TO STUDY ANTHELMINTIC ACTIVITY OF CROTON BONPLANDIANUM WHOLE PLANT

Vilas B. Ghawate^{*1}, Varsha S. Jadhav¹, Chetan C. Kedari¹, Pratiksha V. Bavane¹, Ashwini B. Belge¹

Author's affiliations

1. Department of Pharmacognosy, Mula education society's college of pharmacy, Sonai-414105, India (MS).

Corresponding author's details

Vilas B. Ghawate^{*1} Head of Department of Pharmacognosy, Mula education society's college of pharmacy, Sonai-414105, India (MS). E-mail id- ghawatevilas@gmail.com

ABSTRACT

The present study was undertaken to evaluate the anthelmintic activity of whole plant of *Croton bonplandinum* in a scientific manner. All parts of plant have been reported to relieve a variety of ailments. The anthelmintic activity was evaluated on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being. The ethanol extract of whole plant of *Croton bonplandinum* (EECB) at 10, 25 and 50 mg/ml caused paralysis followed by death of worms at all tested dose level with standard as Albendazole. The EECB exhibited significant anthelmintic activity at concentration of 10, 25 and 50 mg/ml as compared to reference standard Albendazole.

Keywords: - Anthelmintic, Croton bonplandinum, EECB, Phytochemical, Albendazole.

INTRODUCTION:

Helminthiasis or infection with parasitic worms are the most common infectious agents of human in developing countries and produce a global burden of disease that exceeds better known conditions i.e., tuberculosis and malaria. The disease is highly prevalent particularly in third world countries due to poor management practices. In tropical regions, where prevalence is greatest, simultaneous infection with more than one type of helminths is common. Moreover, human beings can spread these pathogens to previously uninvolved populations through travel, migration & military operations. ^[1, 2, 3]

There are two major phyla of helminthes of which the nematodes includes the major intestinal worms and filarial worms that cause lymphatic filariasis and onchocerciasis, whereas the Platyhelminthes include the flukes, such as the *schistosomes* and the tapeworms, such as the pork tapeworm that causes cysticercosis. The World Health Organization reveals that over two billion people are suffering from parasitic worm infections. It is estimated that by the year 2025, about 57% of the population in the developing countries will be influenced by helminths infections. ^[4]

In recent times focus on plant research has increased all over the world and there is widespread of belief that the green medicines are healthier and harmless than the synthetic ones. Plants are good sources for new, safe, biodegradable and renewable drugs however the use of plants as therapeutic agents in addition to being used as food is age long.

Anthelmintic plants are used traditionally in expelling the worms that is parasitic in nature from the body by stunning or killing them. Anthelmintic medicinal plants are used as good alternatives for the allopathic medicinal agents.

Crontoum bonplandinum (Family-*Euphorbiaceae*) is an important medicinal herb found as a weed throughout India and tropical Asia. ^[5, 6]

It is an erect herb stem much branched, clothed with branched hairs, leaves crowded at top, simple, ovule, flowers in terminal, spikes ,male flowers upper, female flowers lower.

The plant *Croton bonplandianum (Euphorbiaceae)* shows different types of pharmacological activity like to treat liver disorders, skin diseases including ring worm infections, to cure swelling of body, bronchitis and asthma. The seeds are used to treat jaundice, acute constipation, abdominal dropsy and internal abscesses it contains different chemical constituents like alkaloids, flavonoid, Saponin, steroid, resins, and phenol. ^[7, 8, 9]

MATERIALS AND METHODS:

Plant Material:

The whole plant *Croton bonplandianum* was collected from the local areas of Ahmednagar District and was authenticated by Dr. Ashok Tuwar, HOD of Botany department at MES's Arts, Commerce and Science College, Sonai. Prepared herbarium was deposited at Department of Pharmacognosy, MES's College of Pharmacy, Sonai.

Preparation of the extract:

The whole plant was dried under shade, reduced to moderately coarse powder, loaded into Soxhlet extractor and were subjected to successive extraction by using four different solvents such as petroleum ether (90%), ethyl acetate, ethanol, water. The dried extracts were stored in a well closed, air tight and light resistant borosil glass container. ^[10, 11]

Preliminary Phytochemical Screening:

In order to determine presence of various phytoconstituent a preliminary phytochemical study with plant extract was performed.

