

**INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY****Phytochemical and Antimicrobial Study of *Jatropha curcus* Root**

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ABSTRACT

This study investigated the use of *Jatropha curcus* as a medicinal plant used in treating many microbial infections. The lack of scientific standardization as well as possible therapeutic alternatives against antibiotic resistant bacterial and fungal infections indicates strong need for continuous effort to validate the use of plant material as alternative therapy regimens with similar or higher antibiotic beneficial properties. The present study describes the phytochemical activities and antibiotic properties of *Jatropha curcus* extracts against *S. aureus*, *E. coli*, *Klebsiella*, *Shigella* sp, *Candida albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. kfyer*. The phytochemical analysis was carried out using hexane, ethyl acetate and methanolic extracts of root bark of the plant using standard methods. The antibacterial potency was initially determined by agar well diffusion method for crude extracts of *Jatropha curcus* followed by quantitative evaluation of antibacterial activity by Minimum inhibitory concentration and Minimum bactericidal/fungicidal concentration. Phytochemical screening of all the extracts revealed the presence of alkaloid, flavonoids, carbohydrate, triterpenes and tannins. Steroid was absent in hexane and ethyl acetate but present in methanol extract. Anthraquinones was absent in all the extracts. Sensitivity test result showed that all the test isolates were sensitive to hexane, ethyl acetate and methanolic extracts at 500mg/ml. *Staphylococcus aureus*, *Shigella* sp., *Candida krusei* were found to have Minimum Inhibitory and Minimum Bactericidal/Minimum Fungicidal Concentrations of 31.25mg/ml and 62.5mg/ml respectively in all the extracts. Thus this plant is a potential candidate for drug development for the treatment of diseases caused by these pathogens.

Keywords: *Jatropha curcus*, phytochemical analysis, Minimum inhibitory and Minimum bactericidal/fungicidal concentration

1.0 INTRODUCTION**1.1 Traditional Medicine**

The traditional treatment and control of diseases by the use of available medicinal plants by rural communities will continue to play significant roles in medical health care implementation in the developing countries (Ekundayo *et al.*, 2011). The

use of synthetic medicines as therapeutic agents however is limited, due to various challenges such as poor drug solubility, stability, adsorption and high toxicity. In addition, some of these drugs are expensive and generally unavailable to citizens of developing countries, especially those residing in the rural areas (Sule *et al.*, 2011). The shortfalls in the

use of chemotherapeutic agents as control agents of bacterial diseases, further encourages the use of plants as a form of alternative medicine. The drug resistant bacteria and fungal pathogens further complicate the treatment of infectious diseases in immune compromised AIDS and cancer patients. Multi-drug resistant organisms are associated with nosocomial infections (Hidron *et al.*, 2008). Thus infectious diseases represent a critical problem to health and they are the main cause of high morbidity and mortality rates worldwide (WHO, 2012). Until recently, research and development efforts have provided new drugs in time to treat bacteria that developed resistance to older antibiotics. As bacterial antibiotic resistance continues to exhaust the supply of effective antibiotics, a global public health disaster appears likely. Substitutes from the nature to the antibiotics are becoming the prime need of the society in the present and in future.

1.2 Pharmacological Significance of *Jatropha curcus*

Jatropha curcus L. (Euphorbiaceae) or physic nut is an all-purpose, zero-waste perennial plant (Datta, 2007). This plant natively occurs in India, Africa and North America. The genus name *Jatropha* is derived from the Greek *iatros* (doctor) and *trophé* (food) which implies medicinal use. Natural products isolated from *Jatropha curcus* are found to be the excellent source of synthetic and traditional herbal medicine. The therapeutic efficacies of many indigenous plants for various diseases had been described by traditional herbal medicinal practitioners. The past decade has seen a considerable change in opinion regarding ethnopharmacological therapeutic application. The presence of various life sustaining constituents in plants has urged scientist to examine these plants with a view to determine potential properties. *J. curcus* is one of the promising ethnomedicinal plants used in Asia and Africa for solving health problems (Lans, 2001). The plant is a source of folk remedy for alopecia, anasarca, ascites, burns, carbuncles, convulsions, cough, dermatitis, diarrhoea, dropsy, dysentery, dyspepsia, eczema, erysipelas, fever, gonorrhoea, hernia, incontinence, inflammation, jaundice, neuralgia, paralysis, parturition, pleurisy, pneumonia, rash, rheumatism, scabies, sciatica, sores, stomachache, syphilis, tetanus, thrush, tumors, ulcers, uterosis, whitlows, yaws, and yellow

