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Antibacterial Activities, Phytochemical Analysis, and Genotoxicity Test of Aqueous and Methanolic leave extracts of *Jatropha tanjorensis*

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Abstract: The leaves of *Jatropha tanjorensis* have been found to have important application both in traditional medicine and as an edible vegetable in Nigerian soups. The need to substantiate the widely acclaimed antibacterial potentials of *Jatropha tanjorensis* leaf informed the present study. Two extract types of *Jatropha tanjorensis* leaf: aqueous extract of *Jatropha tanjorensis* (JAE) and methanolic extract of *Jatropha tanjorensis* (JME) were prepared and analysed for antibacterial activities against clinical isolates of *Staphylococcus aureus*, *Bacillus cereus*, *Enterobacter species*, and *Pseudomonas aeruginosa* using the agar-well diffusion technique. The plants extracts preparations, the qualitative phytochemical analysis, were performed using standard methods.The minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were also determined using standard methods. The genotoxicity test of the extracts was determined according to Organisation for Economic Cooperation Development (OECD) 420, (2001) guidelines. The result of the phytochemical analysis indicated the presence of tannin, terpenoids, flavonoids, carbohydrate, alkaloids, proteins and amino acids, cardiac glycosides, saponin, and glycoside, in JAE. The result of antibacterial analysis revealed that both leaf extracts of *Jatropha tanjorensis* were inhibitory to all the clinical isolates used in this study, indicating that both extracts have broad spectrum activity. The zone of inhibition (cm) ranged between 2.00± 0.41 and 1.37± 0.05 with JME having the highest inhibition against *Staphylococcus aureus*, While JAE had the lowest against *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) value ranged between 12.50mg/ML and 0.781mg/mL. The minimum bactericidal concentration value ranged between >50.00mg/mL and 0.781mg/ML. The genotoxicity result revealed from the gel images that JAE, and JME had some effects on the rat DNA, these extracts cut the DNA into pieces (plate 1 C and D). It also shows slight effect on the human DNA (plates 3 C and D) if exposed to the exracts for longer hours. These same extracts had no effects on the other DNA analysed (plates 2A-D).

Keywords: *Jatropha tanjorensis*, Antibacterial, aqueous extracts, methanolic extracts, Phytochemistry, toxicity.

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Introduction

There is a growing global burden of multidrug-resistant (MDR) bacteria, which are increasingly resistant to commonly available antibacterial agents, posing a serious threat to public health (Butler & Paterson, 2020). Pathogenic resistance to antimicrobial drugs, particularly antibiotics, continues to rise, and without a corresponding increase in the development of new therapeutic agents, the management of infectious diseases will become increasingly challenging (Ardal et al., 2019). This escalating resistance crisis has resulted in reduced effectiveness of many conventional antibiotics and has intensified the search for alternative antimicrobial agents, especially from natural sources such as medicinal plants (Adindu et al., 2016; Agu et al., 2013; Obianom et al., 2023).

Several studies conducted in Nigeria and other developing regions have demonstrated the antimicrobial potential of plant-derived compounds against pathogenic microorganisms. For instance, Adindu et al. (2016) reported significant phytochemical and antimicrobial activity in *Cola gigantea*, while Agu et al. (2013) demonstrated the antibacterial effects of citrus seed extracts. Similarly, Ubaoji et al. (2020) and Obianom et al. (2023) highlighted the therapeutic relevance of plant extracts through phytochemical characterization and antimicrobial evaluation. These findings are consistent with reports on the antibacterial and antifungal efficacy of medicinal plants such as *Carica papaya*, *Allium cepa*, *Allium sativum*, and *Ocimum gratissimum* (Awah et al., 2017; Anazodo et al., 2024; Orji et al., 2025). In addition, environmental and hospital-based studies have documented the widespread occurrence of pathogenic and drug-resistant microorganisms, emphasizing the urgent need for novel antimicrobial agents (Awari et al., 2023; Awari et al., 2024; Umeoduagu et al., 2023a). Obasi et al. (2024) further reported high levels of resistance among *Pseudomonas aeruginosa* isolates to commonly used β-lactam antibiotics, reinforcing concerns regarding the declining efficacy of standard antimicrobial therapies.

Jatropha tanjorensis is an ethno medicinal plant widely used in African traditional medicine for the treatment of infections and other ailments. Despite its extensive folkloric use, scientific evidence validating its antimicrobial efficacy and safety profile remains limited. Previous studies on medicinal plants within the Euphorbiaceae family have demonstrated diverse biological activities, including antimicrobial, antioxidant, hypoglycaemic, and haematological effects (Omoregie & Osagie, 2011). However, concerns regarding the potential toxicity of latex-containing plants in this family necessitate careful scientific evaluation of their therapeutic applications.

Medicinal plants have long been recognized for their therapeutic potential and continue to serve as valuable alternatives to synthetic drugs, particularly in resource-limited settings where access to conventional healthcare is restricted (Christian et al., 2021; Obeagu et al., 2023). The affordability and availability of herbal remedies have contributed significantly to their widespread utilization in developing countries. Moreover, several Nigerian studies have documented the successful application of plant extracts in managing microbial infections affecting food crops, clinical specimens, and consumer products (Agu et al., 2014; Agu et al., 2016; Awah et al., 2016; Umeoduagu et al., 2023b).

