



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(1): 203-205
Received: 17-11-2015
Accepted: 18-12-2015

Somnath De
Department of Biotechnology,
PanskuraBanamali Collage,
West Bengal, India.

Dulal Chandra Das
Department of Botany, Raja
Narendranal Khan Womens'
College, West Bengal, India.

Tanusri Mandal
Bioconversion Laboratory,
Department of Biotechnology,
Vidyasagar University, West
Bengal, India.

***In-vitro* anthelmintic activity of *Cardanthera difformis* Druce whole plant methanolic extract in Indian adult earthworm**

Somnath De, Dulal Chandra Das, Tanusri Mandal

Abstract

Different extracts of *C. difformis* were taken for anthelmintic activity against Indian earthworm *Pheretima posthuma*. Two concentrations (50 and 100mg/ml) of various extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole (20 mg/ml) was used as reference standard and carboxy methyl cellulose (0.5%) as a control group. Dose dependent activity was observed in the plant extracts but methanolic extract exhibited more activity as compared to others. The anthelmintic activity of *Cardanthera difformis* whole plant extract has therefore been demonstrated for the first time.

Keywords: *Cardanthera difformis*, *Pheretima posthuma*, Methanolic extract.

1. Introduction

During the last few decades, there has been an increasing interest in the study of traditional plants and their medicinal value in different parts of the world [1]. Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tapeworm. These worms inhabit in the gastrointestinal tract and may also burrow into the liver and other organs. Infected people excrete helminth eggs in their faces, which then contaminate the soil in areas with inadequate sanitation [2]. Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Helminthic infections are one of the most widespread infections in humans, affecting a huge population of the world, this infections cause enormous hazard to health and resulting in undernourishment, anaemia, eosinophilia and pneumonia. As per the reports of WHO most of the drugs used against these worms are synthetic but these synthetic drugs are out of reach of millions of people and have a lot of side effect [3]. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases [4].

Fasciola hepatica is well known as liver-fluke, responsible for fascioliasis in humans and ruminants in temperate regions of the world including Bolivia, Chile, Cuba, Ecuador, Egypt, France, Peru, Portugal, Spain, Vietnam etc. [5-6]. The flatworm damages liver parenchyma and bile ducts of its host like sheep, cow, buffalo, man, may be responsible for death of the host [7-8]. Anthelmintics obtained from medicinal plants can be utilized for the treatment of parasitic infections effectively [9]. Several plants including *Alpinia nigra*, *Anogeissus leiocarpus*, *Dregea volubilis*, *Ficus benghalensis*, *Flemingia vestita*, *Piper sp.*, *Zanthoxylum rhetsa* have been tested for their anthelmintic efficacy [10-15]. The plant *Cardanthera difformis* which is chosen, is a weed. It is a tropical aquarium plant under the family *Acanthaceae* and common known as water wisteria, used as environmental ornaments, found in marshy habitats on the Indian subcontinent including Bangladesh, Bhutan, and Nepal [16].

No report was found regarding anthelmintic activity of *Cardanthera difformis* till the date. The purpose of the present work was to investigate the anthelmintic activity of methanolic and aqueous extract of *C. difformis* (MELC & AELC) leaves in Indian earthworm.

2. Material and Method

2.1 Plant material

Cardanthera difformis Druce has been selected for experiment tools. It is collected in the month of march, 2015 from Paschim Medinipur district (Latitude- 22°25'00" to 22°57'00"

Correspondence

Tanusri Mandal
Bioconversion Laboratory,
Department of Biotechnology,
Vidyasagar University, West
Bengal, India.

north, Longitude- 87°11' east, Altitude- 23 meters from mean sea level), West Bengal, India and it is available in any season of year.

2.2 Experimental worms

All the experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with normal saline water to remove all fecal matters. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used in all the experimental protocol

2.3 Preparation of extracts

The leaves of *Cardanthera difformis* were dried under shade and crushed in an electric blender to form coarse powder and subjected to Soxhlet extraction (Continuous hot extraction) by using methanol and water as solvent. The extracts were concentrated by rotary evaporator and used for testing anthelmintic activity.

2.4 Administration of Albendazole

Albendazole (20 mg/ml) was prepared by using 0.5% w/v of CMC as a suspending agent as administered as per method of extract.

