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ANTIBACTERIAL ACTIVITY-GUIDED ISOLATION AND CHARACTERISATION OF THE COMPOUND FROM *Acacia Senegal* PLANT LEAVES

***[1]Ahmed Hamisu, [2]Abubakar Sani, [2]Harisu Mikailu, [2]Khadija H. Wada, [3]Kabir Salsabilu and [1]Muazu Ibrahim**

[1]Department of Chemistry and Industrial Chemistry, Federal University Dutsin-ma, Katsina State, Nigeria.
[2]Department of Pure and Industrial Chemistry, Umaru Musa Yar'adua University, Katsina, Nigeria.
[3]Department of Chemistry, Federal University of Petroleum Resources Effurun Delta State, Nigeria.

*Corresponding authors' email: ahamisu3@fudutsinma.edu.ng

ABSTRACT

The increasing prevalence of antibiotic-resistant bacterial strains such as Salmonella typhi and Staphylococcus aureus has necessitated the search for alternative antimicrobial agents, particularly from plant sources. This study investigates the phytochemical composition and in vitro antibacterial activity of leaf extracts from *Acacia senegal* medicinal plant traditionally used in the treatment of infectious diseases. Crude extract was obtained via ethanol maceration and subsequently fractionated using solvents of increasing polarity. Phytochemical screening revealed the presence of tannins, terpenoids, phenolics, steroids, and saponins in *A. Senegal*. Antibacterial activity was assessed using the agar diffusion method against clinical isolates of *S. typhi* and *S. aureus*. The results demonstrated that chloroform and hexane extracts of *A. Senegal* exhibited strong inhibitory effects against *S. typhi* (zones up to 24.5 mm). (43 mm at 100 mg/ml) Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays confirmed the potency of the extracts, particularly methanol and ethanol fractions. Bioactive compounds were isolated and characterized using thin-layer chromatography (TLC), column chromatography, Fourier-transform infrared spectroscopy (FT-IR), and nuclear magnetic resonance (^{1}H & ^{13}C NMR). The major compound isolated from *A. Senegal* was identified as lupeol, a pentacyclic triterpenoid, based on characteristic NMR signals (δC 151.01, 109.33, 79.03 ppm; δH 5.30–5.35, 4.95–5.05, ~3.35–3.60 ppm). These findings validate the ethnomedicinal use of the plant and suggest its potential as sources of novel antibacterial agents.

Keywords: *Acacia Senegal,* Antibacterial Activity, Phytochemical Analysis, Antibiotic Resistance

INTRODUCTION

Bacterial infections remain a major global health concern, with increasing morbidity and mortality attributed to the rapid emergence of multidrug-resistant strains (WHO, 2023). The growing prevalence of antimicrobial resistance (AMR) has compromised the efficacy of conventional antibiotics, underscoring the urgent need for alternative therapeutic agents (Ventola, 2015). Medicinal plants are a rich source of bioactive compounds, many of which exhibit antibacterial, antifungal, and antiviral properties, providing a promising avenue for the discovery of novel antimicrobial agents (Cowan, 1999; Kuete, 2010).

Acacia senegal (L.) Willd., commonly known as the Gum Arabic tree, is a drought-tolerant leguminous plant widely distributed in semi-arid regions of sub-Saharan Africa. Traditionally, the leaves, bark, and gum of *A. Senegal* are used for the management of gastrointestinal disorders, wound infections, and febrile illnesses (Ibrahim et al., 2020; Siddig et al., 2018). Previous studies have reported that extracts from various parts of *A. Senegal* possess antibacterial, antifungal, antioxidant, and anti-inflammatory activities, attributed to its diverse secondary metabolites, including flavonoids, tannins, saponins, terpenoids, and phenolic compounds (Ahmed et al., 2020; Ali et al., 2012).

Despite its traditional use and documented biological activities, there is limited research isolating and characterizing specific bioactive compounds responsible for the antibacterial effects of *A. Senegal*. The n-hexane fraction of the leaves, in particular, has been suggested to contain lipophilic compounds with potent antimicrobial properties, but detailed spectroscopic characterization and evaluation against clinically relevant bacteria remain scarce.

