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Antibacterial Activity of Leaf Extract of *Guiera senegalensis* on Some Selected Bacteria (*Staphylococcus aureus, Escherichia Coli* And *Pseudomonas aeruginosa)*

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Abstract

The phytochemical Screening and Antibacterial Activities of leaf Extracts of Guiera senegalensis were investigated. The phytochemical analyses according to standard screening tests using conventional protocols revealed the presence of tannins, saponins, alkaloids, and steroids in methanolic leaf extract while tannins, saponins, flavonoids and steroids were present in ethanolic extract of the leaf part of the plant. Agar diffusion sensitivity test of leaf extract of the plant using methanol and ethanol were investigated on three bacterial isolates. The extracts generally exhibited marked antibacterial activities on Staphylococcus aureus and E.coli but had no effect on Pseudomonas aeruginosa. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the leaf extract onStaphylococcus aureus and E. coli were100mg/ml and 200mg/ml, respectively, which revealed high antibacterial activities. These results obtained from this research work justified the traditional use of Guiera seneagalensis leaves in the treatment of diarrhea.

Keywords: Antibacterial Activity, Bacteria, Leaf Extract, *Guiera senegalensis*

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Introduction

Natural products from many plants have been evaluated and used in the management and treatment of several diseases. The emphasis on searching in primary tropical forest habitats for new drugs has meant that plants growing in other habitats have been ignored or overlooked. Yet, in relation to other types of plants, there has been significant evidence that weeds are relatively high in bioactive secondary compounds and are thus likely to hold promise for drug discovery. In fact, while it is likely that important new drugs remain hidden in plants in primary tropical rainforest, they may also lie hidden in those belonging to other ecosystems (Stepp, 2004). On the other hand, secondary natural compounds in plants are important for a variety of ecological functions, including the chemical defense against herbivores and other attacks such as those by fungi and bacteria; and could be a source of interesting antimicrobial compounds. Thus, our continued interest in invasive plants and weeds as potential sources of pharmacologically important compounds, and as previously reported (Mihigo *et al.*, 2015),

Infectious disease represents a major cause of death accounting for approximately one half of all deaths in tropical countries (Iwu *et al.*, 1999). In particular, gastrointestinal disease is the most frequent cause of childhood morbidity and adult mortality in many parts of the developing world. The presence of enterobacteria in Foodstuffs and water is a common cause of diarrhea and dysentery among the infant population (Viera *et al.*, 2001). The increase in antibiotic resistance causing to these disease and in nosocomial and community acquired infections, has renewed interest for new strategies and incentives towards research on treatment, prevention and development of new drugs against these microbial diseases (Iwu *et al.*, 1999; Viera *et al.*, 2001). Over the years, the World Health Organization advocated that countries should interact with traditional Medicine practitioners with a view to identifying and exploring aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (Akinyemi *et al.*, 2005). Phytomedicine derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infection (Iwu *et al.*, 1999). About 80% of the rural population in Nigeria depends on it as a

primary health care. This represents a potential pharmaceutical market and is a motivator for research into new drugs.

Guinea senegalensis is a tropical shrub of the family Combretacea (Hutchinson and Dalziel, 1954), which is used in traditional medicine in Northern Nigeria. It is used by local herdsmen in the treatment of Trypanosomiasis (Wurochekke and Nok, 2004). The plant continues to be one of the plants used by local livestock farmers, traditional healers and Fulani herdsmen in the treatment of snakebite in Northern Nigeria (Sallau *et al.*, 2005). The plant is used in folkloric medicine in the treatment of dysentery, diarrhea, stomach upset and hemorrhoids in local communities in Nigeria.

Material and Methods

Collection and Identification of Plant Materials

The fresh leaves of *Guiera senegelensis* used were collected from various parts of Gombe Metropolis. The leaves were identified at the Botany Unit Herbarium of the Department of Biological Sciences, Gombe State University, Nigeria.

Preparation of Plant Material

The *Guiera senegelensis* leaves were dried under shade for seven days and were grounded into fine powder using motor and pestle. The powder was then stored in an appropriate container until it is required.

