

Full Length Research Paper

Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn)

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The *in vitro* antimicrobial activity of crude ethanolic, methanolic and water extracts of the stem bark of *Jatropha curcas* were investigated. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 5 to 12, 8 to 20 and 0 to 8 mm for ethanol, methanol and water extracts respectively. The minimum inhibitory concentration (MIC) of the ethanol extract was between 0.5 and 6.25 mgml⁻¹ while that of methanol extract ranged from 0.5 to 10 mgml⁻¹. The minimum bactericidal concentration (MBC) for ethanol extract ranged between 2.0 and 12.50 mgml⁻¹, while that of methanol ranged from 2.0 to 20 mgml⁻¹. Again all the extracts exhibited appreciable activity against all the fungal species investigated. The zones of inhibition exhibited by the extracts against the test fungal species ranged between 15 and 18, 15 and 20 and 5 and 10 mm for ethanol, methanol and water extracts respectively. Phytochemical screening revealed the presence of saponin, steroids, tannin, glycosides, alkaloids and flavonoids in the extracts. The ability of the crude stem extracts of *J. curcas* to inhibit the growth of bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections.

Key words: *Jatropha curcas*, antimicrobial activity, phytochemical screening, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC).

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Their role is two fold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases (Iwu, 1993). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). *Jatropha* species belong to the family Euphorbiaceae and are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America (Burkill, 1994). *Jatropha 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, jaundice, guinea worm sores and joint heumatism (Irvine, 1961; Oliver-Bever, 1986). Fagbenro-Beyioku (1998) inv-

estigated 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 cytotoxicity (Matsuse et al., 1999). Previous works have shown that many *Jatropha* species possess antimicrobial activity (Aiyela-agbe et al., 2000; Aiyelaagbe, 2001). Several studies have confirmed the antimicrobial efficacy of different *Jatropha* species; however, there is insufficient information regarding the antimicrobial activities of *J. curcas* Linn. Whatever limited information available on the medicinal properties of *J. curcas* is mostly on the leaf extracts of the plant. In this paper, the antimicrobial property of crude extracts of the stem bark of *J. curcas* has been studied as part of the exploration for new and novel bio-active compounds.

MATERIALS AND METHODS

Plant materials and preparation of extract

Fresh stem bark of *Jatropha curcas* was collected from a local farm in Benin City, Edo State, Nigeria in the month of August, 2008 and was identified by the Botany Department of Ambrose Alli University,

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Ekpoma, Nigeria. Voucher sample was prepared and deposited in the Herbarium for reference. The fresh stem bark was air-dried to constant weight, pulverized in a mill (Pye Unicam, Cambridge, England) and stored in an air-tight container for further use. Exactly 250 g of the pulverized plant material was cold extracted in ethanol and methanol separately. Another 250 g of plant material was extracted in water for 4 days with occasional shaking (Harborne, 1998). Ethanol and methanol used were of analytical grade. The separated extracts were then filtered through Whatman's No. 1 filter paper and the ethanol and methanol filtrate were separately concentrated to dryness *in vacuo* using a rotary evaporator to remove the ethanol and methanol. The aqueous extract was lyophilized to obtain a dry powder extract.

Test microorganisms

The test microorganisms used in this study (bacteria: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus epidermidis*, *Shigella dysenteriae*, *Micrococcus kristinae*, *Klebsiella pneumonia*, *Bacillus cereus*, *Bacillus subtilis*, *Proteus vulgaris* and *Serratia marcescens*; fungi: *Trichophyton longifusus*, *Candida glabrata*, *Fusarium solani*, *Microsporium canis*, *Aspergillus flavus*, *Candida albicans*, *Aspergillus niger* and *Penicillium notatum*) were obtained from the culture collections of the National Institute on Aging at Harbor Hospital, Baltimore, Maryland, USA. The bacterial isolates were first sub-cultured in a nutrient broth (Oxoid) and incubated at 37°C for 18 h while the fungal isolates were subcultured on a Sabouraud dextrose agar (SDA) (Oxoid) for 72 h at 25°C.

