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Full Length Research Article

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF COMBARATACAE (*GUIERA SENEGALENSIS*) ETHANOL LEAF EXTRACT

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ABSTRACT
Guiera senegalensis is a well-known traditional medicinal plant in Africa, whose leaf extracts are used for the treatment of diseases and wounds. This study aimed to assess the phytochemical screening and antibacterial activity of the ethanolic leaf extract of *Guiera Senegalensis.* The plant material was macerated with ethanol to obtain crude ethanol leaf extract. The phytochemical screening revealed the presence of tannins, saponins, anthraquinone, alkaloids, phenolic, and flavonoid compounds. Thin-layer chromatography of the crude extract was carried out to ascertain the various fractions contained in the extract. In addition, column chromatography was carried out on the extract using ethanol as the mobile phase and silica gel as the stationary phase. Fractions from the extracts were collected, and the most active compound was used in the antibacterial studies. The FT-IR studies of the active compound revealed the presence of OH, CH, C=C, and C-O-C functional groups with the corresponding peaks of 3324, 2922, 1610, and 1073 cm^{-1}, respectively The antibacterial assay of the active compound was conducted which inhibited the growth of S. aureus and E.coli by measuring the zone of inhibition of difference concentration ranging from 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25mg/ml respectively, Ciprofloxacin was used as positive control in both S. aureus and E.coli, antibacterial activities in S.aureus showed zone of inhibition of 14 mm, 12 mm, 9 mm 7 mm, respectively and 32 mm at control. Also, E. coli showed 15 mm, 12 mm, 10 mm, 9 mm, respectively, and 29 mm at the control.

Keywords: Antibacterial; Column; Inhibition; *Guiera senegalensis;* Photochemical.

INTRODUCTION

Phytochemicals (from the Greek word *phyto*, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans beyond those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plant's 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 phytochemicals (Aylate *et al*., 2017; Sokan-Adeaga *et al*., 2023). It is clearly known that plants and microalgae have roles in the protection of human health when their dietary intake is significant (Muhamad *et al*., 2023; Zango *et al*., 2023; Sobri *et al*., 2024). More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics, and chemical characteristics. About 150 phytochemicals have been studied in detail (Roy and Datta, 2019; Onuh and Pathak, 2024; Rodríguez-Negrete *et al*., 2024). In wide-ranging dietary sources, phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs, and spices (Hossain *et al*., 2025). Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, beans, legumes, and soybeans are common sources (El-Saadony *et al*., 2025). Phytochemicals accumulate in different parts of the plants, such as in the leaves, stems, flowers, fruits or seeds (Shahidi, Ambigaipalan and Chandrasekara, 2017; El-Saadony *et al*., 2025). Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking, and growing conditions. Phytochemicals are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Brindha, 2016). It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect humans against diseases (Tariq *et al*., 2021; Yang, 2022).

Phytochemicals are found in medicinal plants such as *Guiera senegalensis.* The *Guiera senegalensis,* locally known as 'Sabara' or 'Koren magani' or 'Barbarta' in Hausa Language of Northern Nigeria, belongs to the family Combrataceae. It is widely distributed in the savannah region of West and Central Africa (Mathieu, Taïbou, and Aminata, 2018; Awé *et al*., 2024). The plant has been reported to have numerous traditional medicinal uses, such as treatment of dysentery, diarrhea, and malaria fever (Awé *et al*., 2024). It has also been reported to be used to manage leprosy, abdominal pains, epilepsy, depression, cold and cough, snake bite, syphilis, hypertension, diabetes, breast cancer, and Jaundice (Adam *et al*., 2024; Kabir, 2024). Phytochemical screening for *Guiera senegalensis* showed a significant number of secondary metabolites, namely anthraquinones, terpenoids, saponins, alkaloids, flavonoids, tannins, and terpenoids (Al Shafei, Elshafie, and Nour, 2016). Its cyanogenic glycosides were assayed in different organs of the plant, such as leaves, fruits, and stem bark (Al Shafei, Elshafie, and Nour, 2016). This research aimed to investigate the phytochemical and antibacterial efficacy of the ethanolic leaves extract of *Guiera senegalensis*. The study, therefore, **reports** the phytochemical screening and antibacterial

activity of the leaf extracts of *Guiera senegalensis* against some selected microorganism strains.

MATERIALS AND METHODS

Sample collection

The fresh leaves of *Guiera senegalensis* were collected at Makada village, Batagarawa Local Government, Katsina, Katsina state. The sample was authenticated in the Department of Basic and Applied Sciences, Hassan Usman Katsina Polytechnic, Katsina, Nigeria. The leaves of *Guiera senegalensis* were sorted out 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 a motor and pestle.

