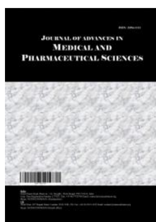

Journal of Advances in Medical and Pharmaceutical Sciences

21(4): 1-11, 2019; Article no.JAMPS.52494
ISSN: 2394-1111

Antimicrobial Activity of Methanolic, Aqueous and Partially Purified Protein of Young and Matured Leaves of *Guiera senegalensis* (Moshi Medicine)

M. K. Jiyil[1*], C. E. Mafuyai[1], M. I. Shago[2] and H. M. Inuwa[3]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Jos, Plateau State, Nigeria.
[2]Department of Chemistry, Yobe State University Damaturu, Yobe State, Nigeria.
[3]Department of Biochemistry, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author MKJ designed the study, performed the statistical analysis, Author HMI wrote the protocol, Author MIS managed the analyses and the literature of the study. Author CEM managed the literature searches. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/JAMPS/2019/v21i430140
Editor(s):
(1) Dr. Palmiro Poltronieri, National Research Council of Italy, CNR-ISPA Institute of Sciences of Food Productions, Operative Unit in Lecce , Rome.
(2) Dr. Julius Olugbenga Soyinka, Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.
(3) Dr. Erich Cosmi, Associate Professor, Department of Woman and Child Heath University of Padua, Italy.
Reviewers:
(1) Senthil Kumar Raju, Swamy Vivekanandha College Of Pharmacy, India.
(2) Hideharu Shintani, Chuo University, Japan.
Complete Peer review History: https://sdiarticle4.com/review-history/52494

Original Research Article

Received 02 September 2019
Accepted 06 November 2019
Published 15 November 2019

ABSTRACT

Background: Microorganisms have evolved defence mechanisms against antimicrobial agents and have become resistant to some antibiotics.

Aim of the Study: This study aimed to evaluate the antimicrobial activity of methanol as well as that of the aqueous and partially purified protein of young and matured leaves of *Guiera senegalensis*.

Study Duration: This study was conducted on 30th January, 2015 at the Department of Biochemistry, Faculty of Science, Ahmadu Bello University Zaria, Nigeria.

Methodology: Antimicrobial activity was determined using disc diffusion and broth dilution techniques, Gel chromatography techniques were used to fractionate the crude protein.

**Corresponding author: E-mail: kirwej@unijos.edu.ng, jiyilk@yahoo.com;*

Results: The Test isolates were *Bacillus subtilis, Staphylococcus aureus, E. coli, Salmonella typhimurium and Candida albicans*. All the extracts were sensitive to most of the isolates except *Candida albican* (fungus).
The crude and partially purified proteins were active against the Gram positive bacteria. The maximum zone of inhibition (42.00±1.00 mm) was observed in the methanol extract of a young leaf against *Staphylococcus aureus* at 100 mg/ml. The methanol extracts exhibited minimum inhibitory concentration (MIC) at a range of 6.25 mg/ml and 12.5 mg/ml and minimum bactericidal concentration (MBC) at 12.5 mg/ml and 25 mg/ml. The young leaf was more active than the matured leaf. Quantitative phytochemicals showed high amounts of saponins (26.20% and 19.66%) in matured and young leaves respectively.
Conclusion: This research justifies the traditional claim of *Guiera senegalensis* leaves for therapeutic purposes. The leaves can be used to remedy diseases caused by bacterial agents.

Keywords: Antimicrobial; guiere senegalensis protein; phytochemistry.

1. INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths, killing people every day. Infections due to a variety of bacterial etiologic agents are most common in recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [1].

Traditional medicine is a comprehensive term used to refer to both traditional medicine systems such as traditional Chinese medicine, and to various forms of indigenous medicine [2].

Plant materials remain an important resource to combat serious diseases in the world. According to WHO [3], 80 % of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. Antibiotic resistance among pathogenic bacteria is increasing at an alarming rate, and at the same time, few new antibiotics reach the market. Today, resistant and multi-drug resistant (MDR) pathogenic strains are widespread facing bigger problems when treating many common bacterial infections [4].

