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Phytochemical investigation and antimicrobial screening of local chewing stick- *Homanium africanum* against selected oral bacterial isolates

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Abstract: Most chewing stick plants are reported to be rich in phyto constituents and mineral composition apart from exerting mechanical cleansing and dislodging oral pathogens. This study is aimed at investigating the phytochemical, proximate, mineral composition, and antimicrobial activity of the local chewing stick (*Homanium africanum*) against selected oral bacterial isolates. The phytochemical, proximate, and mineral analyses were evaluated using standard methods and procedures. The oral bacterial isolation was carried out using the pour plate method, while the antibacterial assay was done using the agar diffusion method. Glycosides, alkaloids, phenolics, saponins, and terpenoids were present in the three plant samples (dried plant powder, methanol extract, and hexane extract). Carbohydrate content of 53.97±8.6% was recorded as the highest, followed by crude fibre (44.51±11.5%), ash (13.27±3.5%), moisture content (6.99±1.3%), crude fat (4.06±1.6%), and the least was the crude protein content (1.35 ±1.1%), while calcium, sodium, potassium, and zinc were among the elements detected. The oral bacterial isolates (*S. mutans, L. bulgaricus, K. pneumonia*, *P. vulgaris,* and *E. coli*) were all susceptible to the inhibitory effect of the methanol extract. The most susceptible was *L. bulgaricus* with a minimum inhibitory concentration of 12 mg/mL, followed by *S. mutans* (14 mg/mL), *P. vulgaris* (16 mg/mL) while *E. coli* and *K. pneumonia* had 18 mg/mL. This finding corroborates and validates the local use of the plant stem as a chewing stick or teeth cleanser, mostly in rural areas.

Introduction

Homalium africanum (Hook. f.) Benth, family -*Flacourtiaceae,* it is a forest tree growing up to 25-30 meters tall, though sometimes a shrub 2-5 metres tall. The short, straight bole is up to 40 cm in diameter; it is slightly fluted, with branches from quite low down, and it is found in the rainforest of West Africa [1, 2]. Some chewing-stick plants (*Homalium africanum*) are reported to not only exert mechanical cleansing and dislodging of oral microorganisms, including bacteria, but also, for their immensely beneficial elemental and proximate phytochemicals. These compounds expand the usefulness of chewing stick plants to the entire body system while leaving the oral cavity and teeth clean and healthy. According to Velavan [3], phytochemicals or otherwise referred to as plant chemicals are chemical compounds produced by plants, generally to help them resist microbial infections, and consumption by insects and other animals [4, 5]. Plant chemicals include compounds assayed for as anti-nutrients, elemental composition, proximate content, vitamins, and all organic structural, physiological, as well as secondary metabolites of plants [6, 7].

The popularly assayed phytochemicals are actually anti-nutritive secondary metabolites. Antinutrients commonly found in plant foods have both adverse effects and health benefits. For example, phytic acid, lectins, phenolic compounds (tannins), saponins and enzyme (amylase and protease) inhibitors have been shown to be of immense health or medicinal value; however, they reduce the availability of nutrients and cause growth inhibition, while others have been reported to be poisonous, of course, depending on the amount or dose applied. In another regard, a phytochemical that are of health benefits with respect to mopping up free radicals are called antioxidants. Therefore, any of various bioactive free-radical scavenging chemical compounds found in plants is referred to as an antioxidant, considered to be beneficial to human health [8-10]. A related description of phytochemicals is biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans beyond those attributed to macronutrients and micronutrients. These compounds represent a class of medicinal compounds such as alkaloids, tannins, glycosides, saponins, etc. It is important to note that the groupings of phytochemicals are not mutually exclusive, nor are they arbitrary. When a phytochemical is associated with compounds or substances of natural or synthetic origin that interfere with the absorption of nutrients, and act to reduce nutrient intake, digestion, and utilization, and may produce other adverse effects, they are referred to as antinutrients.