2.5 Administration of extract

The suspension of methanolic and aqueous extract of *Cardanthera difformis* of different concentration (50,100 mg/ml) were prepared by using 0.5% w/v of CMC as a suspending agent and final volume was made up to 10 ml for respective concentration. Albendazole was used as standard. Groups of approximately equal size worms consisting of two earthworms individually in each group were released into in each 10 ml of desired concentration of drug and extracts in the petridish.

2.6 Experimental design

The anthelmintic activity was performed according to the method [17]. On adult Indian earth worm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. *Pheretima posthuma* was placed in petridish containing two different concentrations (50 & 100 mg/ml) of methanolic & aqueous extract of leaves of *Cardanthera difformis*. Each petridish was placed with 2 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with reference compound Albendazole (20 mg/ml) treated samples.

3. Results and Discussion

The data revealed that the methanol extract showed anthelmintic activity at a concentration of 100 mg/ml, whereas the aqueous extract also showed paralysis and death at similar concentrations. The other test concentrations of both the extracts showed marked degree of anthelmintic activity. The anthelmintic effect of extracts is comparable with that of the effect produced by the standard drug albendazole. Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. Hundreds

of millions if not billions of human infections by helminthes exist worldwide and increased world travel and immigration from the developing countries. However tremendous advances has been made during the previous decade and substantial number of synthetic precursors have been derived to cope up the damage caused by parasite, but unfortunately no effective medicine has been developed so far. Moreover the problems associated with the use of such drugs like some serious side effects and development of resistance drives the severity of infection to the next level. These factors paved the way for herbal remedies as alternative anthelmintics. Evaluation of activities of medicinal plants claimed for possessing the anthelmintic property is getting the attention these days. Screening and proper evaluation of the claimed medicinal plants could offer possible alternatives that may be both sustainable and environmentally acceptable. The results of this study have shown promising anthelmintic activity suggesting the possible use of *Cardanthera difformis* extracts in intestinal nematode control.

Table 1: Anthelmintic potency of methanolic and aqueous extract of *Cardanthera difformis*

Extract	Concentration (mg/ml)	<i>Pheretima posthuma</i>	
		Paralysis (P)	Death (D)
Control (0.5% CMC)	-	-	-
Standard (Albendazole)	20 mg/ml	28.50 ± 1.80	58.90 ± 5.65
Methanolic extract	50 mg/ml	24.30 ± 1.30	56.45 ± 1.45
	100 mg/ml	21.22 ± 1.02	43.51 ± 1.05
Aqueous extract	50 mg/ml	21.40 ± 1.56	53.10 ± 1.08
	100 mg/ml	10.40 ± 0.10	41.01 ± 0.10

All values represent mean ± SD; n=2 in each group. Comparisons made between standard versus treated groups, $P < 0.05$ was considered significant

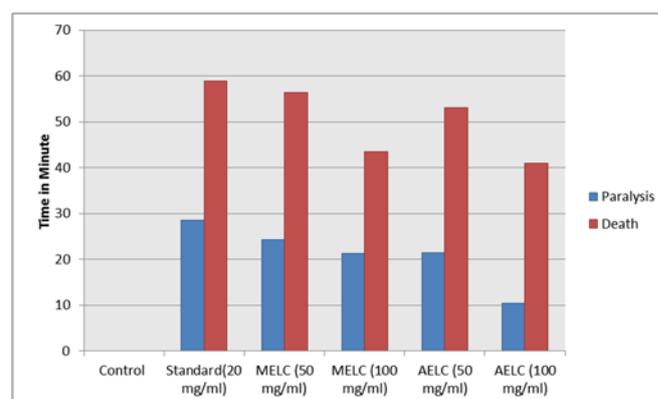


Fig 1: Anthelmintic activity of *Cardanthera difformis* leaves on *Pheretima posthuma* MELC stands for methanolic extract, AELC stands for aqueous extract.

