



Comparison of Antibacterial Properties of Solvent Extracts of Different Parts of *Jatropha curcas* (Linn)

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ABSTRACT

Recent reports have suggested the urgent need for the development of alternative antibacterial substances of natural origin for the treatment of infections because of the growing cases of bacterial resistance to many available synthetic antibiotics. *Jatropha curcas* (*J. curcas*) belongs to the family Euphorbiaceae and has been widely reported for its medicinal values. It is used in traditional folklore medicine to cure various ailments such as skin infections, gonorrhoea, and jaundice and fever. Almost every part of *J. curcas* has been reported to have medicinal importance. In the present study, the aqueous, ethanol and pet-ether extracts of the root, stem bark and leaves of *J. curcas* were evaluated in vitro for antibacterial activities using *Salmonella typhi* (*S.typhi*) and *Escherichia Coli* (*E.coli*) as test organisms. The results were compared in order to identify the extract with the strongest growth inhibition properties. Preliminary phytochemical screening of the extracts revealed the presence of bioactive compounds with demonstrated antimicrobial properties. The ethanol extracts showed superior antibacterial activities compared with both the aqueous and pet-ether extracts. The root, stem bark and leaf ethanol extracts demonstrated comparable antibacterial activities against *S. typhi* and *E. coli*. Thus the root, stem bark and leaf of *J. curcas* are potential candidates in the search for potent antibacterial agents from medicinal plants.

Key Words: Anti-bacterial activity, *Jatropha curcas*, MIC, MBC, Phytochemical screening, Euphorbiaceae

INTRODUCTION

Herbal medicine, also called botanical medicine or phytomedicine refers to the use of a plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Long practiced outside of conventional medicine, herbal medical practices has increasingly become more significant in primary health care as improvements in analysis and quality control along with advances in chemical research demonstrates how valuable they are in the treatment and prevention of diseases.

Historically, medicinal plants have provided a source for novel drug compounds, and plant derived medicines have made large contributions to health needs of many societies. Thus medicinal plants have become the base for the development of a medicine or a natural blue print for the development of new drugs.

Several reports have suggested the urgent need for the development of alternative antibacterial substances of natural origin for the treatment of infections because of the growing cases of bacterial resistance to many available synthetic antibiotics. Thus, plants with medicinal potentials can be evaluated for the purpose of identifying those that may be potent against infectious organisms and hence useful in treating ailments caused by human pathogens.

Many of these plants contain large varieties of chemical substances referred to as secondary metabolites which have significant biological effects on humans.

Jatropha curcas belongs to the family *Euphorbiaceae* and has been widely reported for its medicinal values. It is used in traditional folklore medicine to cure various ailments such as skin infections, gonorrhoea, and jaundice and fever^{1,2}. *J. curcas* Linn is commonly called physic nut, purging nut or pig nut. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, guinea worm sores and joint rheumatism^{3,4}. Fabrenro-Beyoku⁵ investigated and reported the anti-parasitic activity of the sap and crushed leaves of *J. curcas*. The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity⁶. Sanni *et al.*⁷ reported antibacterial, antitumor and antiinsect activities of this plant. Several other works have also shown that many other *Jatropha* species possess antimicrobial activity^{8,9}. The root methanolic extracts of the plant was shown to exhibit anti-diarrhoeal activity in mice¹⁰. In addition, Mujumdar and Misar¹¹ revealed that the root extract from *J. curcas* showed anti-inflammatory activity on local inflammatory induced in albino rat. Naengchommong *et al.*²³ reported the isolation of two lathyranes from *J. curcus* while Aiyelaagbe *et al.*¹³ isolated diterpenoids from the same plant and also reported its antibacterial activity on some bacterial isolates. The sap from *J. curcas* is employed for treating sores, cleaning teeth and toothache¹⁴. The anti-microbial activities of the crude extract of the bark has also been reported¹⁵.

In Ghana, various communities use different parts of *J. curcas* to cure various ailments. In some parts of northern Ghana, the decoction of the roots is used for the treatment of gonorrhoea, diarrhoea and rheumatism. The latex of the plant is also used to arrest bleeding, toothache, and wound healing. The leaf decoction is also used to treat typhoid fever and diarrhoea.

