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Phytochemical and antibacterial studies of methanolic extract and fractions of *Guiera senegalensis* leaves

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The aim of the research study was to carry out phytochemical and antibacterial studies of methanolic leaves extract and its fractions of G*uiera senegalensis*. The plant material was collected, identified, shed dried and pulverized to fine powder using pestle and mortar. The powdered plant material was subjected to maceration using methanol to obtained crude methanol leaf extract which was then partitioned using *n*-hexane, chloroform ethyl acetate and *n*-butanol. The extract and the fractions obtained were subjected to phytochemical screening using standard procedure, to detect the presence of secondary metabolites. The antibacterial assay of the extract and its fractions against *S. aureus*, *B. subtilus*, *P.mirabilis* and *S. typhyrium* were investigated using agar well diffusion method at different concentration (100 – 12.5 mg/mL). The phytochemical screening revealed the presence of various secondary metabolites which varies in the extract and fractions. The extract and its fractions showed significant ($p< 0.05$) antibacterial activities against all the test isolates with the methanol extract having the highest mean zone of inhibition ranging from 2.00±1.00d-24.00±1.00* mm then followed by ethyl acetate fraction with the mean zone of inhibitions from 4.00±1.00d-22.00±1.00* mm the n-butanol fraction and the chloroform fraction had mean zone from 4.00±1.00d-18.50±1.00* mm and 4.00±1.00d-17.60±1.00d mm respectively while n-hexane fraction recorded lower mean zone of inhibition from 4.00±1.00a-15.00±1.00* The standard drug Ciprofloxacin had mean zone of inhibition from 16.20±0.00-39.0±0.00 mm. The most sensitive organism was *S. aureus*, while the least sensitive organism was *S. typhyrium*. The study has validated the ethnomedicinal claim for the use of this plant in treatment of antibacterial infections.

Keywords: Phytochemical screening *Guiera senegalensis*, Ciprofloxacin, Antibacterial isolates.

1. Introduction

The prevalence and clinical pattern of skin disorders are known to vary with climatic factors and cultural habits. There are reports highlighting the prevalence and pattern of skin disorders in various geographical locations (Patil *et al.,* 2012). Although hospital based figures may not give a true representation of the prevalence, they may suggest the burden of the illness necessitating measures to combat them in the community. (Okoro and Emeka, 2013).

Skin diseases are common and cause considerable morbidity worldwide. Lack of awareness of symptoms among the majority of lay people and lack of knowledge about skin diseases among first- and second-line health care providers have contributed to underestimations of prevalence. Household surveys (including people not seeking treatment) before the year2000 report point prevalence rates of 27%–53%, while it was 62%–87% after 2000. (Taal *et al.,*2015).

In Nigeria, most studies on the pattern of skin diseases are hospital based and there is paucity of data from rural communities. The few studies carried out in rural communities in Nigeria were carried out mainly among school children. Reports from studies in rural communities in Cameroun and India have shown that infections are among the major skin diseases documented (Akinkugbe *et al.,* 2016).

Medicinal plants are of great importance to the health of individuals and communities. It is estimated that there are about 700,000 species of tropical flowering plants that have medicinal properties. Their actions include: antibacterial, antifungal, antiviral, antihelminthic and anticarcinogenic among others. These medicinal values lie in some chemical substances they contain (Bako *et al.,* 2014).

Several traditional medicinal plants, including *Guiera senegalensis* (plate 1), a shrub that grows well in sub-Saharan Africa have been candidates for research because of their perceived medicinal properties. *Guiera senegalensis* has been used in Northern part of Nigeria and elsewhere in traditional medicine as a cure for infections and wounds (Mohammed *et al.,* 2016). The importance of *Guiera senegalensis* in traditional medicine became more apparent with the recent increase in fungal infections in Africa, and elsewhere. Extracts of Leaves, shoots and galls of *Guiera senegalensis* were found to be useful against bacteria and fungi infections (Al Shafei *et al.,* 2016). *Guiera senegalensis* belongs to *combretacea*e family which consists of trees or shrubs, sometime climbing plants, comprising about 20 genera and 500 species (Siddig Hamad *et al.,* 2017). *Guiera senegalensis* has numerous traditional medicinal applications, for instance, its leaves are employed for various internal diseases, prevention of leprosy, dermatoses, as tonic, infusions as diuretic, for stomach ache, cough and so on (Siddig Hamad *et al.,* 2017).

