



Antimicrobial Activity of Root Extracts of *Albizia chevalieri* on some selected Medical Isolates

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Abstract

Albizia chevalieri is a tree with a long list of folklore therapeutic claims many bacterial species have been reported to develop resistance to antibiotics. Hence the need to search for natural products remedy to this problem cannot be over emphasized. This study was aimed to determine the antimicrobial effect of the plant. The plant material was extracted with distilled water, chloroform, acetone and methanol. Phytochemical screening was carried out to detect for the presence of phytoconstituents. Agar well diffusion was employed to determine the antimicrobial activity, where the inhibitory level of the extracts was found to be promising nearing the positive control, *Escherichia coli* being inhibited the following concentrations of (17, 15, 18, and 16% *Staphylococcus aureus* being inhibited (29, 19, 14 and 11% inhibition) and *Candida albicans* being inhibited 15, 8, 12 and 10% inhibition). Phytochemical screening of the root extracts of *Albizia chevalieri* revealed the presence of secondary metabolites in good amounts. Being four extracts showed broad spectrum of activity. Low minimum inhibitory concentration, minimum bactericidal concentration (125 and 250mg/ml respectively) and minimum fungicidal concentration values ranging from 31.25 and 62.5mg/ml while a high Minimum Inhibitory Concentration, Minimum Bactericidal Concentration and Minimum Fungicidal Concentration values ranges from 125 and 250mg/ml respectively. With this result, it shows that Acetone extract and Methanol extract have more effect on the bacterial isolates than Chloroform extract and distilled water extract. Therefore, the plant can be explored by pharmaceutical industries for drugs production and also in textile industries for making dyes.

Keywords: Antimicrobial, Concentration, Extract, Inhibition, Phytochemical, sensitivity.

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Introduction

Medicinal plant is any plant in which one or more of its parts contain substances (phytochemical) that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. About 80% of the world medicines are originally derived from plants sources especially those found in tropical regions. Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because

the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. Phytochemicals, have been recognized as the basis for traditional herbal medicine practiced in the past and are currently in vogue in parts of the world. Most of the knowledge about plants that have useful properties comes from people living in each locality. These natives pass on the knowledge

from generation to generation making the use of techniques available for them to perform plant extractions, such traditional methods of metabolite extraction include, boiling with water, cold infusion, burning into ashes and mixing with oil. During the technological advancement of the last century, contemporary method of extraction utilizes principles based on polarity and alteration of pH. This method provides chances to quantify and to study the environmental factors that regulate the synthesis of such chemical compounds. Furthermore, medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking for remedies and health approaches free from side effects caused by synthetic chemicals (Dubey *et al.*, 2004). In addition, medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Recently, considerable attention has been paid to eco-friendly and bio-friendly plants, which can prevent and cure different human diseases (Dubey *et al.*, 2004).

Albizia is a large genus of trees, of the pea family (Fabaceae), native to warm regions of the old world. The plant *Albizia chevalieri* is a tree that grows up to 12 m height or a shrub under harsher conditions of dry savannah from Senegal, Niger and Nigeria. It has an open and rounded or umbrella-shaped canopy, bark pale-grayish, twigs pubescent with white lenticles, leaves with 8 to 12 pairs of pinnate and 20 to 40 pairs of leaflets each was reported to contain alkaloids and also tannins sufficient for use in tanning in Nigeria and Senegal. The common Hausa name is kasari, is a tree of the dry deciduous forest. Found in well-watered places, sandy terraces, not gregarious, nor common. *Abizia chevalieri* leaf is used in Borno-North eastern Nigeria as purgative, dysentery, diarrhoea,

taenicide and also remedy for coughs (Le Houèrou, 2009).

