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Research Article

Diplazium Esculentum Fronds Extract: A Promising Source Of Anthelmintic Compounds Against Pheretima Posthuma - Phytochemical Insights

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ABSTRACT

This studywas to determine the phytochemical composition of Diplazium esculentum and evaluate its in-vitro anthelmintic properties against Pheretima posthuma using extracts from the fronds of Diplazium esculentum. Helmintcs is an infectious disease caused by worms that is also known as Helminths. These affect human health globally by causing infection. Anthelmintics designed to inhibit the growth of parasite worm or kill it.*Diplazium esculentum* is known as edible fern,lingra,fiddlehead ,vegetable fern etc.for the identification of bioactive compounds to conduct phytochemical screening of *Diplazium esculentum* with employing verious tests to detect the presence of compouds - Alkal-oids, Tannin, flavonoids, phenolics, terpenoids, ster-oids, and sap-onins, of *Diplazium esculentum* extracts. Extraction from the fronds of *Diplazium esculentum* via using the soxchlet Apparatus and the ethanolic and aqeous extract are extracted out.The yield of aqueous extact is highr then ethanol extract. These extracts are used for in-vitro anthelmintics activity againts Pheretima posthuma that is commonly known as earthworm that is found in moist soil rich in organic matter.The concentrations (10,25,45 mg/ml) are studies to examine the anthelmintcs activity.during the experiment observe the mortility, paralysis and death time of worm.The distilled water is uses as vehical and the albendazole use as standard with same concentrations.Reasult:Thia study shows that *Diplazium esculentum* has valuable source of bioactive compouds.*Diplazium esculentum* natural source for anthelmincs and the aqueous extract display the anthelmintcs activity albeit to a lesser extent compared to the ethanol extract.

Keywords:*Diplazium esculentum*, worms,Extract,etanolic,aqueous, Pheretima posthuma,Anthelmintcs activity etc.

Introduction:

Worm infection-related disease, helminthiasis, is a major global health concern that is more common in tropical areas. (semwal et.al.2023) Its extensive incidence is mostly caused by factors including poor sanitation, a lack of clean water supplies, poverty, and low levels of education, particularly in developing nations. Helminthiasis affects the population worldwide. It is more common in those places that have inadequate environmental and personal hygiene standards. (semwal et.al.2023,singh et.al.2008)
Medication used to remove parasitic worms (helminths) from the body by killing or stunning them is referred to as an anthelminthic medicine, or anthelmintic. These medications are essential for managing and treating helminthiasis and assisting in reducing the disease's impact on afflicted communities. (Singh et.al.2008,Dewivedhi et.al.2009)
Diplazium esculentum (Family- Athyriaceae) is distinguished by its creeping and branching ferns. (semwal et.al.2023) The Garhwali people often use the plant, which is often found in the Uttarakhand region, for medical purposes. Young leaves that are coiled in a circle are used as vegetables and to treat pains, fever, dyspepsia, diarrhoea, cough, and asthma. (semwal et.al.2021, Kaushik et.al.2011) It is also used as an insect

and pest repellant and as an antidysentery. Young leaves and rhizome infusions are used to treat hemoptysis and constipation, while the tips of the fronds are used as health tonics. (semwal et.al.2023,Zannah et.al.2017) sources of medicinal substances dating back thousands of years. Historically, medicinal treatments could only be derived from natural products. (Zannah et.al.2017) More than half of them want to use still-natural compounds. *Diplazium esculentum* also referred to as "Lingra," is important to the people who live in hills and surrounding areas. (semwal et.al.2021,Zannah et.al.2017) This plant has high concentrations of flavones, triterpenoids, phenols, and steroids. Its dried rhizomes are used as pesticides, while leaves are consumed as vegetables. Apart from that, a plant is used for cough and hemoptysis and vegetable shoots contain antioxidant qualities and many other pharmacological actions. (semwal et.al.2023,Kaushik et.al.2011,Zannah et.al.2017)

Material and method –

The *Diplazium esculentum* plant was sourced from the local market in Dehradun, Uttarakhand region, in August, which coincides with the rainy season. To verify the authenticity of the collected specimens, authentication was conducted by the Department of Botany SDM Government PG College Doiwala (Dehradun).