fever. Root is used in decoction as a mouthwash for bleeding gums and toothache. It is also used for eczema, ringworm, and scabies (Perry, 1980; Debnath and Bisen, 2008).

1.3 Antimicrobial Activity of *Jatropha curcus*

Jatropha curcus grows well particularly in the Northern part of Nigeria and can be used to provide cheap source of antibiotic and disinfectant due to its antimicrobial activities that have been reported (Igbinosa, *et al.*, 2009). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing countries (WHO, 2002). Previous studies have reported that *J. curcus* exhibits antimicrobial activity (Igbinosa *et al.*, 2009; Ekundayo *et al.*, 2011; Namuli *et al.*, 2011). The search for new antibacterial drugs of natural origin is urgently needed in the light of growing cases of microbial resistance to the available synthetic antibiotics (Iwu *et al.*, 1999; Wurochekker *et al.*, 2008; Krishnaiah *et al.*, 2009).

1.4 Hypotheses and Research Design

The present study therefore, attempts to determine the phytochemical contents of the extracts from root of *Jatropha curcus* in relation to their antimicrobial activity on some common pathogens. The aim is to contribute to scientific knowledge on the development of potential human uses for *J. curcus* root, either as drug, food or pharma-food. Therefore, the objective of the study is to ascertain the antimicrobial and phytochemical activity of the extracts of *J. curcus* roots.

2.2 METHODS

2.2.1 Study Area

The research was carried out in Microbiology Laboratory, Bayero University Kano with geographical coordinates of 13⁰²1N, 42⁰ 4'E.

2.2.2 Collection and Identification of Plant material

Jatropha curcus root was collected in March, 2017 from Babura Local Government Area of Jigawa State and conveyed for identification and authentication at the Herbarium unit of Department of Plant Biology, Bayero University, Kano in which a voucher's numbers BUKHAN60 was assigned to the plant.

2.2.3 Phytochemical Screening of extracts of *Jatropha curcus* Root

The hexane, ethyl acetate and methanol extracts were subjected to phytochemical screening using standard method as described below (Evans, 2009).

2.2.3.1 Test for Saponins

Frothing Test:

About 0.5g of the extracts was shaken with water in a test tube followed by warming on a water bath. Frothing which persists on warming was taken as an evidence for the presence of saponins (Evans, 2009).

2.2.3.2 Test for steroids/ terpenes

Lieberman-Buchard test:

A small portion of the extracts was dissolved in chloroform. Equal volume of acetic anhydride was then added, followed by concentrated sulphuric acid down the side of the test tube. The solution was observed for the presence of a brown ring at the interphase which indicates the presence of steroids/triterpenes (Evans, 2009).

2.2.3.3 Test for Flavonoids

Shinoda Test:

Exactly 0.5g of the extracts was dissolved in 5ml 95% ethanol, warmed and filtered. Three (3) pieces of magnesium chips were added followed by five drops of concentrated hydrochloric acid. The appearance of a pink, orange or red to purple color was used as an indication for the presence of flavonoids (Evans, 2009).

