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Antibacterial activity of the stem bark of *Jatropha curcas* L. against four bacteria species

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Abstract. The antibacterial activity of the aqueous and methanolic crude stem back extracts of *Jatropha curcas* L. against four bacteria species (*Pseudomonas aeruginosa*, *Staphyloccus aureus*, *Salmonella typhi* and *Escherichia coli*) was investigated, using agar diffusion technique. At concentrations ranging from 10 - 40 mg/mL, the methanolic crude extract showed activity against the four bacteria (*P. aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *E. coli*) from 2 mm - 20 mm, after 24 h incubation. The aqueous crude extract showed no activity against all the bacteria species investigated. Minimal inhibitory concentration of the crude extract was found to be between 10 - 20 mg/mL. Similarly, the minimal bacteriocidal concentration was between 20 - 25 mg/mL. The phytochemical constituents of the crude extracts include alkaloid, cardiac glycosides, carbohydrates, terpenes, flavonoids, steroids and anthraquinone. More extraction solvents should be employed to ascertain the antibacterial potentials and the phytochemical constituents of the plant.

Keywords: Antibacterial, Activity, Crude extract, Minimal inhibitory concentration, Minimal bacteriocidal concentration.

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1. Introduction

Jatropha curcas L. is a species of flowering plant in the spurge family Euphorbiaceae. It is a native of American tropics, most likely Mexico and Central America (Dharmander, 2003). *J. curcas* exist in sub tropical countries and has a wide spread distribution in Nigeria especially in the Middle Belt Region of the country (Aliero et al., 2006). The plant is a poisonous semi evergreen shrub or small tree, with a height of 6 m (20 feet) and is resistant to high degree of aridity, allowing it to be cultivated in the deserts (Kowalski and Kedzia, 2007). *J. curcas* has been grown in Nigeria for over 50 years.

The plant is commonly used for medicinal purposes. It has a large green to pale green leaves and the flowers are divided into male and female produced on the same inflorescence, averaging 20 male flowers to each female flower. Fruits are produced in winter or they may be several crops during the year if soil moisture is good and temperature is sufficiently high. The seeds mature when the capsule changes from green to yellow (Ashafa et al., 2008). The plant can grow on water lands and grow on almost any terrain even on gravelly, sandy and saline soils. It can also thrive in poor and stony soils. This study therefore was an attempt to determine the antimicrobial potentials of the stem bark extract against four bacterial species namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Samonella typhi* and *Escherichia coli*.

Materials and methods

Collection of plant materials

Stem bark of *J. curcas* was collected from different locations in Bosso, Minna, Niger State in the month of May, 2010. The plant was authenticated at the Botany Unit, Federal University of Technology, Minna, Niger State (herbarium voucher sample was deposited for reference). The bark of the plant was removed by scrapping with a clean knife and it was air dried and ground. Thirty gram (30 g) of the powdered stem bark was mixed with 500 mL of methanol. Similarly, 30 g of the powdered stem bark of the plant was mixed with 500 mL of distilled water for 24 h respectively. The methanol and distilled water mixtures were filtered using Whatsman filter paper. The filtrate were collected separately and concentrated in a steam bath evaporator at 60 °C - 70 °C. The concentrated filtrate were stored in the refrigerator at 40 °C for further investigation.

Test microorganisms

The test organisms in this study include *Staphylococcus aureus*, *P. aeruginosa*, *E. coli* and *Salmonella typhi*. The organisms were collected from the Microbiology Laboratory of the Federal University of Technology, Minna and were identified through biochemical tests.

Susceptibility test

The antibacterial activities of the crude extracts were determined in accordance with the agar well diffusion method described by Abalaka et al. (2011). The bacterial isolates were first grown in a nutrient broth for 6 h and standardized to 0.5 McFarland standard before inoculation on to the media. Wells were then bored onto the agar using a sterile 4 mm diameter cork borer. The crude extracts at 1 - 4 mg/mL were introduced into the wells for about 30 min. The plates were incubated for 24 h. Observation for zones of inhibition after 24 h was done. The activities of the crude extracts were compared to that of chloramphenicol (control) at a concentration of 1 - 4 mg/mL, respectively, and the results were recorded.

Determination of Minimum Inhibitory Concentration (MIC)

Tube dilution method was used. Concentration of the crude extract ranging from 1 - 4 mg/mL was aseptically introduced into four test tubes containing nutrient broth and the test organisms. The mixtures were incubated at 37 °C for 24 hours. The lowest amount of extract that inhibited the growth was considered as the MIC (Abalaka et al., 2011).

Determination of Minimal Bactericidal Concentration (MBC)

The minimal bactericidal concentration was determined by culturing the content of the tube that showed no visible growth in MIC. This was done by plating on fresh agar medium devoid of any antibiotic or the crude extracts. The plates were incubated at 37 °C for 24 h and the results were recorded (Abalaka et al., 2011).

