The antibacterial efficiency of dental powder, toothpastes, mouth rinses, charcoal, table salt and chewing sticks against *Streptococcus* and *Lactobacillus acidophilus*.

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**ABSTRACT**

Chewing sticks and other means of obtaining oral health are widely used throughout Africa. But how does the usage of materials compare to the conventional use of fluoride toothpastes for oral hygiene? The aim of the study was to comparatively evaluate the antibacterial efficacy of traditional oral care practices (chewing sticks, dental powders, mouth washes, table salt, and charcoal) and conventional toothpaste against two bacteria strains of public health significance. Standard microbiological and analytical methods were used. *Lactobacillus acidophilus* ATCC 314TM and *Streptococcus mutans* ATCC 25175 were obtained from the American Type Culture Collection Centre and appropriately reactivated. The stem cuttings of chewing stick (*Zanthoxylum zanthoxyloides* and *Massularia acuminata*) were extracted using Soxhlet apparatus. Antibacterial activity of the extracts were done using a modified Kirby-Bauer disk diffusion technique, the minimum inhibitory concentration and minimum bactericidal concentration were carried out using micro dilution technique of double fold dilution. Antibacterial susceptibility testing was done and multiple antibiotic resistance index of the bacterial strain was evaluated thereafter. The results showed that the toothpaste sample labelled B had the highest zone of inhibition (18.00±0.10 cm) and (21.00±0.87 cm) in at a 100% concentration for *L. acidophilus* and *S. mutans* respectively. The two chewing stick samples used in the study had antibacterial activity at 100% concentration for both strains. The mouthwash used in the study tend to have the highest antibacterial activity against *S. mutans* and *L. acidophilus* having a diameter (mm) zone of inhibition 26.00±0.20 cm and 24.00±0.95 cm at 100% concentration respectively. Mouthwash sample met the Clinical Laboratory Standard Institute criterion for reporting the result as sensitive ≥20. Gentamicin, Cefazidime and Meropenem were sensitive to both *S. mutans* ATCC®25175 and *L. acidophilus* ATCC®314. Both bacterial strains used in the study had an index greater than 0.2 which symbolizes that they are of public health importance.

**Keywords:** Chewing sticks, Dental and oral care, *Lactobacillus acidophilus*, *Massularia acuminata*, *Streptococcus*, *Zanthoxylum zanthoxyloides*
INTRODUCTION

Oral health has a significant impact on overall quality of life and well-being. With the rising prevalence of oral disorders, the global demand for effective and cost-efficient preventative and treatment products has grown (Wu et al., 2001). In Nigeria, conventional practices such as the use of fluoride-based dental pastes and mouthwashes are becoming less popular in rural communities as traditional chewing sticks become more prevalent. Chewing sticks contain volatile oils, tannic acid, sulphur, and sterols, which contribute to anti-septic, astringent, and bactericidal properties that help reduce plaque formation, provide anti-caries effects, eliminate bad odor, improve the sense of taste, and cure many systemic diseases, according to Halawany (2003); Tubaishat et al. (2005); Hooda et al. (2011). Very common examples are Zanthoxylum zanthoxyloides, Massularia acuminata, and Vernonia amygdalina. Therefore, it is no news that tooth brushing with toothpaste is a commonly practiced form of oral hygiene in most countries of the world (Pannuti et al., 2003) and the triumph of any toothpaste, lies on its proficiency to removed and eliminate pathogenic oral microflora (Oviasogie et al., 2015).

A wide variety of chemicals with antimicrobial activities have been added to toothpastes in order to produce a direct inhibitory effect on plaque formation (Pannuti et al., 2003; Fine et al., 2006). Toothpastes that contain fluoride have been proven to protect teeth against attack from bacteria (Vohra et al., 2012). It is also found naturally in certain substances including food and drinking water (WHO, 1986). Thus, antimicrobial toothpastes which augment mechanical plaque removal may provide an effective means to maintain good oral hygiene.

