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Efficacy of *Jatropha curcas* Leaf Extract on Some Isolates Associated with Surgical Wounds

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study is to determine the antibacterial efficacy of *Jatropha curcas* leaf extract against some isolates associated with surgical wounds.
Study Design: This was a hospital-based study conducted in 2017 in the Department of medical microbiology unit of Bingham university teaching hospital and the national veterinary research institute (NVRI) both institutions are located in Jos Plateau State Nigeria.
Methodology: A total of twenty (20) isolates from clinical specimens of surgical wounds were used. The following bacteria were identified using biochemical analysis: *Staphylococcus aureus* (15), *Pseudomonas aeruginosa* (2), and *Klebsiella pneumonia*e (3). Using standard methods of ethanol and aqueous extraction techniques, the leaves of the *Jatropha curcas* were examined for phytochemical content. The minimum inhibitory concentration (MIC) and minimum bactericidal

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concentration (MBC) of the isolate were determined using the broth dilution method, while the antibacterial activity of the isolate was observed using the agar well diffusion techniques.
Results: The result of the phytochemical extraction shows the presence of the following Alkaloids (secondary metabolite) Saponins, Tannins, glycosides, Flavonoids, Oxalate, Terpenoids, Resins and steroids. The ethanol extract of *Jatropha curcas* exhibited antimicrobial activity against all the test bacteria with higher activity recorded with *Staphylococcus aureus* (15 mm) followed by *Klebsiella pneumonia* (12 mm) and *Pseudomonas aeruginosa* (10 mm). Aqueous extract of *Jatropha curcas* leaves did not exhibit significant antimicrobial activity against all the test isolates when compared to Ethanol Extraction However Aqueous extract of *Jatropha curcas* had a slight antimicrobial activity against only *Staphylococcus aureus in the range of (*5 mm to 7 mm). The MIC value for the ethanol extract of *Jatropha curcas* ranged from 100mg/ml to 200mg/ml while the MIC value for the aqueous extract was 200mg/ml.
Conclusion: The result of the present study shows that the ethanol and aqueous extracts of *Jatropha Curcas* leaves possess some antibacterial activities. The ethanol extract had higher antimicrobial activity than the aqueous extract on surgical wounds. It could therefore be inferred that these leaves contain bioactive constituents which can effectively inhibit the growth of some microorganisms. The plant could be used as an alternative therapy in the treatment of surgical wounds.

*Keywords: Jatropha curcas; surgical wounds; ethanol extract; aqueous extract; phytochemicals; Minimum Inhibitory Concentration (*MIC*); Minimum Bactericidal Concentration (MBC).*

1. INTRODUCTION

Surgical wounds are caused by incisions. Incisions are cuts made during surgery. If these cuts are not treated appropriately, they may turn into wounds that become infected after surgery. Each year, surgical wounds endanger millions of lives and contribute to antibiotic resistance [1]. Most wound infections manifest within the first 30 days following surgery and may be accompanied by pus.The majority of surgical site infections are caused by bacterial colonization that originates from the normal skin flora, bacteria from the environment, contaminated surgical instruments, or from the contaminated hands of healthcare providers. Individuals at risk of a surgical wound infection include the immunocompromised persons, those on intravenous catheters, poorly controlled diabetes, obese or an individual that has surgery that lasts longer than two hours [2]. Microorganisms associated with surgical wound infections include yeast C*andida* specie, S*taphylococcus aureus* which is the most frequent organism isolated: other organisms include P*seudomonas aeruginosa, Bacillus* species and *Escherichia coli* [3].

Staphylococcus aureus is an emerging major pathogen causing surgical wound infections. *Staphylococcus aureus* is a gram-positive, round-shaped facultative anaerobic bacterium that is frequently found in the respiratory tract and on the surface of the skin. They are the common causes of skin infections, abscesses, sinusitis and food poisoning. Other diseases associated with *Staphylococcus aureus* are bacteraemia, sepsis and osteomyelitis. In a healthcare setting, there is the risk of more serious *Staphylococcus aureus* infection because most patients often have weakened immune systems [4].

Pseudomonas aeruginosa is a gram-negative, aerobic, spore-forming rod-shaped bacterium capable of causing a variety of infections in both immune-competent and immune-compromised hosts. They are found in the soil, water, skin and hospital environment. It is a multidrug-resistant pathogen which is associated with various diseases in humans, animals and plants. The versatility of the organism enables it to colonize, infect and damage tissues of those with reduced immunity [5].