The reagents and Thin Layer Chromatography (TLC) precoated plates, Silica gel, were purchased from Merck Pharmaceutical. The reagents were of analytical grade. Mueller Hintor Agar was obtained from Bristol Scientific Company Limited. The incubator was a Eurotherm Model No. 2216E incubator. Ciprofloxacin was used as a positive control and obtained **from** Merck Pharmaceuticals

Test Organisms

The test organisms for antimicrobial analysis were two bacteria. Pure isolates of these organisms were obtained from the Department of Microbiology, Katsina State Orthopaedic Hospital, Katsina, Nigeria. The isolates include *Salmonella, Staphylococcus aureus,* and *Escherichia coli.*

Preparation of the Ethanolic Extracts Using the Maceration Method

For the ethanolic extract preparation, 10 g of the powdered sample was dissolved in 100ml of ethanol in a conical flask that contain magnetic bar, and it was covered with a foil and set on a magnetic stirrer for regular stirring. After 24 hours, the mixture was filtered using 11 µm Whatman filter paper. The filtrate was stored in a refrigerator, **while** the residue **was allowed to dry and repeatedly extracted with** the same volume of solvent. The combined filtrate was concentrated using rotary evaporation to a low volume (15 mL) and exposed to dryness at room temperature (27 ˚C) to afford the solid crude extract, which was weighed.

Phytochemical Screening Procedure:

The phytochemical screening was conducted to detect the presence of key secondary metabolites in the leaf extract of the *Guiera senegalensis*. These include compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds. The analyses were carried out using the standard procedures (Egbuna *et al*., 2018; Balamurugan, Fatima, and Velurajan, 2019; Nagori *et al*., 2025).

Alkaloids

For the alkaloid determination, 10 mg of the extract was stirred with 5 mL of 1% aqueous hydrochloric acid on a water bath and filtered. 2 mL of the filtrate was divided into two portions. To the first portion, 1 drop of Mayer's reagent was added, and yellow color precipitate was observed. To the second portion, 1 drop of Wagner's reagent was observed to give a reddish- brown precipitate (Momoh *et al*., 2021).

Saponins

For the analysis of saponins, 10 mg of extract was diluted with distilled water and made up to 20 mL. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm (2 cm) layer of foam indicates the presence of saponins-(Momoh *et al*., 2021).

Phenolic Compounds

To test the presence of phenolics, 10 mg of extract was dissolved in 5 ml of distilled water, and then a few drops of 5% ferric chloride solution were added. A dark green color indicates the presence of a phenolic compound (Momoh *et al*., 2021).

Anthraquinones

The crude extract was taken into a dry test tube. Then 1.0 ml of chloroform was added and shaken for 5 min, and it was then shaken with an equal volume of 10 % ammonia solution. A pink violet or red color in the ammonia layer (lower layer) indicated the presence of anthraquinone (Momoh *et al*., 2021).

Terpenoids

For the terpenoids, the crude extract was added to a few ml of chloroform. Then a few ml of concentrated H_2SO_4 were added, and two layers were formed. The reddish-brown coloration in the interface indicated the presence of terpenoids (Momoh *et al*., 2021).

Tannins

To test the presence of tannins, the crude extract was added to 2.0 ml of distilled water and boiled in a test tube, and three drops of 10% of $FeCl_{3\ were}$ also added. The brownish green color indicates the presence of tannins (Momoh *et al*., 2021).

Flavonoids

The crude extract was added to 5.0 ml of distilled water and boiled for 5 min. Three drops of 20 % NaOH solution were added. The color changes from colorless to yellow. Then, 5 drops of 1 % of HCl were added to the mixture. The presence of flavonoids was interpreted by observing the decolorization of the yellow color (Momoh *et al*., 2021).

Steroids

The crude extract was dissolved in 1.0 ml of chloroform, then added slowly 2.0 ml of concentrated sulfuric acid, H_2SO_4. Two layers were formed, a lower layer which is in yellow color with green fluorescence, and a reddish-brown color on the upper layer, which was interpreted as a steroid ring (Momoh *et al*., 2021).