Many African homes cleaned their teeth in the morning by using chewing stick until they acquire brush-like ends. The World Health Organization recommends chewing sticks for oral cleanliness, and some of them, or their extracts, have been employed in ethno-medical treatment of oral illnesses (Ndukwe et al., 2005). Nigerians basically employ two methods to remove debris (Plaque) from the tooth and these methods are either through the use of tooth brush and paste or by use of parts of various plants native to West Africa, referred to as “African Chewing Sticks” (Bankole et al., 2012). Meanwhile, a large proportion of persons living in rural areas as well as a few who are also in the urban areas make use of the natural toothbrush known as “chewing sticks. Ojo et al. (2007) defined chewing sticks as parts of higher plants which are cut into suitable lengths and used for the maintenance of oral hygiene. They are important Non Timber Forest Product (NTFP) widely used for dental cleaning in the tropical West Africa (Akande and Hayashi, 1998).

Plants from which chewing sticks are derived are abundant and diverse in Nigeria rural communities. Several studies have demonstrated the antiplaque and antibacterial actions of extracts of these Nigerian chewing sticks (NCS) against oral bacteria, such as Streptococcus mutans (Wolinsky and Sote, 1984), Streptococcus mitis and oral anaerobes (Rotimi and Mosadoni,, 1987), which are the organisms commonly implicated in dental caries and orodental infections. The merits and demerits of this method have been established in literatures but there are contrasting opinions on the efficacy of the various substance(s) used in tooth cleansing and plaque removal. Oviasogie et al. (2016) posited that herbal toothpaste products had better antibacterial activity compared to ordinary fluoridated toothpaste counterparts while in a separate study by Oviasogie et al. (2015), they opined that the antibacterial efficacy of toothpastes was better than the use of natural miswak or chewing sticks (although they used bitter leaf and guava chewing sticks). Some persons are in the habit of using specialized dental formulated powder to cleanse the teeth and eliminate bad breath while some individuals prefer the use of mouthwash and others employ some crude ancient methods such as using charcoal and table salt. The efficacy of some of the listed methods for tooth cleansing has not...
been fully elucidated. This study, therefore, seeks to comparatively evaluate the antibacterial efficacy of toothpastes, mouth washes, dental powder, salts, chewing sticks and charcoal against two strains of public health significant bacteria.

MATERIALS AND METHODS
The 7 communities selected for the study and their location with respect to the reservoir are shown in Fig 1. The area

MATERIALS
Dental powder, salts, charcoal, toothpastes and *Zanthoxylum zanthoxyloides* and *Massularia acuminata* chewing sticks were purchased from New Benin Market, Benin City. Reference strains of odontopathogens (*Streptococcus mutans* ATCC 25175 and *Lactobacillus acidophilus* ATCC 314TM) were obtained from VSR international limited, Lagos, Nigeria. The reference strains which came in lyophilized Kwik stick form and were kept in the refrigerator until it was ready for use according to manufacturer’s specification.

PLANT COLLECTION AND IDENTIFICATION
Fresh *Zanthoxylum zanthoxyloides* and *Massularia acuminata* leaf sticks obtained from stem cuttings were harvested together with the leaves and identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

PREPARATION AND EXTRACTION PLANT MATERIAL
The chewing sticks were chopped into smaller pieces of irregular sizes and dried in an aerated oven at 50°C according to the methods delineated by Oviasogie *et al.* (2016) but with certain modification. The dried chewing sticks were further pulverized using the British milling machine (Gallahap) into powder. The pulverized samples were labelled and kept separately in a sterile, dry screw-capped bottles which were stored in a dry cool place. Using the Soxhlet apparatus, the extraction was carried out in 99.9 % methanol. Following the process of Mustapha *et al.* (2015), 100 g of each crushed chewing stick sample were extracted separately using 0.9 L of 100 % methanol. Each chewing stick’s pulverized sample was placed in its own porous cellulose thimble. The thimble was suspended above the flask containing the solvent (900 ml of methanol) below a condenser in the extraction chamber. The flasks were heated and the solvent evaporated and moved up into the condenser where it was converted to liquid that trickles into extraction chamber containing the sample. At the end of the extraction process which lasted for about 3 hrs, the flask containing the solvent and liquid was removed. The liquids in the various flasks were concentrated to paste level at a temperature of 98°C using crucibles on water bath. They were then scooped into well labeled universal bottles and stored in the refrigerator until ready for use. The phytochemical components of extracts of these stem cuttings were done according to Khalid *et al.* (2018).

