



## Comparative investigation of anthelmintic activity using various extracts of *Cassia tora* plant parts *in vitro* study

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**Abstract** : Parasitic infection including helminthiasis is a critical serious problem in the tropical region. Helminths produce serious problem in human and other animals around the world specifically to the third world countries. As per WHO, only few drugs are frequently used in the treatment of these parasite infections. Albendazole, Piperazine citrate are the commercial anthelmintic drugs available. The drug is poorly absorbed and efficacy depends on transit time in the gastrointestinal tract. The toxicity is extremely low, but the drug has not been studied in children under two years of age. There is increasing demand of natural anthelmintics. Hence present study ethyl acetate, acetone, methanol and aqueous extracts of *Cassia tora*, seed and seed cover were investigated for their phytochemical followed anthelmintic activity against *Eisenia foetida*. Three concentrations (1, 2.5, 5 mg/ml) of each extract were studied it involves the determination of time of paralysis and time of death of the worms. The gradual increased in a dose exhibited, a gradual increase in the activity. Phytochemical analysis revealed presence of flavonoids as one of the chemical constituent. It was observed that all extracts exhibit positive response to certain degree of anthelmintic activity in dose dependent manner. Aqueous and methanol extracts displayed significant anthelmintic activity at highest concentration of 5mg/ml which is significant in case of seed extracts compare to seed cover. It was concluded from the experimental details that the plant revealed noteworthy anthelmintic activity. The data were verified as statistically significant by using two way ANOVA at 1% level of significance.

**Keywords** : *Cassia tora*, anthelmintic activity, *Eisenia foetida*, seed and seed cover extracts.

### Introduction

Growing interest has promoted researcher to screen scientifically various claims regarding properties and use of medicinal plant materials. Presently both common consumers and health care professional seek updated, authorities information towards safety and efficacy of recommended medicinal plant as drug to its use<sup>1</sup>. Parasitic infection including, Helminthiasis is a critical serious problem in the tropical regions including the Asian countries which affects more than two billions of people worldwide. Helminths produce serious problem in human and other animals around the world specifically to the third world countries<sup>2</sup>. The WHO estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worm also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on anthelmintic drug is poor<sup>3</sup>. The WHO estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worm also infect livestock and crops, affecting food production with a resultant

economic impact. Despite this prevalence of parasitic infections, the research on anthelmintic drug is poor<sup>4</sup>. The WHO estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worm also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on anthelmintic drug is poor<sup>5</sup>. The WHO estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worm also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on anthelmintic drug is poor<sup>6</sup>. The WHO estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worm also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on anthelmintic drug is poor<sup>7</sup>. However, during the last few years, an explosive increase of interest in antioxidant property of cassia species and correlation with bioactivity such reports are available in the literature<sup>8</sup>.

*Cassia Tora 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<sup>9,10</sup>. An ethanolic extract of seeds was evaluated by Patil *et al* for its hypolipidemic activity on triton induced hyperlipidemic profile<sup>11</sup>. Some synthetic phenolic anthelmintics e.g. niclosamide, oxiclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation<sup>12</sup>. Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death<sup>10</sup>. In literature survey anthelmintic activity of *Cassia tora* against *Pheretima posthuma* and *Ascaridia galli* is reported<sup>12</sup>. The present study involves comparative investigation of phytochemicals followed by the anthelmintic activity of various extracts of seed and seed cover against *Eisenia foetida* using saline as control with a view to justify the use of the plant in the treatment of helminths.

## Experimental

### 1. Plant materials and preparation of extracts:

*Cassia tora* was collected from Western Pune, Maharashtra, India. The taxonomic identification was carried out with the help of Flora of Botany Presidency and Flora of Maharashtra (Singh *et al*. 2000) and herbaria was prepared by following standard methods. The specimen was also compared with the authentic herbaria of BSI, Western circle Pune, Maharashtra, India for confirming the identification, its number is BSI/WRC/Cert./2015/SG02. Air shade dried and pulverized plant material was used. Extracts were prepared using weighed (50gm) sample powder in the measured volume (50ml) of solvents like, ethyl acetate (H1), acetone (H2), methanol (H3) and water (H4) by refluxing at its boiling point temperature for 6 hours. Vacuum dried extracts are used for the experiment. Solvents used after distillation.

### 2. Phytochemical screening:

Qualitative assay, for the presence of plant phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins were carried out on following standard procedure<sup>13,14</sup> and using analytical grade reagents.

