Analysis of Anthelmintic activity of Chlorophytum borivilianum Santapau & Fernandez against Earthworm

(Eisenia foetida)

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ABSTRACT: Anthelmintic properties of saponin extract of Chlorophytum borivilianum sant. & Farnandez (Liliaceae) was studied. The present investigation was carried out to find out the effect of Root Extract of Chlorophytum borivilianum for its anthelmintic activity against the Indian adult earthworm, Eisenia foetida. Methanol, acetone, Chloroform and water were used to prepare the extract. In present study Methanolic extract (ME), Crude saponin extract (CSE) and purified saponin fractions (PSF) were taken as test solution. Piperazine citrate and 2% w/v gum acacia in distilled water were used as standard and control respectively. Time of paralysis and time of death of the worms were considered as the parameters to assess the anthelmintic action. The result suggest that PSF found to be more active in compare to ME and CSE. The PSF confirmed paralysis as well as death of Eisenia foedita in less time as compare to piperazine citrate.

Key words: Safed Musli, Chlorophytum borivilianum, Anthelmintic, Medicinal plants, Methanol, Piperazine, Eisenia foetida.

1. Introduction:

Since the time immemorial, our traditional system of medicine and folklore claiming that medicinal plant as whole or their parts are being used for different type of disease successfully. Parasitoses have been of great concern to the medical field for centuries and the helminthes still cause considerable problems for human beings and animals. Most of the medicinal preparation now a day available in the market are either not effective up to the mark or has developed resistance resulting in reoccurrence again. Plant derived drug serve as prototype to develop more effective and less toxic medicines. The helmentic activity was evaluated on adult Indian earthworm, Eisenia foetida due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Vidyarthi RD .1977 & Thorn et al 1977). Helmintic infections are now being recognized as cause of much chronic ill health and sluggishness amongst the tropical people. More than half of the population in the world suffers from worm infection of one or the other (Vigar Z.1984)4. Traditional system of medicine reports the efficacy of several natural products eliminating helmenthic & (Dwivedi S et al.2007) 5. The plant Chlorophytum borivilianum Santapau and Fernandes belongs to Liliaceae family. The plant is a short, rhizomatous herb, distributed in tropical & sub-tropical regions at an altitude of 1500 mts of the world. It is used as a natural aphrodisiac, improves immunity in human body. It has also been proved very

effective for alleviating diabetes, arthritis and many other gynecological problems in women. A very few research have been reported on anthelmentic activity of root tubers of this plant. Therefore, it is worth conducting an investigation on the anthelmentic activity of root extract of C. borivilianum . The root contains carbohydrate , phenolic compounds , saponins and alkaloids . Saponins are reported to have anthelmintic activity hence current research work was undertaken to prove the efficacy of *Chlorophytum borivilianum* root tuber extract against *Eisenia fetida* (*Red Worm*) .

1. Material and Methods:

2.1. Plants Materials:

Roots of *Cholorphytum borivilianum* Sant. & Farnandez were purchased from local market of Haridwar , Uttarkhand , India and authenticated by Dr. Kishan Pal at the Dept. of Microbiology , Shri Venkateswara University , U.P , India . Root tubers were made free from aerial parts and wiry rootlets and thoroughly washed , peeled and stored in airtight container for further research work

2.2. Preparation of the Extract:

The tubers was washed , dried , powdered and deffated by petroleum ether . Marc then extracted with methanol for 3 hours at $55\ 0^{o}\text{C}$ which is further concentrated to give methanol extract (ME) . In order to get crude Saponins , ME extract was again dissolved in methanol and then acetone was added (1:5 v/v) to precipitate the Saponins as described by Yan et al . The precipitates were dried under

Vacuum . The Whitish amorphous powder thus obtained as a crude saponin extract (CSE) .

2.3. Isolation of Pure Extract:

To get the pure saponin fraction (PSF), certain amount of CSE was fractionated with silica gel – 60 (230-400 mesh) column chromatography and eluted successfully with chloroform –methanol –water (70:30:10) as described by Favel $et\ al$ · Eluted fraction were combined to give saponin fraction (PSF) .

3. Analysis of Anthelmintic Activity:

3.1. Animal (Eisenia foetida):

I have selected Indian adult earthworm *Eisenia foetida* (Sav.) (Oligochaeta, Lumbricidae) to conduct my experiment owing to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings .

Scientific Name: Eisenia fetida

Common Name: Red worm_, Red Wiggler, Tiger Worm, Manure Worm, Stink Worm, Fish Worm, Dung Worm, Fecal Worm, Striped Worm, Angleworms, Bandlings Habitat: Usually found in garden compost but also occurs in wet, decaying leaf litter, organic-rich soils and manure heaps

Size: Typical size of adult is 6 cm

The earthworm (Eisenia foetida) collected from Local Garden and washed with normal saline to remove all feacal matter were used for the anthelmintic study.

