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Scanning electron microscopy study of *Raillietina echinobothrida* Megnin, 1880 (Cestoda) and *Syphacia obvelata* Rudolphi, 1802 (Nematoda) exposed to *Cyperus compressus* (L.) (Cyperaceae) and its GC-MS study

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The roots of *Cyperus compressus* (Cyperaceae) have traditionally been used to treat intestinal helminthiasis in India. This study was designed to examine the abnormalities caused on *Raillietina echinobothrida* Megnin, 1880, and *Syphacia obvelata*, Rudolphi, 1802, on exposure to the root methanolic extract of *C. compressus*, using scanning electron microscopy. Worms were placed in extract concentration of 30mg/mL and on paralysis, worms were dehydrated in acetone grades and images were taken. The extract was then processed for GC-MS study to detect the compounds present in it. The images portrayed damaged microtriches in *R. echinobothrida* and loss of annulations and wrinkled cuticle were observed in *S. obvelata*. Compared to the damages caused by the reference drug, the results were milder. GC-MS study revealed 30 prominent peaks where 7-Methyl-Z-tetradecen-1-ol acetate, was the most abundant compound which was detected at retention time 20.48 and with a relative abundance of 60.49. Its efficacy could be contributed to these detected compounds in the extract.

Keywords Anthelmintic, *Cyperus compressus*, GC-MS, *Raillietina echinobothrida*, *Syphacia obvelata*

In recent times, plant-based medicines have been on the rise as an alternative form of treatment[1]. The marginalised people in several countries use medicines of natural origin to treat their sick[2]. Currently, these medicinal herbs are also being marketed as supplements as a source of nutrients and to treat multiple ailments[3]. *Cyperus compressus* (Cyperaceae) is one such medicinal plant used in India. It is a common weed and is known to possess multiple healing properties. It has been shown to possess both *in vitro* and *in vivo* efficacy against cestode, trematode and nematode parasites[4,5]. The damaging effect of this plant in the tegument of *Gastrothylax crumenifer* (Trematoda) has already been elaborately studied[5]. *Cyperus* spp. have been used to treat menstrual disorders, respiratory infections, and blood disorders. In addition, they are also known to possess anti-inflammatory, antioxidant and antimicrobial properties. Several phytochemicals such as zierone, *α*-cyperone, germacrene D, caryophyllene oxide, *α*-pinene, cyperotundone, mustakone, and *α*-corymbolol have been isolated from this plant already making it a phytochemical rich plant which are responsible for its multiple effects[6]. GC-MS studies have been conducted to establish the chemical profile of plants[7] and is a very good tool to detect aromatic compounds[8]. There are however no reports of chemical profiling of *C. compressus* using GC-MS or any other techniques. Ethno-medical traditions prove that they have a great therapeutic value and are important bioresources[9]. This study was therefore undertaken to examine the effect of *C. compressus* root extract on *Raillietina echinobothrida* (Cestoda) a fowl tapeworm and *Syphacia obvelata* (Nematoda) a mice pinworm and also the detect the phyto-compounds present in it using GC-MS. This study will add to the existing medicinal plant pharmacopeia on the medicinal properties of *C. compressus*.

Materials and Methods

Scanning electron microscopy study

Live worms were collected from freshly sacrificed animals (vide Member Secretary, IEC-Animal Models, NEHU, Shillong, dated December 04, 2014). and placed in 30 mg/mL concentration of the methanolic extracts prepared using Soxhlet apparatus. Worms (n=5) were placed in petridishes in triplicates and the experiment was carried out in an incubator at 37˚C[4]. On attainment of paralysis, the worms were washed in buffer and then dehydrated using acetone grades. Worms were then dried in tetramethylsilane (TMS), coated in gold, and imaged in Hitachi TM 4000 Plus SEM.

