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ORIGINAL ARTICLE

The Toothache Plant (*Acmella oleracea*) Exhibits Anthelmintic Activity on Both Parasitic Tapeworms and Roundworms

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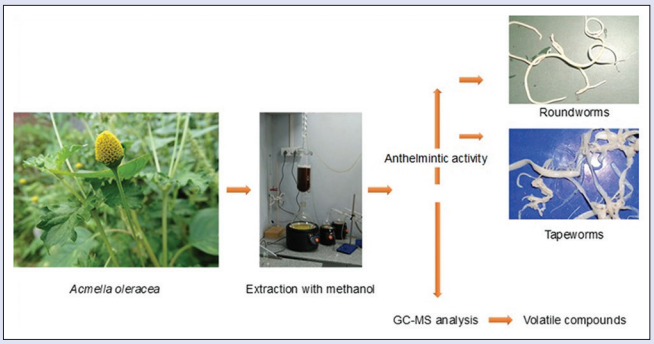
ABSTRACT

Background: Among its many uses, *Acmella oleracea* (L.) R. K. Jansen as an anthelmintic agent for general intestinal helminthiasis is remarkable because of its indigenous usage in the Mizo traditional medicine. However, the rationale has not been established. **Objectives:** The objective of this study is to perform chemical analysis of the plant extract using gas chromatography-mass spectrometry (GC-MS) and to test the anthelmintic activity on intestinal helminths. **Materials and Methods:** Methanol extract of the whole plant was prepared and volatile compounds were analyzed using GC-MS. Anthelmintic activity was studied by survival test on the cestode *Raillietina echinobothrida* and the nematode *Ascaridia galli*. Anthelmintic effects were examined using scanning electron microscopy. **Results:** Thirteen compounds were detected, with methyl *n*-hexadecanoate (palmitic acid) as the major constituents. N-Alkylamides such as N-isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide and N-(2-phenylethyl) non-2(E)-en-6, 8-diynamide were present. The plant extract was significantly effective ($P < 0.05$) at all concentrations tested on both the parasites and showed dose-dependent activity similar to that of albendazole. On the cestode, tegumental shrinkage, erosion of microtriches, and destruction of suckers with clumping of the spines were observed. The nematode was also extensively deformed with its lips collapsed, warty surface on the head, and contracted cuticle on the main body. **Conclusion:** *A. oleracea* contains bioactive compounds that have broad-spectrum activity on cestode and nematode parasites.

Key words: *Acmella oleracea,* anthelmintic activity, cestode, nematode, scanning electron microscopy

SUMMARY

- *Acmella oleracea* has a wide range of medicinal and culinary applications. The Mizo people uniquely use it for the treatment of intestinal helminthiasis. Chemical analysis of the methanol extract was performed using gas chromatography-mass spectrometry. Among the compounds detected N-alkylamides such as N-isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide and N-(2-phenylethyl) non-2(E)-en-6, 8-diynamide were known to be among the major bioactive compounds in different species of *Acmella*. The plant extract caused significant concentration-dependent activity on both the tapeworm (cestode) *Raillietina echinobothrida* and the roundworm (nematode) *Ascaridia galli*. Scanning electron microscopy revealed shrinkage of the tegument, erosion of microtriches, and deformity of the suckers on the cestode. Damaged lips and sensory organs and abnormal contraction of the cuticle were seen on the nematode. Our findings support the anthelmintic property of the plant as used in Mizo traditional medicine.



Abbreviations used: °C: Degree centigrade; Da: Dalton; DMSO: Dimethylsulfoxide; eV: Electron volt; GC-MS: Gas chromatography-mass spectrometry; H: Hour; kV: Kilovolt; m: Meter; M: Molarity; Mg: Milligram; Min: Minute; Ml: Milliliter; Mm: Millimeter; Ml: Microliter; µm: Micrometer; m/z: Mass-to-charge ratio; PBS: Phosphate-buffered saline.