Klebsiella pneumoniae is a gram-negative rod shaped facultative anaerobic bacteria found in the water, soil; plants, insects and other animals including humans [6] The primary reservoirs for the transmission of *Klebsiella species* are the gastrointestinal tract of humans and the hands of hospital personnel. They are mostly associated with nosocomial infections because of their ability to spread rapidly in the hospital environment [7]. *Klebsiella* bacteria have developed resistance recently to the class of antibiotics known as Carbapenem. They cause different kinds of illnesses such as pneumonia, soft tissue infections, blood stream infections, meningitis, and urinary tract infections [6].

Plants are rich sources of natural products most of which have been extensively used for human welfare and in the treatment of various diseases. Medicinal plants can be defined as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products [8] *Jatropha curcas* is a multi-purpose drought-resistant perennial plant belonging to the Euphorbiaceae family which is gaining a lot of economic importance because of its several potentials in industrial application and medical values. The Common names in English are physic nut, Barbados nut, poison nut, bubble bush or purging nut. [9]. *Jatropha curcas* is a source of several secondary metabolites of medical importance. The leaves, fruits, latex and bark contain cyanogenic glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins that exhibit a wide range of antibacterial and antifungal activities [10].

2. METHODOLOGY

2.1 Study Design

This was a hospital-based study conducted in the Department of Medical Microbiology unit of Bingham University Teaching Hospital and National Veterinary Research Institute (NVRI) Vom; both institutions are located in Jos Plateau state. Jos is a city in the middle belt of Nigeria, with a population of about 900,000 residents based on the 2006 census. BUTH is a private tertiary institution while NVIR is a federal research institution. The study design was to investigate the antibacterial efficacy of *Jatropha curcas* leave extract against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* associated with surgical wounds.

2.2 Collection and Identification of *Jatropha curcas*

The leaves of the *Jatropha curcas* plant were collected at Farin Gada, Jos North LGA of Plateau State in November 2017. The leaves were authenticated at the herbarium of the Federal College of Forestry Jos with the voucher number FHJ341.

2.3 Preparation and Ethanol Extraction of *Jatropha curcas*

The plant sample was air-dried, crushed and blended into smaller pieces to enhance the penetration of the extracting solvents into the plant cells, thus facilitating the release of the active ingredients. One hundred grams (100g) of powdered *Jatropha curcas* leaves were weighed using a weighing balance into a 1000ml capacity conical flask. 700 millilitre of ethanol and 200 millilitre of water were added to the samples. The conical flask containing the mixtures was then placed on a shaker for 24 hours. After 24 hours of shacking and mixing, it was then filtered using a muslin cloth. The filtrate was then filtered again using suction pressure with the aid of a vacuum pump. The filtered extract was concentrated using the rotary evaporator equipment after which they were dried on an evaporating dish at a temperature of 50°C to 60°C to a semi-solid form. A sticky semi-solid greenish substance was obtained from the sample. The extract was stored in a well-corked universal bottle [11].

2.3.1 Solvents used for ethanolic extraction

The two solvents used for *Jatropha curcas* leaves extraction were

(i) Water
(ii) Alcohol

2.4 Aqueous Extraction

The dried powdered leaves of *Jatropha curcas were* prepared by addition of 150g of the powdered plant material in 1500 ml of distilled water in a sterile flask. The solution was kept at room temperature for 3 days and swirled to ensure effective mixing. The preparation was subsequently filtered using a What-man filter paper and evaporated to dryness using a carbonate oven set at 45^{o} C. The dried extract was preserved at 4^{o}C till required for further use.

2.5 Phytochemical Analysis of the Extract

The extract was screened for the presence of alkaloids, saponins, flavonoids, tannins, glycosides, resins, terpenes and steroids.

Picture of *Jatropha curcas* plant

2.5.1 Test for alkaloids

Exactly 0.5g of the extract was weighed and placed in two tubes to which 3 ml of 1% HCL was added and stirred over a steam bath. The preparation was filtered and the following tests were carried out on the acidified filtrate. Filtrates were treated with Mayer's regent (Potassium Mercuric iodide). the Formation of a brown creamy precipitate indicated the presence of alkaloids.

2.5.2 Test for saponins (Froth Test)

The 0.5g of the extract was mixed vigorously with 5 ml of distilled water and shaken vigorously. The presence of frothing which persisted on warming indicated the presence of Saponins.

2.5.3 Test or flavonoids

0.5g of the plant extract was de-tanned with acetone in a water bath. The mixture was filtered while hot and allowed to cool. To this 5ml of 20% sodium hydroxide (NaOH) was added. The appearance of a yellow solution indicated the presence of flavonoids.