ANTIBIOGRAM ASSAY
All the bacterial species were tested for resistance or sensitivity to different antibiotics using the standard disc diffusion method (Kirby Bauer test). For the disc diffusion assay, bacterial strains were grown between 18 and 24 hrs on Mueller-Hinton agar, harvested and then suspended in 0.85 % sterile physiological saline solution adjusted to a 0.5 McFarland turbidity standard, corresponding to $10^8$ cfu mL$^{-1}$. The inoculum was streaked onto plates of Mueller-
Hinton agar using a sterile cotton swab impregnated with appropriate antibiotics produced by Oxoid Limited, UK, which include: Ciprofloxacin (5µg), Cefazidime (30 µg), Sulfamethoxazole (23.75 µg), Azithromycin (15 µg), Vancomycin (30 µg), Meropenem (10 µg), Amoxicillin/clavulanic acid (20/10 µg), and Gentamicin (10 µg) respectively. The results were recorded after 24 h of incubation at 37°C. The diameter of the zone of inhibition around each disc was measured and interpreted as Resistant (R), Intermediate resistant (I) or Sensitive (S) in accordance with the recommended standard established by the Clinical Laboratory Standards Institute (2017). This however served as a positive control for the quality control strains.

**PREPARATION OF TOOTHPASTES, DENTAL POWDERS, SALT, CHARCOAL AND CHEWING STICKS FOR ANTIBACTERIAL ACTIVITY**

Four (04) samples each of toothpastes, dental powder, salt, charcoal and test chewing sticks were tested for their ability to exert antibacterial effect on *Streptococcus mutans* and *Lactobacillus acidophilus*. A series of dilution in double folds were made in four sterile cryogenic with 1.8 ml capacity. Using a calibrated micropipette, sterile distilled water was used as the diluent which was placed in all four sets of tubes. For the extracted plant materials, 0.1 g of the extract (corresponding to 100 mg) was weighed and placed in a cryogenic bottle before the addition of 1000 ml of sterilized distilled water to make a concentration of 100 mg/ml. This concentration was taken as the highest concentration which was further diluted or varied to 20 mg/ml, 4 mg/ml and 0.8 mg/ml. The same process was repeated for dental powder, salt and charcoal samples with differing concentration similar to the plant extract.

**ANTIBACTERIAL ACTIVITY OF TOOTHPASTES, DENTAL POWDER, CHARCOAL, SALT AND CHEWING STICKS**

The Kirby Bauer disc diffusion assay according to the methods delineated by Bauer et al. (1996) was employed for antibacterial testing of the toothpastes and the chewing sticks. Filter paper disc of 6 mm size was cut using a two-hole puncher (office puncher). The paper discs were wrapped in aluminum foil and sterilized at 121 °C for 25 mins using a pressure of 15 psi. The paper discs were aseptically placed using a sterile forceps into the respective concentrations of the toothpastes, charcoal, salts, dental powder, and the chewing stick extracts which were allowed to stand for 3 hrs before they were aseptically transferred using forceps onto Mueller Hinton Agar plates already seeded with the bacterial strains equivalent to 108 cells of McFarland standard. The inoculated plates and the discs containing the respective concentrations of samples were incubated at 37 °C for 18 -24 h. The diameter zones of inhibition were measured and recorded using a meter rule (strictly ensuring that parallax error was avoided by taking several measurement of a particular sample).

**DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION**

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MIC) of the plant extracts, Charcoal, salts, mouth rinses, dental powder and toothpastes were determined using the method described by Vinothkumar et al. (2010) with certain modifications. The least concentration with zones of inhibition was diluted in double fold using Tryptone Soya Broth (Oxoid) and to each of the tubes, equal volume of the test organism (equivalent to 108 McFarland standard) was added and incubated at 37 °C for 24 hrs. Controls were prepared by inoculating tubes without the extracts but with the cell suspensions. A loop from the MIC broth was streaked onto Mueller Hinton agar
plates and left to incubate for another 24 hrs. the concentration with no observable growth following the line of streaking was regarded as the minimum bactericidal concentration (MBC).

MULTIPLE ANTIBIOTIC RESISTANCES (MAR) INDEX
The multiple antibiotic resistance (MAR) index was calculated for each isolate using Krumpernam's (1983) procedures, which involved dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested.

