



## ***In vitro* anthelmintic activities of *Cassia tora* stem bark**

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### **ABSTRACT**

Pune region of Maharashtra is inhabited by several tribes and they are using several plants or plant-based preparations for the treatment of various ailments in their traditional system of medicine. During our course of studies on ethnomedicine of this region, the plant being used as anthelmintic is root of *Cassia tora* (Caesalpiniaceae) This plant has a wide reputation among natives of being curative for intestinal-worm infections in the form of aqueous extract. Based on this, an attempt has been made to evaluate the anthelmintic potential of this plant. Methanolic and aqueous extracts of *Cassia tora* stem bark were investigated for anthelmintic potential against earthworm (*Pheretima posthuma*). Various concentrations (25-100 mg/ml) of methanolic and aqueous plant extract were tested in the bioassay and different parameters such as determination of paralysis and time of death of worms were recorded. Both extracts exhibit significant anthelmintic activity at highest concentrations of 100 mg/ml and 75 mg/ml. Albendazole (40 mg/ml) was used as reference standard and distilled water as control.

**Keywords:** Anthelmintic activity; *Pheretima posthuma*; *Cassia tora*.

### **INTRODUCTION**

Helminth infections are among the most common infections in humans, affecting a large population of the world. Although the majority of infections due to worms are generally limited to tropical regions and pose a great threat to health and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia (Bundy, 1994). Parasites diseases causes severe morbidity affecting mainly population in endemic areas with major economic and social consequences (Tagbota and Townson, 2001). The gastrointestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases (Sondhi et al., 1994) ; hence there is an increasing demand towards natural anthelmintics.

*Cassia Tora* L. Caesalpiniaceae, is a wild crop and grows in most parts of India as a weed. According to Ayurveda the leaves and seeds are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardio tonic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders (Ayurvedic Pharmacopeia of India, 2001). Chemical components of roots *Cassia tora* are 1,3,5-trihydroxy-6-7-dimethoxy-2-methylanthroquinone and  $\beta$ -sitosterol. Seeds contain naphtho-alpha-pyrone-toralactone, crysofenol,

physcion, emodin, rubrofusarin, Chrysophonic acid-9-anthrone. Leaves contain emodin, tricontan-1-01, stigmasterol,  $\beta$ -sitosterol- $\beta$ -D-glucoside, freindlen, Palmitic, stearic, succinic and d-tartaric acids uridine, quercitrin (Nadkarni, 2007), Anthelmintic (Deore et al., 2009, John et al., 2009), Antioxidant (Yen and Chuang, 2000), Antishigellosis (Awal and Shamim, 2004) , Hypolipidemic (Patil et al., 2004) , antifungal (Ahmad et al., 2002) , Antibacterial (Kitanaka et al., 1988), anti-platelet aggregation (Yun , 1990) , hepatoprotective (Wong et al., 2009 , Wong, 1988), cAMP-phosphodiesterase inhibitory activities (Shibata et al., 1969), antifungal, anti-inflammatory (Maity, 1998), estrogenic and antiestrogenic (Halawany, 2007), anti-mutagenic (Young, 2002) activities has been evaluated. It also shows negative regulation of aluminum-responsive citrate efflux by an anion channel antagonist activity (Yang, 2006). Literature survey revealed that stem bark extract has yet not been screened for anthelmintic activity. Hence, the present study was undertaken to evaluate the anthelmintic property of *Cassia tora* stem bark.

### **MATERIAL AND METHODS**

#### **Plant material**

The stem bark of *C. tora* was collected from the Abhinav college of B Pharmacy campus, Narhegoan (MAHARSHTRA) in September 2009. The plant material were identified and authenticated in Botanical Survey of India, Koregaon park, Pune (Ref BSI/WRC/Tech/2009/658 and Voucher herbarium specimens was deposited in the Department of Pharmacognosy of our College. The plant material was dried in sunlight, pulverized, passed through sieve no.

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40 and stored in air tight container and used for further extraction.

