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Pytochemical Screening, Antimicrobial Activity and Cytotoxicity Effects of Extract of *Guiera senegalensis* Leaves

***Momoh, H., Olaleye, A. A., Sadiq, I. S. and Mohammad, A.**
Department of Chemistry, Federal University Dutse, Jigawa State, Nigeria
***Correspondence Email:** momohhajara@yahoo.com

ABSTRACT

The leaves of *Guiera senegalensis* extracts were extracted using solvents of increasing polarity in order to investigate preliminary phytochemical constituents, antimicrobial activity and cytotoxic effect of the crude extracts. The results of the phytochemical screening indicated the presence of cardiac glycosides, saponins, flavonoids, tannins, steroids, terpenoids and alkaloids. While the antimicrobial activity of the extracts against ten pathogenic organisms revealed moderate to good inhibition of six of the ten bacterial and fungal species. Generally, the ethyl acetate extract appeared to have the highest zone of inhibition (24 mm) and best minimum inhibitory concentration (2.5mg/ml) against *Staphylococcus aureus*. The cytotoxicity effects revealed that the chloroform extracts exhibited the highest lethality on brine shrimp larvae at LC_{50} value of 58.908μg/ml.

Keywords: Antimicrobial activity, Crude extracts, Cytotoxicity, *Guiera senegalensis*

INTRODUCTION

Herbal therapy has been used over generations to manage diseases ranging from fever, cough, abdominal pains, conjunctivitis, diarrhoea, sexually transmitted infections and malaria (Banfi *et al.*, 2014; Momoh and Idris, 2014a). These herbal preparations are normally administered as aqueous concoctions of the plant barks, leaves or roots. (Van Vuuren and Naidoo, 2010). The aforementioned disease conditions are due to infection by pathogenic microorganisms such as bacteria, protozoa and fungi. Unfortunately, these infections especially in tropical and subtropical developing countries have been reported to be increasing at an alarming rate. (Fenner *et al.*, 2005; Momoh *et al*, 2017). This could be as a result of indiscriminate use of anti-microbial drugs which lead to the emergence of microbial resistance to some of the synthetic drugs (Momoh *et al.,* 2015). Considering that microorganisms are gradually developing resistance to the existing drugs, coupled with the high cost and unfavourable side effects of some of those drugs necessitates the need for the development of new affordable, effective antimicrobial agents with less side effect (Momoh *et al.*, 2017). Therefore, it becomes favourable to focus on medicinal plants to harness their potentialities. (Momoh *et al.*, 2017).

Guiera senegalensis locally known as "*Sabara*" or "*Barbarta*" in Hausa Language of Northern Nigeria belongs to the family combretaceae, It is widely distributed in the savannah region of west and central Africa (Sombie *et al.,* 2011). *G. senegalensis* has been reported to have numerous traditional medicinal uses such as treatment of dysentery, diarrhea and malaria /fever (Jigam *et al.*, 2011). It has also been reported to be used to manage leprosy (Jigam *et al.*, 2011), abdominal pains, epilepsy (Somboro *et al.*, 2011), depression (Maria *et al.*, 2012), cold and cough, snake bite (Shatima *et al.*, 2012) and eczema (Somboro *et al.*, 2011), syphilis (Jigam *et al.*, 2011), hypertension, hypotension and diabetes (Shatima *et al.*, 2012), breast cancer and Jaundice (Fadimu *et al.*, 2014).

In this study, we reported the phytochemical screening, antimicrobial activity, and cytotoxicity of the leaf extracts of *Guiera senegalensis* against some selected microorganism strains.

MATERIALS AND METHODS

Collection and identification of plant materials

The leaves of *G. Senegalensis* were collected from Federal University Dutse, Nigeria. It was authenticated at the herbarium unit, Biological Sciences Department, Bayero University Kano-Nigeria with a specimen voucher number. BUKHAN32 *G. senegalensis.* Theleaves were air dried, pulverized using wooden motor and pestle and stored until extraction.

