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Phytochemistry and Antimicrobial Assay of *Jatropha curcas* Extracts on Some Clinically Isolated Bacteria - A Comparative Analysis

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Abstract: Increasing awareness of multi-drug resistant strains of bacteria and the hazards associated with the use of synthetic antimicrobial agents has accelerated investigations involving plant extracts as possible alternative drugs. The phytochemical analysis of the methanol extract of the leaf, stem bark and root of *Jatropha curcas* linn were carried out using standard methods and revealed the presence of alkaloids, flavonoids, saponins, glycosides, tannins, terpenoids, resins and steroids in varying abundances. The antibiotic assay was determined *in vitro* on the following bacteria viz; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella typhi* by agar diffusion cup method. Inhibition zone diameters were determined in triplicate after incubation at 37°C for 24 hours. It was shown that the methanol extracts of *J. curcas* are active against both gram-positive and gram-negative organisms with extracts of the root and stem bark having wider activity than the leaf extract. This study is a contribution towards the use of screened herbal extracts and phyto-drugs to cure diseases like diarrhea, intestinal tract, throat, skin and other microbial infections. By this study, methanolic extracts of *J. curcas* have shown great potential for use as herbal drug.

Key words: Agar diffusion · Antimicrobial · Phytodrugs · Phytochemistry · Inhibition zone · *Jatropha curcas* · Methanolic extract

INTRODUCTION

Consumer's attitude towards hygiene and active lifestyle has created a rapidly increasing market for a wide range of synthetic and biofunctional products with antimicrobial activity, which in turn has stimulated intensive research and development [1, 3]. Due to the relatively lower incidence of adverse reactions of natural products in comparism with synthetic pharmaceuticals, they can be exploited as an attractive ecofriendly alternative for antimicrobial therapy. As a result, the number of biofunctional products with antimicrobial property has increased considerably over the last few years [2, 4]. In near future, biomedical products and phyto-drugs will perhaps be the largest application as antimicrobial agents [5, 6].

Besides, plant based antimicrobial represent yet a vast untapped source for medicines and hence, further exploration is needed. Medicinal plants are finding their way into pharmaceuticals, cosmetics and food supplements. This increasing interest on traditional ethnomedicine may lead to discovery of novel therapeutic agents. *Jatropha curcas* linn belongs to the family Euphorbiaceae and are used in traditional folk medicine to cure various ailments in Africa, Asia and Latin America [7]. It is a perennial drought-resistant shrubs or tree which grows up to a height of 5m distributed in Brazil, India, Jamaica, Panama, Salvador, Central and South America, South-east Asia and Africa [8, 9]. The interest in the scientific investigation of *J. curcas* is based on the claims of its effective use for the treatment of many diseases [10]. It has been reported that *J. curcas* latex is active against

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Staphylococcus spp. and *Escherichia coli* and active in the treatment of sores, ulcers and inflamed tongues [10]. *Jatropha* species (*J. integerrima, J. podagrica, J. nudicaulis, J. berlandieri)* are ornamental plants locally referred to as 'Omengwa' due to its common use in hastening cassava fermentation in Ibagwa-Aka and other parts of the South-eastern Nigeria.

Biomolecules of plant origin appear to be one of the alternatives for the control of antibiotic resistant human pathogens [11]. In this regard, plants have given the western pharmacopia about 7000 different pharmaceutically important compounds and a number of top-selling drugs of modern time, eg. quinine, artemisinin, taxol, camptothecin etc [12]. Until natural products have been approved as new antibacterial drugs, there is urgent need to identify novel substances active against highly resistant pathogens. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this research has been to evaluate the phytochemistry and antimicrobial-efficacy of *Jatropha curcas* extract on some clinically important multi-drug resistant bacteria.

MATERIALS AND METHODS

Plant Materials: The fresh leaves, stem bark and roots of *Jatropha curcas* were collected from Ibagwa-Aka, Igbo-Eze South Local Government Area of Enugu State (August-October, 2012). The plant was identified and confirmed using standard manuals by Mr. J.C. Onyeukwu of Plant Science and Biotechnology Department, University of Nigeria, Nsukka.