Phytochemical screening plays a crucial role in identifying bioactive compounds responsible for the therapeutic properties of medicinal plants. Standard qualitative tests such as Mayer's, ferric chloride, froth, Keller–Killian, ninhydrin, iodine, and Borntrager's methods are commonly employed for detecting alkaloids, tannins, saponins, glycosides, proteins, carbohydrates, and anthraquinones (Okaiyeto et al., 2019). Quantitative phytochemical analysis using gas chromatography–flame ionization detection (GC–FID) further enables precise characterization of active constituents (Buss & Butler, 2010).

The antibacterial activity of plant extracts is routinely evaluated using agar-well diffusion techniques (Oso et al., 2018), while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are determined through dilution and plate methods (Adegoke et al., 2010). Furthermore, genotoxicity assessments are essential for establishing the safety of herbal formulations and are commonly conducted using standardized DNA extraction and analysis protocols.

Given the increasing prevalence of MDR pathogens and the documented antimicrobial potential of medicinal plants, there is a compelling need for systematic evaluation of traditionally used herbs. Therefore, this study aims to investigate the antibacterial activity, phytochemical composition, and toxicity profile of aqueous and methanolic leaf extracts of *Jatropha tanjorensis* against antibiotic-resistant bacterial strains. The findings of this study are expected to contribute to the growing body of knowledge on plant-based antimicrobial agents and support the development of safe, effective, and affordable alternatives to conventional antibiotics.

Materials and Methods

Sources and Identification of Plant Material

Fresh leaves of *Jatropha tanjorensis* were collected from Port Harcourt Road Fegge Onitsha, in Anambra State Nigeria. The leaves were sent to the Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu Univerisity, Uli Campus, Anambra State, Nigeria, for taxonomic identification and authentication.

Plant Extract Preparation

Fresh leaves of *Jatropha tanjorensis* were rinsed with clean water, and then air-dried for 14 days at room temperature. The leaves were destalked and pulverized into coarse powder using industrial blender. Twenty (20 g) of the powdered leaves of the test plant was then weighed and placed into a 500 mL conical flask containing 200 mL distilled water and 300 mL methanol, mixed and macerated for 72 hrs. The aqueous and methanolic extracts were double filtered using a muslin cloth and then through a filter paper (Whatman no. 1). The filtrates were then concentrated to dryness in a water bath at 45 °C. The extracts were stored in a desiccator until needed (Oso *et al*., 2018).

Preparation of Extract Concentrations

Stock solutions (100 mg/mL) of the aqueous and methanolic extracts of *Jatropha tanjorensis* was prepared by weighing 1 g of the extracts and dissolving in 10 mL of dimethyl sulpho-oxide (DMSO). Thereafter, different concentrations were obtained from the stock solutions as described by Oso *et al*. (2018).

Test Organisms

The test organisms used in this study include *Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter species*. and *Bacillus cereus* isolates. These isolates were obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria. They were re-subcultured in Nutrient Agar slant contained in Bjou bottles and preserved in refrigerator at 4 ℃ for further analysis.

Inocula Preparation

Each bacterial strain was sub-cultured overnight at 35 ℃ in Mueller-Hilton agar slants. The bacterial growth was harvested using 5 mL of sterile saline water; its absorbance was adjusted at 600 nm and diluted to attain viable cell count of 1.5 X 10^8 CFU/mL using spectrophotometer (Mostafa *et al*., 2018).

Phytochemical Analysis

The qualitative and quantitative phytochemical screening of the aqueous and methanolic leave extracts of *Jatropha tanjorensis* were performed by the following standard methods as described by Okaiyeto *et al*. (2019), Karthigaiselvi and Rameshwari (2016) with slight modifications, and Gas Chromatographic ionization Detection(GC-FID) respectively. The obtained result was qualitatively expressed as positive (+) or negative (-).

Antibacterial Activity of the Plants Extracts

The antibacterial activities of the crude extracts were determined using the standard agar-well diffusion method by the method of Oso *et al*. (2018). The viable cell count of 1.5 X 10^8 CFU/mL for *S. aureus*, *Pseudomonas aeruginosa*, *Enterobacter* sp. and *Bacillus cereus*isolates were inoculated onto the surface of Muller Hinton agar using sterile swab sticks. After 30 minutes of inoculation, wells were made using sterile 9 mm cork borer and subsequently 100 µL of each stock concentration of the different crude extracts of *Jatropha tanjorensis* and were placed inside the labelled wells of each Petri dish plates and was done in triplicates. A control using dimethyl sulfoxide (DMSO) was also prepared and introduced into the well. Also, wells loaded with 1 mg/mL of Ciprofloxacin antibiotic was used as positive control. The plates were kept in the fridge at 4 ℃ for 2 h to permit plant extracts diffusion. The plates were finally incubated at 37℃ for these test bacteria and observed for zones of inhibition after 24 h. After incubation, observed clear zones of inhibition around the wells containing plant extract were measured using a metric ruler.

