



IN-VITRO EVALUATION OF ANTHELMINTIC ACTIVITY OF CROTON TIGLIUM SEED EXTRACTS

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ABSTRACT

Background: Helminthes have been common cause of concern and pose many problems to human beings and animals. Many medicinal plants claimed to possess anthelmintic activity and can be effectively used against these types of disorders. *Croton tiglium* plant commonly known as Jamalgota was traditionally used to treat Helminthiasis. **Objective:** To test Anthelmintic activity of aqueous and petroleum ether extracts of *Croton tiglium* seeds. **Materials and Methods:** Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. The activity was checked in aqueous and petroleum ether extracts at three different concentration (25, 50 and 75mg/ml) and results were expressed in terms of paralysis time and death time for worms. The results were compared with standard solution, Albendazole (20 mg/ml & 40mg/ml). **Results:** Both the extract showed significant anthelmintic activity. The effect was dose dependant and shortest time taken for paralysis and death was observed in case of Petroleum ether extract at 75 mg/ml concentration. **Conclusion:** It was concluded that seeds of *Croton tiglium* have potential anthelmintic activity.

Keywords: Anthelmintic, Aqueous extract, *Croton tiglium*, Petroleum ether extract

INTRODUCTION:

Helminthiasis or infection due to parasitic worms, affect almost two billion people worldwide causing malnutrition, blindness, debility, disfigurement and death.⁽¹⁾ The half of world suffering from bacterial and helminthes infection, due to poor sanitation, poor family hygiene, malnutrition, and crowded living conditions.⁽²⁾ Due to discovery and development of anthelmintic physician now have effective, and in some cases, broad spectrum of agents that will cure or control most infections caused by flukes or intestinal helminthes.⁽³⁾ Development of resistance to most of the commercially available antihelminthiasis became a severe problem worldwide.⁽⁴⁾ Medicinal plants are the source of great economic value in the Indian subcontinent.

Herbal medicines are of great importance as about 75–80% of the whole population rely on natural products because of better cultural acceptability, better compatibility and fewer side effects. Nowadays multiple drug resistance has been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, the uses of antibiotics are associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reaction.

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. The plant *Croton tiglium* Linn. (*Euphorbiaceae*) commonly known as Jamalgota and Jepak, is a small evergreen tree, with 5-7 m high, found throughout tropical India. Traditionally it is used as a remedy for skin disorders, dysentery, diarrhea, purgative, colds, fever, worms, ascities, and intracranial hemorrhage and in snake poisoning.^[5] The Present work was undertaken to screen anthelmintic potential of petroleum ether and aqueous extract of seeds of plant *Croton tiglium* in adult earth worms.

MATERIALS AND METHODS:

Plant material:

The seeds of *Croton tiglium* were purchased from the local market of Pune. These were authenticated from Agharkar Research Institute, Pune.

Experimental worms:^{[6] [7] [8] [9]}

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. They were collected from local Nursery. The earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. Adult earthworms were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity

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Preparation of extracts:

Aqueous extract:

Seeds of *Croton tiglium* were powdered and then soaked in water for 48 hrs. It was filtered through muslin cloth and filtrate was evaporated to get dry extract. It was stored in airtight container

Petroleum- ether extract:

Powder of seeds was soaked in Petroleum-ether for 1 day. Then it was filtered through muslin cloth and concentrated. The extract was stored in air tight container and was used for anthelmintic activity. Preliminary phytochemical analysis was carried out to find of nature of chemical constituents present in extract.

Standard solution:

Albendazole (20 mg/ml & 40mg/ml) was administered as standard solution.

Test solution:

The suspension of petroleum ether extracts (PEE) was prepared by using Tween 80 as surfactant at different concentration (25, 50 and 75mg/ml) and final volume was made up to 10 ml for respective concentration. The different concentration (25, 50 and 75mg/ml) of aqueous extracts (AE) were also prepared. All the extracts and the standard drug solution were freshly prepared before starting the experiments.

Experimental design:⁽¹⁰⁾

The anthelmintic activity was carried out in 9 groups; each group consists of 6 worms and worms were released into 10 ml of desired formulations. Group 1 were the control worms placed in normal saline; Group 2-4 received petroleum extract of *Croton tiglium* seeds at 25, 50 and 75mg/ml concentrations; Group 5-7 were treated with aqueous extract of various (25, 50 and 75mg/ml) concentrations.

