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ORIGINAL RESEARCH ARTICLE

Isolation, Structural Characterization and Bioactivities of Antioxidant and Antimicrobial Compounds from *Prosopis africana* Stem Bark

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ABSTRACT
Prosopis africana, also known as African mesquite, is the only species of Prosopis indigenous to tropical Africa. It has vast social, economic, cultural, medicinal and agricultural values. In this research, the Antioxidant and antimicrobial activities of *Prosopis africana* were determined. 500g of the grounded stem bark of *Prosopis africana* was extracted with n-hexane and ethanol. The Antioxidant activities were evaluated using the DPPH free radical scavenging assay. The ethanolic extract showed greater antioxidant activity than the n-hexane extract, with IC50 values of 6.91 µg/mL and 671.81 µg/mL, respectively. Microbial activity was determined using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) method, were the two extracts inhibited the growth of tested bacterial strains; *Ralstonia solanacearum*, *Xanthomonas campestris*, Methicillin resistant *Staphylococcus aureus*, and *E. coli* in the ranges of 21mm,15mm,21mm,19mm for the ethanolic extract and 18mm,14mm,17mm,15mm for n-hexane extract. Purification of the bioactive compound was done using column chromatography. The structure of the compound was elucidated on the basis of spectroscopic analysis, including Fourier-transform infrared spectroscopy, 1H NMR, and 13C NMR. This lead to the Isolation and identification of a bioactive compound, namely palmitic acid. This study supported the traditional use of *Prosopis africana* in traditional medicine, and the results revealed that the ethanolic extract possesses stronger antioxidant and antimicrobial activity than the n-hexane extract.

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Prosopis africana, Antioxidant, Antimicrobial, Hexane, Ethanol

INTRODUCTION
Prosopis africana stem bark is widely used in traditional African medicine, with research increasingly validating its antioxidant and antimicrobial properties. Recent studies have focused on isolating, characterizing, and evaluating the bioactivities of its phytochemicals, supporting its potential as a source of therapeutic agents. Advanced techniques such as LCMS, NMR, and chromatographic methods have identified a diverse array of compounds in the stem bark, including phenols, flavonoids, alkaloids, saponins, terpenoids, steroids, glycosides, and peptides. Notable isolated compounds include 7,3',4'-trihydroxy-3-methoxyflavanone, dehydroabietic acid, caffeate, linalool, quinovic acid, quercetin, catechin, and apigenin, many of which are linked to strong bioactivities (Elmezughi et al., 2013; Yanda et al., 2022; Ibrahim et al., 2025; Abubakar et al., 2024; Olujimi, 2023; Abubakar et al., 2023).

Historically, the majority of new drugs have been derived from natural products (secondary metabolites) and from compounds derived from them (Lahlou, 2013). Drug discovery from medicinal plants has mainly relied on biological activity-guided isolation methods which have led to the discovery of important drugs. Many higher plants contain metabolites with different biological properties (Lahlou, 2013).

The present trend requires that the bioactivity of many medicinal plants against common pathogens be scientifically established (Mohammed et al., 2017; Adamu et al., 2022, 2018; Mohammed et al., 2016b, 2016a; Mohammed et al., 2017; Muhammad et al., 2024; Salisu and Shema, 2019; Salisu et al., 2019).

In this regard, the present work focused on providing scientific information on the phytochemical composition and antioxidant and antimicrobial activities of n-hexane and ethanol extracts of the stem of *Prosopis africana* against clinical isolates of *Ralstonia solanacearum*, *Xanthomonas campestris*, methicillin-resistant *Staphylococcus aureus*, and *E. coli*, respectively.

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MATERIALS AND METHODS

Extraction of the Plant Material

The stem bark of *Prosopis africana* was collected from Katsina state, Nigeria, and it was authenticated in the Herbarium at the Department of Plant Biology, Bayero University Kano, with accession number BUKHAN 0193. The plant sample was air-dried and pounded into powder. 500g of the powdered sample was successively extracted with *n*-hexane (1.5L) and ethanol (1.5L) for ten days with each solvent. It was then filtered using a Whatman filter paper. The filtrates were concentrated using a rotary evaporator (Buchi R200, Switzerland) and allowed to dry at room temperature to yield the *n*-hexane and ethanol extracts labeled PAHX and PAET, respectively (Pandey and Tripathi, 2013).

