Gogoi et al.
The Journal of Basic and Applied Zoology (2022) 83:31
https://doi.org/10.1186/s41936-022-00296-5

The Journal of Basic and Applied Zoology

RESEARCH

Open Access

Ultrastructural studies on effect of *Caesalpinia bonducella* and *Croton joufra*, traditionally used anthelmintics, on *Hymenolepis diminuta* and *Syphacia obvelata*

Shyamalima Gogoi[1*], Amar Deep Soren[2] and Arun K. Yadav[1]

Abstract

Background: The leaves of *Caesalpinia bonducella* and *Croton joufra* are traditionally used anthelmintics by the Mishing tribe in Assam, India. In vitro studies have shown them to be effective as anthelmintics. This study evaluates their effects on the tegument of *Hymenolepis diminuta* (Cestoda) and the cuticle of *Syphacia obvelata* (Nematoda) using scanning electron microscopy. The effects were compared with negative control parasites and the one exposed to reference drugs (positive control). Parasites were collected from freshly necropsied animals and were exposed to 30 mg/ml of the methanolic leaf extract of the two plants. Paralysed worms were then processed for ultrastructural studies as per standard methods.

Results: Adult and juvenile *H. diminuta* exposed to extracts of both the test plants showed damaged scolex, suckers and altered tegument. *S. obvelata* treated with *C. bonducella* showed damaged apical region, closed mouth, and a damaged cuticle. Worms exposed to *C. joufra* showed deformations in the apical region with closed mouth, loss of cephalic papillae, distorted lips and damaged cuticle.

Conclusions: In conclusion, the findings of this study demonstrate that the extract of these plants acts via a tegumental/cuticular mode. This study also validates the traditional knowledge system of the Mishing tribe in Assam, India.

Keywords: Anthelmintic, *Caesalpinia bonducella*, *Croton joufra*, *Hymenolepis diminuta*, Mishing, *Syphacia obvelata*

Background

Helminthiasis is a commonly occurring health problem in developing countries like India, where there is lack of proper sanitation and open defecation is rampant (Samuel et al., 2017). In India, several communities are known to use herbal remedies to treat helminthiasis (Deori & Yadav, 2016; Nath et al., 2017; Soren & Yadav, 2021a). These herbal remedies play a very important role in the treatment of various ailments in several cultures across the globe (Juvatkar & Jadhav, 2021). The Mishings are the second largest tribes in Assam and are known to inhabit Lakhimpur, Sonitpur, Dibrugarh, Sibsagar and Jorhat districts of Assam (Assam Info, 2020). Individuals of this tribal community use the leaves of *Caesalpinia bonducella* (L.) Roxb (Caesalpiniaceae) and *Croton joufra* Roxb. (Euphorbiaceae) to treat the intestinal worm infections.

Caesalpinia bonducella commonly known as “fever nut” is a large prickly shrub found abundantly in India, Burma and Ceylon (Subbiah et al. 2019). It has been reported to be used as a medicinal plant in folklore and Ayurveda (Kannur et al. 2012; Manikandaselvi et al.

*Correspondence: lima.gog4434@gmail.com

[1] Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya 793022, India

Full list of author information is available at the end of the article

2015). It is known to possess anti-inflammatory, anti-diabetic, anti-mitotic, anti-microbial and free radical scavenging activities (Subbiah et al. 2019). It has been shown to be effective against nematodes and cestodes (Gogoi & Yadav, 2016). *C. joufra* is a small tree found in India, Bangladesh, Bhutan, Myanmar and Vietnam. It is also known to possess anthelmintic activity against cestodes (Gogoi & Yadav, 2017). It is one of the least studied species of the genus *Croton*.

Ultrastructural studies using scanning electron microscopy (SEM) are a widely used method to study the ultrastructural alterations due to anthelmintics on the body surface of helminth parasites in extensive detail (de Oliveira et al. 2012). Since in vitro studies on the effects of *C. bonducella* and *C. joufra* have revealed significant anthelmintic efficacy compared to reference drugs, this study was undertaken to investigate the ultrastructural changes in the parasites exposed to these plant extracts. It is hoped that the findings of the present study will assist in evaluating the extent of damages the extract of these medicinal plants may incur on the surface of test parasites.

