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PHYTOCHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITIES OF INDIGENOUS CHEWING STICK (*Anogeissus leiocarpus*) Stem

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ABSTRACT

The stems from Anogeissus leiocarpus are commonly used as chewing sticks in Northern Nigeria. If properly used, the chewing sticks have proven to be effective in removing dental plaque due to mechanical cleaning and enhanced salivation. Chewing sticks from other plants have been shown to display antimicrobial activities against a broad spectrum of microorganisms. However, there is limited information available in Northern Nigeria on the chemical composition, antimicrobial properties and the ability of the plants under study to prevent bacterial adhesion to tooth surface. Therefore, the purpose of this study was to ascertain the phytochemical and antibacterial properties of Anogeissus leiocarpus and correlate the results obtained to their ethnomedicinal uses as chewing sticks. Powdered stem was exhaustively extracted using methanol at room temperature for 72 hours. Antibacterial activities of the methanol extract was assessed using the agar well diffusion methods against the oral pathogens, Staphylococcus aureus, Streptococcus salivarius, Streptococcus pyogenes, Streptococcus mutansand Streptococcus sanguinis. Acute toxicity study was achieved using Lorke method. Phytochemicals which include flavonoid, steroid, triterpenes, alkaloids, tannins, carbohydrate, glycoside, phenols were detected in the extracts while anthraquinones was absent. The antibacterial results revealed that, the methanol extract had promising antibacterial activity. S. aureuswas found to be the most susceptible bacteria at 500mg/ml with inhibition of 22 mm, Streptococcus salivarius, Streptococcus pyogenes and Streptococcus sanguinis were inhibited at 16 mm while Streptococcus mutans showed inhibition of 14 mm. The extract have MIC and MBC of 31.25 mg/ml and 62.5 mg/ml respectively against all the tested clinical isolates. The LD_{50} of Anogeissus leiocarpus was found to be greater than 5000 mg /kg and could be considered safe for consumption.

Keywords: Anogeissus leiocarpus, Phytochemical, Toxicity, Antibacterial activity

INTRODUCTION

Different methods of oral hygiene are used in different countries and cultures. In Africa, the two preferred methods used are the toothbrush-toothpaste and the use of chewing sticks which are used by both adults and children (Moola, 2014). The common plants from which chewing sticks are sourced in Northern Nigeria are *Anogeissusleiocarpus*, *Azadirachtaindica, Albiziachevalieri, Pseudocedrelakotschyi* and *Diospyrossp.*. The choice between these methods is influenced by personal preferences, perceived effectiveness, availability and the medicinal properties (Aderinokun *et al*., 1999), whereas agreeable taste and anti-plaque activity are also considered by many. A study by Odongo *et al*. (2011) revealed that the choice of plants used as sources of chewing stick is mainly driven by the high fiber content of their stems and branches. Chewing stick users mention various reasons for choosing a stick over the toothbrush and toothpaste method. Among the reasons includes; ease of access (proximity), availability, reliability, efficiency, no cost and age long practice (Odongo *et al*., 2011). Moreover, the use of chewing sticks has been recommended by the World Health Organization (WHO) as an effective tool for oral hygiene (Cai *et al*., 2000). The sticks can be used fresh or may be dried and kept for later use.

To prepare a stick, the plant part is cut into a suitable size, cleaned and the bark removed on the side to be used for cleaning (Moola, 2014). When the stick is chewed, the fibers at the end become loose, forming a rough "brush" which cleans the teeth surface. Continued chewing on the stick dislodges particles between the teeth and stimulates blood circulation in the gums (Moola, 2014). Chewing also increases saliva production; the later acts as a natural mouthwash that rinses away bacteria and creates an inhospitable environment for them to flourish (Hoque *et al*., 2007).

