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### *In-Vitro* Anthelmintic Activity of *Diplazium esculentum* (Retz.) Swiss Rhizome Extract

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Parasitic diseases cause severe morbidity by affecting population in endemic areas with major economic and social consequences. More than half of the population of the world suffers from various types of infection and majority of cattle suffer from worm infections. So there is a need to find new drugs to treat parasitic diseases. Ethanolic, aqueous and petroleum ether extract from the rhizome of *Diplazium esculentum* (Retz.) Sw (Family- Athyriaceae) was investigated for their anthelmintic activity against *Pheretima posthuma*. Three concentrations (10, 25 and 50 mg/ml) of each extracts were studied, which involved the determination of time of paralysis and time of death of the worm. It was found that all the extracts exhibited significant anthelmintic activity. Piperazine citrate in same concentration as that of extract was included as standard reference and distilled water as control. Results showed that the ethanolic extract was more potent as compared to other extracts as it took less time to cause paralysis and death of the earthworms as compared to standard reference drug.

**Keyword:** Anthelmintic Activity, *Diplazium esculentum*, Pteridophytes, *Pheretima posthuma*,

#### 1. Introduction

Helminthiasis is the condition resulting from worm infestation, and is one of the major prevalent diseases in the world, particularly in the tropical countries. Lack of adequate sanitary facilities and supply of pure water coupled with poverty and illiteracy are some of the factors responsible for wide spread nature of this disease in the developing countries.

Helminthiasis is prevalent globally (1/3 of the world's population harbours them), but is more common in developing countries with poorer personal and environmental hygiene<sup>[1,2]</sup>. Anthelmintics or anthelmintics are drugs that expel parasitic worms (helminthes) from the body, by either stunning or killing them. <sup>[3]</sup> The

gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics<sup>[4]</sup>. *Diplazium esculentum* (Retz.) Sw (Family- Athyriaceae) consists of creeping and branched rhizome; scales brown, lanceolate and upto 7 to 15 mm in length; stripes fragile, straw coloured, 10-35 cm long, lamina variable, broadly lanceolate to subdeltoid with acuminate apex, decomponently pinnae and stalked, distantly placed, 6-15 × 2-4.7 cm ascending with slender, naked, greenish rachides, pinnules, upto 15 × 6 mm, sub deltoid, cut down into ultimate oblong, narrow segments with dentate margin, secondary rachides minutely pubescent, veins forked; sori

minute, indusium thin, membranous; spores dark brown.

The plant is common in Uttarakhand region and is frequently used by the Garhwali peoples for medical purpose. The circinate young leaves are used as vegetables (lingra). The rhizome is considered as strong haemoptysis,

used in cough, asthma, phthisis, fever, dyspepsia, stomachache, diarrhea and as antidysenteric, insect and pest repellent. Young tips of fronds are used as tonics for health. Decoction of rhizome and young leaves are useful haemoptysis and constipation<sup>[5-9]</sup> and used as antibacterial<sup>[10]</sup>.

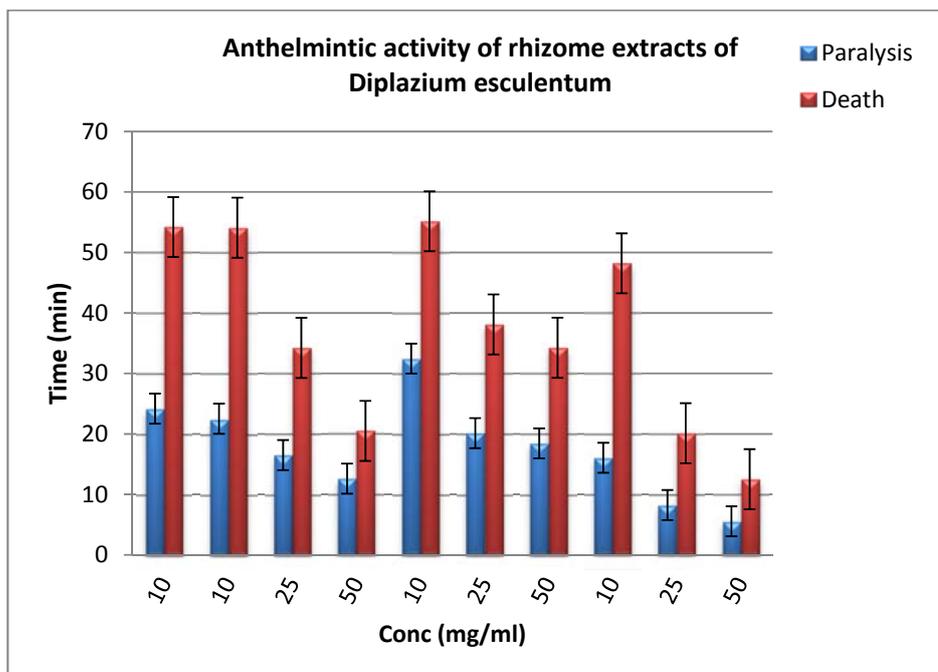


Fig-1: Graphical Presentation of *In Vitro* Anthelmintic activity of rhizome extracts of *Diplazium esculentum* against *Pheretima Posthuma*

