of chewing sticks reimbursement, which established that these sticks contain natural ingredients, which are beneficial for oral health [30, 31, 32]. It has been reviewed that it contains ascorbic acid, tri-methylamine, chloride, fluoride, silica, resins, and salvadorine, which have proved potency to heal the infiamed and bleeding gums, produce stimulatory effect on gingiva, remove tartar, and other stains from the teeth, re-mineralize dental hard tissue, whitens teeth, provide enamel barrier, and increase salivary flow, respectively. In addition, chewing sticks also contains volatile oils, tannic acid, sulphur and sterols which attribute to anti-septic, astringent and bactericidal properties that help reduces plaque formation, provides anti-carious effects, eliminates bad odor, improves the sense of taste, and cure many systemic diseases [33, 34, 35]. Even with the many toothbrushes being invented nowadays, chewing stick is being used by many people all over the world, especially among Muslims because it has religious and customary values. Chewing stick is also practiced by many people in developing countries because of the availability, low cost, simplicity and/or traditional culture.

2. Material and methods

2.1. Source of materials

A Bundle of chewing stick *Salvadora persica* (Miswak) was bought from a seller and wrapped in a paper foil. Two locally made dental powder (N-sol and Dr. Agnes nwamma) were bought from a shop in Umuahia. Five brands of toothpastes (Close-Up, Oral-B, Colgate, MacClaen and Pepsodent) were bought from a shop in Umuahia.

2.2. Preparation of crude extract

The chewing stick was grinded to powder. About 200 g of the powder were separately soaked in 400ml of 95% ethanol and sterile distilled water each, in reagent bottles and stoppered. This was allowed to stand for 14 days to permit full extraction of the active ingredients. The fluids were then filtered using Whatman No1 filter paper. The extracts were rotary dried to obtain the concentrate. It was then kept in fridge prior to use as described by [36].

2.3. Sample collection, preparation and Isolate identification

Six sterile bijou bottles were used to collect saliva from three males and three females (a boy and a girl within the age of 3-6 years old; labelled AM and AF, a young man and a woman within 30-40 years old; labelled BM and BF, an elderly man and woman at their sixties labelled CM and CF) aseptically, this was done early in the morning before they had their mouth wash or ate anything

Tenth - fold serial dilutions were prepared (by adding 1ml of the saliva to 9 ml of 0.1% sterile peptone water tube and then 1 ml was taken from this tube to another one containing 9 ml of sterile peptone water and so on). This was done to decrease bacteria concentration and to get a proper plate count within 30-300 colonies.

0.1 ml of aliquot from appropriate dilutions was inoculated into brain heart infusion agar, blood agar and casein nutrient agar by spread plate technique using a glass rod. The inoculum were well labelled and incubated at 37 °C for 24 hours. The distinct colonies were observed from all the cultured plates having within 30-300 number of colonies and were subcultured to obtain pure isolates.

The required test organisms were identified using gram staining technique and biochemical tests. The biochemical tests carried out were catalase, coagulase, oxidase, indole, citrate, urease, sugar fermentation and hydrogen sulfide test.

2.4. Preparation of different concentration for chewing stick extract, dental powder and toothpastes solutions

5 g of the chewing stick extract was added to 10 ml of 70% Dimethyl Sulfoxide (DMSO) to get a solution of 500 mg/ml, this was diluted subsequently to get 3 different concentrations of 250, 125 and 62.5 mg/ml.

5 g of each of the dental powder was added to 10 ml of 70% Dimethyl Sulfoxide (DMSO) to get a solution of 500 mg/ml, this was diluted subsequently to get 3 different concentrations of 250, 125 and 62.5 mg/ml.

5 g of each of the toothpastes was added into 10 ml of sterile distilled water to get a concentration of 500mg/ml, and was subsequently diluted to get 3 different concentrations of 250, 125 and 62.5 mg/ml.

