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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF METHANOLIC AND AQUEOUS STEM EXTRACTS OF *Guiera senegalensis* AGAINST BACTERIAL PATHOGENS

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ABSTRACT

With the growing resistance of pathogens to conventional antibiotics, there is an increasing interest in exploring alternative treatments derived from medicinal plants, particularly those rich in bioactive compounds. Guiera senegalensis, a medicinal shrub native to West and Central Africa, has traditionally been used to treat gastrointestinal disorders, fever, and rheumatism. This study aimed to investigate the presence of bioactive compounds and antimicrobial properties of the stem extract of G. senegalensis, with a particular focus on its efficacy against bacterial infections.Phytochemicals were extracted through cold maceration using methanol and distilled water as solvents. The antimicrobial effects of the extracts on clinical isolates of Salmonella typhi, Escherichia coli, and Pseudomonas aeruginosa were evaluated via the agar disc diffusion method.Phytochemical analysis of both the methanol and aqueous extracts revealed the presence of alkaloids, flavonoids, tannins, and saponins. The methanol extract exhibited higher concentrations of flavonoids, tannins, and saponins, whereas the aqueous extract contained more alkaloids. Antimicrobial assays demonstrated that both extracts inhibited all tested bacterial strains, with the methanol extract demonstrating significant efficacy against E. coli, with an inhibition zone of 25 mm at 500 µg/mL. The minimum inhibitory concentration (MIC) values ranged from 6.25 to 500 µg/mL, indicating that these extracts may serve as alternative treatments for infections, particularly those resistant to antibiotics.These findings substantiate the therapeutic potential of G. senegalensis as a source of antimicrobial agents, corroborating its traditional use in treating infectious diseases.

Keywords: Aqueous extract, Antimicrobial properties, *Guiera senegalensis*, Methanol extract, Phytochemicals.

INTRODUCTION

Historically, people have turned to natural remedies to improve their health, and modern medicine largely relies on drugs sourced from natural products (Bhandari, 2024). A variety of antimicrobial agents have been discovered from both synthetic and natural origins to treat and manage infections. However, only a small fraction of these antimicrobial agents from natural products are available in the global market (Hayashi *et al.,* 2013). The rise of bacteria resistant to multiple drugs has further complicated the availability and affordability of many antibiotics currently prescribed worldwide (Lin *et al.,* 2022). This situation reduces treatment effectiveness, increases illness and mortality rates, and raises healthcare costs (Lin *et al.,* 2022). The problem is even more severe in low-income nations due to the lack of effective monitoring systems, laboratory diagnostics, and financial barriers to accessing suitable antimicrobials (Vitry *et al.,* 2020). Researching new antibiotics from natural products is a vital part of modern medicine, aiming to tackle the socioeconomic and health issues caused by microbes resistant to multiple drugs (Sathishkumar, 2024).

Guiera senegalensis, belonging to the Division Magnoliophyta, Order Myrtales, Family Combretaceae, and Genus Guiera, is often known as Senegal guiera or "Sabara" in Hausa. This (semi-) evergreen shrub typically grows to a height of 1–3 m, and is notable for its black glandular spots. Its bark is fibrous, with a texture that ranges from smooth to finely scaly, and its color varies from gray to brown, whereas the young branches are soft and covered with hair. Wood is characterized by a coarse grain and knotted texture, being short and hard, with a whitish or reddish tint. The leaves are grey-green, arranged in opposite pairs, with a short, hairy petiole measuring 2–5 mm, and an oblong-elliptical blade. The flowers are small and yellowish-green, and the linear fruit, which is 3-4 cm long, is topped by a persistent perianth and is densely silky and velvety. *Guiera senegalensis* flourishes in areas with low rainfall and light and dry soils, and is commonly found across Western Africa, from Mauritania and Senegal to Nigeria, Cameroon, and East Africa, including Egypt and Sudan (Somboro *et al.,* 2011).

G. senegalensis holds a significant place in traditional African medicine due to its therapeutic benefits for various ailments. The leaves are particularly known for their ability to treat conditions like jaundice, diabetes mellitus, hypertension, cough, arthritis, enteritis, diarrhea, and malaria. After drying, the roots are ground into a powder to treat wounds, including diabetic ulcers, skin inflammation, and other injuries (Alshafei *et al.,* 2016). In addition to its medicinal roles, *G. senegalensis* is also used in cosmetic production, as a component in animal feed, and as a fuel source (Somboro *et al.,* 2011).

