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ORIGINAL RESEARCH ARTICLE

in vivo Antibacterial Activity of *Acacia nilotica* Phenolics Againts Extreme Multidrug Resistant *Pseudomonas aeruginosa* Strains

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ABSTRACT

Multidrug-resistant Gram-negative bacteria have long posed serious social, health, and economic issues in Nigeria. *Pseudomonas aeruginosa,* a nosocomial pathogen, has always been a major healthcare problem globally, especially with its elevated capacity to resist novel, strongest antibiotics and other therapeutic agents. This necessitates the development of novel therapeutic agents for the treatment and management of multidrug-resistant strains. This research aimed to evaluate the potential of *Acacia nilotica* plant-based phenolics as antibacterial agents against extreme drug-resistant *Pseudomonas aeruginosa* clinical isolates from Kaduna State metropolis. Ethanol was used as a solvent to extract *Acacia nilotica* (L.) leaves. Thin-layer chromatography (TLC), column chromatography, and spectral analyses were used to isolate the phenolics from the plant extract. Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FTIR) spectroscopy were used to characterize the fraction. Using microbiological, biochemical, and molecular methods, eleven highly resistant strains of *Pseudomonas aeruginosa* were identified after being isolated from clinical samples. Their antibiogram profile was determined using Antibiotic disc diffusion method. The biochemical effects of the plant extract phenolic fraction were assessed *in vivo* against extreme drug-resistant bacteria. Data obtained showed that tannins, saponins, alkaloids, flavonoids, and phenols were present in the plant extract at varying concentrations (ranging from 0.06–14.47 mg/g dry wt). The bioassay revealed that the phenolic fraction of *Acacia nilotica* (L.) has significant biological therapeutic activity against the extremely drug-resistant *Pseudomonas aeruginosa* strains in this study. Phenolics from the *Acacia nilotica* (L.) plant may serve as a substitute active pharmaceutical ingredient for treating *Pseudomonas aeruginosa* strains resistant to drugs.

ARTICLE HISTORY

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Acacia nilotica, Phenolics, Chromatography, Bioassay, Resistance

INTRODUCTION

Pathogenic bacterial infection has always been an important clinical and pharmaceutical issue in Nigeria (Ibtihaj *et al.,* 2021) and other parts of the world, more importantly, when it involves human pathogens of high clinical and environmental importance, especially the opportunistic human pathogens like *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Ruiz-Roldan *et al.,* 2021).

Numerous nosocomial bacteria have recently become more important because of their increased resistance to broad-spectrum antibiotics. Drug-resistant *P. aeruginosa* and methicillin-resistant S. aureus have become bacteria of serious medical, economic, and social concern (Abdel-Hamid *et al.,* 2020). Using intricate regulatory systems to detect diverse cues, these bacteria may adjust to various conditions within the host to modulate resistance and virulence. They can use a wide range of virulence and resistance factors to overcome the entire host's immune and defence system barriers (Wang *et al.,* 2022).

The primary cause of biofilm-related infections of indwelling medical devices, which contribute significantly to the high annual cost of healthcare waste in Nigeria, is *Pseudomonas aeruginosa* (Pandukur *et al.,* 2020). Luckily, the use of ethnomedicinal plants like *Acacia nilotica* to treat and manage infections caused by multidrug-resistant pathogens is opening the way for a safer, cost-effective pharmacological solution. However, extracts from plant materials are composed of a variety of phytochemical compounds, some of which are detrimental to biological systems when taken. This necessitates the extraction and identification of the exact bioactive phyto-compound for the treatment and management of such recalcitrant infections. In this study, *Acacia nilotica* phenolic

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compound is used as potential antibacterial agents against extreme multidrug-resistant *P. aeruginosa*.

MATERIALS AND METHOD

Plant Material Extraction

Acacia nilotica leaves were collected from the Biological Garden of the Nigerian Defence Academy, Kaduna. After which, the samples were identified, sterilized, and pulverized before extraction. A portion of 400 g was macerated in a maceration bottle for 14 days using 500 ml of ethanol. The mixture was then dried-up using a rotary evaporator (Hassan *et al.,* 2021).

Extraction and Purification of Phenolic Compounds

Bio-compound Analysis

Standard techniques as outlined by Sofowora *et al.* (1993), were used to qualitatively and quantitatively assess the phytometabolic content of the plant extract for the presence of various phytochemicals.

Tin Layer Chromatography

This was carried out according to the method employed by Chinyere *et al.* (2016), in which all extracts were cleaned up, followed by tin layer chromatography using silica gel-G, a solvent system of ethanol/hexane (8:2 v/v), and garlic acid as the standard. A qualitative phytochemical analysis was then carried out to all the individual fractions collected. All fractions that tested positive for phenolic compounds, with Rf values equivalent to those of the standard, were then subjected to column chromatography and further fractionated. A spectroscopic scan was carried out on the standards and all the positive fractions to ascertain their individual wavelength of maximum absorption.

Column Chromatographic Fractionation

Silica gel column chromatography was used to separate the phyto-constituents from the plant fractions. For fractionation, a vertical borosilicate glass column measuring 40 mm wide by 60 mm long was employed. Prior to packing, the column was dried and rinsed with acetone. Using a glass rod, a piece of glass wool was positioned at the base of the column. The top of the glass wool was covered with sea sand (particle size 50–70) up to a height of 1 cm. The solvent was used to rinse the sand particles away. After shutting off the stopcock, hexane was poured into the column until it reached 3/4 level. The packing material was then 200 g of silica gel with a mesh size of 60–120. To help with proper column packing, a silica slurry was prepared with hexane and poured from the top of the column about two-thirds of the way down, while the solvent was simultaneously drained. The extract fractions were eluted with the solvents as they flowed down the sides of the column from the top. Using solvents of low polarity to high polarity (solvent system: methanol, ethanol, ethyl acetate, chloroform, and hexane) in different ratios at a flow rate of 1 ml/min, the gradient elution method was used to separate the fractions (Panel *et al.,* 2018).

Determination of Total Phenolic Compounds

Gallic acid was used as the standard in the Folin–Ciocalteu test to assess the total phenolic content of the plant extract fractions. In short, 1 mL of diluted FC reagent (1:10) was combined with 100 µL of various test sample concentrations. One millilitre of a 7.5% (w/v) sodium carbonate solution was added to the mixture after 10 minutes, and the mixture was then incubated for 90 minutes in the dark. At 725 nm, the absorbance was then measured. Ten milligrams of gallic acid and 10 millilitres of methanol were combined to prepare the standard solution, which has a concentration of 1000 mg/L. The standard's concentration variation is created by adding 0.1, 0.2, 0.4, 0.6, and 0.8 millilitres of the standard solution to a 10-millilitre volumetric flask. 0.5 mL of Folin-Ciocalteu reagent is added to each variation, and the mixture is left for 5 minutes. A 1 mL solution of sodium carbonate is then added, and it is diluted with distillated water until it reaches the limit. Two hours are spent waiting for the combination. The mixture's absorbance is then measured at a wavelength of 765 nm. A calibration curve is created using the absorbance value, and a regression formula is derived from the standard solution. Gallic acid equivalents were used to express the phenolic content, which was determined using the calibration curve (mg GAE/g DW) (Atwaa *et al.,* 2022).

Antioxidant Activity Test

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) technique, as described by Panel *et al.* (2018), was used to accomplish this. The plant fraction test solution was prepared in conjunction with a negative control solution. Vials were filled with 1 millilitre of each concentration of the test solution, 2.5 millilitres of DPPH (0.1 mM), and 0.1 millilitre of DPPH solution after 30 minutes. Additionally, this work was completed in a dark room. At a wavelength of 517 nm, the absorbance from the concentration of each test solution and the negative control was then determined. The following formula was used to determine the absorbance and percentage of inhibition:

% inhibition= [(Absorbance of negative control – Absorbance of sample)/ Absorbance of negative control] x 100%

Antibiotic Sensitivity Assay for the Resistant Clinical Isolates of *Pseudomonas aeruginosa*

Eleven resistant strains of clinically isolated *P. aeruginosa* bacteria were collected from numerous general hospitals in Kaduna state metropolis, Nigeria. After which, the pathogens are reconfirmed using microbial, biochemical, and molecular confirmation analyses (Gellatly and Hancock, 2013; Breidenstein et al., 2011; Mohammed et al., 2017a). Disc diffusion bioassay was performed on all 11 confirmed isolates using industrial-grade antibiotics (Hassan *et al.,* 2023; Salisu et al., 2017).

