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Anthelmintic efficacy and preliminary phytochemical investigations of *Cassia mimosoides* (L).: Unexplored plant

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Abstract: The traditional claims of various parts of any species of *Cassia* are claimed as anthelmintics. The present study was investigated the anthelmintic activity of *Cassia mimosoides* leaves against earthworm and roundworm. Five different concentrations (5, 15, 25, 50, 75 mg/ml) of aqueous, chloroform, acetone, and petroleum ether extracts were tested against worms. It involved the determination of time of paralysis (P) and death (D) of the worms. The aqueous extract showed a more significant effect on paralyzing the worms. The effect of extracts on the paralysis and death of the *Pheretima posthuma*, according to the results indicated as aqueous > Chloroform > Petroleum ether >Acetone extracts. The effect of extracts on the paralysis and death of the *Haemonchus contortus*, according to the results indicated as aqueous > Acetone > Petroleum ether > Chloroform extracts. Piperazine citrate was used as a reference standard drug were normal saline water as a control. Leaves of *Cassia mimosoides* of family Caesalpinaceae showed as a good remedy in worm infections. The results of *in vitro* trials of *Cassia mimosoides* proved its anthelmintic property at a higher dose.

Key-words: (Cassia mimosoides* L., Anthelmintic, *Pheretima posthuma, Haemonchus contortus.)

1. Introduction:

Some members of Caesalpinaceae are traditionally used as vermifuge drugs. *Cassia mimosoides* of family Caesalpinaceae is a wild plant and grows in most parts of India as a weed. The leaves of *C. mimosoides* are used in the treatment of asthma, typhoid fever, stomach problems etc. The roots and seeds are useful in whooping cough and antispasmodic, seeds used as stimulating drink (Kirtikar and Basu, 1998). Prusty et al. (2011) investigated anti-ulcer activity and extracts of *C. mimosoides.* Pharmacologically this plant is not explored much.

This species of *Cassia* is not studied for its anthelmintic property. Not a single example of published data regarding anthelmintic activity found during review though it is reported as an anthelmintic (Asolkar et al., 1965). In the present study, this plant has been tested for the first time for its anthelmintic activity against worms in India.

2. Materials and methods:

2.1 Collection and Identification plant material

Fresh leaves of *Cassia mimosoides* were collected from G.V.I.S.H. campus, Amravati (M.S.) in the month of Nov 2015. The plant was identified and authenticated by Dr. Milind Sirdesai**,** and a voucher specimen was deposited in the herbarium of Department of Botany, Dr. Babasaheb Aambedkar, Marathwada University, Aurangabad.

2.2 Extraction of plant material

The extracts were prepared as per the method described by Thimmaiah (1999). The collected plant material was washed, shade dried and crushed to produce coarse powder. The crude powdered was subjected to extract exhaustive extraction with different solvents like acetone, chloroform, petroleum ether, and water by using Soxhlet apparatus. These extracts were concentrated under reduced pressure in oven to yield semisolid mass. Stored for further analysis in cool and dry place. The different concentrations of extracts (5, 15, 25, 50, 75 mg/ml) were prepared for study. The extracts were used for anthelmintic activity against *Pheretima posthuma* and *Haemonchus contortus.*

2.3 Preliminary photochemical screening

The leaf powder was subjected to preliminary phyto-chemical tests to detect the presence of various phyto-constituents. A phytochemical screening was carried out as per the standard methods (Kokate et al 1998, Kulkarni and Apte, 2000, Sadasivam and Mnickam, 2005, Thimmaiah, 1999). Responses to various tests were denoted by +, ++ and +++; indicating weak, moderate and strong reactions respectively.

3.Organisms

Pheretima posthuma and Haemonchus contortus.

4. Drugs and chemicals

Piperazine citrate, acetone, chloroform, petroleum ether.

5. Evaluation of anthelmintic activity:

The anthelmintic assay was carried as per the method of Deore et al (2009) 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 (Deore et al 2009, Dash et al 2002). Earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro because of its easy availability (Szewezuk et al 2003).

