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Research Article

BRINE SHRIMP CYTOTOXIC ACTIVITY OF 50% AQUEOUS ETHANOLIC LEAF EXTRACT OF *CALOTROPIS PROCERA* R. BR

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ABSTRACT

Objective: The objective of this work entails a preliminary screening of 50% aqueous ethanolic leaf extract of *Calotropis procera* for locating antitumor activity.

Methods: 50% aqueous ethanolic extract, obtained from dried powdered plant material of *C. procera* was partitioned sequentially in petroleum ether, benzene, and chloroform. Each fraction thus obtained was concentrated in a vacuum evaporator. The residual mass was collected separately and dissolved in propylene glycol. The three samples were subjected to brine shrimp cytotoxic assay to locate if there may be any positive response of antitumor activity.

Result: It was found that the sample obtained from chloroform extract responded positively in brine shrimp test and showed lethal concentration (LC_{50} at which 50% individual dies) at the concentration of 5 mg/ml. Benzene extract showed LC_{50} at the concentration of 15 mg/ml and petroleum ether showed LC_{50} at the concentration of 20 mg/ml.

Conclusion: Proves the presence of antitumor phytochemicals in *C. procera.*

Keywords: Antitumor compounds, Brine shrimp, *Calotropis procera*, Cytotoxic activity.

INTRODUCTION

Interest toward active phytochemicals is gaining worldwide acceptance to formulate nontoxic, antihazardous, and cost effective management for different therapeutic approaches. *Calotropis procera* R.Br. belongs to the family Asclepiadaceae, is an important medicinal plant whose leaves and roots have multiple uses. It is known by various names such as swallow wort, dead sea apple, sodom apple, or milkweed, commonly used and known as Arka or Madar. Telugu name is Jilledachetta and in English calotrope, calotropis, dead sea fruit, desert wick, giant milkweed, mudar fiber, rubber bush, rubber tree, sodom apple, and swallow wort. The root extract of *C. procera* has been found to produce a strong cytotoxic effect on COLO 320 tumor cells [1]. A derivative of a cardenolide isolated from the root barks of *C. procera* shows a strong cytotoxic effect on several human cancer lines, a high *in vivo* tolerance to tumor growth and prolonged survival in the human xenograft models of nude mice *LIVER CANCER* [2]. The latex of the plant has been extensively studied and found responsible for cytotoxic, procoagulant, anti-inflammatory, and abortifacient activities [3,4]. Ethanolic extract of its flowers and aqueous and organic extracts of its dried latex (DL) also exhibit strong anti-inflammatory activity in animal models of acute and chronic inflammation [5-7]. The present work has been done with 50% aqueous ethanolic leaf extract of *C. procera* to assess antitumor activity by Brine Shrimp assay [8] - an internationally accepted, less expensive, simple protocol for assaying antitumor action.

MATERIALS AND METHODS

Preparation of plant extract

Healthy *C. procera* plant leaves were collected from Bamanpukur, Sree Mayapur, Nadia during the month of June - July 2011. The collected plant leaves were washed thoroughly with the distilled water. Plants were sun dried. 100 g of the powered plant material was soaked in 1 L. Of 50% aqueous ethanol for 5 days and then filtered. The residue was repeatedly washed with 50% aqueous ethanol and filtered until the extract became colorless. The filtrate was evaporated under reduced pressure in a vacuum evaporator to a deep brown sticky substance. Each residual mass thus obtained was dissolved in propylene glycol and was partitioned over benzene, petroleum ether, and chloroform. The brine shrimp cytotoxic assay was done by using different concentrations of the samples.

Brine shrimp lethality assay

Brine Shrimp cytotoxicity assay was done following the method of Meyer *et al.*, 1982. About 1 g of *Artemia salina* (Linnaeus) cysts (Sanders Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) was properly aerated in 1 L capacity glass container (separating funnel) containing filtered seawater (30 ppt NaCl solution, pH about 8.2). Incubation was done at room temperature (25-29°C). After 48 hrs of incubation, newly hatched free-swimming pink-colored nauplii were harvested from the bottom under continuous illumination of fluorescence lamp. When the nauplii were floated on the surface, they were collected, and these 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 a watch glass. The sufficient aeration to the solution of watch glass was ensured. In each watch glass, some nauplii were transferred, and the set up was allowed to remain for 24 hrs, under constant illumination of florescent lamp. Numbers of survived nauplii were counted with a hand lens in 3 hrs interval. Three replicates were prepared for each dose level and after 24 hrs LC_{50} values were determined, based on the percent mortality, statistical software SPSS 13.