Although less extensively studied, the stem bark of *G. senegalensis* may exhibit antimicrobial activity. Numerous studies have demonstrated the antimicrobial properties of *Guiera senegalensis* extract. Elmalik *et al.* (2022) demonstrated that ethanolic leaf extracts exhibited antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans*. Similarly, Momoh *et al.* (2021) observed that an ethyl acetate extract of *G. senegalensis* leaves produced the largest zone of inhibition (24 mm) and the most effective minimum inhibitory concentration (2.5 mg/mL) against *Staphylococcus aureus*. The antimicrobial efficacy of these extracts was attributed to the presence of bioactive phytochemicals, including tannins, alkaloids, flavonoids, saponins, steroids, and terpenoids.The root extracts of *G. senegalensis* have also shown antibacterial activity against clinical isolates, including *E. coli* and *S. aureus*. Alshafei *et al.* (2016) highlighted the antimicrobial potential of the root extracts, suggesting their applicability in treating infections caused by these pathogens. Sadiq *et al.* (2024) reported that fractions of the stem bark tested against *E. coli, S. aureus*, *Streptococcus pneumoniae, P. aeruginosa, Salmonella typhi*, and *Klebsiella pneumoniae* displayed varying degrees of inhibition. The antimicrobial properties of stem bark are thought to be due to the presence of phytochemicals, such as alkaloids, tannins, flavonoids, and saponins (Umma *et al.,* 2023).

Phytochemical investigations of *Guiera senegalensis* have revealed a diverse array of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic acids. Notably, the leaves contain bioactive galloylquinic acids, which contribute to antimicrobial properties. These compounds were isolated and characterized by column chromatography and high-performance liquid chromatography (HPLC) (Bouchet & Pousset, 2000). The presence of these bioactive constituents is believed to contribute significantly to the documented antimicrobial, anti-inflammatory, and anti-diarrheal properties of plants, as highlighted in previous studies by Somboro *et al.* (2011).

Although *Guiera senegalensis* has been traditionally used for medicinal purposes and some preliminary scientific evidence supports its benefits, there is a notable absence of thorough clinical studies to confirm its effectiveness and safety. Most current research has concentrated on in vitro studies, with only a few in vivo or human clinical trials. Additionally, there are concerns regarding possible genotoxic effects, emphasizing the necessity for comprehensive toxicological assessments (Alshafei *et al.,* 2016). These gaps highlight the critical need for further investigation to thoroughly evaluate the therapeutic potential and safety of *G. senegalensis* to validate its traditional applications and incorporate them into contemporary pharmacological practices. This study focused on analyzing the bioactive compounds and antibacterial properties of *G. senegalensis* stem extract against certain bacterial pathogens.

MATERIALS AND METHODS

Study Area

The study was conducted at the Biological Sciences Laboratory of the Federal University of Kashere, situated in Kashere, within the Akko Local Government Area of Gombe State, Nigeria. Kashere is located at an elevation of 431 m above sea level, with a recorded population of 77,015, according to the 2006 census. The exact geographical coordinates of Kashere are 9°9′12″N and 11°01′04″E (Kolawole *et al*.,2024).

Sample Collection

Whole stems of *Guiera senegalensis* were collected from the botanical garden at the Federal University of Kashere's campus, located in the Akko Local Government Area of Gombe State. A taxonomist identified and authenticated the plant samples, which were further validated at the Department of Biological Sciences at the Federal University of Kashere.

Preparation of Plant Extracts

The freshly collected stem of *Guiera senegalensis* were washed with tap water and then distilled water. Subsequently, the stems were allowed to air-dry at a constant room temperature of 25°C for 14 days. After drying, they were ground into a fine powder using a mixer-grinder and then stored in containers that were sealed to prevent air exposure (Ahmed *et al.,* 2022)

Extraction of Plant Material

The stem powder of *Guiera senegalensis*, weighing 50 g, was subjected to extraction in a 250 mL solution using two separate flasks, one containing methanol and the other distilled water, over a duration of seven days. The resulting extracts were filtered through Whatman No. 1 filter paper and subsequently evaporated to dryness at 40°C in a hot water bath to produce stock solutions. Following evaporation, the extracts were stored in airtight containers at 4°C in a refrigerator until use (Mafuyai *et al.,* 2019).