Thin Layer Chromatography

Thin-layer chromatography (TLC) was carried out on the sample (Figure 1) using a pre-coated silica gel aluminium sheet (TLC silica gel 60 F_{254}, Merck). The Samples were spotted on the TLC plates using a capillary tube. The spotted TLC plates were developed in suitable solvent systems in a Chroma tank, allowed to dry and visualized under UV light (254nm and 365nm). The plates were later stained with iodine vapour until a dark drown color appeared. The column fraction's profiles were monitored by TLC to confirm the similarities of elutes based on the number of spots on the plates (Sonam *et al.*, 2017).

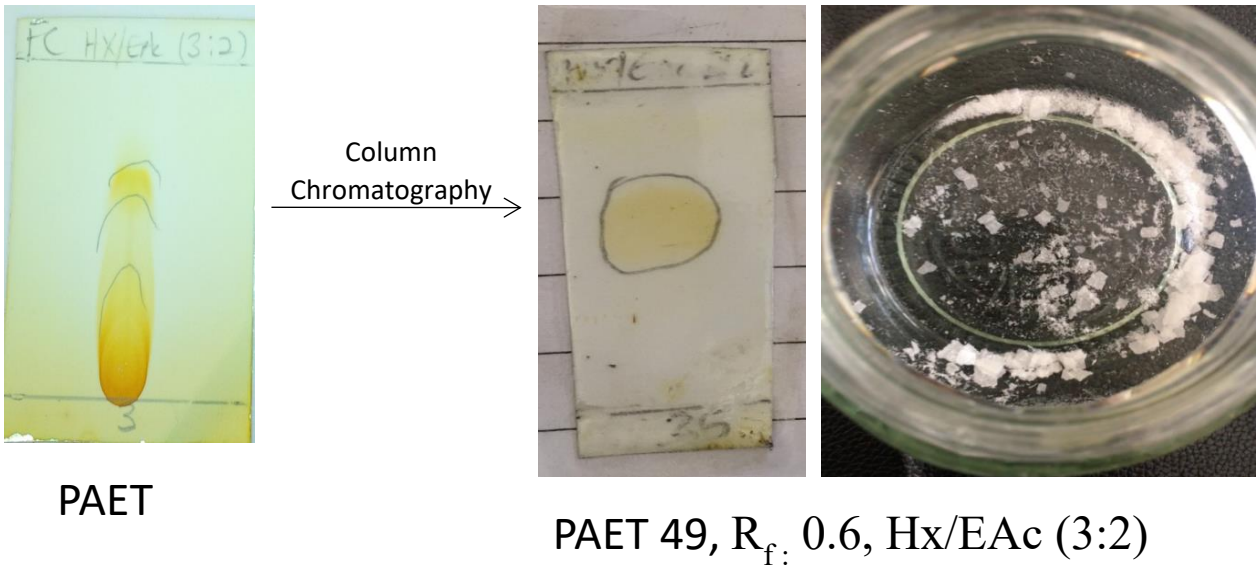

Figure 1: Thin Layer Chromatography

Column Chromatography of Ethanol Extract (PAET)

All the chemicals and solvents used in the present study were of Analytical Reagent grade. The column (2.5cm diameter by 160cm length) was packed with silica gel (60: 260 mesh size). 170g, washed with *n*-hexane. About 15g of the ethanolic extract (PAET) was mixed with a small amount of silica gel and loaded into the packed column; a small amount of silica was added on top of the sample. The column was eluted with solvents of different polarities, by increasing the polarity, starting with n-hexane, n-hexane and chloroform, chloroform, chloroform and ethyl acetate, ethyl acetate, and then methanol. Eluents of about 20ml were pooled in collection bottles, which resulted in about 103 fractions. Fractions of the same TLC profile were combined together. Two major fractions were further purified.

Isolation of compound PAET-49

Similar fractions of PAET-49 were combined and purified using a column with silica gel (50g), and it was eluted using a polarity gradient of n-hexane, n-hexane & chloroform, chloroform, chloroform & ethyl acetate, ethyl acetate, ethyl acetate & methanol and then methanol.