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INTRODUCTION

The toothache plant or paracress, *A. oleracea* (L.) R. K. Jansen, is an annual herb belonging to the family Asteraceae. It is known in different ethnic practices for its medicinal properties and culinary usages.[1] It is consumed either cooked or used raw as a vegetable or food seasoning because of its unique menthol-like minty flavor. The nickname toothache plant is given owing to its practical use in dental healthcare.[2] It is among one of the most versatile medicinal plants with a wide variety of uses such as in the treatment of anemia, cancer, constipation, diuresis, high (febrile) fever, flatulence, inflammation, liver abscess, peptic ulcer, toothache, and ulcer.[3,4] It is also regarded as an effective therapy in severe malaria and has been shown to be effective on malarial parasites.[5] In addition, it is being used in Indian medicine as a remedy for impotency and as aphrodisiac, as well as for treating articular rheumatism, dysentery, snakebite, and tuberculosis.[6]

The plant extract has been successfully tested for analgesic, antimicrobial, antioxidant, diuretic, larvicidal, and mosquitocidal activities.[3] It also specifically induces increased proliferation of macrophage concentration

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in the blood, indicating its ability to enhance immunomodulatory activity of professional phagocytes.[7] In experimental mice, the plant extract also suppresses neutrophilic inflammation in the lungs.[8] Its high antipyretic activity against Brewer's yeast-induced pyrexia supports its use as a therapy for high fever and inflammation.[9] As an insecticide, it has been shown to be effective against the insect-pest *Tuta absoluta*,[10] and vectors of important infectious diseases, including *Aedes aegyptii*,[11] *Anopheles,* and *Culex* species.[12]

To the Mizo people, the whole plant is a common vegetable and is boiled in water to be served as side dish. In their traditional medicine, the plant is a convenient remedy for gastrointestinal pains, headaches, dysentery, oral and dental infections, rheumatism, and stuttering in children. In households and agricultural fields, its pungent odor is used to ward off mosquitos and other insects. In addition, the watery extract of the whole plant is used as a stupefying agent in traditional community fishing.[13] Perhaps, the most unique application is as a deworming agent for both tapeworm and roundworm intestinal helminthiases. To evaluate this acclaimed broad-spectrum anthelmintic property is the aim of this study.

MATERIALS AND METHODS

Preparation of plant material

A. oleracea was harvested from a plantation in Ngopa, a village in Champhai district, Mizoram, India, located between 23.8861°N and 93.2119°E. The specimen was identified at the Botanical Survey of India, Shillong, India and catalogued (accession no. PUC-A-17-1) in the herbarium section of Pachhunga University College, Aizawl, Mizoram. The aerial parts of the plant were dried in the shade at room temperature. Extraction was done in a 5 l Soxhlet apparatus using methanol as the solvent for 72 h. The slurry of extract was concentrated by removing and recovering the solvent in a vacuum rotary evaporator (Buchi Rotavapor® R-215) under reduced pressure. The final semi-solid extract was stored in a refrigerator at 4°C until further use.

Chemicals and drugs

All chemicals were standard analytical grades procured from HiMedia Laboratories Private Limited, Mumbai, India. Acetonitrile for gas chromatography (GC) was a product of Merck Life Science Private Limited, Mumbai, India. Albendazole (Zenlee) was a product of UNI-PEX Pharmaceutical Private Limited, New Delhi, India.

Gas chromatography-mass spectrometry analysis

A. oleracea methanol extract was analysed in a single quadrupole GC-mass spectrometry (GC-MS) system (Thermo Scientific TRACE™ 1300 ISQ™ LT). The plant extract was dissolved in acetonitrile in a ratio of 50 mg in 3 ml. A nonpolar column TR-5MS (260F142P) was used as a stationary phase. The dimension of the column was 30 m × 0.25 mm × 0.25 µm with film thickness of 0.25 µm. The injector port was set at a temperature of 250°C. The oven temperature was initially set at 70°C for 2 min and incrementally raised by 10°C up to 250°C. Helium was used as a carrier gas and was released into the oven chamber at a constant flow rate of 1 ml/min. Sample injection was done in a volume of 1 µl in split mode and the splitting ratio was maintained at 1:50. The mass spectrometer was run with ionization electron energy of 70 eV. Ion source and transfer line temperature were set at 250°C. The total running duration was 55 min. Mass ratio (m/z) was scanned up to 1100 Da. The final chromatogram was generated with Thermo Scientific™ Xcalibur™ software. Compounds were identified on the basis of their retention time, chemical formula, and molecular weight from libraries of Wiley Registry™ (10) and National Institute of Standards and Technology database.

