

Beautyberry (*Callicarpa arborea*) as an Antiparasitic Agent Against *Raillietina echinobothrida*, an Intestinal Tapeworm

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History

- Submission Date: 25-10-2019;
- Review completed: 09-11-2019;
- Accepted Date: 03-12-2019.

DOI : 10.5530/pj.2020.12.11

Article Available online

<http://www.phcogj.com/v12/i1>

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ABSTRACT

Background: The beautyberry (*Callicarpa arborea* Roxb.) is member of the family Lamiaceae and is native to Asia. It is used in different traditional medicines for the treatment of debilitating ailments including cancer, dermatitis, diabetes mellitus, gastritis, helminthiasis, and pyrexia. A couple of pentacyclic triterpenes and phytosterols have been reported from this plant. **Objective:** We aimed to investigate the antiparasitic potentials of *C. arborea* bark extract. We used an intestinal tapeworm, *Raillietina echinobothrida*, for its convenience in handling and established nature as a model helminth parasite. **Materials and Methods:** The extract of the stem bark was prepared using methanol. Tapeworms were treated *in vitro* with varying concentrations of the extract. Survival values were statistically analysed. Treated tapeworms were fixed and processed for scanning electron microscopy. **Results:** *C. arborea* bark extract showed dose-dependent antiparasitic similar to that of albendazole. Scanning electron microscopy revealed damaging effects all over the body of the tapeworm. There was general shrinkage of the tegument on the scolex, neck region and strobila. Microtriches were completely removed throughout the tegument. Suckers and rostellum on the scolex were also deformed. **Conclusion:** Our data shows that *C. arborea* is a promising source of antiparasitic principles.

Key words: *Callicarpa arborea*, Scanning electron microscopy, Tapeworm, Tegument.

INTRODUCTION

Pharmaceutical drugs for helminth infections are on the verge of utter uselessness as a consequence of pervasive drug resistance in all major helminth parasites, especially in livestock animals. Every anthelmintic drug is facing an irrevocable nosedive in terms of effectiveness.¹ The situation is alarming and prompts for an urgent call to seek new drugs.^{2,3} Global strategic programmes on mass drug administration to eliminate infections are unsatisfactory and not completely successful.⁴ As it turns out, helminth infections are now the most prevalent infectious diseases in humans. As of the latest WHO reports, soil-transmitted helminths infect 1.5 billion people,⁵ while schistosomiasis alone accounts for another 220 million cases,⁶ thereby surpassing malaria (at 219 million cases), which has always been the predominant infection and leading cause of health crisis.

Callicarpa arborea Roxb. is a perennial medium-sized tree belonging to the family Lamiaceae and is found in China, Nepal, Bhutan, India, Sri Lanka, Bangladesh, and South-East Asian region. In India, Bangladesh and Nepal, the bark is used for the treatment of skin diseases, fever, indigestion,⁷ and boils.^{8,9} In ayurvedic medicine, it is a medication for rheumatism and paralysis.¹⁰ Among the Adi tribes of northeast India, it is a valuable medication for toothache and scorpion sting.¹¹ It is most well-known as an effective remedy for diabetes mellitus,¹² and this property is attributed to its ability of enhancing insulin secretion and metabolism of liver glycogen.¹³ The hydro-alcoholic extract of the

stem bark was demonstrated as effectively reducing blood-sugar level in streptozotocin-induced diabetic rats.¹⁴ There are only very few reports on the chemical analysis of the plant. Maslinic acid was isolated from the bark.¹⁵ This pentacyclic triterpene from other sources has been shown to exhibit antitumor, antidiabetic, antioxidant, cardioprotective, neuroprotective, antiparasitic and growth-stimulating activities.¹⁶ Chemical detection showed the presence of bauerenol, betulinic acid, and β -sitosterol in the bark,¹⁷ and epilupeol, β -sitosterol, and ursolic acid in the leaves.¹⁸

In the Mizo traditional medicine, the plant is called *hnahkiah* and its bark juice is used for the treatment of gastric disorder, dysentery, vomiting,¹⁹ and haemorrhage²⁰ including general cuts and wounds.²¹ Among the Mizo and Andhra Pradesh tribal people, it is consumed twice a day to cure intestinal helminth infection.²² So far, this potentially valuable medicinal plant has received no attention on its antiparasitic property. Therefore, it is worthwhile to examine its efficacy and effects against an intestinal tapeworm, *R. echinobothrida*.