2.5.4 Test for tannins

0.5g of the plant extract was dissolved in 1 ml of distilled water, shaken and then filtered. To the filtrate, ferric chloride ($FeCl_3$) reagent was added. A blue-black colouration indicated the presence of tannins.

2.5.5 Test for cardiac glycosides

The plant extract (0.1g) was dissolved in 1 ml of glacial acetic acid containing one drop of $FeCl_3$. The solution was then interlayered with 1 ml of sulphuric acid. A brown ring at the interface indicated the presence of cardiac glycosides.

2.5.6 Test for resins

To 0.5g of the plant extract, 5 ml of boiling ethanol was added. The mixture was filtered and mixed with 4ml o 1% aqueous HCl. The formation of a resinous precipitate indicated the presence of resin.

2.5.7 Test for steroids and terpenes

The plant extract (0.1g) was dissolved in 1 ml of chloroform. To this, 1ml of acetic anhydride and 2 drops of conc. H_2SO_4. A pink colour which turns bluish green indicated the presence of steroids and terpenes.

2.6 Preparation of Culture Medium and Inoculation

The culture media used for the laboratory analysis were nutrient agar, chocolate agar, blood agar, Macconkey agar and Mueller Hinton agar. All were prepared following the manufacturer's instructions. The wound swabs collected from patients with surgical wounds at Bingham University Teaching Hospital were cultured onto a Nutrient agar, Blood agar, Chocolate agar and Macconkey agar plate. The inoculated plates were then incubated for 24-48 hours at 37°C. The suspected colonies were then subcultured onto a nutrient agar slope to get pure culture.

2.7 Identification of Organisms

The following biochemical test was used to identify the bacteria: gram staining reaction, catalase test, coagulase test, oxidase test, citrate test, methyl red test and Indole test.

2.8 Preparation of Plants Extracts Concentrations

A sample of 4g of the aqueous extract of *Jatropha curcas* leaves was dissolved in 10ml sterile distilled water obtaining a stock solution of 400mg/ml, which was used to make two-fold double dilutions to give six extract concentrations (400, 200, 100, 50, 25 and 12.5mg/ml). The same procedure was repeated for the Ethanol extract using sterile dimethyl sulfoxide (DMSO) as the solvent. These were used as the extracts for the antimicrobial sensitivity test.

2.9 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility test was done using the agar well diffusion techniques. *Jatropha curcas* leave extracts were tested on the test isolates.Mueller Hinton Agar was evenly inoculated with the test isolates over the entire surface of the agar plate, holes were bored using a sterile cork borer, and the different concentrations of the extract were aseptically placed into the wells and allowed to diffuse. The inocculated plates were incubated overnight the bacterial growth around each well was observed and the zone of inhibition was measured in (mm) 500mg of Ciprofloxacin antibiotics was used as a positive control.

2.10 Assessment of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using a standard two-fold dilution broth methodology. A stock solution of each active extract for both ethanol and aqueous extraction *Jatropha curcas l*eaves were serially diluted in six test tubes with Mueller Hinton broth to obtain a concentration of 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5mg/ml in concentration. A standardized inoculum for each bacterial strain was prepared to give an inoculum size of approximately 5 x 105 CFU/ml in each tube. The tubes were then kept at 37°C for overnight incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain. Positive and negative cultures were also prepared. The lowest concentration of the extract that did not show any visible bacterial growth was recorded as the MIC of the extract for that microbial species. The level of growth of the test organism was compared by observing the turbidity in each test tube using the control medium as a guide.

2.11 Minimum Bactericidal Concentration (MBC)

The tubes with no growth after 24 hours were subcultured onto a freshly prepared Mueller Hinton agar and incubated for 24 hours. The tubes were observed for growth after 24 hours, the lowest concentration from which the microorganism did not grow was recorded as the minimum bactericidal concentration (MBC).The level of growth of the test organism was observed using the negative and positive control medium as a guide.

