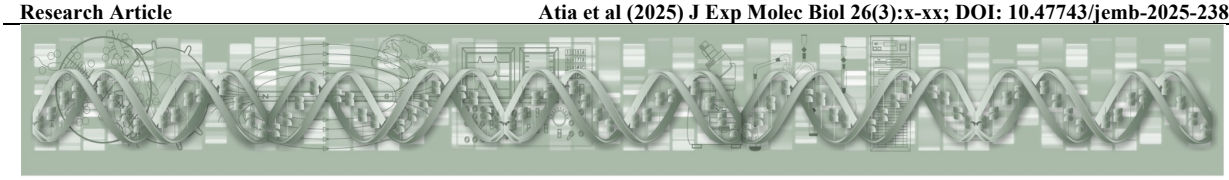

BIOACTIVITY AND PHYTOCHEMISTRY OF GUIERA SENEGALENSISJ.F.GMEL (COMBRETACAE*)*

Amna H. A. Atia[*] [1], Salar Hafez-Ghoran[2], P. D. Douanla[3] Sobia. Ghani[2], and S. Yousuf[2]

1 Department of Microbiology and Parasitology, Medicinal and aromatic plants and traditional medicine research institute (MAPTMRI), National center for research, Khartoum-Sudan

2 H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

3 Department of Organic Chemistry, Faculty of Science, The University of Yaoundé-1, P.O. Box 812, Yaoundé, Cameroon

Corresponding author: Amna Atia **E-mail:** hamadamna6@gmail.com /AmnaAtia@ncr.gov.sd

Abstract:

The phytochemical constituents of *Guiera senegalensis*, a widely used traditional medicinal plant in Africa, were investigated for their potential biological activities. Ethanolic extracts of *G. senegalensis* leaves were fractionated, and the resulting fractions were analyzed using GC-MS and NMR techniques. Forty compounds were identified by comparing their spectral data with published references. The hexane extract exhibited promising anti-leishmanial activity against *Leishmania major*, with an IC_{50} of 16.69 ± 0.3 µg/mL. The ethyl acetate fraction showed moderate activity (IC_{50} = 89.63 ± 0.6 µg/mL), while dichloromethane fractions were inactive at concentrations exceeding 100 µg/mL. The extract demonstrated limited antibacterial activity, inhibiting only *Bacillus subtilis* with a 60.31% inhibition rate, and showed no antifungal effects. Cytotoxicity testing on 3T3 cells revealed less than 50% inhibition, indicating minimal toxicity at tested concentrations. These findings support the ethnopharmacological relevance of *G. senegalensis* and highlight its potential as a source of anti-leishmanial drug candidates.

Keywords: *Guiera senegalensis,* spectral analysis, Bio-assay, Anti-leishmanial activity.

Introduction:

Natural products remain a vital source of chemical diversity for drug discovery, with medicinal plants offering a rich repository of bioactive compounds, including crude extracts, essential oils, and secondary metabolites (1). In Africa, many traditionally used medicinal plants remain understudied despite their therapeutic potential.

Guiera senegalensis, a shrub belonging to the *Combretaceae* family, is widely used in traditional African medicine, particularly across the Sahel region. It is employed in treating wounds, snakebites, gastrointestinal disorders, coughs, and neurological conditions such as Alzheimer's disease (2). These traditional uses have prompted increasing scientific interest in its phytochemistry and pharmacological properties. Previous studies have reported antioxidant, neuroprotective, and antibacterial effects from the ethanolic leaf extract, attributed to compounds such as flavonoids, alkaloids, and galloylquinic acid derivatives (3). GC-MS analysis has identified over 50 compounds in *G. senegalensis*, including significant concentrations of flavonoids and alkaloids (4). These phytochemicals are known to exert antibacterial and antifungal activities, primarily through mechanisms like membrane disruption and enzyme inhibition (5).

Received 3 April 2025; Reviewed 10June 2025; Accepted 09 September 2025; Published Online 09 September 2025; Published xx September 2025

Given the increasing threat of antimicrobial resistance and the global burden of leishmaniasis, there is an urgent need to identify novel therapeutic agents. This study investigates the phytochemical profile of *G. senegalensis* leaves and evaluates the biological activities of its extracts and fractions, with a focus on anti-leishmanial, antibacterial, and antifungal properties.

We hypothesize that the ethanolic leaf extract contains bioactive phytochemicals such as flavonoids, alkaloids, and terpenoids that exhibit antimicrobial and antiparasitic effects through mechanisms including membrane disruption, microbial enzyme inhibition, and interference with parasite metabolism. This study aims to isolate and identify these compounds and assess their biological activities to elucidate their potential modes of action.