Extraction of Plant Material

Twenty five (25) grams of the powdered leaf materials was extracted with 250 ml of methanol and ethanol using a soxhlet extractor and the filtrates were separately evaporated at 40^0C. These were labeled as Methanol and Ethanol soxhlet extracts (SPE) (Fatope *et al.*, 1993).

Phytochemical Screening

Test for Tannins:

About 0.5 g of the extracts were mixed thoroughly with 10 ml distilled water and then filtered; 5 ml of the filtrates was added to 1 ml of 5% Ferric Chloride solution. The appearance of blue- black, greenish or blue green precipitate indicated the presence of tannins (Ciulci, 1994).

Test for Flavanoids
A few drops of concentrated Hydrochloric acid were added to a small amount of extracts of the plant material. Immediate development of red colour indicated the presence of flavonoids (Sofowora, 1993).

Test for Saponins:
About 0.1 g of powdered plant materials was boiled with 10 ml of water for 5 minutes then filtered. After cooling, 5 ml of filtrate was then diluted with water and shaken vigorously. The formation of persistent foam indicated presence of saponin (Sofowora, 1993).

Test for Steroids
About 1 ml solution of the extracts was added to 1 ml sulphuric acid. The appearance of red colour indicated the presence of steroid (Sofowora, 1993).

Test for Alkaloids:
About 0.5 g of the extracts was stirred with 5 ml of 1% hydrochloric acid on a steam bath and filtered. 1 ml of the filtrate was then treated with few drops of Mayer's reagent. A white or creamy white precipitate considered as an indication for the presence of alkaloids (Ciulci, 1994).

Preparation of Sensitivity Disc
Sensitivity discs of about 6 mm in diameter were punched from Whatman's no. 1 filter paper using a file punch, then put onto Bijou bottle. The sensitivity discs were then sterilized in an autoclave at 121^{0}C for 15 minutes, and then allowed to cool. Sensitivity discs were prepared by weighing the appropriate amount of the extracts and dilution in Dimethyl-Sulphoxide (DMSO) followed by placing the improvised paper discs in the solution such that each disc absorb 0.01 ml to make the potency of 100 µg, 200 µg and 300 µg (Valekubia *et al.*, 2001, Akinyemi *et al.*, 2005).

Test Isolates
Clinical isolates of *Staphylococcus aureus*, *E. coli* and *P. aeruginosa* were collected from Gombe State Specialist Hospital and maintained in agar slants in refrigerator (10°C) prior to use. Appropriate

confirmatory Biochemical tests such as gram staining, urease, citrate and indole were carried out on each isolate.

Microscopy Procedure for the Bacterial Isolates

The microscopic examination of the organisms was carried out according to the method described by Cheesebrough(2006).

Preparation of smear

1-2 drops of normal saline was dropped on a clean slide. A loopful of the test organism was then transferred onto the normal saline and spread over a small area and allowed to air-dry. The dried smear was heated fixed by passing the slide through the Bunsen flame 2-3 times.

Gram staining

The slide was flooded with Crystal violet solution for up to a minute, washes off with distilled water and drained. The slide was then flooded with iodine solution and allowed to act as mordant for 1min, wash up with distilled water and rained. The slide was flooded with 95% alcohol for 10Sec. and washed off with Distilled water. The slide was flooded with Safranin solution for 30 sec. washed off and allowed to air-dry, the back of the slide was cleaned with a blotting paper. All slides were viewed under the microscope using oil immersion lens to confirm the isolates.

Biochemical Tests

Catalase test

2–3 ml of the hydrogen peroxide solution was poured into a test tube. Using a sterile wooden stick or a glass rod (notanichrome wire loop), remove several colonies of the test organism and was immersed in the hydrogen peroxide solution and the result was recorded.

Urease test

A dense 'milky' suspension of the test organism in 0.25 ml saline in a small tube. A urease tablet was added and the tube was closed and incubated at 35–37°C (in a water bath for a quicker result) for up to 4 hours or overnight.

Oxidase test

A piece of filter paper was placed in a clean Petri dish and 2 or 3 drops of freshly prepared oxidase reagent was added. A glass rod was used to transferred a colony of the test organism and smear it on the filter paper. The result was observed and recorded.

Indole test

The test organism was inoculated in a bijou bottle containing 3 ml of sterile peptone water. Incubated at 35–37 °C for 24- 48 h. 0.5 ml of Kovac's reagent was added and shaken gently. The result was carefully observed and recorded.

