

Qualitative Phytochemistry and Antibacterial Resistance Pattern of Leaves and Stem Bark Extracts of *Jatropha curcas*

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Abstract *Jatropha caurcas* (Euphorbiaceae) is used in folklore medicine to cure various ailments in Africa, Asia and Latin America. Present study investigated the phytochemical components and antimicrobial activities of *Jatropha caurcas* against some selected clinical isolates. The leaf and stem bark was macerated successively in ethanol and aqueous solvent for 24 hrs each. Qualitative phytochemistry screening of aqueous and ethanolic solvent extracts of plant materials was conducted using standard methods. The antibacterial susceptibility assay was carried out on three clinical isolates by preparing discs of a standard concentration of aqueous extracts of *Jatropha caurcas*. Result obtained indicates the presence of some secondary metabolites which are glycosides, flavonoids, phenols, tannin, steroids, reducing sugar and terpenoids. It was observed that alkaloids and saponins were absence in aqueous extract of leaves and stem bark solvent extract but present in stem bark extracts. However, ethanolic leaves extract contains no alkaloids and saponins. Susceptibility pattern of *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella dysenteriae* was measured to be 17mm, 23mm and 24mm respectively. Present investigation could serve as a panacea for emergence of multidrug resistance bacteria and also, the analysis of the secondary metabolites will provide the pharmacognosy and drug development sector preliminary information about the phytochemical constituent of *Jatropha caurcas* (Euphorbiaceae).

Keywords: *Jatropha caurcas*, phytochemical, antibacterial susceptibility, extracts

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1. Introduction

Jatropha curcas commonly called "Barbados nuts," or "purging nut," it is also known as "Itiakpa" by the Urhobos, "Ipalapa" in Yoruba, "Binidazugu" by the Hausas, "olulu-idu/uru" by the Igbos and "omangba" by the Igedes in Benue State. These plant is one of the most important sources of medicine. Plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds [11]. Extracts of plants were used for the treatment of various diseases and this forms the basis for all traditional systems of medicine [11].

Plants are rich source of secondary metabolites with interesting biological activities (inhibition of certain pathogens). In general, these secondary metabolites are of an important source with a variety of structural arrangements and properties [3]. Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides [15,16].

The treatment and control of diseases by the use of these active compound in *Jatropha curcas* within a

locality will continue to play significant roles in medical health care implementation in the developing countries [22]. Haslam *et al.* (1989) reported that plant extracts and their products are used in many parts of the world as the active principles in herb remedies. They are used locally in the treatment of infections, many centuries before scientific studies were discovered. Therefore, because of the increasing rate of antibiotic resistant organisms, there is need to screen for new active compound in plant that may serve as an emerging novel antibiotics.

The major aims of these studies seek to investigate the type of phytochemical components present in this plant (*Jatropha curcas*) and an attempt to examined it antimicrobial strength against some selected clinical isolates.

2. Material and Methods

2.1. Samples Procurement

The plant samples i.e the leaf and stem bark that was used in this research work was collected in the month of

March 2016 from the Institute of Agriculture Research (IAR) Zaria. The samples were identified in the department of Biological Sciences Ahmadu Bello University Zaria and a voucher specimen number 22873 was assigned, the plant samples were taken to faculty of pharmacy, department of pharmacognosy and drug development where it was air dried for two weeks after which it was grounded into fine powder using pestle and mortar, the dried samples were kept in tight bottles.

2.2. Preparation of Plant Extracts

Grounded plant sample (200g) of the leaf and stem bark was macerated successively in ethanol and aqueous for 24 hrs each. The extracts were then filtered under vacuum and the filtrates concentrated at 46°C using a rotary evaporator. The extracts were evaporated to dryness on a water bath. The extracts were stored in airtight sample bottles and kept in a desiccator until required.

