- Short communication

Antimicrobial and Cytotoxic activities of *Jatropha curcas* (Euphorbiaceae)

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The crude extracts and two purified compounds JC-1 and JC-2 isolated from the stem bark of *Jatropha curcas* were screened for antibacterial, antifungal and cytotoxic activities. The petroleum ether, ethyl acetate and methanol extracts and compounds isolated from the ethyl acetate extract were studied for their antimicrobial activity. Cytotoxic activity was determined only with the EtOAc soluble extractives. The ethyl acetate extract showed promising antibacterial activity against all the gram positive and gram negative bacteria. The isolated compounds JC-1 and JC-2, whose structures were not elucidated, inhibited the growth of most of the microbial strains. The ethyl acetate extract also showed promising antifungal activity against *Candida albicans*, *Aspergilus niger*, *Candida oryzae* and *Saccharromyces cerevisiae.* The cytotoxicity of the ethyl acetate extract towards brine shrimp nauplii was also studied, where it showed the LC_{50} value of 19.95 µg/ml.

A wide variety of interesting biological and pharmacological activities have been reported for the secondary metabolites of *Jatropha curcas* belonging to the family Euphorbiaceae. *J. Curcas* is locally known as Ratanjyot or Jangli erandi. The plant is now widely spread throughout arid and semi arid tropical regions of the world.[1,2] *J. Curcas* is widely distributed in Nilphamari and some other districts of Bangladesh. Traditionally this plant is used for treating dysentery and diarrhea.[3] *J. Curcas* showed antibacterial activity against *Staphylococcus aureus, Escherchia coli* and *Pseudomonas aeruginosa.*[1,4] The latex of *Jatropha* contains an alkaloid known as "Jatrophine" which is believed to have anti-cancer properties.[1,5,6] It is also used for skin diseases, rheumatism and for sores on domestic livestock.[1,6] Previous phytochemical studies with this plant resulted in the isolation of an alkaloid, atherospermidine and a steroid, stigmasterol. As this plant is medicinally valuable, the present work was undertaken to study its antimicrobial and cytotoxic activities elaborately.

The stem bark of *J. curcas* was collected from the forest of Nilphamari district of Bangladesh. The sun-dried stem bark was ground mechanically and extracted in a Soxhlet apparatus successively with petroleum ether, ethyl acetate and methanol. The extracts were then concentrated in vacuo using a Buchii rotavapor. The EtOAc extract was then fractionated by vacuum liquid chromatography (VLC) over silica gel. Pure compounds were then isolated and purified from different fractions using different types of chromatographic techniques.

The *in vitro* antibacterial and antifungal activities of the crude extracts as well as the isolated purified compounds were determined by the disc diffusion technique.[8] Thirteen bacterial strains, which included

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five gram positive and eight gram negative organisms and seven fungi, were collected from the Department of Microbiology and Institute of Nutrition and Food Sciences, University of Dhaka. Nutrient agar media was used for the culture of bacteria and potato dextrose agar media was used for the culture of fungi. In brief, a measured amount of the test sample was dissolved in definite volumes of $CHCl_3$[9] to give solutions of known concentration (µg/ml). The sterile Matricel (BBL, Cocksville USA) filter paper discs were impregnated with known amounts of the test substances and dried. Standard ampicillin disc (l0µg/disc) and disc on which $CHCl_3$ was absorbed and dried (blank disc) were used as positive and negative controls, respectively. Griseofulvin (l00µg/disc) was used as standard for antifungal activies.

The discs were then placed in petridishes (120 mm in diameter) containing Mueller- Hinton agar media seeded with the test organisms using sterile cotton swabs. The plates were then incubated at 37^0C for 24 hours. The antimicrobial activities were measured from the zone of inhibition expressed in mm.[10] All experiments were carried out in triplicate and the mean of the readings were recorded. The cytotoxic activities were performed by Brine shrimp lethality test.[11]

The antimicrobial and antifungal activities of the petroleum ether, EtOAc and methanol extracts of *J. curcas* were determined against thirteen bacterial strains and seven fungi. The results were compared with those produced by the standard antibiotic, ampicillin trihydrate BP. The results are summarized in Table 1. All the tested strains showed sensitivity toward ethyl acetate extract. *Bacillus megaterium, B. subtilis* and *Sarcina lutea*, showed promising sensitivity (16-18 mm) to ethyl acetate extract, while *Staphylococcus aureus* and *B. cereus* demonstrated mild sensitivity towards EtOAc extract. On the other hand, most of the gram-negative bacteria showed strong sensitivity toward ethyl acetate extract except *Vibrio parahemolyticus* and *Salmonella paratyphi.* The growth of *S. typhi*, *Pseudomonas aeruginosa* and *Vibrio minicus* were not inhibited by the pet. ether extract. Moreover the methanol extract did not show any antimicrobial activity.

It was found that all the gram positive bacterial strains exhibited promising sensitivity (Table 1) against compounds JC-1, JC-2 except *Bacillus cereus.* Among the gram negative organisms, JC-1 showed strong inhibitory activity against *Shigella dysenteriae, Vivrio minicus* and *Shi. Boydii,* whereas JC-2 showed strong inhibition of growth of *Salmonella paratyphi* and *Pseudomonas aeruginosa.*

The crude EtOAc and MeOH extracts and column fractions 14, 15 were investigated against fungi at 3mg/disc and the result is summarized in Table (1). The ethyl acetate extract and its column fraction 14, 15 showed moderate zone of inhibition against some of the test organisms, except *Aspergilus fumigatus, Rhizopus oryzae* and *Candida krusii.* The MeOH extract did not show activity. The isolated compound JC-1 exhibited mild inhibitory activities against *Candida albicans.* JC-2 demonstrated prominent zone of inhibition against *Rhizopus oryzae.* Both the compounds did not reveal any activity against *Aspergilus fumigates, A. niger* and *Candida krusii* (Table 1).