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Gas Chromatography-Mass Spectrometry (GC-MS)

C. compressus methanol extract was analysed in a Thermo Scientific TRACE™ 1300 ISQ™ LT GC-MS system. The plant extract was dissolved in acetonitrile (50 mg in 3 mL) and a nonpolar column TR-5MS (260F142P) was used as a stationary phase (30m×0.25mm×0.25µm). Helium was used as a carrier gas and was released at the rate of 1mL/min. The MS was run with ionisation electron energy of 70eV for 55 min and mass ratio (m/z) was scanned up to 1100Da. The chromatogram was generated using Thermo Scientific™ Xcalibur™ software and the compounds present were identified based on the retention time, chemical formula, and molecular weight from libraries of Wiley Registry™ (10) and National Institute of Standards and Technology database.

Results and Discussion

Scanning electron microscopy study

The extract caused partial damages in the microtriches of *R. echinobothrida*. Microtriches appeared broken and damaged. Suckers appeared open and the tegument showed regularly arranged segments with mild shrinking. Other structures appeared normal in architecture. PZQ exposed worms also showed damaged microtriches, and compression of segments (Fig. 1). Likewise, the extract treated *S. obvelata* worms showed mild annulation loss, with intact and open mouth region and lips. The cuticle also appeared wrinkled compared to the control worms. No other deformities or irregularities were observed. ABZ treated worms also showed similar deformities (Fig. 2).

Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical constituents analysed from GC-MS showed 30 prominent peaks (Fig. 3). The most abundant compound was 7-Methyl-Z-tetradecen-1-ol acetate, which was detected at retention time 20.48 with relative abundance of 60.49. Other major compounds detected were 5-Hydroxymethylfurfural (retention time 3.20, relative abundance 52.77), Phorbol (retention time 18.41, relative abundance 49.69), 6-Acetyl-á-d-mannose (retention time 3.05, relative abundance 49.44), Octadecanoic acid (retention time 17.15, relative abundance 45.94), and 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (retention time 25.54, relative abundance 44.90). The list of other compounds detected are displayed in Table 1.

SEM has been used as a preliminary study to determine the effect of the extracts on the outer body

