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SHORT COMMUNICATION

# In vivo anthelmintic activity of *Clerodendrum colebrookianum* Walp., a traditionally used taenicidal plant in Northeast India

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**Abstract** Although there are several effective drugs available for the treatment of intestinal helminths, the fact remains that they continue to remain out of reach to a vast majority of people in the world, especially in developing countries. On the other hand, there are a great many herbal remedies that are effective against common intestinal worms and are easily available to common people in developing countries. *Clerodendrum colebrookianum* Walp. (Lamiaceae) is a perennial shrub which is native to South and Southeast Asia. Traditionally, the leaves of this plant are used by the indigenous people of Northeast India as a remedy for the treatment of intestinal tapeworm infections. The aim of this study was to evaluate and authenticate the anthelmintic efficacy of *C. colebrookianum* leaf extract in experimentally induced *Hymenolepis diminuta* (a zoonotic tapeworm) infections in Wistar rats. The efficacy of the plant extract was assessed by monitoring the eggs per gram of faeces (EPG) counts and worm recovery rates of experimental animals, following treatment with the leaf extract of this plant at three different doses, i.e. 200, 400 and 800 mg/kg body weight, each given singly for 5 days. The results obtained revealed that the leaf extract of *C. colebrookianum* possesses a dose-dependent efficacy against the larval, immature and adult stages of *H. diminuta*. However, the efficacy of the extract was found to be considerably high only against the adult stages of the parasite. For this stage, a single 800-mg/kg dose of extract, given for 5 days, resulted into 68.42 % reduction in the EPG counts and 62.50 % reduction in the worm counts in the extract-treated group of animals, as compared to the control. The reference drug, praziquantel (5 mg/kg, single dose), however, showed slightly better efficacy and caused 95.16 and 87.00 % reductions in the EPG and worm counts of treated animals, respectively. Unlike the adult stages, the efficacy of the plant extract was recorded to be comparatively low against the larval and immature stages of the parasite, as the treatment of animals with the highest dose of extract (800 mg/kg) could cause only 37.50 and 54.00 % reductions in worm counts, respectively. The experimental evidence obtained in this study suggests that leaves of *C. colebrookianum* possess significant anthelminthic properties and supports their use against intestinal tapeworm infections in traditional medicine.

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## Introduction

Intestinal helminths are one of the most common causes of infections in humans, especially in tropical and subtropical countries. The cost of harbouring these parasites in terms of human misery and economic loss is incalculable (Savioli et al. 1992). Although there are many effective drugs available for the treatment of intestinal helminthiasis, the fact remains that they remain out of reach to a majority of people in the world, especially in African and Asian countries (WHO 2007). On the other hand, there are a great many herbal remedies that are effective against common intestinal worms and are easily available and affordable to common people in developing countries (WHO 2002). India is known for its vast resources of medicinal plants, and many of these plants have long been used in local traditional medicine as a cure for intestinal worm infections (Tandon et al. 2011; Yadav and Temjenmongla 2011, 2012; Yadav and Tangpu 2012). Recent studies on some Indian anthelmintic plants that are

used traditionally to cure helminthic diseases reveal that there are a number of medicinal plants which possess significant efficacy against a range of helminth parasite species (Nisha et al. 2007; Zahir et al. 2009; Kamaraj et al. 2010, 2011; Hossain et al. 2012). Most notable among these plants are *Flemingia vestita* (Fabaceae), *Achyranthes aspera* (Amaranthaceae), *Anisomeles malabarica* (Lamiaceae), *Gloriosa superba* (Liliaceae), *Ricinus communis* (Euphorbiaceae), *Houttuynia cordata* (Saururaceae), *Solanum myriacanthum* (Solanaceae), *Lasia spinosa* (Araceae), to name but a few, which revealed significant efficacy against a number of helminth species and have potential for development as natural anthelmintics (Das et al. 2004; Zahir et al. 2009; Yadav and Temjenmongla 2011, 2012 Yadav and Tangpu 2012).

