

## Anthelmintic activity of ripe fruit extract of *Solanum myriacanthum* Dunal (Solanaceae) against experimentally induced *Hymenolepis diminuta* (Cestoda) infections in rats

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**Abstract** Although there are many effective drugs available to treat intestinal worms, the fact remains that they remain out of reach to a majority of the population in many areas of the world. On the other hand, traditional plant-based remedies continue to be an important therapeutic aid for treating worm infections throughout the world, especially in the developing nations. *Solanum myriacanthum* Dunal is a perennial shrub that is used in the folk medicine of Tangkhul Naga tribe of India for treating intestinal worms. This study evaluates the anthelmintic activity of its ripe fruit extract using experimental *Hymenolepis diminuta* (a zoonotic tapeworm) infections in albino rats. The efficacy of extract was adjudged by monitoring the eggs per gram (EPG) count of parasite as well as by the direct count of surviving worms in the intestine following treatment with methanol fruit extract of this plant to different groups of rats harbouring *H. diminuta* infections. The plant extract showed a dose-dependent reduction of both EPG as well as worm counts for all the developmental stages of *H. diminuta* in rats. However, the effects of the extract were more apparent on the adult stages than larval or immature stages of the parasite. Against the adult stage, a single oral dose of 800 mg/kg of extract, given for 3 days, showed 60.49% reduction in the EPG counts and 56.60% reduction in the worm counts in the extract-treated group as compared to untreated control. In comparison, the reference drug praziquantel (5 mg/kg) showed 51.81% and 70.00% reduction in the EPG and worm counts, respectively. The LD<sub>50</sub> (oral) of the extract was determined to be 3,093.24 mg/kg in rats, and no significant changes were

observed in the values of serum glutamate oxalate transaminase, serum glutamate pyruvate transaminase, cholesterol and total protein between the extract-treated and control groups of animals. These findings indicate that ripe fruits of *S. myriacanthum* possess significant anthelmintic property, without any adverse effects to the experimental animals. This may provide a scientific rationale for the traditional use of this plant against intestinal worms.

### Introduction

Intestinal helminthic infections, such as ascariasis, trichuriasis, hookworm and tapeworm infections, continue to be a cause of major concern to human health in several parts of the world, particularly in the developing nations, causing malabsorption, diarrhoea, anaemia and other states of poor health (Hotez et al. 2007). Globally, over 3.5 billion people are infected with intestinal worms, of which children between 5–15 years account for the highest infection rate of about 400 million cases of worm burden that are mainly attributed to poor sanitation and hygiene (Luong 2003). In India, infections with these parasites are regarded as amongst the most common public health problems, particularly in rural areas and urban slums (Naish et al. 2004). Data from recent surveys in the country reveal an overall prevalence of 22–85% of soil-transmitted helminthiasis in children of age group 4–15 years (Qadri 2008). Although there are several effective drugs for the treatment of intestinal helminthiasis, the fact remains that they remain out of reach of majority of the people in the world, as about one third of the global population still lacks regular access to affordable essential medicines (WHO 2004). And, according to the World Health Organization (WHO), in India itself, about 50% to 65% of the total population (i.e.,

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about 38% of the world population) does not have regular access to essential medicines (WHO 2004). In this connection, traditional medicines, based largely on the use of plants or plant extracts, offer a major and accessible source of health care to a large section of the people in India (Anonymous 2000; WHO 2002). A perusal of the literature reveals that parasitic infections are among those ailments that traditional healers confidently treat and against which an enormous variety of plant-based traditional remedies do exist (Githiori et al. 2005; Tandon et al. 2011).

