

PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITIES OF *A. CHEVALIERI* HAMS (FABACEAE) STEM

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ABSTRACT

Albizia chevalieri Hams (Mimosaceae/Fabaceae), mostly found in the Northern Sahel Savannah region of Nigeria as well as in Nigér and Senegal is a tree of the acacia type with a long list of folklore therapeutic claims that include its use as purgative, taenicidal, cough remedy, dysentery, cancer, diabetes mellitus, tuberculosis and snake bite remedy. However, many bacterial species have been reported to develop resistance to antibiotics commonly prescribed for dental infections. Therefore, the need to search for natural products for remedy to this problem cannot be overemphasized. The stem was collected, dried and powdered. The powder was thereafter extracted with distilled water and subsequently with methanol. Phytochemical screening was carried out using standard methods. Agar well diffusion, agar dilution and spread plate methods were employed to determine the zone of inhibition, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and rate of killing respectively. Phytochemical screening of methanol and aqueous extracts of *Albizia chevalieri* stem revealed the presence of phenol, alkaloid, saponins, tannins, steroids, triterpenes, flavonoids and carbohydrate as secondary metabolites. The two extracts showed broad spectrum of activity but the aqueous extract had larger zones of inhibition of 32 mm against *S. mutans* while methanol extract had higher zone of inhibition on *S. aureus* (24 mm). Low MIC and MBC values ranging from 6.25 mg/ml and 12.5 mg/ml respectively. The results obtained also provided scientific evidence for the use of *A. chevalieri* in folklore medicine in the treatment of tooth infections.

Keywords; *Albizia chevalieri*, Dental infections, Zone of inhibition, Minimum inhibitory concentration, Minimum bactericidal concentration.

INTRODUCTION

Nigeria has a rich tradition of herbal medicine with its diverse cultures and traditions. Traditional medicine practices are a main source of livelihood for a significant number of herbalist who depend on it as their source of income. High population growth rate and poverty coupled with dwindling economic reserves in the country make Nigerians resort to more affordable sources for their immediate health needs (Ekeanyanwu, 2011). As the population increases, demand for herbal medicine will increase. Nigeria has established national and state traditional medicine boards for regulation of herbal medicine practice and to promote cooperation and research. In order to provide affordable health care services especially, to those who cannot afford orthodox medicine, several state

governments through their traditional medicine boards have tried to institutionalize the use of traditional medicine. Antimicrobial drug resistance is a global challenge for the 21st century with the emergence of resistant bacteria strains worldwide (Furin *et al.*, 2011). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy *et al.*, 2005; Rojas *et al.*, 2006).

Albizia chevalieri Harms (Mimosaceae) is a tree of acacia type native to tropical and subtropical regions including Nigeria and Niger Republic, with loose balls of whitish fragrant flowers and flat brown pods (Maydell, 1986). Ethnobotanical survey

conducted in the cause of this research revealed the use of the root and stem-barks of *A. chevalieri* for therapy against tuberculosis, toothache, inflammations and snake bite remedy. It has been established that phytochemists believed that tropical plants are understudied and contain many undiscovered secondary metabolites with therapeutic potentials. These facts necessitated the need to investigate phyto-constituents of *A. chevalieri* for bioactivity. Credibility for the choice of this plant is supported from its ethnobotany; owing to the fact that researchers have found that chances of success in finding useful drugs can be increased threefold if the search for a medicinal plant is concentrated on plants used for medicinal purposes by indigenous peoples of regions who have preserved their traditional culture (Rodriguez and West, 1995). Meanwhile, ethnobotanical survey conducted in the cause of this research revealed the use of the root and stem-barks of *A. chevalieri* for therapy against tuberculosis, toothache, inflammations and snake bite remedy among the Zuru people of Kebbi State, Nigeria (Sylvester, 2016).

Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional system of medicine relatively cheaper than modern medicine. Many works have been carried out with the aim of knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to antibiotics and other chemotherapeutic agents to which many infectious microorganisms have become resistant.

Dental disease is one of the globally affecting diseases. Herbal medicine is effective because they interact with specific chemical receptors within the body. Herbal medicines have less side-effect in comparison with orthodox medicines (Ishnava, 2018). There is lack of information about the effect of herbal medicine treatments on dental health. Herbal medicine treatment in dental diseases they are used as antibacterial agents, anti-inflammatory agents, sedative and anxiolytics (Ishnava, 2018). The natural phytochemical could offer an effective to antibiotics and represent approach to prevention and therapeutic strategies for various dental diseases. The major problem is the lack of information about the effects of herbal medicine on dental disease (Ishnava, 2018). Therefore the need to evaluate the phytochemical and antibacterial activity of *Albizia chevalieri* stem. Herbal medicine may vary in their effectiveness; therefore, it is necessary to select herbal medicine very carefully. Herbal medicine and their extract can be used as adjuvant in dental disease treatment. Achieving the objectives of this research will potentiate the use of extract from this plant in formulations. This research therefore, aimed at identifying biologically active metabolites and the determination of antibacterial activity of *Albizia chevalieri* against dental pathogens.