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and roundworm (*Haemonchus contortus*) collected from Mominpura abattoir of Amravati. *H.contortus* is commonly used as animal model to evaluate the anthelmintic activity of medicinal plants (Ciulei, 1982, Jabbar et al., 2006)The worms washed with normal saline to remove all faecal matter and were used for the anthelmintic activity. The worms were divided into six groups containing four worms of both types. The experiment was performed at the dose of various concentrations i.e., 5, 15, 25, 50, 75 mg/ml of each extract in distilled water. Saline water as a control group I and standard reference drug piperazine citrate treated as group II. Determination of time of paralysis and time of death of the worms was observed. Time for paralysis was recorded when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death was concluded when the worms lost their motility completely while dipped in warm water (50 ^{0}c) followed with fading away of their body colors.

6. Statistical Analysis

Results were analyzed statistically by ANOVA two factors with replications. The data were expressed as Mean value with standard deviations. All assays were carried out in quadruplicated. Statistical analysis was performed in excel. To test the effects of various extracts and their study as well as various concentrations. Of different extracts, the data collected by experimentation is analyzed by using F test (ANOVA).

Table 1. Phytochemical analysis of *Cassia mimosoides*

S.N.	Test	Response	Intensity	Inference
1	**Alkaloids**			
	a) Mayer's Reagent	White ppt	+++	Present
	b) Dragendorff's Reagent	White ppt	+++	Present
	c) Wagner's Reagent	White ppt	++	Present
2	**Anthraquinones**			
	Test – a	Red colour	++	Present
	Test – b	Red colour	+	Present
	Test – c	Violet colour	++	Present
3	**Simple Phenolics**			
	Test a) With NaoH	Red	+++	Catechol present
4	**Flavonoids**			
	a) Shinoda Test	Pink	+++	Present
	b) Flavononol Test	Magenta colour	+++	Present
	c) Flavanol Test	Yellowish Green	++	Present
	d) Flavone, Flavonol	Orange	++	Present
	Flavonone Test			
	e) Flavanone (Rao & Sheshadri	Yellowish Brown	+++	Present
5	**Tannin** Test a- Test b-	 White ppt Green colour	 +++ +++	 Present Present
6	**Saponins**	Froth Formation	+++	Present
7	**Steroid/ Triterpenoid Test** Test a Test b Test c	 Red colour Pink colour Brown colour	 +++ +++ ++	 Present Present Present

(Test was noted as +++ Strong, ++ Moderate, + Weak, -Negative)

Tables 2: Anthelmintic activity of *Cassia mimosoides* against earthworm and roundworm