The novelty of this study lies in its integrative approach, combining GC-MS and NMR-based phytochemical profiling with targeted bioactivity screening. Unlike prior studies, our work systematically links specific chemical fractions of *G. senegalensis* to anti-leishmanial and antimicrobial effects, providing new insights into its pharmacological potential.

Materials and Methods:

Plant Collection and Authentication:

Guiera senegalensis leaves were collected from Darfur, Western Sudan, and authenticated by a taxonomist at the Herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTRI), National Centre for Research (NCR), Khartoum, Sudan. A voucher specimen was deposited (Voucher No. KHT-SD-2022/43).

Extraction Procedure

The plant material was washed, air-dried, ground into powder, and stored at room temperature. One kilogram of powdered leaves was extracted with 80% ethanol using the maceration method described by Harborne (6). The extract was filtered, evaporated under reduced pressure, and the yield was calculated. Residues were stored at 4 °C in tightly sealed glass vials for further analysis.

Fractionation, Compound Isolation, and Purification

The crude ethanolic extract was partitioned sequentially using solvents of increasing polarity: n-hexane, dichloromethane (DCM), and ethyl acetate (EtOAc), using a separating funnel. Each fraction was concentrated, weighed (n-hexane: 14.29 g; DCM: 32.46 g; EtOAc: 38.14 g), and stored for chromatographic separation.

Column chromatography was performed using silica gel and a gradient solvent system (n-hexane–EtOAc–MeOH). Fractions (F1–F24) were collected and further purified by semi-preparative HPLC (RP-C18 column), using MeOH:H_2O (80:20) at a flow rate of 4 mL/min for 20 minutes. Isolated compounds were collected in pre-weighed, labeled vials and analyzed by thin-layer chromatography (TLC) (7).

The bio-active compounds were identified by GC-MS, and Nuclear magnetic resonance (NMR) spectral data. GC- MS were conducted using:

Gas chromatography- mass spectrum (GC-MS) analysis: Mass-Hunter GC/MS (Agilent Technologies, Santa Clara, CA, USA), equipped with a ZEBRON –ZB-5 column (430C: 30 m × 320 µm × 0.25 µm) and operated in electron ionization (EI) mode at 70 eV with a scan MS1 range of 40–700 m/z. Helium was used as the carrier gas (1.5 mL/min). The temperature of the inlet was set to 250 ◦C. The column temperature initiated at 50 ◦C for 5 min, and then was programmed to rise to 200 ◦C at the rate of 7 ◦C/min for 20 mi, then to raise 300 ◦C at the rate 7 ◦C/ min, for 30 min

Nuclear magnetic resonance (NMR) spectral data: One – dimensional (l^{1}H NMR), **two – dimensional(**2D), **and** ^{13}**C NMR,** spectra were recorded in CDCl3 or MeOD on AVANCE III 500 MHz spectrometer (Bruker, Switzerland) at 500 MHz using tetramethylsilane as an internal standard. The chemical shifts were given in δ (ppm). Their spectral data were then

compared with published values and further conformed using NIST/EPA/NIH Mass spectral database (NIST11) to NIST MS search program v.2.0 g. (8).

Biological Assays:

Cell Line and Cytotoxicity screening

The cytotoxicity of the extracts and fractions was evaluated using 3T3 mouse fibroblast cells via the MTT colorimetric assay (9). Cells were seeded in 96-well plates and incubated with varying concentrations of test samples. After 24 hours, MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) solution was added to each well and incubated for 4 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 540 nm using a micro-plate reader (Spectra Max Plus, Molecular Devices, CA, USA).

Cytotoxicity was expressed as the concentration causing 50% growth inhibition (IC_{50}). Percent inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = 100 - \left(\frac{\text{mean of O.D of test compound} - \text{mean of O.D of negative control}}{\text{mean of O.D of positive control} - \text{mean of O.D of negative control}}\right) \times 100$$

OD test: Mean optical density of cells treated with test compound

OD negative: Mean OD of negative control (e.g., untreated cells = 100% viability)

OD positive. Mean OD of positive control (e.g., a known cytotoxic agent like doxorubicin = 0% viability). Data were processed using Soft Max Pro Software (Molecular Devices, USA).

