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Evaluation of Bacteriostatic Effect of Methanolic Extract of *Guiera senegalensis* on Some Clinical Bacteria

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Abstract – *Guiera senegalensis (family: Combretaceae) leaves have been in use for quite long period of time, as traditional medicine in rural areas of developing nations such as Nigeria. It has purportedly been used in the treatment of tumours and related infections by the Nigerian natives especially those in the northern part of the country with no scientific evidence supporting such. It has equally been used therein to cure pyrexia through ingestion of the leaves aqueous extract soaked in water. This study aimed to determine the preliminary phytochemical components and antibacterial activity of the methanol extract of the leaves of G. senegalensis against three clinical bacterial isolates of Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae using standard methods of analysis. The phytochemical analysis revealed the presence of bioactive compounds which includes alkaloids, flavonoids, saponnins and tannins. Highest activity of the leaves extract was recorded at 100µg/mL concentration on K. pneumoniae with 20mm diameter of the inhibition zone, while the lowest activity was on E. coli at 10µg/mL concentration with 9.0mm zone of inhibition. However, this does not portends from the MIC and MBC that the methanolic extract of Guiera senegalensis leaves is bactericidal. The MIC recorded was 25µg/mL, but when seeded on an agar medium to determine the MBC, growth was observed in all the plates. This indicates that the methanolic extract of G. Senegalensis could not kill the bacteria tested, but retard their growth, hence bacteriostatic, and not bactericidal.*

Keywords: Phytochemical, Antimicrobial, Bacteriostatic, Extract, Isolates.

1.0 INTRODUCTION

The chemical compounds produced by plants, most especially medicinal and aromatic species during usual metabolic processes are called 'Phytochemicals'. These chemical compounds are referred to as 'secondary metabolites" [1] and are of different classes which include flavonoids, alkaloids, tannins, saponins, phenols, steroids etc [2]. Apart from those

constituents, such species contain other chemical substances. The aforementioned phytochemicals act as agents to prevent undesirable side effects of the main active substances or assist in assimilation of the other main substances.

As reported earlier, there is upsurge interest on the phytochemical/bioactive compounds activity present in plant species by plant scientist across the globe [3, 4]. Diverse species of plant organs extract have been analyzed for their phyto-active compounds [3]. These natural products are normally found in significant quantities in plant leaves, fruits, seeds as well as stems. These bio-active compounds are responsible for providing medical effect, preventing or delaying the oxidation of products by free radicals scavenging and reducing oxidative stress [5]. Previous findings have indicated the presence of such compound in *Guiera senegalensis* leaves [6, 7], while [8] determine the antimicrobial effect of the leaves.

Guiera senegalensis belongs to the family Combretaceae, a shrub plant of savannah region of Central and West Africa including Nigeria. The species is commonly called 'Sabara' in Nothern Nigeria and is very well known in its native areas. *G.* Senegalensis leaves are small in size and widely being used in traditional medicine for the remedy of many ailments/diseases which includes gastrointestinal pain and disorder, dysentery, diarrhea, respiratory infections, fever, rheumatism and also act as anti-malarial agent [9]. While other organs of the plant like root and bark are used for treatment of abdominal pain and dysenteric diarrhea [10]. This species extract is also used in veterinary medicine on certain animal species by adding into their diets. It is designed to increase body weight, milk secretion and reproductive capacity of veterinary animals as communicated by Somboro, Patel [11].

Evenly, *G. Senegalensis* leaves extract was found to be of pharmacological properties such as anti-microbial effects. The extracts possess anti-inflammatory, analgesic, antiemetic, as well as earlier stated anti-microbial properties. It has also been used locally as traditional medicine by many rural folks in Gombe. For these reasons, there is a strong need for further investigation of the phyto-constituents and antimicrobial potential of the leaves of this species that grow in Akko, Gombe state, Nigeria. Therefore, this study was undertaken to determine the presence of some phytochemicals from methanolic extract of *G. Senegalensis* leaves and also to investigate biological activity of the leaves, precisely focused on the anti-microbial effect(s).

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves of *G. senegalensis* were collected from the plants at Bomala village, Akko local government, Gombe State, Nigeria. The leaves were identified at herbarium of Department of Biological Sciences, Gombe State University (GSU) Nigeria with voucher number 0371 and kept as reference specimen for future use.

2.2 Preparation of Aqueous Extract

The collected leaves were heat dried at ~32^{o}C for 5 days. The dried leaves were ground into fine powder using mortar and pestle, sieved to mesh size of 150 pm and transferred to appropriate containers. Methanol 80% was used as solvent for percolation of the *G. senegalensis* leaves [3, 12]. About 25g of powdered leaves was transferred into 250mL of

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80% methanol and was continuously extracted by maceration at room temperature with shaking at regular intervals for 2-weeks [7]. The homogenized mixture was filtered and the residue was discarded. The filtrate was then transferred to water bath and subjected to evaporation at 40°C. The greenish extract obtained was stored at -4°C prior to use.

