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Research Article

Evaluation of Cytotoxic and Anthelmintic Activities of The Methanolic Extract of *Thevetia peruviana*

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ABSTRACT

This study screened the cytotoxic, and anthelmintic effects of methanol-extracted bark of yellow oleander (*Thevetia peruviana)*. Cytotoxicty was determined against brine shrimp nauplii and LC_{50} of the plant extract was determined 3.9 µg/ml. A reputed cytotoxic agent vincristine sulphate (LC_{50} value 0.839 µg/ml) was used as positive control. And in comparison with vincristine sulphate, it can be said that the methanolic extract has significant cytotoxic activity. The anthelmintic activity of methanolic extract was carried out against adult earthworm *Pheretima posthuma*. Albendazole was used as standard reference drug. In this test five different concentration of extract was used. The test showed that the methanolic extract at higher concentration 50 mg/ml showed moderate anthelmintic activity in comparison with reference standard albendazole at concentration of 20 mg/ml.

Key words: Cytotoxic, Anthelmintic Activities, Methanolic, *Thevetia peruviana.*

INTRODUCTION

Medicinal plants have been used by human beings from time immemorial for healing different ailments. This practice still continues, even after the advent of modern allopathic medicine. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other, were used for medicinal purposes.[1] It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%.[2]

In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. In recent years, secondary plant metabolities (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents.[3]

Thevetia peruviana belongs to the family Apocynaceae plant has been referred with different names as Digoxin, Lucky Nut, Nerium oleander, Yellow Oleander. This plant is native of Central & South America, but now frequently grown throughout the tropical and sub-tropical regions. It is a small ornamental tree which grows to about 10 to 15 feet high. The leaves are spirally arranged, linear and about 13 to 15 cm in length. Flowers are bright yellow and funnel-shaped with 5 petals spirally twisted. The fruits are somewhat globular, slightly fleshy and have a diameter of 4 to 5 cm. The fruits, which are green in color, become black on ripening. Each fruit contains a nut which is longitudinally and transversely divided. All parts of the plant, particularly the seeds are poisonous owing to the presence of cardiac glycosides or cardiac toxins which act directly on the heart. Ingestion of these plant parts could lead to death. The whole plant exudes in a milky juice which is very poisonous. The absorption of the equivalent of two *Thevetia peruviana* leaves may be sufficient to kill a 12.5 kg child. All parts of the plant contain the milky juice. Many cytotoxic compounds have been investigated in *Thevetia peruviana are* Cardiac glycosides, Thevetin A & B, Thevetoxin, Peruvoside, Ruvoside and Nerifolin are found in *T. Peruviana.*[4]

The Barks of yellow oleander might possess anthelmintic and mainly cytotoxic activities. The purpose of the present study was to find out the anthelmintic and cytotoxic activities of methanol-extracted barks of yellow *oleander.*

MATERIALS AND METHODS

Collection of Plant: The bark of *Thevetia peruviana* was collected from Chowmahany, Noakhali, the coastal region of Bangladesh on July 2011 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No. 31059).

Extraction of plant constituents (cold extraction): The collected samples were cleaned, air dried and ground into powder. The dried powder (430 gm) was extracted with 1300 ml of methanol (ratio 1:3). The extract was filtered and the filtrates were evaporated to dryness under normal environmental condition and finally get methanol extract at 25.37 gm.

Cytotoxic Activity Screening

Experimental Model

Brine shrimp lethality bioassay was performed for cytotoxic activity screening.

Drugs and chemicals

1. DMSO (dimethyl sulfoxide)

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Table 1: Brine shrimp lethality bioassay of ethanol extract of yellow oleander leaves

Methanolic Extract				Vincristine sulfate			
Conc. (µg/ml)	log C	Percent Mortality	LC_{50} (µg/ml)	Conc. (µg/ml)	Log C	Percent mortality	LC_{50} (µg/ml)
400	2.60206	100		40	1.60206	100	
200	2.30103	100		20	1.30103	90	
100	2	100		10	1	90	
50	1.69897	100	3.9	5	0.69897	80	
25	1.39794	80		2.5	0.39794	70	0.839
12.5	1.09691	80		1.25	0.09691	70	
6.25	0.79588	50		0.625	-0.20412	50	
3.125	0.49485	40		0.3125	-0.50515	30	
1.5625	0.19382	40		0.15625	-0.80618	20	
0.78125	-0.10721	20		0.078125	-1.10721	10	

