# ANTHELMINTIC ACTIVITY OF ROOT EXTRACT OF *RAPHANUS SATIVUS*

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## Summary

The hydro-ethanol extract of roots of *Raphanus sativus* (HERS) was investigated for anthelmintic activity using earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*). Various concentrations (10-50 mg/ml) of root extract were tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug whereas DMF (Di-methyl formamide) as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant anthelmintic activity at highest concentration of 50 mg/ml. The result showed that root extract possesses vermicidal activity and found to be effective as an anthelmintic.

**Key words:** Anthelmintic activity, *Pheretima Posthuma. Ascardia Galli*, *Raillietina spiralis*, *Raphanus sativus*

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## Introduction

Helminthiasis, or worm infestation, is one of the most prevalent disease and one of the most serious public health problems in the world. Hundreds of millions if not billions of human infections by helminthes exist worldwide and with increased world travel and immigration from the developing countries (1). Modern medicines are gaining less attention due to their limited availability and affordability in human intestinal helminthesis. Thus, most of the world's population depends to a greater extent on traditional medical remedies.

*Raphanus sativus* is an annual herb, consumed as vegetable. It belongs to the family Brassicaceae. In India, root of the plant is used for cure of many diseases like gall bladder trouble, diabetes, hepatitis and gastrointestinal disorders. Fresh radish has been reported to increase the protein digestibility. Roots, flowers and pods of the plant are active against gram-positive bacteria like *Staphylococcus aureus* and *Bacillus subtillis* (2). It decreases blood glucose levels in diabetic rats (3). It improves the histopathology of colon mucosa in the rats fed high fat diet (4). Gastrointestinal and uterine tone modulatory activities of *R. sativus* are reported (5). However, literature survey revealed that the root extract has yet not been screened for its traditional claim of anthelmintic activity and hence an attempt has been made in the present study to investigate an anthelmintic activity of hydro ethanol extract of roots of *Raphanus sativus*.

## Materials and Methods

### Plant materials and preparation of the extract

Fresh roots of *R. sativus* were obtained from local market and its authenticity was confirmed by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. Hydro ethanol extract was prepared after chopping the roots into thin slices and drying. Dried roots were crushed to powder and soaked with 70% ethanol for 4 days. After that, extract was filtered and dried in rotary flash evaporator. The yield was 8% of the dried weight. The extract was formulated as 10, 20 and 50 mg/mL solutions in DMF (dimethyl formamide) and investigated for anthelmintic activity.

### Drugs and chemicals

Piperazine citrate (Glaxo Smithkline) was used during the experimental protocol.

### Phytochemical screening

Freshly prepared hydro-ethanol extract of the roots of *R. sativus* (HERS) was subjected to preliminary phytochemical screening for detection of major chemical constituents (6).

### Investigation of anthelmintic activity

The extract of the roots of *R. sativus* (HERS) was investigated for its anthelmintic activity against earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and

roundworms (*Ascaridia galli*). Various concentrations (10, 20 and 50 mg/mL) of the extract was tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. The anthelmintic activity was carried as per the method of Ajaiyeoba et al (7) with minor modifications. The anthelmintic activity was evaluated on the adult Indian earth warm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* (8-10). Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The earthworms of 5-7 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. Use of *Raillietina spiralis* and *Ascaridia galli* as a suitable model for screening of anthelmintic drug was advocated earlier (11, 12).

**Table 1. Anthelmintic activity of hydro-ethanol extract of the roots of *Raphanus sativus* (HERS)**

| Group | Treatm-ent | Conce-ntration (mg/ml) | *Pheretima posthuma* (Earthworm) | | *Raillietina spiralis* (Tapeworm) | | *Ascardia Galli* (Rounrworm) | |
|---|---|---|---|---|---|---|---|---|
| | | | P | D | P | D | P | D |
| I | DMF | - | - | - | - | - | - | - |
| II | HERS | 10 | 28±0.59 | 38±0.64 | 31±0.13 | 61±1.73 | 59±1.56 | 71±1.94 |
| III | HERS | 20 | 19±0.98 | 29±0.95 | 21±0.62 | 38±0.90 | 40± 1.04 | 50±1.34 |
| IV | HERS | 50 | 09±0.18 | 11±0.51 | 12±0.21 | 22±0.73 | 23±0.53 | 28±1.12 |
| V | PZC | 10 | 07±0.05 | 08±0.93 | 07±0.24 | 13±0.78 | 10±0.61 | 18±0.85 |

Each value represents mean ± SEM (n=6). DMF: Di-methyl-formamide, PZC: Piperazine citrate, P: Time taken for paralysis of worms (min), D: Time taken for death of worms (min).

Test samples of the extract was prepared at the concentrations, 10, 20 and 50 mg/ml in DMF and six worms i.e. *Pheretima posthuma, Raillietina spiralis* and *Ascaridia galli* of approximately equal size (same type) were placed in each 9 cm petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg/ml) was used as reference standard and DMF as control. This procedure was adopted for all three different types of worms (13-16). 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 shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 $^{0}$C). All the results were shown in Table.1 and expressed as a mean ± SEM of six worms in each group.

## Results and Discussion

From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. The root extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration, for all three types of worms. However, extract exhibited

prominent activity at lower concentration (10 mg/ml) against all three types of worms. Evaluation of anthelmintic activity was compared with reference standard piperazine citrate, which was more potent than the seed extract activity (Table.1).

Preliminary phytochemical screening of extract revealed the presence of flavonoids, tannins and other phenolic compounds. Phenolic and tannin compounds show anthelmintic activity by binding to glycoprotein on the cuticle of the parasite and thus lead to death of the worm (17, 18). It is possible that phenolic contents in the extract of roots of *R. sativus* produced similar effects.

From the above results, it is concluded that *Raphanus sativus* roots used by tribals traditionally to treat intestinal worm infections, showed promising anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

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