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Research Article

BRINE SHRIMP CYTOTOXIC ACTIVITY OF 50% ALCOHOLIC EXTRACT OF CROTON BONPLANDIANUM BAILL.

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ABSTRACT

Objective: To study the presence of anti-tumorous compounds in *Croton bonplandianum.*
Methods: 50 % ethanolic extract, obtained from dried powdered plant material of *C. bonplandianum* was assayed for its Brine shrimp cytotoxic activity.
Results: It was found that the extract was very effective against brine shrimp and shows LC_{50} at the concentration of 46.7mg/lit (38 mg/lit -55 mg/lit).
Conclusions: It proves the presence of anti-tumorous compounds in *C. bonplandianum*.

Keywords: Brine shrimp, cytotoxic activity, *Croton bonplandianum*, anti-tumorous compounds.

INTRODUCTION

The Croton bonplandianum is a common roadside and waste land weed of Euphorbiaceae family. The plant has been reported by many authors for its various activities e.g. genotoxicity and antimicrobial activity [1]; cardio and hepatotoxic effect [2]; antifungal and antibacterial activity [3] etc.; beside all these activities Islam et al. [4] reported this plant (methanolic extract) for its antitumor activity based on potato disc and radish seed bioassays method.

In vivo lethality in a simple zoologic organism can be used as a convenient monitor for screening and fractionation in the discovery and monitoring of bioactive natural product [5]. It is possible to detect and then monitor the fractionation of cytotoxic, as well as3PS (P388) (in vivo murine leukemia) active extracts using the brine shrimp lethality bio-assay rather than more tedious and expensive in vitro and in vivo antitumor assays. The brine shrimp assay has advantages of being rapid (24 hours), inexpensive, and simple (eg, no aseptic techniques are required). It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of sample (2–20 mg or less). Furthermore, it does not require animal serum as is needed for cytotoxicities. [5]

Not only that, there is positive correlation between brine shrimp toxicity and 9KB (human nasopharyngeal car-cinoma) cytotoxicity (p = 0.036 and kappa =0.56). And the brine shrimp test was being used as a prescreen for a panel of six human solid tumor cell lines at the Cell Culture Laboratory of the Purdue Cancer Center [5]. This is a internationally accepted bioassay for screening of antitumor compounds [6].

So the present work incorporates the cytotoxic effect of 50% aqueous ethanolic extract of Croton bonpladiaum on Brine shrimp. Here 50 % aqueous ethanol was used as extract media, instead of methanol (as used by Islam et al) because it was cheap, easily available and non-toxic.

MATERIALS AND METHODS

Preparation of plant extract

Healthy Croton bonplandianum plants were collected from the campus of the University of Kalyani during the month of June –July 2011. The collected plants were washed thoroughly with the distilled water. Plants were sun dried and powdered with electric blender. 100 gm. of the powered plant material was soaked in 1000 ml. of 50% aqueous ethanol for overnight and then filtered. Residue was repeatedly soaked with 50% aqueous ethanol and filtered until the extract became colourless. The filtrate was evaporated under reduced pressure in a vacuum evaporator to a deep brown sticky substance. This material was used for the study with required dilution. The vehicle used was propylene glycol.

Brine Shrimp Lethality Assay

About 1 g of Artemia salina (Linnaeus) cysts (Sanders Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) was aerated in 1 L capacity glass container (separating funnel) containing filtered seawater (30 ppt NaCl solution, pH about 8.2). Air pump was fitted to the water to ensure complete aeration of the cysts. After 48 hours of incubation at room temperature (25-29°C), under continuous illumination of fluorescence lamp newly hatched free-swimming pink-coloured nauplii were harvested from the bottom. As the cyst capsules floated on the surface, this collection method ensured pure harvest of nauplii. The freshly hatched free-swimming nauplii were used for the bioassay. The assay system was prepared with 10 ml of filtered seawater containing chosen concentration of extract and 1% yeast extract (for feeding) in watch glass. Conducting the experiment in watch glass ensure the sufficient aeration to the solution. In each watch glass, 20 nauplii were transferred and the setup was allowed to remain for 24 h, under constant illumination of florescent lamp . Number of survived nauplii were counted with a hand lens in 3 hours interval. Three replicates were prepared for each dose level and after 24 hours LC50 values were determined, based on the per cent mortality, using probit regration by statistical software SPSS 13.

