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Original Research

ScopeMed

In vitro and *in vivo* anthelmintic effects of *Caesalpinia bonducella* (L.) Roxb. leaf extract on *Hymenolepis diminuta* (Cestoda) and *Syphacia obvelata* (Nematoda)

Shyamalima Gogoi, Arun K. Yadav

ABSTRACT

Background: Leaves of *Caesalpinia bonducella* (L.) Roxb. have been traditionally used as an herbal remedy to treat the intestinal helminthic infections in traditional medicine of India. **Aim:** This study was undertaken to evaluate the potential *in vitro* and *in vivo* anthelmintic effects of *C. bonducella* leaf extract against *Syphacia obvelata* (Nematoda) and *Hymenolepis diminuta* (Cestoda). **Materials and Methods:** The *in vitro* anthelmintic activity of the extract was investigated on adult worms of *S. obvelata* (Nematoda) and *H. diminuta* (Cestoda) in terms of physical motility and mortality of parasites. The *in vivo* study was performed in *H. diminuta*-rat model and *S. obvelata*-mice model, by monitoring the egg per gram of feces count and worm count of animals following the treatment with different doses of plant extract. **Results:** The study recorded significant and dose-dependent anthelmintic effects of the extract on both the parasites. In the *in vitro* study, 30 mg/ml concentration of extract caused mortality of *H. diminuta* in 2.5 ± 0.2 h and *S. obvelata* in 3.57 ± 0.16 h. In the *in vivo* study, the extract showed a comparatively better efficacy on *S. obvelata*, where its 800 mg/kg dose revealed 93% reduction of worm load in mice, as compared to 85% worm load reduction of *H. diminuta* in rats. **Conclusions:** The findings suggest that leaf extract of *C. bonducella* possesses significant anthelmintic effects and supports its use as an anthelmintic in traditional medicine. This appears to be the first report of *in vivo* anthelmintic activity of *C. bonducella* against these parasites.

KEY WORDS: Anthelmintics, *Caesalpinia bonducella,* helminthiasis, *Hymenolepis diminuta*, soil-transmitted helminthiasis, *Syphacia obvelata*

Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya, India

Address for correspondence:
Arun K. Yadav,
North-Eastern Hill University, Shillong, Meghalaya, India.
E-mail: akynehu@hotmail.com

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INTRODUCTION

Intestinal helminths or soil-transmitted helminths (STH) are among the most widespread infections and affect more than 2 billion people worldwide [1]. India has the highest burden of STH in the world, with comparatively more prevalence in rural areas and infections encountered more often in children of school-age or younger age between 2 and 14 years at its highest rate. STH are mainly comprised the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*). Although the intestinal helminth infections are relatively less pathogenic to human beings as compared to other infectious agents, cases of heavy worm burdens often lead to malnutrition, anemia, stunted growth, and other intestinal disorders in infected subjects [2]. The global strategy to control the STH is to control the morbidity due to worms applying mass drug administration (MDA) in endemic areas by two standard anthelmintic drugs: Albendazole (ABZ) and mebendazole [3]. However, despite the availability of safe and effective drugs, presently only about 40% of the people at risk of STH is under MDA coverage at global level [4]. In particular, in many African and Asian countries, including India, the anti-STH drugs do not reach in all endemic regions in a sufficient manner. In addition, since currently there are only limited number of drugs available against intestinal worms, the threat of drug resistance in STH is also posing a big challenge [5].

From ancient times, intestinal helminth infections have been treated by several herbal medicines, based on the traditional beliefs of different cultures. The herbal medicines are also easily available and affordable by a large section of the people in rural regions. In the recent past, a number of studies have been made to validate the anthelmintic effects of medicinal plants. For example, Abdel-Ghaffar *et al.* [6] reported that extracts prepared from coconut, onion, garlic, fig, date tree, chicory, ananas, and cistrose possess significant effects on cestodes, (*Hymenolepis diminuta*, *Hymenolepis microstoma*, and *Taenia taeniaeformis*)

and trematodes (*Fasciola hepatica and Echinostoma caproni*). Similarly, leaf extracts of *Adhatoda vasica* and *Clerodendrum colebrookianum* have also been found to be effective against *H. diminuta* infections in rats [7,8]. Recently, Deori and Yadav [9] have also reported the anthelmintic effects of stem bark extract of *Oroxylum indicum*, a traditional anthelmintic plant of India, on larval and mature worms of *H. diminuta*, a zoonotic tapeworm.