Guiera senegalensis leaves are widely administered for pulmonary and respiratory complaints, for coughs, as a febrifuge, colic and diarrhea, syphilis, beriberi, leprosy, impotence, rheumatism, diuresis and expurgation. In Northern Nigeria powdered leaves are mixed with food as a general tonic and blood restorative and also to women as a galactagogue. In Ghana and other West African Countries, leaves are used to treat dysentery and fever due to malaria¬ (Jigam *et al.,* 2011).

Phytochemical screening for *Guiera senegalensis* showed significant number of secondary metabolites namely anthraquinones, terpenoids, saponins, alkaloids, coumarins, mucilages, flavonoids, tannins and cardiotonic. Its cyanogenic heterosides were assayed in different organs of the plant, such as leaves, fruits, roots, and stem bark (Siddig Hamad *et al.,* 2017). This research was aimed at investigating the phytochemical and antibacterial efficacy of methanolic leaves extract of *Guiera senegalensis*,

Plate 1: A close view of *Guiera senegalensis* (Siddig *et al.,* 2017).

2. Materials and Methods

2.1 Sample collection and Identification

The leaves of *Guiera senegalensis* was collected from Ruggar-Lima, Kware Local Government area of Sokoto State, Nigeria. The sample was identified at the Herbarium Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto and was given a specimen number (UDUH/ANS/0145), packed in a polythene bags and then transported immediately to the Biology Laboratory for further treatments.

2.2 Sample preparation and extraction

Fresh leaves of *Guiera senegalensis* was rinsed with tap water and shade dried in an open air, and then grounded into powdered form. One thousand grams (1000 g) of the powdered using pestle and mortar sample was macerated with 8 L of methanol with occasional agitation for 72hours, the extract was filtered and the solvent evaporated with rotary evaporator at 40 ^{o}C to obtain crude methanol leaf extract of *Guiera senegalensis*. (250 g) of the extract was suspended in 800 mL of distilled water which was then filtered and partitioned with solvent of increasing polarity to obtain n-hexane (HF), chloroform (CF), ethylacetate (EF) and n-butanol (BF) fractions.

2.3 Phytochemical screening

Various chemical tests was conducted on the methanol extract and it's fractions to identify the presence of secondary metabolite such as alkaloids, flavonoids, tannins, saponins, terpenoids, Cardiac glycoside phenols and steroid according to the method described by Evans (2002).

2.4 Antibacterial Studies

2.4.1 Preparation of nutrient agar plates

The nutrient agar plates were prepared by suspending 28 g of nutrient agar powder in 1000 mL of distilled water. The mixture was then heated while stirring to fully dissolve all components. The dissolved mixture was autoclaved at 121 for 15 minutes and allowed to cool. The nutrient agars poured into each plate and leave the plates on the sterile surface until

the agar becomes solidified. The lid of each Petri dish was replaced and the plates were stored in a refrigerator (Proom *et al.*, 1950).

2.4.2 Test strains

Authentic pure cultures of pathogenic bacteria of Gram-positive (*Staphylococcus aureus* and *Bacillus subtilus)* and Gram-negative (*Proteus mirabilis* and *Salmonella typhyrium*) bacterial strains were used in the study. The organisms was sub-cultured on Mueller Hinton Agar medium, incubated at 37°C for 24 h and stored at 4°C in the refrigeration to maintain stock culture. The Gram-positive and Gram-negative bacteria were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C and centrifuged at 10,000 rpm for 5 mins, pellet was suspended in double distilled water and the cell density were standardized by UV spectrophotometer (Soniya, 2009).

2.4.3 Preparation of control solution

Stock solutions of Ciprofloxacin (5 mg/ mL) was prepared by dissolving 50 mg of the powder in 10 mL of distilled water from which 0.05 mg/mL (50 µg/ mL) working solution was prepared.

2.4.4 Preparation of crude extract and fractions of G. senegalensis

Stock concentrations of 100 mg/mL was prepared with 10 % dimethyl sulfoxide (DMSO) by dissolving 0.5 g (500 mg) each of the crude extract and fractions (*n*-Hexane, chloroform ethylacetate, and *n*-butanol) in 5 mL of 10 % DMSO two-fold serial dilution was carried out to obtain three solutions of concentrations of 50, 25 and 12.5 mg/ mL.