Acute Toxicity Study

Using the conventional procedure outlined by Lan *et al.* (2017), an acute toxicity investigation of the phenolic fraction was conducted. The dry-matter content of the extract and fractions (1000 mg/kg body weight) was used to calculate the dosage (Health, 2018). Five groups (10 mice each), including a control group and three treatment groups, were randomly selected from the mice. Following an overnight fast, the treatment groups received 1000 mg/kg of plant extracts or phenolic fractions orally in 5 small doses over 12 hours, whereas the control group received distilled water. Every hour for 24 hours following the dose, changes in skin, hair, eyes, respiration, motor activity, convulsions, tremors, salivation, diarrhoea, sleep, and indicators of toxicity and mortality were noted. For ten days in a row, additional observations were conducted. At the conclusion of the observation, the animals were ultimately put to death by CO2 euthanasia in the Euthanasia-Chamber EUTH1A (Orchid Scientific, Nashik-422010, India). For relative weight and histological analysis, the internal organs were collected and preserved in 10% formalin.

Determination of the Infectivity Dose

The sterile test tubes were set up serially. Six sets of test tubes were filled with 9 millilitres of sterile distilled water, and the first tube was filled with 1 millilitre of an extreme multidrug-resistant bacterial culture, creating a 1:10 dilution. The remaining five tubes underwent the same process. Each dilution was poured onto the nutrient agar medium in a mililiter. Observable colonies were counted, and the count was adjusted for the dilution factor after incubation for 18 to 24 hours at 37°C. One millilitre of each relevant dilution was administered orally to the mice. For a week, they were watched for signs of infection. The dosage that would have the greatest clinical impact on the animal was determined (Olaya and Lozano, 2010).

Animal Bioassay

Extremely drug-resistant bacterial strains were employed. The infectivity doses of the various organisms were administered orally to the mice. Three groups of ten mice each one control group, an infected group, and a treatment group, were randomly allocated to the mice. Following an overnight fast, eight tiny extract fractions were administered orally to the treatment groups at a dose of 1000 µg/g over 16 hours, whereas the control group received distilled water. Every hour for 24 hours following dosing, general changes (alterations in the skin, hair, eyes, mucous membranes, respiratory, circulatory, autonomic, and central nervous systems, motor activity, convulsion, tremors, salivation, diarrhoea, lethargy, or sleep), as well as indications of toxicity and mortality, were noted. These observations continued for seven days in a row. Finally, the animals were put to death in the Euthanasia-Chamber EUTH1A (Orchid Scientific, Nashik-422010, India) using CO2 euthanasia. For relative weight and histological analysis, the internal organs were collected and preserved in 10% formalin (Ayomide *et al.,* 2019; Usman et al., 2025).

Clinical Observations and Survival

During the seven days of the acute toxicity trial, each mouse was monitored daily. At the same time every day, clinical symptoms were noted both before and after the dose was administered. Motor activity, tremors, convulsions, posture, stereotypical movements (such as excessive grooming) or strange behaviour (such as walking backwards), watery eyes and dilated eyelids, skin changes (such as cyanosis), salivation, diarrhoea, dehydration, aggressiveness, runny nose, and difficulty breathing were among the clinical observations. After that, the data and the control were contrasted (Periferakis *et al.,* 2022).

Body Weight

The mice's body weights were measured at regular time intervals during the entire testing process using the formula:

Weight gain (%) = $[(Mf - Mi)/Mi]$ X 100
Where: *Mf*: final weight, *Mi:* initial weight (Ugwah *et al.,* 2019)

Relative Organ Weight

The mice were sacrificed by CO_2 euthanasia in the Euthanasia-Chamber EUTH1A (Orchid Scientific Nashik-422010, India) and vital organs like the liver, heart and kidney were separated, weighed using an electric weighing balance, and compared with the organs of the control group, using the formula (Tabarraei *et al.,* 2019):

Relative organ weight (%): [((Absolute visceral weight (g))/ (Body weight on the day of surgery (g)))] X 100

Food and Water Consumption

The animals' daily food and water intake was recorded. Before being given to each group, the amount of food and water was measured (Abotsi *et al.,* 2011). The amount of food and water that the animals had consumed during the day was estimated by gathering and weighing the leftovers at the end of the day. Water consumption was monitored in millilit**re**s per day, while feeding was assessed in grams per day (Upadhyay *et al.,* 2019).

Haematological and Biochemical Study

Prior to the test, the mice were given chloroform anaesthesia, fasted overnight, and had a blood sample taken. At the time of sacrifice, two milliliters of blood were drawn from the major vein in the mouse's neck. Different blood components were transferred to tubes containing trisodium EDTA or lithium heparin as anticoagulants. After centrifuging heparinized samples at 1,900 × g for 10 minutes at 4°C, the plasma supernatants were transferred to new tubes for analysis of blood biochemistry using a Toshiba Medical Systems, Co., Ltd., TBA-120FR automatic chemical analyzer (Tochigi, Japan). Haematological analysis of EDTA blood was performed with the XT-2000iV (SYSMEX, Co., Ltd., Kobe, Japan) (Yan and Chunying, 2018).

Table 1: *Acacia nilotica* Extract Physical and Biochemical Properties

EXTRACTION		PHYTOCHEMICAL		
			Qualitative	Quantitative (mg/g dry wt)
Total yield (g)	67.03	Terpenoids	+	1.32 ± 1.74^{b}
Yield (%)	13.41	Tannins	+	1.36 ± 1.82^{b}
Colour	Dark greenish	Alkaloid	+	1.53 ± 2.85^{b}
Texture	Gummy	Saponins	+	1.32 ± 2.75^{b}
		Flavonoid	+	1.97 ± 1.64^{c}
		Phenols	+	11.92 ± 1.15^{d}
		Cardiac glycoside	_	_
		Anthraquinones	+	0.06 ± 0.94^{a}
		Anthocyanins	-	-
		Coumarins	+	NT

Key:
+ = Presence - = Absence NT: Not Tested
The mean ± standard deviation (SD) represents the values. Values with distinct superscripts in each column indicate statistical differences ($p<0.01$).

Table 2: Thin Layer Chromatography of Gallic Acid

S/N	Standard	R_f Value
1	Gallic Acid	0.921
		0.960

Table 3: Thin Layer Chromatography Fractionation of Ethanol Leaf Extract of *Acacia nilotica*

S/N	FRACTIONS	FRACTION DISTANCE (cm)	Rf VALUE
1	First	13.7	0.96*
2	Second	13.0	0.92*
3	Third	12.4	0.87*
4	Fourth	10.4	0.73
5	Fifth	8.3	0.58
6	Sixth	6.4	0.45
7	Seventh	2.2	0.15
8	Eighth	2.1	0.15
9	Ninth	1.1	0.08

Key:
Solvent font: 14.2cm; *: Fractions with phenolic compounds activity

Table 4: Antioxidant Activity Test and Total Phenolic Content of Column Chromatography Products of *Acacia nilotica* (L.) Leaf Extract

Fractions	IC50 (mg/L) (λ = 517 nm)	Total phenolic content (mg/L GAE) (λ = 765 nm)
I	121.29	241.72
Ii	80.17	992.73
Iii	30.58	3127.83*
Iv	43.34	2344.60*
V	78.25	1145.05
Vi	519.82	-
Vii	742.87	-

Key:
* : Phenolic Compound Fraction

Histopathology Study

Pathological cardiac tissue alterations were evaluated using hematoxylin and eosin (HE) staining. Neutral-buffered formalin (10%) was used to fix heart tissue samples. Following conventional procedures, preserved lung samples were cut, fixed in paraffin, sectioned at 3 μm, mounted on glass slides, stained with HE, and covered with a coverslip (Yan and Chunying, 2018).

Statistical Analysis

The mean ± standard deviation of three determinations was used to display the results. Additionally, one-way analyses of variance (ANOVA) were performed on the means using SPSS. A difference was deemed statistically significant if its value was $P<0.05$.

Table 5: Spectroscopic Scan for Wave Length of Maximum Absorption

Sample	Fractions	Maximum Wave Spectra(nm)
Garlic Acid	Standard	292.50
A. nilotica (L.)	Iii	295.02
	Iv	301.50