Anti-Leishmanial Activity:

The anti-leishmanial activity of *G. senegalensis* extracts, fractions, and isolated compounds was evaluated against *Leishmania major* promastigotes (ATCC 50155) using a micro-plate-based assay (10). Promastigotes were incubated with test samples in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS). Parasite viability was assessed by counting motile cells using a Neubauer chamber. IC_{50} values were calculated using EZ-Fit software based on dose-response curves (11)

Screening of plant extract on Amastigotes:

To evaluate intracellular activity, macrophage monolayers were prepared in 24-well plates (1×10^6 cells/well) and infected with *L. major* promastigotes at a ratio of 5:1 (parasite: macrophage). After 48 hours of incubation, non-internalized parasites were removed, and infected macrophages were treated with test extracts. The following parameters were calculated:

Infection Rate (IR) = Number of infected macrophages in 100 macrophages

Multiplication Index (MI): = Number of amastigotes in test culture / 100 macrophages x 100

Number of amastigotes in control / 100 macrophages

Antibacterial Activity:

Preparation of Bacterial Inoculum:

Antibacterial activity was tested against five bacterial strains (*E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *S. typhi*) using the Microplate Alamar Blue Assay (MABA), as described by Sarker. (12). Bacterial suspensions were adjusted to 0.5 McFarland standard (~10^8 CFU/mL), and diluted 1:10 in nutrient broth.

Microdilution Assay: 100 µL of nutrient broth was dispensed into each well of a sterile 96-well plate. Serial two-fold dilutions of extracts were prepared, followed by 100 µL of bacterial suspension and 10 µL of resazurin dye (0.01% w/v). Ciprofloxacin was used as the positive control. Plates were incubated at 37 °C for 18–24 h. Color change was visually inspected and quantitatively measured at 570 nm and 600 nm.

MIC Determination: The **Minimum Inhibitory Concentration (MIC)** was defined as the lowest concentration of the extract or fraction at which no visible color change (i.e., retention of blue color) occurred, indicating effective inhibition of bacterial growth.

$$\text{Percentage Inhibition (\%)} = \left(\frac{\text{A control} - \text{A blank}}{\text{A control} - \text{A test}}\right) \times 100$$

***In-vitro* Anti-fungal activity Bioassay (Preliminary Screening)**:

The antifungal activity of *G. senegalensis* leaf extracts and fractions were evaluated against six fungal strains*, Aspergillus fumigatus* (ATCC 46645), *Aspergillus niger* (clinical isolate), *Candida albicans* (SC5314), *Candida glabrata* (ATCC 2001), *Fusarium lini* (environmental isolate), *Microsporum canis* (ATCC 36299), and *Trichophyton rubrum* (clinical isolate) using the broth microdilution method. (13), with minor modifications.

Preparation of Fungal Inoculum:

Fungal strains were cultured on Sabouraud Dextrose Agar (SDA) and incubated at 28 °C for 48-72 hours. Spores or yeast cells were harvested using sterile saline solution containing 0.05% Tween 80 and adjusted to a final concentration of approximately1 × 10^6 spores/ml, using a hemocytometer.

Microdilution Assay:

In sterile 96-well micro-titer plates, 100 µL of Sabouraud Dextrose Broth (SDB) was dispensed into each well. Serial two-fold dilutions of the test extracts and fractions were prepared in the wells to achieve different concentrations. Subsequently, 100 µL of fungal inoculum was added to each well, with final volume 200 µL /well. The plates were incubated at 28°C for 48 hours under sterile conditions.

Assessment of Fungal Growth:

After incubation, fungal growth was assessed visually and by measuring linear growth (in mm). Additionally, 20µL of 0.01% (w/v) Resazurin solution was added to each well as a viability indicator. Blue-to-pink color shift indicated fungal viability and metabolic activity.

Determination of Antifungal Activity:

Antifungal efficacy was determined by calculating the percentage inhibition of fungal growth compared to the negative control. The % inhibition was calculated using the following formula:

$$\% \text{ inhibition} = 100 - \frac{\text{Linear growth in test (mm)}}{\text{Linear growth in Control (mm)}} \text{ x } 100$$

The Minimum Inhibitory Concentration (MIC) Determination:

The MIC was defined as the lowest concentration of the test extract or fraction that resulted in no visible fungal growth or no color change in the resazurin dye, indicating complete growth inhibition.

