



Phytochemical Evaluation and Antibacterial Potential of Ethanolic Leaf Extract of *Guiera Senegalensis* (J.F.Gmel.)

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Abstract

The leaves of *Guiera senegalensis* are reported to possess medicinal properties in Nigerian folklore. These properties are linked to the bioactive constituents of the plant. This study investigated the phytochemical constituents and antibacterial activity of the ethanolic leaves extract of *G. senegalensis*. Powdered leaves were subjected to maceration with 90% ethanol. The crude extract obtained was sticky in texture and brownish-green in color. Phytochemical screening using standard methods revealed the presence of phenolics, tannins, saponins, terpenoids, steroids, alkaloids, and flavonoids, while anthraquinones were absent. Antibacterial activity was evaluated using the agar disc diffusion method against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The results showed that *E. coli* was highly susceptible at 250 mg/ml with the highest zone of inhibition (16 mm), while *S. aureus* showed moderate susceptibility with a 14 mm zone of inhibition at the same concentration. The findings suggest that *G. senegalensis* is a promising source of naturally occurring antibiotics, thereby justifying its ethnomedicinal use.

Keywords: Antibacterial; Bioactive compounds; Crude extract; Ethnomedicinal; Medicinal plants and Phytochemicals

Introduction

Guiera senegalensis (locally called "Sabara" in Hausa) is a perennial shrub that grows up to 3–5 m depending on habitat [1]. The specie is widely distributed across West and Central Africa, including Nigeria, Senegal, Gambia, Mali, Niger,

Burkina Faso, and Ghana [2]. Traditionally, the plant is prescribed for cough, respiratory congestion, fever, hypertension, hypotension, and venereal diseases, and has also been used against malaria [3, 4].



Figure 1: *Guiera senegalensis*

Morphologically, *G. senegalensis* presents an ash-grey stem with fibrous bark, bearing short petioled, oval leaves with black spots on the underside, giving the plant its distinctive silver green appearance [5]. It thrives in Sudanian-Sahel regions, growing in sandy, leached, or exhausted soils, and is commonly associated with overgrazed areas [6]. Flowering occurs almost throughout the year, when it is leafy. Often blooms twice a year, during the dry season and the rainy season. Each flower has a calycinal tube ovoid, welded to the ovary. This tube is topped by a bellflower blade with 5 teeth screened black and persistent points to fruiting. The ligulform corolla is composed of 5 petals also riddled with black spots. The stamens are 10 on two rows of 5, all inserted on the calyx. The ovary has a single box containing 4 to 6 eggs [7].

The plant grows primarily in Sudanian Sahel area, on soils sandy, leached or exhausted, fallow and dry stations. Plant pioneer disseminated by cattle in the fallow land, it is also indicative of

overgrazing. It is found from Nigeria, Senegal to Cameroon to Sudan. Widespread and common, locally gregarious and very abundant [8].

G. senegalensis is considered by traditional practitioners of Nigeria, Burkina Faso, Senegal, and Mali as a panacea; both its medicinal properties are significant and varied. The usual form of preparation for internal use is in decoction or mixed with food preparations. *G. senegalensis* leaves are widely administered for pulmonary and respiratory complaints, for coughs, as a febrifuge, colic and diarrhea, syphilis, beriberi, leprosy, impotence, rheumatism, diuresis and expurgation [9, 10].

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plants color,

aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals [11, 12]. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [13].

The exact classification of phytochemicals could have not been performed so far, because of the wide variety of them. In recent year Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, phenolics, flavonoids and glucosides [14].

Pathogenic bacteria are divided into gram-positive and gram-negative based on their differences in cell wall. Those bacteria with a thick peptidoglycan layer and no outer lipid membrane are known as gram positive. E.g. *Staphylococcus aureus* etc. While those bacteria with a thin peptidoglycan layer and an outer lipid

membrane is known a gram negative. E.g. *Escherichia coli* etc. [15].

Materials And Methods

Sample collection and treatment

Fresh leaves of *Guiera senegalensis* were collected on 10th August, 2023 at Umaru Musa Yar'adua University Katsina Campus; the plant was identified by a botanist at the Department of Biology, Umaru Musa Yar'adua University Katsina, Katsina State Nigeria. The leaves of *Guiera senegalensis* were sorted out from the plant and then washed with fresh water to remove unwanted particles. The plant samples were then air dried for 14 days and the leaves were crushed into powder using motor and pestle, and stored in polythene bags for future use.

Preparation of the ethanolic extracts (using maceration)

Fifty grams (50 g) of the powdered sample was dissolved in 600 cm³ of 90 % ethanol into stoppered container. After 68 hours 55 minutes, the mixture was filtered using whatman filter paper into conical flasks. The filtrate was concentrated by placing the flasks into the water bath at 60 °C. The resulting extract was cooled at room temperature and the remaining solvent escaped.