Isolation of Active Compounds

TLC chromatography

The TLC was performed as a free assay to observe the fractions present in the leaves of the *Guiera senegalensis*. The TLC of the crude leaf extracts was conducted on a precoated TLC plate using ethanol as the mobile phase. The results showed yellow and green fractions, with green being more prominent. The main purpose of this TLC analysis was to compare the results obtained with those from the column chromatography for confirmation

Column Chromatography

Column chromatography was performed using a wet-loaded silica gel column. Weighed 20 g of silica gel into a 250 mL beaker and mixed it with ethanol (the TLC solvent) to form a slurry, which was allowed to stand for 2 minutes to remove air bubbles, followed by

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packing the column bottom with sand and a cotton-wool bed. The slurry was loaded into the column after vigorous stirring, and the column walls were rinsed with the solvent mixture to push down any stock silica gel; the stopcock was opened to initiate drainage and ensure proper packing, and closed when the solvent front reached the top of the silica gel, yielding a column about 10 cm long and 2 cm in diameter. To load the sample, 0.5 g of ethanol extract was dissolved in ethanol, and a small amount of silica gel was added to form a slurry, which was then placed onto the packed bed; the column sides were rinsed to push the sample down gently without disturbing the bed, and an additional cotton-wool plug was placed on top. Ethanol was poured as the mobile phase to begin elution, the stopcock was opened to allow the mixture to pass through the column, and four fractions were collected in separate test tubes, with the most active fraction selected for FTIR characterization and further antibacterial testing.

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy of the ethanolic extracts was carried out in order to determine the types of functional groups present. The IR spectra of *Guiera senegalensis* leaf extract were recorded using a Perkin Elmer System 2000 (Waltham, MA, USA), with a wavelength range of 4000 to 400 cm^{-1} at 4 cm^{-1} resolutions and 16 scans. The potassium bromide technique (KBr) was applied to the samples with the proportion of 1:20 (w/w).

Antibacterial Activities

Preparation of Mueller Hinton Agar (MHA) Media

3.9 g of Mueller-Hinton agar was measured. The MHA powder was dissolved in 100 mL of distilled water in 500ml conical flask, with constant stirring of the mixture to ensure proper mixing. After stirring, the MH agar solution was boiled using an electric heating plate at 100°C, and then the MH agar solution was autoclaved for sterilization at a temperature of 121°C for 15 minutes. After, the hot sterilized MH agar solution was allowed to cool for at least 5 minutes, and then poured into the petri-plates in the laminar air flow cabinet. Each of the petri-plates contained approximately 25mL 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. Clinically isolated Escherichia coli and Staphylococcus aureus were streaked into (MHA) agar surfaces using a sterile wire loop.

From the four different concentrations of the plant leave extracts which were 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL, two pieces of 11µm Whatman filter paper were immersed into each of the respective plain containers containing four different concentrations and allowed to soaked for 20 minutes, using sterilized forceps and wire loop, one pieces of Whatman filter paper from the four different concentrations was arranged 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. After the 24-hour incubation, a clear zone was observed as a zone of inhibition, which was measured in millimeters (mm). Antibacterial activity was calculated by measuring the clear zone produced in millimeters.

RESULTS AND DISCUSSION

The extraction of 10 g of the *Guiera senegalensis* afforded a yield of 2.36 g of the crude extract, and the percentage (%) yields are presented in Table 1. This implies that there are more polar compounds in the leaves of *Guiera senegalensis* than non-polar compounds

Table 1.The percent yields of the ***Guiera senegalensis*** ethanolic leaf extract

Solvent	Weight (g)	Yield (%)	Color
Ethanol	2.36	23.6	Green

Table 2. The qualitative analysis of phytochemical constituents of the *Guiera senegalensis* ethanolic leaves extract

Phytochemical Constituents	**Results**
Alkaloids	**++**
Tannins	**+**
Saponins	**+**
Flavonoids	**+**
Terpenoids	**++**
Steroids	**++**
Anthraquinone	**++**
Phenolic	**++**

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

The qualitative phytochemical analysis of the ethanolic extract of *Guiera senegalensis* showed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, Anthraquinone and phenolic. As **shown** in Table 2, this finding is similar to Momoh *et al.,* who reported the results of phytochemical screening of *Guiera senegalensis,* indicating the presence of cardiac glycosides, saponins, flavonoids, tannins, steroids, terpenoids, and alkaloids. Also, the antimicrobial activity of the extracts against ten pathogenic organisms revealed moderate to good inhibition of six of the ten bacterial and fungal species (Momoh *et al*., 2021). In addition, the ethyl acetate extract appeared to have the highest zone of inhibition (24 mm) and best minimum inhibitory concentration (2.5 mg/mL) against Staphylococcus aureus. The cytotoxicity effects revealed that the chloroform extracts exhibited the highest lethality on brine shrimp larvae at LC50 value of 58.908 µg/mL.