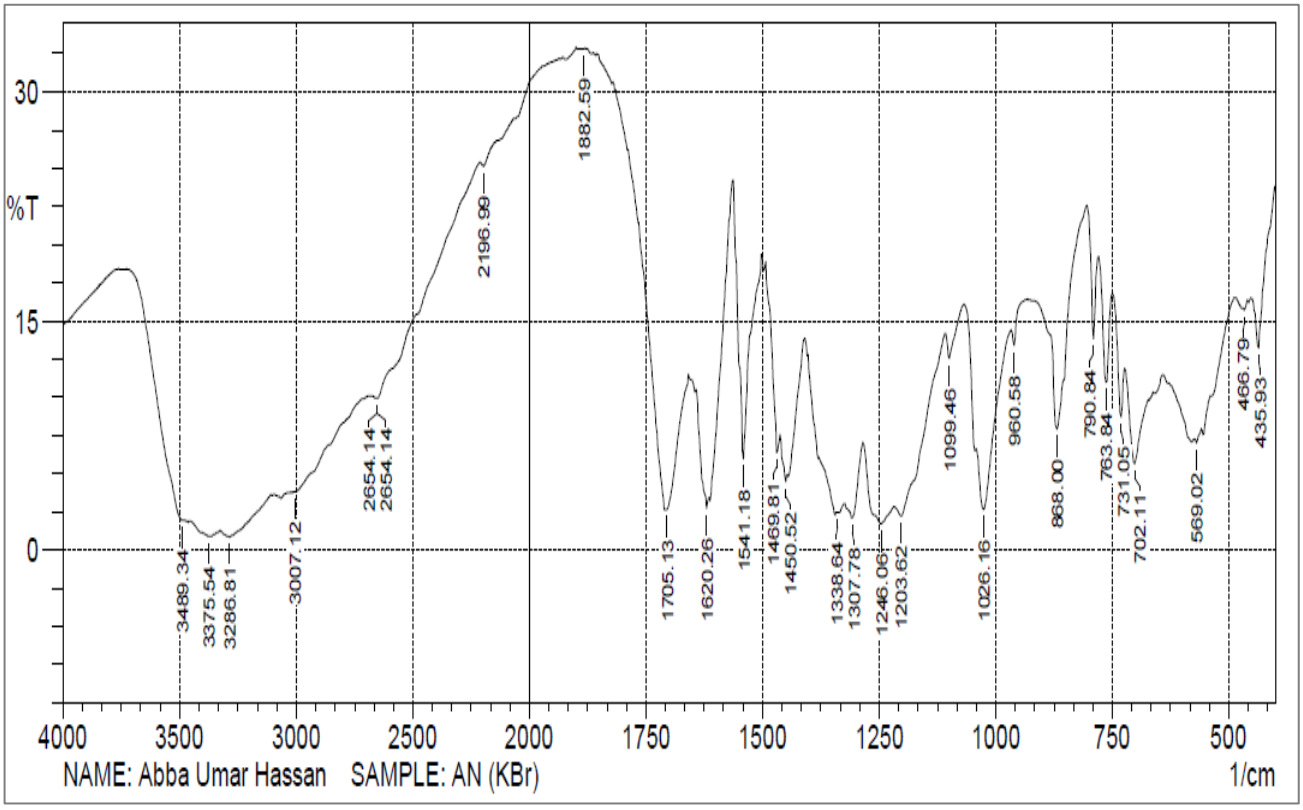


Figure 1: FTIR micrograph of *A. nilotica* (L.) phenolic fraction

Table 6: Probable Functional Groups Obtained from the FTIR analysis of *A. nilotica* (L.) phenolic Fraction

No.	Peak	Intensity	Area	Bond (types of vibration)	Functional Group
1	569.02	7.039	15.279	Stretching (halo)	C-Br
2	702.11	5.645	54.839	Bending (alkene)	C=C
3	731.05	8.688	24.507	Stretching (aromatic)	Ar-Cl
4	763.84	11.003	24.196	Stretching (mono)	-R-Cl/Br
5	790.84	13.781	18.628	Bending (alkene)	C=C
6	868	7.928	93.945	Stretching (esters)	epoxide ring
7	960.58	13.368	28.144	Bending (esters)	C-O-C
8	1026.16	2.67	85.652	Free (hydroxyls)	-OH
9	1099.46	12.556	32.637	Stretching (phenol)	Ar-OH
10	1203.62	2.216	134.145	Stretching (vinyl ether)	C-O
11	1246.06	1.708	106.429	Stretching (amine)	C-N
12	1307.78	2.104	56.884	Stretching (sulfone)	S=O
13	1338.64	2.464	27.293	Stretching (sulfonamide)	S=O
14	1450.52	4.427	19.427	Bending (alkane, methyl group)	C-H
15	1469.81	6.366	29.673	Stretching (olefins)	Cyclic HCs
16	1541.18	5.964	52.073	Stretching (nitro)	N-O
17	1620.26	2.733	35.578	Stretching (α, β unsaturated ketone)	C=C
18	1705.13	2.658	58.427	Stretching (conjugate aldehyde)	C=O
19	1882.59	32.796	5.6	Bending (aromatic)	C-H
20	2196.99	25.164	84.104	Stretching (alkyne)	CΞC
21	3489.34	1.946	219.85	Stretching (amide)	N-H

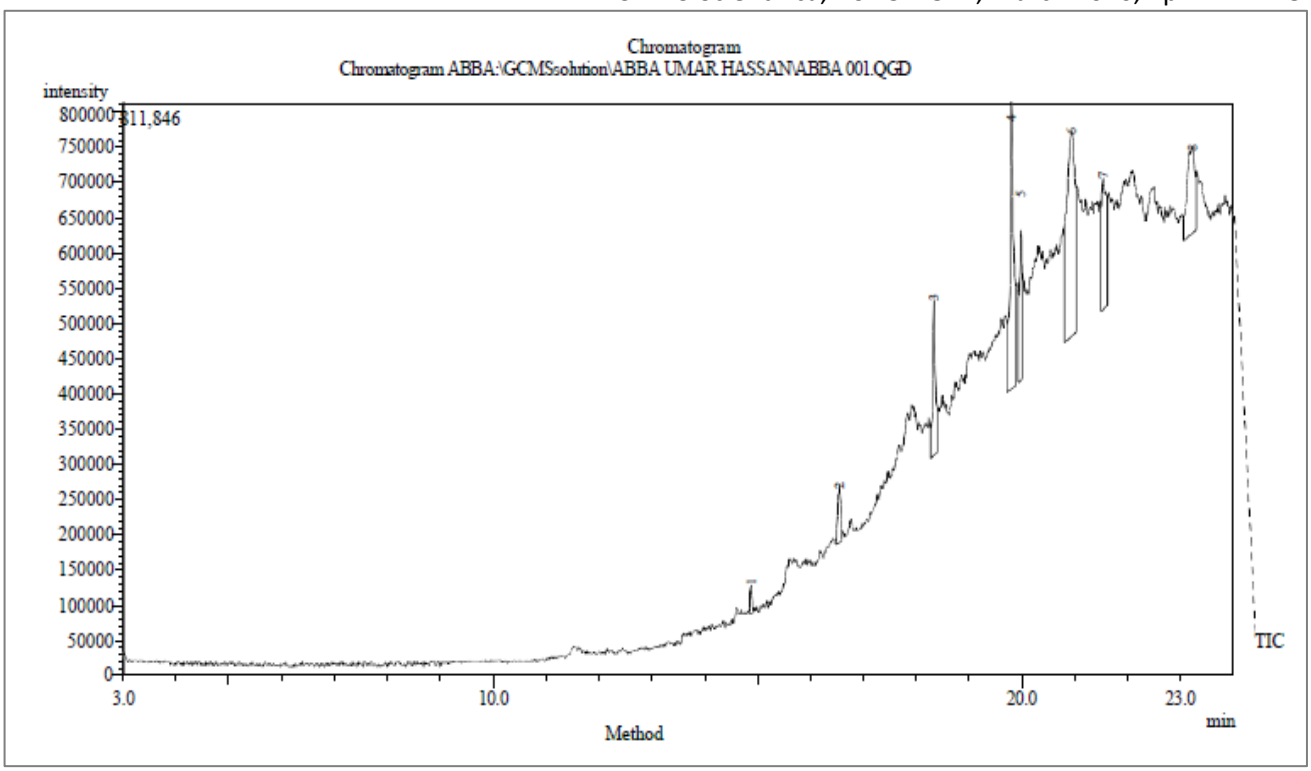


Figure 2: *Acacia nilotica* (L.) Phenolic Fraction GC-MS Micrograph

RESULTS

Plant Material Extraction and Processing

The extraction, qualitative, and quantitative phytochemical characteristics of the ethanol extract of *Acacia nilotica* (L.) are displayed in Table 1. Numerous phytochemicals, including tannins, saponins, alkaloids, flavonoids, phenols, terpenoids, quinones, phytosterols, and phlobatanins, were found in the plant extracts after qualitative screening. According to quantitative phytochemical tests, the plant extract contains a relatively high concentration of phenolic compounds.

Phyto-Phenolic Extraction and Fractionation

Thin Layer Chromatography of the three Active Plants Extracts

Acacia nilotica (L.) plant extracts were subjected to Thin-Layer Chromatography fractionation using gallic acid as a phenolic standard (Table 2). The crude plant extract was subjected to TLC fractionation, using gallic acid as a phenolic standard, with R_f values of 0.921 and 0.960. Fractions with the same R_f value as the standards, which also tested positive for phenolic compounds, were selected and pooled together to be further fractionated using TLC (Table 3).

Antioxidant Activity and Total Phenolic Content of Column Chromatography Products

The results of the antioxidant activity analyses revealed that the TLC phenolic-positive fractions of A. nilotica (L.) had an IC_{50} value of 28.53 mg/L, indicating excellent antioxidant activity. One hundred and twenty (120) fractions were collected from the column chromatography fractionation, antioxidant activity and total phenolic content were determined on all the fractions as shown in Table ... Moreover a spectral scanning analysis was also carried out on the individual phenolic positive column fractions, with lowest IC50 and highest phenolic content, to ascertain their compound-mix content similarity (Table 4).