Methods

Plant material

Fresh leaves of the plant were collected from natural habitats, washed and shade dried. They were then powdered and extracted in methanol using a Soxhlet apparatus. Herbaria of the plants were submitted to the Department of Botany, North-Eastern Hill University (NEHU), where they were identified by a taxonomist and voucher numbers were allotted (*Caesalpinia bonducella*—NEHU-12034; *Croton joufra*—NEHU-12035).

Animals and collection of parasites

Animals were procured from the animal room of the Department of Zoology, NEHU, infected with parasites, and maintained in the laboratory. *H. diminuta* (Cestoda) infection was maintained in Wistar rats, whereas *S. obvelata* (Nematoda) infection was maintained in Swiss albino mice. All experiments on animals were conducted strictly with the approval from the Institutional Ethics Committee (IEC) of animal models, of the institute and comply with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and the International Guiding Principles for Biomedical Research Involving Animals. Juvenile and adult *H. diminuta* were collected from freshly necropsied infected rats, whereas *S. obvelata* were collected from freshly necropsied infected mice maintained in the laboratory. Parasites exposed to plant extracts were placed in petri dishes containing phosphate-buffered saline (PBS) inside an incubator at 37 °C.

Scanning electron microscopy (SEM) studies

SEM was used in order to observe the probable effects of *C. bonducella* and *C. joufra* leaf extracts/reference drugs on body surfaces of test worms, the adult and juvenile *H. diminuta* and *S. obvelata*. Parasites were placed in triplicates in 30 mg/ml concentrations of each of the plants' methanolic extract dissolved in PBS. Specific concentrations of the doses were already established in previous studies (Deori & Yadav, 2016; Gogoi & Yadav, 2016). To compare the effects, parasites were placed in reference drugs albendazole (5 mg/ml), for nematode and praziquantel (1 mg/ml), for cestode parasites. The paralysed worms were then fixed in 3% formaldehyde for 4–12 h. Following fixation, they were washed in 0.1 M phosphate buffer (pH-7.2) thrice and placed in the same buffer for 30 min. Samples were then dehydrated through acetone graded series (30%, 50%, 70%, 80%, 90%, 95% and 100%) for 15 min twice per grade at 4 °C. The worms were then dried using tetramethylsilane (TMS) (Dey et al. 1989) and coated with gold–palladium. They were then observed under JSM-6360 (JEOL) scanning electron microscope SEM at 15 kV.

Results

Effects of plant extracts on adult *Hymenolepis diminuta*

Surface topographical observations on *H. diminuta* control worms depicted the normal contour of body, with intact suckers on the scolex and unilateral uniformly shaped tegument (Fig. 1a, b, c). Adult worms treated with PZQ (1 mg/ml) revealed mild damage of the scolex and tegument. Suckers appeared closed and erosion of tegument throughout the body surface was seen (Fig. 1d, e, f).

Compared to the control group, parasites treated with 30 mg/ml of *C. bonducella* extract showed altered contours throughout the body surface in the form of irregular architecture, such as wrinkled scolex, withered suckers, eroded microtriches and distorted tegument (Fig. 1g, h, i). Similarly, worms exposed to 30 mg/ml concentration of *C. joufra* extract revealed shrunken scolex with extensively eroded suckers, irrevocable destruction of the surface and disruption (Fig. 1j, k, l).

Effects of plant extract on newly excysted juveniles of *Hymenolepis diminuta*

The control juveniles showed intact body architecture, normal suckers and microtriches (Fig. 2a, b). In contrast, the worms treated with reference drug PZQ (1 mg/ml) showed fully altered body architecture, clumped suckers and the microtriches were poorly visible due to clumping and disorganization in their structures (Fig. 2c, d).

