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Evaluation of in vitro Cytotoxic, Anthelmintic, Anti-inflammatory and Antioxidant activity of Ethyl acetate fraction of *Hymenocallis littoralis*

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ABSTRACT: The aim of the present study was to evaluate the in-vitro cytotoxic, anthelmintic, anti-inflammatory and antioxidant activity of ethyl acetate fraction of Hymenocallis littoralis. The preliminary phytochemical analysis of the crude extract of Hymenocallis littoralis (bulb) clearly demonstrated the presence of phyto-active compounds, which were responsible for the observed cytotoxic, anthelmintic, anti-inflammatory and antioxidant activities. We used ethyl acetate to execute phytochemical screening. We found mild to moderate cytotoxicity in brine shrimp lethality bioassay, promising anthelmintic activity, dose dependent anti-inflammatory activity using in vitro anti-inflammatory egg albumin denaturation assay and mild antioxidant activity in DPPH free radical scavenging assay.

Keywords: Hymenocallis littoralis, cytotoxic, anthelmintic, anti-inflammatory and antioxidant activities.

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1. INTRODUCTION

Over centuries, natural remedies were availed to treat human disease and till now half of all pharmaceuticals are derived from natural products and in the case of cancer this proportion surpasses 60% [1].Though the term natural product refers to any naturally occurring substances, generally it means a secondary metabolite. The biochemical basis of using natural products to control human disease came into focus after the discovery of cardiac glycosides and important bioactive alkaloids, e.g. digoxin, ergotamine, reserpine, etc.

It is estimated that about 25% of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use. Of the total 252 drugs in WHO's essential medicine list, 11% is exclusively of plant origin. World Health Organization (WHO) claimed that 80% of people still rely on plant-based traditional medicines for their primary health care [2].

Hymenocallis littoralisis a bulbous, herbaceous plant which belongs to Amaryllidaceae family. It is also known as 'beach spider lily' due to the spider like appearance of its flowers[3]. Generally, the plant is 0.5 to 0.7 m in height and 0.7 m in width [4]. It has ornamental flowers and leaves. It is native to Mexico to Peru and Brazil [5]. Hymenocallis littoralis is known to contain bioactive constituents such as alkaloids, tannins, flavonoids and phenolic compounds. So, they possess a wide variety of pharmacological actions e.g. antiviral, anticancer, antiparasitic, antibacterial, antioxidant, anti-inflammatory, anthelmintic activity [6],[7],[8].Three alkaloids lycoricidine, narciclasine and pancratistatin exhibited significant antitumor activity [9].

In this experimental study, the ethyl acetate extract of whole bulb of *Hymenocallis littoralis* is was used and in vitro cytotoxic activity, anthelmintic, anti-inflammatory and antioxidant activity was observed.

2. METHODS AND MATERIALS

2.1. Collection

The fresh bulb of *Hymenocallis littoralis* was collected from Mithapanir Chora sea beach, Teknaf, Cox's bazar, Bangladesh in 2021. Later, it was identified by a taxonomist of Bangladesh National Herbarium and the accession number was 94818.

2.2. Preparation of plant extract

The plant (whole bulb) was collected and washed thoroughly with clean water to remove the dirt. Then the sample was cut into small pieces and introduced to a blender along with ethyl acetate. After blending, the mixture was passed through a mesh to separate the yellow-colored liquid portion. This liquid was put in a vacuum desiccator to dry and the plant extract was ready for further use.

2.3. Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is considered as a simple, high throughput cytotoxicity test for preliminary assessment of toxicity of bioactive compounds [10]. It is widely used in the evaluation of toxicity of natural plant extracts. Basically, it is a preliminary toxicity screen for further experiments on mammalian animal models rather than any critical conclusions [11].

The cytotoxicity is measured based on the killing ability of test materials on brine shrimp Artemiasalina. Michael et al., first proposed this method, though this method was further modified by several others [12]. Here, we used the method developed by Gillian E. Lewis et al., 1995 [13]. It is one of the most convenient cytotoxicity tests as it is simple and easy to operate, inexpensive and require only a small amount of test material.

Control solution- Equivalent amount of distilled water and tween 80+5ml simulated sea water

Standard solution- 30ppm potassium dichromate solution made with distilled water+5ml simulated sea water

Sample solution- Different concentrations of sample was prepared [Table 01].