In the search for plant-based anthelmintics, extracts of several medicinal plants have been tested and proved to be effective against many helminth parasites (Akhtar et al. 2000; Tagboto and Townson 2001; Tandon et al. 2011; Abdel-Ghaffar et al. 2011; Klimpel et al. 2011). *Solanum myriacanthum* Dunal (Solanaceae), vernacularly known as Changranlotei, is a thorny shrub of about 0.5–1.5 m height. It is native to Central and South America, but has been naturalized in parts of tropical Asia, including India (Babu and Hepper 1979). This plant bears globose fruits, 2–3 cm in diameter, which are green in the beginning and then turn light yellow at maturity. All parts of *S. myriacanthum* have traditionally been used by several indigenous communities in India and the neighbouring countries to treat a variety of diseases and ailments since time immemorial. In the folk medicine of Apatani and Nyishi tribes of Arunachal Pradesh, the seeds of *S. myriacanthum* have been employed to relieve toothache (Kala 2005; Srivastava and Nyishi Community 2010). Among the Naga tribes of Nagaland state, fruits of *S. myriacanthum* are burnt on fire and kept in contact with the teeth for some time to kill the germs (Rao and Jamir 1982). Similarly, many tribes in the northeast India consume the root decoction of this plant for the treatment of malarial fever (Bora et al. 2007; Srivastava and Nyishi Community 2010). Also, a paste prepared from the fruits of this plant is applied on the abdomen to relieve stomachache in the Tangkhul tribe of Manipur state (Tangpu et al. 2007). In Bangladesh, the Chakma tribe consumes the fruits of *S. myriacanthum*, believing them to function as a natural aphrodisiac (Roy et al. 2008). Recently, it was also reported that Nyishi tribes in Arunachal Pradesh consume the decoction of powdered dried fruits of a closely related species, namely *Solanum kurzii*, for getting complete relief from worm infestations (Srivastava and Nyishi Community 2010). In a previous *in vitro* study, it was shown that extract of ripe fruits of *S. myriacanthum* possess a good activity against a bovine filariid, *Setaria cervi* (Tangpu and Yadav 2003).

During our course of studies on anthelmintic plants of northeast India, it was revealed that fruits of *S. myriacanthum* have a good reputation among the native Tangkhul Naga tribe of Manipur for curing intestinal worm infections

(Tangpu et al. 2007). A literature survey revealed no reports on anthelmintic effects of this plant against intestinal helminths. Therefore, this study was undertaken to evaluate the anthelmintic activity of *S. myriacanthum* fruit extract against experimentally induced *Hymenolepis diminuta* (a zoonotic cestode) infections in rats. For this purpose, we studied the efficacy of plant extract against different developmental stages of parasite, i.e. larval worms [days 2–4 post-infection (p.i.)], immature worms (days 8–10 p.i.) and adult worms (days 21–23 p.i.).

## Materials and methods

### Pilot study

A pilot survey was conducted in the Ukhrul district of Manipur in India on the use of anthelmintic plants by interviewing some local traditional healers, called Haori-Khanong. A brief questionnaire was administered for collecting data on the reasons for using this plant, frequency of its use and its perceived efficacy as a cure for intestinal worms.

### Plant material and preparation of extract

The ripe fruits of *S. myriacanthum* were collected from Paoyi village in Manipur, India. The plant material was identified by a plant taxonomist at the Department of Botany, NEHU, Shillong, and a voucher specimen (No. AKY–216) has been deposited in the Department of Zoology, NEHU, Shillong. The fruits were dried under shade and powdered for extraction with methanol in a Soxhlet extractor at 40°C. The resulting suspension was decanted out discarding the remnants, and the filtrate was further concentrated in a rotary evaporator under reduced temperature and pressure for removal of the solvent. The percentage yields (*w/w*) of the crude extract was 7.56%. The extract was stored in plastic vials at +8°C until use.

### Reference drug

Praziquantel (PZQ, Distocide®) was used as the reference drug and was manufactured by Shinpoong Pharmaceutical Co., Ltd., Seoul, Korea. Plant extract and PZQ solutions were prepared fresh in 0.9% phosphate-buffered saline before administration to experimental animals.