Caesalpinia bonducella (L.) Roxb., (Caesalpiniaceae), called as "*Letaguti/letaiguti*" in Assam, India, is a prickly shrub of about 20 m height [Figure 1a]. In addition to India, this plant is also distributed in other Southeast Asian countries such as China, Thailand, Philippines, Indonesia, and Malaysia, particularly in the waste ground and coastal areas and up to an altitude of 1000 m. The crude extracts prepared from different parts of *C. bonducella* have been widely used in folk medicines for treating various diseases such as, pneumonia [10], filarial infections [11] and have also been reported to possess hypoglycemic, antihyperglycemic, and hypolipidemic effects [12]. Interestingly, in Mishing tribe of Assam, India, *C. bonducella* leaves [Figure 1b] are taken as decoction, infusion or as vegetable to treat the intestinal helminth infections. In India, an anthelmintic herbal medicine prepared from the leaves of this plant is called as *Kusere* [Figure 1c]. In view of the extensive use of this plant in traditional medicine and considering the few significant scientific studies that has been carried out on this plant against intestinal helminths, this study was undertaken to evaluate the anthelmintic effects of *C. bonducella* leaf extract, using model parasites *Syphacia obvelata* (a rodent pinworm) and *H. diminuta* (a zoonotic cestode).

MATERIALS AND METHODS

Chemicals and Standard Drugs

All the chemicals used were of standard analytical grade, purchased from Merck, India. ABZ (Ambalal Sarabhai Enterprises Ltd., Vadodara) and praziquantel (PZQ) (Distocide, Chandrabhagat Pharma Pvt. Ltd., Mumbai, India) were used as reference drugs to compare the plant's anthelmintic efficacy.

Figure 1: *Caesalpinia bonducella* (a) Whole plant in its natural habitat, (b) close-up of plant leaves, (c) *Kusere*, the indigenous anthelmintic medicine prepared from plant leaves

Plant Material and Preparation of Extract

The plant material was collected in October 2011 from Dhakuakhana, Lakhimpur district of Assam, and authenticated by a plant taxonomist. A voucher specimen (No. NEHU-12034) has been deposited in Ethnopharmacology and Parasitology Lab, Department of Zoology, NEHU, Shillong. The field-collected leaves were washed, dried under shade, and powdered to a fine grade for extraction with methanol in a Soxhlet extractor at 40°C. The final yield of extract was 3.61% (w/w), from the fresh material.

Phytochemical Screening

The phytochemical analysis of *C. bonducella* methanol leaf extract was carried out to confirm the presence of various secondary metabolites, as described by Harborne [13], Trease and Evans [14], and Sofowra [15]. The presence of phytochemicals in trace or abundance was judged by the color intensity of the test results.

Experimental Animal Models

Swiss albino mice of both sex weighing 20-25 g, and Wistar rats of both sex weighing 180-200 g, were used in the study. Experimental animals were kept at standard room temperature with 12 h light and dark cycle and fed standard rodent feed (Pranav Agro Industries Ltd., Delhi) and water *ad libitum*. Before the extract testing, all the animals were given 2 mg/kg dose of PZQ and 5 mg/kg dose of ABZ for 3 days, and their fecal samples were examined to confirm that they are free from any intestinal helminth infections. After acclimatization for 7 days, the natural infections of *S. obvelata* were identified in mice by the use of perianal cellophane tape, as described by Meade and Watson [16], with slight modifications. While infection of *H. diminuta* was maintained in Wistar rats as described by Tangpu *et al.* [17].