The juvenile worms exposed to *C. bonducella* extract at 30 mg/ml concentration showed considerable damages to

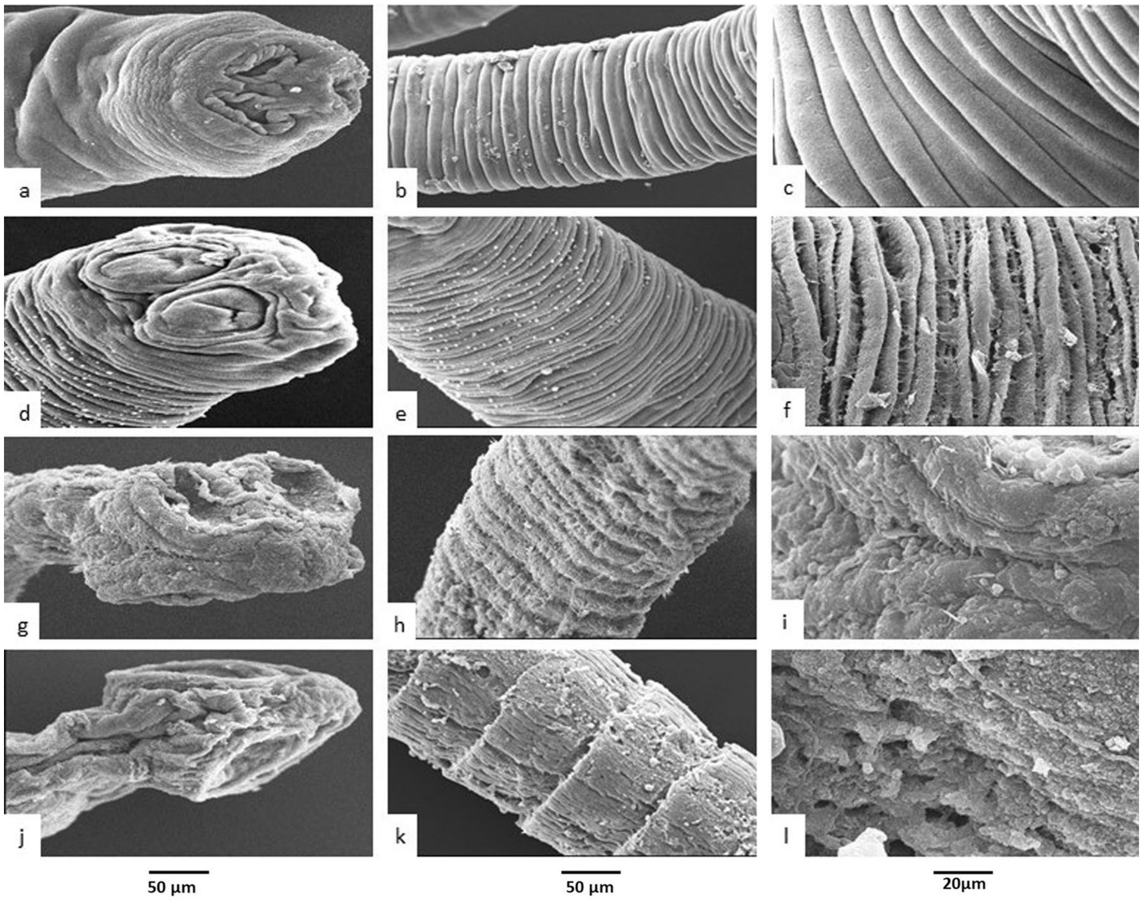

Fig. 1 Scanning electron micrographs of adult *H. diminuta*. **a**, **b**, **c** Control worms, showing scolex with normal suckers and intact tegument; **d**, **e**, **f** Worms exposed to PZQ (1 mg/ml), showing distorted suckers, contracted tegumental architecture, but normal microtriches; **g**, **h**, **i** Worms exposed to *C. bonducella* extract (30 mg/ml), showing shrunken suckers, eroded microtriches and distorted tegument; **j**, **k**, **l** Worms exposed to *C. joufra* extract (30 mg/ml), showing shriveled scolex, shrunken suckers, and irrevocable destruction over the tegumental surface

