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ORIGINAL PAPER

Anthelmintic Efficacy and Phytochemical Screening of Ethanolic Extract of the Discarded *Schumannianthus dichotomus* Stem-a Waste Product of the Local Handicraft Industry of Cooch Behar, West Bengal, India

Manjil Gupta[1,2] · **Rachita Saha**[1] · **Subrata Saha**[1] · **Pradip Kumar Kar**[1]

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Abstract

Purpose This study aims to explore the anthelmintic potential of the ethanolic extract derived from the discarded stem of *Schumannianthus dichotomus* on the cestode *Raillietina* spp. Additionally, phytochemical screening of the extract seeks to elucidate the presence of bioactive compounds responsible for the observed anthelmintic activity.

Methods *Raillietina* spp., the model parasite, was collected from the intestine of freshly slaughtered fowl and treated with different doses of ethanolic extract and fractions of *Schumannianthus dichotomus* for motility assays to determine the most efficacious dose. Changes in the ultrastructure of the worms were investigated through TEM and SEM. Qualitative and quantitative analysis of phytochemicals in the crude extract as well as GCMS analysis of the ethyl acetate fraction were also done.

Results The worms showed dose dependent reduction in motility and survival. The most efficacious dose and fraction were determined to be 20 mg/ml and ethyl acetate fraction respectively. Changes in tegument and internal structures were evidenced by SEM and TEM observations. The crude extract was found rich in alkaloids, flavonoids and terpenoids. GCMS analysis of the ethyl acetate fraction identified five major compounds out of which Phthalic acid, di(2-propylpentyl) ester may be the major bioactive component responsible for the anthelmintic activity.

Conclusion Our study firmly establishes the anthelmintic potential of the waste part of *Schumannianthus dichotomus* and prospects its valorisation.

✉ Pradip Kumar Kar
karpradip@gmail.com

[1] Parasitology Laboratory, Department of Zoology, Cooch Behar Panchanan Barma University, Cooch Behar, West Bengal, India

[2] Department of Zoology, Dinhata College, Dinhata, Cooch Behar, West Bengal, India

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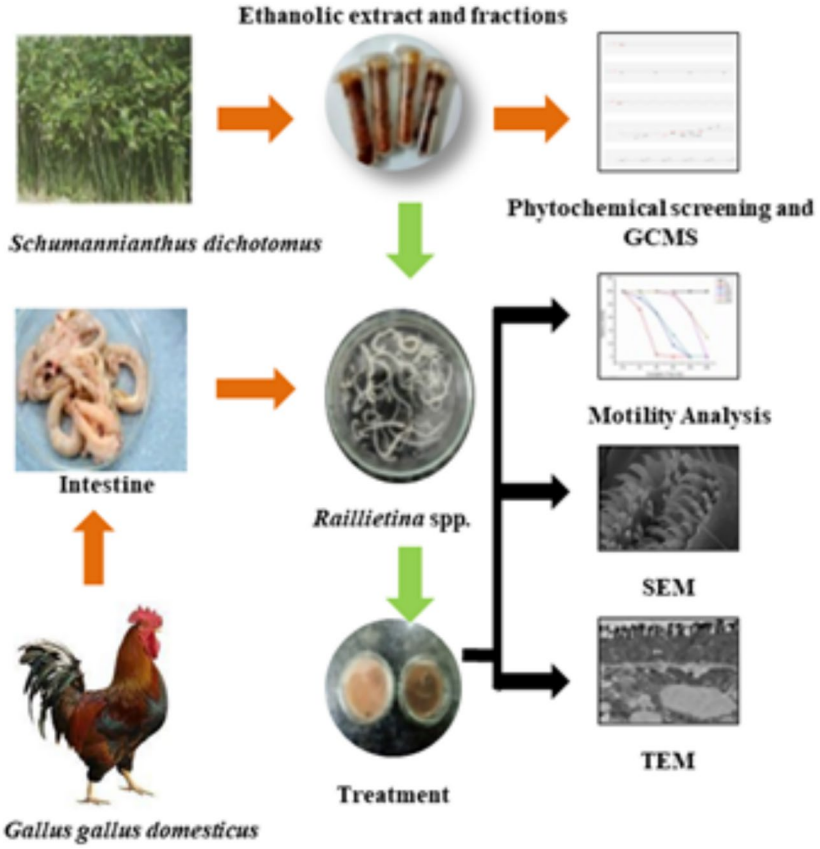
Graphical Abstract



Keywords *Schumannianthus* · *Raillietina* · Anthelmintic · Phytochemical · Ultrastructure · GC–MS

Statement of Novelty

In this paper, we have established the anthelmintic potential of the ethanolic extract of discarded stem of Schumannianthus dichotomus, a waste product from the local handicraft industry in Cooch Behar, West Bengal, India by morphological and ultrastructural studies. Additionally, we have characterized the extract for qualitative and quantitative analysis of phytochemicals that may be responsible for the anthelmintic effect. This is the first study on the discarded part of the stem of Schumannianthus dichotomus. This knowledge could aid in creating anthelmintic drugs. Additionally, using the plant's discarded stem could offer sustainable solutions for managing helminth infections in chickens, benefiting rural communities that raise livestock non-intensively for extra income.

Introduction

Agriculture stands as one of the most substantial biological sectors, yielding extensive biomass that serves as a crucial input for the bioeconomy. To meet the escalating demands of a burgeoning global population, there has been a noteworthy increase in both livestock and crop production, consequently leading to the generation of agricultural wastes [1]. On an annual basis, India contributes significantly to solid waste production, with agricultural wastes accounting for a substantial portion ranging from approximately 350 to 990 million tonnes per year [2]. Traditionally, certain crop residues have found applications in combustion, animal fodder, roof thatching, composting, soil mulching, as well as in the production of matchsticks and paper. The valorisation of agricultural wastes presents an opportunity to generate value-added products, support farmers' livelihoods, create job opportunities for the youth, and promote sustainability in agriculture [3].

Agricultural wastes serve as abundant reservoirs of bioactive compounds and phytochemicals, contributing to the development of sustainable practices in waste utilization [4, 5]. These compounds, derived from various agricultural residues, exhibit diverse biological activities and potential applications in pharmaceutical, nutraceutical, agrochemical industries [6] and have demonstrated antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, showcasing their pharmacological significance [7]. Harnessing these bioactive compounds from agricultural wastes not only aligns with the principles of green chemistry and circular economy, contributing to waste valorisation and sustainable resource management [6, 8], but also promotes the development of eco-friendly and economically viable processes for the production of bioactive-rich extracts with potential health and industrial benefits [9].

Schumannianthus dichotomus is a rhizomatous shrub belonging to the family Marantaceae growing in some low-lying wetland areas of India, Bangladesh, Thailand, Myanmar, and Malaysia [10]. In the Cooch Behar district of West Bengal, India, parts of Assam, India and many parts of Bangladesh, the plant is cultivated to produce a type of mat locally called "*Sital pati*" (meaning cool mat) which is popular for its utility, durability, and cultural significance. Apart from mats, the plant is used to create prayer seats, schoolbags, handbags, etc. [11, 12]. Craft preparation involves utilizing the stems and branches of the plant. The harvested stems, known as *bets*, are washed, and subsequently spliced using a sharp knife. Each *bet* is longitudinally split into two parts, referred to as *chittor*. The *chittor* can be further divided longitudinally into two or more segments, and these segments can be split lengthwise, resulting in two slips—the outer *saloi* used for crafting *bets* and the inner *maji* which is discarded (Fig. 1). This discarded part of the stem is sometimes used as a tying or thatching material, but is mostly dumped around the households and adjoining areas as waste [10].