The earthworm of 4-5 cm in length and 0.1 to 2.5 in width were used for all the experimental protocol

3.2. Experimental

Anthelmintic activity of *Chlorophytum borivilianum* of different root extract namely as Methanolic Extract (ME), Crude Saponin Extract (CSE), purified Saponin fractions (PSF) were tested against the Earthworm Eisenia foetida. Various concentration (10-30 mg/ml) of each extract were tested in the bioassay, Which involved determination of time of paralysis and time of death of the worms. The anthelmintic assay was carried as per the method of Ajaiyeoba *et al* with minor modifications.

In this experiment , six groups of five earthworms were taken in to $30\,$ ml solutions of piperazine citrate , ME , CSE

and PSF extracts of tubers of *Chlorophytum borivilianum* in distilled water. Piperazine citrate was included as standard drug and distilled water as control.

3.3. Observations:

After releasing the earthworm in the 30 ml solutions of different root extract of *Chlorophytum borivilianum* Sant. Farnandez observation were made for the time taken to paralysis and death of individual worms . Time for paralysis was noted when no movement could be observed except when the worms were shaken vigorously . Death was concluded when the worm lost their mortality followed with fading away of their body colours .

4. Result and Discussion:

It is observed that the crude extract of *Chlorophytum borivilianum* contain Saponins as the chief chemical Constituents and presence of Saponins have established biological evidence to be an anthelmintic agent. The result of present evaluation of anthelmintic activity of Saponins of *Chlorophytum borivilianum* Sant.& Fernandez on Indian earthworm (*Eisenia foetida*) is depicted Table – I for time taken for paralysis and death of earthworms after treatment with test drugs .

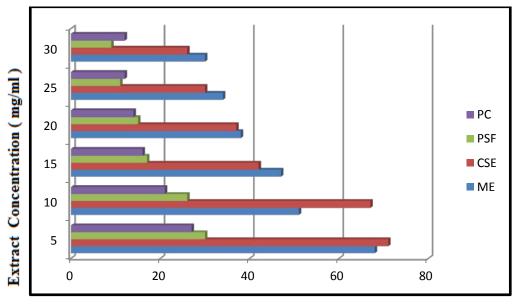
From the result shown in Table – I , the predominant effect of paperzine citrate on the earthworm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis . Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produce hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis .

The comparative data for different fractions for the time paralysis and time of the death of *Eisenia foetida* have been depicted in the Figure no. 1 and 2.

From all the three figures it has been calculated and analyzed that PSF found to be more active and potent in compare to ME and CSE . The PSF confirmed paralysis as well death of *Eisenia foetida* in less time (23.19 min.) as compare to piperazine citrate (25.88) especially at higher concentration of 25 mg/ml

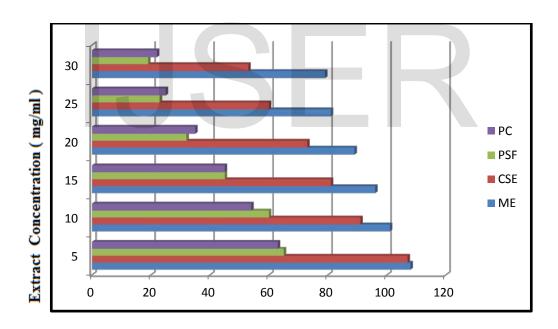
Table: -1 Anthelmintic Activity of roots extract of *C. borivilianum* Sant. & Fern.