Phytochemical screening of different extracts from *Croton bonplandianum* indicates the presence of various phytoconstituents such as alkaloids, flavonoids, glycoside, terpenes and tannins.

Animals Used:

Indian adult earthworms *Pheretima posthuma (Annelida)* were used to study anthelmintic activity. The earthworms were collected from local place. Earthworms were washed with normal saline to remove the faecal matter. The earthworms of 3-5 cm in length and 0.1 - 0.2 cm in width were used for all experimental protocols. The earthworms resemble both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity.

Drugs and Chemicals:

Albendazole, Ethyl acetate, Ether, Ethanol (Qualigen Fine Chemicals, Mumbai)

Anthelmintic Bioassay:

The Earthworm *Pheretima posthuma (Annelida, Megescolecidae)* was used for evaluation of the anthelmintic activity of crude extract using reference substance for comparison. The worms were procured from local suppliers and maintained at MES's College of pharmacy, Sonai.

Evaluation of Anthelmintic Activity:

Anthelmintic activity was assessed using earthworms by the reported methods with slight modification. Samples for anthelmintic activity were prepared by dissolving 2.5 gm dried crude extracts in 25 mL 1% gum acacia solution prepared in normal saline (vehicle). To obtain a stock solution, different working solutions were prepared to get a concentration range of 10, 25 and 50 mg/mL. ^[12-15]

The anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human being. The activity of crude petroleum ether, ethyl acetate, ethanol and aqueous extract of whole plant of *Croton bonplandianum* was determined by using four groups of approximately equal size Indian earthworms consisting of six earthworms in each group were used for the study^[16, 17]. Pet ether extracts having the four groups as follows.

Group-I- Pet ether Extract (10 mg/ml)

Group- II-Pet ether Extract (25 mg/ml)

Group-III- Pet ether Extract (50 mg/ml)

Group-IV- Albendazole (Std.) (25 mg/ml)

Likewise four groups were made for each extract i.e. ethyl acetate, ethanol and aqueous extract respectively. Observations were made for the time taken to paralyze and /or death of individual worms. Paralysis was said to occur when the worm do not move even in normal saline death was concluded when the worms lost their motility followed with fading away of their body color. ^[18, 19]

Statistical Analysis:

The values are expressed as mean SEM the results were analysed for statistical significance using one way ANOVA followed by Dunnett's test. P<0.05 was considered significant.

Table No. 1 Anthelmintic	activity of petroleum	ether extract of	whole plant of Croton
bonplandianum.			

Sr.No.	Groups	Dose mg/ml	Time of paralysis (min) <u>+</u> SEM	Time of death (min) <u>+</u> SEM
1	Pet.ether Extract-1	10	91.33 <u>+</u> -4.24 ns	75 <u>+</u> 5.62ns
2	Pet.ether Extract-2	25	64.16 <u>+</u> 4.31ns	72.16 <u>+</u> 6.87ns
3	Pet.ether Extract-3	50	61.3 <u>+</u> 6.08	67.33 <u>+</u> 3.90*
4	Albendazole (std)	25	35.16 <u>+</u> 3.21	50.00 <u>+</u> 2.82

Table No. 2 Anthelmintic activity of ethyl acetate extract of whole plant of *Croton* bonplandianum

Sr.No.	Groups	Dose mg/ ml	Time of paralysis (min) <u>+</u> SEM	Time of death (min) <u>+</u> SEM
1	Ethyl acetate Extract-1	10	63.83 <u>+</u> 4.08ns	86.5 <u>+</u> 9.12ns
2	Ethyl acetate Extract-2	25	62.0 <u>+</u> 9.45ns	83.66 <u>+</u> 5.22ns
3	Ethyl acetate Extract-3	50	44.5 <u>+</u> 6.81*	61.5 <u>+</u> 5.20*
4	Albendazole (Std.)	25	35.16 <u>+</u> 3.21	50.00 <u>+</u> 2.82

