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RESEARCH ARTICLE

Comparative *in-vitro* evaluation of anthelmintic property of leaves and rhizome of *Costus pictus* D. Don against albendazole

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ABSTRACT

Background: In developing countries, helminthic infections pose a great threat to public health. Traditional medical remedies using plant products as antiparasitic agents have been in trail throughout the world accounting to limited availability and affordability of modern medicine. More recently, the medicinal plant *Costus pictus* D. Don has been extensively studied by researchers because of its diverse biochemical properties. **Objective:** This study was designed to analyze the plant for its primary constituents and to evaluate the anthelmintic property of methanolic and aqueous extract of leaves and rhizomes of *C. pictus* D. Don in different concentrations and compare it against the standard drug albendazole using earthworm *Pheretima posthuma* as an animal model. **Materials and Methods:** Anthelmintic property was tested by exposing the worms (18 groups with 3 worms each) one after another to different concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml) of methanolic and aqueous extracts of leaves and rhizomes of *C. pictus* D. Don. The absence of movement even after dipping the worm in warm water was noted as time of paralysis, and death was noted when its body color became pale. **Results:** The results of our study showed both the methanolic and aqueous extract of leaves and rhizomes in all concentrations were toxic to the worms. However, methanolic extract of rhizomes at a concentration of 100 mg/ml showed more potency when compared to albendazole (20 mg/ml). **Conclusions:** Methanolic extracts of leaves and rhizomes of *C. pictus* D. Don have potent dose-dependent anthelmintic effect when compared to their aqueous extract.

KEY WORDS: *Costus pictus* D. Don; Anthelmintic Property; *Pheretima Posthuma*; Albendazole

INTRODUCTION

Helminthiasis is one of the most prevalent and preventable infections in developing and poorly developed countries. According to the WHO, more than 1.5 billion people around the globe are infected with soil-transmitted helminthic infections. Anemia, malnutrition, eosinophilia, and pneumonia are the major health issues related to these helminthic infections.[1] Of all the 350 species of helminths, nematodes such as *Ascaris lumbricoides* (round worm), *Trichuris trichura* (whipworm), Nector American; *Ancylostoma duodenale* (hookworm), *Strongyloides stercoralis* (thread worm), and *Taenia solium* (tapeworm) are the most common causative agents of soil-transmitted helminthiases. Hotez et al., in their study stated that the global prevalence of these helminthiases were 807 million for *A. lumbricoides*, 604 million for *T. trichura*, 576 million for Nector americane; *A. duodenale,* and 30-100 million for *S. stercoralis* with the highest prevalence in developing regions of Asia, Africa, and Latin America.[2]

As of now, albendazole, praziquantel, invermectin, diethyl carbamazine, and mebendazole are some common drugs used

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in the treatment and prevention of parasitic worm infections. The high coast and limited availability of these medicines warrants the search of other economic anthelmintic agents.[3] Traditional medicine in developing countries, like India, have been using plants and plant-derived agents for centuries as they hold a greater promise in providing effective and economic treatment against parasitic infections, especially in rural population.

Many indigenous plants have been reported to possess anthelmintic properties. *Costus pictus* D. Don, a novel medical plant belonging to the family Zingiberaceae, has gained interest among researchers for its diverse biochemical properties. As not much research is done on the reason for anthelmintic property of this plant, an attempt has been made to analyze the plant for its primary constituents and to evaluate the anthelmintic property of methanolic and aqueous extract of leaves and rhizomes of *C. pictus* D. Don in different concentrations and compare it against the most commonly used standard drug albendazole.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of 1 year old *C. pictus* D. Don were collected during summer from the local gardens of Pondicherry. The specimen was identified and authentication certificate issued by the Department of Botany, Annamalai University, Chidambaram (No. 326). Voucher specimen of the plant is preserved in the Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth University, Puducherry.

Preparation of Plant Extract

The leaves and rhizomes after washed thoroughly with water were air dried in shade for 5 days and powdered. The powdered material was soxhalated with methanol and distilled water separately for obtaining methanolic and aqueous extract of leaves and rhizomes. The extract was then evaporated to complete dryness using rotary evaporator. The final content was stored separately in airtight brown capped bottle in a refrigerator.

Experimental Worms

Owing to anatomical and physiological resemblance to human intestinal roundworm, earthworms have been used extensively for basic evaluation for newer anthelmintic agents by researchers.[4] Moreover, all clinical anthelmintics are toxic to earthworms as well. In our study, we used adult earthworm (*Pheretima posthuma*) collected from moist soil and the same was confirmed by a local zoologist. The worms of equal size (3-5 cm length and 0.1-2 cm width) were cleaned off its adhering fecal matter with tap water and later used for the study.[5]

Drugs and Chemical

Methanol was procured from Changshu Yangyuan Chemicals and Albendazole from GlaxoSmithkline.

Evaluation of Anthelmintic Activity

The drug and extract solutions were freshly prepared and poured in a separate petri dish before starting the experiment. The extracts were dissolved in minimum amount of 0.5% carboxymethyl cellulose (CMC) and the final volume adjusted to 10 ml with distilled water. The corresponding concentration was expressed in term of mg of extract per ml of solvent (mg/ml). The procedure was performed with one worm at a time.

The earthworms were divided into 18 groups (3 worms in each group). The first group served as control received 0.5% CMC in distilled water, and the second group served as standard control received albendazole (20 mg/ml) suspended in 0.5% CMC. The remaining worms were randomly distributed into 16 groups. They were exposed to different concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml) of methanolic and aqueous extracts of *C. pictus* D. Don suspended in 0.5% CMC.

