

Short Communication

EVALUATION OF *IN-VITRO* ANTIHELMINTIC PROPERTIES OF THE EXTRACT OF *RUSSELLIA EQUISETIFORMIS* (SCHLECHT. AND CHAM.) SCROPHULARIACEAE

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ABSTRACT

Objective: The present study was aimed to evaluate the *in vitro* anthelmintic activity of *Russelia equisetiformis* leaves in comparison with anthelmintic drug Piperazine citrate.

Methods: *In vitro* Anthelmintic activity of different solvent extracts (petroleum ether, chloroform, ethyl acetate and methanol) of *Russelia equisetiformis* were carried out on mature *Pheretima posthuma*.

Results: The results of the study revealed that all the solvent extracts produced a significant anthelmintic activity in a dose-dependent manner. Methanol extract offered a good anthelmintic activity at 15 mg/ml concentration, where the time of paralysis was 15 and death was observed at 27 min, followed by ethyl acetate extract. The negative control DMF did not show any activity and positive control standard piperazine citrate recorded death at 13 min and paralysis at 10 min.

Conclusion: Despite ample evidence of anthelmintic properties of *R. equisetiformis* through validated experimental data, further studies on *Russelia equisetiformis* is required to recognize the active principle responsible for the anthelmintic activity.

Keywords: *Russelia equisetiformis*, *Pheretima posthuma*, Anthelmintic, Solvent extracts.

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Helminthiasis is a serious parasitic infection that causes serious problems in human beings in tropical regions including the Asian countries. Helminthes produce a serious problem in human and other animals around the world specifically to the third world countries [1]. According to World Health Organization (WHO), two billion people are suffering from parasitic worm infections [2] of which, 450 million have significant morbidity to their infection, the majority of them being children. Helminthiasis are also one of the most important animal diseases worldwide, inflicting heavy production losses in grazing animals especially in developing countries leading to mortality, chronic infections that cause reduced productivity, fertility growth, milk and meat production in animals [3]. A variety of several clinical symptoms arises due to this infection of helminths including dysentery, diarrhoea, nausea-vomiting, loss of appetite and weight, acidity and sometimes anemia, respiratory symptoms, dermatological consequences, etc. [1, 4].

Anthelmintic drugs are used to control helminth infections, but they are associated with many side effects [5]. Frequent use of anthelmintic drugs leads to the development of resistance in helminths due to several factors such as initial resistance, allele frequency, treatment frequency and refugia. Herbal medicine is still the main source of medicine and about 75-80% of the population, particularly in developing countries relies on herbal medicine. Plant anthelmintics have been in the forefront due to the awareness in medicinal and scientific communities about the importance of them and studying herbal medicine can serve to validate and enhance existing local anthelmintic. There are numerous reports on medicinal plants with anthelmintic properties that are safe for use and ingest [6-9] but there are no reports on the anthelmintic activity of *Russelia equisetiformis*. The extracts of the plant used in the present study are known to possess greater number of secondary metabolites with many biological activities like antimicrobial, antioxidant, cytotoxicity [10], including treatment of malaria, cancer and inflammatory diseases [11]. Hence, the present study was aimed to investigate the anthelmintic potential of crude leaf extracts of *R. equisetiformis* at different concentrations on Indian earthworm (*Pheretima posthuma*) taking Piperazine citrate as a standard drug as the plant contains numerous ethnopharmacological importance.

Fresh leaves of *Russelia equisetiformis* (Schlecht. & Cham.) were collected in and around Mysore (12.30° N, 76.65° E), Karnataka, India and authenticated at Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore. The fresh leaves of *Russelia equisetiformis* were dried under shade. The dried leaves were cut into small pieces and powdered in a hand mill. Solvent extraction was carried out using Soxhlet extraction apparatus. Powdered leaf material (100 g) was placed in a porous thimble in the upper chamber. In the lower boiling flask, the extracting solvent (200 ml) was added. The flask was heated by the thermostat controlled heating mantle. The round bottom flask was filled successively with different solvents in the following order petroleum ether, chloroform, ethyl acetate and methanol based on polarity from low to high. The temperature was adjusted based on the boiling point of the solvents. The solvent was heated to reflux and extracted. The sample filled in the thimble was extracted with the non-polar to polar solvents successively till colorless extract was obtained. The solvent extract was concentrated separately under reduced pressure. After complete evaporation of the solvent, each of these solvent extracts was weighed and preserved in brown air tight bottle at 5 °C until further use [12].

The anthelmintic assay was carried out as per the method of [13]. The assay was performed *in vitro* using adult earthworm (*P. posthuma*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation of the anthelmintic activity. Test samples of the extract was prepared at the concentrations of 5, 10 and 20 mg/ml in DMF and six worms, i.e., *P. posthuma*, of approximately equal size (3-5 cm in length and 0.1-0.2 cm in width) were used for all the experimental protocol) were placed in each Petri dish containing 25 ml of above test solution. Piperazine citrate (5 mg/ml) was used as reference and DMF as control [14]. All the test solutions and standard drug Piperazine citrate (5 mg/ml) solution were prepared freshly before starting the experiments. Observations were made for the time taken to set paralysis and death of the individual worms. Meantime for the paralysis (in min) was noted when there was no movement, except when the worm was shaken vigorously, time of death (in min) was recorded after ascertaining

the worms neither moved when shaken vigorously nor when dipped in warm water (50 °C).

