



Antibacterial activity of *Jatropha curcas* against Isolates of Clinical Origin

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Abstract: The antibacterial activities of ethanol, methanol and aqueous extracts of *Jatropha curcas* leaves were observed in vitro against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* was analyzed using agar well diffusion method. The pattern of zone of inhibition varied with different plant extract, the solvent used, and the organism tested. The antibacterial activities of the methanolic extract were significantly higher ($p < 0.05$) and the most susceptible organism was *Pseudomonas aeruginosa* at the lowest concentration. The Minimum Inhibitory Concentration (MIC) exhibited by *Pseudomonas aeruginosa* in the ethanolic extract and aqueous extract was 5mg/ml and 75mg/ml respectively. *Jatropha curcas* proved to be effective over the use of antibiotics by inhibiting the activity of *Pseudomonas aeruginosa* which was resistant when tested with standard antibiotics. The antibacterial activity of the extract could be enhanced if the components are purified. This plant therefore holds a promise as a potential source of new drug for treating infections caused by these clinical pathogens.

Keywords: Antibacterial, *Jatropha curcas*, clinical pathogens

Introduction

The antimicrobial characteristics of medicinal plants are due to compounds like alkaloids, flavonoids, phenolic compounds, tannins, resins, gum, fatty

acids, saponins and steroids. Many of the plant materials used in traditional medicine are generally proved more effective and relatively cheaper than modern medicine [1]. For ages,

thousands of species of medicinal plants used globally have contributed many ingredients to help fight diseases and illnesses. Over 80% of the world's population particularly in developing world relies on medicinal plants as sources of medicine for their primary health care [2]. Phytochemicals have been attracting much interest as natural alternatives to synthetic compounds because the antimicrobial properties are of great importance in curative treatments. *Jatropha curcas* is becoming a very useful economic resource both in agriculture, phytomedicine development and development of new lead compounds [3, 4].

In spite of various researches as regards the antimicrobial activity of plant extracts, little has been developed in comparison to modern medicine. The pharmacological activities of medicinal plants provide clues to synthesize less expensive antimicrobial chemicals that are relatively safe to man and limit the supply of synthetic chemicals. These plants are used as antimicrobial agents and several works have been carried out by scientist to find out a scientific basis [5] and some of these plants include *Jatropha curcas*, *Moringa oleifera*, *Senna occidentalis*. Antimicrobial agents are widely employed to cure bacterial diseases [6]. Current social trends in health care show a definite movement towards the use of natural remedies like medicinal plants and away from chemotherapeutic regimens [7].

Medicinal plants like *Jatropha curcas* have played a major role in the treatment of various diseases including bacterial and fungal infections. The extracts of many *Jatropha*

species displayed potent cytotoxic, anti-tumor and anti-microbial activities in different assays. The latex of *J. curcas* also showed anti-bacterial activity against *Staphylococcus aureus* [8], however the antimicrobial activity of the other parts has not been fully investigated. The aim of this study is therefore to investigate the effectiveness of *Jatropha curcas* against some selected microorganisms of clinical origin which are established to cause infections.

Materials and methods

Collection and Identification of plant Sample

Jatropha curcas leaves were collected from Farm, Sango, in Ilorin west Local Government area of Kwara State, Nigeria. It was identified properly and authenticated at the Botany unit of the Department of Plant Biology, University of Ilorin. The leaves were washed with distilled water and air dried. The dried leaves were grinded into powder and stored in a tightly covered container.

Collection of test organisms

Pure culture of *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were obtained from the Medical Laboratory of the Microbiology and Parasitology unit of the University of Ilorin Teaching Hospital and used as test organisms.

Preparation of plant extracts

The fresh leaf sample was sundried and ground into fine powder and kept in a plastic container until use

Extraction

Aqueous and ethanolic extraction of the plant material was prepared as described by [9]. The aqueous and ethanolic extracts of the plant material

was carried out by suspending 25g of the finely ground leaf in 125ml of 95% ethanol and 250mls of distilled water respectively. A preliminary test has shown that the extract shared greater activity at 80°C than at 28°C, so the aqueous extraction was done at 80°C in a water bath for 11/2 hours. The ethanolic extraction was done at 28+1°C for 120 hours by subjecting it to agitation on rotator shaker at 200 rpm. The resulting aqueous extract suspension were filtered with Whatman filter paper and evaporated to dryness at 45°C in an oven.

