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Phytochemical Analysis, Antioxidant and Antimicrobial Activities of *Guiera senegalensis* Methanol Extract

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ABSTRACT

Medicinal plants are widely used by the populace for their everyday health needs. The presence of phytochemical compounds is believed to be the basis of the observed pharmacological properties of medicinal plants. The aim of this research is to investigate the phytochemical constituents, antioxidants and antimicrobial activities of *Guiera senegalensis* (whole plant) plant. Phytochemical analysis (qualitative and quantitative) was carried out by following standard procedures. Free radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP) were employed to determine the antioxidant activities of the methanol extracts of the plants, while paper disc agar diffusion method was used to determine the antimicrobial activity of the extracts. The result from the phytochemcal analysis revealed that the plant extract contains alkaloids, flavonoids, steroids, saponins, terpenoids, anthraquinones and tannins. Glycosides were not detected. The quantitative analysis results showed that, the plant extracts have appreciable amounts of phytocompounds with the highest value of 286.22 mgGAE/100 g total phenol. Extract showed high antioxidant activity of 84.15 % scavenging activity using DPPH and 13.05 % reducing power with FRAP. Results from the antimicrobial activity showed that, the plants extracts can inhibit bacteria growth of *Escherichia coli, Shigella spp, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus spp, Klebsiella pneumonia* and *Salmonella typhi.* The highest inhibition zone of 43 mm at 500 µg/mL was observed for *Streptococcus spp.* and *Shigella spp*. Extracts exhibited lower zones of inhibition for antifungal activity. Consequently this study lends support to the therapeutic use of *Guiera senegalensis* in traditional medicine.

Keywords: Phytochemicals, Antioxidant, Antimicrobial, *Guiera senegalensis*

INTRODUCTION

Medicinal plants are plants with therapeutic or beneficial pharmacological effects on both humans and animals. Majority of people, about 80 % of the world populations rely on plants derived medicines for their health care (WHO, 2018). In spite of improved healthcare in the United State and Europe, millions of their people are turning back to traditional herbal medicine to prevent or treat many illnesses; also to overcome resistance of many human pathogens to conventional antibiotics that often have side effects (WHO, 2018). Many African plants are used in traditional medicine as antimicrobial or antioxidant agents but only few have been documented. Plants synthesize a wide range of chemicals that are classified into primary or secondary metabolite. Secondary metabolites have some valuable biological properties like antioxidant activity, antimicrobial, anticancer, detoxification, hormone metabolism modulation and so forth. Phytochemicals antioxidants and antimicrobial activities have attracted the attention of plant scientist due to development of new and sophisticated techniques for analysis and the demand for new drugs (Selvakunar and Anoop, 2016)

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Guiera senegalensis is a semi-ever green shrub that grows up to 3-5 m tall with spindly bole or many branched from base. All parts are covered with black glandular dot, bark fibrous more or less smooth to fine scaly gray to brown. Flowers are bisexual, regular petals, and creamy white to yellowish densely short hair. The Plant is widely distributed in West Africa. In Burkina Faso, the gall is used to treat fowl pox and have antiseptic and antifungal activities (Sanago *et al.,* 2012). Preliminary tests on *Guiera senegalensis* suggests its usefulness in traditional medicine. In Sudan, nearly 80, 000 people in Ghubaysh village use the plant as medicine. Random survey was conducted on selected group of people (126), where 66 were male and 60 female with age range between 20-50 years. The study showed that boiled and soaked leaves are used for treating many diseases, while dried roots are used for wound treatment. Common ailment treated by leaves extract are jaundice (51.5 %) and 48.5 % showed it can be used to treat diabetes mellitus, hypertension, cough, arthritis, diarrhea, malaria. *Guiera senegalensis* is also used in cosmetics, animal feed and fuel (Nabaa *et al*., 2016).

Nabaa *et al*. (2016) reported that *Guiera senegalensis* water extracts of the leaves has no inhibition activity against all the tested fungal strains; *Stemphyllum solani, Aspergillus flavus, Trichoderma viride, Penicilliun sp, Fusaruim verticillatum, Cladosporium cladosporioide* and *Fusarium solani*. Similarly, the toxicity test using brine shrimp suggest that the leaves of the plant are apparently not toxic. Phytochemical tests of *G. senegalensis* leaf extract indicated the presence of alkaloid, flavonoid, terpenoid, tannin, carbohydrate, protein, steroid and saponin. The result of this study suggests that the medicinal plant extract may be safe for use in treatment of the various diseases (Nabaa *et al*., 2016).