Anthelmintic test

In vitro anthelmintic activity was studied on two intestinal parasites, namely a tapeworm *Raillietina echinobothrida* and a roundworm *Ascaridia galli.* The helminth parasites were recovered and collected from the intestines of freshly sacrificed local fowls, *Gallus gallus domesticus.* The plants extract was prepared in solutions of varying concentrations such as 1.25, 2.5, 5, 10, and 20 mg/ml by dissolving them in 0.9% neutral phosphate-buffered saline (PBS) supplemented with 1% dimethylsulfoxide (DMSO). Corresponding concentrations of albendazole were also prepared as standard references. Control media consisted of only PBS with 1% DMSO. Batches of two worms were selected for each test, which were performed in triplicates. They were incubated in a biological incubator maintained at a constant temperature of 37°C ± 1°C.

Anthelmintic efficacy was assessed in terms of duration of survival. Data were presented as statistical means ± standard deviation. Significance of the anthelmintic activity was determined using Student's *t*-test and the level of significance was considered when *P* value was < 0.05.

Scanning electron microscopy

Helminths treated with 20 mg/ml of the plant extract were selected for scanning electron microscopy. They were fixed in 10% cold-buffered formaldehyde at 4°C for 4 h. 0.1 M sodium cacodylate (pH 7.2) was used as buffer. Secondary fixation was done with 1% osmium tetroxide (OsO_4) at 4°C for 1 h. The specimens were dehydrated through increasing

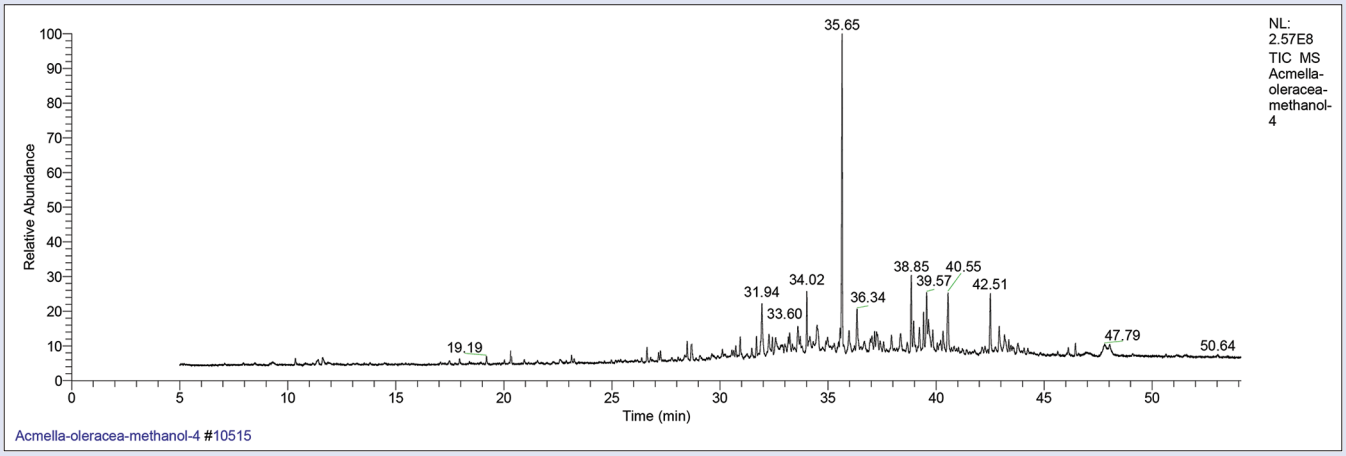

Figure 1: Gas chromatogram of the methanol extract of *Acmella oleracea.* Total retention time is 55 min

concentrations of acetone up to pure acetone. They were then treated with tetramethylsilane, $Si(CH_3)_4$, for 15 min and left to dry in air-drying chamber at 25°C. They were mounted on metal stubs and sputter coated with gold in JFC 1100 (JEOL Ltd., Tokyo, Japan) ion-sputtering chamber. Finally, they were observed under a JSM-6360 scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an electron accelerating voltage of 20 kV.