Extraction of plant materials

The air dried powdered leaves (270g) was soaked in a 2 litre conical flask containing 95% methanol (1.5L) stoppered and kept for one week. The mixture was filtered and the filtrate was concentrated using rotary evaporator. To the concentrated methanol extracts was added warm water and stirred, the water soluble portion was decanted, this was repeated severally for exhaustive

extraction, hexane was then added to the water soluble portion and mixed thoroughly in a seperatory funnel and allowed to settle after which the lower layer was gently drained and the upper layer was collected, concentrated, dried and labelled as the hexane fraction. To the remaining water soluble portion the same procedure was repeated using chloroform then ethyl acetate, each fraction was concentrated, dried, weighed and labelled.

Phytochemical screening

A portion of each of the four extracts was subjected to preliminary phytochemical screening using standard methods described below.

Test for saponins

About 0.1g of the extract was shaken with water in a test tube. Frothing was observed which persisted for 1 minute which indicated the presence of saponins. (Silva *et al*., 1998).

Test for glycoside

Little quantity of the extract, 5ml of conc. H_2SO_4 was added and boiled for 15 min. This was then cooled and neutralized with 20% KOH. Three drops of ferric chloride solution was added to one of the portions, and a green to black precipitate observed indicated phenolic aglycone as a result of hydrolysis of glycoside.(Cannel, 2000).

Test for flavonoids

***Sulphuric acid test*:** Little quantity of the extract was dissolved in 1ml concentrated sulfuric acid and a colour changed was observed which indicated the presence of flavonoids (Silva *et al.,* 1998).

Lead Acetate Test: A small quantity of the extract was dissolved in water and filtered. Few drops of 10 % lead acetate was added to 5mls of the filtrate. A buff coloured precipitate observed indicate the presence of flavonoids.(Cannel, 2000).

***Sodium Hydroxide test*:** 2ml of the extract was dissolved in 10% aqueous sodium hydroxide solution and filtered to give yellow colour, a change in colour from yellow to colourless on addition of dilute HCl was observed which indicated the presence of flavonoids (Cannel, 2000).

Test for Alkaloids:-Small quantity of the extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath and filtered. 2ml of the filtrate was divided into two portions. To the first portion 1 drop of Mayer's reagent was added and yellow color precipitate observed. To the second portion 1 drop of Wagner's reagent was observed to give a reddish- brown precipitate (Silva *et al*., 1998).

Test for Tannins:-A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride was added to the filtrate, formation of green precipitate was observed which indicated the presence of tannins (Trease and Evans, 1996).

Test for Anthraquinones:-Small quantity of the extract was shaken with 10 ml of benzene, the content was filtered, and 5ml of 10% ammonia solution was added to the filtrate, the mixture was shaken. No colour change was observed in the ammoniacal layer (Lower phase) which indicated presence of free anthraquinone (Cannel, 2000).

Test for Steroids

Little quantity of the extract was dissolved in 1ml chloroform and 1ml of concentrated sulfuric acid was added down the test tube to form two phases. Formation of yellow coloration was taken as an indication for the presence of steroids (Silva *et al.*, 1998).

Antimicrobial Assay

The antimicrobial activity of the ethyl acetate, chloroform, methanol and n-hexane of *Guiera senegalensis* plant extract was determined against ten pathogenic microbes obtained from the Department of Microbiology, Federal University Dutse.

Preparation of the Extracts

0.2g of the extract was weighed and dissolved in 10mls of DMSO to obtain a concentration of 20 mg/ml. This was the concentration of the extract used to determine the antimicrobial activity of the plant. Following the standard procedure of the diffusion method, Mueller-Hinton agar was prepared as the media for the growth of the microbes according to manufacture instruction. After sterilization at 121°C for 15min, the media was poured into sterile petri dishes, cooled and allowed to solidify. Pure cultures of the bacterial organisms were inoculated on to Mueller Hinton Agar and incubated for 24h at 38^{0}C. About 5 discrete colonies were asceptically transferred using sterile wire loops into tubes containing sterile normal saline (0.85% NaCl) and were adjusted to a turbidity of 0.5 MacFarland Standard. Thesuspensions were then inoculated on the surface of sterile Mueller –Hinton Agar plates using sterile swabs. A sterile 6 mm diameter cork borer was used to make holes (wells) into the set of inoculated Mueller-Hinton Agar. The wells were filled with 0.1ml of solution of the extract of the concentration of 20mg/ml. The plates were incubated for 24h at 38^{0}C, while the fungi were incubated at 34^{0}C for 48h after which the medium was observed for zone of inhibition of growth, the zone was measured with a transparent ruler and result was recorded in millimetres (Momoh *et al,* 2017).