This study therefore aimed to isolate, characterize, and evaluate the antibacterial activity of bioactive compounds from the n-hexane leaf fraction of *A. Senegal*, with particular focus on *Salmonella typhi* and *Staphylococcus aureus*. Identification of specific bioactive constituents may provide insights into the plant's therapeutic potential and contribute to the development of plant-derived antibacterial agents against resistant pathogens.

Plate 1: Picture of *Acacia Senegal* Leaf

MATERIALS AND METHODS

Plant Material Collection and Identification

Fresh leaves of *Acacia senegal* were collected from Baure town, Katsina State, Nigeria. The plant was identified by a taxonomist at Umaru Musa Yar'adua University, Katsina, and a voucher specimen (UMYUH 1802) was deposited at the University's Herbarium. Leaves were air-dried at room temperature for three days, pulverized, and stored in sterile airtight containers until extraction.

Extraction and Fractionation

150 g of powdered leaves were macerated in 700 mL of ethanol for 2 weeks at room temperature with occasional stirring. The crude extract was filtered and concentrated to dryness using a water bath, yielding 38 g of ethanol extract (AEF).

The crude extract was fractionated sequentially with solvents of increasing polarity: n-hexane (AHF), chloroform (ACF), ethyl acetate (AEAF), methanol (AMF), and water (AAF). Each fraction was concentrated, weighed, and stored at 4°C until use (Fatope et al., 1993; Haris *et* al., 2025).

Preliminary Phytochemical Screening

Qualitative phytochemical tests were performed on all fractions for flavonoids, tannins, alkaloids, terpenoids, phenolics, saponins, steroids, and glycosides following standard procedures (Trease & Evans, 2002; Shahid & Anwarul, 2009).

Bacterial Strains and Culture Conditions

Clinical isolates of Staphylococcus aureus and Salmonella typhi were obtained from the Microbiology Department, Umaru Musa Yar'adua University. Cultures were maintained on nutrient agar slants and subcultured prior to experiments.

Antibacterial Activity

Preparation of Extract Concentrations

Stock solutions of each extract fraction were prepared in distilled water to 100 mg/mL and serially diluted to obtain concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78 mg/mL.

Agar Diffusion Assay

Antibacterial activity was assessed using the agar disc diffusion method. Sterile 6 mm filter paper discs were impregnated with 20 µL of each extract concentration, air-dried, and placed on Mueller-Hinton agar inoculated with test organisms. Discs with ciprofloxacin (30 µg/mL) and DMSO served as positive and negative controls, respectively. Plates were incubated at 37°C for 24 h, and zones of inhibition were measured in millimeters (Baker & Silverton, 1993; Mukhtar & Tukur, 2000).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC values were determined using the tube dilution method, with extract concentrations ranging from 0.78 to 100 mg/mL. Tubes showing no visible growth were recorded as MIC. MBC was determined by subculturing from MIC tubes onto fresh agar plates; the lowest concentration showing no colony growth was recorded as MBC (Baker & Silverton, 1993).

Isolation and Purification of Bioactive Compounds

The n-hexane fraction, exhibiting the highest antibacterial activity, was subjected to thin layer chromatography (TLC) using hexane:ethyl acetate (4.5:0.5) to identify fractions with distinct Rf values. Column chromatography on silica gel was performed with stepwise gradient elution (5–20% ethyl acetate in n-hexane). Fractions containing single TLC spots were combined, concentrated, and dried to yield the purified compound.

Spectroscopic Characterization

FT-IR Analysis

Fourier-transform infrared (FT-IR) spectra were recorded on an Agilent Cary 630 FT-IR spectrophotometer using KBr pellets in the range 4000–650 cm^{-1} to identify functional groups.

Nuclear Magnetic Resonance (NMR) Analysis

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DBX-400 MHz spectrometer using $CDCl_3$ as solvent. Chemical shifts (δ, ppm) and multiplicities were analyzed to determine the structure of the isolated compound.

RESULTS AND DISCUSSION

Physical Properties of Extract Fractions

The yield, color, and texture of *A. Senegal* leaf fractions are summarized in Table 1. The highest yield was obtained with the aqueous fraction (AAF, 61%), while the methanol fraction (AMF) gave the lowest yield (21%). Most extracts were granular in texture and varied from dark brown to brown in color.

