

In Vitro* Evaluation Of Anthelmintic Activity Of *Gymnema sylvestre* Leaves Against *Pheretima posthuma

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Abstract

Gymnema sylvestre has been used as a traditional medicinal plant to prevent diabetes. The present study was undertaken to evaluate anthelmintic activity of hot and cold hydroalcoholic extracts of *Gymnema sylvestre* leaves against *Pheretima posthuma*. Various concentrations (25-500 mg/ml) of hot and cold hydroalcoholic extracts of *Gymnema sylvestre* were evaluated in the bioassay involving determination of time of paralysis (P) and time of death (D) of the worms. Albendazole was used as standard anthelmintic drug and distilled water was used as control. The results of present study indicated that the hydroalcoholic extracts significantly exhibited the paralysis in worms and also caused death of worms in dose dependent manner, among which hot maceration extract showing more significant results when compared with the cold maceration extract. Further studies have to be done to isolate the active principles responsible for the activity.

Key words:

Anthelmintic, *Gymnema sylvestre*, *Pheretima posthuma*, Albendazole.

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INTRODUCTION:

Infections with helminths or parasitic worms affect more than two billion people worldwide. In regions of rural poverty in the tropics, where prevalence is

greatest, simultaneous infection with more than one type of helminth is common [1]. Primarily as a result of stepped-up advocacy by the World Health Organization (WHO), the World Bank and smaller nongovernmental organizations such as the London-based Partnership for Child Development (PCD), there is increasing appreciation for the impact of helminth infections on the health and education of school-aged children [2]. Helminthosis plays a crucial role in the small ruminant production leading to enormous economic losses particularly in areas where extensive grazing is practiced [3]. Helminths may be limited solely to the intestinal lumen or may involve a complex process with migration of the adult or immature worm through the body before localization in a particular tissue. Complicating our understanding of the host– parasite relationship and the role of chemotherapy in helminth-induced infections is the complex life cycle of many of these organisms, whereas some helminths have a simple cycle of egg deposition and development of the egg to produce a mature worm. Helminths can be divided into the following major groups: cestodes (flatworms), nematodes (roundworms), trematodes (flukes) and less frequently, Acanthocephala (thorny-headed worms) [4]. Development of resistance to most of the commercially available anthelmintics became a severe problem worldwide. Moreover, these drugs are unaffordable, inaccessible or inadequately available to the resource-poor farmers of the developing countries [5]. Synthetic anthelmintics produce side effects like vomiting, headache, abdominal pain and diarrhea [6]. Anthelmintics from the natural sources may play a key role in the treatment of these parasite infections and can avoid resistance to synthetic agents.

Gymnema sylvestre (Asclepiadaceae) leaves, commonly known as “Gudmar” is a large woody, much branched climber with pubescent young parts in dry forest up to 600 mts height. *Gymnema sylvestre* leaves have been widely used in Ayurvedic traditional medicine. Leaves of the plant are used as

antidiabetic [7], antiinflammatory [8], antiarthritic [9], antiobesity [10], woundhealing[11] astringent, bitter, acrid, thermogenic anodyne, digestive and liver tonic. Tannins, flavonoids and saponins are the chief chemical constituents present in *Gymnema sylvestre* [9]. *Gymnema sylvestre* a plant used in the Ayurvedic medicine as anti-helmentic but it is clinically not proved. The active principles are flavonoids which show selective anti helmintic activity.

MATERIALS AND METHODS:

Drugs and Chemicals:

Albendazole (Micro Lab. Ltd., Goa), Normal saline and Ethanol (Merk specialities Pvt Ltd, Mumbai).

Plant collection and Authentication:

The dried leaves of *Gymnema sylvestre*, belonging to Family Asclepiadaceae were collected from RIPER medicinal garden, Anantapur (Andhra Pradesh), and authenticated by Dr.J.Raveendhra reddy, M.pharm, PhD., Department of Pharmacogonosy, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur and voucher specimen (riper 15/11) preserved in department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur.

Plant Extraction:

Leaves were dried at room temperature (25-35°C) and powdered with the help of an electric grinder. The fine material was subjected to extraction, successively by hot and cold maceration using hydroalcoholic mixture (60 water: 40 ethanol) as solvent. The extracts were allowed to dry at 100°C in a water bath. The percentage yields of the different successive extracts were 12.4% and 14.8% respectively.

Worms Collection:

Indian earthworms *Pheretima posthuma* were collected from the water logged areas of soil worms were obtained from, local place of Anantapur.

Qualitative Phytochemical Analysis:

A systematic and complete study of crude drugs should include a complete investigation of both

primary and secondary metabolites derived from the plant metabolism. Different qualitative chemical tests were performed for establishing the profiles of given extracts for their nature of chemical composition [12], [13]. The hydroalcoholic extracts obtained as above were subjected to qualitative chemical test for identification of various phytoconstituents [14].

