Clinical Phytoscience

ORIGINAL CONTRIBUTION Open Access

Studies on the anthelmintic potentials of the roots of *Asparagus racemosus* willd. (Asparagaceae)

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Abstract

Background: The Santhal tribe in Assam, India use the roots of *Asparagus racemosus* (Asparagaceae) as a deworming remedy. The study aimed to investigate the anthelmintic credentials of this plant, using two representative groups of helminth parasites.

Methods: The *in vitro* testing was conducted against *Hymenolepis diminuta* (cestode) and *Syphacia obvelata* (nematode). Parasites were exposed to 10, 20 and 30 mg/ml concentrations of plant extract, and efficacy was adjudged on the basis of parasites paralysis and mortality. *In vivo* efficacy was examined using *H. diminuta*-rat and *S. obvelata*-mice models where animals were administered 125, 250 and 500 mg/kg doses of extract.

Results: *In vitro* assay, against *H. diminuta* revealed that at 30 mg/ml concentration the extract showed almost a comparable efficacy with that of reference drug praziquantel (PZQ) (1 mg/ml). The *in vitro* efficacy of extract against *S. obvelata* was however lower than *H. diminuta*. *In vivo* studies against *H. diminuta* at 500 mg/kg revealed 53.88 and 24 % reduction in eggs per gram (EPG) and worm counts respectively. Against *S. obvelata* the extract showed 26.61 and 30.93 % reduction for the same.

Conclusions: The findings of this study present suggest that the roots of *A. racemosus* are effective against intestinal helminthic infections and justifies its use as an anthelmintic in the traditional medicine of the Santhals.

Keywords: Anthelmintic, *Asparagus racemosus*, *Hymenolepis diminuta*, *Syphacia obvelata*

Background

Soil-transmitted helminthiasis (STH) or intestinal helminthic infections are important public health problems in tropical and sub-tropical countries, and infect approximately one quarter of the world's population [1]. The WHO 2030 global targets aim to achieve and maintain elimination of STH morbidity, in pre-school and school age children, where they are mainly implicated for malnutrition and physical impairment [1]. There are some effective drugs against these parasites, nevertheless, the existing pharmacopoeia is not considered satisfactory to tackle any future risks of drug-resistance. Folk medicine systems are common practices in several indigenous communities in the world. Especially, in developing countries, the plant-based traditional medicines appear to hold a great promise for the treatment of numerous common diseases. India is well known for its various popular traditional medicine systems, which are widely practiced throughout the country.

Asparagus racemosus Willd. (Asparagaceae), commonly called Satavar or as Kedar among the Santhal tribe, is a spinous shrub. This plant is found in Thailand, Myanmar, Nepal and Bhutan. In India, it is distributed in the states of Assam, Kerala, Kashmir, Maharashtra and Odisha [2]. In the system of Ayurvedic medicine, *A. racemosus* has found numerous important uses. Some of its Ayurvedic usages include its use as antineoplastic,

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antitussive, antihepatotoxic, galactagogue, and aphrodisiac agent [3–7]. In India, the use of this plant against intestinal helminths has been documented in the states of Jharkhand, Kashmir and Assam[8–10].

Experimental studies on this plant have shown that besides its efficacy as anti-dyspepsia and anti-ulcerogenic agent [6] this plant has been found to reverse memory loss [11] and also possesses immunoadjuvant, antioxidant, and anti-diarrheal properties [12–14]. The chemical studies on this plant have revealed presence of important steroidal saponins, called shatavarins, in its roots [15, 16]. Besides, an isoflavone, 8-methoxy-5,6,4′-trihydroxyisoflavone 7-o-β-d-glucopyranoside, has also been isolated from the roots of *A. racemosus* [17]. Owing to its multiple uses in traditional medicine, *A. racemosus* is highly demanded in Indian markets, but the supply is considered inadequate.

During a recent ethnomedicinal survey in Assam, India to collect information about anthelmintic plants, it emerged out that root decoction of *A. racemosus* is commonly used by the natives of the area to cure intestinal worm infections. In order to scientifically validate the aforesaid claims of the people, a systematic ethnomedicinal field survey was conducted to document some key facts about the usage pattern of this plant in the study area. In the present study, investigations on the *in vitro* and *in vivo* anthelmintic potentials of methanolic root extract of *A. racemosus*, involving a cestode, i.e. *Hymenolepis diminuta*-rat and a nematode, *Syphacia obvelata*-mice models was carried out, so as to comment about the usefulness of this plant as anthelmintic.