Ethical Standards

All experiments were performed in accordance with Indian Animal Ethics Committee regulations. All the experiments on animals were performed following the approval from the Institutional Animal Ethics Committee (Animal Models) of North-Eastern Hill University, Shillong.

Acute Oral Toxicity Study

The acute oral toxicity study was conducted according to the guidelines of Organization for Economic Cooperation and Development (OECD) revised up-and-down procedure for acute oral toxicity testing [18]. First, a limit dose of 2000 mg/kg body weight of plant extract was used in five female Swiss albino mice. Animals were dosed individually and observed for adverse clinical signs (e.g., tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma, gait, and posture) or mortality during

first 30 min and then periodically during first 24 h. If the first animal survived, then four additional animals were given the same dose of extract at 48 h of interval, and all the animals were kept under observation for 14 days. The lethal dose, 50% (LD_{50}) was predicted to be above 2000 mg/kg if three or more animal survived in the experiment. Similarly, a limit dose of 5000 mg/kg was also tested using almost similar experimental protocols, except that herein the treatment of animals was done by 5000 mg/kg dose of extract, involving first only a single mouse and later two more additional animals, following which the dosing of further animals was terminated [18].

In Vitro Anthelmintic Assay

The adult *S. obvelata* was collected from naturally infected mice, whereas the adult *H. diminuta* specimens were obtained from laboratory maintained infection in Wistar rats. The adult specimens of test parasites were washed several times in Hank's solution. The test worms ($n = 5$) were then kept in Petri dishes containing 10, 20, and 30 mg/ml concentrations of plant extract in Hank's solution and put inside an incubator at 37 ± 1°C. ABZ (5 mg/ml) and PZQ (1 mg/ml) were used as reference drugs. One set of worms ($n = 5$), maintained in Hank's solution, was kept as control. The anthelmintic efficacy of extract was determined in terms of physical motility of test worms, as evidenced by their paralysis or mortality [19]. The experiments were performed 3 times with five number of test worms per concentration of plant extract.

In Vivo Anthelmintic Assays

For *S. obvelata*, infection in experimental mice was detected by the cellophane tape test. Infected animals were then divided into five groups, consisting of five animals in each group. Group I of mice served as control, whereas, Groups II, III, and IV of mice were treated with 200, 400, and 800 mg/kg doses of extract for 5 days. The Group V of mice was given 20 mg/kg dose of a reference drug, ABZ for the same duration. A cellophane tape preparation was obtained from each mouse, consecutively for 3 days before treatment and after treatment of plant extract to determine the eggs per gram of feces (EPG) count. For this, to each mouse, a single piece of cellophane tape was firmly applied to its anal region and sampling was done during 13:00-14:00 h. The length of tape varied between 25 and 30 mm. These tape samples were attached to the standard 25 mm × 75 mm microscope slides, and characteristics *S. obvelata* eggs were counted under a microscope [16]. Finally, the EPG of feces was calculated in all groups of animals to work out the reduction in EPG counts in treated groups [6]. On day 8 post-treatment, all the animals were sacrificed to determine their respective worm load.

Against *H. diminuta*, the efficacy of extract was evaluated against the larval and adult stages. Four cysticercoid larvae of *H. diminuta* were orally given to each Wistar rat to induce infection. For each stage, rats were divided into five groups, comprising of five animals per group. Against the larval stage of parasite, the first group of animals was used as control. The Groups II, III, and IV of animals were orally given 200, 400, and 800 mg/kg dose of extract, respectively, on day 2-4 post inoculation (p.i.) of cysticercoids. The animals of Group V received 5 mg/kg of reference drug, PZQ for the same duration. From day 18 p.i., fecal samples of rats were collected from each cage, and the EPG of feces counts was done for three consecutive days, i.e., days 18-20 p.i. On day 31 p.i., rats were sacrificed and the percentage reduction in worm count was also determined [17].

For the adult stages of parasite, almost similar methods were followed, except that there were some differences in days of treatments and EPG counts. Herein, extract treatment was done between day 21 and 23 p.i. of cysticercoids, and the EPG counts were undertaken between day 18 and 20 of p.i. (pre-treatment period) and during days 34-36 p.i. (post-treatment period).

