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Anthelmintic Activities of the Crude Extracts of *Allium cepa* Bulbs and *Elletatria cardomomum* Seeds

Abhijeet Bidkar*, Mudreka Ghadiali, Chirag Patel, Manoj Aswar, Deepa Sanghai, Pallavi Adate

Dept of Pharmacognosy, Sinhgad Institute of Pharmacy, Narhe, Pune-411041.

ABSTRACT

Crude extracts of *Allium cepa* bulbs and *Elletatria cardomomum* seeds show strong anthelmintic activity on *Pheretima posthuma* (earthworm). The ethanolic extracts of *Allium cepa* are more potent than the corresponding aqueous extracts; while, the aqueous extracts of *Elletatria cardomomum* are more potent than the corresponding methanolic extracts. The anthelmentic activity has a linear relationship with the dose of the extract used. For all the extracts the earthworms were paralysed within 20 mins and died within 30 mins of exposure. The extracts of *Elletatria cardomomum* are found to be more potent than the extracts of *Allium cepa*.

Keywords: Anthelmentic; *Allium cepa* bulbs; *Elletatria cardomomum* seeds; earthworms.

Corresponding author
Email: abhijeet_bidkar@rediffmail.com

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INTRODUCTION

Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent particularly in third world countries due to poor management practices [1]. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminths against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics. A number of medicinal plants have been used to treat parasitic infections in animals [2]. Several workers have investigated the anthelmintic activity of various plant extracts and observed good results [3-7].

In Indian folk medicine, the bulb of *Allium cepa* is used to treat dysentery, fever, chronic bronchitis, insect bites, stings, skin diseases [8]. The bulb of *Allium cepa* and seeds of *Elletatria cardomomum* has shown dysenteric action along with anti-microbial and anti fungal activity [9-11]. Tannins have been found to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. Tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [12]. These observations therefore support the use of *Allium cepa* and *Elletatria cardomomum* in herbal cure remedies.

However both the bulb extracts of *Allium cepa* and seed extracts of *Elletatria cardomomum* have not been scientifically investigated for anthelmintic activity keeping in mind the potential medicinal uses.

The present study was therefore conducted to evaluate the in vitro anthelmintic activity of extracts of bulbs of *Allium cepa* and seeds of *Elletatria cardomomum*. Earthworms were used to test the anthelmintic activities since they bear anatomical and physiological resemblance to the intestinal roundworm parasites of human beings [13, 14].

MATERIAL AND METHODS

Plant Materials

Bulbs of *Allium cepa* were collected from the local markets of Maharashtra, India, in September 2010. Seeds of *Elletatria cardomomum* were collected from Vadgaon, Pune Maharashtra, India in September 2010. Specimens of these bulbs (MBP-001) and seeds (MBP-002) weres submitted and authenticated by Agharkar Research Institute. The bulbs were then reduced to small pieces and the seeds were finely powdered.

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Animals

Indian adult earthworms (*Pheretima posthuma*), 3-5 cm long and 0.1-0.2 cm wide were used for the anthelmintic study. They were collected from moist soil and washed with normal saline to remove all fecal matter.

Drugs and Chemicals

Albendazole (BANDY, Mankind Pharma Ltd., New Delhi), Petroleum ether (60-80^{o}C) A.R. (PCL, Pune), 95% Ethanol (PCL, Pune), methanol (PCL, Pune), Saline water (Claris Life sciences Ltd., Ahmedabad) and distilled water were procured from the local market and used as such.

Extraction Methods

For *Allium cepa*

The onions were washed with clean sterile distilled water and allowed to air dry for one hour. The outer coverings were manually peeled off and the aqueous and ethanolic extracts were obtained from the bulbs by the method used by earlier workers [9].

Exactly 200gms of fresh onion bulbs were blended and soaked in 100ml of distilled water for 24hrs. The pulp obtained was left in a clean sterile glass container and shaken vigourously to allow for proper extraction and was filtered using muslin cloth. The filtrate was concentrated using distillation to give the aqueous extract.

The ethanolic extract was obtained in a similar fashion by soaking the blended onion bulbs in 100ml of 95% ethanol instead of water and concentrating the filtrate by air drying rather than distillation. The color and yields of the aqueous and ethanolic extracts thus obtained are reported in Table 1.

