SHORT COMMUNICATION

BRINE SHRIMP LETHALITY AND ANTIMICROBIAL PROPERTIES OF VARIOUS EXTRACTS OF AMOORA ROHITUKA

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Amoora rohituka (Syn. *Aphanamixis polystachya*; Bengali name- Rayna, Pitraj, Family-Meliaceae) is a medium size tree, distributed in the sub-Himalayan tract and outer hills, central India, Pakistan, Nepal, Bhutan, Bangladesh, Myanmar and Srilanka [1]. It is a reputed plant for its medicinal uses. Root is useful in treating of abdominal complaints, body tumors, constipation, rheumatism, anthelmintic, diabetes, and enlargement of liver and spleen [2]. As a part of our continuing effort to study the chemical and pharmacological aspects of the medicinal plants of Bangladesh, we investigated *A. rohituka* in respect of brine shrimp lethality bioassay with the crude extract, pet-ether and chloroform fractions of the plant. The crude extract and fractions were also tested for antimicrobial activities against bacteria and fungi.

Stem bark of *A. rohituka* was collected from Comilla, Bangladesh in the month of August, 2005 and identified by Professor Abdul Jabbar, Emeritus Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka- 1000, Bangladesh. A voucher specimen has been deposited in the Phytochemical Laboratory of the Department of Pharmaceutical Chemistry, University of Dhaka. The pure culture of the strains of gram positive bacteria (*Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus*), gram negative bacteria (*Escherichia coli, Salmonella typhi, Shigella dysenteriae, Vibrio mimicus* and *Vibrio parahemolyticus*) and three fungi (*Candida albicans, Aspergillus niger* and *Sacharomyces cerevacae)* were collected from the Institute of Nutrition and Food Science (INFS), University of Dhaka. The bark of *A. rohituka* was first dried in sun and cut into pieces (800 g) and was soaked with ethanol (2.5 L). A portion of the concentrated ethanol extract (5 g) was fractionated by using modified Kupchan partition method [3] into pet ether, carbon tetrachloride, chloroform and aqueous fractions and evaporated to dryness to get pet ether extract (1.5 g), carbon tetrachloride extract (0.04 g), chloroform (1.5 g) and aqueous materials.

In our assay, brine shrimp eggs were hatched in simulated sea water to get nauplii. Sample solutions were prepared by dissolving the test materials in pre-calculated amount of DMSO. Ten nauplii were taken in vials containing 5 mL of simulated sea water. The samples of eight different concentrations as 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL and 3.125 µg/mL were added to the premarked vials with a micropipette. Survivors were counted after 24 hours.

The assay was performed using three replicates. These data were processed in a simple program for probit analysis to estimate LC_{50} values with 95% confidence intervals for statistically

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significant comparisons of potencies. Vincristine sulphate is used as the positive control for this study. Antibacterial and antifungal activity studies were carried out by disc diffusion method [3,4]. Kanamycin (30 μg/disc) was used as the reference drug for antibacterial assay, and nystatin (50 μg/disc) was used for antifungal activity study.

The brine shrimp test (BST) represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumour properties [5]. Following the procedure of lethality of the crude extract, pet ether and chloroform fractions were determined and the summary of the result is expressed in Table 1. The LC_{50} values of crude extract, pet ether and chloroform soluble fraction found to be 4.365 μg/mL, 5.370 μg/mL and 10.00 μg/mL respectively (Table 1). The positive control vincristine sulphate showed LC_{50} at a concentration of 0.563 μg/mL. From the results of the brine shrimp lethality bioassay it can be well predicted that both the crude extract and pet ether soluble fractions possess cytotoxic principles. The chloroform extract was also found to be considerable cytotoxic activity. Ethanolic extract showed the highest potency (LC_{50} 4.365 μg/mL) among all the fractions and compounds. Comparison with positive control vincristine signifies that cytotoxicity exhibited by the crude extract and pet ether soluble fraction might have mild antitumor and pesticidal activity. However this cannot be confirmed without further higher and specific tests.

The zone of inhibition produced by the crude extract, pet-ether and chloroform fractions were found to be 11 to 17 mm, 7 to 13 mm, and 7 to 12 mm, respectively at a concentration of 400 μg/disc. The crude extract showed moderate activity against *B. cereus* (17 mm), *B. subtilis* (14 mm), *S. aureus* (12 mm), *E. coli* (13 mm), *S. typhi* (13 mm), *S. dysenteriae* (16 mm), *V. mimicus* (16 mm), *V. parahemolyticus* (11 mm), *C. albicans* (14 mm), *A. niger* (11 mm), and *S. cerevacae* (15 mm).

Table 1 Effect of crude extract, pet ether and chloroform soluble fractions on shrimp nauplii

Conc. (C) (μg/mL)	Log C	% Mortality			LC_{50} (μg/mL)			Vincristine sulfate			
		Crude	Pet ether	CF	crude	Pet-ether	CF	Conc. (C) (μg/mL)	Log C	% Mortality	LC_{50} (μg/mL)
400	2.602	100	90	90	4.365	5.370	10.0	40	1.602	100	0.563
200	2.301	100	90	80				20	1.301	90	
100	2	90	80	70				10	1.000	90	
50	1.699	80	70	70				5	0.698	80	
25	1.398	70	70	60				2.5	0.397	70	
12.5	1.097	60	60	50				1.25	0.096	70	
6.25	0.796	60	50	40				0.625	-0.204	50	
3.125	0.495	40	40	40				0.3125	-0.505	30	

The pet ether fraction exhibited mild activity against *B. cereus* (13 mm), *B. subtilis* (12 mm), *S. aureus* (10 mm), *E. coli* (11 mm), *S. typhi* (10 mm), *S. dysenteriae* (9 mm), *V. mimicus* (9 mm), *V. parahemolyticus* (8 mm), *C. albicans* (7 mm), *A. niger* (7 mm), and *S. cerevaceae* (8 mm). The chloroform extract exhibited mild activity against *B.cereus* (12 mm), *B. subtilis* (11 mm), *S. aureus* (9 mm), *E. coli* (8 mm), *S. typhi* (7 mm), *S. dysenteriae* (9 mm), *V. mimicus* (12 mm), *V. parahemolyticus* (7 mm), *C. albicans* (10 mm) and *A. niger* (10 mm). The results were summerized in the **Table 2**.

Table 2 Antimicrobial activity of the crude sample, pet ether and chloroform soluble fractions of *A. [illegible]*

Test bacteria and fungi	Zone of inhibition in mm			
	Crude extract (400 µg/disc)	Pet-ether fraction (400 µg/disc)	Chloroform fraction (400 µg/disc)	Reference*
Gram positive				
B. cereus	17	13	12	34
B. subtilis	14	12	11	36
S. aureus	12	10	9	32
Gram negative				
E. coli	13	11	8	32
S. typhi	13	10	7	20
S. dysenteriae	16	9	9	32
V. mimicus	16	9	12	25
V. parahemolyticus	11	8	7	24
Fungi				
C. albicans	30	7	10	30
A. niger	11	7	10	26
S. cerevacae	15	8	11	20

* Kanamycin at 30 µg/disc for antibacterial and nystatin at 50 µg/disc for antifungal assay.

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