

Research Article

In-vitro Comparative Study of Anthelmintic Activity of *Mukia maderaspatana* and *Sida cordata*

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ABSTRACT

Mukia maderaspatana, a leafy vegetable is reported to possess antihyperglycemic, anthelmintic, antihypertensive, vasodilatory, antihyperlipidemic, hepatoprotective and many other activities. *Sida cordata* is given in dysentery, dysuria and some cases of helminthic diseases. The present research work is an attempt to highlight the current clinical and experimental evidences available in literature on the extent of its potentials to protect against parasite infections. The aqueous and ethanolic extracts of both the plants were tested here for their anthelmintic activity on the common Indian earthworm, *Pheretima posthuma*. Various concentrations of the extracts (2.5, 5, 10, 25, 50 mg/ml) respectively were screened for their anthelmintic activity using the standard drug Albendazole. The parameters estimated here were the paralysis time and the death time. When the doses of the extract are increased, a gradual increase in the anthelmintic activity is observed. The aqueous extracts of both the plants showed good activity when compared to that of ethanolic extracts. The highest action was obtained for the aqueous extract of *Sida cordata*. In conclusion, it was confirmed that the aqueous and ethanolic extracts of both *Mukia maderaspatana* and *Sida cordata* showed prominent anthelmintic activity.

Keywords: *Mukia maderaspatana*, *Sida cordata*, Anthelmintic activity, Albendazole.

INTRODUCTION

Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production. Anthelmintics from the natural sources may play a key role in the treatment of these parasite infections¹. Despite the prevalence of parasitic worms, anthelmintic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases have little money to invest in drug discovery or therapy. This prompts concern, as anthelmintic resistance has been widely reported in livestock and it may also only be a matter of time before this phenomenon occurs in parasites of humans². *Mukia maderaspatana* (Cucurbitaceae) is also commonly called as Bilari. Plant pacifies vitiated vata, pitta, constipation, burning sensation, dyspepsia, flatulence, anthelmintic and dental pain³. It is reported as expectorant and has been used traditionally from a long

time for a number of ailments. The tender shoots and bitter leaves are used as a gentle aperient and prescribed in vertigo and biliousness. *M. maderaspatana* has been shown to exert hepatoprotective, anti-inflammatory, and antiarthritic activities⁴. *Sida cordata* (Malvaceae) is a species of *Sida* commonly called as long-stalk. The roots are astringent, thermogenic and are useful in fever, uropathy and arthritis. The leaves are good for diarrhoea and are also used as anthelmintics. The flowers and ripe fruits are refrigerent and are useful in relieving burning sensation, hyperdiuresis, anthelmintic, pectoral lesions and promoting strength⁵. However, the anthelmintic activity of *Mukia maderaspatana* and *Sida cordata* has not so far been scientifically proved, so the present study was carried out to assess the anthelmintic activity by using their aqueous and ethanolic extracts.

MATERIALS AND METHODS

Plant material

The whole plant of *Mukia maderaspatana* belonging to family *Cucurbitaceae* and leaves, flowers and fruits of *Sida cordata* belonging to family *Malvaceae* were collected from local area of Guntur district (India) and was

identified and authenticated by Dr. Sreenivasa Prasanna, M. Pharm., PhD, M.L.College Of Pharmacy, Singarayakonda and voucher specimens (MPESFPS-01/12 and MPESFPS-02/12) were preserved in the department of Pharmacology, Malineni Perumallu Educational Society's Group of Colleges, Guntur, India.

Drugs and chemicals

Albendazole (Micro Lab.Ltd., Goa), normal saline, chloroform and ethanol were used.

Animals

Healthy adult Indian earthworms *Pheritima posthuma* (Annelida, Megascolecidae) was used for evaluating the Anthelmintic activity due to its anatomical and physiological resembles with the intestinal round worm parasites of human beings. Because of easy availability, earthworms have been used extensively for the preliminary *in vitro* evaluation of anthelmintic activity. All earthworms were of approximately equal size. They were collected from local place, washed and kept in water⁶.

Extraction procedures

Preparation of aqueous extract of *Mukia maderaspatana*

The whole plant parts of *Mukia maderaspatana* were extracted by maceration process. In this a total amount of 30 g powdered plants were macerated in 200ml of distilled water for 48 hours with occasional stirring for every 3 hours. At the end, the extract was passed through a filter paper and filtrate was evaporated under reduced pressure on water bath to obtain crude. After cooling 2 drops of chloroform drops are added for preservation. Condensed extracts were weighed and stored in air-tight containers at 4°C till further investigation.

Preparation of ethanolic extract of *Mukia maderaspatana*

30 gm of whole plant of *Mukia maderaspatana* was taken in a soxhlet and 200 ml of 90% ethanol was added. It was refluxed for 7 hours and filtered through muslin cloth while hot. The alcohol extract was dried under vacuum. After cooling 2 drops of chloroform drops are added for preservation. Condensed extracts were weighed and stored in air-tight containers at 4°C till further investigation.