Brine shrimp eggs were hatched for 48 hours in simulated sea water to get the nauplii. For this purpose, we used 35gm of sea salt with 1liters of water and 5 gm of brine shrimp eggs. A light source was added to allow hatching of the brine shrimps. After 48 hours, the nauplii began to hatch. Using a Pasteur pipette, we counted 10 nauplii and added to the control, the standard and sample (C1-C8) marked test tubes.

Table 01: Preparation of sample for brine shrimp lethality bioassay.

Test tube	Concentration (μg/mL)	Simulated sea water (mL)	Total volume
C1	6.25	4	5
C2	12.5	4	5
C3	25	4	5
C4	50	4	5
C5	100	4	5
C6	200	4	5
C7	400	4	5
C8	800	4	5

After 48 hours, by counting the dead nauplii in each tube, the percent mortality rate was calculated by using the following equation:

% death= (number of dead nauplii/number of total nauplii)x100

Finally, median lethal concentration, LC_{50} was calculated from the concentration vs percent mortality rate curve.

2.4. In vitro anti-inflammatory egg albumin denaturation assay

By using in vitro anti-inflammatory egg albumin denaturation assay, it can be identified whether an agent has the ability to stop or hinder the denaturation of the egg albumin. Denaturation is a process by which the secondary and tertiary structure of protein as well as the biological function is disturbed. Substances having anti-inflammatory activity are able to stabilize the protein structures. It is one of the anti-inflammatory mechanisms of commonly used NSAIDs [11].

Preparation of 1% egg albumin solution-At first, fresh hen’s egg was collected. Then 1% egg albumin solution was prepared by transferring 1ml of the translucent portion after careful cracking to 100 ml distilled water ensuring that the water must be cold during this preparation.

Phosphate buffer solution was prepared using distilled water, sodium chloride, sodium phosphate, potassium chloride and potassium phosphate.

Control solution-0.2 ml egg albumin + 2.8 ml of phosphate buffered saline+ 2 ml of distilled water.

Standard solution- 0.2 ml egg albumin +2.8 ml of phosphate buffered saline +2 ml of diclofenac sodium

Sample solution-0.2 ml egg albumin +2.8 ml of phosphate buffered saline + 2 ml of sample extracts of different concentrations prepared according to Table 02.

The mentioned mixtures (control, standard, and samples) were then incubated at 37±2ºC for 15 minutes and were heated in a water bath at 70±2ºC for 5 minutes. The absorbance values were measured at 660 nm after cooling by a UV-Vis spectrophotometer where distilled water was used as a blank.

2.5. Anthelmintic activity

Helminthiasis is one of the major causes of substantial morbidity and mortality, especially in tropical regions. Due to the resistance of the conventional anthelmintic medicines and high cost, the evaluation of anthelmintic activity of medicinal plants is crucial [14]. In this study, it was observed that the anthelmintic activity of Hymenocallis littoralis for determining anthelmintic activity described by Suelen Carolina Silva Soares et al., 2018 technique [15].

Control –Distilled water was used.

Table 02: Preparation of sample for in vitro anti-inflammatory egg albumin assay.

Test tube	C1	C2	C3	C4	C5	C6	C7
Concentrations (μg/mL)	7.8125	15.620	31.25	62.5	125	250	500

Standard- Diclofenac Sodium at a concentration of 10mg/ml was used

Sample- Plant extract of different concentrations was prepared as showed in Table 03.

Table 03: Preparation of samples for anthelmintic activity.

Test tube	C1	C2	C3	C4	C5	C6
Concentrations (μg/mL)	100	200	400	600	800	1000

Worms were randomly allocated in eight groups. Each earthworm was discharged into control, standard and sample solutions of various concentration. Then we measured the time taken by each earthworm to become paralyzed and die. By analyzing this time, it was identified whether our plant extracts had any promising anthelmintic activity or not.

2.6. Antioxidant assay

The determination of radical scavenging activity by DPPH was performed by the process described by Prakash (2001) [16]and absorbance of the assays were measured in 517 nm.

