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CYTOTOXIC ACTIVITY OF CHLOROFORMIC EXTRACTS OF *Xylocarpus mekongenesis* (Lamk.) M. Roem. and *Cerbera manghas*

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ABSTRACT

Hoque MM, Sabrin F, Alam SMN, Khatun MA, Billah MM (2012) Cytotoxic activity of chloroformic extracts of *Xylocarpus mekongenesis* (Lamk.) M. Roem. and *Cerbera manghas. J. Innov. Dev. Strategy.* 6(2), 29-34.

Xylocarpus mekongenesis and *Cerbera manghas*, two widely distributed woody plants of the Sundarbans are reported to possess the valuable sources of several phytochemicals and have been used in traditional medicine for the treatment of a number of diseases. The present study was undertaken to evaluate the cytotoxic potential of the chloroformic stem and leaf extracts of these two plants. The chloroformic stem extract of *X. mekongenesis* showed the highest 50% mortality of lethal concentration (LC_{50}) which was 7.29µg/ml. The LC_{50} values obtained chloroformic extracts of *X. mekongensis* leaf and *C. manghas* stem were 13.04 µg/ml and 13.33 µg/ml, respectively. The preliminary results suggested that these two woody plants possessed potential cytotoxic compounds with a promise for further investigation.

Key words: *cytotoxic activity, chloroformic extracts, Xylocarpus mekongenesis, Cerbera manghas, mangrove plants, the Sundarbans*

INTRODUCTION

Plants serve as one of the major sources of therapeutic agents still today. A number of key drug molecules, e.g. morphine (narcotic analgesic), taxol (anticancer) and artemisinin (antimalarial) have come from plant sources over the years of investigation and research, which have transformed modern medicine to the state we observe at the present time. The unique inherent chemical diversity arising from plant sources are still considered to be one of the major potential sources of bioactive compounds which could be used directly or their structural features could be exploited for new drug discovery and development (Sarker *et al*. 2011).

The Sundarbans mangrove forest, located to the southern region of Bangladesh, offers a unique and uncommon combination of flora. However, unlike other mangrove forests, the Sundarbans faces the possibility of extinction due to global warming, natural disasters and various manmade conditions. The plants of mangrove origin often produce chemical classes of compounds that may not be found in terrestrial plants as the conditions are extreme. Therefore, two basic factors rationalize and encourage the study of the chemical constituents of mangrove plants (Bandaranayake 2002). Firstly, mangroves are abundantly available in tropics and subtropical parts of the world. They can grow where no other vascular plants can. The mangroves experience stressful conditions such as violent environments, high concentration of moisture, high and low tides of water, and abundant living microorganisms and insects. Therefore, they thrive in a very peculiar environment and serve as a bridging ecosystem between freshwater and marine systems. These have imposed several modifications in these plants which lead to an unusual morphology and physiology and the path of photosynthesis in mangroves is different from other glycophytes. They possess modifications to establish water and salt economy. There are modifications or alterations in other physiological processes such as carbohydrate metabolism or polyphenol synthesis and due to this reason; they may have chemical compounds, which protect them from these destructive elements. The second reason is that numerous mangrove plants have been used in folklore medicine, and recently, extracts from mangroves and mangrove-dependent species have proven activity against human, animal and plant pathogens but only limited investigations have been carried out to identify the metabolites responsible for their bioactivities. This opens up the possibilities for evaluating a number of 'untapped' mangrove species for their medicinal properties, and if necessary appropriate measures for preventing extinction (Sarker *et al.* 2011).

Bioactive natural products are those chemical compounds produced by living organisms with possible biological effects on other organisms. This includes therapeutic activity for diseases of humans and animals, toxic activity responsible for causing human and animal diseases, and selective, biodegradable toxicity to help combat responsible harmful agricultural pests (Colegate and Moleneux, 2008). Plants biosynthesize a wide variety of secondary metabolites as their metabolic processes, most of which are biologically active and could be used potentially to treat diseases. In fact, plants have acquired evolutionary measures of defense mechanisms to combat "enemies" like herbivores, parasites and microbes through producing these bioactive molecules. Due to this inherent property of producing bioactive compounds, plants have long been used traditionally in the treatment of various ailments, and a number of ancient medicine systems, e.g. the Ayurvedic, the Unani, the traditional Chinese Medicine (TCM), predominantly rely on the uses of plants.

