

Phytochemical screening and antimicrobial activity of ethanol and aqueous extracts of stem of *Glyphaea brevis* (spreng.) monachino on oral microorganisms

Oshomoh EO and *Idu M

Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin city, Edo State. Nigeria

ABSTRACT

The phytochemistry and antimicrobial activities of the aqueous and ethanolic extracts of the *Glyphaea brevis* stem was investigated. The results from this study indicate that the antimicrobial activities of the stem of *G. brevis* are microbe specific. The stem showed a significantly higher antimicrobial activity against *Staphylococcus aureus* and *Streptococcus mutans* even at a lower concentration of 3.13 mg/ml. Phytochemical screening of the plant part reveal the presence of alkaloids, flavonoids, anthraquinones, saponins, steroids, cardiac glycosides, phlobotannins and carbohydrates in both extracts. While tannins and terpenoids were absent in extracts. The potency of the antimicrobial activities of *Glyphea brevis* plant has made the stem suitable for better dental care and cleansing.

Keywords: Potency, dental care, periodontal disease, medicinal plant.

INTRODUCTION

Studies have shown that chewing sticks are at least as effective as tooth brushes in maintaining oral hygiene.^{[1-4][5]} Reported that Africans that use chewing sticks have fewer carious lessons than those using tooth brushes, and their use has been encouraged by the World Health Organization.^[6] Most of these chewing sticks have been shown to have significant antimicrobial activity against a broad spectrum of microorganisms.^[7] Described the activity of several plant extracts against *Streptococcus mutans*, a carcinogenic organism. Since then, several investigators including^{[8][9]} as well as^[10] have made similar reports of the antimicrobial activity of chewing stick extracts.

Secondary metabolites flavonoids, lignoids and tannins are well distributed in the plant kingdom. Their structures prevent oxidative damage, many of their beneficial effects in animal and human organisms are reported as anti-aging, anti-inflammation,^[11] anti-carcinogenic^[12-13]

anti-mutagenic^{[14][15]} anti-ulcer and anti-atherogenic effects and as the inhibitors of human low density lipoprotein oxidation.

Recent interests in chewing sticks and their extracts have focused on their effects on organisms that are involved in oral infections.^[16] Have reported the isolation of compounds active against aerobic and anaerobic periodontopathic bacteria from *Ceanothus americanus* plant employed by Native Americans in the treatment of these conditions.^[17] Also reported some Nigerian chewing sticks exhibited strong activities against a broad spectrum of bacteria including those that are involved in both medical and dental morbidity. The study also showed that some of the chewing stick extracts demonstrated activity against antibiotic resistant organisms. So they can be viewed as sources of novel lead substances with potential therapeutic or preventive application.

Utilization of non-timber forest products (NTFP) is gaining importance in the tropical world because of their commercial importance to the host community.^[18] They further stated that chewing sticks are important NTFP widely used for dental cleaning in tropical Africa. Chewing sticks also impact varying taste and sensation such as a tingling peppery taste, a bitter taste and numbness.^{[19][20]} Posited that chewing sticks, in addition to providing

*Corresponding author.

Tel: +2348050607009

E-mail: macdonaldidu@hotmail.com

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mechanical stimulation of the gums, also destroy microbes; these advantages of the chewing sticks over the conventional toothpaste and brush has been attributed to the strong teeth of Africans.^[21]

^[18]Reported that some of the chewing sticks being used are obtained from the following plants: *Garcinia manni*, *Musalaria acuminita*, *Terminalia glaucescens*, *Anogeisus leiocarpus*, *Pseudocedrela kotschy*, *Zanthoxylum gilleti* and *Azadirachta indica*.

Investigation further revealed that some of these chewing sticks possess anti-microbial activity against oral microbial flora such as *Staphylococcus aureus* and *S. auricularis*,^[18] *Candida albicans*, *Aspogillus flavus*, *Microsporium gypseum* and *Trichophyton metagrophytes*.^[22]

Benefits derived from using medicine obtained from plants are that they are relatively safer than synthetic alternative by offering profound therapeutic benefits and more affordable treatment.^[23–24]

It has been found that some drugs are synthesized from plants, and it is estimated that plant materials are present in, or provide the models for more than 50% of western drugs.^[25] Chewing sticks with antimicrobial activity could become a potential source of new drugs for oral diseases. Oral microorganisms are known for their pathogenesis in tooth decay, gingivitis, periodontitis, and their ability to cause teeth loss.^[26]

The twigs of *G. brevis* are chewed to clean teeth. *G. brevis* of the family tiliaceae are herbs, shrubs and trees, they are mostly found in tropical region. They are tap rooted with tall and erect stem may be simple or branched with mucilage. The flowers are regular, bisexual or hypogenous; the leaves are stipulate, alternate, simple, entire or toothed. It is valued as vegetable^[27] and put into various therapeutic uses such as treatment of hepatitis and poisoning,^[28] has anticonvulsant properties.^[29] *G. brevis* possess antioxidant properties which are related to the therapeutic activities of the plant.^[30] Plants constitute highly available and low-cost sources of antioxidants.