RESULTS

Tables 1-3 clearly showed that after solvent partitioning of 50% aqueous ethanolic leaf extract using chloroform, benzene, and petroleum ether, it was found that the sample obtained from chloroform portion was most effective against brine shrimp with LC_{50} at a concentration of 5 mg/ml, followed by benzene portion with LC_{50} at a concentration of 15 mg/ml. Petroleum ether portion showed LC_{50} at a concentration of 20 mg/ml.

Table 1: Brine shrimp lethality assay of the sample prepared from benzene extract of *C. procera*

Sample concentration (mg/ml)		No. of survivals after 0 hrs	No. of survivals after 3 hrs	No. of survivals after 6 hrs	No. of survivals after 9 hrs	No. of survivals after 12 hrs	No. of survivals after 15 hrs	No. of survivals after 18 hrs	No. of survivals after 21 hrs	No. of survivals after 24 hrs	LC_{50}
0	Mean±SD	20±0.00	20±0.00	20±0.00	20±0.00	20±0.00	20±0.00	19.66±0.57	19.33±0.57	19±0.00	
1	Mean±SD	20±0.00	19.66±0.57	19.33±1.15	18.66±1.15	18.33±1.52	18.33±0.57	18±1.00	17.66±1.15	17±1.00	
3	Mean±SD	20±0.00	19±1.00	18.66±1.52	18.33±1.15	18±2.00	17.66*±1.52	17*±1.00	16.6*±1.15	16*±1.00	
5	Mean±SD	20±0.00	18±1.00	18±1.00	17.66±1.15	17.33±0.57	15.33*±0.57	15*±1.00	14*±1.00	13.66*±0.57	
10	Mean±SD	20±0.00	17.66±1.15	17±1.00	16.66±1.15	16±1.00	15.66*±1.15	14.33*±0.57	13.6*±0.57	12.66*±0.57	
15	Mean±SD	20±0.00	16.66±1.15	16.33±1.52	16.33±0.57	15.6*±1.52	14.66*±1.52	13.66*±0.57	12*±0.00	10*±1.00	(15 mg/ml)
20	Mean±SD	20±0.00	16.66±1.15	16±1.00	15.33±1.52	14.3*±1.52	13.33*±1.52	12.33*±1.52	11.3*±0.57	9.33*±1.15	
25	Mean±SD	20±0.00	14.33*±0.57	12.33*±1.52	11.33±1.52	10*±1.73	8.33*±1.52	8*±1.00	6.33*±0.57	5.33*±0.57	
30	Mean±SD	20±0.00	13.33*±1.52	10.66*±1.15	8.33*±0.57	5.66*±1.15	5*±1.00	3.33*±0.57	2.33*±1.15	1*±1.00	
SE			±0.81	±0.95	±0.8	±1.14	±0.83	±0.75	±0.68	±0.68	
CD at 5% level			1.71	2.00	1.77	2.40	1.74	1.58	1.43	1.43	

*Indicates significance at ($p<0.05$) in respect of control. LC_{50} seems to be 15 mg/ml of the extract, *C. procera: Calotropis procera*, SD: Standard deviation, SE: Standard error, LC_{50}: Lethal concentration

Table 2: Brine shrimp lethality assay of the sample prepared from chloroform extract of *C. procera*

Sample concentration (mg/ml		No. of survivals after 0 hrs	No. of survivals after 3 hrs	No. of survivals after 6 hrs	No. of survivals after 9 hrs	No. of survivals after 12 hrs	No. of survivals after 15 hrs	No. of survivals after 18 hrs	No. of survivals after 21 hrs	No. of survivals after 24 hrs	LC_{50}
0	Mean±SD	18±0.00	18±0.00	18±0.00	18±0.00	18±0.00	18±0.00	18±0.00	18±0.00	18±0.00	
1	Mean±SD	18±0.00	17.66±0.57	17.33±0.57	17±1.00	16.66±0.57	16.33±0.57	16±1.00	15.33±0.57	14.66±1.15	
3	Mean±SD	18±0.00	16.66±0.57	15.66±0.57	14.66*±1.15	13.66*±1.15	12.66*±1.15	12.33*±1.52	11.66*±0.57	10.66*±1.15	
5	Mean±SD	18±0.00	14.66±1.15	14±1.00	12.66*±1.15	12*±1.00	10.66*±1.15	10.33*±0.57	9*±1.00	8.66*±0.57	5 mg/ml
10	Mean±SD	18±0.00	13.66±0.57	11.33*±1.52	10.33*±1.52	9.66*±1.15	8.66*±1.15	8*±1.00	6.66*±1.15	6.33*±1.15	
15	Mean±SD	18±0.00	11.33*±1.15	10.33*±1.15	9.33*±0.57	8.33*±0.57	7*±1.00	6.33*±0.57	5.33*±0.57	3.66*±1.52	
20	Mean±SD	18±0.00	10.66*±1.15	9.66*±1.15	8.33*±0.57	6.66*±1.15	4.66*±1.15	3.33*±1.52	2.33*±0.57	1*±1.00	
SE			±0.69	±0.79	±0.79	±0.73	±0.79	±0.83	±0.66	±0.85	
CD at 5% level			1.48	1.65	1.70	1.57	1.70	1.79	1.42	1.83	