Antimicrobial susceptibility test of some standardized antibiotics

The antibiotics used for this study had been prepared into multiple discs kit containing different antibiotics. The plate diffusion technique was used for the antibiotic sensitivity test. Broth cultures of the organisms were swabbed on sterile Mueller Hinton agar plates. The multiple antibiotic discs were then placed on the agar surface after solidification and pressed using sterile forceps to ensure complete contact with agar. The plates were incubated at 37°C for 24hrs and after incubation the diameter of the inhibition zones were measured and recorded [7]. The antibiotics used and their corresponding concentrations are as follows: Gentamycin (10 µg), Tetracycline (30 µg), Erythromycin (5 µg), Ceftriaxone (30 µg), Cloxacillin (5 µg), Nitrofurantoin (300 µg), Cotrimoxazole (25 µg), Augmentin (30 µg), Ofloxacin (5 µg), Amoxicillin (25 µg), Nalidixic acid (30 µg), Cefuroxime (30 µg) and Ceftazidime (30 µg).

Phytochemical screening of extracts of *Jatropha curcas*

The phytochemical screening of the extracts was done on the aqueous extracts and the powdered specimens using standard procedure as described by [8] and [10]. The following qualitative tests were carried out as follows:

Test for tannins

About 0.5 gram of dried powdered samples was boiled in 20mL of water and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration.

Test for phlobatannins

The aqueous extract of each sample was boiled with 1% aqueous hydrochloric acid. Observation of deposits of a red precipitate was taken to indicate the presence of phlobatannins.

Test for saponins

Two grams of each powdered sample was boiled in a water bath and filtered. The boiled samples were one milliliter of filtrate was mixed vigorously to form a stable persistent froth. A formation of emulsion was observed after mixing the froth with about 3 drops of olive oil to indicate the presence of saponins.

Test for flavonoids

The method described by [10] was used to determine the presence of flavonoids. Five milliliters of diluted ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H₂SO₄. A yellow coloration which disappeared after some minutes indicates the presence of flavonoids.

Test for steroids

Two milliliters of acetic anhydride was added to 0.5g ethanol extract of each sample with 2mL of H₂SO₄. A colour change of violet to green is indicative of the presence of steroids.

Test for terpenoids

The Salkowski test was used to test for the presence of terpenoids. Five milliliter of each aqueous extract was mixed with 2mL of chloroform and 3mL of concentrate H₂SO₄. A reddish brown colouration of the interface was used to indicate the presence of terpenoids.

Test for cardiac glycosides

Five milliliter of each aqueous extract was treated with 2mL of glacial acetic acid which contains one drop of ferric chloride solution. One milliliter of H₂SO₄ was later added. The formation of a brown ring indicates the presence of cardenoids.

Quantitative Determinations

To determine the quantity of each of the constituents, 2g of each powdered sample was first defatted with 100mL of diethyl ether for 2h using a soxhlet apparatus.

Determination of Minimum Inhibitory Concentration (MIC) of the Extract:

The broth dilution method was used to determine MIC. Varying concentrations of the extracts were used which, ranged from 5mg/ml – 200mg/ml each concentration contain 0.1ml was added to each 9ml of nutrient broth containing 0.1ml of standardized test organism of bacterial cells. The tubes were incubated aerobically for 24hours at 37°C. Controls were equally set up by using solvent and test organisms without the extract.

Effect of varying concentrations

Varying concentrations of the extracts were prepared in sterile test tubes. Six different concentrations were prepared i.e. 150mg/ml, 100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml and 5mg/ml. To prepare these concentrations, dilution factor was first determined, and the concentrations were obtained by diluting each of the extract with the corresponding solvent in a known fraction. The effect of these concentrations on the test organisms was checked for by inoculating the test organisms on the appropriate media. Six equidistance holes were bore on the inoculated plates using a sterile cork-borer. Then each of the concentrations was dispensed making use of a sterile syringe, into the hole (well labeled). The plates were left on the work bench for 10-15 minutes to allow proper diffusion. The plates were then incubated for 24 hours at 37°C. After incubation, concentrations that have zone of clearance around it was observed and recorded in 'mm'.