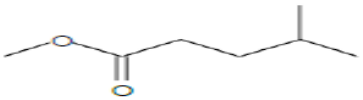
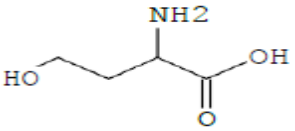
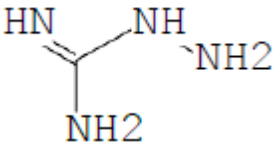
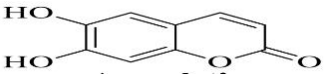
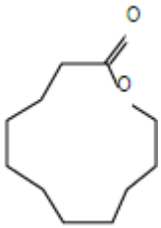
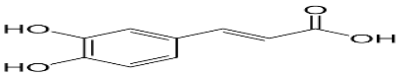
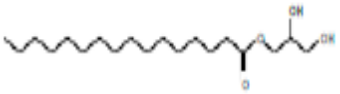
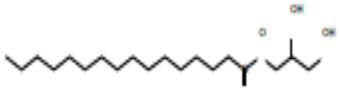
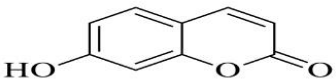
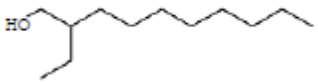
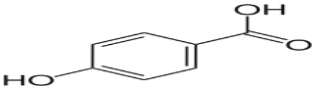
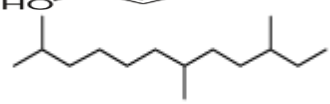
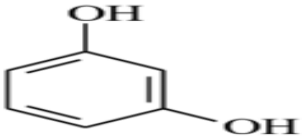
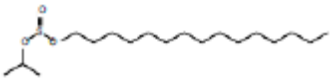
The Phenolic Fraction of Acacia nilotica as determined by Fourier Transform Infrared (FTIR) spectroscopy

The presence of several functional groups, including OH, -COOH, -CH2, and C=O, was detected by Fourier transform infrared spectroscopy of the A. nilotica phenolic fraction based on the IR absorption bands. It also indicates the presence of alcohols, aromatic compounds, amino acids, and carbonyl compounds from the individual functional groups (Table 6 and Figure 1). The phenolic fraction of Acacia nilotica (L.) leaf extract was shown to contain functional groups such as Ar-Cl, C-Br, Cyclic HCs, and CΞC, in addition to the more prominent groups, such as methyl and hydroxyl groups.

Gas Chromatogrphy-Mass Spectrometry (GC-MS) Analyses of *Acacia nilotica* (L.) Phenolic Fractions

The GC-MS analysis of the phenolic plant fraction revealed the presence of various compounds, with different chemical structures, such as amino-guanidine, undecanoic acid, 13-tetradece-11-yn-1-ol, dodecyl alcohol, z,z-8,10-hexadecadien-1-ol, hexadecanoic acid, 2,3-dihydroxypropyl ester, and methyl n-octanoate. Phenolic compounds like esculetin, 3, 4-dihydroxycinnamic acid, trans caffeate, para-hydroxybenzoic acid and resorcinol were also found present in the individual fractions (Table 7 and Figure 2).

Table 7: Probable Peaks Obtained from the GC-MS Analysis of *A. nilotica* (L.) Phenolic Fraction

Hit	Entry	Formula	Mol. Weight	Compound Name	Compound Structure
1	5268	C7H14O2	130	Pentanoic acid Methyl 4-methylpentanoate	
2	4980	C4H9NO3	119	Homoserine Butyric acid, 2-amino-4-hydroxy	
3	401	CH6N4	74	Aminoguanidine Hydrazinecarboximidamide Guanidine, amino	
4	98778	C9H6O4	178	Esculetin, 6,7-dihydroxycoumarin cichorigenin 6,7-dihydroxy-2H-1-benzopyran-2-one	
5	32119	C11H20O2	184	Undecanoic acid, hydroxy-, lactone Oxacyclododecan-2-one	
6	27524	C9H8O4	180	3,4-dihydroxybenzeneacrylicacid Caffeic acid	
7	117520	C19H38O4	330	Palmitin, 1-mono-	
8	14413	C12H26O	186	1-Dodecanol	
9	117518	C19H38O4	330	Hexadecanoic acid, 2,3-dihydroxypropyl ester	
10	23649	C9H6O3	162	Hydragine 7-hydroxy-2H-1-benzopyran-2-one Skimmetine	
11	33459	C12H26O	186	2-Ethyl-1-decanol	
12	131223	C7H6O3	138	4-hydroxybenzoate Para-hydroxybenzoic acid	
13	48605	C15H32	212	2,7,10-Trimethyldodecane	
14	112218	C8H6O2	110	Resorcinol 1,3-dihydroxybenzene	
15	119650	C18H38O3S	334	Sulfurous acid, pentadecyl 2-propyl ester	

16	96229	C14H12O4	244	Piceatannol Astringinin

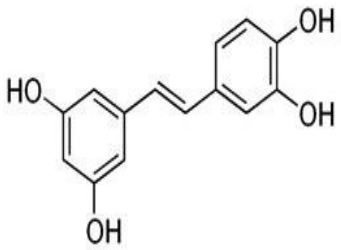


Table 8: Result for the Sequencing of the 16S rRNA Gene the eleven *Pseudomonas aeruginosa* Resistant strains

Strain	*Pseudomonas aeruginosa* Percentage Identity (%)	E-value	Query Cover (%)	NCBI Accession No
R1	98.7	-	100	PV763251
R2	100	-	100	PV778243
R3	99.60	-	100	PV778511
R4	100	-	100	PV778512
R5	99.50	-	100	PV778513
R6	100	-	100	PV778514
R7	99.80	-	100	PV778515
R8	100	-	100	PV778516
R9	100	-	100	PV778517
R10	99.81	-	100	PV778518
R11	100	-	100	PV778519

Key: - : 0.0

Table 9: Resistant Strains Infectivity Dose

Organisms	Cfu/ml	Days infection set in
R1	1.2×10^3	1
R2	1.1×10^3	1
R3	8.2×10^2	1
R4	1.1×10^2	1
R5	1.42×10^2	1
R6	8.9×10^2	2
R7	1.6×10^3	1
R8	1.4×10^3	1
R9	1.4×10^3	1
R10	1.48×10^3	2
R11	1.5×10^2	1

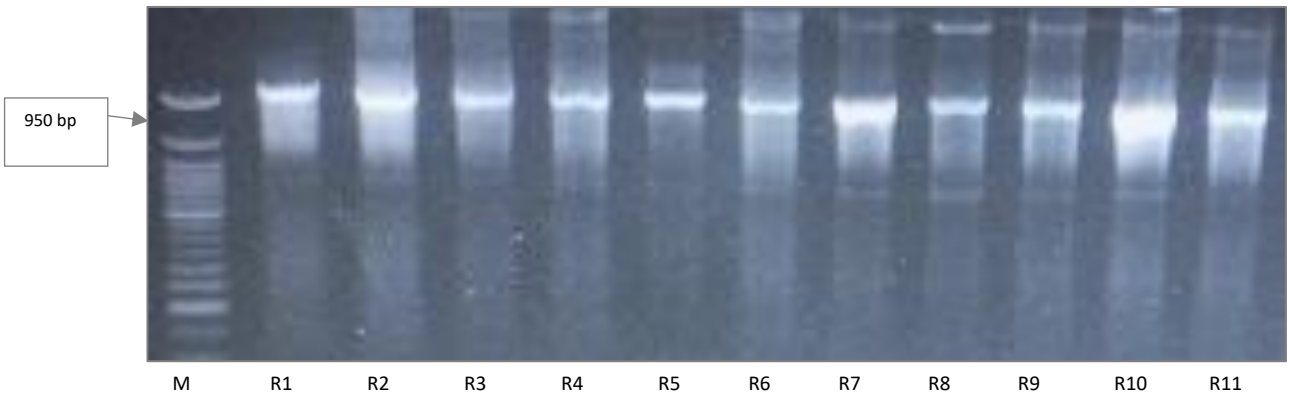


Plate 1: 16S ribosomal RNA Gel Electrophoresis of the Resistant Bacteria (M: Molecular maker)

Antibiotic Sensitivity Assay for the Resistant Clinical Isolates of *Pseudomonas aeruginosa*

The bioassay of the eleven *P. aeruginosa* resistant strains shows that all the isolates are multi-drug resistance to industrial-grade standard antibiotics (Figure 3). Imipenem, which belongs to the carbapenem class, showed the least resistance among the test isolates. Plate 1 shows the 16S ribosomal RNA Gel Electrophoresis of the Resistant Bacteria.