Research on the physiochemical properties of the rhizome, stem and leaves of *Schumannianthus dichotomus* have highlighted the tensile strength of its epoxy composites [13], scavenging behaviour [14], adsorbent property [15] and potential for phytoremediation [16, 17]. The leaf and twig extracts of the plant has also shown remarkable phytotoxic activity and compounds like syringic acid, methyl syringate, schumannione, 8-(5-oxo-2,5-dihydrofuran-2-yl) octanoic acid (ODFO) and (E)-6-hydroxy-2,6-dimethylocta-2,7-dienoic acid (8-carboxylinalool) have been isolated in different studies [10, 18, 19]. Ethnobotanical studies report the use of the plant parts among the Dimasa tribe of Assam, India [20], Garo tribes of Meghalaya, India [21], Southern Thailand [22] and Bangladesh [23] to cure a variety of ailments like fever, urinary tract infections, stomach ache, ear ache, gingivitis and intestinal worms. Experimentally, the rhizome and leaf extracts have shown hepatoprotective, hypoglycaemic, anti-nociceptive and antipyretic effects [24, 25]. Most of the studies have used the rhizome and leaves for the extraction of phytochemicals. Among the few that have utilised the stem, none of the studies on the putative medicinal properties have used the discarded part of the stem for the extraction of bioactive components.

Parasitic worms, or helminths, are responsible for chronic and often fatal diseases with significant socio-economic ramifications worldwide [26]. Human infections caused by these worms affect approximately 14 million individuals

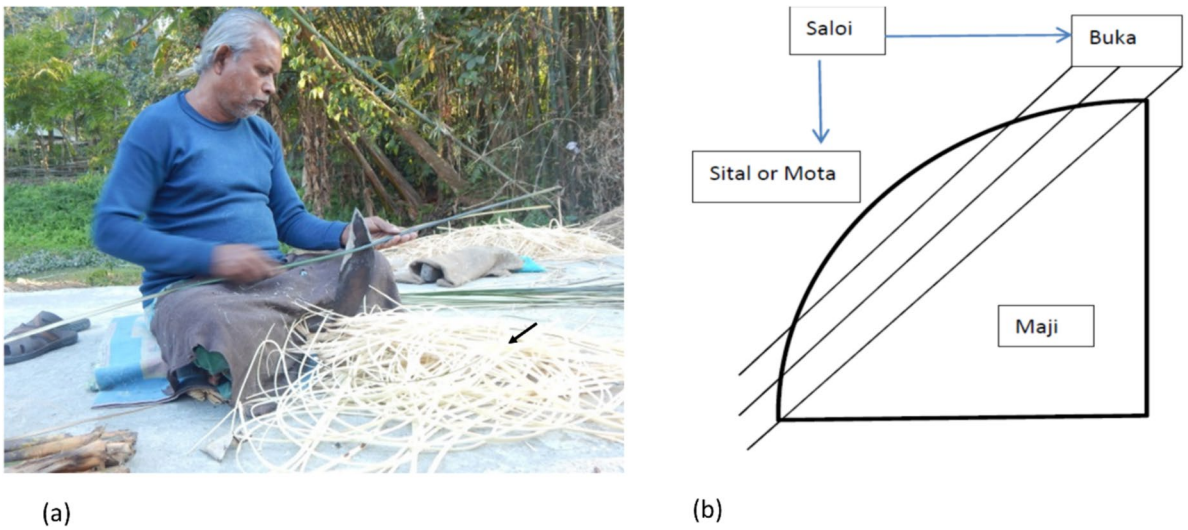


Fig. 1 **a** Photograph of a *Sitalpati* craftsman slicing the stem of *Schumannianthus dichotomus*. Arrow indicates the discarded/waste part of the stem **b** Cross section of a half *chittor* (half slice of the stem). *Saloi* is the external part used for handicrafts which may further be divided into the *sital/mota* part and the *buka* part. The inner part of the stem called *maji* is discarded

globally, collectively termed neglected tropical diseases (NTDs) [27]. In agricultural animals, parasitic diseases result significant economic losses annually on a global scale [28]. Domestic poultry, a primary source of dietary protein worldwide through meat and eggs, plays a crucial role in nutrition [29]. Despite advancements in poultry industry productivity, helminth infections caused by nematodes, cestodes, and trematodes remain significant challenges to production efficiency [30]. Cestodes, particularly *Raillietina* spp., account for 66% of global helminth infections, with *Raillietina* spp. being prevalent in numerous prevalence studies [31]. Pathogenicity associated with *Raillietina* infections in poultry includes intestinal villi disruption, degeneration and necrosis of intestinal epithelial cells, parasitic granulomas, enteritis, anemia, weight loss, and decreased egg production [32]. Accidental *Raillietina* spp. infections in humans have been reported due to the inadvertent ingestion of cysticercoid-infected ants or beetles [33, 34]. *Raillietina* spp. is therefore used as a model organism for the study of anthelmintic activity in many studies [35–42].Widespread use of over-the-counter anthelmintics has resulted in drug resistance in many animal populations [43]. In numerous underdeveloped regions, livestock producers lack access to basic veterinary services and medications, leading to the continued utilization of medicinal plants in conjunction with veterinary pharmaceuticals to treat animal ailments. Therefore, traditional, indigenous, or ethnomedicinal knowledge of local populations can offer valuable insights for the development of anthelmintic remedies derived from natural products and many compounds isolated from such studies have shown promising anthelmintic potential [44, 45].

Whole worm-based assays are frequently employed for the identification of novel anthelmintic compounds, evaluating features such as worm motility, morphological changes, ATP production, and enzyme activity [46, 47]. This study aims to explore the anthelmintic potential of the ethanolic extract derived from the discarded stem of *Schumannianthus dichotomus* through morphological and ultrastructural analyses. Additionally, phytochemical screening of the extracts seeks to elucidate the presence of bioactive compounds responsible for the observed anthelmintic activity. Validating the anthelmintic efficacy of the extract sourced from the plant's waste material, specifically the stem, holds promise for valorising this waste by recognizing its medicinal properties. This could lead to further avenues of research for drug discovery and applications, benefiting both local communities and global initiatives.

Materials and Methods

Collection of Plant Material and Preparation of Ethanolic Extract and its Different Fractions

The freshly discarded stem of *Schumannianthus dichotomus* was collected from the local craftsmen of the Ghughumari village (26.280552^0N, 89.461233^0E) of Cooch Behar district, West Bengal, India in March–April each year from 2018 to 2023. The plant was identified and preserved at the herbarium of the Department of Botany, University of North Bengal, Darjeeling, West Bengal with accession number 12339. Following collection, the plant materials underwent fine chopping, were air-dried for 24 h and then immersed in ethanol (100 g in 500 ml) for 15 days with regular stirring. The solution was filtered using Whatman Filter Paper (No. 14) and then subjected to drying through a Rotary Vacuum Evaporator (Buchi Rotavapor R-100). Post-drying, the crude extracts were stored under refrigeration at 4 °C until further use. Approximately 2–2.5 g of plant extract were obtained from 100 g of plant material soaked in 500 ml of ethanol.

To separate different fractions of the ethanolic extract, solvents with varying polarities such as Hexane, Chloroform, Ethyl acetate, and n-Butanol were employed using a separating funnel and fractional distillation process [41]. A 50 ml aqueous solution of crude extract (2.5 gm in 50 ml distilled water) was mixed with 50 ml of Hexane and allowed to stand for 2 h. The Hexane fraction was then separated from the upper phase using the liquid–liquid separation technique. This process was repeated thrice using Chloroform (lower phase), Ethyl acetate, and n-Butanol. The final volume of each fraction (150 ml) was collected and subsequently dried using a Rotary Vacuum Evaporator (Buchi Rotavapor R-100). From 2.5 g of crude extract, approximately 0.15 g, 0.19 g, 1.2 g, and 1 g of Hexane, Chloroform, Ethyl acetate, and n-Butanol fractions were obtained, respectively.

Collection of Parasites

Recently acquired intestines from *Gallus gallus domesticus* were sourced from a nearby market. Live worms, specifically *Raillietina* spp., were dissected out from the intestine, kept in 0.9% Phosphate-Buffered Saline (PBS) at pH 7.4 in petri dishes subsequently maintained in an incubator set at 37 ± 1 °C throughout the experiments. Worms with approximately the same weight (about 20 mg) and vigour were used for the experiments.

