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Anthelmintic Activities of Polypodium Decumanum Leaf-Extracts

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Abstract Various herbal plants used in traditional medicines contain a vast array of substances that can be used to treat various disease and complications, the present study deals with anthelmintic activity of different extracts of leaves of Polypodium decumanum, a potential medicinal plant. The crude plant extracts of leaf part exhibited anthelmintic activity. Two concentrations (30mg/ml and 40mg/ml) of each extract were studied, which involved for the determination of time of paralysis and time of death of the test worms. It was found that ethyl acetate extracts exhibited significant anthelmintic activity followed by ethanolic extract and aqueous extract in causing paralysis and death of earth worms.

Keywords: *Polypodiun decumanum, anthelmintic activity, piperazine citrate*

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1. Introduction

Helminthes infections are among the most common infections in humans, affecting a large population of the world. Although the majority of infections are due to worms, generally limited to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting large population in endemic areas [1,2]. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore is a foremost problem in the treatment of helminthes diseases. Hence, there is an increasing demand towards natural anthelmintics. There is economic impact of parasitic gastroenteritis, which is caused by mixed infection with several species of stomach and intestinal roundworms. Parasitic diseases cause morbidity including lymphatic filariasis, onchocerciasis and schistosomiasis [3].

Development of anthelmintic resistance against helminthes is reported in number of countries which gives a clear indication that control programs based exclusively on their use are not sustainable. The development of integrated programs to control helminthes is vital, but such control programs require viable alternatives to the use of anthelmintics. Medicinal plants have served through ages, as a constant source of medicaments for the exposure of a variety of diseases. The history of herbal medicine is almost as old as human civilization [4,5]. The plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides. A number of medicinal plants have been used to treat parasitic infections in man and animals.

The predominant effect of piperazine citrate on worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis [6]. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol has shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [7,8]. Another possible anthelmintic effect is that tannins bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of parasite and cause death.

The aim of this research was to identify the anthelmintic effect of the Polypodium decumanum plant extract obtained from the leaves of the plant.

2. Materials and Methods

The Polypodium decumanum used in this study was procured from the forest of Sikkim and was authenticated from the Regional Research Institute, Bangalore. The anthelmintic activity was performed on adult earthworm (*Eisenia foetida*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

Each groups consisted of six adult earthworms (*Eisenia foetida*):

1st group - Vehicle (Normal saline)
2nd group - Standard drug (Piperazine citrate)

3rd group - Ethyl acetate extract of *Polypodium decumanum* (30 mg/ml)
4th group - Ethyl acetate extract of *Polypodium decumanum* (40 mg/ml)
5th group - Ethanol extract of *Polypodium decumanum* (30 mg/ml)
6th group - Ethanol extract of *Polypodium decumanum* (40 mg/ml)
7th group - Aqueous extract extract of *Polypodium decumanum* (30 mg/ml)
8th group - Aqueous extract of *Polypodium decumanum* (40 mg/ml)

2.1. Preparation of Aqueous Extract

The dried powder (70g) was extracted with water for 72hr and the same was dried on water bath [9].

2.2. Preparation of Ethanol and Ethyl Acetate Extract

The dried powder (70g) extracted in a soxhlet apparatus using ethanol (95%) and ethyl acetate, at a temperature range of 45°C to 60°C. The filtrate was evaporated to dryness at reduced pressure in vacuum evaporator [9].

2.3. Experimental procedure

Ethanol, ethyl acetate and aqueous extracts from the leaves of Polypodium decumanum were investigated for anthelmintic activity against *Eisenia foetida*. Test samples of three extracts (ethyl acetate, ethanol and aqueous) were prepared at the concentrations of 30 mg/ml and 40 mg/ml in 25 ml of normal saline. Six worms of approximately equal size were placed in petridish containing above solution of extracts. Piperazine citrate (10 mg/ml) was used as reference standard and normal saline as control. The concentrations (30mg/ml and 40 mg/ml) of each extract were tested by bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate was used as standard reference and saline water as control. The Anthelmintic assay was carried as per the method with minor modifications [10]. The assay was performed on adult earthworms, *Eisenia foetida* due to its anatomical and physiological resemblance with that of intestinal round worm parasite of human beings [11,12]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* [13]. The earthworms were collected from moist soil and washed with normal saline to remove all faecal matter and were used for the anthelmintic study. The earthworms of 6-8 cm in length and 0.2-0.3 cm in width were used for all experimental protocol.