A study on the antimicrobial and phytochemical analysis on fraction of *Guiera senegalensis* from Alasan Tumbuwal Nigeria was carried out by Simon and Aminu (2016). The leaf sample was extracted using maceration technique with petroleum ether (PE), dichloromethane (DCM) and methanol. The crude extract of pet-ether proved to be active against *Escherichia coli* and *Pseudomonas aeruginosa* with inhibition zones of about 14 mm and 21 mm respectively. DCM extract was only active against *Salmonella typhi* while methanol extract showed no activity against the entire organism under test. Column chromatography fraction of the crude extract from PE and DCM gave 17 and 19 fraction respectively. On the basis their TLC profile, the fractions of PE and DCM were grouped into three.. Second fraction of pet-ether (PE2) showed activity on the entire test organism except *Salmonella typhi*. On the other hand, first fraction (D1) from DCM extract was active against *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. D2 was active on all the microbes whereas D3 was active against *Pseudomonas aeruginosa* and *Salmonella typhi*. The phytochemical tests revealed the presence of steroid, saponins, flavonoid and alkaloid (Simon and Aminu, 2015).

Report by Abdulmalik *et al*. (2019) showed that the methanolic extract of *Guiera senegalensis* revealed the presence of anthraquinones, alkaloids, flavonoids, cardiac glycoside, saponins, steroids/terpenoids, phenolic compounds and tannins but glycosides were not detected. The antioxidant activities of *Senna occidentalis* and *Guiera senegalensis* leaf extracts with DPPH were found to be 43.468-72.564 % and 47.748-66.154 % respectively. The 50 % that is inhibitory concentration (IC_{50}) of *Guiera senegalensis* were found to be 58.55 µg/ml,

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while that of *Senna occidentalis* was 77.565 µg/ml. (Abdulmalik *et al.*, 2019). Several factors are known to affect the therapeutic efficacy of medicinal plants which include but not limited to the age of plant, time of collection, extraction method, geographical location and the general climatic conditions of area. Consequently, there is need to carry out the phytochemical screening, antioxidant and antimicrobial peoperties determination of *Guiera senegalensis* methanol leaf extract from Gombe state, Nigeria.

MATERIALS AND METHODS

Study Area

Guiera senegalensis samples were collected from Billiri local government area of Gombe state and was transported in cleaned polyethene bag and sacks to the Chemistry Department of Gombe State University. It was identified and authenticated by a botanist at Botany Department of Gombe State University. It was air-dried at room temperature.. The sample was pulverized into powdered form using mortar and pestle.

Extraction using Maceration Method

The powder plant samples was weighed (500 g) and soaked in methanol for a week with occasional shaking to facilitate the extraction of phytocontituents. It was decanted and filtered using No.1 whatman filter paper and then concentrated under reduced pressure with rotatory evaporator until a semi solid residue was obtained.

Qualitative Phytochemical Screening

The qualitative phytochemical tests were carried out according to well established protocols. Wagners test was used for the presence of alkaloids (Musa *et al*., 2018). Similarly, froth test was used for Saponins (Musa *et al*., 2018). The formation of blue black precipitate using 1% $FeCl_3$ was used as a test for tannins (Trease and Evans, 2002). Test for flavonoids was carried out according to the method reported by Kwaji *et al.* (2018). Salkowski's method was used for the presence of terpenoids (Sonali *et al.,* 2015), anthraquinones (Musa *et al*., 2018) and Glycosides (Sonali *et al.*, 2015).

Quantitative Phytochemical Determination

Estimation of total alkaloids

Crude extract (10 mg) was weighed and 20 ml of methanol: ammonia (68:2) was added. After 24 hrs, the ammoniacal solution was decanted. The procedure was repeated thrice to obtain maximal extracts. The extracts were concentrated and then treated with 1 M HCl and kept over-night. The acidic solution was extracted with 20 ml of $CHCl_3$ thrice. The organic layers were then pooled and evaporated to dryness after which it was basified with NaOH solution to pH-12 and then extracted with 20 ml $CHCl_3$ again. The acid layer was evaporated and dried over absorbent cotton. The fraction was weighed and expressed as mg/100 g Atropine sulfate.

Estimation of total flavonoids

The method is based on the formation of the flavonoid - aluminium complex which has an absorption maximum (λ_{max}) at 415nm. 100 µl of the sample extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % $AlCl_3$ in methanol and a drop of acetic acid, and then diluted with methanol to 5 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 100 ml of sample extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard quercetin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicate.