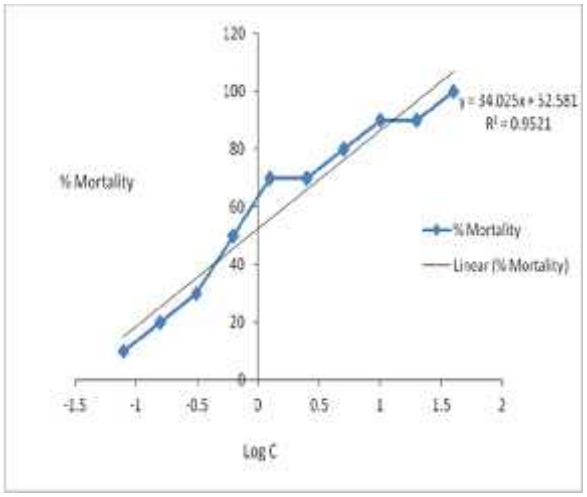

Fig 1: Effect of vincristine sulfate on shrimp nauplii

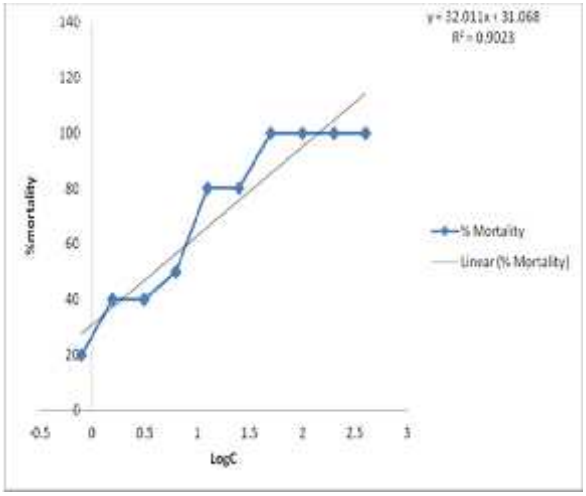

Fig 2: Effect of methanolic extract on shrimp nauplii

Table 2: Paralysis and Death time of worms for Sample solution

Group	Conc. (mg/ml)	Sl. no	Paralysis time (min.)		Death time (min.)	
			Individual	Mean±S.E.M.	Individual	Mean±S.E.M
I	10	01	99	101.33±1.19	108	109.67±0.98
		02	101		109	
		03	104		112	
II	20	01	89	91.33±0.98	98	100±0.82
		02	92		101	
		03	93		101	
III	30	01	66	66.33±0.72	78	80.33±0.98
		02	67		81	
		03	69		82	
IV	40	01	76	79±1.741	87	89.67±1.44
		02	79		89	
		03	82		93	
V	50	01	41	42.67±0.72	56	57.67±0.72
		02	43		58	
		03	44		59	

SEM = Standard Error Mean

2. Vincristine sulfate

Cytotoxic Activity: The cytotoxicity assay was performed on brine shrimp nauplii as reported in Meyer *et al.*1982.[5,6,7,8] Brine shrimp nauplii were obtained by hatching brine shrimp eggs in artificial sea-water (38gm sodium chloride dissolved in 1000 ml distilled water) for 48 hours.

Dissolution of extract was performed in artificial sea-water by using DMSO (dimethyl sulfoxide). Each 5 ml solution of ten different concentrations (0.78125, 1.5625, 3.125,

Table 3: Paralysis and Death time of worms for Standard and Control

Group	Concentration (mg/ml)	Sl. no	Paralysis time (min.) Individual	Mean±S.E.M.	Death time (min.) Individual	Mean±S.E.M.
VI (Albendazol)	20	01	17	17.67±0.54	47	48±0.47
		02	17		48	
		03	19		49	
VII (control)	0	01	--		--	
		02	--		--	
		03	--		--	

6.25, 12.5, 25, 50, 100, 200, 400 µg/ml) of the extract was taken in ten different beakers containing ten living brine shrimp nauplii. The assay is performed using three replicates and observed for mortality after 24 hours.