Preparation of Plant Materials: The leaves, stem bark and root of the plant were washed with distilled water, air-dried to constant weight and separately pulverized into powder using Thomas Wiley Laboratory mill, model 4. The powdered plant materials (1000 g each) were extracted with 7.0L of methanol by cold maceration at room temperature for seven days. The extracts were filtered with chess cloth and then with Whatman No.1 filter paper to obtain a clear filtrate. This clear filtrate was concentrated *in vacuo* with a rotary evaporator (Buchi, CH-9Z30 Switzerland) and dried by evaporation at room temperature. The extracts were stored in the refrigerator at about 5°C throughout the period of experiments.

Test Organisms: Clinical isolates of the test microorganisms were obtained from the University of Nigeria Medical Centre, Nsukka which were donated to the Department of Microbiology, University of Nigeria, Nsukka and include; *staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella typhi*.

Phytochemcial Analysis: The phytochemcial analysis of the extracts were performed using standard methods [13, 14].

Antimicrobial Assay: The agar diffusion cup method [15] were used for the antimicrobial assay. The seeded agar in the plates were bore holed using 6 mm diameter cork bore. 0.3 ml each of the extract concentration was introduced into the hole and allowed to diffuse for 5-10 minutes before incubation at 37°C for 24 hours. The inhibition zone diameters (IZD) were determined and recorded for further analysis. Amplicillin was used as a control.

RESULTS

Table 1 shows the analysis and relative abundance of phytochemicals in different parts of *Jatropha curcas*. Results revealed that the root and stem bark contained alkaloids, glycosides, saponins and terpenoids in high abundance. Steroids and tannins were in moderate amount in the root. Tannins were very low in stem bark but the leaf showed moderate abundances for all the phytochemicals present.

Tables 2 to 4 shows the *in vitro* antimicrobial analysis of the methanol extract of different parts of *Jatroph curcas*. Results revealed that the root and stem bark had higher inhibition zone diameters than the leaf extract.

The leaf extract showed activity only against *E. coli* and *Ps. aeruginosa*. The inhibition was found to be maximum against *staph. aureus*, followed by *E. coli*¸ *B. subtilis* and *Ps. aeruginosa* while *Sal. typhi* was resistant to all the extracts concentrations.

DISCUSSION

The screening of secondary metabolites and antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents [15, 16]. Reports have also shown that most of the

Table 1: Qualitative phytochemcial screening of methanol extract of various parts of *Jatropha. curcas* linn.

	Plant part/relative abundance		
	Root	Stem bark	Leaf
Alkaloids	+++	+++	++
Glycosides	+++	+++	++
Saponins	+++	+++	++
Tannins	++	+	++
Flavonoids	++	++	++
Resins	++	+++	++
Terpenoids	+++	+++	++
Steroids	++	++	++
Proteins	-	-	-
Fats and oil	-	-	-

Key: +++ = high abundance, ++ = moderate abundance, + = low abundance, - = Not detected.

Table 2: Inhibition Zone Diameters (mm) at various concentrations (mg/ml) of the Methanol extract of *J. curcas* root.

	200	*100*	*50*	*25*	*12.5*	*6.25*	*3.125*	*1.5625*
Test organism								
Staph. aureus	13.10±0.6	12.00±0.00	9.80±0.11	8.20±0.11	7.03±0.03	6.00±0.00	4.93±0.33	3.57±0.12
E. coli	10.00±0.00	9.10±0.06	7.53±0.09	6.00±0.00	5.23±0.15	4.00±0.00	3.03±0.03	+
B. subtilis	10.00±0.00	9.00±0.00	8.03±0.03	7.00±0.00	6.10±0.06	5.00±0.00	4.10±0.06	3.00±0.00
Ps. aeruginosa	8.06±0.06	7.17±0.09	6.00±0.00	5.00±0.00	4.00±0.00	3.00±0.00	+	+
Sal. Typhi	+	+	+	+	+	+	+	+

Key: + means growth or No activity.