Motility Test on Parasites

The worms were initially divided into the following groups namely- control (C) (incubated in only PBS without plant

extract), praziquantel treated (PZ) (incubated in PBS with 0.05 mg/ml Praziquantel), and crude ethanolic extract treated (CEE) (incubated in PBS with increasing doses of the crude ethanolic extract viz. 5 mg/ml, 10 mg/ml, 20 mg/ml and 50 mg/ml respectively). The doses were selected and standardized by treating the worms with gradually increasing range of concentrations of the extract. We initially treated the worms at 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 5 mg/ml, then with 2.5 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml and then with 5 mg/ml, 10 mg/ml, 20 mg/ml and 50 mg/ml. The last range was selected based on lower and paralysis and death times of the worms. Praziquantel, a common anthelmintic drug, served as the reference drug in these experiments. Each petri dish had three worms and each group i.e. C, PZ, CEE (5 mg/ml), CEE (10 mg/ml), CEE (20 mg/ml) and CEE (50 mg/ml) had three replicates. The experiments were repeated 5 times on separate days. The parasites were monitored at a time interval of 1 h, 3 h, 6 h, 12 h and 24 h for recording paralysis and death. The evaluation of motility scores required inspecting the worms through a stereomicroscope, employing criteria derived from a technique used by Lorsuwannarat et al. (2014) [48] and described by Kiuchi et al. (1987) [49]. The scoring system comprised the following- 3: active movement throughout the entire body (indicating no paralysis), 2: movement observed in specific body parts (indicating partial paralysis), 1: immobile but moves when transferred to warm PBS at 40 °C (indicating complete paralysis) and 0: immobile and does not move even when transferred to warm PBS at 40 °C (indicating death). The same set of experiments were also performed with four more groups formed by incubating worms in PBS with equal doses (20 mg/ml) of the hexane fraction (HXF), chloroform fraction (CHF), ethyl acetate (EAF) fraction and n-butanol fraction (BUF) of the ethanolic extract respectively in PBS. The minimum effective concentration and most efficacious fraction were determined and worms threated with them were used for further examination through SEM and TEM.

Relative Motility (RM)

The relative motility (RM) values were calculated using the following formula [49]:

$$\text{Motility Index(MI)} = \frac{\Sigma nN}{N} \times 100$$

$$\%\text{RM} = \frac{\text{MI test}}{\text{MI control}} \times 100$$

where, n = motility score, N = number of tapeworms with the score of ''n''.

The worms in the control group (C) had an RM value of 100. The worms in the groups treated with drugs and extracts had RM values lower than 100. Lower RM values indicated stronger anthelmintic activity.

Survival Index

Survival indices reflect the proportions of viable tapeworms at a specific time following treatment. Worms assigned a motility score 0 were considered deceased, while those with scores of 3, 2, or 1 were considered alive. The survival indices were computed using the provided formula, with a survival index of 0 indicating the absence of surviving flukes.

$$\text{Survival Index (SI)} = \frac{\text{Number of live flukes}}{\text{Number of all flukes}} \times 100$$

IC_{50}

In our experiments, the IC_{50} value represents the concentration of extract needed to achieve 50% inhibition of parasite movement after a 6-h in vitro incubation in the drug and extracts. We used the ED50V10 Excel add-in program, which calculated IC_{50} values based on RM values at various concentrations of the extract [48].

Scanning Electron Microscopy (SEM)

For Scanning Electron Microscopy (SEM), the control and treated parasites underwent fixation in 10% Neutral Buffered Formalin (NBF) at 4 °C for 4 h. Subsequently, they were thoroughly washed in double-distilled water, subjected to dehydration through acetone grades, underwent critical-point-drying (CPD) using liquid CO_2, were coated with a layer of gold palladium, and finally examined using a JEOL-JSM-35 CF scanning electron microscope [50].

Transmission Electron Microscopy (TEM)

Both control and treated worms underwent fixation in Karnovosky's fixative, consisting of 4% paraformaldehyde and 1% glutaraldehyde, in 0.1 M sodium phosphate buffer (pH 7.4). Subsequently, they were processed for Transmission Electron Microscope (TEM), specifically the TECNAI G20 HR-TEM, using the standard method for detailed ultrastructural studies [51, 52].

Qualitative Test for Phytochemicals

The crude ethanolic extract was used for the qualitative detection of phytochemicals following standard protocols [53].

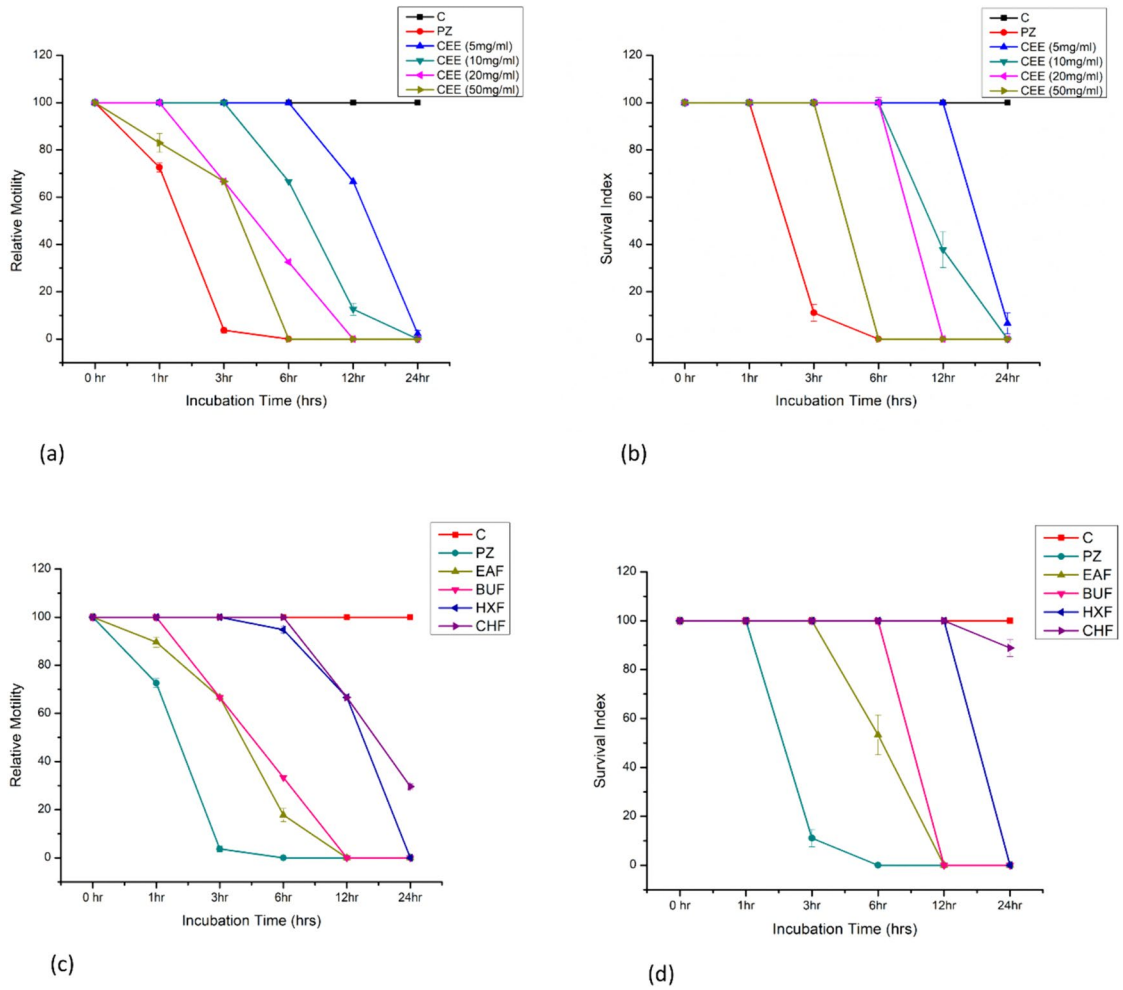


Fig. 2 Relative motility (RM) and survival index (SI) values recorded in *Raillietina* spp. after treatment with (**a**, **b**) different doses of crude ethanolic extracts of *Schumannianthus dichotomus* and (**c**, **d**) its different fractions at different time intervals after incubation (C = Control, Treated with: PZ = Praziquantel, CEE = Crude ethanolic extract, EAF = Ethyl acetate fraction, BUF = n-Butanolic fraction, HXF = Hexane fraction, CHF = Chloroform fraction). Data are expressed as mean ± SE

Test for Carbohydrates

Two drops of an alcoholic solution of α-naphthol to was added to 2 ml of plant sample extract. The mixture was shaken thoroughly and gradually a few drops of concentrated sulfuric acid (H_2SO_4) was added along the test tube's sides. The presence of a violet ring signifies the existence of carbohydrates.