2.3. Detection of Phytochemical Compounds in Plant Extracts

Qualitative phytochemistry screening of aqueous and ethanolic extracts of plant materials was conducted using standard methods described by Harborne, [6]; Sofowora, [17] and Ayoola *et al.*, [2].

2.3.1. Detection of Alkaloids (Mayer's Reagent Test)

To 2ml of the extracts, few drops of dilute Hydrochloric acid was added to acidify the sample, 2ml of Mayer's reagent i.e Mercury Chloride (HgCl_2) and potassium iodide (KI) in distilled water was added. Formation of white to yellow precipitate indicates the presence of alkaloids.

2.3.2. Detection of Glycosides (Keller-Killani Test)

Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. It was under layered with 1ml of concentrated sulphuric acid. A brown ring at the interface will indicate a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may be formed just gradually throughout the thin layer.

2.3.3 Detection of Flavonoids

To 2ml of extracts, few drops of concentrated NaOH solution was added. Formation of intense yellow colour which becomes colourless on addition of dilute HCl, indicates the presence of flavonoids.

2.3.4. Detection of Saponins

To 2ml of extract was shaken with 5ml of distilled water and the solution was shaken vigorously for 30 seconds. Stable persistent frothing indicates the presence of saponins.

2.3.5. Detection of Phenols (Ferric Chloride Test)

To 2ml of extracts, few drops of Ferric Chloride solution were added. Formation of bluish black colour indicates the presence of phenols.

2.3.6. Detection of Tannins

To 2ml of extracts, 1ml of FeCl_3 was added. A blue-green or blue-black coloration indicates the presence of tannins.

2.3.7. Detection of Steroids

To 2ml of extracts, few drops of concentrated H_2SO_4 will be added. Red colour indicates the presence of steroids.

2.3.8. Detection of Reducing Sugar

To 2ml of extracts, 3-5 drops of Fehling solution A and B were added, and boiled for 30 seconds. Brick-red colour indicates the presence of reducing sugar.

2.3.9. Detection of Terpenoids

To a portion of extracts, equal volume of acetic acid anhydride was added and mixed gently. Blue-green colour indicates the presence of terpenoids.

2.4. Preparation of Extracts for Antibacterial Screening

Blended leaf and stem bark of *Jatropha curcas* powder was dispensed into 100 ml of sterilized distilled water and 5, 10, 15, 20, 25 g respectively such that we have 5g/100ml, 10g/100ml, 15g/100ml, 20g/100ml and 25g/100ml. Each concentration bottle was labelled and left on a mechanical shaker overnight at room temperature.

2.5. Preliminary Antibacterial Screening:

The activity was tested, using the disk diffusion method on clinical isolates of *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella dysenteriae* which are common pathogens that could be easily encountered. These clinical isolates were obtained from Department of Microbiology and Chemical Pathology, Ladoke Akintola University of Technology Teaching Hospital, Oyo State, Nigeria. Sterilized blank disks of 6 mm diameter were punched from qualitative filter paper sheets and dipped into the prepared crude *Jatropha curcas* extract (Pundir *et al.*, 2010). The disks were allowed to dry in the laminar flow. The direct colony suspension method was used by selecting at least three to five well-isolated colonies of the same morphological type from an agar plate culture of each of those clinical isolates. The top of each colony was touched with sterile swab and transfer the growth into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth. The tubes were incubated for 18 to 24 hrs.

Turbidity of the same standard was archived through dilution of highly turbid samples. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension, rotated the swab several times and pressed firmly on the inside wall of the tube above the fluid level. This removes excess fluid from the swab. The dried surface of MHA plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking all other clinical isolates on MHA plates. The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganisms (Kumar, *et al.*, 2007). The disks containing different concentrations of the crude extracts were aseptically placed on the seeded plates and then placed in an incubator. The inhibition zones were measured after 24 h of incubation at 37°C. All tests were run in duplicate, (Obiukwu and Nwanekwa, 2009).