STATISTICAL ANALYSIS
The Statistical Package for Social Scientists (SPSS, version 21.0) was used for the analysis of the data obtained. Two-way ANOVA test was used to determine the level of significance of the test at 95 % confidence limits or 5 % level of significance.

RESULTS
The result of the percentage yields of chewing stick material with methanolic extraction using Soxhlet apparatus is shown in Table 1. There was a percentage yield of 5.99 % for Zanthoxylum zanthoxyloides (ZZ) stem chewing stick extract. Concerning the Massularia acuminata (MA) stem material, leaving only 8.07 % material recovered from the round bottom flask after extraction. Table 2 reveals the microbial purity of the crude extracts of chewing stick as well as other materials used in the study. And after 3 days on agar plates, there was no form of microbial growth present as can be seen by no visible growth of culturable bacteria after several days of incubation except for the charcoal samples.

The qualitative phytochemical screening of MA and ZZ stem and bark are shown in Table 3 below. Reducing sugar was found to be absent in both plant materials tested in the study. However, saponins, flavonoid and tannins were found to be present in both plant materials analyzed in the study. The antibacterial sensitivity or activity of dentifrice formulations against Streptococcus mutans is shown in Table 4. Toothpaste sample labelled B had the highest zone of inhibition (18.00±0.10 mm) in diameter at a concentration of 100 %. At the least concentration (0.8 %) for the same sample, the inhibition zone was 8.00±0.00 mm. Toothpastes sample D had the least inhibition against the bacterial isolate with diameter of inhibition of 14.00±0.00 mm. The antibacterial sensitivity or activity of dentifrice formulations against L. acidophilus is also shown in Table 4. Toothpaste sample labelled B had the highest zone of inhibition (21.00±0.87 mm) in diameter at a concentration of 100 %. At the least concentration (0.8 %) for the same sample, the inhibition zone was 8.00±0.83 mm. Toothpastes sample D has the least inhibition against with diameter of inhibition of 8.00±0.00 mm. There was no statistical significant difference (P>0.05) in the activities of the toothpaste samples C and D when compared with one another against the tested bacterial strain (L. acidophilus). There was a significant difference in the activities of toothpaste samples B when compared with C and D (P<0.05). Toothpastes sample labelled A also had significant activity compared to samples C and D (P<0.05) but not B.

The antibacterial efficacy of chewing stick extracts against S. mutans and L. acidophilus strains is shown in Table 5 below. The two chewing stick samples used in the study had considerable antibacterial activity at 100 % concentration. The chewing stick ‘ZZ’ had an inhibition zone in diameter of about of 14.00 ± 0.20 at 100% concentration. The
chewing stick MA had similar zone of inhibition in diameter of 16.00 ± 0.20 at the same concentration. There was no statistical difference (P>0.05) in the antibacterial efficacy of the chewing sticks (MA and ZZ) against the test bacterial strain *S. mutans* used in the study.

In this study, mouth wash, table salt and charcoal were also evaluated for their antibacterial efficacy against the tested quality control bacterial strains. The Table 5 also revealed the antibacterial activity of the aforementioned substances against quality control bacterial strains. The mouthwash used in the study had most antibacterial activity against the tested bacteria strains with *S. mutans* having a diameter (mm) zone of inhibition of 26.00±0.20 mm at 100 % concentration and 8.00±0.00 mm at 0.8 % concentration. Similar trend was obtained for *L. acidophilus* having diameter of 24.00±0.95 mm at 100 % concentration and 0.00±0.00 mm at 0.8 %. Dental powder also had appreciable zone of inhibition or clearance at 100 % concentration for both bacterial strains used in the study. Table salt and wood charcoal had the least antibacterial effect on the selected bacterial strains employed in the study.

There was however no significant difference between the antibacterial effect of table salt and charcoal on the bacteria strains used in the study (p>0.05).