Test for Proteins

The Biuret test involved mixing 2 ml of an aqueous solution of the extract with 1 ml of 40% NaOH and then adding 2 drops of $CuSO_4$. A violet colour indicates presence of proteins.

Test for Steroids

Steroid presence was detected by adding glacial acetic acid and concentrated sulfuric acid (H_2SO_4) to the aqueous extract, resulting in a green colour at the liquid interface.

Test for Phenols

For phenolic compounds, a 10 ml methanolic extract was mixed with 4–5 drops of 2% ferric chloride ($FeCl_3$) solution. A colour change in the solution, ranging from green to blue, signals the presence of phenolics.

Test for Alkaloids

A few drops of Mayer's reagent {prepared by dissolving 1.36 g mercuric chloride ($HgCl_2$) and 5 g potassium iodide

Table 1 Relative motility (RM) and survival index (SI) values recorded in *Raillietina* spp. after treatment with different doses of crude ethanolic extracts of *Schumannianthus dichotomus* and its different fractions at different time intervals after incubation (C = Control, Treated with: PZ = Praziquantel, CEE = Crude ethanolic extract, EAF = Ethyl acetate fraction, BUF = n-Butanolic fraction, HXF = Hexane fraction, CHF = Chloroform fraction). Data are expressed as mean ± SE

Treatment	Incubation Time									
	1 h		3 h		6 h		12 h		24 h	
	RM	SI	RM	SI	RM	SI	RM	SI	RM	SI
C	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
PZ	72.59 ± 1.89	100.00 ± 0.00	3.70 ± 1.17	11.11 ± 3.51	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CEE (5 mg/ml)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	66.67 ± 0.00	100.00 ± 0.00	2.22 ± 1.48	6.67 ± 4.44
CEE (10 mg/ml)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	66.67 ± 0.00	100.00 ± 0.00	12.59 ± 2.51	37.78 ± 7.54	0.00 ± 0.00	0.00 ± 0.00
CEE (20 mg/ml)	100.00 ± 0.00	100.00 ± 0.00	66.67 ± 0.00	100.00 ± 0.00	32.59 ± 0.74	100.00 ± 2.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CEE (50 mg/ml)	82.96 ± 3.99	100.00 ± 0.00	66.67 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
EAF	89.63 ± 2.16	100.00 ± 0.00	66.67 ± 0.00	100.00 ± 0.00	17.78 ± 2.72	53.33 ± 8.16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
BUF	100.00 ± 0.00	100.00 ± 0.00	66.67 ± 0.00	100.00 ± 0.00	33.33 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
HXF	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	94.81 ± 1.48	100.00 ± 0.00	66.67 ± 0.00	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CHF	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	66.67 ± 0.00	100.00 ± 0.00	29.63 ± 1.17	88.88 ± 3.51

(KI) in water, adjusting the volume to 100 ml)} were added to 2 ml of ethanolic extract in a test tube. The formation of a white creamy precipitate confirms the presence of alkaloids.

Test for Tannins

Ferric chloride ($FeCl_3$) solution (0.1%) was added to 10 ml of the ethanolic extract. A blue-black precipitate confirms presence of tannins.

Test for Saponins

Saponins were assessed by shaking 5 ml of the ethanolic extract with sodium bicarbonate ($NaHCO_3$) solution. The stable froth formation confirms the presence of saponins.

Test for Flavonoids

To identify flavonoids, 2 g the extract was extracted with 10 ml of ethyl acetate and 1 ml of 10% diluted ammonia (NH_3) solution was added and shaken. The development of a yellow colour indicated flavonoid presence.

Test for Terpenoids

The extract was combined with 2 ml of chloroform, followed by the careful addition of 3 ml of concentrated H_2SO_4 to form a distinct layer. A positive indication of the presence of terpenoids was observed as the interface developed a reddish-brown coloration.

Quantification of phytochemicals

The flowing procedures were followed for the quantitative estimation of the phenols, alkaloids and flavonoids in the crude ethanolic extract [53, 54]

Determination of Total Phenolic Content

The Folin-Ciocalteu method was used to calculate the crude ethanolic extract's total phenolic content (TPC). Gallic acid standard solutions containing 100–500 µg/ml of water were made. A standard or extract solution (1 mg/ml) was combined with 50 µl of distilled water. In a 96-well plate, this mixture was mixed with 50 µl of a 1 M sodium carbonate (Na_2CO_3) solution and 10% Folin-Ciocalteu's (F–C) phenol reagent. As a blank, distilled water was utilized. For 60 min, the reactions were incubated at room temperature with protection from light. A Multi-mode Reader (SYNERGY|LX, Biotek) was used to detect absorbance at 750 nm. The total phenolic content was expressed in Gallic Acid Equivalents (GAE) per mg of dried plant. All determinations were conducted in triplicate.

Table 2 Table showing pairwise results of Dunn's post hoc test with relative motility (RM) values values recorded in *Raillietina* spp. after treatment with different doses of crude ethanolic extracts of *Schumannianthus dichotomus* at different time intervals after incubation (C = Control, Treated with: PZ = Praziquantel, CEE = Crude ethanolic extract; * = value significant at $p \leq 0.05$). Only pairwise comparisons with C at each time interval is shown

Incubation Time	Doses Compared	Z statistics	p value (raw)	P value (Bonferroni corrected)
1 h	C-PZ	3.576	0.0003*	0.0052*
	C-CEE (5 mg/ml)	0	1	1
	C-CEE (10 mg/ml)	0	1	1
	C-CEE (20 mg/ml)	0	1	1
	C-CEE (50 mg/ml)	2.848	0.0044*	0.0660
3 h	C-PZ	3.924	0.0001*	0.0013*
	C-CEE (5 mg/ml)	0	1	1
	C-CEE (10 mg/ml)	0	1	1
	C-CEE (20 mg/ml)	2.453	0.0142*	0.2127
	C-CEE (50 mg/ml)	2.453	0.0142*	0.2127
6 h	C-PZ	3.745	0.0002*	0.0013*
	C-CEE (5 mg/ml)	0	1	1
	C-CEE (10 mg/ml)	1.404	0.1602	1
	C-CEE (20 mg/ml)	2.341	0.0192*	0.2887
	C-CEE (50 mg/ml)	3.745	0.0002*	0.0027*
12 h	C-PZ	3.859	0.0001*	0.0017*
	C-CEE (5 mg/ml)	0.965	0.3346	1
	C-CEE (10 mg/ml)	1.930	0.0537*	0.8047
	C-CEE (20 mg/ml)	3.859	0.0001*	0.0017*
	C-CEE (50 mg/ml)	3.859	0.0001*	0.0017*
24 h	C-PZ	3.892	0.0001*	0.0015*
	C-CEE (5 mg/ml)	2.676	0.0075*	0.1119
	C-CEE (10 mg/ml)	3.892	0.0001*	0.0015*
	C-CEE (20 mg/ml)	3.892	0.0001*	0.0015*
	C-CEE (50 mg/ml)	3.892	0.0001*	0.0015*