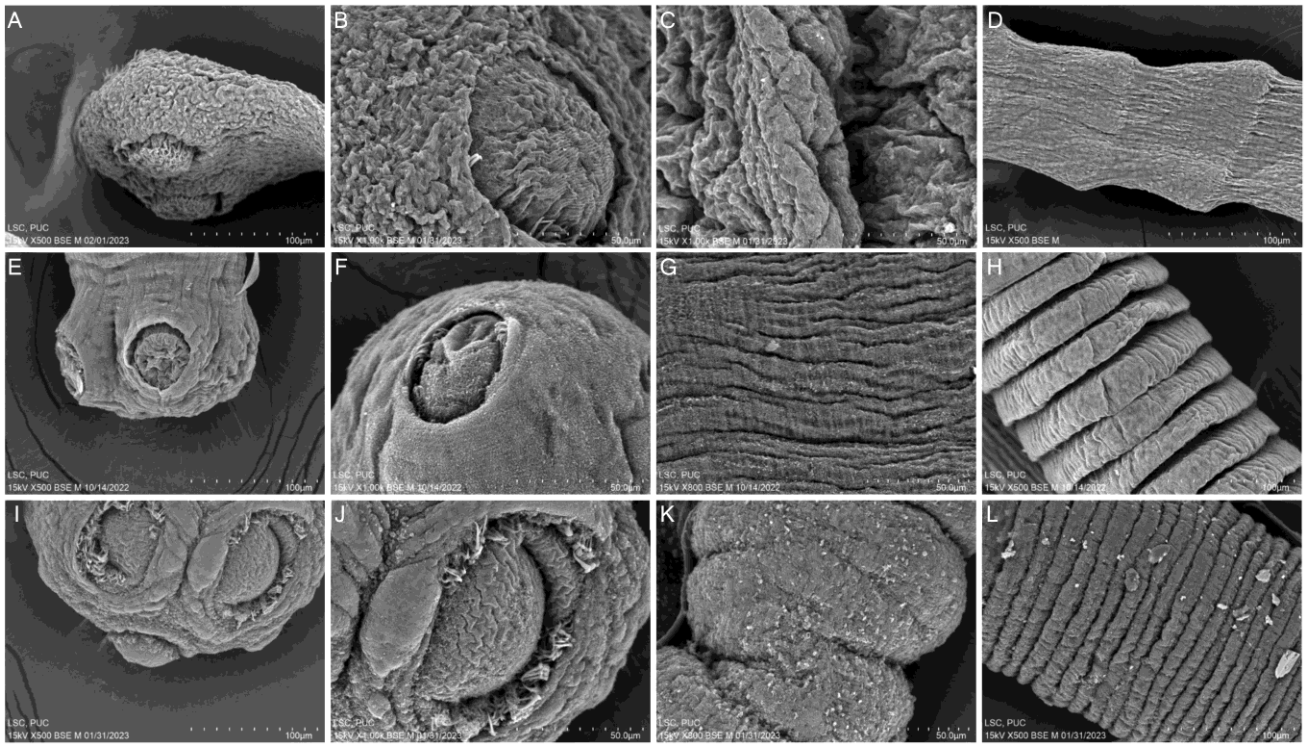

Fig. 1 — Effect of extract on *R. echinobothrida*. (A-D) Control worms showing normal architecture; (E-H) extract treated worms (I-L) PZQ treated worms

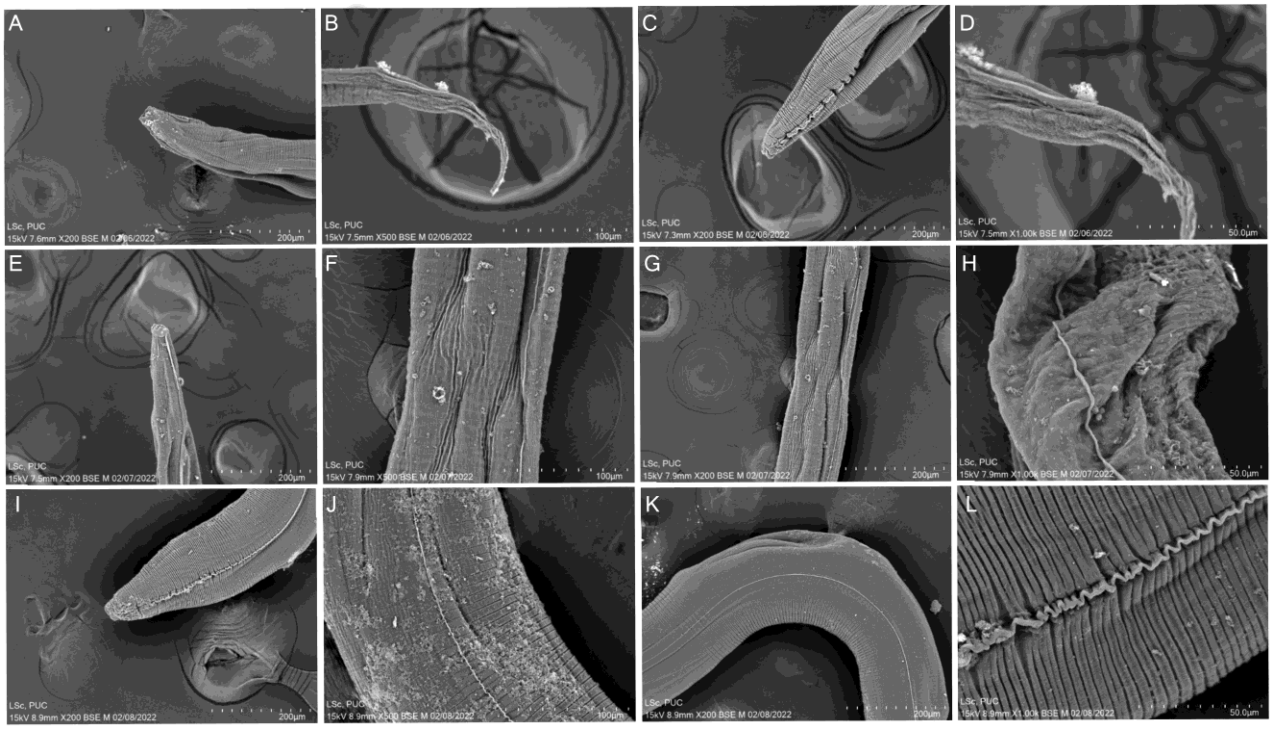

Fig. 2 — Effect of extract on *S. obvelata.* (A-D) Control worms showing normal architecture; (E-H) extract treated worms (I-L) ABZ treated worms