Preliminary Phytochemical Screening

The analysis of phytochemicals in the plant samples was performed using the methodologies specified by Sofowora (1993) and Das *et al.* (2010). This involved a series of tests, including Wagner's test to detect alkaloids, ferric chloride test for the presence of phenols, gelatin test to identify tannins, lead acetate test for flavonoids, foam test to determine saponins, and acetic acid test for steroids.

Test Organisms

Bacterial isolates were obtained from hospitalized patients at the Microbiology Laboratory of the Federal Teaching Hospital Gombe. The isolates, specifically *Salmonella typhi*, Pseudomonas aeruginosa, and *E. coli*, were subsequently sub-cultured on nutrient agar plates for 24 hours and stored at 4°C (Oladipo *et al.,* 2018).

Antimicrobial Activity of the *G. Senegalensis* Stem Extracts

The agar disc diffusion method was used as reported by Odonye *et al.* (2021) to evaluate the antibacterial activity of the *G. senegalensis* stem extract against three pathogenic bacterial strains: *E. coli*, *S. typhi*, and *P. aeruginosa*. The agar disc diffusion method was chosen due to its sensitivity and reproducibility.The extract was dissolved in dimethyl sulfoxide (DMSO), sterilized by filtration, and stored at 4°C. Sterile test tubes were prepared: one with only nutrient broth, and the other with nutrient broth plus the test organism . Test tubes contained 1 ml extract, 2 ml nutrient broth, and 1 ml test organism solution .To compare the effects, augmentin, a standard antibiotic, was used to assess the zones of inhibition produced by the bacterial strains. In distilled water, four different concentrations (500, 250, 125, and 6.25 µg/mL) of the stem extract and standard antibiotics were prepared in distilled water. The bacterial strains were inoculated on sterile Mueller-Hinton agar plates, with a control using augmentin maintained at 37°C for 3 h. After incubation at 37°C for 18–24 h, the inhibition zones surrounding the discs were measured in millimeters. The antibacterial activity of the extracts was determined by measuring the size of the inhibition zones, including the disc diameter, with zones smaller than 9 mm considered inactive. The mean diameters of the inhibition zones were calculated in triplicates (Odonye *et al.,* 2021).

Determination of Minimum Inhibitory Concentration of *Guiera senegalensis* Stem Extracts

Crude stem extracts of *Guiera senegalensis* were tested in triplicate to determine the minimum inhibitory concentration (MIC) for each bacterial test organism. In sterile test tubes, different concentrations of the extracts (500 µg/mL, 250 µg/mL, 125 µg/mL, and 6.25 µg/mL) were mixed with 1 ml of the extract, 2 ml of nutrient broth agar, and 1 ml of test organisms, which had been previously adjusted to a 0.5 McFarland turbidity standard for bacterial isolates. Two control test tubes were prepared: one with only nutrient broth, and the other with nutrient broth plus the test organism. Following inoculation, test tubes were placed in an incubator at 37°C for 18–24 h. The turbidity level in each tube was then assessed to evaluate microbial growth (Mafuyai *et al.,* 2019).

RESULTS AND DISCUSSION

Results

The preliminary phytochemical screening results for the aqueous and methanolic extracts of *Guiera senegalensis* are summarized in Table 1. The presence of flavonoids, alkaloids, tannins, and saponins was confirmed in both extracts, with notable differences in the concentrations of these compounds. Steroids were absent in the aqueous extracts.

Table 1: Preliminary Phytochemical Screening of Methanolic and Aqueous Extracts of *Guiera senagalensis*

S/N	Parameter	Methanol Extract	Aqueous Extract
1	Alkaloids	+	++
2	Flavonoids	++	+
3	Tannins	+++	++
4	Steroids	+	-
5	Saponins	+++	++

+++ = strongly present, ++ = Moderately present , + = mildly present , - = Absent

The results indicated that the bacterial isolates exhibited different degrees of sensitivity to the aqueous extract of *Guiera senegalensis* across various concentrations (500, 250, 125, and 6.25 µg/mL), as shown by the inhibition zones in Table 2, which expanded at higher concentrations. All tested isolates were responsive to the positive control (Aug) at 30 µg/mL, but *Salmonella typhi* was particularly sensitive to the extract at 500 µg/mL, with a 20 mm inhibition zone.