Groups	Treatments	Conc. (mg/ml)	*Pheretima posthuma* (Earthworm)		*Haemonchus contortus* (Roundworm)	
			P (min)	D (min)	P (min)	D (min)
I	Normal saline	_	_	_ _	_	_ _
II	Standard (Piperazine citrate)	5	13.55±2	17.5±3.8	9.6±1.14	14.3±2.4
		15	12.57±2.7	15.6±2.3	7.5±2	11.5±1.2
		25	10.5±2.3	13.75±2.8	6±0.9	7.1±1.70
		50	6.7±1.70	9±1.8	3.9±0.8	5.5±1.2
		75	**3.25±0.5**	**4.8±0.8**	**3±0.8**	**4.6±1.6**
III	Acetone (CUAC)	5	47.25±4.8	49±5.2	17±5.4	22±3.3
		15	44.5±7.8	48±8.16	14±2.1	18.2±1.7
		25	40.7±7.8	43.7±8.9	11.75±2	16.5±1.2
		50	36.5±6.1	41±9	10.5±1.2	15±1.8
		75	**32.2±5.7**	**34.5±5.7**	**7.2±1.7**	**10.5±1.2**
IV	Chloroform (CUCHL)	5	22.5±1.2	28.2±3.3	17.7±3	22±3.2
		15	21.7±2.6	25.2±4.1	14±2.1	17.5±1.7
		25	19.5±6.8	24.5±4.5	7.5±3.4	17.2±3.3
		50	16.5±1.2	22.25±4.19	6.7±0.9	16.2±3.5
		75	**15±2.1**	**21±3.5**	**4.2±0.9**	**14.5±4.2**
V	Petroleum ether (CUPE)	5	31±7	36.7±6.7	15±7.5	23.5±6.9
		15	29.2±2.5	35.5±6.2	10.5±2	20±2.9
		25	24.2±4.9	31.2±5.2	7.2±2.2	18.5±1.2
		50	22.2±8.7	29.7±4.9	6.7±0.9	16.2±2.5
		75	**17.7±10**	**27.5±9.8**	**5.2±2.2**	**14.5±2.6**
VI	Aqueous (CUAQ)	5	24±2.1	32.2±5.3	11.7±1.7	13.5±1.7
		15	20.25±2.5	28.5±7.7	6.5±1.2	10.5±0.5
		25	20±2.16	25±1.8	4.2±1.8	10±1.8
		50	19±3.5	21.7±2.9	3.7±1.5	8.2±1.2
		75	**15±0.8**	**20±3.4**	**3.5±1.2**	**6.2±0.9**

Values are expressed in mean ± SD (n=4); - no paralysis, -- no death

Table 3a: ANOVA for paralysis of *P. posthuma* (*C. mimosoides*)

Source of Variation	SS	df	MS	F	P-value	F crit
Extracts	10286.74	4	2571.685	105.8918113	4.64311E-30	2.493696004
Concentrations	1526.284	4	381.571	15.71158378	2.2135E-09	2.493696004
Interaction	142.336	16	8.896	0.366302076	0.986310976	1.780229913
Error	1821.4475	75	24.28596667			
Total	13776.807	99				

Table 3b: ANOVA for death of *P. posthuma* (*C. mimosoides*)

Source of Variation	SS	df	MS	F	P-value	F crit
Extracts	10389.56	4	2597.39	86.65308108	2.418E-27	2.493696
Concentrations	1575.09125	4	393.7728125	13.13689028	3.7E-08	2.493696
Interaction	148.84	16	9.3025	0.310346266	0.9943874	1.7802299
Error	2248.09375	75	29.97458333			
Total	14361.585	99				

Table 3c: ANOVA for paralysis of *H. contortus* (*C. mimosoides*)

Source of Variation	SS	df	MS	F	P-value	F crit
Extracts	562.54	4	140.635	21.60430135	6.92582E-12	2.493696004
Concentrations	1153.48375	4	288.37093	44.29944633	4.85765E-19	2.493696004
Interaction	114.31	16	7.144375	1.097516482	0.37291738	1.780229913
Error	488.21875	75	6.5095833			
Total	2318.5525	99				

Table 3d: ANOVA for death of *H. contortus* (*C. mimosoides*)

Source of Variation	SS	df	MS	F	P-value	F crit
Extracts	1721.7756	4	430.4439	63.1862807	2.87164E-23	2.493696004
Concentrations	930.0006	4	232.50015	34.12946435	3.27899E-16	2.493696004
Interaction	66.3824	16	4.1489	0.609030724	0.867038503	1.780229913
Error	510.9225	75	6.8123			
Total	3229.0811	99				