Table 3: Inhibition Zone Diameters (mm) at various concentrations (mg/ml) of the Methanol extract of *J. carcas* stem bark.

Concentration	*200*	*100*	*50*	*25*	*12.5*	*6.25*	*3.125*	1.5625
Test Organism								
Staph. aureus	10.13±0.08	9.133±0.13	8.00±0.00	6.27±0.15	5.03±0.03	4.10±0.06	3.00±0.00	+
E. coli	12.00±0.00	11.00±0.00	9.17±0.08	7.23±0.07	6.00±0.00	4.63±0.08	3.77±0.14	+
B. subtilis	11.07±0.07	10.00±0.00	8.60±0.06	6.10±0.10	5.00±0.00	4.00±0.00	3.00±0.00	+
Ps. aeruginos	10.06±0.07	9.13±0.13	7.06±0.07	6.03±0.03	5.10±0.10	4.00±0.00	3.06±0.07	+
Sal. Typhi	+	+	+	+	+	+	+	+

Key: + means growth or No activity.

Table 4: Inhibition Zone Diameters (mm) at various concentrations (mg/ml) of the Methanol extract of *J. carcas* leaves.

Concentration	*200*	*100*	*50*	*25*	*12.5*	*6.25*	*3.125*	1.5625
Test organism								
Staph. aureus	+	+	+	+	+	+	+	+
E. coli	8.10±0.10	7.00±0.00	6.10±0.06	5.20±0.06	4.00±0.00	3.00±0.00	+	+
B. subtilis	+	+	+	+	+	+	+	+
Ps. aeruginosa	6.07±0.07	5.00±0.00	4.00±0.00	3.00±0.00	2.67±0.09	+	+	+
Sal. Typhi	+	+	+	+	+	+	+	+

Key: + means growth or No activity.

antibacterial principles were either polar or non-polar and were extracted only through the organic solvent medium [17]. Hence, the present investigation suggested that the organic solvent extraction method is also suitable to determining antimicrobial assay.

On the basis of the results obtained from the present investigation, we conclude that the methanol extract of *Jatropha curcas* linn had significant *in vitro* antimicrobial activity. Again, the graim-positive bacteria were more susceptible to the extracts than gram-negative bacteria. Possibly because of the presence of outer membrane that serves as an effective barrier in gram-negative species [18, 19].

The presence of secondary metabolites; alkaloids, glycosides, saponins, tannins, flavonoids terpenoids, resins and steroids in *Jatropha* species which are known to have antimicrobial activity have been reported [20-22].

The presence of tannins, saponins, steroids, alkaloids, flavonoids and glycosides in the methanol extract of *J.curcas* stem bark has been reported[20] which

is in agreement with the present study. However, the present study contradicts[22] who reported the absence of tannins and flavonoids in the methanol extract of *J.curcas* stem bark. Table 1 shows that the root and leaf methanol extracts also contain these secondary metabolites at varying abundances.

The results of antimicrobial assay as presented in table 2 to 4 showed that the root and stem bark methanol extracts were active against both gram-positive and gram-negative bacteria while the leaf extract was active against (gram-negative) *E. coli* and *Ps. aeruginosa* with inhibition zone diameters of 8 mm and 6 mm respectively. These results agree with those of [21], using the filter paper disc method, their data indicated that gram-negative *E. coli* was the most sensitive strain among the tested organisms with ethanolic extract of *J.curcas* leaves, with inhibition zone of 11 mm. It also showed moderate levels of antimicrobial activity against *Pseudomonas spp.* with inhibition zone diameter of 6 mm for *Ps. aeruginosa*. The little discrepancy in inhibition zone diameter for *E. coli* could be due to differences in extracting solvent.

CONCLUSION

In order to halt the trend of increased emerging and resistant infectious disease, it will require a multi-prolonged approach that includes the development of new drugs. Obtained results may provide a support to the use of plant in traditional medicine. Based on this, a further chemical and pharmacological investigation to isolate and identify the chemical constituents in *Jatropha curcas* linn and to screen other potential bioactivities is hereby recommended.

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