Table 1: Color and yield of extracts obtained

	Allium cepa		***Elletatria cardomomum***	
Extract	**Color**	**%yield**	**Color**	**%yield**
Aqueous	Dark brown	3.5%	Dark brown	2.0%
Methanolic	--	--	Dark green slurry	2.5%
Ethanolic	Pale white	3.1%	--	--

For *Elletatria cardomomum*

Four hundred g of the fresh seeds of *Elletatria cardomomum* were powdered and defatted with petroleum ether (40-60˚C). The defatted residue was dried at 55 ^{o}C for 30 minutes. The dried residue was solvent extracted using methanol in a soxhlet apparatus. The extract was then concentrated by distilling off the methanol. The residue obtained is the

methanolic extract, whose anthelmentic activity has been tested in this study. The methanolic extract obtained from another batch of 400 g of the fresh seeds of *Elletatria cardomomum* was processed further to obtain the aqueous extract. The methanolic extract was macerated with 300 ml water for 6 days. This solution was filtered and the filtrate concentrated by distillation to obtain the aqueous extract. The color and yields of the aqueous and methanolic extracts of *Elletatria cardomomum* obtained, are also shown in Table 1.

Preliminary Phytochemicals analysis

During preliminary phytochemical screening tests were mainly concluded to carbohydrates, flavonoids, proteins, glycosides, saponins, fats and oils, alkaloids, steroids and tannins. The constituents are reported in Table 2.

Table 2: Results of preliminary phytochemicals analyses of the extracts obtained

	***Allium cepa* extract**		***Elletatria cardomomum* extract**	
Phytoconstituents	**Water**	**Ethanolic**	**Water**	**Methanolic**
Carbohydrates	+ve	-ve	-ve	-ve
Flavanoids	-ve	+ve	+ve	+ve
Proteins	-ve	+ve	-ve	-ve
Glycosides	-ve	+ve	-ve	+ve
Saponin	-ve	+ve	-ve	-ve
Fats & Oils	-ve	-ve	+ve	-ve
Alkadoids	-ve	-ve	+ve	+ve
Steroids	-ve	-ve	-ve	-ve
Tannins	+ve	+ve	+ve	+ve

EXPERIMENTAL

The in-vitro anthelmintic activity was determined by dissolving known weights of each extract in distilled water to form 20, 30, 40 and 50 mg/ml solutions. Earthworms were released into 10 ml of each of these solution extracts and the time for paralysis and death was recorded. The results, which reflect the anthelmentic activity of the extracts of *Allium cepa* and *Elletatria cardomomum* are shown in Figures 1 and 2, respectively. Earthworms were also released into 10 ml of standard albendazole (10 mg/ml) and normal saline (control) solutions for comparison.

The 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 [7].

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RESULT AND DISCUSSIONS

Extract yield and characteristics

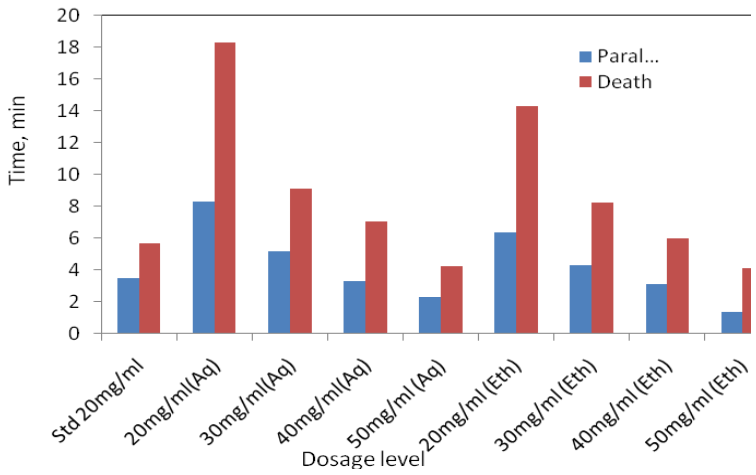
Table 1 shows that aqueous and ethanolic extracts of *Allium cepa* can be obtained in comparable yields by using simple maceration technique. The aqueous extracts of both the plant materials were found to be dark brown and the ethanolic extract of *Allium cepa* was found to be the lightest in color. Table 2 shows that the aqueous extract contains carbohydrates and tannins and is devoid of flavanoids, saponins, glycosides, steroids, fats and oils, proteins and alkaloids. On the other hand the ethanolic extract was found to contain glycosides, flavanoids, saponins, proteins and steroids and is devoid of carbohydrates, fats and oils and alkaloids.

Table 1 also shows that the aqueous and methanolic extracts *Elletatria cardomomum* can be obtained in comparable yields. The aqueous extract was found to contain flavanoids, fats and oils, alkaloids and tannins while the methanolic extract contained only flavanoids, alkaloids and tannins. It is also interesting to note that the phytoconstituent present in the aqueous extracts of *Elletatria cardomomum* are quite different from those present in the aqueous extract of *Allium cepa*.

