

In vitro Anthelmintic activity of *Cassia tora*

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Abstract: Alcohol and aqueous extracts from the seeds of *Cassia tora* were investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascaridia galli*. Three concentrations (25, 50 and 100 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worm. Both the extracts exhibited significant anthelmintic activity at highest concentration of 100 mg/ml. Piperazine citrate in same concentration as that of extract was included as standard reference and distilled water as control. The anthelmintic activity of alcohol and aqueous extracts of *Cassia tora* has therefore been demonstrated for the first time.

Keywords: Anthelmintic Activity, *Ascaridia Galli*, *Cassia tora*, *Pheretima Posthuma*,

Introduction

Cassia Tora L., (*Cassia obtusifolia* L.), Caesalpinaceae, 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, cardiogenic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders (1,2). Chemical component of *Cassia tora* are anthraquinones (3); chrysophanol, emodin, obtusifolin, obtusin, chryso-obtusin, aurantio-obtusin, and their glycosides. Naphthopyrones 4(4); rubrofusarin, nor-rubrofusarin, rubrofusaringentiobioside. Toralactone, torachryson. Roots contains 1, 3, 5-trihydroxy-6-7-dimethoxy-2-methylanthroquinone and beta-sitosterol. While Seeds contains Naphtho-alpha-pyrone-toralactone, chrysophanol, physcion, emodin, rubrofusarin, cchrysophonic acid-9-anthrone. Emodin, tricontan-1-0l, stigmasterol, β -sitosterol- β -D-glucoside, freindlen, palmitic, stearic, succinic and d-tartaric acids uridine, quercitrin and isoquercitrin are isolated from leaves. Antibacterial 5, anti-platelet aggregation (3), hepatoprotective (6,7), cAMP-phosphodiesterase inhibitory activities (8), antifungal, antiyeast, antiinflammatory (9), estrogenic and antiestrogenic (10), Hypolipidemic (11), antimutagenic (12) antioxidant (14) activities has been evaluated. Literature survey revealed that the plant extract has yet not been screened for its traditional claim of anthelmintic activity (1). Therefore the objective of this work was to explore the anthelmintic properties of *Cassia tora* seeds.

Methods

Extraction of Plant Material

The fresh seeds of *Cassia tora* (CT) were collected in the months of July-August from the local market of Amravati, Maharashtra state, India, and authenticated by the authority of the botany department, VMV, Amravati. A voucher specimen was submitted at Institute's herbarium department for future reference. Dried seeds were ground to coarse powder. Powder was first defatted with pet. Ether and then extracted with ethanol which is further evaporated to dryness to obtain alcoholic extract. Aqueous extract were obtained by maceration for 24 hours.

Experimental

Alcohol and aqueous extracts from the seeds of *Cassia tora* were investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascaridia galli*. Various concentrations (10-100 mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate was included as standard reference and distilled water as control.

The anthelmintic assay was carried as per the method of Ajaiyeoba et al. (15) 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 (16,17,18,19) Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro (20,21,22,23,24)

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all 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. *Ascaridia galli* worms are easily available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of anthelmintic drug was advocated earlier (25,25,27). In the first set of experiment, six groups of six earthworms were released in to 50 ml of solutions of piperazine citrate, aqueous and alcoholic extracts of seeds of *Cassia tora* (25, 50 and 100 mg/ml each) in distilled water. Piperazine citrate was used as reference standard while distilled water as control.

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. Death was concluded when the worms lost their motility followed with fading away of their body colors. Same experiment was done for *Ascaridia galli* worms only the difference was solutions were prepared in normal saline solutions.

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 piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis.

The alcoholic seed extract of *Cassia tora* demonstrated paralysis as well as death of worms in a less time as compared to piperazine citrate especially at higher concentration of 100 mg/ml. While water extract also shown significant activity. Phytochemical analysis of the crude extracts revealed presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show anthelmintic activity (28). Some synthetic phenolic anthelmintics e.g. niclosamide, oxiclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (29). It is possible that phenolic content in the extracts of *Cassia tora* produced similar effects.

Conclusion

In conclusion, the traditional use of seeds of *Cassia tora* as an anthelmintic have been confirmed as the seed extracts displayed activity against the worms used in the study. Further studies need to establish the mechanisms (S) of action are required.

Table no. 1 Anthelmintic activity of extracts of *Cassia tora*

Extracts of LS	Concentration mg/ml	Pheretima posthuma		Ascaridia galli	
		P	D	P	D
AE	25	89.03± 0.4	120.21± 0.6	54.15 ± 0.76	74.5 ± 0.34
	50	72± 0.3	108.98± 0.1	46.2 ± 0.21	60.2 ± 0.11
	100	64.73± 0.8	95.63± 0.1	27.5 ± 0.18	48.5 ± 0.48
AE	25	65± 0.14	72± 0.44	64.04 ± 0.9	79.5 ± 0.23
	50	43± 0.21	66± 0.11	49.7 ± 0.1	68.2 ± 0.1
	100	23± 0.9	33± 0.45	34.2 ± 0.6	45.75 ± 0.23
PC	25	1.5 ± 0.7	54.5 ± 0.4	41.23 ± 0.14	54.5 ± 0.4
	50	0.9 ± 0.12	30.2 ± 0.1	29.75 ± 0.5	30.2 ± 0.1
	100	0.5 ± 0.17	18.5 ± 0.8	20.05 ± 0.9	23.5 ± 0.8
Control	-	-	--	-	-

Where AE: Aqueous extract, EE: Alcoholic extract, PC: piperazine citrate
P: Time taken for Paralysis (min), D: Time taken for Death of worms (min)

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