Statistical Analysis

All the results are represented as mean±standard error of the mean using GraphPad Prism (version 4.5) software. *In vitro* data were analyzed using Student's *t*-test and *in vivo* study; data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test with $P < 0.001$ being considered statistically significant.

RESULTS

Phytochemical Studies

The qualitative phytochemical tests revealed that methanol leaf extract of this plant contains terpenoids, tannins, alkaloids, flavonoids, glycosides and reducing sugars as major secondary metabolites. On the basis of color intensity of the test results, it was observed that tannins and flavonoids are present in abundance in the extract, whereas the others are present only in trace amounts.

Acute Oral Toxicity

Oral administration of *C. bonducella* leaf extract at single limit doses of 2000 and 5000 mg/kg caused no signs of toxicity or morality in all the treated mice. During the observation period of 2-week, all the dosed animals were found to be healthy and normal without any apparent symptoms of adverse effects.

In Vitro Studies

In the present study, *C. bonducella* leaf extract showed a dose-dependent activity against nematode *S. obvelata* and cestode *H. diminuta*. Exposure to varying concentrations of extract led to a significant reduction ($P < 0.001$) in paralysis and mortality time of test worms [Figure 2]. At 30 mg/ml concentrations of extract, the mean paralysis and mortality time of *S. obvelata* were observed to be 3.57 ± 0.16 h, which compared very well with the efficacy of a standard drug, ABZ, which showed a mean paralysis and mortality time of worms of 2.37 ± 0.21 h and 3.57 ± 0.16 h, respectively. While for *H. diminuta*, exposure to 30 mg/ml concentration of extract showed paralysis of worms in

4.86 ± 0.16 h and mortality in 2.5 ± 0.2 h. Herein, the reference drug PZQ revealed paralysis of worms in 1.46 ± 0.52 h and mortality in 3.46 ± 0.23 h. On the other hand, physical activity of nematode and cestode control worms was recorded till 36 ± 0.57 h and 32.44 ± 0.23 h, respectively. Thus, *in vitro* studies showed that exposure of test worms leads to an early paralysis, followed by mortality of parasites.

In Vivo Studies

In the *in vivo* study against *S. obvelata* in mice, following treatment with different doses of plant extract a significant decrease was observed in the EPG counts of animals during the post-treatment periods [Table 1]. During the first 3 days of study, the pinworm eggs were detected in the cellophane-test of mice all groups. However, during consecutive 5 days administration of the highest dose of plant extract (800 mg/kg dose) to the animals, the number of eggs dropped significantly from 20.8 ± 2.14 to 5.1 ± 0.78 in animals. In comparison, in the ABZ treated animals, the number of eggs in cellophane-test reduced from 18.7 ± 0.88 to 2.4 ± 0.33 during the pre- and post-treatment periods. In contrast, no EPG reduction was observed in control animals and the same increased slightly from 25.8 ± 1.19 to 26.7 ± 1.41, during the pre- and post-treatment periods. In addition, the oral administration of 800 mg/kg dose of *C. bonducella* extract to *S. obvelata* naturally infected mice for 5 days also revealed a significant worm reduction of 93% as compared to control animals. Herein, the efficacy of extract was found to be quite comparable with the reference drug ABZ (20 mg/kg) which showed an EPG reduction of 97% [Table 1].

In the *in vivo* assay of plant extract against larval stages of *H. diminuta*, the highest dose of extract, i.e. 800 mg/kg, caused 84.38% reduction in EPG counts and 80% reduction in worm counts of animals [Table 2]. The effects of extract were almost similar with that of treatment of animals with 5 mg/kg dose of reference drug, PZQ, wherein reductions in EPG count and worm counts were recorded to be 82.60% and 85%, respectively. Further, the *in vivo* testing of extract against the adult stages of parasite also showed almost similar pattern, as in case of testing of extract against the larval stages of parasite. The animals treated with 800 mg/kg dose of extract showed 91.53% reduction in the EPG counts at the post-treatment