OBJECTIVES OF THE STUDY

This study is aimed at screening the phytochemistry and evaluates the antimicrobial activities, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) levels of the aqueous and alcoholic extracts of

G. brevis used as chewing stick against oral microorganisms which are responsible for dental caries.

MATERIALS AND METHODS

Collection and identification of plant material

The stems of *Glyphea brevis* plants were collected from Edo North Senatorial District of Edo State. The plants were identified by Dr J. F Bamidele of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State.

Preparation and extraction of plant material

The fresh stems of *Glyphaea brevis* were cut from the plants, rinsed in water and spread on trays and dried under the sun. The plant materials were then transferred to the oven set at 45°C for 20–30 minutes before being reduced to fine powder with the aid of a mechanical grinder. The powdered plant materials were then collected and stored in a tightly covered glass jars and kept for further studies.

For ethanol extraction, 100 g of the powdered stem and root materials were soaked in 600 mls of ethanol. The resultant solution was filtered using Whatman filter paper No 1 after 48 hours under room temperature (25°C). For aqueous extraction, 100 g of the powdered stems and root materials were boiled in 600 mls of water for 24 hours after which the resultant solutions were filtered using Whatman filter paper No 1.

The two extracts were concentrated through evaporation process using a water bath set at 100°C. The extracts were then stored in a refrigerator until required for use.

Preparation of stock solution of extracts

Fresh stock (known concentration) solution of the extract was prepared for each experiment. To prepare a required concentration of the extract, a specific weighed amount of the concentrated extract was dissolved completely in an appropriate volume of distilled water. To prepare 100 mg/ml concentration of extract, 1 gm of either of the extract was dissolved in 10 ml of distilled water in a sample bottle, corked and shaken vigorously to obtain a homogenous solution.

Phytochemical screening

The phytochemical tests were carried out on the aqueous and ethanol extracts using standard procedures as described by.^{[31][32]}

Antimicrobial investigation

Source of microorganisms

Pure stock cultures of *Staphylococcus aureus*, *Staphylococcus auricularis*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Candida albicans*, *Aspergillus flavus*, *Microsporium gypseum* and *Bacillus subtilis* isolated from patients with dental diseases were obtained from the Department of Medical Microbiology, Department of Dentistry University of Benin, and University of Benin Teaching Hospital (UBTH). These pure isolates were used and maintained in slants of Nutrient Agar (NA), Blood Agar (BA) and Potato Dextrose Agar (PDA) at 4°C until when needed for further studies.

Microbial inoculums preparation for susceptibility testing

The inocula of the bacterial isolates were prepared by growing each pure isolate in nutrient broth at 37°C for 24 hrs. The fungal isolates were grown in Potato dextrose broth at 28±2°C for 48 hrs.

After incubation, 1 ml of the diluted cultures of the microbial isolates in normal saline using a Pasteur pipette was inoculated unto the solidified nutrient agar at 40°C for bacteria and Potato dextrose agar for fungi.

Antimicrobial assay

Antimicrobial activity was evaluated by noting the zone of inhibition against the test organisms.^[35] Two colonies of a 24-hour plate culture of each organism were transferred aseptically into 10 ml sterile normal saline in a test tube and mixed thoroughly for uniform distribution. A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of oven-dried Nutrient agar, blood agar for bacteria and Potato dextrose agar plates for fungi, respectively. Three (3) adequately spaced wells of diameter 4 mm per plate were made on the culture agar surface respectively using a sterile metal cup-borer. 0.2 ml of each extract and control were put in each hole under aseptic condition, kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly. Conventional antibiotics were used as positive controls for bacteria and fungi respectively; distilled water was used as the negative control. The plates were then incubated at 37°C for 24 hours for the bacterial strains and at 28°C for 72 hours for fungal isolates. The zones of inhibition were measured and recorded after incubation. Zones of inhibition around the wells indicated antimicrobial activity of the extracts

against the test organisms. The diameters of these zones were measured diagonally in millimeter with a ruler and the mean value for each organism from the triplicate cultured plates was recorded. Using the agar-well diffusion technique, an already made gram positive and gram negative (Asodisks Atlas Diagnostics, Enugu, Nigeria) standard antibiotic sensitivity disc bought from a laboratory chemical equipment store in Benin city was used as positive control for bacteria while Ketoconazole was used as positive control for fungi. Distilled water was used as negative control for all the test organisms.

