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INVESTIGATION OF PRELIMINARY CYTOTOXIC ACTIVITY OF ETHANOLIC STEM AND LEAF EXTRACTS OF *Amoora cucullata*

S.M.T. KABIR[1], F. SABRIN[2], M.N. HASAN[3], K.D. ISLAM[1] AND M.M. BILLAH[1]

[1]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh; [2]Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh; [3]Genetic Engineering and Biotechnology Department, Jessore science and Technology University, Jessore, Bangladesh.

Corresponding author & address: Dr. M. Morsaline Billah, E-mail: morsaline@yahoo.com
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ABSTRACT

Kabir SMT, Sabrin F, Hasan MN, Islam KD, Billah MM (2012) Investigation of preliminary cytotoxic activity of ethanolic stem and leaf extracts of *Amoora cucullata. J. Innov. Dev. Strategy.* 6(2), 24-28.

Amoora cucullata (amur), a member of Meliaceae family is a typical mangrove tree and traditionally used as a therapeutic agent to treat diarrhoea and inflammation like diseases. In order to find out the aqscientific basis of such uses, the present study was undertaken to determine the cytotoxic potential of this plant. Brine shrimp lethality assay was employed to determine the preliminary cytotoxic activity of ethanolic extracts obtained from stem and leaf of this plant. The ethanolic stem extract exhibited a potential LC_{50} value at 10µg/ml identical to the value obtained from standard drug. Thus, these preliminary experimental findings support ethno-medicinal uses of this plant and demand further investigation in isolating active principle.

Keywords: *cytotoxic effect, ethanolic extract, Amoora cucullata, sundarbans, mangrove*

INTRODUCTION

The brine shrimp lethality bioassay was initially proposed by Michael *et al.* (1956) and modified by others. Since its introduction, this *in vivo* lethality test has been successively employed as a basis for frontline screening. This assay thus could be backed up by more specific and more sophisticated bioassays once the active compounds have been isolated. As an assay, this method is rapid (24 h), simple (e.g., no aseptic techniques are required), could be easily mastered, inexpensive, and requires small amounts of test material (Ghisalberti *et al.* 1990). Recent development in the assay procedure for the bioactive compounds and natural product extracts was able to indicate general toxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, pesticidal etc. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at higher dose. Thus, *in vivo* lethality in a simple zoological organism (brine shrimp or *Artemia salina* Leach nauplii) can be used as a convenient monitor for the screening and fractionation in the discovery of new bioactive natural products (Anderson *et al*. 1988).

Amoora cucullata (Local name: Amur), a member of Meliaceae family is a rare and endangered tree species of the Sundarbans. It has been found that the plant parts of this tree species has been evaluated for a few biological activities. Traditionally, *A. cucullata* is used in folk medicine for the treatment of diarrhoea and inflammatory diseases (Boonyapraphat and Chockchaicharaenphorn, 1998). The juice prepared from the leaves of this tree has been reported to possess antibacterial activity and is extensively used for the treatment of dysentery, skin and cardiac diseases (Kirtikar and Basu, 1999). Related species *A. rohituka* and *A. chittagonga* was reported to possess significant antitumor and antibacterial properties (Chan *et al.* 2011). In particular, *A. rohituka* (stem) exhibited significant *in vitro* antibacterial activity. The extracts also demonstrated mild antifungal effect (Chowdhury *et al.* 2003). However, there remains the opportunity to evaluate the preliminary cytotoxic activity as a further basis for anticancer and antitumor properties of the ethanolic stem and leaf extract of Amur.

METHODS

Preparation of brine

Sixty gram sea salt (pure NaCl 20gm and table salt 40gm) was accurately weighed, dissolved in distilled water to make the final volume one liter and then filtered off to get a clear solution.

Preparation of stock solution

Ten milligram of the ethanolic extracts of *A. cucullata* was weighed accurately and then taken into 10ml separate volumetric flask and two to three drops of Tween-80 was added before dissolving. The final volume was made 2ml with saline water. The concentration of the stock solution was 5µg/µl.

