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In-vitro Screening for Anthelmintic Potential of *Crotalaria retusa* on *Pheretima posthuma*

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ABSTRACT

Objective: Aim of the present study is to evaluate the anthelmintic potential of *Crotalaria retusa* leaf extract on *Pheretima posthuma*.

Methods: Extracts of *Crotalaria retusa* leaves prepared by Soxhlet method. The extracts were screened for phytochemicals such as alkaloid, flavonoid, tannin, steroids, etc. aqueous (AECR) and ethanolic (EECR) extracts were tested for anthelmintic activity using Indian adult earthworm (*Pheretima posthuma*).

Results: The observation showed that EECR gave shorter paralysis and death time at 100 mg/mL as compared to AECR. Mean±SEM values were calculated for both extract and standard. EECR showed anthelmintic activity in a dose-dependent manner taking the shortest time for paralysis.

Conclusion: The result demonstrated that both the investigational extracts showed significant anthelmintic activity ($P<0.05$) for the time taken for paralysis and death when compared to standard drug mebendazole.

Keywords: *Crotalaria retusa*, Anthelmintic, *Pheretima posthuma*, phytochemical.

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1. INTRODUCTION

Helminthiasis is a parasitic infection, mostly affects poor populations of the world[1]. Its prevalence is directly associated with sub-standard housing and lack of proper sanitation [2,3]. Helminthiasis is generally occurred by the transmission of parasitic helminths through ingestion of drinking water, uncooked meat, and contaminated vegetables[4]. Ascaris limbricoides, Ancylostoma duodenale, Trichuris tritura, schistosomes and filarial worms, are common helminths responsible for causing helminthiasis in human[5]. a large variety of chemotherapeutic drugs known as anthelmintic, that used to treat helminthiasis like albendazole, mebendazole, praziquantel[6]. these anthelmintic drugs have some serious problems, which includes drug resistance and severe side effects such as fever, stomache, and urticaria[7,8].

Numerous herbs have been screened for their anthelmintic potential and scientific data confirmed their considerable potential against helminths[9]. Some of the phytoconstituents isolated from herbs and reported for anthelmintic activity such as curcumin, β-sitosterol, Stigmasterol β-D Glucoside, epiisopilosine, alkaloids [10-14].

In the present study, *Crotalaria retusa* (**family: *Fabaceae***) was evaluated for anthelmintic activity. *Crotalaria retusa* (**Figure 1**) is also known as rattle weed (in English)[15]. it is commonly found in India and traditionally used to treat various ailments such as cough, dyspepsia, fever, cardiac disorders, stomatitis, dysentery, and scabies[16]. Some efforts have been done in isolation of active principle of *Crotalaria retusa* seed and few alkaloids were identified such as retusine, retusamine, retronecine N-oxide[17].

Figure 1: ***Crotalaria retusa***

2. MATERIAL AND METHODS

2.1 Collection of Plant material

The leaves of plant ***Crotalaria retusa*** were collected from korba district, chhattisgarh, India during the month of December, 2017. These leaves (both young and mature) were washed thoroughly with water and dried under the shade then coarsely powdered and stored in airtight containers for further study. The plant was identified by Department of Botany, Government V.Y.T. P.G. (Autonomous) College of Science, Durg (C.G.).

2.2 Preparation of plant extract

Leaves were shade dried and pulverized into coarse particles and extracted with solvents (water and ethanol) using Soxhlet apparatus[18]. The extracts were then evaporated to dryness to obtain alcoholic and aqueous extract[19,20].

2.3 Phytochemical screening

The selected plant was analyzed for preliminary phytochemical analysis for active principle such as flavonoids, alkaloid, saponin, steroids, and tannin[21,22].

2.4 Selection of earthworm

Due to the anatomical and physiological resemblance of *Pheretima posthuma* with the roundworm of human beings; this was selected for the study[23,18]. The earth worms were collected from moist soil of Raipur, Chhattisgarh, India and washed with normal saline to remove the dirt and fecal material. Furthermore, the earthworms in the size range of 3-5 cm length were used for the experimental work and others were discarded[24].