Antinutrients are found in their highest concentrations in grains, beans, legumes, and nuts, but can also be found in leaves, roots, and fruits of certain varieties of plants. The major antinutrients found in plant-based foods are phytates, tannins, lectins, oxalates, *etc*. Antinutrients in vegetables, whole grains, legumes, and nuts are a concern only when a person's diet is composed exclusively of uncooked plant foods. Oxalate, for instance, prevents calcium from being absorbed in the body by binding with it; Phytate is present in grains, nuts and seeds, while peppers, eggplants, and tomatoes contain lectins. Phytate consumption may lead to lower mineral absorption and lectins are able to cause various reactions in the body. However, some vital elements (magnesium, sodium, zinc, etc.) are found to co-exist with bioactive chewing stick plant phytochemicals such as alkaloids, flavonoids, glycosides, etc and also as an oral hygiene plant, their proximate assay reveals various levels of carbohydrate, proteins, fibers, etc., all of which are of immense benefits not only to the plants but also to the end user or consumers whose teeth becomes well cleansed by the mechanical dislodging of the teeming microbial population in the oral cavity as well as residual health benefits since chewing sticks are used without necessarily involving serious mouth rinsing and in the process [11], bioactive compounds are extracted by the salivary juice which are partly often swallowed. Chewing sticks could be obtained from over 300 plant species but the most commonly preferred are those having a good flavour, texture and a recognized effect on the teeth and supporting tissues. Plants that are used as chewing sticks include: *Salvadora persica, Garcina manni, Terminalia glaucescens, Anogeissus leiocarpus, Pseudocedrela kotschi, Xanthoxyllum gilleti, Acacia arabica, Juglans regia, Alnus glutonisona, Antidesma venosum, Capparis decidua, Albizia coriaria, Aegles marmelos, Fagara zanthoxyloides, Balanites aegyptiaca, Boscia coriacea, Berchemia discolor, Cadaba farinose, Dobera glabra, Citrus spp., Cordia sinensis, Cupressus lusitanica, Dodonia angustifolia, Euclea schimperi, Olea europia, Rhus abyssinica, Rhamnus staddo, Sterospermum kunthianum, Salix subserrata, Cassia Vinnea, and Azadirachta indica*. Plants used are very carefully selected for such properties as foaminess, hardness, bitterness, numbness or a tingling peppery taste. Among the selected plants, certain species are more popular than others (**Plates 1 & 2**). *Anogeissus leiocarpus* is a graceful tree of the Sahel to forest zones, with straight tapering boles branching, often gregarious and effectively killing out grasses. The leaves serve as fodder to livestock and used in traditional medicine as a remedy for many ailments of livestock and man, including helminthiasis, schistomiasis, leprosy, diarrhoea, and psoriasis [12, 13].

It is generally accepted that oral hygiene maintenance through regular removal of dental plaque and food deposits is an essential factor in the prevention of dental caries and periodontal disease. Methods for oral hygiene vary from country to country and from culture to culture. Despite the widespread use of toothbrushes and toothpastes, natural methods of tooth cleaning using chewing sticks selected and prepared from the twigs, stems or roots of a variety of plant species have been practised for thousands of years in Asia, Africa, the

Middle East and the Americas (**Table 1**). Selected clinical studies have shown that chewing sticks, when properly used, can be as efficient as toothbrushes in removing dental plaque due to the combined effect of mechanical cleaning and enhanced salivation. It has also been suggested that antimicrobial substances that naturally protect plants against various invading microorganisms or other parasites may leach out into the oral cavity, and that these compounds may benefit the users by protection against cariogenic and periodontopathic bacteria. Some clinical epidemiological studies are in support of this, and many laboratory investigations have suggested the presence of heterogeneous antimicrobial components extractable using different chemical procedures. A few recent studies have identified some of the active antimicrobial compounds. Today, chewing sticks are still used in many developing countries because of religion and/or tradition, and because of their availability, low cost and simplicity. The World Health Organization (WHO) also encourages their use. The Year 2000 Consensus Report on Oral Hygiene (CROH) states that chewing sticks may have a role to play in the promotion of oral hygiene, and that evaluation of their effectiveness warrants further research.

Plate 1: *Homalium africanum* in its natural habitat

Plate 2: Dried chewing stick

Table 1: Different names of chewing stick/country

Name	Country/ethnicity
1. Miswak, Siwak or Arak	Arab nations (Middle East)
2. Datun	India, Pakistan
3. Miswaki	Tanzania
4. Sithiou	Senegal
5. a) Aswaki	Nigeria - Hausa
b) Pako	Nigeria - Yoruba
c) Atu	Nigeria - Igbo
d) Ukpakon	Nigeria - Bini
e) Atho	Nigeria - Urhobos