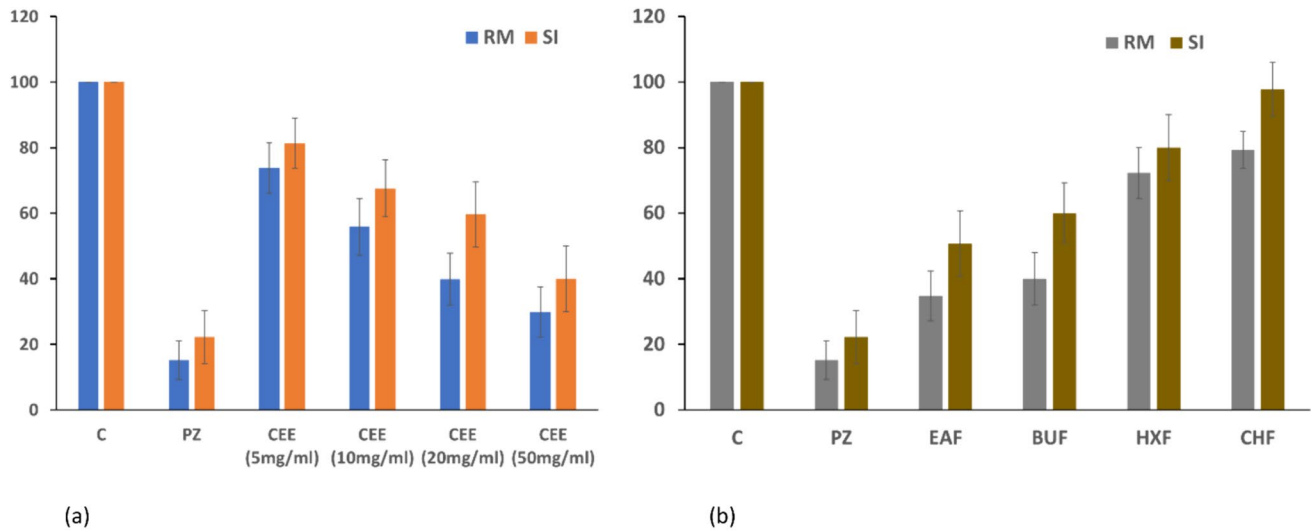


Fig. 3 Combined relative motility (RM) and survival index (SI) values recorded in *Raillietina* spp. after treatment with (**a**) different doses of crude ethanolic extracts of *Schumannianthus dichotomus* and (**b**) its different fractions at different time intervals after incubation (C = Control, Treated with: PZ = Praziquantel, CEE = Crude ethanolic extract, EAF = Ethyl acetate fraction, BUF = n-Butanolic fraction, HXF = Hexane fraction, CHF = Chloroform fraction). The RM and SI values from all time points of each dose were pooled and expressed as mean ± SE

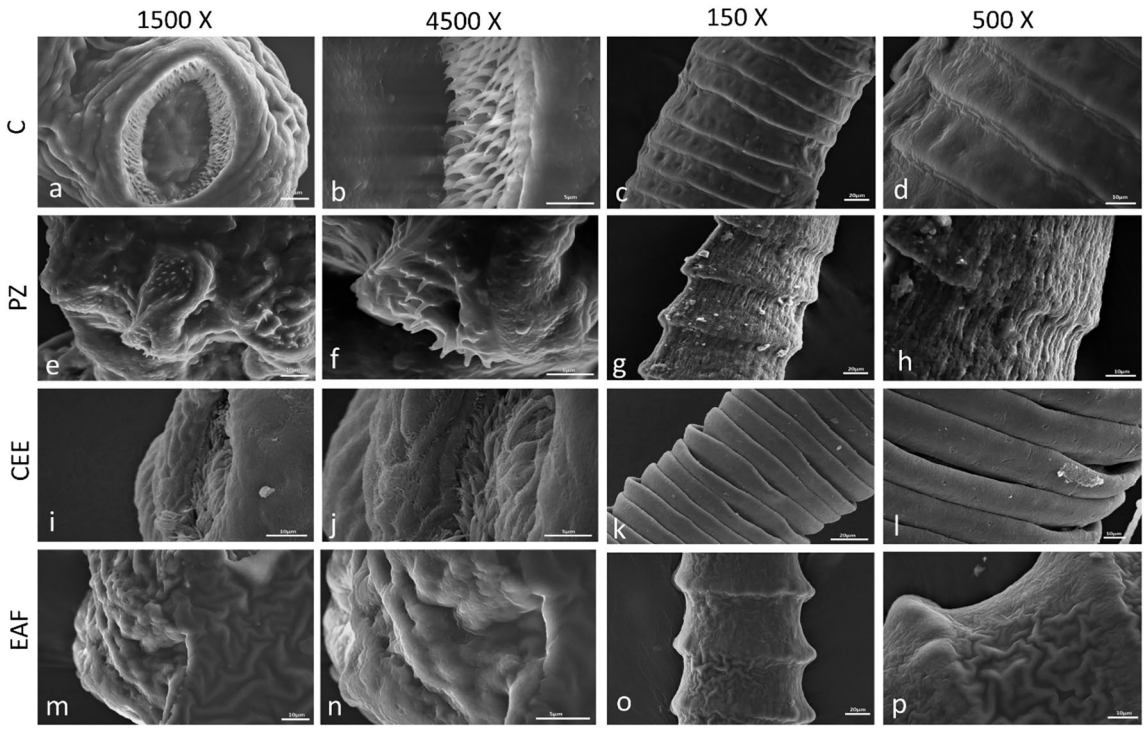


Fig. 4 SEM images of head showing the sucker region (a, b, e, f, i, j, m, n) and proglottids (c, d, g, h, k, l, o, p) of control and treated *Raillietina* spp at different magnifications. a–d: Control, e–h: PZ treated, i–l: CEE treated, m-p: EAF treated. Scale bars: a, d, e, h, i, l, m, p = 10 µm; b, f, j, n = 5 µm; c, g, k, o = 20 µm

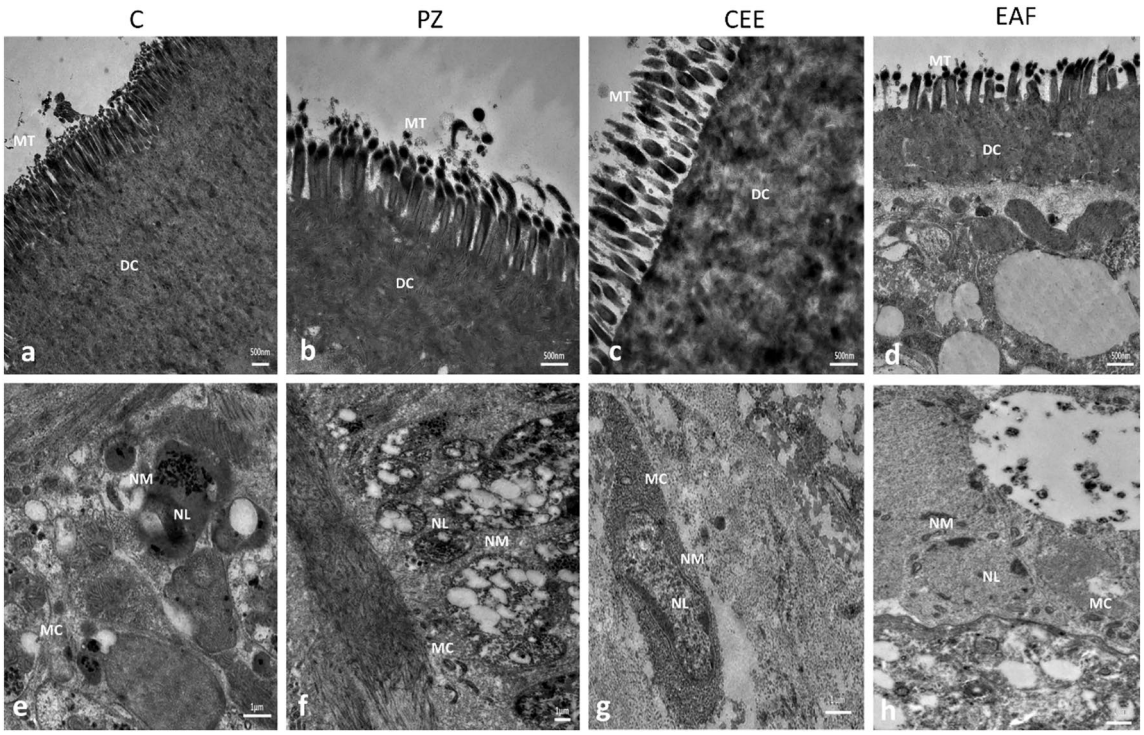


Fig. 5 TEM images of control (**a**, **e**) and treated (**b**–**d**, **f**–**h**) *Raillietina* spp. a–d: showing microtrix layer (MT) and distal cytoplasm (DC); e–h: showing nuclear membrane (NM), nucleolus (NL) and mitochondria (MC). Scale bars: a–d = 500 µm, e–h = 1 µm

