ANTIBACTERIAL EFFICACY OF *JATROPHA CURCAS* LEAF EXTRACT ON SOME BACTERIAL SPECIES ASSOCIATED WITH SURGICAL WOUNDS.

Comment [h1]:

Comment [h2]: Replace bacterial species with isolates

ABSTRACT

AIM: This study is aimed at determining the antibacterial potentials of *Jatropha curcas* leave extract against some bacterial species associated with surgical wounds.

Comment [h3]: 'Potentials' is an incorrect word

Study Design: This was a hospital-based study conducted 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: Twenty (20) isolates obtained from clinical specimen of surgical wounds, were used: Biochemical analysis identified the following bacteria to be present *Staphylococcus aureus* (15) *Pseudomonas aeruginosa* (2) and *Klebsiella pneumonia* (3). The phytochemical analysis of the *Jatropha Curcas (J. Curcas)* leaves was carried out using standard methods of ethanol and aqueous methods of extraction. The agar well diffusion techniques was used to determine the antimicrobial activity of the isolate *in-vitro* while the broth dilution method was used to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Result: The result of the phytochemical extraction shows the presence of the following Alkaloids (secondary metabolite) Saponins, Tannins, glycosides, Flavonoids, Oxalate, Terpenoids, Resins and Steroid. 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 *Klebsiellapneumonia* (12 mm) and *pseudomonas aeruginosa* (10 mm) all in diameter. Aqueous extract of*J.Curcas* leaves did not exhibit significant antimicrobial activity against all the test isolates when compared to Ethanol extraction However Aqueous extract of *J.Curcas* had a slight antimicrobial activity against only *Staphylococcus Aureus in the range of (*5 mm to 7 mm) in diameter. 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 possesses 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 treatment of surgical wounds.

Keywords: *Jatropha Curcas*, surgical wounds, ethanol extract, aqueous extract, phytochemicals, minimum inhibitory concentration and Minimum Bactericidal Concentration (MBC).

1. INTRODUCTION

Surgical wounds are caused by incisions. Incisions are cuts made during surgery. These cuts if not properly taken care can develop into wounds leading to an infection after surgery. Surgical wounds pose a threat to millions of lives each year and contribute to the spread of antibiotic resistance [1]. Most wound infections show up within the first 30 days after surgery which may be accompanied by pus. The pus can be painful and irritating. Majority of the surgical site infections are caused by bacteria colonization originating either from a normal flora on the skin, bacteria from external

environment, contaminated surgical tools and contaminated hands of health care providers. Individual at risk of a surgical wound infection include, the immune- compromised, 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* speciesand*Escherichia Coli* [3]

Comment [h4]: In 'coli' c should be in small case

Staphylococcus aureus is an emerging major pathogen causing surgical wound infections. S. 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 S. 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 system [4]

Pseudomonas aeruginosa is a gram negative, aerobic, spore forming rod shaped bacterium capable of causing variety of infections in both immune-competent and immune-compromised host. 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 species is a gram-negative, rod shaped facultative anaerobic bacteria found in water, soil, plants, insects and other animals including human [6].The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment, they cause nosocomial outbreaks [7]. *Klebsiella* bacteria have developed antimicrobial resistance most recently to the class of antibiotics known as Carbapenem.They cause different types of healthcare-associated infections.*Klebsiella pneumonia* normally lives inside human intestines, where it doesn't cause disease. But if the get into other areas of the body, it can lead to a range of illnesses, including pneumonia, bloodstream infections, meningitis, and urinary tract infections [6].

Plants are a rich source of natural products most of which have been extensively used for human welfare and 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]. The Common names in English include physic nut, Barbados nut, poison nut, bubble bush or purging nut. *Jatropha Curcas* is a multipurpose 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 [9]. J*atropha 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 medical properties. The plant products exhibit antibacterial and antifungal activities. *Jatropha Curcas* plant is designated as an energy plant and the use of its oil as biodiesel is a promising and commercially viable alternative to diesel oil [10].

Comment [h5]: 'Curcas c should be in small case'

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 *JathrophaCurcas* leave extract against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella* species 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, Plateau State, Nigeria.

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 was 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 decoction of dried powdered leaves of *Jatropha curcas*was prepared with distilled water by soaking 150g of the dried plant material in 1500 ml of distilled water (boiled to 100^{o}) for 24 hours. 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.

2.5.1 Test for Alkaloids

Exactly 0.5g of the extract was weighed and placed in two tubes to which 3ml 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). Formation of a yellow colored precipitate indicated the presence of alkaloids.

2.5.2 Test for Saponins (Froth Test)

The 0.5g of the extract was mixed vigorously with 5ml 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 on a water bath. The mixture was filtered while hot and allowed to cool. To this 5ml of 20%Sodium hydroxide (NaOH) was added. Appearance of a yellow solution indicated the presence of flavonoid.