The time taken for paralyzes and death of all the worms was observed individually. The time of paralysis was confirmed when no movement of any sort could be observed except when the worms were shaken vigorously. The time of death was confirmed after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C) and when the body color turned pale.[6]

RESULTS

Primary phytochemical analysis for the presence of carbohydrates, proteins, phenols, flavonoids, steroids, alkaloids, tannins, glycosides, saponins, ascorbic acid, and triterpenoids was carried as per the methods described by Hariprasad and Ramakrishnan[7] and Bhargava et al.[8] with few modifications.

The results of our study showed the methanolic extract of *C. pictus* D. Don leaves and rhizome had more phytochemical constituents when compared to aqueous extract. Phenols and quinones were absent in both methanolic and aqueous extract of *C. pictus* D. Don rhizomes (Tables 1 and 2).

DISCUSSION

The anthelmintic potency of the extracts is inversely proportional to the time taken for paralysis or death of the

Table 1: Primary phytochemical analysis of methanolic and aqueous extracts of leaves and rhizomes of *C. pictus* D. Don

Plant constituents	Methanolic leaf extract of *C. pictus* D. Don	Methanolic extract of *C. pictus* D. Don rhizome	Aqueous leaf extract of *C. pictus* D. Don	Aqueous extract of *C. pictus* D. Don rhizome
Carbohydrate	+	++	+	++
Proteins	+	+	+	+
Phenols	++	-	+	-
Flavonoids	++	++	+	+
Steroids	+	+	+	+
Alkaloids	++	++	+	+
Tannins	++	++	+	+
Glycosides	++	++	+	+
Saponins	+	+	+	+
Ascorbic acid	++	+	++	+
Triterpenoid	++	++	+	+
Quinones	+	-	+	-

+: Present, ++: Present in high concentration, -: Absent, *C. pictus*: *Costus pictus*

Table 2: Anthelmintic activity of methanolic and aqueous extract of leaves and rhizomes of *C. pictus* D. Don

Experimental groups	Concentration (mg/ml)	Earthworm (*P. posthuma*)	
		Time taken for paralysis (P) in minutes (mean±SEM)	Time taken for death (D) in minutes (mean±SEM)
Control (0.5% CMC)	-	-	-
Albendazole (standard control)	20	10.93±0.25	13.98±0.38
Methanolic leaf extract of *C. pictus* D. Don	25	23.10±0.99	26.18±0.45
	50	17.87±0.44	19.02±0.33
	75	13.59±0.21	14.70±0.31
	100	11.61±0.21	12.72±0.14
Methanolic extract of *C. pictus* D. Don rhizome	25	19.81±0.34	21.49±0.59
	50	13.63±0.39	16.15±0.93
	75	11.54±0.24	13.70±0.61
	100	10.16±0.29	13.06±0.28
Aqueous leaf extract of *C. pictus* D. Don	25	35.79±0.28	38.17±0.59
	50	30.08±0.65	32.19±0.93
	75	16.51±0.30	18.35±0.59
	100	13.59±0.27	15.07±0.59
Aqueous extract of *C. pictus* D. Don rhizome	25	20.54±0.49	23.54±0.82
	50	15.76±0.67	17.26±0.62
	75	13.57±0.22	14.83±0.26
	100	11.02±0.24	13.61±0.21

CMC: Carboxy methyl cellulose, *P. posthuma*: *Pheretima posthuma, C. pictus: Costus pictus,* SEM: Standard error of mean

worms. It is notable from our study that though all the extracts were toxic to the worms, methanolic extract of *C. pictus* D. Don rhizomes had more potent anthelmintic activity with reference to the standard drug albendazole. Moreover, it was also observed that there was a dose-dependent anthelmintic activity of all the extracts, i.e. more the concentration greater the potency. A study by Srivastava et al., on another species of Costus, *Costus speciosus Koen* also showed the same result with methanolic and aqueous extracts of root and rhizome. However, in their study, aqueous extract showed a more significant effect in terms of paralysis of worms.[6] Another study by Thomas and Devi done to evaluate the anthelmintic property of hydro-alcoholic extract of *C. pictus* D. Don rhizome tested against piperazine citrate on adult earthworm; *Eudrilus eugeniae* also proved the anthelmintic effect of the rhizome.[9]

Williams et al., in their study reported that plant-derived tannins have a definite anthelmintic effect on free living/ non-infective stages of adult human intestinal parasites.[10]

Our research also showed a higher concentration of tannins in methanolic extracts when compared to aqueous extracts of leaves and rhizomes of *C. pictus* D. Don (methanolic extract of rhizome: 2.8 mg, methanolic leaf extract: 2.2 mg, aqueous extract of rhizome: 1.3 mg, and aqueous leaf extract 1.2 mg). This may be the reason for increased anthelmintic activity of methanolic extract of *C. pictus* D. Don rhizomes and leaves when compared with their aqueous extracts.

Plant-derived cysteine proteinase digests the proteins in the cuticle of adult gastrointestinal nematodes. However, our study did not focus on the presence of cysteine proteinase or the damage caused by them on the worm setting as a limitation for our study.[11]

As earthworms are the basic model for evaluating anthelmintic activity, further studies are required on animal and human models and to establish the pharmacological rationale for using this plant as anthelmintic drug.

CONCLUSIONS

Standing by the aim of this study, methanolic extracts of leaves and rhizomes of *C. pictus* D. Don have definite dose-dependent anthelmintic effect as tested on the preliminary model used. A multifaceted treatment approach for helminthic infections including both complementary alternative and conventional treatment would be a better strategy in producing optimal health benefits. Furthermore, considering social factors such as aversion toward modern medicine, economic, and health burden, *C. pictus* D. Don will be a potent, effective, and economic anthelmintic agent provided further studies are done focusing on the precise mechanism of action and *in vivo* study with other animal models.

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