Oral health is fundamental to the general wellbeing and relates to the quality of life that affects the functions of the oral cavity, dental and soft tissues of the face (Moola, 2014). Millions of people around the world are affected by diseases and conditions of the oral cavity. These effects include pain, bad breath, and difficulty in speaking, chewing and swallowing and in some cases death can result (Moola, 2014). Pathogens causing oral diseases are evolving at a faster rate and most have developed resistance to drugs used clinically. Because of this, oral diseases continue to be a major health problem worldwide (Moola, 2014). Dental caries and periodontal diseases are among the most important global oral health problems. Although gum disease is the most common cause of tooth loss in adults, the early signs and symptoms which include bleeding gums and loose teeth are often seen by many as part of the aging process (Moola, 2014). Naturally, humans are a host of a variety of microorganisms. Over 750 species of bacteria are found in the oral cavity alone and many of these play a role in causing oral diseases. While some of these microorganisms are harmful, others help in preventing diseases by fighting diseases causing germs in the mouth. There is a link between oral diseases and the activities of microbial species that form part of the microbiota of the oral cavity. Among other microorganisms involved in the development of dental caries are *Streptococcus mutans*, *S. sanguinis*, *S. sobrinus* and *Lactobacilli* (Palombo, 2009). Periodontal diseases have mainly been associated with *Actinomyces*, *Actinobacillus*, *Streptococcus* and C*andida* species (More *et al*., 2008). The *S. mutans* strains make up at least 90% of what affects the oral cavity negatively (Moola, 2014), and it is also known to cause pneumonia, sinusitis, and meningitis. S. *mutans* and *S. sanguinis* are members of the human indigenous biota belonging to the genus *Streptococcus* of spherical Gram-positive bacteria (Moola, 2014). They form part of the bacterial community in plaque which is the main cause of dental disease. On average, *Streptococci* bacteria account for less than 10% of bacteria species in the plaque, indicating that other bacterial species play a role in caries formation (Moola, 2014). *Streptococcus* and other bacterial species metabolize sugar in the mouth to produce organic acids, mainly lactic acid that dissolves the calcium phosphate in teeth which leads to tooth decay (More *et al*. 2008; Palombo, 2009; Moola, 2014).

Anogeissusleiocarpus (DC.) Gill & Peer is adeciduous tree species that can grow up to 15 - 18 m of height and measure up to 1 m diameter (Ahmad, 2014). Bark greyish and scaly, branches often drooping and slender, leaves alternate, ovate lanceolate in shape, 2 - 8 cm long and 1.3 - 5 cm across (Ouedraogo *et al*., 2013). The leaves are acute at the apex and attenuate at the base, pubescent beneath. Inflorescence globose heads, 2 cm across, yellow; the flowers are bisexual, petals absent. Fruits are globose, cone like heads; each fruit is broadly winged, dark grey, 3 cm across. It can reproduce by seeds as well as vegetative propagation (Mukhtar *et al.,* 2017). The plant is popularly known as African birch, Axle wood tree (Victor *et al*., 2013); "Marke", "Faringamji" in Hausa, "Kojoli" in Fulfulde, "Kukunchi" in Nupe, "Annum" in Kanuri, "Ainy" or "Orin-odon" in Yoruba and "Atara" in Igbo language of Nigeria (Victor *et al.,* 2013). The plant has been reported to used traditionally for the treatment of different ailments including the treatment of diabetes, ulcers, general body pain, blood clots, asthma, coughing, jaundice, pile and tuberculosis (Abubakar *et al.,* 2017), Malaria, Trypansomiasis, Helminthiasis and dysenteric syndrome (Okpekon, 2004) and also, it is used against stomach infections and fungal infections such as dermatitis and Mycosis (Batawila *et al*., 2005). Recent studies have revealed that the plant exhibits a variety of pharmacological activities including Antiplasmodial (Akanbi, 2012), Antioxidant and hepatoprotective (Victor and Grace, 2013) Leishmanicidal (Shuaibu, 2008a) Anthelmintic (Agaie and Onyeyili, 2007; Ademola and Eloff, 2011) Trypanocidal (Shuaibu, 2008b) antimicrobial (Taiwo *et al.,* 1999; Elegami, 2002).