Methods

Plant material

Plant material was collected from natural habitats in Kokrajhar, Assam, India and identified by a taxonomist. Herbarium was made and submitted to the Department of Botany herbarium museum and assigned a voucher number (NEHU-12098). A copy of the herbarium has been retained in the Parasitology and Ethnopharmacology Lab. The taxonomic status of plant was also verified with the database of Kew working list of all known plant species (www.theplantlist.org). The roots were washed in tap water, air-dried and powdered in a blender. The powdered plant material was extracted in methanol using Soxhlet apparatus. The yield of extract was 7.98 % (w/w).

Experimental animals

Albino rats of either sex (Wistar strain), weighing about 180 to 220 g, and Swiss mice of both either sex, weighing about 25 to 30 g were used to perform *in vivo* experiments. Animals were maintained in acrylic cages and had *ad libitum* access to food and water. *S. obvelata* infection was maintained in Swiss mice as described by Gogoi and Yadav (2016) [18]. Infection of *H. diminuta* was maintained in Wistar rats as described by Tangpu et al. (2006) [19].

All the experiments on laboratory animals were done after the approval from the Institutional Ethics Committee (Animal models), North-Eastern Hill University, Shillong, vide, Member Secretary, IEC, NEHU, letter dated December 4, 2014. All experiments on animals adhere to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines, the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines and the EU Directive 2010/63/EU for animal experiments.

Ethnomedicinal survey

Firstly, data was collected from traditional healers, called as Ojhas, and local people, using a closed type semi-structured questionnaire, about different medicinal plants used against intestinal-helminthic infections in different villages of Kokrajhar district, Assam, India. The study involved 300 respondents, interviewed from April, 2014 to April, 2015. The questionnaire included queries such as, the local names of various plants that are used by the natives against worm infections, the form of their preparations, their dosage, perceived efficacy, and opinion about adverse effects.

Anthelmintic assays

In vitro study

H. diminuta and *S. obvelata* were collected from infections maintained in rats and mice respectively in the laboratory. The parasites were washed in phosphate-buffered saline (PBS) and exposed to 10, 20 and 30 mg/ml concentrations of extract in separate petridishes (n = 5) inside an incubator at 37 ± 1 °C. A group of worms (n = 5) were placed in PBS alone and served as negative control. In addition, another set (n = 5) was placed in reference drugs, praziquantel (PZQ) (1 mg/ml) and albendazole (ABZ) (5 mg/ml). Observations for parasites paralysis and mortality were made at regular time intervals [20].

In vivo study

For *in vivo* testings in *H. diminuta*, rats were orally fed with 5 cysticercoid larvae to induce infection. Animals were divided into 5 groups (n = 5). Group 1 served as negative control and was administered only the vehicle. Group 2 to Group 4 of animals were administered with 125 mg/kg, 250 mg/kg, and 500 mg/kg doses of extract to animals. Group 5 of animals served as positive control and were administered PZQ at 5 mg/kg concentration. Eggs per gram (EPG) counts were done counts were done 3 days prior to and after the dosings. Animals were

sacrificed on the 29th day post-inoculation to calculate the worm burden.

For *in vivo* testings in *S. obvelata*, mice were kept in infected bed for 15 days. Establishment of infection was confirmed by cellophane tape test. Animals were divided into 5 groups, with 5 animals in each group. Group 1 served as negative control and was administered only the vehicle. Group 2 to 4 of animals were given 125 mg/kg, 250 mg/kg, and 500 mg/kg doses of extract. Group 5 of animals were administered ABZ (20 mg/kg). EPG count was done for 3 days prior to and after dosing. The animals were dosed for 5 days and they were sacrificed for worm recovery count on day 11. The percentage reductions in EPG and worm counts were done as described by Kozan et al. (2006) [21].

The prescribed dose administered by practitioners to their clients was taken as the median dose i.e., 250 mg/kg body weight (b.w.) and two doses, one exponentially lower (125 mg/kg b.w.) and the other higher (500 m g/kg b.w.) to the median dose were selected.

Statistical analysis

The experimental data are represented as mean ± standard error of mean (SEM). The *in vitro* data were analyzed by student's *t*-test and the *p* value ≤ 0.05 was considered to be statistically significant between the values from control and treated groups. The data from *in vivo* tests was analyzed by one-way analysis of variance (ANOVA), followed by Tukey's test. The *p* value ≤ 0.05 was considered to be statistically significant. Statistical calculations were done using OriginPro 8.