Statistical Analysis:

All experiments were conducted in triplicate, and the results are presented as mean ± **standard deviation (SD). The inhibitory concentration (IC_{50})** values for both anti-leishmanial and cytotoxicity assays were calculated using dose response curves generated in Microsoft Excel. Absorbance readings at 540 mm were used to assess cell viability following treatment with varying concentration of plant extracts and fractions. Percentage inhibition (% inhibition) was calculated relative to untreated controls, and non-linear regression was applied to IC_{50} values. The IC_{50} was defined as the concentration of the test sample that resulted in 50% inhibition of parasite or cell viability. A p-value **<0.05 was considered statistically significant. For antibacterial and antifungal assays,** for antibacterial and antifungal assays, the percentage inhibition of microbial growth was calculated relative to untreated controls. Differences between treated and control groups were considered statistically significant at **p < 0.05.**

Results:

Isolation and Identification of Bio-active compounds:

The bioactive compounds in *G. senegalensis* were identified through Gas Chromatography-Mass Spectrometry (GC-MS) analysis and Nuclear Magnetic Resonance (NMR) spectral data (1H, 2D, 13C NMR), along with mass spectral data and comparison with previously

published data from the National Library of Medicine. The fractions of the ethanolic extract of *G. senegalensis* were analyzed using (^{1}H and ^{13}C NMR), mass spectral analysis, and GC-MS chromatography. Known compounds (40) were identified (**Table 1**. and **Figures (1-40) supplementary file**), by comparing their spectral data with previously reported data in the literature. As pure Compounds: **7 (**43 mg), Flavasperone (14) **(figure 1.), 10**(34.5mg) as Ethyl gallate (3) **(figure 2.),**, **32**(22 mg) as 1-Eicosanol, **(37)** as β –Amyrin, **38** (31 mg), as α-Amyrin (15) **(figures 3, 4@ 5),** and **40** (4.65mg) as Daucosterol (16) **(figure 6),** while the other compounds were obtained as mixtures **(figure 7).**(Supplementary File).

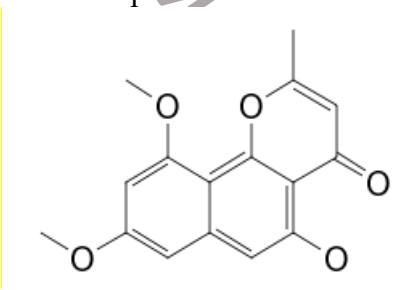

Figure 1. Structure of pure compound Flavasperone in *G senegalensis J.F.Gmel*

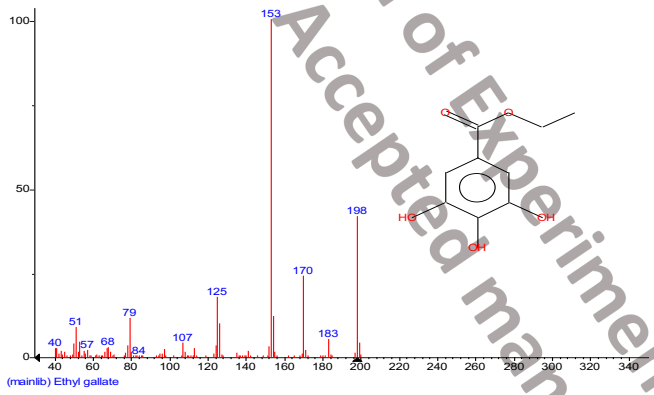

Figure 2. Mass spectrum of pure compound Ethyl gallate of hexane fraction.

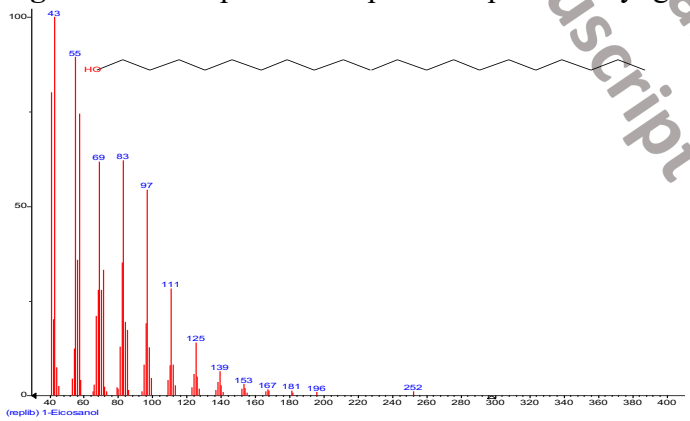

Figure 3. Mass spectrum of pure compound 1-Eicosanol of hexane fraction.