Determination of free radical scavenging activity using DPPH (2,2-Diphenyl-2-picrylhydrazyl)

The free radical scavenging activity of the extract and isolated compounds from *Prosopis africana* was measured in terms of hydrogen donating of radical scavenging ability using the stable DPPH radical method. Each test extract and isolated compound were made in to stock solution (10mg/ml) which was then further diluted to final concentrations of 1000, 500, 250, 125, 61.5, 31.25, 15.6, and 7.8µg/ml in methanol using dilution formula. 2 mg of the DPPH was dissolved in 100ml of methanol in a dark bottle to give a concentration of 50µM and kept in the refrigerator until use.160µL of 50µM solution of DPPH was added to 40µL of each concentration in a micro plate and incubated for 30mins in the dark. After 30mins the Absorbance was measured at 517nm using a spectrophotometer. The radical scavenging activity was calculated using the following equation (Nimmi and George, 2012)

$$\%AA = \frac{AS-AB}{AB} \text{ X } 100$$

Where:

AA = Antioxidant activity

AS = Absorbance of sample

AB = Absorbance of blank

Anti-Bacterial Assay

Agar well Diffusion Method

The anti-typhoid activities of the extracts were tested using the agar well diffusion according to the method described by Muhaidat *et al.* (2015). The standard bacterial species (three strains of *Salmonella typhii*, STI, STII and STIII) were cultivated in nutrient broth and incubated at 37 °C for 18 h. Bacterial cultures were brought to turbidity equivalent to about 0.5 McFarland turbidity standard and inoculated (0.2 ml each) onto nutrient agar media. Afterwards, wells (8 mm diameter each) were bored in the agar using a sterile cork borer (NO.4) and the agar discs were removed. Each of the extracts (20 mg/L) was dissolved in dimethyl sulphoxide (DMSO) and diluted to final concentrations of 1000, 500, 250, and 125 µg/mL. An aliquot (100µl) of each test concentration was placed into a well with a standard Pasteur pipette, and the plate was held for 1 hr at room temperature for diffusion of extract into the agar. Subsequently, the plate was incubated for 18 hr at 37 ºC. After incubation, the diameters of the zones of inhibition were measured to the nearest mm. Three replicates were performed, and results were recorded (Abakar *et al.*, 2017).

Minimum Inhibitory Concentration (MIC)

20g of nutrient broth was weighed and transferred into a one-litre volumetric flask, dissolved and then the solution was made to the mark using distilled water. 1ml of broth was added to clean labelled test-tubes, covered with cotton wool and the solution was steam sterilized in an autoclave (RAU-530D) instrument at 120 °C and 15 atmosphere pressure for 15-20 minutes. The broth was allowed to cool. 1ml of 1000µglml for each extract was added to test tubes corresponding to the concentrations, and then serial dilutions were employed to obtain the other concentrations. 0.1ml of the test organism was added to the test tubes and incubated for 24hours. A positive control containing only broth and the test organism was also prepared. A test-tube containing broth only is the negative control. After 24hours the test-tubes were observed for the formation of turbidity or growth of the tested microorganisms. The lowest concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the minimal inhibitory concentration or MIC. The test is only valid if the positive control shows growth and the negative control shows no growth.

Minimum Bactericidal Concentration (MBC)

The concentrations that showed no bacterial growth from the MIC result were tested for minimum bactericidal concentration. 28 g of nutrient agar was weighed and transferred into a one-liter volumetric flask, dissolved and then the solution was made to the mark using distilled water. The solution was then steam sterilized in an autoclave at 120 °C and 15 atmospheric pressure for 15 minutes. 15 ml of the sterilized nutrient agar solution was dispensed into each of the sterile Petri dishes, after cooling at 25 °C to act as a nutrient medium for the growing bacteria. The bacterial strain was streak on the media with the help of a wire loop. After an overnight incubation, the bacterial growth was observed. If a clear area of no growth is observed, the tested sample is bactericidal, and where growth is observed is bacteriostatic.

RESULTS AND DISCUSSION

Isolation and Characterization of PAET-49

Repeated column of fractions (49-55) yielded an oily yellow substance fraction as PAET-49 which is soluble in hexane and chloroform. R_f = 0.7 (Hexane/Chloroform, 2:3). The IR spectrum (Figure 2) indicated the presence of O-H bond at 3335 cm^{-1}, saturated C-H stretching at 2918 cm^{-1} and C=O group of carboxylic acid at 1704cm^{-1}.