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, 2006).

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 stem of *Albizia chevalieri* were collected from local farm in March, 2018 at Madobi Local Government Area, Kano state. The plant was identified and authenticated in the

herbarium of the Plant Biology Department of Bayero University, Kano, Kano State, Nigeria and was compared with a voucher specimen number.

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) each of the powdered stem was added to 500ml each of methanol and distilled water. Each was allowed to stand for 3 days at room temperature ($28 \pm 2^\circ\text{C}$), with hourly agitations. Each 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°C until all the solvent evaporated.

Qualitative Phytochemical screening of Aqueous and Methanolic extract of *Albizia chevalieri* Stem

The plant extract was subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described below.

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 heat metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red color indicates presence of flavonoids (Evan, 2009).

Test for Alkaloid

Wagner's Test: Few drops of Wagner's reagents were added to a portion of the extract, whitish precipitate indicates the presence of alkaloid (Evans, 2009).

Test for Steroid and Triterpenes

Liebermann-Burchard's test:

Equal volume 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 Glycoside

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 was added to the portion of the extract. A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate (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).

Preparation of McFarland Turbidity Standard

McFarland standard are used as a reference to adjust the turbidity of microbial suspension so that the number of bacteria will be within a given range. Firstly, BaCl_2 (1% w/v) and H_2SO_4 (1% v/v) were prepared by dissolving 1g of BaCl_2 in 100ml of sterile distilled water and 1ml of concentrated H_2SO_4 in 99ml of sterile distilled water respectively to serve as stock solutions for the preparation of the McFarland standard. From the stock solutions, 0.5 McFarland scale was prepared by adding 9.95ml of (1% v/v) H_2SO_4 to 0.05ml of (1% w/v) BaSO_4 whose density is equivalent to 1.5×10^8 CFU/ml approximate cell density of bacteria. The Barium Sulphate suspension in 6ml aliquots were transferred into screw-cap tubes, tightly sealed, and stored at room temperature in order to prevent loss by evaporation. This was subsequently used for comparison with the turbidity of the bacterial (Cheesbrough, 2010).

Antimicrobial Susceptibility Test

Mueller Hinton agar was used for the antibacterial susceptibility testing. It was prepared according to manufacturer's instructions by suspending 38g of medium in 1000ml distilled water, sterilized at 121°C , and cooled to room temperature prior to dispensing in petri dishes.

Preparation of different Concentrations of the Plant Extract

This was carried out according to the method described by Srinivasan *et al.* (2009). Stock solution of the plant extracts were prepared by adding 0.1g of each crude plant extract in 1ml dimethyl sulphuroxide (DMSO). From each of the stock solutions, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml concentrations were prepared using two-fold serial dilution

method. These concentrations were labelled and kept in bijou bottles for subsequent use.

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 Clinical isolates to Plant extracts.

The antimicrobial activity of *Albizia chevalieri* crude extracts (Methanol and aqueous) against the test bacteria was evaluated using agar well diffusion method for susceptibility testing (Srinivasan *et al.*, 2009). Sterile molten Mueller-Hinton agar was inoculated with 0.1ml of standardized inoculum of each bacterium (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Wells of 6mm size were made with sterile cork borer into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml each of the crude extracts were dispensed into wells of inoculated plates. The prepared plates were then left at room temperature for 10 minutes, allowing the diffusion of the extracts into the incubation at 37 °C for 24 hrs in an incubator. The diameter of inhibition zones (DIZ) were measured and expressed in millimetres after incubation. The mean values of the diameter of inhibition zones were calculated to the nearest whole number. DMSO was used as negative control. Commercially available standard antibiotic, ciprofloxacin was used as positive control parallel with the extracts.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was evaluated on plant extracts which showed activity on any bacteria organism. The method used was the tube dilution method (Adesokan *et al.*, 2007). The plant extracts were serially diluted from the solutions of 50 mg/ml to obtained varying concentration. The concentrations were; 25

mg/ml, 12.5 mg/ml, 6.25mg/ml, and 3.125 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 and another set of test tubes containing only Mueller Hinton broth were used as negative control, 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 °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. Growth of bacteria on solid media indicated that particular concentration of the extract was unable to inhibit the bacteria. The lowest concentration of extract showing no growth indicated the amount of extract in grams per milliliter to which the organism is susceptible. This was the minimum inhibitory concentration (MIC).

