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Anthelmintic potential of *Helicteres isora* bark extract against *Pheretima posthuma*

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable research work in which authors have demonstrated the anthelmintic potential of *H. isora* bark extract against *P. posthuma*. The activity was assessed by comparing paralysis and death time with standard. The methanolic extract of *H. isora* bark showed potent anthelmintic activity against *P. posthuma*.
Details on Page 315

ABSTRACT

Objective: To evaluate anthelmintic potential of *Helicteres isora* (*H. isora*) bark extract in Indian adult earthworms.
Methods: The *H. isora* bark and Indian adult earthworms (*Pheretima posthuma*) were collected and authenticated by approved taxonomist. Earthworms were grouped and treated with extract at concentration of 10, 20 and 50 mg/mL, albendazole of 10 mg/mL as standard and normal saline as a control. The paralysis time and death time was considered as indicator of anthelmintic activity.
Results: All the extracts showed concentration dependent activity but significant activity was observed at 50 mg/mL. The extract showed better activity at concentration of 50 mg/mL with paralysis time (12.54 min) and death time (16.55 min) when compared to standard albendazole.
Conclusions: The study revealed that the methanolic extract of *H. isora* bark have potent anthelmintic activity against Indian adult earthworms.

KEYWORDS

Anthelmintic, Earthworm, *Helicteres isora*, Paralysis

1. Introduction

Helminth infections are amongst the most common infections in humans, affecting huge amount of people worldwide. Although most of infections due to worms are usually in tropical regions and create a great threat to health and contribute to the prevalence of anaemia, eosinophilia malnutrition, and pneumonia[1]. Helminthiasis is mainly due to human body part contaminated by worms such as pinworm, tapeworm or roundworm. Normally, the worms live in the gastrointestinal tract but may also reside into the liver and other organs while infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with poor sanitation[2]. The gastrointestinal helminths have a potential resistance to currently available anthelmintic drugs. Therefore there is a leading problem in treatment of helminths infections[3].

Helicteres isora (*H. isora*) is a shrub or small tree belonging to family Sterculiaceae. It spreads rapidly with stem measuring 1-5 inches in diameter, reaching a height of 5-15 feet. This species is native to Asia and Australia[4]. The roots and bark are expectorant, demulcent, hypoglycemic and useful in colic, constipation, diabetes, gastropathy, snake bite, scabies, diarrhea and dysentery. The fruits are astringent, stomachic, refrigerant, and useful in griping of bowels, flatulence of children and antispasmodic effect[5]. *H. isora* bark contains chloroplast pigments, phytosterols, hydroxyl carboxylic acid, orange-yellow colouring matter, saponins, phlobotannis, sugar and lignins[6]. In the indigenous system of medicine, *H. isora* has been used for antipyretic, anti-inflammatory, antioxidant, antidiabetic, anticancer, antimicrobial, antinoceptive, hepatoprotective and cardiotonic activity[5,6]. It is manifest that the plant has great potentials in treating various diseases. *H. isora* bark has yet not been explored for anthelmintic activity. Therefore, the present study was planned to evaluate the anthelmintic activity of *H. isora* bark extract.

2. Materials and methods

2.1. Plant material

The bark of *H. isora* was collected from Sindagi village (Kalamnuri) in the District Hingoli, of Maharashtra State of India.

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Plant materials were authenticated by Dr. V. R. Marathe, Department of Botany, N. E. S. Science College, Nanded, Maharashtra, India.

2.2. Drugs and chemicals

Albendazole (Micro Lab. Pvt. Ltd., Goa), and all organic solvents and chemicals were purchased from Himedia Pvt. Ltd., Mumbai and were of analytical grade.

2.3. Preparation of crude extract

Bark of *H. isora* was rinsed well with tap water and distilled water and kept under shade for drying. Dried material coarsely powdered using mortar and pestle followed by oven dry and further reduced to powder using an electric blender and stored in air tight glass container. The powder was subjected to solvent extraction so that 20 g of powder was extracted in 100 mL of methanol solvent for 5 h. Dried methanolic extract was used for evaluation of anthelmintic activity.

2.4. Phytochemical screening

The phytochemical study of the extract was carried out using standard procedures[7,8]. Bark of *H. isora* plant was primarily intended for the phytochemical analysis and detection of major chemical constituents were carried out.

2.5. Earthworms collection and authentication

The earthworms *Pheretima posthuma* (*P. posthuma*) were used to study the anthelmintic activity. Earthworms were collected from S.R.T.M. University Campus, Nanded. Worms were authenticated by Dr. S. P. Chavan, Associate Professor, School of Life Sciences, S.R.T.M. University, Nanded, Maharashtra, India. The worms were washed with normal saline to remove all the fecal matter. The earthworms *P. posthuma* having 4-5 cm in length and 0.1-0.2 cm in width weighing 0.8-3.04 g were used for all experiment procedure. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence where used to study the anthelmintic activity[9].

2.6. Preparation of test drug and reference drug

Extracts for *in vitro* study were prepared at the concentrations of 10, 20 and 50 mg/mL. Samples of methanolic extract were prepared by dissolving 100, 200, and 500 mg crude extract of each in 1 mL dimethylsulfoxide and made the volume up to 10 mL with normal saline solution and final concentration of samples achieved were 10, 20, and 50 mg/mL, respectively. Normal saline solution was used as control and albendazole was used as the standard drug for this study[10].