Phytochemical analysis of the plant extract

The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, steroid, alkaloids and glycosides in accordance with Trease and Evans (1989) and Harborne (1998) with little modification.

Antibacterial activity

The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method described by Irobi et al. (1994). The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (10^6 cfu ml⁻¹). Two hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar (Oxoid). Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 100 µl of the crude extract at 10 mg ml⁻¹ were introduced into the wells, allowed to stand at room temperature for about 2 h and then incubated at 37°C. Controls were set up in parallel using the solvents that were used to reconstitute the extract. The plates were observed for zones of inhibition after 24 h. The effects were compared with those of streptomycin and ampicillin at a concentration of 1 mg/ml and 10 µg ml⁻¹ respectively.

Antifungal activity

The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) (Oxoid) at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. The harvested fungal spores and bacterial isolates were standardized to an OD_{600nm} of 0.1 before use. One hundred microliter of the standardized fungal spore suspension was evenly spread on the SDA (Oxoid) using a glass spreader. Wells were then bored into the agar media using a st-

erile 6 mm cork borer and the wells filled with the solution of the extract taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the media. Plates were incubated at 25°C for 96 h and later observed for zones of inhibition. The effect of the extract on fungal isolates was compared with amphotericin B and miconazole at a concentration of 1 mg/ml.

Minimum inhibitory concentration (MIC)

The estimation of MIC of the crude extracts was carried out using the method of Akinpelu and Kolawole (2004). Two-fold dilutions of the crude extract was prepared and 2 ml aliquots of different concentrations of the solution were added to 18 ml of pre-sterilized molten nutrient agar and SDA for bacteria and fungi respectively at 40°C to give final concentration regimes of 0.050 and 10 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry under laminar flow before streaking with 18 h old bacterial and fungal cultures. The plates were later incubated at 37°C for 24 h and at 25°C for up to 72 h for bacteria and fungi respectively, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Minimum bactericidal concentration (MBC)

The MBC of the plant extracts was determined by a modification of the method of Spencer and Spencer (2004). Samples were taken from plates with no visible growth in the MIC assay and sub-cultured on freshly prepared nutrient agar plates and SDA plates, and later incubated at 37°C for 48 h and 25°C for 72 h for bacteria and fungi respectively. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates. The MBC was not determined for water extract since the antibacterial activity was low.

RESULTS AND DISCUSSION

Antibacterial activity

All three extracts of the plant tested showed varying degree of antibacterial activities against the test bacterial species (Table 1). The antibacterial activities of the ethanol and methanol extracts compared favourably with that of two standard antibiotics (streptomycin and ampicillin) and have appeared to be broad spectrum as its activities were independent on gram reaction. The inhibition zone for *Klebsiella pneumonia* was much less (0 - 8 mm) as compared to other bacteria. The methanol extract (inhibition zone 8 - 20 mm) was found to be more effective than the ethanol extract (inhibition zone 5 - 12 mm) against all the organisms. The water extract showed low antibacterial activity with inhibition zones ranging between 0 and 8 mm for different bacteria tested. The minimum inhibitory concentration (MIC) of the ethanol extract for different organisms ranged between 0.5 and 6.25 mg ml⁻¹, while that of the methanol extract ranged between 0.5 and 10 mg ml⁻¹. Also the MIC of streptomycin control ranged between 0.065 and 0.5 mg ml⁻¹ (Table 2).

Table 1. Antibacterial activities profile of three extracts from the stem bark of *J. curcas*.