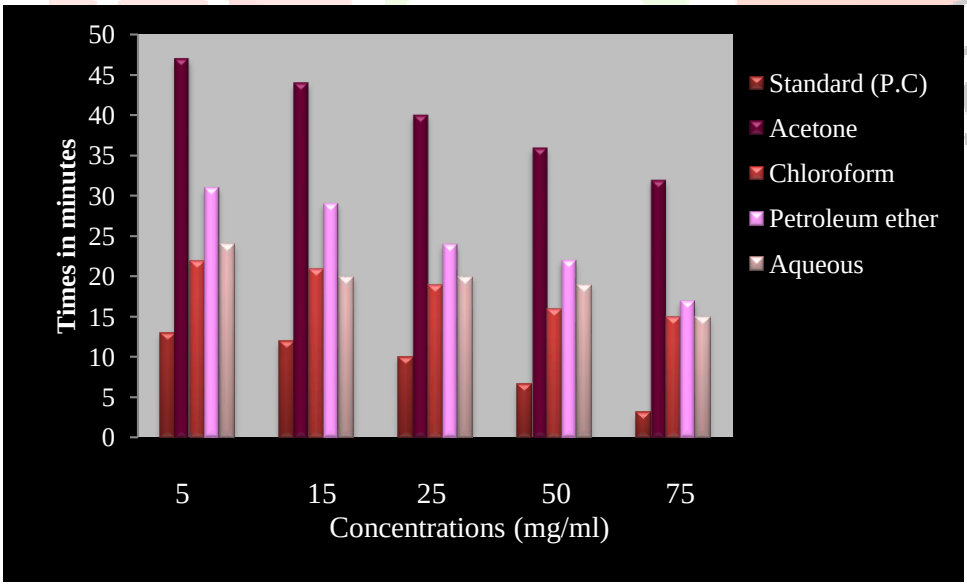

Fig 1. Comparative *in vitro* anthelmintic effect of different concentrations of *C. mimosoides* against illustrating paralysis time of *P. posthuma*.

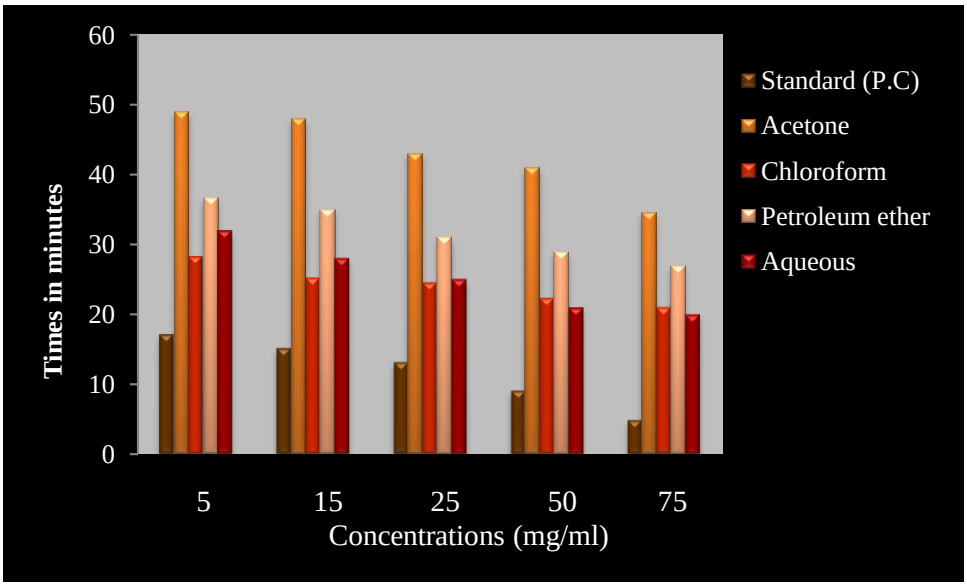

Fig 2. Comparative *in vitro* anthelmintic effect of different concentrations of *C. mimosoides* against illustrating death time of *P. posthuma*.

