



CYTOTOXIC SCREENING OF *Entada scandens* SEEDS ON BRINE SHRIMP

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Abstract

In Ayurveda, *Entada scandens* is known as the Gil. It is used in the treatment of human pathologies such as inflammation, hemorrhage and cancer. The present study was designed to evaluate the cytotoxicity of ethanol extract of *Entada scandens* seeds. The brine shrimp lethality test showed the significant cytotoxic activity of the seeds extract (The LC₅₀ & LC₉₀ values were 10 µg mL⁻¹ and 59.77 µg mL⁻¹ respectively). The obtained result support the traditional uses of the seeds and require further investigation to identify the chemical constituents responsible for cytotoxicity.

Key words: Cytotoxicity, *Entada scandens*, Brine shrimp

Introduction

Since disease, decay and death have always co-existed with life, the early man had to think about disease and its treatment at the dawn of human intellect. Thus the human race started using plants as a means of treatment of disease and injuries from the early days of civilization on earth and its long journey from ancient time to modern age the human race has successfully used plants and plant products as effective therapeutic tools for fitting against disease and various other health hazards.

Sword bean (*Entada scandens*) is a tree climber that belongs to Mimosoideae, a subfamily of Leguminosae. It plays important role in the treatment of human pathologies such as inflammation, hemorrhage and cancer.¹

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, pesticidal, etc.²

Bioactive compound are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus, in-vivo lethality of an extract against a simple zoological organism (brine shrimp napulii) can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products.³

Materials and methods

Collection of plant materials and preparation of plant extract

The seeds of *Entada scandens* was collected from Rangamati, Bangladesh in the month of May, 2009 and the plant was identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka. The voucher specimen was deposited at the Pharmacy Discipline of Khulna University Bangladesh. The seeds are very strong with an air pocket. The seeds were cut into small pieces and then slashed to

coarse powder with the help of mechanical grinder. The powder materials were stored in a suitable container. About 500 gm of powder was extracted by maceration over 20 days with 1200 ml of 80% ethanol. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material and then it was evaporated by using a rotary evaporator to get a viscous mass which was dried to get a dried ethanol extract. The extract thus obtained was used for experimental purposes.

Determination of cytotoxic activity

The brine shrimp eggs were hatched in a conical flask containing brine shrimp medium (300 ml). The flask were well aerated with the aid of an air pump and kept in a water bath at 29 - 30°C. A bright light was left on it. The nauplii hatched within 48 h. The extract was dissolved in brine shrimp medium with addition of few drops of 5% dimethyl sulfoxide (DMSO) to obtain a concentration of 5, 10, 20, 40, 60, 80 and 160 µg mL⁻¹. Each preparation was dispensed into clean test tube in 10 ml volume. For control, same procedure was followed except test samples. A series of same concentration as of sample was prepared for positive control, chloramphenicol. After making the test tube properly, 10 living shrimps were added to each of the test tubes with the help of Pasteur pipette. The test tubes containing the sample, control and positive control were then incubated at 29°C for 24 h in a water bath, after which each test tube was examined and the surviving brine shrimp counted and recorded. From this, the percentage of mortality was calculated at each concentration to determine the LC₅₀ and LC₉₀.⁴

Result

Cytotoxic activity

Brine shrimp lethality bioassay indicates cytotoxicity of the ethanol extract. The extract was found to show lethal activity against brine shrimp nauplii where LC₅₀ and LC₉₀ values were 10 µg mL⁻¹

and $59.77 \mu\text{g mL}^{-1}$.

see Table 1.

see Fig. 1

Discussion

In this bioassay, the crude extract showed lethality indicating the biological activity of the compound present in the extract. Test sample showed different mortality rate at different concentration. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the sample and plot of percent mortality versus log of concentration on the graph paper produced an approximate linear correlation between them. From the graph (figure 1) the concentration at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred were obtained by extrapolation. The LC_{50} & LC_{90} values were $10 \mu\text{g mL}^{-1}$ and $59.77 \mu\text{g mL}^{-1}$ respectively.

The crude extracts were found to show high activity against the brine shrimp nauplii. Therefore the response obtained in this assay suggests that the crude extract may contain antitumor, antimicrobial or pesticide compound. However, this cannot be confirmed without further higher and specific tests. So further researches are essential to get the information about these activities.

Conclusion

According to the above discussion *Entada scandens* contains important chemical constituents that confer upon it as a cytotoxic agent. This could provide a rational for traditional use of anticancer and further research is necessary for elucidating the active principles.

Acknowledgments

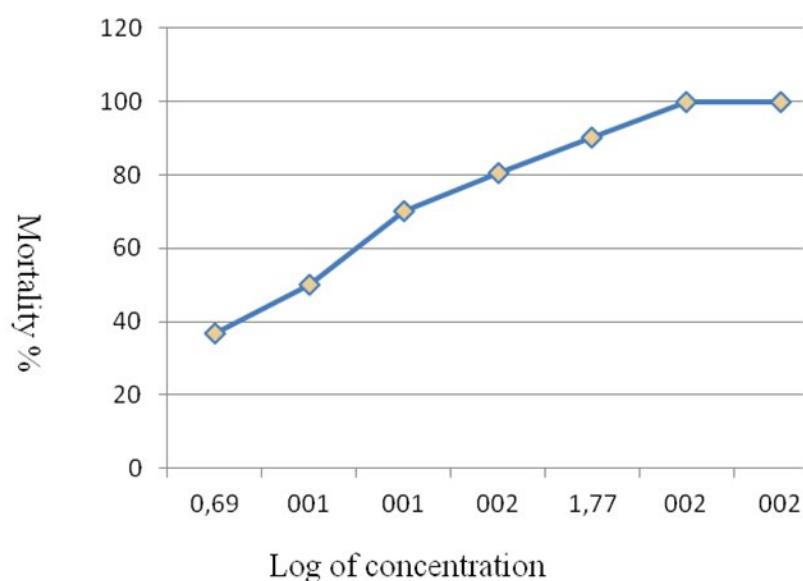
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Conc.($\mu\text{g mL}^{-1}$)	Log (conc.)	No. of alive shrimp			Avg.	Mortality (%)	$\text{LC}_{50} \mu\text{g mL}^{-1}$	$\text{LC}_{90} \mu\text{g mL}^{-1}$
		Test 1	Test 2	Test 3				
5	0.69	6	6	7	6.33	36.7	10	59.77
10	1.00	5	5	5	5.00	50		
20	1.30	3	4	2	3.00	70		
40	1.60	2	2	1	1.66	80.34		
60	1.77	1	1	0	0.66	90.34		
80	1.90	0	0	0	0.00	100		
160	2.20	0	0	0	0.00	100		

Table 1: Result of brine shrimp lethality bioassay of ethanolic extract of *Entada scandens*.Figure 1: Graphical presentation of lethality test for the ethanolic extract of *Entada scandens*