The brine shrimp lethality bioassay was proposed by Michael *et al*. (1956) and modified by others. Since its introduction, this *in vivo* lethality test has been successively employed as an initial assay to be validated by more specific and sophisticated bioassays once the active compounds have been isolated. The bioassay is rapid (24 h), simple, easy to operate, inexpensive, and requires small amounts of test material (Ghisalberti *et al*. 1993). The recent development in the assay procedure for the bioactive compounds and natural product extracts provides information on general toxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, pesticidal etc. Bioactive compounds usually show toxicity at higher doses. In this connection, it is appreciated that 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 nauplii) can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products (Anderson *et al.* 1988).

Xylocarpus mekongenesis (*X. mekongenesis*) (Lamk.) M. Roem. (Meliaceae) and *C. manghas* are two widely distributed woody species of the Sundarbans. *X. mekongenesis*, commonly known as Possur is a glabrous, medium-sized tree found in littoral forests of Bengal, Burma, the Andaman's, the Malay Peninsula and Archipelago, Australia, Fiji and Africa. In Bangladesh, this species are abundant in the north tract, remote from the sea, chiefly in low lying swampy locality, of the Sundarbans, the largest mangrove forest in the world (Kirtikar and Basu, 1999). The traditional uses of *X. mekongenesis* include its use as an astringent and febrifuge and for the treatment of dysentery and diarrhoea (Ghani 1998). Xyloccensin has been isolated from the bark of *X. mekongenesis* (Bandaranayake 2002). The bark and pneumatophore of *X. mekongensis* possess antimalarial, antidiarrhoeal and antinociceptive activities (Bandaranayake 2002; Uddin *et al.* 2005, 2006). Species belonging to the *Cerbera* (Apocynaceae) genus of plants are commonly found on the islands of Southeast Asia and Oceana, and on other lands surrounding the Indian Ocean. The two most frequently encountered species are *C. manghas* and *C. odollam*, which differ only in the color and shape of their respective fruits. Very few of the natural products from this plant were initially examined for biological activity. However, as secondary metabolites have been re-isolated from this and other species, bioassays have played a greater role. The cardenolides obtained from *C. manghas* in recent years have shown a significant level of cytotoxicity (Chang *et al.* 2000). Literature shows that the plant parts of *X. mekongenesis* and *C. manghas* have not evaluated in a great detail for the presence of a number of bioactivities. Traditionally juice extracted from the leaves of *C. manghas* is used in the treatment of rheumatism; the leaves are also used to treat skin diseases (Weiner 1984). The traditional medicinal uses of *X. mekongenesis* include the seeds for treating stomachaches, bark tannin for intestinal ailments (Giesen *et al.* 2006). However there remains the opportunity to determine systematically for possible bioactivities from these plants. The study was carried out to determine cytotoxic activity of chloroformic extracts obtained from stem and leaf obtained from these plants.

MATERIAL AND 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

Seven milligram of the chlorofomic extracts of *C. manghas* and *X. mekongenesis* were 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 1.4ml 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 (Myer *et al*. 1982). 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.

RESULT AND DISCUSSION

In the present study, chloroformic extracts obtained from different plant parts of *X. mekongenesis* and *C. manghas* were evaluated using the brine shrimp lethality bioassay and their cytotoxic activities were determined (Table 1, 2, 3 and 4). These extracts showed a relatively potent lethality indicating the biological activity of the compound present in the extracts. Test samples showed different mortality rates at different concentrations and the mortality rate of brine shrimp increased with the increment in concentration of the extracts and the plot of percentage mortality against logarithmic concentration of samples showed approximate linear relationship between them (Figure 1, 2, 3 and 4).

Table 1. Brine shrimp lethality bioassay of chloroformic extracts of *X. mekongenesis* stem