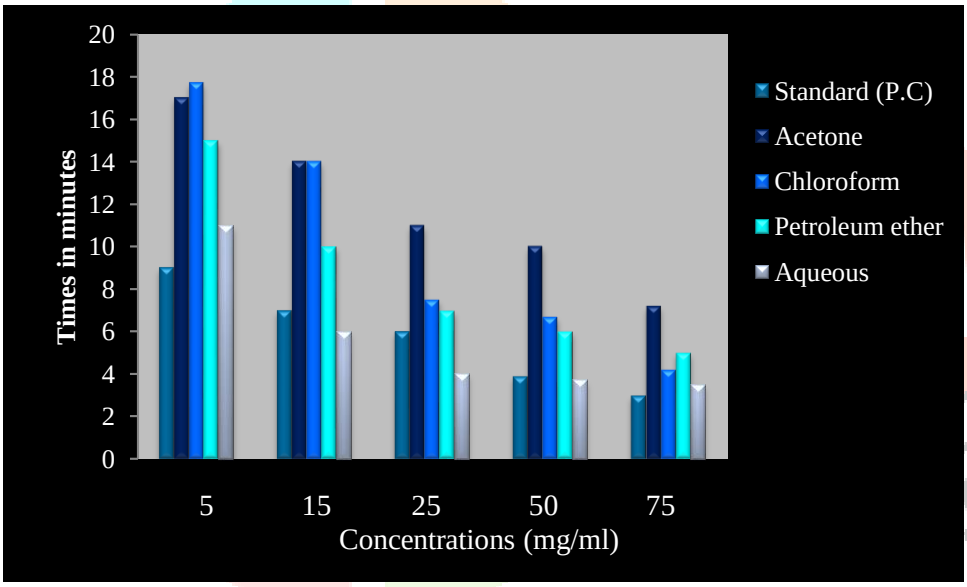

Fig 3. Comparative *in vitro* anthelmintic effect of different concentrations of *C. mimosoides* against illustrating paralysis time of *H. contortus*.

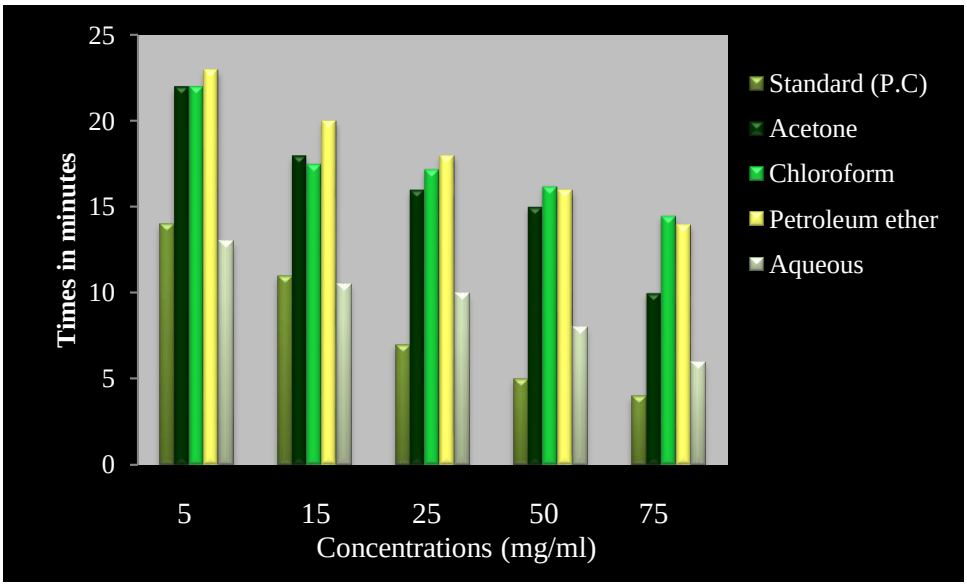

Fig 4. Comparative *in vitro* anthelmintic effect of different concentrations of *C. mimosoides* against illustrating death time of *H. contortus.*

7. Result And Discussion

The aqueous extracts showed significant anthelmintic effect causing death of the worm at all concentrations. However, response of worms in case of paralysis, there was significant variations among the results observed which is produced by the different extracts at different concentrations (75, 50, 25, 15, 5 mg/ml). The aqueous extract showed more significant effect on paralyzing the worms. The effect of extracts on the paralysis and death of the *P. posthuma*, according to the results (**Table 2, Fig1 and 2**) indicated as aqueous > Chloroform > Petroleum ether > Acetone extracts. The effect of extracts on the paralysis and death of the *H. contortus*, according to the results (**Table 2, Fig 3 and 4**) indicated as aqueous > Acetone> Petroleum ether > Chloroform extracts. In particular, aqueous extract exhibited an increased paralytic as well as helminthiatic effect over Piperazine citrate at the given experimental concentrations (**Table 2**).

