

ANTI-HELMINTHIC POTENTIAL OF SOLVENT EXTRACTS OF INDIGENOUS
BOTANICALSRashmi Jain¹, Shubham^{2*}, Shabista Ansari⁵, Deepali Aswal⁵, Abhishek Mathur^{3,4}

¹Bench Biologist, National Institute of Biological, Noida, India; ²Maharaj Vinayak Global University, Jaipur, India; ³National Centre of Fungal Taxonomy, New Delhi, India; ⁴CBTPL, Ghaziabad, India, ⁵SGI, Dehradun, India.

Address of Corresponding Author: E-mail: shubhambiochem@gmail.com; abhishekmthr@gmail.com

ABSTRACT: Ethno botanical studies reveal that the indigenous knowledge of a community is a key player in the identification of medicinal plants and such plants have been often tested by generations of indigenous people. In the present investigation, the hydro-alcoholic extracts of leaves of *Azadirachta indica* (Neem) and *Swertia chirayta* (Chirayta) were assessed for anti-helminthic potential against helminths (earthworms were used as model) at 10, 50 and 100 mg/ml. No anti-helminthic potential was observed at 10 mg/ml of dose of the hydro-alcoholic extracts of *Swertia chirayta*. The hydro-alcoholic extracts of both the plants showed significant anti-helminthic activity on selected worms at higher doses. Hydro-alcoholic leaves extracts of *Azadirachta indica* (Neem) was found to be more active as compared to hydro-alcoholic whole plant extracts of *Swertia chirayta* (Chirayta) at concentration of 100 mg/ml. It was observed that with the variation in dose, the death time and paralysis time of the worms' decreases. The results indicated that extracts possessed dose dependent anti-helminthic activity. The results were compared to Piperazine citrate and Albendazole (15 mg/ml). The hydro-alcoholic extracts demonstrated paralysis as well as death of worms in a less time in comparison to the standard drugs. The anti-helminthic activity of the extracts indicates the presence of active principle responsible for anti-helminthic activity.

Key words: Anti-helminthic potential, hydro-alcoholic extracts, *Azadirachta indica*, *Swertia chirayta*

INTRODUCTION

Helminthiasis is one of the most important animal diseases worldwide that can cause heavy production losses in grazing animals. The disease is prevalent all over the world especially in developing countries (Ibrahim M.A. et al., 1984) and is always associated with poor management practices and inadequate and inappropriate control strategies. An integrated approach is required for the effective control of helminths which includes strategic and tactical use of anthelmintics which remains the corner stone to this end and careful management of grazing lands including control of stocking rates and appropriate rotation strategies. Role of vaccinations is also vital for the control of various parasitic diseases as in the case of lungworms. However, various problems have emerged with the use of anthelmintics and among them; resistance against various species of helminths is of utmost importance to different anthelmintic compounds and classes, as well as chemical residue and toxicity problems. In addition, recognition of the antigenic complexity of parasites has slowed vaccine development. For these various reasons, interest in the screening of medicinal plants for their anthelmintic activity remains of great scientific significance despite extensive use of synthetic chemicals in modern clinical practices all over the world. The plant kingdom is known to provide a rich source of botanical anthelmintics, antibacterials and insecticides. A number of medicinal plants have been used to treat parasitic infections in man and animals. However, their scientific evaluation as compared to commercial anthelmintics is limited. Plants with anthelmintic activity have been reviewed (Githiori J.B. et al., 2004).

In the beginning, most of the *in vitro* researches regarding anthelmintic activity of plants, their different extracts or oils have been based on their toxic effects on earthworm, *Pheritima posthuma*. Most of these substances which are toxic to earthworms produce a primary irritation or agitation that results in the withdrawal of the worm from the neighborhood of the poison.

By asset of this effect, anti-helminthics doubtless often drive out the parasite when the concentration does not get sufficiently higher to kill the worm. Some workers have also used hookworms, *Haemonchus contortus*, and tapeworms and/or *Ascaris lumbricoides* for the evaluation of *in vitro* anthelmintic tivity of different plant materials. A modified egg hatch assay (Chartier C. et al., 2001) is often used to evaluate the effect of plant products against eggs of *Haemonchus contortus* or other trichostrongylids. Some other researchers conducting *in vitro* studies have used an alteration of the larval development assay (LDA) or larval motility tests which are commonly used for testing of resistance of parasites to anthelmintics (Leathwick D.M. et al., 2001; Muhammad G. et al., 2004; Cox P.A., 2001; Dhar D.N. et al., 1982). In the present study, some traditional medicinal plants were screened for anti-helminthic potential in lieu for isolation of natural molecules which can be utilized to formulate the anti-helminthic drugs and show no side symptoms and resistance.

MATERIALS AND METHODS

The chemicals and reagents used were of Analytical grade and were procured from Ranchem and CDH. Standard anti-helminthic drugs (positive controls), viz. Albendazole and Piperazine citrate were procured as gift samples from Ranbaxy Health Care Pvt. Ltd., Gurgaon.