Test bacteria	Zone of inhibition (mm) (Mean ± SD)				
	Ethanol (10 mgml ⁻¹)	Methanol (10 mgml ⁻¹)	Water (10 mgml ⁻¹)	ST (1 mgml ⁻¹)	AMP (10µml-1)
<i>Staphylococcus aureus</i>	10 ± 0.4	20 ± 0.2	5 ± 2.0	20 ± 0.3	0 ± 0
<i>Pseudomonas aeruginosa</i>	12 ± 0.5	16 ± 1.1	4 ± 1.1	24 ± 1.5	0 ± 0
<i>Escherichia coli</i>	11 ± 0.2	14 ± 0.2	7 ± 0.5	0 ± 0	15 ± 0.4
<i>Streptococcus faecalis</i>	9 ± 1.5	11 ± 1.2	2 ± 0.1	23 ± 1.0	16 ± 0.1
<i>Staphylococcus epidermidis</i>	11 ± 1.0	15 ± 2.1	2 ± 1.3	22 ± 0.5	0 ± 0
<i>Shigella dysenteriae</i>	12 ± 0.0	16 ± 1.3	4 ± 0.2	25 ± 1.5	0 ± 0
<i>Micrococcus kristinae</i>	11 ± 0.8	12 ± 0.1	8 ± 1.5	15 ± 0.5	17 ± 1.5
<i>Klebsiella pneumonia</i>	5 ± 0.3	8 ± 0.3	0 ± 0	0 ± 0	0 ± 0
<i>Bacillus cereus</i>	10 ± 1.0	16 ± 1.5	5 ± 0.8	20 ± 1.1	0 ± 0
<i>Bacillus subtilis</i>	12 ± 0.5	14 ± 1.1	5 ± 1.2	22 ± 1.8	0 ± 0
<i>Proteus vulgaris</i>	14 ± 0.2	14 ± 0.5	7 ± 0.1	18 ± 1.2	22 ± 1.2
<i>Serratia marcescens</i>	11 ± 1.1	15 ± 0.1	3 ± 1.4	25 ± 0.4	15 ± 0.5

ST- Streptomycin; AMP-Ampicillin

Table 2. The MIC and MBC regimes of the extracts of the stem bark of *J. curcas*.

Test bacteria	Ethanol (mgml ⁻¹)		Methanol (mgml ⁻¹)		Water (10 mgml ⁻¹)	ST (mgml ⁻¹)
	MIC	MBC	MIC	MCB	MIC	MIC
<i>Staphylococcus aureus</i>	5.00	10.0	5.00	10.00	Na	0.065
<i>Pseudomonas aeruginosa</i>	5.00	10.0	5.00	10.00	Na	0.5
<i>Escherichia coli</i>	5.00	10.0	5.00	10.00	Na	Nd
<i>Streptococcus faecalis</i>	2.50	5.00	5.00	10.0	Na	0.065
<i>Staphylococcus epidermidis</i>	0.50	2.00	0.50	2.00	Na	0.065
<i>Shigella dysenteriae</i>	5.00	10.0	10.0	20.00	Na	0.25
<i>Micrococcus kristinae</i>	1.00	4.00	5.0	10.00	Na	0.5
<i>Klebsiella pneumonia</i>	5.00	10.0	10.0	20.00	Nd	Nd
<i>Bacillus cereus</i>	0.50	2.00	10.0	20.00	Na	0.065
<i>Bacillus subtilis</i>	5.00	10.00	6.25	12.50	Na	0.0625
<i>Proteus vulgaris</i>	5.00	10.00	5.0	10.00	Na	0.25
<i>Serratia marcescens</i>	6.25	12.50	10.0	20.00	Na	0.5

ST- Streptomycin; MIC- minimum inhibitory concentration; MBC- minimum bactericidal concentration; Nd- not determined; Na- not active at 10 mgml⁻¹, the highest concentration test.

The minimum bactericidal activity (MBC) of the extract for different bacteria ranged between 2.0 and 12.50 mgml⁻¹ for the ethanol extract and for the methanol extract ranged between 2.0 and 20 mgml⁻¹ (Table 2). Water extract was not active against any of the organism at 10 mgml⁻¹ which was the highest concentration tested. Generally, the methanol extract was more active than other extracts. This may be attributed to the presence of soluble phenolic and polyphenolic compounds (Kowalski and Kedzia, 2007).