2.5 Drug and chemical

Ethanolic and aqueous extract of Crotalaria retusa (EECR and AECR), were used as test drug and Mebendazole was used as a standarddrug [23]. All the reagents, solvents and chemicals used in the screening were of analytical grade[25].

2.6 Experimental method for anthelmintic screening

The anthelmintic screening was carried out as per methods developed by scientists with some modifications[23]. total of 45 earthworms was collected and separated in groups (each group: 5 earthworms). Solutions of test extract (ethanolic and aqueous extract) and the standard drug were prepared in distilled water (20ml) at a concentration of 25, 50, and 100 mg/mL. The earthworms were cleaned with normal saline solution and exposed to different concentrations of respective standard and test solutions in Petri dishes. The time of exposing worms in to test solutions was recorded and observation made for mortality of earthworm. in the present study time taken for paralysis and mortality were recorded by shaking Petri dishes. the mortality of earthworm was also confirmed by observing faded body color[23,26].

2.7 Statistical analysis

The results are expressed as mean±SEM of five worms in each group. Comparisons have been made between standard against test treated group. $P<0.05$ was considered significant.

3. RESULTS

3.1 Phytochemical screening:

Phytochemical screening indicated the presence of glycoside, Saponins, Alkaloids, Sterols in extracts.

3.2 Anthelmintic screening:

The observation showed in table 1 suggested that EECR gave shorter paralysis and death time at 100 mg/mL as compared to AECR. Mean±SEM values were calculated for the both extract and standard. EECR showed considerable anthelmintic effect in a dose-dependent manner by taking shortest time for paralysis (29.03±0.55) and death (38.17±0.28) at 100 mg/mL concentration. In the case of AECR, the dose of 100 mg/mL showed shortest time of paralysis (41.19 ±0.33) and death (69.33±0.56).it is observed that both the investigational extracts showed the significant anthelmintic activity ($P<0.05$) for time taken for paralysis and death when compared to standard drug mebendazole.

Table 1: Anthelmintic Activity of *Crotalaria retusa* leaf extract

Groups	Drug treatments	Concentration (mg/mL)	Time (in minute)	
			Time taken for paralysis	Time taken for death
	Control	-	-	-
I	Mebendazole	25	40.70±0.45	49.35±0.25
II	Mebendazole	50	31.84±0.37	37.11±0.20
III	Mebendazole	100	20.69±0.54	27.30±0.15
IV	EECR	25	48.06±0.47	60.28±0.28
V	EECR	50	40.59±0.51	45.27±0.19
VI	EECR	100	29.03±0.55	38.17±0.28
VII	AECR	25	70.13±0.42	105.23±0.31
VIII	AECR	50	63.28±0.23	80.13±0.11
IX	AECR	100	41.19±0.33	69.33±0.56

EECR- Ethanolic extract of *Crotalaria retusa*, AECR- Aqueous extract of *Crotalaria retusa*

4. DISCUSSION

The results of preliminary phytochemical screening implicated the presence of alkaloid, saponin, tannin, and steroids, in both extracts. In anthelmintic screening, the alcoholic extract of leaves of *Crotalaria retusa* demonstrated significant paralysis and maximum death of worms was observed in the least time at a concentration of 100 mg/ml. the considerable result indicated that extracts possess some precious alkaloids which might be the reason for its anthelmintic activity. In this modern era, there are numerous of anthelmintic drugs are available that produces anthelmintic action on helminths by inhibition of polymerization of β-tublin and some other mechanism action[27]. These synthetic drugs also causing some serious side effects. Hence, it is very needful to develop anthelmintic drugs with the least side effect. According to the observation of our study, it has been found that *Crotalaria retusa* showed significant anthelmintic activity as compared to the standard drug. Further investigations are required to determine the therapeutic potential against helminthiasis by *in-vivo* model and elucidate the mode of action of anthelmintic action of its isolated alkaloidal active principles.

5. CONCLUSION

Scientific data of study revealed that *Crotalaria retusa* leaf possesses considerable anthelmintic potential. Hence, this approach will lead to the development of new formulations with lesser side effects and improved activity for the treatment of Neglected tropical diseases such as helminthiasis.

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Conflict of interest statement

I declare that I have no conflict of interest.

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