MATERIALS AND METHODS

Preparation of plant extract

Callicarpa arborea barks were collected from Aizawl, Mizoram, India. The plant specimen was identified at the Botanical Survey of India, Kolkata, India, and is catalogued (C-01-18) at Pachhunga University College, Aizawl, India. The barks were washed with distilled water and dried under room temperature

Cite this article: Lalthanpuii PB, Lalchhandama K. Beautyberry (*Callicarpa arborea*) as an Antiparasitic Agent Against *Raillietina echinobothrida*, an Intestinal Tapeworm. Pharmacogn J. 2020;12(1):66-70.

(23-25°C). The dried samples were crushed in an electric blender. Extract was prepared in a 5-L Soxhlet apparatus using methanol as a solvent. The extract was concentrated by recovering the solvent in a vacuum rotary evaporator (Buchi Rotavapor® R-215). The final yield was 5.16%. It was then refrigerated at 4°C for further use.

Chemicals and drug

All chemicals were standard analytical grades. Osmium tetroxide, sodium cacodylate and tetramethylsilane were supplied from Merck India, Mumbai. Methanol was procured from SD Fine-Chem Ltd., Mumbai. All other chemicals were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Albendazole (ZENTEL®) was a product of GlaxoSmithKline Pharmaceuticals Ltd., Mumbai, India.

In vitro survival test

Efficacy of albendazole and *C. arborea* leaf extracts were assessed by survival test on helminth parasite, *Raillietina echinobothrida* Mégnin, 1881. Live tapeworms were dissected out and recovered from the intestines of local fowls (*Gallus gallus* Linnaeus, 1758). They were collected in neutral phosphate-buffered saline (PBS) maintained at 37±1°C in a microbiological incubator. Incremental concentrations, viz. 1.25, 2.5, 5, 10 and 20 mg/ml, of the plant extract was prepared by dissolving the pre-weighed extract in PBS supplemented with 1% dimethylsulfoxide (DMSO) in separate culture plates. A set of two tapeworms were introduced into each culture plates. In addition, similar treatment was done for albendazole as a reference drug (with manufactured dosage of 20 mg/ml). One set of tapeworms was maintained as control in a medium that contained only PBS with 1% DMSO. The duration of survival was assessed from the onset of complete paralysis, i.e. when there was complete loss of motor activity upon agitation such as dipping in lukewarm PBS (45°C). Each test was performed in triplicates.

All experimental data were normalised against the control and were presented as means plus or minus the standard deviation of the mean (± SD). The efficacy of albendazole and the plant extract were compared against the control by Student's *t*-test, and the level of significance was considered when *p* value was less than 0.05.

Scanning electron microscopy

Tapeworms treated with 20 mg/ml of the plant extract were processed for scanning electron microscopy. After complete paralysis in the culture media, they were washed with PBS and treated with 10% cold-buffered formaldehyde at 4°C for 4 hr. The fixative was buffered with 0.1 M sodium cacodylate (pH 7.2). Secondary fixation was done with 1% osmium tetroxide (OsO₄) buffered using the same buffer at 4°C for 1 hr.

The fixed specimens were dehydrated through increasing concentrations of acetone and finally in pure acetone. They were then treated with tetramethylsilane, Si(CH₃)₄, for 0.25 hr and left to dry in air-drying chamber at 25°C. The different parts were scrupulously selected and were mounted on metal stubs. Then, they were sputter coated with gold in JFC-1100 (JEOL Ltd., Tokyo, Japan) ion sputtering chamber. Finally, they were observed under a scanning electron microscope (JSM-6360, JEOL Ltd., Tokyo, Japan) at an electron accelerating voltage of 20 kV.

