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Antibacterial Activity of *Guiera senegalensis* Root extracts against some Clinical Isolates

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Abstract
Guiera senegalensis is a medicinal plant used for the treatment of various illnesses. The study was aimed to investigate the phytochemical constituents and antibacterial activity of Guiera senegalensis root extracts. Extraction was conducted with the aid of soxhlet Extractor. Phytochemical screening was carried out using conducted by conventional method. The antibacterial activity of the extracts was investigated using agar well diffusion method while broth dilution method was used to determine minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the extracts. The phytochemical screening of the extracts revealed the presence of saponin, flavonoid, alkaloid, tannin, steroid, terpenoid and glycoside. The antibacterial activity of the extract depends on its concentration and types of isolates. Highest zone of inhibition is demonstrated by S. aureus (14.25 mm), E. coli recorded 13.33 mm while Salmonella was least sensitive with 12.66 mm. The ethanol extract was more active against the isolate with average zone of inhibition of 15.58 mm followed acetone has 13.16 mm while ethyl acetate extract has an average zone of inhibition of 11.83 mm. it is concluded that the extracts of G. senegalensis possessed antibacterial agents

Keywords*: Antibacterial activity, bacteria, G. senegalensis, Phytochemistry*

Introduction

Guiera senegalensis is one of the medicinal plants used for the treatment and control of diseases which belongs to the Combretaceae family. It is also distributed in the Savannah region of West and Central Africa, Senegal, Nigeria, Chad, Gambia, Mali, Guinea, Guinea-Bissau, Niger, Burkina Faso, Mauritania and Ghana (Anka *et al.*, 2020). It is usually occur as a shrub or a small tree of 3 to 5m height relying on the habitat. Its grey to brown spindly bike consists of numerous knots that send out branches. The leaves are grey-green colour and oval shape which are 3 to 5cm long and 1.5 to 3.0cm broad are arranged opposite or sub opposite on the stem (Anka *et al.*, 2020).

The *Guiera senegalensis* has extensively been used as a source of traditional medicine.

The different part of this plant has lots of useful properties. The root are used for treatment gonorrhea, tooth and stomach ache (Ogbeba *et al.,* 2017).wounds, injuries and different skin conditions, including inflammation of skin (Alshafei *et al.,* 2016).The leaves are widely administered for pulmonary and respiratory complaints, for cough as a febrifuge, colic and diarrhea, syphilis, against dysentery, rheumatism, hypertension, eczema, beri-beri, leprosy, gastro enteritis, impotence, epilepsy, dieresis, expiration, bronchitis, tuberculosis, fever, cold, asthma (Mamman and Isah, 2013; Denou *et al*., 2016). It is moreover, used as a poultice on tumors and against the Guinea work, kidney diseases, abdominal pain, joint problems, diabetes mellitus and increasing high blood sugar levels (Alshafei *et al*., 2016).

Many phytochemical studies of different organs of *Guiera senegalensis* indicated the presence of bioactive compounds such as alkaloids, tannins, terpenoids, saponin, flavonoids, counmarins, mucilage, amino acid, ascorbic acid, anthraquinones, elastin, cardio tonic and cyano genicheteroside. The root concoction is used to cure diarrhoea, dysentery and microbial infections (Mamman and Isah, 2013). The plants continues to be one of the plants used by local livestock farmers, traditional healers and Fulani herdsmen in the treatment of snake bites in northern Nigeria. Phytomedicine derives from plants have shown great promise in the treatment of intractable infectious disease including opportunistic AIDS infections (Iwu *et al.,* 1990). About 80% of the rural population in Nigeria depends on it as a primarily Healthcare. This represents a potential pharmaceutical market and is an incentive for research in to new drugs.

Materials and Methodology

Sample collection

Fresh root of *Guiera senegalensis* was collected from Tamburawa, Dawakin kudu local government, Kano State, Nigeria. The root were placed in sterile polyethene bag and transported to the laboratory for further extraction using soxhlet extraction procedure. The *Guiera senegalensis* root was identified at Herbarium section at Plant Biology Department, Bayero University Kano. The identification number is BUKHAN 0032.

Extraction Procedure

About 25g of the sample was extracted using Soxhlet Extractor with 250 ml of the solvent namely ethanol, Acetone and ethyl acetate respectively. This was followed by the filtration and the filtrate was evaporated in using rotary evaporator until the extract is recovered. The extract was diluted using Dimethylsulfoxide to produce different concentration of the extract.