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Estimation of total saponin content

About 2 mg of extract was weighed in a 250 ml beaker and 100 ml of isobutyl alcohol was added. The mixture was agitated using orbital shaker for 5 hours to ensure uniform mixture. It was then filtered with Whatman No.1 filter paper into a 100 ml beaker containing 20 ml of 40 % saturated solution of $MgCO_3$. The mixture obtained was filtered again using Whatman No.1 filter paper. The filtrate (1 ml) was taken in a 150 ml volumetric flask using pipette and 2 ml of 5 % iron (III) chloride ($FeCl_3$) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min for color to develop. The absorbance was read against the blank at 380 mm.

Estimation of total phenols

A 0.5 g sample was homogenized in 10x volume of 80 % ethanol and centrifuged at 6000 rpm for 20 min. The procedure was repeated twice. The supernatants were combined and evaporated to dryness. The residue was then dissolved in 20 ml of distilled water. Several aliquots were pipetted out and the volume in each test tube was made up to 3 ml with distilled water. Folin-ciocalteau reagent (0.5 ml) was added and equal volume of $NaCO_3$. The tubes were placed in a boiling water bath for exactly one minute. The test tubes were cooled and the absorbances were read at 600 nm in a spectrophotometer against a blank. A standard gallic acid solution (0.2- 1 ml) with concentration range of 2.0 – 10 µg/mL were also treated as above and the standard gallic acid graph was plotted (Oladummoe *et al*., 2016).

Estimation of steroids

0.1ml and 0.2 ml of triple acid extract was taken and a set of standards (0.5 to 2.5 ml) were taken and made up to 5 ml with ferric chloride diluting reagent. A blank was prepared simultaneously by taking 5.0 ml diluting reagent. Then add 4.0 ml of concentrated sulphuric acid to each tube. After 30 minutes' incubation, intensity of the colour developed was read at 540 nm. Standard calibration curve was obtained from various diluted concentrations of cholesterol expressed as mg/100 g.

Estimation of tannin

Weigh 0.5 g of the powdered sample and transfer to 250ml conical flask. Add 75 ml of water. Heat the flask gently and boil for 30 mins. Centrifuge at 2000 rpm for 20 mins and collect the supernatant in 100 ml volumetric flask and make up the volume. Transfer 1ml of the sample extract to 100 ml volumetric flask containing 75ml water. Add 5ml of Folin-Denis reagent, 10 ml of sodium carbonate solution and dilute to 100ml with water. Shake well. Read the absorbance at 700 nm after 30 mins. Prepare a standard graph using 0-100 µg tannic acid.

Antioxidant Activity

Free Radical Scavenging Activity

The determination of the free radical scavenging activity of the crude extract was carried out using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) as described by Kwaji *et al*. (2018) with slight modification. Various concentrations of 500, 400, 300, 200 and 100 µg/ml were prepared. 0.1 ml of DPPH was added to 2.5 ml solution of the extract or standard and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow will then be measured at 518 nm on a spectrophotometer (CECIL CE7400). The decrease in absorbance is then converted to percentage antioxidant activity (AA %) using the following formula:

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% AA= 100 {[(AbS $_{sample}$ AbS $_{blank}$)/ AbS $_{control}$ x100

Blank= methanol (1.0 ml) +sample solution (2.0 ml)

Negative control= DPPH (1.0 ml, 0.25ml) + Methanol (2.0 ml)

Positive control= Ascorbic acid was use as standard

The scavenging reaction between (DPPH) and an antioxidant (H-A) will be written as;

DPPH + H-A DPPH-H + (A)

Purple light yellow

Ferric reducing antioxidant power (FRAP)

Various concentrations of 500, 400, 300, 200 and 100 µg/ml of the crude extract or standard was prepared. 0.1 ml of each concentration was taken in test tubes then 2.5ml of phosphate buffer (0.2 M) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1 %) solutions were added into the test tubes. The reaction mixture was incubated for 20 minutes at 50^0 C to complete the reaction. 2.5 ml of trichloro acetic acid (10 %) solution was added into the tubes. The total mixture was centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant solution was withdrawn from the mixture and mixed with 2.5 ml of distilled water. 0.5 ml of ferric chloride ($FeCl_3$), (0.1 %) solution was added to the diluted reaction mixture, then the absorbance of the solution was measured at 700 nm using spectrophotometer against blank (blank contain the same solution without extracts). The graph of the absorbance against sample concentration was plotted and the reducing power was calculated.

