

# In vitro Evaluation of Anthelmintic Activity of *Gymnema sylvestre* Plant

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## ABSTRACT

The evaluation of anthelmintic activity of *Gymnema sylvestre* was performed by bioassay method where hot and cold hydroalcoholic extracts were used against earthworm (*Pheretima posthuma*). The anthelmintic assay was carried out as the 50ml formulations containing five different concentrations of each cold and hot hydroalcoholic extracts (25, 50, 100, 250 and 500mg/ml in distilled water) were prepared and six worms (same type) were placed in them. Time for paralysis and death time was noted. Albendazole (20mg/ml) was used as a reference standard, while normal saline as the control.

## INTRODUCTION

Helminths are parasitic worms, they are most common infectious agents of humans in developing countries and produce a global burden of disease. Helminths have plagued humans since before the era of our earliest recorded history.<sup>1</sup> There are two major phyla of helminths. The nematodes (roundworms) include the major intestinal worms, also known as soil transmitted helminths. Whereas platyhelminths (flatworms) include flukes also known as trematodes and tapeworms. It is estimated that approximately one-third of almost three billion people that live on less than two US dollars per day in developing regions of Sub-saharan Africa, Asia, and America are affected with one or more helminth.<sup>2</sup> The high medical, educational and economic burden of helminth infections provides an important rationale for launching a global assault on parasitic worms.<sup>3</sup> However, the tools we currently have for controlling worm infections are limited, of the 1,556 new chemical entities marketed between 1975 and 2004, only four drugs-albendazole, oxfamiquine, praziquantel and ivermectin were developed to treat helminthiasis.<sup>4</sup>

Nowadays multiple drug resistance has been developed due to the indiscriminate use of the antimicrobial drugs commonly used for treatment of infectious diseases. It can also cause hypersensitivity, immune-suppression and allergic reactions. Recently the indiscriminate use of anthelmintic produced toxicity in humans. Hence the development and discovery of new anthelmintic are being derived through plants. Herbal medicine is still the main source of medicine and about 70-80% of the whole population, mainly in developing countries for primary healthcare because of better cultural acceptability, better compatibility, with the human body and fewer side effects. *Gymnema sylvestre* also known as madhunashini (Sanskrit) and cherukurinja (tamil) belonging to the family of Asclepiadaceae is a vulnerable species is a slow growing, perennial, medicinal woody climber

found in central and peninsular India. It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine.<sup>5</sup> In addition, it possesses wound healing,<sup>6</sup> anti-inflammatory,<sup>7</sup> anti-obesity,<sup>8</sup> treatment of snake bite<sup>9</sup> and anthelmintic properties. Flavonoids, tannins and saponins are the chief constituents of *Gymnema sylvestre*.<sup>10-12</sup> *Gymnema sylvestre* is used in ayurvedic medicine as anthelmintic but it is not clinically proved. The active principles are flavonoids which show selective anthelmintic activity.

## MATERIALS AND METHODS

### Drugs and chemicals

Albendazole, normal saline (Abaris healthcare private limited) and ethanol.

### Plant collection and authentication

The leaves of *Gymnema sylvestre* belonging to the family of Asclepiadaceae were collected from the herbal garden of Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai. The plant was authenticated Dr. P. Jayaraman (Director, Plant Anatomy Research Centre, Tambaram).

### Plant extraction

Leaves were dried at room temperature (25 to 35 °C) and powdered with the help of a electrical grinder. The fine material was subjected to extraction, successfully by hot and cold hydroalcoholic mixture in the ratio of (water60:40 ethanol) as solvent. The extract was allowed to dry at 100 °C in water bath. The percentage yield of the different successive extracts was 30 and 40% respectively.

### Worms collection

Indian earthworms *Pheretima posthuma* of nearly equal size (8 to 10 cm) were collected from the water-logged areas from herbal garden of SRI RAMACHANDRA INSTITUTE OF HIGHER EDUCATION AND RESEARCH.

