

Cytotoxic Activity Evaluation of *Euphorbia granulata* Forssk

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Abstract: *Euphorbia granulata* Forssk is a medicinal plant used in many countries for treating different ailments. The present investigations were focused on cytotoxic activity of this plant using brine shrimp lethality bioassay. 2% lethality was recorded with negative control and 0.01mg/ml while no lethality occurred with 0.1 and 1.0mg/ml of extracts. The lowest toxic level (2.5% lethality) noted was 2mg/ml while the high toxic level (97.5% lethality) was 25mg/ml. The extract had LD₅₀ of 16.21mg/ml against brine shrimp larvae. The overall results suggest that the extracts had no toxicity at standard concentrations, but toxic at high dose above 2 mg.

Key words: *Euphorbia granulata* • Cytotoxicity • Lethality • Brine Shrimp

INTRODUCTION

Cancer cells proliferate at the expense of normal body cells and ultimately damage the surrounding tissues leading to malfunction of organ and ultimately death [1, 2]. After coronary diseases cancer is the leading cause of death and according to an estimate 10 million cases of different forms of cancer are reported every year [3]. Toxicity of chemotherapeutic agents to brine shrimps nauplii has direct correlation with human nasopharyngeal carcinoma [4]. Originally developed for the insecticidal potential of a product the brine shrimp lethality assay is now a day's an easy, time saving, effective and internationally accepted method for screening anticancer potential [5].

To see the cytotoxic effect of plant extract [6] used MTT cancer assay for oil extract from *Croton matourensis* and *Croton micans* against LoVo, X-17 (colon carcinoma). It was seen that HeLa cancer and control normal cell showed moderate cytotoxic effects only against cancerous cells and not against control. It was reported [7] that essential oil from *Origanum majoricum*, *Origanum compactum* and *Origanum applii* showed cytotoxicity against brine shrimp with LD₅₀ value of 10 to 29 ppm as compared to aqueous extracts which showed LD₅₀ equal to water (negative control), clearly indicates the cytotoxicity of the aqueous extract. Ethanolic extract from

different parts of *Polyalthia longifolia*, *Trachyspermum ammi* and *Elaeocarpus serratus* when used against brine shrimp larvae proved cytotoxic with mild to moderate effect [8]. *Viscum articulatum* collected from five different plants was extracted with ethanol were used against brine shrimp larvae. All the five extracts showed very potent effects at 1000µg/ml against the brine shrimp larvae [5].

Essential oils [9] from leaves of *Cymbopogon citratus* had pronounced cytotoxic effect at low dose (150µl/ml) as compared to *Cymbopogon nardus* which appeared comparatively less cytotoxic at higher doses (450µl/ml). A similar study [10] on Iranian medicinal plants stated that methanolic extracts showed cytotoxicity against MCF7 cells. Sixteen plants from Bangladesh [11] with water, dichloromethane, *n*-hexane and methanol for their cytotoxicity against mouse fibroblasts (normal), humane gastric, colon and breast (cancerous) cells. It was noted that extracts of *Limnophila auriculata* (aqueous), *Hygrophila auriculata*, *Hibiscus tiliaceus* (methanolic) were cytotoxic against breast cancerous cells but were not active against normal mouse fibroblasts. Other plants extract showed low or no activity while *Blumea lacera* (extracted with methanol) was the most potent (IC₅₀ 0.01 to 0.08 µg/ml) of all the plants studied. The study of Merghoub [12] reported that extracts from *Ormenis mixta*, *Retama monosperma* and *Inula viscosa* showed remarkable cytotoxic activity against two cell lines.

MATERIALS AND METHODS

Brine shrimp lethality assay (BSA) used by McLaughlin and Rogers [13] was followed with some adjustments. Artificial sea water (ASW) was prepared by 3.8grams of sea salt in 100ml of distilled water.

Eggs Hatching: A 20 × 8 cm light proof tray was divided with perforated plastic band into two 1:3 unequal portions. The tray was filled with enough ASW to cover the hole in the partition wall. The entry of light to larger part was blocked with a plastic cover. 10mg of Brine shrimp (*Artemia salina*) eggs were added to the larger portion of the tank and covered with a lid to prevent light. The open end of the tank was illuminated with lamp so that phototrophic nauplii (Brine shrimp larvae) may move towards the light through the pore in the partition. The setup was left to hatch at room temperature for 48hrs.

Technique: 100ml ASW was prepared in a beaker, filtered and poured into the water tank. From stock solution (100mg/ml) of *E. granulata*, three dilutions of 5000 µg, 500µg and 50µg/ml in DMSO were prepared. 1ml from each of the above samples was poured into test tubes and allowed to evaporate to dryness. After dryness 3ml of ASW was added into each test tube and 10 nauplii were added with the help of disposable Pasteur pipette.