3. RESULTS

Table 1. Phytochemical constituents of the aqueous and ethanol extract of *Jatropha curcas*

Secondary Metabolites	Ae	Ee
Saponins	+	++
Tannins	+	+++
Alkaloids	+	++
Cyanogenic glycosides	+	++
Flavonoids	+++	+
Oxalate	-	+++
Terpenoids	+	+
Resins	+	+
Steroid	-	+

Key: Ae = Aqueous extract, Ee = Ethanol extract, (+) = Present in small amount, (++) = Present in moderate amount, (+++) = Present In high amount, (-) = absent

This shows the presence of secondary metabolites present in the *Jatropha Curcas* leaves. They include tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides, Terpenoids, Rennins and Steroids. The results were interpreted according to the intensity of the colour observed and they were qualified with the present in high amount (+++), Moderate amount (++), present in low amount (+) and absent (-). Most of the (+++) was seen in ethanol extracts while aqueous extracts had more of the (+).

Table 2. Antimicrobial activity of aqueous extract of *Jatropha curcas* on test microorganisms

Test organism	Diameter of Zone of inhibition (mm)							
	Concentration (mg/ml)						Control (mg/ml)	
	400	200	100	50	25	12.5	+ve	-ve
Staphylococcus aureus	7	5	-	-	-	-	24	-
Pseudomonas aeruginosa	-	-	-	-	-	-	18	-
Klebsiella pneumoniae	-	-	-	-	-	-	20	-

Key: Reference drug = Ciprofloxacin, positive control drug for bacteria.

It was observed that only *staphylococcus aureus* was inhibited at the concentration of 400mg/ml with zone inhibition of 7mm.

Table 3. Antimicrobial activity of Ethanol extract of *Jatropha curcas* on test microorganisms

Test organisms	Diameter of Zone of inhibition (mg/ml)							
	Concentration (mg/ml)						Control (mg/ml)	
	400	200	100	50	25	12.5	+ve	-ve
Staphylococcus aureus	15	10	7	-	-	-	24	-
Pseudomonas aeruginosa	10	6	-	-	-	-	18	-
Klebsiella pneumoniae	12	8	6	-	-	-	20	-

Key: Reference drug = Ciprofloxacin, positive control for bacteria

Jatropha curcas recorded 15mm as the highest zone of inhibition at the concentration of 400mg/ml against *staphylococcus aureus* and 6mm as the lowest zone of inhibition at the concentration of 100mg/ml against *Klebsiella.*

Table 4. Minimum Inhibitory Concentration (MIC) of aqueous extract of *Jatropha curcas* against the test organisms

Test organisms	Concentration (mg/ml)						MIC
	400	200	100	50	25	12.5	
Staphylococcus aureus	+	+	-	-	-	-	200
Pseudomonas aeruginosa	-	-	-	-	-	-	>400
Klebsiella pneumoniae	-	-	-	-	-	-	>400

Key: (+) = inhibition (-) = no inhibition

The MIC of the aqueous extract was only observed against *Staphylococcus aureus* at 200mg/ml of concentration.

Table 5. Minimum Inhibitory Concentration (MIC) of ethanol extract of *Jatropha curcas* against the test organisms

Test organism	Concentration (mg/ml)						MIC
	400	200	100	50	25	12.5	
Staphylococcus aureus	+	+	+	-	-	-	100
Pseudomonas aeruginosa	+	+	-	-	-	-	200
Klebsiella pneumoniae	+	+	+	-	-	-	100

Key: (+) = inhibition (-) = no inhibition

The MIC of the ethanol extract of *Jatropha curcas* was recorded against *Staphylococcus aureus* and *Klebsiella* species at 100mg/ml of concentration.

Table 6. Minimum Bactericidal Concentration (MBC) of aqueous extract of *Jatropha curcas* against the test organisms

Test organisms	Concentration (mg/ml)						MBC
	400	200	100	50	25	12.5	
Staphylococcus aureus	-	-	-	-	-	-	>400
Pseudomonas aeruginosa	-	-	-	-	-	-	>400
Klebsiella pneumoniae	-	-	-	-	-	-	>400

Key: (+) = MBC (-) = No MBC

The MBC of the aqueous extract of *Jatropha curcas* did not show any bactericidal effect against the test isolates.

Table 7. Minimum Bactericidal Concentration of Ethanol Extract of *Jatropha curcas* against the test organisms

Test organisms	Concentration (mg/ml)						MBC
	400	200	100	50	25	12.5	
Staphylococcus aureus	+	+	-	-	-	-	200
Pseudomonas aeruginosa	+	-	-	-	-	-	400
Klebsiella pneumoniae	+	+	-	-	-	-	200

Key: (+) = MBC (-) = No MBC

The MBC of the ethanol extract of *Jatropha curcas* showed some level of bactericidal effect on the test isolates, The MBC had its highest concentration against *Pseudomonas aeruginosa* at 400mg/ml and the lowest concentration was against *Staphylococcus aureus* and *Klebsiella pneumoniae* at 200mg/ml respectively.