Table 1: Yield and Physical Properties of *A. Senegal* Leaf Fractions

Fractions	Weight(g)	Yield (%)	Texture	Color
AEF	36.0g	24.00%	Granular	Dark Brown
ACF	15.0g	50.00%	Gummy	Dark Brown
AHF	4.30g	43.00%	Granular	Dark Brown
AMF	2.10g	21.00%	Granular	Dark Brown
AAF	6.10g	61.00%	Granular	Brown
AEAF	3.40g	34.00%	Granular	Brown

AHF= *A.senegal* n-hexane fraction, ACF= *A.senega* chloroform fraction, AEAF= *A.senega* ethyl acetate fraction, AEF= *A.senegal* ethanol fraction, AAF= *A.senegal* aqueous fraction, AMF= *A.senegal* methanol fraction

Phytochemical Screening

Qualitative analysis of the leaf fractions revealed the presence of saponins, steroids, terpenoids, phenolics, and tannins in the n-hexane fraction (AHF), while flavonoids, alkaloids, and glycosides were absent (Table 2).

Table 2: Phytochemical Constituents of *A. Senegal* Leaf Extracts

Phytochemicals	***A. Senegal* leaves Extract**
Flavonoids	-
Tannis	+
Alkaloids	-
Terpenoids	+
Phenolics	+
Saponins	+
Steroids	+
Glycosides	-

Key: + Phytochemicals Detected, - Phytochemical Not Detected

Antibacterial Activity (Zones of Inhibition)

The n-hexane fraction (AHF) exhibited the highest antibacterial activity against S. aureus (29 mm) and S. typhi (24.5 mm) at 100 mg/mL (Table 3). Activity decreased with lower concentrations. Ethyl acetate fraction showed minimal inhibition, while aqueous fraction displayed moderate activity against S. aureus but no inhibition against S. typhi.

Table 3: Zone Of Inhibitions (Mm) Of *A. Senegal* Extract against *S.Aureu and Salmonella*

Fractions	**Conc. (mg/mL)**	**Organisms**	
		S. aureus	*Salmonella*
		Mean Zone of Inhibition (mm) ± SD	
AEF	100	20.5	18.5
	50	13.5	13.5
	25	NA	13.5
	12.5	NA	12.5
	6.25	NA	11
	3.12	NA	9.5
	1.56	NA	9.0
	78	NA	NA
ACF	100	24	11.5
	50	22	10.5
	25	16	9.0
	12.5	9.5	7.5
	6.25	8.5	7
	3.12	7.5	NA
	1.56	NA	NA
	0.78	NA	NA
AHF	100	29	10
	50	14,5	10
	25	14	NA
	12.5	NA	NA
	6.25	NA	NA
	3.12	NA	NA
	1.56	NA	NA
	0.78	NA	NA
AMF	100	23	24.5
	50	10.5	21
	25	8.5	18.5
	12.5	8.5	16.5
	6.25	NA	16
	3.12	NA	14.5
	1.56	NA	13.5
	0.78	NA	13.5
AAF	100	19.5	NA
	50	14	NA
	25	11	NA
	12.5	10.5	NA

Fractions	Conc. (mg/mL)	Organisms	
	6.25	10	NA
	3.12	10	NA
	1.56	NA	NA
	0.78	NA	NA
AEAF	100	12	9.0
	50	NA	9.0
	25	NA	8.5
	12.5	NA	8.0
	6.25	NA	8.0
	3.12	NA	7.0
	1.56	NA	NA
	0.78	NA	NA
+ve control		30	28
-ve control		NA	NA

AHF= *A.senega*l *n*-hexane fraction, ACF= *A.senegal* chloroform fraction, AEAF= *A.senegal ethyl* acetate fraction, AEF= *A.senegal* ethanol fraction,AAF= *A.senegal aqueous fraction, AMF= A.senegal methanol fraction*, -ve control = DMSO and +ve control = Ciprofloxacin.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values of the fractions against both bacterial strains are summarized in Table 4. The n-hexane fraction (AHF) exhibited potent activity against S. typhi with MIC of 0.78 mg/mL and MBC of 25 mg/mL.