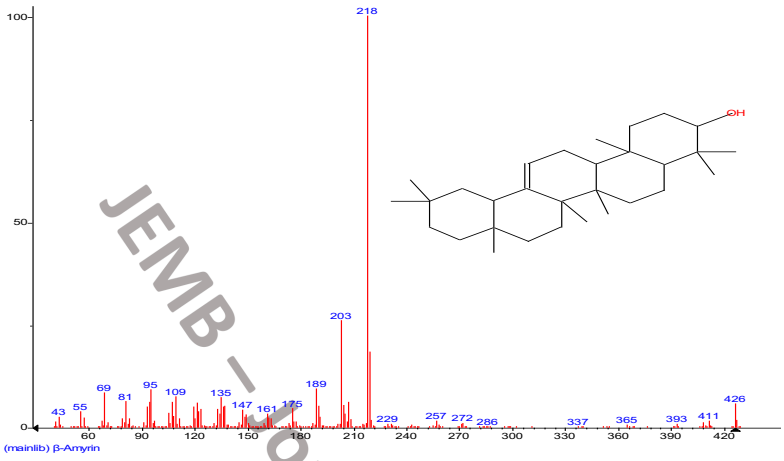

Figure 4. Mass spectrum of pure compound β-Amyrin of hexane fraction.

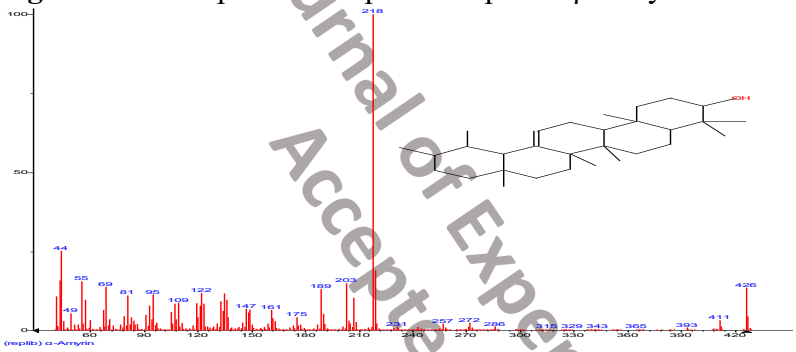

Figure 5. Mass spectrum of pure compound α-Amyrin of hexane fraction.