RESULTS

Analysis of the survival test of *R. echinobothrida* after treatment with *C. arborea* bark extract and albendazole is presented in Table 1. Untreated tapeworms in the control media thrived well for 74.03 hr. Both albendazole and the plant extract were effective at all concentrations tested, i.e. at 1.25, 2.5, 5, 10 and 20 mg/ml, and showed concentration-dependent activity (Figure 1). Albendazole was more active than the plant extract. It took 23.76 ± 1.93, 20.24 ± 0.58, 16.30 ± 0.66, 12.15 ± 0.61, and 4.39 ± 0.88 hr respectively to completely kill the tapeworms. *C. arborea* bark extract took longer time, taking 68.28 ± 2.03, 61.68 ± 1.72, 43.19 ± 1.71, 35.36 ± 2.23, and 25.70 ± 2.36 hr to kill the tapeworms at the same concentrations.

Figure 2 is an image of *R. echinobothrida* from scanning electron microscopy showing the anterior end of the body. The terminal knob-like scolex and the adjoining body segments (proglottids) of the neck region are visible. The apical depression is the rostellum which showed complete collapse and folding into a creased lip-like structure. Just behind the rostellum are two rounded suckers. Both the suckers are also wrinkled and lost the otherwise smooth contour. The tegument on the scolex and neck is entirely crumpled indicating severe body contraction due to destruction of the tegumental and muscle layers. A close-up view of the upper sucker is shown in Figure 3. There are no signs of the spines and their attachment region along the rim of the circular sucker is left barren. The central and surrounding teguments are constricted and distorted.

The main body (strobila) of the tapeworm consists of a chain of body segments. Toward the neck region are immature and developing segments (Figure 4). The body segments are thin along the transverse loop of the body. The transverse borders of the segments are entirely distorted by irregular creases. A magnification of the same body part reveals total loss of hairy microtriches, indicating complete destruction of the absorptive and sensory organs (Figure 5). Mature body segments are shown in Figure 6. All the body segments show massive shrinkage due to contraction of the tegument. No intact microtriches could be identified implying that they are completely removed (Figure 7).

Table 1: Efficacy of albendazole and an extract of *C. arborea* bark on the tapeworm *R. echinobothrida* in normalised values with respect to control.

Media	Dose (mg/ml)	Normalised survival time in hr (± SD)	t value	t critical value
Control	0	100.00 ± 2.56	-	-
Albendazole	1.25	023.76 ± 1.93	58.32	2.26*
	2.5	020.24 ± 0.58	74.53	2.45*
	5	016.30 ± 0.66	77.66	2.45*
	10	012.15 ± 0.61	81.85	2.45*
	20	004.39 ± 0.88	86.57	2.45*
<i>Callicarpa arborea</i> bark extract	1.25	068.28 ± 2.03	23.04	2.23*
	2.5	061.68 ± 1.72	30.46	2.26*
	5	043.19 ± 1.71	45.26	2.26*
	10	035.36 ± 2.23	46.66	2.23*
	20	025.70 ± 2.36	52.29	2.23*

*Significantly different at *p* < 0.05 in comparison with control (0 treatment) group; *n* = 6.

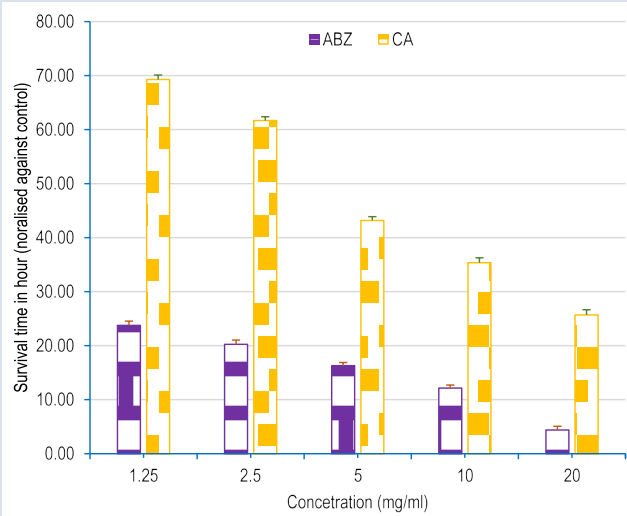


Figure 1: Graph showing the concentration-dependent activity of albendazole (ABZ) and *C. arborea* extract (CA) against *R. echinobothrida*.