Table 4: MIC and MBC of *A. Senegal* Fractions

Fractions	*Salmonella typhy*		*Staphylococcus aureus*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
AEF	1.56	100	50	100
ACF	6.25	100	3.12	-
AAF	-	-	3.12	50
AEAF	3.13	50	100	-
AMF	0.78	25	12.5	-
AHF	50	100	25	-

Key: - = No turbidity,AHF= *A.senegal n*-hexane fraction, ACF= *A.senegal* chloroform fraction, AEAF= *A.senegal* ethyl acetate fraction, AEF= *A.senegal* ethanol fraction, AAF= *A.senegal aqueous fraction, AMF= A.senegal methanol fraction*

Thin Layer Chromatography (TLC) and Rf Values

TLC analysis of the n-hexane fraction showed four distinct spots (N1–N4) with Rf values ranging from 0.15 to 0.70, suggesting the presence of multiple components. The isolated compound (N50) had a single TLC spot with Rf = 0.38, indicating purity.

FT-IR Spectroscopy Results of the Isolated Compound

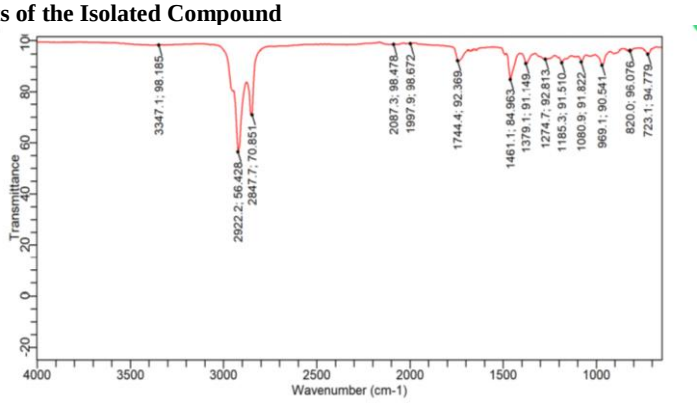

Plate 2: FTIR Spectrum of the Isolated Compound

FT-IR analysis of the isolated compound revealed absorption bands corresponding to hydroxyl (O–H, 3347 cm^{-1}), carbonyl (C=O, 1744 cm^{-1}), olefinic (C=C, 1613 cm^{-1}), and aliphatic (C–H, 2922 cm^{-1}) functional groups, consistent with a pentacyclic triterpenoid structure.

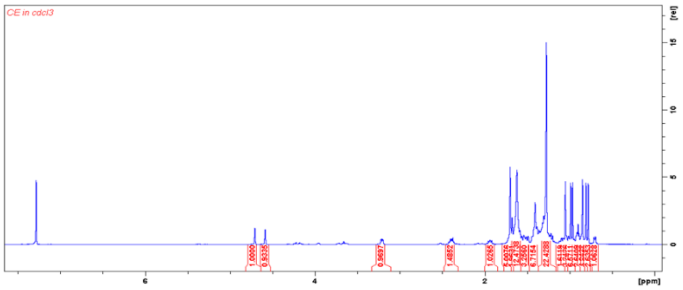

Plate 3: HNMR Spectrum of the Isolated Compound

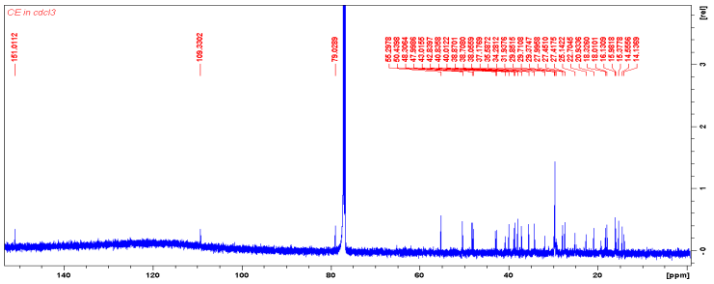

Plate 4: 13CNMR Spectrum of the Isolated Compound

^{1}H NMR spectra displayed signals for olefinic protons (δ 5.30–5.35 ppm), exomethylene protons (δ 4.95–5.05 ppm), and multiple aliphatic protons (δ 0.90–2.25 ppm), indicating a triterpenoid skeleton with hydroxyl substitution

^{13}C NMR spectra exhibited 30 distinct carbon resonances, including oxygenated tertiary carbon (C-3, δ 79.03 ppm) and olefinic carbons (C-20, δ 151.01 ppm; C-29, δ 109.33 ppm), confirming the structure as lupeol.

