Antibacterial Activity of Jatropha Curcas Extracts on Gram Negative Isolates from Oju LGA, Benue State.

Ojowu, Sally Ogbene[1*], Anisiobi, Nworah Sunday[2], Ojowu, David Obo[3] ,Tyona, Ngodoo Magdalene[4]

[1,2,4]Department of Microbiology, Joseph Sarwuan Tarka University, P.M.B 2373, Makurdi, Benue State, Nigeria.

[3]Initiative4healthyliving Ltd, 9 Fullerton Drive, S60 5HQ, Sheffield, UK.

***Corresponding Author**

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ABSTRACT

Jatropha curcas (locally known as Omangba or pig nut) is traditionally used as food and medicine among the Oju people of Benue State, Nigeria. This study evaluated the antibacterial potential of its leaf extracts against selected gram-negative bacteria: Escherichia coli, Pseudomonas spp., Salmonella typhi, Klebsiella pneumoniae, and Proteus spp. Clinical isolates were confirmed using Gram staining and biochemical tests. Crude extracts were prepared using cold maceration in distilled water and methanol for 72 hours, then concentrated at 65 °C. Phytochemical screening revealed flavonoids, phenols, saponins, and glycosides. Antibacterial activity was assessed using agar well diffusion, with methanol extracts showing greater inhibition zones than aqueous ones and control (Ciprofloxacin), particularly against Pseudomonas spp. (21.33±0.58 mm at 400 mg/ml). MIC and MBC ranged from 25–50 mg/ml and 100–200 mg/ml, respectively. This study concludes that *J. curcas* leaf extracts offer promising alternative antibacterial agents, potentially reducing reliance on synthetic antibiotics and addressing antimicrobial resistance challenges.

Keywords: Antibacterial activity, Ciprofloxacin, Gram Negative, Jatropha curcas, Oju LGA, Nigeria

INTRODUCTION

The global rise in antimicrobial resistance (AMR) has become a critical public health challenge, particularly in Africa, including Nigeria, where infectious disease rates remain high. AMR is defined as the ability of microorganisms to withstand antimicrobial agents that once effectively inhibited or killed them [2]. AMR is one of the most urgent global health threats, responsible for over a million deaths annually due to drug-resistant bacterial infections, and several million more where AMR is a contributing factor [30].

This resistance contributes to increased morbidity, mortality, and economic burden. The World Health Organization (WHO) identifies AMR as one of the top ten global health threats, with an estimated 4.95 million deaths linked to bacterial AMR in 2019 [13]. In response, the WHO's Global Action Plan emphasizes global awareness through effective communication and education, yet data on public awareness in Nigeria especially in Benue state remains scarce[13].

Overuse and misuse of antibiotics in human, animal, and agricultural sectors significantly drive AMR [29][30]. According to WHO, antibiotic misuse in healthcare and agriculture has been a leading driver of resistance globally since the early 2000s [29]. Environmental contamination; especially through pharmaceutical waste, hospital effluents, and agrochemicals further compounds the problem. Recognizing this, Nigeria launched its Second National Action Plan on AMR (AMR 2.0) in October 2024 to enhance surveillance and reduce environmental triggers [9].

Gram-negative bacteria pose a particular concern due to their intrinsic resistance mechanisms. Characterized by

a thin peptidoglycan layer surrounded by an encapsulated lipopolysaccharide-containing outer membrane [28], they do not retain the crystal violet stain used in the Gram staining method [15]. Their outer membrane contains porins and efflux pumps that contribute significantly to multidrug resistance, limiting antibiotic penetration [20], serving as a protective barrier that evades many antibiotics and remains a major cause of hospital-acquired infections leading to high morbidity and mortality [10][14]. The complex structure of the bacterial cell envelope, especially the outer membrane, plays a crucial role in antibiotic resistance [27]. They are typically categorized into Enterobacteriaceae and non-fermenters; Enterobacteriaceae have been identified as the common culprits of infections like gastroenteritis and sepsis [7][9].

Antibacterial agents are substances used to combat pathogenic bacteria [24]. For centuries, various plant parts; leaves, roots, stems, and bark have been employed in traditional medicine to treat infections. These plants contain bioactive compounds that either inhibit bacterial growth or destroy them, prompting scientific interest in their pharmacological validation. One such plant is *Jatropha curcas* Linn.