Preparation of aqueous extract of *Sida cordata*

The leaves, flowers, fruits of *Sida cordata* was collected, dried under shade and powdered.

25g of the powder was extracted in 200 ml of distilled water for 3 days in the room temperature with intermittent shaking. After incubation, the extract was filtered through filter paper and then it was concentrated. After cooling 2 drops of chloroform was added for preservation. Condensed extracts were weighed and stored in air-tight containers at 4°C till further investigation.

Preparation of ethanolic extract of *Sida cordata*

The leaves, flowers, fruits of *Sida cordata* was extracted by soxhlation process by using 200ml of ethanol. In the soxhlation procedure, a total amount of 25 gm powdered plant material was soxhlated for 6 hours. This cycle may be allowed to repeat many times, over hours⁷. The extract was filtered and then it was concentrated. After cooling 2 drops of chloroform drops are added for preservation. Condensed extracts were weighed and stored in air-tight containers at 4°C till further investigation.

Phytochemical analysis

Preliminary phytochemical screening of aqueous and ethanolic extracts of *Mukia maderaspatana* and *Sida cordata* was performed for the detection of the constituents that were responsible for the activity.

Anthelmintic assay

The Anthelmintic activity was evaluated on adult Indian earthworm by the reported methods with slight modification.^[8] 50 ml formulations containing *Mukia maderaspatana* and *Sida cordata* in five different concentrations of aqueous extract of its various fractions (2.5, 5.0, 10, 25 and 50 mg/ml) and ethanolic extract of its various fractions (2.5, 5.0, 10, 25 and 50 mg/ml) were prepared and six worms (same type) were placed in it. The wide range of dose was taken to establish the relationship between dose and pharmacological activity and also to find out the minimum and maximum dose that can be better therapeutically effective in comparison to standard drug. 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 were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C) followed with fading away of their body colour. Albendazole (20 mg/ml) was used as reference standard. A separate beaker with saline solution which was used as a vehicle in the study was also kept as a negative control.

RESULTS

The extracts obtained for both the plants showed better yields. Preliminary phytochemical screening of aqueous and ethanolic extracts of both *Mukia maderaspatana* and *Sida cordata* revealed the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, and polyphenols (Table – 1) in both the plants. In extra, *Mukia* showed the presence of saponins.

Anthelmintic assay

Both the aqueous and ethanolic extracts of *Mukia maderaspatana* and *Sida cordata* showed anthelmintic activity in dose dependent manner as shown in table – 2. As the dose increased, there is an increase in the anthelmintic action. Among all the extracts, the aqueous extract of *Sida cordata* showed very good anthelmintic activity. The decreasing order of the anthelmintic activity of different extracts is as follows.

Aqueous extract of *Sida* > Aqueous extract of *Mukia* > Ethanolic extract of *Sida* > Ethanolic extract of *Mukia*.

The results of the activity were represented in the form of graphs. The fig – 1 indicates the anthelmintic activity of aqueous and ethanolic extracts of *Mukia maderaspatana* on Indian Earthworm *Pheretima posthuma* where as the fig – 2 indicates the anthelmintic activity of aqueous and ethanolic extracts of *Sida cordata* on Indian Earthworm *Pheretima posthuma*.

DISCUSSION

Earthworms are invertebrates which are composed of many segments. They do not have bones and move by contracting and relaxing the body segments in sequence. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer⁹. All anthelmintics essentially kill worms by either starving them to death or paralyzing them because worms have no means of storing energy, they must eat almost continuously to meet their metabolic needs. Any disruption in this process results in energy depletion. Interfering with feeding for 24 hours or less is sufficient to kill most adult parasites. Parasites will also die if they become paralyzed and temporarily lose

their ability to maintain their position in the gut. Preliminary phytochemical screening of aqueous and ethanolic extracts of both *Mukia maderaspatana* and *Sida cordata* revealed the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, and polyphenols in both the plants. In extra, *Mukia* showed the presence of saponins.

The possible mechanism of action of tannins may be interference with energy generation by uncoupling oxidative phosphorylation or they may interfere with glycoprotein of cell surface or they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death¹⁰. Alkaloids may act on central nervous system and caused paralysis of the earthworm. The effect would be due to presence of alkaloids which may suppress the transfer of sucrose from the stomach to the small intestine together with its antioxidant effect which is capable of reducing the nitrate generation which could interfere in local homeostasis which is essential for the development of helminths.^[11] Albendazole binds to free β -tubulin, inhibiting polymerisation and thus interfering with microtubule dependent glucose uptake by the worms¹². Thereby the presence of all these active chemical constituents in the plants leads them to show a better anthelmintic activity.

CONCLUSION

From the above results, it was concluded that the aqueous extract of *Sida cordata* showed more potent anthelmintic activity than that of the any other extracts of *Mukia maderaspatana*. Further work will emphasize the isolation and characterization of active principles responsible for anthelmintic activity and to establish the effectiveness and pharmacological rationale for the use of *Mukia maderaspatana* and *Sida cordata* as an anthelmintic drug.

