

Phytochemical, Antibacterial and Characterization of 9-methoxy-12-phenyl-dodec-10-enoic acid-3,4,5-trihydroxy-6-methyl-tetrahydropyran-2-ylester Isolated from *Acacia macrostachya* leaves

Garba, Umar^{1&2}, Faruq, U. Z.², Uba, A.³, Nasir, F.³, Yusuf, A.J.³, Kwazo, H.A.⁴ and Gayari, S.M.⁴

¹Department of Chemistry Shehu Shagari University of Education, Sokoto Nigeria.

²Department of Energy and Environmental Chemistry, Usmanu Danfodiyo University, Sokoto Nigeria

³Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto Nigeria.

⁴Department of Chemistry Shehu Shagari College of Education, Sokoto, Nigeria

*Corresponding Author's Email & Phone No.: umar.garba@ssues.edu.ng +234(0)8032932684

Received on: August, 2025 Revised and Accepted on: September, 2025

Published on: October, 2025

ABSTRACT

Acacia macrostachya is plant usually a tree belong to family *Mimosaceae* (*Fabaceae*), it is popularly known as "Gardaye" in Hausa Language (Sokoto). *A. macrostachya* is one of such useful plants in African traditional medicine. It is used to treat numerous diseases such as malaria with convulsion, diarrhea, inflammatory diseases, snake bite, anti-diabetic, and cancer among others. The aim of this research is conduct phytochemical screening; antibacterial studies of *Acacia macrostachya* leaves and characterizes the isolated compound from *A. macrostachya* leaves. The plant were collected from "Lungu" bush in Shagari local government area 12°38'N 5°0'E and authenticated at herbarium Department of Pharmacognosy and Ethnopharmacy Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria with Voucher number PCG/UDUS/Faba/0016. One kilogram of the powdered plant materials was methanol which gave the extract yields of 131.72g (13.17%). The phytochemical screening reveals the presence of alkaloids, steroids, flavonoids, cardiac glycosides, tannins, saponins, carbohydrate and phenolics. Antibacterial effects on the extracts at various concentration (200 mg/cm³, 100 mg/cm³, 50 mg/cm³ and 25 mg/cm³, against *S. Typhi*, *S. dysenterae*, *E. coli*, *P. aeruginosa* were studied. The extract was then subjected to column chromatography which leads to the isolation of compound UGP₂. The isolated compound was characterized using FTIR, and Nmr. The study showed that *A. macrostachya* leaves have medicinal potential which could help in the treatment of bacterial infections that affect human and animal respectively.

Keywords: *Acacia macrostachya*, Anti-bacteria, isolation, characterization, extract.

1.0 INTRODUCTION

Bacterial infections are thought to be the cause of over 8 million fatalities worldwide; they place a significant burden on the public health sector (WHO, 2018; GBD, 2019; Fred *et al.*, 2020). Infectious diseases caused by different bacteria, especially, *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli*, *Pseudomonas aeruginosa* among others and multiple resistances to antibacterial agents have become global problems largely due to the indiscriminate use of antimicrobial drugs commonly employed for the treatment of these infectious diseases (UNEP, 2024; Yusuf *et al.*, 2015). *Acacia macrostachya* is a plant, usually a tree belonging to the family *Leguminosae* (*Fabaceae*). It is popularly known as "Gardaye" in the Hausa Language (Sokoto). It is one of such useful plants in African traditional medicine (Ken, 2023). It is a prickly shrub growing to about 5 m high and it can adopt a longer climbing habit, become more tree-like and sometimes as much as 15 metres tall (Butler, 2019; Garba *et al.*, 2023; Ken, 2023), and widely distributed in savanna areas in West Africa (Bukil 1995; Barfour *et al.*, 2021). *Acacia macrostachya* plant has reportedly been used in ethnomedicines for the treatment

of a variety of illnesses, such as prostate enlargement, diarrhea, inflammatory diseases, snake bites, anti-diabetic, anti-cancer (Chika *et al.*, 2022; Coulibally *et al.*, 2020; Ganame *et al.*, 2020). Extensive literature searches reveal that no work has been done on the phytochemical, antibacterial properties and characterization of isolated compound from the leaves of *Acacia macrostachya*. This research, therefore, focuses on the phytochemical, antibacterial study and characterization of isolated compound from *Acacia macrostachya* leaves.



Plate I: *Acacia macrostachya* Plant

2.0 MATERIALS AND METHODS

2.1 Sample Collection, Identification, Drying and Powdering of Plant Material

The sample of *Acacia macrostachya* Leaves was collected from “Lungu” bush in Shagari local government area (12°38'N 5°0'E) of Sokoto State. It was identified at the Department of Pharmacognosy and Ethnopharmacy Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria with Voucher number PCG/UDUS/Faba/0016. The leaves of *A. macrostachya* were washed with distilled water to remove dirt, shade dried, grounded to powder and preserved in polyethene bag.



Figure 1: Map of sample collection area

2.2 Extraction of Plant Material (Maceration)

One kilogram (1 Kg) leaf powder of *A. macrostachya* was extracted successively with hexane, ethyl acetate and methanol (1:5) using a maceration method for 10 days with constant agitation. The extracts obtained were then filtered using muslin cloth, followed by filter paper (OCOME No 103, 240 mm) and the solvent was evaporated at 40°C using a rotary evaporator. (Buhari *et al.*, 2022; Muhammad *et al.*, 2022; Sani *et al.*, 2020).

2.3 Phytochemical Screening

The standard procedure for phytochemical screening described by Garba *et al.* 2025 and Muhammad *et al.* (2022) was used in this research. These are as follows:

a. Test for Alkaloids

2 grams of crude extracts was dissolved with 20 cm³ of distilled water. 10 cm³ of the crude extracts were diluted with 5cm³ of (1 % HCl) in a boiling tube, the mixture were boiled on a water bath.

- i. **Dragendorff's Test:** Three (3) drops of Dragendorff's reagent were added to the 2 cm³ crude extracts in the test tube. The formation of a reddish-brown precipitate indicated the presence of alkaloids.
- ii. **Wagner's Reagent:** Three (3) drops of Wagner's reagent were added to crude

extracts (2 cm³) in a test tube, and the formation of a dark brown precipitate indicated the presence of alkaloids.

- iii. **Mayer's Reagent:** Three (3) drops of Mayer's reagent were added to crude extracts (2 cm³) in a test tube. An orange precipitate was obtained which indicated the presence of alkaloids.

b. Test for Anthraquinones

Borntrager's Test: To 2 cm³ crude extracts, 2 cm³ of 10% ammonia solution was added. The formation of rose-pink (reddish-purple) coloration confirmed the presence of Anthraquinones.

c. Test for Cardiac glycosides (Keller Kiliani)

A small quantity of crude extract (50 mg) was dissolved in 1 cm³ of glacial acetic acid containing five (5) drops of ferric chloride solution. The solution was then transferred to a clean test tube and 2 cm³ of sulfuric acid was added. A formation of a brown ring at the interface indicates the presence of cardiac glycoside

d. Test for Steroids/Terpenes

- i. **Liebermann-Buchard's Test:** A small quantity of the crude extracts was diluted with chloroform (2 cm³), then acetic anhydride (5 cm³) and concentrated sulfuric acid (2 cm³) was also added to the solution. The formation of green coloration showed the presence of steroids.