Table 2: The Antibacterial Activity of the Aqueous Extract of *Guiera senegalensis* against Selected Bacterial Isolates

Test Organisms	Concentrations (µg/mL) Zones of Inhibition (mm)				Positive Control (Aug)
	500µg/mL	**250 µg/mL**	**125 µg/mL**	**6.25 µg/mL**	**30 µg/mL**
Salmonella typhi	20.0 ± 0.12	7.0 ± 0.05	11.0 ± 0.08	10.0 ± 0.04	27.0 ± 0.10
E.coli	11.0 ± 0.09	8.0 ± 0.03	7.0 ± 0.06	6.0 ± 0.02	25.0 ± 0.07
Pseudomonas aeruginosa	12.0 ± 0.11	8.0 ± 0.04	6.0 ± 0.03	6.0 ± 0.01	26.0 ± 0.08

Aug= Augmentin

The findings of this study demonstrated that the bacterial isolates exhibited varying degrees of sensitivity to the methanol extract of *Guiera senagalensis* at different concentrations (500, 250, 125, and 6.25 µg/mL), with enhanced activity noted at higher concentrations. Nonetheless, all tested isolates were susceptible to the positive control (Aug) at 30 µg/mL. Notably, at 500 µg/mL, *E. coli* was susceptible to the extract, exhibiting a 25 mm zone of inhibition, as shown in Table 3

Table 3: The Antibacterial Activity of the Methanolic Stem Extract of *Guiera senegalensis* Against Selected Bacterial Isolates

Test Organisms	Concentrations (µg/mL) Zones of Inhibition (mm)				Positive Control (Aug)
	500 (µg/mL)	**250 (µg/mL)**	**125 (µg/mL)**	**6.25 (µg/mL)**	**30 (µg/mL)**
Salmonella typhi	12.0 ± 0.25	11.0 ± 0.18	9.0 ± 0.12	7.0 ± 0.09	23.0 ± 0.30
E.coli	25.0 ± 0.32	12.0 ± 0.20	8.0 ± 0.10	7.0 ± 0.08	30.0 ± 0.35
Pseudomonas aeruginosa	11.0 ± 0.22	10.0 ± 0.15	8.0 ± 0.11	6.0 ± 0.07	26.0 ± 0.28

Aug= Augmentin

Table 4 presents the minimum inhibitory concentration (MIC) values for the aqueous stem extract of *Guiera senegalensis*, illustrating its efficacy against various bacterial isolates. The findings indicated that the minimum inhibitory concentration (MIC) for *Salmonella typhi* was between 250 and 500 µg/mL, for *Escherichia coli* it ranged from 6.25 to 500 µg/mL, and for *Pseudomonas aeruginosa* it ranged from 125 to 500 µg/mL.

Table 4: The Minimum Inhibitory Concentrations of Aqueous Stem Extract of *Guiera senegalensis* on Bacteria Isolates

Test Organisms	Minimum Inhibitory Concentrations (µg/mL)			
	500µg/mL	**250µg/mL**	**125µg/mL**	**6.25µg/mL**
Salmonella typhi	+	+	-	-
E.coli	+	+	+	+
Pseudomonas aeruginosa	+	+	+	-

+ = Turbid (presence of Growth) - = Not Turbid (Absence of Growth)

Table 5 shows the minimum inhibitory concentration (MIC) values for the methanol stem extract of *Guiera senegalensis* evaluated against various isolates. The findings showed that the minimum inhibitory concentration (MIC) for *Salmonella typhi* was between 250 and 500 µg/mL. In contrast, *Escherichia coli* had an MIC ranging from 6.25 to 500 µg/mL, and *Pseudomonas aeruginosa* had an MIC that fell within the 125–500 µg/mL range.