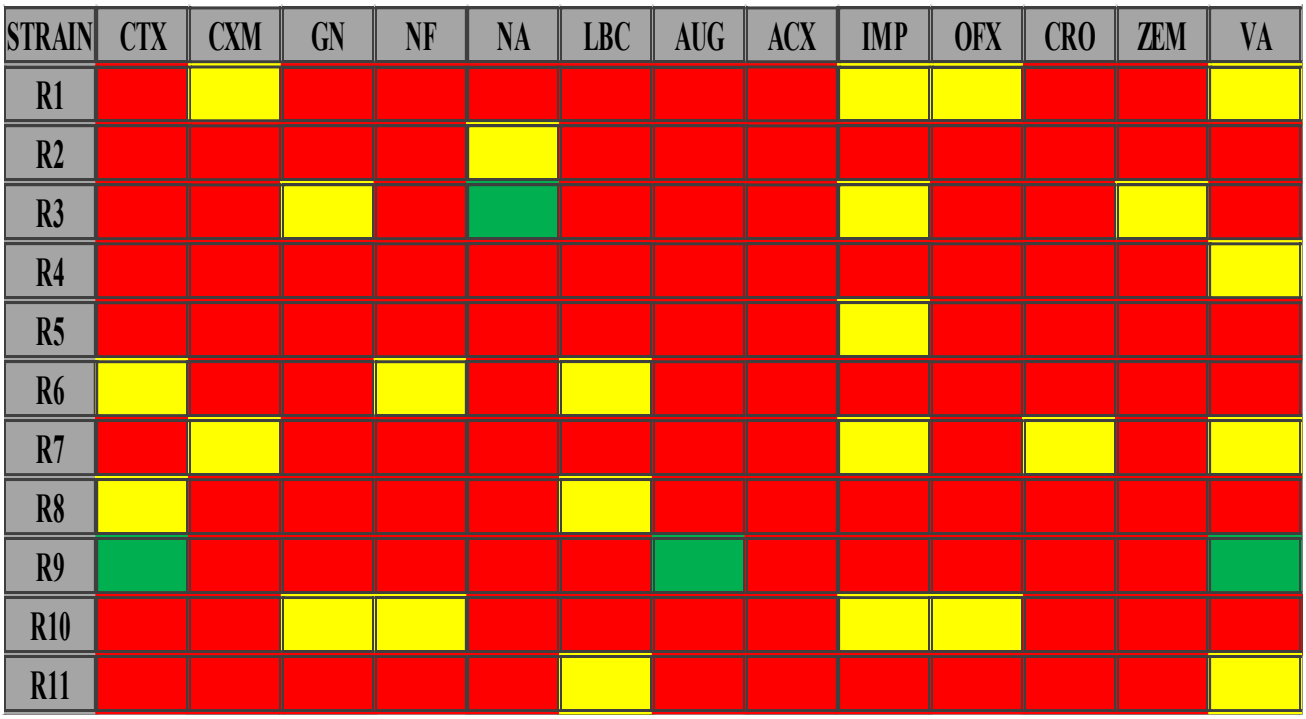


Figure 3: Antibiotic sensitivity assay heat-map for *Pseudomonas aeruginosa* resistant strains (Green: Sensitive, Yellow: Intermediate & Red: Resistant)

Table 10: Acute Toxicity Assays' Impact on Mice's Food Intake, Water Consumption, and Body Weight in Response to an Extreme Drug-Resistant *P. aeruginosa* Infection

Group	Average daily intake of food (g)	Average water intake (ml/day)	Weight of Body		Survival Rate (%)
			Real weight in grams	Gain weight In grams	
Control	3.74 ± 1.21	5.05 ± 1.22	22.14 ± 0.74	0.78 ± 1.57	100
R1	3.20 ± 2.51	4.71 ± 1.82	20.48 ± 1.49	0.03 ± 1.92	10
R2	1.02 ± 1.72*	1.63 ± 1.73*	22.27 ± 0.15	-	10
R3	0.62 ± 1.09*	2.53 ± 1.82*	21.52 ± 2.05	-	0
R4	0.97 ± 1.51*	4.01 ± 2.07	20.36 ± 1.93	1.13 ± 1.52*	0
R5	0.24 ± 1.05*	2.71 ± 1.13*	22.90 ± 1.87	1.03 ± 0.94*	0
R6	2.51 ± 1.72	4.92 ± 1.24	24.52 ± 1.37	-	0
R7	1.35 ± 0.26*	2.81 ± 1.83*	14.62 ± 2.18	-	0
R8	2.65 ± 0.26	3.72 ± 0.52	20.67 ± 1.13	-	0
R9	1.05 ± 1.62*	1.07 ± 0.98*	21.39 ± 1.05	0.68 ± 1.14*	10
R10	1.45 ± 0.75*	2.53 ± 0.63	22.62 ± 1.13	-	30
R11	1.18 ± 1.34*	4.62 ± 1.24	23.72 ± 1.31	-	0

Means ± SD are used to display values. Asterisks were used to indicate values that differed substantially from the control group's corresponding value ($p < 0.05$).

Acute Toxicity Assay for the Extreme Multi-drug Resistant Bacterial Strains in Experimental Mice

The acute toxicity assay conducted on all the extreme multidrug-resistant bacteria revealed that all the isolates have a low infectivity dose, especially the R4, R5, and R11 resistance strains (Table 9). These strains were shown to have an adverse effect on the animals' food and water intake (Table 10). There was a significant ($p < 0.05$) weight loss of 7.52 g in animals infected with strain R7, compared to the control group average body weight. The animals in the control group had a 100 % survival rate, while those in the infected groups had a very low or no survival rate (0-30 %). Infected group R10 and S_{72} have the highest survival rates of 30% each. Behavioral responses, general appearance, inflammatory symptoms, and mortality were observed in both infected and control groups at regular time intervals, as shown in Table 11. Numerous adverse symptoms and negative behaviours were observed in the infected groups, such as watery eyes, loss of appetite, difficulty breathing, and unformed stool. Photomicrographs of heart tissue from most infected groups showed an altered cellular arrangement of inflammatory tissue (Plate 2 and 3).

Acute toxicity bioassay on some haematological parameters revealed that most of the resistant isolates had detrimental effects on the red blood cell, platelet, and white blood cell concentrations in the infected animals

(Table 12). There was a significant change in some of the parameters studied. The blood total protein and creatinine levels were greatly impaired by infection with most of the resistance strains used, including the animals infected with strains R4, R5, and R11, which exhibit extreme drug resistance in *P. aeruginosa* (Table 13). The pathogenic infection manifests differently in the infected individual group, producing distinct inflammatory patterns in the animal's biochemical and haematological parameters; significant increases or decreases were observed in all tested parameters, depending on the infecting strain.

Table 11: Symptoms Observed in Albino Mice when Infected with the Extreme Multi-Drug Resistant Strains

Group	**Infectivity dose (**Cfu/ml**)**	**24 hours**	**48 hours**	**72 hours**	**96 hours**	**120 hours**	**144 hours**	**168 hours**
Control		*	*	*	*	*	*	*
R1	1.2 x 10^3	A, NS, EW	EW, A, NS, FS	W, NS, LA	W, RD, NS	W, RD	W, RD	W
R2	1.1 x 10^3	EW, A, NS	NS, W, LA, US	W, LA, US, NS	-	-	-	-
R3	8.2 x 10^2	FS, A, EW	W, NS, FS	HL, W, US	HL, RD, W	-	-	-
R4	1.1 x 10^2	NS, W, EW	RD, C, W, US, LA	HL,W, O	-	-	-	-
R5	1.42 x 10^2	Fs, A, EW	US, RD, LA	HL, C, W	-	-	-	
R6	8.9 x 10^2	A, NS, FS, EW	W, FS, NS	W, C, US, RD	-	-	-	-
R7	1.6 x 10^3	EW, A, FS	LA, US, W	W, LA, US	HL, W, RD	-	-	-
R8	1.4 x 10^3	NS, A, FS	LA, W	C, US	-	-	-	-
R9	1.4 x 10^3	A, EW, FS, NS	W, LA	HL, RD	-	-	-	-
R10	1.48 x 10^3	A, FS, NS, EW	A, US, EW, NS	W, EW, US	W, RD, LA	W	W	W
R11	1.5 x 10^2	W, EW, NS, FS	C, W, RD	HL, W	-	-	-	-

KEYS: C- convulsion; FS- Formed stool; US- Unformed stool; A- Active; W- Weak; RD- respiratory distress; EW- Eaten well; LA- Loss of appetite; NS- Normal skin; HL: hair loss; O- obesity, *- No symptoms observed Effect of Infection with the Isolates on the Heamatological and Biochemical Parameters of the Mice

Table 12: Acute Toxicity Assays' Impact on Mice's Haematological Parameters Due to the Severe Drug-Resistant *P. aeruginosa* Infection