Control- 2 ml of methanol solution + 3 ml of 0.004% DPPH solution in methanol

Standard- 2 ml of ascorbic acid of different concentrations + 3 ml of 0.004% DPPH solution in methanol

Sample- 2ml of sample extracts of different concentrations+ 3 ml of 0.004% DPPH solution in methanol [Table 04]

Aforementioned mixtures (control, standard, samples) were kept in a dark place at room temperature for 30 minutes and then their absorbances were measured using a UV-Vis Spectrophotometer at 517 nm against methanol as a blank.

3. RESULTS AND DISCUSSION

3.1. Brine Shrimp lethality bioassay

In brine shrimp lethality bioassay, the crude ethyl acetate extracts were screened for probable cytotoxic activity [Table 05]. The concentration at which 50% mortality of brine shrimp nauplii occurred were determined. In this study, LC50 was calculated from regression equation, $y = 31.242x + 10.968$ ($R^2 = 0.9556$)that was obtained from the graphical plot using the data and compared against the reference standard and a cytotoxic drug, potassium dichromate. The LC_{50} value of potassium dichromate was 0.93 (

g/ml) while our plant extracts showed LC50 value of 1.24 (μg/ml)[Table 05] [Figure 02].

Table 05: Calculation of LC_{50} of *Hymenocallis littoralis*.

Test tube	Concentration (µg/mL)	Log C	Number of live nauplii in 24 hours	Mortality %	LC_{50} (µg/mL)
C1	6.25	0.795	6	40	
C2	12.5	1.096	6	40	
C3	25	1.397	4	60	
C4	50	1.698	4	60	
C5	100	2.000	3	70	17.75
C6	200	2.301	2	80	
C7	400	2.602	0	100	
C8	800	2.903	0	100	

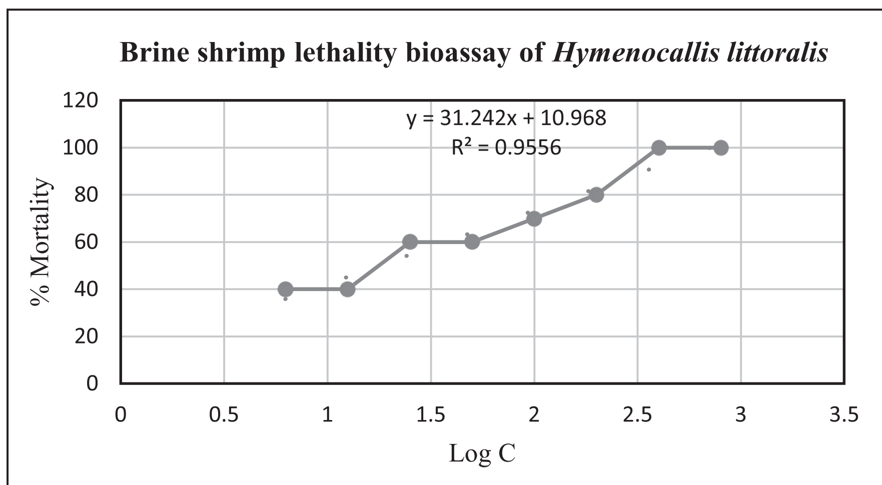

Figure 02: Brine shrimp lethality bioassay of *Hymenocallis littoralis*.

3.2. In vitro egg albumin anti-inflammatory bioassay

Observations of the dose dependent inhibition pattern i.e. as the sample concentration is increased so as their respective percent inhibition. Among all the concentrations, 125 (µg/ml), 250 (µg/ml) and 500 (µg/ml) showed percent inhibition higher than the standard. We can conclude that they have promising anti-inflammatory activity [Table 06].

Table 06: Calculation of anti-inflammatory activity of *Hymenocallis littoralis*.

Test Sample	Concentration (μg/mL)	Absorbance	% Inhibition
Control	-	0.495	-
Standard	Diclofenac Sodium	0.170	71.67
C1	7.8125	0.311	37.17
C2	15.620	0.200	59.69
C3	31.25	0.194	90.40
C4	62.5	0.183	63.03
C5	125	0.112	77.37
C6	250	0.087	82.42
C7	500	0.061	87.68

3.3. Anthelmintic activity

Individual worms are observed for the time taken to paralyze and die. The dose dependent paralysis and death time assay of earthworms was seen in an ascending order, higher the sample concentration, higher the paralysis and death time. 800 (μg/mL) and 1000 (μg/mL) showed most promising anthelmintic activity when compared with standard (albendazole) [Table 07]

Table 07: Calculation of anthelmintic activity of *Hymenocallis littoralis*.