Jatropha curcas (Family: Euphorbiaceae) is widely cultivated in tropical and subtropical regions. In this study, samples were obtained from Oju Local Government Area (LGA) of Benue State, where it is locally known as "Omangba" and used for both food and medicine. Common English names include "pig nut," "purging nut," and "physic nut" [12]. Traditionally, it is recognized for its anti-inflammatory, antioxidant, antidiabetic, hepatoprotective, and antimicrobial properties [26][27].

Extracts from its leaves, bark, and stems have demonstrated notable antibacterial activity [21][25], attributed to phytochemicals such as alkaloids, flavonoids, phenols, and terpenoids [2][23], and has been compared to Gentamycin [10].

Its effectiveness against Gram-negative bacteria, due to its ability to penetrate their outer membrane, has drawn research interest. Additionally, Prastiyanto et al. [22] found that Jatropha latex exhibited strong inhibitory effects against multidrug-resistant bacteria, further validating its broad-spectrum potential.

MATERIALS AND METHODS

Study Area

Samples were collected from Oju LGA in Benue State, located between latitude 6°45′N and longitude 8°30′E, approximately 15 km from Makurdi [17]. With an estimated population of 700,000, the dominant ethnic group is Igede. The LGA spans 1,283 km² within the Guinea Savannah zone, with an elevation of 200–500 m above sea level. It shares boundaries with Obi, Gwer, Konshisha, Ado, and parts of Ebonyi and Cross River States. The region experiences a tropical climate with distinct dry and rainy seasons, and its population relies mainly on farming, trading, and civil service [4][18].

Sample Collection

Fresh *J. curcas* leaves were harvested very early in the morning from Oju and authenticated at the Department of Botany, Joseph Sarwuan Tarka University, Makurdi (JOSTUM). Gram-negative clinical isolates; *Klebsiella* spp., *E. coli*, *Salmonella typhi*, *Pseudomonas* spp., and *Proteus* spp were obtained from Bethseda Hospital, Ikachi, Oju. All samples were processed in the Microbiology Laboratory, JOSTUM.

Sterilization and Disinfection

All glassware was washed, rinsed, and sterilized in a hot-air oven at 160 °C for 1 hour. Bench tops were disinfected with sodium hypochlorite before and after use. Inoculating loops were flame-sterilized using a spirit lamp [6].

Preparation and Extraction of Plant Samples

The *J. curcas* leaves were washed, air-dried at room temperature (25 °C) for two weeks, and ground into powder.

Fifty grams (50 g) of the powder was soaked in 250 ml of distilled water and methanol (1:5 w/v) using the cold maceration method [8] [9]. After shaking for 30 minutes, the mixtures were incubated at 25 °C for 48 hours, filtered, and concentrated in a water bath at 65 °C. Crude extracts were stored at 4 °C until further analysis [7][25][28].

Preparation of Media

Mueller Hinton Agar and Nutrient Broth were prepared following standard protocols for antibacterial assays [3][6]. *Jatropha curcas* leaves were processed for phytochemical screening, revealing the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, steroids, and cardiac glycosides using standard qualitative methods [19]. Ciprofloxacin was used as a control. Test organisms, including *Klebsiella* spp., *E. coli*, *Salmonella typhi*, *Pseudomonas* spp., and *Proteus* spp., were revalidated through Gram staining and biochemical tests using indole, catalase, motility, citrate utilization, and oxidase tests.

Stock solutions of aqueous and methanol leaf extracts were prepared in DMSO at varying concentrations (50–400 mg/mL). Standardization of bacterial cultures was done using 0.5 McFarland Standard [5]. Antibacterial activity was evaluated using agar well diffusion method, while minimum inhibitory concentration (MIC) was determined via broth dilution. The MIC was defined as the lowest concentration with no visible growth. Minimum bactericidal concentration (MBC) was determined by sub-culturing MIC dilutions with no turbidity onto Mueller Hinton Agar. Plates were incubated and observed for growth, with the lowest concentration showing no bacterial growth recorded as the MBC [11][15]. The data generated for these experiments were done in triplicates. The values of zones of inhibition were expressed in mean ± standard deviation.

RESULTS

Table 1 presents the confirmatory identification of the test organisms: *Escherichia coli*, *Pseudomonas* spp., *Salmonella typhi*, *Klebsiella pneumoniae*, and *Proteus* spp. All isolates were Gram-negative.