Antimicrobial peptides (AMPs), are important part of the innate immune system, made up of small molecules that may present antibacterial, antifungal, antiparasitic, and antiviral activity [5]. Usually these molecules are composed of 10–50 amino-acid residues, and arranged in different groups depending on the amino-acid composition, size, and conformation [6]. AMPs have promising therapeutic properties, they kill microbes rapidly, have broad activity-spectra and there are few reports of emerging bacterial resistance, and therefore much effort is focused on finding potential novel antibacterial drugs among AMPs.

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases.One approach is to screen local medicinal plants for their antimicrobial properties.

The organic solvent extracts of *G. senegalensis* are widely used for pulmonary and respiratory diseases, for coughs, colic and diarrhea, syphilis, beriberi, leprosy, impotence, rheumatism, diuresis, dysentery, gastrointestinal pain and disorder, rheumatism and fever [7].

In view of this, the antimicrobial activity of the methanolic and aqueous extracts and partially purified protein of the *G. senegalensis* will be determined. This will increase the chances of finding new therapeutic agents for the treatment of diseases caused by pathogens.

2. MATERIALS AND METHODS

This study was conducted on 30th January 2015 at the Department of Biochemistry, Faculty of Science, Ahmadu Bello University Zaria, Nigeria.

2.1 Sample Collection

Fresh leaves and roots of *Guiera senegalensis* were collected from Giwa, Zaria Local Government Area of Kaduna State, Nigeria. They were authenticated at the Haberium Department of Biological Sciences, Ahmadu Bello University Zaria Kaduna State.

Voucher Number of *Guiera senegalensis*: 1823.

2.2 Preparation of Extracts

The fresh leaf of *G. senegalensis* were washed with distilled water and dried at room temperature for one month. They were pulverized using a mechanical grinder. The powdered plant material 50 g was extracted in methanol (300 ml) and cold water (300ml) as detailed below according to method [8].

2.2.1 Methanol extraction of dried leaves of *G. senegalensis*

Exactly 50 g of dried leaf powdered was extracted in a soxhlet apparatus with 300 ml methanol. The solution of the methanol extract was gently evaporated to dryness in a water bath at 40ºC in fume cupboard. The resultant crude extract was transferred into airtight sample bottles and kept at 4ºC until when required.

2.2.2 Cold water extraction of dried leaf *G. senegalensis*

Exactly 50 g of dried leaf powdered was soaked in 300 ml distilled water in a 500 ml sterile conical flask with a constant stirring using magnetic stirrer. The mixture was allowed to stand at room temperature for 48 hours, after which it was filtered using No. 1 Whatman filter paper. The filtrate was gently evaporated to dryness in a water bath at 40ºC and stored in a refrigerator at 4ºC until when required.

2.3 Quantitative Phytochemical Constituents of *Guiera senegalensis*

Total alkaloids, flavonoids,Tannins,cyanogenic glycosides, oxalate, phytates and saponins were determined from the dried leaves and roots samples using the method described [9]

2.4 Preparation of Stock Solution of Extract

The stock solutions of the four extracts, methanol extracts of young and matured leaves,were prepared by dissolving 1.0 g of each extract in 10 ml of sterile distilled water to give a concentration of 100 mg/ml. The stock solutions were reconstituted to graded concentrations of 50 mg/ml, 25 mg/ml and 12.5 mg/ml using two-fold dilution. They were well labelled and stored at 4ºC until when required.

2.5 Preparation of Test Organisms

The stock bacterial and fungal isolates used were obtained from Ahmadu Bello University Teaching Hospita Zaria, Kaduna State. Fresh pure plates of the test organisms were made from the isolate cultures obtained on agar slants. The isolates were sub-cultured on selective and differential solid media and re-identified using colony morphology, gram reaction, motility test, haemolytic activity and biochemical tests namely catalase, bile solubility, litmus milk, citrate, oxidase and fermentation of sugars- mannitol, lactose and sorbitol [10]. With the aid of sterile wire loop, colonies of fresh cultures of the different bacterial isolates were picked and suspended in 5 ml nutrient broth in a well-labelled sterile 10 ml bijou bottles. They were incubated at 37ºC for 24 hours.