The inhibitory effect of the extract of *J. curcas* against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by these pathogens. The non-activity of the water extract against most bacterial strains investigated in this study is in agreement with previous

works which show that aqueous extracts of plant generally showed little or no antibacterial activities (Koduru et al., 2006; Aliero et al., 2006; Ashafa et al., 2008; Aiyegoro et al., 2008). Mujumdar et al. (2001) also reported that the crude methanol extract from the root of *J. curcas* exhibited anti-diarrhea activity in mice through the inhibition of prostaglandin biosynthesis and the reduction of osmotic pressure. Recently, Aiyelaagbe et al. (2007) reported that the presence of some secondary metabolites in the root extract of *J. curcas* inhibited some microorganisms isolated from sexually transmitted infections.

Antifungal activity

The three extracts showed broad antimycotic activity aga-

Table 3. Antifungal activity profile of three extracts from the stem bark of *J. curcas*.

Test fungi	Zone of inhibition (mm) (Mean \pm SD)				
	Ethanol (10 mgml ⁻¹)	Methanol (10 mgml ⁻¹)	Water (10 mgml ⁻¹)	Miconazole (1 mgml ⁻¹)	Amphotericin B (1 mgml ⁻¹)
<i>Trichophyton longifusus</i>	15 \pm 0.1	15 \pm 2.5	8 \pm 0	19 \pm 1.2	20 \pm 0.5
<i>Candida glaberata</i>	13 \pm 0.1	18 \pm 2.0	10 \pm 0	21 \pm 1.1	25 \pm 0.8
<i>Fusarium solani</i>	15 \pm 1.1	18 \pm 1.5	10 \pm 0	22 \pm 0.2	20 \pm 1.0
<i>Microsporum canis</i>	12 \pm 0.5	15 \pm 1.2	5 \pm 0	20 \pm 1.2	22 \pm 0.2
<i>Aspergillus flavus</i>	15 \pm 0.2	18 \pm 1.0	5 \pm 0.1	27 \pm 0.1	25 \pm 0.4
<i>Candida albicans</i>	18 \pm 0.5	20 \pm 0.5	10 \pm 0	30 \pm 1.0	28 \pm 2.2
<i>Aspergillus niger</i>	14 \pm 1.5	16 \pm 1.5	8 \pm 0	25 \pm 0.2	25 \pm 0.5
<i>Penicillium notatum</i>	15 \pm 0.6	19 \pm 1.1	5 \pm 0	23 \pm 2.1	27 \pm 1.1

inst the tested fungal isolates at a final concentration of 10 mgml⁻¹ (Table 3) and the performance of the three extracts were similar to the antibacterial activity. The susceptibility of these fungi to *J. curcas* extracts is significant, as most of these fungi have recently been implicated in cases of immuno-compromised patients who frequently develop opportunistic infections (Portillo et al., 2001). Generally the methanol extract had the highest activity against both bacterial and fungal isolates. This was followed by the ethanol extract and the least was observed in the water extract. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum anti-microbial potential of *J. curcas*, which makes the plant a candidate for bioprospecting for antibiotic and antifungal drugs.

Phytochemical screening

Investigations on the phytochemical screening of *J. curcas* stem bark extracts revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *J. curcas*. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with proline-rich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). These observations therefore support the use of *J. curcas* in herbal cure remedies. Li and Wang (2003) reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *J. curcas* has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of

tannins in *J. curcas* supports the traditional medicinal use of this plant in the treatment of different ailments.

Another secondary metabolite compound observed in the stem bark extract of *J. curcas* was alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori et al., 1994). Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). Just et al. (1998) revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in *J. curcas* extracts and has supported the usefulness of this plant in managing inflammation. Steroidal compounds present in *J. curcas* extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001). Quinlan et al. (2000) worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann et al. (2004) also confirmed the antiviral property of steroids. Flavonoids, another constituent of *J. curcas* stem bark extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angiogenic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002). Different parts of *J. curcas* contain the toxic alkaloids curcin and phorbol ester which prevent animals from feeding on it. Hence, the presence of these compounds in *J. curcas* corroborates the antimicrobial activities observed. It is concluded that *J. curcas* stem bark could be a potential source of active antimicrobial agents, and a detailed assessment of its *in vivo* potencies and toxicological profile is ongoing.

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