Almost every part of *J. curcas* has medicinal importance. Despite the extensive reports on the medicinal potentials of *J. curcas*, the efficacies of the extracts with potent anti-bacterial activities have not been compared. The overarching aim of the present study is to determine the phytochemical constituents present in the aqueous, ethanol and petroleum ether extracts of the root, stem bark and leaf of *Jatropha curcas*. The growth inhibitory activities of the various extracts on *Salmonella typhi* and *Escherichia Coli* have been evaluated and compared in order to determine the extract with the greatest anti-bacterial potential.

MATERIALS AND METHODS

Plant Materials

Fresh leaves, stem bark, and roots were collected in the month of November in Gognia, a suburb of Navrongo and were identified by Dr. Walter Kpikpi of the Department of Applied Biology, Faculty of Applied Sciences, University For Development Studies. The samples were air dried. The stem bark and the roots were pulverized in a mill. The leaves were also made into powder. The samples were stored in an air-tight container for further use.

Preparation of Extracts

Exactly 200g each of the pulverized stem bark and roots were cold extracted in ethanol (95%), distilled water and petroleum ether separately for five days with occasional shaking¹⁶. Exactly 250g each of the powdered leaves were also extracted separately in cold using ethanol (95%), distilled water and petroleum ether for five days with occasional shaking. The extracts were then separately filtered through Whatman's No. 1 filter paper and the filtrates were concentrated to dryness *in vacuo* using a rotary evaporator to remove the solvents.

Phytochemical Screening

The tests were carried out using standard procedures in accordance with Trease and Evans¹⁷ and Harborne¹⁶ with little modification.

Test for alkaloids

About 1ml of the plant extract was stirred with few drops of 1% HCl on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with 1 drop of Mayer's reagent. Turbidity of the extract filtrate on the addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract.

Test for saponins

About 2ml of the extract was measured into a test tube and shaken vigorously. Test becomes positive if characteristic honeycomb froth persists for at least 30 minutes.

Test for cynogenic glycosides

About 2ml of the extract was measured into a test tube and 1ml of chloroform added to it. A piece of picric acid paper was then inserted into the test tube just above the extract and folded over the rim of the tube. The test tubes was then stoppered and warmed at about 35°C in a water bath for about 30 minutes. A change in colour of the yellow picric paper to various shades of red indicates the presence of cynogenic glycosides.

Test for reducing sugars

About 0.5ml each of Fehling's solutions A and B were measured into a test tube. About 0.5ml of the extract was added to the solution and heated in a water bath. A brick-red precipitate denotes the presence of reducing sugars.

Test for polyuronides

About 2ml of acetone was measured into a test tube. About 2ml of the filtered extract was added to the acetone in the test tube. Positive test is observed if the solution precipitates.

Test for phenolics

About 2ml of the extract was measured into a test tube and three drops of ferric chloride solution added. A positive test is confirmed if the solution turns blue, blue-black, green or blue-green and precipitates.

Test for flavonoids

About 3 drops of dilute NaOH was added to 1ml of the extract. An intense yellow colour was produced in the plant extract which becomes colourless on addition of few drops of dilute HCl indicates the presence of flavonoids.

Test for tannins

About 0.5ml of the plant extract was heated in a steam bath for about 5 minutes. About 2 drops of 5% FeCl₃ was then added. Presence of greenish precipitate indicated the presence of tannins.

Test for anthracenosides

About 5ml of the extract was transferred into a separating funnel and about 3 drops of diethyl ether added to it and shaken gently and allowed to stand for about 10 minutes. About 2ml of the diethyl ether portion was measured into a test tube. 2ml of Ammonium hydroxide was added and shaken gently. A cherish-red colour of the alkaline solution indicates the presence of anthracenosides.

Test for triterpenes and phytosterols

About 5ml of the diethyl ether portion (same procedure from the test for anthracenosides) was evaporated to dryness. The residue was dissolved in 0.5ml acetic anhydride and then in chloroform (0.5ml). The solution was transferred to a dry test tube and 2ml of concentrated sulphuric acid added to it and shaken gently. A brownish-red colour was observed for the presence of triterpenes and a green colour was observed for the presence of phytosterols. If triterpenes and phytosterols are absent, no colour will be observed as compared with the control or reference.

Bacteriological Analysis*Preparation of Muller-Hinton agar*

About 19g of Muller-Hilton agar was weighed into a 500ml conical flask. 500ml of distilled water was added and agitated. The content was heated to boil on a hot plate with a magnetic stirrer until the powder dissolves completely in the water. The media was then poured into a media bottle, corked and was autoclaved at 121°C for 15 minutes. The media was allowed to cool and stored in the refrigerator until use.

