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Research Article

BRINE SHRIMP LETHALITY ACTIVITY OF *EUPHORBIA HIRTA* LINN.

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ABSTRACT

Brine shrimp larva has been used as a bioassay for a variety of toxic substances. Brine shrimp lethality assay has applied to plant extracts in order to facilitate the isolation of biologically active compounds. This method is used for the determination of LC_{50} values of herbal extracts. In this study, the plants *Euphorbia hirta* Linn which is reported in traditional literature for its anti-tumor activity, had been selected to test for brine shrimp lethality activity. Petroleum ether, Diethyl ether, Chloroform, Ethyl acetate, Acetone, Ethanol (successive) extracts and aqueous and ethanolic extract were collected and used for activity. The result showed that the LC_{50} values of ethanolic extract of the whole plant are found 118.88 µg to possess significant cytotoxic activity

Keywords: Brine shrimp lethality activity, Euphorbiaceae, *Euphorbia hirta* Linn.

INTRODUCTION

The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of the crude extracts as well as isolated compounds to access the toxicity towards brine shrimp. The activity also provides an indication of possible cytotoxic properties of the test materials. Brine shrimp nauplii has previously utilized in various bioassay systems[1].

Euphorbia hirta L is popularly known as *Euphorbia pilulifera* family Euphorbiaceae. It is commonly called Dudhni, Australian asthma herb, Queensland asthma weed, Pills bearing spurge, Cat's hair, Hairy spurge. Recent findings suggested that several thousands of plants have been known with medicinal applications in various cultures[2]. The whole plant has also been reported to possess antibacterial, antiamoebic, antifungal, antiviral, spasmolytic, antidiarrhoeic, sedative, anxiolytic, analgesic, antipyretic, anti-inflammatory, antimalarial and Diuretic properties[3-13].

MATERIALS AND METHODS

Plant material

Euphorbia hirta Linn was collected from various geographical region of Sangli districts (Maharashtra). Authentication was get done by Dr. Mrs. U. S. Yadav HOD of Botany department, Willingdon college of Science, Sangli Specimen vouchers of plants were also kept with number EH1 for future reference.

Preparation of extracts

The whole plant of *Euphorbia hirta* Linn were shade dried at 37^{0}C to 40^{0}C and coarsely powdered through mesh 20 and defatted with Petroleum ether (60-80). The powdered plant material was extracted with the various solvents such as Petroleum ether (40-60), Diethyl ether, Chloroform, Ethyl acetate, Acetone, Ethanol (successive), using soxhelet apparatus and aqueous extract was obtained by maceration method.

Table 1: Brine Shrimp lethality bioassay of various extract of whole plant of *Euphorbia hirta* Linn

Drugs	Conc. of extract	Total no. shrimps used/tube	Shrimp Survived			Total No. of Shrimp Survived	% inhibition	LC_{50} (µg)
			T1	T2	T3			
Petroleum ether	100µg	10	6	8	6	20	33.33	>1000
	500 µg		4	6	8	18	40.00	
	1000 µg		8	4	6	18	40.00	
Chloroform	100µg	10	8	8	6	22	26.66	>1000
	500 µg		6	2	8	16	46.66	
	1000 µg		6	4	6	16	46.66	
Diethyl ether	100µg	10	6	6	4	16	46.66	580.83
	500 µg		6	6	8	20	33.33	
	1000 µg		2	6	2	10	66.66	
Acetone	100µg	10	6	4	2	12	60.00	450.71
	500 µg		2	2	0	04	86.66	
	1000 µg		0	2	2	04	86.66	
Ethyl acetate	100µg	10	8	6	6	20	33.33	803.66
	500 µg		6	8	6	20	33.33	
	1000 µg		0	6	6	12	60.00	
Ethanol	100µg	10	7	5	4	16	46.66	198.27
	500 µg		4	6	2	12	60.00	
	1000 µg		3	2	3	8	73.33	
Aqueous	100µg	10	10	10	9	29	3.33	>1000
	500 µg		10	10	8	28	6.66	
	1000 µg		10	9	10	29	3.33	
Ethanolic	100µg	10	6	4	5	15	50.00	118.88
	500 µg		3	6	4	13	56.66	
	1000 µg		3	4	3	10	66.66	

From the Table 1 it is evident that the ethanolic extract was found to significant activity against brine shrimp nauplii and LC_{50} was found 118.88 µg/ml.

Hatching the brine shrimp

Brine shrimp eggs (*Artemia salina*, Sanders) were hatched in artificial sea water prepared from artificial sea salt 38 g/l and supplemented with 6 mg/l dried yeast. The two unequal compartments plastic chamber with several holes on the divider was used for hatching.

Later on the eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. After 48 hours incubation at room temperature, nauplii (larvae) were collected by pipette from the illuminated side[14].

Cytotoxicity bioassay [15, 16]

Cytotoxicity study was carried out using the standard procedure as described by McLaughlin 1991. Brine shrimps (*Artemia salina*) were hatched using eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 h. After hatching, active *nauplii* free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5 ml of brine solution. In each experiment, 0.5 ml. of the plant extract was added to 4.5 ml of brine solution and maintained at room temperature for 24 h under the light and surviving larvae were counted. Vehicle treated used as control for the test. Test solutions were used in sets of three tubes per dose.

Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} values were obtained from the best-fit line plotted as concentration verses percentage lethality.

RESULTS & DISCUSSION

Although the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to acess the bioactivity of the plant extracts. The variation in BSLA results (Table no.1) observed in the amount and kind of cytotoxic substances (e.g. tannins, flavonoids, triterpenoids, or coumarins) present in the crude extracts. Moreover, this significant lethality of the crude plant extracts (LC_{50} values less than 1000 ppm or µg/mL) to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigation. In the present study ethanolic extract of the plant of *Euphorbia hirta* Linn showed remarkable activity which may be due to presence of tannin, flavonoids, terpenoids which is tested by chemical test.

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