Coagulase test

Each test organism was inoculated in a test tube containing 0.2ml of plasma and 1.8ml of normal saline. It was then Incubated at 37°C for 24-48hr. 3-4 drops of 3% hydrogen peroxide was added. The solution was carefully observed and result recorded.

Preparation of Turbidity Standard

Barium sulphate suspension at 1.0% w/v was prepared as follows. One percent (1% v/v) solution of sulphuric acid was prepared by adding 1ml of concentrated H_2SO_4 in 99cm^3 of water. One percent (1% w/v) solution of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride in 50cm^3 distilled water. Barium chloride solution (0.6cm^3) was added to 99.4cm^3 of sulphuric acid solution to yield 1.0% w/v barium suspension. The turbid solution formed was transferred into a test tube as the standard for comparison (Cheesebrough, 2000).

Standardization of Inoculum

A loopful of each of the test isolates was picked using sterile wire loop and emulsified onto 3.4 ml of sterile physiological saline. The turbidity of the suspension was then matched with that of 0.5 McFarlands standard (Chessbrough, 2004).

Sensitivity Testing

Using sterile swab stick, standardized inoculum of each isolate was swabbed onto the surface of Mueller Hinton agar in separate Petri dishes. Disc of the extracts was placed onto the surface of the

inoculated media, then the plates were inverted and allowed to stand for 30 minutes for extracts to diffuse into the agar, after which the plates were incubated aerobically at 35°C for 18hours. Zone of inhibition formed around each of the extracts and standard antibiotic discs 'was measured using meter rule (National Committee for Clinical Laboratory Standards, NCCLS, 1999).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MIC) of the extracts were determined using the tube dilution method (Baker and Silverton, 1993). Dilution of the plant extracts were incorporated in nutrient broth in 1: 1 ratio Initial rough estimates of the MIC values of the plant extracts against the test organisms were estimated to determine the range of MIC values. Consequently, the following concentrations were prepared for each extract, using the dilution formula: 400, 200, 100, 50, 25, 12.5, 6.25mg/ml. In addition, 0.1ml of standard suspension of the test organisms was added to each tube. The tubes were incubated at 37°C for 24 hours. A tube containing extract and growth medium without inoculum was included to serve as control. The presence of growth (turbidity) or absence of growth (clear solution) at the end of incubation period was recorded. The lowest concentration of the extracts showing no growth was regarded as the minimal inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration, (MBC) were determined by sub culturing the last test dilution that showed visible growth (turbidity) and all others in which there were no growth on a fresh extract solid medium and incubated for further 24 hours. The highest dilution that showed no single bacterial colony was taken as the minimum bactericidal concentration (MBC) as reported by Baker and Silverton(1993).

Results

The *Guiera senegalensis* leaves were screened for the presence of bioactive components and the extracts of these leaves were prepared using methanol and ethanol and then antibacterial activity of extracts were carried out on clinical isolates (*Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined and the results obtained were presented in the Tables below:

Table 1: Physical Properties of Guiera senegalensis Extracts

Physical Parameters	Ethanol	Methanol
Weight extracted (g)	25	25
Weight of extract (g)	7	8
Percentage yield (%)	28	32
Color	Green	Green
Texture	Smooth	Smooth

Key: g = gram
% = percentage

Table 2: Phytochemical Screening of *Guiera senegalensis* Extracts

Phytochemical	Methanol	Ethanol
Tannins	+	+
Saponins	+	+
Flavonoids	-	+
Alkaloids	+	-
Steroids	+	+

Key: + = presence of metabolite
- = absence of metabolite

Table 3: Antibacterial Activity of *Guiera senegalensis* of Leaf Extracts on Bacterial Isolates

Plant extract	Concentration	*Staphylococcus aureus*(mm)	*Escherichia coli* (mm)	*Pseudomonas aeruginosa*(mm)
Methanol	100	13.5	12.5	-
	200	15.5	14.5	-
	300	21.5	19.5	-
Ethanol	100	12.5	13.5	-
	200	14.0	16.5	-
	300	18.0	19.5	-

Key: - = negative
µg = microgram
Mm = millimeter zone of inhibition

Table 4: Minimum Inhibitory Concentration (MIC) of the Test Isolates against the Leaf Extracts of *Guiera senegalensis*

