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Phytochemical Composition and Broad-spectrum Antimicrobial Activity of *Guiera senegalensis* Methanolic Leaf Extract Against Clinical Isolates

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ABSTRACT

Background: The growing threat of antimicrobial resistance has intensified the search for alternative therapies using natural sources. *Guiera senegalensis*, a medicinal shrub widely used in African traditional medicine, has shown promising antimicrobial properties due to its rich phytochemical content.

Objectives: This study evaluated the phytochemical composition and the in vitro antibacterial and antifungal activities of the methanolic leaf extract of *G. senegalensis* against selected bacterial isolates.

Methods: Fresh leaves of *G. senegalensis* were collected, shade-dried, and extracted by maceration in 70% methanol for 72 hours. Phytochemical testing was conducted using standard qualitative methods. Antibacterial activity was assessed using the agar disk diffusion method on Mueller-Hinton agar, while antifungal activity was evaluated using the agar well diffusion method on potato dextrose agar (PDA). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth microdilution and tube dilution methods, respectively.

Results: Phytochemical testing revealed the presence of flavonoids, tannins, saponins, glycosides, and steroids, while alkaloids were absent. Antimicrobial activity was concentration-dependent, with *E. coli* and *S. aureus* showing the highest bacterial susceptibility (zones of inhibition up to 29 mm) and *F. oxysporum* exhibiting strong antifungal sensitivity (zone of inhibition= 25 mm at 500 mg/mL). MIC values ranged from 31.25 to 125 mg/mL for bacteria and fungi, while MBC values ranged from 62.5 to 250 mg/mL.

Conclusion: The methanolic leaf extract of *G. senegalensis* demonstrated high antibacterial and antifungal activity, particularly against gram-positive bacteria and filamentous fungi, supporting its potential as a natural antimicrobial agent.

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Introduction

The global rise in antimicrobial resistance, related to both bacterial and fungal pathogens, has become an urgent public health concern, threatening the efficacy of existing therapies, leading to more severe and difficult-to-treat infections, increased mortality rates, and higher healthcare costs [1]. As conventional antibiotics and antifungal agents continue to lose effectiveness due to resistance, there is a growing interest in alternative therapeutic methods, particularly herbal medicines. Historically, plants have served as critical reservoirs of bioactive compounds with potent pharmacological properties, many of which have been successfully translated into modern drugs [2]. One of these plants is *Guiera senegalensis*, abundant in the savanna and semi-arid zones of West and Central Africa [3]. Known locally as "Sabara" among the Hausa and "Geloki" among the Fulani populations of Nigeria, *G. senegalensis* has long been used in African traditional medicine to treat a wide variety of ailments, including bacterial infections, fungal diseases, malaria, and respiratory disorders [3, 4]. Various parts of this plant, particularly the leaves, roots, bark, and galls, are used in traditional remedies, either alone or in combination with other herbs, to treat conditions ranging from gastrointestinal infections and skin inflammation to systemic febrile illnesses [4].

Phytochemical study of *G. senegalensis* has revealed a diverse array of secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, saponins, and phenolic compounds, many of which have been shown to have antimicrobial properties [4, 5]. These bioactive constituents contribute not only to the plant's defense mechanisms but also to its therapeutic potential in human and veterinary medicine. In particular, its leaf extract has shown inhibitory activity against a variety of microbial species, suggesting that *G. senegalensis* can be a valuable source of natural antimicrobial agents [5].

Despite its extensive traditional use and reported bioactivity, there is a paucity of well-designed scientific studies that simultaneously evaluate the antibacterial and antifungal efficacy of *G. senegalensis* against clinically relevant pathogens. This study aims to evaluate the phytochemical composition as well as the in vitro antibacterial and antifungal activities of the methanol leaf extract of *G. senegalensis* against clinical isolates.

Materials and Methods

Materials

The chemicals and reagents used in this study included: Ammonium chloride, ammonium hydroxide, chloroform, perchloric acid, sodium chloride, sodium hydroxide, DPPH, distilled water, dilute hydrochloric acid (HCl), benzene, nutrient agar, Mueller-Hinton agar, Simmon's citrate agar, nutrient broth, Kovac's reagent, and hydrogen peroxide.

Bacterial and fungal isolates were obtained from the Federal University of Health Sciences Teaching Hospital, Azare, Nigeria. The isolates were transported to the Microbiology Laboratory, Sa'adu Zungur University, Bauchi State, for re-identification and further analysis.