Extract Activity

Figure 1 which is a plot of the time for paralysis and death for the extracts of *Allium cepa* shows that all the extracts possess anthelmintic activity. At a dose of 20mg/ml the aqueous extract caused paralysis after 8.32 min and death after 18.30 min; while the ethanolic extract caused paralysis in 6.40 min and death in 14.30 min. Figure 1 also shows that, increasing the dose results in a remarkable increase in the anthelmentic activity for both the extracts. However at each dosage level the anthelmentic activity of the ethanolic extract is found to be superior to the aqueous extract.

Figure 1. Comparison of activity of *Allium Cepa* extracts



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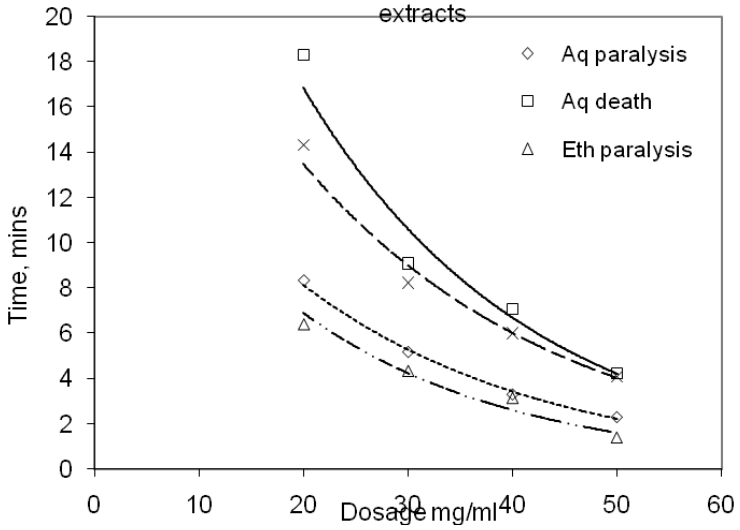
The time required for paralysis and death is plotted against the extract in Figure 2. The anthelmentic activity is found to increase exponentionally with an increase in dosage and is well correlated (R^2 >0.9) with the equations shown below.

Figure 2. Change in activity with dosage of *Allium Cepa* extracts



For Aqueous Extract

Time required for paralysis = 19.18 Exp (-0.04*dosage) (1)
Time required for death = 42.54 Exp (-0.04*dosage) (2)

For Ethanolic Extract

Time required for paralysis = 18.35 Exp (-0.04*dosage) (3)
Time required for death = 30.41 Exp (-0.04*dosage) (4)

The above equations show that for both the extracts, the time for paralysis and death decrease at the same exponential rate (-0.04) with increase in dosage level.

Though, both the extracts have anthelmentic properties, their activities are somewhat low as compared to the commercially available Albendazole, which can be used as a standard for comparison. The equations presented in this study show that higher dosage levels (in the range of 40 to 50 mg/ml) of the extracts are required to exhibit anthelmentic activity comparable to that exhibited by the standard (20 mg/ml).

Figure 3 compares the the time taken for paralysis and death by the aqueous and methanolic extracts of *Elletatria cardomomum*. The figure clearly shows that both these extracts possess anthelmintic activity. Figure 4 clearly shows that, increasing the dose results in a remarkable increase in the anthelmentic activity for both the extracts. The aqueous extract is found to take a significantly longer time to cause paralysis, than the methanolic extract at a

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dosage of 20 mg/ml. However, at a higher dosage level, the anthelmintic activities of both extracts are comparable. At 20 mg/ml, the aqueous extract requires a significantly longer time to induce death as compared to the methanolic extract. At 20 mg/ml, the anthelmintic activity (in terms of time required to induce paralysis as well as death) of the standard drug, albendazole, is found to be far superior to both the aqueous as well as methanolic extracts. Furthermore, the anthelmintic activity of the methanolic extract is superior to that of the aqueous extract.