- ii. **Salkowski Test:** The crude extracts (0.5 g) were dissolved in chloroform (5 cm³) and concentrated sulfuric acid (2 cm³) was then added to the mixture. Formation of a red precipitate indicated the presence of steroids.

e. Test for Flavonoids

Ferric Chloride Test: Two (2 cm³) of 5% ferric chloride solution were added into a test tube containing 5 cm³ crude extracts. The formation of green precipitate showed the presence of flavonoids.

f. Test for Saponins

Two (2) cm³ of the extracts were diluted with 5 cm³ of distilled water in a 100 cm³ measuring cylinder and shook vigorously, and then allowed to stand for 15 minutes. Persistent foaming indicated the presence of saponins (Muhammad *et al.*, 2022).

g. Test for Tannins

Ferric Chloride Test: Distilled water (5 cm³) was added to 2 cm³ of the crude extracts. The mixture was heated to boil for five (5) minutes, two drops of 5% ferric chloride was then added. Formation of greenish precipitate indicated the presence of tannins.

2.4 Anti-Bacterial Test

Bacterial isolates used in this research were obtained from the Microbiology Department of Usmanu Danfodiyo University, Sokoto, these are *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli* and *Pseudomonas aeruginosa*.

2.4.1 Isolation and Identification of Bacteria

The bench space and practical shelves were cleaned with 70% alcohol swabs before the commencement of the experiment (bench work). The samples were inoculated immediately on sheep blood agar and MacConkey agar plates. Inoculated plates were incubated at 37°C for 24 h. After incubation, a growth pattern was observed on agar plates and isolated colonies proceeded for identification, whereas others were subculture to obtain pure growth. After pure growth, microscopy such as gram stain was done to differentiate the gram-positive and gram-negative bacteria. Phenotypic methods have been used for the identification of bacteria. Isolates were identified by colony morphology and microscopic features. For confirmation, biochemical tests (e.g., catalase, coagulase, DNase, oxidase, triple sugar iron, motility, urease, citrate, indole, methyl red, and Voges–Proskauer) were used for other gram-negative bacteria (Muazzam *et al.*, 2025).

2.4.2 Susceptibility Test

The antibacterial activity of the extracts was determined via a susceptibility test using an agar diffusion method. Two hundred milligrams per centimeter cubes (200 mg/cm³) of stock concentration were prepared by dissolving 1g of *A. macrostachya* leaves extracts in 5 cm³ dimethyl sulfoxide (DMSO) solution. Muller Hinton Agar (Growth Media) was prepared according to the manufacturer's instructions "38 g were suspended in 1 liter of distilled water, the media were boil so as to dissolve completely and sterilized by auto-clove at 121°C for 15 minutes." Poured onto sterile petri dishes and were allowed to cool solidified, inoculated and incubated for 24 hours. The solidified sterile medium was seeded with a 0.1 cm³ standard inocula of the test bacteria; the inoculum was spread over the surface of the medium with the aid of a sterile swab stick. Wells were bored into the solidified inoculated nutrient agar plate using a cork borer of 6 mm diameter. The wells were filled with a 0.1 cm³ DMSO solution of the extract and allowed to diffuse for 1 h. Incubation of the inoculated medium was made at 37°C for 24. Ciprofloxacin disc was used as a reference for antibacterial. The test was conducted twice and the average zone of inhibition around the wells was recorded in millimeters (mm) and used as the antibacterial activity of *A. macrostachya* leaves extracts (Uba *et al.*, 2020; Muhammad *et al.*, 2022).

2.4.3 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the *A. macrostachya* leaves extracts was determined using a Broth dilution method. Muller Hinton broth was prepared according to the manufacturer's instructions; (39.0 g in 1 liter of distilled water mix and heated to boil and autoclave at 15 psi pressure at 121°C for 15 minutes). 2 cm³ of the medium were dispensed into 96 well micro-titre plates and sterilized at 121°C for 15 minute, and then were allowed to cool. Dilution of the organism suspension was done continuously using sterile normal saline until the turbidity matched that of Mc-Farland's scale by visual comparison. At that point, the concentration of the test microbes was about 1.5x10⁸cfu/cm³. Two-fold serial dilution of the *A. macrostachya* leaves extracts was performed in the sterile broth to obtain a concentration of 25 mg/cm³, 50 mg/cm³, 100 mg/cm³ and 200 mg/cm³ respectively. 0.1 cm³ of the standard inoculum of the test microbe was then inoculated into the different concentrations of the extracts in the broth, incubated at 37°C for 24h after which, the plate was observed for turbidity (growth) and the lowest concentration of the extracts in the sterile micro-titre plate which shows no turbidity was measured as the minimum inhibition concentration (Luhata and Toyonobu 2021; Alex-Asaolu *et al.*, 2024)

2.4.4 Determination of Minimum Bactericidal Concentration (MBC)

The MBC was carryout to determine whether there is complete death of test microbes or just growth inhibition. Muller Hinton agar broth was prepared, sterilized at 121°C for 15 minutes and was then transfer into sterile petri dishes to cool and solidify. The content of the MIC in the serial dilution were sub-cultured into the prepared medium and was incubated at 37°C for 24h; the plates was observed for colony growth; the MBC was the plate with lowest concentration of the extract in serial dilution without colony growth (Yusuf *et al.*, 2015; Alex-Asaolu *et al.*, 2024).

2.5 Chromatographic Technics

2.5.1 Thin-layer Chromatography

Thin-layer chromatography was carried out on methanol extract using TLC silica gel 60F254 by using one-way ascending techniques. The plates were cut with scissors and marked with pencil about 1 cm from the bottom of the plate. The extract was dissolved in methanol and capillary tubes were used to uniformly apply the dissolved sample to the plate and were allowed to dry. The plates were developed in a chromatographic tank using different solvent systems. The plates were dried and

visualized under normal daylight, ultraviolet light (254 nm and 366 nm) and also by spraying 10% sulfuric acid, followed by heating at 105°C for 5–10 minutes in an oven. The R_f values were calculated using the following equation:

$$R_f = \frac{\text{Distance travel by spot}}{\text{Distance travel by the solvent}} \dots\dots\dots 1$$

2.5.2 Column Chromatography

The most active extract (methanol), 12.0 g, was mixed with 3.0 g silica gel (60-120 mesh) and eluted in a silica gel-packed column (5 X 75 cm) using different solvent systems started with chloroform (100%), Chloroform: methanol (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, 50:50) and finally methanol (100%). Two hundred and ninety-nine (299) fractions of 30 cm³ were collected and combined based on their TLC profile into twenty-seven (27) fractions coded UG1-UG27. Fraction UG27 was subjected to further purification with Sephadex LH-20.