2.5.1 Determination of preliminary antimicrobial activity of extracts

The antimicrobial activity of the extracts were determined using agar well diffusion test and broth dilution technique [11].The antimicrobial activity of the plant extracts was tested on four standard bacteria species and fungus namely; *Bacillus subtilis, Staphylococcus aureus, Eschericia coli*, *Salmonella typhimurium* and *Candida albicans* in the Microbiology Laboratory, Faculty of Sciences Ahmadu Bello University Zaria. These were standard laboratory cultures whose susceptibility on commonly used antibiotics was already established*, Staphylococcus aureus* and *Bacillus subtilis* represented gram positive bacteria while *Eschericia coli* and *Salmonella typhimurium* represented gram negative bacteria.

2.5.2 Agar well diffusion assay

The agar well diffusion technique [12] was the standard method used to determine the antibacterial activity of the bioactive compounds. Briefly in the method, the media of Mueller hinton agar (Becton Dicknson M.D USA) and while the potatoes dextrose agar was prepared and treated according to manufacturer's guidelines, where 38 g of mueller hinton agar was mixed with one litre of distilled water and enclosed in a container and autoclaved at 121ºC for 15 minutes. The media was later dispensed into 90mm sterile agar plates (Oxoid, UK) and left to set for bacterial assay while 19 g of potatoes dextrose agar was mixed with one litre of distilled water and autoclaved at 121ºC for 15 minutes for fungus assay. The agar plates were

incubated for 24 hours at 37ºC to confirm their sterility. Absence of growth after 24 hours showed that the plates were sterile. The Sterile agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each organism evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The culture plate then had at most 4 wells of 6 mm diameter and 5 mm depth made into it using a sterile agar glass borer. Ciprofloxacin was used as a positive control for bacteria and ketoconazole for fungus. Approximately 0.2 ml of the bioactive test compound of concentration 100 mg/ml,50 mg/ml, 25 mg/ml and 12.5 mg/ml was suspended in the wells and thereafter inoculated plates/culture were incubated for 24 hours at 37ºC. The plates/cultures were examined for the presence of inhibition zones around each well.

Antimicrobial activity was determined from the zone of inhibition around the wells. Single readings were carried out. Non-active compounds did not show any inhibition zone. The zones of inhibition were measured using a ruler and a pair of divider and results were reported in millimetres (mm). All zone diameters were considered important since the extracts from the plants were still crude. A zone size interpretive chart was then drawn to show the different plant extracts and their corresponding inhibition zone diameter to the nearest millimetre.

2.5.3 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 [13].The plant extracts were serially diluted from the solutions of 50 mg/ml to obtained varying concentration .The concenration were; 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml.doubling dilutions of the extract were incorporated in Muller Hinton broth (Oxoid, UK) and then inoculated with 0.1 ml 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 37ºC for 24 hrs. After incubation period, the presence or absence of growth on each tube was rated using the following scale: - = no growth, + = scanty growth, ++ =moderate growth, +++ =heavy growth. a loop full from each tube was further sub cultured on nutrient agar to comfirmed weather 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 millilitre to which the organism is susceptible. This was the minimum inhibitory concentration(MIC).

2.5.4 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 37ºC for 24 h. The least concentration that did not show any growth after incubation was regarded as the MBC [14].

2.6 Partial Purification of Antimicrobial Proteins/Peptides from Young and Matured Leaves of *G. senegalensis*

Antimicrobial proteins and peptides was analysed using method of Bibiana and Selvamani [15] with slight modification in buffer concentration. The fresh leaves and roots samples of 50 g each were homogenized using 0.1 M phosphate phosphates buffer, pH 7.4 and then filtered. The crude sample was centrifuged at 10,000 rpm for 30 mins. The crude extracts were saturated with 80% ammonium sulfate. The saturated extract was subjected for dialysis. After dialysis these samples were subjected to spectrophotometric analysis to determine the concentration of the protein. The supernatant was subjected to gel filtration chromatography using Sephadex G-15 Approximately 40 fractions (3.0 ml each) were collected at the flow rate of 1 ml/21 seconds with potassium phosphate as eluting buffer and absorbance (OD) was measured at 280 nm.

2.7 Antimicrobial Assay of Crude and Partial Purified Proteins Extracts

Purified protein fractions obtained after gel filtration chromatography were also tested for their antimicrobial activity by agar well diffusion method. The fractions showing maximum antimicrobial activity were then taken for SDS-PAGE to determine the molecular weight of the protein. Antimicrobial activity was expressed in arbitrary units (AU/ml). One AU was defined as

the reciprocal of the highest level of dilution resulting in a clear zone of growth inhibition [16].