Making Plant Extracts

First, clean the fronds of *Diplazium esculentum* with tap and distilled water, which was done three or four times. (semwal et.al.2023) The plant components that had been cleaned were then dried in the shade and then dried again in an oven. (semwal et.al.2023,Alekhya el.al.2013) The dried plant material was crushed into a rough powder using a mortar and pestle. Then store it in an air-tight Vials at room temperature for the experiment. When the extraction process started the dried frond powder was filled into the extractor of the soxhlet apparatus with (ethanol and aqueous)solvents (500 ml), and a portion of the coarsely ground material (50 gm) was extracted in stages over three days, with the temperature kept between 50 and 60°C.when extraction process completed then the extracts filter by the Whatman filter. (Rey et.al.2020, Semwal et.al.2021)After filtration, the filtrate was concentrated and dried through the water bath at 50-70 °C temperature. After that weight, the dried extract was stored in storage vials and kept in the fridge for further study. (semwal et.al.2023,Alekhya et.al.2013) The yield percentage of extracts(ethanolic- 3.20and aqueous-8.03) determine by the following equation-

$$\text{Yield Percentage} = \text{Weight of dried plant material}/\text{Weight of dried extract} \times 100$$

Figure 1: These are Pictures of the Extraction (Ethanolic and Aqueous) of fronds of *Diplazium esculentum* and Evaporation of extract by a water bath.

Drugs and Chemicals
Albendazole
Ethanol
Distilled water
DMSO

Collection of worms
The worms are collected from the agriculture field of Dev Bhoomi Uttarakhand University, Dehradun (U.K)

Anthelmintics activity
Indian earthworms were used to measure the anthelmintic activity. Approximately three earthworms of the same size were used in each of the three groups of earthworms used in the experiment.(Dey et.al.2010,Alekhya et.al.2013,Kappus et.al.2003,Srinivasa et.al.2006) Various quantities of plant extracts (10, 25, and 45 mg (about half the weight of a business card), or half a business card's weight)/ml), serve as a control, and the reference standard Albendazole (10 mg (about the weight of a grain of table salt), or a grain of table salt)/ml in distilled water) were examined with each group of earthworms. (Semwal et.al.2023,9Raul et.al.2012,Chandra et.al.2014,Sreejith et.al.2013,Mahato et.al.2014) It was noted how long it took for each worm to become paralyzed and die. (Mahato et.al.2014,Srinivasa et.al.2006)

Even after the worms were submerged in regular saline worm water, no movement was seen, and this was considered paralysis. When the worms stopped moving and their body colour changed, it was recognized that they had died. (Dadde et.al.2012, Srinivasa et.al.2006) These findings were noted to assess how well the reference standard and the tested extracts performed. (Raul et.al.2012,Kumar et.al.2018,Zannah et.al.2017,17Koasalge et.al.2009)

REMEMBER: ethanolic extract was diluted in dimethyl sulfoxide (DMSO). Albendazole dissolves in DMSO when the aqueous extract is dissolved in distilled water. Using the parasite *Pheretima Posthuma,* was the approach employed in the experiment to determine the fronds of *Diplazium esculentum* are the source of its anthelmintic activity.