Antimicrobial Activity

Antibacterial activity/Antifungal activity

Paper disc diffusion method was adopted for antimicrobial susceptibility test. Clinical isolates: *Escherichia coli, Shigellia spp, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus Klebsiella pneumonia* and *Salmonella typhi,* and five fungi; *Aspergillus flulaccus, Aspergillus niger, Aspergillus fumigatus, Candida albicans and Rhizopus oryza* were incubated in Mueller Hinton agar for bacteria and potato dextrose agar for fungi at 37^0C for 24 hours and 48 hours respectively. The cultures were standardized to McFarland turbidity standards. About 25 ml of sterile Mueller Hinton agar was poured into 100 ml of petri dish and was allowed to solidify. The microorganisms prepared in McFarland standard was smeared unto agar surfaces using sterile swap stick. Punched sterile filter disc was immersed into the plants extracts of different concentration (500 ug/ml, 400, 300, 200 and 100) and placed on the agar surfaces together with standard commercially manufactured disc of ofloxicin 5ug/ml. The plates were incubated at 37^0C for 24 hours and 48 hours for bacteria and fungi respectively. After which the zone for bacteria and fungi growth inhibition were examined and measured in millimeters (mm). (Kwaji *et al*., 2018).

Determination of MIC, MBC and MFC

The minimum inhibitory concentration (MIC) of the crude extracts of the plants was estimated for each of the test organism in duplicates. To 1 ml of various concentration of the extracts (500, 250, 125 and 65), 2 ml of nutrient broth agar for bacteria and potato dextrose agar for fungi was added and then 1 ml of test organisms previously diluted to 0.5 McFarland turbidity standard for bacterial isolate and 10^6 cfu/ml for fungi isolates were introduced into the sterile test tubes. A test

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tube containing only nutrient broth and another containing nutrient broth with test organism to serve as control was introduced. Test tubes containing bacterial cultures were then incubated at 37^0C for 18-24 hours while those containing fungal spores' culture were incubated at 48 hours at room temperature. The tests tubes were then examined for microbial growth by observing the turbidity of each tests tube.

To determine the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC), for each set of tests tubes in the MIC determination from those which did not show any turbidity indicating no microbial growth, about 0.5 ml of the broth was collected and inoculated on sterile nutrient agar and potato dextrose agar for bacteria and fungi organisms respectively. These were then incubated at 37^0C for 18-24 hours while those containing fungal spores' culture were incubated at 48 hours at room temperature. After incubation the concentration at which no visible growth of microbial was seen was noted as the MBC and MFC (Kwaji *et al*., 2018)

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RESULTS AND DISCUSSION

Qualitative Phytochemical Screening Results

Table 1 shows the qualitative phytochemical screening of the methanol extract of *Guiera senegalensis* (whole plant). The result revealed the presence of alkaloids, flavonoids, tannins, steroids, saponins, anthraquinones, glycosides and terpenoids. This fairly agrees with the one reported by Nabaa *et.al*, (2016) and Abdulmalik *et al.* (2019). Phytochemical constituents such as alkaloids, flavonoids, tannins, steroids, saponins, anthraquinones, glycosides, terpenoids and others are secondary metabolites of plants that serve as defense mechanism to humans and animals hence, this explain the demonstration of the medicinal properties of the plants and its pharmacological activities such as anti-inflammatory, antioxidant, antimicrobial, analgesic. (Ogbeba*et al*., 2017)

Table 1: Qualitative Phytochemicals of *Guirea sensgalensis* Methanolic Extract.

Secondary Metabolites	**G.S.M.E**	**Reported literature** (Nabaa *et.al*., 2016)	**Reported Literature** (Ogbeba*et al*., 2017)
Alkaloids	++	+	-
Flavanoids	++	+	+
Tannins	++	+	+
Steroids	++	+	+
Saponins	++	+	+
Antraquinones	+		+
Glycosides	++		-
Terpenoid	+	+	+

Key: + means presence of phytochemical - means not detected.

G.S.M. E= *Guirea senegalensis* Methanol Extract

Quantitative Phytochemical Analysis Results

Quantitative phytochemical composition reveals that the plants contain a reasonable amount of secondary metabolite that were screened in this research. Total phenols was found to be much higher with value of 286.22 mg/100 g of Gallic acid equivalent. Oxalate and alkaloids were found to have the least concentration of 19.31 mg/100 g gravimetric

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and 25.73 mg/100 g of atropine sulfate in the methanol extract (Table 2).

Table 2: Quantitative Analysis of *Guiera senegalensis* Methanolic Extract.