Determination of Minimum Inhibitory And Minimum Bacterial Concentrations Of The Plants Extracts

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h and 48 h of incubation. Using the tube dilution technique, 1 g of the extracts of *Jatropha tanjorensis* and *Dialium guineense* were dissolved in 10 mL sterile nutrient broths containing 0.2 % triphenyltetrazolium chloride (*TTC*) redox indicator; this gave 100 mg/mL. Thereafter, two fold serial dilutions were made from the original stocks of 10 mL using Muller Hinton broth to achieve the following concentrations: 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.781 mg/mL (Adegoke *et al*., 2010). Having obtained different dilutions and concentrations, 0.1 mL of standardized test organisms were inoculated into each diluted tubes and incubated at 37 ℃ for 24 h for the test bacterial strains. Using test organisms without extracts, positive controls were equally set up. The lowest concentration of the extracts that inhibited the growth of the test organism was recorded as the MIC. Bacterial tubes showing no visible growth from the MIC testwere sub - culture into nutrient agar and incubated at 37 ℃ for 24 h. The lowest concentration of the extracts yielding no growth on subculturing was recorded as the minimum bactericidal concentration (MBC) for bacteria as described by Adegoke *et al*. (2010).

Genotoxicity Test

The genotoxicity test was determined using Comet Assay methodology, and following the Organization for Economic Development Cooperation (OECD)(2000) guidelines.

Table 1: Qualitative phytochemical profile of the different plant extracts.

Component	JAE	JME
Anthroquinone	-	-
Tannins	+	+
Terpenoids	+	+
Flavonoids	+	-
Carbohydrate	+	+
Alkaloids	+	-
Protein and amino acid	+	-
Cardiac glycoside	++	_
Saponin	++	-
Glycoside	+	-

Key: JME = *Jatropha tanjorensis* methanolic extract; JAE = *Jatropha tanjorensis* aqueous extract; (+)= presence; (-) = absence.

Table 2: Gas chromatographic profile of the phytochemical components of *Jatropha tanjorensis* aqueous extract

RetTime (min)	Area (pA*s)	Amount (ppm)	Grp	Name
2.617	210.53412	29.60385	1	Kaempferol
3.055	129.44804	18.18869	1	Catechin
4.567	5.96399	8.29861e-1	1	Quercetin
4.768	6.57157	9.00292e-1	1	Genistein
6.160	7.40762	1.03277	1	Luteolin
6.379	37.41651	5.23845	1	Ferulic acid
7.069	4.36262	6.04803e-1	1	Artemetin
7.282	1.61948	2.17169e-1	1	Gallocatechin
7.459	6.36053	8.85062e-1	1	Flavone
7.630	14.59382	4.03981	1	Reveratrol
7.884	5.53773	7.67988e-1	1	Lunamarin
8.010	2.62720	3.60245e-1	1	Retusin
8.517	1.12811	1.47978e-1	1	Nobeletin
8.880	9.34233	3.30432	1	Ellagic acid
9.326	10.89357	4.52147	1	Tangeretein
9.742	3.83840	5.29527e-1	1	Epicatechin
9.905	130.06775	19.27924	1	Vanillic
10.130	5.21132	7.21139e-1	1	Hesperidin
10.295	9.28244	5.29419	1	Butein
10.425	1.65181	2.10675e-1	1	Apigenin
11.168	4.50330	6.23899e-1	1	Naringenin
11.477	5.26400	8.30262e-1	1	Myricetin
11.769	2.89167	6.96406e-1	1	Hesperidin
12.310	3.36374	5.64143e-1	1	Diadzin
12.563	2.44619	3.34740e-1	1	Isorhamnetin
13.038	1.87221	2.53243e-1	1	Maricetin
13.483	3.23720	4.44739e-1	1	Epicatechin
14.101	27.88656	7.91145	1	Daidze
Totals :		107.83641		