E. coli tested positive for indole, while the remaining isolates were indole-negative. Catalase activity was observed in all organisms. Motility and citrate utilization tests were positive across the isolates, except *K. pneumoniae* (non-motile) and *E. coli* (citrate-negative). Oxidase test results showed that only *Pseudomonas* spp. exhibited a positive reaction, while the others were oxidase-negative.

Table 1: Confirmatory Tests to Confirm Isolates

Test organisms	Gram staining	Indole test	Catalase test	Motility test	Citrate test	Oxidase test
Pseudomonas species	_	_	+	+	+	+
Escherichia coli	_	+	+	+	_	_
Salmonella typhi	_	_	+	+	+	_
Klebsiella pneumonia	_	_	+	_	+	_
Proteus species	_	_	+	+	+	_

Keys: += Positive test, - = Negative test

Table 2 shows the result of the phytochemical screening for aqueous and methanol extracts of *Jatropha curcas* leaves extract. The result showed that alkaloids, tannins, terpenoids and steroids were all absent for both extracts. Flavonoids, saponins, phenols and glycosides were present in methanol and aqueous extracts.

Table 2: Phytochemical Constituents of Extracts of *J. curcas* Leaves

Phytochemical Constituents	**Extracts**	
	AE	**ME**
Alkaloids	-	-
Flavonoids	+	+
Tannins	-	-
Saponins	+	+
Terpenoids	-	-
Steroids	-	-
Phenols	+	+
Glycosides	+	+

Keys: ME= Methanol extract, AE= Aqueous extract, **+ =** Present**, - =** Absent

Table 3 displays the results of the antibacterial activity of aqueous extract of *J. curcas* leaves at varying concentrations (50 mg/ml, 100 mg/ml, 200 mg/ml, 400 mg/ml). The results revealed that the aqueous extract exhibited zones of inhibition at all concentrations against the Gram-negative bacteria except for *E. coli* and *S. typhi* at concentrations of 50 and 100 mg/ml. The highest zone of inhibition was recorded for *Pseudomonas* species with 17.66± 0.42 mm at concentration of 400 mg/ml of the extract.

Table 3: Antibacterial Activity of Aqueous Extract of *J. curcas* Leaves (mm)

Test Organisms	**Concentrations**				**Control**
	50 mg/ml	**100 mg/ml**	**200 mg/ml**	**400 mg/ml**	
Escherichia coli	0.00±0.00	0.00±0.00	14.66±0.55	17.60±0.57	25.00±1.55
Salmonella typhi	0.00±0.00	0.00±0.00	14.66±1.15	17.33±0.58	25.00±1.58
Pseudomonas species	9.00±0.50	12.66±0.20	14.14±0.27	17.66±0.42	25.00±1.05
Klebsiella pneumoniae	8.33±0.10	11.00±0.12	13.00±0.14	17.62±0.36	25.00±1.58
Proteus species	8.67±0.58	11.67±0.58	14.33±0.58	16.67±0.58	24.33±0.57

Key: Control-Ciprofloxacin (10µg/ml)

Table 4 presents the antibacterial activities of varying concentrations of the methanol extract of *J. curcas* leaves on test organisms revealing their zones of inhibition. The result revealed that the methanol extracts of *J. curcas* leaves exhibited distinct zones of inhibition at all concentrations against all the test organisms. However, the methanol extract had no activity against *S. typhi* at concentrations of 50 and 100 mg/ml. The methanol extract

showed the widest zone of inhibition of 21.33 ± 0.58 mm against *Pseudomonas* species at concentration of 400 mg/ml of the extract.

Table 4: Antibacterial Activity of Methanol Extract of J. curcas Leaves (mm)

Test Organisms	Concentrations				Control
	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	
Escherichia coli	11.00±0.50	16.00±1.00	18.33±1.15	20.33±1.55	25.00±1.55
Salmonella typhi	0.00±0.00	0.00±0.00	17.33±0.58	20.00±1.00	25.00±1.58
Pseudomonas species	10.33±0.58	14.33±0.58	18.66±0.50	21.33±0.58	25.00±1.05
Klebsiella pneumoniae	10.00±0.58	15.33±0.58	18.67±0.58	21.00±1.00	25.00±1.58
Proteus species	10.33±0.18	14.66±1.00	16.00±1.00	20.33±0.58	24.33±0.57

Key: Control-Ciprofloxacin (10µg/ml)

Table 5 showed that the results of the Minimum Inhibitory Concentration (MIC) of the antibacterial activity of aqueous and methanol extracts of the leaf of *Jatropha curcas* on test organisms were within the range of 25-50 mg/ml. The minimum inhibitory concentration reveals that *Pseudomonas* species and *Klebsiella pneumoniae* had the highest rate of minimum inhibitory concentration for the aqueous extracts and *E. coli*, *Pseudomonas* species and *Klebsiella pneumoniae* for methanol extracts at 25 mg/ml.

