Caliphate Journal of Science & Technology (CaJoST)

ISSN: 2705-313X (**PRINT**); 2705-3121 (**ONLINE**) **Research Article**

Article Info

Received: 1st May 2020

Revised: 14th June 2020

Accepted:18th June 2020

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Cite this: *CaJoST*, 2020, 2, 182-189

Preliminary Phytochemical Analysis and Antibacterial Activity of *Guiera senegalensis* Leaf Extract

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Guiera senegalensis is a medicinal plant used in Nigerian folk medicine for the treatment of malaria, acute gastroenteritis, leprosy and dysentery. The leaf extracts were investigated for their phytochemical constituents and antibacterial activity. Extraction was carryout successively using n-hexane, ethyl acetate and methanol with the aid of Soxhlet extractor. Preliminary phytochemical analysis was carried out on the extracts and the results revealed the presence of tannins, terpenoids, flavonoids, alkaloids, anthraquinones, cardiac glycosides and phlobatannins. The antibacterial activity of the n-hexane, ethyl acetate and ethanol extracts were investigated *in vitro* at various concentrations using agar well diffusion method. The results revealed that n-Hexane extract showed zone of inhibition of 30 mm at 100 mg/ml against *Escherichia coli* while it gave zone of inhibition of 26 mm at 50 mg/ml against *Pseudomonas aeruginosa*. The ethyl acetate extract showed the highest zone of inhibition of 17mm at 12.50 mg/ml against *Streptococcus pneumoniae*. It also gave zone of inhibition of 21 mm at 100 mg/ml and 50 mg/ml against *Pseudomonas aeruginosa*. The methanol gave zone of inhibition of 15 and 11 mm at 100 mg/ml against. *Streptococcus Pneumoniae* and *Pseudomonas aeruginosa* respectively. The result of this research has justified the folkloric usage of the plant's leaf for the treatment of microbial infections.

Keywords: *Phytochemicals, antibacterial activity, Guiera senegalensis*

1. Introduction

Interest in plants are on the increase due to their pharmaceutical, nutritional and cosmetic applications. They represent the source of active ingredients known long time ago by their traditional used for medical purposes (Marie et al., 2017). Plants can be considered as an origin of natural ingredients useful in medicine and other purposes. Plants are rich in active compounds (secondary metabolites) such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are present in their organs such as leaves, flowers, bark, seeds, fruits, and root (Marie et al., 2017). Extraction processes of these metabolites are related to the difference in solubility of the compounds present in a mixture of solvent. The beneficial actions of these phytoconstituents typically come from the synergic role of these secondary products (Tonthubthimthong et al., 2001). Natural products are products from plants, microbes and animals. Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cosa et al., 2006).

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyan et al., 2006). Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men have turned to ethnopharmacognosy (Sombié et al., 2011) They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activities such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity have been reported from many plants extracts (Sasidharan et al., 2011; Karuo et al., 2005).

Guiera senegalensis, (family; Combrataceae), is one of the most important West African medicinal plants, often used to treat a variety of microbial infections. The most frequently used