### Animals

Male and female Wistar rats weighing 100–120 g body weight were used. They were acclimatized for 15 days in the laboratory prior to use for experiments. During this

period, the stool samples of animals were routinely examined to ensure that they do not harbour any intestinal worms. Animals were maintained under standard environmental conditions and fed with rodent diet (Pranov Agro Industries Ltd., Delhi) and water ad libitum. Proper care was taken to protect the welfare of the experimental animals, and all the experiments were performed according to the rules laid down by the Institutional Animal Care and Use Committee.

#### Maintenance of *H. diminuta* infection

The life cycle of *H. diminuta* was maintained in the laboratory in Wistar rats, using flour beetle *Tribolium confusum* as the intermediate host (Dixon and Arai 1991). In brief, the gravid segments of tapeworm were scratched smoothly on to the filter papers inside the petri dishes, and the beetles were allowed to feed on flour for 72 h. These beetles were then maintained at room temperature for at least 12–14 days for the cysticercoid larva to develop. Cysticercoids were collected by dissecting the beetles and inoculated to uninfected rats for initiation of infection. After 18–20 days, eggs of *H. diminuta* were detected in the faeces of rats, which were mixed with flour powder and fed to the beetles to continue the life cycle in the laboratory.

#### Anthelmintic assay

Efficacy of plant extract was evaluated against three life cycle stages of *H. diminuta* (larval, immature worms and adult worms). For each developmental stage, animals were divided into five groups, six animals per group. Each animal was then orally inoculated with five cysticercoids, using a blunt feeding needle, and maintained in a separate cage.

Against the larval stage of the parasite, the first group of animals was used as control and given only vehicle. The animals belonging to groups 2, 3 and 4 were orally administered with 200, 400 and 800 mg/kg body weight dose of plant extract on days 2–4 p.i. of cysticercoids. The fifth group of animals received 5 mg/kg, p.o. dose of reference drug PZQ for the same duration. From day 18 p.i., 1 g of fresh faeces was collected from each cage of the treated and control rats for counting the eggs per gram of faeces (EPG), using the modified McMaster method (Anonymous 1977), for three consecutive days, i.e. on days 18–20 p.i. Finally, an autopsy was performed by chloroform anesthesia killing of the animals on day 31 p.i. and worms from the intestine were collected. Accordingly, the percentage reduction in worm count was determined from the number of worms recovered divided by number of cysticercoids inoculated, as described previously by Tangpu et al. (2006).

For the immature stages of parasite, almost similar experimental protocols were followed, except that treatment

of experimental animals with plant extract and reference drug was undertaken on days 8–10 p.i. of cysticercoids. Against the adult stages of parasite, the extract and PZQ were given on days 21–23 p.i. of cysticercoids, and an EPG count of animals was undertaken between days 18–20 (pretreatment period) and days 34–36 (post-treatment period). The percentage reduction in the EPG counts was calculated as per the criterion of Iqbal et al. (2004). On day 37, all the experimental animals were sacrificed and the numbers of worms remaining in their intestine were counted to calculate the percentage reduction in worm burden as described above.

#### Acute toxicity study

##### *Determination of median lethal dose*

The rats were divided into seven groups, comprising of six animals in each group. Group 1 of animals served as control and received only vehicle. Groups 2–7 of animals were orally administered with 100, 200, 400, 800, 1,600 and 3,200 mg/kg dose of plant extract. The general signs and symptoms of toxicity, intake of food and water, and mortality of experimental animals was observed for 72 h post-administration of the extract (Tangpu and Yadav 2004). From these observations, the median lethal dose (LD50) of the extract was calculated using SPSS software (SPSS Inc., Chicago, IL, USA).