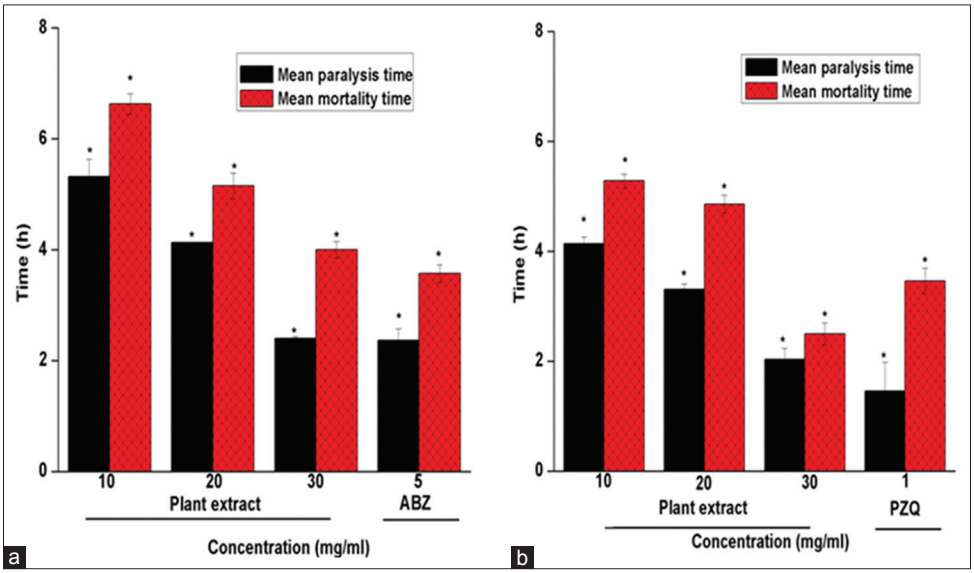

Figure 2: *In vitro* anthelmintic effects of *Caesalpinia bonducella* leaf extract investigated in terms of physical motility and mortality on adult parasites, (a) *Syphacia obvelata*, (b) *Hymenolepis diminuta*. Physical activity of control worms for respective experiment was recorded as 36 ± 0.57 h and 32.44 ± 0.23 h. Data are presented as mean ± SEM. **P* < 0.001, as compared to control, Student's *t*-test. SEM: Standard error of the mean

Table 1: Effects of *C. bonducella* leaf extract* on rodent pinworm, *S. obvelata* in naturally infected mice (*n*=5)

Groups (mg/kg×day×dose)	EPG (mean±SEM)		Worm count at necropsy		Percentage reduction in worm count
	Pre-treatment (Days 1-3)	Post-treatment (Days 9-11)	Min-Max	Mean±SEM	
Control	25.5±1.19	26.7±1.41	180-200	208.0±11.5	0
Plant extract					
200×1×5	18.5±0.87	12.0±0.85[a]	100-180	146.0±12.8[b]	29.8
400×1×5	22.5±1.09	11.2±0.66[a]	100-200	136.0±22.2[b]	34.6
800×1×5	20.8±2.14	5.1±0.78[a]	12-20	15.4±1.6[b]	92.6
ABZ					
20×1×5	18.7±0.88	2.4±0.33[a]	1-7	7.0±1.90[b]	96.6

Values are presented as the mean±SEM; One-way ANOVA followed by Tukey's test; *Administration of plant extract and reference drug ABZ on days 4-8 post EPG count; [a]*P*<0.05 compared to pre-treatment, [b]*P*<0.001, as compared to control. SEM: Standard error of the mean. ANOVA: Analysis of variance, EPG: Eggs per gram, ABZ: Albendazole¸ *C. bonducella*: *Caesalpinia bonducella, S. obvelata: Syphacia obvelata*

period, compared to 80.55% reduction by 5 mg/kg dose of PZQ [Table 3].