Preparation of standard solution

Sterile chloramphenicol (0.5%) eye drop was used as a standard and the concentration of this solution was maintained 5µg/µl.

Hatching of the brine shrimp

Sea water was taken in a small tank and shrimp eggs were added to the one side of the tank and the side was covered. The shrimps were allowed for two days to hatch and mature as nauplii (larvae). The hatched shrimps were attracted to the lamp through the perforations in the dam and taken for bioassay.

Application of test solution and brine shrimp nauplii to the test tubes

Fourteen out of 30 clean test tubes were taken to study the effects of the samples in seven concentrations (two test tubes for each concentration) and fourteen test tubes were employed for control test. Four ml of sea water was given to each of the test tubes. Then specific volumes (5, 10, 20, 40, 80, 160 and 320 µl) of samples were transferred from the stock solutions to the test tubes to get (2.5, 5, 10, 20, 40, 80, and 160 µg/ml) concentration with a micropipette. For the control experiments, same volume of chloramphenicol (as in the sample test tubes) was taken in the two test tubes. Finally, 10 living shrimps were placed inside each of the test tubes with a Pasteur pipette (Myers *et al*. 2000). For the standard, specific volumes (5, 10, 20, 40, 80, 160, 320 µl) of standard samples were transferred from stock solutions to the rest fourteen test tubes to get final sample concentration of (2.5, 5, 10, 20, 40, 80, and 160 µg/ml) respectively with a micropipette.

Counting of nauplii

After twenty-four hours of incubation, the test tubes were observed and the number of survived nauplii in each test tube counted and the result were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for the extracts.

RESULTS AND DISCUSSION

In the cytotoxicity assay based on the lethality of brine shrimp, the crude extract of *A. cucullata* leaves and stems showed lethality indicating the biological activity of the compound present in the extracts.

Test samples showed different mortality rate at different concentrations. The mortality rate of brine shrimp showed the increment with the increase in concentration of the extracts and plot of percent age mortality against logarithmic concentration showed almost a linear relationship. From this plot, the concentrations at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred were obtained by extrapolation. Table 1 and 2 summarize the results obtained from brine shrimp lethality bioassay. It is evident from Figure 1; the LC_{50} value corresponds to approximately 1 in the logarithmic scale which obtains the value 10 µg/ml upon transformation.

Table 1. Brine shrimp lethality bioassay of ethanolic extract of *A. cucullata* stem

Sample Concentration (µg/ml)	Logarithmic Concentration	Treatment-1	Treatment-2	Average no. of alive shrimp (sample)	Average no of alive shrimp (control)	Standard deviation	Standard error	% Mortality
2.5	0.398	9	8	8.5	10	0.707107	0.5	15
5	0.699	8	6	7		1.414214	1	30
10	1	5	5	5		0	0	50
20	1.3	3	2	2.5		0.707107	0.5	75
40	1.6	1	2	1.5		0.707107	0.5	85
80	1.9	1	1	1		0	0	90
160	2.2	0	0	0		0	0	100