Collection of Plant material

The plants were selected on the basis of information of traditional healers. The plants, *Azadirachta indica* (Neem) and *Swertia chirayta* (Chirayta) belonging to families, Meliaceae and Gentianaceae respectively were selected for the study. The herbariums of plant material were prepared and were further identified by Dr. Ajai Swami, Chinmaya Degree College, Haridwar (U.K). Leaves of Neem and whole plant material of Chirayta were dried under shade and ground to form the fine powder.

Preparation of Solvent Extracts

Each of the powdered plant material was soaked in approximately 400 ml of hydro-alcoholic solvent (50 % v/v), on an electrical shaker for three hours at room temperature and then left to stand overnight. The mixtures were filtered into conical flasks using Whatmann filter paper No. 1. The filtrate was then concentrated on a rotary evaporator at 50°C to yield semi-solid masses whose weights were determined. The extracts were then stored in a refrigerator at 4°C (Akhtar M.S. et al., 2000).

Anti-helminthic Bioassay

Healthy adult Indian earthworms, *Pheretima postuma*, (Annelida, Megescolecidae) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Iqbal Z. et al., 2001a; 2001b) were used in the study.

Assessment of Anti-helminthic activity

Anti-helminthic activity was assessed using earthworms by the reported methods with slight modifications (Iqbal Z. et al., 2004). Samples for anti-helminthic activity were prepared by dissolving 2.5 gm dried crude extracts in 25 ml of 1 % N-saline (0.85 % Sodium chloride) solution. N-saline was used as the vehicle/control. To obtain a stock solution, different working solutions of the extracts were prepared to get a concentration range of 10, 50 and 100 mg/ml. The anti-helminthic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human being (Iqbal Z. et al., 2005). The anti-helminthic activity of the extracts was determined (Iqbal Z. et al., 2006a). Six earthworms were soaked separately within the petridishes in hydro-alcoholic plant extracts within 5 groups at 0 time. The time (in minutes) of paralysis and death of earthworms were recorded after placing in the extracts. Paralysis is meant by the motility of the earthworms after their placement in the extracts and controls. The death time shows the non-motility/death of the worms.

Groups subjected for the study:

I Group: Vehicle/Negative Control: 1 % N-saline (0.85 % Sodium chloride)

II Group: Positive Control: Piperazine citrate (15 mg/ml)

III Group: Positive Control: Albendazole (15 mg/ml)

IV Group: Test: Hydro-alcoholic extract of *Azadirachta indica* (Neem)- 10 mg/ml

V Group: Test: Hydro-alcoholic extract of *Azadirachta indica* (Neem)- 50 mg/ml

VI Group: Test: Hydro-alcoholic extract of *Azadirachta indica* (Neem)- 100 mg/ml

VII Group: Test: Hydro-alcoholic extract of *Swertia chirayta* (Chirayta)- 10 mg/ml

VIII Group: Test: Hydro-alcoholic extract of *Swertia chirayta* (Chirayta)- 50 mg/ml

IX Group: Test: Hydro-alcoholic extract of *Swertia chirayta* (Chirayta)- 100 mg/ml

RESULTS AND DISCUSSION

Anti-helminthic assay

Hydro-alcoholic extracts of leaves of *Azadirachta indica* (Neem) and *Swertia chirayta* (Chirayta) showed significant anti-helminthic activity on selected worms at higher doses much more effectively. Hydro-alcoholic leaves extracts of *Azadirachta indica* (Neem) was found to be more active as compared to hydro-alcoholic whole plant extracts of *Swertia chirayta* (Chirayta) at concentration of 100 mg/ml. The hydro-alcoholic extract of *Swertia chirayta* (Chirayta) did not showed anti-helminthic activity unlike to that of *Azadirachta indica* (Neem) at 10 mg/ml. The results indicated that extracts possessed dose dependent anti-helminthic activity. The hydro-alcoholic extracts demonstrated paralysis as well as death of worms in a less time as compared to Piperazine Citrate and Albendazole (15 mg/ml). The anti-helminthic activity of the extracts indicates the presence of active principle responsible for anti-helminthic activity. The results of anti-helminthic activity are recorded in Table 1 and Figure 1 (a & b).

Table 1: Anti-helminthic activity of plant extracts and standard drugs

Group	Treatment	Concentration (mg/ml)	Paralysis time (minutes)	Death time (minutes)
1	Vehicle: 1 % N-Saline	--	No activity	No activity
2	Positive Control: Piperazine citrate	15 mg/ml	23.3±0.6	27.3±0.5
3	Positive Control: Albendazole	15 mg/ml	25.6±0.6	29.5±0.5
4	Hydro-alcoholic extract of <i>Azadirachta indica</i> (Neem)	10 mg/ml	28.17±0.2	35.07±0.2
		50 mg/ml	17.15±0.2	23.43±0.2
		**100 mg/ml	10.12±0.2	20.15±0.2
5	Hydro-alcoholic extract of <i>Swertia chirayta</i> (Chirayta)	10 mg/ml	56.03±0.2	75.02±0.2
		50 mg/ml	36.05±0.2	45.02±0.2
		*100 mg/ml	25.14±0.2	55.12±0.2

Note: *Extracts (100 mg/ml) showing anti-helminthic activity in dose dependent manner;

** Potent extract; Level of significance-p<0.5



Figure 1(a): Earthworms motility (paralytic movement) at different doses of botanicals extracts and control

The results were found to be significant at p<0.5. Hydro-alcoholic extracts of leaves of *Azadirachta indica* and hydro-alcoholic extracts of whole plant of *Swertia chirayta* showed significant anti-helminthic activity on worms. The extracts were found to have potent anti-helminthic activity in dose dependent manner. Hydro-alcoholic extract of *Azadirachta indica* found to be more active as compared to *Swertia chirayta* at 100 mg/ml.