Table 5 shows the antibacterial sensitivity interpretation criteria based on the Clinical Laboratory Standard Institute's (2017) report. No toothpaste or chewing stick extract sample satisfied the CLSI requirement for reporting sensitive results (diameter of inhibition zone 20 mm). In terms of their different zones of inhibition, all toothpaste and chewing stick extract were classified as intermediate or resistant (see Tables 5 and 6). Only the mouthwash used in the study had zones of inhibition greater than 20 mm, indicating that it is sensitive at 100 percent concentration. *Streptococcus mutans* ATCC®25175 was resistant to ciprofloxacin, vancomycin, azithromycin, and sulfamethoxazole, but sensitive to gentamicin, ceftazidime, and meropenem according to Table 5. Azithromycin, vancomycin, and sulfamethoxazoles were all resistant to the *Lactobacillus* strain. Table 6 reveals the minimum inhibitory concentration and minimum bactericidal concentrations of toothpastes and chewing sticks against quality control bacterial strain used in the study. Following the micro dilution technique employed for MIC as well as MBC assay, toothpastes samples B and C had MIC and MBC values of 1:1 (that is 100 % concentration respectively). Only the mouth wash as well as the dental powder used in the study had antibacterial activity with a lower concentration compared to the other substances tested in the study. The MAR index of the quality control bacterial strains is shown in Figure 1. The results revealed that the bacterial strains were obtained from sources were antibiotics have been used and they are of public health importance. Both bacterial strains used in the study had an index greater than 0.2 which symbolizes that they are of public health importance.

| Table 1: Percentage yield of plant parts after Soxhlet extraction process |
|---------------------------------|-----------------|-----------------|
| Plant extract | Amount of material in grams (g) | Percentage yield (%) |
| Before extraction | After extraction | |
| *Zanthoxylum zanthoxyloides* | 107.76 | 6.46 | 5.99 |
| *Massularia acuminata* | 121.97 | 9.84 | 8.07 |
Table 2: Microbial purity of the plant extract after soxhlet extraction

| Extracts               | Presence of colonies (+) | Incubation period (days) |
|------------------------|--------------------------|--------------------------|
|                        |                          | 1 | 2 | 3 |     |
| Zanthoxylum zanthoxyloides | -                        | - | - | - |     |
| Massularia acuminata   | -                        | - | - | - |     |
| Mouthwash              | -                        | - | - | - |     |
| Table Salt             | -                        | - | - | - |     |
| Charcoal               | +                        | + | + | + |     |
| Dental powder          | -                        | - | - | - |     |

+ = presence of colonies, - = absence of colonies

Table 3: Qualitative phytochemical screening of ZZ stem and bark

| Phytochemicals          | Plant extracts               |
|-------------------------|-------------------------------|
|                         | Zanthoxylum zanthoxyloides    | Massularia acuminata        |
| Alkaloid                | +                             | +                           |
| Carbohydrate            | -                             | -                           |
| Reducing sugar          | -                             | -                           |
| Saponins                | +                             | +                           |
| Tannin                  | +                             | +                           |
| Flavonoid               | +                             | +                           |
| Cardiac glycosides      | -                             | +                           |

+ = Present, - = Absent

Table 4: Antibacterial sensitivity of the test oral wash materials against Streptococcus mutans and L. acidophilus

| Toothpastes               | Zone of inhibition (mm)±S. D |
|--------------------------|-----------------------------|
|                          | Concentration in percentage (%) | 100 | 20 | 4 | 0.8 |
|                         |                             |
| Streptococcus mutans     |                             |
| Toothpaste A             | 14.00±0.87 a                | 10.00±0.59 a                | 0.00±0.00 a | 0.00±0.00 a |
| Toothpaste B             | 18.00±0.10 a                | 14.00±0.35 a                | 12.00±0.49 a | 8.00±0.00 a |
| Toothpaste C             | 16.00±0.15 a                | 12.00±0.05 a                | 18.00±0.00 a | 8.00±0.00 a |
| Toothpaste D             | 14.00±0.00 a                | 8.00±0.00 a                 | 0.00±0.00 a | 0.00±0.00 a |
| Mouthwash                | 26.00±0.20 a                | 18.00±0.00 a                | 12.00±0.00 a | 8.00±0.00 a |
| Table Salt               | 10.00±0.10 b                | 8.00±0.15 b                 | 0.00±0.00 b | 0.00±0.00 b |
| Charcoal                 | 10.00±0.15 b                | 8.00±0.00 b                 | 0.00±0.00 b | 0.00±0.00 b |
| Dental powder            | 16.00±0.30 ab               | 12.00±0.65 ab               | 8.00±0.00 ab | 0.00±0.00 ab |
| Zanthoxylum zanthoxyloides | 14.00±0.20 a            | 8.00±0.00 a                 | 0.00±0.00 a | 0.00±0.00 a |
| Massularia acuminata     | 16.00±0.30 a                | 10.00±0.65 a                | 8.00±0.00 a | 0.00±0.00 a |