the microtriches, shrunken and closed suckers. However, extensive damaged was not observed in the body architecture, as compared to the worms treated with reference drug ABZ (Fig. 2e, f). On the other hand, the juvenile worms exposed to 30 mg/ml concentration of *C. joufra* extract revealed more substantial damages to the overall body contour in the form of shrunken body, damaged sunken suckers and corroded microtriches when compared to the control worms (Fig. 2g, h).

Effects of plant extracts on adult *Syphacia obvelata*

SEM observations on adult *S. obvelata* control worms showed normal anterior region with three lips surrounding the mouth opening, provided with cephalic labial papillae, uniformly distributed striations, and bifurcated, symmetrically longitudinal septa on the surface (Fig. 3a–c). Adult worms exposed to ABZ (5 mg/ml) revealed apical damage with a closed mouth, crumpling of cuticle and deepening of annulations grooves (Fig. 3d–f).

In contrast, the adult worms treated to *C. bonducella* (30 mg/ml) leaf crude extract showed damaged apical region, closing of mouth, where aggregates of extract were observed. Also, a partially damaged cuticle, with visible annulations was noticed, which however, did not appear to be deeply indented (Fig. 3g–i).

Similarly, worms exposed to *C. joufra* leaves extract (30 mg/ml) showed shrinkage and rupture of the apical region with closing of mouth, loss of cephalic papillae and distorted lips. Damage to the body architecture was also observed in the form of shedding of cuticle, damage to the cuticular striations and annulations with shrunken body (Fig. 3j–l).

Discussion

In vitro anthelmintic effects of medicinal plants on parasites give a better picture of the anthelmintic mode of action when the activity is supported by SEM. In the recent time, SEM studies have been carried out by some

