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Anthelmintic potential of three ornamental tree

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Abstract
Bambusa vulgaris Schrad. Ex. Wendl. (Poaceae), *Cocos nucifera* Linn. (Arecaceae), *Saraca indica* Linn. (Caesalpiniaceae) are traditional ornamental trees possess immense medicinal properties. *C. nucifera* used in chronic fever, in hemicranias, gravels, worms, wound and vomiting, acid gastritis. *S. indica* used as a treasured remedy for uterine and menstrual distress, uterine haemorrhage, menorrhagia and uterine fibroid. It is also used in leucorrhoea, internal bleeding, haemorrhoids and hemorrhagic dysentery. *B. vulgaris* Leaves are beneficial in sexually transmitted diseases, measles, Mycobacterium tuberculosis and used to treat various inflammatory conditions. Water preserved in Bamboo tubes used to cure jaundice. The present study explores the anthelmintic potential of 50 % hydroethanolic leaf extract these three ornamental trees at three different concentrations i.e., 20, 50, 80 mg/ml against Indian earthworms (*Pheretima posthuma*). Time taken for paralysis and death was determined and the results were compared with that of standard drug albendazole (100 mg/ml). Among all three plants extracts *Bambusa vulgaris* was found to be highly effective against earthworms (*Pheretima posthuma*) and give significant results. At three different concentrations i.e., 20, 50, 80 mg/ml *Bambusa vulgaris* cause paralysis in 13.45, 10.52, 08.40 minutes and corresponding death in 20.05, 14.12, 12.30 minutes. At high concentration i.e., 80 mg/ml, it causes paralytic effect more quickly and death time was shorter. Potency of extracts was found to be inversely proportional to the time taken for paralysis and death of individual worms.

Keywords: Medicinal plant extracts, anthelmintic activity, *in vitro*-assay, albendazole

Introduction
Anthelmintics also termed as vermifuge or vermicides, are the drugs which expel parasites from host through killing and stuning mechanism. If these drugs kill effectively the parasites. They also cause side effects. Now a day's resistance against chemotherapeutics by nematodes has also become a major issue of discussion [1]. Additionally these drugs too costly to use in developing countries.

Nature is a big store house of remedies for ailments of mankind. Due to toxic effects of synthetic drugs, there's a great need to invent new bio-active constituents and plants can complete this need effectually. Anthelmintic derived from plants have very little or no toxicity, wide area of spectrum and are more environmentally compassionate [2]. Secondary metabolites isolated from plants by different isolation and purification techniques play major role as alternative to synthetic drugs. Now a day's herbal medicine capture huge market due to low cost and there easy availability in developing and poor countries. Due to meager medical and economical support its become big task for pharmaceutical companies to market new botanicals including anthelmintics. Helminthiasis is a problem of low economic status countries which have little or no money to control these infections. There's a great need to develop new molecules against helminth parasites because of resistance against presently used drugs. Plants can effectively help in ridding all these difficulties [3].

Recently we tested 50 % hydroethanolic leaf extracts of three ornamental trees *Bambusa vulgaris* (bans), *Cocos nucifera* (coconut), *Saraca indica* (ashoka) at different concentrations (20, 50, 80 mg/ml) for anthelmintic activity against earthworm (*Pheretima posthuma*) in comparison to Albendazole 100 mg. These three Ornamental trees basically grown for decorative purposes in gardens and landscape design projects.

Materials and Methods
Drugs and chemicals
The drug Albendazole was procured from Apple Biotech, Ludhiana, Punjab on gratis basis along with complete analytical data. All organic solvents and chemicals purchased from SD fine chemical limited, Mumbai. All were analytical grade.

Plant material

The leaves of *Bambusa vulgaris* collected from Technocare Nursery, Baddhowal, Punjab, Leaves of *Cocos nucifera* collected from Banglore, Karnataka, leaves of *Saraca indica* collected from road side near Deepak Hospital, Ludhiana, Punjab. All the plant parts were identified and authenticated by Dr. Sunita garg, NISCAIR, Delhi through ref. number NISCAIR/RHMD/Consult/2018/3246-47-1, NISCAIR/RHMD/Consult/2018/3215-16-2, NISCAIR/RHMD/Consult/2018/3215-16-3.

Preparation of Extract

All plant parts were dried in shade and grinded into coarsely powdered form and stored in air tight closed container for further research work. Each plant part powder was extracted with 50% ethanol through maceration technique. Minimum 200g quantity of each plant part was taken and kept in contact with solvent in a closed container for 3 days. Frequent agitation was done to dissolve matter properly. The extract was filtered after 3 days and concentrated using rotary evaporator. The yield was hydroethanolic extract of each plant was found to be 7.04 % w/w dark green (*B. vulgaris)*, 4.2 % w/w greyish green (*C. nucifera)*, 12.16 % w/w reddish brown (*S. indica)*, with reference to shade dried plant material. This hydroethanolic extract used for the evaluation of anthelmintic activity at three different concentrations i.e., 20, 50, 80 mg/ml.

Earthworm collection and authentication

Healthy Indian adult earthworm (*Pheretima posthuma*) were collected from campus garden of Mata Ganga Hostel, G.H.G. Khalsa College of Pharmacy, Gurusar Sadhar, Ludhiana during rainy season and washed with normal saline water and used for study. Earthworms, 4-6 cm in length and 0.1-0.2 cm in width were used for all the experimental procedures due to its anatomical and physical resemblance with intestinal parasites of human beings [4, 5].