Type	Concentration (μg/mL)	Time taken for paralysis (min)	Time taken for death (min)
Control	-	-	-
Standard	Albendazole	32	40
C1	100	80	91
C2	200	77	91
C3	400	71	80
C4	600	67	75
C5	800	45	47
C6	1000	40	47

3.4. Antioxidant activity

Usually, lower the absorbance, higher the free radical scavenging activity.

Here, the absorbance of control at 517 nm was 0.537.

We can conclude that *Hymenocallis littoralis* extract have some antioxidant property [Table 08] as their IC_{50} value was close to the IC_{50} value of ascorbic acid [Table 09].

Table 08: Calculation of IC_{50} of *Hymenocallis littoralis*.

Type	Concentration (µg/mL)	Mean absorbance	% Inhibition	IC_{50}
Control		0.763		
C1	31.25	0.631	17.30	177.91
C2	62.5	0.508	33.42	
C3	125	0.345	54.78	
C4	250	0.229	69.99	
C5	500	0.116	84.80	

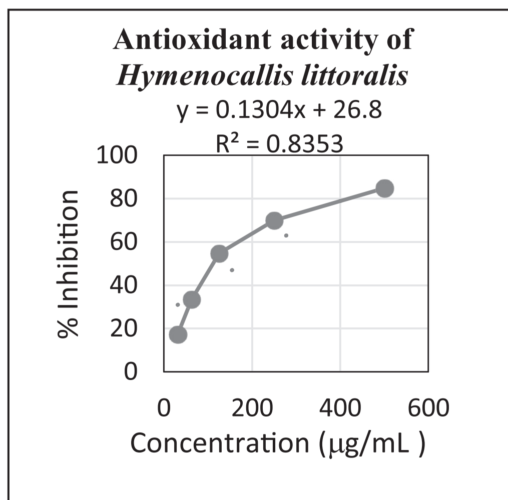

Figure 03: Antioxidant activity of *Hymenocallis littoralis*.

Table 09: Calculation of IC_{50} of Ascorbic acid.

Type	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50}
S1	31.25	0.452	15.83	
S2	62.5	0.386	28.1	
S3	125	0.236	56.05	172.21
S4	250	0.134	75.1	
S5	500	0.047	91.25	

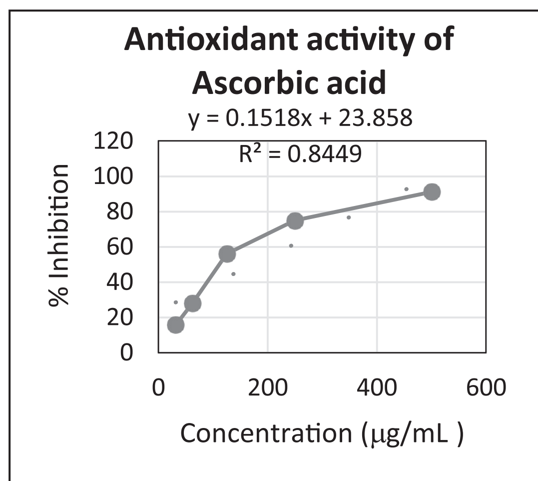

Figure 04: Antioxidant activity of Ascorbic acid.

4. CONCLUSIONS

Brine shrimp lethality bioassay- *Hymenocallis littoralis* extract showed cytotoxicity when compared with potassium dichromate.

Anthelmintic activity- *Hymenocallis littoralis* extract has dose dependent anthelmintic property when compared with albendazole.

Anti-inflammatory activity- Few concentrations of *Hymenocallis littoralis* extract have promising anti-inflammatory activity when compared with diclofenac sodium.

Antioxidant activity- *Hymenocallis littoralis* extract have a mild antioxidant activity when compared with ascorbic acid.

5. RECOMMENDATIONS

Further study can be done with specific lead compound of the crude extracts.

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