Figure 3. Comparison of activity of *Elletatria cardomomum* extracts

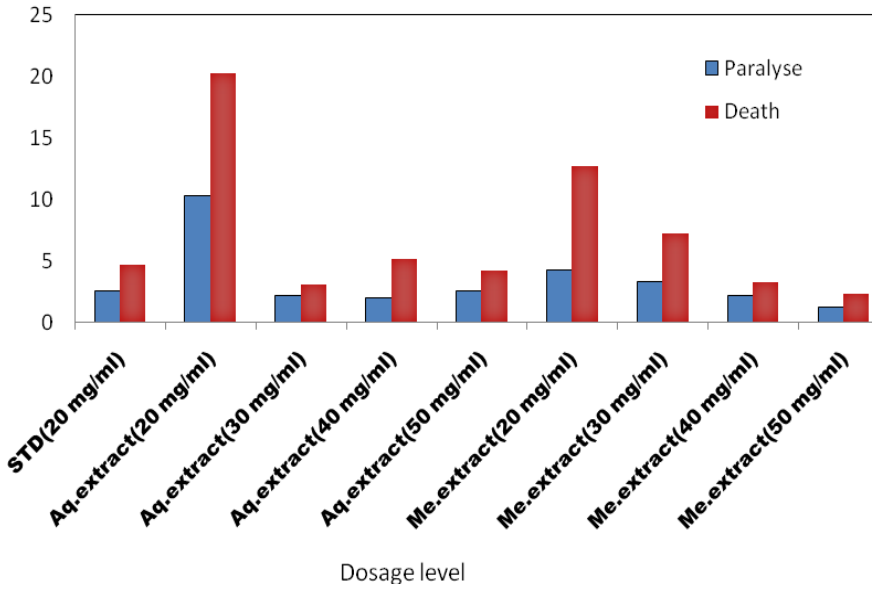
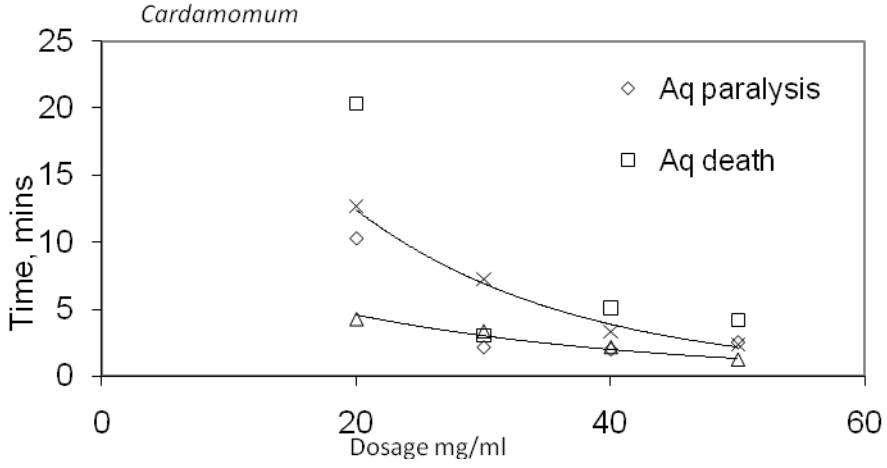


Figure 4. Change in activity with dosage of *Elletatria Cardamomum*



Over the range studied the anthelmentic activity of the non aqueous (methanolic) extract of *Elletatria cardomomum* can be satisfactorly (R^2 value > 0.90) correlated exponentionally with time (Equations 5 and 6 shown below) as is the case with ethanolic

extract of *Allium cepa*

For methanolic extract:-

Time required for paralysis=10.62 Exp(-0.04*dosage) (5)
Time required for death = 39.96 Exp(-0.06*dosage) (6)

Interestingly, Equation 5 indicates that the time required for paralysis changes with dosage levels follows a trend comparable to that observed for the extracts of *Allium cepa* (Equations 1 through 4). It is also interesting to note that, the anthelmintic activity of the aqueous extract of *Elletatria cardomomum* is slightly inferior to that of the *Allium cepa* at a dosage of 20 mg/ml; however, at higher doses the activity values become comparable.

CONCLUSION

The aqueous and non-aqueous solvent (ethanol or methanol) extracts of the Bulbs of *Allium cepa* and the seeds of *Elletatria cardomomum* are found to have good anthelmintic activity when tested on earthworms. At 20 mg/ml the aqueous extracts of *Allium cepa* are more effective anthelmintics than those of *Elletatria cardomomum*. The non-aqueous extracts are found to be more active than the aqueous extracts for both plant materials. For the range studied, the change in activity with dosage of the aqueous and ethanolic extracts of *Allium cepa* and the methanolic extracts of *Elletatria cardomomum* can be faithfully correlated with exponential relationships. All the extracts studied are less active than Albendazole. Since the two solvents extract different constituents from the same material, extracting with other solvents might hold the key to obtaining a product having activity comparable to that of the commercially available product (Bendex). Bulbs of *Allium cepa* and seeds of *Elletatria cardomomum* hold great potential as source of active anthelmentic agents and in depth analysis of its active components needs to be undertaken in future studies.

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