Though more attention is being paid to oral and dental hygiene all over the world, tooth decay and gum diseases are increasing daily. Inadequate cleaning of the teeth to remove plaque plays a key role in the development of these problems [14]. An intermittent combination of a chewing stick with a modern toothbrush seems to be the perfect solution to this problem. This is because plants have played an important role in the treatment of infectious diseases caused by many germs for centuries. Despite the emergence and popularity of artificial toothbrushes today, chewing sticks have steadily and increasingly enjoyed recommendations and the WHO proposed and re-emphasized the use of these sticks as an effective tool for oral hygiene after their effect against certain germs that cause dental problems was studied [15]. The use of plants with antimicrobial properties and mechanical cleansing of the oral cavity is desirable and research to substantiate such claims is imperative [4]. Despite various research on the *Homalium* species, information on the antimicrobial and phytochemical properties of *H. africanum* is scarce. However, the plant is used in traditional medicine to treat bacterial, fungal and viral related infections [15]. It is used as a chewing stick for oral-dental hygiene [16-20]. The use of antimicrobial chewing sticks to help in the control of oral biofilms difficult to reach by brushing and flossing especially biofilm of bacteria contained in oral mucosal reservoirs has warranted the search for new bioactive plants. This study is aimed at investigating the phytochemical constituents and antimicrobial screening of the local chewing stick, *Homanium africanum*, against selected oral bacterial isolates.

Materials and methods

Collection and identification of samples: The stem of *Homalium africanum* bearing the leaves and flowers was sourced from local dealers in Evwreni Kingdom of Ughelli-North Local Government Area, Delta State, Nigeria. The plant bearing leaves and flowers was separated and authenticated in the Department of Plant Biology and Biotechnology, University of Benin, and a voucher specimen of the plant was deposited in the Herbarium and voucher number UBHH264 (*H. africanum*) was assigned.

Treatment of plant sample: The stem of the plant was washed with distilled water, dried in the laboratory for three weeks, cut into smaller pieces and then air-dried again for two weeks before milling. The powdered sample was stored in air-tight bottles for subsequent analysis.

Extraction procedure: One hundred and ninety-eight grams of the ground stem was exhaustively extracted with 2500 mL of hexane and methanol solvents, respectively, using a Soxhlet extractor equipped with a reflux condenser for about 10 hrs till the solvent leaving the thimble was clear. The two extracts were concentrated separately using a rotary evaporator on a water bath (75ºC) to give the crude extracts.

Phytochemical screening of plant extracts: Phytochemical screening was performed on a portion of the dried powder, hexane and methanol extracts of *Homalium africanum* to identify the presence of constituents like alkaloids, tannins, saponins, glycosides, flavonoids, and steroids using standard procedures [21-23].

Moisture content: The moisture content was determined using the weight difference method [24]. The sample (5.0 g) was weighed into a clean dry crucible of known weight. The crucible with its content was oven dried at 105ºC for over 3 hrs a drying interval, until a constant weight was achieved. It was then removed after the first 3 hrs and weighed at 30 min-intervals until a constant weight was achieved. The sample was cooled in a desiccator, re-weighed using an analytical balance and the percentage moisture content was calculated as the loss in weight in percentage.

Total ash: The total ash value was determined using the dry ashing method [24]. Six clean crucibles were labelled 1-6 and 2.0 g of the samples were weighed into each crucible. Three of the crucibles with its contents were heated in the muffle furnace at 700ºC for 3 hrs until white (light grey) residue was obtained. The muffle furnace was switched off, allowed to cool and all the crucibles were removed, cooled in a desiccator and reweighed. The percentage ash was calculated for the three replicates with reference to the air-dried content of the other three crucibles.

Crude protein: A modified method of micro-Kjeldahl as described by AOAC [24] was used for crude protein determination. Procedure for digestion: 3.0 g each of the defatted samples was separately weighed into a pre-weighed micro-Kjeldahl digestion flask together with a few anti-bumping granules. 2.0 g of catalyst mixture ($CuSO_4$: Na_2SO_4: SeO_2, 5: 1: 2 w/w) was added to each flask and then 10.0 mL nitrogen-free concentrated H_2SO_4 was added to each flask. The flasks were placed in an inclined position on a heating mantle in a fume cupboard. Digestion was started at a temperature of 30°C until frothing ceased and then heating was increased to 50°C for another 30 min and finally at full heating (100°C) until a clear solution was obtained. Simmering was continued below the boiling point for another 30 min to ensure complete digestion and conversion of nitrogen to ammonium sulphate. After digestion was completed, samples were allowed to cool and then transferred quantitatively to 100 mL volumetric flasks with washing and cooling to room temperature. Volumes were made up to mark with distilled water. 5.0 ml of the filtrate from the digest was transferred with the aid of a 10.0 ml pipette into a 25.0 ml standard flask. 2.5 ml of the alkaline phenate was added and the solution was shaken to mix properly. Then, 1.0 ml of sodium potassium tartarate was added, shaken properly, followed by the addition 2.5 ml of sodium hypochlorite. There after the solution was made up to the 25.0 ml mark with distilled water and the absorbance of the resultant solution was measured with the aid of UV/visible spectrophotometer, at 630 nm. The Nitrogen standards were treated the same way as the sample.