Determination of minimum inhibitory concentrations (MICs) of the extracts

The lowest concentration of the extracts that will inhibit the growth of test organisms is the Minimum Inhibitory Concentration (MIC). The initial concentration of the plant extract (100 mg/ml) was diluted using double fold serial dilution by transferring 5 ml of the sterile plant extract (stock solution) into 5 ml of sterile Normal saline to obtain 50 mg/ml concentration.^[34] Different concentrations were prepared from the crude extract by doubling dilution in distilled water. The different concentrations were 50, 25, 12.5, 6.25, 3.125, 0.625, and 0.3125 mg/ml respectively. Each dilution was introduced into nutrient agar plates, blood agar plates and potato dextrose agar plates already seeded with the respective test organism. All test plates were incubated at 37°C for 24 hrs for bacteria and 28°C ± 2°C for 72 hrs for fungi. The minimum inhibitory concentration (MIC) of the extracts for each test organism was regarded as the agar plate with the lowest concentrations without growth.^[33]

Minimum bactericidal concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of the plant extracts were determined by the method described by.^[35-36] Samples were taken from plates with no visible growth in the MIC assay and subcultured on freshly prepared nutrient agar plates, blood agar plates and Potato dextrose agar plates and later incubated at 37°C for 48 hours and 28 ± 2°C for 72 hours for bacteria and fungi respectively. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plate.

Determination of the antibiotic susceptibility of bacteria isolates

The disc diffusion method^[37] was used for the determination of microbial sensitivity. The antibiotic discs employed were: septrin, chloranphenicol, sparfloxacin, ciprofloxacin, amoxicillin, augmenting, gentamicin,

pefoxacin, ofloxacin, streptomycin, zinnacef and recophin. The zones of inhibition were measured and interpretation was in accordance with manufacturer's instructions.

RESULTS

In Table 1 the results of the phytochemical analysis of aqueous and ethanolic stem extracts of *G. brevis* revealed the presence of some secondary metabolites such as alkaloids, flavonoids, cardiac glycosides, anthraquinones, phlobotannins, steroids, Carbohydrates and Saponins. Tannins and terpenoids were absent in the aqueous and ethanol extract of *G. brevis*.

Table 2 shows the antimicrobial properties of the ethanol extract of the *G. brevis* on the test microorganisms. All the test organisms were sensitive to the ethanol extracts at a concentration of 100 mg/ml. The activities of the ethanol extracts on all the tested organisms were significantly different from one another. The highest zone of inhibition was recorded against *A. flavus* with a sensitivity diameter of 15.13 ± 0.09 mm, while the least sensitive was recorded against *M.gypseum* with a sensitivity diameter of 6.63 ± 0.03 mm.

Plant extracts were more susceptible to *A. flavus* (fungus) followed by *S. auricularis* (gram +ve), *S. mutans* (gram +ve), *B. subtilis* (gram +ve rod bacteria), *C. albicans* (fungus), *S. pyogenes* (gram +ve), *S. aureus* (gram +ve) and *M. gypseum* (fungus) respectively. Table 2 also revealed that the antimicrobial activity of the aqueous extract of *G. brevis* plant extracts were significantly different from one to another on each organism. *G. brevis* (stem) did show antimicrobial

activity in all the tested oral microorganisms with the highest sensitivity of 13.80 ± 0.06 mm against *M. gypseum* and the least sensitivity of 4.20 ± 0.12 mm against *S. auricularis* at the test concentration of 100 mg/ml.

Table 2 indicates the comparison of the effect of the aqueous and ethanol extracts of *G. brevis* on the test organisms. It was revealed that the ethanol extract has the highest antibacterial and antifungal activity against all the tested oral microorganisms with inhibition diameters of 14.03 ± 0.03 mm (*S. auricularis*, gram +ve bacteria) and 15.13 ± 0.09 mm (*A. flavus*, fungus) respectively at 100 mg/ml.