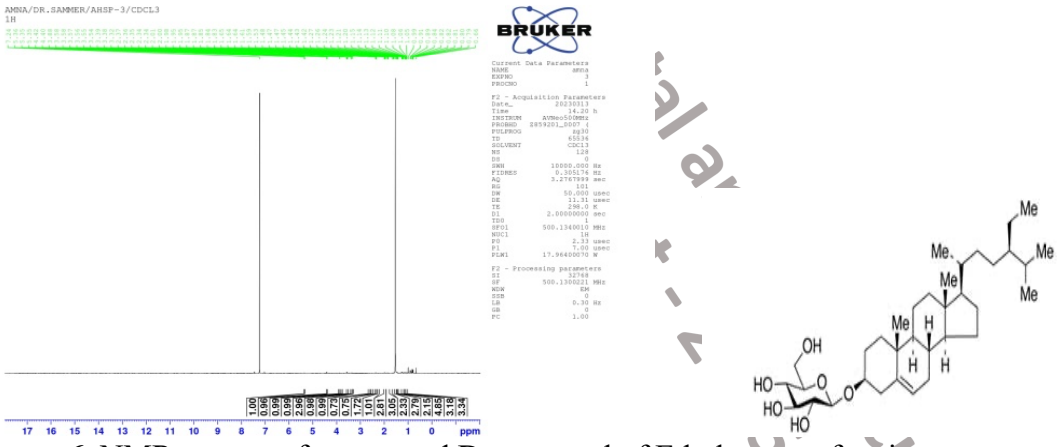

Figure 6. NMR spectra of compound Daucosterol of Ethyl acetate fraction

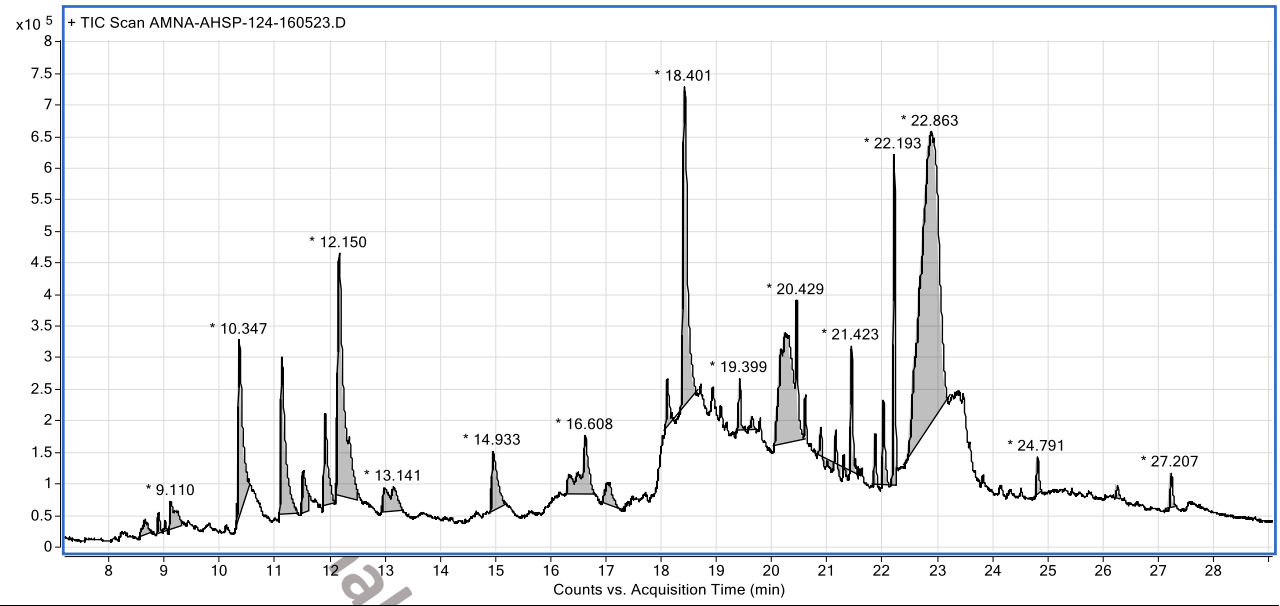

Figure 7. Mass spectrum of compounds mixture in *Guiera senegalensis J.F.Gmel (Combretacae*) leaves using GC-MS analysis.

Cytotoxicity screening:

The cytotoxicity of the *G. senegalensis* extract was assessed alongside standard drugs (Table 1), and demonstrated less than 50% cytotoxicity against 3T3 cells, indicating no significant toxicity at the tested concentrations.

Table 1: Cytotoxicity of *G. senegalnesis* extract on 3T3 (Mouse fibroblast) cell evaluated by assay (MTT BI).

Key: IC_{50} < 50 µg\ml: High toxic, > 50 µg/ml: no toxic *Control = standard drug/s (Doxorubicin) was used as the control positive at 0.1±0.02 µg/mL.

Test compound\s	**Concentration (µg\ml)**	**% inhibition**	**IC_{50} µg\ml± SD**
G. senegalensis crude extract	30 µg\ml	5.0%	In active (non- cytotoxic)
Doxorubicin(Control)	30 µg\ml	89.9%	0.2

***In vitro* assessment of anti-leishmania activity**

The hexane fraction exhibited the highest anti-leishmanial activity against *L. major* promastigotes, with an IC_{50} of 16.69 ± 0.3 µg/mL. The ethyl acetate fraction had moderate activity (IC_{50} = 89.63 ± 0.6 µg/mL), while the DCM fraction and crude extract were inactive (IC_{50} > 100 µg/mL). Standard drugs amphotericin B and pentamidine had IC_{50} values of 3.39 ± 0.03 and 4.39 ± 0.01 µg/mL, respectively.

Table 2. *In vitro* anti-*leishmania* activity (*leishmania major) Promastigotes* of extract/ fractions of *G. senegalensis* and standard drugs.

Test compound\s		**IC_{50} µg\ml± SD**	
crude extract		>100	In active
fractions	hexane	16.69±0.3	Significant active
	DCM	>100	In active
	Ethyl acetate	89.63 ±0.6	Low activity

Compound			
Standard Drug\s	Amphotericin B	3.39±0.03	Highly potent (Ref.)
	Pentamidine	4.39±0.01	Highly potent (Ref.)

Antibacterial activity:

The ethanolic extract of *G. senegalensis* showed **moderate antibacterial activity** against *Bacillus subtilis* (60.31% inhibition), but was **inactive** against the other strains. In contrast, standard antibiotics exhibited **broad-spectrum potency** with over 89% inhibition. The selective activity against *B. subtilis* suggests a potential affinity for Gram-positive bacteria, likely due to phytochemicals such as flavonoids or alkaloids. While less potent than conventional drugs, the extract's activity supports further **fractionation and optimization** to identify and enhance its active constituents (**Table 2.**).

Table 3. percentage inhibition of *G. senegalensis* of test bacterial strain vs. standard antibiotics.

