

EVALUATION OF *IN VITRO* ANTHELMINTIC ACTIVITY OF METHANOLIC EXTRACTS OF DIFFERENT PARTS OF *SPERMACOCE ARTICULARIS* L.F.

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ABSTRACT

Present study was undertaken to investigate the anthelmintic potential of methanolic extracts of leaves, stem and roots of *Spermacoce articularis* L.f. on adult Indian earthworm *Pheretima posthuma*. Various concentrations (12.5, 25, 50 and 100 mg/ml) of methanolic extracts were evaluated for anthelmintic activity by recording the time required for paralysis and death of worms. Piperazine citrate (10 mg/ml) was used as reference standard and 1% acacia in normal saline water as a control group. The results of present study indicated that the methanolic extracts of leaves, stem and roots of *Spermacoce articularis* significantly exhibited dose dependent vermifugal activities. The methanolic extracts of leaves exhibited maximum anthelmintic activity as compared to methanolic extracts of stem and roots. The preliminary phytochemical analysis indicated the presence of various phytoconstituents in all the tested extracts.

Keywords: Anthelmintic activity, *Spermacoce articularis*, Piperazine citrate, *Pheretima posthuma*

INTRODUCTION

The use of medicinal plant products for treatment of various acute and chronic diseases is gaining increasing importance around the globe¹⁻⁴. 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 anthelmintic agents to the people⁵⁻⁸. Anthelmintics are drugs that either kill (vermicides) or expel (vermifuge) infesting helminths⁹. These are used to eradicate or reduce the number of helminthic parasites in the intestinal tract or tissues of the body¹⁰. *Spermacoce articularis* L.f. (Rubiaceae) is known as 'Madanaghanti' in Ayurveda and is a commonly known weed in India. The herb is used traditionally for the treatment of various diseases like ophthalmia, inflammation of eye and gums, blindness, carache, fever, spleen complaints, sores, conjunctivitis¹¹. Leaves extract is used as an astringent in haemorrhoids and gall stones, seeds as demulcent, in diarrhoea and dysentery, roots are prescribed as a mouthwash to cure toothache, decoction of the herb is used in the treatment of headache. The weed is rich in calcium and phosphorus; principally contains beta-sitosterol, ursolic acid, iso-rhamnetin and D-mannitol¹²⁻¹³. Purpose of the study is to evaluate the *In-vitro* anthelmintic potential of herb *Spermacoce articularis* L.f.

MATERIALS AND METHODS

Plant material

The whole herb of *Spermacoce articularis* L.f. was collected from the local areas of Gurgaon district, Haryana, in the month of September, 2010. The herbarium of this plant was identified and authenticated by Dr. H.B. Singh, Head, Raw Materials, Herbarium and Museum Division, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, vide reference no. NISCAIR/RHMD/Consult/-2010-11/1530/128, Dated Sept 21, 2010.

Preparation of extract

Fresh leaves, stem and roots of *Spermacoce articularis* L.f. were collected and air dried in shade at room temperature. Dried parts of the plant were ground to coarse powder individually. Powdered plant materials were first defatted with petroleum ether and then extracted with methanol using Soxhlet extractors. All the extracts were further dried at low temperature under reduced pressure and used for the present study.

Phytochemical screening

The methanolic extracts of leaves, stem and roots of *Spermacoce articularis* L.f. were screened for the presence of various

phytoconstituents such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, amino acids, steroids and triterpenoids¹⁴.

Worm collection and authentication

Adult Indian earthworms (*Pheretima posthuma*) were used for the evaluation of *in vitro* anthelmintic activity. They were collected from Agronomy Department of Chaudhary Charan Singh Haryana Agriculture University (CCSHAU), Hisar (Haryana) and authenticated by Dr. Thakral (Senior Scientist), Agronomy Department, CCSHAU, Hisar. Then all collected worms were washed with normal saline to remove all the faecal matter and used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol.

Drugs and chemicals

Piperazine citrate (GlaxoSmithkline, Mumbai). All other chemicals or solvents used were of analytical grades.

Sample preparation

Test samples for *in-vitro* study were prepared by dissolving and suspending 2.5 gm of methanolic extracts of each part of herb in 1% acacia and the volume was adjusted to 25 ml with normal saline to obtain a stock solution of concentration of 100 mg/ml, from this stock solution further dilutions were prepared to obtain concentrations range of 12.5, 25 and 50 mg/ml.

Evaluation of anthelmintic activity

The anthelmintic activity was performed on adult Indian earthworm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. These are easily available and suitable model for screening of anthelmintic drugs¹⁵⁻¹⁷. The *in-vitro* studies were performed according to the method of Ghosh et al.¹⁸. The worms were divided into fourteen groups containing six earthworms of approximately equal sizes placed in petridishes for each concentration separately. 50 ml suspension of methanolic extracts (12.5, 25, 50 and 100 mg/ml) of leaves, stem and roots respectively were used as test samples, piperazine citrate (10 mg/ml) as reference standard while 1% acacia in normal saline as control group were poured into the petridishes. Observations were made for the time taken to paralyse or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color and was ascertained by transferring it into a beaker containing hot water at 50°C, which stimulated and induced

movements if the worms were alive¹⁹. The results were shown and expressed as mean \pm SEM of six worms in each group.