2.5. Phytochemical Analysis

Phytochemical screening were carried out on the ethanol and aqueous chewing stick *Salvadora persica* (Miswak) to determine the presence of the following constituents: alkaloids, terpens, flavonoids, phenol, tannins, saponins, reducing sugars and glycosides using the method described by [37].

2.6. Preparation of McFarland Standard

A 0.5 McFarland standard was prepared by mixing 0.05 ml of barium chloride dihydrate, with 9.95 ml of 1% sulfuric acid [38]. The standard was compared visually to a suspension of bacteria in sterile saline.

2.7. Antimicrobial Susceptibility Testing

The antimicrobial quality was determined using modified agar well diffusion. This method was carried out by spreading 0.1 ml of each of the test isolates from an overnight broth culture onto a prepared brain heart infusion agar plate. A sterile 8 mm cork-borer was used to cut one central and four wells at equidistance in each of the plates. 0.2 ml of the dental powder and chewing stick extract dilutions was introduced into each of the four wells with the same volume of 70% DMSO introduced into the central well, serving as control. This method was repeated on the toothpastes dilutions with the same volume of sterile distilled water serving as a control, and the plates were allowed to stay on a horizontal surface for one hour to enable the substances to diffuse before incubating at 37 ºC for 24 hours. Zones of inhibition were measured in mm after incubation to determine the antibacterial efficacy. All experiments were performed in duplicate. Labelling was made clearly and carefully.

2.8. Statistical Analysis

The data were analysed using Anova IBM® SPSS® statistics version 21. One-way ANOVA was used to compare the mean value of the outcome variable followed by post hoc test. The significance level was set at P =.05.

3. Results

Table 1 Number of occurrence of test organisms

Sample name	Isolate designation	Organism
AM	AM_1	*Lactobacillus spp*
AF	AF_1	*Lactobacillus spp*
BM	BM_1	*Lactobacillus spp*
	BM_2	*Porphyromona spp*
	BM_3	*Staphylococcus spp*
BF	BF_1	*Porphyromons spp*
	BF_2	*Streptococcus spp*
CM	CM_1	*Porphyromona spp*
	CM_2	*Staphylococcus spp*
CF	CF_1	*Porphyromona spp*
Percentage occurrence = Number of positive isolates/Number of total isolates obtained × 100/1		
Percentage occurrence of *Streptococcus spp* = 1/10 × 100/1 = 10%		
Percentage occurrence of *Streptococcus spp* = 2/10 × 100/1 = 20%		
Percentage occurrence of *Porphyromona spp* = 3/10 × 100/1 = 30%		
Percentage occurrence of *Lactobacillus spp* = 4/10 × 100/1 = 40%		

Key: AM = Sample from a male between the age of 3 to 6 years old; AF = Sample from a female between the age of 3 to 6 years old; BM = Sample from a male between the age of 30 to 40 years old; BF = Sample from a female between the age of 30 to 40 years old; CM = Sample from a male between the age of 60 to 70 years old; CF = Sample from a female between the age of 60 to 70 years old

Table 1 shows the percentage occurrence of required test organisms. A total of six (6) samples were examined and out of these contained ten (10) bacterial isolates. In these ten (10) isolates, *Streptococcus spp* occurred once, *Staphylococcus spp* occurred twice, *Porphyromona spp* occurred four times and *Lactobacillus spp* occurred three times. These gave a percentage occurrence of; Streptococcus *spp* (10%), *Staphylococcus spp* (20%), *Porphyromona spp* (40%) and *Lactobacillus spp* (30%).

Table 2 Phytochemical analysis of chewing stick extracts

Phytochemicals	Ethanol extract	Aqueous extract
Alkaloids	++	+
Terpens	++	+
Flavonoids	-	-
Phenol	+	-
Tannins	+	+
Saponins	+	++
Reducing sugars	-	-
Glycosides	++	+

Key: ++ = highly present**; + =** moderately present**;** - = absent

Table 2 shows the phytochemical constituents of ethanol and aqueous extracts of chewing stick *Salvadora persica* (Miswak). This showed the presence of alkaloids, terpens and glycosides in higher concentrations in ethanol extract than in aqueous extract. Saponin in higher concentration in aqueous extract than ethanol extract. Presence of tannins in both extracts. Presence of phenol in ethanol extract but absent in aqueous extract. Absence of flavonoids and reducing sugars on both extracts.