Table 3 revealed that only *S.mutans* was sensitive to the plant extract at the test concentration of 3.125 mg/ml with inhibition zone of 2.87 ± 0.12 mm, it also revealed the highest susceptibility when compared with other test organisms at all concentrations except at 12.5 mg/ml and 100 mg/ml where *S. aureus* showed the highest zone of inhibition of 5.73 ± 0.07 mm, while *A. flavus* recorded the highest susceptibility to the plant extract at the highest concentrations of 100 mg/ml with inhibition zone of 13.37 ± 0.09 mm closely followed by *S. auricularis* with inhibition zone of 13.20 ± 0.12 mm.

Table 4: present the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) values of the ethanol extracts of *G. brevis*. The ethanol extract of the plant at various concentrations showed minimum inhibitory concentration (MIC) at 6.25 mg/ml against *S. mutans* and *S. aureus*, in addition the extract indicate MIC at 12.50 mg/ml against *S. auricularis* and 25.00 mg/ml extracts against *S. pyogenes*, and *B. subtilis*, while for the fungi 25 mg/ml extracts of

Table 1. Phytochemical screening of the aqueous and ethanol extracts of *G. brevis* plant stem used as chewing stick.

Test Plant Extracts										
Chemical components										
<i>G. brevis</i> (stem)	Cardiac									
	Alkaloids	Flavonoids	Anthraquinones	Saponins	Tanins	Glycosides	Steroids	Terpenoids	Phlobatanins	Carbohydrates
Aq	+	+	+	+	-	+	+	-	+	+
Et	+	+	+	+	-	+	+	-	+	+

KEY: + = Present, - = Absent, Aq = Aqueous, Et = Ethanol

Table 2. Zone of inhibition of Aqueous and Ethanol extracts (100 mg/ml) of *G. brevis* against selected oral pathogens.

Test Organisms								
<i>G.brevis</i> (stem)	<i>S. aureus</i>	<i>S. auricularis</i>	<i>M. gypseum</i>	<i>S. pyogenes</i>	<i>S. mutans</i>	<i>B. subtilis</i>	<i>A. flavus</i>	<i>C. albicans</i>
Aq	6.01 ± 0.03	4.20 ± 0.12	13.80 ± 0.06	5.20 ± 0.58	5.37 ± 0.72	6.67 ± 0.07	7.00 ± 0.06	5.00 ± 0.06
Et	9.13 ± 0.03	14.03 ± 0.03	6.63 ± 0.03	10.50 ± 0.06	12.27 ± 0.09	11.30 ± 0.09	15.13 ± 0.09	11.00 ± 0.58

NB: Means \pm S.E.M; n = 3, Means \pm S.E.M within a row are significantly different, P < 0.01.

- = No inhibition. Aq = Aqueous, Et = Ethanol

Table 3. Zone of inhibition in mm of various concentrations of the ethanol extract of *G. brevis* on test organisms.

Test organisms	Concentration of extract (mg/ml)						Sterile distilled water
	3.125	6.5	12.5	25	50	100	
<i>S. aureus</i>	–	3.27 ± 0.09	5.73 ± 0.07	7.30 ± 0.12	8.63 ± 0.09	10.03 ± 0.15	–
<i>S. auricularis</i>	–	–	3.30 ± 0.06	4.70 ± 0.12	6.37 ± 0.12	13.20 ± 0.12	–
<i>S. pyogenes</i>	–	4.03 ± 0.09	5.13 ± 0.09	6.53 ± 0.09	7.47 ± 0.09	10.30 ± 0.06	–
<i>S. mutans</i>	2.87 ± 0.12	4.13 ± 0.09	5.30 ± 0.12	7.57 ± 0.09	9.13 ± 0.09	11.23 ± 0.09	–
<i>M. gypseum</i>	–	–	–	3.23 ± 0.09	4.70 ± 0.06	5.87 ± 0.09	–
<i>B. subtilis</i>	–	–	–	3.03 ± 0.12	3.50 ± 0.06	5.23 ± 0.09	–
<i>A. flavus</i>	–	2.10 ± 0.06	3.10 ± 0.07	5.10 ± 0.09	8.53 ± 0.03	13.37 ± 0.09	–
<i>C. albicans</i>	–	–	–	3.10 ± 0.06	4.57 ± 0.09	5.47 ± 0.09	–

NB: Means ± S.E.M; n = 3; Means ± S.E.M within a row are significantly different, P < 0.01.
 – = No inhibition.

Table 4. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) in mg/ml of the ethanol extracts of *G. brevis* stem against the test organisms.