##### *Serum biochemical tests*

The samples of blood were collected from extract-treated (800 mg/kg) and control group of animals after 72 h and processed for collection of serum, as per the standard methods. The serum was assayed for glutamate oxalate transaminase (SGOT; EC 2.6.1.1), glutamate pyruvate transaminase (SGPT; EC 2.6.1.2), cholesterol and total protein, as per the methods of Strickland et al. (1961), Allain et al. (1974) and Henry et al. (1974), using a semi-automated biochemical analyzer (Bayer).

##### *Statistical analysis*

The experimental results were expressed as the mean ± standard error of the mean (SEM). 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

During our course of studies on anthelmintic plants of northeast India, we came across many medicinal plants which are used by the native tribal populations to cure

**Table 1** Anthelmintic activity of *S. myriacanthum* fruit extract (administration of extract on days 2–4 post-inoculation with five cysticercoids per rat) against larval stages of *H. diminuta* infections 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	32,406±616	–	5.00±0.00	0.00
Plant extract				
200 mg/kg	22,639±647*	–30.14	3.50±0.21*	30.00
400 mg/kg	23,383±763*	–27.85	3.33±0.21*	33.40
800 mg/kg	16,222±1350*	–49.95	2.17±0.31*	56.60
Praziquantel				
5 mg/kg	3,739±2365*	–88.47	0.67±0.42*	86.60

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

intestinal worms (Tandon et al. 2011). This study was undertaken to evaluate the anthelmintic efficacy of ripe fruits of *S. myriacanthum* (a medicinal plant used by the Tangkhul Nagas tribe of Manipur to cure worm infections), employing *H. diminuta* (Cestoda)–rat experimental model. This animal model has widely been used to evaluate the efficacy of several anticestodal agents (Siles-Lucas and Hemphill 2002; Abdel-Ghaffar et al. 2011).

In this study, the effects of *S. myriacanthum* fruit extract were studied against three different life cycle stages of *H. diminuta*. The results obtained revealed that the extract possesses a dose-dependent and moderate to moderately high level of efficacy ( $p<0.05$ ) against various stages of the parasite (Tables 1, 2 and 3). For all tested developmental stages of the parasite, the extract showed its highest efficacy against the adult stages than the larval or immature stages of parasite. Against the larval stage, a single 800-mg/kg oral dose of plant extract, given for 3 days, revealed only 49.95% reduction in EPG counts and 56.60% reduction in worm count, as compared to the control group. In comparison, the reference drug PZQ, given at 5 mg/kg dose for the same duration, showed 88.47% reduction in the EPG counts and 86.60% reduction in the worm count (Table 1). The efficacy of the extract was also found to be comparatively low against the immature stages of parasite. At 800 mg/kg dose of the extract, though the reduction in EPG counts was 57.86%,

reduction in worm count was only 40.00%, as compared with control group. On the other hand, PZQ-treated group of animals showed 78.69% and 80.00% reduction in EPG and worm counts, respectively (Table 2). Interestingly, the plant extract showed a significant activity (in terms of reduction in EPG and worm counts) against the adult stages of *H. diminuta*. The animals treated with 800 mg/kg dose of the extract, for three consecutive days, revealed 56.60% reduction in worm count as compared to the control. In this case, the reduction in worm count by 5 mg/kg dose of PZQ was only slightly higher (70.00%) than that of the plant extract. In a similar manner, animals administered with the same dose of extract showed up to 60.49% reduction in the EPG count between pre and post-treatment periods, compared to only 51.81% by the reference drug PZQ. On the other hand, not much difference was observed in the EPG counts of control group of animals between the pre- and post-treatment periods (Table 3).

We assume that the anthelmintic efficacy of plant extract may be due to the presence of secondary metabolites, particularly the alkaloids. Solanaceae are known for possessing a diverse range of alkaloids, like solasodine, solakhasanin, solamargine and khasinin (Weissenberg 2001). And, many previous studies have assigned the anti-parasitic effects of medicinal plants to these alkaloids (Athanasiadou and Kyriazakis 2004; Li et al. 2011).