RESULTS

Chemical analysis using gas chromatography-mass spectrometry

Gas chromatogram of the methanol extract of *A. oleracea* is shown in Figure 1 and the corresponding chemical identification based on mass spectra is given in Table 1. The presence of 13 compounds was confirmed. Methyl *n*-hexadecanoate (palmitic acid) was by far the most abundant compound with relative abundance of 99.5%, followed methyl (8E, 11E)-8,11-octadecadienoate (30.5%). Hexadecanoic acid, oplopanone or 1-((1S,3aR,4R,7S,7aS)-4-hydroxy-7-isopropyl-4-methyloctahydro-1H-inden-1-yl) ethanone, 6,10, 14-trimethylpentadecan-2-one, (Z)-18-octadec-9-enolide, N-isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide, and N-(2-phenylethyl) non-2(E)-en-6, 8-diynamide were detected in moderate amounts.

Anthelmintic efficacy

The antiparasitic efficacy of albendazole and *A. oleracea* methanol extract on *R. echinobothrida* is given in Table 2. Worms maintained in control media survived up to 74.03 ± 1.89 h. Concentration-dependent anthelmintic efficacy was noted at all concentrations tested. Albendazole killed all the tapeworms in 20.40 ± 1.17, 17.59 ± 1.43, 14.99 ± 0.43, 12.07 ± 0.49, and 08.99 ± 0.45 h at the concentrations of 1.25, 2, 5, 10, and 20 mg/ml, respectively. At similar concentrations, the plant extract took 51.18 ± 1.41, 45.57 ± 1.00, 39.01 ± 1.31, 30.83 ± 1.25, and 18.42 ± 0.95 h to kill the tapeworms.

Albendazole was highly effective on the roundworm, as shown in Table 3. *A. galli* survived relatively longer, up to 187.01 ± 6.77 h in control media. Albendazole killed all the roundworms at 56.94 ± 1.76, 43.57 ± 1.25, 28.86 ± 1.28, 18.01 ± 2.73, and 4.01 ± 1.00 h at the concentrations of 1.25, 2, 5, 10, and 20 mg/ml. The plant extract was less potent but nonetheless effectively killed all the roundworms at 173.89 ± 2.58, 171.68 ± 2.23, 155.24 ± 2.26, 135.70 ± 2.26, and 112.17 ± 0.88 h at similar concentrations.

Anthelmintic effects

Scanning electron microscopic analysis of the structural changes on *R. echinobothrida* after treatment with 20 mg/ml of *A. oleracea* methanol extract is shown in Figures 2-5. In Figure 2, the scolex with oval-shaped suckers and an apical rostellum is shown. The general body surface called tegument is severely shrunken. In Figure 3, a sucker is magnified showing circular rows of spines, the parasitic holdfast devices. Most of the spines are clumped. Large portions on the top and bottom are eroded with total loss of the spines. The body segments (proglottids) are more wrinkled [Figure 4]. Some portions show scar formation indicating complete erosion of the microtriches [Figure 5].

The effects on *A. galli* are most prominent on the head, as shown in Figure 6. The otherwise dome-shaped and smooth lips are totally disfigured and collapsed. The smooth cuticle is reduced to warty surface. The sensory organs, amphids, are lost. Shrinkage is the most prominent in the posterior region of the body where annulations (transverse rings) are distorted [Figure 7] and the tail end is also abnormally contracted with a series of transverse folds [Figure 8].