Albizia chevalier 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. Ethnobotanical survey conducted in the cause of this research revealed the use of the root and stem-barks of *Albizia 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 phytoconstituents of *Albizia chevalieri* for bioactivity. Credibility for the choice of this plant is supported by 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. Meanwhile, ethnobotanical survey conducted in the cause of this research revealed the use of the root and stem-barks of *Albizia chevalieri* for therapy against tuberculosis, toothache, inflammations and snakebite 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 the 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 (Sylvester, 2016). The study was aimed to determine the antimicrobial activity

of *Albizia chevalieri* on some selected medical isolates, with the following Objectives; to extract *Albizia chevalier* using maceration method, to determine qualitative phytochemical screening, to determine antimicrobial activity of the plant extracts on selected medical isolates

Materials and Methods

Plant collection, Authentication, Preparation and Extraction

The roots of *Albizia chevalieri* was collected at Kurba North, Yamaltu Deba L.G.A. Gombe State. They were taxonomically authenticated by a taxonomist at the herbarium unit of the Department of Biological Science, Gombe State University. A voucher specimen (649) was deposited there for future reference.

The roots were washed under a running tap water to eliminate dirt and other foreign particles that may be present, they were air-dried at room temperature (27-30° C) away from direct sun light for three weeks and were later pulverized into coarse powder using mortar and pestle, and into the fine powder using an electric grinder, the plant materials were then stored in air-tight containers until use.

Extraction Procedure

Maceration method was used in the extraction of the root of the plant, 150g of the root powder was percolated with 500ml of chloroform, acetone, methanol (95%), Ethanol (95%) and distilled water, the mixtures were allowed to stay for three days with regular stirring, after which the mixtures were filtered using Whatman's filter paper No. 1. and the residue were separated from the liquid, then the filtrates were then evaporated using water bath thereby living behind the extracts (crude extract), the extracts were then kept for analysis (Prashant *et al.*, 2011).

Qualitative Phytochemical Screening

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins were performed on the plant extract as described by Tiwari *et al.*, (2011).

Preparation of Meyer's Reagent

The procedure was carried out as described by Mayer, (2012). 1.35g of mercuric chloride was dissolved in to 10ml of distilled water in a conical flask. 5g of potassium iodide (KI) was also dissolved in 10ml of distilled water in different conical flask. The solution was mix up and stir in a volumetric flask.

Test for Alkaloids

One gram of *Albizia chevalieri* root extract was dissolved in 1% of hydrogen chloride inside test-tube for 24 hours. Few drops of Meyer's reagent were added. Formation of yellow coloured precipitate indicates the presence of alkaloid (Tiwari *et al.*, 2011).

Test for Flavonoids

Exactly 0.5 gram of the plant extracts was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids Tiwari *et al.*, 2011.

Test for Tannins

Exactly 1% of gelatin solution containing sodium chloride was added to 0.5 g of the plant extract. Formations of white precipitate indicate the presence of tannins (Tiwari *et al.*, 2011).

Test for Saponins

Exactly 0.5 g of plant extract was diluted into 6ml of distilled water in a test tube. The mixture was shaken vigorously for 15 minutes. Formation of persistent foam, confirms the presences of saponins Tiwari *et al.*, 2011).

Microbiological Analysis

Characterization was done by initial examination of the colonies on the plate, physiological examination such as staining reactions and biochemical test were also carried out to aid identification of the bacterial and fungal isolates (Cheesebrough, 2006).

Preparation of Stock and Standard Solutions of the Plant Extract

The stock solution was prepared by taking 5g and diluting it with 10ml of DMSO making it 500% concentration, furthermore varying concentrations of the solutions were made from the stock culture using distilled water. 500, 250, 125, 62.5 and 31.25% respectively. The controls were set up with equivalent quantities of water (Cheesebrough, 2010).

Preparation of MacFarland Turbidity Standards

Exactly 1% solution (w/v) of anhydrous barium chloride (BaCl_2) and 1% solution (v/v) of sulfuric acid (H_2SO_4) were made. The two solutions were mixed in test tube (0.6 ml of 1% BaCl_2

99.4 ml of 1% H_2SO_4) to obtain 0.5 McFarland standard. The bottle containing the McFarland standard was tightly sealed and stored at room temperature in the dark (Cheesebrough, 2006).