2.6. Interpretation of Antibacterial Activities

Antibacterial activity was recorded at 5g/100ml, 10g/100ml, 15g/100ml, 20g/100ml and 25g/100ml of the crude extract of both Aqueous and ethanolic solvent to inhibit the growth of the tested clinical isolates as indicated in Table 2

3. Results and Discussion

Table 1. Qualitative photochemistry of *Jatropha curcas* in ethanolic and aqueous extracts

Secondary metabolites	Ethanolic Extract		Aqueous extracts	
	Stem bark	Leaf	Stem bark	Leaf
Alkaloids	+	–	–	–
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	–	–	–
Phenols	+	+	+	+
Tannis	+	+	+	+
Steroids	+	+	+	+
Reducing sugar	+	+	+	+
Terpenoids	+	+	+	+

Key: + = Positive
– = Negative

Result of the various phytochemical constituents present or absent in both ethanol and aqueous extract of *J.*

curcas is shown in Tables 1. The ethanolic extract of stem bark as presented in Table 1 shows the presence of Alkaloids, Glycosides, Flavonoids, Saponins, Phenols, Tannis, Steroids, Reducing sugar and Terpenoids while all these phytochemicals are also present in ethanolic extract except for Alkaloids and Saponins. Presence of these compound in this research contradict the work of Wakirwa *et al.* (2012). However, the variation observed could be justified by different geographical location of the plant. It is also worthy to note that presence of these compound can made them to have some beneficial biological properties [10].

Table 1 showed the aqueous extract of both the stem bark and leaf, Secondary metabolites present in stem bark and leaf include Glycosides, Flavonoids, Phenols, Tannis, Steroids, Reducing sugar and Terpenoids. Alkaloids and Saponins are found to be absent in both the leaf and the stem bark. The presence of the observed compound are in line with the findings of Obasi *et al.* (2011). The study by Namuli *et al.* [1] confirmed the fact that stem bark, root bark and kernel meal of *J. curcas* contained compounds with antibacterial activities which made them potential source of antibacterial compounds. There is therefore the need for explicit exploitation of these compounds in inhibiting emerging infectious micro-organisms. These has led to further analysis by subjecting the crude extract of both stem bark and leaf to antibiotics sensitivity testing.