2.5.4 Test for Tannins

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

2.5.5 Test for cardiac Glycosides

The plant extract (0.1g) was dissolved in 1ml of glacial acetic acid containing one drop of $FeCl_3$. The solution was then interlayered with 1ml 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, 5ml 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 1ml of chloroform. To this, 1ml of acetic anhydride and 2 drops of conc. H_2SO_4. A pink color 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 instructions.The wound swabs collected from patients with surgical wounds at Bingham University Teaching Hospital was 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 sub-cultured 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 sulphur oxide (DMSO) as the solvent. These were used as the extracts for the antimicrobial test.

2.9 Antimicrobial susceptibility testing

The antimicrobial activities of Jatropha Curcas leaves extracts were tested on the test isolates using the agar-well diffusion method. A growth medium normally (Mueller Hinton Agar) were first evenly seeded with the test isolates of interest over the entire surface of the agar plate, holes were bored using a sterile borer, each of which different concentrations of the extract were then aseptically placed into the wells bored and allowed to diffuse, after an overnight incubation, the bacterial growth around

each well was observed and the zone of inhibition was measured in (mm) and compared to a standard interpretation chart used to categorize the susceptibility of the isolate using ciprofloxacin antibiotics as a positive control.

Comment [h6]: Concentration of ciprofloxacin not mentioned.

2.10 Assessment of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using standard two-fold dilution broth methodology. A stock solution of each active extract for both ethanol and aqueous extraction of j. curcas leaves were serially diluted in six test tubes with Mueller Hinton broth to obtain a concentration of 400mg/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 so as to give an inoculum size of approximately 5 x 105CFU/ml in each tube. The tubes were then kept at 37°C for an 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 Determination of Minimum Bactericidal Concentration (MBC)

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

Comment [h7]: No statistical analysis done.

3. RESULT

Table.1: Phytochemical constituents of the Aqueous and Ethanol Extract of *Jatropha curcas.*

phytochemical analysis of *Jatropha Curcas* leaves highlighted the presence of tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides, Terpenoids, Rennin's and Steroids. The results were interpreted according to the intensity of the colour observed and they were qualified with the present in high amount (+++), Present in Moderately amount (++), present in low amount (+) and Absent (-). Most of the (+++) was seen in ethanol extracts while aqueous extracts had more of the (+) for example, Alkaloids, tannins and Oxalate were more in ethanol extracts than in aqueous extracts.

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.

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

It was observed that only *staphylococcus aureus* was inhibited with 7mm as the highest zone of inhibition at the concentration of 400mg/ml and 5mm as the lowest zone of inhibition at concentrations of 200mg/ml.

Test organism	**Diameter of Zone of inhibition (mm)**							
	Concentration (mg/ml)						Control (mg/ml)	
	400	200	100	50	25	12.5	+ve	-ve
S. aureus	7	5	-	-	-	-	24	-
P. aeruginosa	-	-	-	-	-	18	-	
Klebsiella species	-	-	-	-	-	20	-	

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

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

Jatropha Curcas had 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*

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

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

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

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

Test organisms	**Concentration (mg/ml)**						**MIC**
	400	**200**	**100**	**50**	**25**	**12.5**	
S. aureus	+	+	-	-	-	-	200

P. aeruginosa	-	-	-	-	-	-	>400	
Klebsiella species	-	-	-	-	-	-		>400

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

Table 5: Minimum Inhibitory Concentration (MIC) of Ethanol Extract of *Jatropha curcas*against the test organisms

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

Test organism	**Concentration (mg/ml)**						**MIC**
	400	**200**	**100**	**50**	**25**	**12.5**	
S. aureus	+	+	+	-	-	-	100
P. aeruginosa	+	+	-	-	-	-	200
Klebsiella species	+	+	+	-	-	-	100

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

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

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

Test organisms	**Concentration (mg/ml)**						**MBC**
	400	**200**	**100**	**50**	**25**	**12.5**	
S. aureus	-	-	-	-	-	-	>400
P. aeruginosa	-	-	-	-	-	-	>400
Klebsiella species	-	-	-	-	-	-	>400

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

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

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 Pneumonia* at 200mg/ml respectively.