**Table 2** Anthelmintic activity of *S. myriacanthum* fruit extract (administration of extract on days 8–10 post-inoculation with five cysticercoids per rat) against immature stages of *H. diminuta* infections 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	31,561±1,060	–	4.67±0.33	6.60
Plant extract				
200 mg/kg	16,783±696*	46.83	4.00±0.00	20.00
400 mg/kg	14,228±1065*	54.92	3.50±0.34**	30.00
800 mg/kg	13,300±1280*	57.86	3.00±0.26**	40.00
Praziquantel				
5 mg/kg	6,728±3025*	78.69	1.00±0.45*	80.00

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

**Table 3** Anthelmintic activity of *S. myriacanthum* fruit extract (administration of extract on days 21–23 post-inoculation with five cysticercoids per rat) against adult stages of *H. diminuta* infections 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
	Pretreatment (days 18–20)	Post-treatment (days 34–36)			
Control	33,111±1,289	31,678±1,056	-4.33	5.00±0.00	0.00
Plant extract					
200 mg/kg	32,989±1,191	24,872±1283*	-24.61	4.00±0.26**	20.00
400 mg/kg	32,111±1,476	15,856±1965*	-50.62	3.00±0.26***	40.00
800 mg/kg	32,283±1,378	12,756±1380****	-60.49	2.17±0.31***	56.60
Praziquantel					
5 mg/kg	30,978±1,781	14,928±1492****	-51.81	1.50±0.56***	70.00

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

With an estimated 1,400 species, *Solanum* is the largest genus in Solanaceae and one of the medicinally important groups of plants. As many as 44 species of *Solanum* are reported to occur in India, of which 9 are considered to be of high economic value and have been classified in the traded list of medicinal plants (Anonymous 2000). A perusal of literature reveals that besides *S. myriacanthum*, either the leaves or fruits of few other *Solanum* spp., namely *Solanum lycocarpum*, *Solanum surattense*, *Solanum khasianum*, and *S. kurzii* have also been used in the folk medicines of a variety of cultures worldwide as a cure for intestinal worms. Many workers, therefore, have attempted to evaluate the anthelmintic efficacy of different *Solanum* species, using various experimental models (Costa et al. 2008; Jarald et al. 2008; Nayak et al. 2009; Kamaraj and Rahuman 2010).

Costa et al. (2008) studied the effects of aqueous leaf extract of *S. lycocarpum*, collected from two localities of Brazil, against *Vampirolepis nana* (a tapeworm of man/mice, now known as *Hymenolepis nana*) and *Aspiculuris tetraptera* (a pinworm of mice) in naturally infected mice.

An oral administration of 10% of *S. lycocarpum* leaf extract for three consecutive days eliminated 28.4–29.2% of adult *V. nana* as compared to untreated control. Whereas, at the same concentration, the efficacy of plant extract was noted to be almost negligible (3.70–4.18%) against the pinworms. Compared to our findings, the anthelmintic efficacy of *S. lycocarpum* extract was relatively lower than *S. myriacanthum*.

We assume that these differences in the efficacies of two *Solanum* species perhaps may be due to the variations in the quantities of chemical constituents present in these two *Solanum* species or may also be due to the differences in the concentrations of extract used to test their efficacy.

Similarly, Nayak et al. (2009) studied the in vitro anthelmintic activity of aqueous and ethanolic fruit extracts of *S. surattense* Linn. (now considered as a synonym of *Solanum virginianum* L.), using earthworms (*Pheritema posthuma*) as test worms. In this study, all extracts of both the solvents were able to show anthelmintic activity (at 10 mg/ml concentration) and the activities were comparable with the standard drugs piperazine citrate and albendazole. A look into the literature, however, reveals that it is only in the very beginning stages that studies on anthelmintic activity of medicinal plants employed earthworms as a test worm (Tandon et al. 2011). Merely on the basis of some morphological similarity with parasitic worms, it was often argued that substances which kill and/or are toxic to earthworms may also bring similar actions in parasitic worms. It may, however, be noted here that except for few morphological similarities, earthworms do not share any anatomical or physiological resemblance with parasitic worms. The reported efficacy of *S. surattense* should therefore be considered in the light of the above facts.

