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# *In vitro* studies on anthelmintic activity of modern drugs and aqueous extracts of *curcuma aromatica* and *coscinium fenestratum*

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## ABSTRACT

The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India. In the present investigation, we have subjected five commercial anthelmintic drugs and aqueous extracts of two medicinal plants namely *Curcuma aromatica* and *Coscinium fenestratum* to anthelmintic activity using earthworm model. The results revealed the potential of the plant extracts in affecting worms. Mebex was more effective against worms whereas Piperazine was least effective in killing worms. Extracts exhibited dose dependent activity. Among extracts tested, *Coscinium fenestratum* was found to affect worms to a greater extent followed by *Curcuma aromatica*. The study was carried *in vitro* and experiments in animal models could possibly reveal the potential of the plants to inhibit worms. The anthelmintic activity of aqueous extracts of plants could be due to the active principle present in the extracts.


## KEYWORDS

Anthelmintic drugs;
*Curcuma aromatica;*
*Coscinium fenestratum;*
Anthelmintic activity;
*Pheretima pasthuma.*

## INTRODUCTION

Helminths are recognized as a major problem to livestock production throughout the tropics. Parasitic helminths affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature. The parasitic gastroenteritis is caused by mixed infections with several species of stomach and intestinal worms, which results in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity. Although some synthetic drugs are available to control such kind of infections, but due to their high cost and untoward effects, the development of more effective and safe drugs from reasonably less expensive natural sources is the main consideration. This can rationally be approached through the study of indigenous traditional plant remedies[1]. The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India. It is in the context that people consume several plants or plant derived preparations to cure helminthic infections[2].

*Coscinium fenestratum* Colebr. belongs to the family Menispermaceae and is a critically endangered dioecious medicinal liana found in Western ghats of India. It is commonly known as Daru haridra in Sanskrit,

Ceylon wood in English and Arisina balli and Marmanjal in Kannada. The stem of the plant is used in curing several diseases and disorders like diabetes, wounds and ulcers, fever, jaundice, snake bite, piles etc in ethnomedicine. The chief constituent of *Coscinium* is the yellow crystalline alkaloid, berberine. *Curcuma aromatica* belongs to the family Zingiberaceae and is found distributed in India, Malaysia, South Asia, and Java. It is known as Wild Yellow Ginger in English. It is recognized as a medical herb with strong antibiotic properties. The rhizome is used to treat several types of ailments in the body including cancer. It contains aromatic volatile oils that possess several important physiological functions[3].

In the present study, we explored scientifically the anthelmintic potential of aqueous extracts of two traditionally used medicinal plants *Curcuma aromatica* and *Coscinium fenestratum* and substantiate the folklore claims.

## MATERIALS AND METHODS

### Collection and identification of plant materials

The plant materials of *Curcuma aromatica* (rhizome) and *Coscinium fenestratum* (stem) were purchased from local shops and authenticated to their identity by Department of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga and voucher specimens were deposited in the department for future references.

### Preparation of aqueous extract of plant materials

The plant materials were dried and mechanically powdered. 10g of powdered material was added to 100ml of distilled water taken in a beaker and boiled for about half an hour. The contents were filtered and the filtrate was condensed to 1/3$^{rd}$ of the original volume. The condensed aqueous extract was kept in refrigerator and used for the anthelmintic and antibacterial studies. For anthelmintic study, extract concentration of 3% and 5% in normal saline was used.

### Screening of aqueous extracts of *c. aromatica* and *c. fenestratum* for anthelmintic activity

### 1. Selection and collection of worms for the study

Indian adult earthworms (*Pheretima pasthuma*) collected from the local earthworm breeder in the outskirts of Shivamogga city were used for the Anthelmintic study. Equal sized (8±1 cm) worms were selected for the study. The worms were washed with normal saline to remove all the extraneous matter.

### 2. Standard drug

Five anthelmintic drugs purchased from local medical shops were employed as standard drugs. Piperazine citrate (750mg/5ml) manufactured by GlaxoSmithKline Pharmaceutical Limited, Bangalore, was used as reference standard for anthelmintic study. The function of the anthelmintic drugs like Piperazine is to cause paralysis of worms so that they are expelled in the feces of man and animals. The dilutions of drugs namely 1%, 3% and 5% in normal saline were employed to assess anthelmintic activity. Other anthelmintic drugs were used in 1% and mentioned in the TABLE 1.

### 3. Plant extract concentrations used

For anthelmintic study, concentrations namely 3% and 5% of aqueous condensed plant extract in normal saline were used.

### 4. Control

0.85% normal saline was used as control. It was prepared by dissolving 0.85 g NaCl in minimum volume of distilled water and the final volume was made up to 100ml with distilled water.

### 5. Method

In this study, Indian earthworm model was selected as the earthworms are easily available and used widely for the initial evaluation of anthelmintic activity of compounds. The assay was performed on adult Indian earthworm due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. The worms were washed well with saline to remove extraneous matter. Various dilutions of standard drug (Piperazine) and test (3% and 5% of

**TABLE 1: Standard drugs used**

| Name | Active principle | Concentration |
|---|---|---|
| Bandy | Albendazole | 200mg/5ml |
| Bandy plus | Albendazole and Ivermectin | 200mg Albendazole+ 1.5mg Ivermectin/5ml |
| Albendazole oral suspension | Albendazole | 200mg/5ml |
| Mebex | Mebendazole | 100mg/5ml |
| Piperazine citrate | Piperazine citrate | 750mg/5ml |

aqueous extracts) were prepared in normal saline (0.85%). Different concentrations of standard drug and test in normal saline were poured into respective labeled Petri plates (50 ml in each plate) and 6 worms of equal size (or nearly equal) were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur *when the worms were not able to move even in normal saline*. Death was concluded *when the worms lost their motility followed with fading away of their body colors*[4]. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased[2].

