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In-vitro evaluation of anthelmintic activity of barks of *Caesalpinia sappan*

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Abstract

The present study was undertaken to evaluate anthelmintic activity of ethanol and aqueous extracts of bark from *Caesalpinia sappan* against *Pheritima posthuma*. The different concentrations (10mg/ml, 25mg/ml and 50 mg/ml) of both extracts were studied in the bioassay, which involved determination of time of paralysis and time of death of the worms. The results of present study indicated that ethanol and aqueous extracts were significantly demonstrated paralysis, and also caused death of worms especially at higher concentration of 50 mg/ml, as compared to standard reference Albendazole (10 mg/ml). In conclusion, the *C. sappan* as an anthelmintic have been confirmed.

Key words: *Caesalpinia sappan*, anthelmintic, *Pheritima posthuma* Albendazole.

Introduction

Gastrointestinal nematodes have been found to be of great economic importance in domesticated livestock throughout the world because of their adverse effects on productivity. Livestock producers have generally derived substantial benefits from the use of anthelmintics in controlling livestock parasitosis. In developing countries, small scale farmers have a limited access to the commercially available anthelmintics and veterinary services either due to their non-availability or high costs. Most of the farmers, therefore, have to rely on the ethnoveterinary medicine as in some other parts of the world[1].

Caesalpinia sappan, Linn. (Fabaceae) locally known as sappan-wood found almost throughout India mainly in TamilNadu and Kereala. The bark used for tuberculosis, diarrhea,

anthelmintic, antibacterial, dysentery, postpartum tonic[2]. A review of the literature revealed that the anthelmintic activity of barks of *C. sappan* has not been subjected to scientific evaluation. The present study was carried out in an experimental animal model to report the anthelmintic property on barks of this plant.

Material and methods

Plant material

The bark of *Caesalpinia sappan* were collected in the month of August 2008 from the ABS Botanical Garden, Salem-Dist, Tamil Nadu. The collected material was authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai.

Preparation of extract

The dried and powdered bark (250 gm) were successively extracted on a Soxhlet apparatus, employing hexane, ethanol and distilled water respectively. The extracts were further concentrated under reduced pressure with a rotary evaporator. Bark of *C. sappan* yielded 1.5%, 26.89% and 17.58% w/w powdered extract with hexane, ethanol and distilled water respectively.

Worms

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil of the Dam, Krishnagiri (T.N.) and washed with normal saline to remove all the faecal matter, were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites human beings[3, 4].

Table 1: *In-vitro* anthelmintic activity of ethanol and aqueous extract of *Caesalpinia sappan*

Treatment	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Ethanol Extract	10	14.40±0.44	17.73±0.20
	25	11.01±0.51	13.68±0.91
	50	7.80±0.57	9.80±0.89
Aqueous Extract	10	19.44±0.47	22.73±0.50
	25	15.19±0.33	18.27±0.32
	50	11.38±0.40	14.55±0.28
Albendazole	10	4.23±0.51	6.13±0.24
Normal Saline	-	-	-

Results are expressed as Mean ± SEM from six observations; Control worms were alive up to 24 hrs of observation.

Anthelmintic assay

The ethanol and aqueous extracts of *C. sappan* were dissolved in minimum amount of DMF and the volume was adjusted to 50 ml with saline water. All drugs and extract solutions were freshly prepared before starting the experiment. 50 ml formulations containing three different concentrations (10, 25 and 50 mg/ml in saline water), each of crude ethanol and aqueous extract were prepared and six worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for

death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50 °C. Albendazole (10 mg/ml) was used as reference standard while normal saline as the control[5-7].

Results and Discussion

From the table 1, it is evident that ethanol and aqueous extracts of *C. sappan* exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death with 50 mg/ml concentration. The ethanol extract caused paralysis of 7.80 min. and time of death of 9.80 min., while aqueous extract revealed paralysis of 11.38 min and time of death of 14.55 min. respectively against the earthworm *Pheretima posthuma*. The standard drug Albendazole at 10 mg/ml concentration showed the same at 3.67 and 5.01 minutes, respectively. Table reveals that ethanol extract of bark of *C. sappan* showed the best anthelmintic activity. These parts required the least time for causing paralysis and death of the earthworms.

The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the feces of man and animals. The extracts not only demonstrated this property, they also caused death of the worms, especially at 50 mg/ml as compared with the Albendazole. In conclusion, the study has shown that, alcoholic extracts of *C. sappan* barks have significantly determined anthelmintic activity. Further studies are in process to identify the possible phytoconstituents responsible for anthelmintic activity.

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