Results

Ethnomedicinal survey

In ethnomedicinal study, *A. racemosus* was found to be the third most commonly used anthelmintic plant in the study area, after two other plant species, *Sesbania sesban* (L.) and *Cyperus compressus* Linn. Of 300 respondents interviewed in this work, about 80 % individuals were recorded to use the roots of this plant as a deworming remedy. It was also observed that about 72 % of respondents consume one dose of the root infusion of this plant for about a week to cure worm infections. However, only 56 % of the interviewed persons considered it to be highly efficacious as a deworming remedy. Over and above, 82 % respondents had a view point that using this plant preparation does not cause any adverse effect to the user. An infusion of *A. racemosus* is prepared by grinding fresh roots which is then fed to patients or they are either made into pellets and administered (Fig. 1).

Anthelmintic assays

In vitro study

The extract showed dose-dependent effects ($p < 0.0001$) against *H. diminuta*. Notably, 30 mg/ml concentration of extract revealed almost a comparable efficacy with that of reference drug PZQ at 1 mg/ml concentration. However, the *in vitro* efficacy of extract against *S.*

Fig. 1 Fresh roots of *Asparagus racemosus*

obvelata was comparatively lower than 5 mg/ml concentration of reference drug, ABZ. Against *H. diminuta*, at the highest dose, parasites showed mortality in 5.03 ± 0.05 h, whereas parasites placed in PZQ showed mortality a little later, i.e. in 5.96 ± 0.01 h (Fig. 2). Control worms survived till 42.15 ± 0.20 h. Against *S. obvelata*, at the same concentration of extract, the parasites showed mortality in 11.70 ± 0.04 h, whereas parasites placed in ABZ showed mortality much earlier (7.23 ± 0.01 h) (Fig. 3). Herein, the control worms showed physical activity until 34.16 ± 0.007 h.

In vivo study

The administration of *A. racemosus* extract to rats, harboring *H. diminuta* infections, showed a significant reduction ($p < 0.001$) in EPG counts and worm recovery rate, in a dose-dependent manner. The animals treated with 500 mg/kg dose of extract, for 5 days, showed 53.88 % reduction in EPG counts, compared to EPG counts of pre-treatment period, while, the reduction in worm count was recorded as 24 %. The treatment with PZQ (5 mg/kg) showed 87.08 and 84 % reductions in EPG counts and worm counts, respectively, while, EPG and worm counts in control group maintained a uniform trend throughout the observation period (Table 1). The administration of extract to mice, carrying *S. obvelata* infections, showed a significant decrease in EPG counts of animals during the post-treatment period. During the first 3 days (pre-treatment EPG), eggs were detected in the cellophane test of all animals. However, after 5 days of treatment, 26.61 and 25.93 % reduction in EPG counts and worm counts were recorded in animals treated with 500 mg/kg dose of extract. On the other hand, ABZ (20 mg/kg) showed greater decrease in EPG counts (78.57 %) and worm counts (94.11 %) of animals, at necropsy. There was no noticeable variation observed in EPG counts of animals in control group, between the pre-and-post treatment periods (Table 2).

Discussion

Numerous studies have been conducted to investigate the anthelmintic effects of medicinal plants used in traditional medicine, which have not only involved different parasite species, but have also used an array of experimental models to validate the therapeutic potentials of plant species [22]. While some researchers have used