2.2.3.4 Test for Tannins

Ferric chloride Test:

Exactly 0.5g of the sample (extracts) was stirred with 10 ml distilled water and filtered. Two drops of 1% ferric chloride solution was added to 2 ml of the filtrate. Formation of a blue-black (Hydrolysable/Gallitannins) or green or blue-green (Condensed/Cathehic tannins) precipitate was used as an indication for the presence of phenolic compounds (Evans, 2009).

2.2.3.5 Test for Alkaloids

About 0.5 g of the extracts was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath and filtered. Ammonia solution was added to the filtrate until it was basic then chloroform was added and shaken gently to allow separation. The chloroform layer was collected then diluted HCl added and

shaken gently for separation. The aqueous layer was divided into three.

To the first portion, few drops of freshly prepared Dragendorff's reagent was added and observed for formation of orange to brownish precipitates. To the second portion, one drop of Mayer's reagent was added and observed for formation of white to yellowish or cream color precipitates. To the third portion, 1ml of Wagner's reagent was added to give a brown or reddish or reddish-brown precipitates, in the presence of alkaloids (Evans, 2009).

2.2.3.6 Test for anthraquinones

Borntrager's Test:

A small portion of the sample (extracts) was shaken with 10ml of benzene, the content was filtered and 5ml of 10% ammonia solution was added to the filtrate, then the mixture was shaken. Formation of a pink, red or violet colors in the lower part of the aqueous layer indicates the presence of free anthraquinones (Evans, 2009).

2.2.3.7 Test for Cardiac glycosides

Keller-Killiani test:

About 0.5g of the sample (Extracts) was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interphase indicates the presence of a deoxy sugar characteristic of cardenolides (Evans, 2009).

2.2.3.8 Test for Carbohydrates

Molisch Test:

To small portion of the extract in a test tube, few drops of Molisch reagent was added and concentrated sulphuric acid was then applied down the side of the test tube to form a lower layer, a reddish colour ring at the interphase indicates presence of carbohydrate (Evans, 2009).

2.2.4 Antimicrobial Bioassay

2.2.4.1 Preparation of Stock Solution

Plant extracts were dissolved in 1ml dimethyl sulphoxide (DMSO); 0.5g of each extract was dissolved in 1ml of the solvent. Half (0.5) ml of the extract was introduced in to bijour bottles to make 500mg/ml concentration. Half ml of DMSO was added in to the remaining stock solution making 1ml,

0.5ml was taken and placed in to another bottle containing 1 ml DMSO and labeled 250mg/ml, 0.5ml of DMSO was added, and another 0.5ml was taken and placed in to another bottle and labeled 125mg/ml. Another 0.5ml DMSO was added to the stock solution and taken to the next bottle and labeled 62.5mg/ml (Yusha'u and Sadis, 2011).

2.2.4.2 Preparation of Turbidity Standard

Barium Sulphate (1% w/w) standard suspension was used as turbidity standard. This was prepared as follows; one percent (1% w/v) solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid in to 99ml of distilled water. One percent (1% w/v) solution barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride solution in 50ml distilled water, then 0.6ml of the barium chloride solution was combined with 99.4ml of sulphuric acid solution to yield 1% w/v barium sulphate suspension. The turbid solution formed was transferred in to the test tube as the standard for comparison (Cheesbrough, 2005).

2.2.4.3 Standardization of Inoculum

Using inoculum loop, enough material from an overnight culture of the test organism was transferred in to a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National committee for clinical laboratory standard (NCCLS, 2008).

2.2.4.4 Sensitivity Test

The bacterial and fungal solution (inoculum) was aseptically seeded on a sterilized N.A and SDA media respectively. A 6mm in diameter cork borer was used to bore four holes on the media. The holes were labeled according to the extract concentration (i.e. 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml). A sterilized micro-pipette was used to take 0.1ml of the 500mg/ml extract concentration and transferred into its labelled hole on the N.A media and SDA media. The same procedure was used on the remaining extract concentration; it was undistributed for at least an hour for the extract to effectively diffuse into the media. The plate was

incubated at 37°C for 24 hours before observation and measurement of zone of inhibition (NCCLS, 2008). Ciprofloxacin 500mg/ml was used as positive control and D.M.S.O was used as negative control. The process was carried out in triplicate.