Group	RBC (× 10^6cell/mm^3)	PLT (×10^3cell/mm^3)	WBC (×10^3cell/mm^3)	Survival Rate
Control group	6.95 ± 0.98	362.14 ± 4.21	2.02 ± 0.77	10
R1	4.62 ± 1.53	293.02 ± 1.03	2.56 ± 0.65	10
R2	1.62 ± 0.32*	300.52 ± 8.63	3.52 ± 1.53*	10
R3	2.61 ± 1.13*	164.65 ± 1.72*	2.58 ± 1.38	0
R4	5.62 ± 2.13	214.02 ± 2.72	4.62 ± 1.25*	0
R5	4.92 ± 1.76	105.52 ± 1.76*	4.32 ± 1.78	0
R6	3.62 ± 0.71*	281.86 ± 3.21	3.62 ± 1.13*	0
R7	6.97 ± 2.18	161.42 ± 2.73*	2.71 ± 0.16	0
R8	5.63 ± 2.81	113.60 ± 3.24*	2.86 ± 1.58*	0
R9	3.53 ± 0.16*	268.94 ± 1.90	3.82 ± 0.71*	10
R10	1.65 ± 0.86*	359.67 ± 1.96	2.38 ± 2.01	30
R11	1.63 ± 2.12*	123.04 ± 3.21*	6.42 ± 1.25*	0

Key: RBC: red blood cells, PLT: platelets, WBC: white blood cells
Means ± SD are used to display values. Asterisks were used to indicate values that differed substantially from the control group's corresponding value ($p < 0.05$).

Acute Toxicity Bioassay of *Acacia nilotica* Leaf Extract Phenolic Fraction on the Experimental Mice

Behavioural responses, phenotypic appearance, inflammatory symptoms, mortality, haematological and biochemical parameters were evaluated in all animals fed the plant extract phenolic fraction. All animals survived the period of the three fractions administration (Table 14). Moreover, there were no treatment-related infections,

symptoms, behavioural changes, or mortality in any of the groups. The haematological and biochemical parameters were also within the normal range for the control non-fraction-fed group (Table 15). Photomicrographs of the animal's heart tissue also showed a normal tissue cellular arrangement (Plates 2, 3, 4 and 5).

Table 13: Effects of the Extreme Drug Resistance *P. aeruginosa* Infection on Serum Biochemical Parameters in Mice using Acute Toxicity Assays

Group	Total proteins (g/dl)	Glucose (mg/dl)	Creatinine (mg/dl)	Survival Rate
Control group	4.47 ± 1.39	56.43 ± 1.03	0.35 ± 1.94	100
R1	3.20 ± 1.63	55.82 ± 1.92	0.31 ± 0.12	10
R2	5.27 ± 0.52*	53.92 ± 1.56	0.04 ± 1.62*	0
R3	6.95 ± 1.63*	68.52 ± 1.25*	0.28 ± 1.20	0
R4	5.62 ± 1.78*	21.53 ± 1.12*	1.73 ± 1.13*	0
R5	6.72 ± 2.51*	64.72 ± 2.65*	0.97 ± 0.98*	0
R6	3.67 ± 1.13	32.12 ± 1.43	0.26 ± 1.63	0
R7	5.86 ± 1.43*	68.15 ± 1.45*	0.27 ± 2.44	0
R8	3.47 ± 0.98	51.62 ± 2.78	0.30 ± 1.05	0
R9	4.73 ± 1.84	50.42 ± 1.83	0.37 ± 1.25	0
R10	4.53 ± 1.06	55.98 ± 2.61	0.38 ± 1.27	30
R11	8.63 ± 1.35*	72.49 ± 2.51*	1.45 ± 1.82*	0

Means ± SD are used to display values. Asterisks were used to indicate values that differed substantially from the control group's corresponding value ($p < 0.05$).

Table 14: Effects of *A. nilotica* (L.) Leaf Extract Phenolic Fraction on Food Intake, Water Consumption and Body Weight of Mice by Acute Toxicity Assays

Group	Average food intake (g/day)	Average water intake (ml/day)	Body weight		Survival Rate
			Actual weight (g)	Weight Change (%)	
Control group	3.39 ± 0.13	4.76 ± 1.27	21.07 ± 1.25	0.80 ± 0.81	100
A. nilotica	3.82 ± 1.20*	3.64 ± 1.12	21.72 ± 1.20	1.03 ± 1.13	100

Means ± SD are used to display values. Asterisks were used to indicate values that differed substantially from the control group's corresponding value ($p < 0.05$).

Table 15: Effects of *A. nilotica* (L.) Leaf Extract Phenolic Fraction on Hematological Parameters in Mice by Acute Toxicity Assays

Group	RBC ($\times 10^6$cell/mm^3)	PLT ($\times 10^3$cell/mm^3)	WBC ($\times 10^3$cell/mm^3)	Survival Rate
Control group	5.72 ± 1.16	354.02 ± 1.94	3.06 ± 1.23	100
A. nilotica	5.84 ± 1.20	351.02 ± 1.98	2.73 ± 1.06	100

Key: RBC: red blood cells, PLT: platelets, WBC: white blood cells
Means ± SD are used to display values. Asterisks were used to indicate values that differed substantially from the control group's corresponding value ($p < 0.05$).

Table 16: Impact of Phenolic Fraction of *A. nilotica* (L.) Leaf Extract on Serum Biochemical Parameters in Mice via Acute Toxicity Assays

Group	Total proteins (g/dl)	Glucose (mg/dl)	Creatinine (mg/dl)	Survival Rate
Control group	4.61 ± 1.20	53.07 ± 1.28	0.41 ± 1.01	100
A. nilotica	4.53 ± 1.42	54.04 ± 1.04	0.44 ± 1.10	100

Means ± SD are used to display values. Asterisks were used to indicate values that differed substantially from the control group's corresponding value ($p < 0.05$).

IN VIVO BIOASSAY OF *A. NILOTICA* (L.) LEAF EXTRACTS PHENOLIC FRACTIONS IN MICE INFECTED WITH EXTREME MULTI-DRUG RESISTANT BACTERIA

Response of the Infected Animals to Treatment with *A. nilotica* (L.) Extracts Phenolic Fraction

During the bioassay treatment, it was observed that the animals responded positively to the treatment (*A. nilotica* (L.)) leaf extracts, phenolic fractions, 1000 µg/ml, an active in *vitro* concentration. The animals infected with different extreme multidrug-resistant bacteria formed stools, became active over time, their feeding rate and psychological behavior restored, and even those with specific inflammation started showing positive therapeutic signs at varying significant rates (Table 17). Microphotographs of the treated animal's heart tissue showed recuperating tissues with no dilation (Plate 6). Toxicological effects of the extremely resistant bacteria were reversed and managed through treatment with the phenolic fraction of *A. nilotica* (L.) leaf extracts. In the experimental groups, behavioural responses were observed, with a 60–100% survival rate.

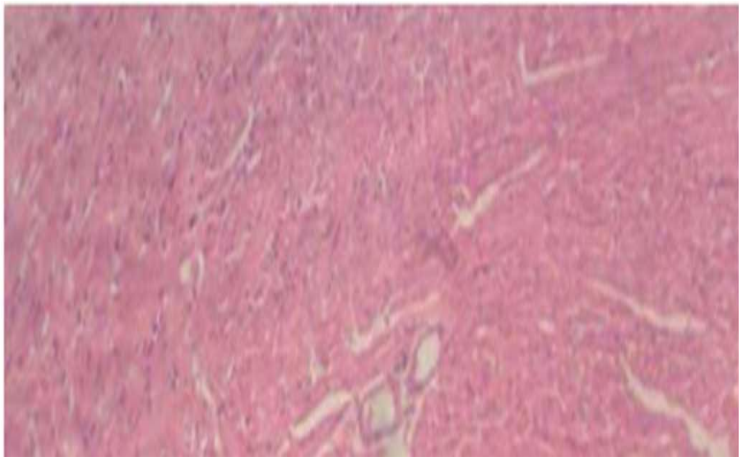


Plate 2: Light micrograph (100X) of a mice heart tissue without dilation (Negative Control Group – *A. nilotica* (L.) Phenolic)

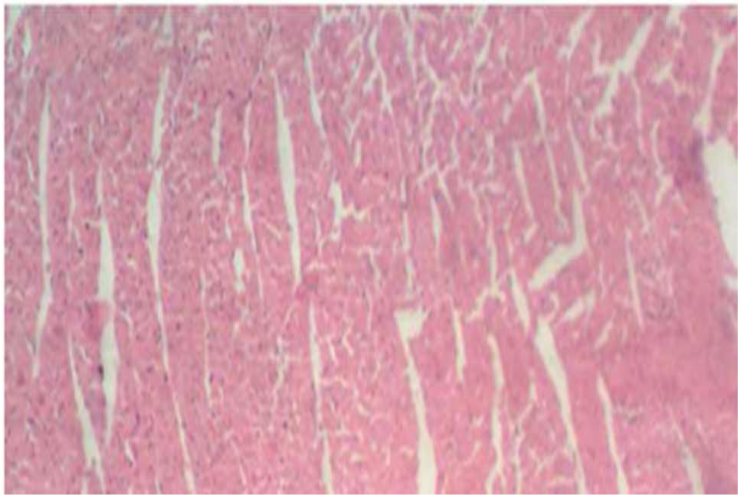


Plate 3: Light micrograph (100X) of a normal heart tissue without dilation (Positive Control Group)