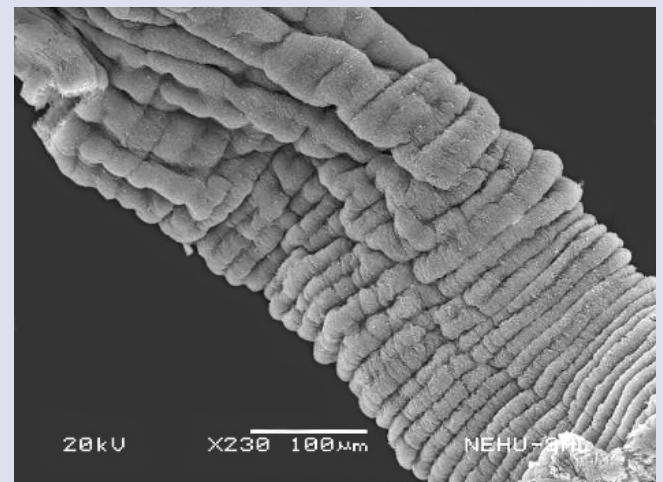


Figure 4: The neck region of *R. echinobothrida* showing a series of creased immature body segments.



Figure 2: Scanning electron microscopy of *R. echinobothrida* treated with *C. arborea* bark extract. Anterior portion of the tapeworm shows an apical rostellum and two suckers in behind. The neck portion with immature body segments is also visible.

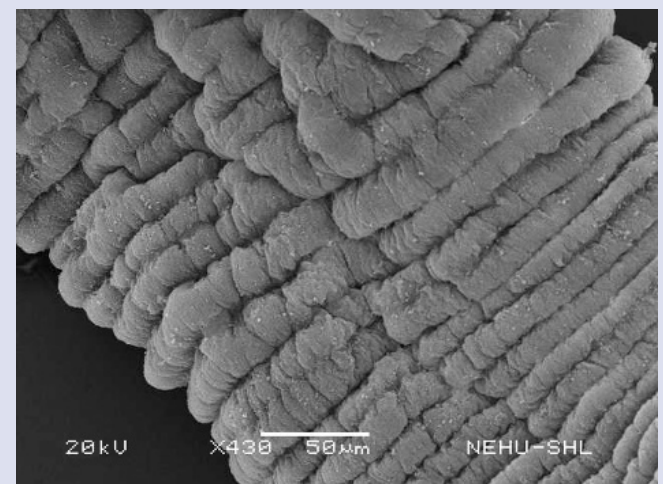


Figure 5: The body segments of *R. echinobothrida* indicating complete loss of microtriches.

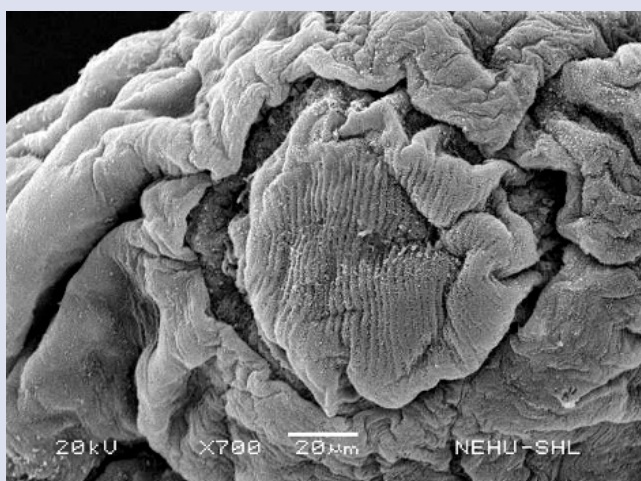


Figure 3: Magnification of the upper sucker of *R. echinobothrida* scolex exposing tegumental shrinkage and loss of spines.