2.2.4.5 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the test organisms was determined using the test tube dilution technique. Nine milliliter (9ml) of the nutrient broth was pipetted in to various test tubes containing concentrations of 2000mg/ml, 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml of the extract, the remaining two as negative (containing only nutrient broth and positive control (nutrient broth and organism) respectively. The overnight culture of the test organisms (0.1ml) aseptically obtained was added to the test tubes with exception of negative control and incubated at 37°C for 24hrs. The least concentration of the extract that did not indicated any visible growth of the incubated organisms in broth culture was taken as the minimum inhibitory concentration (MIC) (Abalaka *et al.*, 2012).

2.2.4.6 Determination of Minimum bactericidal/fungicidal Concentration

The bactericidal and bacteriostatic effect of the extract was determine by sub-culturing the well that showed no growth on the fresh medium and incubated it for 24hrs. If the growth is observed, the extract is considered to have only bacteriostatic effect while on the other hand, if the growth is observed, the extract is said to have bactericidal effect (Abalaka *et al.*, 2012). The data was subjected to mean statistical analysis.

3.0 RESULTS AND DISCUSSION

Phytochemical screening of hexane, ethyl acetate and methanol extracts revealed the presence of alkaloid, flavonoids, carbohydrate, triterpenes and tannins. Steroid was absent in hexane and ethyl acetate but present in methanol extract. Anthraquinones was absent in all the extracts

Table 1: Qualitative Phytochemical Screening of Hexane, Ethyl acetate and Methanolic Root Extracts of *Jatropha curcus*

Metabolites	Inference		
	Hexane	Ethyl acetate	Methanol
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponins	-	+	+
Cardiac glycoside	-	+	+
Tannins	+	+	+
Steroid	-	-	+
Triterpenes	+	+	+
Phenol	-	+	+
Anthraquinones	-	-	-
Carbohydrate	+	+	+

Key: + Present, - Absent

Hexane extract of *Jatropha curcus* root exhibited the highest zone of inhibition against *S. aureus*, *Shigella* and *C. krusei* at 500mg/ml. *Jatropha curcus* root have MICs and MBC of 31.25mg/ml and 62.5mg/ml against *S. aureus*, *Shigella* and *C. krusei*.

Table 2: Inhibition Activity and Minimum Inhibitory/Bactericidal/Fungicidal Concentration of Hexane Root of *Jatropha curcus*

Test organisms	Diameter zone of inhibition/ Concentration (mg/ml)				Ciprof/Fluconazole	DMSO	MIC	MBC
	500	250	125	62.5				
<i>S. aureus</i>	14	12	10	08	41/-	06	31.25	62.5
<i>E. coli</i>	12	10	08	06	40/-	06	-	-
<i>Klebsiella</i>	12	10	08	06	36/-	06	-	-
<i>Shigella</i>	14	12	10	08	38/-	06	31.25	62.5
<i>C.albicans</i>	10	08	06	06	-/40	06	-	-
<i>C.glabrata</i>	10	08	06	06	-/38	06	-	-
<i>C. krusei</i>	14	12	10	08	-/37	06	31.25	62.5
<i>C.tropicalis</i>	10	08	06	06	-/38	06	-	-
<i>C.kfyer</i>	12	09	07	06	-/39	06	-	-

Key: - Not tested for MIC/MBC/MFC because there was no inhibition during Agar Well Diffusion.

Ethyl acetate extract of *Jatropha curcus* root exhibited the highest zone of inhibition (14mm) against *S. aureus*, *E. coli*, *Shigella* and *C. krusei* at 500mg/ml. *Jatropha curcus* root have MICs and MBC of 31.25mg/ml and 62.5mg/ml against *S. aureus*, *E. coli*, *Shigella* and *C. krusei*.