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## Preparation of test sample

Samples for *in vitro* study were prepared by dissolving and suspending (0.12,0.25,0.5,1.25 and 2.5g) of each hot and cold hydroalcoholic extract in 50ml of distilled water at different concentrations ranging from 25, 50, 100, 250 and 500 mg/ml.

## Anthelmintic assay

It was carried out using adult earthworm (*Pheretima posthuma*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation of anthelmintic activity.<sup>13</sup> The 50ml formulations containing five different concentrations of each hot and cold hydroalcoholic extracts (25,50,100,250 and 500mg/ml in distilled water) were prepared. *Pheretima posthuma* was placed in petri dish containing 10ml of the extract.<sup>14</sup> Each petri dish was placed with six worms and observed for paralysis and death. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm is shaken vigorously. The time of death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in normal saline followed with fading away of their body colour and the results were expressed in comparison to the standard drug Albendazole (20 mg/ml).

## RESULTS AND DISCUSSION

From the Table 1, it was evident that hot and cold hydroalcoholic extracts of *Gymnema sylvestre* exhibited anthelmintic activity in dose dependent manner giving the shortest time of paralysis and death with 500 mg/ml concentration. The hot extract caused paralysis in 15 minutes and

time of death 23 minutes, while cold extract revealed paralysis of 24 minutes and time of death 35 minutes against the earthworm *Pheretima posthuma*.

Chemically flavonoids are polyphenolic compounds and they interfere with the energy generation by uncoupling the oxidative phosphorylation which interfere with the glycoprotein of cell surface leads to parasite death.<sup>15</sup> Another possible anthelmintic effect of tannins is that they bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death.<sup>16</sup>

## HOT EXTRACT

From the Figures 1-4, Albendazole standard (20 mg/ml) showed paralysis time in  $42 \pm 0.2$  min and death time in  $54.15 \pm 0.3$  min. In hot extract 25mg showed paralysis time in  $53 \pm 0.5$  min and death time in  $61.02 \pm 0.4$  min, 50mg showed paralysis time in  $49.2 \pm 0.2$  min and death time in  $57.18 \pm 0.6$  min, 100mg showed paralysis time in  $37.23 \pm 0.4$  min and death time in  $43.13 \pm 0.3$  min, 250mg showed paralysis time in  $21.07 \pm 0.6$  min and death time in  $29.11 \pm 0.4$  min and 500mg showed paralysis time in  $15.17 \pm 0.4$  min and death time in  $23.02 \pm 0.6$  min.

## COLD EXTRACT

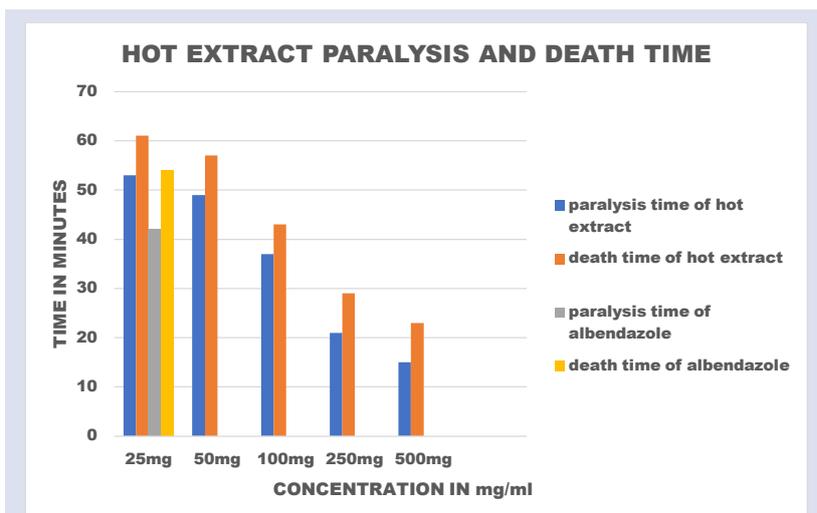
From Figure 5, In cold extract 25mg showed paralysis time in  $55.11 \pm 0.2$  min and death time in  $64.03 \pm 0.3$  min, 50mg showed paralysis time in  $51.33 \pm 0.1$  min and death time in  $59.12 \pm 0.7$  min, 100mg showed paralysis time in  $45.19 \pm 0.3$  min and death time in  $55.05 \pm 0.4$  min, 250mg showed paralysis time in  $36.16 \pm 0.8$  min and death time in  $49.06 \pm 0.6$  min and 500mg showed paralysis time in  $24.17 \pm 0.3$  min and death time in  $35.11 \pm 0.2$  min.