In aqueous extract, at 75 mg/ml concentration for *Pheretima posthuma* showed paralysis and death at 15 ± 0.8 (P) min and 20 ± 3.4 min (D) respectively. *Haemonchus contortus* showed paralysis and death at 3.5 ± 1.2 min (P), 6.2 ± 0.9 min (D) respectively.

In Chloroform extract, at 75 mg/ml concentration for *Pheretima posthuma* showed paralysis and death at 15 ± 5.1 min (P) min and 21 ± 3.5 min (D) respectively. *Haemonchus contortus* showed paralysis and death at 4.2 ±0.9 min (P), 14.5 ±4.2 min (D) respectively.

In Petroleum ether, at 75 mg/ml concentration for *Pheretima posthuma* showed paralysis and death at 17.7 ± 10 min (P) min and27.5 ± 9.8 min (D) respectively. *Haemonchus contortus* showed paralysis and death at 5.2 ± 2.2 min (P), 14.5 ± 2.6 min (D) respectively.

In Acetone extract, at 75 mg/ml concentration for *Pheretima posthuma* showed paralysis and death at 32.2± 5.7 min (P) min and34.5 ± 5.7min (D) respectively. *Haemonchus contortus* showed paralysis and death at 7.2 ± 1.7 min (P), 10.5 ± 1.2 min (D) respectively. Both worms were more sensitive to the aqueous extract of *C. mimosoides*.

The ANOVA are depicted in **Table 3a, 3b, 3c and 3d**. For this ANOVA, the null hypothesis mentioned. The P values for extracts are 4.64311E-30, 2.418E-27, 6.92582E-12, 2.87164E-23 and corresponding F calculated for extracts are 105.89, 86.65,21.60, 63.18. The critical value of F at 5% level of significance at 4 and 75 degree of freedom is 2.49. Since calculated value of F is very large as compared to critical value. Thus, all the null hypothesis are rejected and concluded that the effects of various

extracts are significantly different as well as the effect varies with concentrations and also there is interaction between extracts and concentrations.

As reported earlier the plant has been evaluated for anthelmintic activity for the first time in the India (no such example could be found through literature search), though it is reported in indigenous system of medicine as anthelmintic.

Preliminary phytochemical analysis of *C.mimosoides* showed Alkaloids, Tannins, Anthraquinones, Steroids, Terpenoids, Flavonoids, Phenols and Saponin Comparatively Tannins, Alkaloids, Terpenoids, Phenols and Saponin were observed in more concentration (**Table 1**). The previous phytochemical analysis was not found much during literature survey. Major classes of the plant chemicals include the Terpenoids, Alkaloids and other nitrogen containing metabolites, Phenolic metabolites was reported by Walton and Brown (1999) Nair (2007) and Prajapathi et al. (2007).

The anthelmintic activity of the extracts of *C. mimosoides* may be due to the presence of Polyphenolic compounds and Tannins in plant extracts. The data presented in the table and observations made therefore, lead to the conclusion that the different degree of helminthiasis of the different extracts are due to the level of tannins present in compounds.

8. Conclusion

It may be concluded from the current studies that *Cassia mimosoides* leaves exhibited prominent anthelmintic activity at all doses against both worms. Thus, plant can be used as aanthelmintic drug. Further studies to attempts for isolation and characterization of active compound responsible for such activities are necessary to understand the exact mechanism of action.

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