Parameters	G.S.M.E
Steroids (mg/100g Cholesterol eqv.)	182.3
Saponins (mg/100g Gravimetric)	271.0
Tannins (mg/100g Tannic acid eqv)	252.77
Phytates (mg/100g Phytic acid eqv)	82.37
T. Phenols (mg/100g Gallic acid eqv)	286.22
Oxalate (mg/100g Gravimetric)	19.31
Alkaloids (mg/100g Atropine sulfate eqv)	74.86
Flavonoids (mg/100g Quercetin eqv)	160.25

Key: GSM-*Guiera senegalensis* methanol extract.

Antioxidant Activity

Free Radical Scavenging Activity

Antioxidants compounds have the ability to neutralize any free radicals. DPPH assay is based on the measurement of the loss protons after the reaction with the sample extracts. Table 3 shows the results recorded from the free radical scavenging activity of methanol extracts of *Guiera senegalensis* whole plant. The antioxidant activity of the methanolic extracts of *Guiera senegalensis* showed significant potential. High value of 84.15 % at 500 µg/mL was recorded. Interestingly this is relatively comparable with the standard ascorbic acid (87.92 %) at the same concentration of *Guiera senegalensis* (Table 3). The percentage of free radical inhibition of the sample is concentration dependent that is increase in sample concentration is directly proportional to increase in the activity. The IC_{50} value is a parameter widely used to measure the concentration at which the initial concentration of the sample will decrease by 50 %. This goes for IC_{90} as well. thus, the low value of IC_{50} or IC_{90} means the sample have high antioxidant activity. In this research the IC_{50} (168.074 µM) and IC_{90} (457.719 µM) o*f Guirea senegalensis* were relatively low indicating the high activity of *Guirea senegalensis* extract. This may be as a result of high total phenolic content recorded in the quantitative phytochemical analysis since it is the major plant metabolite responsible for free radical scavenging ability. The result partially agree with the one reported by Abdulmalik *et al.* (2019).

Table 3: Free Radical Scavenging Activity (DPPH) of *Guiera senegalensis* Methanolic Extract..

Concentration µg/ml	AGS	Scavenging Activity% of AGS	Absorbance of control A_0	Scavenging activity% of Control
100	0.104	60.75	0.265	72.07
200	0.086	67.54	0.265	73.96

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300	0.061	76.98	0.265	80.75
400	0.057	78.49	0.265	82.26
500	0.042	84.15	0.265	87.92
	Standard		**GSME**	
IC_{50} µM	131.394		168.074	
IC_{90} µM	424.844		457.719	

Key: GSME- *Guiera senegalensis* methanol extract

Ferric Reducing Antioxidant Power (FRAP)

Table 4 shows the ferric reducing antioxidant power of methanolic extract *Guiera senegalensi.* FRAP assay measured the ability of the sample antioxidant component to reduce iron (+3) Fe^{3+} to iron (+2) Fe^{2+}. The results ranged from 4.45-13.05 µg/ml in *Guiera* nt.

senegalensis. The highest value was (13.05 µg/ml). The reducing power of the plants extracts were relatively low compare to the standard, which ranged from 20.25-50.93 µg/ml at 10-50 µg/ml concentration. In addition, the reducing power is concentration depende

Table 4: Ferric Reducing Antioxidant Power (FRAP) *Guiera senegalensis* Plant Methanolic Extract.

Conc. µg/ml	**Average absorbance GSME**	**Reducing power GSME**	**Conc. SAS** µg/ml	**Average absorbance SAS**	**Reducing power SAS**
100	0.1052	4.45	10	0.478	20.25
200	0.1833	7.76	20	0.569	24.11
300	0.2062	8.73	30	0.655	27.75
400	0.2942	12.46	40	0.849	35.97
500	0.3081	13.05	50	1.202	50.93

Key: GSME- *Guiera senegalensis* methanol extracts and SAS- Standard ascorbic acid

CONCLUSION

The study had shown the presence of alkaloids, flavonoids, steroids, saponins, glycosides and terpenoids in the *Guiera senegalensis* crude extract similar to what is obtainable elsewhere. Quantitative phytochemical analysis revealed that, the plants contain a reasonable amount of secondary metabolite. Total phenols was found to be approximately 286.22 mg/100 g of Gallic acid equivalent. Oxalate and alkaloids were found to have the least concentration of 19.31 mg/100g sample. *Guiera senegalensis* extract antioxidant activity at 500µg/ml was comparable to that of the standard ascorbic acid. This suggests that *Guiera senegalensis* may be a potential source of antioxidant compounds. The study also showed that the plant can inhibit the growth of some bacteria and fungi. These results lends credence to the use of the *Guiera senegalensis* for treatment of some diseases in traditional medicine.

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