Isolate	MIC mg/ml	
	Methanol	Ethanol
Escherichia coli	100	100
Staphylococcus aureus	100	100

Table 5: Minimum Bactericidal Concentration (MBC) of the Test Isolates against the Leaf Extracts of *Guiera senegalensis*

Isolate	MBC mg/ml	
	Methanol	Ethanol
Escherichia coli	200	200
Staphylococcus aureus	200	200

Discussion

High yield of the extracts was obtained at the end of the methanolic soxhlet extraction with extract having greenish and smoothly texture as presented in Table 1. The high yield of the extract was as a result of high solubility of the plant material in the compound. There was no difference in the color of the extract based on the extraction of the solvent with both methanolic and ethanolic extracts having greenish color and smooth texture. The higher yield of methanol extracts was in agreement with the result of William *et al* (2009). The phytochemical screening of methanolic and ethanolic extracts of *Guiera senegalensis* leaf using soxhlet extraction method revealed the presence of tannins, saponins and steroids in all the extracts irrespective of the solvent used for the extraction. Flavanoid was present in ethanolic extract while alkaloid was present in methanolic extract (Table 2). This agrees to some extent with Mudi and Salisu (2009) which showed that Tannins and Saponins present in the plant exhibit similar antibacterial activities. The result is also in agreement with Gbile (1986). The Table 3 showed the result of the antibacterial activity of the plant extracts on*Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The mean zone inhibition sizes on the test organisms showed that *Staphylococcus aureus*had 13.5, 15.5 and 21.5mm at concentrations of 100, 200 and 300mg was more sensitive to the methanolic extract of *Guiera senegalensis* as compared to *Escherichia coli* which had 12.5, 14.5 and 19.5mm at the same concentrations while *Escherichia coli* had 13.5, 16.5 and 19.5mm at concentration of 100, 200 and 300mg respectively was

more sensitive to the ethanolic extracts as compared to *Staphylococcus aureus* which have 12.5, 14.5 and 18mm at the same concentrations. The observed antibacterial effect of the isolate may be due to the presence of Tannins, Flavonoid and Saponins which have been shown to possess antibacterial properties (Osadebe, 2004). This is in agreement with Luttrodt *et al.* (1999), who carried out similar work on plant extracts of *Eucalyptus camaldulensis* against *Staphylococcus aureus* and *Escherichia Coli* and attributed the action of the plant to these active ingredients. However *Pseudomona aeruginosa* was resistant to both extract of *Guiera senegalensis* leaf.

The minimum inhibitory concentration of the extracts for *Escherichia coli* was determined to be 100 and 100mg/ml for methanolic and ethanolic extracts, respectively and that of *Staphylococcus aureus* was also found to be 100 and 100mg/ml for methanolic and ethanolic extracts respectively (Table 4). These showed that minimum inhibitory concentrations of *Escherichia coli* and *Staphylococcus aureus* were the same, which means that the extract of *Guiera senegalensis* contain substances that can inhibit the growth of some microorganisms.This result is in line with that of Williams *et al.* (2009) who reported that leaf extract of *Guiera senegalensis* inhibited the growth of various microorganisms at different concentrations. The minimum bactericidal concentration of the extracts against*Escherichia coli* was determined to be 200 and 200mg/ml for methanolic and ethanolic extracts respectively and that of *Staphylococcus aureus* was also found to be 200 and 200mg/ml for methanolic and ethanolic extracts, respectively (Table 5). These findings showed that minimum bactericidal concentration of *Escherichia coli* and *Staphylococcus aureus* were the same. High minimum bactericidal concentration value is an indication of low activity while low minimum bactericidal concentration value indicates of high activity as reported by Baker and Silverton (1993).

Conclusion

This research work therefore, supports the traditional use of *Guiera senegalensis* leaf extract for the treatment of various infections and demonstrated its role as an antibacterial agent for the treatment of infection caused by *Escherichia coli* and *Staphylococcus aureus*. The

study also proved that *Guiera senegalensis* leaf has the potential for the production of novel antimicrobial drugs for the treatment of bacterial infections caused by *Escherichia coli* and *Staphylococcus aureus*.

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