Crude fat: The crude fat of the sample was determined using petroleum ether Soxhlet extraction of lipid [22, 23]. Exactly 5.0 g of sample was weighed into an oven-dried thimble of pre-determined weight and placed in a Soxhlet extractor connected to the extraction flask containing 500 ml petroleum ether (extraction solvent with 50-70^{o}C boiling point) and a condensing system connected to a Jolambo to supply chilled running water. The process of extraction was allowed to continue until the extraction solvent flowing through the thimble and its content became clear (samples no longer contain lipid). The thimble was then removed from the extraction set-up, oven dried at 90^{o}C. After 3.0 hrs of drying the thimble and its contents were removed from the oven cooled in a desiccator and the weight was recorded.

Crude fibre: 2.0 g of the powdered sample was weighed into a clean dry beaker. 10.0 ml of acetone was added to the sample and the sample was allowed to air dry for 10 min. To the air-dried sample 100 ml of 1.25% sulphuric acid was added; the mixture was heated over a hot plate and allowed to reflux for 30 min. After reflux the mixture was filtered through a muslin cloth rinsing with hot water to neutrality. To the filtrate 100 ml of 1.25% of NaOH was added; the mixture was allowed to reflux again for 30 min. The resultant mixture was filtered and the residue was washed with dilute HCL followed by hot water till it is free from alkali (to neutrality). The residue was oven dried in a crucible of known weight to constant weight. The dried residue was ashed for one hour at 300^{o}C in a muffle furnace. The loss of weight due to ignition was recorded as the weight of crude fiber [21].

Determination of carbohydrate: The soluble carbohydrate content of the sample was determined using the method described previously [21]. In the process, 5.0 g of the sample was dissolved in 250 ml of distilled water and continuously agitated for 3 hrs using a shaker, followed by filtration. 4.0 ml of the filtrate was pipetted into three test tubes while 4.0 ml of distilled water was introduced into a fourth test tube as a blank and 4.0 ml of glucose into a fifth test tube as a standard. To each of the test tubes was added 2.0 ml each of Fehling's solutions (A & B). It was boiled gently in a water bath for 3 min. After which 10.0 ml of freshly prepared 0.10% enthrone reagent was added, stoppered and mixed thoroughly by gently shaking. Each tube was labelled and placed in a test tube rack and allowed to stand for 12 min before transfer to the Spectrophotometer and absorbance read at 630 nm against the blank. Another method is to estimate the carbohydrate content. In this process, the total carbohydrate content of the diet samples is obtained by subtracting the sum of percentage crude protein, crude fat, moisture, fibre and ash from 100.

Mineral analyses: The elemental assay was achieved according to the method [24] in the following process: Wet ashing of sample: 5.0 g of the sample was placed in a Kjeldahl flask and 10.0 mL of mixed acid (nitric acid and perchloric acid mixture at 3: 1) was added to the flask. The flask and its content were mildly heated for 20 min at a temperature of 40^{o}C and then increased to 100^{o}C for another 20 min. The sample was allowed to cool; 20.0 mL of distilled water was added and filtered into a standard flask. It was then made up to 100 ml with distilled water.

Flame Photometer (FP) and Atomic Absorption Spectrometer (AAP): The elements sodium and potassium were assayed using a FP while calcium, magnesium, copper, and zinc were assayed using.

Source of microorganisms: Oral bacterial isolates were obtained from 15 samples collected randomly using a sterile swab stick from 15 students within the Ugbowo campus community of the University of Benin, Benin City, Nigeria. Individuals in the sample population were sensitized on the process of sample collection and the sterile swab had its seal broken and immediately used to obtain the oral swab by rubbing the woolly part over the gum, tongue, teeth and oral buccal mucosa in a tooth-brushing stroke. The swabs were reintroduced into its sterile shield and appropriately labelled. All samples were immediately transported to the laboratory for subsequent analyses.