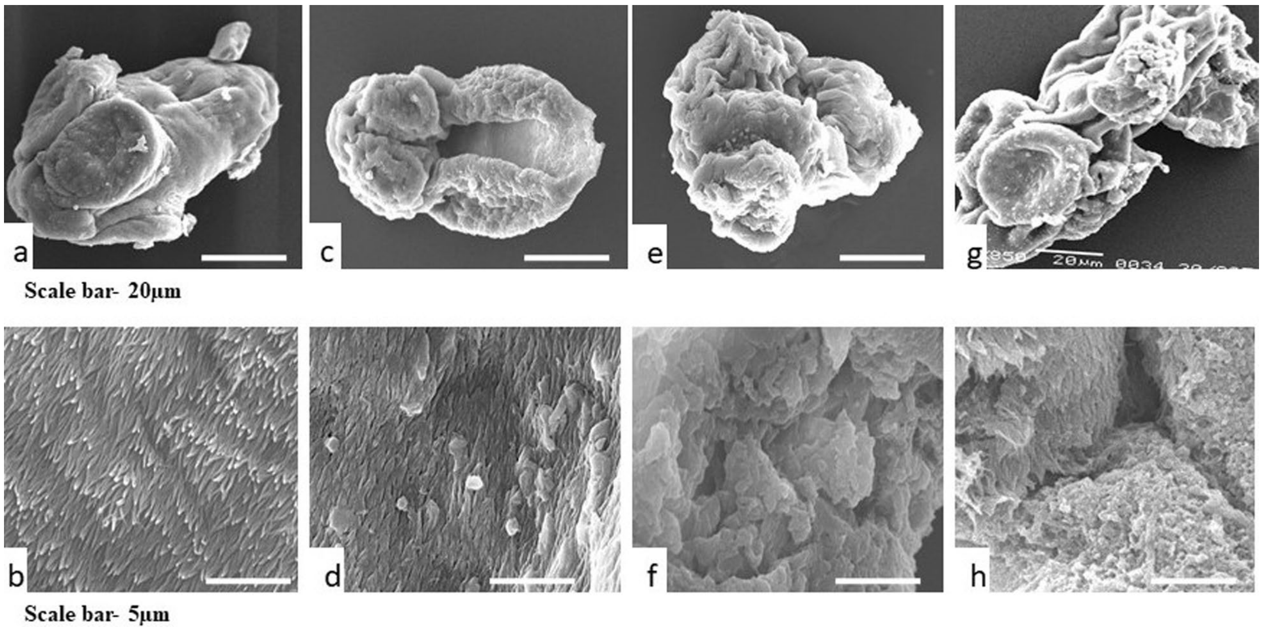

Fig. 2 Scanning electron micrographs of newly excysted juveniles of *H. diminuta*. **a**, **b** Control juvenile, showing intact body contour, suckers and microtriches; **c**, **d** Juveniles exposed to PZQ (1 mg/ml), showing distorted body architecture, suckers and clumped microtriches; **e**, **f** Juveniles exposed to *C. bonducella* extract (30 mg/ml), showing altered body structure, closed suckers and corroded microtriches; **g**, **h** Juveniles exposed to *C. joufra* extract (30 mg/ml), showing shrunken altered body structure, damaged sunken suckers, and corroded microtriches

authors to examine the minute changes in the body surface of the parasites due to anthelmintics (Li et al. 2021; Mrifag et al. 2021). SEM provides important information on the body of helminths and helps to understand the host-parasite relationship, as tegument and cuticle of helminth parasites have been associated with one of several target sites by which the anthelmintic products act (Kundu et al. 2012). Therefore, any destruction or damage caused to the body surface of parasite due to the treatment of drug or extract from any medicinal plant may lead to paralysis and mortality of parasite. Therefore, in the present in vitro study, at the end of physical motility test, dead adult and juveniles of *H. diminuta* and adult *S. obvelata* were processed for SEM to analyze the topographical effects of plants extract on the test parasites.

In SEM study, the control worms revealed a normal architecture, without any alterations to the surface of parasites. On the other hand, parasites treated with *C. bonducella* extract showed changes in the general topography with distortion of tegument, closed and damaged suckers in the cestode and closed anterior region with damaged labial papillae, eroded cuticle in nematode. Parasites treated with the reference drug PZQ and ABZ implicated similar kind of damages, the changes in the annulations grooves of nematode parasite might possibly affect the permeability of cuticle. In a similar study, carried out by Temjenmongla et al. (2015), on the effects of *Psidium guajava* and *Lasia spinosa* extracts on *H. diminuta*, an irreversible damage to the tegument and distortion of suckers was observed. Likewise, Shalaby and Farag (2014) observed corrugated and wrinkled cuticle during their study on in vitro effects of *Allium sativum* oil on nematode *H. contortus*. It is likely that the possible damage to the tegument and cuticle of the worms caused by the plant extract may bring out trans-tegumental mode of actions and diminished the motility.

SEM studies on *C. joufra* extract-treated worms also revealed shriveled scolex with extensively shrunken and eroded suckers, irrevocable destruction of the surface fine topography of the tegument and disruption of muscle in the treated *H. diminuta* worms. On the other hand, the juvenile worms exposed to *C. joufra* extract revealed more substantial damages to the overall body contour in the form of shrunken body, damaged sunken suckers and corroded microtriches when compared to control worms. Similar changes in the body surface of parasites have also been reported for several other species of cestodes. For example, Kundu et al. (2012) studied in vitro anthelmintic effects of *Cassia alata* against *H. diminuta* and noticed that plant extract-treated worms possessed irrevocable destruction all over the general topography of the body with an extensive damage to the scolex and the suckers. In case of juvenile worms, similar findings were also observed by Deori and Yadav (2016), where