*Clerodendrum colebrookianum* Walp. (Lamiaceae) is a perennial shrub which is native to South and Southeast Asian countries like India, China and Thailand (Nath and Bordoloi 1991; Yang et al. 2000). In India, it grows mainly on the moist and waste lands in the north-eastern region. This plant is unique in its genus of about 400 species in having several important medicinal properties, and has been used to treat such ailments as bronchitis, asthma, fever and stomach disorders to getting relief from hypertension, etc., in many Asian countries (Nath and Bordoloi 1991; Shrivastava and Patel 2007; Inta et al. 2008). In traditional Chinese medicine, *C. colebrookianum* has been used to expel toxins by cooling, cooling blood to induce diuresis and purging heat, for a long time (Yang et al. 2000). In experimental studies, the antioxidant and antihyperlipidaemic activities of root and leaf extracts of this plant have been well established in rats (Rajlakshmi et al. 2003; Devi and Sharma 2004). Besides, it has been reported by Devi et al. (2005) that the leaf extract of *C. colebrookianum* has the ability to protect the rat heart against oxidative stress. In the course of our ethnomedicinal field survey in Northeast India, we found that the leaves of this plant have been used by the native people against intestinal tapeworm infections. In an in vitro anticestodal screening of some medicinal plants used by the native tribes in Northeast India, the leaf extract of *C. colebrookianum* revealed a moderate efficacy against *Raillietina echinobothrida*, the intestinal cestode parasite of domestic fowl (Temjenmongla and Yadav 2005). Some recent evidence suggests that although in vitro testing of plant extracts for antiparasitic efficacy may provide good results, it does not always guarantee so in in vivo test systems (Klimpel et al. 2011). It has therefore been advocated to employ suitable in vivo animal models to provide a more accurate reflection of the activity of plant extracts (Klimpel et al. 2011). Keeping this in mind, in the present study, we were interested to evaluate and verify the anthelmintic efficacy of *C. colebrookianum* leaf extract by using an experimental laboratory model for intestinal cestodiasis. The study employed *Hymenolepis diminuta* (a zoonotic tapeworm)-Wistar rat model and tested the efficacy of the plant extract against three developmental stages of the parasite, i.e. larval, immature and adult stages.

## Materials and methods

### Plant material and preparation of extract

The leaves of *C. colebrookianum* (Fig. 1) were collected from their natural habitats in Nagaland (India) and identified by a plant taxonomist, and a voucher specimen (AKY-005) was deposited in the Department of Zoology, North-Eastern Hill University (NEHU), Shillong. The leaves were dried in the shade, powdered and extracted in methanol, using a Soxhlet extractor. The final yield (*w*/*w*) of crude extract was 5.71 %.

### Experimental animals and maintenance of *H. diminuta* infection

Both male and female Wistar rats (100–120 g) were used. Prior to use for experiments, animals were acclimatized for 15 days in the laboratory and had ad libitum access to standard rodent food and water. During this period, the faecal samples of the animals were examined daily to ensure that they are free from any intestinal parasitic infections. *H. diminuta* strain was maintained in the laboratory by cyclical passage through Wistar rats and the flour beetle, *Tribolium confusum* (intermediate host), as described by Tangpu et al. (2006). All experiments involving animals were performed according to the protocols of the Institutional Animal Ethics Committee of NEHU, Shillong.

### Anthelmintic assay

The plant extract was tested against the larval, immature and mature stages of *H. diminuta* in rats. For each developmental stage, the animals were divided into five groups, six animals per group. Each animal was then orally infected with four cysticercoids and maintained in a separate cage. Plant extract and praziquantel (PZQ, the reference drug) solutions were prepared fresh in 0.9 % phosphate-buffered saline. The first group of animals served as untreated control and given only the vehicle. The animals belonging to groups 2, 3 and 4 were treated with 200, 400 and 800 mg/kg body weight doses of plant extract, respectively. The fifth group of animals was treated with 5 mg/kg, p.o. dose of PZQ, the reference drug.