## 2. Materials and methods

The rhizomes of *Diplazium esculentum* were collected from Shyampur, Rishikesh in Uttarakhand region during the month of August and their identity was confirmed by Botanical Survey of India (BSI), Dehradun. The voucher specimen was deposited in the Department for future records.

### 2.1 Preparation of Plant Extracts

Fresh plants parts were washed 2-3 times with tap water and distilled water and then dried under shade and then in oven and lastly powdered with the help of an electric grinder. Coarsely powdered material (500 gm) was extracted successively with petroleum ether (40-60), ethanol and water

by maceration process at room temperature for three days. Then all the extracts were filtered and concentrated with a rotary evaporator and kept in a refrigerator.

### 2.2 Worm Collection and Authentication

Earthworm (*Pheretima posthuma*) have been used widely for the initial evaluation of anthelmintics activity, because of their easy availability and due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity and authenticated by Head,

Department of Zoology, DAV (PG) College, Dehradun.

### 2.3 Drugs and Chemicals

Piperazine citrate (Sharon Biomedicine Pvt. Ltd Dehradun), petroleum ether AR (60-80), ethanol AR (CDH Pvt. Ltd), normal saline water was used as control.

### 2.4 Anthelmintic Activity

The anthelmintic activity was evaluated on adult Indian earthworm. Five groups of Indian earthworms each containing six earthworms approximately of equal size was used for the study. Each group of earthworms were tested with different extract conc. (10, 25 and 50 mg/ml), distilled water (control), and reference standard piperazine citrate (10 mg/ml in distilled water).<sup>[10]</sup> Time taken to paralysis and death of individual worms were observed. Time taken to paralysis was noted when no movement of any sort could be observed. Observations were made for the time taken to paralysis and / or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline water. Death was concluded when the worms lose their motility followed with fading away of their body colour.

### 3. Result and Discussion

From the observation made extracts exhibited more potent activity at higher concentration (50 mg/ml) against *Pheretima posthuma* (earthworm). Evaluation of anthelmintic activity was compared with reference standard piperazine citrate. The ethanolic rhizome extract of *Diplazium esculentum* produced dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which gradually progressed to death as shown in Table 1. The aqueous extract showed significant activity, the petroleum ether extract was the least active among all the extracts.

In the present study, it was observed that the ethanolic extract of *Diplazium esculentum* have exhibited positive and potent response than the other two extracts (petroleum-ether and aqueous) even though all the three extracts were endowed

with anthelmintic property. The order of activity was ethanol extract greater than aqueous extracts greater than petroleum ether extract. The activity revealed concentration dependence nature of the different extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms.

**Table 1:** Anthelmintic activity of rhizome extracts of *Diplazium esculentum*

Test Substance	Conc (mg/ml)	Time taken for paralysis (minutes)	Time taken for death (minutes)
Distilled Water (Control)	-	-	-
Piperazine citrate (Standard)	10	24.18±0.70	54.21±0.23
Aqueous Extract	10	22.58±0.79	54.10±0.12
	25	16.5±0.42	34.22±1.88
	50	12.60±0.25	20.50±1.45
Petroleum ether extract	10	32.45±0.14	55.16±1.36
	25	20.12±0.62	38.10±1.16
	50	18.44±0.55	34.25±1.12
Ethanolic Extract	10	16.05±0.68	48.20±1.52
	25	8.24±1.44	20.10±0.36
	50	5.55±0.50	12.5±0.50

### 4. Conclusion

It could be concluded that the ethanolic extract showed most potent anthelmintic activity. The other two extracts e.g., petroleum ether and aqueous extracts, exhibited lesser anthelmintic activity than the ethanolic extract. The present study revealed that the anthelmintic activity increases with increasing polarity. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anthelmintic activity and to establish the effectiveness and pharmacological rationale for the use of *Diplazium esculentum* as an anthelmintic drug.

## 5. Acknowledgment

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