Sample Concentration (µg/ml)	Logarithmic Concentration (µg/ml)	Treatment-1	Treatment-2	Average no. of alive shrimp (sample)	Average no. of alive shrimp (control)	Standard deviation	Standard error	%mortality	LC_{50} (µg/ml)
2.5	0.398	6	7	6.5	10	0.707107	0.5	35	7.29*
5	0.699	7	5	6		1.414214	1	40	
10	1	4	4	4		0	0	60	
20	1.3	4	3	3.5		0.707107	0.5	65	
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	

extrapolated

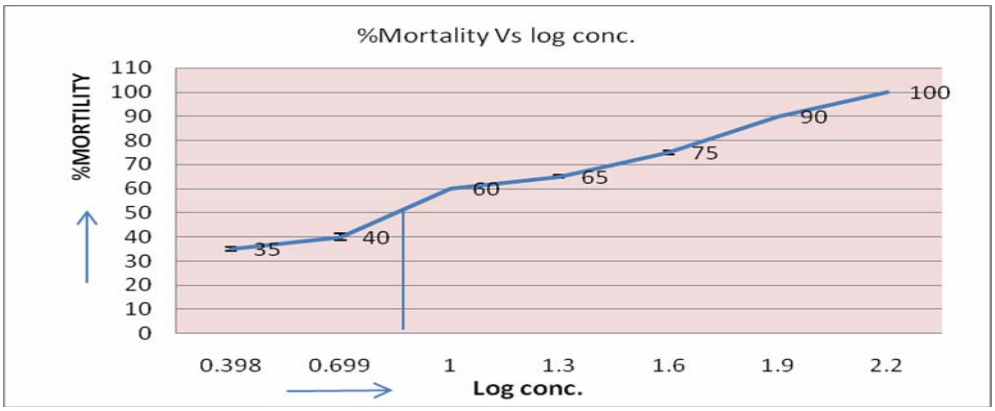

Fig. 1. Cytotoxic activity of chloroformic extract of *X. mekongenesis* stem with LC_{50} value obtained from brine shrimp lethality bioassay. Data were presented as mean± standard error

Table 2**.** Brine shrimp lethality bioassay of chloroformic extracts of *X. mekongenesis* leaf

Sample Concentration (µg/ml)	Logarithmic Concentration (µg/ml)	Treatment-1	Treatment-2	Average no. of alive shrimp (sample)	Average no. of alive shrimp (control)	Standard deviation	Standard error	%Mortality	LC_{50} (µg/ml)
2.5	0.398	8	9	8.5	10	0.707107	0.5	15	13.04*
5	0.699	7	6	6.5		0.707107	0.5	35	
10	1	7	4	5.5		2.12132	1.5	45	
20	1.3	6	5	5.5		0.707107	0.5	45	
40	1.6	2	1	1.5		0.707107	0.5	85	
80	1.9	1	0	0.5		0.707107	0.5	95	
160	2.2	0	0	0		0	0	100	

extrapolated

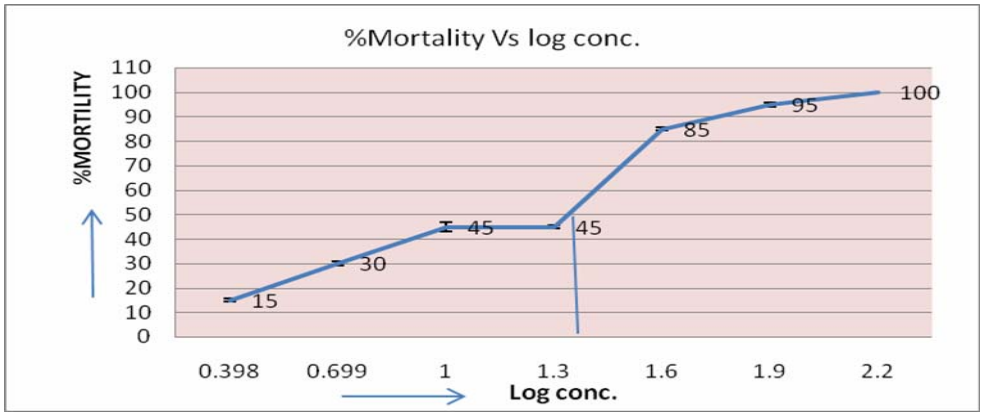

Fig. 2. Cytotoxic activity of chloroformic extract of *X. mekongenesis* leaf with LC_{50} value obtained from brine shrimp lethality bioassay. Data were presented as mean± standard error

Table 3. Brine shrimp lethality bioassay of chloroformic extracts of *C. manghas* stem