2.4.5 Antibacterial Assay

Antibacterial activity of the extracts and its fractions were determined by agar diffusion method as adapted by (Tari *et al.,* 2015). The standardized organisms were uniformly streak unto freshly prepared Mueller Hinton Agar with the aid of a sterile swab stick (cotton swabs). Four wells were punched on the inoculated agar plates using a cork borer. The wells were properly labeled according to the different concentrations of the extract and the fractions prepared. The punched wells were then filled with the extract. The plates were allowed to stay on the bench for 1hour for the extract to diffuse into the agar after which they were incubated at 37^0C for 18-24hours. At the end of the incubation period, the plates were observed for any evidence of inhibition, which appeared as clear zones that were completely devoid of growth around the wells. The diameter of the clear zones was measured with a transparent ruler calibrated in millimeter (mm).

2.5 Statistical Analysis

The results obtained was subjected to the analysis of variance (ANOVA) using SPSS software followed by post hoc test, values were considered significant at $p<0.05$ and the data expressed as mean ± standard deviation

3. Results and Discussion

3.1 Result

3.1.1 Extraction and Fractionation

The extraction of 1000 g of *Guiera senegalensis* afforded a yield of 250 g of the crude extract and the percent yields from the partitioned fractions are presented in Table 1.

Table: 1: Percentage Yield of partitioned fractions

Solvent	Weight(g)	Yield (%)	Colour
Methanol	250.00	25.00	Green
n-hexane	18.00	1.800	Green
Chloroform	12 .00	1.2 .00	Light brown
Ethylacetate	22.67	2.267	Brown
n-butanol	23.46	2.346	Redish brown
Water insoluble	36.40	3.64	Green

3.1.2 Preliminary phytochemical screening of methanol extract and its fractions

Preliminary phytochemical screening of the methanol extract and its fractions (*n*-hexane, chroform, ethylacetate and *n*-butanol) revealed the presence of flavonoids, tannins, saponins, cardiac glycosides, steroids/terpenes, phenol and alkaloid and the result is presented in Table 2.

Table.2: Preliminary phytochemical screening of the methanolic extract and fractions of *G. senegalensis* leaves

Constituent	Test	Observation	ME	HF	CF	EF	BF
Flavonoids	Shinoda	Orange color	+	-	+	+	+
	Sodium hydroxide	Yellow color	+	-	+	+	+
Alkaloids	Mayer's	A cream ppt	+	-	+	-	-
	Dragondoff's	rose red ppt	+	-	-	+	+
Saponin	Frothing	Formation froth	+	-	+	+	+
Tannins	Lead sub-acetate	Cream color ppt	+	-	+	+	+
Triterpenoids/steroids	Salkowki's	Red brown color	+	+	-	-	-
	Lieberman	Purple color	+	+	-	-	-
Phenols	Ferric chloride	A dark green color	+	-	+	+	+
Cardiac glycoside	Keller-Kiliani	A brown ring at interface	+	-	+	+	+

Key += present - = absent; ME=methanol extract; HF=*n*-hexane fraction; EF=ethylacetate fraction and BF=*n*-butanol fraction

3.1.3 Antibacterial assay

The result of antibacterial assay of the methanol extract and its n-hexane, chloroform, ethylacetate, and n butanol fractions against some selected antibacterial isolates are presented in Table 3, 4, 5, 6 and 7 respectively.

Table 3. Antibacterial assay of methanol leaf extract of *G. senegalensis*

Test organisms	Zone of inhibition (mm) Concentration(mg/mL)				Ciprofloxacin (PC)	DMSO	
	100	50	25	12.5	0.05	0.05	
S. aureus	24.00±1.00*	16.00±1.12*	4.00±1.00d	4.00±1.00d	22.20±0.00	0.00	
B. subtilus	13.00±0.06*	20.00±1.00*	16.00±1.00*	13.00±1.00*	47.20±0.00	0.00	
P. mirabilis	18.00±1.00*	15.00±1.00*	8.00±01.00*	4.00±1.00d	33.0±0.00	0.00	
S. typhyrium	12.00±1.00d	10.00±1.00d	4.00±1.00d	4.00±1.00d	16.20±0.00	0.00	

Key: = The values are mean ± SD * ($p< 0.05$) compared with PC, (ANOVA): d Indicates no statistical significant compared with PC (Positive control).