L. acidophilus

| Toothpaste A             | 12.00±0.10 a                | 10.00±0.35 a                | 8.00±0.49 a | 0.00±0.00 a |
| Toothpaste B             | 21.00±0.87 a                | 16.00±0.59 a                | 12.00±0.71 a | 8.00±0.83 a |
| Toothpaste C             | 12.00±0.15 b                | 8.00±0.05 b                 | 0.00±0.00 b | 0.00±0.00 b |
| Toothpaste D             | 8.00±0.00 b                 | 0.00±0.00 b                 | 0.00±0.00 b | 0.00±0.00 b |
| Mouthwash                | 24.00±0.95 a                | 14.00±0.15 a                | 10.00±0.00 a | 0.00±0.00 a |
| Table Salt               | 10.00±0.00 a                | 8.00±0.00 a                 | 0.00±0.00 a | 0.00±0.00 a |
| Charcoal                 | 12.00±0.00 a                | 8.00±0.00 a                 | 0.00±0.00 a | 0.00±0.00 a |
| Dental powder            | 16.00±0.35 a                | 10.00±0.13 a                | 8.00±0.00 a | 0.00±0.00 a |
| Zanthoxylum zanthoxyloides | 12.00±0.55 a            | 8.00±0.10 a                 | 0.00±0.00 a | 0.00±0.00 a |
| Massularia acuminata     | 14.00±0.25 a                | 10.00±0.20 a                | 0.00±0.00 a | 0.00±0.00 a |

Key: Values are mean ±SE of triplicates, TP A-D = are toothpaste samples, Similar alphabets along each column indicate no significant difference.
Table 5: Antibacterial sensitivity result interpretation based on CLSI standard 2017

| Sample code | Concentration in percentage (%) | ABC  |
|-------------|---------------------------------|------|
|             | 100  | 20   | 4    | 0.8  |      |
| **S. Mutans** |      |      |      |      |      |
| Tp-A        | R    | R    | R    | R    | na   |
| Tp-B        | I    | R    | R    | R    | na   |
| Tp-C        | I    | I    | R    | R    | na   |
| Tp-D        | R    | R    | R    | R    | na   |
| Zz (Stem)   | I    | R    | R    | R    | na   |
| Ma (Stem)   | I    | R    | R    | R    | na   |
| Mouthwash   | S    | I    | R    | R    | na   |
| Table Salt  | R    | R    | R    | R    | na   |
| Charcoal    | R    | R    | R    | R    | na   |
| Dental powder | I   | R    | R    | R    | na   |
| CIP         | na   | Na   | Na   | na   | R    |
| AMC         | na   | Na   | na   | na   | I    |
| CAZ         | na   | Na   | na   | na   | S    |
| AZM         | na   | Na   | na   | na   | R    |
| VA          | na   | Na   | na   | na   | R    |
| MEM         | na   | Na   | na   | na   | S    |
| CN          | na   | Na   | na   | na   | S    |
| RL          | na   | Na   | na   | na   | R    |
| **L. acidophilus** |        |      |      |      |      |
| Tp-A        | R    | R    | R    | R    | na   |
| Tp-B        | S    | I    | R    | R    | na   |
| Tp-C        | R    | R    | R    | R    | na   |
| Tp-D        | R    | R    | R    | R    | na   |
| Zz (Stem)   | R    | R    | R    | R    | na   |
| Ma (Stem)   | R    | R    | R    | R    | na   |
| Mouthwash   | S    | I    | R    | R    | na   |
| Table Salt  | R    | R    | R    | R    | na   |
| Charcoal    | R    | R    | R    | R    | na   |
| Dental powder | I   | R    | R    | R    | na   |
| CIP         | na   | Na   | na   | na   | S    |
| AMC         | na   | Na   | na   | na   | S    |
| CAZ         | na   | Na   | na   | na   | S    |
| AZM         | na   | Na   | na   | na   | R    |
| VA          | na   | Na   | na   | na   | R    |
| MEM         | na   | Na   | na   | na   | S    |
| CN          | na   | Na   | na   | na   | S    |
| RL          | na   | Na   | na   | na   | R    |