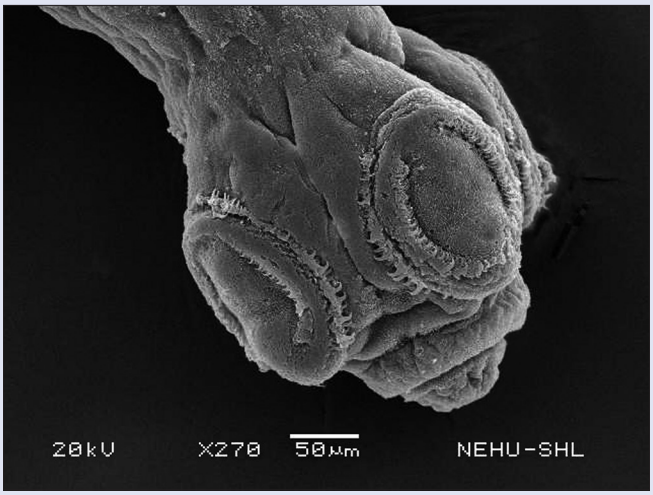

Figure 2: Scanning electron microscopic image of the anterior end of *Raillietina echinobothrida* treated with *Acmella oleracea* extract. The scolex with two sucker and an apical rostellum is visible. Shrinkage marked by tegumental folds can be seen. 270×, scale bar = 50 µm

Table 1: List of compounds detected from *Acmella oleracea* methanol extract using gas chromatography-mass spectrometry

Retention time (min)	Relative abundance (%)	Compound	Formula	Molecular weight (g/mol)
19.19	7.2	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl	$C_7H_9NO_2$	139
31.94	22.5	1-((1S,3aR,4R,7S,7aS)-4-Hydroxy-7-isopropyl-4-methyloctahydro-1H-inden-1-yl) ethanone	$C_{15}H_{26}O_2$	238
33.60	15.9	1-Heptatriacotanol	$C_{37}H_{76}O$	536
34.02	25.7	6,10,14-Trimethylpentadecan-2-one	$C_{18}H_{36}O$	268
35.65	99.5	Methyl *n*-hexadecanoate (palmitic acid)	$C_{17}H_{34}O_2$	270
36.34	20.7	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568
38.85	30.5	Methyl (8E,11E)-8,11-octadecadienoate	$C_{19}H_{34}O_2$	294
39.57	25.7	(Z)-18-Octadec-9-enolide	$C_{18}H_{32}O_2$	280
40.55	25.4	N-isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide	$C_{16}H_{25}NO$	247
42.51	25.3	N-(2-Phenylethyl) non-2(E)-en-6, 8-diynamide	$C_{17}H_{17}NO$	251
46.45	11.1	3,8,8-Trimethoxy-3-piperidyl-2,2-binaphthalene-1,1,4,4, tetrone	$C_{28}H_{25}NO_7$	487
47.79	10.9	Lup-20 (29)-en-3-ol, acetate, (3a) (lupeol acetate)	$C_{32}H_{52}O_2$	468
50.64	7.6	2,4,6,8,10-Tetradecapentanoic acid, 9a-(acetyloxy)-1a, 1b, 4,4a, 5,7a, 7b, 8,9a-decahydro-4a, 7b-dihydroxy-3-(hydroxymethyl)-1, 1,6,8-tetramethyl-5-oxo-1H-cyclopropa[3,4] benz[1,2-e] azulen-9yl ester, [1aR-(1aa, 1ba, 4aa, 7aa, 7ba, 8a, 9a, 9aa)]	$C_{36}H_{46}O_8$	606

DISCUSSION

A. oleracea is known to contain important compounds such as α-and β-amyrinester, miricilic alcohol glycosides, sitosterol, saponins, amides, stigmasterol, and triterpenes which are attributed the wide range of pharmacological properties.[14] Hexadecanoic acid or palmitic acid detected in *A. oleracea* in the present study is already known to have anti-inflammatory activity.[15] In addition to this compound, N-isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide and N-isobutyl-2E, 6Z, 8E-decatrienamide recorded here are also reported from other species *Acmella*.[16] These N-alkylamides are the principal bioactive compounds in these plants. In fact, N-isobutyl-2E, 6Z, 8E-decatrienamide is established to be the main N-alkylamide in the genus *Acmella*, *Welelia parviceps* and *Heliopsis longipes* and is attributed to most of the pharmacological properties of these plants. It has been shown to have analgesic, neuroprotective, antioxidant, antimutagenic, anti-cancer, anti-inflammatory, antimicrobial, and insecticidal activities.[17]