The extracts were found to have potent and significant activity in comparison to standard traditional anti-helminthic drugs, Albendazole and Piperazine citrate at 100 mg/ml. The results are in accordance with the previous studies reporting *Azadirachta indica* as anti-helminthic drug (Iqbal Z. et al., 2006b; 2006c). Although lesser studies are reported on the anti-helminthic potential of *Swertia chirayta* but some of the previous studies done on this aspect reported its anti-helminthic potential (Gaur R.D., 1999). Further studies are however needed to isolate the active principle from extracts of *Swertia chirayta* responsible for anti-helminthic activity.



Figure 1(b): Earthworms non-motility in extracts and control (non paralytic movement/ death)

REFERENCES

- Akhtar M.S., Iqbal Z., Khan M.N. and Lateef M. (2000). Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo-Pakistan subcontinent. *Small Rumin. Res.* Vol. 38, 99–107.
- Chartier C., Soubirac F., Pors I., Silvestre A. (2001). Prevalence of anthelmintic resistance in gastrointestinal nematodes of dairy goats under extensive management conditions in southwestern France. *J. Helminthol.* Vol.75, 325–330.
- Cox P.A. (2000). Will tribal knowledge survive the millennium. *Science.* Vol. 287, 44–45.
- Dhar D.N., Sharma R.L. and Bansal G.C. (1982). Gastrointestinal nematodes in sheep in Kashmir. *Vet. Parasitol.* Vol. 11, 271–277.
- Gaur, R.D. (1999). *Flora of the District Garhwal, North West Himalaya (with Ethnobotanical Notes)*, Transmedia, Srinagar, Garhwal.
- Githiori J.B., Hogland J., Waller P.J. and Baker R.L. (2004). Evaluation of anthelmintic properties of some plants used as livestock dewormers against *Haemonchus contortus* infection in sheep. *Parasitology.* Vol. 129, 245–53.
- Ibrahim M.A., Nwude N., Ogunsusi R.A. and Aliu Y.O. (1984). Screening of the West African plants for antihelminthic activities. *ILCA Bulletin.* Vol. 17, 19–23.
- Iqbal Z., Munir M.A., Khan M.N., Akhtar M.S. and Hassan. (2001a). In vitro inhibitory effects of *Sorghum bicolor* on hatching and moulting of *Haemonchus contortus* eggs. *Int. J. Agri. Biol.* Vol. 3, 451–453.
- Iqbal Z., Nadeem Q.K., Khan M.N., Akhtar M.S. and Waraich F.N. (2001b). In vitro anthelmintic activity of *Allium sativum*, *Zingiber officinale*, *Curcubita mexicana* and *Ficus religiosa*. *Int. J. Agri. Biol.* Vol. 3: 454–457.
- Iqbal Z., Lateef M., Ashraf M., and Jabbar A. (2004). Anthelmintic activity of *Artemisia brevifolia* in sheep. *J. Ethnopharmacol.*, 93: 265–268.
- Iqbal Z., Lateef M., Jabbar A., Muhammad G., and Khan M.N., (2005). Anthelmintic activity of *Calotropis procera* (Ait.) Ait. F. flowers in sheep. *J. Ethnopharmacol.* Vol. 102, 256–261.
- Iqbal Z., Lateef M., Akhtar M.S, Ghayur M.N. and Gilani A.H. (2006a). In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. *J. Ethnopharmacol.* Vol. 106, 285–287.
- Iqbal Z., Lateef M., Jabbar A., Ghayur M.N. and Gilani A.H. (2006b). In vitro and in vivo anthelmintic activity of *Nicotiana tabacum* L. leaves against gastrointestinal nematodes of sheep. *Phytoth. Res.* Vol. 20, 46–48.
- Iqbal Z., Lateef M., Jabbar A., Ghayur M.N. and Gilani A.H. (2006c). In vivo anthelmintic activity of *Butea monosperma* against *Trichostrongylid* nematodes in sheep. *Fitoterapia.* 77, 137–140.
- Leathwick D.M., Pomroy W.E. and Heath A.C.G. (2001). Anthelmintic resistance in New Zealand. *New Zealand Vet. J.* Vol. 49, 227–235.
- Muhammad G., Abdul J., Khan M.Z. and Saqib M. (2004). Use of neostigmine in massive ivermectin toxicity in cats. *Vet. Hum. Toxicol.* Vol. 46, 28–29.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY



Email : ijabpt@gmail.com

Website: www.ijabpt.com