Sample Concentration (µg/ml)	Logarithmic Concentration (µg/ml)	Treatment-1	Treatment-2	Average no. of alive shrimp (sample)	Average no. of alive shrimp (control)	Standard deviation	Standard error	%Mortality	LC_{50}(µg/ml)
2.5	0.398	6	8	7		1.414214	1	30	
5	0.699	6	6	6		0	0	40	
10	1	5	6	5.5		0.707107	0.5	45	
20	1.3	4	4	4	10	0	0	60	13.33*
40	1.6	3	4	3.5		0.707107	0.5	65	
80	1.9	2	1	1.5		0.707107	0.5	85	
160	2.2	1	0	0.5		0.707107	0.5	95	

**extrapolated*

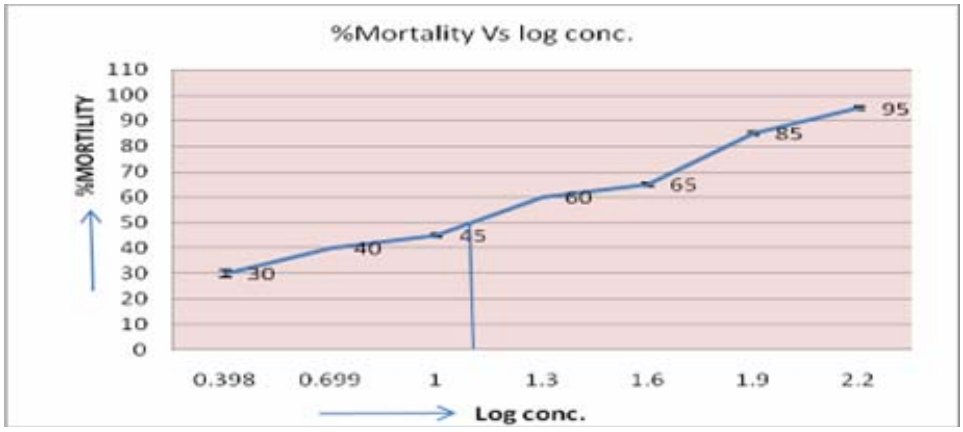

Fig. 3**.** Cytotoxic activity of chloroformic extract of *C. manghas* stem with LC_{50} value obtained from brine shrimp lethality. Data were presented as mean± standard error

Table 4. Brine shrimp lethality bioassay of chloroformic extracts of *C. manghas* leaf

Sample Concentration (µg/ml)	Logarithmic Concentration (µg/ml)	Treatment-1	Treatment-2	Average no. of alive shrimp (sample)	Average no. of alive shrimp (control)	Standard deviation	Standard error	%Mortality	LC_{50}(µg/ml)
2.5	0.398	9	9	9		0	0	10	
5	0.699	9	8	8.5		0.707107	0.5	15	
10	1	8	7	7.5	10	0.707107	0.5	25	45.76*
20	1.3	7	7	7		0	0	30	
40	1.6	6	5	5.5		0.707107	0.5	45	
80	1.9	4	3	3.5		0.707107	0.5	65	
160	2.2	3	3	3		0	0	70	

** extrapolated*

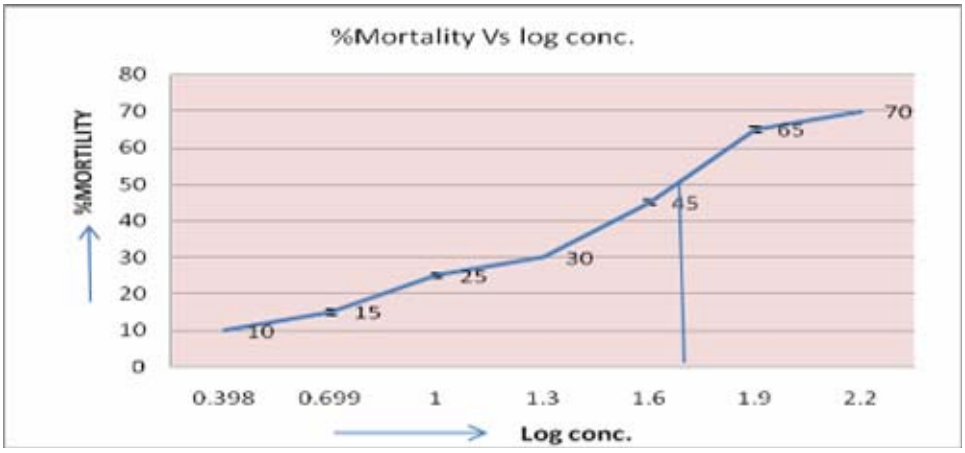

Fig. 4. Cytotoxic activity of chloroformic extract of *C. manghas* leaf with LC_{50} value obtained from brine shrimp lethality. Data were presented as mean± standard error