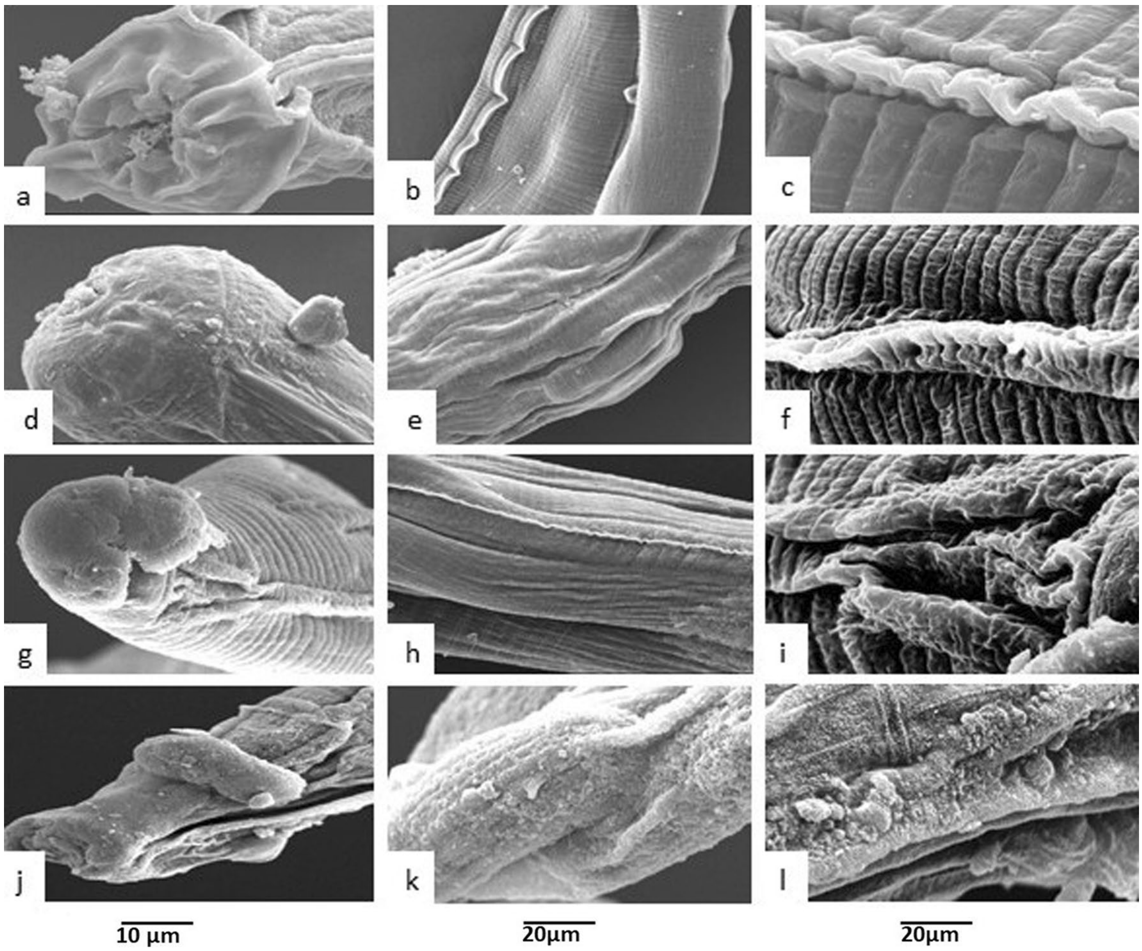

Fig. 3 Scanning electron micrographs of adult *S. obveleta*. **a** Control worm in apical view showing three lips with cephalic vesicles, anterior cervical alae and disposition of sub median cephalic papillae; **b**, **c** Control worm representing fine transverse striations in cuticle; **d**–**f** worms exposed to albendazole (5 mg/ml) representing a close cephalic end and distorted tegument; **g**–**i** worms exposed to *C. bonducella* (30 mg/ml) leaves extract showing damaged apical end and cuticular disruption; **j**–**l** worms exposed to *C. joufra* leaves extract (30 mg/ml) showing damaged apical region and loss of cephalic papillae

extract-treated juvenile worms showed a shrunken bodies and substantially damaged suckers and microtriches.

