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Cytotoxic studies on medicinal of plants of *Calatrophis procera* and *Euphorbia hirta* latex

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Abstract

Brine shrimp lethality bioassay (BSLB) is considered as a useful tool for preliminary assessment of cytotoxicity studies. The present study was aimed to assess the cytotoxicity properties of both plants of *Calatrophis procera* and *Euporbia hirta* latex. The cytotoxic activity of both plants of *C. procera* and *E. hirta* latex was tested using the test animals of *Artemia salina* (Brine shrimp) bioassay. The percentage of mortality of both extracts was observed by LC50 values by the Finney probit analysis method. The results of both these plants have good cytotoxicity activities.

Keywords: Brine shrimp test, Cytotoxicity, Medicinal plants, *Calotraphis procera*, *Euphorbia hirta*

1 INTRODUCTION

Natural products have been used for the treatment of several diseases. The plants have been used for medicinal purposes throughout human history, and the first pharmaceuticals were derived from medicinal plants [1]. About 80% of all medicines used to treat human and animal illness were obtained from the leaves, barks, roots and rhizome. Now about 70% of the modern drugs prepared for natural products [2]. Approximately 700 drugs derived from natural products were approved by New Chemical Entities (NCEs) [3]. Biodiversity and traditional medical knowledge had provided useful lead compounds for cancer chemotherapy, as exemplified in the discovery of the vinca alkaloids (vincristine and vinblastine), taxols (paclitaxel and docetaxel), camptothecin and etoposide [4-5].

The medicinal herb of *Calotrophis procera* is belongs to family Asclepiadaceae. The latex of *C. procera* is a rich source of proteins that have anti-inflammatory, anti-nociceptive and selective cytotoxic and anti-tumorigenic properties [6]. Euphorbia hirta belonging to the family Euphorbiaceae, which is possessed antibacterial, anthelmintic, antiasthmatic, sedative, antispasmodic, antifertility, antifungal and antimalarial properties [7].
The brine shrimp lethality bioassay represents a rapid, inexpensive, and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and antitumor properties. The bioassay is considered to be a very useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, toxicity of plant extracts, heavy metals, and cytotoxicity testing of dental materials. In the present study was evaluated in the cytotoxicity activities on the both plants of *C. procera* and *E. hirta*.

2 MATERIALS AND METHODS

2.1 Preparation of Extracts

The fresh latex of *Calotrophis procera* and *Euphorbia hirta* were collected from local habitat in the early morning. The latex was diluted immediately in dimethylsulfoxide at 10% v/v. The quality of the solution was determined by homogeneity and the absence of precipitates.

2.2 Brine Shrimp Lethality Bioassay (Cytotoxicity Test)

2.2.1 Hatching the brine shrimp

Brine shrimp eggs were hatched in artificial sea water prepared from commercial sea salt 40gms/Lr and supplemented with 6mg/L dried yeast. The two unequal compartments plastic chamber with several holes on the divider was used for hatching. The eggs were sprinkled into

the larger compartment which was darkening, while the smaller compartment was illuminated. After 48hr incubation at room temperature (25-29°C), nauplii (larvae) were collected by pipette from the lighter side, whereas their shells were left in another side. The free nauplii in each well were counted under a stereoscopic microscope after 24 exposures. Five replicates were used for each treatment and control. The percentages of hatchability were calculated by comparing the number of free nauplii in each treatment with the number of free nauplii in the control. Later the percentage of hatch inhibition (HI%) was calculated as follows: HI (%) = % Hatchability in the control − % Hatchability in each treatment.

2.2.2. Brine shrimp lethality bioassay

The method is attractive, because it is very simple, inexpensive and sensitive [8]. 10 nauplii were drawn through a glass capillary and placed in test tubes containing 10 ml of artificial seawater solution and 0.5 ml of the diluted plant extract (Table-1) was added to it and maintained at room temperature for 24h under constant aeration and a light source. The test was also carried out on control (artificial sea water). The test animals of *Artemia* mortality in both treated and control was recorded after 24h and the percentage of mortality calculated.

$$\% \text{ Mortality} = \frac{\text{- mortality at control}}{\text{100 - mortality at control}} \times 100$$

2.3 Statistical Analysis

The lethal concentrations, LC_{50} concentrations (ppm) 50% larvae of *Artemia* showed mortality, 95% confidence limit of upper and lower confidence levels were calculated by Probit analysis (SPSS, version 11.5).

3 RESULTS AND DISCUSSION

The result of the brine shrimp lethality assay of both extracts was represented in the table-1. The latex of *C. procera* was observed by maximum cytotoxicity represented in the table-1. The medicinal plants of *C. procera* and *Euphorbia hirta latex* were observed by 50% of mortality of tested animals of Artemia for 24h seen in the table-2. The latex of *C. procera* and *Euphorbia hirta were good cytotoxicity activity was observed.* The medicinal values of Euphorbiaceae family members have been reported in the antiviral and antitumor proprieties [9-13]. Earlier studies, Sokmen (2001) who conducted different extracts of plant parts and callus cultures of 10 medicinal plants almost all extract showed a moderate activity [14]. BSLA is inadequate in determining the mechanism of action of the bioactive substances in plants nor is it specific for antitumor activity, it provides a preliminary screen that can be supported by a more specific bioassay, once the active compounds have been isolated [15]. Plants found to be toxic to brine shrimp are likely to be a good candidate for anti-cancer research [Ramachandran,2011]. The conclusion of the present study observed that both plants are good cytotoxicity effect. Further investigations of both plants on the potent crude extract to find out the cytotoxic potential compound through a bioassay guided separation and also to find out the mechanism of action is suggested by this study.

Table-1: Cytotoxicity studies on *Calotraphis procera* and *Euphorbia hirta* latex on lethal mortality of Brine shrimp

Dose/Conc.	Total	Dead (No.0f Death)	
		C.Procera	*E.hirta*
0.00	10	0	0
0.10	10	1	1
0.20	10	2	2
0.40	10	6	4
0.80	10	7	5
1.00	10	9	7
5.00	10	10	10

Table-2: Probit analysis of *Calotraphis procera* and *Euphorbia hirta* latex on lethal mortality of Brine shrimp

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Calotrphis procera	-.151	.272	-.553	.580	-.685	.383
	Euphorbia hirta	.405	.250	1.620	.105	-.085	.894
	Intercept	-2.563	1.685	-1.521	.128	-4.248	-.878

a. PROBIT model: PROBIT(p) = Intercept + BX

Covariances and Correlations of Parameter Estimates

Parameter Estimates

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		CP	EH	Natural Response
PROBIT	VAR00003	.074	-.796	.333
	VAR00004	-.054	.062	.220
	Natural Response	.005	.003	.004

Covariances (below) and Correlations (above).

Natural Response Rate Estimate

	Control Group		Estimate	Std. Error
	Number of Subjects	Number of Responses		
PROBIT	10	0	.000	.060

Chi-Square Tests

		Chi-Square	df[a]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	.316	3	.957

a. Statistics based on individual cases differ from statistics based on aggregated cases.

Cell Counts and Residuals

	Number	CP	EH	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	.000	.000	10	0	.052	-.052	.005
	2	1.000	1.000	10	0	.105	-.005	.010
	3	2.000	2.000	10	0	.199	.001	.020
	4	6.000	4.000	10	0	.322	.078	.032
	5	7.000	5.000	10	1	.553	.247	.055
	6	9.000	7.000	10	1	1.383	-.383	.138
	7	10.000	10.000	10	5	4.899	.101	.490

4 ACKNOWLEDGEMENTS

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