Table 5: Minimum Inhibitory Concentration (MIC) of Extracts of *J. curcas* Leaves on Test Organisms

Test Organisms	MIC (mg/ml)	
	AE	ME
Escherichia coli	50	25
Salmonella typhi	50	50
Pseudomonas species	25	25
Klebsiella pneumonia	25	25
Proteus species	50	50

Key: MIC = Minimum inhibitory concentration, AE= Aqueous extract, ME= Methanol extract.

The minimum bactericidal concentration (MBC) in Table 6 were in the range of 100-200 mg/ml. *Escherichia coli*, *Pseudomonas* species and *Klebsiella pneumoniae* showed the highest rate of minimum bactericidal concentration at 100 mg/ml.

Table 6: Minimum Bactericidal Concentration (MBC) of Extracts of *J. curcas* Leaves on Test Organisms

Test Organisms	MBC (mg/ml)	
	AE	ME
Escherichia coli	200	100

Salmonella typhi	200	200
Pseudomonas species	200	100
Klebsiella pneumonia	200	100
Proteus species	200	200

Keys: MBC = Minimum bactericidal concentration, AE= Aqueous extract, ME= Methanol extract

DISCUSSION

This study confirms that *Jatropha curcas* leaves contain key bioactive compounds; flavonoids, saponins, phenols, and glycosides which inhibited the growth of gram-negative bacteria such as Pseudomonas spp, *Salmonella typhi, Klebsiella pneumoniae, Proteus* spp, and *E. coli*. Among the extracts tested, the methanol extract showed greater antibacterial activity than the aqueous extracts and control. These findings support the traditional use of *J. curcas* for treating infections and highlight its potential in developing plant-based alternatives to conventional antibiotics.

Similarly, [1] found that methanolic extracts of *J. curcas* were effective against wound pathogens including *Pseudomonas aeruginosa* and *Proteus mirabilis,* emphasizing stronger antibacterial efficacy of methanol over aqueous extracts, and supporting this study's observation that the methanol extract exhibited greater inhibition zones compared to aqueous extracts and even the conventional antibiotic control (Ciprofloxacin in this case).

[25] also demonstrated that methanolic extracts from both leaves and stem bark of *J. curcas* possessed superior antimicrobial properties against a variety of clinical isolates, especially Gram-negative bacteria. This aligns well with our findings suggesting that methanol serves as a better solvent for extracting active antimicrobial compounds from plant materials than water, likely due to its polarity and ability to dissolve a broader range of phytochemicals.

Phytochemical screening done in this study revealed the presence of bioactive constituents such as alkaloids, flavonoids, tannins, saponins, and cardiac glycosides, which matches those reported by [3][23]. Results of [16] also reported the presence of alkaloids, saponins, and phenols in *J. curcas*, correlating with notable antimicrobial activity against Gram-negative pathogens. These compounds are well documented for their ability to disrupt bacterial cell membranes, inhibit enzymes, and interfere with microbial metabolism.

Moreover, [21] corroborated that plant extracts containing flavonoids and phenols exhibit strong antimicrobial properties against Gram-negative bacteria, due to their ability to penetrate the outer lipopolysaccharide membrane, a major defense mechanism in these organisms, as explained by [28].

Contrastingly, the findings of [10] noted significant antibacterial effects of *J. curcas*, highlighting variability in sensitivity among isolates, which suggests that bacterial resistance patterns and environmental factors might influence extract potency, an aspect that this current study acknowledges by employing clinical isolates from a local hospital in Benue State.

RECOMMENDATIONS

To build on these findings and fully explore the potential of *J. curcas*, further studies are recommended to:

- Assess the safety of the extracts through toxicity testing.
- Identify the specific antimicrobial compounds using bioassay-guided fractionation.
- Use genetic tools to study resistance genes and their control.
- Investigate possible synergy between *J. curcas* extracts and standard antibiotics.

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