Table 3 Results of the qualitative test for different phytochemicals present in the ethanolic extract of *Schumannianthus dichotomus* ("−" = absent, "+" = present, "++" = present in higher concentration)

Name of Phytochemicals	Tests	Present/Absent
Carbohydrates	Molish's Test	−
Proteins	Biuret Test	+
Steroids	Concentrated sulphuric acid test	+
Phenols	2% Ferric chloride ($FeCl_3$) test	++
Alkaloids	Mayer's test	++
Tannins	Ferric chloride ($FeCl_3$) test	+
Saponins	Foam test	+
Flavonoids	10% Diluted Ammonia Test	++
Terpenoids	Salkowski test	+

Determination of Total Flavonoid Content

To assess the total flavonoid content of (TFC) the crude ethanolic extract, the aluminium chloride colorimetric test was employed. In a nutshell, a mixture was prepared by combining 2 ml of distilled water, 0.15 ml of sodium nitrite solution (5% $NaNO_2$, w/v), and 0.5 ml aliquots of the extract and a standard solution (0.01–1.0 mg/ml) of rutin. After 6 min, 0.15 ml of a solution containing 10% aluminum chloride ($AlCl_3$, w/v) was added. Following an additional 6 min of standing time, 2 ml of sodium hydroxide solution (4% NaOH, w/v) was introduced into the mixture. The final volume was adjusted to 5 ml with the immediate addition of distilled water, ensuring thorough mixing, and left to stand for an extra 15 min. The absorbance of each mixture was measured at 510 nm using a multimode reader (SYNERGY|LX, Biotek), comparing it to a blank sample of the same mixture without the extract. TFC was determined using the rutin calibration curve and expressed as mg rutin equivalent per gram of material. All determinations were conducted in triplicate.

Determination of Total Alkaloid Content

The total alkaloid content (TAC) was assessed utilizing the reaction between alkaloids and bromocresol green (BCG). The plant extract (1 mg/ml), dissolved in 2 N HCl, was filtered. The pH of the phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. Subsequently, 5 ml each of the BCG solution and phosphate buffer were added to 1 ml of this solution of the plant extract in a separating funnel. Vigorous shaking facilitated the extraction of the complex with chloroform. After collecting the extract in a 10 ml volumetric flask, chloroform was used to dilute it to volume. The absorbance of the chloroform complex was quantified at 470 nm in a multi- mode reader (SYNERGY|LX, Biotek). All measurements were conducted in triplicate.

Table 4 List of compounds with SI, RSI, retention time and relative area identified from the GCMS analysis of ethyl acetate fraction of the ethanolic extract of *Schumannianthus dichotomus*

Sl No	Compound	SI	RSI	Retention Time (mins)	Relative Area (%)
1	Propanoic acid, 3-chloro-, 4-formylphenyl ester	743	915	11.381	2.23
2	2-Heptadecenal	515	882	13.102	2.76
3	Diethyl Phthalate	889	922	14.027	2.31
4	Phthalic acid, butyl hept-4-yl ester	818	933	16.228	2.55
5	Hexadecanoic acid, methyl ester	861	873	16.531	2.83
6	n-Hexadecanoic acid	825	870	17.517	13.01
7	2,6,10,14-Tetramethylpentadecan-6-ol	470	888	18.204	9.13
8	9-Eicosenoic acid, (Z)-	608	856	19.632	0.41
9	2-Heptadecenal	555	845	19.700	0.21
10	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	518	920	19.837	2.34
11	9-Eicosenoic acid, (Z)-	753	912	19.881	2.92
12	Octadecanoic acid	855	874	20.075	5.26
13	1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-7-nitro-	523	777	21.578	3.17
14	1,1,1,5,7,7,7-Heptamethyl-3,3bis(trimethylsiloxy)tetrasiloxane	510	911	21.677	3.11
15	Phthalic acid, di(2-propylpentyl) ester	910	921	23.710	38.83
16	Silane, diethylnonyloxypropoxy-	525	822	28.105	2.34
17	Squalene	911	920	29.438	6.58

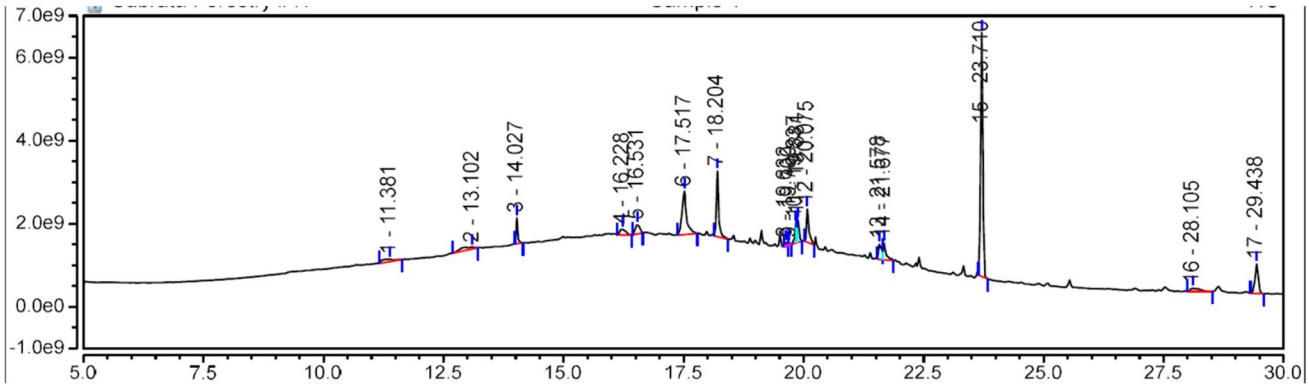


Fig. 6 Gas chromatogram of the ethyl acetate fraction of the ethanolic extract of *Schumannianthus dichotomus*

GCMS

The most efficacious fraction i.e. the ethyl acetate fraction of the ethanolic extract was further subjected to GCMS analysis following standard protocols [55]. An Auto System TRACE 1310 Gas Chromatograph (Thermo Scientific, US) coupled with an ISQ 7000 Single Quadrupole Mass Spectrometer (Thermo Scientific, US) was utilized, employing the electron impact ionization (EI) method. A fused silica capillary column with dimensions of 30 m length, 0.25 mm diameter, and a film thickness of 0.25 µm (TG1MS) was employed. Specific run conditions were set for the GC, with the injection port temperature programmed at 250 °C, and the column temperature ranging from 70 to 250 °C at a rate of 10 °C/min increase. Helium served as the carrier gas at a constant pressure of 100 kPa and a flow rate of 1 ml/min. Samples dissolved in methanol were thoroughly analyzed within the range of 60–550 amu. The obtained results were compared using the National Institute of Standards and Technology (NIST) 2017 Spectral library search program. Chemical compounds were identified using the NIST match factor or similarity index (SI) and reverse match factors or reverse search index (RSI) of the mass spectra available in the NIST GC–MS Library 2017. Identification followed the NIST library guidelines, which categorize match factor (SI) and reverse match factor (RSI) thresholds for mass spectral match as follows: > 900—excellent match, 800–900—good match, 700–800—fair match, and < 600—poor match.