**Table 1: In vitro evaluation of anthelmintic activity of *Gymnema sylvestre*.**

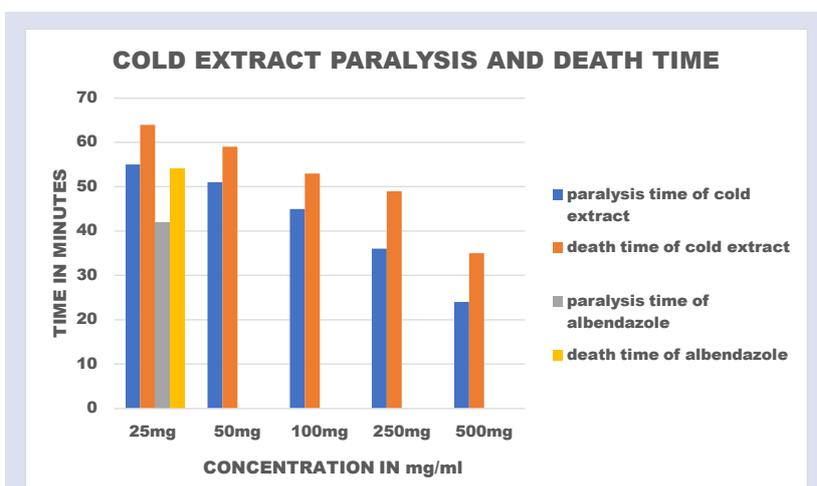
Group	Solution	Concentration in Mg/MI	Time taken for paralysis (Mins)	Time taken for death (Mins)	Time between paralysis and death (Mins)
1.	Control	-	-	-	-
2.	albendazole	20	$42.2 \pm 0.2$	$54.15 \pm 0.3$	$11.95 \pm 0.1$
3.	Hot extract	25	$53.05 \pm 0.3$	$61.02 \pm 0.4$	$7.97 \pm 0.1$
		50	$49.12 \pm 0.2$	$57.18 \pm 0.6$	$8.06 \pm 0.4$
		100	$37.23 \pm 0.4$	$43.13 \pm 0.3$	$5.9 \pm 0.1$
		250	$21.07 \pm 0.6$	$29.11 \pm 0.4$	$8.04 \pm 0.2$
		500	$15.17 \pm 0.4$	$23.02 \pm 0.6$	$7.85 \pm 0.2$
4	Cold extract	25	$55.11 \pm 0.2$	$64.03 \pm 0.3$	$8.92 \pm 0.3$
		50	$51.33 \pm 0.1$	$59.12 \pm 0.7$	$7.79 \pm 0.6$
		100	$45.19 \pm 0.3$	$55.05 \pm 0.4$	$9.86 \pm 0.1$
		250	$36.16 \pm 0.8$	$49.06 \pm 0.6$	$12.9 \pm 0.2$
		500	$24.17 \pm 0.3$	$35.11 \pm 0.2$	$10.94 \pm 0.1$