Bacteria culture

Salmonella typhi and *Escherichia coli* were first sub-cultured in a nutrient agar and incubated at 37°C for 24 hours at the microbiology laboratory.

Antimicrobial susceptibility test

The agar well diffusion method was used. The Muller-Hinton agar media was poured in the sterilized petri dishes and allowed to solidify. About 0.1ml each of the *Salmonella typhi* and *Escherichia coli* was spread uniformly over the surface of the Muller-Hinton media with a sterile glass rod spreader. A hole was bored by 5mm cork borer in the middle of each inoculated agar plate. About 0.1ml of each extract (10mg/ml in DMSO) was pipetted into the respective holes. Ciprofloxacin which was used as a control. Triplicates of plates were allowed to stand for about 1 hour to allow the extracts to diffuse into the media. The plates

were then incubated upside down at 37°C for 24 hours. The diameters (mm) of the zones of inhibition were measured from underneath the plates using a pair of dividers and a ruler and their means were also recorded.

Determination of Minimum Inhibitory Concentrations (MIC)

Various concentrations of extracts ranging between 2.0 and 10.0 mg/ml were introduced into different test tubes; each tube was inoculated with an overnight culture of *S. typhi* and *E. coli* diluted to give a final concentration of 10⁶ cells per ml. The tubes were incubated at 37°C for 24 h. The least concentration of extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the minimum inhibitory concentration (MIC) in each case¹⁸.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC of the plant extracts was determined by a modification of the method of Spencer and Spencer¹⁹. Samples were taken from plates with no visible growth in the MIC assay and sub-cultured on freshly prepared nutrient agar plates, and later incubated at 37°C for 48 h. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

Statistical Analysis

Data collected in the study are expressed as the mean \pm standard error of mean (S.E.M.)

RESULTS AND DISCUSSION

Phytochemical Screening

The extracts showed variations in the type of phytochemicals present. Phytochemical analysis revealed the presence of reducing sugar in all the extracts. Polyuronides, Cynogenic glycosides and Anthracenosides were however absent in all extracts. (Table 1). Ethanol extract of leaves, aqueous extracts of root and leaf showed the presence of saponins. These phytochemicals are biologically active and therefore may aid the antibacterial activity of *J. curcas*. Alkaloids were found to be present in all ethanol and aqueous extracts. Alkaloids were also found to be present in the leaf pet-ether extract. Alkaloids have been reported extensively for their anticancer activities²⁰. Phenolics were found only in the stem bark ethanol extract. The presence of these phenolic compounds may have contributed to the antibacterial properties of the stem bark ethanol extract and thus the usefulness of this plant in herbal medicament. Flavonoids were present only in the leaf aqueous extract. Phytosterols were also present only in the stem bark ethanol extract. Phytosterols have cholesterol reducing properties and may act in cancer prevention²¹. Triterpenes were found in the root and leaf ethanol extracts as well as in the root aqueous extract. Triterpenes have demonstrated antibacterial activities²². The presence of this phytochemical thus supports the antibacterial potentials of *J. curcas*. Tannins are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery²³. Therefore its presence may have a significant effect in the inhibition of the test organisms as demonstrated by ethanol and pet-ether extracts. The absence of many of the phytochemicals in both aqueous and pet-ether extracts may be responsible for the little antibacterial activity shown by these extracts. The activity of the aqueous extracts against the bacterial strains investigated in this study is consistent with previous works which show that aqueous extracts of the plant generally showed little or no antibacterial activities^{24,25,26,27}.

Antibacterial Activity

Root extracts of the various solvents exhibited antibacterial activity on the test organisms with the leaf ethanol extract demonstrating the highest activity (Zone of inhibition = 12.20 \pm 0.10mm against *S. typhi*). (Table 2). The result is consistent with the type phytochemicals present in the root ethanol extract. All the stem bark extracts, with the exception of the stem bark pet-ether extract, showed some activity on both test organisms. The stem bark ethanol extract again demonstrated the highest antibacterial activity in this category. (Zone of inhibition = 12.00 \pm 0.60 against *E. coli*). The leaf ethanol extract showed significant antibacterial activity on both test organisms. (Zone of inhibition = 13.00 \pm 0.50mm against *S. typhi*; 12.80 \pm 0.30mm against *E. coli*). The result reveals that the ethanol extracts of the plant have greater antibacterial potential than the aqueous and pet-ether extracts. It further reveals that the root, stem bark and leaf ethanol extracts have comparable antibacterial activities on the test organisms.