Table 3. Thin Layer Chromatography of *Guiera senegalensis* ethanolic leaf extract

Sample	**Result**
Guiera senegalensis leaf extract	Green, Yellow, reddish brown, orange

The TLC result indicated the separation of the leaf extract into four distinct fractions: a yellow band, a green band, reddish brown, and orange. Among these, the green fraction was found to be the predominant, while the other fractions appeared in smaller proportions. This suggests that the crude sample contained a major component and at least minor components, which were successfully resolved on the TLC plate. The difference in band intensities correlates with the relative abundance of each compound in the mixture.

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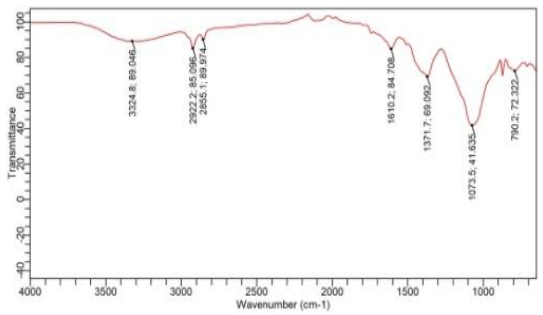
These findings are in agreement with the general principle of the TLC separation, where compounds migrate at different rates depending on their polarity, affinity to the stationary phase (silica/alumina), and solubility in the mobile phase (solvent system). According to Stahl *et al*., TLC can effectively distinguish compounds by producing bands of different colors and Rf values, reflecting differences in polarity and molecular structure (Ashworth and Stahl, 2013).

Table 4. Column Chromatography *Guiera senegalensis* ethanolic leave extract

Sample	Result
Guiera senegalensis extract	Green, Yellow, reddish brown, orange

From the column chromatography of the extract, it also produced four distinct fractions, characterized by green, yellow, orange, and reddish brown. Thus, the green fraction appeared most prominently similar to the TLC, while the other fractions were present in smaller proportions. The dominance of the green fraction suggests that it represents the major active compound(s) within the leaves, while the other fractions might likely correspond to secondary or minor phytochemical constituents. This result is consistent with the principle of column chromatography, where compounds are separated based on their polarity, adsorption to the stationary phase, and differential solubility in the mobile phase. The strong elution of the green fraction indicates a compound of higher polarity, which is typical of many biologically active flavonoids and phenolic compounds that have been reported in *Guiera senegalensis*.
Findings from previous studies support this observation. Harborne *et al*. reported that the green coloration in plant chromatographic fractions is often associated with flavonoids and tannins, which are abundant in senegalensis species (Harborne, 1998). In agreement, Edeoga *et al*. identified green fractions in chromatographic separations of medicinal plants as rich in flavonoid compounds with known antimicrobial and antioxidant activities (Edeoga, Okwu, and Mbaebie, 2005). Similarly, Orwa *et al*. revealed that *Guiera senegalensis* contains a high concentration of flavonoids and condensed tannins, which often appear as greenish fractions during chromatographic separation (Orwa, 2009).



FTIR spectrum of the *Guiera senegalensis* leaf extract

The broad O–H stretching band at 33240 cm^{-1} suggests the presence of hydroxyl groups, which are characteristic of phenols and flavonoids, known for their strong antioxidant properties. This band is commonly observed in FT-IR spectra of plant extracts, particularly those containing phenolic compounds, flavonoids, or terpenoids (Singh *et al*., 2022). The broad nature of this band suggests the presence of hydrogen bonding between molecules. Similar O-H stretching vibrations have been reported in various plant extracts, including those from *Guiera senegalensis* (Ahmed *et al*., 2022).

The absorption bands at 2822 cm^{-1} are attributed to the C-H stretching vibrations of methyl ($-CH_3$) and methylene ($-CH_2-$) groups, respectively. These bands are indicative of the presence of aliphatic compounds, such as fatty acids, waxes, or terpenes, in the Guiera senegalensis extract (Ahmad *et al*., 2024). These bands are consistent with the presence of aliphatic hydrocarbons, which have been reported in plant extracts (Ahmad *et al*., 2024).

The C=C stretching vibration at 1610 cm^{-1} confirms the presence of aromatic rings, which could be attributed to flavonoids, alkaloids, or polyphenols (Ahmad *et al*., 2024). These compounds play a crucial role in antibacterial activities (Ahmad *et al*., 2024).. This band is similar to those reported in studies on plant extracts containing aromatic compounds (Ahmed *et al*., 2022).