*Indicates significance at ($p<0.05$) in respect of control. LC_{50} seems to be 5 mg/ml of the extract, *C. procera: Calotropis procera*, SD: Standard deviation, SE: Standard error, LC_{50}: Lethal concentration

Table 3: Brine Shrimp lethality assay of the sample prepared from petroleum ether extract of *C. procera*

Sample concentration (mg/ml)		No. of survivals after 0 hrs	No. of survivals after 3 hrs	No. of survivals after 6 hrs	No. of survivals after 9 hrs	No. of survivals after 12 hrs	No. of survivals after 15 hrs	No. of survivals after 18 hrs	No. of survivals after 21 hrs	No. of survivals after 24 hrs	LC_{50}
0	Mean±SD	24±0.00	24±0.00	24±0.00	24±0.00	24±0.00	24±0.00	24±0.00	24±0.00	23.66±0.57	
1	Mean±SD	24±0.00	24±0.00	24±1.00	24±1.00	24±1.00	23.66±0.57	23±1.00	22.33±0.57	22±1.00	
3	Mean±SD	24±0.00	24±0.00	24±1.00	23.33±0.57	22.66±1.15	22±1.00	20.66±1.15	20.33±1.52	20.33±1.15	
5	Mean±SD	24±0.00	23.33±0.57	22.33±0.57	22±1.00	21.33±0.57	20.33±0.57	19.33±0.57	18.33±0.57	17.33*±0.57	
10	Mean±SD	24±0.00	22.33±0.57	21±1.00	20±1.00	19.33±0.57	19*±1.00	18.66*±0.57	17.66*±1.15	16.66*±1.15	
15	Mean±SD	24±0.00	21±1.00	20±1.00	19±1.73	18.66±1.15	18*±1.00	17.33*±1.52	16.33*±0.57	15.66*±1.15	
20	Mean±SD	24±0.00	19.33*±0.57	17.66*±1.15	16*±1.00	15.33*±1.52	14.66*±1.15	13.33*±0.57	12.33*±0.57	11.66*±0.57	20 mg/ml
25	Mean±SD	24±0.00	17.66*±0.57	15.66*±1.15	14.3*±0.57	13*±0.00	11.33*±1.15	9.33*±0.57	8*±1.00	5.66*±1.15	
30	Mean±SD	24±0.00	15.33*±0.57	12.33*±0.57	9.33*±1.15	7.66*±0.57	4.66*±0.57	2.33*±0.57	0.66*±0.57	0.00±0.00	
SE			±0.44	±0.62	±0.68	±0.66	±0.70	±0.62	±0.68	±0.80	
CD at 5% level			0.93	1.32	1.43	1.40	1.47	1.32	1.43	1.68	

*Indicates significance at ($p<0.05$) in respect of control. LC_{50} seems to be 20 mg/ml of the extract, *C. procera: Calotropis procera*, SD: Standard deviation, SE: Standard error, LC_{50}: Lethal concentration

DISCUSSION

The brine shrimp cytotoxicity assay is an easy and simple bioassay to detect bioactivity of phytocompounds. This procedure has been used to establish cytotoxic activity of 50% alcoholic extract of *Croton bonplandianum Baill* in our laboratory [9]. In the present study, lethality of the nauplii was counted by comparing the mean surviving larvae of the test and control set. All the experimental sets in this study responded positively in Brine shrimp cytotoxic assay. All the results obtained from different concentrations of benzene; chloroform and petroleum ether extracts are statistically significant in respect of control set. By ANOVA, it is clear that this lethality rate always crossed 95% confidence level (significant at 0.05 level). From Tables 1-3, it is clear that active principles obtained from chloroform extract showed the highest activity over all the other treatments. The significant lethality of benzene, chloroform, and petroleum ether extracted samples to brine shrimp lethality suggests the presence of potent cytotoxic phytocompounds in *C. procera.*

CONCLUSION

50% aqueous ethanolic extract of *C. procera* responded positively in brine shrimp cytotoxic assay. Hence, *C. procera* may be designated as a specimen that may be indexed as a source for obtaining antitumor principle.

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