Minimum Inhibition Concentration

The minimum inhibition concentration of the extract was determined using the broth dilution

method. Mueller Hinton broth was prepared 10mL was dispensed in to test tube and the broth was sterilized at 121°C for 15mins, the broth was allowed to cool. Mc- farland's turbidity standard scale number 0.5 was prepared to give turbid solution.

Normal saline was prepared 10ml was dispensed in to the sterile test tube and the test microbe was incubated at 37°C for 6hrs.Dilution of the test microbe was done in the normal saline until the turbidity reached that of the Mc-forland's scale by visual comparison at this point the test microbe has a concentration of about 1.5 x 10^8cfµ/ml. Two fold serial dilution of extract was done in to sterile broth to obtain the concentration of 20mg/ml, 10mg/ml, 5mg/ml 2.5mg/ml. the initial concentration was obtained by dissolving 0.2g of the extract in 10ml of sterile broth. Having obtained the different concentration of the extract in the sterile broth 0.1ml of the microbe in the normal saline was then introduced in to the different concentration. Incubation was made at 37℃ for 24hrs. Minimum inhibition concentrations were recorded as the lowest concentration of extract in the broth which showed no turbidity (Momoh *et al*., 2017b).

Minimum Bactericidal Concentration and Minimum Fungicidal Concentration

Mueller-Hinton agar was prepared, sterilized at 121ºC for 15mins, poured into sterile petri dish and was allowed to cool and solidify. The content of the Minimum Bactericidal Concentration (MIC) in the serial dilution were then sub cultured on to the prepared medium , incubation was made at 37°C for 24hrs, after which the plates were observed for colony growth, Minimum Bactericidal Concentration (MIC) / Minimum Fungicidal Concentration (MFC) were the plates with lowest concentration without colony growth (Momoh *et al*, 2017b).

Brine shrimp Lethality Test

16g of *Artemiasalina* eggs containing sea salt were dissolved in 500 ml of distilled water as instructed in the container label. The shrimps were then allowed to hatch and mature for 48h at room temperature. Clean test-tubes were labelled for each concentration of plant extract and 5ml volume was marked with a masking tape.20mg of each test extract was seperately dissolved in 2ml methanol from which 500µl, 50µand 5µl of each solution was transferred into vials corresponding to 1000, 100 and 10µg/ml respectively. Potassium dichromate was use as the positive control which was dissolved in distilled water. The vials (9 per test fraction) and one control were allowed to evaporate to dryness at room temperature overnight. About 50µl of DMSO was added to each test-tube per 5ml of sea water. When the brine shrimp larvae (nauplii) have matured, they were separated into different beakers of sea water using Pasteur pipette. 1ml of sea water was added to each test tube before DMSO toxicity affects their suits. 10 shrimps per test-tube were added with the help of Pasteur pipette (30 shrimps per dilution) and the final volume in each test tube was adjusted by sea water to the 5ml mark. After 24hrs the number of surviving shrimps were counted with the aid of a magnifying glass and recorded. The data was analysed using probit analysis to determine LC_{50} values and 95% confidence intervals by a software package IBM SPSS (Momoh et al. 2017a; Hamidi *et al.,* 2014).

RESULTS AND DISCUSSION

Extraction of 270 g of the dried leaves of *G. senegalensis* with a sequence of solvents in increasing order of polarity yielded comparable amount of the crude extract indicated in Table 1.The Table showed that generally the more polar solvent indicated that the more polar solvent gave the highest percentage yield, which signified that the extract contains more polar secondary metabolite such as alkaloid, phenolic compounds, cardiac glycoside and saponins.