Characterization and identification of isolates: The bacterial isolates were identified by standard protocol [25] based on colonial appearance, microscopy and biochemical characteristics. Essentially, sampled swab sticks

in their casing received 3.0 ml of normal saline by injection, carefully agitated and used for serial dilution. Aliquots (0.1 mL) of dilution factor 10^{-3} were plated out in nutrient agar and incubated at 38ºC for 24 hrs. After which, emerging discrete colonies were enumerated, colonial morphology was observed and further sub-cultured into other selective and differential media such as MacConkey agar, blood agar, nutrient agar, MRS agar and mannitol salt agar. All petri-plates were appropriately incubated and cultures of distinct colonies were Gram-stained for microscopy and biochemical characterisation (coagulase, citrate, indole, urea, motility and sugar fermentation tests). Suspected *Streptococcus* spp. was sub-cultured on blood agar and *Lactobacillus* spp. were isolated on MRS agar. The *Lactobacillus* MRS agar culture plates were incubated in anaerobic Jars at 38ºC for 48 hrs. Characterised and identified isolates were grown on agar slants and stored in the refrigerator at 4ºC prior to antibacterial assay. The reference bacterial cultures included *S. mutans*, *E. coli, L. bulgaricus, P. vulgaris,* and *K. pneumoniae*.

Antibacterial assay of the plant extracts: The modified agar well diffusion method described was used to determine the antibacterial sensitivity/potency of the extract against test isolates [18, 19]. In the process, wells of 6.0 mm in diameter were made into previously seeded Mueller-Hinton agar plates using a flamed (sterilized) cork borer. Prior to seeding, isolates stored in slants were sub-cultured into nutrient broth, incubated overnight and diluted (1: 100) to achieve 0.5 MacFarlane and turbidity standard (containing approximately 10^6 cfu/mL). Sterile swab sticks were then dipped into the standardized bacterial suspension and gently spread over (seeding) the surface of the agar plates in even strokes to obtain a uniform growth pattern across the entire surface of the plate. This was achieved by rotating the plate 90 degrees followed by 45 degrees with continuous streaking, and finally by streaking around the diameter of the agar. The 6.0 mm wells were filled with equal volumes (0.1 mL) of the stock concentration and lower dilutions of the sample corresponding to 300, 150, 75, and 30 mg/mL concentrations. The same quantity of 10.0% Tween-80 and 10.0 µg/mL ciprofloxacin served as negative and positive controls, respectively. All plates were incubated at 38ºC for 24 hrs in an upright position to allow proper diffusion of extracts. All experiments were in triplicate. After incubation, the absence or presence of microbial growth around the wells was observed on the plates and the diameter of clear zones was measured and the mean inhibitory zone diameters (IZDs) were calculated.

Determination of MICs of the extract against test organisms: The modified broth dilution method described previously [19-21] was used to determine the MICs of the extracts against the test organisms. Varying concentrations of the selected antimicrobial agent ranging from 1-30 mg/mL were constituted in 10.0 ml of Mueller-Hinton broth in sterilized capped tubes/bottles. Exactly 100 µL of the overnight broth culture of the test organisms diluted one in hundred-fold, corresponding to 0.5 McFarland turbidity standard was added. In each round of the experiment, a tube without the extract but with same volume of broth and inoculum served as control. All tubes were appropriately incubated at 38ºC for 24 hrs. After incubation, tubes were observed for growth by examining for turbidity. In all cases, the lowest concentration of the extract at which there was no observable bacterial growth was recorded as the MICs.

Determination of minimum bactericidal concentration (MBC) of the extract: The plates with no visible growth following MIC determination were plated into fresh nutrient agar plates using a flamed wire loop. Three MIC experimental tubes with progressively higher concentrations than the MIC were considered. All plates were appropriately incubated at 38ºC for 24 hrs. The MBC was taken for the minimum tube concentration in which no growth of bacteria was observed [25].

Results

This experimental study indicated the presence of terpenoids, saponins, glycosides, phenolics, flavonoids and alkaloids in the dried plant powder and two extracts screened except tannins as shown in **Tables 2 and 3**. **Table 4** indicates the proximate assay, which includes carbohydrate content, crude fibre, moisture content,

crude fat and crude protein. While mineral contents of the plant are shown in **Table 5**. The percentage occurrence of oral bacterial isolates obtained from the sampled population of students is given in **Table 6** and **Figure 1** was used to depict the antibacterial activities of the methanol extract at different concentrations against the test organisms. The MICs and MBCs of the methanol extract of *H. africanum* on bacterial isolates are presented in **Table 7**.

