



Evaluation of anthelmintic activity of methanolic extract of *Asystasia gangetica*

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Abstract

Methanolic extract of the leaves of *Asystasia gangetica* were screened for its anthelmintic activity against earthworms (*Pheretima posthuma*) and roundworms (*Ascaridiagalli*). The parameters like the time of paralysis and the time of death were determined by using the extract at the concentrations of (10-100 mg/ml). The extract exhibited significant anthelmintic activity at highest concentration of 100 mg/ml as compared with piperazine citrate (10 mg/ml) as standard reference and distilled water as control.

Key-Words: Anti-helminthic activity, *Pheretima posthuma*, *Raillietina spiralis*, *Ascaridiagalli*

Introduction

Asystasia gangetica also known as Chinese Violet belongs to Acanthaceae family. Leaves are opposite petioles, flowers are pale purple blue to violet or lime white in colour, and capsules are 2.5 – 3.5 cm wide at the base and the seeds are 5mm diameter. Traditionally this plant is used for anthelmintic activity, in swelling, rheumatism, ear diseases etc¹. Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent particularly in third world countries due to poor management Helminthiasis practices^[2]. A number of medicinal plants have been used to treat parasitic infections in man and animals.^[3, 4, 1] The plants are known to provide a rich source of botanical anthelmintics^[5, 6]. The assay was performed on adult Indian earthworm, *Pheretima posthuma* and roundworms (*Ascaridiagalli*) due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings^[7, 8]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro^[9, 10, 11]. The objective of the present research was to prove traditional anthelmintic use of the plant.

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Material and Methods

Preparation Plant material

The leaves of *Asystasia gangetica* Linn.(Acanthaceae), was collected from Tirumala hills, Andhra Pradesh in the month of December 2010. The plant material was authenticated by National institute of Herbal Science (PARC/2010/542), west Tambaram, Chennai.

Preparation of extract

The leaves of the plant was dried in shade and made to fine powder using a laboratory mill. The dry powder is extracted with methanol using maceration process for 48hours.

Phytochemical tests

The preliminary phytochemical tests revealed the methanolic extract of the leaves shows the presence of alkaloids, phenols, tannins, flavonoids, terpenoids, saponins. The results are tabulated in Table 1.

Experimental Animals

Adult earthworms (*Pheretima posthuma*) and Roundworm (*Ascaridia galli*) were used to evaluate anthelmintic activity *in vitro*. Earthworms collected from moist soils of Herbal Garden, Sri Ramachandra University, Porur, Chennai, was authenticated by Manidharman Biotech Pvt Ltd, Porur, Chennai, was 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. Roundworms were obtained from intestine of freshly slaughtered fowls. Infested intestines of fowls were collected from the local slaughter house and washed with normal saline solution to remove all the faecal matter. These

intestines were then dissected and double distilled water as control ^[12, 13, 14, 15].

This procedure was adopted for all different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were collected and kept in normal saline solution. The average size of round worm was 5-7 cm.

Chemicals

- Piperazine Citrate (Glaxo)
- Double distilled water

Anthelmintic Activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al* ^[26]. The assay was performed *in vitro* using adult earthworm (*Pheretima posthuma*) as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings for preliminary evaluation of anthelmintic activity ^[16,17,18]. Use of *Ascaridia galli* species as a suitable model for screening of anthelmintic drug was advocated earlier ^[19, 20]. Test samples of the extract was prepared at the concentrations, 10, 20,30,40 and 50 mg/ml in distilled water and six worms i.e. *Pheretima posthuma*, *Ascaridia galli* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg /ml) was used as reference standard was advocated earlier ^[21, 22]. Test samples of the extract was prepared at the concentrations, 10, 20, 30, 40, 50, 60 and 70 mg/ml in distilled water and six worms i.e. *Pheretima posthuma*, *Ascaridia galli* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10mg /ml) was used as reference standard and double distilled water as control ^[12, 13, 14, 15]. This procedure was adopted for all different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were collected and kept in normal saline solution. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50⁰ C). All the results were shown in Table.2 and expressed as a mean \pm SEM of six worms in each group.

Results and Discussion

From the above study it was seen that the methanolic extract of *Asystasia gangeticum* showed dose

dependent anti helminthic activity showing maximum efficacy at 70mg/ml for two types of worms as compared to a standard drug piperazine citrate (Table 2). Higher concentration of extract produced paralytic effect much earlier and the time taken for death was shorter for all types of worms. Therefore, further study must be carried out so that the general people can get actual benefit from this important medicinal plant.

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Table 1: Phytochemical screening of methanolic extract of *Asystasia gangetica*

Phytochemicals	Methanolic Extract
Alkaloids	+
Flavonoids	+
Phenols	+
Tannins	+
Terpenoids	+
Saponins	+
Sterols	-
Glycosides	-
Carbohydrates	-

+ = present, - = absent

Table 2: In – vitro anthelmintic activity of methanolic extract of *Asystasia gangetica*

S/No:	Groups	Concentration mg/ml	<i>Pheretima posthuma</i> groups		<i>Ascaridia galli</i> groups	
			Time taken for Paralysis (P)in mins Mean & SEM	Time taken for Death (D) in mins Mean & SEM	Time taken for Paralysis (P)in mins Mean & SEM	Time taken for Death (D) in mins Mean & SEM
1.	Control	--	--	--	--	--
2.	Methanolic extract	10	50±0.6	70±0.22	52±0.33	63±1.39
		20	42±0.10	58±0.26	49±0.45	55±0.49
		40	35±0.25	50±0.62	43±1.12	46±1.2
		60	26±0.4	42±0.11	30±0.62	43±0.43
		80	18±0.15	35±0.25	21±0.55	38±0.12
		100	12±0.7	30±0.79	16±0.25	33±0.1
3.	Piperazine citrate	10	24±1.25	58±0.68	14±1.70	42±1.10