Statistical Analyses

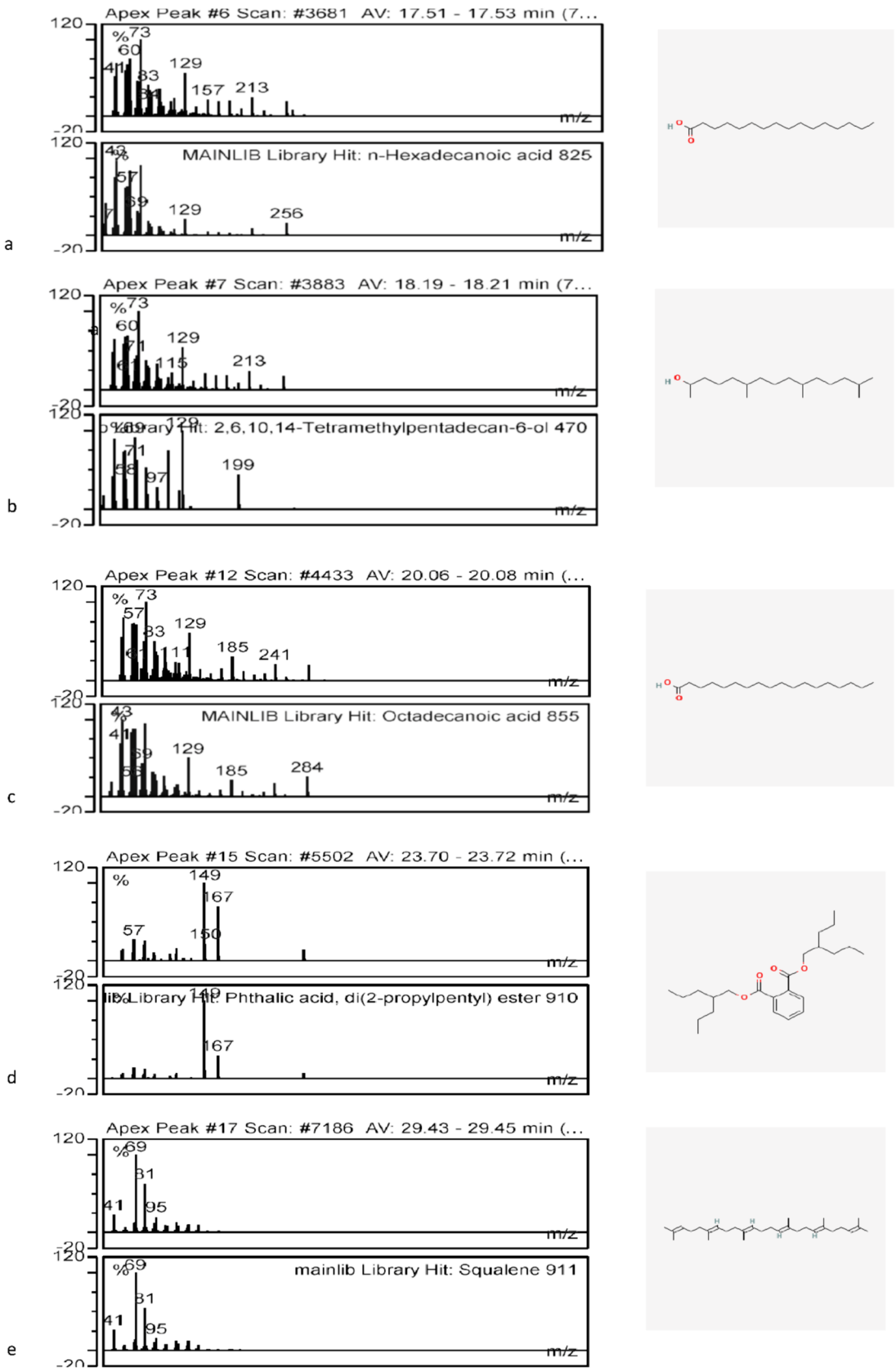
The motility experiment followed a completely randomized design with three replications and five repeats. Statistical analysis was performed using the R based software Jamovi (Version 2.2.5) [56]. The Kruskal Wallis test was conducted followed by Dunn's post hoc test at a significance level of 0.05 to assess differences among the RM values of different treatment and doses. The concentration needed for 50% growth inhibition (IC_{50} value) was determined using a regression equation calculated by the ED50V10 Excel add-in program.

Results and Discussion

Results of Motility Test on Parasites

As depicted in Fig. 2a, 2b and Table 1, all worms within the control group exhibited active movement and remained alive throughout the experimental duration (RM = 100, SI = 100). In the group subjected to 0.05 mg/ml praziquantel treatment, the flukes experienced reduction in RM values at 1 h and 3 h and become completely immobile at 6 h. All the other groups treated with increasing doses of the crude ethanolic extract viz. 5 mg/ml, 10 mg/ml, 20 mg/ml and 50 mg/ml started showing a gradual reduction in RM values at and after 12 h, 6 h, 3 h and 1 h respectively. Worms treated with 5 mg/ml of the extract were alive throughout the experimental duration. Worms treated with 10 mg/ml, 20 mg/ml and 50 mg/ml showed complete immobility at 24 h, 12 h and 6 h respectively. Correspondingly, SI values of praziquantel treated parasites reduced to 11.11 at 3 h and touched 0 at 6 h. Worms treated with 5 mg/ml, 10 mg/ml, 20 mg/ml and 50 mg/ml of the crude ethanolic extract started showing a gradual reduction in SI values after 12 h, 6 h, 6 h and 3 h respectively. Results of Kruskal Wallis test and Dunn's post hoc test indicate that significant difference in the RM values of the treated and control worms are observed at 6 h, 12 h and 24 h for CEE doses 50 mg/ml, 20 mg/ml, 10 mg/ml respectively (Table 2). PZ shows significant difference in RM values from C from 1 h. We considered 20 mg/ml as the most efficacious dose and used it for further studies.

The RM and SI values of the worms treated with 20 mg/ml each of hexane fraction, chloroform fraction, ethyl acetate fraction and n-butanolic fraction of the crude ethanolic extract are shown in Fig. 2c, d. It is clearly evident that the


Apex Peak #6 Scan: #3681 AV: 17.51 - 17.53 min (7...
MAINLIB Library Hit: n-Hexadecanoic acid 825
a
Apex Peak #7 Scan: #3883 AV: 18.19 - 18.21 min (7...
2,6,10,14-Tetramethylpentadecan-6-ol 470
b
Apex Peak #12 Scan: #4433 AV: 20.06 - 20.08 min (...
MAINLIB Library Hit: Octadecanoic acid 855
c
Apex Peak #15 Scan: #5502 AV: 23.70 - 23.72 min (...
Phthalic acid, di(2-propylpentyl) ester 910
d
Apex Peak #17 Scan: #7186 AV: 29.43 - 29.45 min (...
mainlib Library Hit: Squalene 911
e
m/z

◂**Fig. 7** MS spectra along with the reported structural formula of the five major compounds identified through the GCMS analysis of ethyl acetate fraction of the ethanolic extract of *Schumannianthus dichotomus* [**a**: n-Hexadecanoic acid, **b**: 2,6,10,14-Tetramethylpentadecan-6-ol, **c**: Octadecanoic acid, **d**: Phthalic acid, di(2-propylpentyl) ester, **e**: Squalene]

ethyl acetate fraction is more efficacious than the other three fractions.

The overall anthelmintic effect of the different doses of CEE as well as its different fractions is depicted in Fig. 3 where the RM and SI values of all the time points are pooled together. The absolute IC50 value was calculated to be 21.76 ± 0.07 mg/ml at 6 h of incubation time [34].

SEM Observations

In control worms, the scolex and proglottids exhibit a smooth surface adorned with dense microtriches, as illustrated in Fig. 4a–d. The suckers appear smooth with clearly defined hooks. Conversely, in worms subjected to the plant extract, discernible distortions manifest in the head region, characterized by tegument folding, eversion of the suckers, and the absence of encircling hooks. Within the proglottids, the tegument displays a folded and cracked appearance, as depicted in Fig. 4i–p. Comparable observations were also noted in worms treated with praziquantel Fig. 4e–h. Notably, the extent of damage is more pronounced in worms treated with both praziquantel and the ethyl acetate fraction.