These data are processed in a simple program for probit analysis to estimate LC_{50} values with 95% confidence intervals for statistically significant comparisons of potencies. Vincristine sulfate was used as positive control. A negative control group was also prepared containing sea water and 100µl DMSO.

Anthelmintic Activity Screening

Experimental Model

Adult earthworms (*Pheretima posthuma) were* used to evaluate anthelmintic activity in *vitro*. Earthworms were collected near the swampy water along Noakhali Science and Technology University road, Sonapur, Noakhali. All the worms were washed with normal saline to remove all fecal matters. The earth worms (*Phertima posthuma*) 5–7 cm in length and 0.3–0.4 cm in width weighing 0.8–3.04 g were used for all experiment protocols.

Drugs and chemicals

1. Albendazole

Anthelmintic Activity: The anthelmintic assay was carried out as per the method of Ajaiyeoba et al. 2001.[9] with minor modifications. The assay was performed in *vitro* using adult earthworm (*Pheretima posthuma)* owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity (Gboladet*al.* 2008). [10, 11, 12, 13]

Test samples of the extract was prepared at the concentrations, 10, 20,30,40 and 50 mg/ml by dissolving 200, 400, 600, 800 and 1000 mg of extract in 20 ml of distilled water in volumetric flask. Albendazole (20 mg/ml) was used as reference standard and distilled water as negative control. Earthworms were dividing into seven groups each containing three worms in Petri dish. In five groups extract solution was applied. And one is for reference and one is for negative control. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^0C).

RESULTS AND DISCUSSIONS

Cytotoxic Activity: Cytotoxic activity of yellow oleander bark extract was determined by brine shrimp lethality assay. Percentage mortality of brine shrimp at ten different concentrations of crude extract was shown lethality in a dose dependent manner.

More specifically, 10%, 20%, 30%, 50%, 70%, 70%, 80%, 90%, 90% and 100% mortality was observed at 0.78125, 1.5625, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400 µg/ml , respectively (Table 1, and Figure 2). This is might be due to increase in active compound concentration and thus exhibiting concentration dependent activity.

The LC_{50} value of the methanolic extract is 3.9µg/ml. And the vincristine sulfate (Table 1, and Figure 1) showed LC_{50} at concentration of 0.839µg/ml.

From the results of the brine shrimp lethality bioassay it can be well predicted that the methanolic extract possess cytotoxic principles.

Comparison with positive control vincristine signifies that cytotoxicity exhibited by the methanolic extract might have significant antitumor activity. However this can not be confirmed without further higher and specific tests.

Anthelmintic Activity: Anthelmintic activity of yellow oleander bark extract was determined by observing Paralysis and Death time of Earthworm (*Pheretima posthuma)* (Table 2, and 3).

From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death. The methanolic extract of *Thevetia peruviana* showed anthelmintic activity in dose-dependent manner. Aqueous extract demonstrated paralysis as well as death of worms in a much more time even in higher concentration of 50 mg/ml (paralysis and death time was 42.67±0.72 minutes and 57.67±0.72 minutes) as compared to albendazole especially at lower concentration of 20 mg/ml (paralysis and death time was 17.67±0.54 minutes and 48±0.47 minutes). So it can be said that the methanolic extract of the bark of *Thevetia peruviana* used by tribals traditionally to treat intestinal worm infections, showed moderate anthelmintic activity.

CONCLUSION

From the result of brine shrimp lethality bioassay it can be well predicted that the methanolic extract has significant cytotoxic activity in comparison with reference standard. The results suggest the probable use of the plant in preparing recipes for tumor-related ailments and the methanolic extract of the bark of *Thevetia peruviana* showed moderate anthelmintic activity. Further investigation is required to establish in-vitro in-vivo correlation to reveal the accurate pattern of anthelmintic activity of *Thevetia peruviana.*

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