The phytochemical screening of the extracts of *Guiera senegalensis* indicated the presence of various classes of secondary metabolites as shown in Table 2. The methanol extract showed the presence of cardiac glycosides, tannins, steroids, terpinoids, saponins, flavonoids and alkaloid. While the n-hexane extract showed the presence of steroids, terpenoids, alkaloids and tannins, the chloroform extract revealed the presence of saponins, alkaloids, flavonoids, cardiac glycosides, saponins, tannins, steroids. While the ethyl acetate extract revealed the presence of tannins, saponins, steroids, and terpenoids. Therefore the phytochemical screening of the methanol, n-hexane, chloroform and ethyl acetate extract showed the presence of most secondary metabolites tested. This study corroborates the work of Ifigen *et al.* (2019) who reported similar result except that the cardiac glycoside was absent in his report. The presence of a good number of vital secondary metabolite could be linked to the its effectiveness in treating several diseases (Bieu *et al.*, 2016).

Antimicrobial activity of the extract was tested against seven tester bacterial strains namely *Shigella dysentriae, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogens, klebsiella pneumonia, Escherichia coli, and Salmonella typhi* and two fungal strains *candida albican and Candida tropicalis*. The activity of the extracts (Table 3) revealed good zones of inhibition against five out of the seven tested bacterial. It was observed that the extracts were active against *Candida albican, Shigella dysentriae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhi* whereas none of the extracts was active against *Streptococcus pyogens,*

klebsiella pneumonia, Candida tropicalis, and *rhizopus* (Table 3). Generally, the results showed that the zones of inhibition of the extracts against the tested organism ranged from 16-19 mm (n-hexane), 20-23 mm (chloroform), 22-26 mm (ethyl acetate) and 20-22 mm (methanol). The result of the minimum inhibitory concentration (MIC) shows that the ethyl acetate extract inhibited the growth of all tested microorganisms at concentration (2.5-5mg/mL), whereby the higher MIC value was observed for then-hexane extract at 10mg/ml against all the tested organisms (Table 4). The chloroform extracts inhibited the growth of all tested organisms at concentration 5mg/ml and methanol extract showed MIC value range (5-10mg/ml).The result of the Minimum Bactericidal Concentration(MBC and Minimum Fungicidal Concentration (MFC) of the chloroform, n-hexane, ethyl acetate and methanol extracts as shown in Table 5 indicated that the MBC/MFC for the n-hexane extract,was20mg/ml against all the test organisms and chloroform extract was10mg/ml; but for the ethyl acetate extract, it was found to be 10mg/ml against *Pseudomonas aeruginosa, Escherichia coli, S. typhi* and *C. albicans* and5mg/ml against *S. aureus* and *S. dysenteriae,* The MBC/MFC of methanolic extract was10mg/ml against *S. typhi* and *S. dysentriae* and 20mg/ml against, *Pseudomonas aeruginosa, Escherichia coli, C. albicans and S. aureus*.

The result of cytotoxicity test (Table 6) as obtained from the brine shrimp lethalty test of the extracts revealed that the plant has varying degree of activity against the brine shrimp larvae (nauplii). It was found that the chloroform extracts gave the highest activity LC_{50} value 58.908µg/ml followed by 128.311µg/ml for the hexane extract, 823.0µg/ml for the ethyl acetate extract. The methanol extract was found to be inactive (LC50 value = 1000µg/ml). These range of LC50 values is in agreement with Adoum,2009 who reported similar ranges with LC50 values of less than 60 µg/ml as remarkably active and those near and above 1000 µg/ml as inactive.

Table 1: Yield of Crude Extract of *G. senegalensis* Leaves

S/N	Samples	Weight (g)	Solvent	Weight of crude extract (g)	% recovery yield
1	*Gueira senegalensis* (leaves)	270	n-hexane	6.6	2.444
			Chloroform	5.2	1.925
.			Ethyl acetate	2.3	0.851
			Methanol	45.6	16.851

Table 2: Phytochemicals screening of extractsof *G. semegalensis*leaves

Extract	HE	CM	EA	ME
Alkaloids	+	+	-	+
Tanins	-	-	+	+
Saponins	-	+	+	+
Terpenoid	-	-	+	+
Flavonoid	-	+	-	+
Cardiac glucoside	+	+	-	+
Steroids	-	-	+	+

KEY: + =Present, - = Absent, HE = Hexane extract, CM = Chloroform extract, EA= Ethyl acetate extract, ME = Methanol.