TEM Observations

Transmission electron microscopic studies revealed that the control worm exhibited normal features across its surface, proximal and distal cytoplasm adorned with microtriches, basal lamina, nucleus enclosed by a double nuclear membrane, nucleolus, chromatin granules, and typical mitochondrial characteristics (Fig. 5a, e). However, parasites subjected to praziquantel treatment displayed severe damage in the microtrix layer and distal cytoplasm, accompanied by swollen nuclei featuring anomalous singlet nuclear membranes (Fig. 5b). Furthermore, these treated parasites exhibited deformed mitochondria lacking cristae (Fig. 5f). Similarly, exposure to the crude plant extract led to complete erosion of the distal cytoplasm, exposing the basal lamina of the microtriches to the outer surface (Fig. 5c). Additionally, disintegration of the nucleolus and observation of swollen nuclei with uneven nuclear membranes were noted. Most mitochondria in these treated parasites displayed deformities (Fig. 5g). *Raillietina* spp. treated with the ethyl acetate fraction exhibited similar detrimental effects, including fully damaged microtriches and distal cytoplasm, rupture of the nuclear membrane, condensation of chromatin granules, and disintegration with vacuolization of mitochondrial membranes (Fig. 5d, h).

Qualitative Test for Phytochemicals

The results depicted in Table 3 indicate the presence of a host of different phytochemicals including proteins, steroids, phenols, alkaloids, tannins, saponins, flavonoids and terpenoids. Judging by the intensity of the reaction products formed it was likely that phenols, alkaloids and flavonoids were present in relatively higher concentration.

Quantification of Phytochemicals

The phenolic compound content in the crude extract, expressed in gallic acid equivalent, was determined to be 262.99 ± 6.9 (SD) mg/g of crude extract, using the regression equation of the calibration curve ($y = 1347.6x—10.09$, $R^2 = 0.9834$). The concentration of flavonoids (mg/g) in rutin equivalent was found to be 816.41 ± 31.71 (SD), calculated from the regression equation of the calibration curve ($y = 5465.6x$, $R^2 = 0.9988$). Additionally, the total alkaloid amount, expressed in atropine equivalent, was determined to be 951.83 ± 86.85 mg/g of plant extract, utilizing the regression equation of the calibration curve ($y = 706.49x + 16.566$, $R^2 = 0.9815$).

GCMS

The initial gas chromatogram of the ethyl acetate fraction of the ethanolic extract of *Schumannianthus dichotomus* identified 64 compounds. However, an area percentage composition greater than 0.21% was used to analyse the results, which yielded a list of 17 compounds (Table 4 and Fig. 6). The identified compounds are mostly fatty acids and their esters, esters of dicarboxylic acids, and terpenoids. The five major compounds identified based on maximum relative area are Phthalic acid, di(2-propylpentyl) ester (38.83%), n-Hexadecanoic acid (13.01%), 2,6,10,14-Tetramethylpentadecan-6-ol (9.13%), Squalene (6.58%) and Octadecanoic acid (5.26%) (Fig. 7). The relative area of Phthalic acid, di(2-propylpentyl) ester (38.83%) is much higher in comparison to the other compounds.

Discussion

In our study, the worms treated with CEE exhibited a dose-dependent decrease in both RM and SI, suggesting the promising anthelmintic efficacy of *Schumannianthus dichotomus.* In comparison, the control worms endured significantly longer than those subjected to either praziquantel or plant extracts under identical conditions, which is reflected in the

greater RM and SI values recorded in the control worms. Similar results have been reported in *Raillietina* spp [57] and other parasites in similar experiments [48]. Notably, among the fractions of the ethanolic extract, those treated with the Ethyl acetate fraction displayed the most substantial decrease in RM and SI.

The tegument of cestodes serves as a multifunctional surface at the interface between the host and parasite, providing digestive, absorptive, and protective functions [58, 59]. Ultrastructural examinations have unveiled the presence of diverse microtriches throughout the scolex region and in both immature and mature proglottids, indicating their involvement in nutrient absorption across the entire body surface [60]. The efficacy of most anthelmintic drugs relies on their passive diffusion through this external tegumental layer. Consequently, alterations in tegumental ultrastructure serve as a dependable indicator of a compound's anthelmintic activity [61]. Scanning electron microscopy (SEM) studies have revealed distortions in the smooth texture of the tegument, as well as cracking and folding in treated worms, which are likely to impede nutrient uptake and energy metabolism in the parasite. Similar tegumental alterations caused by phytoproduct treatments have been documented by numerous researchers [35, 41, 42, 57, 62–64]. The TEM images reveal notable damage to various cellular components in the phytoproduct-treated worms, including the microtrich layer, nuclear membrane, chromatin, and mitochondria. These observations suggest that the phytoproducts disrupt the energy metabolism of the worms and impair the nucleus's function. Additionally, the plant extract distorts the surface topography and subcellular structures. These findings align with previous studies utilizing different plant extracts [39, 41, 42, 57, 65, 66].

Most of the products isolated from different plants that have shown anthelmintic activity either in vitro or in vivo mainly belong to the class of fatty acids, tannins, phenols, flavonoids, alkaloids and saponins [67–72]. Earlier studies carried out with the methanolic extract of the leaves and twigs of *Schumannianthus dichotomus* yielded two phenolics namely syringic acid and methyl syringate and a butanolide named Schumannione, all of which have shown phytotoxic potential [18, 19]. Another study conducted on the discarded stem of the plant identified two compounds with phytotoxic potential namely 8-Carboxylinalool, a cyclic monoterpene acid, and 8-(5-oxo-2,5-dihydrofuran-2-yl) octanoic acid (ODFO), a butanolide [10]. Out of the five major compounds identified in our study, two are fatty acids, and the other three are phthalate, terpenoid alcohol and triterpene respectively. Phthalic acid, di(2-propylpentyl) ester is a phthalate reported in extracts of bacteria [73, 74], fungi [75] and some plants [76–78]. The compound has reported cytotoxic [78] and anti trypanosomal activity [79]. Both n-Hexadecanoic acid and Octadecanoioc acid are commonly found in different plant extracts that have reported anthelmintic activity [80–85]. The anthelmintic activity of 2,6,10,14-Tetramethylpentadecan-6-ol, a terpenoid alcohol, is reported in a few plant extracts [86]. Squalene, a triterpene has been isolated in the ethanolic extract of an edible mushroom [87] as well as the anthelmintic decoction of pumpkin seeds [88]. So, the anthelmintic effect of the ethyl acetate fraction can be attributed to the presence of a combination phytochemicals with reported anthelmintic activity (Fig. 7). Phthalic acid, di(2-propylpentyl) ester may be the major bioactive component responsible for the anthelmintic activity and further studies are required to explore its anthelmintic potential.

Conclusion

The ethanolic extract and the ethyl acetate fraction derived from the discarded stem of *Schumannianthus dichotomus* exhibit notable anthelmintic activity. The crude extract is particularly abundant in phenols, alkaloids, and flavonoids, which may contribute to its biological activity. Phytochemical screening of the ethyl acetate fraction reveals higher concentrations of phthalates, fatty acids, and terpenoids, among which Phthalic acid, di(2-propylpentyl) ester emerges as a potential major bioactive component responsible for the anthelmintic activity. Our findings confirm the anthelmintic potential of *Schumannianthus dichotomus* extract. However, further investigations are warranted to precisely identify the active constituents of both the ethanolic extract and the ethyl acetate fraction, as well as to elucidate their mechanisms of action. This knowledge could facilitate the development of anthelmintic drugs. Moreover, there is potential in utilizing the discarded stem of the plant to formulate solutions for managing helminth infections in chickens, providing a sustainable, integrated, and localized approach to addressing the global issue of helminthiasis in livestock, particularly those reared through non-intensive methods by rural communities as a supplementary source of income. Additional biological activities of the waste can also be explored to valorize it to the full potential.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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