4. Conclusion

The results of the present study clearly indicated that the crude methanol extract of *Cardanthera difformis* did produce anthelmintic activity against Indian earthworm *Pheretima posthuma*. The plant possesses significant anthelmintic activity at 100 mg/ml concentration measured by time taken for paralyse / death of the earth worms. The current investigation leads to conclusion that the leaves of *Cardanthera difformis* have potent anthelmintic activity when compared with the conventionally used drug. The results did not, however, exclude the possibility that doses of

the extract with lower anthelmintic activity in this study might be efficacious against other species of helminths. Further studies using *in vivo* models and to isolate active constituents from extract are required to carry out and established the effectiveness and pharmacological rational for the use of *Cardanthera difformis* as an anthelmintic drug.

Druce leaf extracts from West Bengal, India. Int. J phytomedicine. 2013; 5:446-451.
17. Ghosh T, Maity TK, Bose A, Dash GK. Indian J nat Product. 2009, 16-19.

5. Reference

1. Prashant KR, Dolly J, Singh KR, Gupta KR, Watal G. Glycemic properties of *Trichosanthes dioica* leaves, Pharm Biol. 2008; 12:894-899.
2. Idika IK, Okonkwo EA, Onah DN, Ezech IO, IheagwamCN, Nwosu CO. Efficacy of levamisole and ivermectin in the control of bovine parasitic gastroenteritis in the sub-humid savanna zone of southeastern Nigeria. Parasitol. Res. 2012; 111:1683-7.
3. Bundy DA. Immunoepidemiology of intestinal helminthic infection I: The global burden of intestinal nematode disease. Trans Royal Soc Trop Med Hyg. 1994; 8:259-261.
4. Sondhi SM, Shahu R. Magan Archana. Indian Drugs, 1994; 31:317-320.
5. Kirchhofer C, Vargas M, Braissant O. Activity of OZ78 analogues against *Fasciola hepatica* and *Echinostomacaprioni*. Acta Tropica. 2011; 118:56-62.
6. Nguyen TGT, Le TH, Dao THT. Bovine fasciolosis in the human fasciolosis hyperendemic Binh Dinh province in Central Vietnam. Acta Tropica. 2011; 117:19-22.
7. Milligen FJV, Cornelissen JBWJ, Gaasenbeek CPH, Bokhout BA. A novel *ex vivo* rat infection model to study protective immunity against *Fasciola hepatica* at the gut level. J Immunol Methods. 1998; 213:183-90.
8. Keiser J, Morson G. *Fasciola hepatica*: *Fasciola hepatica*: Surface tegumental responses to *in vitro* and *in vivo* treatment with the experimental fasciolicide OZ78. Exp Parasitol. 2008; 119:87-93.
9. Carvalho CO, Chagas ACS, Cotinguiba F. The anthelmintic effect of plant extracts on *Haemonchus contortus* and *Strongyloides venezuelensis*. Vet Parasitol. 2012; 183:260-8.
10. Agaie BM, Onyeyili PA. Anthelmintic activity of the crude aqueous leaf extracts of *Anogeissus leiocarpus* in sheep. Afr J Biotech. 2007; 6:1511-5.
11. Aswar M, Aswar U, Watkar B, Vyas M, Wagh A, Gujar KN. Anthelmintic activity of *Ficus benghalensis*. Int J Green Phar. 2008; 2:170-2.
12. Hossain E, Chandra G, Nandy AP, Mandal SC, Gupta JK. Anthelmintic effect of a methanol extract of leaves of *Dregea volubilis* on *Paramphistomum explanatum*. Parasitol Res. 2012; 110:809-14.
13. Roy B, Swargiary A. Anthelmintic efficacy of ethanolic shoot extract of *Alpinia nigra* on tegumental enzymes of *Fasciolopsis buski*, a giant intestinal parasite. J Parasit Dis. 2009; 33:48-53.
14. Tandon V, Pal P, Roy B, Rao HSP, Reddy KS. *In vitro* anthelmintic activity of root-tuber extract of *Flemingia vestita*, an indigenous plant in Shillong, India. Parasitol Res. 1997; 83:492-8.
15. Yadav AK, Tangpu V. Therapeutic efficacy of *Zanthoxylum rhetsa* DC extract against experimental *Hymenolepis diminuta* (Cestoda) infections in rats. J Parasit Dis. 2009; 33:42-7.
16. Das DC, De S, Bhattacharya S, Das M. Antibacterial and phytochemical analysis of *Cardanthera difformis*