Table 2: Phytochemical screening of pulverized stem of chewing-stick plant (*Homalium africanum*)

S/N	Phytochemicals	Dry powder
1	Glycosides	+
2	Saponins	+
3	Steroids	+
4	Flavonoids	+
5	Tannins	-
6	Cyanogenetic glycosides	-
7	Alkaloids	+

Keys: + = Present; - = Absent

Table 3: Phytochemical screening of methanol and hexane extracts of *Homalium africanum*

S/N	Phytochemicals	Methanol extract	n-Hexane
1	Glycosides	+	-
2	Saponins	+	+
3	Steroids	+	+
4	Flavonoids	+	-
5	Tannins	-	
6	Cyanogenetic glycosides	-	-
7	Alkaloids	-	+

Keys**:** + = Present - = Absent

Table 4: Proximate Assay of *H. africanum* powdered sample

S/N	Parameter	Content
1	Moisture Content	6.99±1.3%
2	Ash Content	13.27±3.5%
3	Crude Fat	4.06±1.6%
4	Crude Fibre	44.51±11.5%
5	Crude Protein	1.35±1.1%
6	Carbohydrate	53.97±8.6%

Table 5: Mineral analysis of *H. africanum* extracts

S/N	Parameter	mg/100g
1	Copper	0.09±0.008
2	Iron	2.65±0.161
3	Sodium	7.37±0.618
4	Potassium	118.67±0.047
5	Calcium	382.4±1.88
6	Magnesium	3.22±0.039
7	Manganese	1.18±0.051
8	Zinc	2.95±0.04
9	Lead	BDL
10	Cadmium	BDL
11	Nickel	BDL
12	Chromium	BDL

Keys: Mean± Standard error; BDL = Below detectable level

Table 6: Percentage occurrence of oral bacterial isolates obtained from the sampled population

Isolates	No. of isolates	Frequency of occurrence
Streptococcus mutans	12	37.5%
Lactobacillus bulgaricus	8	25%
Klebsiella pneumonia	5	15.6%
Escherichia coli	5	15.6%
Proteus vulgaris	2	6.3%

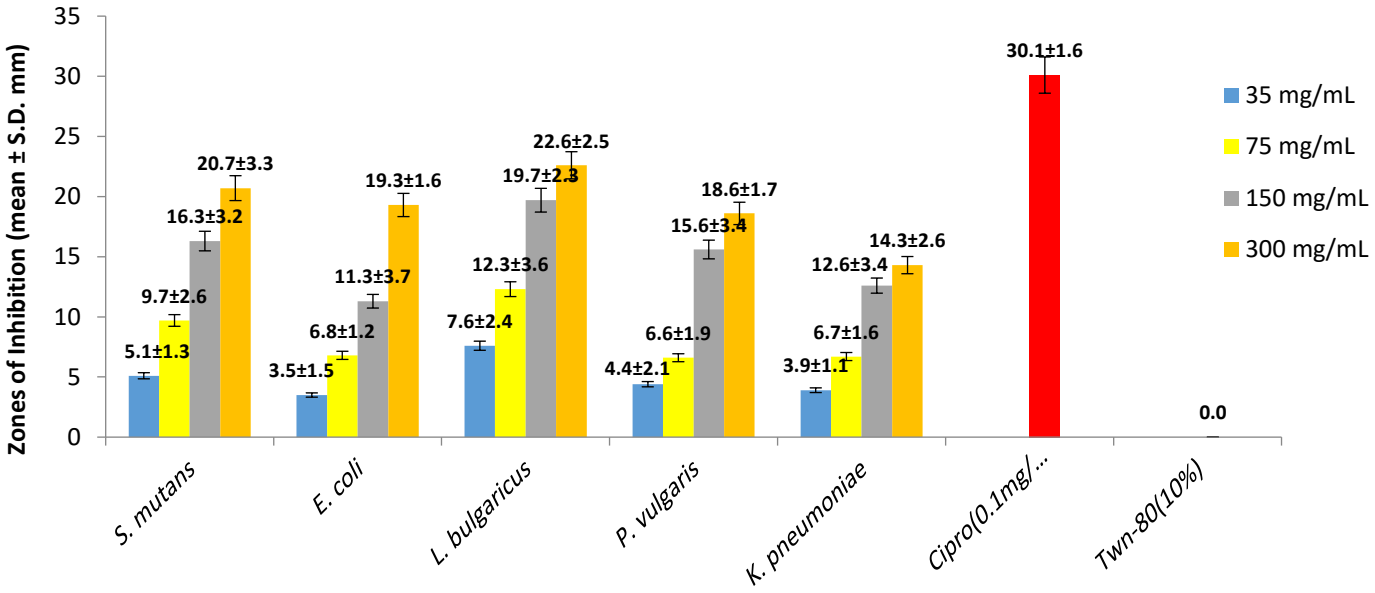

Figure 1: Antibacterial activities of the methanol extract at different concentrations against the test organisms