The earthworms were divided into eight groups containing six earthworms in each group. All the extracts and standard drug solution were freshly prepared in normal saline before starting the experiments. Different extracts and standard drug solutions were poured in different petri plates. All the earthworms were released into 10ml of formulation as follows: Ethanol, ethyl acetate, aqueous extract and Piperazin citrate in two different concentrations. Observations were made for the time taken to paralysis and death of worms [14].

Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility when dipped in warm water (50ºC) followed with fading away of their body colors. Time of paralysis was noted when no movement was observed except when the worms were shaken vigorously. All the readings were taken in triplicate. Then all the extracts were compared with the standard by observing the paralysis time and death time of earthworms on different extracts.

3. Results and Discussion

Anthelmintic activity results revealed that different extracts of *Polypodium decumanum* were found to exhibit significant Anthelmintic activities against earth worm. Two doses 30 mg/ml and 40 mg/ml of ethyl acetate, ethanolic and aqueous extracts were taken to observe the paralysis time (PT) and death time (DT) of earthworms with these doses (Table 1).

Among the Ethyl acetate extract of two concentrations of 30 mg/ml show the Paralysis time (PT) and Death time (DT) is 8.42 minutes and 11.9 minutes and for concentration of 40 mg/ml PT and DT is 3.43 and 7.16 respectively (Figure 1, Figure 5).

Ethanol extract of two concentrations of 30 mg/ml show the Paralysis time (PT) and Death time (DT) is 12.70 minutes and 17.23 minutes and for concentration of 40 mg/ml PT and DT is 10.36 and 15.9 minutes respectively (Figure 1, Figure 6).

Table 1. Evaluation of Anthelmintic activity

Treatment	Dose (mg/ml)	*Polypodium decumanum* Extract	
		Paralysis Time (Mean±SEM)	Death Time (Mean±SEM)
Control	Normal Saline (25ml)	_	_
Piperazine citrate	10	21.66±0.88	61.33±1.33
Ethyl acetate extract	30	8.42±0.81**	11.9±0.20**
	40	3.43±0.29**	7.16±0.12**
Ethanol extract	30	12.70±0.35**	17.23±0.11**
	40	10.36±0.08**	15.9±0.20**
Aqueous extract	30	54.2±0.11**	66.85±0.20**
	40	22.23±0.11	28.16±0.12**

Each value represent Mean±SEM, n=5. One-way ANOVA followed by Dunnet test through Instat software, compare all vs. standard applied. Statistically significant at **P<0.01, *P<0.05.