Key: PA= peak area, min= minutes, ppm= part per minute,

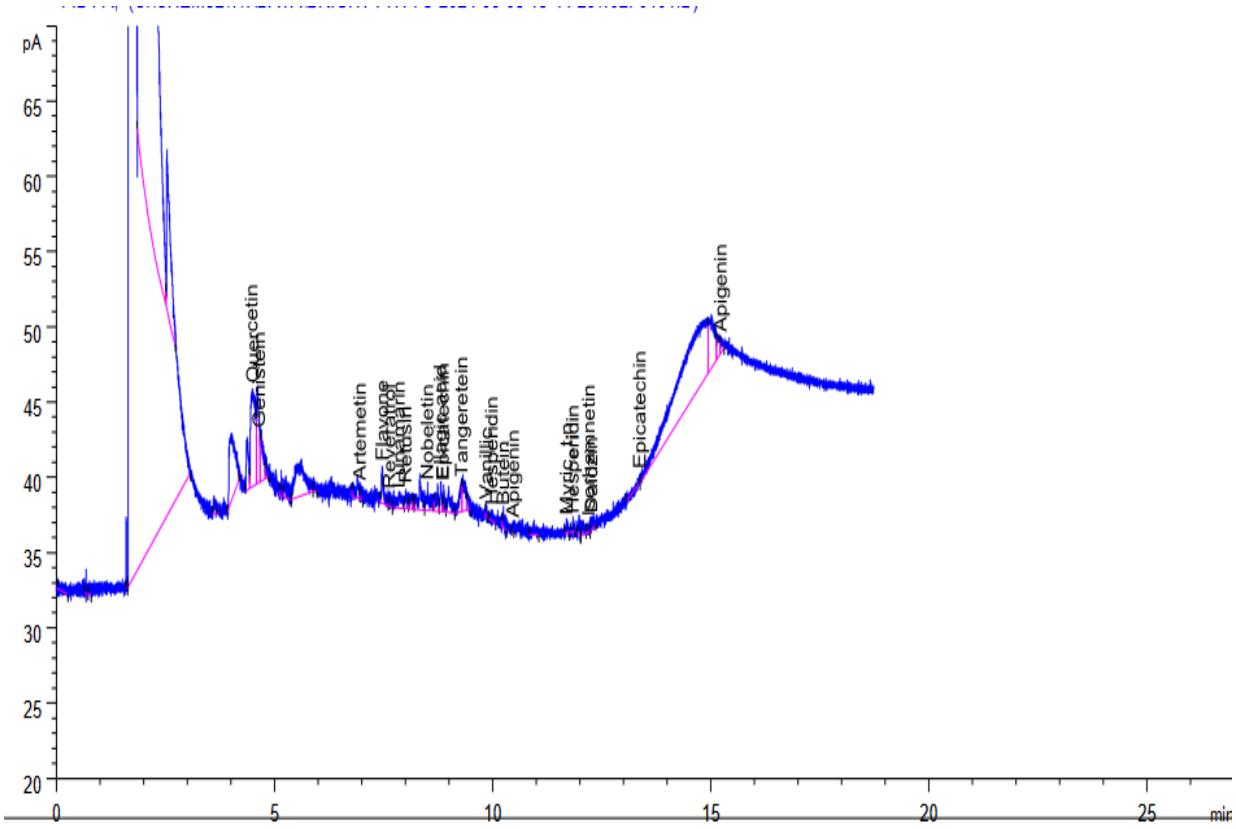
Table 3: Gas chromatographic profile of the phytochemical components of *Jatropha tanjorensis* methanolic extract.

RetTime (min)	Area (pA)	Amount (ppm)	Grp	Name
3.925	6.66480	9.29131e-1	1	Kaempferol
4.030	320.64548	45.01862	1	Butein
4.447	1031.88184	145.04649	1	Catechin
5.172	20.19850	2.83066	1	Qercetin
6.254	15.20751	2.12932	1	Luteolin
6.646	13.46197	1.88053	1	Nobeletin
7.219	4.36695	6.05412e-1	1	Artemetin
7.695	1.11425	1.42723e-1	1	Baicalin
8.264	2.98019	4.10139e-1	1	Daidzin
8.401	3.44543	4.75319-1	1	Retusin
9.159	2.13474	2.90926e-1	1	Ellagic acid
9.515	174.46471	24.51309	1	Gallocatechin
10.156	3.09193	4.25431e-1	1	Vanillic
10.695	1.39242	1.86560e-1	1	Naringenin

11.540	-	-	1	Apigenin
11.907	2.37106	3.23203-1	1	Hesperidin
12.265	-	-	1	Isorhamnetin
12.738	30.40840	4.26606	1	Maricetin
13.521	-	-	1	Epicatechin
14.123	-	-	1	Daidzein
14.607	137.45218	19.25054	1	Genistein
15.115	12.0535	8.30262e-1	1	Apigenin
15.785		-	1	Lunamarin
16.787	-	-	1	Reveratrol
17.312	1.61300	2.16625e-1	1	Tangeretein
17.398	1.19933	1.58277e-1	1	Epicatechin
18.351	1.31555	1.66032e-1	1	Naringin
18.617	1.55022	2.10416e-1	1	Hesperidin
Totals :		251.15947		