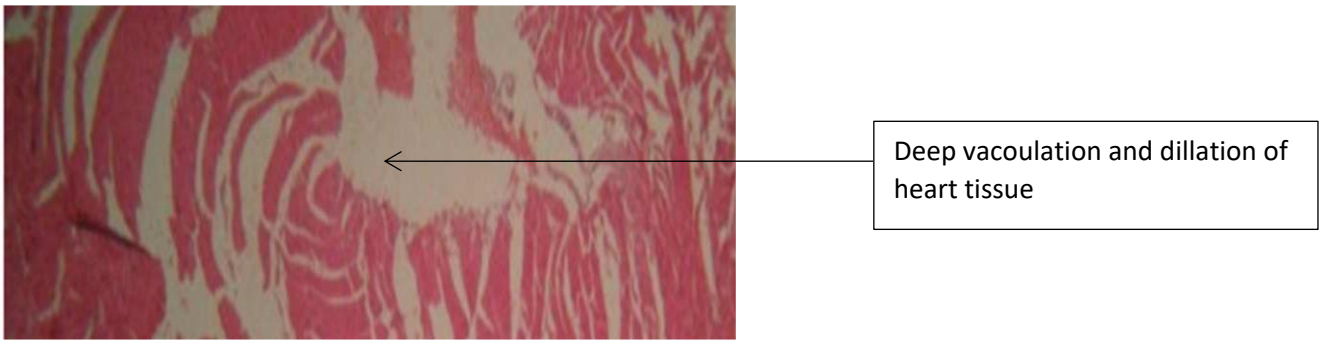


Plate 4: Mice Heart Tissue infected with strain R5 (100X)

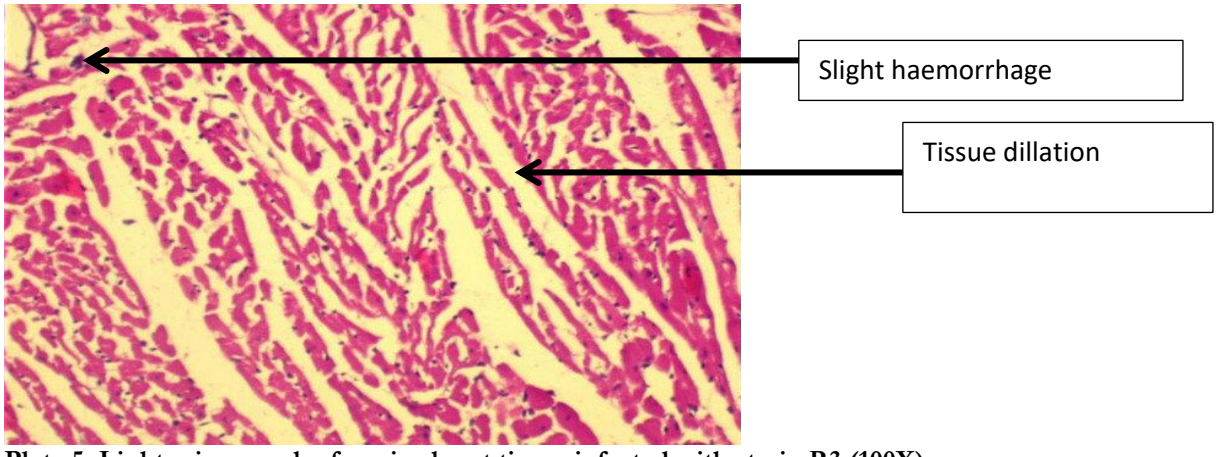


Plate 5: Light micrograph of a mice heart tissue infected with strain R3 (100X)

Table 17: Response of the Multi-drug Resistant Isolates Infected Albino Mice to *A. nilotica* (L.) Leaf Extract Phenolic Fraction

Group	Infectivity dose (Cfu/ml)	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours	Survival Rate (%)
Control		*	*	*	*	*	*	100
R1	1.2 x 10^3	A, NS, EW	LA, W, NS, FS	W, NS, LA, US	A, FS	EW, A, NS	A, FS, EW	90
R2	1.1 x 10^3	EW, A	W, LA, US	LA, FS, NS	EW, A	A, NS, FS	EW, FS, A	100
R3	8.2 x 10^2	FS, A, EW	W, RD	W, FS	NS, W	A, FS	A, EW	90
R4	1.1 x 10^2	NS, W, EW	RD, C, W, US	LA,W, O	W, US	EW, W	A, FS	70
R5	1.42 x 10^2	Fs, LA, W	US, RD, LA	HL, W	W, US	A, EW	EW, FS	70
R6	8.9 x 10^2	A, FS, EW	W, US, LA	W, LA, US	LA, A	FS, EW	A, EW, FS	80
R7	1.6 x 10^3	EW, W, US	LA, US, W	A, LA	A, FS	FS, A	EW, FS	80
R8	1.4 x 10^3	NS, A, FS	LA, RD	W, US	EW, FS	A, NS, EW	A, FS	90
R9	1.4 x 10^3	EW, FS	NS, W,LA	US, W	FS, EW	A, NS, FS	A, EW	90
R10	1.48 x 10^3	A, FS, NS, EW	A, US	A, EW	FS, A	A, FS	A, EW	100
R11	1.5 x 10^2	W, EW, FS	W, RD, US	US, W	W, LA	A, FS	EW, A	70

KEYS: C- convulsion; FS- Formed stool; US- Unformed stool; A- Active; W- Weak; RD- respiratory distress; EW- Eaten well; LA- Loss of appetite; NS- Normal skin; HL: hair loss; O- obesity, *- No symptoms observed

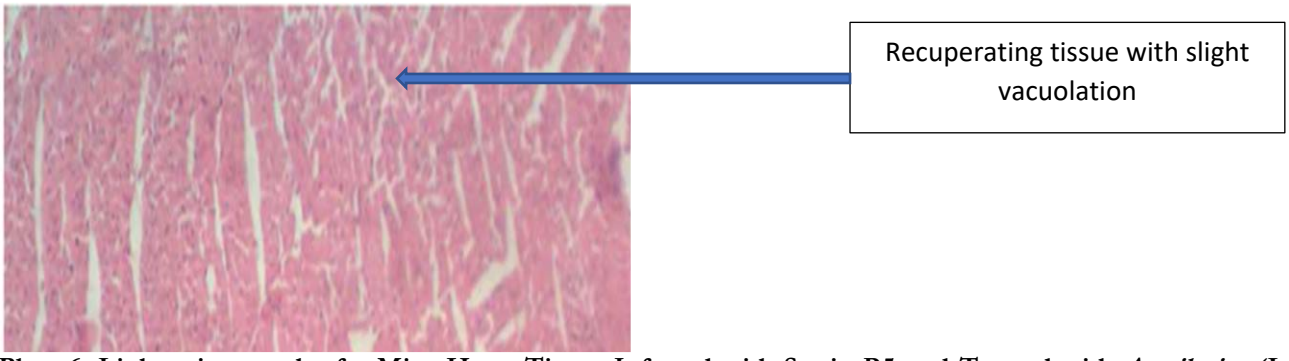


Plate 6: Light micrograph of a Mice Heart Tissue Infected with Strain R5 and Treated with *A. nilotica* (L.) Fraction (100 X)

Table 18: Heamatological parameters of the Multi-drug Resistant Isolates Infected Animals Treated with *A. nilotica* (L.) Leaf Extract Phenolic Fraction

Group	RBC (× 10^6cell/mm^3)	PLT (×10^3cell/mm^3)	WBC (×10^3cell/mm^3)	Survival Rate
Control group	6.32 ± 1.13	365.01 ± 1.62	2.33 ± 1.20	100
R1	5.15 ± 0.72	326.25 ± 1.03	1.96 ± 1.35	90
R2	6.10 ± 1.25	348.76 ± 2.57	2.20 ± 0.24	100
R3	5.15 ± 1.98	364.26 ± 0.84	1.94 ± 0.13	90
R4	4.01 ± 1.03*	350.63 ± 1.25	3.34 ± 0.53*	70
R5	4.12 ± 0.81*	352.26 ± 0.20*	0.75 ± 2.64*	70
R6	5.97 ± 0.18	363.62 ± 2.03	2.06 ± 0.69	80
R7	6.23 ± 0.82	334.10 ± 1.37	1.84 ± 1.25	80
R8	5.68 ± 1.83	204.42 ± 1.98*	1.80 ± 1.74	90
R9	6.37 ± 2.01	368.28 ± 3.01	2.24 ± 1.06	90
R10	5.53 ± 1.25	370.045 ± 0.48	3.74 ± 1.32*	100
R11	5.15 ± 1.20	245.24 ± 1.34*	0.76 ± 0.53*	70

Key: RBC: red blood cells, PLT: platelets, WBC: white blood cells

The values are shown as means ± SD. An asterisk (*$p < 0.05$) was used to indicate a value that differed significantly from the control group's equivalent value.

