

PHYTOCHEMICAL SCREENING AND ANTHELMINTIC ACTIVITY OF METHANOLIC EXTRACT OF *IMPERATA CYLINDRICA*

*PARVATHY N.G, PADMA.R, RENJITH.V, KALPANA. P. RAHATE, SARANYA T.S.

Amrita School of Pharmacy, AIMS Health Sciences Campus, Ponekkara PO., Kochi, Kerala 682041. Email: parvathy_narayan06@yahoo.com

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ABSTRACT

The study aimed to investigate potential *in vitro* anthelmintic activity of methanolic extract of roots of *Imperata cylindrica* (Poaceae) against Indian earthworms *Pheretima posthuma*. Various concentrations (10-80 mg/50ml) of the extract were tested, which involved determination of time of paralysis and time of death of the worms. The methanolic extract exhibited a maximum anthelmintic activity ($p < 0.01$) comparable to standard drug albendazole (1000 mg/50ml). The extract showed anthelmintic activity in dose dependant manner giving shortest time of paralysis (P) and death (D) with 80mg/ml concentration and caused paralysis in 3.3 min and death in 6.0 min. The Preliminary phytochemical analysis indicated the presence of various phytoconstituents in the extract of which tannins and saponins might have contributed for the potent anthelmintic activity.

Keywords: *Imperata cylindrica*, Anthelmintic activity, *Pheretima posthuma*, Tannins, Saponins.

INTRODUCTION

Helminths are endo-parasites which are recognized as a major constraint to livestock production in the tropical countries and elsewhere. They are mostly present in the human intestines either in large or small intestine and produce infections. Such types of infestation produced by worms are called as Helminthiasis.^{1,2} Parasitoses have been concerned to the medical field for centuries and the helminthes are still the cause of considerable problems for human beings and animals. Anthelmintics are drugs which may kill (Vermicide) or expel (Vermifuge) infesting helminthes.³ Chemotherapy is the only treatment and effective tool to cure and control helminth infection, as effective vaccines against have not been developed so far. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites.⁴ Thus the studies on the herbs and the phytochemicals focused in extracting anthelmintic constituents like tannins, phenols and other phytoconstituents. This work mainly focused on the anthelmintic activity of *Imperata cylindrica* due to the presence of tannins and phenols.

The family Poaceae formerly known as Graminae comprising 600 genera and 9000-10000 more species of grasses is an economically important family which includes lawn and forage grasses, staple food grains, cereal crops and bamboo which is widely used in construction.⁴

Imperata cylindrica is known as Thatch grass or Cogon grass (English) and Dabh (Hindi).⁵ It is a perennial, monocot plant. It is an important drug of Tripanchmool and used in urinary calculi, retention of urine, diabetes, cardiac disorders, gout, common cough and cold, anemia.⁶ The edible part of the plant includes young inflorescence and young shoots which are cooked and fibrous root contain starch and sugar but pleasant to chew. Apart from edible use, the medicinal uses include antibacterial, anthelmintic, astringent. It is effective in conditions like arthritis, diarrhea, dysentery, gonorrhoea, cancer, diuretic, emollient, febrifuge, restorative, sialogogue, styptic and tonic.⁷ These plants are also used for soil stabilization stuffing, thatching, paper industry⁸ and also in weaving.⁴ The major chemical constituents includes carbohydrates, glycosides, flavanoids, triterpenoids.⁹⁻¹⁰

MATERIALS AND METHOD

Plant material

The plant *Imperata cylindrica* was collected from the local regions of Ernakulam and was authenticated. The roots were separated and

washed thoroughly with sufficient water. It was dried under shade for 2 weeks and powdered in an electrical blender.

Preparation of the extract

The powdered roots of *Imperata cylindrica* was defatted with petroleum ether. The marc was successively extracted with chloroform, dichloromethane, acetone, methanol by cold maceration process for one week. The aqueous extract was prepared by boiling the powder with successive volumes of water and combined. The macerated pulp from each solvent was filtered through a coarse sieve and the filtrate was concentrated in rotary vacuum evaporator.

Preliminary Phytochemical study

Preliminary phytochemical screening of the extracts was performed by standard methods for the presence of phytochemical constituents.¹¹⁻¹²

Anthelmintic activity

The anthelmintic activity was carried as per the method of Ajaiyeoba et al. with minor modifications.¹³

Animals

Indian adult earthworms (*Pheretima posthuma*) were used to study the anthelmintic activity as these worms resemble both anatomically and physiologically to the intestinal round worms.¹⁴⁻¹⁶ The earthworms were collected from the swamp areas and washed with normal saline to remove all the mud and fecal matter. The adult earthworms of 6-7 cm in length and 0.2-0.4 cm in width were used for the investigation.

Preparation of Test Sample

Samples for experiment were prepared by dissolving the methanolic extract of *Imperata cylindrica* (MEIC) to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 10, 20, 40 and 80 mg/50 ml in distilled water.

Albendazole (1000mg/50 ml) was used as reference standard.

Method

The anthelmintic activity of the methanolic extract of *Imperata cylindrica* (MEIC) was carried out by methodology followed by Singh et al¹⁷ and Bhabani SN et al¹⁸ with modifications. Six groups of approximately equal size earthworms consisting of four earthworms in each group were used for the present study. Group I served as control and treated with normal saline. Group II served as standard and treated with albendazole. Group III, IV, V and VI were treated with 10, 20, 40 and 80 mg/50 ml of MEIC respectively.

All the observations were made from the time taken to paralysis and death of individual worms. Paralysis was stated when the worms did not revive in normal saline. Death was concluded when the worms lost their mortality followed with fading away of their body colour. The study was carried out in triplicate for each concentration of the extract.¹⁹

RESULTS

Preliminary phytochemical screening revealed the presence of carbohydrates, glycosides, alkaloids, saponins, tannins, flavanoids and steroids (Table 1).