Table 4: Antibacterial assay of *n*-hexane fraction *of G. senegalensis*

Test organisms	Zone of inhibition (mm) Concentration(mg/mL)			Ciprofloxacin (PC)	DMSO	
Organisms	100	50	25	12.5	0.05	0.05
S. aureus	15.00±1.00*	9.00±01.00*	8.00±1.00d	4.00±1.00d	29.20±0.60	0.00
B. subtilus	13.60±1.00d	8.00±1.00*	8.00±1.00*	4.00±100*	31.20±0.06	0.00
P. mirabilis	14.60±0.60*	10.00±1.00*	3.00±1.00d	0.00±1.00d	21.00±0.00	0.00
S. typhyrium	15.00±1.00*	9.00±1.00*	7.00±1.00*	5.00±1.00d	33.2±0.06	0.00

Key: = The values are mean ± SD * ($p< 0.05$) compared with PC, (ANOVA): d indicates no statistical significant compared with PC (Positive control).

Table .5 : Antibacterial assay of chloroform fraction of *G. senegalensis*

Test organisms	Zone of inhibition (mm) Concentration(mg/mL)			Ciprofloxacin (PC)	DMSO	
Organisms	100	50	25	12.5	0.05	0.05
S. aureus	15.00±1.00*	10.00±1.00*	8.00±1.00*	4.00±1.00d	26.00±0.60	0.00
B. subtilus	17.60±1.00d	10.00±1.00*	4.00±1.00	00.00±1.00	35.00±0.60	0.00
P. mirabilis	12.00±1.00*	8.00±1.00*	00.00±1.00	00.00±1.00*	20.00±0.00	0.00
S. typhyrium	5.00±1.00*	00.00±1.00d	6.00±1.00d	00.00±1.00d	19.00±0.60	0.00

Key: = The values are mean ± SD*($p< 0.05$) compared with PC, (ANOVA): d indicates no statistical significant compared with PC (Positive control).

Table 6 Antibacterial assay of ethylacetate *G. senegalensis*

Test organisms	Zone of inhibition (mm) Concentration(mg/mL)		Ciprofloxacin (PC)		DMSO	
Organisms	100	50	25	12.5	0.05	0.05
S. aureus	10.00±1.00*	13.00±1.00*	10.00±1.00*	7.00±1.00d	26.20±0.60	0.00
B. subtilus	17.60±1.00d	14.00±1.00*	10.00±1.00*	4.00±1.00*	42.20±0.60	0.00
P. mirabilis	22.00±1.00*	14.00±1.00*	12.00±1.00*	5.00±1.00*	34.00±0.00	0.00
S. typhyrium	16.00±1.00*	12.00±1.00d	9.00±1.00d	4.00±1.00d	31.20±0.60	0.00

Key: = The values are mean ± SD * ($p< 0.05$) compared with PC, (ANOVA): d indicates no statistical significant compared with PC (Positive control).

Table 7. Antibacterial assay of n-butanol fraction of *G. senegalensis*

Test organisms	Zone of inhibition (mm) Concentration(mg/mL)		Ciprofloxacin (PC)		DMSO	
	100	50	25	12.5	0.05	0.05
S. aureus	12.00±1.00*	8.00±1.12*	6.00±1.00*	4.00±1.00d	34.30±0.06	0.00
B. subtilus	16.00±0.06*	12.00±1.00*	8.00±1.00d	4.00±1.00*	36.30±0.06	0.00
P. mirabilis	18.50±1.00*	16.00±1.00*	10.00±01.00*	4.00±1.00*	39.0±0.00	0.00
S. typhyrium	12.00±1.00d	8.00±1.00d	4.00±1.00d	4.00±1.00*	24.30±0.06	0.00

Key: = The values are mean ± SD * ($p< 0.05$) compared with PC, (ANOVA): d Indicates no statistical significant compared with PC (Positive control).

3.2 Discussion

3.2.1 Percentage yield

The extraction of 1000 g of the powdered sample of *G. senegalensis* using methanol as extracting solvent yielded 250 g of methanol extract. The fractionation of water soluble portion of methanol leaf extract revealed that *n*-butanol fraction has the highest percentage % yield followed by ethylacetate, *n*-hexane, and finally chloroform (Table1). The result signifies that n-butanol offers highest activity while chloroform had the least.

