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# *In vitro* antihelmintic activity of bark extract of *Cinnamomum bejolghota* (Buch.–Ham.) in Indian adult earthworm (*Pheretima posthuma*)

Barnali Gogoi\*, Bibhuti Bhusan Kakoti, Nilutpal Sharma Bora, Priyanka Yadav

Department of Pharmaceutical Sciences, Dibrugarh University, Assam– 786004, India

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### ABSTRACT

**Objective:** To carry out an exhaustive study with a view to substantiate the therapeutic potential of methanolic and aqueous extract of bark of *Cinnamomum bejolghota* (*C. bejolghota*) against *Pheretima posthuma* which is used as experimental model for helminthic.

**Methods:** Thirty–six adult earthworms of 3.5–5.0 cm length were collected from university campus of Dibrugarh. Nine groups containing four worms in each group were divided for the experiment. About 0.9% (w/v) of normal saline solution, 25 mg/mL, 50 mg/mL, 100 mg/mL each of standard drug albendazole and methanolic, aqueous extract of bark of *C. bejolghota* were freshly prepared before commencement of the study. Earthworms were observed for paralysis and death time which is recorded in minute.

**Results:** Crude methanolic extract with concentrations of 25 mg/mL, 50 mg/mL and 100 mg/mL produced dose–dependent paralysis. All results were expressed as the mean±SEM using One–way ANOVA. The difference in values at  $P<0.01$  was considered as statistically significant.

**Conclusions:** From the investigation, conclusion can be drawn that the methanolic bark extract of *C. bejolghota* showed better activity than aqueous extract of the same to treat intestinal worm infections. In comparison with the standard drug albendazole, methanolic bark extract of the plant showed significant antihelminthic activity.

## 1. Introduction

Anthelmintic resistance is a worldwide concern and new plant–derived compounds are being studied for their potential use against gastrointestinal nematodes. Most diseases caused by helminths are of a chronic nature; they probably cause more morbidity and even economic and social deprivation among humans and animals than any single group of parasites[1]. Helminthiasis is a problem affecting a large population of the world. In helminthiasis, a part of the body is infested with

worms such as pinworm, round worm or tape worm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs[2]. The parasitic worms are divided into three groups: cestodes or tapeworms, nematodes or roundworms, and trematodes or flukes[3]. Parasitic diseases may cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis[4]. Most developing countries are poverty prone, malnourished and don't follow minimum sanitary hygiene conditions which is the major cause of worm infections[5]. Helminthes infections lead to deficiency diseases like malnutrition, anemia, and weakening of the immune system[6]. The present synthetic anti–helminthic agents produce various side effects and are not cost effective. The broad spectrum antihelmintic drug albendazole is noted to produce

\*Corresponding author: Barnali Gogoi, Department of Pharmaceutical Sciences, Dibrugarh University, Assam– 786004, India.

Tel: 09435865691

E–mail: barnali.gogoi88@gmail.com

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nausea, vomiting, dizziness and gastrointestinal irritation in some patients[7]. As a result, there is an increase in demand for using herbal medicine as antihelmintic agent. Herbal drugs are relatively affordable and have lesser side effects compared to synthetic ones. The anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* (*P. posthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings and easy availability[8,9].

*Cinnamomum bejolghota* (*C. bejolghota*) belonging to the family Lauraceae is commonly known as Patihunda or *Naga dalchini* and is an important ethnomedicinal plant of Assam. It is a medium- to large-sized 6–8 m tall evergreen tree with aromatic leaves, stem bark and panicle, distributed in the central and outer parts of eastern Himalayas up to an altitude of 2100 m, and also in Andaman Islands. Bark is brownish-white, brittle, cream-white (inside), turning darker-brown on exposure, and 4–8 mm thick. In Assam, the plant is well distributed in the Jorhat, Sibsagar, Golaghat, Nowgong and Kamnir Districts. It also grows in the Khasi, Garo and Jaintia Districts of Meghalaya and in a few places of Nagaland in Northeast India. *C. bejolghota* is known locally by different names such as “Pati-Hunda,” “Naga-dalchini,” “Seerang-esing,” “Sami-jong” and “Tejpat-manbi” among the different ethnic groups. Also, it was observed that the bark, which was sold at the local markets, was used traditionally in the region as a spice. The bark and its infusions have local medicinal use for the treatment of a cough, cold, toothache, liver complaints, gall stones and a mouth freshener[10]. In Assam, it is traditionally believed by the folk people that the bark of the plant is used as vermicide.

This plant is widely used by various communities in Assam; however, no detailed study or reports along with scientific evidence are available with this important plant. Work has been carried out with the methanolic and aqueous extract of bark of *C. bejolghota* with a view to investigate its anthelmintic activity against *P. posthuma* using albendazole as a positive control drug.

## 2. Materials and methods

### 2.1. Plant material

The bark of *C. bejolghota* was collected from Jorhat, Rowriah, Assam during the month of July, 2013. The

plants were identified and authenticated by Dr A. A Mao, Botanical Survey of India, Shillong. A voucher specimen is kept in Department of Pharmaceutical Sciences, Dibrugarh University, Assam for future references. The barks were dried under shade for 15 d, coarsely powdered and stored in air tight containers protected from humidity and sunlight for further study.

