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In-vitro anthelmintic activity of various plant extracts against *Pheretima posthuma*

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ABSTRACT: The aim of the present study was to evaluate the anthelmintic activity of various plant extracts using *Pheretima posthuma* as test worms. Various concentrations of all extracts were tested. The time of paralysis and time of death were studied and the activity was compared with Albendazole as reference standard and normal saline as a control group. Dose dependent activity was observed in all theses extracts but the aqueous extract of *Garcinia indica* (Kokam) and *Trachyspermum ammi* (*ajwain) exhibited significant anthelmintic activity as evidenced by decreased paralyzing time and death time. The results support the use of these plants as an anthelmintic agent.*

I. INTRODUCTION

Helminth infections are among the most widespread infections in humans, affecting a large proportion of the world's population. Although the majority of infections due to helminths are generally restricted to tropical regions, in developing countries they pose a major threat to public health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia (Bundy D. A., 1994). Anthelmintics or antihelminthics are drugs that expel helminth parasitic worms (helminths) from the body, either by stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing). Anthelmintics are drugs that either kill or expel infesting helminths and the gastrointestinal tract is the abode of many helminths, although some also live in tissues, or their larvae migrate into tissues. However they have shown the development of resistance. The gastro-intestinal helminthes becomes resistant to currently available some broad spectrum anthelmintics (benzimidazoles, levamisole, vermectins) and also some narrow spectrum wormers such as the salicylanilides (closantel). Therefore there is a foremost problem in treatment of helminthes diseases (Sondhi *et al.,* 1994). Anthelmintic resistance is a major problem for the control of many parasitic nematode species and has become a major constraint to livestock production in many parts of the world. Because of increasing anthelmintic resistant and impact of conventional anthelmintic on the environment, it is important to look for alternative strategies against gastrointestinal nematodes. Hence there is an increasing demand towards natural anthelmintics. The literature survey reveals that use of herbs could be one of the major options to control these pathologies. (Bhattacharjee, 2004). Due to the prevalence of parasitic infections and the developed resistance of some anthelmintic drugs is now an enclosing area in the field of research.

Therefore an attempt has been made to evaluate anthelmintic activity of various locally available plants on adult earthworm *Pheretima posthuma.*

II. METHODOLOGY

A. Plant Material

Leaves of *Moringa oleifera, Garcinia indica, Carica papaya, Psidium guajava* and *Calotropis procera* were collected from local area of Ratnagiri in the month of September 2015. The plant and plant material were identified and authenticated in Department of Botany of Gogate Jogalekar College, Ratnagiri. The collected leaves were air-dried under the shade in laboratory for 3-4 days. After complete drying, leaves were pulverized, passed through sieve no. 40 and stored in air tight container and used for further extraction.

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B. Preparation of extract

Aqueous extract (Maceration method)

The aqueous extracts were prepared by dissolving 100g of powdered plant material in 500 ml of distilled water in a glass percolator. It was allowed to macerate for 24 h at room temperature and the brew was filtered using Whatman number one filter paper. The combined filtrate was then concentrated in a water bath to ensure the complete evaporation of the solvent. The extract was dried and used as a powder. The percentage yield of extract was found to be 3.56 percent.

C. Worm Collection:

The Indian Adult earthworm *Pheretima posthuma* (Annelida) was collected from Behere Wormicompost Farm, Pawas, Dist. Ratnagiri. Earthworm were washed with normal saline solution to remove all the faecal matter and kept in normal saline solution. The average size of earthworm was 4-6 cm. The Earthworms were identified in Dept. of Zoology, Gogate Jogalekar College Ratnagiri and services of veterinary practioners were utilized to confirm the identity of worms.

D. Preparation of test sample:

Samples for in-vitro study were prepared by dissolving the extracts in normal saline to obtain different working solutions such as 20, 40, and 60mg/ml.

E. Anthelmintic Assay:

The anthelmintic assay was carried as per the method of Ajaiyeoba E. O. *et al.* (2001) with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Vidyarthi, 1967; Chatterjee, 1967). Due to their ready availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro (Sollman, 1918; Jain *et al.*, 1972; Dash *et al.*, 2002). The earthworms of 4-6 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. Various concentrations (20-60mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference and normal saline as control. The concentration of the standard drugs were prepared in 2%v/v Tween80 in normal saline to give 20mg/ml. Test samples of the extract was prepared at the concentrations, 10, 20 and 50 mg/ml in distilled water and six worms of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. The worms were divided into the respective groups containing six-earth worms in each group. All the prototypes and the standard drug solution were freshly prepared before commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation. They were observed for their spontaneous motility and evoked responses. Observations were made for the time taken to paralyse and death of individual worms. Time for paralysis was noted when no movements of any sort could be observed except when the worm were shaken vigorously. Death was conducted when the worms lost their motility followed with fading away of their body

III. RESULTS AND DISCUSSION

The crude extracts samples, which were used to evaluate anthelmintic activity, showed variable times at different concentrations and the mean time values were calculated for each parameter. However, when observed the response of worms in case of paralysis, there was significant variation among the results produced by the different extracts at different concentrations like 20, 40 and 60mg/ml. All the results were shown in Table.1 and expressed as a mean ± SEM of six worms in each group.

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In the present study, it was observed that the higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. Aqueous extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 60 mg/ ml concentration. Evaluation of anthelmintic activity was compared with reference standard Albendazole (Table.1).

The different extracts exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) in *Garcinia indica* (Ratamba) with 60mg/ml concentration. The aqueous extract of it caused paralysis of 1 min and time of death of 5 min. *Tachyspermum ammi* (Ajawan) with 40mg/ml concentration revealed paralysis of 6 min and death of 11 min against the earthworm *Pheretima posthuma*. The reference drug Albendazole showed paralysis of 4 min and death of 51 min. The predominant effect of Albendazole on worm is to cause a flaccid paralysis which results in expulsion of the worm by peristalsis.

Table 1 : Anthelmintic activity of various plant extracts

Groups	Concentration (mg/ml)	Time taken for paralysis (P) in min. (Mean & SD)	Time taken for death (D) in min. (Mean & SD)
Moringa oleifera(Shevaga)	20	83±1.7	115±0.3
	40	20±1.20	65±0.54
	60	22±0.56	70±0.38
Garcinia indica (Ratamba)	20	5±0.67	13±0.23
	40	5±0.5	7±0.17
	60	1±0.25	5±0.4
Tachyspermum ammi (Ajawan)	20	7±0.54	15±0.87
	40	6±0.28	11±0.56
	60	9±0.34	9±0.08
Carica papaya (Papita)	20	100±0.47	180±0.34
	40	79±0.86	85±0.74
	60	11±0.12	26±0.63
Psidium guajava (Guava)	20	10±0.73	57±0.4
	40	6±0.2	40±0.24
	60	5±0.96	36±0.72
Calotropis procera (Rui)	20	23±0.54	42±0.81
	40	6±0.3	20±0.37
	60	5±0.5	36±0.26
Albendazole	10	4±0.45	51±0.59

III. CONCLUSION

From the above results it is concluded that aqueous extract of *Garcinia indica* (Ratamba) and *Tachyspermum ammi* (Ajawan) have a potent anthelmintic activity when compared with conventionally used drug. The wormicidal activity of these extracts against earthworm suggests that it is Effective against parasitic infections of humans. 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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