Table 3: Zone of Inhibition of extracts of *G. semegalensis*leaves and Standard Drugs (mm).

TEST ORGANISMs	HE	CM	EA	ME	CPX	FCZ
Streptococcus pyogens	0	0	0	0	30	0
Staphylococcus aureus	18	22	24	21	33	0
Psedomanasaeriginosa	18	21	23	20	32	0
Shigelladysentriae	17	21	23	22	38	0
Salmonella typhi	16	20	22	21	40	0
Escheriria coli	18	23	24	21	33	0
Klebsiella pneumonia	0	0	0	0	37	0
Candida albicans	19	21	26	20	0	34
Candida tropicalis	0	0	0	0	0	30

KEYS: 0= no activity, HE= Hexane extract, CM = Chloroform extract, EA = Ethyl acetate extract, ME = Methanol extract, CPX = Ciprofloxacin, FCZ = Fluconazole.

Table 4: Minimum Inhibitory Concentration of extracts of *G.semegalensis*leaves (mg/ml)

Test organisms	Hexane	Chloroform	Ethyl acetate	Methanol
Shigella dysentriae	10	5	2.5	5
Staphylococcus aureus	10	5	2.5	5
Psedomanas aeriginosa	10	5	5	10
Escheriria coli	10	5	5	10
Salmonella typhi	10	5	5	5
Candida albicans	10	5	5	10

Table 5: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of extracts of *G. semegalensis* leaves (mg /ml.)

Test organisms	Hexane	Chloroform	Ethyl acetate	Methanol
Shigelladysentriae	20	10	5	10
Staphylococcus aureus	20	10	5	20
Psedomanasaeriginosa	20	10	10	20
Escheriria coli	20	10	10	20
Salmonella typhi	20	10	10	10
Candida albicans	20	10	10	20

Table 6: Brine shrimp lethalty testextracts of *G. semegalensis* leaves

Extracts	LC_{50}
Hexane	128.311
Chloroform	58.908
Ethyl acetate	823.0
Methanol	>1000

CONCLUSION

The extracts of *Guiera senegalensis* were found to contain secondary metabolites such as, cardiac glycoside, saponins, flavonoids, tannins, steroid, terpenoid and alkaloids. Similarly, when tested against ten clinically important microbes, the extracts were found to be active on most of the microorganisms. The presence of those secondary metabolites could be responsible for the antimicrobial and cytotoxic effects observed in this work. Moreover, the findings here support the traditional uses of *G. senegalensis* parts for the management of various infectious disease caused by the tested microbes. The results form the basis for further work on the isolation of the possible bioactive constituent of this plant.

REFERENCES

Adoum, O. A. (2009) Determination of toxicity levels of some savannah plants using brine shrimp lethality test. *Bayero Journal of Pure and Applied Sciences*, 2(1):135-138

Alshafei, N.K. Ahmed, S.M..Nour, (2016)A Preliminary Observations on the Uses of *Gueira Senegalensis* as a Traditional Medicinal Plants in Western Kordufan, Sudan International *Journal of Applied and Pure Science and Agriculture*. 2 42-48.

Banfi, S., Caruso, E., Orlandi, V., Barbieri, P., Cavallari, S., Vigano, P., . . Chiodaroli, L. J. (2014). Antibacterial activity of leaf extracts from *Combretum micranthum* and *Guiera senegalensis* (Combretaceae). *9*(2), 66.

Cannel, R. J. P. (2000). Methods in Biotechnology. Natural Products Isolation. Humanpres, New Jersy.14-36.

Cushnie, T. P. T and Lamb, A. J. (2005.)``Antimicrobial activity of flavonoids," *International Journal of Antimicrobial Agents,* vol. 26, no. 5, pp. 343-356,

Fadimu, Y.O., Mohammed, I. and Zurmi, S. (2014). Ethnomedicinal survey of anti-typhoid plants in Ijebu Ode Local Government Area of Ogun State,Nigeria. *International Journal of Science and Nature* 2: 332-336.

