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IN VITRO ANTHELMINTIC ACTIVITY OF DIFFERENT EXTRACTS OF ROOT OF CARISSA SPINARUM

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ABSTRACT

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. *Carissa spinarum,* Linn. *(Apocynaceae)* is a small spinous, evergreen shrub growing throughout India in dry regions. Further, *C. spinarum* roots are traditionally used for their purgative properties as well as to treat worm infested wounds in animals. There is no report on pharmacologically evaluated antihelmintic activity of root extract of *C. spinarum* till date. Therefore, in the present study we have investigated the antihelmintic activity of methanolic, aqueous and chloroform extracts of root of *C. spinarum* on *Pheretima posthuma*. The fresh and dried root of *C. spinarum* were collected in the month of November from the Bilaspur region, Chhattisgarh state, India, and the antihelmintic activity was evaluated in terms of time taken to cause paralysis and death of the adult Indian earthworm *Pheretima posthuma*. Piperazine citrate (PC; 10 mg/ml) was included as reference compound. The present investigation revealed that the methanolic (100 mg/ml) and chloroform (50 and 100 mg/ml) extracts have equivalent potency compared to PC (10 mg/ml) in time taken for both paralysis and death of *Pheretima posthuma*. Standardization of each extracts and isolation of phytoconstituents in each extracts is required in the future. Furthermore, the pharmacological studies for antihelmintic activity should be undertaken in other parasites to mimic the exact human helminthesis.

INTRODUCTION: Human intestinal helminthesis is among the most common infectious diseases occurring throughout the developing world. These infections have been associated with low standard of sanitation and the worldwide prevalence lies between 500 million to one billion annually approximately [1, 2, 3, 4, 5]. The helminthes which infect the e. g. Tape worms (Taenia solium), nematodes e. g. hookworm (Ancylostoma duodenale), intestine are cestodes roundworm (Ascaris lumbricoids) and trematodes or flukes (Schistosoma mansoni and Schistosoma hematobolium [6, 7, 8, 9, 10, 11]. It has been well accepted that due to the limited availability and affordability of modern medicines most of the world's population depends to a greater extent on traditional medical remedies [12, 13, 14]. Further, the use of medicinal plant products for treatment of various acute and chronic diseases is gaining increasing importance around the globe [15, 16, 17, 18]. It has been well evidenced that the traditional medicines including plants and plant-derived preparations hold a great promise as source of easily available effective antihelmintic agents to the people [19, 20, 21, 22].

Carissa spinarum, Linn. *(Apocynaceae)* is a small spinous, evergreen shrub growing throughout India in dry regions [23, 24]. It has been well investigated that *C. spinarum* receives less attention in academic literature. Literature survey revealed that *C. spinarum* contains lignans, sesquiterpenes of eudesmane type and several cardiac glycosides [25, 26]. Moreover, it has been reported that *C. spinarum* leaves contain urosolic acid and naringin, root contain caffeic acid and a new germacrane derivative, carenone is isolated from stem [27]. Further, *C. spinarum* produces edible fruits and its roots are traditionally used for their purgative properties as well as to treat worm infested wounds in animals. [28] Furthermore, pharmacological studies revealed that stem extracts of *C. spinarum* possess antioxidant and cardiotonic activity [26, 27]. There is no report on pharmacologically evaluated anthelmintic activity of root extract of *C. spinarum* till date. Therefore, in this present study we have evaluated the antihelmintic activity of methanolic, aqueous and chloroform extracts of root of *C. spinarum* on *Pheretima postuma*.

MATERIALS AND METHODS:

Plant Material: The fresh and dried root of *C. spinarum* were collected in the month of November from the Bilaspur region, Chhattisgarh state, India, and authenticated by Prof. S. D. Dubey, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Science, Banaras Hindu University, Varanasi, India and a voucher specimen (No. 052) has been submitted to the Pharmacognosy Division, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi, India for future reference.

Preparation of Extracts: The dried root of *C. spinarum* were powdered, defatted with petroleum ether and subjected to successive solvent extraction with chloroform, methanol in Soxhlet extractor. Aqueous extract was obtained by cold maceration for 24 hours. All the extracts were further evaporated to dryness before investigation to be carried out. The preliminary phytochemical investigation was carried out for methanolic (MCS), aqueous (ACS) and chloroform (CCS) extracts of root of *C. spinarum*. Further, the percentage of yield of MCS, ACS and CCS were found to be 10.17, 6.21 and 8.79 respectively.