Phytochemical analysis of the plant extract

The crude extract was subjected to phytochemical test to determine the presence of phytochemical constituents. This was done in accordance with methods described by Harbone (1998).

Results

The results of the biochemical tests conducted revealed the following species of bacteria *P. aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *E. coli* (Table 1).

The antibacterial effects and phytochemical constituents of stem bark of *Jatropha curcas* was determined using agar diffusion technique. Phytochemical screening of the plant crude extracts revealed the presence of alkaloids, cardiac glycosides, carbohydrates steroids, terpenes, flavonoids, saponins and tannins.

Table 2 shows the susceptibility pattern of the organisms to the crude extracts of *J. curcas*. The methanolic extract had effect on all the organisms at concentration from 10 - 40 mg/mL and the zone of inhibition ranged from 2 - 20 mm in diameter. The control recorded zone of inhibition ranging 1 - 18 mm.

Table 1. Biochemical characteristics of the four bacteria isolate.

Organism	Cat	Oxi	Cit	Lac	H2S	MR	VP	Nit	Ind	Ure	Mot
E. coli	+	-	-	+	-	+	-	+	+	-	-
P. aeruginosa	+	+	+	-	-	-	-	+	-	-	+
Staphylococcus aureus	+	+	-	+	-	-	-	+	-	+	-
Salmonella typhi	+	-	+	-	+	+	-	+	+	-	+

Cat = Catalase test; Cit = Citrate utilization test; H2S = Hydrogen sulfide production; Lac = Lactose fermentation test; Nit = Nitrate reduction test; VP = Voges proskauer test; MR = Methyl red test; Ind = Indole test; Mot = Motility test; Ure = Urease test; Oxi = Oxidase test.

Table 2. Antimicrobial activity of stem bark of *J. curcas* and the control drug after 24 hours of incubation.

Test Organism	Concentration of the crude extract (mg/mL)	Zones of inhibition for crude extracts (mm)	Zones of inhibition for control drug (mm)
E. coli	40	12	6
	30	10	4
	20	7	2
	10	3	1
P. aeruginosa	40	8	-
	30	6	-
	20	3	-
	10	2	-
Staphylococcus aureus	40	20	18
	30	12	12
	20	8	7
	10	4	2
Salmonella typhi	40	20	18
	30	18	6
	20	12	2
	10	5	2

- = no activity.

Table 3. Minimum inhibitory concentration (MIC).

Test Organism	Crude stem bark extract (mg/mL)	Control (mg/mL)
E coli	20	10
P. aeruginosa	20	-
Staphylococcus aureus	15	15
Salmonella typhi	12	10

- = no activity.

Table 4. Minimal bactericidal concentration (MBC).

Test Organism	Methanolic crude extract (mg/mL)	Control (mg/mL)
E. coli	20	15
P. aeruginosa	25	20
Staphylococcus aureus	25	20
Salmonella typhi	20	20

Table 3 revealed the minimum inhibitory concentration of the crude methanolic extract against the *E. coli*, *P. aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*.

Table 4 shows the results of minimum bacteriocidal concentration (MBC) of the crude methanolic extract against *E. coli*, *P. auruginosa*, *Staphylococcus aureus* and *Salmonella typhi*.

Discussion

In this study, the antibacterial potentials of the methanolic and aqueous crude extracts of the stem bark of *Jatropha curcas* were investigated. The results revealed the presence of alkaloids, tannins, steroids, saponins, carbohydrates and flavonoids. Similar study by Narayani et al. (2012), and Adewumi et al. (2013) consistently reported phytochemical constituents of *J. curcas* to be saponins, alkaloids, steroids and flavonoids. Therefore, the report of previous studies and the present study are comparable. The antibacterial effect of the crude extracts of the *Jatropha curcas* was determined in comparison with the effect of chloramphenicol (control drug) against the test organisms. The crude extracts had more inhibitory effects compared to the antibiotic chloramphenicol (control drug) used. This could be attributed to the presence of phytochemical constituents (synegy) found in the crude extracts of the *J. curcas*. As reported in a study by Adewumi et al. (2013) although this study failed to determine the active compound(s) in the crude extract, the fact that the crude extract exerted inhibitory action against the organisms known with high resistance capabilities to most common antibiotics in the studied area is an indication that it has antibacterial potentials and could be considered as a promising agent for the treatment of diseases associated with the test organisms.

Conclusion

Further studies should be conducted on the acute and sub chronic toxicity of the crude extract. Similarly, determination and characterization of active compound(s) in the crude extracts should be conducted.

Conflict of interest statement

Authors declare that they have no conflict of interests.

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