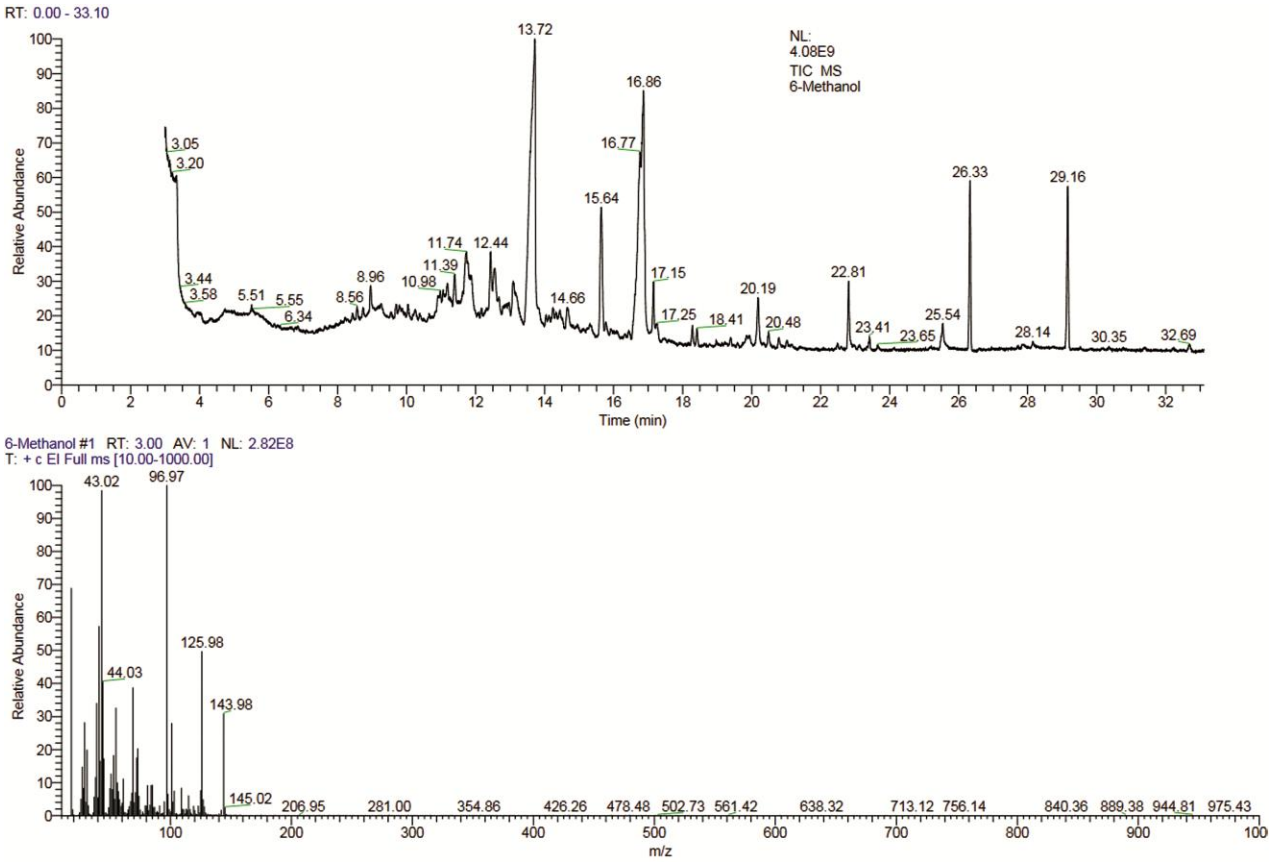

Fig. 3 — GC-MS of *C. compressus*

Table 1 — Compounds detected in the GC-MS study of *C. compressus*

S. No.	Retention Time	Relative Abundance	Compound	Formula	Molecular weight (u)
1	3.05	49.44	6-Acetyl-á-d-mannose	C8H14O7	222
2	3.20	52.77	5-Hydroxymethylfurfural	C6H6O3	126
3	3.44	26.60	Melezitose	C18H32O16	504
4	3.58	14.89	Melezitose	C18H32O16	504
5	5.51	18.10	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	C12H22O11	342
6	5.55	13.12	Dodecanoic acid, 3-hydroxy-	C12H24O3	216
7	6.34	17.86	Dodecanoic acid, 3-hydroxy-	C12H24O3	216
8	8.56	25.64	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	C22H40O2	336
9	8.96	20.24	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	C15H22O	218
10	10.98	19.18	Isoaromadendrene epoxide	C15H24O	220
11	11.39	6.88	Corymbolone	C15H24O2	236
12	11.74	28.28	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	C15H22O2	234
13	12.44	12.93	Isoaromadendrene epoxide	C15H24O	220
14	13.72	64.77	n-Hexadecanoic acid	C16H32O2	256
15	14.66	28.94	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	C23H32O	324
16	15.64	39.84	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	C15H22O2	234
17	16.77	10.39	(Z)-18-Octadec-9-enolide	C18H32O2	280
18	16.86	22.05	trans-13-Octadecenoic acid	C18H34O2	282
19	17.15	45.94	Octadecanoic acid	C18H36O2	284
20	17.25	22.35	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	C23H32O	324
21	18.41	49.69	Phorbol	C20H28O6	364
22	20.19	17.49	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4	352
23	20.48	60.49	7-Methyl-Z-tetradecen-1-ol acetate	C17H32O2	268
24	23.65	21.53	Dodecanoic acid, 3-hydroxy-	C12H24O3	216
25	25.54	44.90	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C21H40O4	356
26	26.33	13.58	cis-15-Tetracosenoic acid, propyl ester	C27H52O2	408
27	28.14	8.48	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	C28H40O10	536
28	29.16	19.77	22-Tricosenoic acid	C23H44O2	352
29	30.35	37.76	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3à,5à)-	C29H43NO3Si	481
30	32.69	6.34	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C21H40O4	356