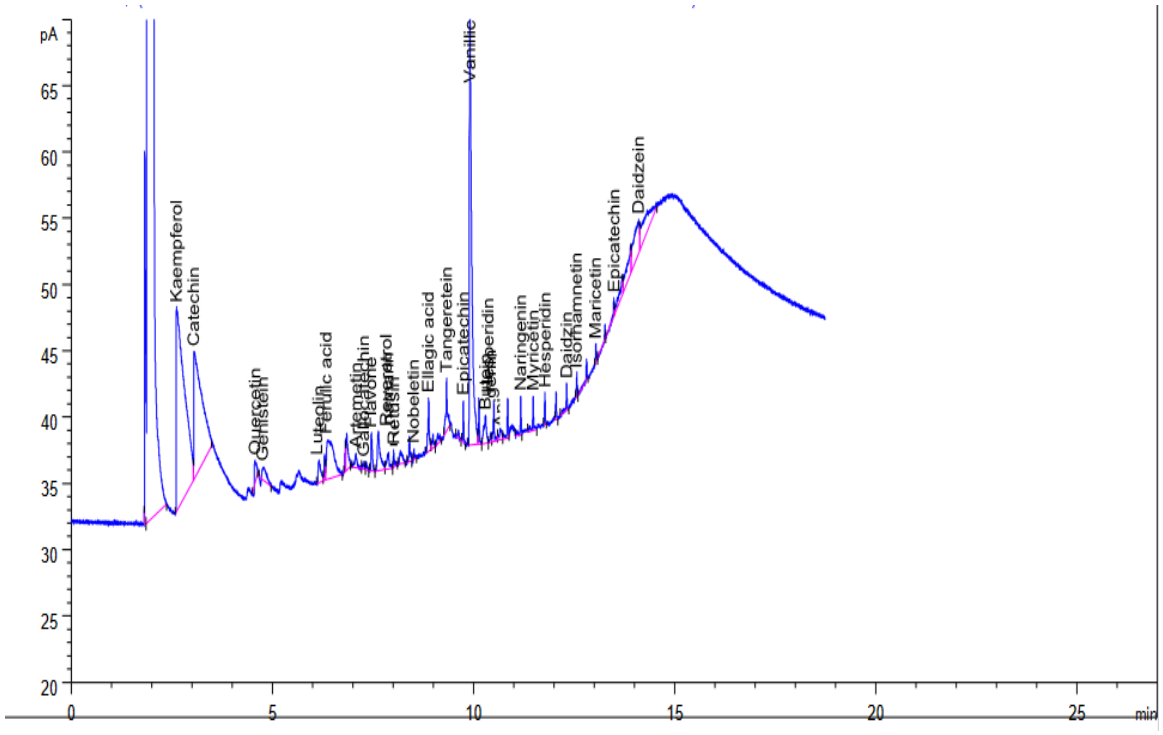
Key: min = minutes; pA = peak area; ppm = part per minute; Grp = group

Figure 1; Gas Chromatogram of *Jatropha tanjorensis* aqueous extract



Key: pA= peak area, min= minutes.

Figure 2: Gas chromatogram of the *Jatropha tanjorensis* methanolic extract.



Key: PA= peak area; min = minutes.

Table 4: Antibacterial activity(cm) profile of the different plant extracts used in this study.

Extract code (100 mg/mL)	*Bacillus cereus*	*Staphylococcus aureus*	*Enterobacter* sp.	*Pseudomoanas Aeruginosa*
JAE	1.85±0.10	1.50±0.00	1.78±0.17	1.37±0.05
JME	1.60±0.08	2.00±0.41	1.50±0.00	1.80±0.16

Key: JME = *Jatropha tanjorensis* methanolic extract; JAE = *Jatropha tanjorensis* aqueous extract; mg/mL = milligram per milliliter; ± plus or minus.

Table 5: Minimal inhibitory concentration (mg/mL) profile of the different plant extracts used in this study

Extract code (100 mg/mL)	*Bacillus cereus*	*Staphylococcus aureus*	*Enterobacter* sp.	*Pseudomoanas Aeruginosa*
JAE	12.50	12.50	1.56	12.50
JME	12.50	0.781	12.50	12.50

Key: JME = *Jatropha tanjorensis* methanolic extract; JAE = *Jatropha tanjorensis* aqueous extract; < = less than. Mg/mL = milligram per milliliter.

Table 6: Minimal bactericidal concentration (mg/mL) profile of the different plant extracts used in this study

Extract code (100 mg/mL)	*Bacillus cereus*	*Staphylococcus aureus*	*Enterobacter* sp.	*Pseudomoanas Aeruginosa*
JAE	>50.00	25.00	12.50	50.00
JME	25.00	12.50	>50.00	25.00

Key: JME = *Jatropha tanjorensis* methanolic extract; JAE = *Jatropha tanjorensis* aqueous extract; mg/ml = milligram per milliliter; > = greater than.

Electrophoregrams of Wistar DNA sample exposed to different concentrations of plants extracts.