### Preparation of extract

Aqueous extract and alcoholic extracts (Maceration method) of powdered material of *Cassia tora* stem bark (200 gm) was kept for maceration with 1000 ml of distilled water for 12 hrs. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be 3.57 percent

### Animals

Adult earthworms (*Pheretima posthuma*) were used to evaluate anthelmintic activity *in vitro*. *Pheretima posthuma* collected from moist soil of the botanical garden were washed with normal saline and used for the anthelmintic study. The earthworms (6-9 cm in length and 0.1-0.2 cm in width) were used. Earthworm were identified in Dept. of Zoology Padmashri Vikhe Patil Science College, Loni, Ahemadnagar and services of veterinary practioners were utilized to confirm the identity of worms

### Drugs and chemicals

Albendazole (CIPLA) was used during the experimental protocol.

## ANTHELMINTIC ACTIVITY

### Anthelmintic bioassay

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al., 2001 with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Kirtikar and Basu, 1999, Vidyarthi, 1967, Thorn, 1977, Vigar 1984, Chatterjee, 1967). Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* (Sollmann, 1918, Jain, 1972, Dash et al., 2002, Szewezuk, 2003, Shivkar and Kumar, 2003). Methanol and aqueous extracts from the stem bark of *Cassia tora* were investigated for their

anthelmintic activity against *Pheretima posthuma*. Various concentrations (10-100 mg/ml) of each extract were tested in the bioassay; Albendazole was included as standard reference and distilled water as control. The assay was performed on *Pheretima posthuma*. The earthworms of 6-9 cm in length and 0.1-0.2 cm in width was used. In the experiment, six groups of six earthworms were released in to 50 ml of solutions of albendazole, aqueous and methanolic extracts of stem bark of *Cassia tora* (25, 50, 75 and 100 mg/ml each) in distilled water. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously nor when dipped in warm water (50°C). Death was concluded when the worms lost their motility followed with fading away of their body colors (Deore et al., 2009). All the results were shown in Table.1 and expressed as a mean  $\pm$  SEM of six worms in each group.

## RESULTS AND DISCUSSION

Preliminary phytochemical screening of alcoholic extract revealed the presence of anthraquinone glycosides, phenolic compounds and steroids while aqueous extract showed presence of glycosides and phenolic compounds. From the results shown in table no. 1, the predominant effect of albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis.

Results obtained indicate that the higher concentration of each plant extract produced paralytic effect much earlier and the time to death was shorter. The results of the current investigation indicate that among the extracts of *C. tora*, the methanolic extract is the most potent one and requires less time to the paralysis and death of the worms as compared to the aqueous extract. Both extracts showed a concentration depended anthelmintic property (Table 1). Both the methanolic and

**Table 1: Anthelmintic activity of *cassia tora* stem bark**

Extract	Concentration (mg/ml)	Time required to paralyse. (min)	Time required to death of worm. (min)
Control	--	--	--
Aqueous extract	25	NA	NA
	50	81.14 $\pm$ 0.05	96.17 $\pm$ 0.4
	75	57.19 $\pm$ 0.21	61.85 $\pm$ 0.28
	100	15.48 $\pm$ 0.26	21.30 $\pm$ 0.37
Methanolic extract	25	140.12 $\pm$ 0.8	147.28 $\pm$ 0.16
	50	51.01 $\pm$ 0.39	61.14 $\pm$ 0.33
	75	43.14 $\pm$ 0.61	49.37 $\pm$ 0.41
	100	11.31 $\pm$ 0.28	16.55 $\pm$ 0.15
Albendazole	40	3.30 $\pm$ 0.21	6.45 $\pm$ 0.07

aqueous extract of *Cassia tora* demonstrated paralysis as well as death of worms especially at higher concentration of 100 mg/ml while 75mg/ml and 50mg/ml concentrations also shown significant activity. The preliminary phytochemical analysis of the extracts has shown the presence of phenolics(Jain,1972) like tannins and saponins as well as alkaloids. Synthetic phenolic anthelmintics like niclosamide interfere with the energy generation in the helminth parasites by uncoupling the oxidative phosphorylation (Thorn,1977, Athnasiadou et al., 2001, Khandelwal,2008, Bate, 1962, Martin1997). Another possible mechanism of action is that they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and by this cause death. Tannins have also been shown to produce anthelmintic activities. From the above results, it is concluded that *Cassia tora* used by tribals traditionally to treat intestinal worm infections, showed significant anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the activeconstituent accountable for anthelmintic activity.

## CONCLUSION

The traditional use of stem bark of *Cassia tora* as an anthelmintic have been confirmed as the stem bark extracts displayed activity against the worms used in the study. Further studies need to isolate and reveal the active compound contained in the crude extracts of *cassia tora* and establish the mechanism of action are required.

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