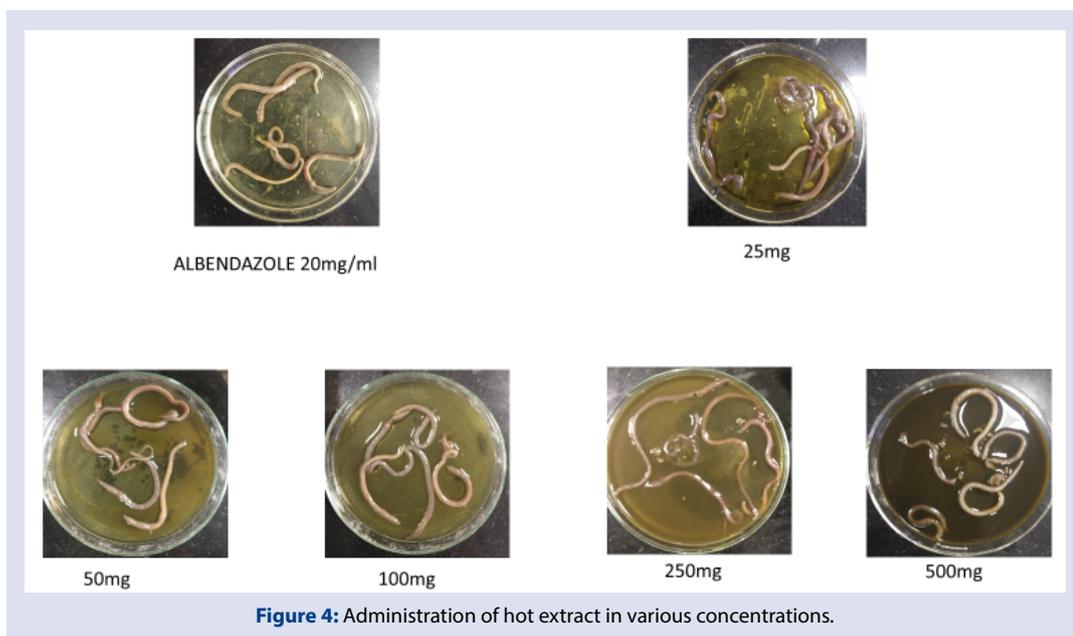
**Figure 1: *Gymnema sylvestre*.**



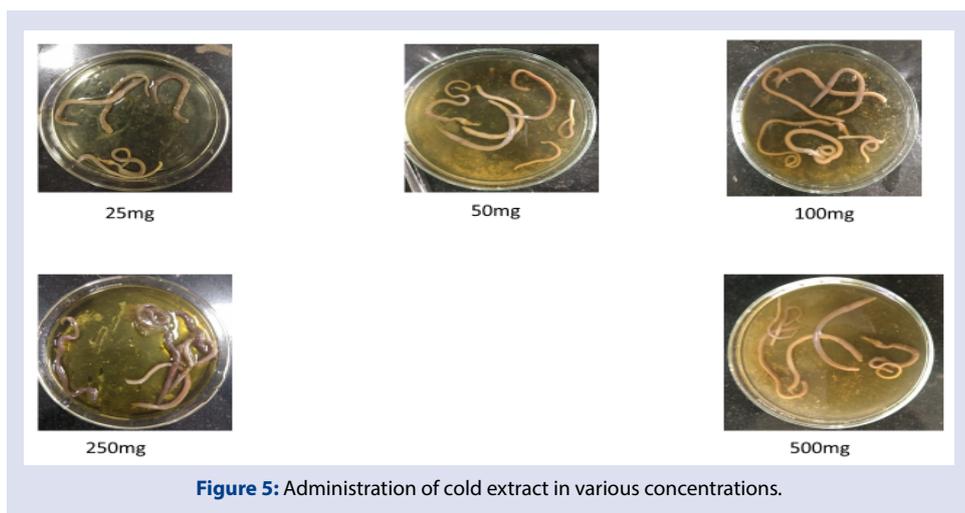
**Figure 2:** *In vitro* evaluation of anthelmintic activity of *Gymnema sylvestre* using hot extract.



**Figure 3:** *In vitro* evaluation of anthelmintic activity of *Gymnema sylvestre* using cold extract.



**Figure 4:** Administration of hot extract in various concentrations.



**Figure 5:** Administration of cold extract in various concentrations.

## REPORT

The graph of hot extract and cold extract expresses the paralysis time and death time at various concentrations of the extract which exhibited anthelmintic activity in dose dependent manner giving the shortest time of paralysis and death with 500 mg/ml concentration. The graph thus helped to identify the concentration at which the paralysis and death occurs in the shortest duration of time. In hot extract the hot extract (Figure 2) caused paralysis in 15 minutes and time of death 23 minutes, while cold extract (Figure 3) revealed paralysis of 24 minutes and time of death 35 minutes against the earthworm *Pheretima posthuma*.