Test organisms	Concentration (mg/ml)						MBC	
	400	200	100	50	25	12.5		
S. aureus	+	+	-	-	-	-		200
P. aeruginosa	+	-	-	-	-	-	400	
Klebsiella spp	+	+	-	-	-	-		200

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

4. DISCUSSION

The phytochemical analysis of *Jatropha Curcas* leaves according to this study shows the presence of secondary metabolites such as tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides. These phytochemicals components are biologically active constituents and might be responsible for the antimicrobial activity of the plant [12]. Alkaloids have been acclaimed for their antimicrobial activities, especially against gram-negative bacteria [13]. In this study, it was observed that alkaloids, tannins and oxalate were in high concentration in the ethanol extraction than in the aqueous extraction. This result is in accordance with the study done in Illorin in 2011 [14] that reported high levels of alkaloids and tannins in the ethanol extraction than in aqueous extraction.Similarly there are some other studies done in Croatia 2004, Nigeria 2010, United States 2011, India 2011 & 2013 and Poland 2013 which recorded ethanolic extraction of plant to be more effective as a therapeutic agent. [15, 16, 17, 18, 19, 20]. Other studies which supported the antimicrobial activity of *Jatropha curcas* plant include studies done in Benin, Illorin, Oshogbo, Abuja and India [21, 14, 24, 26, 22, 25]. The antimicrobial activity of *Jatropha curcas*according to this study has been attributed to the presence of certain phytochemicals such as saponins, tannins, alkaloids and glycosides. This is in agreement with the reports in Illorin and Benin in Nigeria [14, 21]. A study done in Malaysia also attributed the antimicrobial activity of J*atropha curcas*to the presence of alkaloids, flavonoids, saponins and tannins [27]

In this study, the aqueous extract of *Jatropha Curcas* did not show significant inhibition to the tested isolates. There was no inhibition against *Pseudomonas aeruginosa* and *Klebsiella* species however a slight inhibition was observed against *Staphylococcus aureus*, this is in agreement with the study in Illorin in 2011 [14] that reported the aqueous extract of *Jatropha Curcas* latex showing no inhibition against *pseudomonas aeruginosa* but had antimicrobial activity against *Staphylococcus Aureus* although. 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* species[24].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 patterns have been reported to be strain-dependent within a given species [28].The aqueous extracts of *Jatropha Curcas*showed weak antimicrobial activities and this may be attributed to the extraction solvents used, water is more polar, so non-polar compounds may not have been extracted as can be seen from the yield of the aqueous extract leading to bio-active compounds not been extracted by water. This finding above is in support with a report in India in 2010 [30] that reported that water-soluble flavonoids have less antimicrobial significance: But in contrary to the result reported in Slovenia in 2005 [29] which reported that water is a better extraction solvent as compared to ethanol. However, the ethanol extract of *Jatropha Curcas* leaf in this study had significant inhibition against all the test isolates compared to that of the aqueous extract. And this is in agreement with the

report in Illorin in 2011[14] that reported that the ethanol extract of *Jatropha Curcas* latex had antimicrobial activity against *staphylococcus aureus* and *pseudomonas aeruginosa* the similarities could be as a result of the presence of more active ingredients in the ethanol extraction than in the aqueous extract. The reason for this slight discrepancy in the zone of inhibitions may be attributable to a possible difference in the characteristics of bacterial strains in surgical wounds, differences in plant part and differences in extract concentrations used. The same reasons may explain the lower MICs values for stem bark extracts of *Jatropha curcas*reported in Benin in 2009 [21]. The lower the MIC of a plant extract against pathogens, the more desirable it is.The ethanol extracts exhibited antimicrobial activity against all the isolates from surgical wounds at varying concentrations though at high concentrations. In general, the zone of inhibition increased with an increase in the concentration of the extracts while it decreased with a decrease in the concentration of the extract. The strong activities shown by the ethanol extracts could be a result of the extraction solvent (ethanol) or that the compounds in the leaves were less polar since ethanol is polar. The higher activity of the ethanol extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. [31].

5. CONCLUSION

The present study was intended to explore the antimicrobial efficacy of *Jatropha curcas* leave extract on some bacterial species associated with surgical wounds. The bacteria's isolated in this study include staphylococcus aureus, *klebsiella pneumoniae* and *pseudomonas aeruginosa.* It was observed that the ethanolic extraction of *Jatropha curcas* leaves were found to possess more antibacterial activities compared to the aqueous extraction on the test isolates. The ethanolic extraction of *Jatropha curcas* leaves exhibited antimicrobial activity against all the test bacteria though at high concentrations. The MIC value for the ethanol extract of *Jatropha Curcas*was in the range of 100mg/ml against *staphylococcus aureus* and *klebsiella pneumoniae* respectively and 200mg/ml for *pseudomonas aeruginosa*. *Jatropha curcas* could be a promising source of drugs for treatment of wounds though caution need to be taken in the use of these leaves due to its toxicity at certain dosage because of the presence of oxalate which is high in the ethanol extraction.

CONSENT

Not applicable

ETHICAL APPROVAL

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

REFERENCE

Comment [h8]: Most of the References older than 10 years are cited

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Comment [h9]: Cited Literature is not updated

Fig. 1. Picture of *jatropha curcas* plant