surface of parasites and also to study the mechanisms and mode of action[10,11]. Several such plant extracts have been known to cause tegumental damages in the test worms[11,12]. A similar work on *C. rotundus* using *Trichinella spiralis* as a test parasite has also been carried out recently[12]. The study reported similar results where worms showed loss of annulations and damages in the cuticle[13]. Similar damages in the tegument of tapeworms have been reported where the microtriches were found to be damaged in treated worms. The external tegument is an important component of parasites serving various functions such as nutrition, protection, and movement[14]. Hence, its damage can lead to the death of the parasite. Workers had earlier demonstrated that *C. compressus* is known to cause damage to the tegument of trematodes[5]. Likewise, they had also established its anthelmintic efficacy against cestodes and nematodes[4]. Hence, the study suggests that *C. compressus* possess anthelmintic efficacy and could act by damaging the outer protective and nutrition deriving layer of parasites.

GC-MS study is carried out to detect the phyto-compounds present in the plant extract[15]. Some workers had earlier reported that n-Hexadecanoic acid and Cyclopropanepentanoic acid were the major chemical compounds in C. *compressus* from Tamil Nadu[16]. Likewise, from Kerala, 22 components were reported and the plant was found to be rich in sesquiterpeneoids. The major constituents were

guaiane and caryophyllene derivatives[17]. This study is the first report from Assam which showed 30 peaks where n-Hexadecanoic acid, trans-13-Octadecenoic acid and, Octadecanoic acid were also reported in a similar study[16]. These detected phyto-compounds could be responsible for its efficacy.

Conclusion

The study shows that the plant extract is capable of causing damages to the external morphology of parasites. Further studies to isolate the detected phyto-compounds and test them individually for their efficacy and toxicity are warranted.

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Ethical Statement

The use of mice approved by the Institutional Animal Ethics Committee (Animal Models) of North-Eastern Hill University, Shillong (Vide, Member Secretary, IEC, NEHU, dated December 4, 2014).

Conflict of interest

The authors declare that they have no conflict of interest.

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