2.8 SDS-PAGE of Purified Proteins

One dimension SDS-PAGE was carried out following modified method of Laemmli, 1970. SDS-PAGE was run on vertical slab gel system. Proteins were electrophorised on 12% separating gel (0.75 mm thickness) overlaid with 5% stacking gel. A 10% (w/v) stock solution of precipitated protein in deionized water was run in SDS-PAGE [17].

3. RESULTS

The phytochemical analysis of *G. senegalensis* from my analysis showed the presence of alkaloids, saponins, tannins, cyanogenic glycosides, phytic acids, oxalates and flavonoids. High percentage of phytochemicals were observed as showed in Table 1 with saponins of matured and young leaf being 26.20 mg/100 g and 19.66 mg/100 g respectively.

The antimicrobial activity of the following test isolates "*Bacillus subtilis, Staphylococcus aureus, Eschericia coli, Salmonella typhimurium* and *Candida albicans*" indicates that both methanol and aqueous extracts were effective against most of the test isolates compared to purified protein which was effective only to Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). All extracts were ineffective against the fungus; *Candida albicans.* Methanol extract of young leaf showed higher activity against three (3) isolates except *Salmonella typhimurium* while the aqueous extract of same leaf was effective against three isolates except *Staphylococcus aureus.*

The zone of inhibition for methanol extracts of young leaf was 42.00±1.00 mm at a concentration of 100 mg/ml was more than that of the standard drug ciprofloxacin (35.00±1.00 mm) against *Staphylococcus aureus* of same concentration. Both methanol and water extracts exhibited low minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against all the test isolates at a concentration range 6.25 mg/ml to 12.5 mg/ml except *Eschericia coli* and *Salmonella typhimurium* at 25 mg/ml. Methanolic extract of young leaf showed the lowest MIC.

4. DISCUSSION

Pathogenic organisms continue to create problems for humans and natural resources within our environment as a result of the Diseases they cause. Additionally, such organisms tend to develop a resistance against orthodox drugs. However the antimicrobial activity in the test we have conducted affects the isolates *Bacillus subtilis, staphylococcus, E. coli, Salmonella typhimurium* and *Candida albicans*, which indicates that both methanol and aqueous extracts were effective against most of the test isolates when compared to the purified protein that was only effective on Gram positive bacteria(*Bacillus subtilis* and *Staphylococcus aureus*). All extracts were ineffective against the *Candida albicans* fungus. According to the chemical and laboratory standard institute. [18], any plant material should be considered an effective therapeutic agent if its extract produces zones of inhibition ≤ 15.00 mm on the target pathogenic organism. The activity of plant extracts in testing bacteria is normally expressed in vitro, as the zones of inhibition in millimetres (≤10.00 mm) are considered effective zones [19].

Generally, both extracts showed a wide range of antimicrobial activity when compared to the positive control, but a slight difference was observed between the extracts. The methanol extract of the young leaf showed higher activity against three isolates but not *Salmonella typhimurium* and *Candida albicans* while the aqueous extract of the same leaf was also effective against three isolates, but not *Staphylococcus aureus* and *Candida albicans*

Table 1. Quantitative phytochemical screening of young and matured leaves of *G. senegalensis*

Phytochemicals compounds(mg/100 g)	Matured leaf	Young leaf
Tannins	1.20±0.03	1.42±0.03
Flavonoids	12.08±0.06	4.64±0.05
Saponins	26.20±0.05	19.66±0.05
Cyanogenic glycosides	1.51±0.03	0.45±0.02
Phytic acids	1.16±0.04	1.74±0.01
Oxalates	0.24±0.02	0.074±0.00

Table 2. Antimicrobial screening of different concentrations of methanol extracts of matured leaf of *Guiera senegalensis*