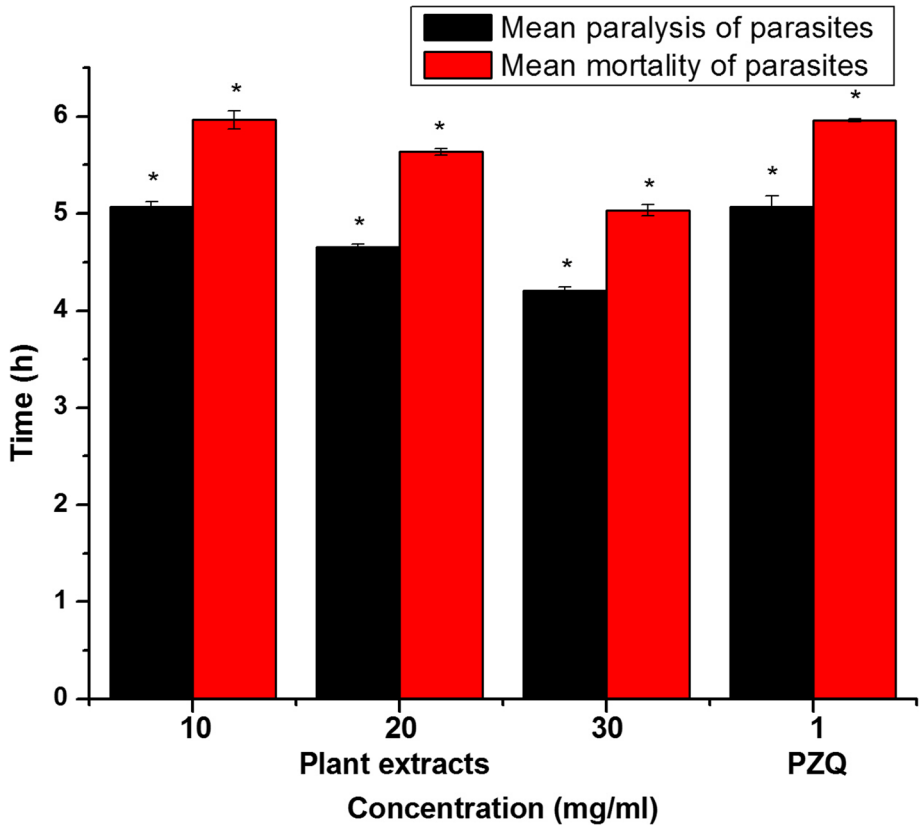

Fig. 2 *In vitro* anthelmintic effects of *A. racemosus* methanolic root extract against *H. diminuta*. Data is expressed as mean ± SEM. *p ˂0.0001 compared with control group, student's *t*-test

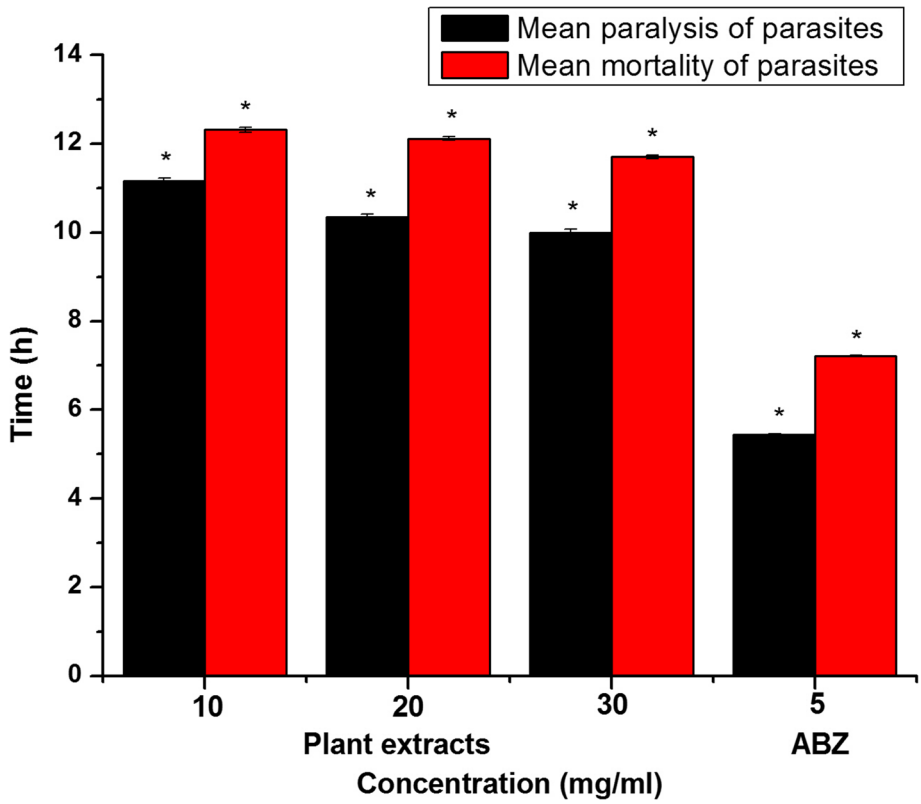

Fig. 3 *In vitro* anthelmintic effects of *A. racemosus* methanolic root extract against *S. obvelata*. Data is expressed as mean ± SEM. *p ˂0.0001 compared with control group, student's *t*-test

only *in vitro* assays [23, 24], other workers have also employed a variety of *in vivo* models to validate the anthelmintic properties of numerous plant species [25, 26]. Nonetheless, a review of literature also reveals that *in vitro* studies alone do not provide a solid scientific basis about the anthelmintic credentials of a substance in question [27]. Therefore, it is now widely recognized that to obtain a more authentic anthelmintic profile of a plant, *in vitro* studies should be followed-up by proper *in vivo* validation processes [18]. Therefore, to provide a better therapeutic profile of *A. racemosus*, in the present study, both the *in vitro* as well as *in vivo* studies on the plant were conducted. In addition, the helminth species used herein were also selected from two different major