Conclusions

Caesalpinia bonducella and *Croton joufra* have already been demonstrated for their in vitro anthelmintic efficacy, and this study further corroborates such biological activity by showing the ultrastructural damages on cestode and nematode parasites caused by the plant extracts. Both the plant extracts caused extensive damages to the parasites, and hence, their use as anthelmintics by the Mishing tribe of Assam can be scientifically justified.

List of Abbreviations

ABZ: Albendazole; ARRIVE: Animal Research: Reporting of In Vivo Experiments; IEC: Institutional Ethics Committee; PBS: Phosphate-buffered saline; PZQ: Praziquantel; SEM: Scanning electron microscopy.

Acknowledgements

The authors thank the faculty at SAIF, NEHU for providing the SEM images. Also, a fellowship awarded to SG by the UGC, New Delhi is duly acknowledged.

Author contributions

AKY conceptualized, supervised the study and finalised the manuscript draft. SG executed the experiments, analyzed the data and wrote the first draft. ADS assisted in the experiments, wrote and edited the first draft. All authors read and approved the final manuscript.

Funding

The study did not receive any funding.

Availability of data and materials

All data generated during this study have been mentioned in this article.

Declarations

Ethics approval and consent to participate

Laboratory inbred mice and rats used were procured from the animal room of North-Eastern Hill University (NEHU). Experiments were approved by the Institutional Ethics Committee (Animal models), NEHU, Shillong after obtaining a written consent (Vide, Member Secretary, IEC, NEHU, dated December 4, 2014). Also, all experiments on animals comply with the ARRIVE guidelines. Prior approval was taken for using the animals and the study on plants and animals was approved by the Research committed of the university.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya 793022, India. [2]Department of Zoology, B. Borooah College, Guwahati, Assam 781007, India.

Received: 8 October 2021 Accepted: 14 June 2022
Published online: 21 June 2022

References

Assam Info. (2020). Mishing people of Assam. https://www.assams.info/assam/mishing-people-of-assam. Accessed 31 August 2021.

de Oliveira, R. N., Rehder, V. L. G., Oliveira, A. S. S., Junior, I. M., de Carvalho, J. E., de Ruiz, A. L. T. G., Jeraldo, V. L. S., Linhares, A. X., & Allegretti, S. M. (2012). *Schistosoma mansoni*: In vitro schistosomicidal activity of essential oil of *Baccharis trimera* (Less) DC. *Experimental Parasitology, 132*, 135–143. https://doi.org/10.1016/j.exppara.2012.06.005

Deori, K., & Yadav, A. K. (2016). Anthelmintic effects of *Oroxylum indicum* stem bark extract on juvenile and adult stages of *Hymenolepis diminuta* (Cestoda), an in vitro and in vivo study. *Parasitology Research, 115*, 1275–1285. https://doi.org/10.1007/s00436-015-4864-6

Dey, S., Baul, T. S. B., Roy, B., & Dey, D. (1989). A new rapid method of air-drying for scanning electron microscopy using tetramethylsilane. *Journal of Microscopy, 156*, 259–261.

Gogoi, S., & Yadav, A. K. (2016). In vitro and in vivo anthelmintic effects of *Caesalpinia bonducella* (L.) Roxb. leaf extract on *Hymenolepis diminuta* (Cestoda) and *Syphacia obvelata* (Nematoda). *Journal of Intercultural Ethnopharmacology, 5*(4), 427–433. https://doi.org/10.5455/jice.20160821024821

Gogoi, S., & Yadav, A. K. (2017). Therapecutic efficacy of the leaf extract of *Croton joufra* Roxb. against experimental cestodiasis in rats. *Journal of Parasitic Diseases, 41*(2), 417–422. https://doi.org/10.1007/s12639-016-0819-9

Juvatkar, P. V., & Jadhav, A. G. (2021). *Caesalpinia bonducella*: A medicinal potential value. *Journal of Pharmacognosy and Phytochemistry, 10*(4), 206–214.