Table 3: Inhibition Activity and Minimum Inhibitory/Bactericidal Concentration of Ethyl acetate Root of *Jatropha curcus*

Test organisms	Diameter zone of inhibition/ Concentration (mg/ml)				Ciprof/Fluconazole	DMSO	MIC	MBC
	500	250	125	62.5				
<i>S. aureus</i>	14	12	10	08	41/-	06	31.25	62.5
<i>E. coli</i>	14	12	10	08	40/-	06	31.25	62.5

<i>Klebsiella</i>	12	10	08	06	36/-	06	-	-
<i>Shigella</i>	14	12	10	08	38/-	06	31.25	62.5
<i>C.albicans</i>	10	08	06	06	-/40	06	-	-
<i>C.glabrata</i>	10	08	06	06	-/38	06	-	-
<i>C.krusei</i>	14	12	10	08	-/37	06	31.25	62.5
<i>C.tropicalis</i>	10	08	06	06	38	06	-	-
<i>C.kfyer</i>	12	09	07	06	-/39	06	-	-

Key: - Not tested for MIC/MBC/MFC because there was no inhibition during Agar Well Diffusion.

Ethyl acetate extract of *Jatropha curcus* root exhibited the highest zone of inhibition (16mm) against *Shigella sp* while 14mm against *S. aureus*, *E. coli*, *Shigella*, *Klebsiella sp.* and *C. krusei*. *Jatropha curcus* root have MICs and MBC of 31.25mg/ml and 62.5mg/ml against *S. aureus*, *E. coli*, *Shigella*, *Klebsiella sp.* and *C. krusei*.

Table 4: Inhibition activity and Minimum Inhibitory/Bactericidal/Fungicidal Concentration of Methanolic Root of *Jatropha curcus*

Test organisms	Diameter zone of inhibition/ Concentration (mg/ml)				Ciprof/Fluconazole	DMSO	MIC	MBC
	500	250	125	62.5				
<i>S. aureus</i>	14	12	10	08	41/-	06	31.25	62.5
<i>E. coli</i>	14	12	10	08	40/-	06	31.25	31.25
<i>Klebsiella</i>	14	12	10	08	36/-	06	31.25	62.5
<i>Shigella</i>	16	13	10	08	38/-	06	31.25	62.5
<i>C.albicans</i>	10	08	06	06	-/40	06	-	-
<i>C.glabrata</i>	10	08	06	06	-/38	06	-	-
<i>C.krusei</i>	14	12	10	08	-/37	06	31.25	62.5
<i>C.tropicalis</i>	10	08	06	06	-/38	06	-	-
<i>C.kfyer</i>	12	09	07	06	-/39	06	-	-

Key: - Not tested for MIC/MBC/MFC because there was no inhibition during Agar Well Diffusion.

4.0 DISCUSSION

The antimicrobial activities of the root extract of *J. curcus* measured by diameter of the zone of inhibition against the test organisms was tested. The methanol extract of *J. curcus* inhibited *S. aureus* and *E. coli* with diameters of zone of inhibition of 14 mm each. The methanolic root extract of *Jatropha curcus* had antimicrobial activity against all the test organisms with mean diameter of zone of inhibition ranging from 10 to 16 mm. The activity was highest against *Shigella* and followed by *S. aureus*, *E. coli*

and *Klebsiella*. The methanol extract of *Jatropha curcus* exhibited the highest activity against *S. aureus* and *E. coli* with MIC of 31.25mg/ml respectively.