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 sub culturing into fresh solid nutrient agar plates. The plates were incubated at 37 °C for 24 h. The least concentration that did not show any growth after incubation was regarded as the MBC (Adesokan *et al.*, 2007).

Results

Results of the physical properties and percentage yields of the plant extracts were presented in Table 1. From the result, methanolic extract produced highest percentage yield while aqueous produced the least. Likewise, methanolic extracts produced highest extract per weight when compared to the aqueous extracts.

Table 1: Physical Properties of *A. chevalieri* Extract

Solvent	Color	Odor	Texture	Weight of Plant Material	Weight of Extracts	% Yield
Aqueous	Brown	Fruity	Solid and dry	50	7.2	14.4
Methanol	Red	Fruity	Solid and dry	50	9.1	18.2

Phytochemical screening of aqueous and methanol extracts revealed the presence of alkaloid, flavonoids, saponins, tannins, anthraquinones and steroid (Table 2).

Table 2: Qualitative Phytochemical screening of aqueous and methanolic stem extract of *A. chevalieri*

Metabolite	Inference	
	Aqueous	Methanolic extract
Alkaloid	+	+
Flavonoid	+	+

Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	+	+
Triterpenes	+	+
Phenol	+	+
Amino acid	+	-
Carbohydrate	+	+

Keys: + Present, - Absent

Table 3 shows antibacterial activity of *A. chevalieri*. The aqueous extract produced highest zone of inhibition of 32 mm against *S. mutans*, 23 mm against *S. salivarius* and 17 mm against *S. aureus* which apparently was the least susceptible. However, for the methanolic extracts the most susceptible bacteria to the extracts is *S. mutans* with zones of inhibition of 25 mm and the most resistant being *S. salivarius* 20 mm while *S. aureus* showed moderate sensitivity with zones of inhibition of 24 mm.

Table 3: Antibacterial Activity of Stem bark Extracts of *A. chevalieri*

Clinical isolates	Diameter of Zones of Inhibition/ Concentration (mg/ml)									
	Methanolic Extracts				Aqueous Extracts				Cpx	DMSO
	100	50	25	12.5	100	50	25	12.5	500	-
<i>S. mutans</i>	25	21	16	13	32	26	22	20	54	0
<i>S. salivarius</i>	20	18	13	10	23	20	17	13	49	0
<i>S. aureus</i>	24	17	14	10	17	13	11	09	59	0

Key: Cpx = Ciprofloxacin, DMSO = Dimethyl Sulphoxide

Table 4. Minimum Inhibitory Concentration of Aqueous and Methanolic extract of *A. chevalieri* against Dental pathogen

Test organisms	MIC		MBC	
	Aqueous	Methanol	Aqueous	Methanol
<i>Streptococcus mutans</i>	6.25	6.25	12.5	12.5
<i>Streptococcus salivarius</i>	6.25	6.25	12.5	12.5
<i>Staphylococcus aureus</i>	6.25	6.25	12.5	12.5

DISCUSSION

In this study, methanol and aqueous extracts have similar composition of phytochemical constituents (i.e. alkaloids, cardiac glycosides, saponins, flavonoid, tannins and steroids/triterpenes) (Table 2) and is attributable to their closeness in polarity. This is in agreement with the findings of Abubakar *et al.* (2015) and Alhassan *et al.* (2014) where similar phytochemicals were reported in the extracts. These primary and secondary metabolites in plants have numerous functions. Crude, pure and isolated alkaloids and their synthetic derivatives have been used as analgesic, antispasmodic and bactericidal agents (Okwu and Okwu, 2004). Flavonoids have been shown to provide antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity (Alan and Miller, 1996). Flavonoid also has immense antioxidant and anti-inflammatory activities because of its ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Okwu, 2004; Okwu and Josiah, 2006). Tanins have been used in the treatment of wounds especially those emanating from

varicose ulcers and hemorrhoids (Njoku and Akumufula, 2007) and is able to stop bleeding during circumcision (Edeoga *et al.*, 2005).

The phytochemical constituents especially the secondary metabolites could be useful as guide to chemotaxonomic markers (Jonathan and Tom, 2008) that will aid in chemo taxonomical classification system and further phylogenetic studies in Fabaceae family.