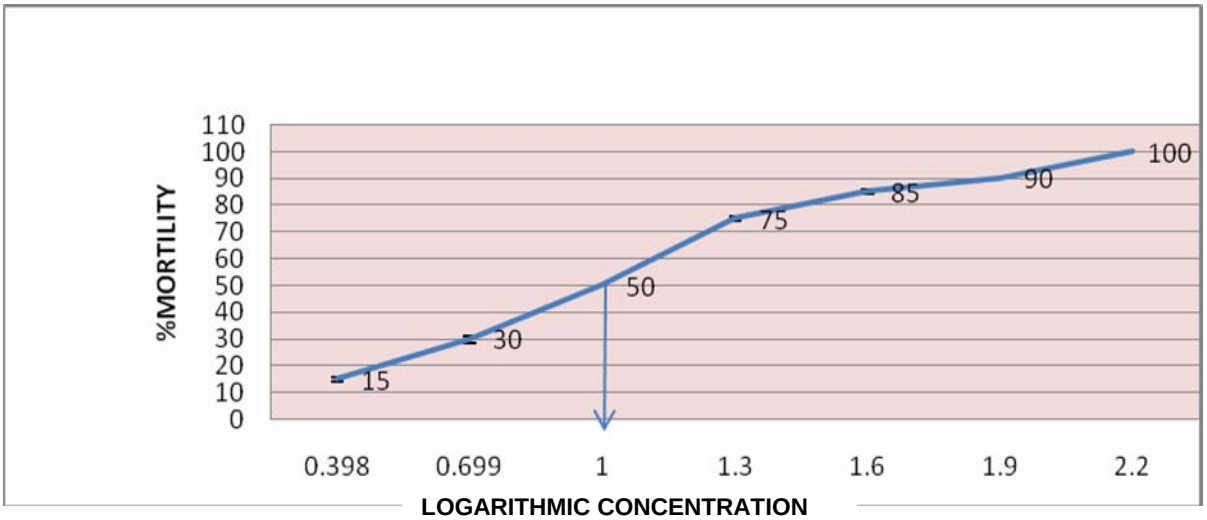

Fig. 1. Percentage mortality and ethanolic extract concentration of *A. cucullata* stem. Data were presented as average ± standard error

Table 2. Brine shrimp lethality bioassay for ethanolic extract of *A. cucullata* leaf

Sample Concentration (µg/ml)	Logarithmic Concentration	Treatment-1	Treatment-2	Average no. of alive shrimp (sample)	Average no. of alive shrimps (control)	Standard deviation	Standard error	%Mortality
2.5	0.398	8	7	7.5	10	0.707107	0.5	25
5	0.699	7	6	6.5		0.707107	0.5	35
10	1	6	6	6		0	0	40
20	1.3	5	5	5		0	0	50
40	1.6	4	1	2.5		2.12132	1.5	75
80	1.9	1	0	0.5		0.707107	0.5	95
160	2.2	0	0	0		0	0	100