Table 3 Mean zone of inhibition (mm) of chewing stick on bacterial isolates

Isolate	Extracts	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Streptococcus spp	AE	5.5 ± 0.71	5.0 ± 1.41	6.5 ± 0.71	3.0 ± 0.00
	EE	13.5 ± 2.12	12.0 ± 2.12	6.0 ± 0.00	3.0 ± 0.00
Staphylococcus spp	AE	_	_	_	_
	EE	_	8.5 ± 0.71	10.0 ± 0.00	8.0 ± 1.41
Porphyromona spp	AE	8.0 ± 1.41	6.0 ± 0.00	3.0 ± 0.00	_
	EE	14.0 ± 1.41	8.5 ± 1.41	6.0 ± 0.00	4.0 ± 0.00
Lactobacillus spp	AE	6.0 ±0.00	4.5 ± 0.71	3.0 ± 0.00	_
	EE	13.5 ± 0.71	8.5 ± 0.71	6.0 ± 0.00	3.5 ± 0.71
Streptococcus spp	CAE	_	_	_	_
	CEE	_	_	_	_
Staphylococcus spp	CAE	_	_	_	_
	CEE	_	_	_	_
Porphyromona spp	CAE	_	_	_	_
	CEE	_	_	_	_
Lactobacillus spp	CAE	_	_	_	_
	CEE	_	_	_	_

Values are mean zone ± S.D of two replicates; **Key: AE** = Aqueous extract; **EE =** Ethanol extract; **CAE =** Control for aqueous extract

Table 3 shows the mean zone of inhibition (mm) of various concentrations of chewing stick *Salvadora persica* (Miswak) bacterial isolates. On *Streptococcus spp*, ethanol extract showed highest zone of inhibition (13.5 mm) at concentration of 500 mg/ml while aqueous extract showed an inhibition of 6.5 mm at 125 mg/ml. The level of Efficacy tends to decrease with decrease in concentration for the ethanol extract. On *Staphylococcus spp*, ethanol extract showed highest zone of inhibition (10.0 mm) at a concentration of 125 mg/ml. While aqueous extract showed no significant zone of inhibition. For *Porphyromona spp* the highest zone of inhibition was with ethanol extract, ranging from 14.0 mm - 4.0

mm at concentration of 500 mg/ml - 62.5 mg/ml, it has a decrease in efficacy with decrease in concentration. While aqueous extract has a range of inhibition of 8.0 mm - 3.0 mm at concentration of 500mg/ml-125 mg/ml, there was no zone of inhibition at concentration of 62.5 mg/ml. The inhibition also decreases with decrease in concentration. On *Lactobacillus spp*, ethanol extract showed inhibition zones ranging from 13.5 mm - 3.5 mm at concentration of 500 mg/ml - 62.5 mg/ml while aqueous extract showed inhibition zones ranging from 6.0 mm - 3.0 mm at concentration of 500 mg/ml - 125mg/ml. However, both chewing stick extracts showed a level of significance (*P*=.05) at all concentrations.

Table 4 shows the mean zone of inhibition (mm) of various concentrations of dental powder on bacterial isolates. On *Streptococcus spp*, Agnes nwamma dental powder showed a highest zone of inhibition (18.5 mm) at 125 mg/ml. While that of N-sol dental powder was 15.5 mm at 500 mg/ml. On *Staphylococcus spp*, Agnes nwamma dental powder showed zones of inhibition ranging from 13.0 mm - 3.0 mm at concentration of 500mg/ml-125mg/ml, while N-sol showed zones of inhibition ranging from 13.5 mm - 8.0 mm at concentration of 500 mg/ml - 250 mg/ml. On *Porphyromona spp*, both dental powder showed zones of inhibition at concentrations of 500 mg/ml - 125 mg/mg with that of Agnes nwamma being 15.5 mm - 8.0 mm and that of N-sol being 14.5mm-6.0mm. On *Lactobacillus spp*, Agnes nwamma showed an inhibition of 18.0 mm - 5.0 mm at concentration of 500 mg/ml - 62.5 mg/ml while N-sol showed inhibition zones of 19.0 mm - 5.5 mm at 500 mg/ml - 250 mg/ml. Both dental powder showed a level of significance (*P*=.05) at all concentrations.