Fresh, mature, and disease-free leaves of *G. senegalensis* were collected from Gadau Campus of Sa'adu Zungur University, Bauchi State, on February 22, 2025, using a sterilized cutlass. The harvested leaves were kept in sterile plastic containers and transported to the laboratory. The plant was authenticated at the university's herbarium with voucher specimen number 002. Leaves were washed with tap water, shade-dried at room temperature for seven days, ground into fine powder using a clean mortar and pestle, and stored in an airtight container until use.

Extract preparation and phytochemical screening

A total of 150 g of the powdered *G. senegalensis* leaves was macerated in 1 L of 70% methanol for 72 hours with stirring every 12 hours. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated in an oven at 45–50 ˚C until complete evaporation. The dried extract was stored in a labelled container for subsequent use. Phytochemical screening of the methanol leaf extract was carried out using standard methods described by Trease and Evans (2002) [6] and Harborne (1998) [7] to detect the presence of alkaloids, flavonoids, saponins, tannins, steroids, and glycosides.

Antibacterial study

All media were prepared following the manufacturer's instructions. A total of 2.8 g of nutrient agar was dissolved in 100 mL of distilled water and autoclaved at 121˚C for 15 minutes. The sterilized medium was poured into sterile Petri dishes and allowed to solidify.

A smear of each isolate was prepared on a clean slide, heat-fixed, and stained sequentially with crystal violet, iodine, alcohol (decolorizer), and safranin. Slides were examined under oil immersion at ×100 magnification.

Biochemical identification

Peptone water inoculated with isolates was incubated at 37 ˚C for 24 hours. A few drops of Kovac's reagent were added. A red or pink ring indicates a positive indole test result. A thick suspension of the isolate was mixed with undiluted human plasma on a slide. Clot formation within 5–10 seconds indicates a positive coagulase test result. A drop of hydrogen peroxide was added to a slide containing a loopful of the isolate. Bubble formation indicates a positive catalase test reaction. Simmon's citrate agar slants were inoculated and incubated at 37 ˚C for 24 hours. A color change from green to blue indicates a positive citrate utilization test result.

Antimicrobial susceptibility testing

The in vitro antibacterial activity was evaluated using the agar disk diffusion method. Bacterial cultures grown in nutrient broth were inoculated onto Mueller-Hinton agar plates. Sterile 6-mm disks were impregnated with different concentrations (500, 250, 125, 62.5, 31.25, and 15.62 µg/mL) of the extract and placed on inoculated plates. Plates were incubated at 37 ˚C for 24 hours, and the zone of inhibition (ZOI) was measured in millimeters. Disks with methanol were used as negative controls.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using the method described by the clinical and laboratory standards institute (CLSI). Serial dilutions of the extract were prepared and inoculated with test organisms in nutrient broth. Tubes were incubated at 37 ˚C for 24 hours. The lowest concentration showing no visible growth was recorded as the MIC [8].

Determination of minimum bactericidal concentration

Tubes showing no growth in the MIC assay were subcultured onto nutrient agar plates. Plates were incubated at 37 ˚C for 24 hours. The lowest concentration showing no bacterial growth was recorded as the minimum bactericidal concentration (MBC).

Antifungal study

Preparation of potato dextrose agar

Potato dextrose agar (PDA) was prepared by dissolving 19.5 g of PDA powder in 250 mL of distilled water, then autoclaving at 121˚C for 20 minutes. Under aseptic conditions, 20 mL of the molten medium was poured into sterile Petri dishes and allowed to solidify. Fungal isolates were inoculated using the spread plate method [9].

Antifungal sensitivity testing

The agar well diffusion method was used for antifungal sensitivity testing. PDA plates were inoculated uniformly with fungal isolates and allowed to dry. A sterile cork borer (6 mm) was used to make four wells and one central control well. Each well was filled with 0.1 mL of the extract at various concentrations. Ethanol served as the control. Plates were incubated at 37 ˚C for 72 hours, and ZOI was measured in millimeters.

Preparation of potato dextrose broth

To determine the MIC for antifungal activity, 6.25 g of potato dextrose broth (PDB) was dissolved in 125 mL of distilled water and autoclaved at 121 ˚C for 20 minutes. The medium was allowed to cool and used for tube dilution assays.

MIC determination

The MIC was determined using the tube dilution method [9]. Serial two-fold dilutions of the extract (from 250 µg/mL down to 1.95 µg/mL) were prepared in PDB. Each tube was inoculated with 0.1 mL of the fungal isolate and incubated at 37 ˚C for 72 hours. The MIC was recorded as the lowest concentration showing no visible fungal growth (i.e. a clear tube).

Results

Qualitative phytochemical analysis of the methanolic leaf extract

Phytochemical screening of the methanolic leaf extract of *G. senegalensis* revealed the presence of tannins, saponins, flavonoids, glycosides, and steroids, while alkaloids were absent (Table 1).