DISCUSSION

This study was aimed at investigating the *in vitro* and *in vivo* anthelmintic effects of a leaf extract from *C. bonducella*, a traditional anthelmintic plant of India, against nematode, *S. obvelata* and cestode, *H. diminuta*. The acute toxicity study in mice revealed that the single limit doses of 2000 mg/kg and 5000 mg/kg of *C. bonducella* leaf extract did not cause any alteration in the animal's behavior, *viz*., breathing, posture, and food and water consumption or neither showed any toxic effects during the whole period of study. These preliminary observations indicate that the extract is non-toxic since LD_{50} value of the extract is calculated to be <5000 mg/kg in mice, which is considered to be safe as per the OECD guidelines [18].

The *in vitro* testing of *C. bonducella* leaf extract against *H. diminuta* and *S. obveleta* showed that following exposure to different doses of extract the parasites reveal an early paralysis and complete loss of body movements as compared to control parasites. Some other similar kind of studies have also investigated the *in vitro* antiparasitic effects of different medicinal plants on adult helminth parasites by evaluating the effects of plant extracts on physical activity of test worms [20-22]. However, in many such studies very often the used concentrations of plant extracts have not always matched appropriately with their dosages used in the *in vivo* assays and thus this has caused some variations in estimating the efficacies of plant extracts in respective assays [23,24]. It is also worth mentioning here that many previous anthelmintic studies on medicinal plants have usually either employed *in vitro* assays [18], or in other cases only *in vivo* models [25] to assess the anthelmintic effects of plant extracts. However, it is only in few cases that an attempt has been made to employ both *in vitro* and *in vivo* tests to evaluate the anthelmintic properties of a specific medicinal plant [9]. Therefore, to achieve a more authenticated scientific data about the anthelmintic properties of plant extracts, it is more worthwhile that, as far as possible, the *in vitro* assays on these plants should also be supplemented by *in vivo* studies.

Our results of *in vitro* testing of extract showed marked effects on nematode parasite with a dose-dependent efficacy ($P < 0.001$) which are in agreement with the findings of Hussain *et al.* [26] against nematode, *Haemonchus contortus* where it has been reported that the leaves extract of *Trianthema portulacastrum* also caused dose and time-dependent anthelmintic effects on test parasites.

Similarly, the present findings of dose-dependent *in vivo* effects of *C. bonducella* extract on *S. obvelata* are in agreement with the findings of Camurça-Vasconcelos *et al.* [27], who investigated the antinematodal properties of *Croton zehntneri* and *Lippia sidoides* essential oils on intestinal nematodes of mice. Likewise, in a study by Kozan *et al.* [28], the highest dose of a Turkish folk medicinal plant, *Jasminum fruticans* also showed 74% reduction

Table 2: Effects of *C. bonducella* leaf extract* on larval stages of *H. diminuta* infections in rats (*n*=5)

Groups (mg/kg×dose×day)	EPG (mean±SEM) Days 18-20	Percentage reduction in EPG counts	Worms count/rat (mean±SEM)	Percentage reduction in worm counts
Control	5866±464	-	3.8±0.20	5.00
Plant extract				
200×1×3	1873±392[a]	−68.07	2.0±0.37[a]	50.00
400×1×3	1790±335[a]	−69.48	1.4±0.24[a]	60.00
800×1×3	916±123[a]	−84.38	0.8±0.37[a]	80.00
PZQ				
5×1×3	1094±348[a]	−82.60	0.6±0.24[a]	85.00

Values are presented as the mean±SEM, one-way ANOVA followed by Tukey's test; *Administration of extract and reference drug PZQ on days 2-4 post-inoculation with four cysticercoids per rat; [a]$P<0.001$, as compared to control. SEM: Standard error of the mean, ANOVA: Analysis of variance, EPG: Eggs per gram, PZQ: Praziquantel, *C. bonducella: Caesalpinia bonducella, H. diminuta: Hymenolepis diminuta*

Table 3: Effects of *C. bonducella* leaf extract* on mature stages of *H. diminuta* infections in rats (*n*=5)