2.7. Anthelmintic activity

The anthelmintic assay was carried out as per the method of Hussain et al. at concentrations of 10, 20, and 50 mg/mL against the Indian earthworms (*P. posthuma*)[11]. Five groups of Indian earthworms, each containing six earthworms approximately of equal size were used for the study. Three groups of earthworms were tested with extract of different concentrations (10, 20, and 50 mg/mL) and one group was treated with 10 mg/mL with reference standard as albendazole and one group was used as control which is treated with normal saline[11]. The anthelmintic activity on earthworm was observed and time required for paralysis and death recorded.

2.8. Statistical analysis

All results are expressed as mean±SEM. Data was compared with ANOVA followed by Dunnette's multiple comparison test. The statistical analysis was conducted with Graphpad Instat Software (Version 3, USA). Values would be considered statistically significant, when $P<0.01$.

3. Results

Preliminary phytochemical screening of extract revealed the presence of carbohydrates, proteins, polyphenols, tannins, flavonoids, alkaloids, saponins and stroids. The extract produced dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which ultimately hasten death of earthworms. Anthelmintic activity with various concentrations of methanolic extract of *H. isora* and reference standard albendazole is shown in Table 1. The methanolic extract at 10, 20, 50 mg/mL concentrations showed paralysis time of 17.52, 15.68, 12.54 min and death time of 26.70, 21.51, 16.55 min, respectively. At highest concentration it produces paralysis and death in short time which is comparable with albendazole. The albendazole treated group at 10 mg/mL showed the paralysis time of 14.71 min and death time of 17.53 min. In control group (normal saline) worms were observed for 24 h and no paralysis or death was found.

Table 1

Anthelmintic activity of *H. isora* bark extract.

Treatment	Concentration (mg/mL)	Time taken in minutes (mean±SEM)	
		Paralysis	Death
Control	Saline	-----	-----
Albendazole	10	14.71±0.213	17.53±0.162
Methanolic extract	10	17.52±0.209**	26.70±0.193**
Methanolic extract	20	15.68±0.225**	21.51±0.218**
Methanolic extract	50	12.52±0.177**	16.55±0.234**

**: $P<0.01$. Values are in mean±SEM, $n=6$, when compared with albendazole. Values of $P<0.01$ were considered as statistically significant.

4. Discussion

The anthelmintic activity of *H. isora* revealed against Indian adult earthworms and it was found to be dose-dependent and significant ($P<0.01$) *in vitro* action. The predominant effect of albendazole on the worms was to cause a flaccid paralysis that result in exclusion of the worm by peristalsis. Albendazole binds to free β-tubulin, inhibiting its polymerisation and hence interfering with microtubule-dependent glucose uptake by the worm. It has a selective inhibitory action on helminth microtubular function, being 250-400 times more potent in helminth than in mammalian tissue[12].

The plant extract consists of several secondary metabolites which are responsible for anthelmintic activity. Preliminary phytochemical screening of *H. isora* revealed the presence of carbohydrates, proteins, polyphenols, tannins, flavonoids, alkaloids, saponins and stroids. Tannins, a polyphenolic compound, have been reported to produce anthelmintic activity[13]. Some synthetic phenolic

anthelmintics (*eg*. niclosamide, oxyclozanide and bithionol) are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation[14]. It is possible that tannins contained in the extract of *H. isora* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastro intestinal tract of host animal and cause death[15]. Alkaloids were reported to cause paralysis by acting on the central nervous system[16]. Therefore, it may be plausible that the relative anthelmintic activity of *H. isora* could be attributed to presence of phytochemicals like tannins and alkaloids. The investigational confirmation obtained in this bioassay could provide a rationale for the traditional use of *H. isora* plant as anthelmintic potential. However, further studies are needed to isolate, characterize and evaluate the actual bioactive components and their mechanism of actions.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The helminth infections are the most common infections worldwide caused by worms. The gastrointestinal helminths have a potential opposing to currently available anthelmintic drugs and also the synthetic anthelmintic drugs cause many side effects. Therefore attempt has been made to evaluate anthelmintic activity of plant drugs as they do not possess any side effects.

Research frontiers

The present research work depicts anthelmintic potential of *H. isora* bark extract against *P. posthuma* and assessed by comparing paralysis and death time with standard.

Related reports

Infection of intestinal parasitic worms such as pinworm, tapeworm or roundworm leads to helminthiasis associated complications. The traditional system of medicine has evidence of effectiveness of herbal drugs in treating various parasitic infections.

Innovations & breakthroughs

The roots and bark of *H. isora* are known to possess expectorant, demulcent, hypoglycemic effect and are also useful in colic, constipation, diabetes, gastropathy, snake bite, scabies, diarrhea and dysentery. In the present study, authors have demonstrated the anthelmentic activity of *H. isora* bark extract against *P. posthuma*.

Applications

The present study shows promising results for anthelmintic efficacy of methanolic extract of *H. isora* bark. This research can promote studies to isolate the compounds which are responsible for showing the anthelmintic effect.

Peer review

This is a valuable research work in which authors have demonstrated the anthelmintic potential of *H. isora* bark extract against *P. posthuma*. The activity was assessed by comparing paralysis and death time with standard. The methanolic extract of *H. isora* bark showed potent anthelmintic activity against *P. posthuma*.

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