<i>G. brevis</i> (stem) (mg/ml)	Test bacteria				
	<i>S. aureus</i>	<i>S. auricularis</i>	<i>S. pyogenes</i>	<i>S. mutans</i>	<i>B. subtilis</i>
MIC	6.25	12.50	25.00	6.25	25.00
MBC	12.50	25.00	25.00	25.00	25.00

NB: Values are means + S.E.M (n = 3);
 Values within a row with different alphabet are significantly different, P < 0.01

Table 5. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) in mg/ml of the ethanol extracts of *G. brevis* plant against the test fungi.

<i>G. brevis</i> (stem) (mg/ml)	Test fungi		
	<i>M. gypseum</i>	<i>A. flavus</i>	<i>C. albicans</i>
MIC	3.125 ^a	12.5 ^b	25.00 ^c
MFC	6.25 ^a	25 ^b	50.00 ^c

NB: Values are means + S.E.M (n = 3);
 Values within a row with different alphabet are significantly different, P < 0.01;

G. brevis was against *C. albicans*. The MBC value was lowest with 6.25 mg/ml against *S. mutans*.

Table 5: Indicate the least MIC value of 3.125 mg/ml against *M. gypseum* a fungus and the MFC value of

6.25 mg/ml of the plant extract against *M. gypseum* and higher concentrations against other fungi.

Table 6: shows the activity of the commercial antibiotics (standard sensitivity disc) on the test bacteria. It revealed a sensitivity zone of inhibition diameter varying from 4.0 mm – 28.3 mm against the bacterial isolates used.

Table 7 revealed that ketoconazole (commercial fungi antibiotic) was active against all the test fungi. It had the highest activity against *M. gypseum* with inhibition diameter of 26 mm, followed by *C. albicans* 24 mm and *A. flavus* 17 mm.

DISCUSSION

The result of qualitative phytochemical screening tests carried out on the aqueous and ethanol extracts of stem

Table 6. Sensitivity zone of inhibition of commercial antibiotics (standard sensitivity disc) on the test bacteria.

Test isolates	Zone of inhibition (in mm) for commercial antibiotics									
	CN	APX	R	CPX	E	SXT	PEF	OFX	S	AM
<i>Staph. Aureus</i>	28.3	7.4	–	24.5	9.7	–	21.6	27.0	–	–
<i>Staph. Auricularicus</i>	27.0	4.6	–	20.9	7.7	–	17.8	28.1	–	10.5
<i>S. pyogenes</i>	22.0	4.1	–	17.9	7.8	–	13.4	20.1	–	9.4
<i>Strept. Mutans</i>	20.6	4.0	–	18.7	8.8	–	14.5	20.9	–	–
<i>B. subtilis</i>	24.8	5.5	–	19.0	8.6	–	11.8	19.9	–	7.7

PEF = Pefloxacin (10 µg/ml), CN = Gentamicin (20 µg/ml), APX = Ampiclox (30 µg/ml)
 OFX = Ofloxacin (10 µg/ml), AM = Amoxicillin (30 µg/ml), R = Rocephin (25 µg/ml)
 CPX = Ciprofloxacin (10 µg/ml), S = Streptomycin (30 µg/ml), SXT = Seprtrin (30 µg/ml)
 E = Erythromycin (10 µg/ml), APX = Ampiclox (10 µg/ml), – = No inhibition

Table 7. Sensitivity zone of inhibition of commercial fungi antibiotics (ketoconazole) on the test fungi.

Test fungi	Ketoconazole (200 mg/ml)
<i>Aspergillus flavus</i>	17 mm
<i>Candida albicans</i>	24 mm
<i>Microsporium gypseum</i>	26 mm

of *G. brevis* revealed the presence of alkaloids, flavonoids, steroids, anthraquinones, cardiac glycoside, saponins, phlobotannins and carbonhydrates while absent of tannins and terpenoids were observed in both extracts.

The presence of bioactive compounds has been known to show medicinal activity as well as exhibit and regulate some physiological activity.^[38-39] Saponins have been reported to be an antifungal agent;^[40] reported the importance of steroids as potent starting material in the synthesis of sex hormones. Some of these bioactive compounds could be said to be responsible for the antimicrobial activity observed in this study. The presence of phytochemicals in plants has been shown to be responsible for the therapeutic activity of plants.^[31]

The aqueous and ethanol extracts of *G. brevis* used showed inhibitory activities against all the test organisms (Table 2 and 3). It was observed that susceptibility increased with increased concentration of the extracts (Table 3). Of all the tested organisms, *A. flavus* recorded the highest susceptibility of 15.13 ± 0.09 mm for ethanol extract while *M. gypseum* recorded the highest susceptibility of 13.80 ± 0.06 mm for aqueous extract both at concentration of 100 mg/ml.