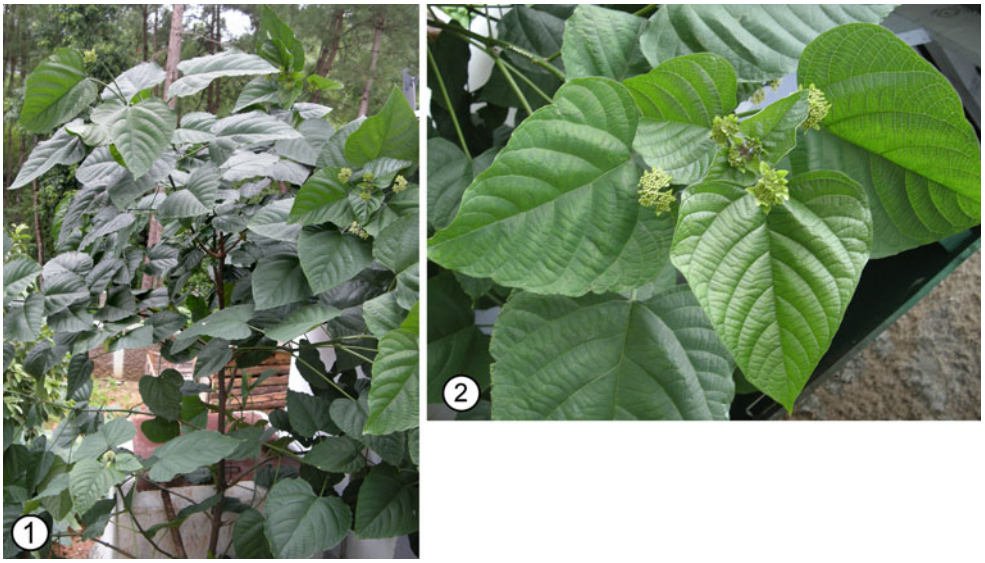


**Fig. 1** *C. colebrookianum* Walp.: shrub (*1*) and leaves with young inflorescence, closer view (*2*)

For the larval stage of the parasite, the animals were given the vehicle, plant extract and PZQ, as per the assigned groups, on days 2–6 p.i. of the cysticercoids. From day 18 p.i., fresh faeces was collected from each cage of the treated and control rats, and the eggs per gram of faeces (EPG) of *H. diminuta* (Fig. 2) were determined (Anonymous 1977), for three consecutive days (days 18–20 p.i.). All animals were then sacrificed under chloroform anaesthesia on day 31 p.i., and worms from the intestine were collected. The efficacy of the extract was determined on the basis of percentage reduction in the faecal EPG counts and worm counts (Rim et al. 1980). For the immature stages of the parasite, almost similar experimental protocols were followed, except that treatment of the experimental animals with the plant extract and reference drug was undertaken on days 8–12 p.i. of the cysticercoids. Against the adult stages of the parasite, the extract and PZQ were given on days 21–25 p.i. of the cysticercoids, and the EPG count of the animals was performed between days 18–20 (pre-treatment period) and days 36–38 (post-treatment period). The percentage reduction in the EPG counts was calculated as per the criterion of Iqbal et al. (2004). All animals were sacrificed on day 39 p.i., and the number of worms in their intestine was counted to determine the percentage reduction in worm counts.

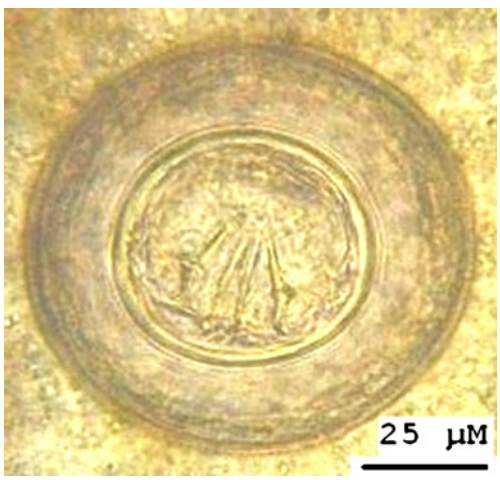


**Fig. 2** Egg of *H. diminuta* with an onchosphere and a thick striated shell

### Statistical analysis

All results were reported as mean±standard error of the mean (SEM). The significance of the differences between experimental and control groups was calculated using Student's *t* test. A *p* value of $<0.05$ was considered statistically significant.

## Results and discussion

In recent years, there has been renewed attention and interest in the use of herbal remedies globally. India is endowed with vast resources of medicinal plants and also has a rich tradition of using them for curing various diseases and ailments. As per an estimate, about 65 % of the rural population in India relies on medicinal plants to meet their primary health care needs (WHO 2002). There are many Indian medicinal plants which have been investigated for their putative anthelminthic efficacy, and a good number of them have also been found to be effective against various helminth parasites (Zahir et al. 2009; Tandon et al. 2011; Yadav and Temjenmongla 2011, 2012; Yadav and Tangpu 2012). Nevertheless,

there are still a large number of medicinal plants in this country, especially in tribal areas, which have long been used against helminthic infections, and without any scientific testimony. It is through scientific studies that the potential of these natural medicines could be gainfully realized for the treatment of helminthic infections. This study investigated the anthelmintic efficacy of the leaf extract of *C. colebrookianum*, a traditional taenicidal plant of Northeast India, using the *H. diminuta*–Wistar rat experimental model. This model has been used before to test a number of plant extracts (Abdel-Ghaffar et al. 2011; Tandon et al. 2011).