Standardization of Inocula

Stock cultures were preserved and maintained at 4°C on slopes (agar slant) of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from stock cultures of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* to different test tubes containing nutrient broth then incubated for 24 hours (overnight) at 37°C, and also 9 ml of distilled water was dispensed in empty test tubes and 1 ml from the overnight cultures were pipette into a test tube containing 9 ml distilled water; further dilution was done to ten (10^{-10}) dilution for the test organisms until the turbidity matches 0.5 McFarland's standards when compared (Cheesebrough, 2006).

Antibacterial Sensitivity Test

Agar well diffusion method was employed as described by Cheesebrough (2006) to test the antibacterial activity of the plant extracts against the clinical isolates. (*Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*). Different plant extracts concentrations used for antibacterial susceptibility test were 31.25, 62.5, 125, 250

and 500 % concentrations respectively (Cheesebrough, 2006).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial/Fungicidal Concentration (MBC)/(MFC)

Minimum inhibitory concentration was determined by serial dilution in test tubes containing 5ml of nutrient broth. 0.1ml of standardized suspension of the test organisms and 1ml of each of the concentrations (500, 250, 125, 62.5, and 31.25%) of the extracts was then introduced into the respective test tubes. Two test tubes labelled as controls containing plant extract plus nutrient broth and nutrient broth plus test organism were set up alongside. All the test tubes were incubated at 37° C for 24 hours (bacteria) and then 3 days (fungi) after which the MIC was determined. MBC/MFC was carried out by sub-culturing all test tubes without an evidence of growth during MIC and incubated at 37° C for 24 hours (bacteria) and 3 days (fungi). (Cheesebrough 2010).

Results and Discussion

The percentage yield of AE (Acetone extract) was found to be 1.13%, CE (chloroform extract) 2.40%, ME (Methanol extract) 1.87%, and DWE (Distilled water extract) 2.53%. This result varies slightly with the report of (Ngbede *et al.*, 2008) who reported percentage yield of distilled water extract to be 14.4%, Methanol extract (18.2%), Acetone (9.14%). This variation could have arisen as a result of differences in the amount of the root powder used in the extraction.

The phytochemical screening in this study shows that methanol and aqueous extracts have similar composition of phytochemical constituents (i.e. alkaloids, cardiac glycosides, saponins, flavonoid, tannins and steroids/triterpenes) (Table 3.2) and is attributable to their closeness in polarity. This is in line 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. 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 that will aid in chemotaxonomical classification system and further phylogenetic studies in Fabaceae family. (Alhassan *et al.*, 2014). 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).

Table 1: Percentage yield of *Albizia chevalieri* sample after extraction.

Extracts	W1 (g)	W2 (g)	Percentage yield (%)
AE	1.70	150	1.13
CE	3.6	150	2.40
ME	2.8	150	1.87
DWE	3.8	150	2.53

Key: Where W1 is the weight of the extract after evaporation of solvent, and W2 is the weight of the plant powder before extraction. AE=Acetone extract, CE= Chloroform extract, ME= Methanol extract and DWE=Distilled water extract, g= gram.

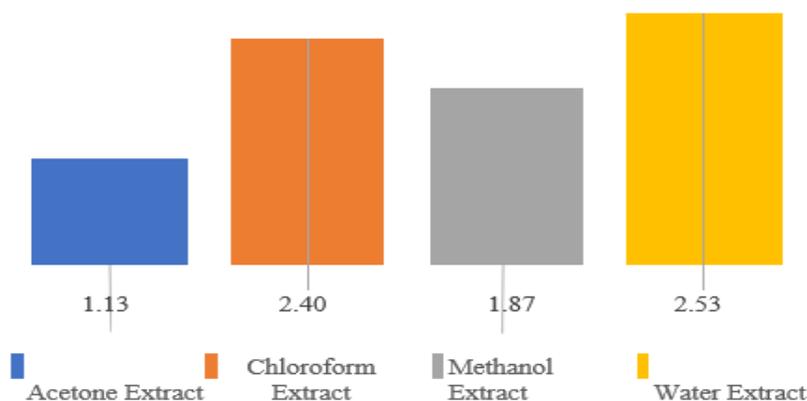


Figure 1: A histogram showing the percentage yield of different extracts of root of *Albizia chevalieri*.