2.3 Phytochemical Screening of Leaves Extract

Phytochemical constituents assay for detection of alkaloids, flavonoids, tannins and saponnins from leaves extract of *G. senegalensis* was carried out at the Department of Biochemistry laboratory, GSU following the routing method described by [13].

2.4 Sensitive Discs Preparation

Whatman No. 1 filter paper was used in the preparation of the sensitivity discs used in the study. Discs of 6mm diameter were punched out of the filter paper using a puncher. Hundreds discs were placed into bijou bottles and sterilized by autoclaving at 121°C for 15minutes. The discs were then dried in an oven at 50°C.

Ten milligram (10,000mg) of the extract of *G. Senegalensis* leaves was dissolved in 1mL of dimethyl sulfoxide (DMSO) to form a stock solution. From the stock solutions, 0.1mL, 0.2mL and 0.5mL aliquots were taken to prepare three different concentrations for each as 1,000µg/mL, 2,000µg/mL and 5,000µg/mL respectively. Another 10mg was equally dissolved in 1mL to serve as 10,000µg/mL concentration. For each concentration, 100 discs were introduced to absorb the solution. Therefore, for 1,000µg/mL concentration, each disc had a potency of 10µg, while for 2,000µg/mL the disc potency was 20µg/disc. A 50µg/disc and 100µg/disc were the disc potencies for 5,000µg/mL and 10,000µg/mL respectively. The prepared discs were left in the solutions for maximum absorption and kept at -4°C prior to next step of analysis [14].

2.5 Test Organism

The test organisms were the bacterial isolates of *Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae* obtained from Medical Microbiology unit of Federal Teaching Hospital Gombe (FTHG), Nigeria. Gram staining and biochemical tests were conducted according to [14] to confirm their identities. The isolates were then maintained in nutrient agar slants at Microbiology laboratory of GSU at -4°C until analysis.

2.6 Standardization of Inocula

Mueller Hinton agar was the medium used for the susceptibility testing. The medium was prepared in accordance with manufacturer's instruction. The medium was sterilized by autoclaving at 121°C for 15minutes and used for the susceptibility testing. A number of colonies of each test isolates was picked-up using sterile wire loop and emulsified onto 3.4mL of sterile physiological saline to make a suspension that matched with 0.5MacFarland turbidity standard as described by [14].

2.7 Antibacterial Susceptibility Test

Bioassay protocol was carried out to determine the antibacterial effect of the leaves extract. The assay was observed using the procedure described by [15]. A sterile swab stick was used

to spread evenly the test organisms onto the sterile Mueller Hinton agar medium. The inoculated plates were allowed to stay for about 3 – 5minutes. The prepared discs of four (4) different concentrations (10µg/disc, 20µg/disc, 50µg/disc and 100µg/disc) were aseptically dispensed onto the inoculated medium within 30minutes. The plates were aerobically incubated in an inverted position at 37°C for 24hours. Septrin was used as positive control, while DMSO as the diluents was used as negative control on each bacterial isolate. After overnight incubation, all inoculated plates were observed for zones of inhibition. Antibacterial activity was recorded based on the mean zone of inhibition diameter >18 using a meter rule [16].

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

Serial doubling dilution using distilled water of the plant leave extract was prepared to determine the minimum inhibitory concentration. Four different concentrations were prepared as 100µg/mL, 50µg/mL, 25µg/mL and 12.5µg/mL. A 2mL of each concentration was pipette into a test tube containing 2mL of nutrient broth. A 0.1ml of the standardized inoculum of the test organism was introduced into the test tube. Alongside these, two test tubes containing plant extract plus nutrient broth and nutrient broth plus test organism as positive and negative control respectively were also prepared. All the tubes were incubated at 37°C for 24hours and thereafter, MIC was determined. Subculture on a nutrient agar medium of the MIC tubes that recorded no growth helps to determine the MBC. The lowest concentration of MIC that was subcultured and still no growth was observed is the MBC [17].

3.0 RESULTS AND DISCUSSION

The preliminary result of extraction showed that higher yield of the extract was obtained and after the methanol extraction, dark green and gummy texture of leaves extract was collected. Table 3.1 summarizes the outcome. The higher yield of the extract was as a result of high solubility of the plant material.

Table 3.1: Physical properties of methanolic percolation extract (MPE) of *Guirea senegalensis* leaves

Physical parameter	MPE
Weight of sample(before percolation)	25g
Weight of extract (after percolation)	2.5g
Colour	Dark green
Texture	Gummy

3.1 Phytochemical Analysis

The result of phytochemical screening of the methanolic leaves extract of *Guirea senegalensis* indicated the presence of the chemicals as shown in Table 3.2. The analysis indicated the presence of all the test compounds including alkaloid, flavonoid, saponnins and tanins. This finding correlates with that of [6] which showed the leaves and root extracts of *Guirea senegalensis* contained the analyzed phytochemicals.