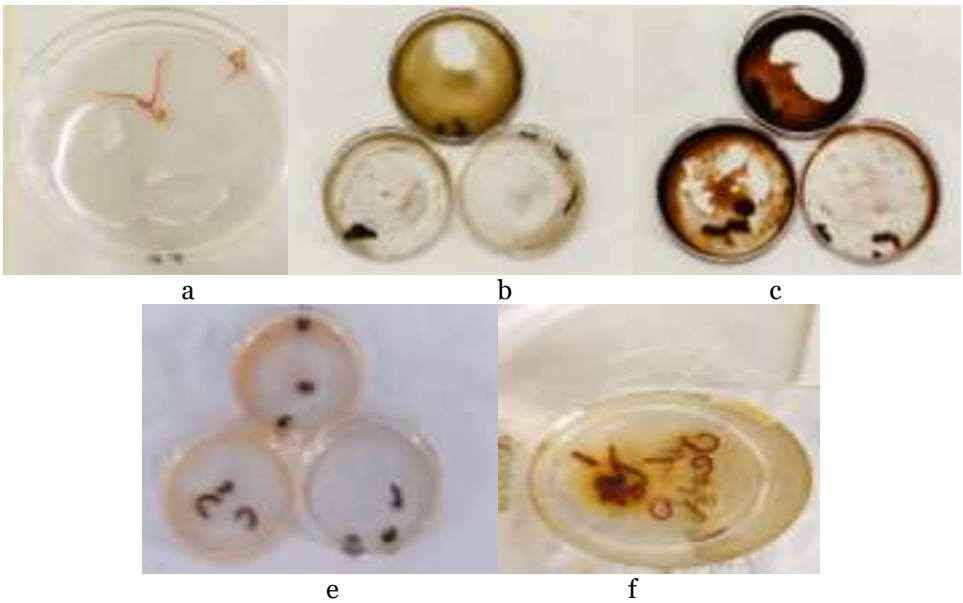

Figure 2: These are picture of Vehicle-group(a), Ethanolic-extract-treated-group (b), Aqueous extract-treated -group (c), Albendazole solution -group (d), Death conformation by warm water.

Phytochemical Analysis: The extracts underwent screening to identify various phytochemicals. The procedure involved specific reagents and reactions, yielding positive indications for the presence of distinct phytochemicals as follows:

Figure 3: pictures of phytochemicals screening of *Diplazium esculentum* Bioactiv compounds.

Phytochemical-Test	Procedure	Positive Reaction
Alka-loids	Add 2 mL of the extracts to Wagner's reagent If a reddish-brown precipitate forms, it suggests the existence of alkaloids. (Ganatra et.al.2013,Kokate et.al.2020,Sreejith et.al.2013)	Reddish-brown precipitate
Carbo-hydrates	Mix 2 mL of the extracts with Benedict's reagent. If an orange-red-precipitate forms, that means reducing sugars are presented. (Shaikh et.al.2020)	Orange-red precipitate
Phen-Ols	Expose 2 mL of the extracts to ferric chloride solution. If the solution turns bluish-black, it suggests the presence of phenols. (Kumar et.al.2018,Sreejith et.al.2013)	Bluish-black coloured solution
Tann-ins	Mix 2 mL of the extracts with a 1% gelatin solution that includes NaCl. If a white precipitate forms, it indicates the presence of tannins. (Shaikh et.al.2020,Kokate et.al. 2020)	White precipitate
Sapon-ins	Dilute 2 mL of the extracts with distilled water and shake vigorously. If a layer of foam measuring about 1 cm (approximately 0.39 inches) forms, it suggests the presence of saponins. (Gandran et.al.203,Kumar et.al.2018,Shaikh et.al.2020)	Layer of foam
Triterp-enes	Expose 2 mL of the extracts to chloroform and concentrate H2SO4. If the solution turns.(Shaikh et.al.2020)	Golden yellow coloured solution
	golden yellow, it suggests the presence of triterpenes. (Shaikh et.al.2020,Raaman et.al.2006,)	
Prote-ins	Expose 2 mL of extracts to concentrated nitric acid. Any variations of a yellow-coloured solution emerge, meaning proteins are present. (Shaikh et.al.2020)	Yellow coloured solution
Free-Amino-Acids	Combine 2 mL of the extract with a solution of 0.25% w/v ninhydrin, then heat the mixture to boiling. The formation of a blue-coloured solution signifies the presence of free amino acids. (Semwal et.al.2021,Shaikh et.al.2020)	Blue coloured solution

Table -This table provides a concise overview of each test, its procedure, and the positive reaction showing the presence of specific phytochemicals.