Concentration of extract/ Standard drugs (mg/ml)	Zone of inhibition of micro organisms (mm)				
	Bacillus subtilis	***Staphylococcus aureus***	***Escharichia coli***	***Salmonella typhimurium***	***Candida albicans***
100	17.33± 0.58[b]	17.67± 0.58 [b]	18.00± 1.00 [b]	16.67± 1.15 [b]	NI
50	15.67±0.58[bc]	16.33± 0.58 [b]	15.33 ±0.58[bc]	15.33± 0.58 [b]	NI
25	15.33±0.58[bc]	14.00 ±1.00c	12.67 ±0.58[bc]	11.33 ±1.00[c]	NI
12.5	12.00±1.00[bc]	12.33± 0.58[c]	12.00± 1.00[bc]	NI	NI
Cipr 100	45.33±1.53[a]	35.00± 1.00[a]	22.33 ±1.53[a]	36.33± 2.08[a]	NI
Keto.100					40.00± 1.00[a]

([a,b,c]) = Means in the same column with different superscripts letter indicates statistically significant differences ($P < 0.05$). Values are mean± standard deviation of triplicate. NI = No inhibition.Diameters of zones inhibition ≥ 10 mm exhibited by plant extract were considered active (Usman et al.,2008).Standard drugs: Cipr = Ciprofloxacin(Antibacterial drug), keto=Ketokonazole (Antifugal drug)

Table 3. Antimicrobial screening of different concentrations of methanol extracts of young leaf of *Guiera senegalensis*

Concentration of extract/ Standard drugs (mg/ml)	Zones of inhibition (mm)				
	Bacillus subtilis	***Staphylococcus aureus***	***Escharichia coli***	***Salmonella typhimurium***	***Candida albicans***
100	18.33± 0.58[b]	42.00± 1.00 [b]	21.67±1.53 [b]	NI	NI
50	16.00± 0.58 [b]	41.00±1.00 [b]	12.33±0.58[b]	NI	NI
25	15.33± 0.58[C]	35.00± 1.00 [C]	12.33±0.58 [C]	NI	NI
12.5	11.67± 0.58 [C]	15.33± 1.00 [C]	12.00±1.00 [C]	NI	NI
Cipr 100	45.33± 1.53 [a]	35.00± 1.00 [a]	22.33 ±1.53[a]	36.33± 2.08 [a]	NI
Keto.100					40.00 ±1.00[a]

([a,b,c]) = Means in the same column with different superscripts letter indicates statistically significant differences ($P < 0.05$). Values are mean± standard deviation of triplicate. NI = No inhibition.Diameters of zones inhibition ≥ 10mm exhibited by plant extract were considered active (Usman et al.,2008).Standard drugs: Cipr = Ciprofloxacin(Antibacterial drug), keto=Ketokonazole (Antifugal drug)

Table 4. Antimicrobial screening of different concentrations of aqueous extracts of matured leaf *Guiera senegalensis*

Concentration of extract/ Standard drugs (mg/ml)	Zones of inhibition of micro organisms (mm)				
	Bacillus subtilis	***Staphylococcus aureus***	***Escharichia coli***	***Salmonella typhimurium***	***Candida albicans***
100	17.33± 0.58 ^{b}	24.33± 3.79 ^{b}	NI	12.00±0.58	NI
50	16.33± 0.58 ^{b}	21.33 ±3.06 ^{b}	NI	11.00±0.00	NI
25	15.33± 0.58 ^{b}	16.00± 1.00^{bc}	NI	11.00±1.00	NI
12.5	11.00± 00^{b}	15.33± 0.58^{bc}	NI	NI	NI
Cipr 100	45.33± 1.53^{a}	35.00± 1.00 ^{a}	22.33 ±1.53 ^{a}	36.33± 2.08 ^{a}	NI
Keto.100					40.00 ±1.00 ^{a}

(^{a,b,c}) = Means in the same column with different superscripts letter indicates statistically significant differences (P< 0.05). Values are mean± standard deviation of triplicate. NI = No inhibition.Diameters of zones inhibition ≥ 10 mm exhibited by plant extract were considered active(Usman et al.,2008).Standard drugs: Cipr = Ciprofloxacin(Antibacterial drug), keto=Ketokonazole (Antifugal drug)

Table 5. Antimicrobial screening of different concentration of aqueous extract of young leaf of *Guiera senegalensis*