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening of methanolic extracts of leaves and stem of *Spermacoce articularis* L.f. revealed the presence of flavonoids, tannins, carbohydrates, saponins, steroids and triterpenoids, while roots showed presence of tannins, carbohydrates, steroids and triterpenoids. The methanolic extracts of leaves, stem and roots has showed anthelmintic activity in dose dependent manner, when the dose of the extracts were increased, a gradual increase in anthelmintic activity was observed. All the extracts of *Spermacoce articularis* L.f. not only showed paralysis but also exhibited significant anthelmintic activity at highest concentrations of 100 mg/ml as shown in Table 1. The shortest time required for paralysis and death of earthworms was with 100 mg/ml of methanolic extracts of leaves as 6.19 ± 0.06 min and 12.15 ± 0.09 min respectively while it was 9.23 ± 0.06 min and 16.22 ± 0.05 min with 100 mg/ml stem methanolic extract respectively. The same concentration of methanolic extracts of roots showed paralysis and death time as 12.29 ± 0.07 min and 23.90 ± 0.16 min respectively. The paralysis time with standard piperazine citrate was 24.16 ± 0.01

min and death time was 61.38 ± 0.97 min after the release of worms in it. The present study has shown that, the methanolic extracts of *Spermacoce articularis* L.f. leaves, stem and roots at the concentrations of 12.5, 25, 50 and 100 mg/ml have been confirmed to have anthelmintic activity. But methanolic extracts of leaves showed most significant anthelmintic activity as compared to the methanolic extracts of stem and roots. Tannins are polyphenolic compounds which were shown to produce anthelmintic activities²⁰⁻²¹. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death²¹⁻²⁴. It is possible that phenolic content in the methanolic extracts of leaves, stem and roots of *Spermacoce articularis* L.f. produced similar effects. This can be attributed to anthelmintic activity of *Spermacoce articularis* L.f. since they also contains tannins. The predominant effect of piperazine citrate on worm is to cause flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation flaccid paralysis²⁵. All the results were comparable with the standard drug piperazine citrate.