The final volume in a test tube was raised to 5ml with ASW so that the tube contain 1000, 100 and 10µg/ml of test samples. 0.30mg/ml potassium dichromate was used as positive control while DMSO as negative control. The experiment was performed in five replicate and was left at room temperature for 24hrs. After 24hrs the nauplii in positive control, negative control and test sample were counted in all test tubes and percent toxicity calculated.

RESULTS AND DISCUSSION

The Table 1 given below shows the results of the brine shrimp lethality bioassay. Table 2 summarizes the toxicity levels of plant extracts. The high toxic level determined was 25mg/ml where 97.5% naupli deaths recorded while the lower toxic level noted was 2 mg/ml with 100% survival. For this plant 16.21 was investigated as the LD50 (Table 1).

Fifty naupli were used in the experiment and only 2% mortality was recorded for negative control (DMSO) and 0.01 mg/ml of extract concentration. 100% survival was observed for 0.1 and 1.0 mg/ml of extract doses. Literature shows that there is a direct correlation between toxicity of a therapeutic agent to brine shrimps nauplii and human nasopharyngeal carcinoma [4]. Cytotoxic property of natural products is also linked to its anticancer potential [14, 15]. For investigating anticancer potential of natural

Table 1: Impact of *Euphorbia granulata* Ethanolic Extracts on Brine Shrimp Larvae

Parameters	NC	Extract concentration used			PC
	DMSO	0.01mg / ml	0.1 mg / ml	1.0 mg / ml	P-dichromate
No of nauplii used	50	50	50	50	50
Nauplii survived	49	49	50	50	0
Dead nauplii	1	1	0	0	50
Percent survival	98	98	100	100	0
LD ₅₀ (µg/ml)	-	16.21	16.21	16.21	-
Upper toxic concentration	-	25.0	25.0	25.0	-
Lower toxic concentration	-	2.0	2.0	2.0	-

The experiment was performed in five replicates for each dilution, NC = negative control, PC = positive control

Note. Log value against probit 5 was 1.21 and its antilog value was 16.21 (LD50)

Table 2: LD₅₀ Calculations for *Euphorbia granulata* for Brine Shrimps Larvae

Groups	Dose (mg)	Log of dose	Total naupli	Dead naupli	Percent dead	Corrected percentage	Probit value
1	2.0	0.30	50	0	0	2.5	3.04
2	8.0	0.90	50	6	12	12	3.82
3	14.0	1.15	50	17	34	34	4.59
4	20.0	1.30	50	38	76	76	5.71
5	25.0	1.40	50	50	100	97.5	6.96

Table 3: Conversion Table from Percentage to Probit Value.

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

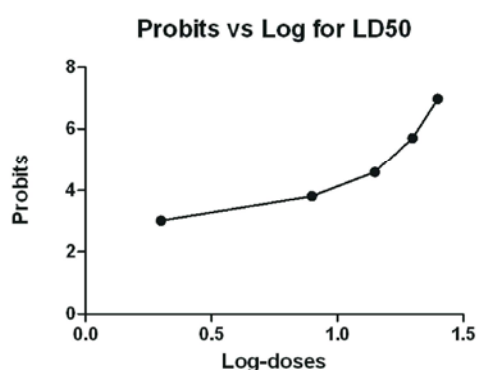


Fig. 1: LD₅₀ determination for *Euphorbia granulata* Extract Against Brine Shrimp Larvae.

products one or two anticancer activities are coupled and usually antitumor and cytotoxic activities follow each other [16]. In the present study standard concentrations of 10 µg, 100 µg and 1000 µg proposed by McLaughlin and Rogers [13] were used for cytotoxic impact of the plant extracts. The results show that the ethanolic plant extracts were not lethal at all the three strengths. Similar results were achieved with negative control. However the positive control (P-dichromate) produced 100% lethality. This suggests that the extract was not toxic at standard concentration proposed by McLaughlin and Rogers [13].

In a separate experiment toxic concentrations of the extract ranged from 1 to 40 mg/ml were determined. The least tolerable level (100% deaths) was noted with 25 mg/ml. The maximum concentration at which no death occurred was taken as highest tolerable level. Thus highest tolerable level determined was 2 mg/ml. After applying the correction factor for both high deaths and lowest death percentage the actual value come out to be 97.5% and 2.5%. Using these and probit values (Table 3), LD₅₀ was determined. Using graphpad prism software 16.21 was determined as LD₅₀ for the extract as shown in the figure 1. This suggests that the extract is not toxic for

brine shrimps. Previously antitumor activity was carried out for *E. granulata* which showed no inhibition of tumor growth.

CONCLUSION

Hence the overall results suggest the non-cytotoxic nature of *Euphorbia granulata*. It further suggests that plant is safe for human consumption even if taken in higher doses.

ACKNOWLEDGMENT

We (authors) appreciative Center of Biotechnology and Microbiology, University of Peshawar, KPK-Pakistan for experimental work support.

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