Concentration of extract/ Standard drugs (mg/ml)	Zones of inhibition of micro organisms (mm)				
	Bacillus subtilis	***Staphylococcus aureus***	***Escharichia coli***	***Salmonella typhimurium***	***Candida albicans***
100	18.00± 1.00^{b}	NI	12.00± 1.00 ^{b}	26 0±.1.00 ^{b}	NI
50	16.00± 1.00^{c}	NI	NI	23.67± 0.58 ^{b}	NI
25	11.67± 1.15 ^{c}	NI	NI	20.33 ± 0.58^{C}	NI
12.5	11.67± 0.58^{c}	NI	NI	18.00± 1.00^{c}	NI
Cipr 100	45.33± 1.53	35.00± 1.00	22.33 ± 1.53	36.33 ± 2.08	NI
Keto.100					40.00 1.00

(^{a,b,c}) = Means in the same column with different superscripts letter indicates statistically significant differences (P< 0.05). Values are mean± standard deviation of triplicate. NI = No inhibition.Diameters of zones inhibition ≥ 10 mm exhibited by plant extract were considered active(Usman et al.,2008).Standard drugs: Cipr = Ciprofloxacin(Antibacterial drug), keto=Ketokonazole (Antifugal drug)

Table 6. Mininmum inhibitory concentration (MIC) of methanol and aqueous extracts of young and matured leaves of *Guiera senegalensis* (mg/ml)

Bacteria isolates	**MT matured leaves(mg/ml)**	**MT young leaves(mg/ml)**	**AQ matured matured(mg/ml)**	**AQ young leaves(mg/ml)**
Bacilus subtilis	12.5	12.5	6.25	12.5
Staphylococcus aureus	12.5	3.125	6.25	_
Eschericia coli	25	50	_	50
Salmonella typhimurium	25	_	_	_

Key: AQ = Aqueous extract, MT = Methanol extract, - = No minimum inhibitory concentration (MIC)

Table 7. Mininmum bactericidal concentration (MBC) of methanolic and aqueous extracts of young and matured leaves of *Guiera senegalensis* (mg/ml)

Bacteria isolates	AQ matured leaves	AQ young leaves	MT matured leaves	MT young leaves
Bacilus subtilis	12.5	25	25	25
Staphylococcus aureus	12.5	_	25	3.125
Eschericia coli	_	100	50	100
Salmonella typhimurium	_	50	50	_

Key: AQ = Aqueous extract, MT = Methanol extract, - = No Minimum Bactericidal Concentration (MBC)

Table 8. Purification of Bioactive protein of the leaf of young and matured *Guiera senegalensis*

Purification steps	Total protein(mg/ml)	Total activity(AU)	Specific Activity(AU/mg)	Recovery (%)	Purification fold
Matured leaves					
Crude protein	14.5	3500	241.38	100	1
80% ammonium sulfate precipitation	8.35	2700	323.35	77.14	1.34
Gel filtration Sephadex	1.2	1500	1250	51.43	5.18
Young leaves					
Crude protein	15.1	4580	303.31	100	1
80% ammonium sulfate precipitation	8.25	4300	521.21	93.89	1.72
Gel filtration Sephadex	1.1	3100	2818.18	67.69	9.29

Table 9. Antimicrobial activity of the leaf and root of the crude and partial purified protein fractions of *Guiera senegalensis*

Samples	*Bacillus subtilis*	*Staphylococcus aureus*	*Eschericia coli*	*Salmonella typhimurium*	*Candida albicans*
Crude extract of matured leaves	++	+++	_	_	_
Crude extract of young leaves	++	+++	_	_	_
Peak of17th fraction of gel filtration of matured leaves	++	++	_	_	_
Peak of 27th Fraction of gel filtration of young leaves	++	++	_	_	_

Key: +++ = very strong activity, ++ = strong activity, + = activity present, −− = active

Both the methanol and aqueous leaf extracts showed a wide range of antibacterial activity, which agreed with similar documented reports [20].

A high zone of inhibition was observed in the methanol extracts of the young leaf when comparing the standard drug ciprofloxacin against *Staphylococcus aureus* with the same concentration. This result is in accordance with the experiment carried out by Chairani and Harfiani [21]. The observed antibacterial effects on the isolates was due to the presence of the secondary metabolites, i.e. the alkaloids, saponins, tannins, cyanogenic glycosides, phytic acids, oxalates and flavonoids, which were found as a result of the phytochemical screening of *G. senegalensis*.