2.6 FTIR Analysis Isolated Compound

The FTIR of the isolated and purified compound was carried out with an Agilent Cary 630 FTIR spectrometer with a base system of high emission of Infrared radiation. The base system is used in conjunction with a 5-bounce diamond ATR sampling accessory of internal reflection element (IRE) crystal. One milligram (1mg) of the compound was encapsulated with 10mg of potassium bromide (KBr) pellet, in order to prepare a translucent sample disc. The sample was placed in a holder in the path of the IR source with a scan range of 4000-400 cm⁻¹ with a resolution of 4cm⁻¹. The detector then read the analog signal and the signal was converted to spectrum. A computer was used to analyze the signal and the peak was identified (Parkkirisamy *et al.*, 2017).

2.7 Nmr spectroscopic analysis of the isolated compound

The isolated and purified compound were transported to united kingdom for nuclear magnetic resonance spectroscopy using Bruker Nmr instrument.

3.0 RESULTS

The table below shows the percentage yield of *A. macrostachya* leaves extract

Table 1: Extract yields from *Acacia macrostachya* leaves

Extracts	Yield (g)	Yield (%)	Colour
Methanol	131.72±0.20	13.17±0.20	Redish-brown

Table 2: Results for Phytochemical Screening of *A. macrostachya* leaves extract

SN	Phytochemical	Results Methanol
1.	Alkaloids (Dragendorff's Test)	++
	Wagner's Test	++
	Mayer's Test	+++
2.	Anthraquinones	-
3.	Cardiac glycosides(keller-killani Test)	+++
4.	Steroids/Terpenes Salkowski's Test	++
	Libermann-Burchard's Test	+
5.	Tannins (FeCl ₃ Test)	+++
	Lead Subacetate Test	+++
6.	Falvonoids (FeCl ₃ Test)	+++
	Shinoda's Test	++
	Alkaline test (10% NH ₄ OH)	++
7.	Carbohydrate Fehling's test	++
8.	Saponins	+++
9.	Phenolics	+++

Key: + = trace, ++ = moderately present, +++ = abundantly present, - = absent

Table 3: Results of in-vitro Antibacterial analysis of *A. macrostachya* Extract
Concentration (mg/ml)/Zone of inhibition (mm)

Isolates	Extract	200mg/ml	100mg/ml	50mg/ml	25mg/ml	+Co ntrol	- 5%DMS O
<i>S. typhi</i>	ME	20.41±0.08	15.18±0.05	13.30±0.10	7.14±0.02	30	-
<i>S. dysenteriea</i>	ME	23.14±0.01	21.20±0.1	17.23±0.15	13.40±0.10	26	-
<i>E. coli</i>	ME	17.30±0.27	14.30±0.15	9.13±0.11	8.07±0.02	23	-
<i>P. aeruginosa</i>	ME	27.22±0.08	21.04±0.01	17.22±0.08	13.40±0.20	26	-
P-value	0.001						

3.1 TLC Profile of some column collection from methanol extract of *A. macrostachya* leaves

The thin-layer chromatographic profile of column collections from methanol extract conducted using Hexane: chloroform (7:3), Hexane: ethyl acetate (8:2), chloroform: methanol (4:1) chloroform: methanol (4:2) as solvent systems reveals various spots after spraying with 10% H₂SO₄ followed by heating at a temperature of 105°C

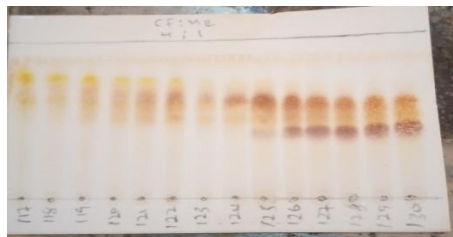


Plate II: TLC of some fraction from *A. macrostachya* leaves during column chromatography

Table 4: Some information about isolated compound

Compound	Solvent Systems	No of Spots	Color of spot after spraying 10% H ₂ SO ₄	Rf Value
UGP ₂ (UGB)	CHCl ₃ :EtOAc (6:1)	1	Pink	0.69



Plate III: TLC for Compound UGP2

Table 5 Fourier Transform Infrared Spectroscopy (FT-IR) for Compound UGP2

Frequency literature (cm ⁻¹)	Frequency of sample (cm ⁻¹)	Intensity	Functional group
2700-3200	2926.0(s)	86.76	C-H(alkane)
2800-3000	2851.40(m)	91.06	C-H (Alkane)
1735-1750	1735.10(w)	96.84	C=O (ester)
1566-1650	1572.90(m)	95.46	C=C (Cyclic alkenes)
1455-1465	1459.30(m)	94.63	C-H (Alkane)
1200-1275	1261.70	95.82	C-O (mono-substitution of benzene)
760-800	797.7(m)	95.59	C=C (alkene)

Key: (m); medium peak, (s); strong peak, (w); weak peak

3.2 ^1H NMR Analysis of UGP2

The ^1H NMR of UGP2 revealed the resonance at δ_{H} 6.70(2H,d, J= 12Hz, H - 2,6), 7.10 (2H,d, J=12Hz, H - 3,5), 7.70 (1H, s, H - 4), 1.26 (2H,d, J =12Hz, H - 2'), 1.96 (2H, s, H - 3'), 1.61 (2H, s, H - 4'), 1.29 (2H, s, H - 5'), 0.80 (2H, s, H - 6'), 0.77 (2H, s, H - 7'), 1.96 (2H, t, H - 8'), 5.80 (1H, s, H - 9'), 4.94 (1H, s, H - 10'), 4.91 (1H, s, H - 11'), 2.30 (2H, s, H - 12'), 2.26 (1H, s, H - 1''), 2.24 (1H, s, H - 2''), 2.17 (1H, s, H - 3''), 3.60 (1H, s, H - 4''), 1.96 (2H, s, H - 5'').