Table 5: The Minimum Inhibitory Concentrations (MIC) of *Guiera Senegalensis* Methanol Stem Extract on Bacteria Isolates

Test Organisms	Minimum Inhibitory Concentrations (µg/mL)			
	500 µg/mL	250 µg/mL	125 µg/mL	6.25 µg/mL
Salmonella typhi	+	+	-	-
E.coli	+	+	+	+
Pseudomonas aeruginosa	+	+	+	-

+ = **Turbid (presence of Growth)** **- = Not Turbid (Absence of Growth)**

Discussion

In the preliminary phytochemical analysis of *G. senegalensis* stem extracts, both aqueous and methanolic solvents were used to identify the presence of key bioactive compounds, such as alkaloids, flavonoids, tannins, and saponins. The methanolic extract is rich in flavonoids, tannins, and saponins, whereas the aqueous extract contains moderate amounts of alkaloids and flavonoids. Notably, the absence of steroids in the aqueous extract suggests limited solubility in water, whereas methanol effectively extracted these compounds. These findings are consistent with those of previous studies by Garba et al. (2018) and Salihu and Usman (2016), who identified these compounds in the root and stem bark extracts of *G. senegalensis*. However, the absence of steroids in the aqueous extract contrasts with the studies by Somboro *et al.* (2011) and Momoh *et al.* (2021), which detected steroids in the leaf extract of both solvents, suggesting potential variations in extraction methods or geographical factors. The strong presence of saponins and tannins in the methanol extract was consistent with the findings of Salihu and Usman (2016), who also reported high concentrations in methanolic leaf extracts. Tannins and saponins are known for their antimicrobial and anti-inflammatory properties, supporting the observed potent antimicrobial activity of the methanol extract, as noted by Gurama *et al.* (2020). Tannins disrupt bacterial cell membranes, while saponins interfere with membrane integrity, enhancing antimicrobial activity .These results reinforce the preference of methanol as an effective solvent for extracting bioactive compounds with significant therapeutic potential.

This study assessed the antimicrobial effects of both aqueous and methanol extracts from *Guiera senegalensis* on *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa* across a range of concentrations (500, 250, 125, and 6.25 µg/mL). The results demonstrated that the antimicrobial activity intensified with increasing concentrations. The aqueous extract was particularly effective against *Salmonella typhi*, achieving a 20 mm zone of inhibition at 500 µg/mL, while the methanolic extract was the most potent against *E. coli*, producing a 25 mm zone of inhibition at the same concentration.These findings align with previous studies, such as Adebiyi *et al*. (2015), which reported strong antimicrobial effects against *E. coli* and *Salmonella typhi* using methanolic root extracts. Salihu and Usman (2016) also observed higher antimicrobial activity in methanolic leaf extracts against *P. aeruginosa*. The stronger activity in methanolic extracts is consistent with the greater solubility of bioactive compounds like alkaloids, flavonoids, and tannins in methanol, as observed in previous studies (Abigail *et al.*, 2024). However, the sensitivity of *Salmonella typhi* to the aqueous extract at 500 µg/mL contrasts with some studies, suggesting that variability is influenced by extraction methods, bacterial strain differences, or geographical factors. This variability may also be attributed to differences in the bacterial cell wall structures or efflux mechanisms (Abigail *et al.*, 2024). Augmentin, the positive control, showed larger inhibition zones for all the isolates, confirming its broad-spectrum efficacy.

The minimum inhibitory concentration (MIC) values for both aqueous and methanolic extracts of *Guiera senegalensis* were evaluated against *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa*. For the aqueous extract, the MIC values ranged from 250 to 500 µg/mL for *S. typhi*, from 6.25 µg/mL to 500 µg/mL for *E. coli*, and from 125 to 500 µg/mL for *P. aeruginosa*.Similar MIC values were observed for the methanol extract with comparable ranges .*E. coli* exhibited the lowest MIC values, likely due to its thinner cell wall structure, which facilitates extract penetration .These results align with previous studies by Garba et al. (2018), which reported similar MIC values for both aqueous and methanolic root and stem extracts against *E. coli* and *P. aeruginosa*. However, Somboro *et al*. (2011) noted that aqueous leaf extract was slightly less effective, particularly against *S. typhi*. Despite this, the current study demonstrated that the aqueous stem extract was still effective at concentrations as low as 250 µg/mL. Notably, *E. coli* showed the lowest MIC values for both extracts, highlighting its higher susceptibility compared to *S. typhi* and *P. aeruginosa*. These findings support the use of *G. senegalensis* in gastrointestinal infections, with methanol extracts showing better efficacy at lower concentrations. These results underscore the preference for methanol in extracting bioactive compounds with antibacterial activity.

CONCLUSION

This study highlights the significant antimicrobial activity of *Guiera senegalensis* stem extracts, particularly methanolic extracts, against bacterial pathogens. Future research should focus on isolating and characterizing bioactive compounds to develop novel antimicrobial agents.

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