### 3. Collection of Animal:

The assay was performed on adult earthworm (*Eisenia foetida*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings<sup>15,16</sup>. Easy availability of earthworms prompts their extensive use for preliminary *in vitro* evaluation of anthelmintic compounds<sup>17</sup>. Earthworms were provided and identified by Dept. of Entomology, College of Agriculture, Pune, and Maharashtra, India. All earthworms were of approximately equal size (15 cm). They were washed with saline before experiment.

### 4. Standard Drugs:

Albendazole was purchased from G.S.K. Pharma Pvt Ltd, Mumbai along with saline NA 0.91 %

NaCl. All the chemical and solvents used were of analytical grade for the experimental protocol.

**Phytochemical Tests:**

Preliminary Phyto-chemical screening of H1,H2,H3,H4 extracts were performed. The results were reported in **Table I**

**Table I: Phytochemical screening of *Cassia tora* plant parts.**

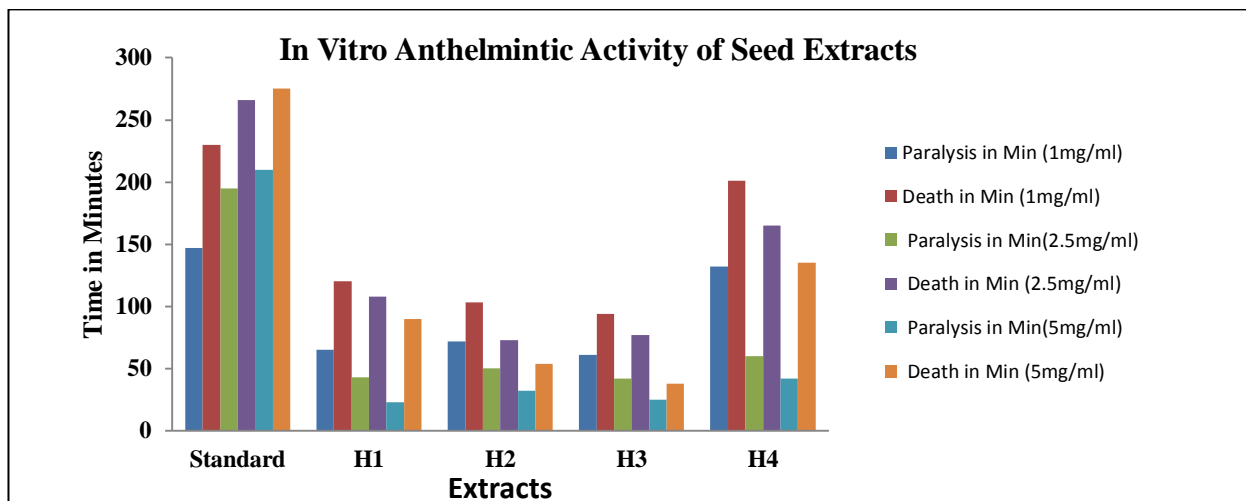
Sr. No.	Test	Seed - Ac	Seed- EA	Seed- MEOH	Seed- Aq	Seed cover - Ac	Seed cover - EA	Seed cover - MEOH	Seed cover - Aq
1	Starch	++	++	++	++	++	++	++	++
2	Protein	++	++	++	++	++	++	++	++
3	Sugar	+	+	+	+	+	+	+	+
4	Alkaloids	+	+	+	+	+	+	+	+
5	Steroids	+	+	+	+	+	+	+	+
6	Tannins	++	++	++	++	++	++	++	++
7	Flavonoids	++	++	++	++	++	++	++	++
8	Phenolics	+	+	+	+	+	+	+	+
9	Glycoside	+	+	+	+	--	+	+	+
10	Saponins	+	+	+	+	+	+	+	+