**Keys**: S= sensitive, R= resistant, I = intermediate, S = resistant. 
Zz = Zanthoxylum zanthoxyloides; Ma (Stem) = Massularia acuminata, Tp=Toothpaste

Legend: CIP; Ciprofloxacin (5µg), CAZ; Cefazidime (30 µg), RL; Sulfamethoxazole (1.25/23.75 µg), AZM; Azithromycin (15 µg), VA; Vancomycin (30 µg), MEM; Meropenem (10 µg), AMC; Amoxicillin/clavulanic acid (20/10 µg), CN; Gentamicin (10 µg). R = Resistant, S = Susceptible, ABC antibiotic concentration
Table 6: Minimum inhibitory concentration and minimum bactericidal concentration of toothpaste and chewing stick extract against *Streptococcus mutans*

| Sample code   | MIC% | MBC% |
|---------------|------|------|
| Toothpaste A  | NA   | NA   |
| Toothpaste B  | 50   | 50   |
| Toothpaste C  | 50   | 50   |
| Toothpaste D  | NA   | NA   |
| Zz stem       | 100  | 100  |
| MA stem       | 100  | 100  |
| Mouthwash     | 50   | 50   |
| Table Salt    | 100  | NA   |
| Charcoal      | 100  | NA   |
| Dental powder | 50   | 100  |

Key: NA=Not applicable, Zz = Zanthoxylum zanthoxyloides; Ma (Stem) = Massularia acuminata