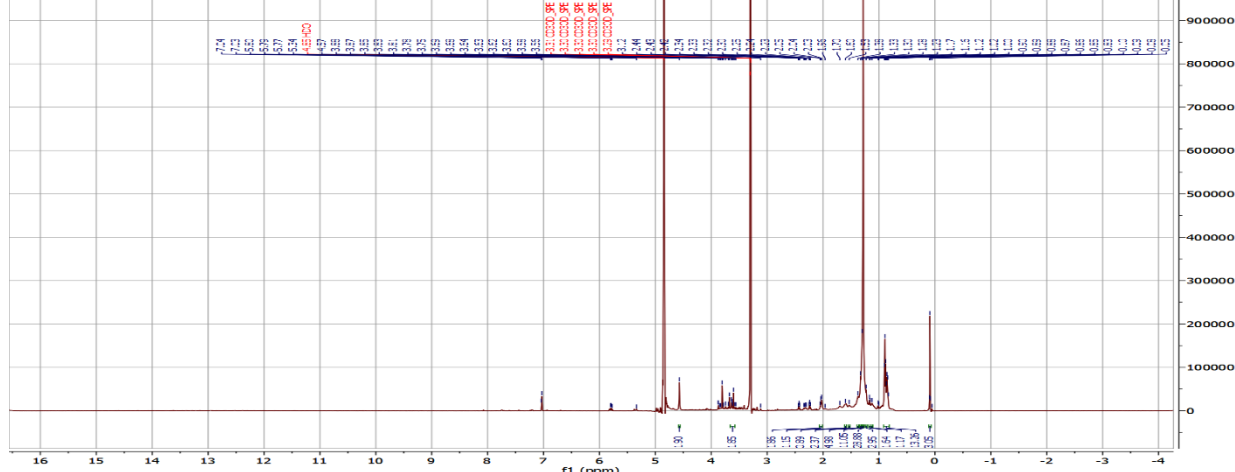


Figure 2: ^1H NMR Spectra of compound UGP 2

3.3 The ^{13}C NMR (600MHz, CD_3Cl_3) of UGP2

The ^{13}C NMR (600MHz, CD_3Cl_3) indicated the presence of 25 carbon atoms; δ_{C} 175.0 (C - 1'), 30.9 (C - 2'), 28.4 (C - 3'), 24.7 (C - 4'), 22.3 (C - 5'), 19.2 (C - 6'), 13.2 (C - 7'), 25.7 (C - 8'), 92.0 (C - 9'), 117.2 (C - 10'), 113.1 (C - 11'), 32.2 (C - 12'), 155.1 (C - 1), 114.0 (C - 2,6), 128.2 (C - 3,5), 138.7 (C - 4), 104.3 (C - 1''), 76.2 (C - 2''), 76.0 (C - 3''), 75.8 (C - 4''), 65.4 (C - 5'').

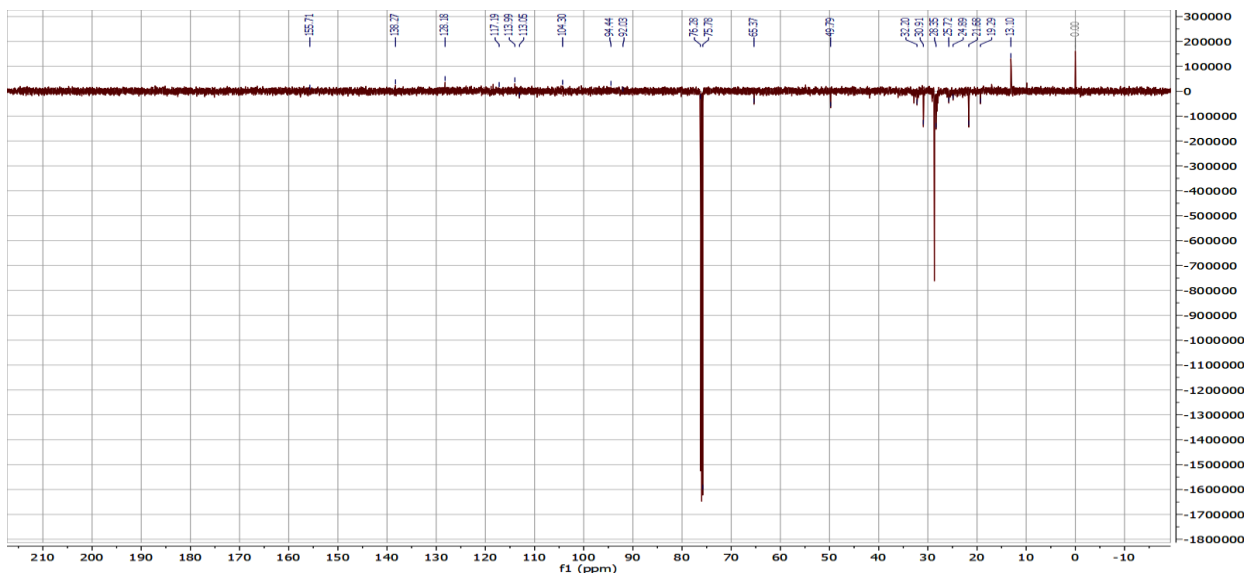


Figure 3: ^{13}C NMR Spectra of compound UGP2

3.4 H-H COSY Spectral Analysis of UGP2

The 2D H-H COSY correlation of UGP2 indicate the proton that are situated at the adjacent carbon. Major correlation include; H3 (7.2) # H4 (7.70) H2 (6.70) # H3 (7.20), H10' (4.94) # H9'(5.80), H9' (5.80) # H8' (1.96), H5'(1.29) # H8'(1.96). As shown in the figure below:

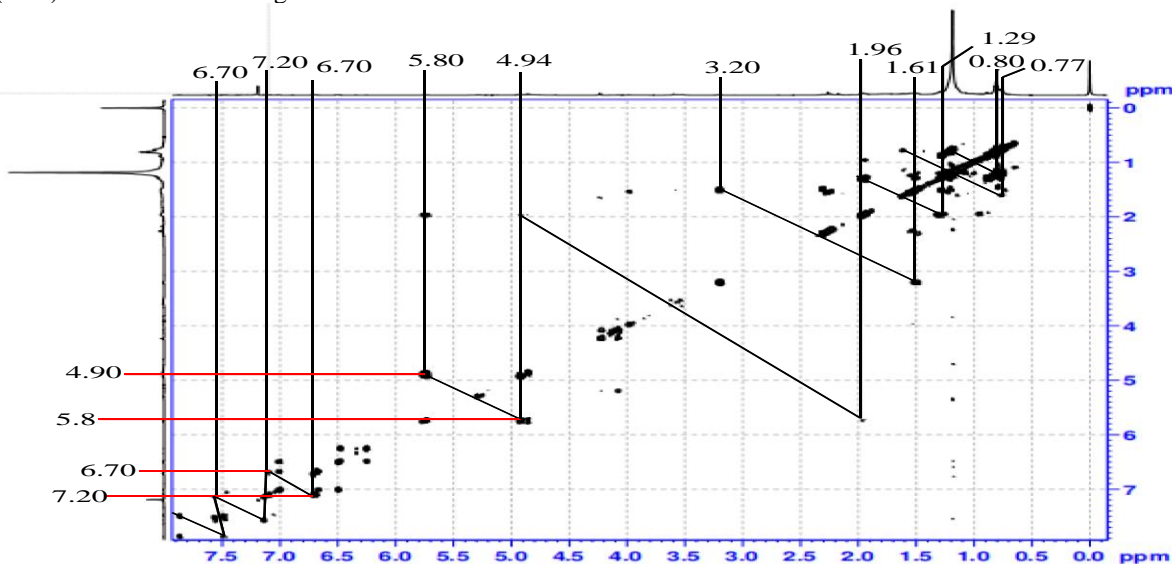


Figure 4: H-H Cosy Spectra of UGP2

3.5 HSQC spectral Analysis of UGP2

The HSQC experiments of UGP2 established the attachment of various protons in their respective carbons. The major correlation observed includes δ 7.70 (H - 4) and 138.7 (C - 4), 7.20 (H - 3) and 128.2 (C - 3), 6.70 (H - 2) and 114.0 (C - 2), 4.91 (H - 11') and 113.1 (C - 11'), 4.94 (H-10') and 117.2 (C - 10'), 0.77 (H - 7') and 13.2 (C - 7'), 1.19 (H - 3') and 28.4 (C - 3') among others. As shown in the figure below:

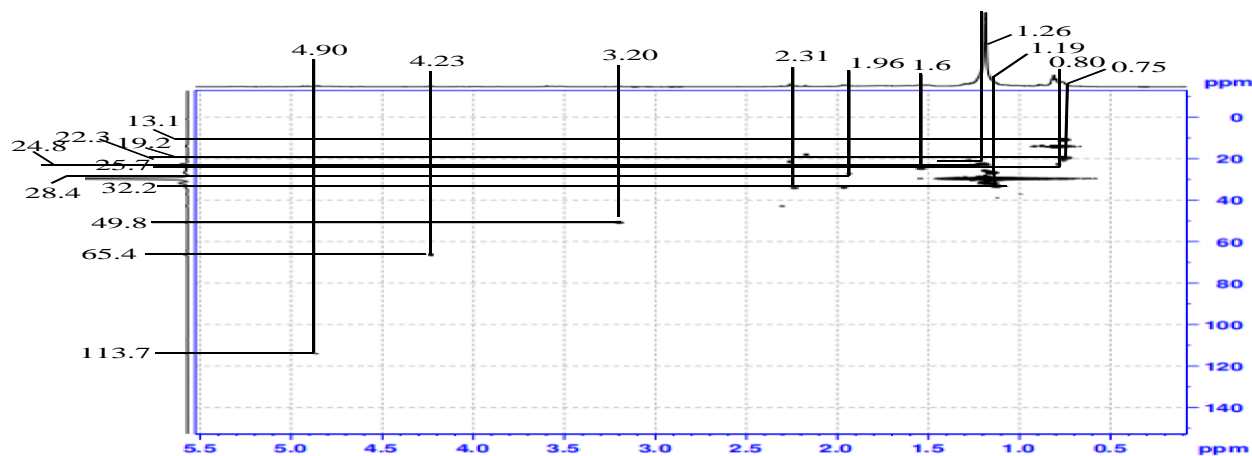


Figure 5: HSQC Spectra of Compound UGP2

3.6 HMBC of UGP2

The HMBC spectrum of UGP2 established the long range connectivity between various units of the molecule. Proton 2.31 (H - 12') correlated with 114.0 (C - 2) and 138.7 (C - 4), 0.77 (H - 7') correlated with 49.8 (C - 9'-OCH₃) and 25.7 (C - 8'), 0.80 (H - 6') correlated with 30.9 (C - 2') and 22.3 (C - 5'), 2.26 (C - 1'') correlated with 175.0 (C - 1') as shown in the figure below:

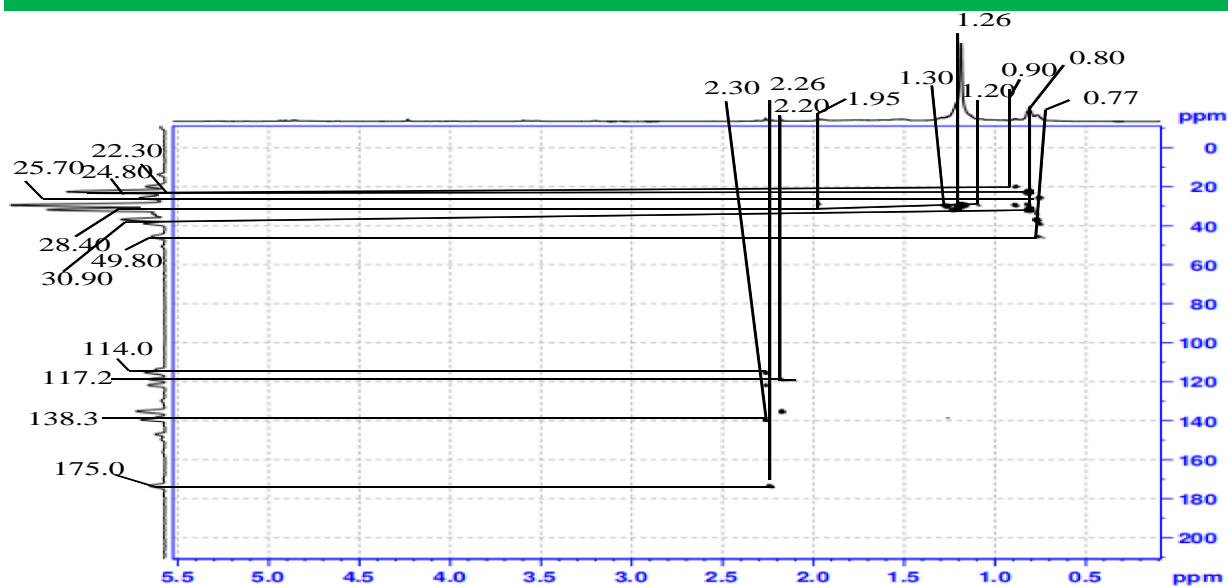


Figure 6: HMBC Spectra of compound UGP2

Table 6 Summary of 1D & 2D NMR of Compound UGP2

POSITION	DEPT	¹³ C	¹ H NMR	COSY	HMBC
1	C	155.1	-	-	-
2,6	CH	114.0	6.70 (2H, d, J= 12Hz)	H-3, H-5	-
3,5	CH	128.2	7.20 (2H, d, J=12 Hz)	H-4	-
4	CH	138.7	7.70 (1H, s)	-	-
1'	C	175.0	-	-	-
2'	CH ₂	30.9	1.26 (2H, d, J=12Hz)	-	C-3'
3'	CH ₂	28.4	1.19 (2H, s)	-	-
4'	CH ₂	24.7	1.61 (2H, s)	-	-
5'	CH ₂	22.3	1.29 (2H, t)	H-8'	-
6'	CH ₂	19.2	0.80 (2H, t)	C-2', C-5'
7'	CH ₂	13.2	0.77 (2H, s)	H-4'	C-8', C-9'- OCH ₃
8'	CH ₂	25.7	1.96 (2H, t)	H-9'	-
9'	CH	92.0	5.80 (1H, s)	H-10'	C-2, C-4
10'	CH	117.2	4.94 (1H, s)	-	-
11'	CH	113.1	4.91 (1H, s)	-	-
12'	CH ₂	32.2	2.30 (2H, s)	-	C-1'
1''	CH	104.3	-	-	-
2''	CH	76.2	2.24 (1H, s)	-	-
3''	CH	76.0	2.17 (1H, s)	-	-
4''	C	75.8	3.60 (1H, s)	-	-
5''	CH ₂	65.4	1.96 (2H, t)	-	-

4.0 DISCUSSION

Extraction yield is affected by the solvent and extraction process (Nortjie *et al.*, 2023). The extraction process used must allow for full extraction of the chemicals of interest while avoiding chemical alteration (Dwived *et al.*, 2023). The methanol extract demonstrated higher percentage yield of 13.17 ± 0.20 % (Table 1). The results obtained was found to be higher than 2.53% *Triumfetta welwitschii* leaves extract and lower than 17.14 for *Guiera senegalensis* as reported by Mombeshora and Mukanganyama, (2019) ; Shehu *et al.* (2025). Hence,