part of the plant is the leaf; due to their antimicrobial activity studies (Tijani et al. 2011). *Guiera senegalensis* is called (Sabara) in Hausa language. It is a shrub and can grow to a height of 3 to 5 m depending on the area of habitat (Adama et al., 2016). The plant is commonly distributed in the savanna region west and central Africa, Nigeria, Gambia, Mali, Niger, Senegal. The Hausa people in Nigeria use the plant for the treatment of fever (Onwulin et al., 2009). The leaves and roots have been reported to be effective against malaria parasites (Etkins, 1988). The leaf infusion had clinically demonstrated to be effective in the treatment of acute gastroenteritis and dysentery. The leaf extract has anti-inflammatory, analgesic and antiemetic properties (Etkins et al., 1982). The leafy decoction of *Guiera senegalensis* is used to relieve aches and pains and to treat fever and malaria, (Somboro et al., 2011; Osibembe et al., 2018). The aqueous and methanol soluble extracts of the leaves were found to exhibit the growth the of *Staphylococcus aureus, Bacillus subtilus Escherichia coli* and *Candida albicans* which are the most causative agents in wound and skin diseases, and gastrointestinal disorders (Sokomba et al., 1983). Traditionally, the roots concoction is used to treat diarrhea, dysentery and microbial infection (Alshafei et al., 2016). *Guiera senegalensis* has reputation in prevention of leprosy, particularly when given to the new born child of leper (Narayana et al., 2011). A simple decoction of the leaves of *Guiera senegalensis* could also be used to treat the opportunistic diseases of AIDS patients (Abubakar et al., 2013). The decoction or powder is mixed with fresh milk and given to pediatric for treatment of diarrhea and skin rashes, where the residues can be applied externally to the affected parts (Personal Communication, 1991). However, the leaves extracts were found to exhibit broad activity against the clinical isolates of *Escherichia coli, staphylococcus aureus* and *Klebsiella* species (Abubakar et al., 2013). The leaves are also used as a poultice on tumors and against Guinea worm (Abubakar et al., 2013; Sule et al., 2002). Other research work done showed the presence of alkaloids (Hyoscyamin [I] and solanine, [II]), tannins, terpenoids menthol, coumarins, saponins, flavonoids (quercetin,[III]), cardiotonics and cyanogenic heterosides which were assayed in various organs of the plant leaves, stem bark, fruits and roots (Somboro et al., 2011). Phytochemical component of *Guiera senegalensis* leaves extract based on crude, aqueous and ethanol extraction methods showed that tannin, flavonoid, saponins, steroid, triterpenes and glycoside were present while alkaloid was absent in all extraction methods (Ogbeba et al., 2017; Ankit et al.,2012; Sule and Mohammed 2009).

Many research works have been carried out with the aim of evaluating antimicrobial and phytochemical constituents of medicinal plants as possible alternatives to antibiotics and other chemotherapeutic agents but resistance still persists. Therefore, the research was aimed at investigating the antibacterial activity of the leaf extracts of *Guiera senegalensis.*

Plate 1. *Guiera senegalensis Tree*

2. Materials and Methods

2.1 Sample Collection and Identification

Guiera sensgalensis leaves were collected in May, 2017 from Kiri town, Taura Local Government Area of Jigawa State. It was identified by a taxonomist in the Biological Science Department, Federal University Dutse, Jigawa State and the Voucher specimen number is FUD/BOT/005 was deposited in the herbarium. The leaves were washed with water to remove dust and dirt. The residual water was evaporated at room temperature and the leaves were allowed to dry under shade for two weeks with regular spreading and turning in order to

prevent the growth of moths. The dried sample was pulverized into fine powder using wooden mortar and pestle. The powdered sample was weighed using analytical balance and kept at room temperature until required further use.

2.2 Extraction of Plant Material

The plant sample (300 g) was subjected to successive exhaustive extraction using n-hexane, ethyl acetate and methanol with the aid of Soxhlet extractor. The extracts were concentrated using a rotary evaporator and the percentage yield of each extract was determined The extracts were then packed in to separate plastic bottles with proper labeling and kept for further use.

2.3 Preliminary Phytochemical Screening

2.31 Test for Flavonoids

(a) Ferric chloride Test: About 5 ml of distilled water was added to the extract and boiled on water bath for about 2 mins and then filtered. 2 ml of the filtrate was obtained in a test tube and few drops of 10% alcoholic ferric chloride solution were added. The formation of dark green colouration indicates the presence of phenolic group (Trease and Evans, 2002).

(b) Lead acetate Test: A small quantity of the extract was dissolved in water and filtered. Few drops of 10% lead acetate was added to 5 ml of the filtrate. A buff coloured, precipitate indicates the presence of flavonoids (Trease and Evans, 2002).

(c) Sodium hydroxide Test: about 2 ml of the filtered extract was dissolved in 10% NaOH to give a yellow coloured solution. A change in color from yellow to colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids (Trease and Evans, 2002).

2.3.2 Test for Reducing Sugar

(a) Fehling's Test: small amount of the powdered leaves was soaked in distilled water and allowed to extract for some time. The mixture was then filtered and filtrate was heated with 5ml of equal volume of Fehling's solution A and B, for few minutes (5 mins). Formation of reddish precipitate of cuprous oxide indicates the presence of free reducing sugar (Trease and Evans, 2002).