Some workers have also attributed the effects of the extracts to the presence of secondary metabolites such as saponins, phenolic acids, tannins and flavonoids [22]. Phenolic acids are highly hydroxylated phenols. Scientific evidence show that increased hydroxylation on phenols result to increased toxicity to pathogens. Also saponins and alkaloids have been shown to be most efficient against gram positive and gram negative bacteria.

The partially purified fractions of the proteins were active against Gram positive bacteria (*Bacillus subtilis and Staphylococcus aureus*). The antimicrobial efficiency depends on several characteristics of the protein or peptide including molecular mass, sequence, charges, conformation, secondary and tertiary structures, presence or absence of disulfide bonds and hydrophobicity [23]. The resistance of Gram negative could be due to the fact that they have an additional outer phospholipids membrane that makes the cell wall impermeable to lipophilic and hydrophilic solutes [24].

Both methanol and water extracts exhibited low minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against all the test isolates. Methanolic extract of young leaf showed the lowest MIC against *Staphylococcus aureus.* Protein fraction of the young leaves showed more activity on *Staphylococcus aureus* compared with other fractions. The low MIC and MBC exhibited by the extracts against *Staphylococcus aureus, E. coli, Salmonella typhimurium* and *Bacillus subtilis* 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 bioactive compounds may be 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 the bacterial cell walls and disrupts microbial membranes. Also some polyphenols and other tannins are attributable to the reduction in enzyme activities of bacteria [25].

The present of low oxalates, tannins, phytate and cyanogenic glycosides also support the safety use of the plant since they are within the range of reported values for leafy vegetables [26].

5. CONCLUSION

The leaf of *Guiera senegalensis* possesses antimicrobial 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The Authors thank the department of Biochemistry, Microbiology and Pharmacognosis Ahmadu Bello University Zaria for their technical support during the period of the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hancock DR, Algozzine B. Doing case study research: A practical guide for beginning researchers. Teachers College Press; 2015.
2. Pan SY, Litscher G, Gao SH, Zhou SF, Yu ZL, Chen HQ, Zhang SF, Tang MK, Sun

JN, Ko KM. Historical perspective of traditional indigenous medical practices: The current renaissance and conservation of herbal resources. Evidence-Based Complementary and Alternative Medicine; 2014.

3. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, AlMazroa MA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. The lancet. 2012;380(9859):2095-128.
4. Seukep JA, Fankam AG, Djeussi DE, Voukeng IK, Tankeo SB, Noumdem JA, Kuete AH, Kuete V. Antibacterial activities of the methanol extracts of seven Cameroonian dietary plants against bacteria expressing MDR phenotypes. Springerplus. 2013;2(1):363.
5. Yang H, Wu H, Hancock G, Clutton G, Sande N, Xu X, Yan H, Huang X, Angus B, Kuldanek K, Fidler S. Antiviral inhibitory capacity of CD8+ T cells predicts the rate of CD4+ T-cell decline in HIV-1 infection. The Journal of Infectious Diseases. 2012;206(4):552-61.
6. Guilhelmelli F, Vilela N, Albuquerque P, Derengowski L, Silva-Pereira I, Kyaw C. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. Frontiers in Microbiology. 2013; 4:353.
7. Mohammed SY. Quantitative phytochemical and elemental analysis of *Guiera senegalensis* leaf extract. Journal of Pharmacognosy and Phytotherapy. 2013;5(12):204-7.
8. Habila AJ, Habila JD, Shode FO, Opoku AR, Atawodi SE, Umar IA. Inhibitory effect of betulinic acid and 3β-acetoxybetulinic acid on rat platelet aggregation. African Journal of Pharmacy and Pharmacology. 2013;7(44):2881-6.
9. Mir MA, Sawhney SS, Jassal MM. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. Wudpecker Journal of Pharmacy and Pharmocology. 2013;2(1):1-5.
10. Natarajan N, Rajikkannu M. Antimicrobial activity of Bacillus cereus strain isolated from Rohu (*Labeo rohita*). Int. J. Curr. Microbiol. Appl. Sci. 2014;3:474-80.
11. Olorunnipa TA, Igbokwe CC, Lawal TO, Adeniyi BA, Mahady GB. Anti-Helicobacter pylori activity of *Abelmoschus esculentus* L. Moench (okra): An in vitro study. Clin Microbiol. 2013;2:132.
12. Yeoh WK, Ali A, Forney CF. Effects of ozone on major antioxidants and microbial populations of fresh-cut papaya. Postharvest Biology and Technology. 2014;89:56-8.
13. Ayankunle AA, Kolawole OT, Adesokan AA, Akiibinu MO. Antibacterial activity and sub-chronic toxicity studies of *Vitellaria paradoxa* stem bark extract. J Pharmacol Toxicol. 2012;7:298-304.
14. Peng W, Han T, Xin WB, Zhang XG, Zhang QY, Jia M, Qin LP. Comparative research of chemical constituents and bioactivities between petroleum ether extracts of the aerial part and the rhizome of *Atractylodes macrocephala*. Medicinal Chemistry Research. 2011; 20(2):146-51.
15. BIBIANA MA, Selvamani P, Latha S. Identification and appraisal of crude protein extracts from south Indian Marine Edible Bivalves for their potential bactericidal Property. Asian J Pharm Clin Res. 2014; 7(1):233-6.
16. Martins S, Amorim EL, Sobrinho TJ, Saraiva AM, Pisciottano MN, Aguilar CN, Teixeira JA, Mussatto SI. Antibacterial activity of crude methanolic extract and fractions obtained from *Larrea tridentata* leaves. Industrial Crops and Products. 2013;41:306-11.
17. Mohankumar A, Selvi ST. Prospective study on antimicrobial protein of Spirastrella inconstans against Methicillin Resistant *Staphylococcus aureus* (MRSA). International Journal of Biological and Chemical Sciences. 2012;6(4):1428-36.
18. Evans NA. Tulathromycin: An overview of a new triamilide antimicrobial for livestock respiratory disease. Veterinary Therapeutics. 2005;6(2):83.
19. Ulukanli Z, Karabörklü S, Cenet M, Sagdic O, Ozturk I, Balcilar M. Essential oil composition, insecticidal and antibacterial activities of Salvia tomentosa Miller. Medicinal Chemistry Research. 2013; 22(2):832-40.
20. Odenigbo MA, Asumugha VU, Ubbor S, Nwauzor C, Otuonye AC, Offia-Olua BI, Princewill-Ogbonna IL, Nzeagwu OC, Henry-Uneze HN, Anyika JU, Ukaegbu P. Proximate composition and consumption pattern of plantain and cooking-banana. Current Journal of Applied Science and Technology. 2013;1035-43.

21. Chairani A, Harfiani E. Efektivitas getah jarak sebagai antiseptik terhadap pertumbuhan *Staphylococcus aureus*, *Eschericia coli* and *Candida* sp. secara In Vitro. Jurnal Kedokteran Universitas Lampung. 2018;2(2):84-92.
22. Shettima AY, Karumi Y, Tijjani MA, Sodipo OA, Idris M. Phytochemical and antidiarrhoeal properties of ethyl acetate root extract of *Guiera senegalensis* JF Gmelin via oral route. Journal of Chemical and Pharmaceutical Research. 2012; 4(10):4604-12.
23. De Souza Cândido E, Pinto MF, Pelegrini PB, Lima TB, Silva ON, Pogue R, Grossi-de-Sá MF, Franco OL. Plant storage proteins with antimicrobial activity: Novel insights into plant defense mechanisms. The FASEB Journal. 2011;25(10):3290-305.
24. Renuka U. Formulation and evaluation of floating controlled release tablets of carvedilol by using natural polymers (Doctoral dissertation, JKK Nattraja College of Pharmacy, Komarapalayam, Tamil Nadu, India); 2017.
25. Sato H, Frank DW. ExoU is a potent intracellular phospholipase. Molecular microbiology. 2004;53(5):1279-90.
26. Kubmarawa D, Andenyang IF, Magomya AM. Amino acid profile of two non-conventional leafy vegetables, *Sesamum indicum* and *Balanites aegyptiaca*. African Journal of Biotechnology. 2008;7(19).

© 2019 Jiyil et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://sdiarticle4.com/review-history/52494