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 hydro alcoholic extract in 50 ml of distilled water, at different concentration ranging from 25, 50, 100, 250 and 500 mg/ml.

Anthelmintic Assay:

The anthelmintic assay was carried out as per the method of Ajayieoba et al. with minor modifications [15]. The assay was performed *in vitro* 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 [16], [17], [18]. The 50 ml 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 was noted when no movement of any sort could be observed except the worms were shaken vigorously. The time for death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in normal saline. Albendazole (20 mg/ml) was used as reference standard, while normal saline as the control.

RESULTS AND DISCUSSION

Table.1 Percentage yield of the extracts from leaves of *Gymnema sylvestre* extracts.

| S. No | Extraction process | Solvent used | Percentage yield |
|-------|--------------------|-----------------|------------------|
| 1 | Hot | Hydro Alcoholic | 14.8% |
| 2 | Cold | Hydro alcoholic | 12.4% |

Table 2: Qualitative Phyto Chemical Analysis of *Gymnema sylvestre* extracts.

| Test | Hot maceration | Cold maceration |
|---------------|----------------|-----------------|
| Alkaloids | + | + |
| Carbohydrates | + | + |
| Flavonoids | + | + |
| Glycosides | + | + |
| Phytosterols | + | + |
| Saponins | + | + |
| Tannins | + | + |

(+) Presence; (-) Absence

Table 3: Anthelmintic activity of herb extract of the *Gymnema sylvestre*

| Test substance | Concentration (mg/ml) | Time taken for paralysis (P) and for Death of worms (D) in min | |
|-----------------|-----------------------|--|-------|
| | | P | D |
| Vehicle | - | - | - |
| Albendazole | 20 | 42.28 | 71.34 |
| Hot maceration | 25 | 13 | 73 |
| | 50 | 12 | 56 |
| | 100 | 11 | 51 |
| | 250 | 08 | 48 |
| | 500 | 03 | 43 |
| Cold maceration | 25 | 18 | 75 |
| | 50 | 14 | 61 |
| | 100 | 12 | 54 |
| | 250 | 09 | 50 |
| | 500 | 04 | 45 |

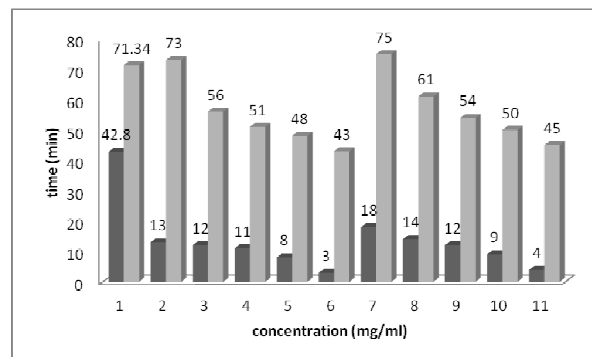


Fig 1. Anthelmintic activity of cold and hot hydroalcoholic extracts of dried leaves of *Gymnema sylvestre* on Indian Earthworm *Pheretima posthuma*.

(Conc.1-Standard Albendazole -20 mg/ml, Conc. 2 to 6 – hot maceration hydroalcoholic extract 2.5, 5, 10, 25, 50 mg/ml, Conc 7 to 11 –cold maceration hydroalcoholic extract 2.5, 5, 10, 25, 50 mg/ml respectively).

From the Table 3, it was evident that hot and cold hydroalcoholic extracts of *Gymnema sylvestre*

exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death with 500mg/ml concentration. The hot maceration caused paralysis in 3min and time of death 43min., while cold maceration revealed paralysis of 4min and time of death of 46min 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 [19]. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death [20]. Saponins mainly act by parallel irritation of mucus membrane that leads to parasite death [21].

Albendazole also has been shown to inhibit the enzyme fumarate reductase, which is helminth-specific. This action may be considered secondary to the effect on the microtubules due to the decreased absorption of glucose. This action occurs in the presence of reduced amounts of nicotinamide-adenine dinucleotide in reduced form (NADH), which is a coenzyme involved in many cellular oxidation-reduction reactions.

CONCLUSION:

From the results, it was concluded that both hot and cold hydroalcoholic extracts of *Gymnema sylvestre* have significant anthelmintic activity and hot macerated hydroalcoholic extract of *Gymnema sylvestre* shown most significant anthelmintic activity when compared to cold macerated hydroalcoholic extract. From results the *Gymnema sylvestre* as an anthelmintic activity have been confirm as it displayed activity against the worm used in present study. Further studies have to be done to isolate the active principles responsible for the activity.

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