Phytochemical Screening of the Extracts

Phytochemical screening of the plant materials was conducted using the method adopted by Tiwari *et al*. (2011). Wagner's test for alkaloid, Ferric chloride test for phenol, gelatin test foe tannin, lead acetate test for flavonoid, foam test for saponin, acetic acid test for steroid, Salkowski test for terpenoid detection, Fehling's test for glycoside

Media Preparation

The Media used was Mueller Hinton Agar. The media was prepared by weighing 39 g of the agar

in to 1000 ml of distilled water and shake it vigorously. The conical flask was plug with cotton wool wrapped with aluminum foil paper then the medium in the flask was sterilized in an autoclave at 121^{o}C for 15minute and allow cooling to about 40^{o}C for 15 minute before dispersing in to Petri dish (Cheesbrough, 2010).

Preparation of Bacterial Culture

Three bacterial strains (two gram negative and one gram positive) including *Escherichia coli, Salmonella typhi,* and *Staphylococcus aureus* were obtained from stock culture in Department of Microbiology, Bayero University. The bacterial isolate were revived in nutrient broth at 37^{0} C for 24 hours and aliquot was diluted with normal saline and standardized with McFarland solution to 1× 10^{6} cfu per milliliter (Satya *et al*., 2012).

Antimicrobial Assay

The agar well diffusion method was used to determine the antibacterial activity of the plant extracts. Standardized organism was inoculated on the surface of Nutrient agar in a sterile Petri dish. A sterile Cork borer 6mm was then used to punch 5 wells in the inoculated agar and the agar was then removed. Four Wells that were formed were filled with different concentrations of the Extract which were labelled accordingly, 1000, 500, 250 and 125 µg/ml. While the 5th well contained the solution used for the research to serve as control, Gentamycin 50 µg/ml was used as control in this research. The plates were then left on the bench for 15 minute for adequate diffusion of the extracts and incubated at 37^{0}C for 24hours. After incubation the diameter of the zones of inhibition around each well were measured to the nearest millimeter (Ali *et al*., 2019).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of the extracts was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2ml of 2000 µg/ml of the extract into a test tube containing 2 ml of Nutrient broth, thus producing solution containing 1000 µg/ml of the extract. The process continue serially up to test tube No. 5, hence producing the following concentrations; 250, 125, 62.5, 31.25µg/ml. Test tube No. 6 do not contain extracts and serve as Control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37°C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity. The least concentration of the extract where there was no growth in tube was taken as the MIC. From each tube that did not show visible growth in the MIC, 0.01ml was aseptically transferred into extract free Mueller Hinton agar plates. The plates were incubated at 37°C for 24 hour. The MBC was recorded as the lowest concentration (highest dilution) of extract that had no growth on agar plates (Kowser and Fatima, 2009).

Results

Phytochemical Screening

The phytochemical screening of ethanol, acetone and ethyl acetate extract of *G. senegalensis*

root shows the presence of saponin, flavonoid, alkaloid, tannin, steroid, terpenoid and glycoside. Flavonoid and steroid were absent in ethanol extract, only alkaloid, flavonoid and steroid were present in ethyl-acetate extract while saponin, steroid, terpenoid and glycoside were present in acetone extract.

Table 4.1: Phytochemical Screening of the Extracts

Phytochemical	Acetone	Ethyl acetate	Ethanol
Saponins	+	-	+
Alkaloids	-	+	+
Flavonoids	-	+	-
Tannins	-	-	+
Steroids	+	+	-
Terpenoids	+	-	+
Glycosides	+	-	+

Key + = Present of phytochemical, - = absent of phytochemical

Antibacterial activity of the Extract

The antibacterial activity of various concentration of acetone, ethyl acetate and ethanol extract of *G. senegalensis* root is presented in Table 2. The antibacterial activity of the extract depends on its concentration and types of isolates. Highest zone of inhibition is demonstrated by *S. aureus* (14.25 mm), *E. coli* recorded 13.33 mm while *Salmonella* was least sensitive with 12.66 mm. The ethanol extract was more active against the isolate with average zone of inhibition of 15.58 mm followed acetone has 13.16 mm while ethyl acetate extract has an average zone of inhibition of 11.83 mm. The zone of inhibition of the control (Gentamicin 50 µg/mL) was 25, 24 and 23 mm for *S. aureus, E. coli* and *Salmonella* respectively.

Table 2: Antibacterial activity of the Extracts against the Isolates

		Concentration (µg/ml)/Zone of inhibition (mm)				
Isolates	**Extract**	**125**	**250**	**500**	**1000**	**Control**
S. aureus	EE	12	15	19	22	25
	EAE	10	11	13	15	
	AE	10	12	15	18	
E. coli	EE	11	13	18	20	24
	EAE	09	11	12	14	
	AE	10	12	14	16	
Salmonella	EE	10	13	16	18	23
	EAE	08	11	12	13	
	AE	10	12	14	15	
P-value	0.0001**					

Key: * = There is no statistical significant difference in the activity of the extracts against the isolates, Hence result is not significant at $p < 0.05$, Control = 50 µg/ml Gentamicin, E.E = Ethanol Extract, EAE = Ethyl-acetate Extract, AE = Acetone Extract.