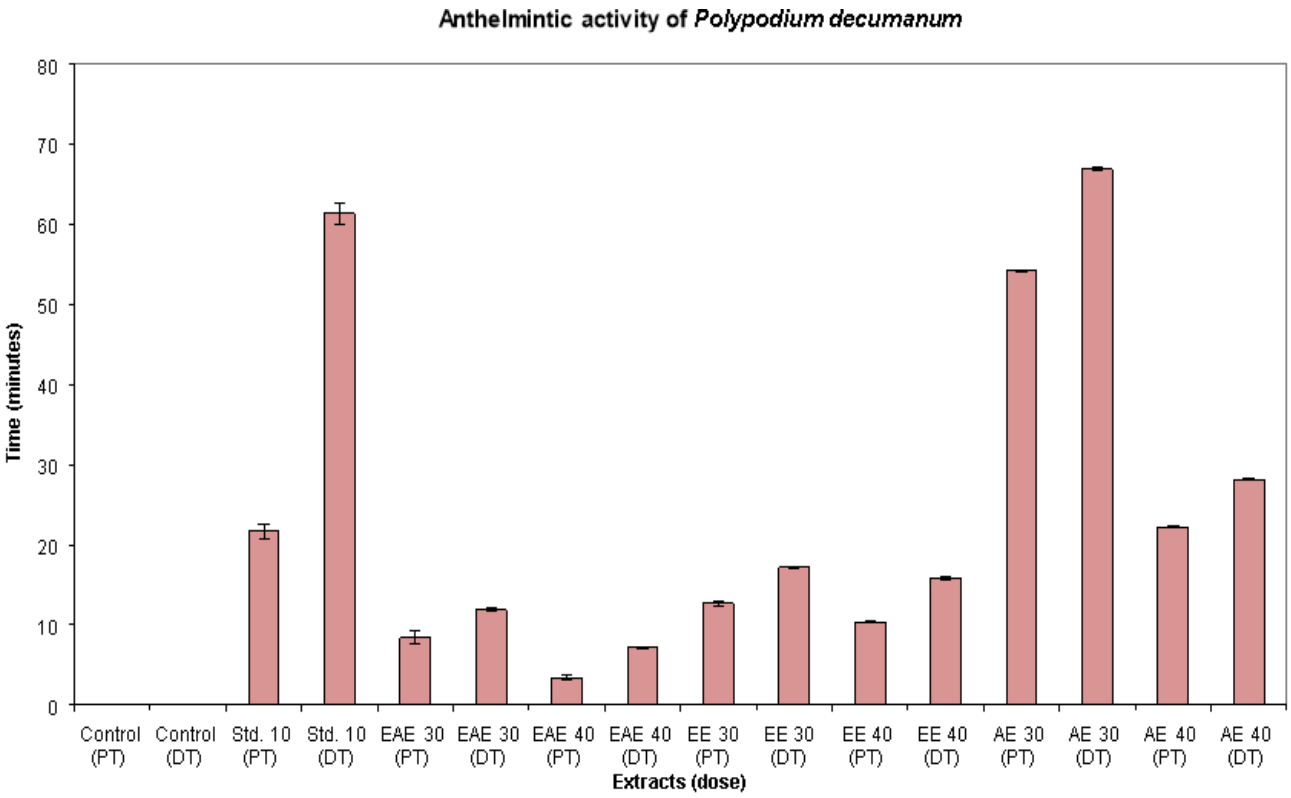

Figure 1. Graphical representation of Anthelmintic activity

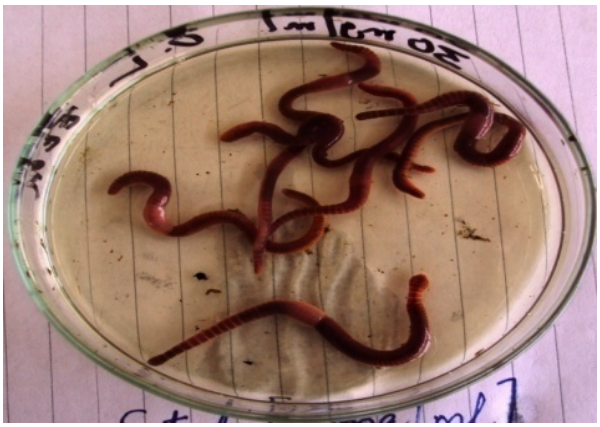

Figure 2. Effect of Piperazine citrate (10mg/ml) extracts of *Polypodium decumanum* on earthworm

Figure 4. Effect of aqueous extracts of *Polypodium decumanum* on earthworms

Figure 3. Effect of normal saline with Tween extract of *Polypodium decumanum* on earthworms

Figure 5. Effect of ethyl acetate extract of *Polypodium decumanum* on earthworms

Figure 6. Effect of ethanol extract extracts of *Polypodium decumanum* on earthworms

Finally the aqueous extract of two concentrations of 30 mg/ml show the Paralysis time (PT) and Death time (DT) is 54.2 minutes and 66.85 minutes and for concentration of 40 mg/ml PT and DT is 22.23 and 28.16 minutes respectively (Figure 1, Figure 4).

Normal saline of 25 ml not show any activity (Figure 1, Figure 2) and 10% solution of Piperazine citrate used as standard show the Paralysis time (PT) and Death time (DT) is 21.66 minutes and 61.33 minutes (Figure 1, Figure 2).

4. Conclusion

Anthelmintic activity was observed on different extracts of *Polypodium decumanum* after studying the acute toxicity on the plant. Two doses 30 mg/ml and 40 mg/ml of ethyl acetate, ethanolic and water extracts were taken to observe the paralysis time (PT) and death time (DT) of earthworms with these doses. It was observed that all the extracts of exhibited dose dependent anthelmintic activity against earthworms. Ethyl acetate extract was more significant followed by ethanol and water extract in causing paralysis and death of earthworms when compared with the standard drug (piperazine citrate, 10 mg/ml). It had been reported that phenolics, flavonoid, diterpenoid, phytosterol are responsible for anthelmintic activity of many plants.

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