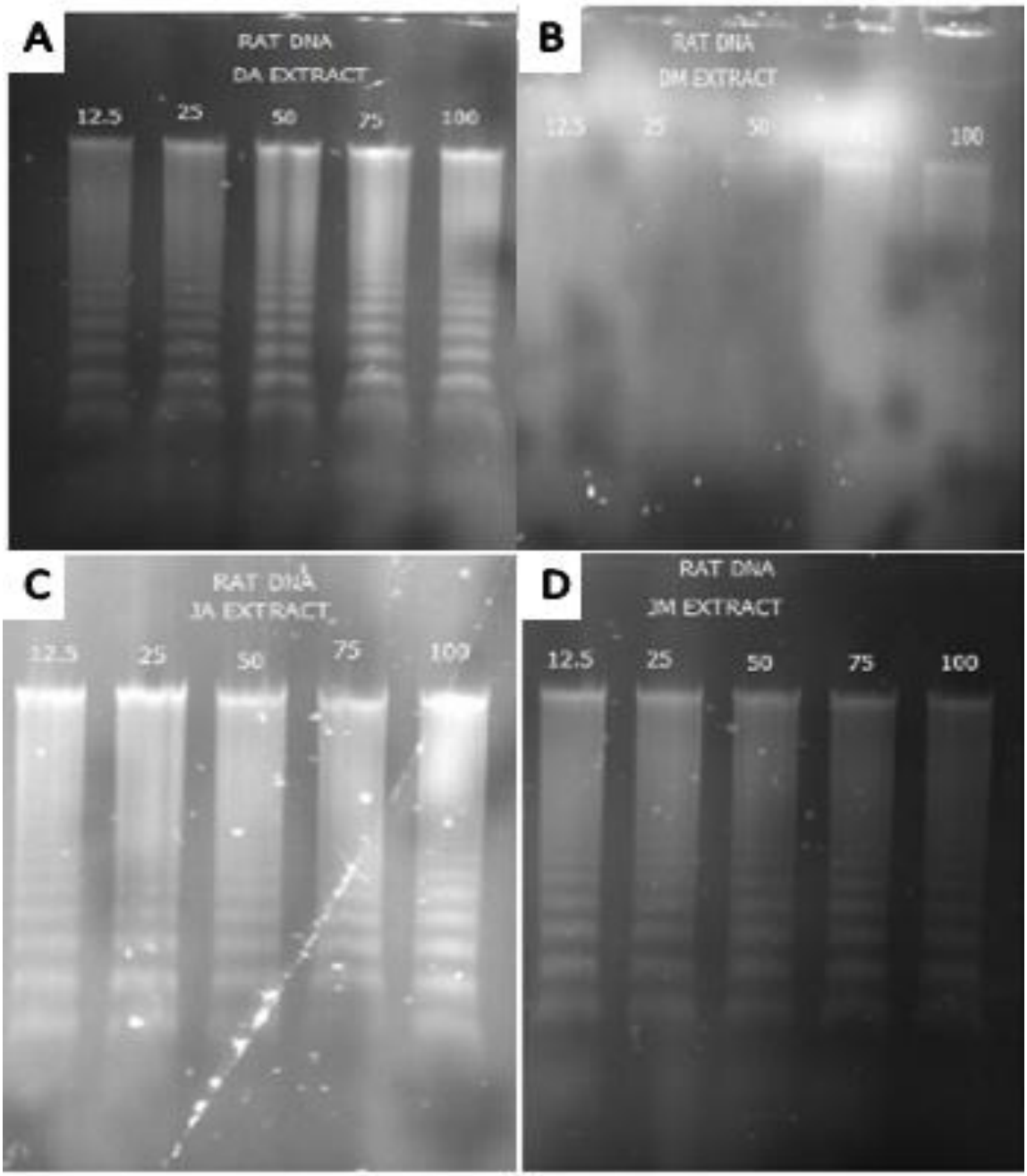


Plate 1(A-D): Electrophoregrams of Wister albino rat DNA sample exposed to different concentrations of(A) DA = *Dialium guineense* aqueous extract; (B) DM = *Dialium guineense* methanolic extract; (C) JAE = *Jatropha tanjorensis* aqueous extract (D) JM = *Jatropha tanjorensis* methanolic extract

Electrophoregrams of plant DNA sample (*Telfairia occidentalis*) exposed to different concentrations of plants extracts.