Group 8 and 9 serves as standard and was treated with Albendazole (20 mg/ml & 40mg/ml). The observations were made for the time taken for paralysis and Death of worm. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with their body colors fading away.

RESULTS AND DISCUSSION:

Preliminary Phytochemical analysis of the petroleum ether extract showed presence of alkaloids, saponins, terpenoids and tannins while aqueous extract showed presence of alkaloids, Flavonoids, carbohydrates, and proteins. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity.

In vitro Anthelmintic activity:

In anthelmintic assay, the petroleum ether and aqueous extracts showed paralysis and death of worms. The effect was dose dependant and shortest time taken for paralysis and death was observed in case of Petroleum ether extract at 75 mg/ml concentration. Both the extracts

showed comparable results as that of standard drug as displayed in Table 1. Aqueous extract at 25 and 50 mg/ml concentration showed better results than petroleum ether extract at same concentration.

Table 1: Anthelmintic Activity of Petroleum and Aqueous Extracts of *Croton tiglium*

Treatment	Concentration mg/ml	Time taken for paralysis (min)	Time taken for death (min.)
Control (Normal Saline)	-	-	-
Petroleum ether extract	25	50.17±0.87	79.33±2.41
	50	39.83± 1.32	60.00±1.46
	75	20.33±0.33	39.17±1.53
Aqueous extract	25	40.67±1.05	75.17±1.10
	50	35.83±1.66	60.17±1.35
	75	30.50±0.76	40.33±1.28
Albendazole	20	68.83±1.57	79.00±1.57
	40	35.00±1.15	39.33±1.11

All Values represents Mean± SEM; n=6 in each group

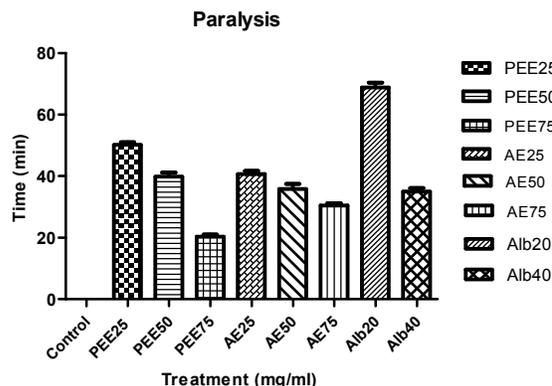


Figure 1: Time taken for paralysis after treatment with different extracts

Where, PEE= Petroleum ether extract; AE= Aqueous Extract; Alb= Albendazole; the number represents concentration

As displayed in figure 1 and figure 2 at 25 and 50 mg/ml concentration petroleum ether extract showed comparable results as that of reference drug Albendazole. At 75 mg/ml concentration of petroleum ether extract, paralysis and death of worms were observed at 20.33 min and 39.17 min respectively which were found to be better as compared to results shown by aqueous extract and the reference drug Albendazole.

Aqueous extract exhibited paralysis and death of worms in 40.67 and 75.17 min respectively at 25 mg/ml concentration which was found to be more effective as compared with that of Petroleum ether extract and reference drug as shown in figure 1, 2. The comparison between treated groups with standards was carried out using one way ANOVA test. All the results were found to be significant with P value less than 0.0001 (P< 0.0001)

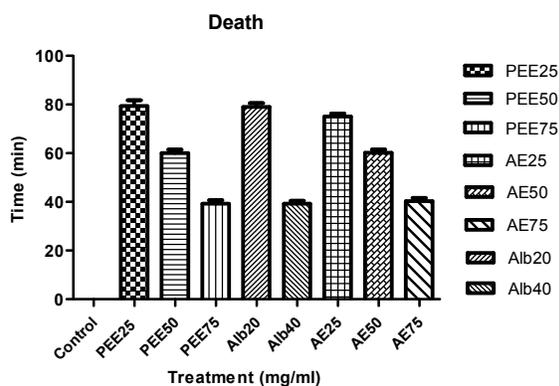


Figure 2: Time taken for Death after treatment with different extracts

Where, PEE= Petroleum ether extract; AE= Aqueous Extract; Alb= Albendazole; the number represents concentration

CONCLUSION:

It was concluded that seeds of *Croton tiglium* showed potential anthelmintic activity. Further research work is needed to isolate phytoconstituent responsible for anthelmintic activity.

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