The presence of these phytochemicals from the leaves extract justifies the tendency of the plant species to be medicinal. These antioxidant compounds were found to be of health effect and are also of commercial values [18, 19]. As communicated by [20] and [21], *Guirea senegalensis* contained Anthroquinolones, ascorbic acid, alkaloids and Tannins and they explain its potentiality. The presence of these phyto-components is very important as they play a vital role as antimicrobial, antidiarrheal and antihelminthetic agents. Precisely, presence of alkaloid in the leaves makes plant to intercalate into cell wall and DNA of pathogens and inhibit the release of autocoid and prostaglandins (antimicrobial). It also possesses anti-oxidative effect, thus reduce nitrate generation which is useful for protein synthesis and suppresses transfer of sucrose from stomach to small intestines. The compound also act on central nervous system as described by [22], while presence of tannins allows for antimicrobial properties (by binding to certain enzymes or inhibit substrate deprivation).

Table 3.2: Phytochemical screening of methanolic extract of *Guirea senegalensis*

Chemical compound	**Test**	**Inference**
Alkaloids	Meyer's reagent test	+ve
Flavonoids	NaOH and dilute acid test	+ve
Saponins	Distilled water test	+ve
Tanins	NaOH test	+ve

3.2 Antimicrobial Potential of Methanolic Extract of Guirea senegalensis Leaves

Sensitivity of the confirmed clinical isolates for *Guirea senegalensis* methanolic leaves extract using disc diffusion method was determined by measuring the zones of inhibition formed around the impregnated discs with different concentrations of the leaves extract. The result of antibacterial activity of *Guirea senegalensis* leaves (refer to Table 3.3) revealed that the extracts possesses bactericidal activity on the clinical isolates used in this study. The isolates tested were *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae*. Comparison of the activity of the extract with that of Septrin (positive control) and DMSO (negative control) shows that the extract may serve as an alternative source of treatment of infections caused by the test organisms.

The antibacterial activity of methanol percolation extract from leaves of *Guirea senegalensis* on *Escherichia coli* at 10µg/disc recorded about 9mm zone of inhibition, while at 20µg/disc was 10mm. At 50µg/disc and 100µg/disc the zones were approximately 11mm and 12mm respectively. On *Staphylococcus aureus* the zone of inhibition measured at 10µg/disc and 20µg/disc were all 10mm, while at 50µg/disc was 11mm and 14mm at 100µg/disc. Inhibition zone on *Klebsiella pneumonea* at 10µg/disc showed 11mm, at 20µg/disc also 11mm, while at 50µg/disc displayed 13mm and at 100µg/disc was 20mm. The negative control i.e. DMSO on *E. coli* and *Klebsiella pneumoniae* the inhibition zone were 0.00mm and 0.00mm in case of *S. aureus*. The Septrin positive control indicated larger zones across the test organisms (on *E. coli* about 25mm, *S. aureus* 24mm and *Klebsiella pneumoniae* 20mm zones of inhibition). Therefore Table 3.3 described the antibacterial activity of methanol extract of *Guirea senegalensis* leaves.

Table 3.3: Antibacterial activity of methanol extract of *Guirea senegalensis* concentration (µg/disc)

	10µg/disc	20µg/disc	50µg/disc	100µg/disc	DMSO (negative control)	Septrin (30µg/disc) positive control
Test Organism	**Zone of Inhibition (mm)**					
E. coli	9	10	11	12	00	25
S. aureus	10	10	11	14	00	24
K. pneumonia	11	11	13	20	00	20

The minimum inhibitory concentration of the leaves extract of *G. senegalensis* was determined at 25µg/mL for all the isolates tested. However, all the test organisms exhibited growth when the contents in the MIC tubes were subcultured onto the nutrient agar medium. This correlates the work of [17] which justifies the fact that leaves extracts of some plants species e.g. *G. senegalensis* are bacteriostatic and not bactericidal as, from the research, MBC was not determined as shown in Table 3.4.

Table 3.4: Antibacterial activity of leaves extract of *G. senegalensis* using Macro Broth Dilution

S/N	Bacteria	Concentration (µg/mL)						
		MIC				MBC		
		100	50	25	12.5	100	50	25
1	*E. coli*	-	-	-	+	***	**	**
2	*S. aureus*	-	-	-	+	***	**	**
3	*K. pneumoniae*	-	-	-	+	***	**	**

Key: MIC= Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration, + = Turbid, - = Not turbid, ** = Growth observed, *** = MBC above 100µg/mL

4.0 CONCLUSION

The screening of the leaves for phytochemicals has showed that the plant leaves contain all the chemicals tested. Result from this study, *G. senegalensis* is a medicinal plant due the possession of those chemical compounds in its leaves, but with a thorough modification. Determination of such compounds from the locally available plant species would be beneficial to the rural people in the under-develop and developing nations, as well as pharmaceutical industries. Thus, the result of this study has provided a scientific evidence to support the acclaimed role of *G. senegalensis* leaves as an antibacterial agent with great emphasis in traditional medicine. However, being bacteriostatic, it is indicative that it does not kill the organisms totally. It rather prevents their growth, which may pave way for their reorganization to develop resistance. The results obtained in this research imply that the leaves extract of this plant species has the potential for the production of modern drug for the treatment of bacterial infections caused by *E. coli, S. aureus* and *K. pneumoniae* but with a lot of modifications to prevent antibacterial resistance.

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