Fenner R, Sortino M, Rates SK, Dall'Agnol R, Ferraz A, Bernardi AP, Albring D, Nör C, Von Poser G, Schapoval E, Zacchino S. Antifungal activity of some Brazilian Hypericum species. Phytomedicine. 2005 Mar 22; 12(3):236-40.

Harborne, J.B. and baxter, H., (1993). *Phytochemical Dictionary:* A *Handbook of Bioactive Compounds from plants.* Taylor and Francis, London. 254-263.

Ifigen, H.I,Mamza A.U, Fasina, K.A,Omoruyi, J.I., IKHUORIA, E.U (2019) Phytochemical analysis of *Guiera senegalensis* J.F. Gmel extract and its antiplasmodial properties on wister albino mice via oral route. International journal of pharmacology, phytochemistry and ethnomedicine 13 35-44.

Momoh, H. and Idris, M.M.(2014a).Anti-plasmodium falciparum activity of extracts from *Dendranthema indicum. Bayero Journal of Pure and Applied Sciences* 7(1): 147-151.

Momoh, H, Idris M.M and Adoum, O. A. (2014b) Evaluation of the antimicrobial and cytotoxic effect of *Dendranthema indicum* (L Desmoul) leave extract IJRDO-Journal of Applied Science Volume-3 (5): 1-8.

Momoh, H., Habila, J. D., Ayo, R. G. and Ndukwe, G. I. (2015a). Clerodendrum Capitatum (Willd) leave extract inhibits bacteria, fungi and *mycobacterium bovis. Scholars Academic Journal of Biosciences* (SAJB), 3(4):386-391.

Momoh, H., Idris, M. M., and Adoum, O. A. (2017a) Evaluation of the antimicrobial and cytotoxic effect of *Dendranthema indicum* (L. Desmoul) leave extract. *IJRDO-Journal of Applied Science*, 3(5):1-8.

Momoh, H., Dambata., M.B and Tahir, M.T (2017b). Phytochemical studies, antimicrobial and antituberculosis evaluation of extracts from the stem bark of *Psorospermum senegalense* (Spach) *World journal of biology and medical science,* 4(2): 32-38.

Momoh, H., Dambata, M. B., and Yau, D. (2017c).Phytochemical Analysis and Antimicrobial Screening of *Psorospermum senegalense* Leaves Extract. *Scholars Academic Journal of Biosciences* (SAJB), 5(6):459-464.

Shettima, Y.A ., Karumi, Y., Sodipo, O.A. (2012). Phytochemical and Antidiarrhoeal Properties of Methanol root Extract of *Guiera senegalensis* J.F .Gmel. *International Research Journal of Pharmacy 11(1): 3-6.*

Silva, G.L., Lee, I. and Douglas, K.A., (1998). Special Problems with Extraction of Plants: Cannel, J.P.R. (ed.). Natural Products Isolation. Humana press publishers, New Jersey (USA). Pp 356-358.

Sombie, P., Hilou ,A., Conulibaly, A..Y, Tibiri, A., Kiendrebeogo, M., and Nacoulma, O.G. (2011). Brain protective and erythrocytes hemolysis inhibition potentials from galls *of Guiera senegalensis* JF Gmel *(Combretaceae*). *Journal of Pharmacology and Toxicology* 4(1): 361-370.

Somboro, A.A., Patel, K., Diallo, D., Sidibe, L., Chalchat, J., Figueredo, G.,Ducki .S., Troin, Y., and Chalard, P. (2011). Anethnobotanical and phytochemical study of the African medicinal plant *Guiera senegalensis* J F Gmel. J Med Plants Res 9: 1639-1651.

Stefano Banfi, Enrico Caruso, Viviana Orlandi, Paola Barbieri, Serena Cavallari Paolo Vigano, Pierangelo Clerici and Luca Chiodaroli (2014). Antibacterial Activity of Leaf Extracts from *Combretum micranthum* and *Guiera senegalensis* (*Combretaceae*). *Research Journal of Microbiology* 9(2): 66-81.

Van Vuuren, S.F. and D. Naidoo, 2010. An antimicrobial investigation of plants used traditionally in Southern Africa to treat sexually transmitted infections. *J. Ethnopharmacol.*, 130: 552-558.