Animals: Indian adult earthworms (*Pheretima posthuma*) were collected from water logged areas and were identified by Dr. R. Kundu, Department of Zoology, Guru Ghasidas University, Bilaspur.

Evaluation of Antihelmintic Activity: The anthelmintic activity was evaluated on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of

human beings. Three different concentrations, each of crude extract of methanolic, aqueous and chloroform (25, 50, 100 mg/ml in distilled water) were prepared and six worms (identical to each other) were placed in it. Observations were made for the time taken to cause paralysis and death of the individual worms. Mean time for the paralysis in min was noted when no movement of any sort could be observed, 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) and Piperazine citrate (PC; 10 mg/ml) [29, 30] was included as reference compound as shown in **Table 1**.

TABLE 1: ANTHELMINTIC ACTIVITY OF DIFFERENT EXTRACTS OF ROOTS OF *C. SPINARUM* ON *PHERETIMA POSTHUMA*

GROUPS	CONCENTRATION (MG/ML)	TIME TAKEN FOR PARALYSIS (MIN)	TIME TAKEN FOR DEATH (MIN)
MCS	25	113.85±2.74*	438.04±3.57*
	50	92.28±1.91*	357.78±1.11*
	100	26.22±0.84	62.90±1.56
ACS	25	97.98±2.68*	465.98±2.21*
	50	90.95±0.97*	435.86±2.57*
	100	86.19±1.86*	396.50±3.00*
CCS	25	90.30±0.98*	286.43±4.74*
	50	29.10±1.25	73.38±2.65
	100	22.95±1.65	63.14±3.32
PC	10	22.46±0.45	63.77±1.57
Control	-	-	-

All the values are Mean±SEM (n = 6). *P<0.05 compared to PC

Chemicals and Reagents: All the chemicals including PC and reagents were procured from local suppliers and were of analytical grade.

Statistical Analysis: The data were analyzed with GraphPad Prism 4 (San Diego, CA). Statistical analysis of data was done by One-way ANOVA, followed by Newman Keuls test. Data are expressed as Mean ± Standard error of mean (S.E.M.). A level of $P<0.05$ was accepted as statistically significant.

RESULTS: Table 1 illustrates the effect of different extracts of root of *Carissa spinarum* (25, 50 and 100 mg/ml) in time for paralysis and death of *Pheretima posthuma*. Statistical analysis by One-way ANOVA showed that there was significant difference in time taken for paralysis [$F(9, 50) = 473.11$, $P<0.05$] of Indian earthworm among groups. Post-hoc test revealed that MCS (100 mg/ml), CCS (50 mg/ml) and CCS (100 mg/ml) groups were not significantly different compared to PC in time taken for paralysis of Indian earthworm, indicating equivalence in potency. Further, all the treated groups except MCS (100 mg/ml), CCS (50 mg/ml) and CCS (100 mg/ml) groups showed significant difference compared to PC (10 mg/ml) in time taken for paralysis of earthworm. Furthermore, statistical analysis by One-way ANOVA showed that there was significant difference in time taken for death [$F(9, 50) = 3957.2$, $P<0.05$] of Indian earthworm among groups. The post-hoc test indicated that the time taken for death of *Pheretima posthuma* was similar to that of the effect observed in time taken for paralysis of earthworm, indicating equivalent potency while compared to PC.

DISCUSSIONS: The present study revealed that the MCS (100 mg/ml) and CCS (50 and 100 mg/ml) have equivalent potency compared to PC (10 mg/ml) in time taken for both paralysis and death of *Pheretima posthuma*. Preliminary phytochemical screening of the extracts

revealed that the presence of terpenoids, flavonoids, alkaloids, tannins, saponins and steroids. It has been well established that PC by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis [31, 32] thus, our drug may have the similar profile of mechanism of action. Further, it has been reported that tannins which are polyphenolic compounds produce antihelmintic activity by binding to glycoprotein on the cuticle of the parasite and thus leads to death of the worm [31]. Therefore, standardization of each extracts and isolation of phytoconstituents in each extracts for antihelmintic activity is required in the future. Furthermore, the pharmacological studies for antihelmintic activity should be undertaken in other parasites to mimic the exact human helminthesis.

CONCLUSIONS: The methanolic and chloroform extract of root of *C. spinarum* showed antihelmintic activity on *Pheretima posthuma*. Therefore, standardization of each extracts and isolation of phytoconstituents in each extracts for antihelmintic activity is required in the future. Furthermore, the pharmacological studies for antihelmintic activity should be undertaken in other parasites to mimic the exact human helminthesis.

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