Table 7: Minimum inhibitory concentrations and minimum bactericidal concentrations of methanol extract of *H. africanum* on bacterial isolates

Bacterial isolates	Test extract MICs (mg/mL)	Test extract MBCs (mg/mL)
S. mutans	14	18
E. coli	18	26
L. bulgaricus	12	15
P. vulgaris	16	22
K. pneuamoniae	18	24

Discussion

Glycosides are found in some medicinal plant and have vast therapeutic efficacy and certain cases, toxic effects depending on the plant of origin and the dose of plant products [28]. Glycobiology has revealed that the glycosidic residue is crucial for the bioactivity of some plants as typified in *H. africanum* revealed by the result of this study. Similar results were reported by Maneerat *et al*. [29]. Some glycosides are of medicinal antimicrobial and toxicological importance [30-32]. Saponin has a bitter taste, foaming property and serves as a mild detergent that solubilises cell permeability barriers and consequent lysing of bacterial and fungal cells [33]. Alkaloids have been investigated for many pharmacological properties including antibacterial, antifungal, antiprotozoal, cytotoxic, antidiabetic and anti-inflammatory properties [34, 35]. The presence of saponins in the powdered plant, methanol and n-hexane extract of the sample is suggestive of the foaming properties usually reported for the chewing stick plant. The proximate assay shows carbohydrate content of 53.97±8.6% as highest followed by crude fibre (44.51±11.5%), ash (13.27±3.5%), moisture content (6.99±1.3%), and crude fat (4.06±1.6%) and the least was the crude protein content (1.35±1.1%) and these values validate its use as an edible chewing-stick plant [35, 36]. This result corroborates the report of Solangai

and Iqbal [37] who conclusively reported ash content of 6.85% to 14.26% for different varieties of the indigenous African chewing-stick plant. However, slightly contrasting values were obtained from some independent researchers who reported higher values of fibre (65.0%), followed by carbohydrate (40.0%), ash (8.0%), moisture (4.0%), and crude protein (2.0%) for another indigenous chewing stick plant-*Massularia acuminata* [38]. Potassium (118.67±0.047) and calcium (382.4±1.88) were detected as major elements which may suggest the role of calcium being responsible for the strong teeth and healthy gums of chewing stick users. This result corroborates the studies of Edesiri *et al*. [39] who carried out a five-year extensive study of exclusive chewing stick plant users as an oral hygiene aid in Ughelli, Delta state of Nigeria. The summary of Edesiri and others [39] revealed that exclusive chewing stick users had stronger teeth and gums against their nylon toothbrush plus paste counterparts. The therapeutic, preventive, and protective properties of the chewing sticks may be due to the combination of all these elements as the process of oral disease development involves a lot of metabolic interplay and interaction among microbial colonies resulting in localized destruction of the tooth and tissues of the oral cavity [39]. Though the mechanism of the clinical effect of elements such as fluoride is still debated, fluoride has been used widely in dentistry for many years for caries prevention. High concentration of Mg in some chewing stick plants has also contributed immensely to oral health. The specific functions of other elements such as sodium (Na) with regard to improved qualities of human teeth are not yet well understood, it is therefore possible that the naturally element rich and fluoridated chewing stick plants may play the same or even better role as the expensive modern nylon brush and toothpastes [38, 40]. Lead, cadmium, nickel, chromium which are heavy metals were below detectable levels in the chewing stick plant (*H. africanum*). Studies have revealed that some heavy metals have long term effect after accumulating in smaller quantities in a process known as bioaccumulation which may in turn result in fatal bio-magnification of toxic heavy metals [40, 41]. However, the absence of heavy metals seems to validate the continuous usefulness of *H. africanum* as a chewing stick plant with high safety as well as health benefits and negligible toxicity. The absence of chromium and lead which are toxic metals shows that the study plant does not pose any health risks. The presence of magnesium in the chewing stick extract is also known to prevent cardiomyopathy, impaired spermatogenesis and bleeding disorders in gums [40]. Iron, a key element was also present in detectable quantities and this helps in the metabolic process of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes. The iron content of the plant was higher than the FAO [42] recommended average dietary allowance (2.37 mg/day). Iron is an essential trace metal that plays numerous biochemical roles in the body, including oxygen binding in haemoglobin and acting as an important catalytic centre in many enzymes [41 42]. The total number of oral bacterial isolates (**Table 5**) was found to be 32 and their percentage occurrence from 15 sampled population of University of Benin students included, 12 *S. mutans* with the highest percentage occurrence of 37.5%, followed by *L. bulgaricus* (8; 25.0%), 5 each of *K. pneumoniae* and *E. coli* both of equal 15.0% and the least occurring *P. vulgaris* (2; 6.3%). This result corroborates the report by Kolapo *et al*. [42] who isolated 38.0% *Streptococcus* spp., 21.0% *Lactobacillus* spp. and 12.0% *Klebsiella* spp. as the predominant species in the plaque of 20 students at Ile-ife, Nigeria. In contrast, Hassan *et al*. [43] isolated *S. mutans*: 45.6%, *Lactobacillus* spp: 15.2%, *K. pneumonia:* 20.0%, *P. aeruginosa*: 6.0% and *S. aureus*: 13.2% from 80 samples collected from the dental clinic of Obafemi Awolowo University Teaching Hospital Complex Ile-Ife, Nigeria. Some predominant oral bacterial isolates include hundreds of the microorganisms of which *Streptococcus* is by far the most dominant compared to other dominant genera of oral microorganisms such as *Lactobacillus* and other bacterial isolates reported in this study. Organisms like *S. mutans* can thrive in the oral cavity due to certain ability to synergise with other cells such as the formation of biofilms thereby creating a plaque ecosystem over time [44]. This study showed the zones of inhibition/inhibitory zone diameters (IZDs) of the chewing stick plants against the test bacterial isolates. The antimicrobial activity of the methanol extracts was evident by the presence of growth inhibitory zones on a seeded agar plate. This zone was measured as an index of the inhibitory action of the extract against the test microorganism. In this study, the extract was considered active since it showed