The ^{1}HNMR data (Figure 3) shows the presence of a proton adjacent to the carbonyl at 2.31 ppm. Aliphatic protons associated with a straight chain were observed in the range of 0.85 to 1.63 ppm.

The ^{13}CNMR spectrum of PAET-49 (Figure 4) supported the presence of aliphatic carbon atoms by the appearance of signals between 13.7 and 37.1 ppm, while the carbonyl carbon associated with carboxylic acid was observed at 179.5 ppm. This is in consistence with Pavia et al. (2015), aliphatic sp^3 carbon typically appears in ^{13}C-NMR spectrum between 10-40 ppm.

Based on the spectroscopic data and literature data comparisons (Bulama *et al.*, 2014). PAET-49 was proposed to be a fatty acid identified as Hexadecanoic acid, commonly known as palmitic acid.

DPPH Radical Scavenging Assay

Free radical scavenging activity of *n*-hexane extract (PAHX), ethanol extract (PAET) and two isolated compounds (PAET- 9B and PAET- 49) from *Prosopis africana* were evaluated using DPPH radical. Ascorbic acid was used as a positive control. All the tested samples showed an inhibitory potential against DPPH free radical. The results are expressed as percentage inhibition (%I) as well as inhibitory concentrations at 50% (IC_{50}). Table 1. The inhibitory concentration IC_{50} is the concentration of the extract or pure compound that is required to inhibit 50% of the DPPH free radical.

In this DPPH assay, the antioxidants present in *Prosopis africana* stem bark extracts of *n*-hexane and ethanol, as well as two isolated compounds, were able to reduce the violet coloured stable 1,1, -diphenyl-2-picryl hydrazyl radical to the yellow coloured 1,1-diphenyl-2-picryl hydrazine. It was observed from the result that the radical scavenging activity was strong in ethanol extract, having a percentage

inhibition of 9892-45.16 % and IC_{50} value of 6.91 µg/ml, compared to the standard ascorbic acid with a percentage inhibition ranging from 96.41-43.74 % and IC_{50} value of 9.25 µg/ml. (Table 1). The isolated compounds PAET-9B and PAET-49 also exhibited good activity with percentage inhibition of 77.52- 16.58% and 68.97-12.33%, and corresponding IC_{50} of 176.73 µg/ml and 216.94 µg/ml, respectively. The results propose that the ethanolic extract of the stem bark of *Prosopis africana* proves to be a potent antioxidant and free radical scavenger and might contain antioxidant substances higher than those of the *n*-hexane extract.