Sr.No	Groups	Dose mg/ml	Time of paralysis (min) <u>+</u> SEM	Time of death (min) <u>+</u> SEM
1	Ethanolic Extract-1	10	49.83 <u>+</u> 3.61ns	69.16 <u>+</u> 3.15*
2	Ethanolic Extract-2	25	43.50 <u>+</u> 2.14*	61.50 <u>+</u> 3.78*
3	Ethanolic Extract-3	50	39.16 <u>+</u> 4.08*	56.33 <u>+</u> 3.29*
4	Albendazole (Std.)	25	35.16 <u>+</u> 3.21	50.00 <u>+</u> 2.82

Table No. 3 Anthelmintic activity of ethanolic extract of whole plant of *Croton bonplandianum*.

Table No. 4 Anthelmintic activity of water extract of whole plant of Croton bonplandianum.

Sr.No	Groups	Dose mg/ml	Time of paralysis (min) <u>+</u> SEM	Time of death (min) <u>+</u> SEM
1	Water Extract-1	10	60.16 <u>+</u> 2.58ns	73.8 <u>+</u> 5.34ns
2	Water Extract-2	25	56.16 <u>+</u> 3.61*	73.6 <u>+</u> 4.94ns
3	Water Extract-3	50	48.50 <u>+</u> 3.36*	54.66 <u>+</u> 2.82**
4	Albendazole (Std.)	25	35.16 <u>+</u> 3.21	50.00 <u>+</u> 2.82

ns p>0.05, *p<0.05, **p<0.01

Value are mean<u>+</u>SEM, n=6

When compared with Albendazole followed by Dunnette multiple comparison tests.

Table No. 5: Phytochemical investigation of Croton bonplandianum.

Phytoconstituents	Water extract	Ethanolic extract	Petroleum ether extract	Ethyl acetate extract
Alkaloids	+	+	-	-
Flavonoids	+	+	-	+
Glycoside	+	+	-	-
Terpenes	-	-	+	-
Tannins	+	-	-	-

(+) indicates the presence of phytoconstituents, (-) indicates the absence of phytoconstituents. As compared other two extract ethanolic extract shows presence of alkaloid, flavonoid, glycoside which shows maximum anthelmintic property.

RESULTS AND DISCUSSION:

Preliminary phytochemical screening of the ethanolic, aqueous extract, petroleum ether extract, and ethyl acetate extract of whole plant showed the presence of alkaloids, glycosides, flavanoids, terpenoids and tannin. Anthelmintic activity of ethanolic extract, aqueous extract, petroleum ether extract, ethyl acetate extract of *Croton bonplandianum* was evaluated. From the observations made when compared to the standard drug Albendazole, the earthworms lost their motility on exposure to ethanolic extract, aqueous extract, petroleum ether extract and ethyl acetate extract of *Croton bonplandianum*. Four extract by using four different solvent, each extract dose 10, 25 and 50 mg/ml concentration produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progresses to death. As compare to other extract ethanol extract shows significance anthelmintic activity due to the presence of alkaloids, flavonoids, and glycosides phytoconstituents.

Mortality was produced by concentration 10, 25 and 50 mg/ ml of ethanol extract within 69.16, 61.5,56.33 sec respectively when compared with standard drug Albendazole which causes death within 50 min respectively. Hemorrhagic and necrotic spots were observed externally on the worms, with the higher concentrations of extracts of Petroleum ether<ethyl acetate<water<ethanol respectively.

In that ethanol shows maximum anthlemintic activity as compared to other extract.

CONCLUSION:

From the present work, it can be concluded that the ethanolic extract, aqueous extract, ethyl acetate extract and petroleum ether extract of the whole plant of *Croton bonplandianum* have potent anthelmintic activity when compared with the conventionally used drugs and by phytochemical screening it can be concluded that due to the presence of phytochemical such as alkaloids, glucosides, flavonoids, tannins *Croton bonplandinum* shows anthelmintic activity. Further studies using in vivo models are required to carry out and establish the pharmacological rationale for the use of *Croton Bonplandianum* as an anthelmintic drug. Apart from this further studies are necessary to isolate the active principles and to formulate the extract into suitable dosage forms to treat the infection.

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CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this research.

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