2.3.3 Test for Cardiac Glycosides: 5 ml of the extract was dissolved in pyridine and a few drops of sodium nitroprusside together with a few drops of 20% sodium hydroxide solution were added. The formation of deep red colour which fades to brownish yellow indicates the presence of cardenolides (Sofowora, 1982).

2.3.4 Test for Saponins

(a) Frothing Test: The extract (0.5 g) was dissolved in 5 ml of distilled water and the shaken vigorously for 2 minutes in a test tube. was shaken with distilled water in a test tube, frothing which persist on warming indicates the presence of saponins (Sofowora, 1982).

2.3.5 Test for Tannins

Ferric chloride test: To 0.5 g of the plant extract, 10 ml of distilled water was added and stirred. This was filtered and a 5 ml of 5% ferric chloride was added. A deep green coloration showed the presence of tannins. A second portion of the filterate was also added to iodine solution, a faint blue coloration formed confirming the presence of tannins (Trease and Evans, 2002).

2.3.6 Test for Alkaloids

(a) Dragendorff's Test: small amount of the extract was stirred with 5ml of 1% aqueous hydrochloric acid on water bath and filtered. To the small portion of filtrate, a few drops Dragendorff's reagent was added, presence of an orange red precipitate indicates the presence of alkaloid (Trease and Evans, 2002).

(b) Mayer's Test: Small amount of the extract was stirred with 5ml of 1% aqueous hydrochloric acid and filtered. To the small portion of filtrate, a few drops of Mayer's reagent was added. The formation of creamy precipitate indicates the presence alkaloid (Trease and Evans, 2002).

2.3.7 Test for Terpenoids

(a) Salkowski Test: 2 ml of chloroform was added to 0.5 g of plant extract, 1 ml of acetic anhydride and 2 drops of concentrated H_2SO_4 was added. A peak colour which changes to bluish green on standing, which indicates the presence of terpenes (Trease and Evans, 2002).

2.4 Antibacterial Analysis

2.4.1 Preparation of stock concentrations of extracts

A stock concentration of 200 mg/ml of each extract was prepared by dissolving 0.4 g of the extract in 2 ml of dimethyl sulfoxide (DMSO). Subsequently, 100, 50, 25 and 12.5 mg/ml concentrations were prepared from the stock using 2-fold serial dilution. (Garba *et al*., 2019).

2.4.2 Clinical Bacterial Isolates

The clinical bacterial isolates of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pneumoniae* were obtained from Department of Microbiology, General Hospital, dutse, Jigawa State.

2.4.3 Standardization of Inocula

The inocula were standardized by dissolving 1-2 colonies of the test bacteria using a sterile wire loop from an overnight culture plates and dissolved in 10 ml of normal saline solutions contained in test tubes. The tubes were vortex mixed for homogeneity and compared with 0.5 McFarland standards (approximate cell count density: $1.5x10^8$) (Garba *et al.,* 2019)

2.4.4 Preparation of culture media

All culture media were prepared according to manufacturers' specifications.

2.4.5 Determination of Antibacterial Activity (Sensitivity Test)

Sterile swab sticks were immersed into the standardized inocula contained in test tubes. Excess fluids were drained by pressing the sticks at the walls of the tubes and swabbed onto prepared Mueller Hinton Agar (MHA) plates. Four wells of approximately 6 mm diameter were cut in each plate using a sterile cork borer. Aliquots of 100µl of various concentrations of the extracts were added into the wells using a micropipette. Standard antibiotic (Septrin 10 mg/cm^3) was prepared and was used as the reference or positive control. The plates were allowed to stand at room temperature for one hour for the extracts to diffuse into the agar and incubated for 24 hours at 37 ºC. Antibacterial activity was determined by measuring the diameter of inhibition zones in millimeter produced against the test bacterial isolates. The experiments were performed in replicates and the mean values were calculated (Garba *et al*., 2019).

2.4.6 Determination of Minimum Inhibitory Concentration (MIC)

Sterile capped test tubes were numbered 1-6. To 2 ml amount of the extracts solution (100 mg/ml) was added to all the other tubes. 1 ml was transferred from the first tube to the second tube. Using a separate pipette then the content was mixed and 1 ml transferred to the third tube with dilution was continue in the same manner to tube number 5, then 1 ml was removed from the tube 5 and discarded. The 6th tube which serves as a control received no extract. A standardized inoculum of the best organism was added to the 6th tube and incubated at 37 ºC overnight. The tubes were examined for visible signs of bacterial growth. The highest dilution without growth was the minimum inhibitory concentration (MIC) (Gamod *et al*., 1983).