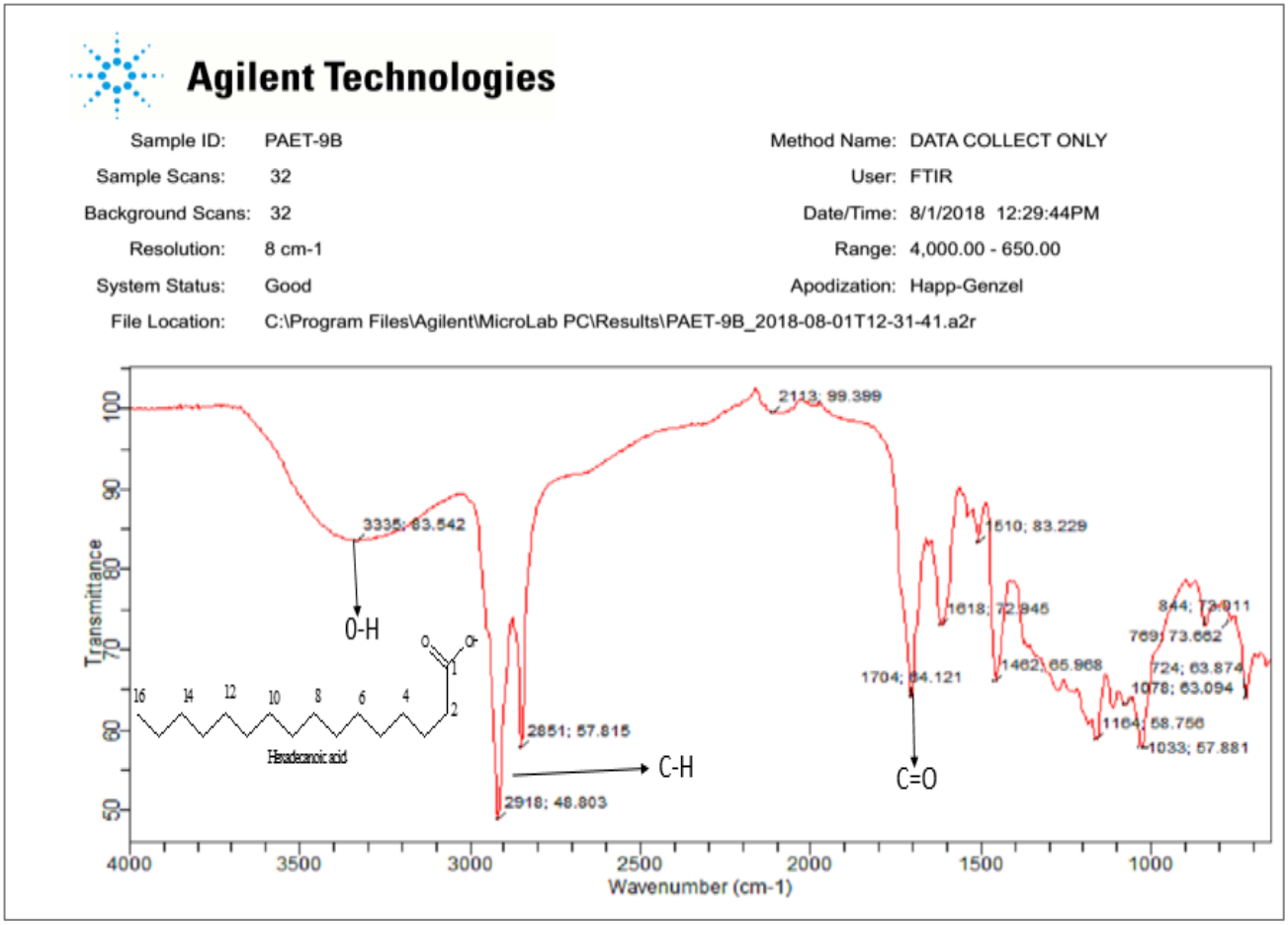

Figure 2: The IR spectrum of the PAET-49

Table 1: Percentage Inhibition and IC_{50} Values of *Prosopis africana.*

Conc (µg/mL)	1000	500	250	125	62.5	31.3	15.6	7.8	IC_{50}
PAHX	54.88	52.21	43.40	41.62	34.42	29.81	42.73	34.09	671.81
PAET	98.92	94.00	94.75	95.96	94.71	93.35	56.04	45.14	6.91
PAET-9B	77.52	67.13	50.79	42.42	28.99	26.55	22.13	16.58	176.73
PAET-49	68.97	66.21	49.25	44.01	39.21	21.37	18.21	12.33	216.94
ASCOBIC	96.41	97.04	96.79	97.03	96.02	94.33	40.11	43.74	9.25

Table 2: MIC and MBC values of *Prosopis africana* extract and isolated compounds against bacterial strains.

SAMPLE	MIC/MBC(µg/mL)	Microorganisms			
		XC	RS	E.coli	MRSA
PAHX	MIC	500	500	250	250
	MBC	1000	1000	500	500
PAET	MIC	250	250	125	125
	MBC	500	500	250	250
PAET-9B	MIC	500	500	250	250
	MBC	1000	1000	500	500
PAET-49	MIC	250	250	250	500
	MBC	1000	500	250	1000
GENTAMICIN	MIC	125	125	125	125
	MBC	125	125	125	125

XC= *Xanthomonas Campesteris*, RS=*Ralstonia solanacearum*, EC= *Escherichia coli*, MRSA=Methicillin Resistance *Staphylococcus aureus*.