A. macrostachya leaves have many polar compounds. The alkaloids content in methanol is higher than that of ethyl acetate followed by hexane extract. Alkaloids are important secondary metabolite which is responsible for anti-tumor, diuretic, anti-viral, analgesic as well as anti-inflammatory effect (Dieu-Hien *et al.*, 2019). Anthraquinones are only presence in ethyl acetate. Anthraquinones plays importance role as anti-inflammatory, antioxidant, antibacterial, antiviral and anticancer (Xin *et al.*, 2022). Cardiac glycosides are presence in both extracts, cardiac glycoside plays

important role as antioxidant and anticancer (Patel, 2016). Steroids/terpene are much higher present in hexane followed by ethyl acetate and methanol respectively. Steroid has an important role in growth development, sexual differentiation and reproduction (Anarado *et al.*, 2020). Methanol extract shows high significance of tannins followed by ethyl acetate. Tannins play an important role in anti-viral, anti-bacterial and anti-parasitic agents and inhibit pathogenic fungi, also associated with many human physiological activities (Muhammad *et al.*, 2022). Flavonoids were found to be higher in methanol extracts followed by ethyl acetate. Flavonoids can be used in the treatment of COVID-19, pregnancy-induced disorders, neurodegenerative disorders; they have shown significant antihypertensive, anticancer, antioxidant and anticholinesterase properties (Mondal and Rahaman, 2020). Carbohydrates are in both ethyl acetate and methanol extracts, carbohydrate are primary source of energy for cells, which provides fuel for metabolic processes and help in regulating blood sugar levels. Finally saponins are found to be only present in methanol extract. Saponins exhibits antibacterial, antifungal and antiviral properties, which help to protect plants from pathogen, it may help to protect against gastrointestinal disorders and promote gut health, antidiabetic effects, and lower cholesterol levels and improve cardiovascular health and inhibit tumor growth (Kareem *et al.*, 2022).

The antibacterial studies for the extracts reveals that methanol extract have high activity against tested bacteria as presented in Table 4.3. For *S. typhi* (20.41±0.08) the results were found to be lower than 24mm for *G. senegalensis* leaves and 22.28± 0.19 for *S. macrostachya* seeds reported by Shehu *et al.* (2025) and Zagga *et al.* (2024). For *S. dysenteriae* (23.14±0.01) this were found to be higher than 17.00mm for *G. senegalensis* leaves and 15.02 ± 0.11 mm for *S. macrostachya* seed as reported by Shehu *et al.* (2025) and Zagga *et al.* (2024). For *E. coli* (17.30±0.27) the result was found to be lower than 20.02 ± 0.11 mm for *G. senegalensis* and 21.22± 0.19 for *S. macrostachya* seed but were found to be higher than 13.1 ±0.27mm for *Acacia catechu* leaf extracts reported by Shehu *et al.* (2025), Zagga *et al.* (2024) and Imran *et al.* (2021) respectively. For *P. aeruginosa* (27.22±0.08) the results were found to be higher than 13 ± 0.02 and 25.00 for *Triumfetta welwitschii* as reported by Muazam *et al.* (2025); Mombeshora and Mukanganyama, (2019) respectively. The minimum inhibitory concentration was found to be 100 mg/cm³. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each micro-organism. The MBC was the plate with lowest concentration of the extract in serial dilution without colony growth. The column chromatographic

separation of the most active extract (methanol) followed by purification led to the isolation of a brown coloured compound coded UGP-2 respectively. The FTIR for compound UGP-2 reveals the presence of alkanes (C-H) at 2926.0 cm⁻¹, C=O (ester) at 1735.10 cm⁻¹ C=C (Cyclic alkenes) at 1572.90 cm⁻¹, C-O (mono-substitution of benzene) at 1261.70cm⁻¹ and alkene (C=C) 797.7 cm⁻¹ respectively. The proton nmr (1H NMR) of UGP-2 exhibited signals for ortho-coupled aromatic hydrogen δ 7.20 (2H, d, J = 12Hz, H – 3, 5; δ 6.70, 2H, d, J = 12Hz, H – 2, 6 given an AA BB aromatic splitting and a proton δ 7.70 (1H, s) assigned to H-4. It also revealed the presence of two olefinic hydrogen (δ 4.91 (1H, s) and δ 4.94 (1H, s) at position H-11' and H-10'. Other resonances were at δ 5.80 (1H, s, H-9'), δ 2.30 (2H, s, H-12'), δ 1.96 (2H, s, H-9'; 2H, t, H-8'), δ 1.61 (2H, s, H- 4'), δ 1.29 (2H, s, H-5'), δ 0.80 (2H, s, H-6'), δ 0.77 (2H, s H-7') among others on the aliphatic chain. Finally, resonances at δ 3.60 (1H, s, H-4''), δ 2.26 (1H, s, H-1''), δ 2.24 (1H, s, H-2''), δ 2.17 (1H, s, H-3'') and 1.96 (2H, s, H-5'') indicating the presence of sugar moiety (Abdullahi *et al.*, 2019). The ¹³C NMR (600MHz, CDCl₃) experiment of UGP-2 revealed the presence of twenty two carbon chemical shift signals of which δ 113.1 and 117.0 were assigned to olefinic carbon at position C - 11' and C-10'. Signals at δ 128.2, δ 114.2 and δ 138.7 were assigned to aromatic methine carbons at position 2, 3 and 4 respectively, while δ 155.1 for the quaternary carbon 1t position 1 of the ring. Signal at δ 175.0 was assigned to a carbonyl group at position 1'. Also signal δ 92.0 on the aliphatic chain was for carbon at position 9' attached to a methoxy group. Other signal on the aliphatic chain were δ 32.2 (C-12'), δ 30.9 (C-2'), δ 28.4 (C-3'), δ 25.7 (C-8'), δ 24.7 (C-4'), δ 22.3 (C-5'), δ 19.2 (C-6'), δ 13.2 (C-7'), among others. Finally, chemical shift at δ 104.3 (C-1''), 76.2 (C-2''), 76.0 (C-3''), 75.8 (C-4'') and 65.6 (C-5'') were characteristics of sugar absorption suggesting the sugar as rhamnose (Abdullahi *et al.*, 2019) H-H COSY of UGP-2 established the correlations between the protons situated in the same environment. There exist cross peaks between protons at δ 6.70 (H-2') and δ 7.20 (H-3') at ortho-coupled position confirmed the assignment of the ring in the structure. The assignment of protons on the aliphatic chain was substantiated via the cross peaks observed between the protons at 1.96 (H-8') and 1.29 (H-5'), and also cross peak observed between the proton at 1.61 (H-4') and 0.77 (H-7'). Furthermore, another cross peak observed on the aliphatic chain between the protons 5.80 (H-9') and 1.96 (H-8'). The HSQC experiment of UGP-2 established the attachment of various protons in their respective carbons. Major correlations observed include δ 7.70 (H-4) and 138.7 (C-4), 7.20 (H-3) and 128.2 (C-3), 6.70 (H-2) and 114.0 (C-2), 4.91 (H-11') and 113.1 (C-11'), 4.94 (H-10') and 117.2 (C-10'), 0.77 (H-

7') and 13.2 (C-7'), 1.19 (H-3') and 28.4 (C-3') among others. The HMBC spectrum of UGP-2 established the long range connectivity between the various units of the molecule. The long range correlation was observed between δ 2.30 (H-12') and 114.0 (C-6) which confirmed the attachment of methoxy group on the aliphatic chain was confirmed via long range correlation observed between δ 0.77 (H-7') and 49.8. Furthermore, there was also long range correlation between δ 2.26 (H-1'') and 175 (C-1') which confirmed the attachment of sugar moiety on the aliphatic chain at C-1' (Bhinderwala *et al.*, 2022).