Table 4 Mean zone of inhibition (mm) of dental powder on bacterial isolates

Isolate	Dental powder	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Streptococcus spp	AN	12.0 ± 1.41	16.0 ± 1.41	18.5 ± 0.71	14.5 ± 0.71
	N-sol	15.5 ±2.12	13.0 ± 1.41	8.5 ± 0.71	_
Staphylococcus spp	AN	13.0 ± 0.00	7.0 ± 0.00	3.0 ± 0.00	_
	N-sol	13.5 ±0.71	8.0 ± 0.00	_	_
Porphyromona spp	AN	15.5 ± 2.12	12.0 ± 1.41	8.0 ± 0.00	_
	N-sol	14.5 ± 0.71	12.5 ± 0.71	6.0 ± 0.00	_
Lactobacillus spp	AN	18.0 ± 1.41	13.0 ± 1.41	10.0 ± 1.41	5.0 ± 0.00
	N-sol	19.0 ± 2.83	11.0 ± 1.41	5.5 ± 0.71	_
Streptococcus spp	CAN	_	_	_	_
	CN-sol	_	_	_	_
Staphylococcus spp	CAN	_	_	_	_
	CN-sol	_	_	_	_
Porphyromona spp	CAN	_	_	_	_
	CN-sol	_	_	_	_
Lactobacillus spp	CAN	_	_	_	_
	CN-sol	_	_	_	_

Values are mean zone ± S.D of two replicates **Key: AN =** Agnes nwamma; **CAN =** Control for Agnes nwamma; **CN-sol =** Control for N

Table 5 shows the mean zone of inhibition (mm) of various concentrations of toothpastes on *Streptococcus spp*. All toothpastes showed highest zones of inhibition at a concentration of 500 mg/ml. That of Close-Up being 19.5 mm, Oral-B; 18.5 mm Colgate; 15.5mm MacClaen; 13.0 mm and Pepsodent; 15.0 mm.The level of inhibition tends to decrease with decrease in concentration for all toothpastes. The toothpastes showed a level of significance (*P*=.05) at all concentrations.

Table 6 shows the mean zone of inhibition (mm) of various concentrations of toothpastes on *Staphylococcus spp*. The zones of inhibition tends to decrease with decrease in concentration. Close-Up showed a zone of inhibition (14.5 mm - 3.5 mm), Oral-B showed zones of inhibition (14.5 mm - 3.5 mm), Colgate showed zones of inhibition (13.5 mm - 3.0 mm), Pepsodent showed the highest zones of inhibition (15.5 mm - 10.5 mm), all at a concentration of 500 mg/ml - 125 gm/ml. MacClean showed no significant zone of inhibition at *P*=.05. The toothpastes showed a level of significance at (*P*=.05) at all concentrations.

Table 5 Mean zone of inhibition (mm) of toothpastes on *Streptococcus spp*

Toothpaste	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Close-up	19.5 ± 0.71	11.5 ± 0.71	8.0 ± 0.71	5.0 ± 0.00
Oral-B	18.5 ± 2.12	14.0 ± 1.41	10.0 ± 1.41	6.0 ± 0.00
Colgate	15.5 ± 2.12	12.5 ± 2.12	5.0 ± 0.00	_
MacClean	13.0 ± 1.41	6.0 ± 0.00	_	_
Pepsodent	15.0 ± 1.41	13.0 ± 1.41	9.5 ± 0.71	5.5 ±0.00
Control A	_	_	_	_
Control B	_	_	_	_
Control C	_	_	_	_
Control D	_	_	_	_
Control E	_	_	_	_