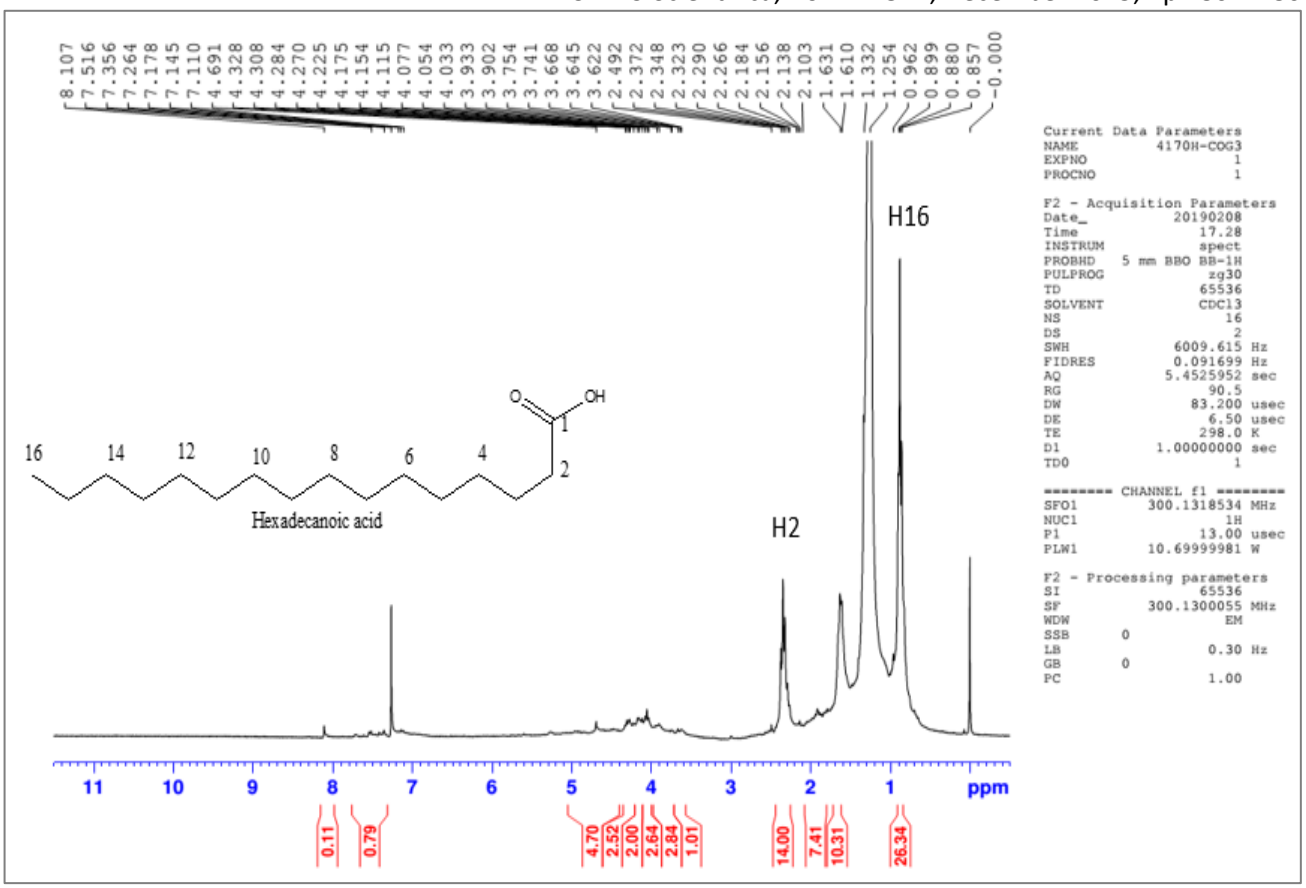

Figure 3: ^{1}HNMR spectrum of PAET-49

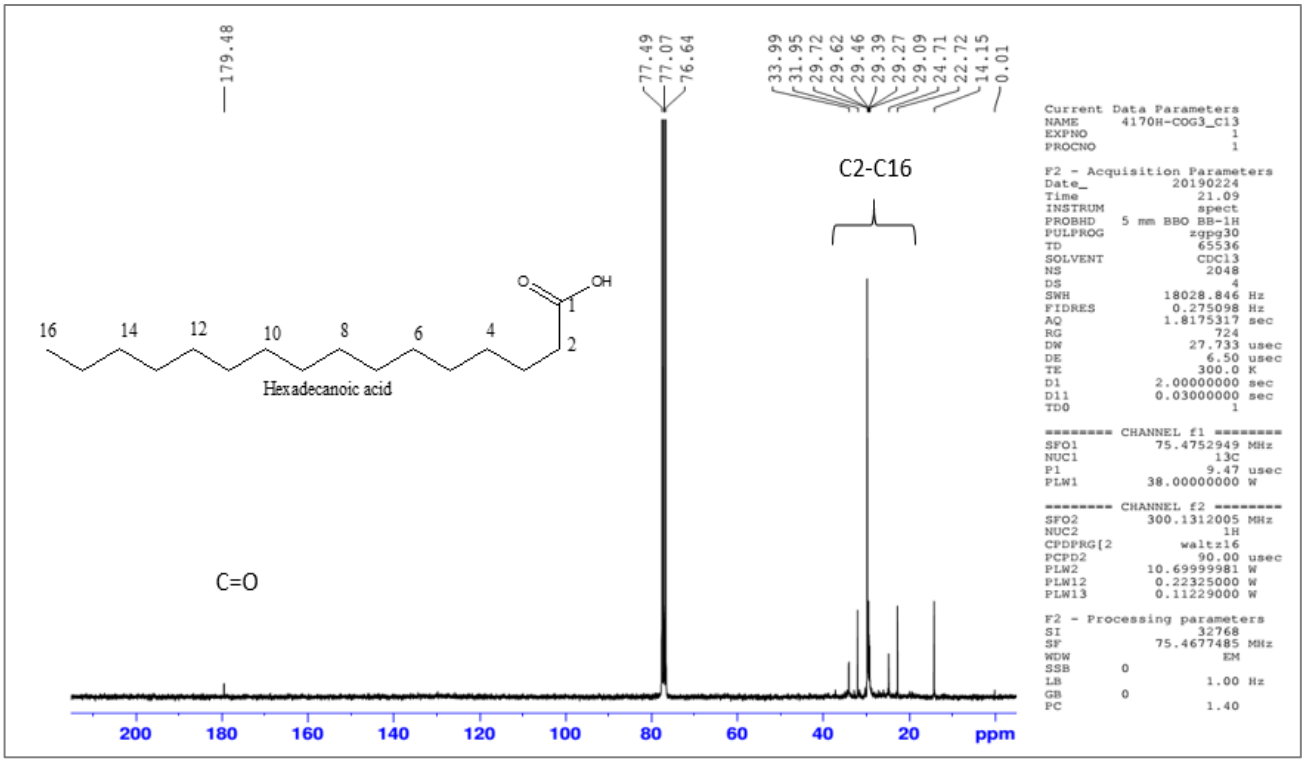

Figure 4: ^{13}CNMR spectrum of PAET-49

The increase in percentage of DPPH radical scavenging activity increases with the increase in the concentrations of the tested samples from *Prosopis africana*. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. (Salawu *et al.*, 2025).

Microdilution Technique

The *n*-hexane and ethanolic extracts, as well as two isolated compounds from *Prosopis africana,* were also tested for antimicrobial activity using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods, targeting the diseases

caused by these bacteria, which include black rot and bacterial wilt in plants and pneumonia and food poisoning in human beings. All the bacterial strains were susceptible to the tested samples. The ethanol extract was found to be more active against the two animal bacteria (Table 2)

CONCLUSION

Research confirms that *Prosopis africana* stem bark contains structurally diverse bioactive compounds with strong antioxidant and antimicrobial properties. These findings support its traditional medicinal use, though further studies on compound isolation, mechanisms, and safety are warranted for therapeutic development.

The powdered stem bark of *Prosopis africana* was extracted with *n*-hexane and ethanol. The extracts were evaluated for antioxidant and antimicrobial activity. The *n*-hexane extract demonstrated a significant activity, while the ethanolic extract exhibited good antioxidant and antimicrobial activities.

On the basis of these results, the ethanolic extract was subjected to chromatographic purification targeting the compounds responsible for the observed activity. This led to the isolation of two compounds identified as 4-(Hexyloxy) phenol and Palmitic acid on the basis of spectroscopic and literature data.

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