

- Short Communication**CYTOTOXIC AND ANTHELMINTIC ACTIVITIES OF METHANOLIC EXTRACT OF *CODIAEUM VARIEGATUM* ROOT**

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Abstract

The present study investigated the cytotoxic and anthelmintic activities of methanol extract of *Codiaeum variegatum* root. Anthelmintic activity was assessed by observation of paralysis and death time after applying different concentrations of the methanol extract; while to evaluate the cytotoxicity of the plant extracts, brine shrimp lethality test method was applied taking vincristine sulphate as positive control. The LC₅₀ value of the extract in the brine shrimp lethality bioassay of *Codiaeum variegatum* root was found to be 1.82µg/ml. The LC₅₀ value of the standard significantly correlated with the cytotoxic activity. In anthelmintic activity, the paralysis and the death time of earthworm at 10mg/ml concentration of *Codiaeum variegatum* root were found to be 42 and 81 minutes respectively, whereas, the standard albendazole 10 mg/ml concentration showed these as 26 and 58 minutes respectively and thus there was correlation with anthelmintic activity.

Key words: Cytotoxic, Anthelmintic, *Codiaeum variegatum*, methanol extract.

Natural products have earned an admirable place in drug discovery and thus screening of plants focusing on bioactivities is essential for exploring the new drug molecules (Hung *et al.*, 2012; Newman & Cragg, 2012). Bangladesh is a tropical country and thus has rich variety of herbs to trees. Even there are lot of applications of plants in the traditional medicines here. Thus the screening of plants for the medicinal compounds is especially important in this tropical country. On the other hand, brine shrimp lethality bioassay has been well-proved by the natural product scientists as a bench-top tool for searching bioactive cytotoxic elements (Meyer *et al.*, 1982). Phytochemistry or plant chemistry deals with these biochemicals, their biosynthesis, turnover and metabolism, natural distribution and biological function (Harbone, 1988).

Most of the existing anthelmintics produce side effects like, abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhoea (Bundy, 1994). Since the ancient times, herbal drugs are used for treating parasitic diseases in human without any side effects. *Codiaeum variegatum* is an ornamental shrub 2-6 m high and having alternate simple leaves, often multi-coloured with a myriad of various shape and sizes depending upon the cultivar.

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Cytotoxic and anthelmintic activities of methanolic extract

The roots of *Codiaeum variegatum* are branched. *Codiaeum variegatum* has been used traditionally for the management of toothache and its leaves are used for fungal infection. Thus the present study was designed to evaluate the cytotoxic and anthelmintic activities of root extract of *Codiaeum variegatum* for finding out the new possibility of drug discovery.

The whole plant was collected from Noyerhat, Ashulia, Bangladesh and then was identified by the National Herbarium of Bangladesh, Mirpur, Dhaka. The voucher specimens of the plants have been deposited in the herbarium for further reference. The underground parts of the collected plants were separated from the aerial parts (leaves & petioles) and have been used for the extraction process.

The *Codiaeum variegatum* collected plant parts were shade-dried and then dried in a hot air oven at reduced temperature (not more than 50 °C) to make suitable for grinding purpose. The powdered plant materials (400gm) were used for cold extraction using methanol (2000ml solvent). The powder plant materials were dissolved in methanol for seven days and then filtered through a cotton plug followed by Whatman filter paper. The extract was then concentrated by using a rotary evaporator under reduced pressure. The crude methanol extract thus obtained was then used for the cytotoxic and anthelmintic screenings.

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds by following Meyer *et al.*, 1982. Measured amount of each of test samples were dissolved in measured amount of pure dimethyl sulfoxide (DMSO) to get stock solutions. Then the solution was subjected to serial dilution with simulate sea water to get the 800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/ml concentrations. 2.5 ml of simulated sea water containing 10 nauplii was added in each of the concentrations. After incubation of 24 h, numbers of dead nauplii were observed. In the present study vincristine sulphate was used as the positive control and simulated sea water was used as the negative control.

The anthelmintic assay was carried out as per the reported method with minor modifications (Vidyadhar *et al.*, 2010). For this assay, adult Earthworm (*Pheretima posthuma*) was taken as the organism due to its anatomical and physiological resemblance with the intestinal round worm parasites of human being. The groups of six equal sized earthworms in each group were kept in 10, 25 and 50 mg/ml concentrations of the extract. Group of earthworms in saline solution was used as negative control group and group of earthworms in Albendazole (10mg/ml) used as the positive control. Observations were made for the time taken to paralysis and death of individual worms. Paralysis as said to occur when no movement of any sort could be observed except the worms was shaken vigorously. Death was concluded when the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C.

The LC₅₀ values of methanol extract of *Codiaeum variegatum* root and the LC₅₀ of vincristine sulphate were determined and the results are presented in Table 1.

Table 1. Brine shrimp lethality bioassay of methanol extract of *Codiaeum variegatum* root.

Sample	Regression line	R ²	LC ₅₀ (µg/ml)
Methanolic extract of <i>Codiaeum variegatum</i> (root)	Y= 0.088X + 49.84	0.37	1.82
Vincristine sulphate (Standard)	Y= 7.717 X + 40.78	0.61	1.19

The methanol extract of *Codiaeum variegatum* root was found to be considerably lethal to Brine shrimp nauplii, with LC₅₀ of 1.82 µg/ml whereas anticancer drug vincristine sulphate showed LC₅₀ value 1.19 µg/ml. From the results of this brine shrimp lethality bioassay it can be well predicted that the methanol extract of *Codiaeum variegatum* root possesses cytotoxic properties.

Earthworm was used in anthelmintic activity determination of the plant extract as mentioned earlier. The methanol extract of *Codiaeum variegatum* root showed moderate anthelmintic activity as shown in table 2. The actions were also found to be dose dependent as shown by the reduction in the time for paralysis as well as the time for death while increasing the concentration of the extract.

Table 2. Anthelmintic activity of the methanol extract of *Codiaeum variegatum* root.

Sample	Group	Concentration (mg/ml)	Time for paralysis (minute)	Time for death (minute)
Albendazole (Standard)	1	10	26	58
	2	10	42	81
Methanolic extract	3	25	35	67
	4	50	28	52
Control (Saline water)	5	10	No paralysis	No death

The methanol extract of *Codiaeum variegatum* root showed significant correlation with cytotoxic property when compared with standard cytotoxic drug vincristine sulphate. Thus further researches can be made to explore any possible cytotoxic agent in this plant. Similarly the prominent anthelmintic activity as observed in dose dependent manner when compared with standard anthelmintic drug albendazole indicated that further exploration can be thought for searching the bioactive molecule present in this root. However, extensive bioactivity-guided research can be employed to isolate the bioactive compounds contained in the root extracts with a target to discover new pharmacological agents.

Acknowledgements

We are grateful to the authority of Gono Bishwabidyalay for providing the facilities to conduct this research work.

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