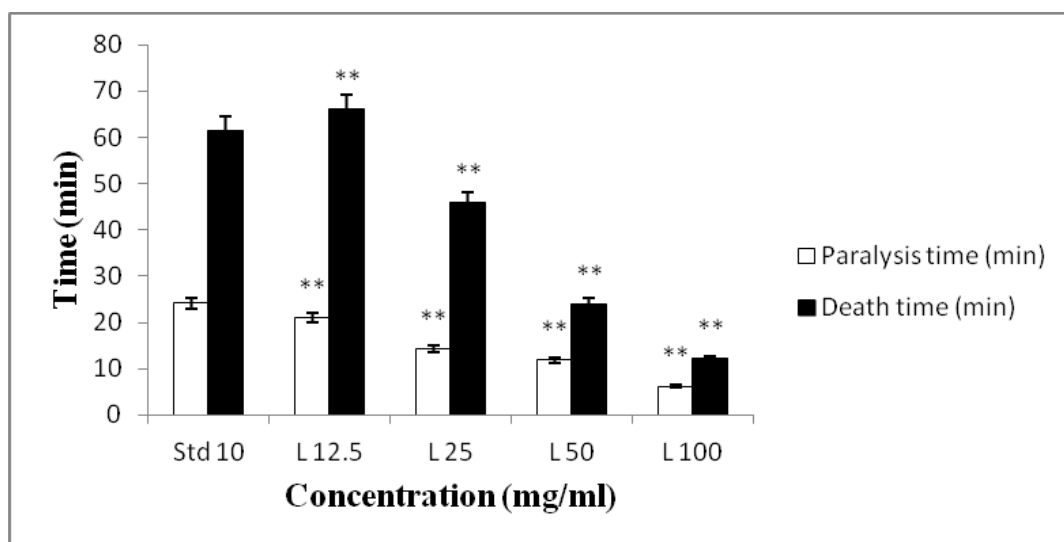


Fig. 1: Paralysis and death time of earthworms in leaves methanolic extract

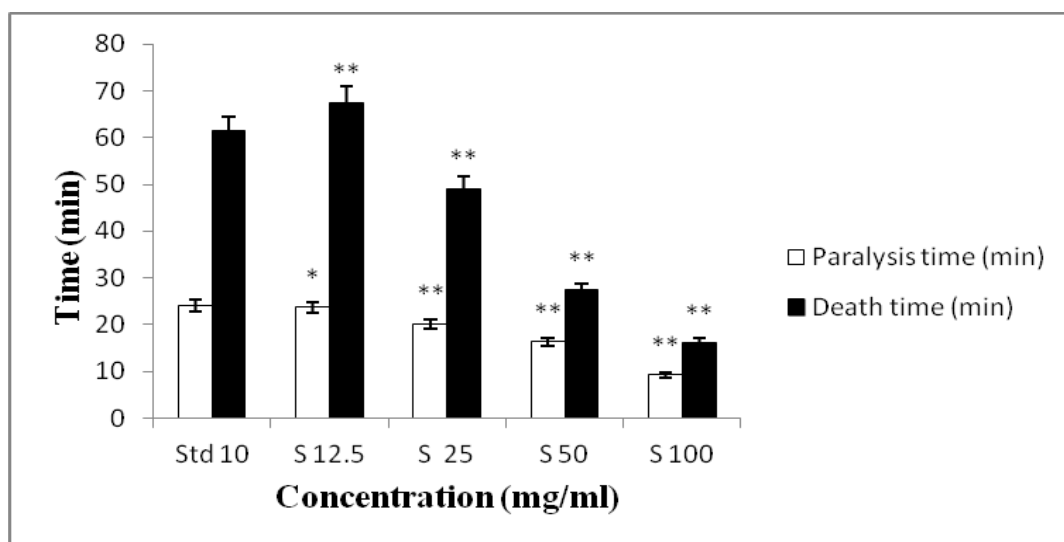


Fig. 2: Paralysis and death time of earthworms in stem methanolic extract

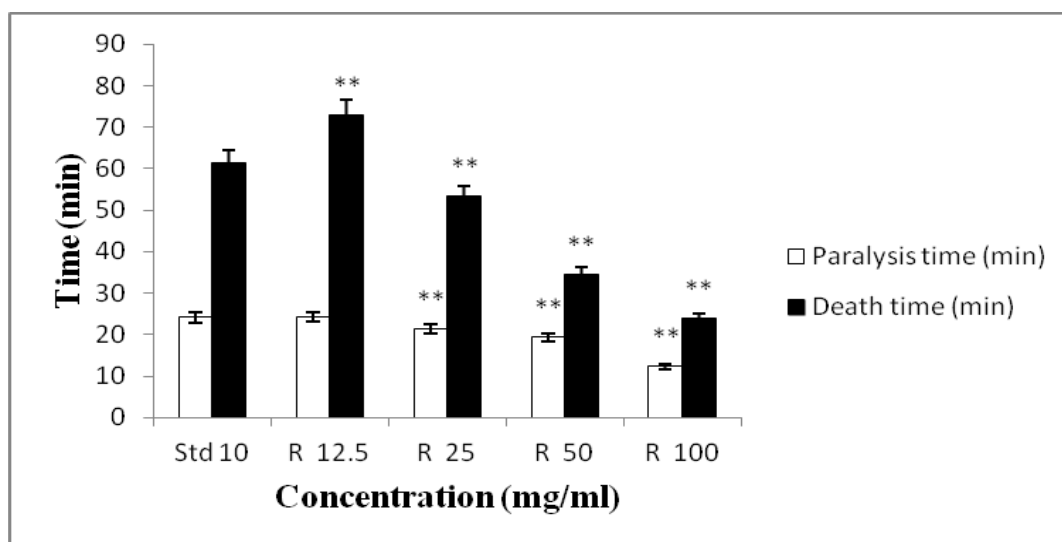


Fig. 3: Paralysis and death time of earthworms in roots methanolic extract

Table 1: Anthelmintic activity of methanolic extracts of leaves, stem and roots of *Spermacoce articularis* L. f.

Test Substance	Concentration (mg/ml)	Paralysis Time (min)	Death Time (min)
Control (1% acacia in normal saline)	--	--	--
Standard (piperazine citrate)	10	24.16±0.01	61.38±0.97
Leaves methanolic extract	12.5	21.03±0.18**	66.03±0.25**
	25	14.36±0.08**	45.89±0.40**
	50	11.95±0.20**	23.97±0.33**
	100	6.19±0.06**	12.15±0.09**
Stem methanolic Extract	12.5	23.73±0.13*	67.57±0.38**
	25	20.22±0.04**	49.12±0.32**
	50	16.35±0.07**	27.49±0.31**
	100	9.23±0.06**	16.22±0.05**
Roots methanolic extract	12.5	24.19±0.04	72.84±1.33**
	25	21.43±0.07**	53.25±1.53**
	50	19.38±0.06**	34.51±0.33**
	100	12.29±0.07**	23.90±0.16**

Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnett's test. Here, n=6 in each group. *P<0.05, **P<0.01.

Statistical analysis

The values were expressed as mean ± standard error of mean (S.E.M.) and statistical analysis was carried out by using one-way analysis of variance (ANOVA) method followed by Dunnett's test. P<0.05 was considered statistically significant when compared with standard references.

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