IZDs of >3.0 mm at the lowest concentration considered. The study showed the plant (*H. africanum*) and the positive control (Ciprofloxacin) were active against the bacterial isolates when compared with the negative control (10.0% Tween-80) which was used in diluting out the various concentrations and it showed no activity. The IZDs results were all concentration dependent as the zones of inhibitions varied with the different extract concentrations and the activity of the extracts was significantly different from one concentration to the other against each organism. An important advantage of the plant extracts is the possession of phytochemicals and useful elements which set it apart from various other health benefits. Also, previous studies reporting the antimicrobial and inhibitory effect of a chewing stick plant noted that the juice extracted from chewing stick plants is reputed to have an antimicrobial effect on oral flora to a varying degree depending on the species of plant used [45, 46].

The IZDs test is a somewhat preliminary potency test that enables the determination of whether an antimicrobial agent is potent or not and also gives an idea of where to begin dilution of an antimicrobial agent for MIC assay. The MIC is defined as the minimum concentration of antimicrobial agent that inhibits the growth of test organisms in a seeded agar during *in vitro* antimicrobial studies and it is particularly useful in setting a benchmark of effective dose concentration. Usually, in selecting medication or antimicrobial doses, the MIC concentration is multiplied four times (4xMIC) to achieve a complete kill or cidal effect even without getting to assay for the cidal concentration [26, 46]. This study shows the MIC and the MBCs of *H. africanum* extract against the oral bacterial isolates. The lower the MIC value the more potent the antimicrobial agent; conversely, the higher the MIC value, the less potent the antimicrobial agent. The antibacterial activities in this study were concentration-dependent and varied with the bacterial species. These variations may be due to genetic differences between the microorganisms and the MICs and MBCs results revealed the most susceptible being *L. bulgaricus* (MIC: 12.0 mg/mL), followed by *S. mutans* (MIC: 14.0 mg/mL), *P. vulgaris* (MIC: 16.0 mg/L), *E. coli* and *K. pneumoniae* with equal MIC of 18.0 mg/mL being least susceptible and this pattern of susceptibility to the inhibitory effect of the plant extract was also repeated as the MBCs results also followed similar pattern to achieve complete destruction or cidal effect on the test organisms. Similar results were reported separately, the inhibitory effect of *H. lettusi* and *M. acuminata* stem extracts on *S. mutans*, *L. sacidophilus*, *C. albicans*, *K. pneumonia* and *P. aeruginosa* which were all susceptible to varying degrees [9, 27]. The juice extracted from chewing stick plants is reputed to have an antimicrobial effect to a varying degree depending on the species of the plant used [45, 46].

Conclusion: The juice extracted from the chewing stick plant (*H. africanum*) has useful medicinal values of phytochemical constituents, proximate composition, and mineral constituents expressing significant *in-vitro* antimicrobial effect to a varying degree depending on the species or genetic makeup of organisms challenged. These validated the relevance and local use of *H. africanum* as a teeth cleanser with numerous health benefits.

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