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Extract	Concentration	Time for	Time for
ME $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Extract			
ME $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		<u> </u>	•	
ME 15 47.62 ± 0.6 96.32 ± 0.44 20 37.93 ± 0.32 89.99 ± 0.2 25 33.67 ± 0.66 81.11 ± 0.69 30 30.32 ± 0.63 79.56 ± 0.47 5 70.98 ± 1.9 107.96 ± 0.6 10 67.66 ± 0.63 90.79 ± 0.06 15 42.57 ± 0.3 81.30 ± 0.12 CSE 20 36.9 ± 0.12 73.90 ± 0.52 25 30.21 ± 0.66 60 ± 0.01 30 26.96 ± 0.52 53.03 ± 0.21 5 30.33 ± 0.2 65.8 ± 0.2 10 26.5 ± 0.7 55.9 ± 0.25 PSF 15 17.9 ± 0.6 45.65 ± 0.8 20 15.20 ± 0.07 32.98 ± 0.98 25 11.5 ± 0.11 23.1 ± 0.5 30 09.46 ± 0.65 19.32 ± 0.32 5 27.22 ± 1.1 63.65 ± 0.7 10 21.99 ± 1.12 53.89 ± 1.35 15 16.92 ± 0.90 45.32 ± 0.4 20 14.01 ± 0.9 34.76 ± 0.6 PC 25 12.5 ± 0.5 25.88 ± 0.4				
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	ME			
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PSF $\begin{array}{c ccccccccccccccccccccccccccccccccccc$				
PSF $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$			26.96±052	53.03±0.21
PSF 15 17.9 ± 0.6 45.65 ± 0.8 20 15.20 ± 0.07 32.98 ± 0.98 25 11.5 ± 0.11 23.1 ± 0.5 30 09.46 ± 065 19.32 ± 0.32 5 27.22 ± 1.1 63.65 ± 0.7 10 21.99 ± 1.12 53.89 ± 1.35 15 16.92 ± 0.90 45.32 ± 0.4 20 14.01 ± 0.9 34.76 ± 0.6 PC 25 12.5 ± 0.5 25.88 ± 0.4 30 10.32 ± 0.32 22.65 ± 063		5	30.33 ± 0.2	65.8 ± 0.2
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		10	26.5 ± 0.7	55.9 ± 0.25
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	PSF	15	17.9 ± 0.6	45.65 ± 0.8
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		20	15.20 ± 0.07	32.98 ± 0.98
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		25	11.5 ± 0.11	23.1 ± 0.5
PC $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		30	09.46±065	19.32±0.32
PC $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		5	27.22 ± 1.1	63.65 ± 0.7
PC $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		10	21.99 ± 1.12	53.89± 1.35
PC 25 12.5± 0.5 25.88± 0.4 30 10.32±0.32 22.65±063		15	16.92 ± 0.90	45.32 ± 0.4
30 10.32±0.32 22.65±063		20	14.01 ± 0.9	34.76± 0.6
	PC	25	12.5 ± 0.5	25.88± 0.4
Control		30	10.32±0.32	22.65±063
	Control		-	-



Paralysis Time (Min.)

Fig No. 1 Comparative data of paralysis time for Eisenia fotida



Death Time (Min.)

Fig. No. 2 Comparative data of death time for Eisenia foetida

Conclusion:

In the present study , we may conclude that the tubers of *Chlophytum borivilianum* can be used as anthelmintic .

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References:

- 1. Sivarajan VV and Balachandran I."Ayurvedic Drugs and their Plant Sources", Oxford & IBH publications co. pvt. ltd, New Delhi, 1994: 314.
- 2. Dash GK, Mishra B, Panda A, Patro CP. and Ganapaty S. Indian . J. Nat, P., 2003; 19(3): 24.
- 3. Vidyasarathi RD, A Text Book Zoology, 14th Edn, S. Chand and Co., New Delhi, 1977: 329.
- 4. Tripathy KD, Essential of Medical Pharmacology, 5th Edn. Reprint Jaypee Brothers Medical Publishers (P) ltd., New Delhi, 2005: 759-760.
- 5. Vigar, Atlas of medical paratology, 2nd Edn., PG publishing home, Singapore, 1984: 216.
- Dwivedi S, Dwivedi SN, Shrivastav S, Dwivedi A, Dwivedi and Kaul S. Reference of Medicinal herbs used in traditional system of medicine, Farmavita.net, 2007, Aug.
- 7. Srinivas GM, Kumar S, Rao S D. and Jayachandran E. Indian Drugs, 2006; 43(4): 343.

- 8. Weyne W, Daniel. "Biostatistics: A foundation for the Analysis in the Health sciences" 7th Edn. Jahn Wiley and Sons tria Pvt. Ltd. Singapore, 2004: 312
- 9. Bhattacharjee SK, Handbook of Medicinal Plants, pointer publication: Jaipur, 4th Edn, 2000, pp.92-93.
- 10. Deore SL and Khadabadi SS, Pharmacognostical Study of plant *Chlorophytum borivilianum*, Biomedicine, 2008, 3 (2 & 4), 239-250.
- 11. Tandon V , Pal P , Roy HS and Reddy KS, *In Vitro* anthelmintic activity of root –tuber extract of *Flemingia vestite* , an indigenous plant in Shillong , India , *Parasitol* Res , 1977 , 83 , 492 -498
- 12. Joshi A, Ganesh R, Sharma A and Audichya P, Sapogenin content determination in *Chlorophytum borivilianum Biontaure*, 2000, 20,47-49.
- 13. Singh P , Gupta P and Singh R , Safed musli as a Divya aushed , Chemistry biology interface , synergestic new frontiers , New Delhi , India , Nov . 2004 ,21-26 .
- 14.
- 15. Ajaiyeoba EO, Onocha PA and Olarenwaju OT, *In Vitro* anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extract, *Pharm Biol*, 2001 39, 217-220
- 16. Jamal M Arif , Effects of Safed musli (
 Chlorophytum borivilianum) on cell kinetics and apoptosis in human breast cancer cell lines ,
 International conference on promotion breast cancer cell lines , International conference on promotion and development of botanicals with International co-ordination : Exploring quality ,
 Safety ,efficacy and regulation , organized by school of Natural Products study , Jadavpur University , Kolkata , India , 25-26 February 2005