Bacterial Strain	% inhibition of test extract/compound	% inhibition of standard drug
Escherichia coli ATCC25922	-	89.20
Bacillus subtilis ATCC23857	60.31%	90.47
Staphylococcus aureus NCTC6571	-	90.37
Pseudomonas aeruginosa ATCC10145	-	92.42
Salmonella typhi ATCC 14028	-	96.62

***Key:** amount of extract 60 mg, Amount of drug: 10 mg, Con. of extract: 3000 µg/ml, Con. of drug: 1000 µg/ml.

Anti-fungal activity:

Based on the growth of various Fungus strains including *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Candida glabarata*, *Fusarium lini*, *Microsporumcanis*, and *Trichophyton rubrum*, their linear growth (in mm) and growth inhibition were summarized in Table 2. Supplementary file).

Discussion:

Phytochemical analysis of *G. senegalensis* revealed rich profile of secondary metabolites, including flavonoids, tri-terpenes (α- and β-amyrin), sterols (daucosterol), and phenolic compounds such as ethyl gallate. These are widely recognized for their antimicrobial and anti-parasitic activities, which likely underpin the bioactivity observed in this study. Flavonoids are known to disrupt microbial membranes, interfere with nucleic acid synthesis, and alter energy metabolism, thereby exhibiting broad-spectrum antimicrobial properties (17). In the context of leishmaniasis, several flavonoids induce oxidative stress and mitochondrial dysfunction in *Leishmania spp.*, leading to apoptotic-like cell death (18). Likewise, tri-terpenes such as α- and β-amyrin have been shown to impair parasite membrane integrity and modulate host immune responses. (19).

Among the tested extract and fractions, the hexane fraction exhibited the most potent anti-leishmanial activity (IC_{50} = 16.69 ± 0.3 µg/mL), supporting the presence of active non-polar constituents particularly tri-terpenoids and sterols. Although less potent than standard drugs

Amphotericin B (IC_{50} = 3.39 ± 0.03 µg/mL) and Pentamidine (IC_{50} = 4.39 ± 0.01 µg/mL), the hexane fraction demonstrated significantly lower cytotoxicity, suggesting a more favorable therapeutic index. Key compounds (α-amyrin, β-amyrin, ethyl gallate, and daucosterol) were successfully isolated and are likely responsible for this activity. Their identification provides a promising foundation for further structural and mechanistic studies.

The extract also displayed selective antibacterial activity, against *Bacillus subtilis* (60.31% inhibition), consistent with the preferential action of flavonoids and alkaloids on gram-positive bacteria (16). While less effective than standard antibiotics (90-96% inhibition).this result supports further bio-guided purification. However, the extract did not exhibit significant antifungal activity, in contrast to earlier studies. The observed antifungal effect was modest and varied by strain, with partial inhibition noted against *Trichophyton rubrum* and *Fusarium lini*, while *Candida albicans* remained resistant. This aligns with previous findings suggesting that antifungal properties in *G. senegalensis* are associated with compounds such as guieranone A and phenolic acids (5). Against *Candida albicans*, remain inconsistent (20). While the extract has demonstrated antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (21).

Importantly, the isolation of specific compounds from the hexane fraction confirms the presence of pharmacologically relevant molecules with selective anti-parasitic activity. These findings position *G. senegalensis* as a promising candidate for the discovery of novel anti-leishmanial agents. Future efforts should focus on detailed mechanistic studies and in vivo validation to assess efficacy, safety, and potential for therapeutic development.

This study has limitations. All bioactivity assays were conducted *in vitro*, and the antimicrobial screening was limited to a select panel of pathogens. Therefore, further research including *in vivo* models and expanded microbial testing is essential to fully establish the clinical relevance and translational potential of *G. senegalensis* derived compounds.

Conclusion:

This study highlights *G. senegalensis* as a promising source of bioactive compounds with selective anti-leishmanial properties. The hexane fraction, in particular, demonstrated significant activity against *Leishmania major* with low cytotoxicity, and the successful isolation of compounds such as α-amyrin, β-amyrin, ethyl gallate, and daucosterol strengthens its potential as a lead for drug development. Although the crude extract exhibited modest antimicrobial activity, its selective effect against Gram-positive bacteria supports further purification and optimization.

Acknowledgement:

The authors express sincere gratitude to Hussin Ebrahim Jamal (HEJ) and the Research Institute of Chemistry at the University of Karachi in Pakistan for their invaluable support in providing the necessary facilities for conducting the analysis and anti-leishmanial studies. Special acknowledgement is owed to all the co-authors who provided assistance in bringing this study to completion.