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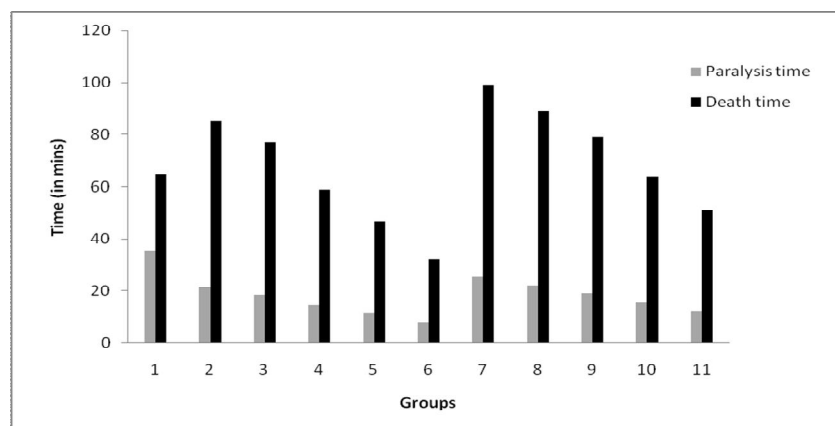
Table 1: Phytochemical screening of aqueous and ethanolic extracts of *Mukia maderaspatana* and *Sida cordata*

Phytochemical Constituents	<i>Mukia maderaspatana</i>		<i>Sida cordata</i>	
	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
Carbohydrates	+	+	+	+
Fixed oils	-	-	+	-
Glycosides	+	+	+	+
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Polyphenols	+	+	+	+
Steroids	-	+	-	+
Saponins	+	+	-	-

+ = Present - = Absent

Table 2: Anthelmintic activity of ethanolic and aqueous extract of whole plant parts of *Mukia maderaspatana* and leaves, flowers, and fruits of *Sida cordata*

S. No	Treatment	Concentration (mg/ml)	Time taken for Paralysis(min)	Time taken for Death(min)
1	Vehicle	-	-	-
2	Albendazole	20	35.4	64.9
3	Aqueous extract of <i>Mukia maderaspatana</i>	2.5	21.6	85
		5.0	18.5	77
		10	14.4	59
		25	11.6	47
		50	7.9	32
4	Ethanolic extract of <i>Mukia maderaspatana</i>	2.5	25.5	99
		5.0	22.3	89
		10	19.1	79
		25	15.5	64
		50	12.3	51
5	Aqueous extract of <i>Sida cordata</i>	2.5	18.4	76
		5.0	15.2	62
		10	11.8	48
		25	8.2	34
		50	5.6	20
6	Ethanolic extract of <i>Sida cordata</i>	2.5	24.6	94
		5.0	21.4	84
		10	18.1	75
		25	14.3	58
		50	11.2	44

**Fig. 1: Anthelmintic activity of aqueous and ethanolic extracts of *Mukia maderaspatana* on Indian Earthworm *Pheretima posthuma***

(Group 1- Standard Albendazole -20 mg/ml, Group 2 to 6 - Aqueous extract 2.5, 5.0, 10, 25, 50 mg/ml respectively, Group 7 to 11 – Ethanolic extract 2.5, 5.0, 10, 25, 50 mg/ml respectively)

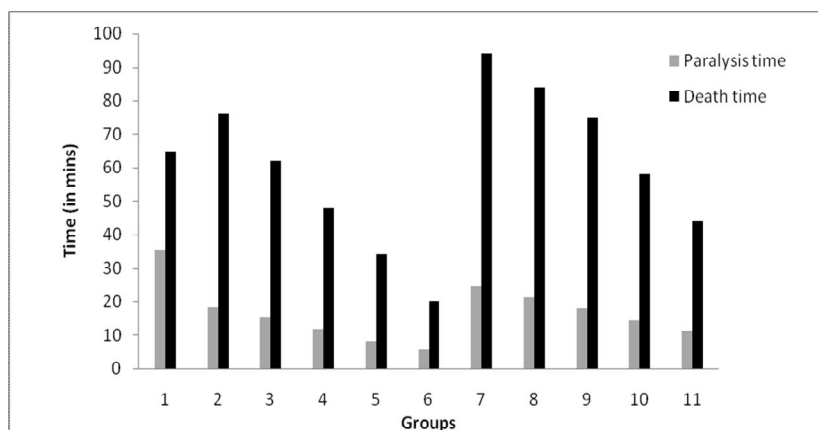


Fig. 2: Anthelmintic activity of aqueous and ethanolic extracts of *Sida cordata* on Indian Earthworm *Pheretima posthuma*

(Group 1- Standard Albendazole -20 mg/ml, Group 2 to 6 - Aqueous extract 2.5, 5.0, 10, 25, 50 mg/ml respectively, Group 7 to 11 – Ethanolic extract 2.5, 5.0, 10, 25, 50 mg/ml respectively)

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