Kannur, D. M., Paranjpe, M. P., Sonavane, L. V., Dongre, P. P., & Khandelwal, K. R. (2012). Evaluation of *Caesalpinia bonduc* seed coat extract for anti-inflammatory and analgesic. *Journal of Advanced Pharmaceutical Technology and Research, 3*(3), 171–175. https://doi.org/10.4103/2231-4040.101010

Kundu, S., Roy, S., & Lyndem, L. M. (2012). *Cassia alata* L: Potential role as anthelmintic agent against *Hymenolepis diminuta*. *Parasitology Research, 111*(3), 1187–1192. https://doi.org/10.1007/s00436-012-2950-6

Li, X., Chen, Q., & Li, Y. (2021). The morphology of the parasitic isopod *Tachaea chinensis* (Isopoda, Cymothoida) revealed through scanning electron microscopy and histological analysis. *Crustaceana, 94*(1), 63–75. https://doi.org/10.1163/15685403-bja10058

Manikandaselvi, S., Vadivel, V., & Brindha, P. (2015). *Caesalpinia bonducella* L. a nutraceutical plant. *Journal of Chemical and Pharmaceutical Research, 7*(12), 137–142.

Mrifag, R., Lemrabott, M. A., El Kharrim, K., Belghyti, D., & Basco, L. K. (2021). *Setaria labiatopapillosa* (Filarioidea, Nematoda) in Moroccan cattle: Atypical localization and morphological characterization of females and microfilariae by light and scanning electron microscopy. *Parasitology Research, 120*(3), 911–918. https://doi.org/10.1007/s00436-020-06966-z

Nath, P., Yadav, A. K., & Soren, A. D. (2017). Sub-acute toxicity and genotoxicity assessment of the rhizome extract of *Acorus calamus* L. a medicinal plant of India. *European Journal of Pharmaceutical and Medical Research, 4*(8), 392–399.

Samuel, F., Demsew, A., Alem, Y., & Hailesilassie, Y. (2017). Soil transmitted Helminthiasis and associated risk factors among elementary school children in ambo town, western Ethiopia. *BMC Public Health, 17*, 791. https://doi.org/10.1186/s12889-017-4809-3

Shalaby, H. A., & Farag, T. K. (2014). Body surface changes in gastrointestinal helminthes following in vitro treatment with *Allium sativum* oil. *Journal of Veterinary Science and Technology, 5*(1), 153–157. https://doi.org/10.4172/2157-7579.100015

Soren, A. D., & Yadav, A. K. (2021a). Studies on the anthelmintic potentials of the roots of *Asparagus racemosus* Willd. (Asparagaceae). *Clinical Phytoscience, 7*, 32. https://doi.org/10.1186/s40816-021-00270-8

Soren, A. D., & Yadav, A. K. (2021b). In vitro anthelmintic efficacy of *Sesbania sesban* var. *bicolor*, *Cyperus compressus* and *Asparagus racemosus* against *Gastrothylax crumenifer* (Trematoda). *Proceedings of the Zoological Society, 74*(3), 262–267. https://doi.org/10.1007/s12595-021-00370-w

Subbiah, V., Nagaraja, P., Narayan, P., & Nagendra, H. G. R. (2019). Evaluation of pharmacological properties of *Caesalpinia bonducella* seed and shell extract. *Pharmacognosy Journal, 11*(1), 150–154. https://doi.org/10.5530/pj.2019.1.25

Temjenmongla, & YadavDeori, A. K. K. (2015). Anthelmintic effects of *Psidium guajava* and *Lasia spinosa* on *Hymenolepis diminuta* (Cestoda): A scanning electron microscopic study. *Journal of Advanced Microscopy Research, 10*(1), 20–23. https://doi.org/10.1166/jamr.2015.1231

Publisher's Note