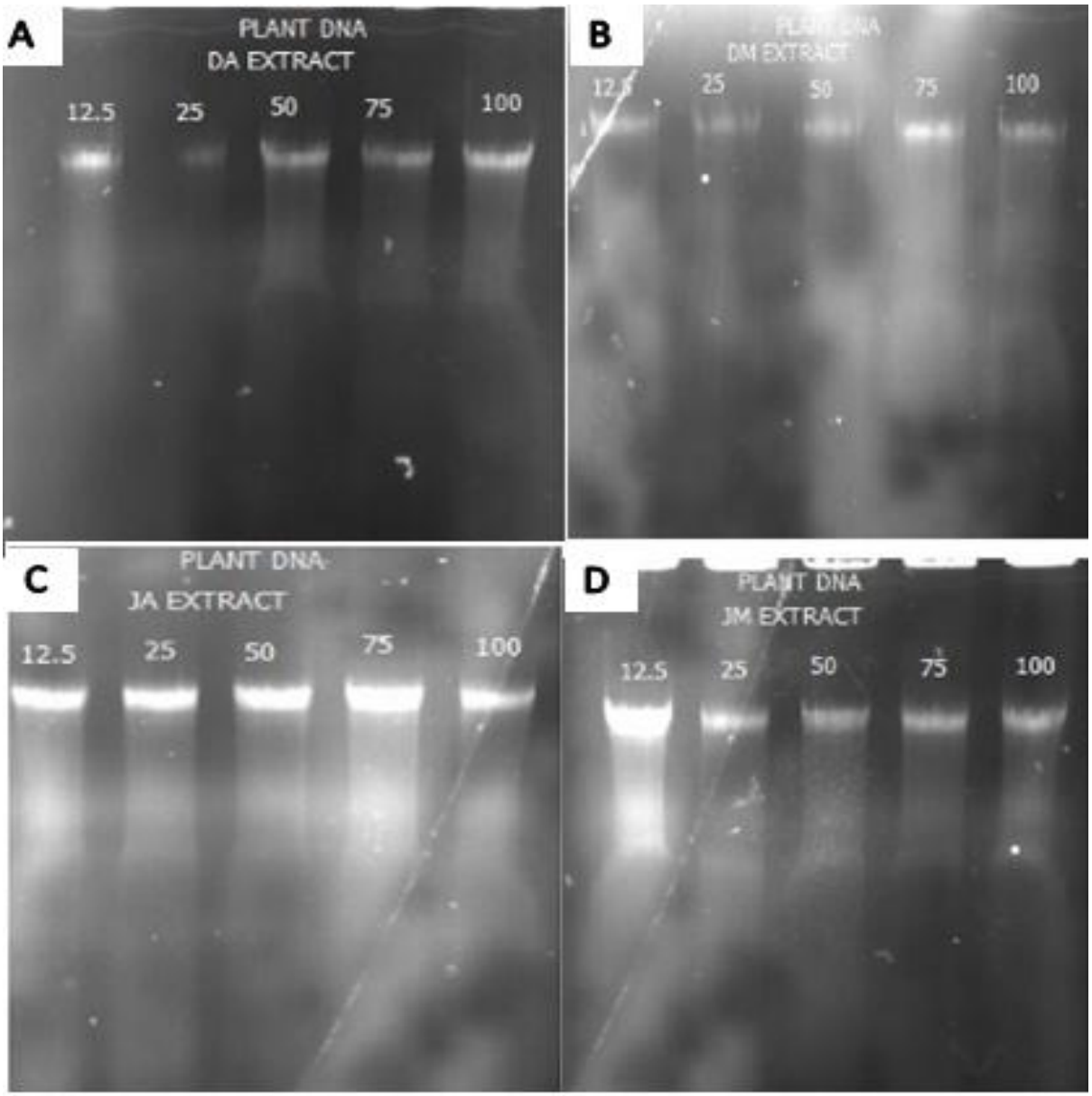


Plate 2(A-D): Electrophoregrams of plant DNA sample (*Telfairia occidentalis***)** exposed to different concentrations of(A) DA = *Dialium guineense* aqueous extract; (B) DM = *Dialium guineense* methanolic extract; (C) JAE = *Jatropha tanjorensis* aqueous extract (D) JM = *Jatropha tanjorensis* methanolic extract

Electrophoregrams of Human DNA exposed to different concentrations of plants extracts.