Table 5. Brine shrimp lethality bioassay of chloramphenicol as a negative control

Sample Concentration (µg/ml)	Logarithmic Concentration (µg/ml)	Treatment-1	Treatment-2	Average no of alive shrimp (sample)	Average no of alive shrimp (control)	Standard deviation	Standard error	%Mortality	LC_{50} (µg/ml)
2.5	0.398	9	9	9	10	0	0	10	13.706*
5	0.699	7	7	7		0	0	30	
10	1	6	5	5.5		0.707107	0.5	45	
20	1.3	4	3	3.5		0.707107	0.5	65	
40	1.6	3	2	2.5		0.707107	0.5	75	
80	1.9	0	1	.5		0.707107	0.5	95	
160	2.2	0	0	0		0	0	100	

** extrapolated*

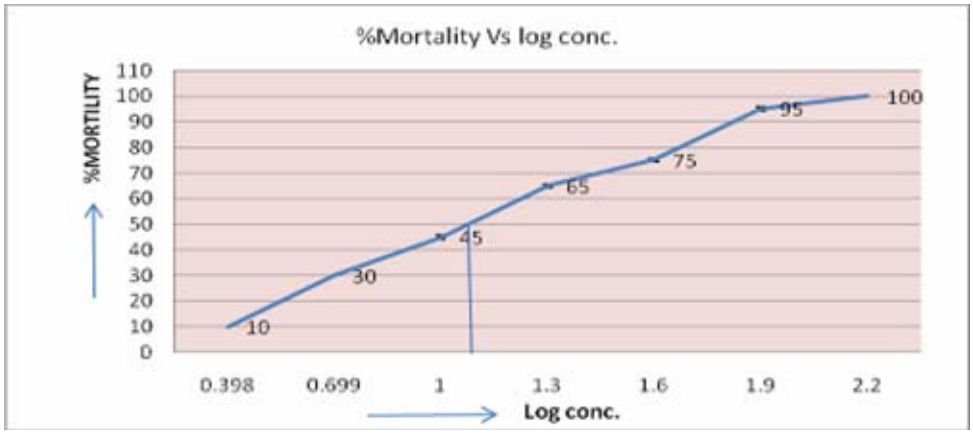

Fig. 5. Cytotoxic activity of chloramphenicol with LC_{50} value obtained from brine shrimp lethality. Data were presented as mean± standard error

The concentration at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred for chloroformic extract of the stem of *X. mekongenesis* can be obtained from Figure 1. It was observed that the chloroformic stem extract showed the LC_{50} at the concentration of 7.29µg/ml. On the other hand, cytotoxic activity of chloroformic extract of *X. mekongenesis* leaf was obtained from the LC_{50} value which was 13.04µg/ml (Figure 2) indicating that the presence of more bioactive compounds related to cytotoxicity in stems than leaves. The methanolic bark extract of *X. granatum* showed promising cell growth inhibitory activity on HepG2 cells due to the presence of bioactive compounds in the extracts while leaf extracts showed no activity (Khairina *et al.* 2011). Haque *et al.* (2007) reported ethyl acetate bark extracts of *X. mollucensis* showed toxicity towards brine shrimp with a LC_{50} value at 12.6µg/ml. Similarly, cytotoxic activity of chloroformic extracts of *C. manghas* stem and leaf were determined from the LC_{50} values as 13.33 and 45.76µg/ml, respectively (Figure 3 and 4). It was obvious from these findings that the chlorofomic extracts of stem contained more cytotoxic bioactive compounds than the extracts from leaf. If these results were compared with control (Table 5 and Figure 5), it was seen that

chloroformic extracts of *X. mekongenesis* stem and leaf contained more bioactive compounds relating to cytotoxicity than the chloroformic extracts of *C. manghas.*

CONCLUSION

The results showed that the chloroformic extracts of *X. mekongenesis* and *C. manghas* stems and leaves possessed differential levels of cytotoxic activity and this clearly indicated the presence of potent bioactive compounds related to cytotoxicity. The findings obtained from this study were promising as it validates the traditional medicinal uses of these plants and this effort could reinforce the concept of ethno-botanical approaches for antitumor and anticancer drug discovery through systematic screening of plants as potential source of bioactive substances.

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