## CONCLUSION

From the results, it was concluded that both hot and cold hydroalcoholic extracts of *Gymnema sylvestre* have significant anthelmintic activity, but hot hydroalcoholic extract shown most significant anthelmintic activity when compared to cold hydroalcoholic extract. From the results, *Gymnema sylvestre* has an anthelmintic activity have been confirmed as it displayed activity against the worm used in the present study.

## FUTURE SCOPE

Further studies have to be done to isolate the active principles responsible for the activities. In addition to that we are going to perform comparative studies on various species of *Gymnema* (almost 49 species) and also to formulate the herbal formulation with anthelmintic property.

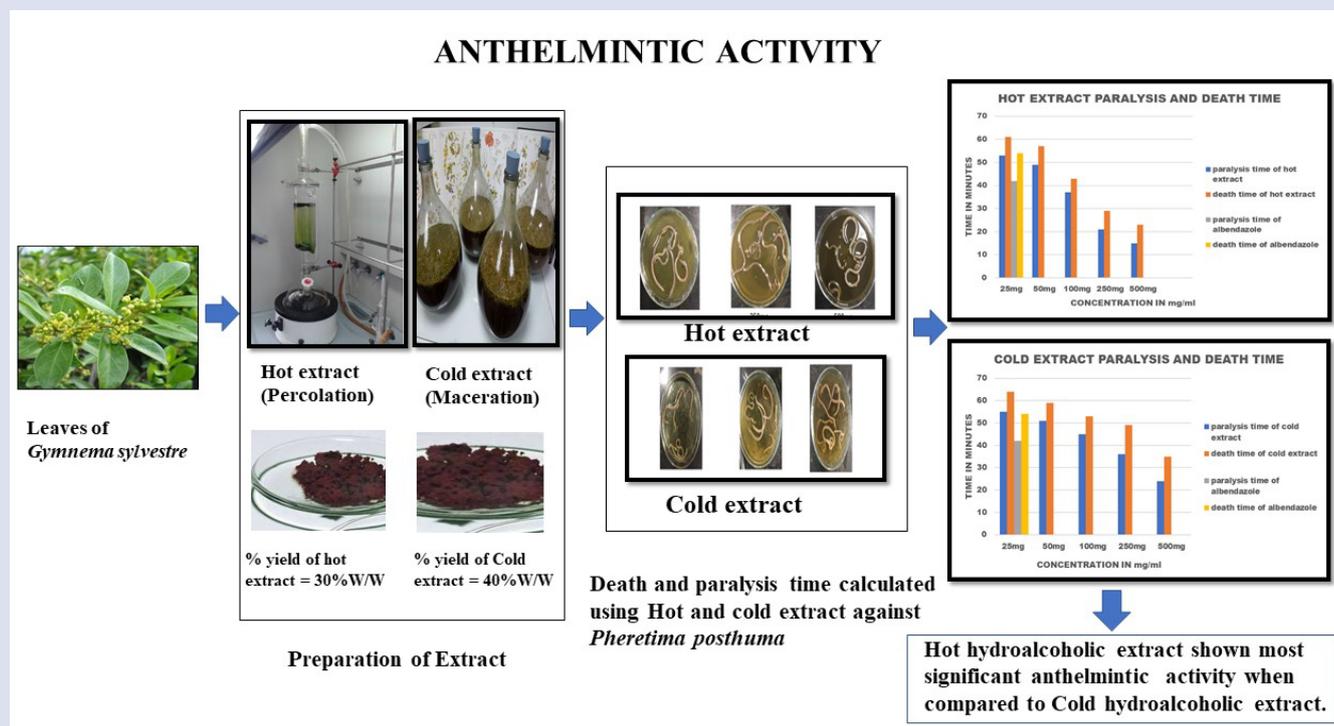
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## GRAPHICAL ABSTRACT



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