In the present study, the *C. colebrookianum* leaf extract showed a dose-dependent efficacy against various developmental stages of *H. diminuta* (Tables 1, 2 and 3). In particular, the efficacy of the extract was observed to be considerably high against the adult stages than the larval or immature stages of the parasite. For the larval stage, a single 800-mg/kg dose of extract, given for 5 consecutive days, revealed 59.47 % reduction in the eggs per gram of faeces counts and 37.50 % reduction in the worm count, as compared to the control. Whereas the reference drug PZQ, given at a 5-mg/kg dose and for the same duration, showed much better efficacy and caused 83.26 and 70.75 % reductions in the EPG and worm counts, respectively (Table 1). For the immature stages of the parasite, the extract showed almost similar trends as in the case of the larval stages. The reduction in the EPG and worm counts of animals by 800 mg/kg dose of extract, given for 5 days, was 64.70 and 54.00 %, as compared to the control. Whereas the reference drug PZQ once again showed a better efficacy and resulted in 88.17 and 83.25 % reductions in the EPG and worm counts, respectively (Table 2). Interestingly, the plant extract showed its most significant efficacy against the adult stages of *H. diminuta*. The animals treated with 800 mg/kg dose of extract showed 68.42 % reduction in the EPG counts at the post-treatment period, compared to 95.16 % revealed by 5 mg/kg dose of PZQ. On the other hand, the animals in the control group maintained almost a uniform trend in their EPG counts during the pre- and post-treatment periods. Furthermore, the same dose of the extract also reduced the worm burden of animals by 62.50 %, as compared to 87.00 % by 5 mg/kg dose of PZQ (Table 3). The findings of the present study are in agreement with some similar studies on in vivo efficacy of plant extracts on cestode parasites. For example, Abdel-Ghaffar et al. (2011) tested combinations of several extracts obtained from plants like coconut, onion, garlic, fig, date tree, chicory, etc., against *H. diminuta* (besides other parasites) and found that among all the tested extract combinations, the combination made from onion (*Allium cepa*) and coconut (*Cocus nucifera*) eliminates all the adult cestodes from their hosts. More recently, Yadav and Tangpu (2012), in an investigation into the anthelmintic effects of the fruit extract of *Solanum myriacanthum* in rats, also reported that the *S. myriacanthum* fruit extract possesses more apparent effects on adult stages than larval or immature stages of the parasite. In another study, the extract of a shrub *Diospyros mollis*, popularly known as Ma-klua in Thailand, was found to be more effective in the elimination of adult *Hymenolepis* worms in mice but not the larvae (Maki et al. 1983).

A perusal of the literature reveals that besides *C. colebrookianum*, a few more species in this genus have also been reported to possess good anthelmintic properties (Garg and Siddiqui 1992; Modi et al. 2010; Siju et al. 2011). For example, *Clerodendrum umbellatum* is traditionally used in Cameroon for the treatment of intestinal helminthiases, and an in vivo study on the leaf extract of this plant showed significant antischistosomal

**Table 1** Effects of *C. colebrookianum* leaf extract on larval stages of *H. diminuta* in rats ($n$=6 in each group)

| Groups | EPG (mean±SEM) (days 18–20) | Percentage reduction in EPG count | No. of worms recovered/rat (mean±SEM) | Percentage reduction in worm count |
|---|---|---|---|---|
| Control | 16,066±465 | – | 3.50±0.34 | 12.50 |
| Plant extract | | | | |
| 200 mg/kg | 12,000±347* | −25.30 | 3.17±0.17 | 20.75 |
| 400 mg/kg | 9,977±388* | −37.89 | 3.00±0.26 | 25.00 |
| 800 mg/kg | 6,511±260 * | −59.47 | 2.50±0.22** | 37.50 |
| Praziquantel | | | | |
| 5 mg/kg | 2,688±243* | −83.26 | 1.17±0.17* | 70.75 |

Administration of extract on days 2–6 post-inoculation with four cysticercoids per rat