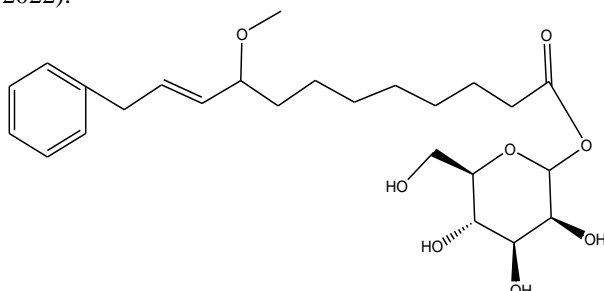


Figure 7: Structure of the isolated compound UGP2

5.0 CONCLUSION AND RECOMMENDATIONS

Acacia macrostachya leaves have shown the presence of secondary metabolite such as alkaloids, anthraquinones, cardiac glycosides, steroids, tannins, and flavonoids. The plants extracts demonstrate activity on the tested bacterial isolates. The most active extract (i.e. methanol) was subjected to column chromatographic techniques which led to the isolation and characterization of compound (9-methoxy-12-phenyl-dodec-10-enoic acid-3,4,5-trihydroxy-6-methyl-tetrahydropyran-2-ylester). Thus the finding of this study validates the antibacterial claim of the *A. macrostachya* leaves in treatment of varieties of illness cause by bacterial species.

The researcher made the following recommended the following:

- Antiviral study of the *A. macrostachya* should be conducted
- Antivenin activity of the plant should be also investigated to validate its ethno-medicinal claim for the treatment of snake bite.
- Further researches should be carryout on other pharmacological activity on *A. macrostachya* leaves

REFERENCES

Abdullahi., S.M., Musa, A.M., Sani, Y.M., Abdullah, M.I & Magaji, M.G. (2019). Isolation of Rutin from leaves

of *Ziziphus mucronata* WILD Rhamnaceae. *Bayero Journal of Pure and Applied Sciences* **12** (1):573-576
Alex-Asaolu, A., Uba, A., Birnin-Yauri, U.A. & Yusuf, A.J (2024). Isolation and Characterization of antimicrobial constituents from the stem of *cissus populnea* Guill. & Perr. *Drugs and Drug candidates* 3:172-183.

Anarado, C.E., Anarado, C.J.O., Umedum, N.L., Chukwubueze, F.M. & Anarado, I.L. (2020) Phytochemical and Antimicrobial analysis of leaves of *Bridelia micrantha*, *Cassia filiformis*, *Euphorbia hirta* and *Securinega virosa*. *Journal of Pharmacognosy and Phytochemistry* **9**(3): 581-587

Barfour, A.F., Mensah, A.Y, Evelyn, A.K., Cynthia, A. D., Anokwah, D Adjei, S Michael K. B. & Merlin L. K. M (2021). Antibacterial, Antibiofilm, and Efflux Pump Inhibitory Properties of the Crude Extract and Fractions from *Acacia macrostachya* Stem Bark. *Hindawi the Scientific World Journal* : 1-10
<https://doi.org/10.1155/2021/5381993>

Bhinderwala, F., Vu, T., Smith, T.G., Kosacki, J., Marshall, D.D., Xu, Y., Morton, M., & Powers, R. (2022). Leveraging the HMBC to facilitate metabolite identification. *Analytical Chemistry* **94**(47):16308-16318 doi:10.1021/acs.analchem.2002902.

Buhari, M.K., Dangoggo, S.M., Mohammed, M. L., Abubakar, H., Mohammed, A .A., Dambatta, M.B. & Yusuf, A.J (2022). Isolation and characterization of some flavonoids from the leaf of *Tapinanthus globiferus* growing on *Acacia nilotica*. *Caliphate Journal of Science and Technology* 1:40-45. DOI: <https://dx.doi.org/10.4314/cajost.v4i1.6>

Burkill, H. M (1995). *The Useful Plants of West Tropical Africa*, Royal Botanic Gardens, Kew, Richmond, UK, 2nd edition.

Bulter, R.A (2019). *World rain forests*. Available on <https://worldrainforests.com/04-rainforest-canopy.html>

Chacko, S. (2022). Bacterial infections caused most deaths in sub-Saharan Africa in 2019: Lancet. Retrieved on 4th march, 2023 from <https://www.downtoearth.org.in/news/africa/bacterial-infections-caused-most-deaths-in-sub-saharan-africa-in-2019-lancet-86209>

Chika, A., Bello, S.O., Yunusa, A., Yahaya, A.B. & Sadiq, A (2022). Ethnobotanical survey of medicinal plants used as remedies for benign prostatic hyperplasia in Sokoto North western Nigeria. *National Journal of Physiology, Pharmacy and Pharmacology*. **12**(4):511-518

Coulibaly, A.C., Esther, W.L.M., Kabre, B., Traore, T.K., Achatde, C., Boly, G.L., Nebie, M.T., Ouedraogo, N., Kiendrebeogo, M. & Sawadogo, R.W (2020). Phytochemical screening, anti-inflammatory and