The objective of the present investigation is to establish some important antibacterial profile and safety margin of *A. leiocarpus* stem with the hope of assisting in its standardization and safety.

MATERIALS AND METHODS

Ethical Approval

Ethical clearance with the number MOH/Off/797/T.I/645 was obtained from the ethical committee of Kano State Hospital Management Board, Kano State Ministry of Health for all the sample collection.

Collection of Clinical Specimen

The consent of patients that presented tooth infections cases were sought before taking their samples (oral cavity). Ten (10) consecutive, non-duplicate samples were collected at the General out Patient (GOP) clinic of the Bayero University Kano in the morning hours.

Plaque samples from their oral cavity were collected in sterile tubes containing 2 ml normal saline using swap stick. Samples were stored in an ice box then transported to the laboratory for processing at Microbiology Laboratory, Bayero University Kano, Kano State, Nigeria. .

Isolation of Bacteria Species

The specimens were cultured on sterile blood agar, chocolate agar and mac-conkey agar plates at 37°C for 24 h in an incubator. Discrete colonies were picked based on their morphology and further sub-cultured on blood agar and chocolate agar to obtain pure strains. The isolated colonies were gram stained and based on their gram reactions were inoculated on different selective media; mannitol salt agar, cetrimide agar, eosin methylene blue agar. Different biochemical tests were conducted (catalase test, coagulase test, oxidase test. All the isolates that grew on selected agar media were then placed on nutrient agar and chocolate agar slants and maintained in a refrigerator at 4°C (Cheesbrough, 2010).

Identification and Characterization of Test Organism using Rapid Test Kits

Identification and characterization of the bacteria was carried out using Microgen Identification Kit (XYZ).The test was performed according to the manufacturer's specifications (API biomerieux). It was performed by adding saline suspension of the test organisms to each of the wells and appropriate wells (1, 2, 3 and 9) were overlaid with sterile paraffin oil. After overnight incubation (18-24 hours) at 37°C, suitable reagents (such as Nitrate A and B, Kovacs, Typtophan deaminase (TDA), Voges-proskauer (VPI and II) were added to wells 8, 10 and 12 for additional tests and colour changes of the different tests recorded. The results were converted into four to eight digits codes depending on the organisms being tested and interpreted using the Microgen Identification Software Package (MID-60) (Sylvester, 2016).

Collection and Identification of Plant Materials

The stems of *Anogeissusleiocarpus* were collected from local farm in March, 2017 at Babura Local Government Area, Jigawa State, Nigeria. The plant was identified and authenticated in the herbarium of the Plant Biology Department of Bayero University, Kano, Kano State, Nigeria and a voucher specimen number (BUKHAN29) was deposited.

Preparation of Plant extracts

The stem of the plant was cleaned, air dried and ground to coarse powder using grinding machine. The powder was stored in air tight containers for further use. Fifty grams (50g) of the powdered stem was soaked into 500ml of methanol. The mixtures were allowed to stand for 3 days at room temperature (28 ±2ºC) with hourly agitations. The extract was sieved through a muslin cloth, filtered through a Whatman (No.1) filter paper, poured unto a clean evaporating dish and placed on a water bath at 50^{o}C until all the solvent evaporated.

Qualitative Phytochemical screening of Methanolic extract of *Anogeissus leiocarpus* Stem

The plant extract was subjected to phytochemical screening in order to identify the phytochemical constituents of the plant.

Tests for carbohydrates

Molish's (General) Test for Carbohydrates: To 1 ml of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

Tests for Saponins

Frothing test: About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponins (Evans, 2009).

Test for Flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2ml of 50% methanol in the presence of heated metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. Appearance of red color indicates the presence of flavonoids (Evan, 2009).

