EFFICACY OF *JATROPHA CURCAS* LEAF EXTRACT ON SOME ISOLATES ASSOCIATED WITH SURGICAL WOUNDS

ABSTRACT

AIM: This study aims at determining 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 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.

Result: 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) all in diameter. 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) 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 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 and 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 pus could be painful and irritating. The majority of surgical site infections are brought on by bacterial colonization that originates from either normal skin flora, bacteria from the environment, contaminated surgical instruments, or contaminated hands of healthcare professionals. Individuals 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* 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 water, soil, plants, insects and other animals including humans" [6]. "The principal pathogenic reservoirs for the 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 pneumoniae* normally lives inside human intestines, where it doesn't cause disease. But if they 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]. "*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 medical properties. The plant products exhibit 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 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 dried powdered leaves of *Jatropha curcas were* prepared with distilled water by soaking 150g of the dried 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.

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 test.

2.9 Antimicrobial susceptibility testing

The antimicrobial activities of *Jatropha curcas* leave extracts were tested on the test isolates using the agar-well diffusion method. A growth medium normally (Mueller Hinton Agar) was 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 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 500mg of Ciprofloxacin antibiotics 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 Determination of Minimum Bactericidal Concentration (MBC)

The tubes with no growth after 24 hours were subcultured 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 level of growth of the test organism was observed using the negative and positive control medium as a guide.

3. RESULT

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

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 (+) 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 at the concentration of 400mg/ml

With zone inhibition of 7mm

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.

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
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.

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**	

Staphylococcus aureus	+	+	-	-	-	-	200
Pseudomonas aeruginosa	-	-	-	-	-	-	>400
Klebsiella pneumoniae	-	-	-	-	-	-	>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**	
Staphylococcus aureus	+	+	+	-	-	-	100
Pseudomonas aeruginosa	+	+	-	-	-	-	200
Klebsiella pneumoniae	+	+	+	-	-	-	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**	
Staphylococcus aureus	-	-	-	-	-	-	>400
Pseudomonas aeruginosa	-	-	-	-	-	-	>400
Klebsiella pneumoniae	-	-	-	-	-	-	>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 pneumoniae* at 200mg/ml respectively.

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

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. These phytochemical components are biologically active constituents and are responsible for the antimicrobial activity of the plant [12].This findings is in agreement with Studies conducted in Oshogbo, Benin Abuja and Illorin [22, 24, 25, 26]. Alkaloids have been reported to have inhibition activity especially against gram-negative bacteria due to their antimicrobial properties [13]. The ethanol extraction of *Jatropha curcas* leaves according to this study yielded secondary metabolites with increased activities and tannins, oxalate were in higher concentration than in the aqueous extraction. This report finding is in agreement with the study done at Illorin in 2011 [14]. Other studies done with in Nigeria that supports ethanol extraction to be more effective include the reports at Illorin and Benin [14, 21]. 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. [15, 16, 17, 18, 19, 20]

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 [14] 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* [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* leaves 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 [29] 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 ethanolic extraction of *Jatropha curcas* leaves was 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 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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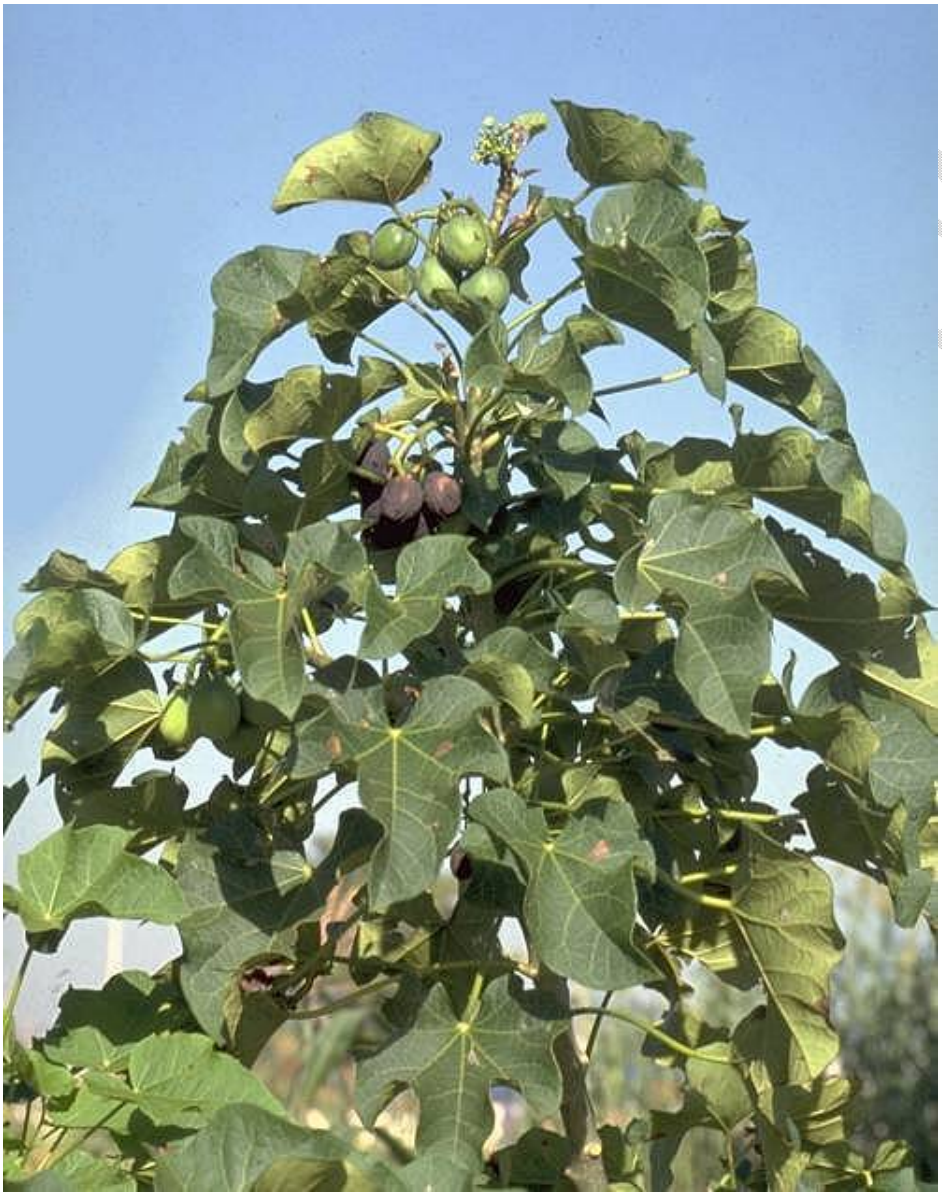

Fig. 1. Picture of Jatropha *curcas* plant