**Anthelmintic activity:**

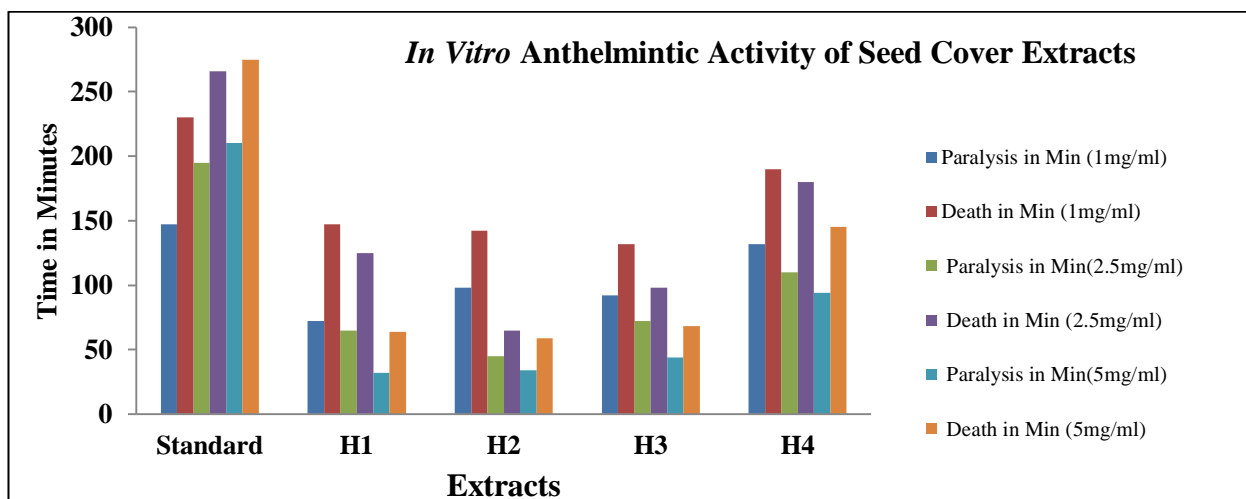
H1,H2,H3,H4 extracts of Plant material investigated for their anthelmintic activity against *Ecinia foetida*. The anthelmintic assay was carried as per the method of Ajaiyeoba<sup>et. Al</sup><sup>18,19</sup> with minor modifications<sup>20</sup>. All the test solutions and standard drug solutions were prepared freshly before starting the experiment. Six groups of earthworms of approximately equal size were released in to solutions of three different concentrations(1, 2.5,5 mg/ml) in petri dishes containing above solutions of extracts. Albendazole was used as reference standard and saline as control. Paralysis and death time of the worm were recorded. Time 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 was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C) followed with fading away of their body colors.

**Results and Discussion:**

Phytochemical analysis of crude extracts revealed the presence of flavonoids, tannins and phenolics as one of the chemical constituent(**Table I**). The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of these plants as anthelmintic. The extracts of leaves displays a significant anthelmintic activity in dose dependent manner(**Graph I and II**). The anthelmintic activity of aqueous and ethyl acetate extracts of seeds as well as seed cover was comparable with that of standard drug. The following trend is depicted as Aqueous>Methanol>acetone >Ethyl acetate in case of seeds as well as seed cover extracts in the said activity. The statistical analysis of anthelmintic activity experiment was performed by Two way ANOVA without replication at 1% level of significance. The p value is less than 0.05 as well as Fcrit less than F value indicates the data is significant in case of seed as well as seed cover (**Table III and IV**).



Graph I: Anthelmintic Activity results of *Cassia tora* of seed



Graph II: Anthelmintic Activity results of *Cassia tora* of seed cover

Table III: Shows Statistical variance of analysis (ANOVA) on anthelmintic activity of *Cassia tora* seed extracts.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	666.125	1	666.125	841.4211	0.00000899	34.11622
Within Groups	425.375	3	141.7917	179.1053	0.000701188	29.4567
<b>Total</b>	<b>1091.500</b>	<b>4</b>				

Table IV: Shows Statistical variance of analysis (ANOVA) on anthelmintic activity of *Cassia tora* seed cover extracts.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	864.000	1	864	12.99248	0.069087	98.50251
Within Groups	342.3333	2	171.1667	2.573935	0.279804	99
<b>Total</b>	<b>1206.333</b>	<b>3</b>				

The traditional medical wisdom is excellent proof of efficacy and safety of medicine. The present report indicates screening of crude extracts of seed and seed cover showed significant in vitro anthelmintic studies to treat intestinal worm infection and it will substantiate the folk claim. To conclude further studies there is need to through phytochemical clinical studies to know the mode of action.

Each value represents mean  $\pm$ SEM(N=6).Data are found to be significant by testing through two way ANOVA without replication at 1% level of significance

### Acknowledgement

The authors are thankful to the Principal, S.P.College and Head, Department of Chemistry,S.P.College, Pune-30 for providing experimentation facilities.

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