2.5 Statistical Analysis

The data obtained was statistically analyzed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical package and the result was reported as mean± standard deviation of value. LSD was applied to determine the direction of the difference between mean at 5% level.

3. Results and Discussion

3.1 Results

3.1.1 Extraction

The percentage yield of each extract is as follow; for n-hexane (8.0%), ethyl acetate (9.3%) and Methanol (9.67%) respectively.

3.1.2 Phytochemical Screening

The result of the phytochemical screening of the n-hexane, ethyl acetate and methanol extracts of Guiera senegalensis is presented in Table 1. The results showed the presence of some primary and secondary metabolites.

Table 1. Phytochemicals screening of Guiera senegalensis extracts

Phytochemicals	*N-Hexane*	*Ethyl acetate*	*Methanol*
Flavonoids	-	+	+
Alkaloids	-	-	+
Tannins	-	+	+
Saponins	-	-	-
Terpenoids	+	+	+
Reducing Sugar	-	+	+
Cardiac Glycoside	+	+	-
Anthraquinones	-	+	+
Phlobatannins	-	+	+

+ = Detected ; - = Not detected

3.1.3 Antibacterial Activity

The results of the antibacterial activity of the extracts are presented in Tables 2, 3 and 4. The extracts inhibited the growth of the organisms at different concentrations.

Table 2. Antibacterial activity of n-hexane leaf extract of Guiera senegalensis

Organisms	Mean Diameter of zones of inhibition (mm) Concentration of extracts (mg/ml)			
	100	50	25	12.5
E. coli	30±0.30	6±0.05	6±0.05	6±0.05
S. Pneumoniae	15±0.20	14±0.04	6±0.05	6±0.04
S. Aureus	6±0.05	6±0.05	6±0.04	6±0.05
P. aeruginosa	16±0.24	26±0.26	24±0.24	21±0.21

Table 3. Antibacterial activity of Ethyl acetate leaf extract of Guiera senegalensis

Organisms	Mean Diameter of zones of inhibition (mm) Concentration of extracts (mg/ml)			
	100	50	25	12.5
E. coli	12±0.16	6±0.04	6±0.04	6±0.05
S. Pneumoniae	12±0.16	11±0.10	11±0.10	17±0.25
S. Aureus	6±0.04	6±0.04	6±0.04	6±0.05
P. aeruginosa	21±0.21	21±0.23	16±0.24	12±0.10

Table 4. Antibacterial activity of Methanol leaf extract of Guiera senegalensis

Organisms	Mean Diameter of zones of inhibition (mm) Concentration of extracts (mg/ml)			
	100	50	25	12.5
E. coli	6±0.04	6±0.04	6±0.04	6±0.04
S. Pneumoniae	12±0.16	14±0.18	6±0.04	6±0.04
S. Aureus	6±0.04	6±0.04	6±0.05	6±0.04
P. aeruginosa	11±0.04	6±0.04	6±0.05	6±0.04

S= Staphylococcus, S= Streptococcus, E= Escherichia, P= Pseudomonas

Table 5. Minimum Inhibitory Concentration (MIC) for E.coli

Organisms	Concentration (mg/ml)					
	100	80	60	40	20	10
n-hexane	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	+
Methanol	-	-	-	-	-	+

Table 6. Minimum Inhibitory Concentration (MIC) for S. aureus

Organisms	Concentration (mg/ml)					
	100	80	60	40	20	10
n-hexane	-	-	-	-	-	-
Ethyl acetate	-	+	-	-	-	+
Methanol	-	+	-	-	-	+

Table 7. Minimum Inhibitory Concentration (MIC) for S. pneumoniae

Organisms	Concentration (mg/ml)					
	100	80	60	40	20	10
n-hexane	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	+	+
Methanol	-	-	-	-	+	+

Table 8. Minimum Inhibitory Concentration (MIC) for *P. aeruginosa*

Organisms	Concentration (mg/ml)					
	100	80	60	40	20	10
n-hexane	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	+	-
Methanol	-	-	-	-	-	+

3.2 Discussion

The result of phytochemical analysis of the leaves extract of Guiera senegalensis was presented in Table 1. The phytochemical screening showed that the leaves extract contained alkaloid only in methanol extract by Dragendorff's test and absence of saponins in all extracts. Tannins, flavonoid, terpenoids and reducing sugar were also found to be presence in the leaves extract. The result of this study correlates with the finding of Mamman et al.