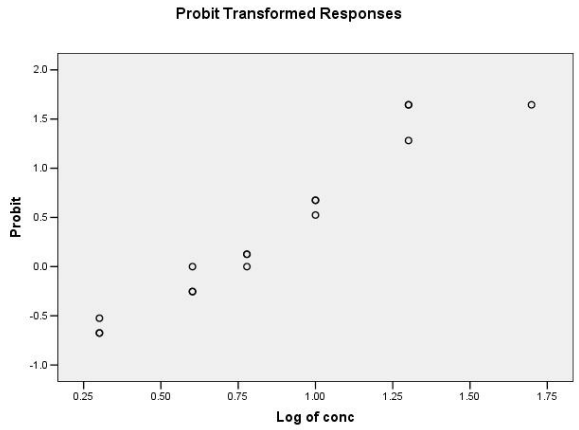
RESULTS AND DISCUSSION

Table 1: Brine shrimp lethality assay of 50% aqueous ethanolic extract of C. bonplandianum.

concentration of the extract		Number of survival of shrimp after								
		0h	**3h**	**6h**	**9h**	**12h**	**15h**	**18h**	**21h**	**24h**
0 mg/ml	Mean	20.00	20.00	20.00	20.00	19.67	19.67	19.67	19.33	19.00
	Std. Deviation	0.00	0.00	0.00	0.00	0.58	0.58	0.58	0.58	1.00
0.02 mg/ml	Mean	20.00	20.00	20.00	18.33	17.00	16.33*	16.00*	15.33*	14.67*
	Std. Deviation	0.00	0.00	0.00	1.15	2.00	1.53	1.00	1.53	0.58
0.04 mg/ml	Mean	20.00	20.00	20.00	16.67*	15.67*	12.67*	11.67*	11.33*	11.33*
	Std. Deviation	0.00	0.00	0.00	1.15	0.58	0.58	1.53	1.15	1.15
0.06 mg/ml	Mean	20.00	20.00	19.33	16.33*	16.00*	14.67*	12.33*	11.00*	**9.33***
	Std. Deviation	0.00	0.00	1.15	2.52	3.00	2.08	1.53	1.00	0.58
0.1 mg/ml	Mean	20.00	20.00	16.67*	11.00*	9.00*	8.00*	7.00*	6.00*	5.33*
	Std. Deviation	0.00	0.00	1.53	1.00	1.73	1.73	2.00	1.00	0.58
0.2 mg/ml	Mean	20.00	20.00	14.67*	9.33*	6.33*	5.00*	4.33*	2.00*	1.33*
	Std. Deviation	0.00	0.00	1.53	2.08	1.15	1.00	0.58	1.00	0.58
0.5 mg/ml	Mean	20.00	20.00	15.67*	11.00*	8.00*	6.00*	2.33*	0.67*	0.33*
	Std. Deviation	0.00	0.00	1.53	1.00	1.00	0.00	0.58	0.58	0.58
1 mg/ml	Mean	20.00	20.00	15.00*	6.33*	5.00*	1.00*	0.33*	0.00*	0.00*
	Std. Deviation	0.00	0.00	1.00	1.15	1.00	1.00	0.58	0.00	0.00
SE				±0.88	±1.18	±1.29	±1.01	±0.96	±0.78	±0.58
CD at 5% level				1.87	2.5	2.74	2.15	2.03	1.66	1.22

*** Indicates significance at (P<0.05) in respect of control. LC_{50} seems to be 0.06 mg/ml. of the extract.**

From the assay it was found (Table 1) that in the control sets almost all the shrimp survived throughout the observed period (24 h). In highest treated concentration 1mg/ml, shrimps began dying only after 6 hours and after 21 hours there was complete lethality of the shrimp. In the concentration of 0.06 mg/ml of plant extract about 50% shrimp remain survived after 24 hour. So LC_{50} value was seems to be 0.06 mg/ml.



LC_{50} (median lethal concentration) values were calculated using the regression line obtained by plotting the concentration against the death percentage on a probit scale, and the results were evaluated with probit analysis (SPSS 13.0). The evaluated LC_{50} was 46.7mg/lit (38mg/lit-55mg/lit)

CONCLUSION

Croton bonplandianum plant showed antitumor property and thus may be utilized for raising antitumor drug.

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