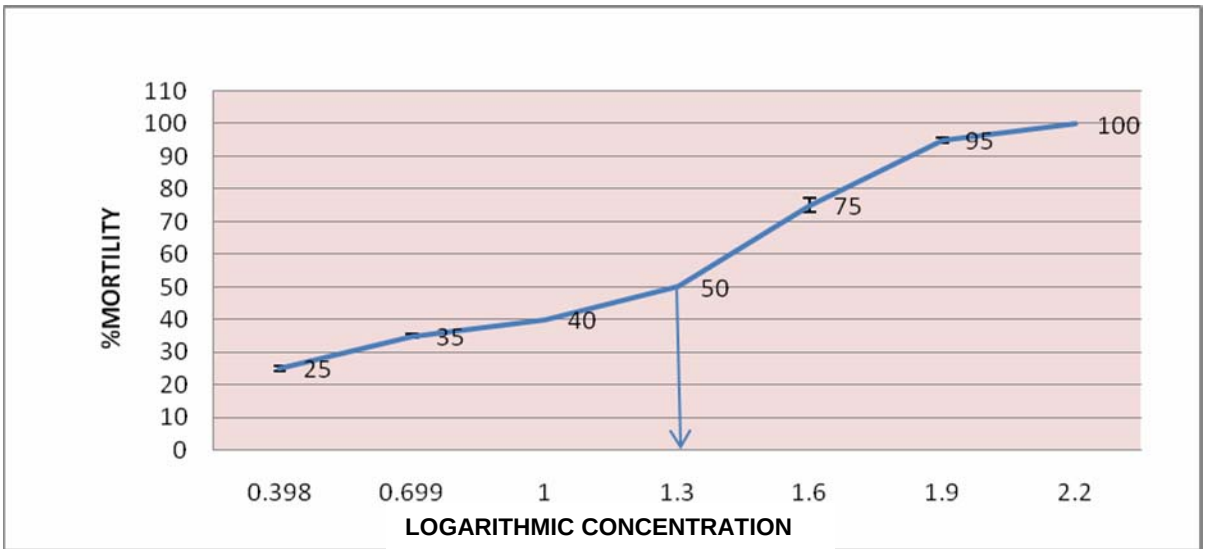

Fig. 2. Percentage mortality and ethanolic extract concentration of *A. cucullata* leaf. Data were presented as average± standard error

The concentration at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred for *A. cucullata* ethanolic leaf extracts can be obtained from Figure 2, which was 1.3µg/ml in a logarithmic scale. Transformation of this value indicated that the value was approximately 20µg/ml.

Table 3. Brine shrimp lethality bioassay of chloramphenicol standard

Sample Concentration (µg/ml)	Logarithmic (Conc.)	Treatment-1	Treatment-2	Average no. of alive shrimp (sample)	Average no. of alive shrimp (control)	Standard deviation	Standard error	%Mortality
2.5	0.398	9	9	9	10	0	0	10
5	0.699	8	7	7.5		0.707107	0.5	25
10	1	5	5	5		0	0	50
20	1.3	4	4	4		0	0	60
40	1.6	3	2	2.5		0.707107	0.5	75
80	1.9	1	1	1		0	0	90
160	2.2	0	0	0		0	0	100

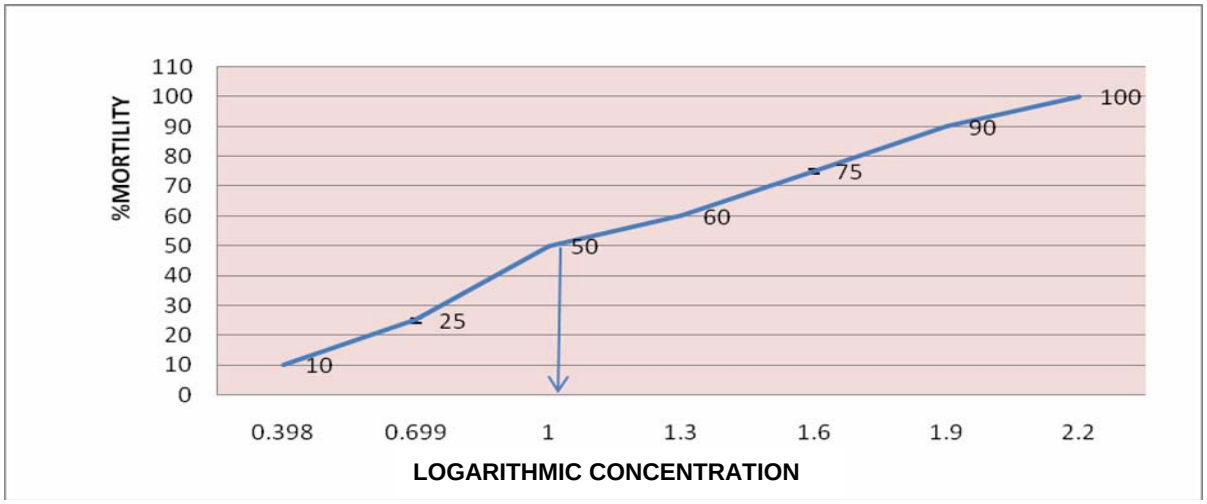

Fig. 3. Percentage mortality and and chloramphenical concentration. Data were presented as average± standard error