Antimicrobial assay of extracts of *Jatropha curcas* using agar well diffusion method

Muller Hilton agar (MHA) was used in carrying out the assay by appropriately inoculating the test organisms which were already pre-adjusted to the 0.5 McFarland's turbidity standard in peptone water. The inoculums were spread all over the surface of the media. Agar well diffusion method was employed. This was done by boring three equidistant holes on the media with the use of sterile cork borer. Appropriate quantity of the three different extracts was dispensed into the holes. The plates were left for 10-15 minutes for diffusion incubated at 37°C for 24 hours. After incubation,

the diameter of zones of inhibition were measured using a meter rule and was recorded in standard unit.

Results

In Table 1, some of the bacteria isolates were sensitive to one or two of the antibiotics used. The isolates used were basically multi-drug resistant. The Gram-positive organism was sensitive only to Ofloxacin but it was resistant to Amoxicillin, Cotrimoxazole, Nitrofurantoin, Nalidixic acid, Augmentin, Tetracycline and Gentamycin. The Gram negative bacteria were sensitive to Gentamycin, Ofloxacin, and Erythromycin and were resistant to Ceftazidime, Cefuroxime, Gentamycin, Ceftriaxone, Erythromycin, Cloxacillin and Augmentin.

Table 2 shows positivity of the phytochemical screening carried out on the ethanolic plant extracts. As shown in Table 3, the MIC values of methanolic extract against *S. aureus* and *S. typhi* were 75mg/ml and

150mg/ml respectively. The MIC values of ethanolic and aqueous extracts obtained against *P. aeruginosa* were 5mg/ml and 75mg/ml respectively. *Escherichia coli* was susceptible to all the extracts. *S. typhi* showed sensitivity to two of the extracts and resistant to one. *S. aureus* was sensitive to ethanolic extract but resistant to methanolic and aqueous extract. *K. pneumoniae* and *P. aeruginosa* were resistance to all the extracts (Figure 1). Figure 3 shows the zone of inhibition of the extracts of *Jatropha curcas* on the isolates at different concentrations. *S. typhi* and *S. aureus* showed sensitivity to varying concentrations of methanolic extract while *E. coli*, *K. pneumoniae* and *P. aeruginosa* were resistant. For both ethanolic and aqueous extracts, *P. aeruginosa* was the most susceptible organism with sensitivity at all concentrations. The other organisms showed no zone of inhibition, hence, were resistant

Table 1: Antibiogram profile of the test organisms used against standard antibiotics

Organisms	AMX	AUG	GEN	COT	OFL	NAL	TET	NIT	CFX	CAZ	CTR	ERY	CXC
<i>S. aureus</i>	R	R	R	R	S	R	R	R	-	-	-	-	-
<i>S. typhi</i>	-	-	S	-	S	-	-	-	R	R	R	S	R
<i>P. aeruginosa</i>	-	-	R	-	R	-	-	-	R	R	R	R	R
<i>E. coli</i>	-	-	S	-	S	-	-	-	R	R	R	S	R
<i>K. pneumoniae</i>	-	-	R	-	S	-	-	-	R	R	R	R	R

Key: R indicates resistance S indicates susceptibility

GEN- Gentamycin, TET- Tetracycline, ERY- Erythromycin, CTR- Ceftriaxone, CXC- Cloxacillin, NIT- Nitrofurantoin, COT- Cotrimoxazole, AUG- Augmentin, OFL- Ofloxacin, AMX- Amoxicillin, NAL- Nalidixic acid, CFX- Cefuroxime, CAZ- Ceftazidime

Table 2: Phytochemical screening of ethanolic extract of *Jatropha curcas*

PHYTOCHEMICALS	RESULTS
Tannins	Present
Saponins	Present
Flavonoids	Present
Anthraquinones	Present
Terpenoids	Present
Alkaloids	Present

Key: Present indicates presence of the phytochemicals in the extract

Table 3: Minimum Inhibitory Concentration (MIC) of the extracts against the isolates

Isolates	Varying concentrations of extracts (mg/ml)						MIC
	150	100	75	50	25	5	
Ethanolic extract							
<i>P. aeruginosa</i>	-	-	-	-	-	-	5
Methanolic extract							
<i>S. aureus</i>	-	-	-	+	+	+	75
<i>S. typhi</i>	-	+	+	-	+	+	150
Aqueous extract							
<i>P. aeruginosa</i>	-	-	-	+	+	+	75