Table 19: Serum biochemical parameters of the Multi-drug Resistant Isolates Infected Animals Treated with *A. nilotica* (L.) Leaf Extract Phenolic Fraction

Group	Total proteins (g/dl)	Glucose (mg/dl)	Creatinine (mg/dl)	Survival Rate
Control group	5.24 ± 1.14	52.73 ± 1.25	0.40 ± 0.12	100
R1	4.78 ± 0.26	50.36 ± 1.07	0.31 ± 0.12	90
R2	5.36 ± 1.27	51.32 ± 1.95	0.45 ± 0.24	100
R3	4.92 ± 1.30	47.52 ± 1.31	0.36 ± 1.13	90
R4	5.03 ± 0.42	51.85 ± 1.32	0.63 ± 1.57*	70
R5	7.64 ± 1.13*	54.02 ± 0.35	0.73 ± 1.33	70
R6	5.24 ± 0.54	51.13 ± 0.53	0.88 ± 0.46	80
R7	6.74 ± 1.32*	50.1 ± 1.05	0.35 ± 1.98	80
R8	4.76 ± 1.20	48.68 ± 1.13	0.466 ± 1.30	90
R9	5.32 ± 1.13	54.02 ± 0.34	0.30 ± 1.27	90
R10	5.03 ± 0.15	55.98 ± 1.32	0.31 ± 1.03	100
R11	5.38 ± 0.53	47.14 ± 0.43	0.91 ± 1.31*	70

The values are shown as means ± SD. An asterisk (*$p < 0.05$) was used to indicate a value that differed significantly from the control group's equivalent value.

Heamatological and Biochemical parameters of the Infected Animals Treated with Phenolic Fractions of *A. nilotica* (L.) Leaf Extracts

Biochemical and haematological parameters of the infected animals treated with the two phenolic fractions showed significant improvements in white blood cell, blood protein, red blood cell, and blood creatinine concentrations, depending on the infected group and the treatment fraction (Tables 18 and 19). The values obtained for the treated groups are close to those of the control; this shows that there are little or no adverse effects or changes in all the blood parameters of the treatment groups tested. This shows the positive attributes of the plant phenolic fraction used.

DISCUSSION

Acacia nilotica (L.) phenolic fractions exhibit antioxidant activity, as indicated by low IC_{50} values, which signifies higher antioxidant activity. The OH, -COOH, -CH2, and C=O functional groups were among those identified in the three plants' phenolic content by Fourier transform infrared spectroscopy (FTIR) analysis. This is consistent with Luvincia's work (Luvincia *et al.,* 2019) on the phytochemical, FTIR, and NMR analysis of the crude extract of Acacia planifrons seeds, which found that the crude extract contained several functional groups, including COOH and -CH2.

Also the GCMS analysis reveals the prsesence of Phenolic compounds like; esculetin (a coumarin), 3, 4-dihydroxycinnamic acid trans caffeate Para-hydroxybenzoic acid (a phenolic derivative of benzoic aicd, soluble in water and chloroform), Skimmetine (an umbelliferone, a hydroxycoumarin), 4-hydroxybenzoate (an isomer of salicylic acid), Resorcinol (a phenol) and Piceatannol (a polyphenolic stilbene) were all indicated to be present in *A. nilotica* (L.) fraction. This corresponds with the research reports by Yadav et al. (2018), Adewole et al. (2013), Karimi and Jaafar (2011), and Sheema et al. (2014), who all reported the presence of one or more of these medically important phenolics, such as para-hydroxybenzoic acid.

In vivo bioassay of the phenolic fraction revealed that all the resistance strains had a high infectivity rate. This might be because the isolates are highly multidrug-resistant strains. A similar result was reported by Ayomide et al. (2019), where *P. aeruginosa* and *S. aureus* showed very high infectivity doses. Effects of the extreme drug-resistant *P. aeruginosa* and *S. aureus* isolates on food intake, water consumption, and body weight of mice revealed a significant reduction in food intake by the animals after infection, with most of the effects manifesting within 24 hours of infection. The group infected with the resistant strain R5 showed the greatest reduction in food intake, from 3.74 ± 1.21 g/day in the positive control to 0.24 ± 1.05 g/day. Similar research findings were reported by Teran-ventura *et al.* (2014), who observed a significant reduction in food intake after the animals were infected with specific pathogenic bacteria. Groups infected with resistance strains R2, R3, R4 and R9 also showed a significant reduction of food intake. Average daily water intake also decreased across the various infected groups. Groups infected with R9 resistance strains, however, showed the lowest water intake of 1.07 ± 0.98 ml/day compared with the normal control group's water intake of 5.05 ± 1.22 ml/day. In a study on oral ingestion of Streptococcus thermophilus, which reduced the severity of small intestine mucositis in mice treated with methotrexate, Tooley et al. (2006) showed a comparable decrease in water intake in albino mice infected with bacteria.

A significant decrease ($p < 0.05$) in the average weight of the animals was recorded during infection bioassay. This may be due to numerous factors, such as diarrhoea, weakness, loss of appetite, and inadequate water intake. In this study, it was observed that the animals lost a lot of their body fluid through diarrhea (unformed stool), while taking little or no water or food; this led to weight loss. Weight loss is a common phenomenon observed in experimental animals infected with pathogenic bacteria, especially Gram-negative virulent bacteria such as *P. aeruginosa.* A similar phenomenon was reported by Ayomide *et al.* (2019).

A higher percentage weight gain was observed in the group infected with the resistance strain R4, which may be attributed to the physical phenomenon of obesity observed in the animals. Obesity is mostly associated with excessive dietary intake or inadequate energy utilization, however there are instances where oral administration of opportunistic bacteria like *P. aeruginosa* were implicated in infection induced obesity. A study by Goodson et al. (2009) found a similar result. Effects of the extreme drug resistance *P. aeruginosa* isolates on serum biochemical parameters indicated a significant increase of blood serum protein in some of the infected animal groups, such as the groups infected with R11 and R3 strains, with a total serum protein of 8.63 ± 1.35 and 6.95 ± 1.63 g/dl, compared to the normal control group total serum protein of 4.47 ± 1.39 g/dl. This phenomenon is referred to as hyperproteinemia, a condition in which the blood contains a high protein concentration, which is normally indicative of disease or infection. Higher blood glucose concentrations were recorded in groups infected with the resistance strains R3, R5, R7, and R11 (with infected group R11 recording the highest glucose level of 72.49 ± 2.51 mg/dl), compared to the control group (56.43 ± 1.03 mg/dl). This may indicate an inflammatory condition affecting the host's carbohydrate metabolism and, probably, the host's pancreatic system (Margot *et al.,* 2014). A similar haematological and biochemical research finding was reported by Ayomide et al. (2019) in their study on the antibacterial activity and toxicological properties of the ethanolic leaf extract of *Carica papaya.* Animals from infected group R8 developed a skin inflammatory condition known as severe ulcerative dermatitis. Those from the R6-infected group developed another condition, referred to as ulcerative pododermatitis, also known as bumblefoot. This is a common bacterial and inflammatory reaction that occurs on the feet of birds, rats, rabbits, and other rodents, caused by drug-resistant *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (Lee *et al.,* 2018). Bahadir *et al.* (2023) reported similar pathological effects of *Pseudomonas aeruginosa* infection in their study titled '*Pseudomonas aeruginosa* keratitis in rats.

Results from the in-vivo bioassay of the phenolic fraction of *A. nilotica* (L.) extract against extreme multidrug-resistant bacteria revealed that all infected groups responded positively to the treatment. The animals treated after infection became active over time, their feeding status was restored to normal, and even those with specific inflammation began showing positive therapeutic recovery signs at varying significant rates. Photomicrographs of the treated animal's heart tissue showed recuperating tissues with no dilation or recuperating dilated/vacuolated tissue. The biochemical and haematological parameters of the infected animals treated with the phenolic fraction indicated significant changes in white blood cell, blood protein, and creatinine concentrations, depending on the infected group and the phenolic fraction used for treatment. This corresponds with the study done by Ayomide *et al.* (2019), where they reported that the treated rats had recuperating heart and intestine, which are close to that of the control, and also with Adeneye's (2014) study, on the sub-chronic and chronic toxicities of African medicinal plants, whose reports are in agreement with this research outcome.

CONCLUSION

Acacia nilotica Phenolic compounds can be used as a source for therapeutic formulations for the treatment of infections caused by multidrug- or extremely resistant bacterial pathogens, such as *Pseudomonas aeruginosa.*

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