The Symmetric deformation peak at 1371 cm^{-1} may indicate the presence of Phenols, Carboxylates, which have potential pharmacological effects, including anti-bacterial and anti-inflammatory activities. This band is consistent with the presence of aliphatic hydrocarbons or phenolic compounds, which have been reported in plant extracts (Ahmad *et al*., 2024).
The ester and ether absorption bands at 1073 cm^{-1} suggest the presence of glycosides or carboxyl compounds, which are common in medicinal plants and may contribute to bioactivity.
This band is similar to those reported in studies on plant extracts containing glycosides or esters (Ahmad *et al*., 2024).

The FT-IR analysis of *Guiera senegalensis* confirms the presence of several bioactive functional groups, including phenols, flavonoids, and alkaloids, which may contribute to the plant's antioxidant, anti-inflammatory, and antimicrobial properties. These findings align with previous phytochemical studies and further support the potential of *Guiera senegalensis* in medicinal and pharmaceutical applications.

Table 5. FTIR peak assignments of *Guiera senegalensis* active components

S/N	Wave number (cm^{-1})	Functional group	Vibration mode	Possible compound
1	3324 cm^{-1}	O–H Stretch	Hydrogen bond	Phenols, Alcohols (Flavonoids, Polyphenols)
2	2922cm^{-1}	C---H Stretch Alkane	Asymmetric stretch	Fatty Acids, Aliphatic Compounds
3	2853cm^{-1}	C---H weak Stretch	Conjunction	Aromatic Compounds
4	1610cm^{-1}	C==C	Conjugated Alkane	Flavonoids, Polyphenols

			/aromatic rings	
5	1371cm^{-1}	C–H Bending	Symmetric deformation	Phenols, Carboxylates
6	1073cm^{-1}	C–O–C Stretch	Ether linkage	Polysaccharides, Flavonoid Glycosides
7	790cm^{-1}	C–H Bending	Out-of-plane bending	Aromatic Rings, Alkenes

Table 6. Antibacterial Activities of *Guiera Senegalensis* Active Components against Gram-Positive Bacteria

Concentration	250 mg/mL	125 mg/mL	62.5 mg/mL	31.25 mg/mg	Control
Zone of inhibition in (mm)				**+ve**	
S. aureus	14 mm	12 mm	9 mm	7 mm	32 mm

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

Table 7. Antibacterial Activities of *Guiera Senegalensis* Active Components against Gram-Negative Bacteria

Concentration	250 mg/mL	125 mg/mL	62.5 mg/mL	31.25 mg/mg	Control
Zone of inhibition in (mm)				**-ve**	
E. Coli	15 mm	12 mm	10 mm	9 mm	29 mm

Key: (-ve) = Negative, positive control = (ciprofloxacin), (mm) = millimeters

This result is similar to the findings of Ifijen et al.; it was observed that the antibacterial activity of the extract was highly significant and comparable with conventional anti-malarial drugs such as Chloroquine (Ifijen *et al.*, 2019). This antibacterial activity could be attributed to the presence of active phytochemicals such as tannins, saponins, anthraquinones, and flavonoids, etc. This explains why it has found its use in traditional medicine among the locals of tropical countries in the treatment of malaria, respiratory congestion, and fever, among others.

It was established that medicinal plants are used for the discovery and screening of the phytochemical constituents, which are very helpful for the manufacturing of new drugs for the treatment of various diseases (Labaran *et al.*, 2024; Musa *et al.*, 2025). The results obtained in the present study have shown that *Guiera senegalensis* leaves have a high concentration of alkaloids and a low concentration of tannins and saponins. These observations and the findings of the present study suggest that *Guiera senegalensis* extract is credited with curing several diseases and infections in Africa (Ali, 2020; Anka *et al.*, 2020; Umma *et al.*, 2023). That is why the determination of the active compound was conducted in order to know whether the active compound in *Guiera senegalensis* is responsible for that activity. Thus, we hope that the active compounds identified by our study in the *Guiera senegalensis* will help treat different diseases of this particular region of Africa. The results could serve for further pharmacological and phytochemical research.

Conclusion

The phytochemical screening of the leaves extract of *Guiera senegalensis* showed the presence of phytochemical constituents such as alkaloids, steroids, flavonoids, phenolics, tannins, saponins, and terpenoids. This might be responsible for the medicinal properties of the plant. The ability of the extract to inhibit the growth and show bactericidal activity against *Staphylococcus aureus* and *Escherichia coli* at concentrations ranging from 250 mg/mL, 125 mg/mL, 62.5 mg/mL, and 31.25 mg/mL revealed that the leaves of *Guiera senegalensis* could be used to cure infections that may be caused by these microorganisms. More research is needed to investigate the toxicological studies of the extracts; further studies on the medicinal properties and phytochemical compounds of the plant is needed to be investigated.

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