Table 1 *In vivo* anthelmintic effects of *A. racemosus* root extract* on *H. diminuta* infections in rats ($n = 5$)

Treatment groups (mg/kgxdosexday)	EPG (mean ± SEM)		Percentage difference in EPG (A-B)	Worm count at necropsy (mean ± SEM)	Percentage reduction in worm count
	Pre-treatment 18–20 days (A)	Post-treatment 26–28 days (B)			
Control	22,318 ± 168	22,688 ± 194	1.63	5.0 ± 0.00	0
Plant extract					
125 × 1 × 5	22,530 ± 148	15,399 ± 142[b]	− 31.65	4.2 ± 0.20	16
250 × 1 × 5	22,620 ± 126	13,643 ± 135[b]	− 39.68	4.0 ± 0.31	20
500 × 1 × 5	22,754 ± 109	10,494 ± 101[b]	− 53.88	3.8 ± 0.37	24
Praziquantel					
5 × 1 × 5	22,850 ± 56.24	2952 ± 38.86[b]	−87.08	0.8 ± 0.20[a]	84

*Administration of plant extract and praziquantel on days 21–25 post-inoculation with five cysticercoids/rat
[a]p<0.001 as compared to control value, [b]p<0.001 as compared to pre-treatment period, one-way ANOVA, followed by Tukey's test

Table 2 *In vivo* anthelmintic effects of *A. racemosus* root extract* on *S. obvelata* infections in mice ($n = 5$)

Treatment groups (mg/kgxdosexday)	EPG (mean ± SEM)		Percentage difference in EPG	Worm count at necropsy		Percentage reduction in worm count
	Pre-treatment (days 1–3)	Post-treatment (days 9–11)		Min – Max	Mean ± SEM	
Control	26.8 ± 1.15	27.8 ± 0.37	3.7	64–117	91.8 ± 9.00	0
Plant extract						
125 × 1 × 5	20.6 ± 0.74	17.6 ± 2.15	−14.56	46–91	71.2 ± 7.80	22.44
250 × 1 × 5	25.4 ± 1.32	21.2 ± 3.2	−16.53	41–102	67.6 ± 10.50	26.36
500 × 1 × 5	24.8 ± 1.85	18.2 ± 3.39	−26.61	36–99	63.4 ± 11.61	30.93
Albendazole						
20 × 1 × 5	28.0 ± 0.70	6 ± 0.70[b]	−78.57	5–6	5.4 ± 0.24[a]	94.11

*Administration of plant extract and albendazole on days 4–8 after pre-treatment EPG
[a]$p<0.001$ as compared to control value, [b]$p<0.001$ as compared to pre-treatment period, one-way ANOVA, followed by Tukey's test

groups, i.e. a cestode, *H. diminuta* and another nematode, *S. obvelata*, that were maintained in two different rodent models.

The findings of ethnomedicinal study revealed that *A. racemosus* is considered as the third most commonly used remedy for intestinal-worm infections in the study area. Further, about 80 % of people in study apprised about the regular use of roots of this plant as a traditional anthelmintic. Likewise, 56 % of the interviewed persons considered it to be highly efficacious against intestinal helminths. However, 82 % respondents also had an opinion that using this plant preparation does not cause any adverse effect to them. Thus, findings of this field study provided a concrete basic lead to put this plant for proper evaluation of its anthelmintic potentials.

In the present study, both the *in vitro* and *in vivo* studies of *A. racemosus* root extract against *H. diminuta* and *S. obvelata* showed a dose-dependent efficacy ($p < 0.001$). The comparative *in vitro* and *in vivo* data analysis of extract revealed that the extract was comparatively more efficacious against cestode, *H. diminuta* than nematode, *S. obvelata*. The *in vitro* efficacy of extract against *H. diminuta* was also almost comparable with that of 1 mg/ml concentration of PZQ, the reference drug. However, in *in vivo* studies against *H. diminuta*, the extract showed a comparatively low efficacy than PZQ (5 mg/kg). On the other hand, the *in vitro* as well as *in vivo* efficacy of extract against the nematode, *S. obvelata* was noticed to be comparatively lower than cestode parasite.