### 2.2. Preparation of methanolic and aqueous extract

About 250 g of powdered crude drug of bark of *C. bejolghota* was extracted by Soxhlet extraction with 1000 mL of methanol for 18 h after pretreatment with petroleum ether. The solvent was recovered by rotary evaporator and stored in desiccator until further use. Aqueous extract was prepared by cold maceration. Preliminary phytochemical tests showed that the methanolic extract of *C. bejolghota* (MECB) contained carbohydrates, glycosides, lignin, saponins, tannins and phenolic compounds and the aqueous extract of *C. bejolghota* (AECB) contained glycosides, saponins, tannins and phenols.

### 2.3. Indian adult earthworm as model for the experiment

All the experiments were carried out in Indian adult earthworms (*P. posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter which were used for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoides* of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic activity. Adult earthworms of approximately 3.5–5.0 cm in length and 0.2–0.5 cm in width were used for the experiment.

### 2.4. Drugs and chemicals

The standard drug albendazole was prepared at three different concentrations of 25 mg/mL, 50 mg/mL and 100 mg/mL in distilled water. Similarly, MECB and AECB were prepared at the concentrations of 25 mg/mL, 50 mg/mL, 100 mg/mL in distilled water and this was used as test drug for the activity.

### 2.5. Evaluation of anthelmintic activity

The anthelmintic activity of methanol and aqueous extracts of *C. bejolghota* was evaluated as per the

method reported by Panda *et al*[11]. Thirty–six Indian adult earthworms were collected, and divided into nine groups containing four worms in each group (Figures 1 and 2). A volume of 10 mL of each different concentration of standard drug albendazole and test drugs MECB and AECB were taken with pipette in nine Petri dishes. Four earthworms were released in each of the nine clean Petri dishes. Earthworms were observed; the time taken for paralysis and death was monitored and documented in minute. Paralysis time was analyzed based on the behavior of the earthworm with no revival of body state in normal saline medium and no movements when shaken vigorously. Death was concluded based on total loss of motility with no movements even when dipped in warm water at 50–60 °C temperature and faded body color.



**Figure 1.** The standard drug albendazole and its effect in paralysis and death.



**Figure 2.** Methanolic extract of *C. bejolghota* and its effect in paralysis and death.

## 2.6. Statistical analysis

The results are expressed as mean±SEM of four worms in each group. Comparisons have been made between standard and test treated group using Dunnett test. The difference in values at  $P<0.01$  was considered as statistically significant.

## 3. Results

The result of anthelmintic activity is depicted in Table 1. It was found that the higher concentration of the extract became faster due to the paralytic effect and shorter due to the death time for all the earthworms. Crude methanolic and aqueous extract of the plants with concentration of 25 mg/mL, 50 mg/mL, 100 mg/mL produced dose–dependent paralysis. Methanolic extract of the barks of *C. bejolghota* showed paralysis and death time at 100 mg/mL in (55.28±0.40, 72.95±0.07) ( $P<0.01$ ) min respectively whereas aqueous extract of the bark of *C. bejolghota* gave paralysis and death time in (70.38±0.54, 74.70±0.42) ( $P<0.01$ ) min as compared to the standard where paralysis and death time was at 100 mg/mL in (35.53±0.33, 52.18±0.16) min respectively.

**Table 1**

*In vitro* anthelmintic activity of MECB and AECB against *P. posthuma*.

Drug treatments	Doses (mg/mL)	Time taken for paralysis (min)	Time taken for death (min)
Standard drug (albendazole)	25	45.63±0.47	61.97±0.47
	50	40.33±0.47	60.32±0.44
	100	35.53±0.33	52.18±0.16
MECB	25	65.52±0.30**	79.07±0.33*
	50	61.93±0.09**	76.17±0.47*
	100	55.28±0.40**	72.95±0.07**
AECB	25	80.63±0.47**	86.63±2.35**
	50	75.43±0.61**	82.87±0.60**
	100	70.38±0.54**	74.70±0.42**

Statistical analysis was carried out with comparisons between standard and treated groups.  $n=4$  was taken in each group. Symbols represent statistical significance: \* $P<0.05$ , \*\* $P<0.01$ .

## 4. Discussion

In the current study, all the tests were performed *in vitro* in Indian adult earthworm (*P. posthuma*) because it has anatomical and physiological resemblance with

the intestinal roundworm parasites of human beings. These parasitic helminths affect mankind and animals causing relentless infections to them. Many synthetic compounds and their derivatives have been developed but the problems associated with the use of such drugs lead to serious side effects. Also sometimes, these parasites develop resistance to the drugs leading to more severe infections. Thus, steps have been taken towards developing herbal medicines as a safer remedy to cure helminths. The results obtained in this study have shown hopeful prospect on anthelmintic activity. This plant could be used by human beings in controlling gastrointestinal nematode infections. Results indicate that time taken for albendazole causing paralysis and death is near to that of paralysis and death time of MECB. Preliminary phytochemical tests showed that the MECB contained carbohydrates, glycosides, lignin, saponins, tannins and phenolic compounds. Some of these phyto-constituents like tannins, phenols *etc.* may cause significant anthelmintic activity. It was reported earlier that tannins and phenolics were known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation or they bind to the free protein of the gastrointestinal tract of the worms and led to death and the presence of flavonoids and polyphenolic compounds were also responsible for anthelmintic activity<sup>[12,13]</sup>. Further studies need to be carried out using *in vivo* models to establish the pharmacological efficacy for the use of *C. bejolghota* as anthelmintic drugs.

### Conflict of interest statement

We declare that we have no conflict of interest.

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