Table 2: *In vitro* survival test of *Acmella oleracea* extract and albendazole on the tapeworm, *Raillietina echinobothrida*

Treatment	Dose (mg/ml)	Survival time (h) in mean±SD	*t*	*t* critical value
Control	0	74.03±1.89	NA	NA
Albendazole	1.25	20.40±1.17*	59.40	2.31
	2.5	17.59±1.43*	58.32	2.27
	5	14.99±0.43*	74.53	2.45
	10	12.07±0.49*	77.66	2.45
	20	08.99±0.45*	81.85	2.45
Acmella	1.25	51.18±1.41*	23.68	2.27
oleracea	2.5	45.57±1.00*	32.58	2.31
	5	39.01±1.31*	37.22	2.26
	10	30.83±1.25*	46.67	2.26
	20	18.42±0.95*	64.31	2.36

*Significantly different at $P<0.05$ in comparison with control; NA; $n=6$. NA: Not applicable; SD: Standard deviation

Table 3: *In vitro* survival test of *Acmella oleracea* extract and albendazole on the roundworm, *Ascaridia galli*

Treatment	Dose (mg/ml)	Survival time (h) in mean±SD	*t*	*t* critical value
Control	0	187.01±6.77	NA	NA
Albendazole	1.25	056.94±1.76*	45.53	2.45
	2.5	043.57±1.25*	51.02	2.57
	5	028.86±1.28*	56.21	2.57
	10	018.01±2.73*	56.69	2.36
	20	004.01±1.00*	65.50	2.57
Acmella	1.25	173.89±2.58*	04.44	2.45
oleracea	2.5	171.68±2.23*	05.27	2.45
	5	155.24±2.26*	10.90	2.45
	10	135.70±2.26*	17.61	2.45
	20	112.17±0.88*	26.85	2.57

*Significantly different at $P<0.05$ in comparison with control; NA; $n=6$. NA: Not applicable; SD: Standard deviation

A. oleracea methanol extract was significantly effective against both tapeworms and roundworms, although the efficacy was lower than that of the standard anthelmintic, albendazole. Structural damages observed on *R. echinobothrida* and *A. galli* are among the signature effects of anthelmintic drugs. Drugs primarily target the body surfaces in helminths as they are the direct interface between the parasites and the host.[18] In cestodes, being devoid of digestive system, the body surface (tegument) with its hair-like microtriches is the primary absorptive and sensory site and for this reason the tegument is always the target site of drugs. Whereas nematodes are comparatively more complex as their body surface (cuticle) is structurally and chemically different. Yet, drugs enter through their body surface as in the cestodes.[19] Albendazole and flubendazole reportedly caused rostellar damages, eruption of swellings or blebs on the tegument, defacement of the microtriches and increased vesiculation on the tapeworm, *Echinococcus granulosus*.[20] It also caused severe contraction, removal of microtriches and tegumental collapse in *R. echinobothrida*.[21] Albendazole-praziquantel combination treatment of *Mesocestoides corti,* resulted in extensive deformity of the suckers, erosion of the tegument, and disintegration of microtriches.[22]

Albendazole with high lipophilicity is among the most diffusible drugs through the cuticle of *Haemonchus contortus* and *Ascaris suum* sulfoxide.[23] Ivermectin also enters through the cuticle in *Caenorhaditis elegans*, *H. contortus,* and *Oncocerca ochengi* to primarily inhibit muscle contraction.[24] Cyclotides, a family of plant peptides, primarily target the cuticle of *H. contortus* and *Trichostrongylus colubriformis*.[25] Natural cysteine proteinases also exert anthelmintic activity by attacking the cuticular proteins in different roundworms.[26] Our observations on the cuticular changes throughout the body of *A. galli* indicate that *A. oleracea* also directly act on the cuticle.