*$p$<0.001 vs. control, Student's $t$ test; **$p$<0.05 vs. control, Student's $t$ test

**Table 2** Effects of *C. colebrookianum* leaf extract on immature stages of *H. diminuta* in rats ($n$=6 in each group)

| Groups | EPG (mean±SEM) (days 18–20) | Percentage reduction in EPG count | No. of worms recovered/rat (mean±SEM) | Percentage reduction in worm count |
|---|---|---|---|---|
| Control | 17,000±435 | – | 3.84±0.17 | 4.00 |
| Plant extract | | | | |
| 200 mg/kg | 14,000±221* | −17.64 | 2.84±0.17** | 29.00 |
| 400 mg/kg | 11,000±168* | −35.29 | 2.00±0.37* | 50.00 |
| 800 mg/kg | 6,000±273* | −64.70 | 1.84±0.48* | 54.00 |
| Praziquantel | | | | |
| 5 mg/kg | 3,200±124* | −81.17 | 0.67±0.21* | 83.25 |

Administration of extract on days 8–12 post-inoculation with four cysticercoids per rat

*$p$<0.001 vs. control, Student's $t$ test; **$p$<0.01 vs. control, Student's $t$ test

activity in mice model (Jatsa et al. 2009). Similarly, *Clerodendrum multiflorum*, which is used as a deworming remedy in West India, also revealed a convincing in vitro efficacy against *Ascaris lumbricoides* and *Taenia solium* (Garg and Siddiqui 1992). Recently, Rahmatullah et al. (2010) reported that in Bangladesh, a root and leaf decoction of *Clerodendrum viscosum* is used to treat helminthic infections. Later, Siju et al. (2011) also demonstrated the presence of significant anthelmintic efficacy in this *Clerodendrum* sp. Likewise, based on their in vitro testing against earthworms (*Pheritema posthuma*), Khare et al. (1982), Mondal et al. (2010) and Modi et al. (2010) also claimed that *Clerodendrum indicum*, *Clerodendrum inerme* and *Clerodendrum infortunatum* also possess good vermicidal properties. It thus emerges from the results of a present in vivo study on the efficacy of *C. colebrookianum* leaf extract against *H. diminuta* in rats and the studies conducted on other *Clerodendrum* spp. mentioned above that some potent anthelmintic principles are widely present in *Clerodendrum* species which ascribe them significant anthelmintic properties.

The aerial parts of *C. colebrookianum* are reported to contain steroids, alkaloids, glycosides, β-sitosterol and daucosterol as the main chemical constituents (Goswami et al. 1996; Yang et al. 2000). It is likely that one or more of these constituents may be responsible for the reported efficacy of this plant, as there are several published reports which attest the anthelmintic actions of medicinal plants to the reported constituents of *C. colebrookianum* (Athanasiadou and Kyriazakis 2004).

In conclusion, the evidence obtained in this study suggests that leaves of *C. colebrookianum* possess significant anthelmintic properties and supports their use against intestinal tapeworm infections. However, there is a need to determine whether this plant is safe for use for the purpose it is employed by people.

**Table 3** Effects of *C. colebrookianum* leaf extract on adult stages of *H. diminuta* in rats ($n$=6 in each group)

| Groups | EPG (mean±SEM) | | Percentage reduction in EPG count | No. of worms recovered/rat (mean±SEM) | Percentage reduction in worm count |
|---|---|---|---|---|---|
| | Pre-treatment (days 18–20) | Post-treatment (days 36–38) | | | |
| Control | 17,844±310 | 17,800±219 | −0.29 | 4.00±0.17 | 0.00 |
| Plant extract | | | | | |
| 200 mg/kg | 17,822±390 | 8,222±249* | −53.87 | 2.84±0.17** | 29.00 |
| 400 mg/kg | 17,788±203 | 7,600±151* | −57.28 | 1.84±0.17** | 54.00 |
| 800 mg/kg | 17,766±395 | 5,611±311* | −68.42 | 1.50±0.22** | 62.50 |
| Praziquantel | | | | | |
| 5 mg/kg | 17,200±336 | 833±90* | −95.16 | 0.50±0.34** | 87.00 |

Administration of extract on days 21–25 post-inoculation with four cysticercoids per rat

*$p$<0.001 vs. pre-treatment value, Student's $t$ test; **$p$<0.001 vs. control, Student's $t$ test

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