Table 1. Qualitative phytochemical analysis of *G. senegalensis* leaf extract

Phytoconstituents	Observation	Inference
Alkaloids	–	Not detected
Tannins	+	Detected
Saponins	+	Detected
Flavonoids	+	Detected
Glycosides	+	Detected
Steroids	+	Detected

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Antibacterial study

Morphological and biochemical identification of bacterial isolates

Ten bacterial isolates were identified through Gram staining and biochemical tests. The results revealed both Gram-positive and Gram-negative organisms with varied morphology and enzymatic profiles. *Staphylococcus aureus* was the most frequently identified species (Table 2).

Antibacterial activity of the extract

The antibacterial activity of the leaf extract was concentration-dependent, with maximum inhibition observed at 500 µg/mL. *Escherichia coli* exhibited the largest ZOI (29 mm), followed by *S. aureus* and *S. pyogenes* (Table 3). No inhibition was recorded at concentrations ≤31.25 µg/mL.

MIC and MBC of the extract

MIC values ranged from 31.25 to 125 µg/mL, while MBC values ranged from 62.5 to 250 µg/mL (Table 4). Gram-positive bacteria were generally more sensitive to the extract than Gram-negative ones. *E. coli* and *Micrococcus luteus* had the highest MBC values (250 µg/mL).

Antifungal study

Antifungal activity of the extract

The methanolic leaf extract of *G. senegalensis* demonstrated dose-dependent antifungal activity against *Aspergillus niger* and *Fusarium oxysporum* (Table 5). Maximum inhibition was observed at 500 µg/mL, with *F. oxysporum* exhibiting a larger ZOI than *A. niger*. No

Table 2. Morphological and biochemical characteristics of bacterial isolates

Isolate	Morphology	Gram Rxn	Cat	Coa	Cit	Ind	Organism
1	Bacilli in clusters	+	+	–	–	–	*B. cereus*
2	Cocci in chains	–	–	–	+	–	*S. pyogenes*
3	Cocci in clusters	–	+	+	+	–	*S. aureus*
4	Bacilli in clusters	–	+	+	+	+	*E. coli*
5	Cocci in chains	–	+	–	+	–	*E. faecalis*
6	Cocci in clusters	+	+	+	+	–	*S. aureus*
7	Cocci in clusters	–	–	–	+	–	*M. luteus*
8	Cocci in clusters	+	+	+	–	–	*S. aureus*
9	Bacilli	–	–	+	+	–	*P. mirabilis*
10	Cocci in clusters	+	+	+	–	–	*S. aureus*

Abbreviation: Cat: Catalase, Coa: Coagulase, Cit: Citrate utilization, Ind: Indole production

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Table 3. Antibacterial activity of *G. senegalensis* extract at different concentrations (ZOI in mm)

Isolate	500 mg	250 mg	125 mg	62.5 mg	31.25 mg	15.62 mg	Control
B. cereus	21	19	19	12	–	–	–
S. pyogenes	24	20	22	15	–	–	–
S. aureus	28	23	20	10	9	–	–
E. coli	29	22	15	–	–	–	–
E. faecalis	26	24	19	12	–	–	–
S. aureus	25	25	18	13	10	–	–
M. luteus	23	25	17	11	–	–	–
S. aureus	19	18	18	12	–	–	–
P. mirabilis	25	21	19	13	–	–	–
S. aureus	24	21	18	11	–	–	–

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inhibition was observed at 15.62 µg/mL or in the control sample.

MIC against fungal isolates

The MIC was determined using broth dilution. For *A. niger*, the MIC was 62.5 µg/mL, while for *F. oxysporum*, the MIC was 31.25 µg/mL (Table 6). No inhibition was observed in the methanol control.

Discussion

This study explored the phytochemical profile and in vitro antimicrobial activity of the methanol-leaf extract of *G. senegalensis*, testing its effects on clinical bacterial and fungal isolates. Phytochemical screening identified flavonoids, alkaloids, saponins, tannins, glycosides, and steroids (Table 1), findings consistent with recent phytochemical profiling reports [3, 5, 10]. These compounds are widely associated with antimicrobial functions, including membrane disruption, enzyme inhibition, and interference with the structural integrity of pathogens [11].