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Table 2: Phytochemicals constituents in the *Albizia chevalieri* root

Phytochemicals/Extracts	ME	CE	AE	DWE
Alkaloids	+	-	+	+
Saponins	+	-	-	+
Phenols	+	+	-	+
Tannins	+	-	-	+
Flavonoids	+	+	+	+

Key: ME= methanol extract, CE= chloroform extract, AE= acetone extract, DWE= Distilled water extract, + =Present, - = Below detectable levels.

Table 3: Anti-bacterial Effect of *Albizia chevalieri* Root Extracts on *Escherichia coli*.

% inhibition	Acetone extract	Chloroform Extract	Methanol Extract	Distilled Water Extract	Positive control
250	17	15	18	16	38
125	14	11	14	14	40
62.5	10	6	11	9	39
31.2	7	0	7	4	39

Key: Where AE=Acetone extract, CE=Chloroform extract, ME= Methanol extract, DWE=Distilled water extract and PC= Gentamycin as Positive control.

Table 4: Inhibitory Effect of *Albizia chevalieri* Root Extracts on *Escherichia coli*.

<i>Escherichia coli</i> Extracts	Concentration of Inhibition (%)			
	31.2%	62.5%	125%	250%
Control	39.25 ± 0.49 ^{ab}	38.50 ± 0.05 ^{ab}	40.00 ± 0.58 ^c	38.00 ± 0.58 ^a
AE	8.00 ± 1.08 ^a	9.50 ± 0.05 ^b	14.00 ± 0.58 ^c	17.00 ± 0.58 ^b
CE	1.75 ± 1.80 ^a	5.00 ± 0.05 ^a	11.00 ± 0.58 ^b	15.00 ± 0.58 ^b
ME	8.25 ± 1.31 ^a	10.50 ± 0.05 ^a	14.00 ± 0.58 ^b	18.00 ± 0.58 ^c
DWE	5.50 ± 1.55 ^a	8.50 ± 0.05 ^a	14.00 ± 0.58 ^b	16.00 ± 0.58 ^b

Key: AE = Acetone extract. CE = Chloroform extract. ME = Methanol extract. DWE = Distilled water extract. ab, a, qb, c.= shows significant difference across the rows

Value are expressed as Mean ±SE. Values with different superscript across the rows differ significantly (P < 0.05).

Table 5: Anti-bacterial Effect of *Albizia chevalieri* Root Extract on *Staphylococcus aureus*

Concentration of inhibition(%)	Acetone extract	Chloroform extract	Methanol Extract	DistilledWater Extract	Positive control
250	29	19	14	11	38
125	24	17	11	9	40
62.5	16	12	9	5	39
31.2	10	7	5	0	39

Key: Where AE=Acetone extract, CE=Chloroform extract, ME= Methanol extract, DWE=Distilled water extract and PC= Gentamycin as Positive control.

Table 6: Inhibitory Effect of *Albizia chevalieri* Root Extracts on *Staphylococcus aureus*

<i>Staphylococcus aureus</i> Extracts	Concentration of Inhibition (%)			
	31.2%	62.5%	125%	250%
Control	39.25± 0.48 ^{ab}	38.50± 0.05 ^{ab}	40.00 ± 0.58 ^b	38.00 ± 0.58 ^a
AE	11.75± 1.80 ^a	15.50± 0.05 ^a	24.00 ± 0.58 ^b	29.00± 0.58 ^c
CE	8.50 ± 1.55 ^a	11.50± 0.05 ^a	17.00 ± 0.58 ^b	19.00± 0.58 ^b
ME	6.25 ± 1.31 ^a	8.50± 0.05 ^{ab}	11.00 ± 0.58 ^{bc}	14.00± 0.58 ^c
DWE	1.50 ± 1.55 ^a	4.50± 0.05 ^a	9.00 ± 0.58 ^b	11.00± 0.58 ^b

Key: AE = Acetone extract. CE = Chloroform extract. ME = Methanol extract. DWE =Distilled water extract. ab,bc, a, b, c. = shows significant difference across the rows. Value are expressed as Mean ±SE. Values with different superscript across the rows differ significantly (P < 0.05).