Although some antibacterial and antifungal activities have been reported previously against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus,*[1,4] such activities have never been studied elaborately. This is the study of antimicrobial activity of *J. Curcas* in Bangladesh.

In the brine shrimp lethality bioassay the crude ethyl acetate extract and the selected fractions exhibited toxicity towards brine shrimp (Table 2). The crude extract was more potent than the selected fractions. The mortality rate of brine shrimp was found to be increased with the increase of concentration of each sample.

Table 1. Antibacterial and antifungal activities of extractives of *J. curcas.*

Name of Bacteria		Zone of inhibition (mm ± SD) after 24 hours of inhibition					
		Pet. ether extract 3mg/disc	EtOAc extract 3mg/disc	MeOH extract 3mg/disc	JC-1 l00µg/disc	JC-2 l00µg/disc	Ampicillin l00 µg/disc
		Gram positive					
1	Bacillus subtilis	08 ± 0.5	18 ± 1	-	18 ± 0.4	16 ± 0.5	21 ± 0.2
2	*B. megaterium*	-	16 ± 0.8	-	17 ± 0.2	19 ± 0.5	20 ± 0.3
3	*B. cereus*	07 ± 0.5	12 ± 0.4	-	07 ± 0.5	06 ± 0.5	19 ± 0.2
4	*Sarcina lutea*	-	17 ± 0.5	-	15 ± 0.3	14 ± 0.5	22± 0.2
5	*Staphylococcus aureus*	07 ± 0.2	11 ± 0.5	-	16 ± 0.5	17 ± 0.6	17 ± 0.6
		Gram negative					
1	*Escherichia coli*	12 ± 0.2	15 ± 0.2	-	11 ± 0.5	10 ± 0.5	18 ± 0.3
2	*Salmonella typhi*	-	17 ± 0.2	-	09 ± 0.8	-	23 ± 0.5
3	*S. paratyphi*	08 ± 0.5	-	-	-	16 ± 0.5	19 ± 0.6
4	*Shigella boydii*	12 ± 0.8	16 ± 0.5		14 ± 0.5	10 ± 0.3	20 ± 0.4
5	*Sh. dysenteriae*	10 ± 0.3	14 ± 0.5	-	16 ± 0.5	-	19 ± 0.3
6	*Pseudomonas aeruginosa*	-	15 ± 0.5	-	10 ± 0.5	17 ± 0.6	22 ± 0.2
7	*Vibrio parahemolyticus*	11 ± 0.2	-	-	09 ± 0.5	09 ± 0.8	16 ± 0.5
8	*V. mimicus*	-	16± 0.6	-	15 ± 0.6	-	18 ± 0.2

Name of Fungi		Zone of inhibition (mm ± SD) after 48 hours of incubation						
		EtOAc extract 3mg/disc	MeOH extrac 3mg/disc	JC-1 l00µg/ disc	JC-2 l00µg/ disc	Fraction 14 3mg/disc	Fraction 15 3mg/disc	Griseo-fulvin l00µg/disc
1	*Aspergilus niger*	10 ± 0.6	-	-	-	08 ± 0.6	12 ± 0.2	19 ± 0.6
2	*As. fumigatus*	-	-	-	-	-	-	-
3	*Rhizopus oryzae*	-	-	08 ± 0.2	14 ± 0.6	-	-	17 ± 0.5
4	*Candida albicans*	09 ± 0.8	-	11 ± 0.8	08 ± 0.5	10 ± 0.5	11 ± 0.2	15 ± 0.3
5	*C. oryzae*	11 ± 0.5	-	08±0.3	-	10 ± 0.6	12 ± 0.4	18 ± 0.5
6	*C. krusii*	-	-	-	-	-	-	13 ± 0.5
7	*Saccharromyces cerevisiae*	12±0.5	-	-	07 ± 0.8	12 ± 0.3	10 ± 0.5	-

"-"= Indicates no zone of inhibition.

Table 2. Determination of LC_{50} in brine shrimp lethality bioassay for extract of *J. curcas*

Sample No.	Concentration of plant extract (µg/ml)	% Mortality of brine shrimp			Mean	LC_{50} (µg/ml)
1	400.00	Expt. 1	Expt. 2	Expt. 3	100	19.95
2	200.00	100	100	100	90	
3	100.00	90	80	90	70	
4	50.00	80	80	70	70	
5	25.00	70	70	70	60	
6	12.50	40	50	40	40	
7	6.25	40	40	30	30	
8	3.13	40	20	20	20	
9	1.56	10	20	20	20	
10	0.0	0	0	0	0	

The percent mortality of the brine shrimp nauplii was calculated for every concentration for each sample. A plot of log concentration of the sample versus percent of mortality showed an approximate linear correlation between them. The LC_{50} value of the crude ethyl acetate extract was 19.95 µg/ml. From this investigation it is evident that this plant may have promising biological activities

including antibacterial, antifungal and cytotoxic properties. Therefore it could be a good source of natural medicine.

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