3.2.2 Phytochemical screening of methanol leaf extract and it fractions

Preliminary phytochemical screening of the methanol leaves extract and it fractions (*n*-Hexane, chloroform, and ethylacetate, and *n*-butanol) of the *leaves* revealed the presence of saponins, tannins, alkaloids, cardiac glycoside, steroid, triterpenoids, phenols and flavonoids, ethylacetate and *n*-butanol fractions indicated the presence of similar constituents including flavonoids, alkaloids, tannins, saponins, phenols, steroid and cardiac glycoside while *n*- hexane contained only steroid, and triterpenoids as preseted in (Table.2).The presence of these phytochemical constituents in the other plants have been reported (Mukhtar *et al*., 2019). These secondary metabolites are thought to be responsible for the pharmacological activities of the plant (Emaikwu *et al*., 2019). Abubakar *et al.,* (2020) reported the presence of saponins, tannins, alkaloids, cardiac glycoside, steroid, triterpenoids, phenols and flavonoids in the ethylacetate and *n*-butanol of the other plant.

3.2.3 Antibacterial studies

The antibacterial activity of methanol extract and it fractions (*n*-hexane, chloroform, ethylacetate and *n*-butanol) exhibited varying antibacterial activity against the test organisms and the activity was concentration dependent. The methanol leaf extract and it fractions exhibited significant ($p<0.05$) antibacterial activity at the graded concentration (100–12.5 mg/cm^3) with mean zone of inhibition ranging from 4.00±1.00d–24.00±1.00* mm against the test organisms (*S.aureus* ,*B. subtilus, P. mirabilis* and *S. typhyrium*). Methanol leaf extract showed the highest mean zone of inhibition against *S.aureus* while n-hexane fraction exhibited the least mean zone against *B. subtilus*. The standard drug ciprofloxacin showed the mean zone of inhibition range (16.20±0.00-39.0±0.00 mm) against all the test organisms*;* the drug showed the highest mean zone of inhibition against *P. mirabilis* and there was a lowest activity against *S. typhyrium* as presented in (Table 3).

Methanol leaf extract showed significant ($p< 0.05$) antibacterial activity against *S. aureus* at 100 mg/mL which was higher than that of ciprofloxacin at 0.05 mg/mL as presented in (Table 3). *n*-hexane fraction indicated a higher antifungal activity against *S. aureus* and *S. typhyrium* at 100 mg/mL (Table 4), similarly, the standard drug ciprofloxacin exhibited higher effect against *P. mirabilis* when compared with the n-butanol fraction at 100 mg/mL and the effect was statistically significant (Table7). Chloroform fraction exhibited significant antifungal activity against *S. aureus* when

compared with standard drugs and the difference is statistically significant (Table 5).

Ethylacetate fraction exhibited the highest antibacterial activity against *P. mirabilis (*Table 5). The highest activity observed by methanol leaf extract might be due to the concentration of moderately polar compounds such as flavonoids and their derivatives that have been reported to possess antibacterial activity (Alemu *et al*., 2017). Methanol leaf extract is a very good antibacterial agent for the treatment of different antibacterial infections caused by *Stemphylium solani, Aspergillus flavus, Trichoderma viride, Penicillium Spp., Fusarium verticillatum, Cladosporium cladosporioides, and Fusarium solani* (Patil *et al.,* 2012).

n-butanol fraction showed the activity against *P. mirabilis* when compared to ciprofloxacin, even though it recorded the mean zone of 12.00±1.00d mm against *S. typhyrium* which was lower than that of ciprofloxacin 24.30±0.06 mm but the difference is not statistically significant(Table.7). However, of all the antibacterial isolates used *S. aureus* was the most sensitive organism to methanol leaf extract and is the most dangerous of all the many common antibacterial isolates.

4. Conclusion

Preliminary phytochemical screening of methanol leaf extract and it fractions (*n*-Hexane, chloroform, ethylacetate, and *n*-butanol) revealed the presence of saponins, tannins, alkaloids, cardiac glycoside, steroid, triterpenoids, phenols and flavonoids. *G. senegalensis* has demonstrated significant antibacterial activity validating the ethnomedicinal claim for the use of the plant in the treatment of antibacterial infections.

Conflict of Interest

The author declares that there is no conflict of interest.

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