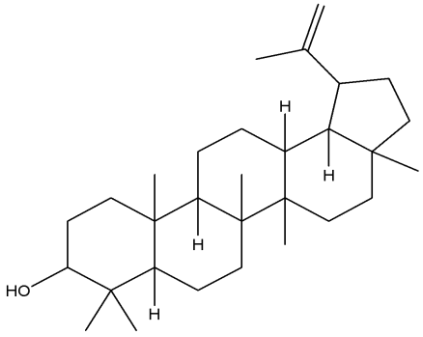

Plate 5: Structure of Lupeol

Discussion

The present study demonstrates that the n-hexane fraction of *Acacia senegal* leaves possesses potent antibacterial activity against *Staphylococcus aureus* and *Salmonella typhi*. The solvent-dependent activity observed, with non-polar fractions (n-hexane and chloroform) showing the highest zones of inhibition, is consistent with previous studies indicating that lipophilic bioactive compounds are efficiently extracted with non-polar solvents (Singh et al., 2016; Ahmed et al., 2018). Methanol and ethanol fractions also exhibited notable activity, whereas aqueous and ethyl acetate fractions showed limited or no inhibition, likely due to the lower solubility of active triterpenoids in polar solvents.

Phytochemical screening revealed the presence of saponins, steroids, terpenoids, phenolics, and tannins in the n-hexane fraction. The antibacterial activity observed can therefore be attributed to these secondary metabolites, particularly terpenoids, which are known to disrupt bacterial membranes and inhibit growth (Cowan, 1999; Ncube et al., 2008). The absence of flavonoids, alkaloids, and glycosides suggests that these classes do not significantly contribute to the activity in the n-hexane fraction.

The MIC and MBC results further confirmed the potency of the n-hexane fraction, with the lowest MIC recorded against S. typhi (0.78 mg/mL) and MBC of 25 mg/mL, indicating strong bacteriostatic and bactericidal properties. These values are comparable to or better than those reported for other medicinal plant extracts, highlighting the potential of *A. Senegal* as a source of antibacterial agents (Adegboye et al., 2019; Ahmed et al., 2020).

Isolation and spectroscopic characterization identified the major bioactive compound as lupeol, a pentacyclic triterpenoid. FT-IR, ^{1}H NMR, and ^{13}C NMR data confirmed its structure, with characteristic hydroxyl, olefinic, and methyl signals consistent with literature (Shwe, 2019). Lupeol has been previously reported to exhibit broad-spectrum antibacterial activity, anti-inflammatory, and antioxidant properties (Fahey, 2005). Its presence in the n-hexane fraction explains the observed strong antibacterial activity, particularly against the gram-negative S. typhi, which is often more resistant to conventional antibiotics.

These findings validate the traditional use of *A. Senegal* in treating bacterial infections and emphasize its potential as a source of plant-derived antibacterial compounds. The isolation of lupeol also provides a basis for further pharmacological studies, including mechanism-of-action investigations, in vivo efficacy, and potential synergistic applications with standard antibiotics.

CONCLUSION

The study demonstrates that *Acacia senegal* leaves possess significant antibacterial activity, with the n-hexane fraction showing the highest efficacy against *Salmonella typhi* and *Staphylococcus aureus*. Phytochemical analysis revealed the presence of saponins, steroids, terpenoids, phenolics, and tannins, which likely contribute to the observed antimicrobial effects. The isolation and spectroscopic characterization of lupeol from the n-hexane fraction confirms it as the major bioactive compound responsible for the antibacterial activity. These findings validate the traditional medicinal use of *A. Senegal* and highlight its potential as a source of plant-derived antibacterial agents, particularly against multidrug-resistant pathogens.Future work should focus on mechanistic studies, in vivo evaluations, and exploring synergistic combinations with conventional antibiotics to fully realize the therapeutic potential of lupeol and other bioactive constituents.

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