Recently, it was also reported that the berries of *S. khasianum* Clarke are used in the treatment of worm complaints among the local people in the Mandsaur district

**Table 4** Effects of *S. myriacanthum* fruit extract on serum biochemical parameters of rats at 72 h post-treatment (data represent mean±SEM,  $n=6$ )

Groups	SGOT (U/l)	SGPT (U/l)	Cholesterol (mg/dl)	Total protein (g/dl)
Control	149.00±4.34	75.83±3.55	122.50±4.22	6.88±0.19
Plant extract (800 mg/kg)	150.67±7.81	86.00±5.14	124.50±1.26	7.72±0.34



of Madhya Pradesh, Central India (Jarald et al. 2008). The berries extract of this plant was tested in vitro against a variety of parasites (tape worm, liver fluke, thread worm and hook worm) of sheep, at 100 and 200 mg/ml concentrations, respectively. The extract was found to be quite effective against all the test parasites. The extract-treated worms got paralyzed first, within 27–30 min, and then showed mortality within the next 16–20 min. In this study, the efficacy of the extract was found to be comparable with piperazine at 15 mg/ml. Although this study attempted to establish the anthelmintic efficacy of *S. khasianum* berries extract, we find there were few shortcomings in its experimental protocols. Firstly, the concentrations of the plant extracts used (100 and 200 mg/ml) in the in vitro testing of plant extract were exceptionally on very higher sides, and secondly, none of the test parasites were identified to the genus or species level.

Very recently, Kamaraj and Rahuman (2010) studied the in vitro larvicidal and ovicidal activity of leaf and seed extracts of yet another *Solanum* species, i.e. *Solanum torvum* on *Haemonchus contortus*. At the maximum concentration tested (50 mg/kg), a 100 inhibition was recorded for ethyl acetate extract of the plant. The extract also showed its antiparasitic effects on some blood-sucking parasites of cattle and goat and also against a digenean fluke of sheep, namely *Paramphistomum cervi* (Kamaraj and Rahuman 2010).

In the acute toxicity study, the LD<sub>50</sub> (oral) of the extract was found to be 3,093.24 mg/kg in rats, which is an indication that the extract could be considered relatively safe for use in the experimental animals. This is in agreement with the findings of Jarald et al. (2008), where the berries extract of *S. khasianum* was also found to be safe when given orally up to the dose of 2,000 mg/kg to the experimental animals. In the serum biochemical profile analysis, the SGOT, SGPT, cholesterol and total protein levels of experimental animals after extract treatment (800 mg/kg) did not revealed any significant differences between the extract-treated and control groups of animals (Table 4). It thus emerges from these preliminary findings that *S. myriacanthum* fruit extract is apparently safe to the experimental animals. However, a more comprehensive investigation is required to remark further on the toxicity of plant extract.

The field data, through the questionnaire, revealed that little less than 60% of the respondents used this plant as a deworming medicine, while 64% of those who did not use it said that it was because of their ignorance about it. Interestingly, while 57% attributed its use to their traditional knowledge about its efficacy, about 36% used it as it was prescribed to them by their local medicine men. Seventeen percent of the respondents depended only on this plant, it was the first priority for about 29% of the respondents.

Lastly, about 10% of the respondents found the plant to be very effective while in the view of about 83% of the respondents, its effectiveness was average.

In conclusion, our study suggests that fruits of *S. myriacanthum* possess significant anthelmintic property, without any adverse effects to the experimental animals. It could therefore be said that belief about the effectiveness of this plant and its use by tribal population does have a scientific rationale based on their traditional wisdom. The plant may therefore be useful against gastrointestinal helminths.

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