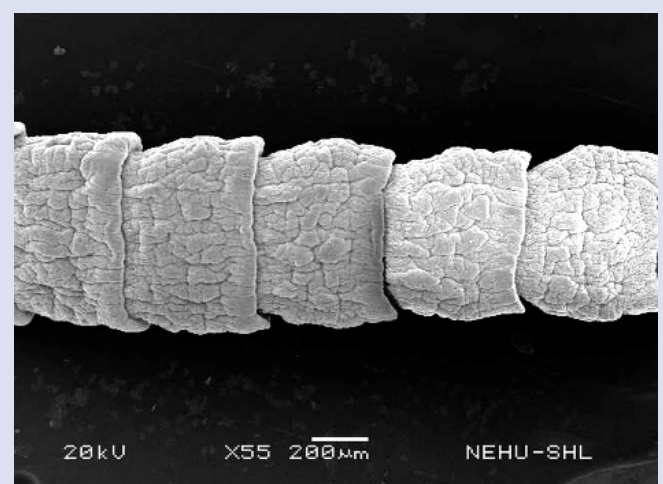


Figure 6: The mature body segments of *R. echinobothrida* exhibiting severe shrinkage.

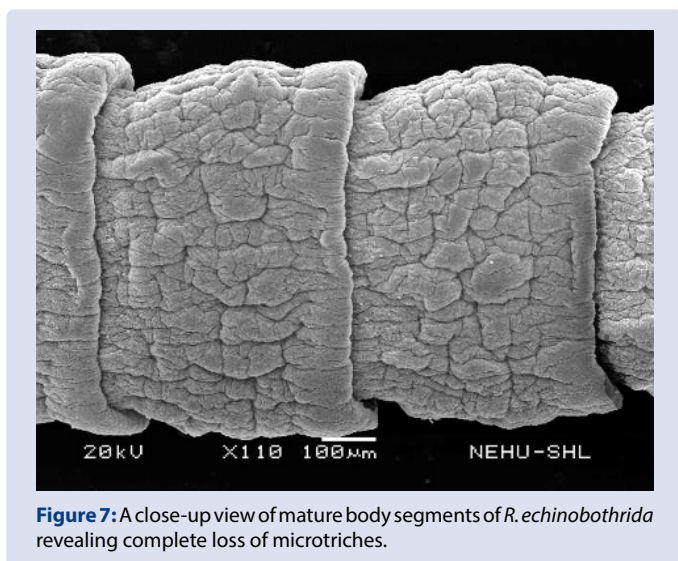


Figure 7: A close-up view of mature body segments of *R. echinobothrida* revealing complete loss of microtriches.

DISCUSSION

Tapeworms are unique helminths in that they have rather simple anatomical architecture being bereft of nervous and digestive systems. Their most elaborate features are in fact the external body surface called tegument. Throughout the body the tegument is overlaid with short and slender hair-like filaments called microtriches (literally meaning “minute hairs” from the Greek words *mikro* meaning small and *thrix* meaning hair). These microtriches are the direct absorptive and sensory organs, and as such they are the primary route of entry of nutrients and drugs. Anthelmintic drugs act on the tapeworm by passively diffusing through the microtriches and the underlying tegument and internal sub-tegument. Thus, their effects are most directly noted as structural damages in these organs.^{23,24} The only areas of the tegument not entirely covered by microtriches are the rims of suckers (spines) and rostellum (hooks) on the head part, the scolex. These spines and hooks are special parasitic adaptations for anchoring on the tissue surfaces, such as intestinal lumen, of the hosts. Anthelmintic drugs also target these organs and normally cause their breakdown.²⁵

The fine morphological structure of *R. echinobothrida* and related species is well understood.^{26,27} In this study, we found that *C. arborea* extract was evidently effective on the *R. echinobothrida* with dose-dependent activity as that of albendazole. The antiparasitic activity was further substantiated by structural damages on the fine body surface. Extensive alterations such as tegumental shrinkage, destruction of the spines and rostellum, and removal of microtriches were clearly the signature effects of an antiparasitic agent.