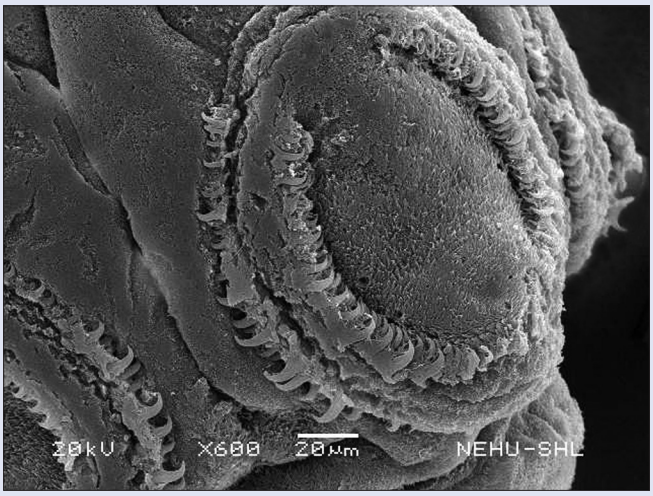

Figure 3: Magnification of the scolex of *Raillietina echinobothrida* treated with *Acmella oleracea* extract. A single sucker is focused, which showed erosion at some points and clumping of the spines. 600×, scale bar = 20 µm

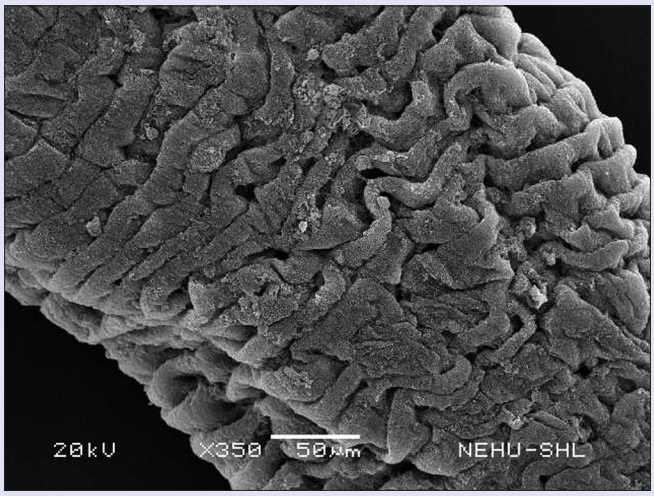

Figure 4: The body proper (strobila) of *Raillietina echinobothrida* treated with *Acmella oleracea* extract. The body segments are all folded and wrinkled. Some areas are clearly eroded. 350×, scale bar = 20 µm

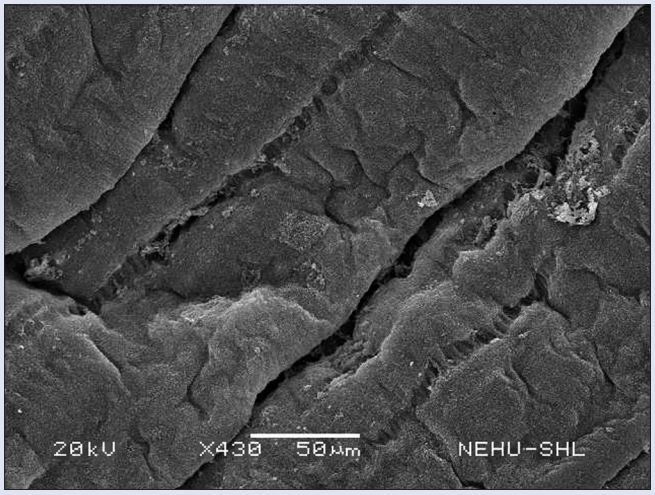

Figure 5: Magnified body segments of *Raillietina echinobothrida* treated with *Acmella oleracea* extract. The tegument is shrunk and fine hair-like structures (microtriches) are completely lost. 430×, scale bar = 50 µm