Table 4. MIC and MBC of *G. senegalensis* extract against bacterial isolates

Isolate	MIC (µg/mL)	MBC (µg/mL)
B. cereus	31.25	62.5
S. pyogenes	31.25	62.5
S. aureus	31.25	125
E. coli	125	250
E. faecalis	31.25	125
S. aureus	31.25	62.5
M. luteus	125	250
S. aureus	31.25	62.5
P. mirabilis	62.5	125
S. aureus	31.25	62.5

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Table 5. Antifungal activity of *G. senegalensis* extract (ZOI in mm)

Concentration (µg/mL)	*A. niger*	*F. oxysporum*
500	20	25
250	20	22
125	17	20
62.5	10	15
31.25	9	12
15.62	0	0
Methanol (Control)	0	0

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Our findings demonstrated concentration-dependent antibacterial activity, with strong inhibition zones at 500 µg/mL (Table 3). *E. coli* was the most susceptible species (29 mm), followed by *S. aureus* and *S. pyogenes*. Antibacterial evaluations from other studies indicate MICs in the range of 40–200 µg/mL and MBCs of 20–200 µg/mL depending on extract type and bacterial strain. In this study, MICs ranged 31.25–125 µg/mL, and MBCs ranged 62.5–250 µg/mL (Table 4). Particularly, Gram-positive isolates, *S. aureus, B. cereus*, and *S. pyogenes*, had the lowest MICs (31.25 µg/mL), indicating heightened sensitivity, consistent with broader trends: Gram-positive bacteria are generally more susceptible to plant extracts due to cell wall structural differences [12]. Moreover, fractionated extracts evaluated by Shehu et al. (2025) revealed that the methanol fraction often produced larger inhibition zones (up to ~24 mm) against *S. aureus* and similar pathogens, confirming the strong activity of unrefined extracts [5]. These comparative data consolidate the antibacterial legitimacy of *G. senegalensis*.

The methanolic extract also exhibited dose-dependent antifungal activity against *A. niger* and *F. oxysporum,* with inhibition zones up to 25 mm and 20 mm at 500 µg/mL, respectively (Table 5). MIC values were 31.25 µg/mL for *F. oxysporum* and 62.5 µg/mL for *A. niger* (Table 6). These findings align with earlier reports by Hadiza et al. (2021), who recorded moderate to good activity against agricultural fungal pathogens using concentrations of 125–250 µg/mL, with zones up to ~14 mm for *S. cruentum* [13].

In a more detailed mechanistic study, an ethyl acetate fraction (EAF) from *G. senegalensis* leaves displayed strong antifungal efficacy against yeasts (*C. neoformans, C. krusei, S. cerevisiae*), achieving MICs as low as 8–16 µg/mL and MFCs up to 125–500 µg/mL, demonstrating potency on par with standard antifungals like pen-

Table 6. MIC of *G. senegalensis* extract against fungal isolates

Concentration (µg/mL)	*A. niger*	*F. oxysporum*
500	–	–
250	–	–
125	–	–
62.5	–	–
31.25	+	–
15.62	+	+
Methanol (Control)	+	+

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conazole and fluconazole [14]. These MIC values (µg range) are significantly lower than those in our crude extract (mg range), highlighting the enhanced efficacy achievable through fractionation. The primary active molecule in that work was guieranone A, possibly aided by chlorophyll-related compounds in the EAF, suggesting that minor phytochemicals may synergistically contribute to antifungal activity.

Conclusion

The methanolic leaf extract of *G. senegalensis* exhibits robust antibacterial and antifungal activity with broader effects on Gram-positive bacteria. These findings validate its ethnomedicinal potential and support its further development as a natural antimicrobial agent, especially in resource-limited settings. It is recommended that future studies focus on isolating and characterizing the active phytochemicals in *G. senegalensis*, particularly those responsible for its strong antibacterial and antifungal effects. In vivo and toxicological evaluations are essential to establish safety and therapeutic potential. Mechanistic studies should explore how the extract disrupts microbial cells.

Ethical Considerations

Compliance with ethical guidelines

The ethical protocol for the study was approved by the Faculty of Basic Medical Sciences' Research and Ethics Committee (FBMSREC), Bauchi State University Gadau (Code: BASUG/FBMS/REC/VOL.08/01047).

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Authors' contributions

Conceptualization: Albashir Tahir, Saddam Ahmad, and Nazif Yakubu; Methodology: Albashir Tahir, and Khadija Abdullahi Kobi; Experimentation: Nazif Yakubu, Aishatu Sani Muhammad, Gideon Makeri Ayuba, and Al-ameen Abubakar Saeed; Writing the original draft: Albashir Tahir,Aminu Samaila Lassi, and Nazif Yakubu; review and editing: Albashir Tahir, Aminu Samaila Lassi, and Nazif Yakubu; Supervision: Albashir Tahir, and Saddam Ahmad; Final approval: All authors.

Conflict of interest

The authors declare no conflict of interest.

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