Key - Not turbid
+ Turbid

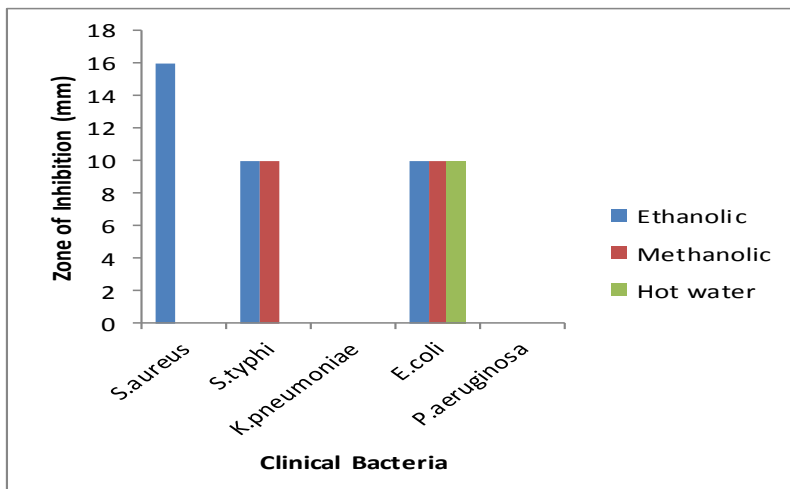
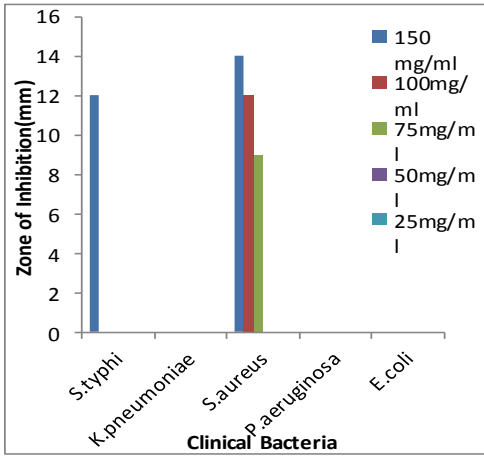
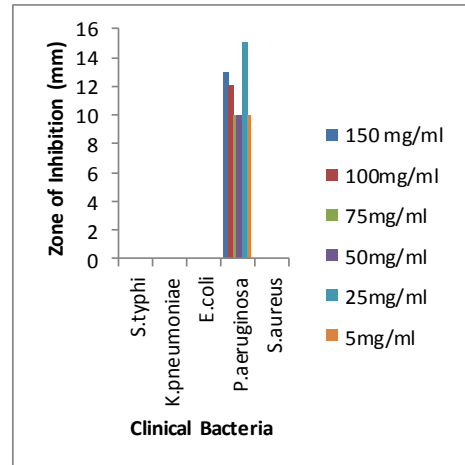


Figure 1: Susceptibility of the clinical isolates to the extracts of *Jatropha curcas*



a.



b.

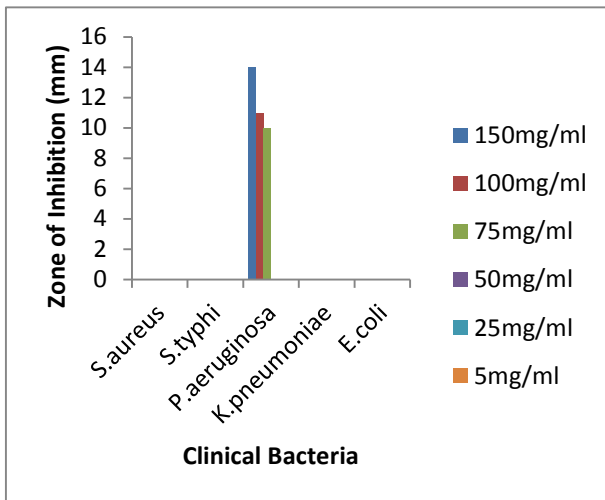


Figure 2: Susceptibility of the clinical isolates to concentrations of extracts *Jatropha curcas*
 a) Methanolic extract b) Ethanolic extract c) Aqueous extract