Test for Alkaloids

Wagner's Test: Few drops of Wagner's reagent was added into a portion of the extract, white precipitate indicates the presence of alkaloids (Evans, 2009).

Test for Steroids and Triterpenes

Liebermann-Burchard's test:

Equal volumes of acetic acid anhydride was added to the portion of the extract and mixed gently. Concentrated sulphuric acid (1 ml) was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the presence of triterpenes while blue or blue green indicates steroids (Evans, 2009).

Test for Cardiac Glycosides

Kella-killiani's test:

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Interphase for purple-brown ring was carefully observed, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 2009).

Test for Tannins

Ferric chloride test:

Exactly 3-5 drops of ferric chloride solution was added to the portion of the extract. A greenish black precipitate indicates the presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitates (Evans, 2009).

Test for Anthraquinones

Borntrager's test:

Exactly 5ml of chloroform was added to the portion of the extract in a dry test tube and shaken for at least 5mins. This was filtered, and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 2009).

Quantitative Determination of Phytochemical Contents of *Anogeissus leiocarpus* Stem

Determination of Alkaloids

About 5g of the sample weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation is completed. The whole solution was allowed to settle, and the precipitates were collected, washed with dilute ammonium hydroxide solution and then filtered. The residue was the alkaloids, which was then dried and weighed (Harborne, 1973).

Determination of Flavonoids

About 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter upper No. 42 (125mm). The filtrate was later be transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipal – Abyazan, 1994).

Determination of Saponins

The method of Obadoni and Ochuko (2001) was adopted. Sample (10g) was transferred into a conical flask and 100ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4hours with continuous stirring at about 55^{o}C. The mixture was filtered and the residue re-extracted with another 200ml, 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^{o}C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n – butanol was added. The combined *n*-butanol extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponins content was calculated.

Determination of Tannins

About 500mg of the sample was weighed into a 50ml plastic bottle and 50ml of distilled water was added and shaken for 1hour on a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferro-cyanide. The absorbance was measure at 120nm within 10mins (Van-Burden and Robinson, 1981).

Determination of Total Phenols

The fat free sample was boiled with 50ml of ether for 15minutes. About 5ml of the extract was pipetted into a 50ml flask, and then 10ml of distilled water was added. About 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added. The sample was made up to mark and allowed to react for about 30 minutes for colour development. This was measured at 505nm.

Antimicrobial Susceptibility Test

Preparation of Extract Concentration

This was carried out according to the method described by Srinivasan *et al.,* (2009). Stock solution of the plant extracts were prepared by adding 0.5g of crude plant extract in 1ml dimethyl sulphuroxide (DMSO). From each of the stock solutions, 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml concentrations were prepared using Two-fold serial dilution method (Srinivasan *et al.,* 2009).

Standardization of bacterial Inoculum

Using inoculum loop, over-night grown agar culture (bacteria) was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National committee for clinical laboratory standard (NCCLS, 2008).

Susceptibility Test of Bacterial isolates to Different Concentrations of the Extracts

The antimicrobial activity of *Anogeissus leiocarpus* crude extract (Methanol) against *Streptococcus mutans, Streptococcus salivarius, Staphylococcus aureus, Streptococcus pyogenes, sanguinis* were evaluated using agar well diffusion method of susceptibility test (Srinivasan *et. al.,* 2009*)*. Mueller-Hinton agar plates was inoculated with 0.1ml of standardized inoculum of each bacterium and fungus respectively (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Three wells of 6mm size were made with sterile cork borer (6 mm) into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml of the crude extracts were dispensed into wells of inoculated plates. . DMSO was used as negative control. Commercially available standard antibiotic, ciprofloxacin and fluconazole were used as positive control parallel with the extracts. The prepared plates were then left at room temperature (37 ºC) for 10minutes, allowing the diffusion of the extracts into the incubation at 37 ºC for 24 hrs in an incubator. The diameter of inhibition zones (DIZ) were measured and expressed in mm.