- Analgesic activities of Root barks from *Acacia macrostachya* Reichenb. Ex DC. (Fabaceae). *Annual Research and Review in Biology* **35**(7):76-83
- Dieu-Hien, T., Dinh, H.N., Nhat T.A .T., Bui, A.V., Tuong., H. D. & Hoang, C. N (2019). Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of *Severinia buxifolia* *Hindawi Journal of Food Quality* **9**(2):1-110 <https://doi.org/10.1155/2019/8178294>
- Dwivedi, S., Singh, S and Singh, J. (2023). Effect of extraction solvent and plant part on the yield of phenolic compounds, plumbagin and biological activity of *Plumbago zeylanica*. *Journal of Pharmacognosy and Phytochemistry* **12**(6): 05-10
- Fred, A.A., Jean, N.I., Brice, E.N.W., Justin, K., Paul, N., Bathelemy, N., Veronique, P.B., Bonaventure, T.N. & Victor K (2020). Antistaphylococcal activity of extracts, fractions and compounds of *Acacia polyacantha* Wild (Fabaceae). *Hindawi. Evidence-Based complementary and Alternative Medicine* :1-10. <https://doi.org/10.1155/2020/2654247>
- Ganame, H.T., Karanga, Y., Ilboudo, O., Wende-Konte H.CN., Sawadogo R.W. & Tapsoba, I. (2020). α -Glucosidase inhibitory and Antiradical properties of *Acacia macroschya*. *European Journal of medical and health sciences* **2**(5):1-6
- Garba , U., Umar, A ., Sa'adu, A ., Abubakar, K ., Umar, Z., & lauwali Mahmud, L. (2025). Phytochemical and Antibacterial Studies of Ethyl Acetate Extract from *Acacia macrostachya* Leaves. *International Journal of Chemistry and Chemical Processes*. 11(4):18-25
- Garba, U., Faruk, U.Z., Uba, A., Nasir, I & Yusuf, A.J. (2023). Phytochemical and antibacterial studies of *Acacia macrostachya* leaves . A Paper Presented at ISCEST X Annual International and 4th Virtual conference 29th November 2023
- GBD(2019): [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(20\)30925-9/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30925-9/fulltext);GRAM: [https://www.thelancet.com/journal/s/lancet/article/PIIS0140-6736\(21\)02724-0/fulltext](https://www.thelancet.com/journal/s/lancet/article/PIIS0140-6736(21)02724-0/fulltext)
- Global Health Data Exchange, GHDx (2022). The Lancet : "One in eight deaths in 2019 linked to bacterial infections, the second leading cause of death globally" Antimicrobial resistant. <https://www.healthdata.org/news-events/newsroom/news-releases/lancet-one-eight-deaths-2019-linked-bacterial-infections-second>
- Imran M, Khan AS, Khan MA, (2021). Antimicrobial activity of different plants extracts against *Staphylococcus aureus* and *Escherichia coli*. *Polim Med.* **51**(2):69–75.
- Kareem, O., Ali, T., Dar, L.A., Mir, S.A., Rashid, R., Nazli, N., Gulzar, T. & Bader, G.N. (2022). Positive Health benefit of Saponins from Edible Legumes: Phytochemistry and pharmacology. *Edible plants in health and Diseases.* 2: 279-298. Doi:10.1007/978-981-16-4959-2_8
- Ken, F (2023). Tropical Plant Database. Tropical.theferns.info 2023-11-29 <tropical.theferns.info/viewtropical.php?id=Senegalia+macrostachya&redir=Acacia+macrostachya
- Luhata, L.P. & Toyonobu, U. (2021). Antibacterial activity of B-sistoterol isolated from leaves of *Odontonema strictum* (Acanthaceae). *Bioorganic Med. Chem Lett.* 48:128248
- Mombeshora ,M & Mukanganyama, S. (2019) Antibacterial activities, proposed mode of action and cytotoxicity of leaf extracts from *Triumfetta welwitschii* against *Pseudomonas aeruginosa*. a *BMC Complementary and Alternative Medicine.* **19**(315):1-12 <https://doi.org/10.1186/s12906-019-2713-3>
- Mondal S. & Rahaman S.T (2020). Flavonoids A vital resource in healthcare and medicine. *International Journal of pharmaceutical pharmacology* **8** (2):91-104 doi:10.15406/ppij2020.08.00285.
- Muazzam,A.,Saleem, S., Faizan, F.M .N., Haq , F.U.I, Ali, G., & Javed, N (2025) Evaluation of the Antibacterial Activity of Acetic Acid in Comparison With Three Disinfectants Against Bacteria Isolated From Hospital High-Touch Surfaces. *Wiley Scientifica* 1-9 <https://doi.org/10.1155/sci5/7598027>
- Muhammad, M.U., Gayari, M.S., Idris, B., Garba, U. & Bello, I (2022) Evaluation of Phytochemical Composition and Antibacterial Activity of *Indigofera hirsuta* Linn. *Arid Zone Journal of basic and Applied Research.* **1**(5):192-203
- Nortjie, E.; Basitere, M.; Moyo, D.; Nyamukamba, P. (2023) Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review. *Plants.* **11**(2011): 1-17
- Pakkirisamy, M., Kalakandan, S.K. & Ravichandran, K. (2017). Phytochemical Screening, GC-MS, FT-IR Analysis of Methanolic Extract of *Curcuma caesia* Roxb (Black Turmeric). *Journal of Pharmacognosy* **9**(6):952-6. DOI : 10.5530/pj.2017.6.149
- Patel, S. (2016). Plant-derived cardiac glycosides: Role in heart ailments and cancer management". *Biomedicine & Pharmacotherapy.* 84: 1036–1041. doi:10.1016/j.biopha.2016. 10.030
- Sani, I., Rabi, U.A., Hassan, S.W., Faruk, U.Z. & Bello, F. (2020). Lethality of *Nja nigricollis* Reinhardt venom and antivenom activity of *Azadirachta indica* A. Juss. Leaf extracts on albino rats. *GSC Biological and Pharmaceutical Science* **12**(2)-080-092



- Shehu, A., Garba, U., Lawal, H.M & Augie, A.L (2025). Phytochemical Composition and Antimicrobial Properties of Guiera Senegalensis Leaves Extract Obtained from Yabo Community in Sokoto State, Nigeria. *International Journal of Science for Global Sustainability*. 11(1):25-32 DOI: <https://doi.org/10.57233/ijsgs.v11i1.778>
- Uba, B., Muhammad, C., Uba, A. & Amali, A.M. (2020). Synthesis, characterization, antibacterial and antifungal activities of Schiff Base complexes of Ni (II) and Cu (II) derived from 2-hydroxy-1-naphthaldehyde and 4-aminophenol. *IAR Journal of Pharmacy Synthesis* 1(1):1-5
- UNEP (2024) United Nation Environmental Programme: "Antimicrobial resistance a global threat" retrieved on 24th may,2024 ; 12:55Pm from <https://www.unep.org/topics/chemicals-and-pollution-action/pollution-and-health/antimicrobial-resistance-global-threat>
- World Health Organization, WHO (2018) (Staphylococcus aureus), Joint WHO/UNICEF statement: Management of potentially serious bacterial infection in young infants aged 0-59 days when transfer to a Hospital facility is not possible.
- Xin, D., Li, H., Zhou, S., Zhong, H & Weiling P.U (2022). Effects of Anthraquinones on Immune Responses and Inflammatory Diseases. *Molecules* 27(12): 3831 <https://doi.org/10.3390/molecules27123831>
- Yusuf, A.J., Abdullahi, M.I., Haruna, A.K., Musa, A.M., Abdullahi, M.S., Ibrahim, Z.Y.Y., Halilu, M.E. & Odiba, O.J (2015). Phytochemical and antimicrobial evaluations of the methanol stem bark extract of *Neocarya macropylla*. *Journal of chemical and pharmaceutical Research* 7(1): 477-481
- Zagga, A.S., Tambuwal, B.Z., Garba, U., Bello, M.A., Achida, B.M and Bande, A.B (2024.). Phytochemical Composition and Antibacterial Properties of *Senegalia macrostachya* Seed Extracts. *Arid Zone Journal of Basic and Applied Research* 3(6): 1-11 doi.org/10.55639/607.02010014Askale