The methanol extract of *J. curcus* root produced diameters of zone of inhibition of 10, 10, 14, 10 and 12 mm against *Candida albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. kfyer* respectively. Thus, the methanol extract of the root showed a broad spectrum of antimicrobial activity against the test

organisms. Falodun *et al.* (2003) had previously investigated antibacterial and antifungal activity of the petroleum ether, butanolic and ethanolic extracts of the root of *J. curcus* against *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, *P. aeruginosa* and *Bacillus subtilis*. The butanolic extract showed a broad spectrum of antibacterial activity against the test organisms at concentration of 100, 150 and 200 mg/ml (Falodun *et al.*, 2003). Antimicrobial activity of medicinal plants has been linked with the presence of these chemical substances in medicinal plants (Cowan, 1999; Cushnie and Lamb, 2005; Ebana *et al.*, 2005). The antibacterial activity of *J. curcus* may be due to the presence of these chemical substances in the leaves (Falodun *et al.*, 2006). The methanolic extracts of *J. curcus* tested in this study showed moderate antibacterial activity against *S. aureus* and *E. coli*. The results of this study were similar to those of Akinpelu *et al.* (2009) who reported strong inhibitory activity against *S. aureus* and *E. coli* in the methanolic extract of the root and leaves of *J. curcus*. Aiyelaagbe *et al.* (2007) reported the antimicrobial activities of some secondary metabolites from the root extract of the plant against some microorganisms associated with sexually transmitted diseases. Igbinosa *et al.* (2009) also reported the antimicrobial activity of the stem bark extracts against 12 bacterial species consisting both gram positive and gram negative organisms while Igbinosa *et al.* (2009) found little or no antibacterial activity in the aqueous extract of the stem bark of *J. curcus*; in this study, the methanolic extract of the root showed moderate antibacterial activity against both *S. aureus*, *Klebsiella pneumoniae*, *Shigella* and *E. coli*. While the differences in results may be due to the varying concentrations of active substances in the root, stem bark and leaves, it may also be due to the different concentrations used (Igbinosa *et al.*, 2009). However, the actual concentration of the extract used in agar well diffusion method employed by Igbinosa *et al.* (2009) may be difficult to determine as the stated concentration is nominal and does not represent the actual amount of substance present in the volume of extract dispensed into agar well. The work of Kisangau *et al.* (2007) showed the possibility of discrepancy in the results of antibacterial activity tested by agar well diffusion method and the paper disc diffusion method. Kisangau *et al.* (2007) found no activity in the water

extract of *J. curcus* when agar well was used but strong inhibitory activity of the extract was seen in the disc diffusion method. The effect of these two methods of antibacterial activity screening of plant extracts may require further study. It is interesting to note that the extracts of the root of *J. curcus* had activity against the *E. coli* isolate which was resistant to Ciprofloxacin, Ampicillin and Tetracycline (Kisangau *et al.*, 2007). A major goal of the antimicrobial screening of medicinal plants is to find substances with novel mechanism of action against drug resistant strains. The results obtained in this study contribute to the scientific validation for the use of these medicinal plants in traditional medicine and serve as a guide for selection of plants with antimicrobial activity for further phytochemical work on isolation and identification of the active compounds. Furthermore, these results show the potential of some of these medicinal plant for development of standardized culturally acceptable herbal medicines for local use as broad spectrum antimicrobial agents.

Tannins and flavonoids have biological activities that are of benefit in the prevention and management of many ailments (James *et al.*, 2007). Earlier, Erah *et al.* (1996) had associated antimicrobial activity with the presence of tannins and flavonoids. Therefore, the presence of tannins and flavonoids in the methanolic extract from the root of *J. curcus* (an indication of the presence of these essential secondary metabolites) might increase its therapeutic potentials and uses. This probably explains the apparent narrow spectrum antimicrobial activity of *J. curcus* root extracts observed in the present study.

5.0 CONCLUSION

The results obtained in this study contribute to the scientific validation for the use of these medicinal plants in traditional medicine and serve as a guide for selection of plants with antimicrobial activity for further phytochemical work on isolation and identification of the active compounds. Furthermore, these results show the potential of this medicinal plant for development of standardized culturally acceptable herbal medicines for local use as broad spectrum antibacterial antifungal agents.

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