Determination of Minimum Inhibitory Concentration (MIC)

The method used was the tube dilution method (Adesokan *et al*., 2007). Thus, the plant extract was serially diluted from 500 mg/ml solution to obtain varying concentration. The concentrations were; 250 mg/ml, 125 mg/ml, 62.5mg/ml, and 31.25 mg/ml. Doubling dilutions of the extract were incorporated in Muller Hinton broth (Oxoid, UK), and then inoculated with 0.1ml each of standardized suspension of the test organisms into the various test tube containing varying concentrations. Another set of test tubes containing only Mueller Hinton broth were used as negative control, and another test tube containing Mueller Hinton broth and test organisms were used as positive control. All the test tubes and controls were then incubated at 37 ^{o}C for 24hrs. After incubation period, the presence or absence of growth on each tube was observed. A loop full from each tube was further sub cultured onto nutrient agar to confirm whether the bacterial growth was inhibited.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by collecting 1ml of broth culture from the tubes used for the MIC determination and subculturing into fresh solid nutrient agar plates. The plates were incubated at 37 ^{0}C for 24 h. The least concentration that did not show any growth after incubation was regarded as the MBC (Adesokan *et al*., 2007).

Acute toxicity studies of methanol extract of *Anogeissus leiocarpus* Stem

Lethal Dose (LD_{50}) Determination

The method of Lorke (1983) was employed. Thus, the phase I involved the oral administration of three different doses of 10, 100 and 1,000 mg/kg of the crude extract, to three different groups of three adult wister albino rats. In a fourth group, three adult male wister albino rats were administered with equivalent/volume of distilled water to serve as control. All the animals were orally administered with the extracts using a curved needle to which acatheter had been fixed. The animals were monitored closely every 30 minutes for the first 3 hours after administration of the crude extracts, and then hourly for the next 6hours for any adverse effects. Then the animals were left for 72 hours for further observation.

When no death occurred, the phase II was employed, only one animal was required in each group. Groups 1–4, animals were orally given 1,500, 2,200, 3250 and 5,000mg/kg dose levels of the crude extract. All the animals were left for observation as in stage one.

RESULTS

The plant material was extracted with methanol to yield brown extract (10.4 g) (Table 1).

Table 1. Mass and Percentage Yield for the Extract of *Anogeissus leiocarpus*

Extract	Mass (g)	Percentage Yield (%)	Extract Appearance
Methanol	10.4	20.8	Brown

Flavonoid, steroid, triterpenes, alkaloids, tannins, carbohydrate, glycoside, phenols were detected in the extract while anthraquinones was absent (Table 2).

Table 2. Phytochemical screening of the whole plant of *Anogeissusl eiocarpus*

Phytochemical	Inference
Alkaloid	+
Flavonoid	+
Saponins	+
Cardiac glycoside	+
Tannins	+
Steroid	+
Triterpenes	+
Phenol	+
Anthraquinones	-
Carbohydrate	+

Table 3 showed the phytochemical contents of *Anogeissus leiocarpus*in the methanol extract. The alkaloids (412 mg/g) was found to have the highest content in the extract while the lowest content was observed insaponins (4.0 mg/g).

Table 3. Quantitative Phytochemical screening of methanolic stem extract of *Anogeissus leiocarpus*

Metabolite	Quantity (mg/g)
Alkaloids	412±0.33
Flavonoids	223±0.40
Saponins	4.00±0.33
Tannins	243.0±0.22
Phenols	12.0±0.50

Antibacterial activity of methanol extract showed inhibition on all the tested organisms *S. aureus* (11-22 mm)*,Streptococcus mutans* (08-14 mm), *Streptococcus salivarius* (10-16 mm), *Streptococcus pyogenes* (11-16 mm), *Streptococcus sanguinis* (10-16 mm)(Table 4). The MIC and MBC of the extract recorded respective values of 12.5 mg/ml and 25 mg/ml against all the tested clinical isolates.