Discussion

Antibiogram profiling of the test organisms showed the organisms used were multi-resistant. The Gram-positive bacterium (*Staphylococcus aureus*) was sensitive only to Ofloxacin but it was resistant to Amoxicillin, Cotrimoxazole, Nitrofurantoin, Nalidixic acid, Augmentin, Tetracycline and

Gentamycin. This is an indication of broad spectrum antibiotic compounds. This may be attributed to the fact that the standard antibiotics as a conventional antibiotic, is a refined and purified product, while extracts of herbal medicines are a mixture of various plant constituents some of which can interfere with antimicrobial activity and are subjected to

degradation and decomposition on storage [11]. The Gram negative bacteria (*S. typhi*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*) were sensitive to Gentamycin, Ofloxacin, and Erythromycin and were resistant to Ceftazidime, Cefuroxime, Gentamycin, Ceftriaxone, Erythromycin, Cloxacillin and Augmentin. Positivity of the phytochemical screening carried out on the ethanolic plant extracts tend to agree with the report of El-Mahmood and Doughari [12] that linked the antimicrobial properties of the plant to the presence of the bio-active secondary metabolites like alkaloids, tannins, saponins, flavonoids, phenols, and glycosides. This finding agrees with previous works of El Di-wani *et al.* [13] who reported the presence of saponins in *Jatropha curcas* leaf. Although absence of alkaloids in *Jatropha curcas* leaf extracts had also been reported by Kubmarawa *et al.* [14] although Igbinsosa *et al.* [15] and Akinpelu *et al.* [16] observed the presence of alkaloids in *J. curcas* stem bark and leaves extracts respectively. These compounds have been associated with medicinal uses for centuries and were reported as the most efficient, therapeutically significant plant substance [17, 18] and exert antibacterial activity through different mechanisms [19, 20].

The Minimum Inhibitory Concentration was carried out only on the organisms that showed zone of inhibitions for ethanolic, methanolic and aqueous extracts respectively at different concentrations. *Pseudomonas aeruginosa* exhibited susceptibility to lesser concentrations of ethanolic extract (5mg/ml for MIC value). *P. aeruginosa* exhibited susceptibility to lower concentrations of aqueous

extract (75mg/ml for MIC). *Staphylococcus aureus* and *Salmonella typhi* exhibited susceptibility to lesser concentrations of the extract (75mg/ml for *Staphylococcus aureus* and 150mg/ml for *Salmonella typhi*). There was no MBC value since there was no growth after extracts of *Jatropha curcas* leaves were plated and incubated. This indicates that there was no cidal effect.

Escherichia coli showed susceptibility to all the extracts while *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were resistant. *Pseudomonas aeruginosa* was the only organism that showed sensitivity at varying concentrations to ethanolic extract and aqueous extract respectively, the other organisms were resistant. *Salmonella typhi* and *Staphylococcus aureus* were sensitive at different concentrations of methanolic extract. *E. coli*, *K. pneumoniae* and *P. aeruginosa* were resistant. High MIC values are indication of low activity while low MIC values are indication of high activity because it is the minimum or the smallest concentration capable of inhibiting the growth of organism. In *Salmonella typhi* and *Staphylococcus aureus* the MIC was obtained as 150mg/ml and 75mg/ml respectively indicating that lower concentrations other than this concentration cannot exert any antimicrobial effect on the organism but can still exhibit at higher concentration as seen in the stock concentration (200mg/ml).

The ability of all the extracts to extricate the phytochemicals and exert antimicrobial activities on one or more of the test organisms shows that they are good solvent for extraction but methanolic extract extricating more

phytochemicals than ethanolic and aqueous extract. This is due to the fact that methanol is a solvent that dissolves all type of compounds either polar, semi polar and non-polar. This gives it the ability to extract the antimicrobial agent from the leaves more than the other solvents.

Conclusion

This study has revealed ofloxacin, gentamycin and erythromycin to be effective against the clinical isolates used. It has further confirmed that the plant extracts could be used for the treatment of various infections and may serve as a good source of novel

bioactive compounds and also the presence of many secondary metabolites in the leaves of *Jatropha curcas*.

Public enlightenment programme to educate people on the hazards of misuse of antibiotics will be beneficial as these antibiotics are a group of drugs that can easily be possessed by many people. Individuals on drug prescription should be educated on the need to follow doses strictly and Government should pass a policy against the unnecessary use and handling of antibiotics.

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