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Full Length Research Paper

Antimicrobial activities and phytochemical screening of pignut (*Jatrophas curcas* Linn.) on some pathogenic bacteria

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The use of antimicrobial agents for the control of pathogenic bacteria is helpful in the treatment of infections and diseases. Hence, there is need to investigate the antimicrobial properties of plant extracts that have not been done. The ethanolic extracts of the leaf and bark of *Jatrophas curcas* Linn. (Euphorbiaceae) were tested for antimicrobial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Salmonella typhi* and *Shigella dysenteriae* by agar well diffusion method. The ethanolic extracts inhibited the above pathogenic bacteria with zones of inhibition ranging from 30.6 to 38.5 mm and the minimum inhibitory concentration (MIC) ranging from 2.2 to 10.0 mm. The leaf and bark extracts of *J. curcas* have high concentration of tannin, saponin, flavonoid, steroid, alkaloid, cardiac glycoside, anthraquinone and terpenoid. The ability of the ethanolic extracts of the leaf and bark of *J. curcas* to inhibit growth of the test bacteria is an indication of its antimicrobial potency which may be employed in treatment of microbial infections.

Key words: *Jatrophas curcas*, antimicrobial agents, minimum inhibitory concentration, ethanolic extracts, pathogenic bacteria.

INTRODUCTION

The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries. The intractable problems of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of noble compound to suppress or possibly eradicate the ever increasing problems of emergence of newer diseases though to be brought under control (Wurochekker et al., 2007). *Jatrophas* species belong to the family Euphorbiaceae and are used in traditional forklore medicine to cure ailments in Africa, Asia and Latin America (Burkill, 1994). *Jatrophas curcas* 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 rheumatism (Oliver-Bever, 2000). Aiyelaagbe (2001) 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. Previous works have shown that many *Jatrophas* species possess antimicrobial activities (Aiyelaagbe, 2001).

The comparison of phytochemicals of old and new growth stem bark and leaves from nine trees used medicinally in Nigeria shows that *J. curcas* contains saponin, tannin, glycoside, steroid, alkaloid and flavonoid (Fasola and Egunyomi, 2005). 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

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mechanisms. Herbs that have tannin as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Kan, 2002). In this paper, the antibacterial potency of ethanolic extract of the leaf and bark of *J. curcas* was investigated.

MATERIALS AND METHODS

Plant materials and preparation of extract

Healthy leaves and bark of *J. curcas* were collected from Apatapiti, Federal University of Technology, Akure, Ondo State in 2009. The leaves and stem bark were taken and homogenised separately with 75% ethanol. The extracts were filtered (Whatman No1 filter paper) and the filtrates were dried at room temperature (Kalimuthu et al., 2010).

Test bacteria

Pathogenic bacterial cultures used in this study; *Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Klebsiella pneumonia, Bacillus subtilis* and *Proteus vulgaris* were obtained from Microbiology Laboratory of Lagos State University Teaching Hospital (LUTH) Surulere, Lagos, Nigeria. The cultures were grown at 37°C for 24 h on nutrient agar.

Phytochemical analysis of the plant extract

The extracts were subjected to both qualitative and quantitative phytochemical tests for plant secondary metatobolites; tannins, phylobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, alkaloids, anthraquinones and phenols according to the method described by Harborne (1998).

Antibacterial screening

The antibacterial activities of the crude plant extract were evaluated in accordance with the agar well diffusion method described by Nair and Chando (2005). The test bacteria were first cultured on nutrient broth for 18 h before use. Nine fold serial dilution was performed on each test bacterium and 0.2 ml of the ninth diluents was dispensed on sterile Petri dishes mixed with molten cooled sterile nutrient agar. The plates were allowed to set. Wells were then bored into the agar using 5 mm diameter sterile cork borer. Approximately 0.2 ml of the reconstituted crude extract was introduced into the wells, allowed to stand at room temperature for about 2 h and then incubated at 37°C for 24 h. Controls were set up in parallel using the solvent (sterile distilled water) that was used to reconstitute the crude extract. The plates were observed for zones of inhibition after 24 h. All the experiment was carried out in triplicate.

Minimum inhibitory concentration (MIC)

The MIC estimation of the crude extract was determined using the methods of Sahm and Washington (1999) and Akinpelu and Kolawole (2004). An aliquot (1 ml) of the extract solution at concentration of 20 mg/ml was added to 1 ml of presterilized nutrient broth. Subsequently, 1 ml from the first test tube was transferred to the second test tube and this continued up to the seventh test tube. Thereafter, 1 ml of 24 h of each test bacterium (1.0×10^6cell/ml) was inoculated into each test tube and mixed thoroughly. The test tubes were then incubated at 37°C for 24 h. The MIC was taken as the lowest concentration that prevented the growth of the test bacterium.

RESULTS

The qualitative estimation of the phytochemicals in the ethanolic extracts of leaf and stem bark of *J. curcas* revealed the presence of tannins, phlobatannins, flavonoids, cardiac glycosides, alkaloids, anthraquinones and total phenols. Stem bark extract has higher percentages of tannins, phlobatannins, flavonoids, cardiac glycosides, alkaloids, anthraquinones and total phenols than the leaf extract of *J. curcas*. However, leaf extract has higher percentages of saponins and steroids than stem bark extract. Terpenoid was present in the stem bark extract but absent in leaf extract.