Table 4. Antibacterial activity of methanol extract of *Anogeissusl eiocarpus* stem

Clinical isolates	**Concentration/Diameter zone of inhibition**						**MIC**	**MBC**
	500	250	125	62.5	Ampicillin	DMSO		
S. aureus	22	17	14	11	32	06	31.25	62.5
S. pyogenes	16	13	11	09	34	06	31.25	62.5
S. mutans	14	12	10	08	30	06	31.25	62.5
S. salivarius	16	14	12	10	32	06	31.25	62.5
S. sanguinis	16	14	12	10	33	06	31.25	62.5

Toxicity study indicate no death recorded in the first phase of the investigated rats. In the second phase, doses of 1500, 2250, 3250 and 5000mg/kg were used and no death was also recorded. The oral median lethal dose (LD_{50}) for the methanol stem extract of *Anogeissus leiocarpus* was therefore estimated to be greater than 5000mg/kg and no sign of behavioural changes were also observed (Table 5).

Table 5. Acute toxicity studies of methanolic stem extract of *Anogeissus leiocarpus*

Treatment	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24hrs
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

DISCUSSION

The phytochemical profile of the methanol extract from the stem of *Anogeissus leiocarpus* showed the presence of flavonoids, saponins, tannins and triterpenes (Table 2). These chemical compounds have been associated with antimicrobial activities (Moola, 2014). Alkaloids are well known for their wide range of pharmacological activities so this finding confirms the potential of *Anogeissusl eiocarpus* as potential antimicrobial agents (Moola, 2014). *Anogeissus leiocarpus* displayed a low saponins content. The presence of saponins in the extract is of importance because saponins are known to be immune boosters (Moola, 2014). Results of the phytochemical screening are in accordance with the popular use of the *Anogeissus leiocarpus* as chewing sticks since the results show the presence of various secondary metabolites that are associated with various pharmacological effects. The presence of saponins in *Anogeissus leiocarpus* stem supports its traditional use for procuring abortion since saponins are linked to the sex hormone oxytocin involved in controlling the onset of labour (Njoku and Akumefula, 2007). The presence of tannins suggest the growth inhibitory effect of these plant extracts on bacteria. A study conducted by Sharall*et al*., (2013) reported the presence of fatty acids and terpenes responsible for bactericidal activity. Hamid *et al.* (2011) also reported that phenolics, flavonoids, saponins and phorbol esters as antimicrobial compounds in *Anogeissusl eiocarpus.* Phytochemicals present in the stem extract was high but moderate antimicrobial activity was observed, probably due to the fact that different phytochemical compounds exert their antibacterial effects differently from one another. A similar result was also reported by Willey *et al.* (2008) that the presence of some secondary metabolites in the root extract of *J. curcus* inhibit microorganisms isolated from sexually transmitted infections. Henrie *et al.* (2009) reported that *J. curcus* root and *Anogeissus leiocarpus* stem extract disrupts bacterial cell membrane by increasing membrane permeability and causing leakage of bacterial contents like nucleic acid and amino acids. Agarwal *et al*.(2012) also states that the presence of metabolic toxins or broad spectrum antimicrobial compounds in *Anogeissus leiocarpus* stem acts against most Gram positive and some Gram negative bacteria especially in solvents methanol, ethyl acetate and cold aqueous. In the same vein, Igbinosa *et al.* (2011) reported that the activities of *J. curcus and Anogeissus leiocarpus* stem to both Gram positive and negative microorganism can be attributed to the presence of phenolic compounds which showed to be powerful antioxidants and free radical scavengers, and those compounds are able to induce reactions of electron transfer which reacts with nitrogen compound in microbial cell like nucleic acid and proteins, this helps as a strong barrier against bacterial infection.