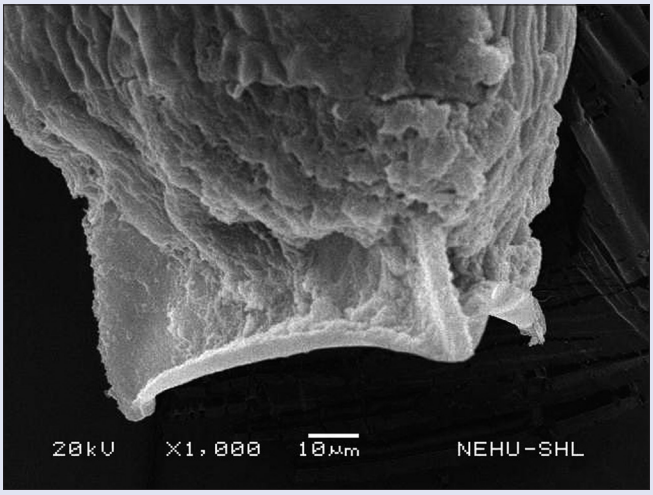

Figure 6: Scanning electron microscopic image of the anterior end of *Ascaridia galli* treated with *Acmella oleracea* extract. The lips are thin and collapsed. There are no signs of amphids and instead the cuticle is wrinkled. 1000×, scale bar = 10 µm

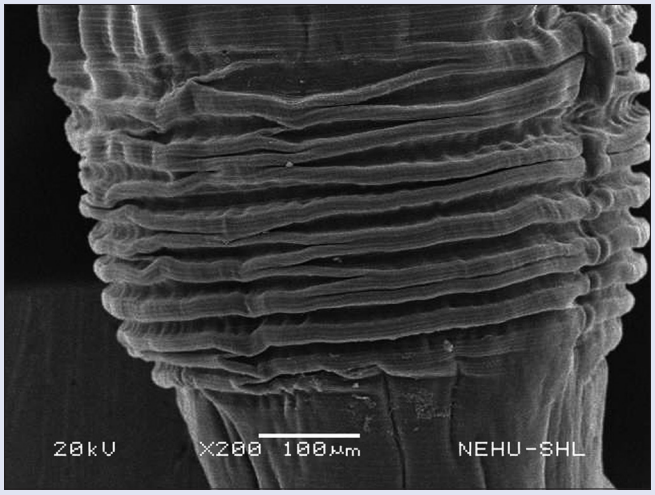

Figure 7: The main body of *Ascaridia galli* treated with *Acmella oleracea* extract. The cuticle is severely contracted and transversely folded. 200×, scale bar = 100 µm

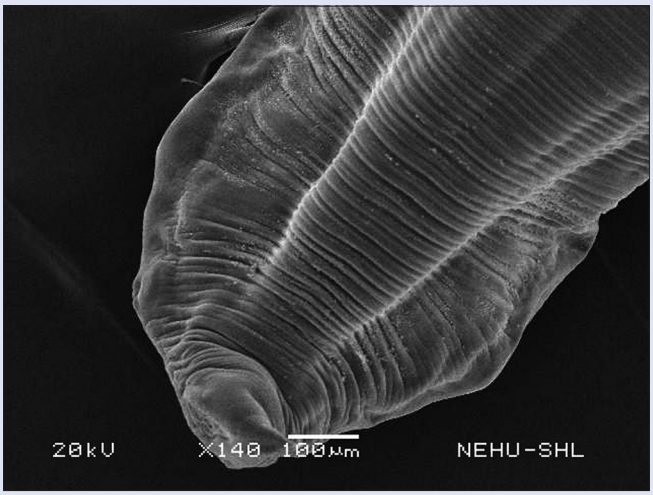

Figure 8: The posterior end of *Ascaridia galli* treated with *Acmella oleracea* extract. The cuticle is also severely contracted and transversely folded. 140×, scale bar = 100 µm

CONCLUSION

A. oleracea is clearly an important anthelmintic plant as is used in Mizo traditional medicine. Due to large biochemical and physiological differences between cestodes and nematodes, drug mostly are helminth specific, i.e., they are active against only either one of them. It is worthwhile to note that the plant studied was effective on both helminths. The presence of N-alkylamides is particularly interesting as these compounds are known for their specific biological activities. Therefore, it would be interesting to isolate individual compounds and further investigate their anthelmintic potentials.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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