Preparation of Wagners reagent

A 1.3 g of iodine crystal and 2.0 g of potassium iodide were dissolved in water in a 100 cm³

volumetric flask and the solution were made up to 100 cm³

Phytochemical screening

Alkaloids

A few drops (2 drops) of Wagner's reagent were added to few cm³ (5 cm³) of plant extract along the sides of test tube. A reddish-Brown precipitate indicates the presence of alkaloid [16].

Saponins

The extract (50 mg) was diluted with distilled water and made up to 20 cm³. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm (2 cm) layer of foam indicates the presence of saponins [17].

Phenolic compounds

The extract (50 mg) was dissolved in 5 cm³ of distilled water and then few drops of neutral 5 % ferric chloride solution were added. A dark green color indicates the presence of phenolic compound [17].

Anthraquinones

The crude extract was taken into a dry test tube. Then 1.0 cm³ of chloroform was added and shaken for 5 min and it was then shaken with equal volume of 10 % ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicated the presence of anthraquinone [18].

Terpenoids

The crude extract was added to few cm³ of chloroform. Then few cm³ of concentrated H₂SO₄ was added, two layers were formed. The reddish-brown coloration in the interface indicated the presence of terpenoids [19].

Tannins

The crude extract was added with 2.0 cm³ of distilled water and boiled in a test tube and three drops of 10 % of FeCl₃ was added. The brownish green coloration indicates the presence of tannins [20].

Flavonoids

The crude extract was added with 5.0 cm³ of distilled water and boiled for 5 min. Three drops of 20 % NaOH solution was added. The color changes from colorless to yellow. Then, 5 drops of 1 % of HCl was added into the mixture. The presence of flavonoids was interpreted by observing the decolorization of the yellow colour [21].

Steroids

The crude extract was dissolved in 1.0 cm³ of chloroform then added slowly 2.0 cm³ of concentrated sulfuric acid, H₂SO₄. Two layers were formed, a lower layer which is in yellow color with green fluorescence and a reddish-brown color on upper layer which was interpreted as a steroid ring [22].

Antibacterial assays

The 90 % ethanolic extract was tested for antibacterial activity by using agar disc diffusion method.

Serial dilution

A 0.5 g of the extracts was measured using weighing balance and dispensed into the clean and sterile plain container containing 2 cm³ of methanol to obtained a concentration of 250 mg/cm³ and labeled as solution A, followed by transferring 1 cm³ of solution A into another plain container containing 1 cm³ of methanol to obtained a concentration of 125 mg/cm³ and labeled as solution B, followed by transferring 1 cm³ of solution B into another plain container containing 1 cm³ of methanol to obtained a concentration of 62.5 mg/cm³ and labeled as solution C, followed by transferring 1 cm³ of solution C into another plain container containing 1 cm³ of methanol to obtained a concentration of 31.25 mg/cm³ and labeled as solution D.

Preparation of Mueller Hinton agar (MHA) media

A 3.9 g of Mueller Hinton agar was weighed. The MHA powder was dissolved in 100 cm³ of distilled water in 500 cm³ conical flask. Stirrer was used to stir the mixture to ensure proper mixing. After stirring, MH agar solution was boiled using electric heating plate at 100 °C, and then MH agar solution was autoclaved for sterilization at temperature of 121 °C for 15 minutes. After autoclave, the hot sterilized MH

agar solution was allowed to cool for at least 5 minutes, and then poured into the four petri-plates in the laminar air flow cabinet. Each of the petri-plates contained approximately 25 cm³ of MHA solution which can only occupy 60-70 % of the petri plate. The Mueller Hinton agar solution was allowed to solidify in the cabinet.

Preparation of the disk and impregnation for the determination of zone of inhibition

From the four different concentrations of complexes which were 250 mg/cm³, 125 mg/cm³, 62.5 mg/cm³ and 31.25 mg/cm³. Sterile disk punched using Whatman filter paper were impregnated into each of the respective plain containers containing four different concentrations and allowed to soak for 20 minutes and dispensed in each of the already seeded plates of *Staphylococcus aureus* and *Escherichia coli*. Ciprofloxacin was used as a bacterial positive control and incubated at 37 °C for 24 hours. Following the 24-hour incubation, a clear zone was observed as zone of inhibition which was measured in millimeters (mm) recorded and tabulated.

Results and Discussion

The qualitative phytochemical analysis of the ethanolic extract of *Guiera senegalensis* showed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, and phenolic while showed the absence of Anthraquinone in (Table 1).

However, the antibacterial activities of the plant leaves extract in (Table 2) showed moderate antibacterial activity against *Staphylococcus aureus* and Ciprofloxacin was used as a positive control.

Also, the antibacterial activities of the plant leaves extract in (Table 3) showed maximum antibacterial activity against *Escherichia coli* and also Ciprofloxacin was used as a positive control.