Values are mean zone ± S.D of two replicates; **Key:** Control A = Control for Close-Up; Control B = Control for Oral-B; Control C = Control for Colgate; Control D = Control for MacClean; Control E = Control for Pepsodent

Table 6 Mean zone of inhibition (mm) of toothpastes on *Staphylococcus spp*

Toothpaste	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Close-Up	14.5 ± 0.71	8.5 ± 0.71	3.5 ± 0.71	_
Oral-B	14.5 ± 0.71	8.0 ± 1.41	3.5 ± 0.71	_
Colgate	13.5 ± 2.12	6.0 ±1.41	3.0 ± 0.00	_
MacClean	_	_	_	_
Pepsodent	15.5 ± 0.71	13.5 ± 0.71	10.5 ± 0.71	_
Control A	_	_		_
Control B	_	_	_	_
Control C	_	_	_	_
Control D	_	_	_	_
Control E	_	_	_	_

Values are mean zone ± S.D of two replicates**; Key:** Control A = Control for Close-Up**;** Control B = Control for Oral-B**;** Control C = Control for Colgate**;** Control D = Control for MacClean**;** Control E = Control for Pepsodent

Table 7 Mean zone of inhibition (mm) of toothpastes on *Porphyromona spp*

Toothpaste	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Close-up	16.5 ± 2.12	8.5 ± 2.12	5.5 ± 0.71	3.5 ± 0.71
Oral-B	14.5 ± 2.12	6.0 ± 0.00	3.0 ± 0.00	_
Colgate	15.0 ± 1.41	8.5 ± 0.71	4.5 ± 0.71	_
MacClean	_	_	_	_
Pepsodent	15.0 ± 1.41	7.5 ± 0.71	4.0 ± 0.00	_
Control A	_	_	_	_
Control B	_	_	_	_
Control C	_	_	_	_
Control D	_	_	_	_
Control E	_	_	_	_

Values are mean zone ± S.D of two replicates; **Key:** Control A = Control for Close-Up; Control B = Control for Oral-B; Control C = Control for Colgate; Control D = Control for MacClean; Control E = Control for Pepsodent

Table 7 shows the mean zone of inhibition of various concentrations of toothpastes on *Porphyromona spp*. Close-up showed the highest zones of inhibition (16.5 mm - 3.5 mm) at a concentration of 500 mg/ml - 62.5 mg/ml. Oral-B, Colgate and Pepsodent showed zones of inhibition at a concentration of 500mg/ml-125mg/ml. That of Oral-B being 14.5 mm - 3.0 mm, Colgate being 14.0 mm - 4.0 mm and Pepsodent being 15.0mm-4.5mm. MacClean showed no significant zone of inhibition at *P*=.05. Only Close-Up showed an inhibition at all concentration. The efficacy of all toothpastes tends to decrease with decrease in concentration. The toothpastes showed a level of significance (*P*=.05) at all concentrations.

Table 8 shows the mean zone of inhibition of various concentrations of toothpastes on *Lactobacillus spp*. Only Close-up showed an inhibition (17.5 mm - 7.5 mm) at all concentrations. Among the toothpastes, Oral-B and Colgate has the highest mean zones of inhibition (19.5 mm) at concentration of 500 mg/ml. The efficacy of all toothpastes tend to decrease with decrease in concentration. The toothpastes showed a level of significance (*P*=.05) at all concentrations.