The extracts of *Russelia equisetiformis* produced a significant anthelmintic activity in a dose-dependent manner as shown in table 1. Methanol extract offered a good anthelmintic activity at 15 mg/ml concentration, where the time of paralysis was 15 and death was observed at 27 min, followed by ethyl acetate extract. The chloroform extract offered maximum anthelmintic activity at 20

mg/ml concentration, and anthelmintic activity was not observed in petroleum ether extract.

The negative control DMF did not show any activity and positive control standard piperazine citrate recorded death at 13 min and paralysis at 10 min. All the extracts except petroleum ether were considered to be potent since 5 mg/ml of the crude extract also showed activity to a longer duration as compared to the standard.

Table 1: Anthelmintic activity of crude extracts of *Russelia equisetiformis*

Treatment	Concentration in mg/ml	Time taken for paralysis (min)	Time taken for death (min)
Petroleum ether extract	5	-	-
	10	-	-
	15	-	-
	20	-	-
Chloroform extract	5	40±0.85	51±0.46
	10	32±0.91	46±0.24
	15	29±0.16	39±0.28
	20	28±0.54	39±0.62
Ethyl acetate extract	5	32±0.24	40±0.26
	10	26±0.50	34±0.72
	15	29±0.48	37±0.18
	20	20±0.62	29±0.54
Methanol extract	5	30±0.32	39±0.40
	10	26±0.58	34±0.18
	15	18±0.20	27±0.26
	20	19±0.36	27±0.52
Control (DMF)	-	No effect till ten hours	No effect till ten hours
Piperazine citrate	5	10±0.16	13±0.12

Values are mean of three independent replicates±Standard Deviation

Gastrointestinal nematodes are chronic, pervasive infections that contribute to widespread morbidity and mortality worldwide. It is estimated to infect over one-quarter of the world's population. The four most common human gastrointestinal nematodes are *Ascaris lumbricoides*, *Trichuris trichiura*, *Nectar americana* and *Ancylostoma duodenale* that are responsible for mortality of 18,000 people and over four million years of life lost per annum due to premature death or disability [15]. In livestock also, the above-mentioned parasites is an important cause of morbidity and mortality, due to which producers use million dollars worth anthelmintic drugs each year which has led to drug resistance in the parasites [16]. Scientific validation of anthelmintic activity has mainly been through *in vitro* studies, and various parasite models are based on the use of free-living nematodes such as *Caenorhabditis elegans* and *Rhabditis pseudocongata* [17]. Other models used are ascarids the rodent nematode *Heligmosomoides polygyrus*, the trematode *Schistosoma mansoni* and cestode *Hymenolepis diminuta* [18] and Indian earthworm *Pheretima posthuma* [19] and *Eisema fetida* [20]. These worm samples are used in most of the anthelmintic screenings as they show anatomical and physiological resemblance with the intestinal round worm parasite of human beings. Regarding overall body plan all the species in the phylum nematode exhibit similarity despite their species in the phylum. Thus, consideration of *P. posthuma* and *C. elegans* for *in vitro* anthelmintic studies seems to be appropriate.

Indigenous system of medicine reports some plants for their anthelmintic efficacy. However, rigorous scientific evaluations comparing their efficacy to commercial anthelmintic are limited. In the present research, different solvent extracts of *R. equisetiformis* were subjected for *in vitro* assay of anthelmintic activity. The results of the present study offered a dose-dependent anthelmintic efficacy. All the extracts except petroleum ether of *R. equisetiformis* were significant in inhibiting the parasite. Likewise, there are a number of reports on the anthelmintic potential of different plant extracts [3, 21-22]. The time taken for paralysis and death in all extracts of *R. equisetiformis* was slightly more than the standard. Ethyl acetate and methanol extracts were more potent than the other extracts where the time of paralysis and death was nearly equal to the standard tested. Anthelmintic activity of *P. betle* and a combination of

different *Piper* species (*P. nigrum*, *P. longum* and *Zingiber*) have shown potent activity where the time of paralysis and death was nearly equal to the standards tested. The results are significant since the crude extracts are a mixture of various ingredients and inert materials while the standard is in pure form and hence the activity of crude extract at 15 mg ml⁻¹ can be considered significant. Ethyl acetate and chloroform extracts were moderate. In several studies based on traditional use, the crude extracts of herbal plants have been tested for their putative anthelmintic properties while in others the active ingredients responsible for the activity have identified and characterized to establish their mode of action. In the context of India, which is rich in diversity, plant-based medicines are an alternate system of medicine among native societies [23]. In this regard, the present investigation reports anthelmintic activity of *Russelia equisetiformis* available locally of which the anthelmintic activity of *E. cotinifolia* is being reported for the first time.

Despite ample evidence of anti-parasitic properties of several plants or plant products, there is still a need to provide validated experimental data of reduction in infection levels to support the view that plants may play a direct role in the sustainable control of helminths infections. Identifying the active compounds in plant extracts, quantifying and estimating their bioavailability in the host are essential steps that strengthen the evidence of the Anthelmintic activity of medicinal plants which is a prerequisite towards achieving the scientific validation of plants for parasite control.

CONFLICT OF INTERESTS

Declared None

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