The result of the antimicrobial susceptibility test showed that the extract had antibacterial activity against the characterized microorganisms isolated from the mouth. This was reflected in the varying zones of inhibition of the individual extracts on the oral pathogens *in vitro* with mean inhibition diameters that ranged from *S. aureus* (11-22 mm)*, Streptococcus mutans* (08-14 mm), *Streptococcus salivarius* (10-16 mm), *Streptococcus pyogenes* (11-16 mm)and *Streptococcus sanguinis* (10-16 mm). This is an indication that the extract possess substances that can inhibit the growth of microorganisms' particularly oral pathogens. Although the phytochemical screen test showed that the plant part from *Anogeissus leiocarpus* contain polyphenols, saponins and tannins which are potentially good antimicrobial agents (Duru and Onyedineke, 2010). Mbanga *et al*., (2013) reported that the aqueous extracts from *D. lycioides* and *Anogeissus leiocarpus* were active against multidrug sensitive and multidrug resistant *S. mutans* isolates.

The MIC and MBC assay procedures are frequently used to evaluate some diverse agents such as antibiotics, antiseptics, disinfectants and chemotherapeutic agents (Andrews, 2001). In this study, the MIC and MBC values of all the tested clinical isolates with methanolic extract of

Anogeissus leiocarpus indicates significant bactericidal activities. This implies the strong efficacy of the extract as stated by Arekemase (2011) that the constituents of the stem of *Anogeissus leiocarpus* contains phenols, flavonoids and some secondary metabolites that are very useful in antimicrobial activity. This moderate MIC exhibited by *Anogeissus leiocarpus* is of great significance because it suggests that this extract can be used in the prevention of dental caries. It also suggests that using *Anogeissus leiocarpus* stem as teeth cleaning agents can kill oral pathogens. It can also indicate that chewing of the stem of *Anogeissusleiocarpus* can be an effective teeth-cleaning agent and thus can be an alternative to conventional toothbrush and toothpaste method since it is cheaper. Inhibition of bacterial adhesion may be associated with the modification of the receptors on the cell surface of the bacteria (Rahim and Khan, 2006). This is an important factor to consider because it means that using these plants as chewing sticks can reduce the adhesion of pathogenic oral microorganisms to the teeth surface and thus limit plaque formation.

In order to determine the safety margin of drugs and plant products for human use, toxicological evaluation was carried out in experimental animals using Lorke's method to predict toxicity and to provide guidelines for selecting a "safe" dose in animals and also used to estimate the therapeutic index (LD_{50}) of drugs (Rang *et al*., 2012). In this study, median lethal dose (LD_{50}) of the methanol extract of the *Anogeissus leiocarpus* stem was carried out orally in rats. The LD_{50} was found to be greater than 5000 mg/kg when administered orally in rats (Table 5) and all the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. These studies showed the extract of *Anogeissus leiocarpus* stem are practically non-toxic when administered using the oral route (Table 6). This is based on the toxicity classification which states that substances with LD_{50} values of 5000 to 15,000 mg/kg body weight are practically non-toxic (Loomis and Hayes, 1996).

CONCLUSIONS

The standardization for the *Anogeissus leiocarpus* stem will be useful for the compilation of suitable pharmacopoeia parameters and also serve as a basis for proper identification and safety usage of *Anogeissus leiocarpus* stem. *Anogeissus leiocarpus* stem possess secondary metabolites which include alkaloids, tannins, flavonoids, cardiac glycosides and saponins. Antibacterial activity showed that the plant contain biologically active compounds which prevent the growth and/or attachment of oral pathogens *S. mutans, S. aureus, S. salivarius, S. pyogenes* and *S. sanguinis*. The study confirms the use of this plant as chewing stick in removing plaque not only mechanically but also with antibacterial and anti-adhesion effects. The Acute toxicity (LD_{50}) of the methanolic extract of *Anogeissus leiocarpus* stem was found to be greater than 5000 mg /kg and is considered safe for use. Nonetheless, further studies are encouraged to evaluate toxicity at much higher doses.

Conflict of interest

None to declare.

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