Anthelmintic Activity

The anthelmintic activity of freshly prepared plant extracts was evaluated as per the method of Sravani and Paarakh [6]. Total thirteen groups (n=4) were made. Out of thirteen groups, four groups were common. Every time volume used to determine paralysis and death time was 20 ml and six earthworms of equal size placed in Petri dish. All the extracts were freshly prepared, properly labeled before starting the experiment and suspended in Tween 80 (0.1%) in normal saline. Observations were made for the time taken to paralysis and death of worms in minutes. Observations were done up to 4 hours of test period. Paralysis means worms movement stop even in normal saline and death means worms lost their integrity, motility by fading away its body color [7]. The results of anthelmintic activity of each plant extract are given under result and discussion in the table 1.

Table 1: Anthelmintic activity of 50 % hydroethanolic extracts of leaves of *Bambusa vulgaris*, *Cocos nucifera*, *Saraca indica*

Groups	Treatments	Paralysis time (minute)	Death time (minute)
1	Naïve control	------->	------->
2	Vehicle	------->	------->
	Albendazole		
3	50 mg/ml	25.12±1.23	38.10±1.43
4	100 mg/ml	18.44±1.12	29.00±1.16
	Bambusa vulgaris		
5	20 mg/ml	13.45±1.21	20.05±1.45
6	50 mg/ml	10.52±2.19	14.12±2.51
7	80 mg/ml	08.40±2.30	12.30±1.26
	Cocos nucifera		
8	20 mg/ml	35.08±1.11	66.10±2.20
9	50 mg/ml	27.17±3.27	47.13±3.11
10	80 mg/ml	21.23±3.21	32.00±1.21
	Saraca indica		
11	20 mg/ml	23.40±0.34	32.40±0.53
12	50 mg/ml	18.17±2.13	28.00±0.21
13	80 mg/ml	12.03±3.11	21.07±1.07

All the results are expressed as mean ± Standard deviation (n=4); naïve control and vehicle worms alive up to 24 hrs of observation; -------> worms were alive

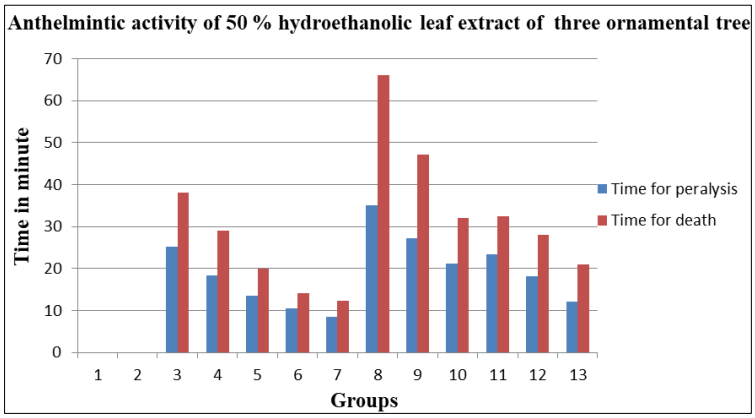

Fig 1: Anthelmintic activity of 50 % hydroethanolic extracts of leaves of *Bambusa vulgaris*, *Cocos nucifera*, *Saraca indica*

Each bar represent as mean ± standard deviation (n=4)
Group 1 (naive control), Group 2 (vehicle 0.1% Tween 80 in normal saline), Group 3 (Albendazole 50 mg/ml), Group 4 (Albendazole 100 mg/ml). Group 5 (*Bambusa vulgaris* 20 mg/ml), Group 6 (*Bambusa vulgaris* 50 mg/ml), Group 7 (*Bambusa vulgaris* 80 mg/ml), Group 8 (*Cocos nucifera* 20 mg/ml), Group 9 (*Cocos nucifera* 50 mg/ml), Group 10 (*Cocos nucifera* 80 mg/ml), Group 11 (*Saraca indica* 20 mg/ml), Group 12 (*Saraca indica* 40 mg/ml), Group 13 (*Saraca indica* 80 mg/ml).

Results and Discussion

The data in table 1 revealed that 50 % hydroethanolic extract of all the three plants produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which gradually produces death. Among all three plants extracts *Bambusa vulgaris* was found to be highly effective against earthworms (*Pheretima posthuma*) and give significant results in comparison to albendazole. At three different concentrations i.e., 20, 50, 80 mg/ml *Bambusa vulgaris* cause paralysis in 13.45, 10.52, 08.40 minutes and corresponding death in 20.05, 14.12, 12.30 minutes. At high concentration i.e., 80 mg/ml, it causes paralytic effect more quickly and death time was shorter. Potency of extracts was found to be inversely proportional to the time taken for paralysis and death of individual worms.

Conclusion

It may be concluded that out of three plant leaf extracts *Bambusa vulgaris* possess potent and significant dose dependent anthelmintic potential. Further studies are required to identify the actual constituents present in extract which are responsible for activity against earthworms. Also to establish the effectiveness and pharmacological rational for the use of *Bambusa vulgaris* as an anthelmintic drug.

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