(2013) which showed that the leaves extract contained anthraquinones, tannins and phlobatannins, flavonoid while saponins were not found in the leaves extract. The result is similar (Onwulin et al., 2009) which show that Guiera senegalensis contained anthraquinones (free anthraquinones and combined anthraquinones), carbohydrates, cardiac glycosides, flavonoids, saponins, terpenes, phenolic compounds and tannins. The presence of this phytochemicals is very important, because they play a vital role as antimicrobial, antidiarrheal and antihelminthetic agent. The presences of alkaloid have anti-diarrhoea effect; Terpenes, which is anti-malarial, antibacterial, antiviral as well as antifungal; Tannins is also known to be anti-tumour; Anthraquinones, which is anti-hemorrhagic; Flavonoids, which inhibits Vibrio cholerae, Strept. Mutans, shigella and viruses (Trease and Evans, 1989; Sadiq et al., 2016)..

The leaf of Guiera senegalensis extracts was tested against some Gram positive and negative bacterial namely: Escherichia coli, Staphylococcus, aureus, Streptococcus pneumoniae and pseudomonas aeruginosa: it showed significant inhibitory effect on them. Based on the results Streptococcus pneumonia and Pseudomonas aeruginosa were found to be more sensitive to the extracts at varying degrees of concentration for the ethyl acetate extract, which was compared to report by (Onwulin et al., 2009) that Staphylococcus aureus was found to be more sensitive to the extracts at varying degrees of concentration of 250, 200, 150, 100 mg/ml. While in this present research Escherichia coli was found to be more sensitive to the extracts of concentration of 100 mg/ml for all the extracts. However, it was reported by (Onwulin et al., 2009) that Escherichia coli was found to be sensitive to the extracts of all concentrations. But in the present research Staphylococcus aureus was not sensitive to all the extracts at varying concentration. The ethyl acetate extract showed high efficacy on almost all the test organisms except Escherichia coli. This may be due to some inhibitory factors during extractions process. The activity of the extracts was shown to increase with the increase in concentration. However higher concentration is required in order to produces significant effect of these leaf extracts on Escherichia coli. Guiera senegalensis possess considerable antimicrobial activity as evidence from their action on the different test organisms. It has been reported to cure skin diseases, leprosy, gonorrhea, stomach pains, asthma, diarrhoea and dysentery (Onwulin et al., 2009). The table 5, 6, 7 and 8 revealed the minimum inhibitory concentration of the three different leaf extracts on the test organisms. The MIC of Streptococcus pneumoniae was 20 mg/ml for the ethyl acetate and methanol extracts. Pseudomonas aeruginosa were 20 mg /ml for methanol and ethyl acetate extracts while 40 mg/ml for ethyl acetate. While the Escherichia coli was 10 mg /ml for the three extracts. While Staphylococcus aureus was 80 mg/ml for the ethyl acetate and methanol extracts. These results implies that all the extracts could attained therapeutic concentration in living host.

4. Conclusion

The leaf extracts contain flavonoids, terpenes, reducing sugar, cardiac glycosides and tannins. These phytochemicals are responsible for the antibacterial activity demonstrated by the extracts. The antibacterial activity demonstrated implies that the leaves extract of *Guiera senegalensis could* be useful in the treatment of infections caused by *Escherichia Coli*, *Streptococcus pneumoniae and Pseudomonas aeruginosa.* Among the four bacteria, *Pseudomonas aeruginosa* is the most sensitive to the extracts.

Acknowledgement

The authors are very grateful to Professor Ali Audu Sani of the department of Chemistry, Federal University Dutse, Jigawa, Nigeria and laboratory technologist for their support and assistance throughout the research work.

Conflict of interest

The authors declare no conflict of interest.

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