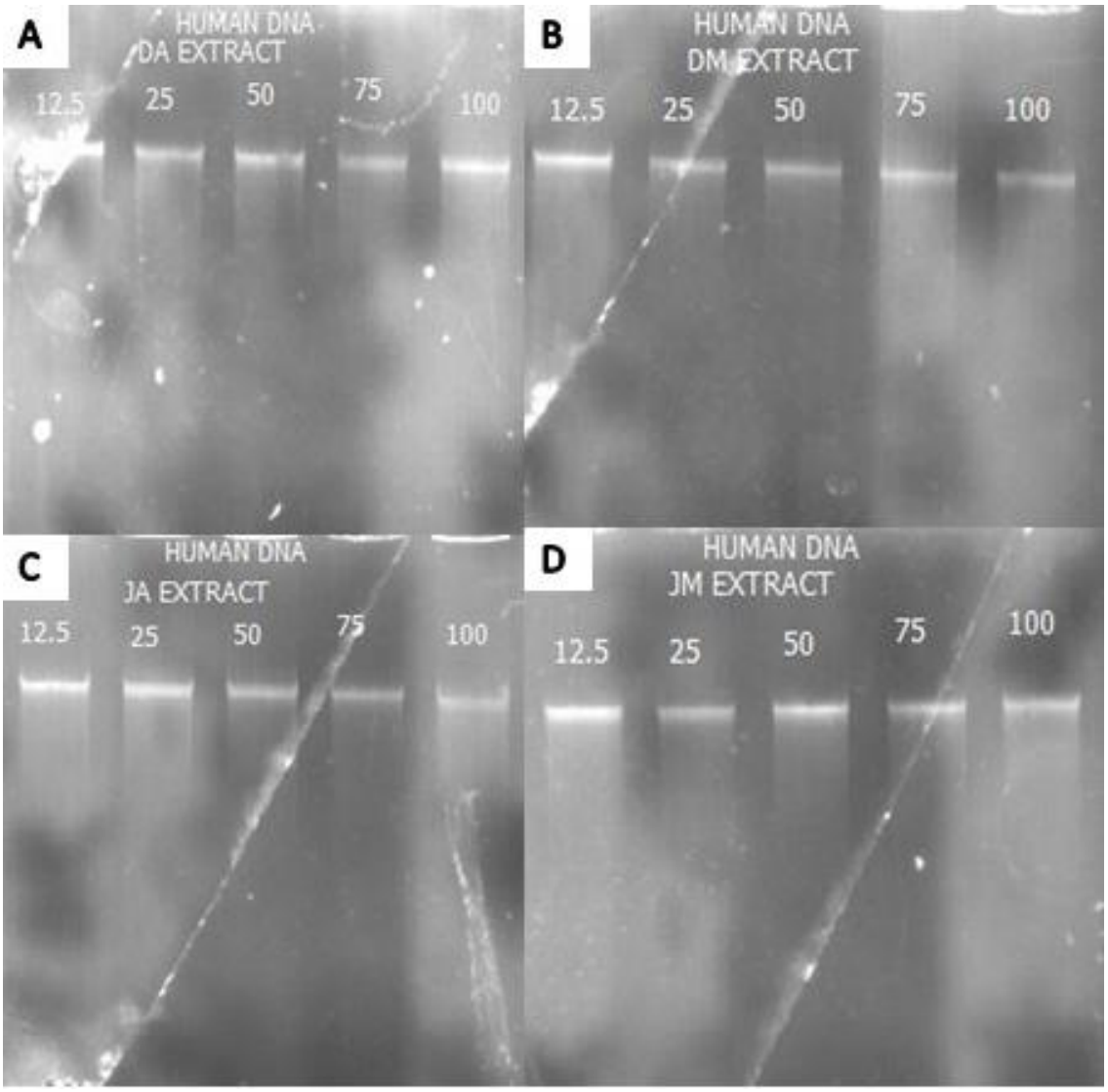


Plate 3(A-D): Electrophoregrams of Human DNA sample exposed to different concentrations of(A) DA = *Dialiumguineense* aqueous extract; (B) DM = *Dialiumguineense*methanolic extract; (C) JAE = *Jatrophatanjorensis*aqueous extract (D) JM = *Jatrophatanjorensis*methanolic extract.

Discussion

In many nations of the World, many green leafy plants are regularly consumed either as vagetables or as medicinal herbs. This study evaluated the antibacterial activity, phytochemical constituents, and genotoxicity effects of methanolic and aqueous leave extracts of *Jatropha tanjorensis*. Methanol is a universal solvent that dissolves all compounds, i.e polar, semi-polar, or non-polar. In the extraction process, the composition, colour, aroma, and extract yields are influenced by the raw material's type, size, maturity level, type of solvent, temperature, extraction time, and extraction method. In addition, the percentage of secondary metabolites in the methanol extract might be strongly influenced by the plant leaves used for extraction, which is generally less than 10% (Van Beek, 1999).

A study by Omoregie and Osagie (2007) on the qualitative phytochemical analysis of *J. tanjorensis* revealed that it contains phytochemical compounds such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones, and saponins which is similar to the findings in this study (Table 1), and also in line with the previous report by Ebana *et al*. 2019, who also reported the presence of three flavonoids, three amines, two associated hydrocarbons, two alkanes, and two alkanoids .

The chromatograms of the aqueous and methanolic leaf extracts of *Jatropha tanjorensis* are shown in figure (1and 2) respectively. The results revealed the quantitative constituents of both extracts.

The results of the antibacterial profile of aqueous and methanolic leave extracts of *Jatropha tanjorensis* are shown in table 2. The result revealed that both extracts demonstrated broadspectrum antibacterial activity. This broadspectrum activity could be attributed to the presence of phenolic compounds which enhances antimicrobial activity in this plant as reported by Besong *et al*., 2016. *Staphylococcus aureus* was more susceptible to JME than JAE.This is similar to a report by Fred *et al*.,(2009) that *Staphylococcus aureus* exhibited sensitivity to aqueous exract of *Jatropha tanjorensis* leaves.

The MIC analysis revealed inhibitory effects of both extract types on the test organisms. However, JAE has proven to be more potent over JME to *Enterobacter species*, as lower MIC value was obtained in JAE than JME. Similarly, JME had lower MIC value than JAE against *Staphylococcus aureus.* Comparing MIC values

help to assess the relative potency of different antimicrobial agents against a particular microorganism.

Both extracts had significant effects on the test organisms, with JAE having 1.85±0.10cm, 1.50±0.00cm, 1.78±0.17cm, 1.37± 0.05cm against *Bacillus cereus, Staphylococcus aureus. Enterobacter species, Pseudomoanas Aeruginosa* respectively. JME had 1.60±0.08cm, 2.00±0.41cm, 1.50±0.00cm, 1.80±0.16cm against *Bacillus cereus, Staphylococcus aureus. Enterobacter species, Pseudomoanas Aeruginosa* respectively.

The results of genotoxicity of different plants extracts revealed from the gel images that JAE, and JME had some effects on the rat DNA, these extracts cut the DNA into pieces (plate 1 C and D). It also shows slight effect on the human DNA (plates 3, C and D) if exposed to the extracts for longer hours. These same extracts had no effects on the other DNA analysed (plates 2A-D).

Conclusion

The findings from this study demonstrate that the aqueous and methanolic leaf extracts of *Jatropha tanjorensis* possess significant antibacterial properties against both Gram-positive and Gram-negative bacterial isolates, supporting their traditional use in the management of infectious diseases. The observed antibacterial effects suggest the presence of potent bioactive phytochemicals capable of inhibiting the growth of pathogenic microorganisms, thereby offering a promising alternative to conventional antibiotics in the face of rising antimicrobial resistance. Toxicological evaluation revealed that the extracts exhibited slight effect on human DNA or no toxicity in the experimental animals, as evidenced by the absence of significant alterations in haematological and biochemical parameters and the lack of observable behavioral or morphological abnormalities. This indicates that both plant's extracts are relatively safe and could be developed further for therapeutic or pharmaceutical applications. Overall, *Jatropha tanjorensis* represents valuable source of natural antimicrobial agents with minimal toxicity risks. Future studies should focus on the isolation and characterization of the specific phytochemicals responsible for the antibacterial effects, as well as detailed chronic toxicity and pharmacokinetic assessments to support safe formulation and clinical usage.

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