Result and discussion

The research revealed that the extracts displayed enhanced effectiveness at higher concentrations (45mg/ml) against *Pheretima posthuma*. Their anthelmintic efficacy was compared with the reference standard albendazole. The ethanolic extract of *Diplazium esculentum* induced dose dependent paralysis, initially causing decreased motility and eventually leading to insensitivity to external stimuli and death. Notably, the aqueous extract also exhibited significant activity. The study emphasized that the ethanolic extract demonstrated stronger and more pronounced anthelmintic effects compared to the aqueous extracts, with the order of activity being ethanolic extract > aqueous extracts. Additionally, the activity of the extracts varied

with concentration, with higher concentrations resulting in greater potency. The potency of the extracts showed an inverse correlation with the time required for paralysis or death of the worms,but the yeild of extract was high of aqueous extract comparision to ethanolic extract. While identyfy the active compounds of Diplazium esculentum via the Phytochemical screening which determined the different chemical of fronds of *Diplazium esculentum* the conducted phytochemical testing found that *Diplazium esculentum* has many beneficial chemical compounds that are actively responsible for the anthelmintics activity and many other. That was important for further study.

Table no.1 In-vitro anthelmintic activity

S.no.	Groups / Test Substance	Concentration in (mg/ml)	Time of Paralysis in (minutes)	Time of Death in (minutes)
1.	Vehicle Treated	-	-	-
2.	Ethanolic extract Treated	10	22.30±0.21	25.12±0.13
		25	20.40±0.40	23.40±0.20
		45	18.09±0.16	20.20±0.9
3.	Aqueous Extract Treated	10	30.40±0.60	35.80±0.57
		25	27.22±0.17	31.90±0.70
		45	23.02±0.9	29.56±0.43
4.	Albendazole Treated (Standard)	10	8.69±0.21	10.09±0.12
		25	5.47±0.16	7.90±0.71
		45	3.40±0.11	5.61±0.49

Table No.2 Analysis of the Chemical Composition of Ethanolic and Aqueous Extracts from *Diplazium esculentum* fronds

Tests	Ethanolic	Aqueous
Alkaloids (Wagner's test)	Active	Active
Phenols (Ferric chloride test)	Active	Active
Tannins (Gelatin test)	Active	Nonactive
Saponins (Froth test)	Active	Active
Triterpenes (Salkowski's test)	Active	Nonactive
Proteins (Nitric acid test)	Active	Nonactive
Carbohydrates - Reducing Sugars	Active	Active
Free amino acids (Ninhydrin test)	Nonactive	Nonactive

Conclusion:

In conclusion, this study aims to unravel the precise chemical compounds driving the anthelmintic properties observed in *Diplazium esculentum* extracts. By identifying and isolating these compounds, researchers endeavour to establish their efficacy and unravel the pharmacological mechanisms underpinning their anthelmintic effects. This research holds significant promise for developing innovative anthelmintic medications derived from natural sources, offering potential alternatives or supplements to current treatments.

The findings of this study revealed that the ethanolic extract of *Diplazium esculentum* exhibited notably superior anthelmintic activity compared to the aqueous extracts. This discrepancy suggests that the effectiveness of anthelmintic properties is intricately linked to the polarity of the solvent utilized for extraction. The higher polarity of the ethanolic extract likely facilitated the extraction of crucial chemical components responsible for the observed anthelmintic activity.

Overall, these insights shed light on the potential of *Diplazium esculentum* as a valuable source of anthelmintic compounds and underscore the importance of solvent polarity in extracting bioactive constituents. This study paves the way for further research into harnessing the therapeutic potential of natural remedies in combating parasitic infections.

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