Chloramphenicol concentration at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred can be obtained from Figure 3, which was 1µg/ml in a logarithmic scale equivalent to 10µg/ml in a normal scale. It is obvious from the activities that the ethanolic extracts of *A. cucullata* stem showed strong cytotoxic activity as this activity was almost equal to the LC_{50} value obtained from chloramphenicol while the ethanolic extracts of *A. cucullata* leaf possessed lower cytotoxicity (LC_{50} value ≈20µg/ml). Rahman *et al.* (2005) first studied the phytochemical properties of *A. cucullata* and isolated five compounds from a hexane extract of the stem bark. Later on, in an effort to determine the preliminary cytotoxic activity of some medicinal plants of Bangladesh, this group evaluated the methanolic extract of *A. cucullata* (Rahman *et al.* 2008). They reported significant brine shrimp lethality and the LC_{50} values were found to be lower than 6.0. Rahman and Rashid (2009) used three different solvent systems, i.e. n-hexane, ethyl acetate and methanol to obtain extractives from *A. cucullata* bark and the cytotoxcity of the samples was evaluated against *A. salina*. The methanolic extract demonstrated highest cytotoxicity having LC_{50} of 0.549µg/ml, whereas the ethyl acetate and n-hexane extract showed LC_{50} of 7.943 and 17.180 µg/ml, respectively. There had been no reports on ethanolic extracts of *A. cucullata* and stem extracts showed more promising results in comparison to leaf extracts which was in good agreement with the previous reports. Therefore the result showed that the preliminary cytotoxic activities exhibited by ethanolic extracts of *A. cucullata* different plant parts were promising and this clearly indicated the presence of potent bioactive cytotoxic compounds. In future, further investigation could lead to the discovery of potent active principle with a promise from antitumor/anticancer drug from *A. cucullata* and shed the light into ethnomedicinal uses of this plant.

CONCLUSION

A detailed pharmacological and toxicological study is required to establish the therapeutic uses of the plant and particularly with active principles. The results obtained from this study were encouraging to certain degree as it validates the traditional medicinal uses of this plant and this effort could reinforce the concept of ethno-botanical approaches for drug discovery through systematic screening of plants as potential source of bioactive substances.

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REFERENCES

Anderson JE, Chang CJ, McLaughin JL (1988) Bioactive components of *Allamanda schottii*. *Journal of Natural Products*. 51(2), 307-308.

Boonyapraphat N, Chockchaicharaenphorn C (1998) "Thai Medicinal Plants," Vol. II, Prachachon Ltd., Bangkok, p. 157.

Chan LL, Gosangari S, Watkin K, Cunningham BT (2011) Cytotoxicity Effects of *Amoora rohituka* and *chittagonga* on Breast and Pancreatic Cancer Cells. Evidence-Based Complementary and Alternative Medicine, Article ID 860605: 8.

Chowdhury R, Hasan CM, Rashid MA (2003) Antimicrobial activity of *Toona ciliata* and *Amoora rohituka.* Fitoterapia, Volume 74, Issues 1-2, pp 155-158.

Ghisalberti EL, Jefferies PR, Vu HTN (1990) Diterpenes from *Eremophila* species, Phytochemistry 29(1), 316-318.

Kirtikar KR, Basu BD (1999) Indian Medicinal Plants, International Book Distributors, India, pp. 553-554.

Michael AS, Thompson CG, Abramovitz M (1956) *Artemia salina* as a test organism for a bioassay. Science 123, 464.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403, 853-856.

Rahman MS, Chowdhury R, Begum B, Rahman KM, Rashid MA (2005) Phytochemical studies of *Amoora cucullata*. The Dhaka University Journal of pharmaceutical Sciences 4(1), 73-75.

Rahman MS, Begum B, Chowdhury R, Rahman KM, Rashid MA (2008) Preliminary cytotoxicity screening of some medicinal plants of Bangladesh. The Dhaka University Journal of Pharmaceutical Sciences 7(1), 47-52.

Rahman MS, Rashid MA (2009) Preliminary antimicrobial and cytotoxic activities of *Amoora cucullata* extractives. Oriental Pharmacy and Experimental Medicine 9(2), 182-185.