## RESULTS AND DISCUSSION

Anthelmintic activity of 1% concentration of drugs tested is depicted in TABLE 2. The time taken for paralysis and death in drugs Bandy, Mebex, Albendazole oral suspension, Bandy plus and Piperazine citrate was found to be 33 and 47, 22 and 35, 47 and 61, 38 and 52 and 78 and 104 respectively. Among drugs tested, Mebex was found to be more efficient against the worms. Among drugs tested, Piperazine was found to be least effective as it took more time to cause paralysis and death of worms.

The anthelmintic activity of different concentrations of standard drug and *C.fenestratum* and *C.aromatica* is shown in TABLE 3. The results revealed dose depended activity. The mean paralysis time in 1%, 3% and 5% Piperazine citrate was found to be minutes 78, 23 and 16 respectively. The average paralysis and death time in 3% and 5% of *C.aromatica* was found to be 118, 184 and 92 and 125 minutes respectively while that of 3% and 5% of *C.fenestratum* was found to be 60, 75 and 49, 63 respectively. From the results, it is arrived that *C.fenestratum* was found to be more effective in killing worms when compared to *C.aromatica*. *C.fenestratum* in 3% and 5% was found to be more effective than 1% of standard drug Piperazine citrate. 5% *C.fenestratum* revealed activity which was found to be close to that of 1% Albendazole.

The origin of many effective drugs is found in the traditional medicine practices and in view of this several workers have undertaken studies pertaining to testing of folklore medicinal plants for their proclaimed anthelmintic activity[2]. Higher plants have yielded a broad spectrum of active compounds, including polythienyls, isothiocyanates, glucosinolates, cyanogenic glycosides, polyacetylenes, alkaloids, lipids, terpenoids, sesquiterpenoids, diterpenoids, quassinoids, steroids, triterpenoids, simple and complex phenolics, and several other classes. Many other antinematodal compounds have been isolated from biocontrol and other fungi. Natural products active against mammalian parasites can serve as useful sources of compounds for examination of activity against plant parasites. The agricultural utilization of phytochemicals, although currently uneconomic in many situations, offers tremendous potential[5]. The anthelmintic activity of *Zanthoxylum alatum* has been found better than Piperazine phosphate against earthworms and could be compared well against roundworms[6]. Methanol extracts of the unripe matured fruits of *Diospyros peregrine*, *Coccinia grandis* leaves and *Schima wallichii* barks were screened against earthworms, tape worms and roundworms. Among the three extracts, *Schima wallichii* showed best activity against all three selected helminthes followed by other two extracts which showed similar spectrum of activity at dose of 10mg/ml. Indian earthworms were more sensitive followed by tapeworms and roundworms[1].

**TABLE 2: Average paralysis and death time in 1% concentration of different anthelmintic drugs (standard)**

| Name of the drug | No.of worms | Average paralysis time in min | Average death time in min |
|---|---|---|---|
| Bandy | 6 | 33 | 47 |
| Mebex | 6 | 22 | 35 |
| Albendazole oral suspension | 6 | 47 | 61 |
| Bandy plus | 6 | 38 | 52 |
| Piperazine citrate | 6 | 78 | 104 |

**TABLE 3: Mean paralysis and Death time in different concentrations of Standard drug and *Coscinium* and *Curcuma* aqueous extracts**

| Compound | Concentration | No. of worms | Mean paralysis time in min | Mean death time in min |
|---|---|---|---|---|
| Piperazine citrate | 1 | 6 | 78 | 104 |
| | 3 | 6 | 23 | 43 |
| | 5 | 6 | 16 | 28 |
| *Curcuma aromatica* | 3 | 6 | 118 | 184 |
| | 5 | 6 | 92 | 125 |
| *Coscinium fenestratum* | 3 | 6 | 60 | 75 |
| | 5 | 6 | 49 | 63 |

Methanol extracts of different parts of *Mentha piperita* and *Lantana camara* against *Pheritima posthuma* was investigated and was found considerable activity by both the extracts at 20mg/ml[4]. Anthelmintic activity of solvent extracts of aerial parts of *Enhydra fluctuans* was reported. The results revealed that ethanolic extract was found to be more potent though all the three extracts *were endowed with anthelmintic activity*[7]. Anthelmintic activity of *Pongamia* glabra was studied. Results showed that ethyl acetate extracts of seeds was most potent followed by petroleum ether extract and the activity could be due to the presence of active principles present in the solvent extracts[8].

## CONCLUSION

The use of plant compounds to treat infections is an age-old practice in a large part of the world, especially in developing countries. Plants constitute major part of traditional practices and have been found to be a rich source of botanical anthelmintics in animals for centuries. The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India. Indigenous system of medicine reports a number of plants for their anthelmintic efficacy. The study undertaken reports the potential of two plants to exhibit inhibitory effects on helminths. Thus, the plants could be used in the control of diseases caused by helminths. The study was carried *in vitro* and experiments in animal models could possibly reveal the potential of the plants to inhibit worms. Further experiments with other solvents may also be undertaken to find the efficacy of the plants against helminths. The anthelmintic activity of aqueous extracts of plants could be due to the active principle present in the extracts.

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