The MIC of the ethanol extracts ranged between 4.00 and 6.00 mg/ml. The MIC of the pet-ether extracts ranged between 8.00 and 10.00 mg/ml (Table 3). The extract with the least MIC is the most potent. The MIC of the aqueous extracts was not determined due to the low antibacterial activity demonstrated by the aqueous extracts. This supports results from previous works. The MBC of the extracts were consistent with the MIC and increased with the MIC. The MBC of the ethanol extracts ranged between 8 and 10mg/ml while those of the pet-ether extracts ranged between 18 and 22mg/ml (Table 4).

CONCLUSION

In conclusion, the aqueous, ethanol and pet-ether extracts of the root, stem bark and leaf of *J. curcas* showed varying inhibitory activities against *Salmonella typhi* and *Escherichia coli*. The phytochemicals present in the extracts play significant roles in inhibiting the test bacteria. The ethanol extracts showed superior antibacterial activities compared with both the aqueous and pet-ether extracts. The root, stem bark and leaf ethanol extracts demonstrated comparable antibacterial activities against *S. typhi* and *E. coli*. This suggests that the root, stem bark and leaf of *J. curcas* may contain similar bioactive compounds and thus, are all potential candidates in the search for potent antibacterial agents from medicinal plants.

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Table 1: Results of phytochemical screening of extracts of *J. Curcas*

Phytochemical Constituents	Ethanol Extracts			Aqueous Extracts			Petroleum Ether Extracts		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Saponins	-	-	++	+++	-	++	-	-	-
Reducing sugars	+++	+++	+++	+++	+++	+++	++	+++	++
Phenolics	-	++	-	-	-	-	-	-	-
Polyuronides	-	-	-	-	-	-	-	-	-
Alkaloids	+++	+	+++	+	-	+	-	-	++
Cynogenic glycosides	-	-	-	-	-	-	-	-	-
Anthracenosides	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	++	-	-	-
Triterpenes	+++	-	++	++	-	-	-	-	-
Phytosterols	-	++	-	-	-	-	-	-	-
Tannins	+++	+++	++	-	-	-	+	++	-

KEY: +++ = Abundant; ++ = Moderately abundant; + = Present; - = Absent

Table 2: Antibacterial profile of extracts of *J. Curcas*

Test organism	Zone of inhibition (mm) (Mean±SEM)									
	Aqueous Extracts			Ethanol Extracts			Petroleum ether Extracts			Control
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	
<i>S. typhi</i>	4.00 ±0.00	3.50 ±0.30	2.60 ±0.40	12.20 ±0.10	10.60 ±0.60	13.00 ±0.50	5.60 ±0.00	7.10 ±0.50	0.00 ±0.00	15.50 ±0.90
<i>E. coli</i>	4.00 ±0.20	7.80 ±0.40	5.90 ±0.00	12.10 ±0.00	12.00 ±0.60	12.80 ±0.30	5.00 ±0.50	0.00 ±0.00	0.00 ±0.00	13.40 ±0.20

Table 3: Minimum Inhibitory Concentrations (MIC) of extracts of *J. curcas*

Test organism	Minimum Inhibitory Concentrations (mg/ml)									
	Aqueous Extracts			Ethanol Extracts			Petroleum ether Extracts			Control
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	
<i>S. typhi</i>	nd	nd	nd	4.00	4.00	4.00	10.00	8.00	nd	2.00
<i>E. coli</i>	nd	nd	nd	4.00	6.00	4.00	nd	nd	nd	2.00

Nd = Not determined

Table 4: Minimum Bactericidal Concentrations (MBC) of extracts of *J. curcas*

Test organism	Minimum Bactericidal Concentrations (mg/ml)								
	Aqueous Extracts			Ethanol Extracts			Petroleum ether Extracts		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
<i>S. typhi</i>	NA	NA	NA	8.00	8.00	8.00	22.00	18.00	NA
<i>E. coli</i>	NA	NA	NA	8.00	10.00	6.00	NA	NA	NA

NA = Not Applicable

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