As broad-spectrum anthelmintics, benzimidazoles are the most versatile and widely used treatment of helminth infections. Their effects and mode of actions are also well understood. Among the most common benzimidazoles, albendazole and flubendazole are demonstrated to cause eruption of swellings or blebs on the tegument, distortion of the entire rostellum, obliteration of the microtriches, and formation of abnormal vesicles on the human tapeworm, *Echinococcus granulosus*.²⁸ A combination therapy of albendazole and praziquantel upon *E. granulosus* and *Mesocostoides corti* resulted in deformation of the suckers including dislocation of the spines, and severe disintegration of the tegument accompanied by erosion of microtriches.^{29,30}

Albendazole alone caused severe shrinkage and tegumental collapse in *R. echinobothrida*.³¹ Suckers were most noticeably destroyed on the scolex while the rostellum remained largely unaffected. In the present study, it is remarkable that both the suckers and rostellum are equally impaired. Another important observation is that efficacious drugs like

praziquantel do not affect the scolex and the neck region of cestodes, implying that they are active as paralytic drugs but not as cestocidal (killing) drugs.³² In contrast, we noted that *C. arborea* bark extract affected indiscriminately the entire body parts on *R. echinobothrida*. This observation indicates that the plant extract has different mode of action and posits the rationale for its potential use in anthelmintic development.

CONCLUSION

Following its traditional usage, *C. arborea* bark extract was tested on the tapeworm *R. echinobothrida* and showed dose-dependent antiparasitic activity as that of albendazole. Scanning electron microscopy revealed structural damages on the tapeworm that indicate antiparasitic effects. There was general shrinkage and constriction throughout the body. The scolex with its suckers and rostellum is completely deformed accompanied by loss of rostellar hooks and sucker spines. The body segments were all wrinkled with their microtriches entirely removed. These findings indicate the antiparasitic efficacy and activity of *C. arborea* and warrant further studies on the plant's bioactive compounds and their mode of action.

CONFLICTS OF INTEREST

None declared.

ACKNOWLEDGEMENT

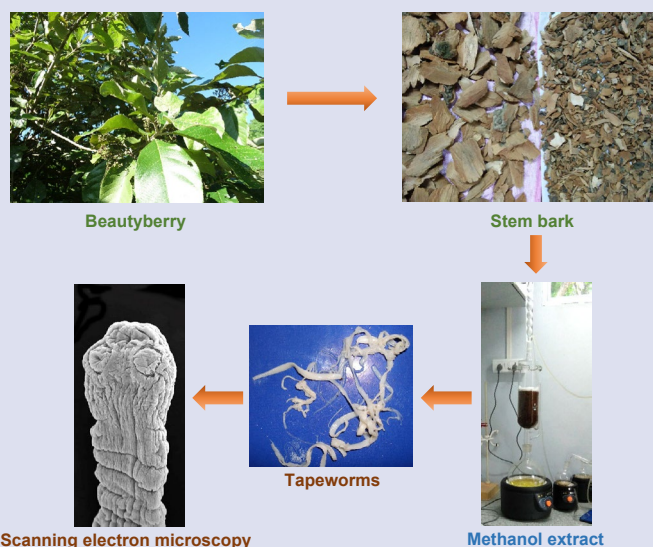
The study is funded by Science and Engineering Research Board (SERB), Government of India (EMR/2016/004053). PBL is a Senior Research Fellow under the project.

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GRAPHICAL ABSTRACT



SUMMARY

- *Callicarpa arborea* bark is a traditional medicine for the treatment of helminth infection. The methanol extract was tested against an intestinal tapeworm, *Raillietina echinobothrida*. It indicated dose-dependent antiparasitic activity against the tapeworm similar to that of albendazole.
- Scanning electron microscopy was used to study the antiparasitic effects. Structural damage was found throughout the body surface of the tapeworm treated with the plant extract.
- Antiparasitic effects include general shrinkage of the tegument, loss of spines on the suckers, and removal of microtriches.
- These antiparasitic effects imply that the plant contains important bioactive compounds, which may have unique chemical and biological properties. This further encourages systematic analyses on the chemical nature and mode of action of the plant extract.

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Cite this article: Lalthanpuii PB, Lalchhandama K. Beautyberry (*Callicarpa arborea*) as an Antiparasitic Agent Against *Raillietina echinobothrida*, an Intestinal Tapeworm. *Pharmacog J*. 2020;12(1):66-70.