References:

1. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020; 83(3):770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

2. Dirar AI, Devkota HP. Ethnopharmacological uses phytochemistry and pharmacological activities of Guiera senegalensis J.F. Gmel. (Combretaceae). J Ethnopharmacol. 2021; 267:113433. https://doi.org/10.1016/j.jep.2020.113433

3. Bhat P, Patil VS, Anand A, Bijjaragi S, Hegde GR, Hegde HV, Roy S. Ethyl gallate isolated from phenol-enriched fraction of *Caesalpinia mimosoides* Lam. promotes cutaneous wound healing: A scientific validation through bioassay-guided fractionation. Front Pharmacol. 2023; 14:1214220. https://doi.org/10.3389/fphar.2023.1214220

4. Zayyan SM, Uba A, Faruq UZ, Izuagie T. Characterization and Phospholipase A_2 Inhibitory Potential of *Guiera senegalensis* Leaves Extracts via Experimental and Computational Approaches. *Preprints* (Preprint). 2024.

5. Moreira R, Ferreres F, Gil-Izquierdo Á, Gomes NGM, Araújo L, Pinto E, et al. Antifungal activity of *Guiera senegalensis*: From the chemical composition to the mitochondrial toxic effects and tyrosinase inhibition. Antibiotics. 2023; 12(5):869. https://doi.org/10.3390/antibiotics12050869

6. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman & Hall; 1998. p. 60–6.

7. Marston A, Hostettmann K. Thin-layer chromatography and bioautography of plant extracts and pure compounds. In: Dey PM, Harborne JB, editors. Methods in Plant Biochemistry: Plant Phenolics. Vol. 1. London: Academic Press; 1991. p. 101–46.

8. Silverstein RM, Webster FX, Kiemle DJ, Bryce DL. Spectrometric Identification of Organic Compounds. 8th ed. Hoboken (NJ): John Wiley & Sons; 2014.

9. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65(1–2):55–63. https://doi.org/10.1016/0022-1759(83)90303-4

10. Croft SL, Coombs GH. Leishmaniasis– current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol. 2003; 19(11):502–8. https://doi.org/10.1016/j.pt.2003.09.008

11. Atta-ur-Rahman R, Chaudhary MI, Thompson W. Bioassay Techniques for Drug Development. Vol. 60. Amsterdam: Harwood Academic Publishers; 2001.

12. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods. 2007; 42(4):321–4. https://doi.org/10.1016/j.ymeth.2007.01.006

13. Kumar S, Yadav A, Yadav M, Yadav JP. Antimicrobial properties of various parts of *Guazuma ulmifolia* Lam. against clinical pathogens. BMC Complement Altern Med. 2016; 16:173. https://doi.org/10.1186/s12906-016-1141-4

14. Abdelwahab GM, Mira A, Cheng YB, Abdelaziz TA, Lahloub MFI, Khalil AT. Acetylcholine esterase inhibitory activity of green synthesized nanosilver by naphthopyrones isolated from marine-derived *Aspergillus niger*. PLoS One. 2021; 16(9):e0257071. https://doi.org/10.1371/journal.pone.0257071

15. Gil Archila E, Rojas-Bautista F, Garcia N, Carvajal Vasquez JA. A promising blueberry from Colombia: Antioxidant activity, nutritional and phytochemical composition of *Cavendishia nitida* (Kunth) A.C.Sm. Heliyon. 2022; 8(5):e09448. https://doi.org/10.1016/j.heliyon.2022.e09448

16. Rajavel T, Mohankumar R, Archunan G, Ruckmani K, Devi KP. Beta sitosterol and daucosterol (phytosterols identified in *Grewia tiliaefolia*) perturb cell cycle and induce apoptotic cell death in A549 cells. Sci Rep. 2017;7(1):3418. https://doi.org/10.1038/s41598-017-03511-4

17. Cushnie TPT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents. 2011; 38(2):99–107.

18. Sen R, Bandyopadhyay S. Activities of flavonoids as potential anti-leishmanial agents: A review. Pharm Biol. 2011; 49(5):442–9.

19. Nery CGC, de Morais SM, da Costa WAK, Pereira PR, Silva ARA, da Silva M, et al. Antileishmanial activity of ursolic acid and oleanolic acid derivatives. Bioorg Med Chem Lett. 2017; 27(7):1791–4.

20. Jiyil MK, Mafuyai CE, Shago MI, Inuwa HM. Antimicrobial activity of methanolic, aqueous and partially purified protein of young and matured leaves of *Guiera senegalensis* (Moshi medicine). J Adv Med Pharm Sci. 2019; 1–11.

21. Saidu T, Galadima M, Jigam A, Abalaka E. Antibacterial and potential toxicological evaluation of leaf extracts of *Guiera senegalensis* against Gram-negative bacteria. J Adv Med Pharm Sci. 2016; 9(1):1–13. https://doi.org/10.9734/JAMPS/2016/25815