Table 8 Mean zone of inhibition (mm) of toothpastes on *Lactobacillus spp*

Toothpaste	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Close-up	17.5 ±0.71	14.5 ± 0.71	10.5 ± 0.71	7.5 ± 0.71
Oral-B	19.5 ± 2.12	10.5 ± 2.12	6.5 ± 0.71	_
Colgate	19.5 ± 2.12	16.0 ± 2.83	14.5 ± 2.12	_
MacClean	14.0 ± 2.83	8.5 ± 0.71	_	_
Pepsodent	12.0 ± 1.41	9.5 ± 0.71	6.0 ± 0.00	_
Control A	_	_	_	_
Control B	_	_	_	_
Control C	_	_	_	_
Control D	_	_	_	_
Control E	_	_	_	_

Values are mean zone ± S.D of two replicates; **Key:** Control A = Control for Close-Up; Control B = Control for Oral-B; Control C = Control for Colgate; Control D = Control for MacClean; Control E = Control for Pepsodent

4. Discussion

In this study, the antimicrobial efficacy of chewing stick *Salvadora persica* (Miswak), dental powder and different toothpastes were tested and examined on selected bacterial isolates, which are *Streptococcus spp, Staphylococcus spp*, *Porphyromona spp* and *Lactobacillus spp*. These isolates were taken from human saliva and have been involved in dental caries . However, the range of effectiveness is concentration dependent and varied against different tested organisms [39]. The percentage occurrence of the bacterial isolates are *Streptococcus* spp (10%), *Staphylococcus spp* (20%), *Porphyromona spp* (40%) and *Lactobacillus spp* (30%).

The evaluation of antimicrobial efficacy of chewing stick *Salvadora persica* (Miswak) extract, showed that it is effective against oral microbes involved in dental caries. However, the aqueous extract of the chewing stick showed no significant zone of inhibition against *Staphylococcus spp* in accordance with a similar work done by [40] who compared the antibacterial activity of chewing sticks and toothpastes commonly used in Kano (Nigeria) on *Staphylococcus* and *Streptococcus* spp. Ethanol extract exhibited higher antimicrobial efficacy than the aqueous extract, this can be due to the absence and concentration level of some constituents such as phenol, alkaloids, terpens and glycosides this is in accordance to the work of [41].

For the dental powder; Agnes nwamma and N-sol, they both showed antimicrobial efficay against *Streptococcus spp*, *Staphylococcus spp*, *Porphyromona spp* and *Lactobacillus spp* with Agnes nwamma being a bit efficacious than N-sol.

Among the toothpastes, Close-up is more efficacious, as also describe by [42] on their work; antimicrobial efficacy of different toothpastes sold in Nigeria. Oral-B, Colgate and Pepsodent are within same range of efficacy. MacClean has the lowest antimicrobial efficacy in accordance with [43].

Comparably, the two dental powder and the toothpastes (with exception of MacClean) showed an equivalent antimicrobial efficacy against the test organisms. With exception of MacClean, the toothpastes tested, has a bit higher antimicrobial efficacy than ethanol extract of the chewing stick but a dominant antimicrobial efficacy than aqueous extract of the chewing stick. This is in accordance with the study carried out by [44] who concluded that toothpastes were more effective in inhibiting cariogenic and pathogenic bacteria than *S. persica.*

5. Conclusion

From the result of the study, it can be concluded that the toothpastes have inhibitory effect on all tested isolates, with exception of MacClean which showed no inhibitory effect against *Staphylococcus spp* and *Porphyromona spp*. Both extracts (ethanol and aqueous) of the chewing stick *Salvadora persica* (Miswak) and the dental powders have inhibitory effect against the tested isolates, however, the aqueous extract of the chewing stick showed no inhibitory effect on *Staphylococcus spp*. Based on the result, the use of chewing stick and locally made dental powder should be encouraged, especially in developing countries where there is lack of funds to acquire toothpastes and other means of health care and services by the population. In terms of dentrifices, inspection should be made on the quality of products by testing the antimicrobial activity of each product brand and improvements should be applied where it is required.

Compliance with ethical standards

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Disclosure of conflict of interest

No competing interests exist.

Authors Contributions

This work was carried out in collaboration between the authors, Authors PCC and PCI designed the study, and wrote the protocol. Authors SCI wrote the first draft of the manuscript. Authors ROU and OSE carried out the statistical analysis. Authors TOO, CA and COA helped with the analysis of the work. The final manuscript was read and approved by the authors.

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