Generally, both extracts showed a wide range of antibacterial activity when compared to the positive control but there was a slight difference between the extracts with respect to the plant's part. Methanol extract of matured stem of *A. chevalieri* showed activity against all the bacteria isolates while aqueous extract of same stem was active against three isolates. The observed antimicrobial effects on the isolates is believed to be due to the presence of tannins, flavonoids and saponins which have shown to possess antimicrobial properties (Yagana *et al.*, 2012). Some workers have also attributed the observed antimicrobial effect of plants extracts to the presence of these secondary metabolites (Radulovic *et al.*, 2013). Some other

workers have identified, that tannins, flavonoids and alkaloids in the extracts of some medicinal plants example *Euphorbia hirta* possess antimicrobial activity (Yadav *et al.*, 2011).thus, The growth inhibition effect of the extracts on the microorganism could be attributed to the presence of bioactive substances such as phenolic acids, tannins and flavonoids as reported by other workers. Phenolic acids are highly hydroxylated phenols, scientific evidence show that increase hydroxylation of phenol result to increased toxicity to pathogens (Yadav *et al.*, 2011).

The diameters zone of inhibition showed a concentration dependent result and the result also showed that the zone of inhibition values of the extracts was far lesser than that of the positive control ciprofloxacin. This may be attributed to the fact that conventional antibiotics are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, while herbal medicinal plants products are still crude, prepared from plant and animal origins and are subjected to contamination and deterioration most of the time (Mahmood and Ameh, 2007).

The MIC and MBC values were generally similar for the aqueous extract against the test isolates compared to those of the methanol extract. All the tested isolates were susceptible to the extracts especially the aqueous extract which showed lowest MIC and MBC values of 6.25 mg/ml to 12.5 mg/ml. This is of great importance as it has been reported that these organisms have developed resistance to many antibiotics, which sometimes makes its clinical management difficult (Adewunmi *et al.*, 2001). This result agrees with the work of Osumah *et al.* (2012) who showed that the root and stem bark extracts and fractions of *A. cordifolia* had more activity against *S. aureus* isolated from fecal and wound samples in Ahmadu Bello University Teaching Hospital Zaria. The differences in the susceptibilities of the isolates to the plant extracts can be related to the cell wall composition of the organisms. Gram – positive bacteria have cell wall composed of peptidoglycan with teichoic acid in between, therefore they are more susceptible than Gram – negative bacteria that have their cell wall surrounded by bi-lipid layers of Gram-negative lipopolysaccharides and lipoproteins, which prevent ready penetration of antibiotics through their cell wall.

Most extracts exhibited MIC and MBC at a low concentration of 6.25 mg/ml and 12.5 mg/ml respectively against all isolates. The low MIC and MBC exhibited by the extracts against *Staphylococcus aureus*, *S. mutants* and *S. salivarius* are of great significance in the health care delivery system, since it could be used as an alternative to orthodox antibiotic in the treatment of infections caused by these microbial pathogens, especially as they frequently developed resistance to known antibiotics. The presence of these biologically active chemicals and antimicrobial amino acids may have been responsible for the antimicrobial activity of these plant extracts. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls

and disrupts microbial membranes (Temitayo *et al.*, 2012). Saponins are surface active agents which alter the permeability of the cell wall of organisms thus facilitating the entry of toxic materials or leakage of vital constituents from the cell (Daniyan *et al.*, 2010). In medicine, saponins are used as hypercholesterolemia, hyperglycemia, antioxidant, anti-cancer, anti- inflammatory agents due to their detergent property (Ngbede *et al.*, 2008). These properties confirm saponins as potent antimicrobial agent. Tannins are polyphenols known to exhibit antibacterial, antiviral and anti-tumor activities. It was also reported that certain tannins are known to inhibit HIV replication selectively and is also used as diuretic (Evans, 2002).

CONCLUSION

The stem of *Albizia chevalieri* possesses antibacterial activity against pathogenic bacteria and may be used in susceptibility cases. These extracts could be used as alternative for commercial orthodox antibiotics for treatment of antimicrobial infections. This study has justified the use of the plant species in the treatment of some bacterial diseases in folkloric herbal medicine.

The stem of *Albizia chevalieri* could be used in the treatment of some diseases caused by bacterial, most especially disease caused by *Staphylococcus aureus* and *Streptococcus mutans*. Further studies are required to advocate its systemic use in infectious diseases. Purification of the extract is also recommended in other to obtain the pure bioactive components for pharmaceutical and other industrial uses.

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