4. DISCUSSION

The phytochemical analysis of *Jatropha curcas* leaves according to this study shows the presence of secondary metabolites which include tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides [12]. These phytochemical components are biologically active constituents and are responsible for the antimicrobial activity of the plant [13]. This findings is in agreement with Studies conducted in Oshogbo, Benin Abuja and Illorin [14,15-18,19]. The ethanol extraction of *Jatropha curcas* leaves according to this study yielded secondary metabolites with increased anti-bacterial activities. Tannins and oxalate were in higher concentration in the ethanol extraction. This report finding is in agreement with the study done at Illorin in 2011 [20]. Other studies done with in Nigeria that supports ethanol extraction to be more effective include the reports at Illorin and Benin [21,22] Similarly investigations conducted outside Nigeria in India in 2011 & 2013, United States in 2011, Poland in 2013 and Croatia in 2004 all found ethanol extraction of plants to be more effective as a therapeutic agent [23-28].

The aqueous extract of *Jatropha curcas* leaves did not show inhibition against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* however a slight inhibition was observed against

Staphylococcus aureus, this is in agreement with the study at Illorin in 2011 [21] It reported that aqueous extract of *Jatropha Curcas* latex had no inhibition against *Pseudomonas aeruginosa* but had antimicrobial activity against *Staphylococcus aureus.* The study finding is in contrast to the report in Oshogbo in 2012 that reported that the aqueous extract of *Jatropha curcas* leaf had antimicrobial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [15]. The disparities in the different reports may be attributable to differences in extract preparation and concentrations, as well as strain differences in the isolates. Microbial antibiotic sensitivity pattern have been reported to be strain-dependent with in a given species [29]

The aqueous extracts of *Jatropha curcas l*eaves in this study showed weak antimicrobial activities and this may be attributed to the extraction solvents used. Water is a polar compound, so non-polar compounds may not have been extracted. The finding above is in contrast with a study at Slovenia in 2005 [30] which reported that water is a better extraction solvent as compared to ethanol.

The Minimum Inhibitory Concentration (MIC) of the aqueous extract of *Jatropha curcas* leaves in this study was observed against *Staphylococcus aureus* at 200mg/ml of concentration while the MIC of ethanol extract was in the range of 100mg/ml against *Staphylococcus aureus* and *Klebsiella pneumoniae* and 200mg/ml against *Pseudomonas aeruginosa*. The lower the MIC of a plant extract against pathogens, the more desirable. Similarly the Minimum Bactericidal Concentration (MBC) of the aqueous extract of the *Jatropha curcas* leaves did not show any bactericidal effect against the test isolates whereas the Minimum Bactericidal Concentration of ethanol extract of the *Jatropha curcas* was observed at 200mg/ml against *Staphylococcus aureus* and *Klebsiella pneumoniae* and 400mg/ml against *Pseudomonas aeruginosa*. The antimicrobial activity shown by the ethanol extracts could be as a result of the extraction solvent (ethanol). Ethanol is a polar solvent, it is possible that compounds in the leaves were less polar and this led to the significant activity that the ethanol extract of *Jatropha curcas* demonstrated. The ethanol extracts exhibited antimicrobial activity against all the bacteria isolates from surgical wounds in this study at varying concentrations. In general, the antimicrobial inhibition increased with an increase in the concentration of the extracts while it decreased with a decrease in the concentration of the extract.

5. CONCLUSION

The present study was intended to explore the antimicrobial efficacy of *Jatropha curcas* leaves extract on some isolates associated with surgical wounds. The bacteria isolated in this study were *Staphylococcus aureus*, *klebsiella pneumonia* and *Pseudomonas aeruginosa.* It was observed that the ethanol extraction of *Jatropha curcas* leaves possess more antibacterial activities on the test isolates compared to the aqueous extraction. The ethanol extraction of *Jatropha curcas* leaves exhibited antimicrobial activity against all the test bacteria. The MIC value for the ethanol extract of *Jatropha curcas* was in the range of 100mg/ml against *Staphylococcus aureus*, *Klebsiella pneumoniae* and 200mg/ml for *pseudomonas aeruginosa*. *Jatropha curcas* could be a promising source of drugs for the treatment of wounds though caution needs to be taken in the use of these leaves due to their toxicity at certain dosages because of the presence of oxalate which is high in ethanol extraction.

ETHICAL APPROVAL

Approvals were obtained from the ethical research committee of Bingham university teaching hospital Jos.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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