As shown in Table 2, MEIC *Imperata cylindrica* displayed significant anthelmintic activity at higher concentrations. The extract showed potent anthelmintic activity in dose dependant manner giving shortest time of paralysis (P) and death (D) with 80mg/50 ml concentration. The extract solution at 80 mg/50 ml concentration caused paralysis in 3.3min and death in 6.0 min, of the earthworms. The reference drug albendazole in 1000mg/50 ml concentration showed paralysis and death at 3.0 min and 6.3 min respectively. The extract produced dose dependent paralysis ranging from loss of motility to loss of response to

external stimuli, which eventually progressed to death. Also as shown in figure 1, increase in concentration of the extracts showed decrease in the paralysis and death time of the worms.

Table 1: Qualitative analysis of various extracts of *Imperata cylindrica*

Phytoconstituents	Dichloromethane	Chloroform	Acetone	Methanol
Alkaloids	+	+	-	+
Carbohydrates	-	+	+	+
Flavanoid	-	+	+	+
Glycosides	-	-	-	+
Proteins	-	-	+	+
Saponins	+	+	+	+
Steroids	-	-	+	+
Tannins				+

Table 2: Anthelmintic activity of *Imperata cylindrica*

Concn. (mg/50ml)	Average time (min)	
	Paralysis	Death
10	71.6 ± 1.76	78 ± 2.85
20	42.4 ± 1.71	50.9 ± 0.73
40	27 ± 0.08	34 ± 0.04
80	3.3 ± 0.57	6 ± 0.09
Standard	3 ± 0.05	6.3 ± 0.57

Values are expressed as Means ± SD for 4 earthworms in each group.

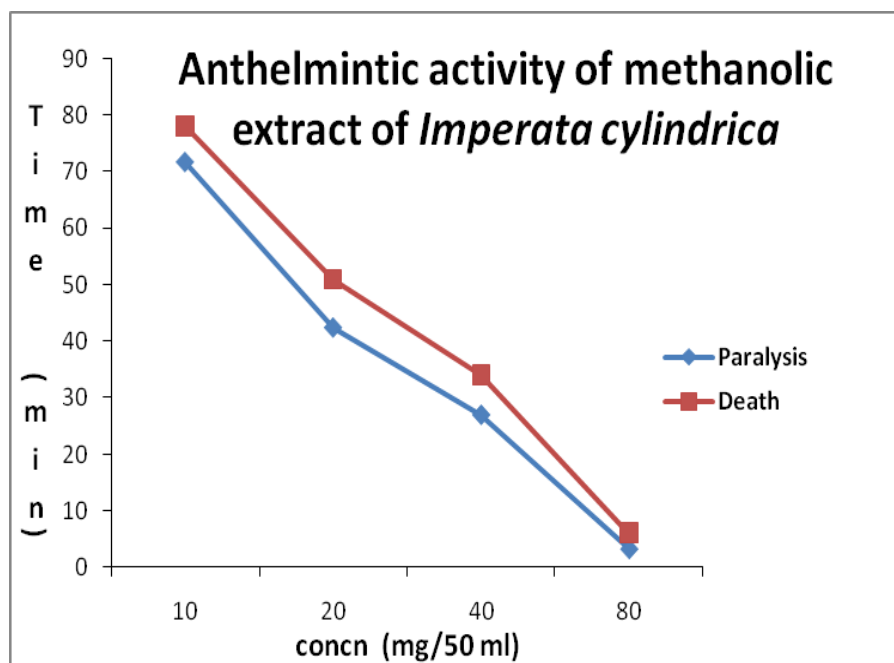


Fig. 1: Anthelmintic activity of methanolic extract of *Imperata cylindrica*

DISCUSSION

The predominant effect of anthelmintic drugs on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole act probably by blocking glucose uptake and depletion of its glycogen stores. They bind to free protein in the gastrointestinal

tract of the host animal or glycoprotein on the cuticle of parasite and cause death.²⁰ Intra cellular microtubules in the cells of the worm are gradually lost. The root extract of *Imperata cylindrica* not only demonstrated paralysis, but also caused death of worms especially at

higher concentration of 80 mg/50 ml, in shorter time as compared to reference drug albendazole.

Phytochemical analysis of the crude extracts revealed presence of tannins as one of the chemical constituent. Tannins are polyphenolic compounds. Tannins were shown to produce anthelmintic activities. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide, bithionol etc are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is possible that tannins contained in the extracts of *Imperata cylindrica* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death.²¹ The phytochemical screening of the extract also revealed the presence of saponins. Recent research addressed that the main biologic activity ascribed to saponins was their membrane permeabilizing property and pore formation which is similar with two conventional anthelmintic drugs such as praziquantel and toltrazuril. That is, they would affect the permeability of the cell membrane of the parasites and cause vacuolization and disintegration of monogenea teguments.²²

In conclusion, the traditional claim of *Imperata cylindrica* as an anthelmintic have been confirmed as the root extracts displayed activity against the worms used in the study. The active constituents responsible for anthelmintic activity are present in the methanolic extracts of the plant. Further studies will emphasize on *in vivo* models to carry out and establish the effectiveness and pharmacological rationale for the use of *Imperata cylindrica* as an anthelmintic drug, to isolate and reveal the active compound (S) contained in the crude extracts of *Imperata cylindrica* and to establish the mechanism of action. The increasing pervasiveness of anthelmintic resistant strains of helminthes and high cost of conventional anthelmintic has created an interest in researching medicinal plants as an alternative source of anthelmintics.²³

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