Table 1: Results for qualitative analysis of phytochemical constituents of the ethanolic extract of *G. senegalensis*

Phytochemical constituents	Results
Alkaloids	++
Tannins	++
Saponins	++
Flavonoids	+
Terpenoids	++
Steroids	++
Anthraquinone	-
Phenolic	++

Key: ++ (highly presence), + (partially presence), - (absence)

Table 2: Antibacterial activities of *Guiera senegalensis* against gram positive bacteria result

Pathogen	250 mg/cm ³	125 mg/cm ³	62.5 mg/cm ³	31.25 mg/cm ³	Control +ve
Zone of inhibition in (mm)					
<i>S. aureus</i>	14	11	9	6	32

Key: (+ve) = positive, positive control = (Ciprofloxacin), (mm) = millimeter

Table 3: Antibacterial activities of *Guiera senegalensis* against gram negative bacteria result

Pathogen	250 mg/cm ³	125 mg/cm ³	62.5 mg/cm ³	31.25 mg/cm ³	Control +ve
Zone of inhibition in (mm)					
<i>E. Coli</i>	16	13	10	9	29

Key: (+ve) = positive, positive control = (Ciprofloxacin), (mm) = millimeter

The sample after collection, preparation, grinding was subjected to maceration for 68 hours 55 minutes and then concentrated to obtain the crude extract. The color was found to be brownish green, texture was oily and sticky.

The phytochemical evaluation in (Table 2) showed the presence of the major secondary metabolites phenolic, alkaloids and terpenoids which account for many of the plant functions such as its metabolism and also showed the presence of tannins, saponins steroids, and flavonoids (which showed a partially presents) while anthraquinone is (absents). In addition, presence of this phytochemicals are accountable for its medicinal uses such as antibacterial, antifungal antidiabetics, antioxidant and antidiarrhea etc. As it was reported by (Lata and Dubey 2010), [23]. That medicinal benefits of plant depend on the quality of the bioactive constituents including tannins, phenolic compounds, alkaloid and flavonoid which are present in the understudied aquatic plant.

The results indicate that the 90% ethanolic extract of *Guiera senegalensis* showed antibacterial activity toward both gram-negative bacteria: *Escherichia coli* and gram-positive bacteria: *Staphylococcus aureus*. The results showed that ethanol extract of the selected parts is highly susceptible against *Escherichia coli* with highest zone of inhibition 16mm at 250 mg/cm³. Ciprofloxacin was used as positive control for all

the bacteria and was very active against all the bacteria.

The findings of this study are consistent with previous reports on the phytochemical composition of *Guiera senegalensis*. Earlier studies have documented the presence of secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, saponins, steroids, and phenolic compounds, which are responsible for the plant's broad range of biological activities by (Ibrahim et al., 2017; Salih et al., 2020), [24, 25]. The absence of anthraquinones in the present study aligns with similar reports, suggesting that this metabolite may not be a common constituent of the species. These bioactive compounds, particularly phenolics, alkaloids, and terpenoids, have been widely recognized for their antimicrobial, antioxidant, and anti-inflammatory effects, thereby supporting the medicinal uses of *G. senegalensis* in traditional healthcare systems.

In terms of antimicrobial activity, the ethanolic extract demonstrated greater susceptibility against *Escherichia coli* than *Staphylococcus aureus*, with inhibition zones of 16 mm and 14 mm, respectively, at the highest tested concentration. This observation corroborates earlier findings by (Bello et al., 2018; Diatta et al., 2021), [26, 27]. Who reported stronger inhibitory effects of *G. senegalensis* extracts against gram-negative bacteria compared to gram-positive species. Such differential activity has been attributed to the

higher permeability of gram-negative bacterial cell walls to certain phytochemicals, especially terpenoids and phenolic compounds. Taken together, the present study not only supports previous research but also strengthens the evidence that *G. senegalensis* possesses promising antibacterial potential, particularly against enteric pathogens such as *E. coli*.

The specific compounds that attribute to the antibacterial activity have not been determined in this study. However, preliminary screening of phytochemicals compounds of the solvent extracts had been carried out. The result showed tannins, terpenoids, Alkaloids, saponins, phenolic, steroids were highly present. Terpenoids are interpreted to be potential antibacterial agents. However, the extract showed moderate activity towards *Staphylococcus aureus* with zone of inhibition 14mm at 250mg/ml. This might be due to the other phytochemical compounds such as flavonoids and polyphenolics compounds also present in the ethanol extract.

Conclusion

The phytochemical screening of the leaf extract of *Guiera senegalensis* showed the presence of phytochemical constituents such as alkaloids, steroids, flavonoids, phenolics, tannins, saponins, and terpenoids. This may be responsible for the medicinal properties of plant. The ability of the extract to inhibit the growth and showed bactericidal activity against *Staphylococcus aureus* and *Escherichia coli* at concentration

ranging from 250 mg/cm³, 125 mg/cm³, 62.5 mg/cm³ and 31.25 mg/cm³ respectively, revealed that the leaves of *Guiera senegalensis* can be used to cure infection that may be caused by these microorganisms.

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