The available published data reveal two more studies related to anthelmintic potentials of *A. racemosus*. Firstly, Kiranmayi et al. (2012) in their study on efficacy of root extract of *A. racemosus* showed that root extract of this plant possesses wormicidal activity and thus, may be useful as an anthelmintic [28]. Similarly, Gupta et al. (2012) also tried to prove the presence of anthelmintic efficacy of this plant based on their experiments on *Pheretima posthuma* (earthworm) [29]. It may however be mentioned here that both these studies were based on testing of extracts on adult Indian earthworms as model organisms, which as such do not bear any anatomical or physiological similarities with parasitic roundworms. Further, in the study by Kiranmayi et al. (2012), the concentration of extract tested was also considerably high, i.e., 100 mg/ml and 200 mg/ml [28]. In the light of these stated facts, it may be concluded that the findings of these two *in vitro* studies about anthelmintic potentials of *A. racemosus* have only limited relevance.

To date, only a few studies on anthelmintic potentials of medicinal plants have backed their *in vitro* assays with *in vivo* studies. For example, Gogoi and Yadav (2016) and Gogoi and Yadav (2017) have supplemented their *in vitro* anthelmintic studies of plants with *in vivo* studies, using *H. diminuta*-rat and *S. obvelata*-mice models [18, 30]. The findings are in agreement with that of Gogoi and Yadav (2016), who in their *in vitro* and *in vivo* efficacy testing of *Caesalpinia bonducella*, against *H. diminuta* and *S. obvelata*, also observed a dose-dependent efficacy of extract [18]. However, in this *in vitro* study, the 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, taking almost similar time duration. However, in *in vivo* study, the same extract showed a comparatively better efficacy on *S. obvelata*, than against *H. diminuta*. Based on these findings, Gogoi and Yadav (2016) suggested that the leaf extract of *C. bonducella* possesses significant anthelmintic effects and supported its use as an anthelmintic in traditional medicine [18].

In another study by Gogoi and Yadav (2017), the *in vitro* and *in vivo* anthelmintic efficacy of *Croton joufra* leaf extract was investigated against *H. diminuta*. The *in vitro* study showed a dose-dependent effect on parasite, and the highest concentration of the extract (30 mg/ml) caused mortality of in worms 1.53 ± 0.12 h,

compared to 3.46 ± 0.10 h shown by PZQ (1 mg/ml). The *in vitro* findings were corroborated by *in vivo* experiments, which showed a very high efficacy of extract against *H. diminuta*. The overall findings of this study suggest that *C. joufra* leaf extract possesses significant anthelmintic efficacy and support its traditional anthelmintic claims [30].

Conclusions

The findings of the present study scientifically validate the common belief of the local people of the Santhal tribe in Assam, that roots of *Asparagus racemosus* are highly effective as a deworming remedy. More precisely, this study suggests that the root extract of this plant species possesses a comparatively better efficacy against cestode than against the nematode parasite, as evidenced by *in vitro* and *in vivo* experimentations.

Acknowledgements

This work was conducted at Department of Zoology, North-Eastern Hill University, Shillong. Authors thank Prof. Larisha Lyndem, Visva Bharati University for assistance in *in vivo* studies. Amar Deep Soren was recipient of a fellowship from University Grants Commission, New Delhi.

Authors' contributions

AKY framed the hypothesis, supervised the experiments and finalized the manuscript. ADS performed the experiments, analyzed data and prepared the first draft of manuscript. All authors read and approved the final manuscript.

Funding

No funding was received from any organization or individual to carry out this study.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Declarations

Consent to participate

Not applicable.

Ethics approval

Experiments on laboratory animals were approved by the Institutional Ethics Committee (Animal models), North-Eastern Hill University, Shillong, vide, Member Secretary, IEC, NEHU, letter dated December 4, 2014. All experiments on animals adhere to the (ARRIVE) guidelines, the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines and the EU Directive 2010/63/EU for animal experiments.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests related to this paper.

Received: 28 November 2020 Accepted: 1 April 2021
Published online: 06 April 2021

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