Table 7: Anti-fungal Effect of *Albizia chevalieri* Root extract on *Candida albicans*.

Concentration of Inhibition (%)	Acetone Extract	Chloroform extract	Methanol extract	Distilled Water Extract	Positive control
250	15	8	12	10	35
125	11	5	9	8	36
62.5	9	0	3	5	36
31.2	4	0	0	0	35

Key: AE=Acetone extract, CE=Chloroform extract, ME= Methanol extract, DWE= Distilled water extract and PC= Gentamycin as Positive control.

Table 8: Inhibitory Effect of *Albizia chevalieri* Root Extracts on *Candida albicans*

<i>Candida albicans</i> Extracts	Concentration of Inhibition (%)			
	31.2%	62.5%	125%	250%
Control	35.50 ± 0.65 ^a	35.50 ± 0.05 ^a	36.00 ± 0.58 ^a	35.00 ± 0.58 ^a
AE	5.50 ± 1.55 ^a	8.50± 0.05 ^{ab}	11.00 ± 0.58 ^b	15.00 ± 0.58 ^c
CE	0.25 ± 0.49 ^a	0.50± 0.05 ^a	5.00± 0.58 ^b	8.00 ± 0.58 ^c
ME	1.00 ± 1.08 ^a	2.50± 0.05 ^a	9.00± 0.58 ^b	12.00 ± 0.58 ^c
DWE	1.50 ± 1.55 ^a	4.50± 0.05 ^{ab}	8.00± 0.58 ^{bc}	10.00 ± 0.58 ^c

Key: AE = Acetone extract. CE = Chloroform extract. ME = Methanol extract. DWE = Distilled water extract. ab, bc, a, b, c. = shows significant difference across the rows. Value are expressed as Mean ±SE. Values with different superscript across the rows differ significantly (P < 0.05).

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Table 9: Minimum Inhibitory Concentration (MIC) of *A. chevalieri* Root Extracts (mg/ml) on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

PlantExtracts	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
AE	250	125	250
CE	125	125	250
ME	500	250	500
DWE	500	250	500

Key: AE=Acetone extract, CE=Chloroform extract, ME= Methanol extract, DWE= Distilled water extract and PC= Gentamycin as Positive control.

Table 10: Minimum Bactericidal/Fungicidal Concentration (MBC)/(MFC) of *A. chevalieri* Root Extract(mg/ml) on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

Plant extracts	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
AE	500	500	500
CE	500	500	250
ME	1000	1000	1000
DWE	1000	1000	1000

Key: AE=Acetone extract, CE=Chloroform extract, ME= Methanol extract, DWE= Distilledwater extract and PC= Gentamycin as Positive control.

Generally, all extracts showed wide range of antimicrobial activity when compared to the positive control but there was a slight difference between the extracts with respect to the plant's part. Methanol root extract of *Albizia chevalieri* showed activity against all the Bacterial and fungal isolates. The observed antimicrobial effects on the isolates are believed to be due to the presence of tannins, flavonoids and saponins which have shown to possess antimicrobial properties. Some workers have also attributed the observed antimicrobial effect of plants extracts to the presence of these secondary metabolites. Some other workers have identified, that tannins, flavonoids and alkaloids in the extracts of some medicinal plants example *Euphorbia hirta* possess antimicrobial activity. thus, the growth inhibition effect of the extractson 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 toincreased toxicity to pathogens. 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 gentamycin. 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/MFC 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. 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. 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 bilipid layers of Gram-negative lipopolysaccharides and lipoproteins, which prevent ready penetration of antibiotics through their cell wall. Most extracts exhibited MIC and MBC/MFC at a low concentration of 31.25 mg/ml and 62.5 mg/ml respectively against all isolates. The low MIC and MBC/MFC exhibited by the extracts against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* 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.

Conclusion and Recommendation

The root of *Albizia chevalieri* possesses antibacterial activity against pathogenic bacteria tested 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. Diseases caused by *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Purification of the extract is recommended in order to obtain the pure bioactive components for pharmaceutical and other industrial uses.

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