The ethanol extracts exhibited more activity, potency and consistency than the aqueous extract. These results support earlier studies which observed that plant extracts in organic solvent provided more consistent antimicrobial activity compared with those extracted in water.^[41-42]

The most sensitive test bacterium was *S. auricularis* in ethanol extract with zone of inhibition of 13.20 ± 0.12 mm at the highest concentration of 100 mg/ml, while the aqueous extract was most active against *M. gypseum* a fungus with zone of inhibition of 13.80 ± 0.06 mm at 100 mg/ml (Table 2 and 3).

Ethanol extract recorded the highest antifungal activity of 15.13 ± 0.09 against *A. flavus* at 100 mg/ml, while *C. albicans* was the least sensitive fungi among the test organisms (Table 2).

The inhibitory effects of the various concentration of ethanol extracts of the plant against *S. Aureus*, *S. Auricularis*,

M. gypseum, *S. mutans*, *B. subtilis*, *A. flavus* and *C. albicans* (Table 3), were significantly even different at the same concentration of the extracts.

It was also observed that the extracts were active when compared with the negative control (sterile distilled water) against all the test organisms (Table 3). The control recorded no visible activity. The positive control (standard sensitivity disc) used on the test bacteria revealed that gentamycin, perfloracin, ampiclox, ofloxacin, ciprofloxacin, and erythromycin had inhibitory effects on all the test bacteria (Table 6). Amoxacillin has activity against *S. auricularis*, *S. pyogenes* and *B. subtilis* with inhibitory diameter of 10.5, 9.4 and 7.7 mm respectively. *S. aureus* was more sensitive to the commercial antibiotics with zone of inhibition of 28.3 mm for gentamycin, 27.0 mm for ofloxacin, 24.5 mm for ciprofloxacin and 21.6 mm for perfloracin.

Rocephin, streptomycin and septrin showed no inhibition zone on any of the tested organisms. Comparatively, the ethanol and aqueous extracts can be said to possess better activity than these since they contain both pharmacological and non-pharmacologically active substances as oppose to the pure active substances contained in the control antibiotics. The effect of the commercial antifungal drug (Ketoconazole) tested at a concentration of 200 mg/ml against the test fungi (Table 7) can be considered not better in activity when compared with the extracts, particularly at the highest tested concentration of 100 mg/ml which was two times lower in concentration than that of the fungal antibiotics. This probably implies that if the concentrations of the extracts were increased, it could lead to increased activity. The MIC values of the ethanol extracts of the plant are given in Table 3.

Antimicrobial substances can be considered as bactericidal agents when the ratio $MBC/MIC \leq 4$ and bacteriostatic agents when the ratio $MBC/MIC > 4$.^[43] For the ethanol extracts tested, the ratio MBC/MIC was ≤ 4 against all the tested bacteria. It suggests that the extracts can be considered as possessing bactericidal effect (Table 4 and 5).

In a related development,^[20] posited that chewing sticks, in addition to providing mechanical stimulation of the gums, also destroy microbes; these advantages of the chewing stick over conventional toothpaste and brushes has been attributed to the strong teeth of Africans.^[21]

CONCLUSION

Dentists are scarce in many parts of Africa, particularly in rural areas. Although diet plays a major role in preventing

dental caries, the practice of dental hygiene is also important. While toothpaste and toothbrushes are widely used by a sector of the population with a high level of formal education, toothpaste consumption is still low and chewing sticks are still in common use in many parts of Africa, particularly in West Africa. Even when people would prefer to use toothbrushes, they do not have access to toothpaste due to high cost or remoteness. Therefore, continued access to popular and effective sources of chewing sticks with anti-bacterial and anti-fungal properties is important as a primary health care measure. The results from these studies provide evidence for the ethnomedicinal uses of the tested plant as chewing sticks. The presence of bioactive substances (secondary metabolites) in the plant could be responsible for their medicinal properties. Although the actual biochemicals could not be ascertained in the scope of the present study, the extracts (ethanol and aqueous) of the plant showed activity against all the test bacteria and fungi. This implies that the plant used for this study possesses both antibacterial and antifungal properties. Therefore, it is recommended as potential chewing sticks for the reduction of dental caries due to the presence of antimicrobial, antifungal and phytochemical agents.

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