Groups (mg/kg×dose×day)	EPG (mean±SEM)		Percentage difference in EPG (A-B)	Worms count/rat (mean±SEM)	Percentage reduction in worm counts
	Pre-treatment Days 18-20 (A)	Post-treatment Days 28-30 (B)			
Control	28992±4761	25233±1939	−12.9	4.0±0.00	0.00
Plant extract					
200×1×3	22966±3885	7186±318[a]	−68.71	1.8±0.31[b]	55.00
400×1×3	20700±2876	4510±428[a]	−78.21	1.2±0.37[b]	70.00
800×1×3	20233±1354	1713±261[a]	−91.53	0.6±0.24[b]	85.00
PZQ					
5×1×3	22125±3590	4303±471[a]	−80.55	0.8±0.20[b]	80.00

Values are presented as the mean±SEM, One-way ANOVA followed by Tukey's test; *Administration of extract and reference drug PZQ on days 21-23 post-inoculation with four cysticercoids per rat; [a]$P<0.001$ compared to pre-treatment, [b]$P<0.001$, as compared to control. SEM: Standard error of the mean, ANOVA: Analysis of variance, PZQ: Praziquantel, *C. bonducella: Caesalpinia bonducella, H. diminuta: Hymenolepis diminuta,* EPG: Eggs per gram

in worm counts as against 93% observed in the present study. Furthermore, the findings of the present study are in agreement with the findings of Shariat *et al.* [29], who reported 95% reduction of *S. obvelata* worms following treatment of infected mice by *Pleurotus eryngii* extract.

Similar findings of dose-dependent anthelmintic effects of various other medicinal plants against cestodes have also been reported by Temjenmongla and Yadav [30] and Kundu *et al.* [31]. In our study, the highest dose of plant extract showed a decline in worm burden of animals by 85%, which was of almost alike to a decrease in worm burden by reference drug PZQ [Table 2]. In related studies by Sapaat *et al.* [32] and Gangwar *et al.* [33], more or less a similar kind of reduction in EPG counts of *H. diminuta* infections in rats was also observed following treatment with extracts from the seeds and fruits of papaya and *Mallotus philippinensis*. Interestingly, in the present study, *C. bonducella* extract showed a better efficacy not only against the adult stages but also almost similar effects on larval stages of the parasite. However, in most other related studies on *in vivo* anthelmintic effects of plants extracts on various developmental stages of *H. diminuta*, it has been reported that plant extracts possess rather more significant effects on adult stages than the larval stages of parasite [8,34].

The phytochemical screening of *C. bonducella* leaf extract revealed the presence of several major chemical constituents, but tannins and flavonoids were present in abundance. It is likely that the potent anthelmintic activity of its methanol extract may be due to the presence of either of these bioactive constituents such as tannins or flavonoids. It is apparent from literature that these plant secondary metabolites have also been used as a primary source of treatment against gastrointestinal helminths for many centuries, and they also exhibit significant anthelmintic activities [23,35]. For example, isoflavones, such as genistein and daidzein, which are found in a number of plants, including in *Flemingia vestita*, have shown significant anthelmintic effects on intestinal helminth parasites [36]. Similarly, condensed tannins, which offer important quality traits to plants, also possess potential anthelmintic effect against gastrointestinal helminth parasites [37]. There are several such studies on the secondary metabolites which suggest that it is very likely that *C. bonducella* leaf extract brings out its anthelmintic effects due to the presence of these secondary metabolites. However, only further studies on isolation and characterization of its active constituents may throw more light on same.

CONCLUSION

From the overall study, it may be concluded that *C. bonducella* leaf extract possesses a significant *in vitro* and *in vivo* anthelmintic effects on both, the nematode *S. obvelata* and cestode *H. diminuta* parasites. However, the actual mode of action of the plant extract has to be determined to draw a complete picture of the active compounds that are responsible for its anthelmintic action. Nevertheless, these findings lend support to the traditional use of this plant as anthelmintic in indigenous medicine. It is hoped that refining of plant formulation in a holistic manner may contribute to the development of a suitable herbal medicine with a low risk of resistance to intestinal helminths.

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