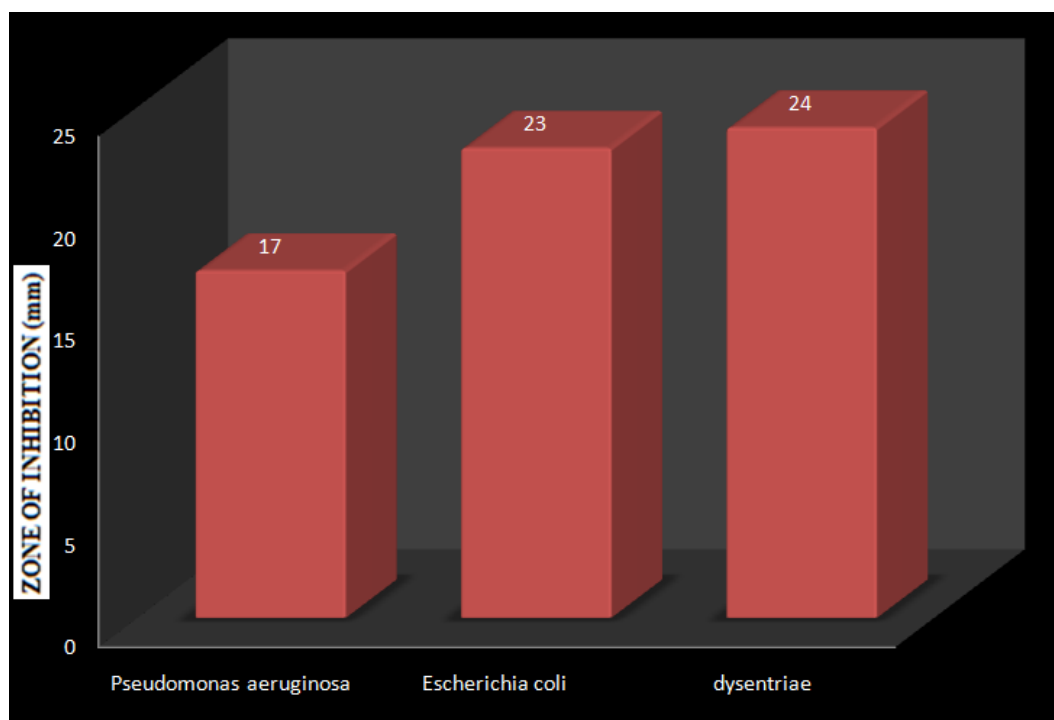


Figure 1. Susceptibility pattern of the tested clinical isolate to the aqueous extract of *J. curcas*

Table 2. (Mean) Minimum inhibition concentration for both Leaves Extract and Stem bark Extract

Clinical isolates	Concentration (grams)	Leaves Extract					Stem bark Extract				
		5	10	15	20	25	5	10	15	20	25
<i>Pseudomonas aeruginosa</i>		17	17	17	16	17	5	6	5	5	7
<i>Escherichia coli</i>		19	17	23	21	20	7	2	5	5	5
<i>Shigella dysentriae</i>		19	19	24	20	22	7	6	5	5	7

*Mean values are measured in (mm)

Table 2 showed the Minimum inhibition concentration (MIC) as the lowest concentration of extract to prevent growths of a particular pathogen, these revealed the relationship between the minimal inhibitory concentrations

of a hypothetical drug and the size of the zone around a disk in which microbial growth is inhibited. The effect of the aqueous extract of *J. curcas* (Leaves Extract) was observed on three clinical isolates of gram-negative

bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella dysenteriae*) as presented in Figure 1. As the sensitivity of the microorganisms to the extract increase, the MIC values decreases and the inhibition zone grows larger.

The crude extract of Leaves Extract from aqueous solvent exhibited some level of susceptibility to the tested isolates compared with Stem bark Extract with values ranging from 17 mm (*Pseudomonas aeruginosa*,) at 5g/100ml, 23 mm (*Escherichia coli*) at 15g/100ml to 24 mm in *Shigella dysenteriae* at 15g/100ml. Observations show that the highest level of inhibition was found in *Shigella dysenteriae* followed by *Escherichia coli*. Crude extracts aqueous solvent of *J. curcas* seeds has previously being reported by Sarin *et al.* [20] to inhibit *E. coli* at 30 mg/ml and 50 mg/ml; *P. aeruginosa* at 50 mg/ml, *S. aureus*, *B. cereus*, and *B. megaterium* at different concentrations except for *Salmonella typhi* which was not inhibited by any of the crude extracts, these findings corroborate with our outcomes. Similar study was also carried out by Kalimuthu *et al.* [10]; Ekundayo *et al.* [22] which was also in line with the present result, findings shows that the inhibitory effects of the aqueous extracts of both stem bark and leaves of *J. curcas* can introduce the plant as a potential candidate for the treatment of ailments caused by these pathogens. However result from stem bark aqueous extract was not encouraging which is an indication of its low potency to inhibit the tested clinical isolates. The antibacterial activities potency of these extract may becomes manipulated when coupled with ethanolic solvent, these has justified our decision in carrying out further analysis on the antibacterial activities of the ethanolic extracts.

4. Conclusion

In general, the results obtained in this finding have established the fact that *Jatropha curcas* leaves and stem bark contains some phytochemicals which can inhibit the growth of the tested pathogenic organisms (bacteriostatic). The significant inhibitory effect of the aqueous extract on *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigella dysenteriae* is an indication that this plant could serve as a potential source of active antimicrobial agents. However, there is need to assess the side effect that might accompany the usage of this plant in folklore medicine.

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