DISCUSSION

Diverse range of bacteria, fungi and protozoa are found in human oral cavity which may cause dental diseases in poor hygiene. The antibacterial activity of chewing stick, toothpastes, mouth wash, dental powders and salt against *S. mutans* ATCCC®25175 and *L. acidophilus* ATCC 314T was evaluated in this study. The in-vitro agar disc diffusion method was adopted in this study because it displayed a relatively direct means of measuring the antimicrobial strength of each product. Thus, the product with the largest zone of inhibition has the strongest antimicrobial properties. In this study, the qualitative Phytochemical screening revealed the presence of flavonoids, saponins, cardiac glycoside and tannins in *Massularia acuminata* (pakuijabu) chewing sticks and flavonoids, saponins and tannins in *Zanthoxylum zanthoxyloid* (pakuijebu). This finding was in consonance with the works of Itemire *et al.* (2013), Taiwo *et al.* (1999).
Adegbolagun and Olukemi, (2010) who identified, cardiac glycoside, saponins, tannins and flavonoids from the plant extract. All samples used in this study were devoid of bacterial isolated and thus microbologically pure except for charcoal. Different brands of toothpastes designated, A, B, C, and D were tested for antibacterial activity against \textit{S. mutans} ATCCr25175 and \textit{L. acidophilus} ATCC 314T. The reason for their activity may be due to the presence of Aloe barbadensis leaf extract and adequate fluoride in each toothpaste, this result is in consonance with work of Adenike and Olubukonla (2010) but with a different strain. This report is in contrast with the view of Oviasogie \textit{et al.} (2015) who opined that these toothpastes had inhibitory effect against the same oral pathogen but a different strain. In the evaluation of chewing stick extract \textit{Massularia acuminata} (MA) and \textit{Zanthoxylum zanthoxyloid} (ZZ) at a concentration of 100 mg/ml and 20 mg/ml had appreciable inhibition zones against the oral pathogens evaluated in this study (\textit{S. mutans} and \textit{L. acidophilus}), this study is in consonant with Osho \textit{et al.} (2011) evaluated the \textit{In Vitro} antibacterial activity of some selected Nigerian medicinal plants including the \textit{zanthoxyum zanthoxyloids} against oral pathogens. It was revealed that the plant materials (chewing sticks) evaluated in the study had appreciable antibacterial activity against \textit{S. mutans} and \textit{L. acidophilus} similar to the report obtained in this study. Akande and Ajao (2011) evaluated the antibacterial effect and chemotherapeutic value of four Nigerian chewing sticks of which \textit{Zanthoxylum zanthoxyloides} and \textit{Massularia acuminata} were used in this study. The findings from their research was consistent with the report obtained in this study as \textit{Massularia acuminata} was more active against the tested bacterial isolates of dental infection compared to \textit{Zanthoxylum zanthoxyloides} used in the study. The antimicrobial activity displayed by \textit{Massularia acuminata} extract could be due to the presence of the bioactive component cardiac glycoside revealed in the phytochemical screening (Table 2). Rotimi and Mosadomi (1987); Osho \textit{et al.} (2011); Osho and Adelani (2012) reported that the chewing stick \textit{Zanthoxylum zanthoxyloid} had antibacterial activity against a plethora of bacteria pathogens implicated in dental caries howbeit at different concentrations. But the best of bacteri al activity was found in the highest concentrations reported in the aforementioned studies which were dubbed as 100 % concentration. These findings are strictly similar to the one reported in this study as there was better activity at 100 %. There was high amount of activity observed for the mouthwash used in the study, Akinyele \textit{et al.} (2014) worked on the comparative study of antibacterial effect of mouthwash and bitter leaf on some bacteria causing tooth decay and made an interesting finding similar to the findings in this study. Akande \textit{et al.} (2004) also reported similar findings to the one obtained in this study as the evaluated mouthwashes for antibacterial activity was found to be effective in terms of zones. Similar report was also obtained in the study carried out by Masadeh \textit{et al.} (2013) on the antibacterial efficacy of some mouthwashes against multi resistant bacterial biofilms. Janan \textit{et al.} (2015) evaluated the antibacterial efficacy of four mouthwashes on \textit{S. mutans} and \textit{Escherichia coli}. Although one of the brands of the mouthwash evaluated was the same with the one used in this study. There was high antibacterial effect of the mouth wash against \textit{S. mutans} which was very similar to the findings obtained in several other findings were also similar to the result obtained in this study for the mouthwash sample. The antibacterial activity of charcoal against the test bacterial strain was in support of the findings of Sung-Hwa \textit{et al.} (2011) who evaluated the antibacterial activity of bamboo charcoal against oral \textit{Streptococcus mutans} and revealed a significant bacterial activity at high concentration of the charcoal but no activity at low concentrations. This corroborated the findings in this study as there was no activity whatsoever at 20 %, 4 % and 0.8 % of charcoal concentration used for activity. The antibacterial activity of dental powder is more or higher compared to salt against the tested bacterial strain (\textit{S. Mutans} and \textit{L. Acidophilus}) used in this study. Simiyu \textit{et al.} (2016) work on effect of homemade dental powder, tumeric and salt on population of \textit{Streptococcus mutans}, reveals
that dental powder has more microbial activity compared to salt which was in consonant with this study. According to Clinical Laboratory Standard Institute (2017) interpretative criteria, all the zone of inhibition was regarded as sensitive greater than (> 20 mm), intermediate between 15 -19 mm or resistance less than (<) 14 mm. Only mouth wash is sensitive for S. mutans ATCCr25175 and L. acidophilus. Following the micro dilution technique employed for MIC as well as MBC assay, toothpastes samples B and sample C had MIC and MBC values of 50 % concentration for both tested bacterial strain. The MIC and MBC of the mouth wash against S. mutans ATCCr25175 and L. acidophilus values were 20 % and 40 % respectively. Both bacterial strains used in this study were both resistant to vancomycin, azithromycin and sulfamethoxazole but they were sensitive to gentamicin, cefazidime and meropenem. The MAR index reveal that the bacterial strain is not just multiple resistant but 0.2 index away from the highest range possible. However, the mouth wash used in this study mouthwash had the highest antimicrobial activities against S. mutans ATCCr25175 and L. acidophilus thus suitable method for mouth cleans.

CONCLUSION/ RECOMMENDATION
According to the findings, mouthwash had a significant inhibitory effect on the oral pathogens (S. mutans and L. acidophilus). It is affordable and frequently available, this could be an excellent alternative to toothpaste in rural regions (especially for plant species with great inhibitory activities against cariogenic bacteria). Having discovered the antibacterial properties of chewing sticks, it is critical to recommend that they be phytochemically described in order to determine the main antimicrobial components.

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