The quantitative estimation of these secondary metabolites showed that tannins were most abundant for both bark (25.4 ± 0.1) and leaves (23.1 ± 0.1) and anthraquinones were least for leaves (0.1 ± 0.0) and terpenoids for bark (0.2) as shown in Table 1. Ethanolic extracts of both leaves and bark of *J. curcas* showed varying degrees of antibacterial activities on all the test bacteria and have appeared to be broad spectrum as the activities were independent on gram reaction as shown in Table 2. Generally, the antibacterial activity of the ethanolic extract of the leaves was higher except for *B. subtilis* and *E. coli* in which bark extract was higher with zones of inhibition of 38.5 and 36.3 mm, respectively. The MIC of the ethanolic leaf extract for the test bacteria ranged between 2.2 and 5.0 mg/ml while it ranged from 2.5 to 10 mg/ml for bark (Table 3).

DISCUSSION

The inhibitory effects of the ethanolic extracts of leaves and bark of *J. curcas* can introduce the plant as a potential candidate for the treatment of ailments caused by these pathogens. The greater inhibitory ability of *B. subtilis* than *E. coli* by stem bark than leaf extract of *Jatrophas* may be due to higher percentages of tannins, phlobatannin, flavonoid, terpenoid, cardiac glycoside, alkaloid, anthraquinone and total phenol. The inhibitory activity of plant extract is largely dependent on the concentration, parts of the plant used and the microbes tested (Kalimuthu et al., 2010). This might be the reasons for the variation in the results obtained. Only saponin and steroid were at higher percentages in leaf than stem extract. These two compounds might be responsible for inhibition of *K. Pneumoniae, S. aureus, S. typhi, P. aeruginosa, P. vulgaris* and *S. dysenteriae.* Igbinosa et al. (2009) reported that the ethanolic extract of the stem bark of *J. curcas* inhibited *B. subtilis, E. coli, P. vulgaris* which is in agreement with this present study. Kalimuthu et al. (2010) also reported the inhibitory ability of the

Table 1. Quantitative estimation (%) of secondary metabolites of leaves and stem bark of *J. curcas.*

Secondary metabolites	Amount of phytochemicals present (mean ± SD)	
	Leaves	Stem bark
Tannins	23.1 ± 0.1	25.4 ± 0.1
Phlobatannins	4.3 ± 0.1	5.1 ± 0.1
Saponins	16.1 ± 0.1	14.3 ± 0.1
Flavonoids	8.2 ± 0.1	11.0 ± 0.1
Steroids	22.1 ± 0.1	20.2 ± 0.1
Terpenoids	-	0.2 ± 0.3
Cardiac glycosides	3.9 ± 0.1	4.3 ± 0.1
Alkaloids	10.0 ± 1.2	12.0 ± 0.2
Anthraquinones	0.1 ± 0.0	1.1 ± 0.3
Total phenols	0.2 ± 0.1	0.6 ± 0.2

- = not present.

Table 2. Antibacterial activities profile of ethanol extract of leaves and stem bark of *J. curcas* at 20 mg/ml.

Test bacteria	Zones of inhibition (mm)	
	Leaves	Stem bark
Bacillus subtilis	33.3	38.5
Escherichia coli	31.3	36.3
Klebsiella pneumonia	33.0	32.6
Staphylococcus aureus	33.0	30.6
Salmonella typhi	34.3	34.0
Pseudomonas aeruginosa	33.0	32.3
Proteus vulgaris	34.0	32.0
Shigella dysenteriae	32.7	32.6

Table 3. The MIC regimes (mg/ml) of the extracts of leaves and stem bark of *J. curcas*.

Test bacteria	Leaves	Stem bark
Bacillus subtilis	5.0	10.0
Escherichia coli	2.5	5.0
Klebsiella pneumonia	2.5	2.5
Staphylococcus aureus	5.0	5.0
Salmonella typhi	2.5	2.5
Pseudomonas aeruginosa	2.2	2.5
Proteus vulgaris	2.5	2.5
Shigella dysenteriae	2.5	2.5

methanolic extract of *in vivo* leaves and *in vitro* derived callus (30 days old) of *J. curcas* against *Pseudomonas, E. coli, Klebsiella* and *S. aureus.*

Ayelaagbe et al. (2007) reported that the presence of some secondary metabolites in the root extract of *J. curcas* inhibited some microorganisms isolated with sexually transmitted infections. Phytochemicals are known to be biologically active and therefore aid the antibacterial property of *J. curcas.* These secondary metabolites exert antimicrobial property through different mechanisms. Tannins have been found to form irreversible complexes with proline rich protein (Shimada, 2006) resulting in the inhibition of protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tannin 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 diarrhoea 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. 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 and leaves extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002). Different parts of *J. curcas* contain the toxic alkaloids curcin and phorbal 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 and leaves could be a potential source of active antimicrobial agents. However, there is need to conduct toxicological assessment of the bark and leaves to ascertain their safety on human.

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