MIC and MBC of the Extracts

The result of minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of the root extracts is presented in Table 3 below. The result showed that the ethanol extract has lower MIC and MBC than the acetone and ethyl-acetate extracts. *S. aureus* has the lowest MIC in both the extract (62.5, 125 and 125 µg/ml ethanol, acetone and ethyl-acetate extract respectively) followed by *E. coli* which has MIC of 62.5, 125 and 125 µg/ml in ethanol, acetone and ethyl-acetate extract respectively, *Salmonella* has MIC of 125 250 and 125 for ethanol, ethyl-acetate and acetone extracts respectively. The MBC of ethanol extracts were 12.5, 125 and 250µg/ml for *S. aureus*, *E. coli* and *Salmonella* respectively. Ethyl acetate has MBC of 500µg/ml for all the isolates while acetone extract has MBC of 250, 250 and 500 µg/ml *S. aureus*, *E. coli* and *Salmonella* respectively.

Table 3: Minimum Inhibitory Concentration (MIC) and MBC of the extracts

Extract	**MIC/MBC**	***S. aureus***	***Escherichia coli***	***Salmonella***
Ethanol	MIC (µg/ml)	62.5	62.5	125
	MBC(µg/ml)	125	125	250
Ethyl-acetate	MIC(µg/ml)	125	250	250
	MBC(µg/ml)	500	500	500

Acetone	MIC(µg/ml)	125	125	125
	MBC(µg/ml)	250	250	500

4.2 Discussion

In this study, the phytochemical screening of ethanol, ethyl acetate and acetone extract of root bark of *Guiera senegalensis* revealed the presence of saponin, flavonoid, alkaloid, tannin, steroid, terpenoid and glycoside. Flavonoid and steroid were absent in ethanol extract, only alkaloid, flavonoid and steroid were present in ethyl-acetate extract while saponin, steroid, terpenoid and glycoside were present in acetone extract. This result correlates with the finding of Williams *et al.* (2009). The antibacterial activity of the different extracts of *Guiera senegalensis* root shows that the extracts were active against the isolates. The inhibitory effects of the extracts could be attributed to the phytochemical components of the crude extracts as reported in previous study by Kubmarawa *et al*. (2007).

The results of antibacterial activity of the extracts against the isolates indicate that the ethanol extracts produces impressive activity, followed by acetone and least activity was demonstrated by ethyl acetate. This finding was in conformity with the finding of Yagana *et al*. (2014) who reported that the extracts of *Guiera senegalensis* inhibited the growth of various microorganisms at different concentration. Previous study by Akinyemi *et al*. (2005) indicated that the crude extracts of the plant were effective against *S. aureus*. In the presence study, the ethanol extract has lower MIC and MBC than the acetone and ethyl-acetate extracts. *S. aureus* has the lowest MIC in both the extract (62.5, 125 and 125 µg/ml ethanol, acetone and ethyl-acetate extract respectively) followed by *E. coli* which has MIC of 62.5, 125 and 125 µg/ml in ethanol, acetone and ethyl-acetate extract respectively, *Salmonella* has MIC of 125 250 and 125 for ethanol, ethyl-acetate and acetone extracts respectively. This also indicated higher activity ethanol extract and higher sensitivity of *S. aureus* to the extract. The higher activity of ethanol extract may be attributed to the better solubility of the phytochemicals in it when compared to acetone and ethyl acetate and this is highly correlated to the polarity of the solvents.

Several studies indicated the use for *G. senegalensis* in traditional medicinal to treat various illness. It is used externally as an antiseptic healing preparation for wounds, stomatitis, gingivitis and syphilitic cankers (Garba *et al*., 2018). The observed antibacterial effect on the isolates is believe to be due to the presence of secondary metabolites such as tannins, flavonoids and saponins which have been shown to possess antibacterial properties (Kubmarawa et al., 2007). The zones of inhibition produced by the extracts against the isolates in the present study have justified the use of the plant by traditional medical practitioners in the treatment of microbial infections.

Conclusion

The results of this study showed that the root bark extract from *Guiera senegalensis* contain

several secondary metabolites such as alkaloid, flavonoid, tannin, saponin and the extracts were active antibacterial agent against some clinical bacterial isolates. This activity of the plant is due to the presence of the phytochemical compounds present in the plant. Therefore, it is recommended that the extract of the plant can be used as a template for production of new antibiotics.

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